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The Cytotoxicity Assessment of Grapefruit Peel Essential Oil and Its Effect on Frying Stability of Sunflower Oil

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ABSTRACT

Background: Frying oil lipids are prone to oxidation, so aromatic plants and their essential oils (EOs) have been applied to prevent this process. This study aimed to incorporate the grapefruit *(Citrus x paradisi)* EO in sunflower frying oil to protect it against oxidation. Before enrichment, a cytotoxicity test was performed to determine the non-cytotoxic concentration of this EO. **Methods:** Cell viability was evaluated using the MTT-based cytotoxicity assay. Various EO concentrations (0.01-0.5 mg/ml) were added to cultured cells Human Umbilical Vein Endothelial Cells (HUVECs) and Human Stellar Hepatic Cell lines LX-2 (SCC064) and incubated for 24 and 48 h. The stability of sunflower oil during frying was assessed by determining several parameters including peroxide value, polar compounds, and free fatty acids. **Results:** The obtained IC_{50} values after 24 h were 0.276 and 0.200 mg/ml for LX-2 and HUVECs cells, respectively, and the results after 48 h were 0.269 and, 0.216 mg/ml, respectively. Following that, the appropriate concentration of EO was incorporated into the sunflower oil. An oxidation acceleration test demonstrated that the lowest concentration of EO provided the best oxidation resistance (14 h 59 min) comparatively to the unfortified sunflower oil (11 h 63 min). The stability of enriched oil polar compounds during repeated frying was also noticed. **Conclusion:** The incorporation of this EO into sunflower oil during a deep-frying process led to a significant increase in its oxidative stability; therefore, it can be used as a food additive.

Introduction

Edible oils have an important role in our diet since they are one of the three main classes of nutrients and a source of energy, vitamins, and essential fatty acids including unsaturated ones which have preventive actions in many cardiovascular diseases and some forms of cancer

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(Bowen *et al.*, 2016). Sunflower crop, which is primarily grown in temperate regions, is the third annual oil crop grown globally after soybean and rapeseed (Salas *et al.*, 2021). Sunflower oil is a pale yellow vegetable oil, practically devoid of flavor, its linoleic acid content is very high (70%), which makes it one of the best sources of highly unsaturated essential fatty acids (Oboulbiga *et al.*, 2023). Vegetable oils including sunflower oil have multiple uses including frying that is one of the most popular processes used by consumers and food industries for various applications, which provides tasty food in a relatively short time (Mitrea *et al.*, 2022, Wenstrup *et al.*, 2014).

Nevertheless, the prolonged use of high temperatures during frying oils may result in ingredients that not only degrade food quality but also encourage the production of some degrading substances (primary and secondary oxidative products) (Aladedunye, 2015). These products might impair edible oils safety and nutritional value, and they might even be harmful to people's health (Zhang *et al.*, 2021). Oxidation is a series of chain reactions that affects all lipids and results from the interaction of unsaturated fatty acids with oxygen (Choe *et al.*, 2019, Ivanova-Petropulos *et al.*, 2015).

The used oil composition, type of food, as well as frying conditions such as temperature, duration of use, the surface-area-to volume ratio of the oil, periodic use, frying capacity (kg of fried food/h), and mode of heat transformation, are involved in frying oil lipid degradation in particular by oxidation (Al Amin *et al.*, 2023, Brühl, 2014).

Many varieties of aromatic plants and their essential oils (EOs) have been applied to prevent this oxidation process. Consequently, searching for natural antioxidants to take the place of synthetic antioxidants has attracted increasing attention in recent years. Meanwhile, plenty of extracts from spices and herbs have been demonstrated to hinder the oxidative degradation of edible oils (Montesano *et al.*, 2018, Singh *et al.*, 2022). Moreover, EOs extracted from spices and herbs not only could reveal antioxidant activity to enhance the oxidative stability and thermal stability of edible oils, but also could ameliorate sensory analysis to increase the acceptability of customers (Chandran *et al.*, 2017).

In this regard, several investigations are interested in using natural antioxidant molecules to address the phenomenon of fatty substances oxidation and its consequences on health (Costa *et al.*, 2021, Hadidi *et al.*, 2022, Ponnampalam *et al.*, 2022). For example, Delfanian *et al*. observed the frying stability of sunflower oil blended with jujube (Ziziphus mauritiana Lam.) leaf extract (Delfanian *et al.*, 2015). Additionally, Sayyari and Farahmandfar (2017) noted improvements in stability when using extracts from pussy willow (Salix aegyptiaca) and EOs (Sayyari and Farahmandfar, 2017). Hashemi *et al*. also studied the oxidative stability of sunflower oil during accelerated storage with Satureja khuzestanica essential oil (Hashemi *et al.*, 2011). Wang et al. (2018) reported that adding Coriandrum sativum L. EOs as a source of natural antioxidants can significantly enhance the oxidative stability of sunflower oil (Wang *et al.*, 2018). In our previous study, grapefruit peel EO increased the oxidative stability of margarine (Kaanin-Boudraa *et al.*, 2021). These EOs were also applied to increase the shelf life of frying baths (Abduh *et al.*, 2016). Hence, in the current study, the antioxidant effect of EO extracted from grapefruit *(Citrus x paradisi*) on refined sunflower oil frying stability was achieved. Beforehand, since there is a rising requirement investigating the security and toxicity of citrus EO peel-based ingredients, a cytotoxicity test was carried out to determine its non-toxic concentration. The stability of sunflower enriched with grapefruit peel EO during frying was tracked by measuring some main parameters like peroxide value, amount of free fatty acids, and polar compounds.

