



Reduction of acrylamide formation in potato chips during deep-frying in sunflower oil using pomegranate peel nanoparticles extract

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Abstract

Lyophilized pomegranate peel nanoparticles extract (LPP-NPsE) is rich in bioactive compounds and could be applied as a natural antioxidant. This study evaluated the effect of using LPP-NPsE as an antioxidant on the oxidative stability of sunflower oil and reducing of acrylamide induction in potato chips during deep frying. LPP-NPsE was incorporated into sunflower oil (1000 mg/kg) while butylated hydroxytoluene (BHT, 200 mg/kg) and tocopherols (1000 mg/kg) were used as a positive control. Peroxide value (PV), total polar compounds (TPCs), free fatty acids (FFAs), and conjugated dienes and trienes were determined during frying to monitor oil stability. LPP-NPsE was effective and had the lowest PV, FFA, and TPC during frying. The acrylamide content was determined using HPLC coupled with a photo diode array detector. The initial value of acrylamide was low (192 mg/kg), while the highest acrylamide content was detected in control deep-fried potato chips (1674 mg/kg) after 20 frying cycles. The reduction (54%) in acrylamide content in potato chips was achieved after the addition of LPP-NPsE. LPP-NPsE could be favorably used as an antioxidant for acrylamide reduction in sunflower oil during deep frying.

Keywords *Punica granatum* · Oxidative stability · Total polar compounds · Fruit by-products · Conjugated dienes · Conjugated trienes

Introduction

Deep frying is a process in which food is completely submerged in heated oil. Fried foodstuffs have desirable color, flavor, and texture, which make deep-frying popular [1–3]. Lipid oxidation, hydrolysis, polymerization and Maillard reactions induced during frying have gained attention from health and food scientists. Complex reactions might occur simultaneously and subsequently [4, 5]. Carbonyls induced

after fatty acids oxidation in the presence of protein residues could constitute several reactant combinations. Deep frying process have the potential to study the effect of lipid oxidation on the induction of carbonyl–amine reaction products.

Since the discovery of acrylamide formation in the thermally processed foods [6], there have been efforts to reduce the acrylamide levels in foodstuffs [7, 8]. Considering the recent EU regulations [9] establishing benchmark levels for acrylamide reduction in foods, an approach to acrylamide mitigation is needed [8].

Toxicity of acrylamide formed in foodstuffs prepared by cooking at high temperatures (above 120 °C) has generated interest. High levels of acrylamide were detected in the heated mashed fried potato [10]. Fried potatoes contain high levels of acrylamide due to the presence of high levels of its precursors in the tuber and to the applied thermal processing. Direct consumer exposure to acrylamide may result from ingestion of high-carbohydrate foods such as potato chips, roasted cereals, and bread. There has been an upward trend in consumption of snacks (i.e., fast foods and drinks) between main meals [11]. Oil absorption could reach 40% in potato chips [12], wherein acrylamide concentration

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in potato chips fried in sunflower oil ranged from 525 to 722 $\mu\text{g}/\text{kg}$ [13].

The effect of antioxidants is among the factors influencing the level of acrylamide in processed foods. Antioxidants decrease lipid oxidation and extend the shelf life of the oils and the fried stuff. Jin et al. [14] reviewed the role of antioxidants on acrylamide formation. Some studies on foodstuffs and model systems mentioned mitigation of acrylamide formation as affected by antioxidants, while others reported no effect or even an increase [15–17]. It could be due to the capability of antioxidants having different functional groups to react with acrylamide precursors leading to either promoting or reducing effects. Some plant extracts rich in phenolics were applied in food products as an alternative source of synthetic antioxidants [18–20]. Urbancic et al. [2] evaluated the impacts of rosemary extract on sunflower oil stabilization and the reduction of acrylamide levels in potato during deep-frying.

Pomegranate (*Punica granatum*) fruit has been used in traditional medicine owing to the presence of bioactive compounds with antimicrobial and immunomodulatory traits [21]. *P. granatum* fruit contains sugars, vitamins, minerals, phenolics, and organic acids. The composition of *P. granatum* fruit is affected by fruit variety, climatic conditions, growing area, soil structure, maturity status, and cultural practice [22, 23]. Peels represent about 50% of the whole fruit [24] and contain high amounts of phenolics such as ellagic tannins, gallic acid, and ellagic acid [25]. Studies described the efficiency of pomegranate peel for the inhibition of lipid oxidation in foods [26, 27].

