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Research Article

Comparative analysis of quality and safety for post-treatment waste cooking oil to explore its validity according to standards for edible oils

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Abstract

The increasing demand for sustainable and cost-effective alternatives to the relatively expensive edible oils, with increasing population and the negative impact of climate change on oilseed crop productivity, raised claims to reuse post-treatment waste cooking oil (WCO) for human consumption, which is prohibited according to official legislation. Therefore, this novel pioneering study aimed to evaluate quality and safety for WCO samples collected from restaurants and households, subjected to two treatments, cornstarch (CS) and bleaching earth (BE), to investigate its validity according to edible oil standards. Physicochemical, toxicological, microbiological, and aroma evaluations with *in vitro* cytotoxicity of pre- and post-treatment WCO against the human embryonic kidney cell line (HEK-2) were investigated. Results ($P<0.05$) of pre- and post-treatment WCO revealed persistent chemical, toxicological, and microbiological contaminants that prevent the possibility for human consumption with the apparent *in vitro* cytotoxicity against HEK-2 with reduced cell viability. The current traditional treatments performed by individuals (CS) and factories (BE) were insufficient to eliminate the inherent hazards, underscoring the continued invalidity of pre- and post-treatment WCO as a safe edible oil according to the established standards for edible oils.

Keywords

bleaching, cytotoxicity, edible oils standards, food quality and safety, HEK-2 cell line, human health, waste cooking oil

Abbreviations

AA – Acrylamide; BaP – benzo[a]pyrene; BE – bleaching earth; CS – cornstarch; FA – fatty acid; FBS – Fetal Bovine Serum; FFA – free fatty acids; HEK-2 – human embryonic kidney cell line; HEPES – (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid); 3-MCPD – 3-monochloropropanediole; MTT – (3-(4,5-dimethyl-2-thiazol-2-yl)- 2,5-diphenyl-2H-tetrazolium bromide); MUFA – mono unsaturated fatty acids; OSI – oxidative stability index; PAHs – polycyclic aromatic hydrocarbons; PUFA – poly unsaturated fatty acids; PV – peroxide value; RI – refractive index; RPMI-1640 – Roswell Park Memorial Institute medium; SDGs – sustainable development goals; SFA – saturated fatty acids; TPC – total polar componuds; USFA – unsaturated fatty acids; WCO – waste cooking oil

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Introduction

The global demand for edible oils has risen sharply due to population growth and increased awareness of nutrition. Edible oils are important for energy and essential fatty acids. However, the rise in consumption has stressed oilseed crop production and raised environmental and sustainability issues with the negative impacts of climate change, leading to lower productivity, higher prices, and limited availability of quality edible oils. Consequently, more people are looking for alternative sources and strategies for sustainably meet the need for edible oils (Abd El-Baset et al. 2024).

Reusing waste cooking oil (WCO), especially post-treatment WCO, which is produced in large quantities by homes and restaurants, is one such tactic. After being used in the kitchen, WCO is usually collected and put through a variety of cleaning and filtering procedures to extend its useful life. Reusing WCO for human consumption raises serious health concerns despite its widespread availability and low cost. This is mainly because of the possibility of hazardous substances like degraded lipids, *trans* fatty acids, polycyclic aromatic hydrocarbons (PAHs), with other toxic and hazardous compounds causing major health risks (Hosseinzadeh-Bandbafha et al. 2022).

Reusing waste cooking oils for human consumption is forbidden by international official laws and food safety regulations, mainly because of the risks involved. Strict standards for edible oils have been set by regulatory bodies like the Food and Drug Administration (FDA), Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), and the Codex Alimentarius Commission (CXS 210-1999) besides the national laws and standards stipulated by individual countries as Egypt (Law No.10 of 1966; ES 8041/2016; ES 50-1/2022). These standards place a strong emphasis on factors like free fatty acids, peroxide value, total polar compounds, microbiological safety, and the lack of harmful contaminants. The purpose of these guidelines is to guarantee the nutritional value, safety, and purity of edible oils meant for human consumption. However, individuals, researchers and industry stakeholders are investigating possible treatments that could lessen these risks and make WCO safer for reuse due to growing economic demands.

Several physical, chemical, and biological treatment strategies have been studied to improve the quality and safety of WCO. Physical adsorbents such as bleaching earth (BE) and natural polymers such as cornstarch (CS) are widely used in refining processes to remove impurities, colors, smells, and some harmful compounds. Bleaching earth (BE), a clay mineral, works largely through adsorption and ion exchange and effectively reduce color and some pollutants (Gharby 2022). In contrast, cornstarch (CS), a natural carbohydrate polymer, can operate as a filtration and adsorptive agent, potentially trapping pollutants and lowering microbial burden (Tyagi et al. 2025). Despite their widespread industrial use in edible oil refining, the efficacy of these treatments, especially when applied to waste cooking oils originating from a variety of culinary sources, is little understood.

The quality and safety of post-treatment WCO, must be thoroughly assessed using extensive physicochemical, toxicological, microbiological, and sensory investigations. Physicochemical parameters such as peroxide value, free fatty acids, and polar compounds provide information on the oil's oxidative stability and breakdown rate (Mitrea et al. 2022). Toxicological assessments are required for the detection of carcinogenic chemicals and other toxicants. Microbiological testing confirms that no pathogenic germs are present, which could cause foodborne sickness (Drewnowska and Swiecicka 2021). Sensory evaluation of aroma aids in determining customer acceptance and detecting unwanted aromas caused by deterioration or contamination (Yang and Boyle 2016). In addition to these traditional assessments, *in vitro* cytotoxicity testing has developed as a useful method for determining the potential health concerns of consuming processed oils using human cell lines (Ghasemi et al. 2021).