Material and Methods

Chemicals and plant material

All chemicals were either from Sigma-Aldrich, Madrid (Spain), Lonza, Basle, (Switzerland), or Merck Millipore, Burlington, Massachusetts

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(USA) and are of analytical grade.

Grapefruits (*Citrus* × *paradisi*, Algerian variety) were from Bejaia region of Algeria, peeled manually by a peeler and the produced fresh peels were hydro-distilled using a Clevenger-style device. The extraction yield was 0.33±0.01%, and the chemical composition of this EO was determined using the direct injection GC-MS technique as reported in our previous study (Kaanin-Boudraa *et al.*, 2021)**.**

Cytotoxicity assay

To assess the toxicity of *Citrus x paradisi* peel EO *in vitro,* the cell line used was Human Umbilical Vein Endothelial Cells (HUVECs) purchased from Lonza (Basle, Switzerland), while Human Stellar Hepatic LX-2 cell line (SCC064) was from Merck Millipore (Burlington, Massachusetts, USA).

The culture medium used for HUVECs was EGM-2 MV, Lonza (Endothelial Cell Growth Medium-2), whereas LX-2SCC064 was cultured in DMEM medium (Minimum Essential Medium, Gluta Max) supplemented with 10% SVF (serum veau foetal), 1% Penicillin-Streptomycin, and 1% Glutamine. The cell cultures were incubated in a humidified oven at 37 °C and with 5% CO_2. Cells were detached using phosphate-buffered saline (PBS10X) and trypsin 1%.

The density used cell seeding was 3 x 10^4 cells per well using 96-well plate. After 24 h of adhesion, they were exposed to increasing concentrations of EO. The latter was dissolved in DMSO stock solution at 100 mg/mL and diluted in DMEM/EGM medium to prepare different concentrations ranging from 0.01-0.5 mg/ml. After 24 h of cell adhesion to the bottom of the well, the culture medium was removed and substituted with 100 µL of each concentration on the 96-well plate containing the cells. Controls were performed without samples but in the presence of solvent. After 24 or 48 h of incubation, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test, a colorimetric indication of cellular mitochondrial activity was used to assess the vitality of the cells (Mosmann, 1983).

The procedure followed to carry out the MTT assay was presented by Mosmann (1983). Briefly, 50 µl of the medium mixture and stock MTT solution at a concentration of 0.5 mg/mL (1/10, *v/v*) was added to all wells followed by the incubation at 37 °C for 3 h and with 5% CO_2. Plates were read at 630 nm in 1 h of adding the DMSO (150 µl). The intensity of coloring is directly proportional to mitochondrial activity. The % viability was estimated as follows:

To calculate the percentage of cell survival, negative control with a 100% survival rate was used. When a compound reduced cell viability to less than 50%, it was deemed to be cytotoxic.

Accelerated oxidation test or "Rancimat test"

A Metrohm 743 Rancimat (Metrohm, Herisan, Switzerland) was utilized in the experiment to measure the effects of *Citrus* × *paradisi* EO on sunflower oil oxidative stability as previously reported in our previous study (Kaanin-Boudraa *et al.*, 2021). The temperature was maintained at 110 °C and air supply was kept at 15 l/h. This technique quantifies the change in conductivity caused by the presence of oxidation products in a heated and oxygenated sample. Afterwards, an interpolated induction time was calculated; the longer the induction time, the better the sample oxidative stability (Metrohm, 2013).

$$\%\,\text{Viability} = \frac{\text{mean absorbance of treated wells}}{\text{mean absorbance of control wells}} \times 100$$

Enrichment of sunflower oil and its frying conditions

Grapefruit EO at 12.5, 25, 50, 75, and 100 µg/g, ethylene diamine tetra acetic acid (EDTA) (25 µg/g), and tocopherol (25 µg/g) were added to sunflower oil. A negative control consists of sunflower oil without antioxidant addition.

The concentration of EO (25 µg/g) which demonstrated the highest induction time in the Rancimat assay was chosen for frying test. This EO was added to 2.5 l of sunflower oil. For frying test, the potatoes were peeled and cut into chips of more or less equal size and then dried with absorbent paper. The test was carried out using an

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electric fryer with a removable lid, with a capacity of 1 kg of frying product and 2.5 l of oil, equipped with a thermostat and a timer. After heating the oil bath to 180 °C, frying was started by introducing the first weight of chips while setting the frying time at 4 min and then the second weight was introduced while timing and this was repeated until the tenth weight was reached. The ratio of the amount of fries to the amount of oil in the fryer was carried out according to the method of Warner and Knowlton (Warner and Knowlton, 1997).