Development of nanotechnology has led to producing valuable by-products in the food and pharmaceutical industries. Nanotechnology was used in food products to enhance safety, quality, shelf life, and nutritional value [28, 29]. Phenolic compounds have many biological traits such as antioxidant and anticancer activities. Nanoencapsulation have been applied for enhancing the bioavailability of phenolic compounds [30]. Balooch et al. [31] synthesized a fungicide nanocomposite using the extract of pomegranate fruit waste and montmorillonite. The synthesized nanocomposite considered as a low-cost valuable by-product in the pomegranate juice processing. Addition of lyophilized pomegranate peel nanoparticles extract (LPP-NPsE) to prevent lipid oxidation, and the effect of LPP-NPsE on acrylamide formation in fried foods has not been explored. The aims of the current investigation were; (i) to prepare LPP-NPsE, and (ii) to evaluate the efficiency of LPP-NPsE as natural antioxidants to stabilize sunflower oil and reduce acrylamide formation during frying of potato chips.

Materials and methods

Materials

Butylated hydroxytoluene (BHT) was purchased from Merck (Darmstadt, Germany). Tocopherols ($\geq 90\%$) and acrylamide ($\text{CH}_2=\text{CHCONH}_2$) (99%, electrophoresis grade) were obtained from Sigma (St-Louis, MO, USA).

Preparation of freeze-dried pomegranate peel nanoparticles

Pomegranate fruit (*Punica granatum* L., cv. Manfaloty) were obtained from a local market (Toukh City, Egypt). Fruits were immediately transported to the laboratory and surface-disinfected by gently immersing for 2 min with 200 $\mu\text{L}/\text{L}$ sodium hypochlorite, then washed with a distilled water. Fruits were manually peeled, and the edible parts were separated. Peels were cut into 1 cm \times 1 cm piece and frozen at -40°C , then dehydrated for 72 h in a freeze-dryer (Labconco 74,200, USA) on 0.120 mbar, chamber temperature at 18°C and a condenser at -85°C . The LPP was transferred to nanoparticles as reported by Khataee et al. [32] with slight modifications. Peels were ground to the range of 100–150 μm using a grinder (Moulinex grinder-Model MC300, France). The particles were crushed using a high-energy planetary ball mill at 320 rpm rotation speed for 120 min to produce nanoparticles. The ratio of ball mass to powder mass was 10:1. The nanoparticles were measured by zetasizer (NanoSight NS300, UK) which was 80 nm. The prepared nanoparticles were kept in a glass bottle until used.

Preparation of lyophilized pomegranate peel nanoparticles extract (LPP-NPsE)

Extraction of LPP extraction particles was performed using an ultrasonic bath (Bandelin Super Sonorex RK-100H) as reported by Tabaraki et al. [19]. LPP and solvent (ethanol 90%) were blended (1 g LPP:20 mL ethanol 90%) and sonicated for 1 h at room temperature in an ultrasonic bath. After extraction, the flask was removed from the bath and cooled at room temperature. The LPP-NPsE was filtered using Whatman No. 1 filter paper and concentrated using rotary evaporator at 40°C . LPP-NPsE was frozen at -40°C , then dehydrated for 72 h in a lyophilizer (Labconco 74,200, USA) at 0.120 mbar, chamber temperature (18°C) and a condenser at -85°C . The LPP-NPsE powder was stored at -80°C .