Therefore, the current study aimed to perform a comprehensive comparative analysis of the quality and safety of post-treatment waste cooking oil compared to fresh and pre-treatment cooking oil to explore its compliance with the established official standards for edible oils, thereby assessing its validity for safe reuse for human consumption or coversion into value-added products. Also, the study aimed to identify the key differences and potential health risks against human cells (HEK-2) as shown in the experimental design (Fig. 1).



Figure 1. Experimental design

Materials and Methods

Materials

Raw materials. Waste cooking oil (WCO) samples were obtained from different restaurants and households in Egypt, cornstarch powder (CS), Engelhard F160 bleaching earth powder (BE), and Whatman No. 1 filter papers were purchased from certified companies registered in the White List of the National Food Safety Authority (NFSA), Cairo, Egypt.

Mammalian cell line. Human embryonic kidney (HEK-2) cells were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland, USA via the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Chemicals and reagents. The essential reagents required by the cell culture assay for growing and maintaining the mammalian cells in laboratory include: Fetal Bovine Serum (FBS), RPMI-1640, HEPES buffer, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were obtained from Lonza (Belgium). Whereas, MTT, standards, chemicals, reagents, and solvents of analytical and HPLC grade were obtained from Sigma-Aldrich, USA.

Methods

Physicochemical treatments of waste cooking oil. Waste cooking oil (WCO) samples were classified into the following three categories according to the type of physicochemical treatment: as-received (non-treated), cornstarch-treated (CS-treated), and bleaching earth-treated (BE-treated).

Preliminary filtration of all samples were carried out to remove resins, and insoluble impurities. Then bleaching of WCO was carried out according to the method described by Almoselhy et al. (2020) with two bleaching materials; cornstarch (CS) as a traditional bleaching material used by individuals at home, and bleaching earth (BE) used in industry by edible oil factories, to compare their effects on the physicochemical characteristics of post-treatment WCO. The bleaching materials; CS and BE were added separately to WCO in a percentage of 2% (w/w) with agitation in a rotary evaporator at 100ºC for 30 min., then the CS-treated and BE-treated WCO samples were cooled to 50ºC, and filtered with Whatman No. 1 filter papers to remove the bleaching material from WCO after bleaching, then transferring the post-treatment waste cooking oil (CS-treated and BE-treated) into dark bottles and were kept in a dry cool place till analysis.

Physicochemical quality parameters of WCO. Waste cooking oil (WCO) samples pre- and post-treatment were evaluated for physicochemical quality parameters. Viscosity was measured according to the method described in ISO 2555:2018 (2018) using a viscometer (Brookfield Rheometer AMETEK DVNext at room temperature, Spindle No. SC4-18 rpm 10), whereas specific gravity (at 25°C) was determined according to the method described in AOCS Official Method Cc 10b-25 (2024). Refractive index (RI) was determined according to the method described in ISO 6320:2017 (2022). Peroxide value (POV) was determined according to the method described in AOAC Official Method 965.33 (2019). Acidity was determined according to the method described in ISO 660:2020 (2020). Rancidity was determined using an in-house method based on the results of acidity and peroxide values. Total insoluble impurities content was determined according to the

method described in ISO 663:2017 (2022), and the soap content was determined according to the method described in ISO 685:2020 (2025). Smoke, flash, and fire points were determined according to the method described in AOCS Official Method Cc 9a-48 (2024). Conjugated diene (primary oxidation product) was measured at 232 nm, whereas conjugated triene (secondary oxidation product) was measured at 270 nm according to the method described in ISO 3656:2011 (2011) using a UV-Visible spectrophotometer, and the total polar compound (TPC) was measured according to the method described in AOCS Official Method Cd 20-91 (2024) using a Testo 270 Cooking Oil Tester. The oxidative stability index (OSI) was determined by the Metrohm Rancimat 679 instrument according to the method described in AOCS Official Method Cd 12b-92 (2024).

Color measurement of WCO. Color measurement of waste cooking oils under study was carried out according to the method described in AOCS Official Method Cc 13e-92 (2024) using a Lovibond tintometer.

Determination of benzo[a]pyrene in WCO. Benzo[a]pyrene was determined in waste cooking oils according to the method described by Bogusz et al. (2004) with slight modification using high-pressure liquid chromatography (HPLC).

Determination of 3-MCPD in WCO. Monochloropropanediole (3-MCPD) was determined in waste cooking oils according to the method described by Almoselhy et al. (2021) using gas chromatography-mass spectrometry (GC-MS/MS).

Determination of acrylamide in WCO. Acrylamide was determined in waste cooking oils according to the method described by Tölgyesi and Sharma (2020) using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Microbiological analysis of WCO. Microbiological analysis of waste cooking oils was performed according to the method described in ISO 16649-2:2001 (2001) for *Escherichia coli*, ISO 6579-1:2017 (2017) for *Salmonella*, and ISO 6888-1:2021 (2021) for *Staphylococcus aureus*.

Sensory evaluation of aroma of WCO. Sensory evaluation of WCO aroma was performed in accordance with the ethical standards of Helsinki Declaration and its later amendments or comparable ethical standards. Twenty panellists (10 women and 10 men, ages 25 to 60) with the affiliation of food science and technology and edible oils and fats categories were chosen on the basis of voluntariness with understanding that they will evaluate only the aroma of WCO samples and record the aroma sensation as positive or negative only as a qualitative test without numerical scores.

Fatty acid composition of WCO. The fatty acid composition of waste cooking oils under investigation was converted into methyl ester according to the method described in ISO 12966-2:2017 (2017), and determined by gas-liquid chromatography instrument (HP 6890N, Agilent Technologies, USA) equipped with a splitless injector with flame ionization detector (FID) and DB-23 capillary column.

MTT assay for evaluation of cytotoxic effects of waste cooking oil (WCO) against HEK-2 cells. *In vitro* MTT assay for the evaluation of cytotoxicity of WCO under different treatments against human embryonic kidney cell line (HEK-2) was performed according to the method described by Mosmann (1983), at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt, applying the following detailed procedures:

Cell line propagation. The cells were cultured in RPMI-1640 media with 10% inactivated fetal bovine serum (FBS) and 50µg/ml gentamycin. The cells were kept at 37°C in a humidified environment with 5% CO_2 and subcultured three times weekly.