A control sample was taken from enriched sunflower oil before frying and considered a control (E0); three others were taken from the fifth (E5) and tenth fry (E10), respectively, and were filtered, cooled, and then put into an oil bottle.

Physico-chemical parameters namely peroxide value (PV) (Ghohestani *et al.*, 2023), free fatty acid content (FFA) (Farhoosh *et al.*, 2012), acid value (AV) (AOCS Official 1998)**,** polar compounds (Schulte, 2004) and oil color were determined for all samples to monitor the alteration of sunflower oil during frying and determinate its stability.

Data analysis

The data were shown as means with standard deviations (SD). The means were compared using one-way and two-way ANOVA analysis of variance (ANOVA) and a post hoc "Tukey HSD test with a *P-value<0.05* using STATISTICA 5.5. The IC_{50} values were determined using the prism dose-response curve by Graph Pad Prism 5.

Results

Cytotoxic effect of Citrus × paradisi peel EO

Studying cytotoxicity informs us about the range of non-toxic doses of EOs that can be used in the enrichment of food products. Hence, to assess the prospective toxic effect of *Citrus × paradisi* peel EO, HUVECs, and LX-2 cell lines were incubated with its various concentrations (0.01-0.5 mg/ml), and the viability was established 24 h and 48 h after the treatment. The concentrations of EO causing 50% inhibition (IC_{50} values) were calculated (**Table 1**). It was noticed that the studied EO reduced cell viability by decreasing cell density from a concentration of 0.05 mg/ml for HUVECs and from 0.1 mg/ml for LX-2, in a dose-dependent manner at 24 and 48 h.

Table 1. IC_{50} values after 24 and 48 h of treatment of LX-2 and HUVECs cell lines with *Citrus x paradisi* peel EO.

Cell line type	IC_{50} (mg/ml) after	
	24 h	48 h
LX-2	0.28 ± 0.08^{a}	0.27 ± 0.07^{a}
HUVECs	0.20 ± 0.07^{b}	0.22 ± 0.07^{b}

The results are the means with standard deviations (mean ± SD); Values with different letters in the same column show a significant difference at P<0.05; n= 3. ***HUVEC****: Human Umbilical Vein Endothelial Cells;* ***LX-2****: Human Stellar Hepatic Cell lines.*

Rancimat assay

The results of the acceleration of oxidation test for sunflower oil enriched with different concentrations of grapefruit EO are shown in **Table 2**. It is quite clear that it was sunflower oil enriched with *C x paradisi* peel EO at 25 µg/g which presented a better stability to oxidation with an induction time of 14 h and 59 minutes. This time was significantly higher compared to the one for sunflower oil without EO (11 h and 63 min). This confirms the antioxidant effect of the EO when added to the sunflower oil which resulted in increasing its resistance effect, and therefore its stability

Evolution of sunflower frying oil physicochemical indices

PV: **Figure 3A** shows changes in PV of oil samples during deep-frying process at 180 °C, it was observed that this PV for a control (sunflower oil without antioxidants) was 0.2 meq O_2/kg, which is in accordance with the standard (≤ 10 meq O_2/kg). Nevertheless, this value increased gradually with the number of frying to achieve a value of 4.6 meq/kg during the fifth frying, then it decreased during the tenth frying to the value of 3.6 meq/kg.

In sunflower oil containing 25 µg/g of grapefruit peel EO, the PV steadily increased, this could be due to the fact that secondary oxidation did not take place, and therefore secondary compounds were not formed.

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Table 2. The induction time with rancimat of sunflower oil enriched with *Citrus x paradisi* peel EO and synthetic antioxidants.

Concentration (µg/g)	EO (12.5)	EO (25)	EO (50)	EO (75)	EO (100)	Sunflower oil without EO	Tocopherol (25)	EDTA (25)
Time (h)	10.89 ± 0.45^{b}	14.59 ± 0.56^{h}	12.07 ± 0.44^{f}	11.89 ± 0.59^{e}	10.57 ± 0.58^{a}	11.63 ± 0.48^{d}	14.32 ± 0.6^{g}	11.23 ± 0.41^{c}

The results are the means with standard deviations (mean ± SD); ***EO****: Essential oil,* ***EDTA****: Ethylene diamine tetra acetic acid; Values with different letters in the same column show a significant difference at P<0.05*

Polar compounds: The content of polar compounds in sunflower oil varied with the number of frying (**Figure 3B**). It was 0% at the beginning, either in sunflower oil enriched or without grapefruit peels EO. Otherwise, it increased at the first frying with 0.3% to reach a value of 4.9% at the tenth one in samples without sunflower oil. Interestingly, the absence of polar compounds (0%) was noticed in sunflower oil enriched with grapefruit peel EO at the first and fifth frying, after that their rate began to increase to reach a value of 0.13%. The studied frying oils enriched by EO of grapefruit peel can be considered to comply with the international standards.