Preparation of potato chips and frying experiment

Fresh potatoes (Spunta) were peeled and sliced to a uniform thickness ($0.5 \times 0.5 \times 8.0$ mm) by a mechanical slicer. Slices were submerged in distilled water at room temperature, then dried with tissue papers. Deep frying was carried out in a deep fryer with a 3-L volume vessel. The LPP-NPsE was added to the sunflower oil at 1000 mg/kg [20]. According to Khalil et al. [33] LPP-NPsE contained 110 to 98 mg punicalagin as an active antioxidant. Tocopherols were added to the oil at 1000 mg/kg, while BHT was added at its legal limits of (200 mg/kg active ingredient). Bringing the temperature to 180 ± 5 °C, the frying process was continued for 10 min. A constant temperature (180 °C) was maintained during the frying process. After heating the oil (3 L), raw potatoes (250 g per batch) were deep fried. After the frying process, the oil was allowed to cool to room temperature. Twenty batches of the pre-fried potatoes were deep-fried over a 10-day period (2 batches per day, a total of 20 frying cycles). Samples were removed for analysis after each frying process. Fresh sunflower oil was not added between the batches and the fryer was left uncovered during the frying operations. Twenty frying cycles were considered as the endpoint because the best oil performance reached ca. 25% polar compounds at this stage, which is close to that of the European standard for spoiled oils [34, 35]. A constant temperature (180 °C) was maintained during two frying cycles each day. After each frying cycle, the oil was allowed to cool to 60 °C and samples (10 mL) were drawn then stored at -20 °C after flushing with nitrogen until analysis. Control without additives was utilized for comparative purposes. The 20 frying cycles was repeated thrice ($n=3$) with different batches of oils and potato chips.

Analysis of deep-fried oil and deep-fried potato

Oil analyses

Peroxide value (PV), free fatty acids (FFAs), saponification value and iodine value of oil samples were determined in the oils before and after deep frying according to the American Oil Chemists' Society official methods [36]. The content of conjugated dienes (CDs) and trienes (CTs) was determined at 232 nm and 268 nm according to Kim and Labella [37] using a Cecil Series 8000 UV/VIS spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Determination of the total polar compounds (TPCs) was carried out according to 2.507 Official Methods of the International Union of Pure and Applied Chemistry [38].

Acrylamide analysis in deep-fried potato chips

The extraction of acrylamide from a deep-fried potato chip was carried out according to Wang et al. [39] using Waters Sep-Pak cartridges (Milford, MA, USA). Since the method was not developed in our lab, the sensitivity and recovery rate of the method was tested. The samples were purified by solid-phase extraction and the solution was centrifuged at $14,500 \times g$ for 20 min at 0 °C to remove lipids. The recovery of acrylamide was higher than 81.0% and the precisions were 2.5–9.85% ($n=3$). Chromatographic analysis was carried out using a Young Lin HPLC, series YL-9100, equipped with a quaternary pump, an autosampler (YL9150), a degasser, and a YL-9160 spectrophotometric detector (photo diode array detector). The detector was set at 202 nm using a reversed phase C_{18} -type column (Hypersil ODS C_{18}) of 4.6 mm \times 15 cm. The flow rate was fixed at 0.5 mL/min and solvent system consisted of acetonitrile:H₂O (70:30, v/v). Mode of the HPLC instrument was isocratic and the injection volume was 20 μ L [40, 41].