Cytotoxicity evaluation using viability assay. For cytotoxicity assays, cells were suspended in media at a concentration of 5×10^4 cell/well in Corning® 96-well tissue culture plates and incubated for 24 hr. The tested compounds which are WCO samples as: non-treated, cornstarch-treated (CS-treated), and bleaching earth-treated (BE-treated), were then placed in 96-well plates (in triplicates) to achieve eight concentrations of each compound. Six vehicle controls with media were run as controls for each 96-well plate. After 24 h of incubation, the MTT test was used to measure the number of viable cells. To begin, remove the media from the 96 well plate and replace it with 100 µl of fresh culture RPMI 1640 medium without phenol red. Next, add 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL

of PBS) to each well, including the untreated controls. The 96-well plates were then incubated for 4 hours at 37°C with 5% CO_2. After removing an 85 µl aliquot of the media, add 50 µl of DMSO to each well and mix thoroughly with a pipette. Incubate for 10 minutes at 37°C. The optical density was then measured at 590 nm using an absorbance microplate reader (Sunrise™, TECAN, USA) to determine the number of viable cells, and the percentage of viability was calculated as [(ODt/ODc)]x100%, where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relationship between surviving cells (Cell Viability %) and WCO Concentration is plotted to produce a survival curve for each cell line after treatment with the WCO. The 50% cytotoxic concentration (CC_{50}), which is the concentration required to produce toxic effects in 50% of intact cells, was determined using Graphpad Prism software (San Diego, CA, USA) with graphical dose-response nonlinear regression curves sketched using the two coordinates representing Concentration of WCO (µg/ml) as X-axis, and Cell Viability (%) as Y-axis.

Statistical analysis. Measurements were performed in triplicate. Data were presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted with SPSS software at $P<0.05$.

Results and Discussion

Physicochemical quality parameters of WCO. Waste cooking oil (WCO) samples pre- and post-treatment were evaluated for physicochemical quality parameters, where it is noticed the variation in significant differences ($P<0.05$) among the different samples of the waste cooking oil pre- and post-treatment, with variation of tests, as some tests showed significant differences, whereas, the others showed no significant differences (Table 1).

Viscosity (Cp) ranged 84.8-80.0, specific gravity ranged 0.9000-0.8970, refractive index (RI) ranged 1.4564-1.4561, peroxide value (PV) ranged 16.53-11.56 $meqO_2.kg^{-1}$ oil, acidity ranged 12.08-10.08 as % oleic acid, with the highest value for non-treated followed by BE-treated, then CS-treated WCO. Rancidity test results were positive for all samples revealing the serious deterioration of oil and its invalidity for reuse for human consumption. Total insoluble impurities content ranged 2.7-1.7%, the soap content ranged 0.0030-0.0028 with the highest value for the non-treated followed by BE-treated, then CS-treated WCO. Smoke point ranged 236-240°C, flash point ranged 330-339°C, fire point ranged 364-368°C with the lowest values for the non-treated followed by BE-treated, then CS-treated WCO without any apparent differences among WCO under different treatments.

Conjugated diene ranged 17.512-14.180, whereas conjugated triene ranged 4.158-3.908, with highest value for the non-treated followed by BE-treated, then CS-treated WCO which all reveals the deterioration of cooking oils with the emergence of primary and secondary oxidation products indicating the invalidity of cooking oils for human consumption, despite the varying values among the different treatments which lessen the effect of primary and secondary oxidations, but the treatments could not remove the considerable oxidation products which had been formed in the waste cooking oil. Whereas, the total polar compound (TPC) ranged 16.9-7.0 with the highest value for non-treated followed by BE-treated, then CS-treated WCO. The oxidative stability index (OSI) was already the same for all WCO samples, as the treatments did not affect the oxidative stability of an oil which is an inherent property for an oil depending on its chemical composition which was not changed by just bleaching process which did not alter the chemical structure of the oil.

Color measurement of WCO. Color measurement of WCO samples under different treatments ranged 36-31 for yellow and 17-7 for red colors, recording the highest value of red color for the non-treated, followed by the BE-treated, then CS-treated waste cooking oil, as the waste cooking oil is characterised by the greater red color (R) which makes it darker than the fresh oil due to the repeated deep frying process that causes the browning of oil due to the Maillard reaction resulting from the interaction between sugars and amino acids in foodstuffs with the frying oil at high temperatures, and thereby, by the continuous formation of Maillard reaction complexes, the waste cooking oil turns into darker color with higher values in the red color compared with the initial fresh cooking oil which has much lower values of red color. The different treatments enhanced the color of WCO with lower value of red color by adsorption of color by bleaching materials (Abd El-Baset and Almoselhy 2023).

