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Bioremoval of Toxic Substances from Edible Oils as Affected by Deep-Fat Frying Process

Y.A. Elhassaneen, [1]H.A. El-Fadaly and N.E. Dewan
Department of Nutrition and Food Science, Faculty of Home Economics,
Minufiya University, Shebin El-Kom, Egypt
[1]Department of Microbiology, Faculty of Agriculture, Mansoura University, Mansoura, Egypt

Abstract: In the present study a trial was carried out to develop a new biotechnique for removal some toxic substances from edible oils as affected by deep-fat frying operation. Wastewater samples from Oil and Soap Company was used as a source of oil using bacteria and very simple technique for treatment the deep-fat frying oils with bacterial isolates was applied. A decrease in many toxic and/or carcinogenic compounds was observed in treated oils included free fatty acids (FFA), peroxide value (PV), malonaldehyde (MDA) and benzo(*a*)pyrene B(*a*)P contents by different rates. The rates of decreasing were increased with the increasing of oil concentration in cultural medium of used bacteria up to 15%. Spectroscopic analysis showed that many absorbance peaks 3420, 3120, 1750 and 970-1000 nm have been disappeared which means the removal of many corresponding toxic compounds included hydroperoxides, polymerization products, carbonyl groups or acids and *trans*-ethylenic double bonds of fatty acids as the result of treatment by *Bacillus firmus*. Results suggested that this biotechnique could be adequately coordinated with the commercial concern and the interests of society or the administration, which aim to improve consumer safety.

Key words: Bioremoval, toxic, carcinogenic, edible oils, deep-fat frying, *Bacillus firmus*

INTRODUCTION

Deep-fat frying represents an important method of food preparation of daily dishes in different countries, (including Egypt), either in home or in restaurants. During this process, several physical and chemical changes occur in the frying oil that may adversely affect on nutritional value and sanitation of foods. These changes include three general chemical reactions may occur simultaneously: hydrolysis, polymerization and oxidation, which produce a great number of potential toxic decomposition products deposit on the surface of the fryer and adsorbed by the food (White, 1991; Arroyo *et al*., 1992; Cuesta *et al*., 1993 and Rabie and Hassan, 1996). Oxidation of hot frying fat is much more important and results in rancidity, which result in undesirable and/or off-flavor food. In this connection, interest in the effect of malonaldehyde, one of the major products of the oxidation of polyunsaturated fatty acids, on human health has been reported by many authors that is mutagenic and carcinogenic (Mukai and Goldstein, 1976; and Shamberger *et al*., 1974). The mutagenicity of malonaldehyde has been demonstrated by the Ames *Salmonella* revertant procedure (Shamberger *et al*., 1979) while its carcinogenicity was observed when painted on the skin of mice (Shamberger *et al*., 1974).

Also, many authors have been paid more attention towards the formation and/or contamination of cooking oils with toxic and/or carcinogenic compounds. Amongst of them, polycyclic aromatic hydrocarbons (PAH) form a very extensive group of substances, which may constitute a significant public health problem. Some substances of this group such as benzo(*a*)pyrene have considerable carcinogenic effects by extensive *in vivo* and *in vitro* studies (Harvey, 1985; Plakunov *et al*., 1987; Hawkins *et al*., 1990; Elhassaneen, 1996; Elhassaneen, *et al*., 1997 and Elhassaneen, 2002). For human, one of the most abundant food sources of PAH is vegetable oil. Elhassaneen and Shaheen (1998) reported that the levels of B(*a*)P in deep-fat frying oil samples collected from the Egyptian restaurant ranged 1.24-2.01 mg kg^{-1} which represent a rate of increasing 526.7% when compared with the control samples. Also, Serag El-Din (2001) found that the fresh corn oil contained 0.21 mg kg^{-1} B(*a*)P, which increased to 2.139 mg kg^{-1} after subjected to deep fat frying operation for 12 h.

All of these data indicated that some products coming from oil degradation during frying and cooking show some toxic and/or carcinogenic effects. These harmful effects restricted the use of recycled cooking oils in formulated feed for animal production because they poses some risks for animal health and, as a consequence of bioaccumulation, for consumer health (Marquez-Ruiz *et al*., 1992; Abed El-Rahman, 1994; Marquez-Ruiz and Dobarganes, 1996 and Elhassaneen, 2002).

In the present investigation a trial was carried out to develop a new microbiological technology for the partial purification of frying oils which has been adequately

coordinated with commercial concern and the interests of society or the administration, which aim to improve consumer safety. Also, the effect of this new technology on the removal of some toxic, carcinogenic compounds formed in edible oils as the result of deep-fat frying operation will be in the scope of this investigation.

MATERIALS AND METHODS

Preparation of deep-frying potato samples: Fresh potato samples (*Solauum tuberosum*, L.) were prepared by deep frying in corn oil at 185±5°C on a laboratory scale such as described by Gordon and Kourimska (1995) as follow: potato slices (c 350 g) were cut into pieces approximately 40-50 mm×10 mm and fried in a pan filled initially with corn oil (4 litre). After heating the oil to 185±5°C the potato slices were added and fried for about 10 mints. The oil was allowed to cool to room temperature after each day of frying operation. Seventy two batches were fried with the oil during a 6-day period (twelve batches/day) and samples were removed for analysis at the end of frying operation. Tamia paste samples were prepared by deep frying in corn oil after cutting into pies approximately 5-7 cm by using the same previous method. Fresh oil was not added between batches, oil samples were cooled to the room temperature and used directly in microbiological treatments.

Microbiological procedures

Sources of oil using bacteria: Wastewater samples were collected from Oil and Soap Company of Sandoup, Dakahlia Governorate coded as OSCS. Samples were collected in one-liter sterile glass bottles for microbiological examination. The examination procedure was carried out according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1992). The physico-chemical properties of this waste was examined in our previous study (El-Fadaly *et al*., 2000).

Cultivation media for microbiology: The following general and specific cultivation media were used for different purposes according to the Bacteriological Analytical Manual for Foods (FDA, 1976). Tryptone glucose yeast agar and nutrient agar (TGY and NA), were used for isolation and total counting of the microbial load of the tested samples. Milk agar (MA), was used for isolation and counting of proteolytic bacteria. Fuchsin lactose agar (Endo C agar) was used to detect the bacterial members of the *Enterobacteriaceae*. Mannitol, Sodium Chloride phenol red agar (MSC), was specifically used for detection of *Staphylococcus sp.* Sabouraud glucose agar medium (SGA) was selectively used for fungi and yeast. Bactoagar F-medium (BFA) is recommended for the detection of *Pseudomonas spp.* Nutrient agar containing the olive oil was used for isolation and counting the lipolytic bacteria. The composition of these culture media was described in Oxoide (1982)

Isolation of obtained microorganisms naturally occurred in the wastewater containing oil: One ml sample of examined industrial wastewater containing oil dispersed in appropriate volume (9 ml) of distilled water and different serial dilutions were made with vigorous shaking. One ml sample was then taken and plated on different cultivation media and appropriate periods of incubation at 37°C according to the purpose required as mentioned above. For isolation the oil using bacteria, different serial dilutions were made with vigorous shaking. One ml sample was taken and plated on nutrient agar containing 1% olive oil at 37°C for 48h. After the incubation period, plates were flooded with concentrated Cu SO_4 solution, Bluish green colonies Surrounded by precipitates were detected as lipolytic bacteria (Mourey and Kilbertus, 1976).

