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Use of Microbiological Methods for Identification of Adulteration of Pure Onion Oil

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ONION oil is considered as an important medical constituent which effect against heart disease, common cold, diabetes, osteoporosis and osteocopic pain. Owing to the high pricing of onion oil, other vegetable oils are used as adulterants in it by dealers and retailers. Thus this study aimed to find a suitable method for detection of onion oil adulteration. Ten mixtures of pure onion and corn oils were investigated to identify adulteration of pure onion oil by following phytochemical assay, physical and chemical properties, as well as the microbiological methods. Results of qualitative phytochemical analysis indicated difficulty and not conformed adulteration of all mixed samples of onion oil. Physical and chemical properties didn't give clearly results. While measurement the diameter of inhibition zone by using some fungal and bacterial strains, as well as microbiological methods have a good results for detection adulteration. This study suggested that microbiological methods is the best and preferred for detection and information adulteration of some commercial onion oils compared with phytochemical analysis and determination of physical and chemical properties.

Keywords: Pure onion**,** Antimicrobial activity, Phytochemical analysis, Physical and chemical properties.

Introduction

Onion (*Allium cepa,* L.) is famous and an important member of the *Alliaceae* family. The genus of *Allium* contents more than 700 species widely grown all over the world (Tepe et al., 2005). Onion is a bulbous plant, cultivated in almost all countries, like India, China, Europe, North America, and also the Mediterranean region.Onion crop are the second most important horticulture after tomato, with current annual production about 101.5 million tones which are equal 9% from 1128 million tonnes of vegetables crops produced in the world. India is the top producer of onion, about 26.4 million tonnes (26%) of total world production (FAO, 2021). There are many kinds of onion, such as green, white, yellow, brown, and red. Onion is usually consumed as fresh, cooked, young green plant or bulbs and fermented also dried onion or extracted oil, is used as commercial products for food flavour.

Several researches reported that onion as a source for extraction the fatty acids and essential oils, like fresh onion bulbs (Benkeblia, 2004; Irkin & Korukluoglu, 2007; Kocic-Tanackov et al., 2012 and Umah et al., 2019), dried onion (Abd El-Salam et al., 2014), dried onion seeds (Topkafa, 2016; Senchi & EL-Inge, 2020), or leaves powder (Efiong et al., 2020). Also, dried onion wastes which include onion skins, two outer fleshy scales roots generated during industrial peeling or damaged bulbs can be used (Beniteź et al., 2011).

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Many researches extracted essential oils from onion can be carried out by different techniques. For examples, Benkeblia (2004); Abdel-Salam et al. (2014); Kocic-Tanackov et al. (2017) and Umah et al. (2019) used steam distillation. Topkafa (2016) extracted seed of onion oil by using a cold press machine, while Bello et al. (2013) and Eflong et al. (2020) used petroleum spirit and n-hexane, respectively. But, Irkin & Korukluoglu (2007) blended onion chopped with distilled water; ethanol and acetone, then filtered the resulted extract by using 0.45 µm pore size cellulose acetate membrane filter under nitrogen gas pressure.

Onion has large and many important medical properties which effective against common cold, heart diseases, diabetes, osteoporosis, cough sore throat, and osteocopic pain (August, 1996). It is rich in proteins, carbohydrates, sodium, potassium, and phosphorus (Lampe, 1999). Many researchers reported that onion extracts have pharmaceutical activities, such as antitumor, anti-diabetic, antioxidant, antimicrobial, anti-allergic, and molluscidal activity (Lampe, 1999; Helen et al., 2000 and El-Meleig et al., 2010). Also, onion flavonoids have beneficial heath effects including, anti-inflammation, anti-carcinogenic, anti-allergenic, antioxidant properties, vasodilatory, and cardio protective (Shon et al., 2004). As well as, reduction of the risk of chronic diseases such as cancer, coronary heart problems, and diabetes (Hirvonen, et al., 2001 and Kosmider & Osiecka, 2004). Onion can be considered as a good source of natural addives for characteristic of flavour and taste, also protection of foods from deterioration because it has activity effect against pathogens and spoilage microorganisms (Carbo & Santas, 2010). Corzo-Martinez et al. (2007) reported that the biological properties of onion and garlic as anticarcinogenic, antimutagenic, antiasthmatic, antimicrobial, antioxidant, prebiotic activity, and immunomodulatory. Cowan (2001) found that onion plants contain wide varieties of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids. Jeffrey & Herbert (2003) mentioned that onion bulbs contain a rich number of phytochemicals, most of them are hydrocarbons and their derivatives.