Table 1. Physicochemical quality parameters of WCO before and post-treatment

Parameter	**Waste cooking oil, WCO**		
	Non-Treated	**CS-Treated**	**BE-Treated**
Viscosity (Cp)	84.8±0.25^{a}	80±0.22^{c}	80.5±0.23^{b}
Specific gravity	0.9000±0.000^{a}	0.8970.000^{c}	0.8980.000^{b}
Refractive index (RI)	1.4564±0.0001^{a}	1.4561±0.0001^{c}	1.4562±0.0001^{b}
Peroxide value – POV, meq.O_2.kg^{-1} oil	16.53±0.03^{a}	11.56±0.03^{c}	12.41±0.03^{b}
Acidity, as % oleic acid	12.08±0.01^{a}	10.08±0.01^{c}	10.30±0.0^{b}1
Rancidity	Positive	Positive	Positive
Insoluble impurities	2.70±0.02^{a}	1.7±0.01^{c}	1.8±0.01^{b}
Soap content	0.0030±0.00^{a}	0.0028±0.00^{a}	0.0029±0.00^{a}
Smoke point, ℃	236±0.08^{c}	240±0.08^{a}	238±0.07^{b}
Flash point, ℃	330±0.03^{c}	339±0.03^{a}	333±0.03^{b}
Fire point, ℃	364±0.06^{c}	368±0.04^{a}	367±0.05^{b}
UV Spectrophotometry			
Conjugated diene	17.512±0.007^{a}	14.180±0.007^{c}	15.505±0.007^{b}
Conjugated triene	4.158±0.007^{a}	3.908±0.007^{c}	3.976±0.007^{b}
Total polar compound (TPC)	16.9±0.21^{a}	7.0±0.13^{c}	10.50±0.23^{b}
Oxidative stability index – OSI, h	10.0±0.06^{a}	10.0±0.06^{a}	10.0±0.06^{a}
Color measurement			
Yellow	36±0.0^{a}	31±0.0^{c}	33±0.0^{b}
Red	17±0.0^{a}	7±0.0^{c}	8±0.0^{b}
Benzo[a]pyrene, µg.kg^{-1}	0.78±0.01^{a}	0.70±0.01^{c}	0.73±0.01^{b}
3-MCPD, mg.kg^{-1}	19.7±0.04^{a}	19.1±0.03^{c}	19.2±0.03^{b}
Acrylamide, µg.kg^{-1}	145±0.14^{a}	108±0.12^{c}	113±0.13^{b}
Microbiological analysis			
Escherichia coli, CFU.g^{-1}	<10	<10	<10
Salmonella spp., presence per 25 g	Absent	Absent	Absent
Staphylococcus aureus, CFU.g^{-1}	<10	<10	<10
Sensory evaluation of aroma	Negative	Negative	Negative

Values are mean ± standard deviation (n=3). Means in the same row with different superscripts are significantly different (P<0.05).

Determination of benzo[a]pyrene in WCO. Benzo[a]pyrene content detected in WCO samples ranged between 0.78-0.70 µg.kg^{-1} within the WCO, recording the highest value (0.78 µg.kg^{-1}) for non-treated, followed by BE-treated (0.73 µg.kg^{-1}) and then CS-treated (0.70 µg.kg^{-1}) which reflects the better bleaching effect of CS compared to BE. Regarding the maximum allowed level of processing contaminants represented by the polycyclic aromatic hydrocarbons (PAHs) group as benzo[a]pyrene, its concentration should not exceed 2.0 µg.kg^{-1} of oils placed on market for final consumer or use as a food ingredient (Commission Regulation (EC) 2023/915). Therefore, the detected values of benzo[a]pyrene will not be considered as potentially hazrdous substances in the WCO under different treatments.

Determination of 3-MCPD in WCO. 3-MCPD (mg.kg^{-1}) detected in WCO samples under different treatments ranged 19.7-19.1, recording the highest value (19.7 mg.kg^{-1}) for non-treated WCO, followed by BE-treated (19.2 mg.kg^{-1}) and then CS-treated (19.1 mg.kg^{-1}) which reflects the better bleaching effect of CS compared to BE in lowering the detected 3-MCPD slightly by adsorbing some negligible content, without profound removal of the previously formed 3-MCPD.

3-MCPD is a serious processing contaminant emerging during the refining process of vegetable oils due to many factors as the high temperature of refining combined with the chlorine-containing compounds originating from fertilizers and irrigation process since growing the oilseed crops.

The accumulation of inorganic chloride in the plant with the bioconversion into organochlorines in fruit, then the formation of liposoluble organochlorines during the sterilization of fruit bunch and processing of oil extraction and refining, results in the reaction between the triacyl glycerol (TAG) as the main form of stored fat in plant, with the liposoluble organochlorines resulting in formation of 3-MCPD which was detected in many refined vegetable oils at high levels (Almoselhy et al. 2021) exceeded the maximum allowed level stipulated by Commission Regulation (EC) 2023/915.

The 3-MCPD content should not exceed 1250 $\mu g.kg^{-1}$ (1.250 $mg.kg^{-1}$) of the oils placed on market for final consumer or use as a food ingredient according to the Commission Regulation (EC) 2023/915. It is concluded the invalidity of reusing post-treatment WCO for food purposes.

Determination of acrylamide in WCO. Acrylamide detected in WCO samples ranged between 145-108 $\mu g.kg^{-1}$ within the WCO, recording the highest value (145 $\mu g.kg^{-1}$) for non-treated, followed by BE-treated (113 $\mu g.kg^{-1}$) and then CS-treated (108 $\mu g.kg^{-1}$) which reflects the better bleaching effect of CS compared to BE. For acrylamide, the benchmark level is varying among different foodstuffs, but its benchmrk level is 500 $\mu g.kg^{-1}$ in the French fries as the most common foodstuff contacting the frying oil, as the fresh cooking oil has no benchmark for acrylamide which is a low molecular weight, highly water soluble chemical compound formed by the naturally occurring elements asparagine and sugars in certain foods when cooked at temperatures above 120°C and with little moisture. It forms mostly in baked or fried carbohydrate-rich foods containing its precursors, like potatoes and cereals (Commission Regulation (EC) 2017/2158). Therefore, the detected levels of acrylamide will not be considered as potentially hazardous substance in the WCO.

Microbiological analysis of WCO. The microbiological analysis revealed the absence of *Salmonella spp*. in all WCO samples, whereas, *Escherichia coli* and *Staphylococcus aureus* were less than 10 $CFU.g^{-1}$ in all WCO samples, showing relatively low microbial load that does not pose significant microbiological health risks.

Sensory evaluation of aroma of WCO. Negative aroma results were recorded for all WCO samples, as all testers declared the rancidity of all samples under treatments due their off-flavors compared.