Acid value: In samples without sunflower oil, the variation in acidity was proportional to the number of frying with significant differences (P<0.05), values of 0.08%, 0.11%, and 0.13% were recorded at the first, fifth, and tenth frying. Nevertheless, when the EO was added to sunflower oil, the stability of acidity during the frying process (0.08%) was observed with no significant difference ($P<0$.05, **Figure 1C**).

Changes in color: The changes in yellow and red colors of sunflower oil before and after frying are shown in **Figures 2A** and **B**, respectively. Before frying, color of the enriched and non-enriched sunflower oil remained within the standard of the company (11/1.4). At the tenth frying, yellow and red colors of non-enriched sunflower oil were not in accordance with the standards. However, yellow color of sunflower oil enriched with grapefruit peel EO remained stable even after the fifth frying (8.4), which increased by one unit (9.5) compared to the difference (six units) recorded in the case of sunflower oil without EO. The EO probably exerted an antioxidant action by preventing the formation of polymers and therefore the degradation of oil.

Discussion

Cytotoxic effect of Citrus × paradisi peel EO

The IC_{50} found for the HUVEC cell line was lower compared to LX-2 cell line. This can be justified by the fact that HUVECS are more sensitive, since they are primary cells of the endothelium of the human umbilical vein, which is considered as the first barrier for the permeability of orally ingested substances. The kind of cell lines is a prominent factor in the variations of IC_{50} values for various cell lines (Ahmadi-Jouibari *et al.*, 2013, Silva *et al.*, 2019). According to the resulting IC_{50} values, the EO of grapefruit from Algeria was less cytotoxic than those from some Iranian *Citrus* peels as reported by Monajemi (Monajemi *et al.*, 2005) .

The cytotoxic impact of EOs extracted from *Citrus × paradisi* peel could be the result of a particular and/or synergistic action of various components that we have already identified in these EOs (Kaanin-Boudraa *et al.*, 2021). Several citrus species, including *C. medica*, *C. sinensis*, and *C. aurantifolia* have shown cytotoxic action against a variety of cell lines as well (Baik *et al.*, 2008, Patil *et al.*, 2009). Limonene is one of the most common naturally occurring monocyclic monoterpene that can be discovered in the oil of *Citrus* fruit peels which has a chemoprotective action against both rodent and human tumors (Crowell, 1999)**.** It was discovered that limonene might trigger apoptosis, and it also can trigger

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phase 1 and phase 2 carcinogen metabolizing enzymes (cytochrome p450) (Sun, 2007). In addition to limonene, it is well-established that myrcene has also cytotoxic effects (Majnooni *et al.*, 2012). However, it should be noted that low concentrations (< 0.1 mg/ml) of the studied EO do not have a toxic effect. Hence, these oils could be employed safely when respecting the dose in the fortification of sunflower oil

Rancimat assay

Edible oils are extremely important in food industry, but there are limited studies devoted to the impact of adding EOs on the oxidative stability of the refined product using the Rancimat test. Nevertheless, evidence from a variety of research methods suggests that some EOs extracted from some plants including thyme, oregano, clove, sage, basil, and fennel may have a positive impact on the antioxidant capacity of edible oils (Amorati and Foti (2012)). For example, Ozcan demonstrated that the EOs of rosemary had high antioxidant activity when combined with poppy oil (Özcan and Arslan, 2011). The same authors found that some spice oils added to sunflower oil could postpone the oxidation process in the following order: summer savory > rosemary > sage > marjoram > oregano > anise > tarragon (Loizzo *et al.*, 2015).