Identification of obtained oil using bacterium: Preliminary identification of the obtained isolate was achieved by Gram reaction, spore formation, motility, capsule formation, as well as the presence of water soluble pigments. Further identification was also conducted following the biochemical tests recommended by Sneath *et al*. (1986).

Maintenance of selected strain: Selected bacterial strain was maintained on nutrient agar (NA) slant at 5°C till use. Prior to use, the microbial cultures were transferred to NA medium and reincubated again at appropriate temperature for 24 h. This process was repeated twice.

Inoculum preparation: Standard inoculum was prepared of bacterial strain by scrapping the growth on slope agar with 5 ml sterile distilled water and transferred into 50 ml appropriate sterilized liquid culture in 250 ml Erlenmeyer flasks which then allowed to grow for 24 h to obtain abundant growth 5 x10^5/ml to be suitable for inoculating the fermentation media for a require purpose.

Fermentation medium: The fermentation medium was prepared by mixing the tested oil with mineral solution containing (1 gl^{-1}): $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO4, $MgPO_4$ and peptone in ratios of 5, 10, 15 and 20%. Erlenmeyer flasks of 250 ml containing 100 ml oil mineral medium then autoclaved at 121°C for 20 min. after that, the experimental flasks inoculated with 10% (V/V) of standard inoculum of tested bacterial strain.

Analytical methods

Infrared spectroscopy: Infrared spectroscopy for oil samples were determined according to the method described by Farag *et al.* (1977). In brief, few drops of oil samples, dissolved in pure-dry carbon tetrachloride, were placed between sodium chloride plates and a slight pressure was applied to obtain a thin film. The sample spectrums (IR) were recorded over the range 4000-600 cm^{-1} using a Pye-Unicam double beam recording spectrophotometer model SP 3-300 infrared spectrometer.

Chemical characteristics of deep-fat frying oils: Peroxide value, free fatty acids and malonaldehyde content were determined according to the methods of A.O.A.C. (1985), Woyewoda *et al.* (1986) and Pearson (1970), respectively.

Polycyclic aromatic hydrocarbons were extracted from heated and deep-fat frying oils samples according to the method mentioned in A.O.A.C. (1995). Throughout this study a SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA, USA) was used with a pump Consta Metvic 4100, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector and a PC 1000 system software. A Hypersil BDS-C18, 5 μm (150 x 4.6 mm; Alltech, Baltimore, USA) column was used for standard and B(a)P samples. Benzo(*a*)pyrene, B(*a*)P standard (Sigma Chemical Co., SL) was dissolved in mobile phase at approximately 1 mg ml^{-1} stock solutions. Standard solutions made from the stock solutions contained approximately 0.5 to 1 mobile phase. Retention time for B(*a*)P standard were determined from the UV detector using isocratic elution program mentioned by Alltech Company catalog-400 (1997) as follow: mobile phase, acetonitrile : water (75:25); flow rate, 1.0 mL min^{-1} and detector, UV at 254 nm.

RESULTS

Microbiological value of tested oil waste: The examined waste containing oil showed a wide range of microorganisms showing high degree pollution of this waste. As shown in Table 1 the values of the total bacterial count was high being 186.6 cfu $x10^5$/ml of the tested waste using NA culture medium. This value became higher in case of using TGY medium being 210.5 cfu $x10^5$/ml. This may be explained by that this waste has pH value 7.5 as shown in our previously work (El-Fadaly *et al.*, 2000). The lipolytic bacteria showed to be the highest group amongst the other tested bacterial groups (Table 1). The same trend of results was previously obtained by El-Fadaly *et al.* (2000). Obtained results proved that the survival of microorganisms in wastewater varied according to the chemical composition, pH, temperature and suspended organic mater. All of these factors affect the microbial enzyme production. This may be explained by the chemical constituents of this waste science the pH value in the neutral range which is suitable for most organisms. Furthermore, the values of both alkalinity and hardness are favorable for most microbes. In addition, the values of sulfate, chloride and biological oxygen demand (BOD) are also help the microbes to survive in this waste (El-Fadaly *et al.*, 2000). On the other hand, both members of *Enterobacteriaceae* and *Staphylococcus spp.* were not detected in tested samples. This means that the conditions of this waste were not favorable to grow and survive such these pathogenic bacteria.

Table 1: Microbiological values of waste containing oil

Media used*	Examined microbes	Colony forming unit cfu x 10^5 / ml
Nutrient agar medium (NA)	Total count	188.3
Tryptone glucose yeast (TGY) extract medium	Total count	205.9
Oil agar medium (OA)	Lipolytic bacteria	155.8
Milk agar medium (MA)	Proteolytic bacteria	72.2
Starch agar medium (SA)	Amylolytic bacteria	35.7
Bacto-agar F-medium (BF)	*Pseudomonas spp*	175.6
Sabouraud glucose agar (SGA)	Fungi and Yeast	70.3 and 28.8

* The composition was as described in Oxoid (1982)

Table 2: Shape of bacterial isolates and their activities by means of MDA and FFA obtained in cultivation medium containing tested potato oil

Isolate no.		Cell shape	MDA mg/kg	FFA µmole/g
1	2	Spore former	0.210	22.618
2	3	Spore former	1.076	24.1297
3	6	Short rod	1.927	24.4920
4	8	Short rod	0.016	33.992
5	10	Coccoid shape	0.195	25.454
6	14	Short rod	8.635	24.689
7	15	Coccoid shape	0.351	25.883
8	23	Spore former	0.101	20.250
9	30	Coccoid shape	1.880	23.540
10	31	Spore former	0.156	35.958

Examination of oil using bacteria: From the proceeding Table, one can noticed that 155.8 isolates obtained from tested waste were able to use oil as a carbon source. A screening program was carried out to select the most potent isolates using oil agar plates. Ten isolates only showed a bluish green color around them. These ten isolates were microscopically examined and obtained results recorded in Table 2. Of these isolates, the spore-forming bacteria showed to be 40% of the total isolate. Either the short rods or coccoid shaped bacteria represent 30% of the total isolate.