After discussion of results (Table 1), it is shown the apparent effect of bleaching materials for improving many parameters in post-treatment WCO with greater effect of cornstarch (CS) as a bleaching material compared to the bleaching earth (BE).

Despite the improvement in some physicochemical characteristics, but these treatments were still insufficient to eliminate the inherent hazards as the high peroxide value, acidity, rancidity, insoluble impurities, conjugated diene and conjugated triene which were out of the values stipulated by the international official standards (CXS 210-1999) besides the national laws and standards stipulated by individual countries as Egypt (Law No.10 of 1966; ES 8041/2016; ES 50-1/2022).

The existance of benzo[a]pyrene, 3-MCPD, and acrylamide, along with the negative aroma, underscores the continued invalidity of pre- and post-treatment WCO as a safe edible oil according to the established standards for edible oils.

The insoluble impurities exceeded the maximum allowed value (0.05%) with the apparent ranicidy determined using an in-house method based on the combined values of acidity and peroxide values. Morevoer the high contents of 3-MCPD exceeding the maximum allowed levels stipualted by Commission Regulation (EC) 2023/915, is considered another significant risk factor with profound hazardous effects that prevent reusing the post-treatment waste cooking oil for human consumption.

It is concluded from the abovementioned results that all waste cooking oil samples were invalid according to the the Codex Alimentarius Commission (CXS 210-1999) besides the national laws and standards stipulated by individual countries as Egypt (Law No.10 of 1966; ES 8041/2016; ES 50-1/2022).

Fatty acid composition of WCO. The fatty acid composition of cooking oils has a considerable impact on both nutritional value and stability. Considering the fatty acid composition of waste cooking oils under investigation (Table 2), the changes in the fatty acid composition were not of considerable amounts among the different treatments.

Table 2. Fatty acid composition of WCO before and post-treatment

Fatty acids, %	**Waste cooking oil, WCO**		
	Non-Treated	**CS-Treated**	**BE-Treated**
C12:0	0.12±0.01[a]	0.11±0.01[b]	0.09±0.01[c]
C14:0	0.94±0.04[b]	0.94±0.04[b]	0.95±0.04[a]
C16:0	38.79±0.05[c]	39.55±0.05[b]	40.20±0.05[a]
C16:1	0.23±0.01[a]	0.20±0.01[c]	0.22±0.01[b]
C17:0	0.09±0.00[a]	0.06±0.00[b]	0.09±0.04[a]
C17:1	0.02±0.00[b]	0.03±0.00[a]	0.02±0.00[b]
C18:0	4.24±0.02[a]	4.18±0.02[b]	4.16±0.02[c]
C18:1	43.49±0.05[a]	42.89±0.05[b]	42.27±0.05[c]
C18:1 *Trans*	0.09±0.00[a]	0.09±0.00[a]	0.09±0.00[a]
C18:2	11.06±0.02[a]	10.99±0.02[b]	10.96±0.02[c]
C18:2 *Trans*	0.02±0.00[a]	0.02±0.00[a]	0.02±0.00[a]
C18:3	0.28±0.00[b]	0.28±0.00[b]	0.30±0.00[a]
C20:0	0.41±0.01[a]	0.40±0.01[b]	0.40±0.01[b]
C20:1	0.16±0.00[b]	0.19±0.00[a]	0.16±0.00[b]
C22:0	0.06±0.00[b]	0.07±0.00[a]	0.07±0.00[a]
ΣSFA	44.65±0.03[c]	45.31±0.03[b]	45.96±0.03[a]
ΣUSFA	55.35±0.03[a]	54.69±0.03[b]	54.04±0.03[c]
ΣMUFA	43.99±0.03[a]	43.40±0.03[b]	42.76±0.03[c]
ΣPUFA	11.36±0.01[a]	11.29±0.01[b]	11.28±0.01[c]

Values are mean ± standard deviation (n=3). Means in the same row with different superscripts are significantly different (P<0.05).
MUFA – Mono unsaturated fatty acids; PUFA – Poly unsaturated fatty acids; SFA – Saturated fatty acids; USFA – Unsaturated fatty acids

Starting with the original waste cooking oil (non-treated) without bleaching, it retains the majority of its original saturated and unsaturated fatty acids. Heat treatment increases free fatty acids and promotes unsaturated fat oxidation with the sequences of Maillard reaction at hot cooking oil. The cornstarch-based bleaching (CS-treated) clarifies the waste cooking oil while slightly increasing the saturated fatty acids, although it may result in loss of unsaturated fat in negligible amounts. In the same way, when using the bleaching earth (BE-treated), it successfully eliminates pollutants and oxidized lipids while retaining the unsaturated fatty acids, albeit heating can result in polyunsaturated fat decreases. In conclusion, there is just a few changes in the fatty acid composition among all samples under different treatment due to the effect of heating on the unsaturated fatty acids undergoing some thermal decomposition.

Evaluation of cytotoxicity of WCO against the HEK-2 cell line. *In vitro* MTT assay measures the cell viability by quantifying the reduction of the MTT reagent (3-(4,5-dimethyl-2-thiazol-2-yl)- 2,5-diphenyl-2H-tetrazolium bromide) to formazan by metabolically active cells. By exposing HEK-2 cells

to various concentrations of WCO, the impact of WCO can be determined, indicating whether it has no effect or it induces cell death or inhibits cell growth, which is critical for understanding its cytotoxicity (Ghasemi et al. 2021).

Results in Tables 3-5 and Figures 2-4 summarize the evaluation of cytotoxicity of WCO samples under different treatments against the HEK-2 cell line. The cell cytotoxicity 50 (CC_{50}) values represent the cytotoxic concentration of WCO required to reduce the cell viability by 50%.

The lower CC_{50} value corresponds to higher cytotoxicity and the higher CC_{50} value corresponds to lower cytotoxicity. Basically, the CC_{50} means the concentration of a substance or compound needed to kill half the cells.