Onion is known for being a good natural flavonoids sources and kaempferol which are found as their glycosides (Fossen et al., 1998). The compounds of onion essential oil are formed from cysteine sulfoxide precursors (Krest & Keusgen, 2002). The biological effect of onion has been commonly ascribed to volatile sulfur containing compounds, like thiosulfinate, which is responsible for the properties of aroma and taste effect (Lanzotti, 2006). Corzo-Martinez et al. (2007) stated that garlic and onion have many biological and medical functions are due to their major organo-sulfur compounds content, such as S-alk(en)yl- L-cysteine sulfoxides and glutamyl cysteines, volatile compounds such as allicin and lipid-soluble sulfure. They added that the biological effect of garlic and onion dedicate to lectins, prostaglandins, fructan, pectin, adenosine, vitamin B_1, B_2, B_6, C, and E, biotin, nicotinic acid, essential amino acids, essential fatty acids, glycolipids, and phospholipids.Eja et al. (2007) mentioned that the volatile fractions of onion essential oil had 90 – 95% of their weight. It contains oxygenated derivatives like monoterpene and sequiterpene hydrocarbons, aliphatic aldehydes, alcohol, and esters. On the other hand, Vazquez-Amenta, et al. (2016) reported that the major volatile compounds in the onion essential oil were dipropyl disulfide (21.31 – 60.4%), dipropyl trisulfide (17.1 – 21.92%), and methyl-5-methylfuryl sulfide (18.3%).While the lower compounds were dimethyl teterasulfide (0.46 – 7.24%), dimethyl disulfide (1.31%), and dipropyl teterasulfide (4.04%).

Benkeblia (2004) extracted essential oils from three types of onion (green, yellow, and red) which were cultivated in Mascara, Algeria by steam distillation and compared their effect at different concentrations on two bacterial and three fungal strains by measure the diameter of the clear zone around disc paper. He found that inhibition zone increased with increasing the concentrations of extracts. Essential oil which was extracted from red onion has strongest effect than those of essential oil extract from green and yellow onion against all bacterial and fungal strains used in this study. Moreover, *Salmonella enteritids* was strongly inhibited compared with *Staphylococcus aureus*. While *Aspergillus niger, Penicillium cyclopium*, and *Fusarium oxysporum* were significally inhibited particularly at low concentrations. Pyun &Shin (2006) extracted essential oil onion by steam distillation and tested their effect on the growth of three fungal strains which belonged to *Trichophyten spp.* by determination of minimal inhibitory concentration (MIC). They found that MIC was 128 µg/mL for these fungal species. Irkin and Korukluoglu (2007) studied effect of three extracts from onion which was cultivated in Turkey

by extraction with water, ethanol and acetone against *Aspergillus niger* growth through determination of inhibitory zone diameters. They found that aqueous and acetone extracts did not show any effect on *A. niger* growth, while ethanolic extract has the highest inhibitory activity. Kocic-Tanackov et al. (2009) used essential oils which were extracted from two types of onion grow in Serbia by steam distillation system. They examined different concentrations from these extracts against growth of three yeast strains and three fungal strains. The inhibition zones of antimicrobial activity were measured with disc paper. They found that during increasing concentration increment the size inhibition zones. They indicated that *Eurotium amstelodami* was the most sensitive mould compared with *Aspergillus tamarii* and *Penicillium griseofulvum*. Santas et al. (2010) obtained methanolic extracts from three varieties of Spanish onion, two white and one yellow onion, then examined these extracts against growth of microbial activity. They found that methanolic yellow onion extract recorded higher diameter inhibition zones with *Listeria monocytogenes, Staphylococcus aureus,* and *Bacillus cereus*, respectively. While *Pseudomonas aeruginosa, Candida albicans,* and *Micrococcus luteus* haven't any inhibition zone with all methanolic extracts which come from two white and yellow onions. Kocic-Tanackov et al. (2012) studied effect of different concentrations of onion essential oils on the mycelium growth of *Aspergillus versicolor.* They mentioned that mycelium dry weight (g) decreased with increasing concentration of onion essential oils. They added that increasing incubation periods from 7 to 21 days has stronger inhibition effect on all fungal growth. El-Taweel (2013) found that increase concentration of essential oil, which extracted from onion blub, the diameter of inhibition zone of *Staphylococcus aureus* incremented. Mnayer et al. (2014) found that *Staphylococcus aureus*, *Listeria monocytogenes,* and *Salmonella typhimurium* were highly sensitive against onion essential oil compared with *Escherichia coli*. Kocic-Tanackov et al. (2017) extracted essential oils from fresh onion using distilled with Clevengen-type apparatus. They studied the effect of different concentrations of this extract on fungal growth namely, *Aspergillus*, *Fusarium,* and *Penicillium* species which were isolated from food. They mentioned that all concentrations used in this study caused the delay or absence of fungal growth, while *Penicillium sp.* growth was different inhibitory effect on the deceleration in the growth rate. Sadeghian et al. (2020) used yellow and white sweet Spanish onion which was cultivated in all over Iran, to obtained aqueous extracts and tested these extracts for dimeter inhibition zones measure with some microbial activity, include four positive and three negative gram bacteria as well as two strains of molds and two strains of yeasts. They found that yellow onion aqueous extract has highest inhibition zone with all examined microbes compared with white onion aqueous extract. While *Lactobacillus casei, Escherichia coli*, and *Pseudomonas aeruginosa* did not recorded any inhibition zones with white onion aqueous extract.This work was carried out to identify the adulteration of pure onion oil through microbiological methods, as well as physical and chemical properties of onion oil.

Materials and Methods

Materials

Pure onion oil was obtained from Al-Nasser Company for Drying Crops, Egypt.

Four commercial onion oil samples (A, B, C, and D) were purchased from Alexandria local markets. All samples were stored in the dark at -20 ˚C until using. Corn oil was purchased from local market for preparing different onion oils concentrations.