Table 3: Morphological and biochemical characteristics of selected bacterial isolate

Performed test		OSCS 23
Motility		+
Spore formation		+,E,C,SNS
Capsule formation		-
Cram reaction		+
Cell dimension (um)		0.9 x 3.0
Tolerance of Nacl (%)		
	2.0	+
	5.0	+
	7.0	+
	10.0	-
Temperature (°C): 5		-
	10	-
	30	+
	37	+
	50	+
	55	+
	65	+
Indole production		-
VP		-
MR		+
Gelatin liquefaction		+
Casein hydrolysis		+
Starch hydrolysis		+
Citrate utilization		-
Propionate utilization		-
Catalase production		+
Urease		-
DNA ase		-
Lipase		+
Gas From glucose		-
Water soluble pigment		-
Growrth in N Broth at PH		
	5.7	-
	6.8	+
Acid from	Glucose	+
	Arabinose	-
	Xylose	-
	Mannitol	+
	Sucrose	+
	Lactose	+
NO_3¬NO_3		-

E,Spore ellipsoidal C,spore center SNS, Sporangium not swollen

Selection of the most potent isolate: Regarding the activity of tested bacteria in using potato oil and the ability to use it as C-source, an experiment was carried and obtained data were also listed in Table 2. Both values of free fatty acids (FFA) and malonaldehyde content (MDA) were measured after 48 h incubation period in a medium containing potato oil. From tabulated data, it could be seen that the isolate No. 23 showed to be the most effective isolate in recycling the used oil. So this sporeformer isolate was selected for further study.

Identification of the most potent isolate: After the microscopic examination, Gram-stain motility test, capsule formation, cell dimension and spore formation were also conducted. In addition, relying upon the standard identification key of Sneath *et al.* (1986), it could identify this isolate OSCS 23 as *Bacillus firmus* as recorded in Table 3. El-Fadaly *et al.* (2000) isolated three strains of the genus *Bacillus* and three strains of the genus *Pseudomonas* from the same source. Their results proved the abilities of these strains to grow on oil containing medium. These strains also showed high activities of lipolytic enzymes.

Effect of bacteriological treatments on chemical characteristic of tested oil: Results in Fig. 1 showed the effect of bacterial treatments on the FFA of corn oil as affected by deep-fat frying process for 12 h. From such data it could be noticed that the FFA content of deep-fat frying oils of heated, potato and tamia were recorded 0.831, 0.708 and 0.763 μg g^{-1} oil, respectively. After treated with *Bacillus firmus*, these values were decreased by different rates ranged 5.05-45.13, 55.79-70.20 and 45.09-51.64% (10% oil concentrations in medium), respectively. These rates were increased with the increasing of oil concentration in medium. When the oil concentration was 15% the rates of decreasing were 25.78-50.18, 30.22-70.48 and 20.32-64.35% for heated, potato and tamia deep fat frying oils, respectively. Therefore, the highest rate of decreasing in FFA content was recorded for potato samples followed by tamia and heated samples, respectively. Also, significant decreasing in FFA contents were observed in all studied incubation periods. The same behavior was relatively observed for PV (Fig. 2).

Bacterial removal of some toxic compounds from tested oil: Some experiments have carried out to examine the efficiency of removal of toxic and/or carcinogenic compounds including malonaldehyde (MDA) and benzo(*a*)pyrene [B(*a*)P] by tested bacterial strain. Results in Fig. 3 showed the effect of bacterial removal treatments on the MDA content of corn oil as affected by deep-fat frying process for 12 h. From such data it could be noticed that the MDA content of deep-fat frying oils of heated, potato and tamia were recorded 8.933, 8.503 and 7.957 mg kg^{-1}, respectively. After treated with *Bacillus firmus* these values were decreased by different rates ranged 31.31-56.81, 33.33-57.38 and 24.90-52.36% (10% oil concentration in medium), respectively. These rates were increased with the increasing of oil concentration in medium. When the oil concentration was 15% the rates of decreasing were 45.18-65.12, 29.64-44.93 and 30.56-56.28% for heated, potato and tamia deep fat frying oils, respectively. Also, significant decreasing in MDA contents were observed in all studied incubation periods. The same behavior was relatively observed for the other toxic and carcinogenic compound i.e. B(*a*)P as shown in Fig. 4. It is clear from such data that the hesitation observed in the values of MDA and B(*a*)P may be due to the growth of used bacteria in new medium containing

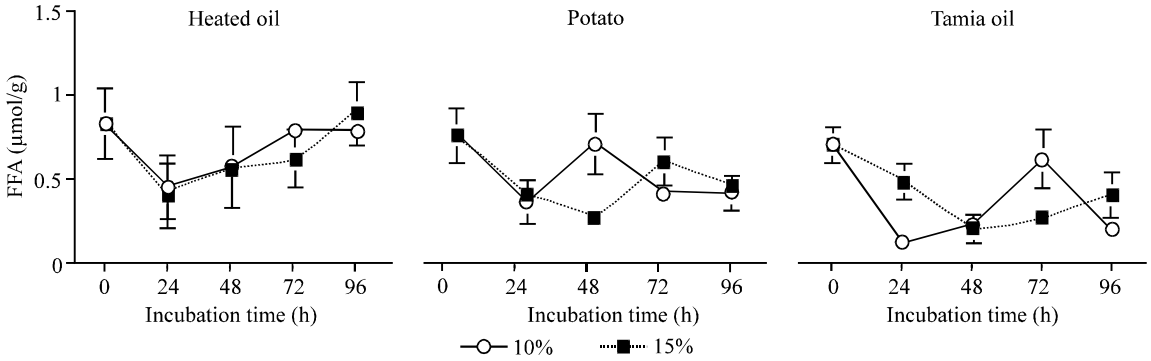

Fig. 1: The effect of bacterial treatment on removal of the free fatty acids (FFA, µmole/g oil) formed in corn oil as result of deep-fat frying process for 12h*

*Potato slices (350 g) were cut into pieces approximately 40-50 x 10 mm while tamia paste were cut into discs approximately 50 mm in diameter and fried in a pan filled initially with 4 liters corn oil. After heating the oil to 185±5°C the potato slices were added and fried for about 10 min/batch. The oil was allowed to cool to room temperature after each day of frying operation seventy two batches were fried with the oil during 6-day period (twelve batches/day). At the end of frying operation, the rest oil was incubated with different bacterial isolates for 24, 48, 72 and 96 h. After that the FFA content was determined such as described in material and methods

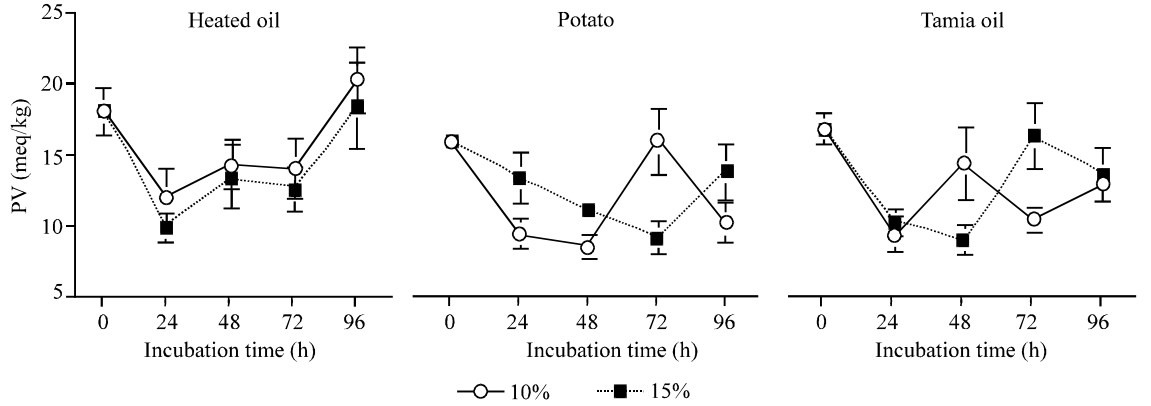

Fig. 2: The effect of bacterial treatment on removal of the peroxides (PV, meq/kg) formed in corn oil as result of deep-fat frying process for 12h*