Table 3 and Fig. 2 reveal the cytotoxic effect of non-treated WCO with the lowest CC_{50} value (238.52 $\mu g.ml^{-1}$) which means its highest cytotoxic effect, followed by BE-treated (Table 4 and Fig. 3) having moderate CC_{50} value (466.10 $\mu g.ml^{-1}$) then CS-treated (Table 5 and Fig. 4) having the highest CC_{50} value (909.38 $\mu g.ml^{-1}$).

Implications for human kidney health (HEK-2 cell line relevance). Starting with the use of the human embryonic kidney (HEK-2) cell line, it is highly relevant, as the kidney is the primary organ in the body for detoxification and is susceptible to serious damage from ingesting food-borne toxicants. Findings of the present study suggest that even in small concentrations, the cytotoxic components in waste cooking oil (WCO) pose a potential risk of nephrotoxicity.

Variation of CC_{50} among the tested oils. Considering the variation in CC_{50} values among WCO under different treatments, the non-treated WCO had the lowest CC_{50} value (238.52 $\mu g.ml^{-1}$) meaning the highest cytotoxicity with the greatest harm to cells, alarming to consider its risk.

Considering the CS-treated WCO, it had the highest CC_{50} value (909.38 $\mu g.ml^{-1}$) meaning the lowest cytotoxicity, with highest viability of HEK-2 cells.

Considering the BE-treated WCO, it had the moderate CC_{50} value (466.10 $\mu g.ml^{-1}$) meaning the moderate cytotoxicity among.

It is noteworthy to mention the serious harmful effects of all waste cooking oil samples under different treatments, as none of the three treatments (non-treated, CS-treated, and BE-treated) samples had the complete safety at the maximum concentration (100%) of WCO aginst the viability of HEK-2 cells.

Even the CS-treated waste cooking oil, which had a CC_{50} value of 909.38 $\mu g.ml^{-1}$ which means the required concentration to kill 50% of cells, as it still having the potential cytotoxic effect to kill 50% of the cells with the concentration of 909.38 $\mu g.ml^{-1}$ approaching 1 $mg.ml^{-1}$, and it is not considered safe enough to confirm the survival of HEK-2 cells under this concentration proving the predominant health risks of ingesting this waste cooking oil (WCO).

Despite the thorough refining processes by individuals using cornstarch (CS), or as applied in the industry using the bleaching earth (BE), it was shown that these treatments enhanced some physicochemical parameters with lighter color, but they were insufficient to create a safe cooking oil.

Eventually when examining the detailed physicochemical analyses along with the *in vitro* MTT assay for cellular metabolic acitivities, it was concluded the failure of waste cooking oil to maintain the survival and full viability of HEK-2 cells (Ghasemi et al. 2021).

The main differences between cornstarch (CS) and bleaching earth (BE) can be described as following: bleaching with cornstarch (CS) is more effective in removing or masking the cytotoxic compounds in CS-treated WCO, as a natural carbohydrate polymer operating as filtering adsorptive agent, potentially trapping pollutants and lowering microbial load (Tyagi et al. 2025).

CS effectively masked the visual degradation of oil by removal of some impurities without complete purification that could mislead consumers into false sense of food safety.

Thereby, the cornstarch (CS) had more efficiency than bleaching earth (BE), despite the traditional use of cornstarch (CS) by individuals at home, but in oil industry, the factories use the bleaching earth (BE).

Bleaching earth (BE), a clay mineral, worked largely through adsorption and ion exchange to reduce color and some pollutants effectively as in the BE-treated WCO (Gharby 2022).

Table 3. Evaluation of cytotoxicity of non-treated WCO against the HEK-2 cell line

Sample concentration, µg.ml^{-1}	Viability, % (3 Replicates) 1st	2nd	3rd	Mean viability, %	Inhibitory, %	S.D. (±)
1000	9.72	8.13	7.69	8.51	91.49	1.07
500	20.35	22.47	19.84	20.89	79.11	1.39
250	49.08	47.56	44.75	47.13	52.87	2.20
125	80.73	78.29	76.18	78.40	21.60	2.28
62.5	91.56	90.34	87.25	89.72	10.28	2.22
31.25	98.12	97.28	96.04	97.15	2.85	1.05
15.6	100.00	99.71	99.38	99.70	0.30	0.31
7.8	100.00	100.00	100.00	100.00	0.00	0.00
0	100.00	100.00	100.00	100.00	0.00	0.00

Non-treated WCO (without bleaching)	CC_{50}, µg/ml – (3 Replicates) 1st	2nd	3rd	Mean CC_{50}, µg.ml^{-1}	S.D. (±)
	246.37	249.07	229.12	**238.52**	8.73