Six culture strains of fungi namely; *Aspergillus flavus* ATCC 5517, *Aspergillus niger* CAIM 147, *Aspergillus niger* DSM 731, *Aspergillus oryzae* NRRL 9362, *Penicillium sp.* and *Rhizopus arrhizus*CAIM 137 were used in this study. Precautions dealing with this kind of fungi were taken in consideration. Five culture strains of bacteria namely; *Escherichia coli* CAIM 193, *Klebsilla penumona* ATCC 12296, *Salmonella serifenbergy* ATCC 18400, *Staphylococcus aureus* NCTC 10783, and *Streptococcus pyogenes* DSM 1576 were used in this study.The strains ATCC were kindly given by the American Type Culture Collection (USA), CAIM from "Cairo Mircen Culture Collection" Microbiology Resource Center Faculty of Agriculture, Ain-Shams University, Egypt; DSM from Deutschs SammLung Von Mikroorganismen, Braunschweig, West Germany,the strain NCTC was brought from National Collection of Type Culture, London, United Kingdom; and NRRL from Northern Regional Research Laboratory (USA).

Methods

Preparation of samples

Different concentrations of corn oil were added to pure onion oil between 5% to 50% as shown in Table 1. The prepared samples were kept under -20 ºC until used.

TABLE 1. The percentage of mixture of corn and onion oils.

No. of Samples	S_I	S_{II}	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
Onion oil%	100	0.0	95	90	85	80	75	70	65	60	55	50
Corn oil%	0.0	100	5	10	15	20	25	30	35	40	45	50

Chemical methods

Physical and chemical characteristics

Physical properties including refractive index (RI) at 20ºC and specific density (SD) at 25°C were measured according to AOCS (1986). The chemical characteristics namely; iodine value(IV), saponification value (SV), acid value (AV) peroxide value (PV) and free fatty acid (%) were determined as described in AOCS (1986).

Phytochemical screening

Qualitative phytochemical components namely; flavonoids, triterpenoids, steroid, cardiac glycosides, and alkaloids (Prashanth & Krishnaiah, 2014), phenolic compounds, tannins, and saponin (Itelima et al*.,* 2016) were tested for all samples according standard methods.

Volatile compounds

Essential oils of onion were diluted in diethyl ester and analyzed to identify and quantify. The oil sample (5 µL) was injected into the gas chromatography (Hewlwett – Packed 5890/ mass spectrometry (Hewlwett - Packed 5989/ HP-GC/MS), apparatus. Qualitative analysis was based on the comparison of retention times and the computer mass spectra libraries using Wiley GC/MS Library and Nist, Tutore Libraries. The percentage composition was computed from the GC peak areas.

Microbiological methods

Culture media, maintenance, enumeration

The studied fungal strains were inoculated on slant of potato dextrose agar (PDA) recommended by Difco Manual (1984), then incubated at 30ºC for 72 hrs. The slants were maintained at -4°C until used. Bacterial strains were prepared by growing cells in slants of plate count agar (PCA) (Oxoid Manual, 1982), then incubated at 37ºC for 24 hrs. The slants were maintained at -4°C until used in this study. Spores suspensions of fungi and bacteria cells growing on and in slants were diluted with 0.1% sterilized peptone water for enumeration (De Moss and Bard, 1957). The number of spores suspension of fungi were between 10^5 to 10^6 CFU/mL, while the bacterial cells number in suspension were 10^2 to 10^3 CFU/mL.

Paper disk diffusion

The antimicrobial activity of pure onion oil, prepared mixture samples (corn and onion oils) and commercial samples of onion oils were evaluated by paper disk diffusion (Yin &Tsao, 1999; Hussain et al., 2011; and Efstratiou et al., 2012). One mL of spores' suspension of each fungal strain was added in a Petri dishe containing liquefied PDA. Also, one mL of cells of each bacterial strain suspension was incubated in PCA medium. Inoculum was evenly spread with media. Sterile 4 mm. filter paper discs (Whatman No.3) containing 30 µl of tested each oil samples were placed on the surface of media after solidification. Sterilized distilled water was taken as a negative control. Dishes containing PDA and PCA were incubated at 28ºC for 48 hr and 37 ºCfor 24 hr, respectively. After the end of incubation periods, the clear zones around the disc papers were recorded in millimeters as the diameter of inhibition zones (DIZ). Duplicate studies were performed for each sample.

Statistical analysis

Data were analyzed using the Statistical Analysis System software package (SAS, 2000). Analyses of variance were performed using ANOVA procedures. Significant differences between mean were determined using Duncan's multiple range test.

Results and Discussion

Ten mixtures of pure onion and corn oils were used in this study to identify adulteration of pure onion oils by following phytochemical analysis, physical and chemical properties compared with microbiological methods.