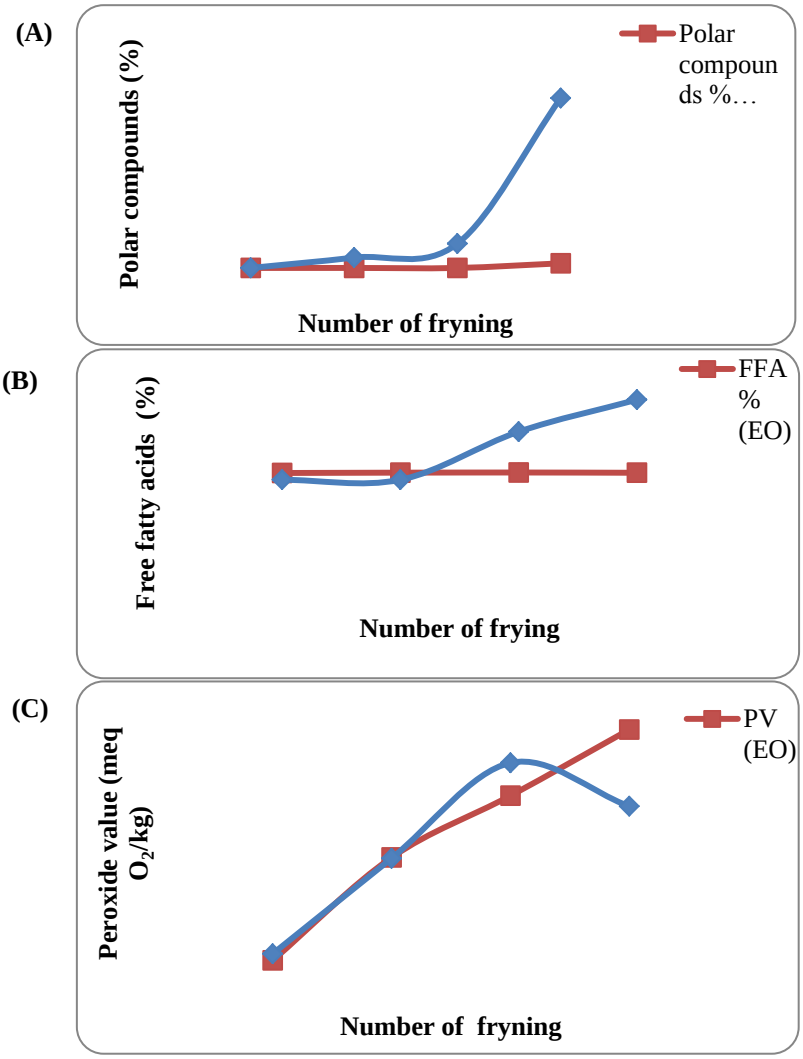

Figure 1. Evolution of peroxide value (A), polar compounds (B), and acid value (C) in sunflower oil fortified with 25 µg/g of grapefruit peel EO as a function of the number of fries comparatively to control. FFA: free fatty acids; SO: sunflower oil; EO: essential oil; PI: peroxide index.

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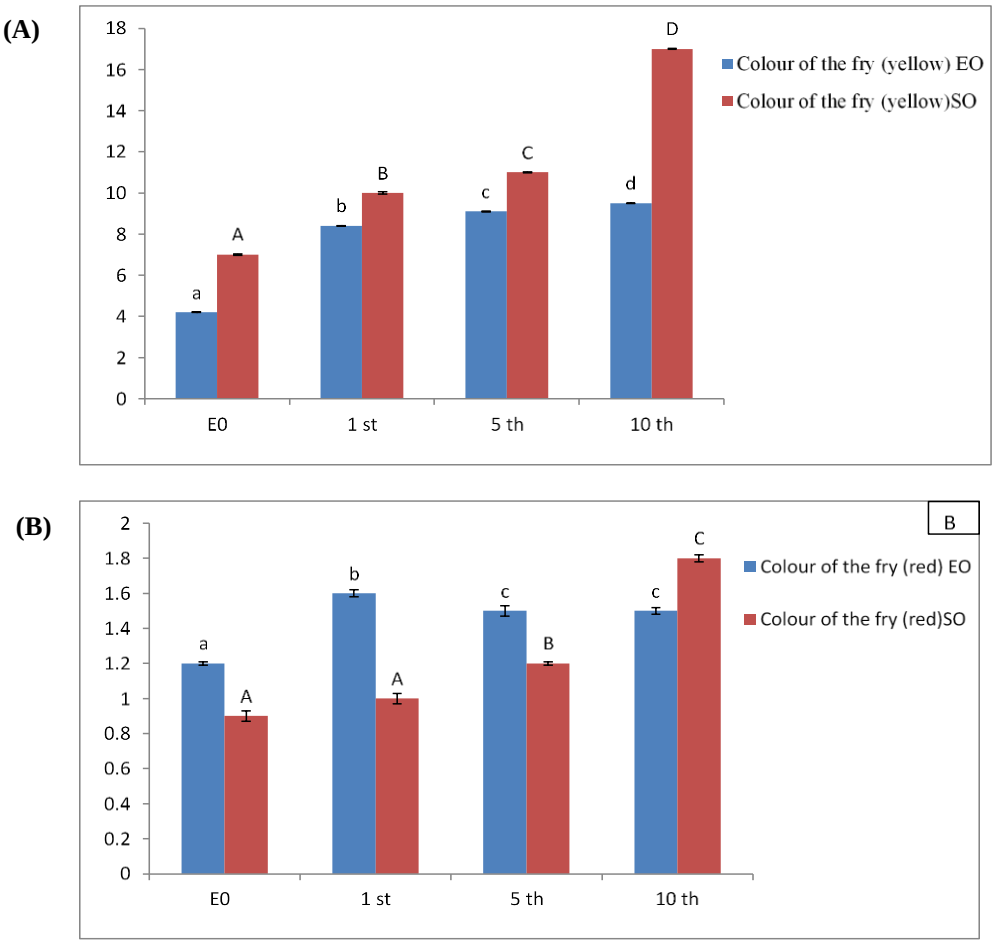

Figure 2. Evolution of yellow (A) and red (B) colors as a function of the number of frying. The bars bearing the same letters do not show any significant differences at $p < 0.05$. SO: Sunflower Oil; EO: Essential Oil.

Evolution of sunflower frying oil physicochemical indices

PV: In oils, the increase of the PV in the first stage can be explained by the occurrence of a primary oxidation that took place during frying leading to the formation of primary compounds such as peroxides and free radicals (Abdulkarim *et al.*, 2007)**.** During frying, the oxidation reactions will be accentuated giving rise to secondary products including aldehydes and ketones which are responsible for the rancid taste (Vitrac *et al.*, 2003) that resulted in this study a decrease in PV (3.6 meq/kg). The formation of peroxides according to a study by Nayak *et al.* affects the nutritional value of oils because their essential fatty acids are similarly destroyed (Nayak *et al.*, 2015).