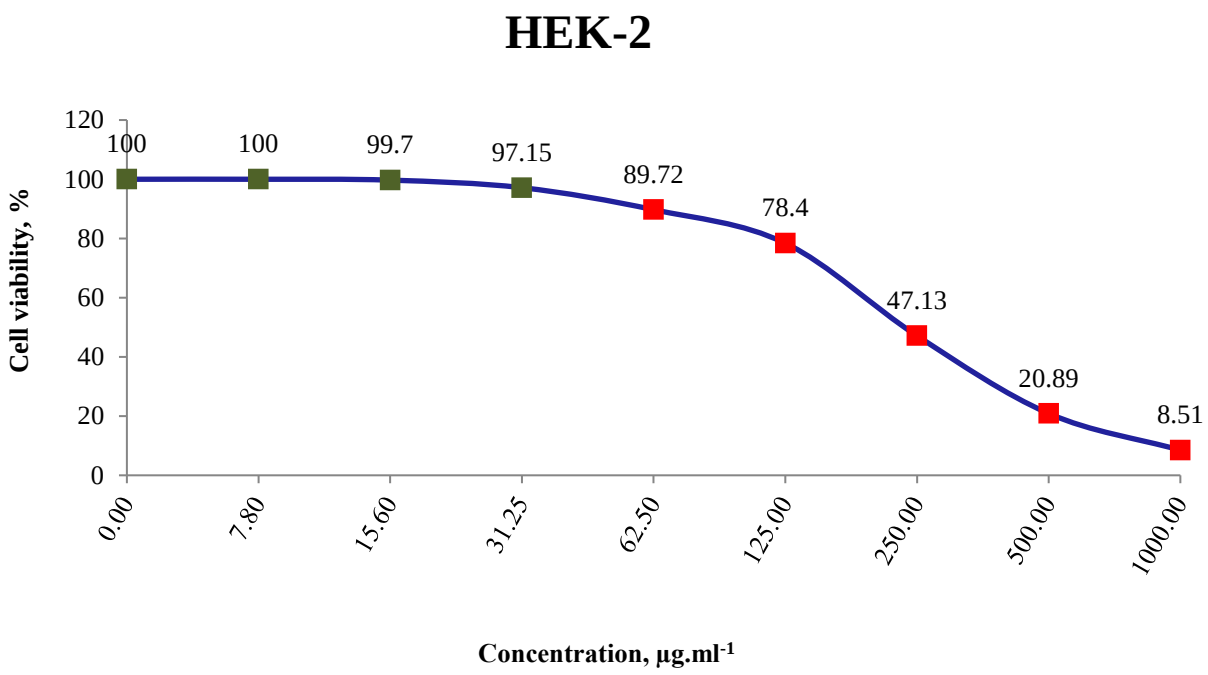


Figure 2. Evaluation of cytotoxicity of non-treated WCO against the HEK-2 cell line

Table 4. Evaluation of cytotoxicity of CS-Treated WCO against the HEK-2 cell line

Sample concentration, µg.ml^{-1}	Viability, % (3 Replicates) 1st	2nd	3rd	Mean viability, %	Inhibitory, %	S.D. (±)
1000	39.78	42.51	43.09	41.79	58.21	1.77
500	86.27	85.92	88.74	86.98	13.02	1.54
250	95.12	94.73	96.23	95.36	4.64	0.78
125	98.79	99.04	99.41	99.08	0.92	0.31
62.5	100.00	100.00	100.00	100.00	0.00	0.00
31.25	100.00	100.00	100.00	100.00	0.00	0.00
15.6	100.00	100.00	100.00	100.00	0.00	0.00
7.8	100.00	100.00	100.00	100.00	0.00	0.00
0	100.00	100.00	100.00	100.00	0.00	0.00

CS-Treated (Bleached with CS)	CC_{50}, µg/ml – (3 Replicates) 1st	2nd	3rd	Mean CC_{50}, µg.ml^{-1}	S.D. (±)
	890.08	913.73	924.32	**909.38**	17.53

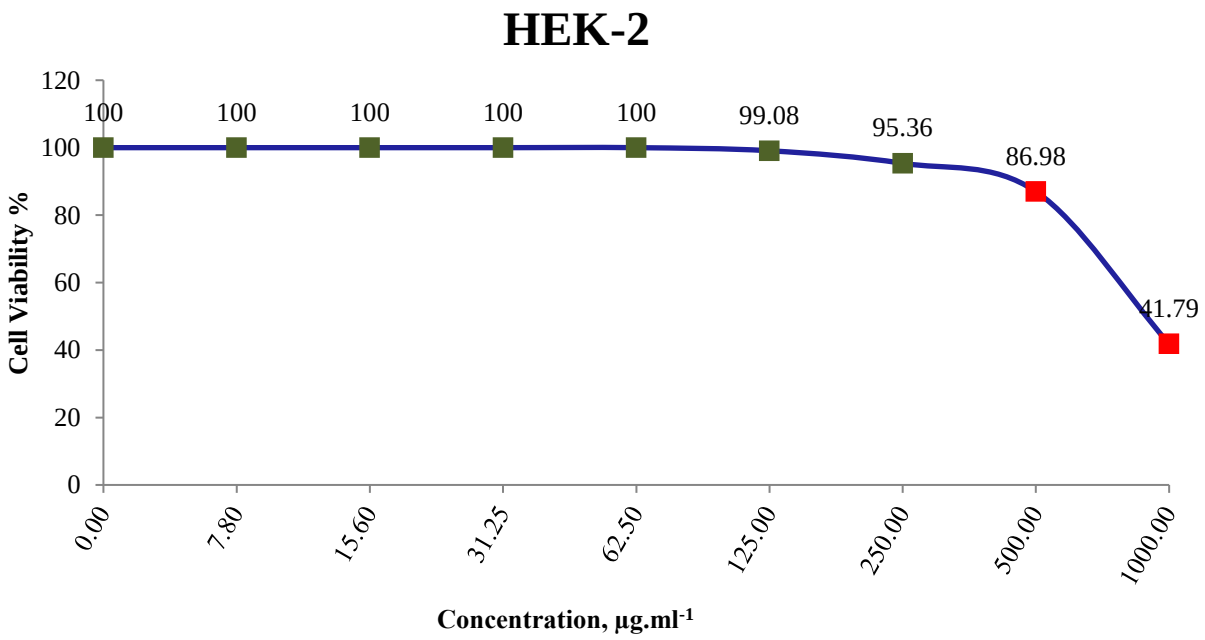


Figure 3. Evaluation of cytotoxicity of CS-Treated against the HEK-2 cell line

Table 5. Evaluation of cytotoxicity of BE-Treated WCO against the HEK-2 cell line