Phytochemical Screening

The qualitative phytochemical analysis of pure onion, corn oils mixtures and commercial onion

oils were analyzed. Results in Table 2 show that samples from S_{10} to S_7 which contained from 50 to 60% onion oils, had only three phytochemical components namely, steroids, cardiac glycosides, and saponins. These results conformed the work done by Dron et al. (1997). While the samples from S_6 to S_1, which contained from 70 to 95% onion oils and pure onion oil (S_I) included the same three phytochemical components beside flavonoid. Therefore, these results indicated that onion oil contained from 35 to 50% corn oil; adulteration was difficulty detected using determination of qualitative phytochemical assay. Efiong et al. (2020) extracted essential oils from three onion species (red, brown, and white) using soxhlet apparatus with n-hexane, they found that each of essential oils from red and brown onion had higher numbers of all phytochemical compounds namely, flavonoids, phenols, tannins, triterpenoids, steroids, cardiac glycosides, and saponins, but alkaloids was absent. While white onion oil contained four phytochemical compounds namely, steroids, cardiac glycosides, alkaloids, and saponins. On the other side, the same results in Table 2 show that commercial samples A, C, and D contained three phytochemical components namely, steroids, cardiac glycosides, and saponines like corn oil and samples from S_7 (35% corn oil) to S_{10} (50% corn oil), while sample B have only triterpenoids.

Physical properties

Table 3 shows that the refractive index of pure onion oil (S_I) was 1.558.These results agree with the results reported by Essentielles (2020). Who stated that refractive index of onion oil ranged between 1.550-1.580. The same results show that refractive index of corn oil (S_{II}) was 1.470 and the increment percentage of corn oil cause decrease refractive index of samples from S_1 to S_{10}.The results values of refractive index of commercial samples C and D recorded 1.470 and 1.471, respectively.But the values of samples A and B were lower than refractive index of all examined samples. These results revealed that refractive index show no significant difference ($p \leq 0.05$) in S_{II} (corn oil) and commercial sample C.While significant difference was found between S_I (onion oil) and all tested samples.

The same table showed that specific density of onion oil recorded 1.1041g/cm^3.This value is in agreement with results reported by Essentielles (2020).He found that specific density of onion oil was ranged from 1.010 to 1.050g/cm^3. On the other hand, Senchi and EL-Inge (2020) found that specific density of onion seed oil was 0.86 g/cm^3. The results in the same table revealed that increasing corn oil with onion oil from S_1 to S_{10} caused a gradual decrease in specific density. Statistical analysis of specific density values obtained from tested samples show relative relation between S_{10} (50% onion oil + 50% corn oil) and commercial samples C and D.While significant deference was found in S_I,samples from S_1 to S_{10} compared with all commercial samples.

Chemical properties

Iodine value is used to measure unsaturated fatty acid of oils. Table 4 shows that iodine value was 123.98 g/100g for onion oil (S_I) and 104.06 g/100g with corn oil (S_{II}).While iodine values ranged between 105.12 to 120.56 g/100g for samples from S_{10} to S_1, respectively. These results indicate that all tested samples are suitable for used with food. Noor and Ikram (2009) compared iodine values between cotton seed oil, corn oil, and sunflower oil, their values ranged between 99 - 119, 103 - 128, and 118 - 141 g/100g, respectively. These results are in agreement with iodine value of the corn oil in this study. On the other hand, results in the same table shows iodine values of samples A and B were 45.68 and 11.42 g/100g, respectively. While values of sample C was 101.52 g/100g and D was 114.21 g/100g. These results were near to mixture of onion and corn oil with S_5 (75% onion oil plus 25% corn oil)and S_4 (80% onion oil plus 20% corn oil). Statistical analysis indicated significant difference of iodine value in S_I with all commercial samples. While difference of iodine values were not significant in sample D compared with S_3 and S_4. Bello et al. (2013) found that iodine value of oil which was extracted from onion wastes and bulb onion by using Soxhlet method was 142.08 and 143.35 g/100g, respectively. These results agree with results in this work.But disagree with results by Senchi and EL-Inge (2020), they found that iodine value of onion seed oil was 98.13 g/100g.

Oils have high saponification values are not suitable for soap production (Bello et al., 2013). The results in Table 4 show that saponification value of S_I and S_{II} was 196.52 and 178.63 mg/g, respectively. Decreasing onion oil ratio, which was added to corn oil, decreases saponification value. These results indicated that saponification value of pure onion oil (S_I) was higher than value of sunflower oil (188 - 194 mg/g) and soybean

oil (192.3 mg/g) which mentioned by Falade et al. (2008). The results in this work (Table 4) showed that saponification value of samples A, C, and D were 106.10, 162.21, and 162.22 mg/g, respectively. These results indicated that these oil samples are not suitable for soap production and also out-off way from the results of all mixture samples used in the present study.Results showed that there was a significant difference in saponification value in S_I, $S_{II,}$ and mixture samples from S_1 to S_{10} and other commercial samples. On contrast no significant differences were found between commercial samples C and D. On the other side, Senchi &Elinge (2020) found that saponification value of oil which obtained from onion seed was 211.54 mg/g.

High acid value indicated that oils may be unstable for edible food at room temperature (Falade et al., 2008). The results in the present work shows low acid values for S_I, S_{II}, mixture samples from S_1 to S_{10} and commercial sample A. While samples C and D recorded the same value (2.011 mg/g). Accordingly, results of statistical analysis showed that there was a significant difference between acid values of S_I, S_{II}, and samples from S_1 to S_{10} with all commercial samples, while no significant differences ($p \leq 0.05$) between samples C and D. Senchi &EL-Inge (2020) stated that acid value in oil which extracted from onion seed by hexane was 5.13 mg/g. This result is ultimately different from all results in the present work.

TABLE 2. Phytochemical analysis of onion & corn oil mixtures and commercial onion oil samples.