*Potato slices (350 g) were cut into pieces approximately 40-50 x 10 mm while tamia paste were cut into discs approximately 50 mm in diameter and fried in a pan filled initially with 4 liters corn oil. After heating the oil to 185±5°C the potato slices were added and fried for about 10 min/batch. The oil was allowed to cool to room temperature after each day of frying operation seventy two batches were fried with the oil during 6-day period (twelve batches/day). At the end of frying operation, the rest oil was incubated with different bacterial isolates for 24, 48, 72 and 96 h. After that the PV was determined such as described in material and methods

these toxic substances. Nevertheless, all values detected for these parameters either up or down, they were in the area below the base line i.e. control samples.

Effect of bacteriological treatments on spectroscopic analysis of tested oil: The spectra showed that the heated control samples (Fig. 5) recorded bands at 3120, 1750, 970-1000 and 680-720 nm which means the formation of polymerization, carbonyl groups or acids, trans-ethylenic double bonds and *cis* isomer of fatty acids. After bacteriological treatments by tested bacterial strain for 24 h the peaks indicating the polymerization and carbonyl groups or acids were removed. The same behavior was observed for the tamia samples such as shown in Fig. 7.

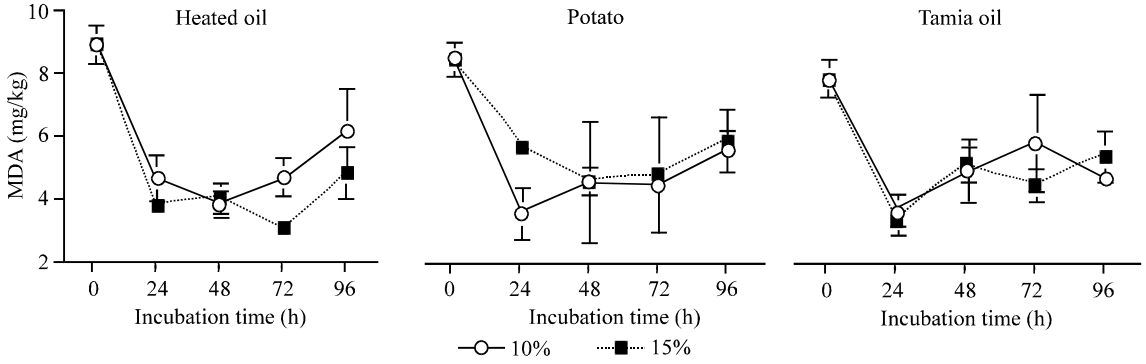

Fig. 3: The effect of bacterial treatment on removal of the malonaldehyde (MDA, mg/kg) formed in corn oil as result of deep-fat frying process for 12h*

*Potato slices (350 g) were cut into pieces approximately 40-50 x 10 mm while tamia paste were cut into discs approximately 50 mm in diameter and fried in a pan filled initially with 4 liters corn oil. After heating the oil to 185±5°C the potato slices were added and fried for about 10 min/batch. The oil was allowed to cool to room temperature after each day of frying operation seventy two batches were fried with the oil during 6-day period (twelve batches/day). At the end of frying operation, the rest oil was incubated with different bacterial isolates for 24, 48, 72 and 96 h. After that the MDA content was determined such as described in material and methods

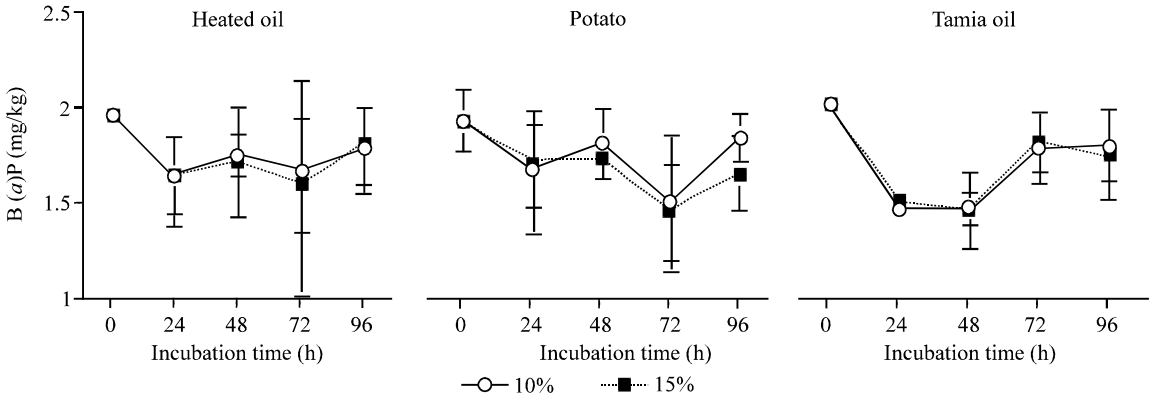

Fig. 4: The effect of bacterial treatment on removal of the benzo (*a*) pyrene [B(*a*)P, mg/kg] formed in corn oil as result of deep-fat frying process for 12h*

*Potato slices (350 g) were cut into pieces approximately 40-50 x 10 mm while tamia paste were cut into discs approximately 50 mm in diameter and fried in a pan filled initially with 4 liters corn oil. After heating the oil to 185±5°C the potato slices were added and fried for about 10 min/batch. The oil was allowed to cool to room temperature after each day of frying operation seventy two batches were fried with the oil during 6-day period (twelve batches/day). At the end of frying operation, the rest oil was incubated with different bacterial isolates for 24, 48, 72 and 96 h. After that the B(*a*) P content was determined such as described in material and methods

In related to potato samples (Fig. 6), the spectra of the control samples recorded one band at 3420 nm more than recorded in heated ones which means the formation of hydroperoxide. After bacteriological treatments by *Bacillus firmus* for 48 and/or 96 h the peaks indicating the hydroperoxide and carbonyl groups or acids were disappeared.