Some findings demonstrated that the addition of natural compounds such as squalene, sterol fraction, quercetin, oryzanol, and ferulic acid improves the stability of vegetable oils at higher temperatures (Gertz *et al.*, 2000). Besides, the research conducted by Blekas indicated that the effects of free sterols and stearyl esters on slowing the degradation of heated oils were quite comparable (Blekas and Boskou, 1986).

Polar compounds: Determining polar compounds during the assessment of the thermo oxidative degradation of frying oils is essential, since it indicates its deteriorated components composition resulted from triglycerides dissociation (Ayouaz *et al.*, 2022, Sánchez-Gimeno *et al.*, 2008, Tsuzuki *et al.*, 2008)**.** According to the Spanish regulation, heated fats may only contain a

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maximum of 25% polar compounds (del Gobierno, 1989). Chen *et al*. stated that if oil contains more than this amount, it is considered unsuitable for consumption or even harmful (Chen *et al.*, 2021).

Acid value: Acid value is a determinant of oil quality and is directly connected to the amount of free fatty acids which are produced from triacylglycerol hydrolysis and oxidation (Sayyad and Ghugal, 2017). Hence, the increase in oil acidity is a result of releasing fatty acids leading to the degradation of its properties (Gertz and Kochhar, 2001, Medina-Valtierra *et al.*, 2017). The stability of fatty acids against oxidation during frying process was probably due to the antioxidant effect of the grapefruit peel EO that was measured in our previous study (Kaanin-Boudraa *et al.*, 2021).

Changes in color: The degradation of edible oils leads to the intensification of their color. Thus, the change of yellow or red color is justified by the alteration of their composition either by the formation of polymers during polymerization reactions of oxidation products or by co-oxidation (Barrera-Arellano *et al.*, 1997). In addition to oxidation, the variation in the color of frying oil would be directly linked to the decomposition of the natural coloring substances present in the oil such as β-carotene (Pénicaud *et al.*, 2011).

The strength of this study is the use of natural products such as grapefruit EOs, by respecting the dose, to reduce oxidation of frying oils and extend their life. However, to profitably exploit this enriched oil, the consumer's opinion is quite important. Hence, it is recommended to a sensory analysis to determine the appreciation of the experts of the oil added with the EO of *C.× paradisi*.

Conclusion

In this study, the cytotoxicity assay has established the non-toxic effect of low concentrations of *C. × paradise* peels EO using HUVEC and LX-2 cell lines. Then, the findings of the oxidation stability test revealed that sunflower oil enriched with EO at 25 µg/g showed better effects with an induction time of 14 h and 59 minutes. Therefore, this predetermined dose was adjusted to follow the effect of the incorporation on the sunflower frying oil over time. The incorporation of grapefruit peel EO into sunflower oil during a deep-frying process led to a significant increase in its oxidative stability. Moreover, this EO had a supplementary valuable impact when added to sunflower oil as it prevented secondary compound formation as revealed by following the peroxide value as a function of the number of frying. The absence of polar compounds was also noticed in sunflower oil enriched with grapefruit peel EO at the first and fifth frying and the acidity during the frying process was stable. Interestingly, the yellow color of sunflower oil enriched with grapefruit peel EO remained stable even after the fifth frying.

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Authors' contributions

G Kaanin-Boudraa and F Brahmi conceived and designed the study, and undertook literature research. All authors participated in experiment and data acquisition. L Grimaud and D Henrion performed data analysis. G Kaanin-Boudraa carried out statistical analysis, F Brahmi, L Boulekbache-Makhlouf, and K. Madani prepared, reviewed, and drafted the manuscript. All authors approved the final version before submission. All authors read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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References

Abduh MY, Rasrendra C, Subroto E, Manurung R & Heeres HJ 2016. Experimental

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[DOI: 10.18502/jnfs.v10i1.17760]

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and modelling studies on the solvent assisted hydraulic pressing of dehulled rubber seeds. *Industrial crops products.* **92**: 67-76.

Abdulkarim S, Long K, Lai OM, Muhammad S & Ghazali H 2007. Frying quality and stability of high-oleic Moringa oleifera seed oil in comparison with other vegetable oils. *Food chemistry.* **105 (4)**: 1382-1389.

Ahmadi-Jouibari T, Nikbakht MR, Mansouri K & Majnooni MB 2013. Cytotoxic effects of the essential oil from Achillea wilhelmsii C. Koch. *Journal of reports in pharmaceutical sciences.* **2 (2)**: 98-102.

Al Amin M, Ali MA, Alam MS, Nahar A & Chew SC 2023. Oxidative degradation of sunflower oil blended with roasted sesame oil during heating at frying temperature. *Grain oil science technology.* **6 (1)**: 34-42.