No. of samples	Flavonoids	Phenolic compounds	Tannins	Triterpenoids	Steroids	Cardiac glycosides	Saponins	Alkaloids
S_I	+	-	-	-	+	+	+	-
S_{II}	-	-	-	-	+	+	+	-
S_1	+	-	-	-	+	+	+	-
S_2	+	-	-	-	+	+	+	-
S_3	+	-	-	-	+	+	+	-
S_4	+	-	-	-	+	+	+	-
S_5	+	-	-	-	+	+	+	-
S_6	+	-	-	-	+	+	+	-
S_7	-	-	-	-	+	+	+	-
S_8	-	-	-	-	+	+	+	-
S_9	-	-	-	-	+	+	+	-
S_{10}	-	-	-	-	+	+	+	-
A	-	-	-	-	+	+	+	-
B	-	-	-	+	-	-	-	-
C	-	-	-	-	+	+	+	-
D	-	-	-	-	+	+	+	-

S_I:Pure onion oil. S_{II}:Corn oil.

S_1 to S_{10}:Onion and corn oil mixtures. A to D: Commercial onion oils.

TABLE 3. Physical properties of onion, corn oil mixtures and commercial onion oil samples.

No. of samples	Refractive Index	Specific Density (g/cm^3)
S_I	1.558^a	1.1041^a
S_{II}	1.470^b	0.9144^b
S_1	1.518^c	1.0053^c
S_2	1.515^d	0.9976^d
S_3	1.511^e	0.9898^{ed}
S_4	1.504^f	0.9823^{fe}
S_5	1.498^g	0.9684^g
S_6	1.493^h	0.9598^g
S_7	1.485^i	0.9436^h
S_8	1.482^j	0.9380^{hi}
S_9	1.477^k	0.9284^j
S_{10}	1.473^l	0.9221^k
A	1.463^m	0.8815^l
B	1.455^n	0.8337^m
C	1.470^o	0.9239^{kn}
D	1.471^b	0.9237^{kn}

S_I:Pure onion oil. S_{II}:corn oil.
S_1 to S_{10}: Onion and corn oil mixtures. A to D:Commercial onion oils.
Values followed by the same letter in the same column are not significantly different ($p \leq 0.05$).

Eka et al. (2009) mentioned that peroxide value used for measures oil rancidity. They stated that values range between 20 to 40 meq/kg could result oil rancidity, while value below 10 meq/kg is characteristic for fresh and good edible oils. Table 4 showed that the results of peroxide value of the S_I (pure onion oil) and sample A recorded below 10 meq/kg. These results indicated that samples can kept for long time without deterioration, while S_{II} (corn oil) and samples from S_1 to S_{10} and also commercial samples C and D might be susceptible to rancidity. The data show significant differences in peroxide values between all tested samples and commercial samples.

Table 4 shows that free fatty acids of pure onion oil (S_I) were found to be 0.819%, which is higher than the value of corn oil (0.409%). Obviously in this study, the increase percentage of corn oil added to onion oil decrease values of free fatty acids. The results obtained from the same table revealed that free fatty acids value of sample a (0.827%) was near with value recorded with S_I.While the values of samples C and D was higher than all samples used in this work. The results show the same trend of significant differences of free fatty acid values in S_I, S_{II} and mixture samples compared with commercial samples, except sample A was no significant difference with S_I.

Accordingly, the results of measured physical and chemical properties revealed significant differences between of S_I, S_{II}, mixture samples compared with commercial samples.Senchi and EL-Inge (2020) stated that free fatty acids, determined as oleic acid, evidence for that oil was edibility or industrial uses. They found that free fatty acids value obtained from onion seed oil was 6.93% and this value was higher than the studied pure onion oil value used in this study and also compared with all commercial samples.

TABLE 4. Chemical properties of onion, corn oil mixtures and commercial onion oil samples.

No. of samples	Iodine value (g/100g)	Saponification value (mg/g)	Acid value (mg/g)	Peroxide value (meq/(meq/kg)	Free fatty acids %
S_I	123.98[a]	196.52[a]	1.162[a]	8.633[a]	0.819[a]
S_{II}	104.06[b]	178.63[b]	0.581[b]	10.424[b]	0.409[b]
S_1	120.56[c]	189.29[c]	0.870[c]	9.482[c]	0.614[c]
S_2	118.02[d]	188.72[d]	0.821[d]	9.555[d]	0.578[d]
S_3	115.48[e]	187.97[e]	0.813[d]	9.630[e]	0.572[ed]
S_4	114.95[f]	187.02[f]	0.791[e]	9.704[f]	0.557[f]
S_5	113.56[g]	186.56[g]	0.765[f]	9.843[g]	0.539[g]
S_6	111.97[h]	185.89[h]	0.732[g]	9.931[h]	0.515[h]
S_7	110.40[i]	184.36[i]	0.685[h]	10.102[i]	0.482[i]
S_8	108.87[j]	182.12[j]	0.660[i]	10.153[j]	0.465[j]
S_9	106.60[k]	180.92[k]	0.639[j]	10.267[k]	0.450[j]
S_{10}	105.12[l]	179.21[l]	0.601[k]	10.337[l]	0.423[k]
A	45.68[m]	106.10[m]	1.73[l]	5.407[m]	0.827[a]
B	11.42[n]	56.23[n]	-[m]	-[n]	-[l]
C	101.52[o]	162.21[o]	2.011[h]	15.476[o]	1.418[m]
D	114.21[ef]	162.22[o]	2.011[h]	36.118[p]	1.418[m]

S_I:Pure onion oil. S_{II}:Corn oil.
S_1 to S_{10}:Onion and corn oil mixtures. A to D: Commercial onion oils.
Values followed by the same letter in the same column are not significant different ($p \leq 0.05$).