DISCUSSION

Deep-fat frying represent an important method of food preparation of daily dishes either in home or in restaurants. During this process, several physical and chemical changes occur in the frying oil that may adversely affect on nutritional value and sanitation of

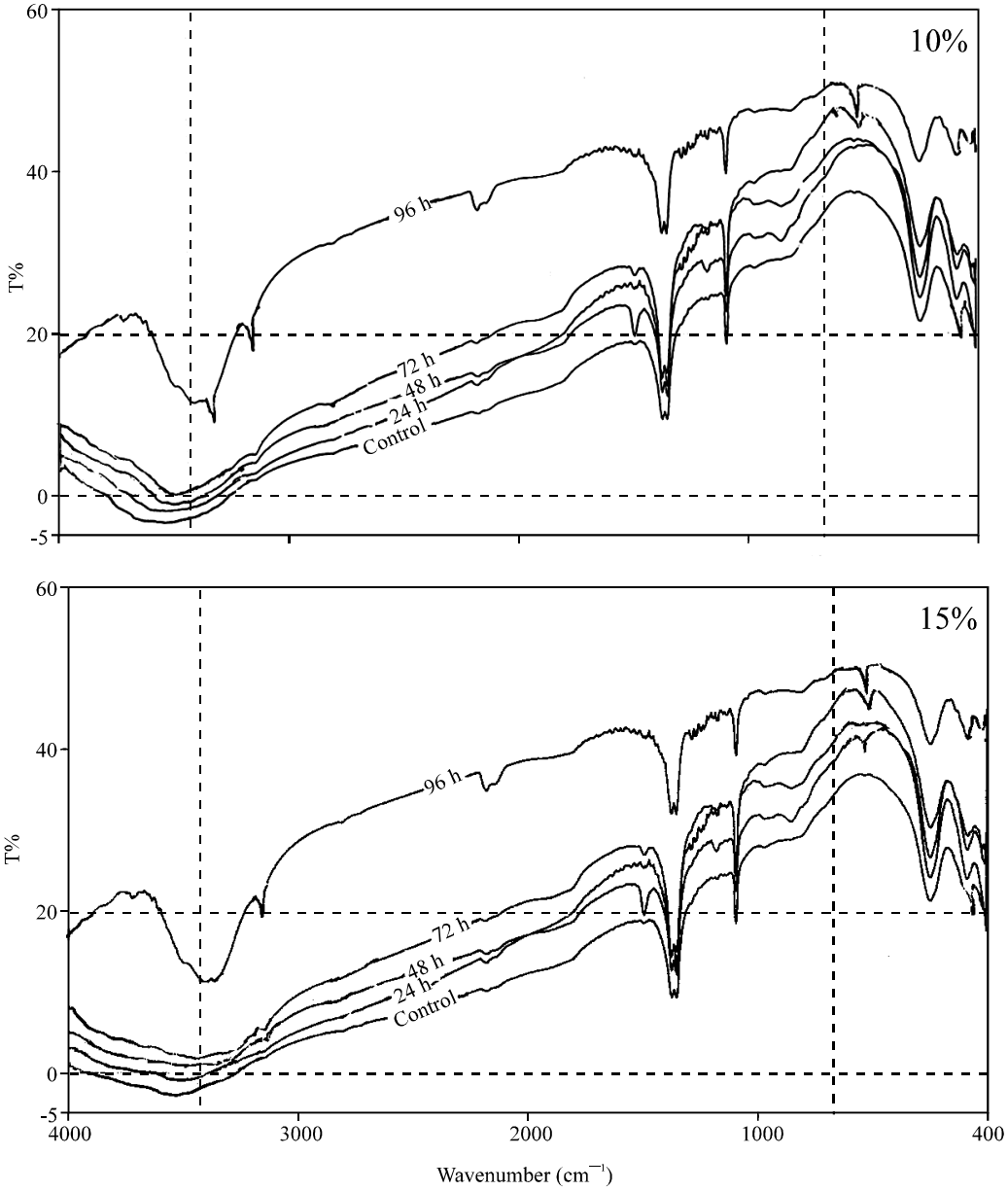

Fig. 5: The effect of bacteriological treatments on the infrared spectroscopic analysis of corn oil as affected by heating process for 12 h

foods. These changes include three general chemical reactions may occur simultaneously: hydrolysis, polymerization and oxidation, which produce a great number of potential toxic decomposition products deposit on the surface of the fryer and adsorbed by the food (White, 1991; Arroyo *et al*., 1992; Cuesta *et al*., 1993 and Rabie and Hassan, 1996). Also, some products coming from oil degradation during deep-fat frying show some toxic and/or carcinogenic effects by using of *in vivo* and *in vitro* studies (Marquez-Ruiz *et al*., 1992; Marquez-Ruiz and Dobarganes, 1996; Abed El-Rahman, 1994 and Elhassaneen, 2002). These harmful effects restricted the use of recycled cooking oils in formulated feed for animal production as a consequence of bioaccumulation, for consumer health. Therefore, many trials have been done relating to the cooking oil recycling process but the field of technology for the purification of cooking oils is not well developed and the work that has been carried out has not been adequately coordinated with commercial concern and the interests of society or the administration, which