Aladedunye FA 2015. Curbing thermo- oxidative degradation of frying oils: Current knowledge and challenges. *European journal of lipid science technology.* **117 (11)**: 1867-1881.

Amorati R & Foti M 2012. Oxidative stability and antioxidant properties of essential oils. *Essential Oils as Natural Food Additives Valgimigli, L., Ed. Nova Science: New York, USA.* 75-95.

AOCS Official 1998. methods and recommended practices of the American Oil Chemists' Society, American Oil Chemists' Society, Champaign, IL, USA.

Ayouaz S, et al. 2022. Impact of incorporating sesame oil (Sesamum indicum L.) in an Algerian frying oil and margarine formulation. *North African journal of food nutrition research.* **6 (14)**: 165-177.

Baik J-S, et al. 2008. Chemical composition and biological activities of essential oils extracted from Korean endemic citrus species. *Journal of microbiology biotechnology.* **18 (1)**: 74-79.

Barrera-Arellano D, Márquez-Ruiz G & Dobarganes M 1997. A simple procedure to evaluate the performance of fats and oils at frying temperatures. *Grasas y Aceites.* **48 (4)**: 231-235.

Blekas G & Boskou D 1986. Effect of esterified sterols on the stability of heated oils. In *The shelf life of foods and beverages* (ed. C. G). Elsevier Amsterdam.

Bowen KJ, Harris WS & Kris-Etherton PM 2016. Omega-3 fatty acids and cardiovascular disease: are there benefits? *Current treatment options in cardiovascular medicine.* **18**: 1-16.

Brühl L 2014. Fatty acid alterations in oils and fats during heating and frying. *European journal of lipid science technology.* **116 (6)**: 707-715.

Chandran J, Nayana N, Roshini N & Nisha P 2017. Oxidative stability, thermal stability and acceptability of coconut oil flavored with essential oils from black pepper and ginger. *Journal of food science technology.* **54**: 144-152.

Chen J, et al. 2021. The formation, determination and health implications of polar compounds in edible oils: Current status, challenges and perspectives. *Food chemistry.* **364**: 130451.

Choe E, Lee J & Min DB 2019. Chemistry for oxidative stability of edible oils. *Healthful lipids.* 558-590.

Costa M, et al. 2021. Polyphenols as antioxidants for extending food shelf-life and in the prevention of health diseases: Encapsulation and interfacial phenomena. *Biomedicines.* **9 (12)**: 1909.

Crowell PL 1999. Prevention and therapy of cancer by dietary monoterpenes. *Journal of nutrition.* **129 (3)**: 775S-778S.

del Gobierno P 1989. Quality standard for heated oils and fats. *Official State Gazette.* **26 (31)**.

Delfanian M, Esmaeilzadeh Kenari R & Sahari MA 2015. Frying stability of sunflower oil blended with jujube (Ziziphus mauritiana Lam.) leaf extract. *Food science nutrition.* **3 (6)**: 548-556.

Farhoosh R, Khodaparast MHH, Sharif A & Rafiee SA 2012. Olive oil oxidation: rejection points in terms of polar, conjugated diene, and carbonyl values. *Food chemistry.* **131 (4)**: 1385-1390.

Gertz C, Klostermann S & Kochhar SP 2000. Testing and comparing oxidative stability of vegetable oils and fats at frying temperature. *European journal of lipid science technology.*

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102 (8- 9): 543-551.

Gertz C & Kochhar SP 2001. A new method to determine oxidative stability of vegetable fats and oils at simulated frying temperature. *Oléagineux, Corps Gras, Lipides.* **8 (1)**: 82-88.

Ghohestani E, Tashkhourian J & Hemmateenejad B 2023. Colorimetric determination of peroxide value in vegetable oils using a paper based analytical device. *Food chemistry.* **403**: 134345.

Hadidi M, et al. 2022. Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products. *Lwt.* **169**: 114003.

Hashemi MB, Niakousari M, Saharkhiz MJ & Eskandari MH 2011. Influence of Zataria multiflora Boiss. essential oil on oxidative stability of sunflower oil. *European journal of lipid science technology.* **113 (12)**: 1520-1526.

Ivanova-Petropulos V, et al. 2015. Characterisation of traditional Macedonian edible oils by their fatty acid composition and their volatile compounds. *Food research international.* **77**: 506-514.

Kaanin- Boudraa G, et al. 2021. Citrus× paradisi essential oil as a promising agent for margarine storage stability: Composition and antioxidant capacity. *Journal of food processing preservation.* **45 (5)**: e15374.

Loizzo MR, Tundis R, Menichini F & Duthie G 2015. Anti-rancidity effect of essential oils, application in the lipid stability of cooked turkey meat patties and potential implications for health. *International journal of food sciences nutrition.* **66 (1)**: 50-57.

Majnooni M-B, et al. 2012. Chemical composition, cytotoxicity and antioxidant activities of the essential oil from the leaves of Citrus aurantium L. *African journal of biotechnology.* **11 (2)**: 498-503.