Volatile compounds

Table 5 shows the concentrations % of volatile compounds identified by GC-MS analysis in pure onion essential oil. The results identify forty one constituents representing about 99.81% of the total essential oils. The highest compounds were di-2-propenyl trisulfide (21.54%), di-2-propenyl teterasulfide (12.02%), di-2-propenyl disulfide (11.06%), and methyl-2- propenyl trisulfide (9.35%). While lower percentage compounds were benzene, (1-butyloctyl) and benzene, (1-pentypheptyl) which recorded the same value (0.28%) followed by octanedioicacid-4-methoxy-dimethyl ester and teterahydrofuran-5-methoxy-2-methyl-2-phenyl (0.32 and 0.33%, respectively). Pyn & Shin (2006) and Romeilah et al. (2010) stated that major volatile compounds in onion essential oil were dipropyl disulfide (16.75%) and trans-propenyl trisulfide (12.46%). The lower concentrations were 2.18 - 0.79% for dimethyl trisulfide and 8.51 - 8.10% for methyl propyl trisulfide, respectively. Results mentioned by Romeilah et al. (2010) agree with percentage of volatile compounds found in Table (5). Kocic-Tanackov et al. (2012) identified twenty one compounds which obtained by hydro-distillation of onion essential oil using GC-MS analysis. They mentioned that the main components were dimethyl-trisulfide (16.64%), methyl-propyl trisulfide (14.21%), methyl-1-propenyl disulfide (13.41%) and methyl-1- propenyl trisulfide (13.02%). Mnayer et al. (2014) found that total compounds were thirty one constituents which represent more than 82.36%. They showed that major compounds were dipropyl-disulfide (30.92%), dipropyl-trisulfide (17.10%), 1-propenyl polydisulfide (7.26%), and methyl propyl-trisulfide (5.20%). Kocic-Tanackov et al. (2017) stated that onion essential oil contained twenty three volatile compounds and the major compounds were dimethyl-trisulfide and methyl-propyl-trisulfide. Generally, the major compounds obtained by previous investigators were different than some results in this work. This may be due

to difference of onion variety, environmental conditions of cultivated counters and extraction methods.

Effect of pure onion, corn oils, and their mixture samples as antimicrobial activity.

Physical and chemical properties of pure onion oil were compared with ten samples which contained different concentration of corn oil. The data obtained in this work doesn't clearly confirming the adulteration of pure onion oil. Therefore, this study depended on the use of some microbial strains to measure diameter inhibition zone (DIZ) of pure onion oil compared with mixture samples contained corn oil and also same commercial onion oils.

TABLE 5. Volatile compounds identified by GC-MS in onion essential oil.

Compounds	Concentration %
Dimethyl trisulfide	1.10
Disulfide, di-2-propenyl or Diallyl disulphide	11.06
Trisulfide, methyl 2-propenyl	9.35
4-Methyl-1,2,3-trithiolane	2.02
Tetrasulfide, dimethyl	1.12
Disulfide, methyl 1-(methylthio)propyl	1.10
Diallyl disulphide	1.85
Trisulfide, di-2-propenyl	21.54
5-Methyl-1,2,3,4-tetrathiane	6.40
Disulfide, methyl 2-propenyl	6.90
Disulfide, methyl 1-(methylthio)propyl	1.90
4-Ethyl-2,3,5,6-tetrathiaheptane	0.41
6-Methoxy-9-thia-bicyclo[3.3.1]non-2-ene	0.75
Tetrasulfide, di-2-propenyl	12.02
Disulfide, 1-(1-propenylthio)propyl propyl	0.95
Disulfide, methyl 1-(1-propenylthio)propyl	2.78
3,4-Dimethylthiophene-2-thiol	1.81
cis-Adamantane-2-carboxylic acid,4-hydroxy-	0.88
Benzene, (1-hexyltetradecyl)-	0.36
Octanedioic acid, 4-methoxy-, dimethyl ester	0.32
Benzene, (1-propyloctyl)	0.34
1,3,5-Trithiane	0.49
2,6-Diisopropylanisole	1.83
4-Ethyl-2,3,5,6-tetrathiaheptane	1.30
Benzene, (1-methyldecyl)-	0.92
Benzene, (1-pentylheptyl)-	0.28
Benzene, (1-butyloctyl)-	0.28
Benzene, (1-propylnonyl)-	0.56
Benzene, (1-ethyldecyl)-	0.39
Benzene, (1-methylundecyl)-	0.42
1-(2-Ethyl-[1,3]dithian-2-yl)-3-methyl-butan-1-ol	1.79
1-Allyl-3-(2-(allylthio)propyl)trisulfane	1.54
Methyl octadec-6,9-dien-12-ynoate	0.35
Methyl 9,11-octadecadiynoate	0.76
2-Ethylidene[1,3]dithiane	0.54
Tetrahydrofuran,5-methoxy-2-methyl-2-phenyl-	0.33
Benzene, (1-methyldodecyl)-	0.49
7,10-Octadecadienoic acid, methylester	0.45
10-Octadecenoic acid, methyl ester	0.47
4-Hydroxy-6-methyl-3-propylhexahydropyrimidin-2-thione	0.46
Phthalic acid, di(2-propylpentyl) ester	1.38