Statistical analysis

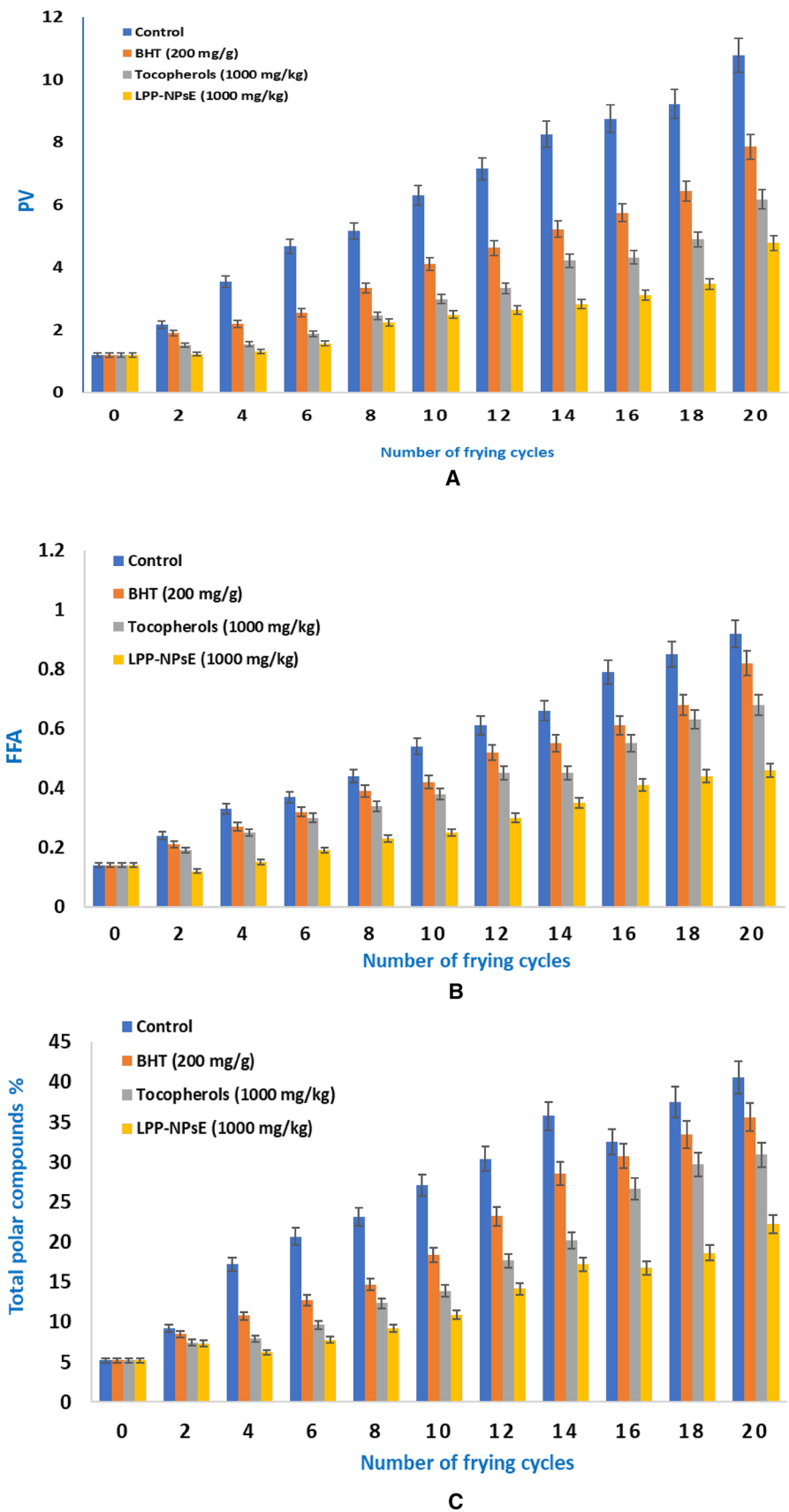
Samples were analyzed in triplicate, and the results were presented as mean \pm standard deviation. Significant differences were calculated using Duncan's multiple range test [42]. Differences were considered to be significant at $p < 0.05$.

Results and discussion

Quality of fresh sunflower oil

Chemical properties of different batches of fresh sunflower oil were evaluated. The values were as follow: PV 1.19 mEq O₂/kg, FFA 0.14% (as oleic acid), TPC 5.20%, iodine value 130.9%, saponification value 190.22 mg KOH/g, CD 1.80, and CT 1.32. FFA is produced mainly by the hydrolysis of oils and fats while the fatty acid content values varied according to exposure to various conditions (i.e. storage and processing). Pal et al. [43] reported that FFA reduced from 1.10 to 0.24% after the refining process. Suliman et al. [44] also reported that refining reduced FFA content from 0.5 to 0.1%. The CD was measured as 1.80%. CD linked with the degree of oxidation occurring in unsaturated oil. Warner and Eskin [45] reported that CD values for vegetable oils range from 0 to 6% upon the source of the oil. In general, the parameters indicated a good quality of fresh sunflower oil used in this study.

Fig. 1 Changes in **a** PV, **b** FFA, and **c** TPC in the sunflower oil during deep-frying of potato chips. Error bars show the variations of three determinations in terms of standard deviation



Effect of LPP-NPsE on the quality of deep-fried oil

Peroxide value (PV)

Hydroperoxides concentration, expressed as PV, versus numbers of frying, are shown in Fig. 1a. At the beginning of the experiment, the PV was 1.19 mEq O₂/kg. The PV of sunflower oil samples enriched with 200 ppm BHT, 1000 ppm tocopherol and LPP-NPsE were 7.85, 6.17 and 4.78 mEq O₂/kg, respectively. The PV of the control sample recorded 10.77 mEq O₂/kg after 20 frying cycles. LPP-NPsE remained the most effective and had the lowest PV during frying. PV determinations for the measurement of peroxides are commonly reported [46–48]. Previous studies reported that pomegranate extract decrease PV during deep frying [49] or under heat conditions [48].