Sample concentration, µg.ml^{-1}	Viability % (3 Replicates) 1st	2nd	3rd	Mean viability, %	Inhibitory, %	S.D. (±)
1000	15.39	16.76	13.81	15.32	84.68	1.48
500	43.91	45.12	44.96	44.66	55.34	0.66
250	82.45	84.13	85.71	84.10	15.90	1.63
125	93.18	94.05	94.29	93.84	6.16	0.58
62.5	99.47	99.18	99.63	99.43	0.57	0.23
31.25	100.00	100.00	100.00	100.00	0.00	0.00
15.6	100.00	100.00	100.00	100.00	0.00	0.00
7.8	100.00	100.00	100.00	100.00	0.00	0.00
0	100.00	100.00	100.00	100.00	0.00	0.00

BE-Treated (Bleached with BE)	CC_{50}, µg/ml – (3 Replicates) 1st	2nd	3rd	Mean CC_{50}, µg.ml^{-1}	S.D. (±)
	460.50	468.73	469.08	**466.10**	4.86

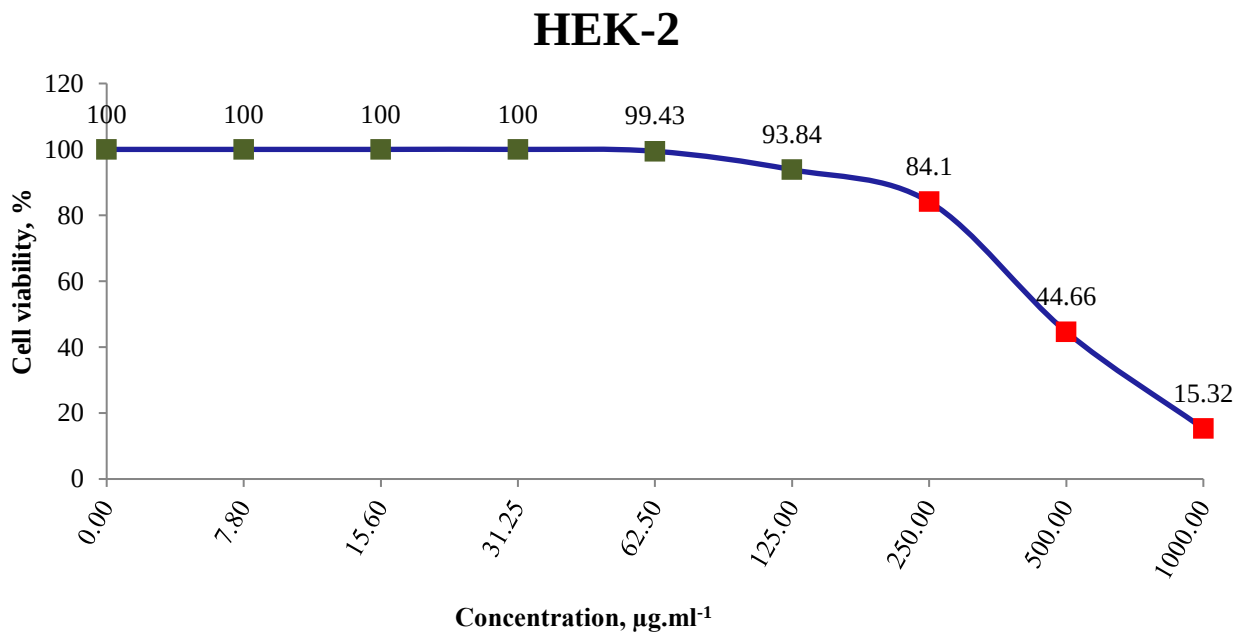


Figure 4. Evaluation of cytotoxicity of BE-Treated against the HEK-2 cell line

Novelty impact statement and significance of the study. The novelty of the current research can be summarized as a novel evidence-based approach integrating systematic process for decision-making by legally prohibiting reuse of waste cooking oil for human consumption after treatment as claimed by indivduals who seek to enhance some parameters as color. But other critical factors driven by the results of the human cell line culture and the emergence of high levels of 3-MCPD along with the inherent hazards confirmed by detailed physicochemical analyses, emphasized the credibilty of the established laws and standards for edible oils.

Aligning the study with the United Nations' Sustainable Development Goals (SDGs). The current research underscores the importance of the achievement of SDG3 (good health and well-being) by illustrating the health hazards associated with the reuse of WCO, which did not meet the quality and safety standards. Also, it emphasizes SDG 12 (responsible consumption and production) by assessment of WCO reuse, claiming the safer management of WCO. Moreover, the study addresses SDG 2 (zero hunger) by claiming the need for effective practices to ensure food security, quality, and safety to protect human health within the sustainable circular bioeconomy system.

Conclusions

The current study successfully demonstrated that the post-treatment waste cooking oil (WCO), whether treated with the traditional cornstarch (CS), as performed by individuals at home, or treated with the bleaching earth (BE), as carried out by factories in industry, does not meet the established quality and safety standards for edible oils. Even with the secondary treatments, persistent chemical, microbiological, and toxicological contaminants remain, posing significant health risks. Moreover, the *in vitro* cytotoxicity evaluation confirmed the potential harm to the human embryonic kidney (HEK-2) cell line, highlighting the inadequacy of these treatments to detoxify the WCO for human consumption. The findings of this pioneering study reinforce the current legislative status prohibiting reusing WCO for human consumption. While pursuit of sustainable and cost-effective edible oil alternatives is essential, post-treatment WCO cannot be considered a valid or safe substitute for fresh edible oils.

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Author Contributions

Conceptualization, R.I.M.A., E.A.M.M., and D.M.M.M.; methodology, R.I.M.A., E.A.M.M., and A.N.A.M.; formal analysis R.I.M.A., E.A.M.M., and A.N.A.M.; investigation, R.I.M.A., E.A.M.M., and A.N.A.M.; resources, R.I.M.A., E.A.M.M., and D.M.M.M.; data curation, R.I.M.A., E.A.M.M., and A.N.A.M.; writing - original draft preparation, R.I.M.A. All authors have read and agreed to the published version of the manuscript.

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The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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