Table 6 shows antifungal activity of pure onion oil (S_I) and different samples which contained from 5% (S_1) to 50% (S_{10}) corn oil. The results exhibited that pure onion oil (S_I) has the highest inhibition effect on all tested fungal strains in the present study. The results show that increasing the percentage corn oil added to pure onion oil decreased diameter inhibition zones gradually. On the other hand, *Aspergillus niger* CAIM 147 was more sensitive to all samples from S_1 (95% onion oil) to S_{10} (50% onion oil). They recorded DIZ 21 and 8 mm. respectively, followed by *A. flavus* ATCC 5517 which recorded DIZ 20 mm. with S_1 and 6 mm. with S_9. While *A. oryzae* NRRL 9362 was less sensitive to all tested samples from S_1 (DIZ, 17 mm.) to S_9 (DIZ, 5 mm.). The same Table illustrated that *Rhizopus arrhizus* CAIM 137and *A. niger* DSM 731was not sensetive to samples S_9 (55% onion oil) and S_{10} (50% onion oil). Corzo-Martinezet al. (2007) and Kocic-Tanackov et al. (2012) stated that percentage of active major and minor sulfide compounds have antifungal activity. These compounds can be found in the onion essential oil high concentrations have major role as the antimicrobial activity. On the other side, some investigators confirmed that compounds present at low concentrations have the key role in antimicrobial activity due to synergistic effect with major compounds (Tajkarimi et al., 2010).

Antibacterial activity of pure onion oil (S_I) and mixture samples from S_1 to S_{10} against bacterial strains is presented in Table 7. Pure onion oil (S_I)has highly effect on each of *E. coli* CAIM 193 and *Salmonella serifenbergy* ATCC 18400 which recorded the same value of DIZ (25 mm.).Followed by *Staph. aureus* NCTC 10783 and *Strep. pyogenes* DSM 1576 which also recorded the same of DIZ (23 mm.). Also, results show that samples from S_8 (60% onion oil) to S_{10} (50% onion oil) were not resistant against growth of *E. coli* CAIM 193. On the other hand, the results illustrate that all bacterial strains (in Table 7) were more affected by all samples used in the present study than fungal strains (in Table 6). These results are in agreement with Wei et al. (1967), Sharma et al. (1979), Pruthi(1980) and Topal (1989).They reported that onion essential oil exhibits more inhibitor activity against bacteria than yeasts and fungi.

The results carried out by some researchers exhibited that same sulfide derivatives in onion essential oil had effect on microbial growth like methyl-5-methyl furyl sulfide, dipropyl disulgide, propyl disulfide, dimethyl trisulfide, and propyl trisulfide (Ye et al., 2013 and Mnayer et al., 2014). These results are agree with results present in table (5), which show that onion essential oil have high concentration of trisulfide di-2-propenyl (21.54%) and disulfide di-2-propenyl (11.06%). Ye et al. (2012) used three bacterial and six fungal strains to evaluate effect of onion essential oil on diameter inhibition zone (DIZ). They found that disc paper contained 100 µL of tested oil recorded 13.4, 17.4, and 13.2

TABLE 6. Antifungal activity of pure onion, corn oils, and their mixtures (30µL) against fungal strains (DIZ mm.).

No. of samples / Fungal strains	Diameter of inhibition zone (DIZ mm.*)											
	S_I	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{II}
Aspergillus flavus ATCC 5517	20	15	13	12	11	10	9	8	7	6	NI	NI
Aspergillus niger CAIM 147	21	19	17	15	14	13	13	12	11	11	8	NI
Aspergillus niger DSM 731	18	14	13	13	12	11	11	10	9	NI	NI	NI
Aspergillus oryzae NRRL 9362	17	13	12	11	10	10	8	7	6	5	NI	NI
Penicillium sp.	18	15	14	12	11	10	9	9	8	8	7	NI
Rhizopus arrhizus CAIM 137	19	18	17	15	14	13	11	11	10	NI	NI	NI

NI: No inhibition zone
***** Diameter of inhibition zone (DIZ) including disc paper diameter 4 mm.

mm. with *E. coli, Staph. aureus,* and *A. niger*, respectively. Their recorded DIZ values aren't near to the results presented in this work. El-Taweel (2013) extracted some bioactive constituents from onion essential oil bulb, then used methanol to obtaine dry-ness matter and use four concentrations of methanolic dry weight for measurement of inhibition zone with *Staph. aureus.* He found that 1000 µg/mL was higher susceptibility than other concentrations on inhibition zone, which reached to 29 mm.While the other concentration (1, 10, and 100 µg/mL.) of this methanolic suspenions of onion oil gave an inhibition zones of 24, 25, and 26 mm, respectively. Abdel-Salam et al. (2014) obtained that essential oil from dry red onion by ethanolic extraction and steam distillation method. They measured the diameter of inhibition zone (DIZ) by using different concentration (20, 40, and 60 µL) against some microorganisms. They found that increasing extracts concentrations increment the DIZ. The microorganisms exhibited higher susceptibility by hydro-distillation compared with ethanolic extract. The disc paper which saturated with 60 µL hydro-distillation onion oil recorded 15, 16, and 11 mm. with *E. coli, Staph. aureus,* and *A. niger*, respectively. Their results aren't agreement with results in this work.The DIZ are varied than the results of many investigators and some results in this study. This difference may be due to variation of fungal and bacterial strains, variety and onion kind, extraction methods, and concentration of onion essential oil which were used for determination of DIZ.