Free fatty acids (FFAs)

The changes in FFA of the oil samples during deep-frying are presented in Fig. 1b. An increase in FFA was measured for all samples. At the end of the frying process, FFA contents in the control sample, as well as BHT, tocopherol and LPP-NPsE, enriched oils were 0.92, 0.82, 0.68 and 0.46 after 20 cycles, respectively. FFA, formed during frying by oxidation and hydrolysis, is used to monitor the quality of frying oil. The rates of this process vary depending on a number of variables, including frying medium and fried foods [50, 51]. The effects of antioxidants on lowering the FFA formation has been reported [52, 53]. The rate of formation of FFA in sunflower oil enriched with LPP-NPsE was lowered by 53% at the end of the deep-frying process compared to the control oil.

Total polar compounds (TPCs)

Figure 1c illustrates that the TPC increased during deep frying. Fried sunflower oil without antioxidants had the highest level of TPC at the end of frying time (40.5%). Mixing sunflower oil with 200 ppm BHT, 1000 ppm tocopherol or LPP-NPsE reduced the TPC. The oil mixed with LPP-NPsE had the lowest level of TPC (22.2%) at the end of the deep-frying process. Polar compounds are formed during deep-frying due to oil hydrolysis, oxidation and thermal reactions that produce FFA, cyclic compounds, dimers, and polymers. The mechanism of thermal degradation of fried oils due to the high temperature and the presence of moisture and air was reported [54, 55]. Fats and oils undergo polymers formation at high temperatures although polymerization also occurs during autoxidation at low temperatures [56]. Control sunflower oil subjected to deep-frying, reached its discard point (TPC 25 g/100 g) after 9 frying cycles, while the addition of BHT, tocopherols, and LPP-NPsE was effective in

reducing TPC. Deep fried oil enriched with LPP-NPsE was more effective than deep fried oil enriched with BHT and tocopherols. LPP-NPsE oil reached discard point after 20 cycles of frying. The results of TPC confirmed the ability of LPP-NPsE to reduce TPC formation in fried sunflower oil. The addition of pomegranate peels extracts enhanced sunflower oil stability during storage [20]. The TPC reduction by addition of antioxidants to sunflower oil was reported in the literature [2, 57] and agreed with our findings.

Conjugated dienes (CDs) and trienes (CTs)

It was found that during deep-frying, the amount of CD and CT gradually increased. The changes in CD are shown in Fig. 2a. Fresh sunflower oil had 3.09% and 1.68% of CD and CT, respectively. High CD value (26.2%) at 232 nm in control sunflower oil was recorded. The CD of sunflower oil enriched with LPP-NPsE was lower than that of oils enriched with BHT and tocopherols. The CD for the oil samples enriched with BHT, tocopherols, and LPP-NPsE were, 15.73%, 12.11% and 8.20%, respectively. CD are generated during thermal oxidation of unsaturated fatty acids to attain more stable radical, generates compounds containing *trans* double bonds and conjugated double bond systems [58].