Medina-Valtierra J, Sánchez-Olmos LA, Carrasco-Marin F & Sánchez-Cárdenas M 2017. Optimization models type box-behnken in the obtaining of biodiesel from waste frying oil using a large-acidity carbonaceous catalyst. *International journal of chemical reactor engineering.* **15 (6)**: 20170072.

Metrohm A 2013. A guide to near-infrared spectroscopic analysis of industrial manufacturing processes, Herisau, Switzerland.

Mitrea L, et al. 2022. The physicochemical properties of five vegetable oils exposed at high temperature for a short-time-interval. *Journal of food composition analysis.* **106**: 104305.

Monajemi R, Oryan S, Haeri-Roohani A, Ghannadi A & Jafarian A 2005. Cytotoxic effects of essential oils of some Iranian citrus peels. *Iranian journal of pharmaceutical research.* **4 (3)**: 183-187.

Montesano D, et al. 2018. Italian Lycium barbarum L. berry: Chemical characterization and nutraceutical value. *Natural product communications.* **13 (9)**: 1151-1156.

Mosmann T 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods.* **65 (1-2)**: 55-63.

Nayak B, et al. 2015. Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from Citrus sinensis peels. *Food chemistry.* **187**: 507-516.

Oboulbiga EB, et al. 2023. Physicochemical, potential nutritional, antioxidant and health properties of sesame seed oil: a review. *Frontiers in nutrition.* **10**: 1127926.

Özcan MM & Arslan D 2011. Antioxidant effect of essential oils of rosemary, clove and cinnamon on hazelnut and poppy oils. *Food chemistry.* **129 (1)**: 171-174.

Patil JR, et al. 2009. Apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles of Citrus aurantifolia. *Food chemistry.* **114 (4)**: 1351-1358.

Pénicaud C, Achir N, Dhuique-Mayer C, Dornier M & Bohuon P 2011. Degradation of β-carotene during fruit and vegetable processing or storage: reaction mechanisms and kinetic aspects: a review. *Fruits.* **66 (6)**: 417-440.

Ponnampalam EN, et al. 2022. The importance of dietary antioxidants on oxidative stress, meat and milk production, and their preservative aspects in farm animals: Antioxidant action, animal health,

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[DOI: 10.18502/jnfs.v10i1.17760]

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and product quality—Invited review. *Animals.* **12 (23)**: 3279.

Salas JJ, Bootello MA, Martínez-Force E, Calerón MV & Garcés R 2021. High stearic sunflower oil: Latest advances and applications. *Oilseeds and fats, crops and lipids (OCL).* **28 (35)**: 3-8.

Sánchez-Gimeno A, Negueruela A, Benito M, Vercet A & Oria R 2008. Some physical changes in Bajo Aragón extra virgin olive oil during the frying process. *Food chemistry.* **110 (3)**: 654-658.

Sayyad AS & Ghugal YM 2017. Bending, buckling and free vibration of laminated composite and sandwich beams: A critical review of literature. *Composite structures.* **171**: 486-504.

Sayyari Z & Farahmandfar R 2017. Stabilization of sunflower oil with pussy willow (Salix aegyptiaca) extract and essential oil. *Food science nutrition.* **5 (2)**: 266-272.

Schulte E 2004. Economical micromethod for determination of polar components in frying fats. *European journal of lipid science technology.* **106 (11)**: 772-776.

Silva AM, Martins-Gomes C, Fangueiro JF, Andreani T & Souto EB 2019. Comparison of antiproliferative effect of epigallocatechin gallate when loaded into cationic solid lipid nanoparticles against different cell lines. *Pharmaceutical development technology.* **24 (10)**: 1243-1249.

Singh AK, Kim JY & Lee YS 2022. Phenolic compounds in active packaging and edible films/coatings: natural bioactive molecules and novel packaging ingredients. *Molecules.* **27 (21)**: 7513.

Sun J 2007. D-Limonene: safety and clinical applications. *Alternative medicine review.* **12 (3)**: 259.

Tsuzuki W, Nagata R, Yunoki R, Nakajima M & Nagata T 2008. cis/trans-Isomerisation of triolein, trilinolein and trilinolenin induced by heat treatment. *Food chemistry.* **108 (1)**: 75-80.

Vitrac O, Trystram G & Raoult-Wack A-L 2003. Frying process and fried products. In *Lipids and dietary fats* (ed. J. Graille). Agri-food science and technology collection: Paris.

Wang D, et al. 2018. Oxidative stability of sunflower oil flavored by essential oil from Coriandrum sativum L. during accelerated storage. *Lwt.* **98**: 268-275.

Warner K & Knowlton S 1997. Frying quality and oxidative stability of high- oleic corn oils. *Journal of the American oil chemists' society.* **74 (10)**: 1317-1322.

Wenstrup MJ, Plans M & Rodriguez- Saona LE 2014. Effect of a novel induction food-processing device in improving frying oil quality. *International journal of food science technology.* **49 (10)**: 2223-2229.

Zhang Y, et al. 2021. Mechanism, indexes, methods, challenges, and perspectives of edible oil oxidation analysis. *Critical reviews in food science nutrition.* 1-15.

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