The present study examined the effect of commercial onion oil (30 µL) on the growth of six fungal and five bacterial strains. Table (8) shows the antifungal and antibacterial activity of the different onion oil samples brought from commercial marks on the diameter inhibition zone (DIZ). The results illustrated that sample A has inhibition effect on all fungal and bacterial strains used in the present work except *E. coli* CAIM 193. The data shows that sample A has the same DIZ of fungal and bacterial strains with sample S_8. These results indicate that sample A contained of 60% onion oil and 40% corn oil. The data in the same table shows that DIZ of samples B and C were near corresponding with the values of DIZ of sample S_5 of fungal and also bacterial strains which contained 75% onion oil plus 25% corn oil. The results showed also that sample D exhibited inhibitory effect on all fungal strains except *A. niger* DSM 731 and *Rhizopus arrhizus* CAIM 137 and bacterial strains except *E. coli* CAIM 193.Results of DIZ confirmed that sample D was corresponding with DIZ of sample S_9 which contained 55% onion oil and 45% corn oil.

Conclusion

Accordingly, above mentioned results, prove that microbiological methods used in this study are the best for information and detection for onion oil adulteration with some vegetable oils like corn oil.

TABLE 7. Antibacterial activity of pure onion, corn oils, and their mixtures (30µL) against bacterial strains (DIZ mm).

No. of samples / Bacterial strains	Diameter of inhibition zone (DIZ mm.*)											
	S_I	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{II}
Escherichia coli CAIM 193	25	24	22	20	19	16	15	13	NI	NI	NI	NI
Klebsilla penumona ATCC 12296	22	20	19	18	17	16	15	14	12	11	10	NI
Salmonella serifenbergy ATCC 18400	25	23	21	19	16	15	14	13	12	11	9	NI
Staphylococcus aureus NCTC 10783	23	22	21	19	17	15	14	14	13	8	8	NI
Streptococcus pyogenes DSM 1576	23	22	21	19	18	16	15	15	11	11	11	NI

NI: No inhibition zone

***** Diameter of inhibition zone (DIZ) including disc paper diameter 4 mm.

TABLE 8. Antimicrobial activity of commercial samples (30µL) against fungal and bacterial strains (DIZ mm).

Microbial strains \ No. of sample	Diameter of inhibition zone (DIZmm*)			
	A	**B**	**C**	**D**
Aspergillus flavus ATCC 5517	7	11	10	7
Aspergillus niger CAIM 147	10	13	12	11
Aspergillus niger DSM 731	9	11	12	NI
Aspergillus oryzae NRRL 9362	6	10	10	5
Penicillium sp.	9	10	11	8
Rhizopus arrhizus CAIM 137	10	12	13	NI
Escherichia coli CAIM 193	NI	15	14	NI
Klebsilla penumona ATCC 12296	11	14	16	10
Salmonella serifenbergy ATCC 18400	12	15	15	9
Staphylococcus aureus NCTC 10783	13	15	14	9
Streptococcus pyogenes DSM 1576	11	16	15	11

NI: No inhibition zone
***** Diameter of inhibition zone (DIZ) including disc paper diameter 4 mm.

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استخدام الطرق الميكروبيولوجية للكشف عن غش زيت البصل النقى

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يعتبر زيت البصل واحد من المركبات الطبية الهامة التى تساعد فى علاج العديد من الامراض مثل امراض القلب , نزلات البرد , الألم و هشاشة العظام. و قد أدى ارتفاع سعره الى لجوء بعض منتجيه و متداوليه الى غشه بخلطه ببعض الزيوت النباتية.

يهدف هذا البحث الى محاولة الكشف عن غش زيت البصل النقى باستخدام بعض الطرق الميكروبيولوجية مقارنة باستخدام تقدير و قياس كلا من الخواص الطبيعية و الكيماوية له. و لذا فقد تم تحضير عشر خلطات من زيت البصل النقى و المضاف اليه نسب مختلفة من زيت الذرة. و قد أجرى قياس و تقدير كلا من الخواص الطبيعية و الكيماوية لهذه الخلطات, و مقارنة ذلك بالنتائج المتحصل عليها بقياس قطر المنطقة المثبطة باستخدام بعض السلالات الفطرية و البكتيرية.

أظهر قياس قطر المنطقة المثبطة لنمو السلالات الفطرية و البكتيرية المستخدمة فى هذا البحث الى الحصول على نتائج أفضل للكشف و تحديد نسبة الغش لعينات زيت البصل النقى المضاف اليه زيت الذرة و ايضا لبعض العينات التجارية و هذا مقارنة بقياس و تقدير كلا من الخواص الطبيعية و الكيماوية لهذه العينات المختبرة.