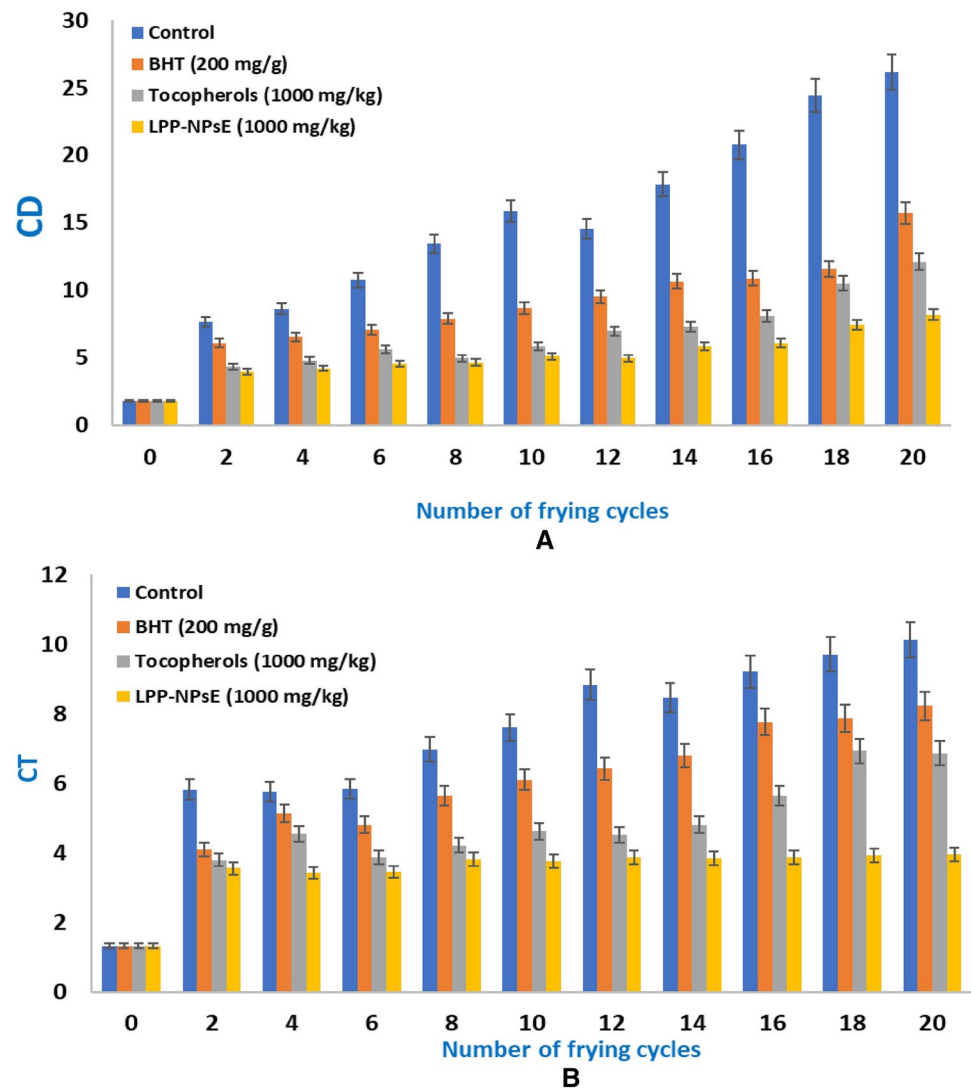
The CD was formed at higher levels than the CT, which agreed with Hamilton [59] and Dostálová et al. [60]. Absorbance at 268 related to the association of CT during thermal treatments. Those changes reflect the induction of oxidation products such as unsaturated β-ketones as well as α- and β-diketones [61]. The LPP-NPsE enriched oil showed low absorbance 268 nm at the end of the deep-frying process. After 20 cycles of frying of potato chips, the CT in sunflower oil for control sample as well as for BHT, tocopherol, and LPP-NPsE enriched oils were 10.12%, 8.23%, 6.87%, and 3.95%, respectively (Fig. 2b).

The antioxidants used in this study (LPP-NPsE, BHT and tocopherols) have an inhibitory effect on CD and CT formation. These data agree with those reported by Kalantzakis and Blekas [62] and Choe and Min [58] who reported that the plant extracts have an inhibitory impact against oxidation of oils heated at 180 °C. On the other hand, our results proved the efficiency of LPP-NPsE in slowing down lipid oxidation and increasing the stability of sunflower oil when exposed to high temperature.

Acrylamide contents in fried potato chips

Acrylamide is an odorless and colorless crystalline solid which have a melting point of 84.5 °C and considered as is a genotoxic, neurotoxic and carcinogenic compound [6]. Acrylamide was determined with a peak detected at 4.07 min (Fig. 3). A good coefficient of correlation of 0.9997 was estimated. The limit of LOD (signal to noise 3) 10.5 μg/kg