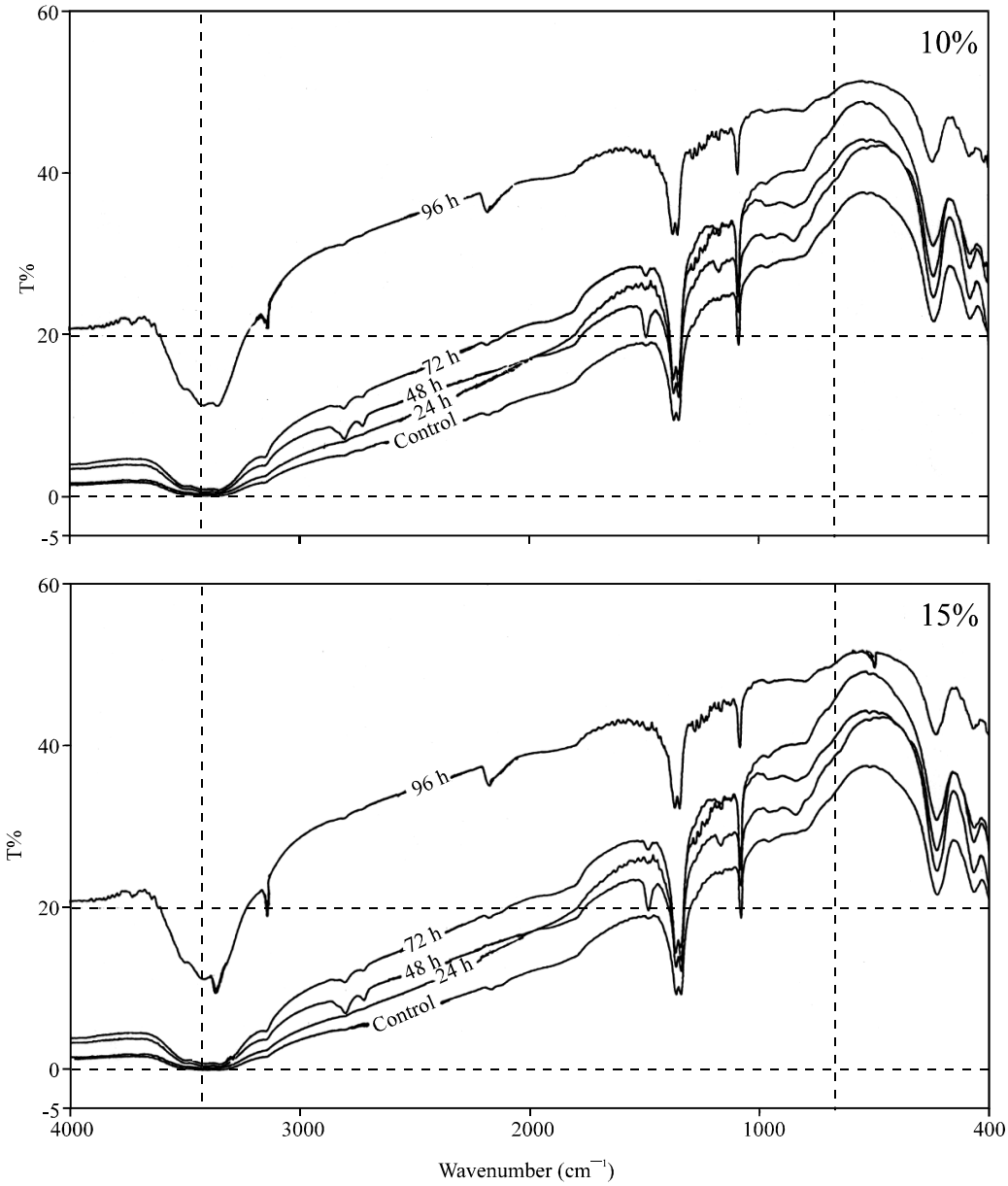

Fig. 6: The effect of bacteriological treatments on the infrared spectroscopic analysis of corn oil as affected by deep-fat frying of potato for 12 h

aim to improve consumer safety (European Parliament, 2001; Farrag *et al*., 2001). Basically, the simplest purification systems consist of agitating waste cooking oils with water and then decanting and/or centrifuging. This very simple procedure may be highly effective for eliminating a large proportion of the water, which accompanies these oils and also for eliminating solid particles or impurities from the fried foods. However, it obviously does not permit proper purification of the oil, as the oil degradation compounds and liposoluble contaminants are retained in the recovered (recycled) oil. Nor have studies been conducted to assess the efficacy of these purification processes on different components of the waste cooking oil. It may be assumed that this purification system might be sufficient for oils with only slight degradation (not exceeding the limit of 25% Polar compounds), but with highly degraded oils, this system would not allow recycled oils to obtain rather demanding minimum quality specifications (European Parliament, 2001).

In the present study a trial was carried out to develop a new microbiological technology for the purification

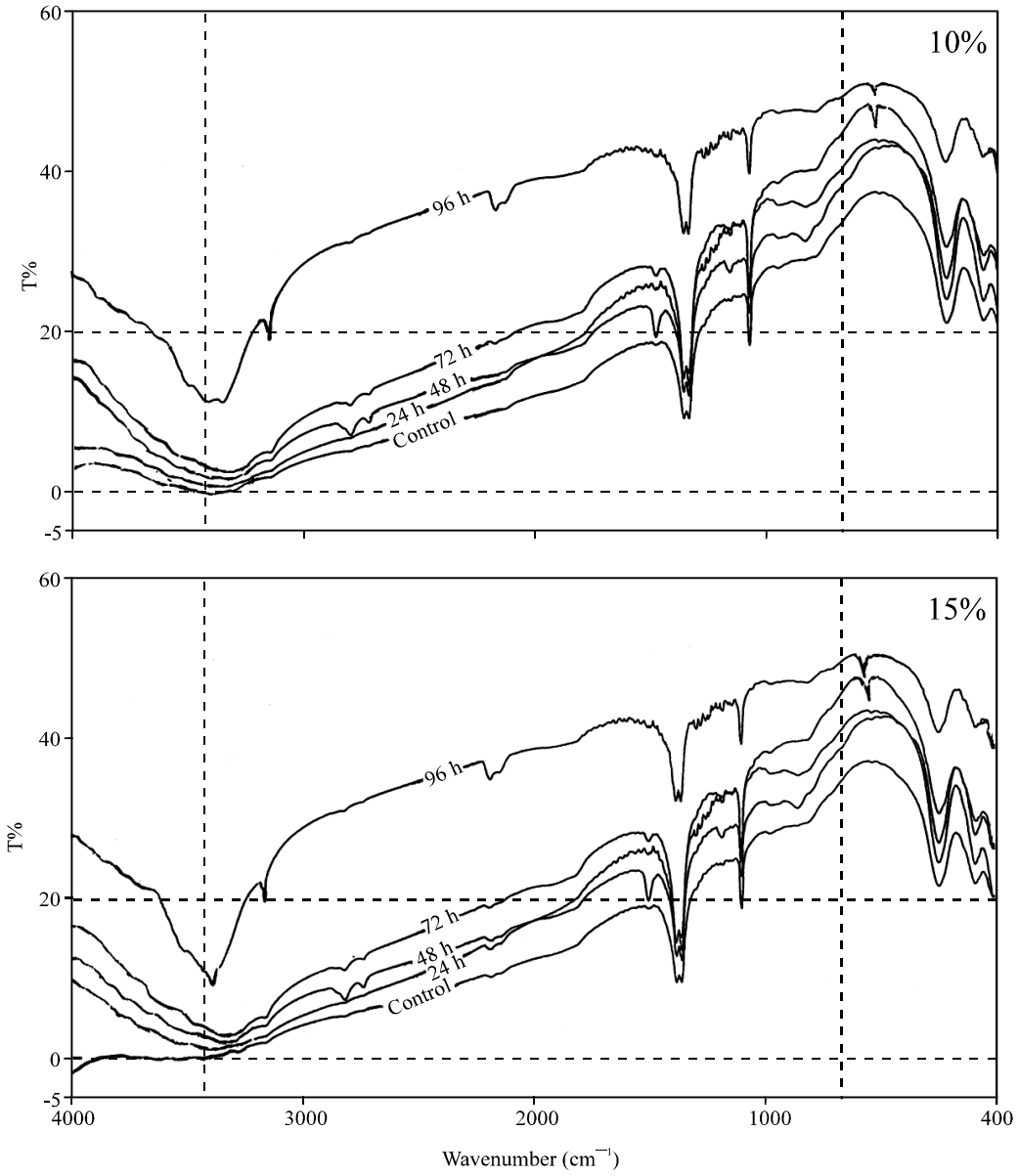

Fig. 7: The effect of bacteriological treatments on the infrared spectroscopic analysis of corn oil as affected by deep-fat frying of tamia for 12 h

of deep-fat frying oils which has been adequately coordinated with commercial concern and the interests of society or the administration, which aim to improve consumer safety. Wastewater samples from Oil and Soap Company was used as a source of oil using bacteria and very simple technique for oil treatment with bacterial isolates was applied. Examination of the oil treated with bacterial isolates by chemical and physical procedures indicated that some toxic and/or carcinogenic products coming from oil degradation during deep-fat frying were partially removed. For examples, free fatty acids (FFA) which developed in oil subjected to heat as the result of hydrolysis, was decreased (Fig. 1). Many studies were undertaken on fat hydrolysis probably because of the ease of measurement of free fatty acids, which arise from this reaction (Rabie and Hassan, 1996; Elhassaneen and Shaheen, 1998; Mohammed, 1999). Also, the same behavior was observed for the PV (Fig. 2) which used as a good parameter for lipid oxidation and rancidity (Elhassaneen and Shaheen, 1998; Mohammed, 1999; Serag El-Din, 2001). The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides.

On the other side, a decreasing in the malonaldehyde content, one of the major products of the oxidation of polyunsaturated fatty acids, in deep-fat frying oil after treated with bacterial isolates was observed (Fig. 3). The effect of malonaldehyde on human health has been reported by many authors that is mutagenic and carcinogenic (Murray *et al*., 1993). The mutagenicity of malonaldehyde has been demonstrated by the Ames salmonella revertant procedure (Shamberger *et al*., 1979) while its carcinogenicity was observed when painted on the skin of mice (Shamberger *et al*., 1974).

One of the most important evidence in the present investigation was the decreasing in polycyclic aromatic hydrocarbons (PAH) content of deep-fat frying oils as the result of bacteriological treatments (Fig. 4). PAH form a very extensive group of substances, which may constitute a significant public health problem, as some substances of this group such benzo(*a*)pyrene have considerable carcinogenic effects by extensive experiments *in vivo* and *in vitro* (Harvey, 1985; Plakunov *et al*., 1987; Hawkins *et al*., 1990; Elhassaneen, 1996; Elhassaneen, *et al*., 1997 and Elhassaneen, 2002). Smokers are exposed to high quantities of these hydrocarbons, as their concentration in tobacco smoke is significant. For non-smokers, however, food constitutes the main cause of exposure to PAH. One of the most abundant food sources of PAH is vegetable oil. It is possible that the high levels of PAH in vegetable oils are due to endogenous production via pyrolysis of lipids, especially sterols (Shibamoto and Bjeldanes, 1993; European Parliament, 2001). Many studies reported that frying, or deep-frying resulted in negligible quantities of endogenous carcinogens, while exogenous treatment with flue gas increased the hydrocarbons, especially benzo(*a*)pyrene (Fritz, 1972; Elhassaneen, 1999; Mohammed, 1999; Serag El-Din, 2001 and Elhassaneen, 2002).

All of the previous data were confirmed by the spectroscopic analysis of treated deep-fat frying oils with bacterial isolated. Infrared spectroscopy is of particular value in the recognition of unusual functional groups and in the study of fatty acids with *trans* double bonds (Belton *et al*., 1988; Lanser and Emken, 1988; Sleeter and Matlock, 1989; Van de Voort *et al*., 1995 and Mossoba *et al*., 1996). As the compounds formed during the oxidation of fat change, it is possible using IR spectroscopy to follow the course of oxidation. The disappearance of many absorbance peaks at 3420, 3120, 1750 and 970-1000 nm means the removal of many toxic compounds corresponding to these including hydroperoxides, polymerization products, carbonyl groups or acids and trans-ethylenic double bonds of fatty acids from the treated oils (Fig. 5-7). Many previous studies indicated that the deep-fat frying of fat may convert the naturally-occurring *cis* fatty acids to *trans* analog which, do not function as essential fatty acids (Elhassaneen and Shaheen, 1998; El-Shafai, 2000 and Serag El-Din, 2001). Some researchers have found that the *trans* fatty acids were not as effective as their cis analogs in lowering blood cholesterol and fat rich in trans fatty acids appeared to promote atherosclerosis (Ensminger *et al*., 1994; Gutteridge and Halliwell, 1994 and Serag El-Din, 2001).

In conclusion, the results of this investigation can constitute a milestone toward the application of simple economical techniques for removal of the toxic, carcinogenic and mutagenic compounds formed in used oil subjected to deep-fat frying operation for a long time period. It may be assumed that this bio-purification system might be partially sufficient for oils with highly degradation and would allow recycled oils to obtain rather demanding minimum quality specifications.

REFERENCES

A.O.A.C., 1985. Official Methods of the Association of Official Analytical Chemists. 14th Ed. Published by the Association of Official Analytical Chemists. Arlington, Virginia, USA.

A.O.A.C., 1995. Official Methods of the Association of Official Analytical Chemists 16th Ed. Published by the Association of Official Analytical Chemists. Arlington, Virginia, USA.

Abed El-Rahman, T.M, 1994. Studies on edible frying oils.M.Sc. Thesis, Fac.of Agri., Kafer El-Sheilk Univ.

Alltech Associates, Inc., 1997. Catalog-400, Chromatography, State College, PA, USA.

Arroyo, R., C. Cuesta, G. Garrido-Polonio, S. Lopez-Varela and F.J. Sanchez-Muniz, 1992. High performance size exclusion chromatographic studies on polar components formed in sunflower oil used for frying. J. Am. Oil Chem. Soc., 69: 557-563.

APHAC, 1992. Standard Health Methods for the examination of water and waste water. American Public Health Association. The 18th Ed., Washington.

Belton, P.S., R.H. Wilson, H. Sadeghi-Jorobachi and K.E. Pears, 1988. A rapid method for the estimation of isolate trans double bonds in oils and fats using fourier transform inferared spectroscopy combined with attenuated total reflectance. Lebensom Wiss, Technol., 21 :153-157.

Cuesta, C., F.J. Sanchez-Muniz and I. Hernandez, 1993. Evaluation of non-polar methyl esters by column and gas chromatography for the assessment of used frying olive oil. J. Am. Oil Chem. Soc., 68: 443-445.

El-Fadaly, H., M. El-Defrawy, F. El-Zawawy and D. Makia, 2000. Chemical and microbiological analysis of certain water sources and industrial wastewater samples in Egypt. Pakistan J. Biol. Sci., 3: 777-781.

Elhassaneen, Y.A. and K.A. Shaheen, 1998. Chemical and toxicological evaluation of deep-fat frying oils used at some Egyptian restaurants. J. Home Econ., 8: 225-242.

Elhassaneen, Y.A., 1996. Biochemical and technological studies on pollution of fish with pesticides and polycyclic aromatic hydrocarbons. Ph.D., Mansoura University, Mansoura, Egypt.

Elhassaneen, Y.A., 1999. Toxicological and biochemical effects of polycyclic aromatic hydrocarbon compounds produced in fish by cooking and processing. Six Arabic conference on Food Science and Technology, March 16-18, Cairo, Egypt.

Elhassaneen, Y.A., 2002. New and quickly biological method for detection the potential chemical toxins and/or carcinogens in foods. Proceedings of 2nd scientific Conference on Foodborne Contamination and Egyptian's Health (24-24 April), Faculty of Agriculture, Mansoura University, Mansoura, Egypt, pp: 371-394.