Fig. 2 Changes in **a** CD, and **b** CT in the sunflower oil during deep-frying of potato chips. Error bars show the variations of three determinations in terms of standard deviation



was detected, while the limit of quantification LOQ (signal to noise 10) was 33.3 $\mu\text{g}/\text{kg}$. It showed that its recovery was 85%. The acrylamide content in deep-fried potato chips is given in Table 1. Final concentrations of acrylamide in potato samples at the end of the frying process were 1674, 1450, 1145, 851 $\mu\text{g}/\text{kg}$ for the control sample, BHT enriched oil, tocopherols enriched oil and LPP-NPsE enriched oil, respectively. LPP-NPsE enrichment resulted in the lowest concentration of acrylamide, a reduction of 54%, which was significantly different from the control sample ($p < 0.05$). The main pathway for acrylamide induction in foodstuffs is Maillard reaction with free asparagine and reducing sugar [63]. Acrylamide was detected in heated foods where its formation was temperature dependent [64]. Potatoes have a high level of asparagine and reducing sugars which are subject to acrylamide formation upon frying (2). In the present work, high levels of acrylamide were measured in the control oil (1674 $\mu\text{g}/\text{kg}$). The relatively low levels of acrylamide in antioxidants enriched oils could be attributed

to the protective action of antioxidants against thermo-oxidative degradation. Addition of LPP-NPsE demonstrated the potent inhibitory effect, compared to the addition of BHT and tocopherols.

Several studies reported the high antioxidant potential of pomegranate peels [28, 65, 66]. Phenolic and flavonoids compounds in pomegranate peels revealed the presence of 23 phenolic compounds and 20 flavonoid compounds [21]. The major phenolic compounds were punicalagin (98.0 mg/g), pyrogallol (45.3 mg/g), ellagic acid (12.5 mg/g), *p*-hydroxybenzoic (7.01 mg/g), and catechol (5.96 mg/g). The major flavonoids were hesperidin (5.04 mg/g), quercetin (3.51 mg/g), and kaemp-3-(2-*p*-coumaroyl) glucose (1.02 mg/g).

Acrylamide amounts were lowered in fried potatoes when rosemary was added to olive or corn oils [67]. Zhu et al. [15] tested the effect of 35 crude plants aqueous extracts and 11 phenolic acids on the formation of acrylamide in an asparagine–glucose model system, finding that 34 out of 35 extracts

Fig. 3 HPLC chromatogram of **a** acrylamide standard and **b** control deep-fried potato