Elhassaneen, Y.A, O.M. Khater and A.A. Morsey, 1997. The effect of vitamins on the cancer initiation induced by some food chemical carcinogenic pollutants *in vitro*. The Second Conference: The Role of Women and Egyptian Associations in Environment Protection and Community Development (25-26 August), Dept. of Home Economics, Faculty of Agriculture, Alexandria University, Egypt.

El-Shafai, M.E., 2000. Chemical and nutritional studies on Mallard reaction occurred in the cooked fish. M.Sc. Thesis, El-Minufiya University, Egypt.

Ensminger, A.H., M.E. Ensminger, J.E. Konlande and J.R. Robson, 1994. The concise Encyclopedia of Food and Nutrition, pp: 799.

European Parliament, 2001. Recycled cooking oils: Assessment of risks for public health. The STOA program, Luxembourg, EP/IV/A/STOA/99/ENVI/01.

Farag, R.S., G.S. Abdel-Malek and A.G. Salib, 1977. Safflower and lettuce oils as potential sources in the preparation of olive oil. Substitutes Chem. Microbial Technol. Lebensom, 5: 113-117.

Farrag, A.A., A. Abdel-Megeid and M.M. El-Said, 2001. Chemical and biological evaluation of reused cottonseed oil after washing or replacement process. Journal of Home Economics, 11: 129-146. Special issue for the Arabic Conference of Home Economics (2-4 September) Faculty of Home Economics, Minufiya University, Egypt.

FDA., 1976. Bacteriological Analytical Manual for Foods. 4th Ed., Food and Drug Administration, Washington, DC.

Fritz, W., 1972. Contamination of foods with carcinogenic hydrocarbons during processing and cooking. Arch. Geschwulst Forsch, 40: 81-90.

Gordon, M.H. and L. Kourimska, 1995. The effect of antioxidant on changes in oils during heating and deep frying. J. Sci. Food Agric., 68: 347-353.

Gutteridge, J.M.C. and B. Halliwell, 1994. Antioxidants in nutrition,Health and disease. Oxford University Press, Walton street, Oxford.

Harvey, R.G., 1985. Polycyclic hydrocarbons and carcinogenesis. ACS Symp. Ser. 283, Am. Chem. Soc., Washington, D.C., USA.

Hawkins, E.W., W.W. Walker, R.M. Overstreet, T.F. Lytle and J.S. Lytle, 1990. Carcinogenic effects of some polycyclic aromatic hydrocarbons on the Japanese Medaka and Guppy in waterborne exposures. The Sci. Total Envi., 94: 155-167.

Lanser, A.C. and E.A. Emken, 1988. Comparison of FTIR and capillary gas chromatographic methods for quantitation of trans unsaturation in fatty acid methyl esters. J. Am. Oil Chem. Soc., 65: 1483-1485.

Marquez-Ruiz, G. and M.C. Dobarganes, 1996. Nutritional and physiologicaleffects of used frying fats. In: Deep frying. Chemistry Nutrition and Practical Applications (Perkins E.G. and Erickson M.D. Ed.). AOCS Press, Champaign IL, USA.

Marquez-Ruiz, G., M.C. Perez-Camino, V. Ruiz-Gutierrez and M.C. Dobarganes, 1992. Absorption of thermoxidized fats: II. Influence of dietary alteration and fat level. *Grasas y aceites,* 43: 198-203.

Mohammed, H.E., 1999. Food safety as influenced by cooking using microwave oven., M.Sc. Thesis, Minufiya University Shebin El-kom, Egypt.

Mossoba, M.M., M.P. Yurawecz and R.E. McDonald, 1996. Rapid determination of the total trans content of neat hydrogenated oils by attenuated total reflection sopctroscopy. J. Am. Oil Chem. Soc., 73: 1003-1009.

Mourey, A. and G. Kilbertus, 1976. Simple media containing stabilized tributyrin for demonstrating lipolytic bacteria in foods and soils. J. Appl. Bacterial., 40: 47-51.

Mukai, F.H. and B.D. Goldstein, 1976. Mutagenicity of malonaldehyde, a decomposition product of peroxidized polyunsaturated fatty acids. Science, 191: 868.

Murray, R.K., D.K. Granner, P.A. Mayes and V.M. Rodwell, 1993. Harper's Biochemistry. 23rd Ed. by Applepton and Lange, London, UK.

Oxoid, 1982. The Oxoid Manual of Culture Media, Ingredients and Other Laboratory Services. 5th Ed., Oxoid Limited.

Pearson, D., 1970. The chemical analysis of food. National College of Food Tech., University of Reading, Weybridge, Ed. by Surry, T. and Churchill, A., ocean fishes, Fish industry, 43: 57-59.

Plakunov, I., T.A. Smolarek, L.D. Fischer, J.C. Wiley and W.M. Baird, 1987. Separation by ion-pair high-performance liquid chromatography of the glucuronid, sulfate and glutathione conjugates formed from benzo(*a*)pyrene in cell cultures rodents, fish and humans. Carcinogenesis, 8: 59-66.

Rabie, M.M. and A.M. Hassan, 1996. Comparative study on the stability of plam oil and local commercial oils during heating and frying processes. The Proceeding of Food Born Contamination and Egyptian's health conference, Mansoura University, pp: 285-297.

Serag El-Din, M.F., 2001. Analysis, occurrence and formation of some toxic compounds in some edible oils as the result of cooking and processing. M.Sc. Thesis, Minufiya University, Shebin El-Kom, Egypt.

Shamberger, R.J., T.L. Andreone and C.E. Willis, 1974. Antioxidants and cancer. IV. Malonaldehyde has initiating activity as a carcinogen. J. Natr. Cancer Inst., 53: 1771.

Shamberger, R.J., C.L. Corlett, K.D. Beaman and B.L. Kasten, 1979. Antioxidants reduce the mutagenic effect of malon-aldehyde and propiolactione. Mutat. Res., 66: 349.

Shibamoto, T. and L.F. Bjeldanes, 1993. Food contaminants from industrial wastes, Heavy metals In: Introduction to Food Toxicology, pp: 129-132.

Sleeter, R.T. and K.G. Matlock, 1989. Automated quantitative analysis of isolated (nonconjugated) trans isomers using fouier transform infared spectoscoy incorporating improvements in the procedure. J. Am. Oil. Chem. Soc., 66: 121-127.

Sneath, P.H., N.S. Mair, M. Sharpe and J.G. Halt, 1986. Bergys Manual of Systematic Bacteriology, Vol. 2 Williams and Wilkins, Baltimore, USA.

Van der Voort, F.R., A.A. Ismail and J. Sedman, 1995. A rapid automated method for the determination of cis and trans content offats andoils by Fourier transform infrared spectroscopy. J. Am. Oil. Chem. Soc., 72: 873-880.

White, P.J., 1991. Methods for measuring changes in deep-fat frying oils. J. Food Technol., 2: 75-80.

Woyewoda, A.D, S.J. Shaw, P.J. Ke and B.G. Burns, 1986. Recommended laboratory methods for assessment of fish quality. Candian Technical Report of Fisheries and Aquatic Science NO.1448.