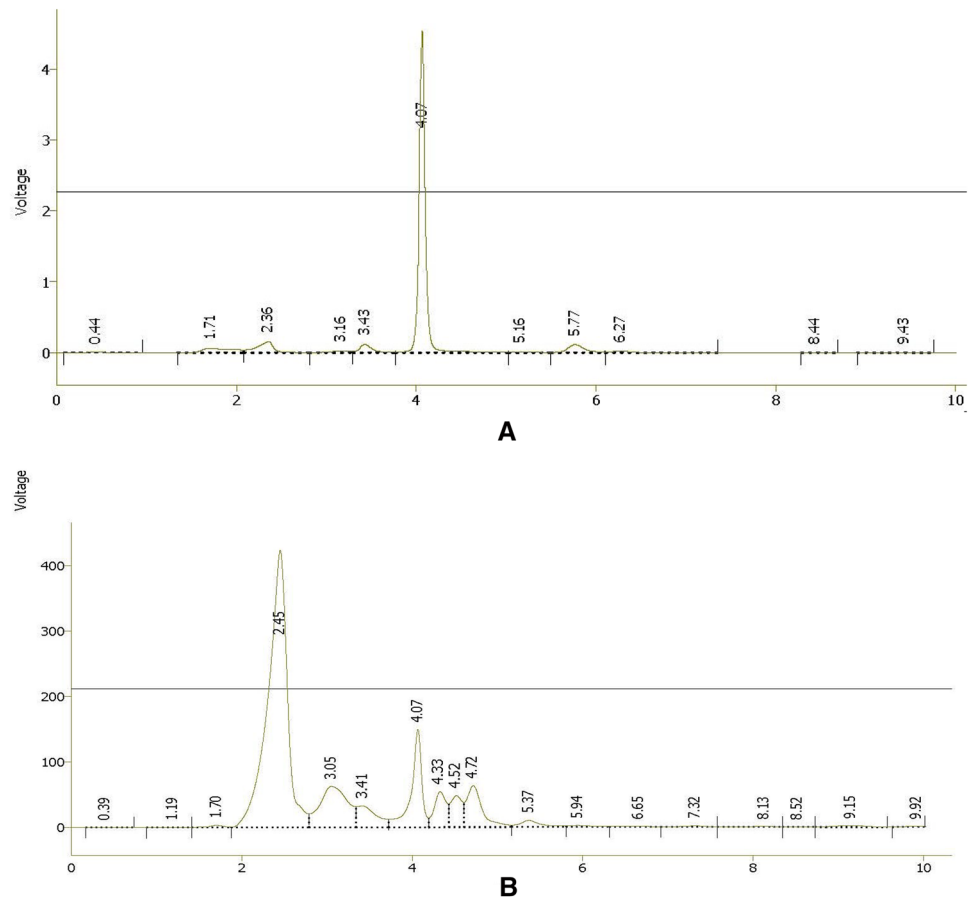


Table 1 Formation of acrylamide ($\mu\text{g}/\text{kg}$) in deep fried potato chips ($n=3$)

Frying cycle	Control	BHT (200 mg/g)	Tocopherols (1000 mg/ kg)	LPP-NPsE (1000 mg/ kg)
0	192 \pm 2.1 ^a	192 \pm 2.1 ^a	192 \pm 2.1 ^a	192 \pm 2.1 ^a
2	236 \pm 3.1 ^a	227 \pm 5.5 ^b	221 \pm 4.4 ^b	228 \pm 6.6 ^b
4	342 \pm 4.1 ^a	322 \pm 6.4 ^b	280 \pm 5.1 ^b	271 \pm 7.3 ^a
6	436 \pm 2.5 ^b	370 \pm 3.7 ^b	354 \pm 5.4 ^a	287 \pm 0.5 ^a
8	563 \pm 1.3 ^b	411 \pm 4.6 ^a	389 \pm 7.5 ^a	314 \pm 3.4 ^a
10	768 \pm 3.2 ^a	546 \pm 3.9 ^b	433 \pm 2.8 ^b	365 \pm 4.3 ^a
12	913 \pm 2.4 ^b	765 \pm 2.8 ^a	634 \pm 4.6 ^b	456 \pm 2.6 ^b
14	1123 \pm 7.1 ^b	945 \pm 1.6 ^a	765 \pm 3.9 ^a	490 \pm 3.9 ^b
16	1343 \pm 4.8 ^b	1134 \pm 3.6 ^a	945 \pm 4.8 ^a	512 \pm 7.1 ^a
18	1558 \pm 4.9 ^b	1328 \pm 6.1 ^a	1038 \pm 4.2 ^a	665 \pm 6.7 ^a
20	1674 \pm 6.7 ^b	1450 \pm 5.3 ^a	1145 \pm 3.1 ^b	851 \pm 5.1 ^b

^{a,b}Mean values in columns with the same letter are not significantly different ($p < 0.05$)

had reduction impact, while 9 phenolic acids inhibited acrylamide formation. Singh et al. [29] reported that nano-herbal drugs extracts exhibited functional traits such as enhancement of bioavailability and solubility, protection from toxicity,

enhancement of stability, and protection from physical and chemical degradation. The present study showed that the synthetic antioxidants did not outperform the plant extracts in the sunflower oil during frying. According to several studies [18, 68], it was shown that plant extract is more effective than synthetic antioxidants in reducing acrylamide formation. Chuda et al. [69] confirmed that controlling the content of carbonyl source and asparagine could reduce the acrylamide content. In the final product, lipids became the main sources of carbonyls [70]. Thus, additions of antioxidants limit carbonyl accumulation [71]. Considering that oxidized lipids induce reactive carbonyls to react with asparagine, we can point out that LPP-NPsE has strong antioxidant activity and have inhibitory effects against acrylamide formation.

From the results of current study, it could be clearly noted that there was a positive correlation between acrylamide content and other oxidation parameters including PV, FFA, TPC, CD and CT.

Conclusion

Studies have reported several efforts to reduce the acrylamide levels in the thermally-processed foodstuffs. Considering the recent EU regulations (2017) establishing benchmark levels for acrylamide in foods, an approach to reduce acrylamide in foods is needed. In the present work, LPP-NPSE was successfully applied as a natural antioxidant to enhance the oxidative stability of sunflower oil and to reduce the formation of acrylamide in potato chips during deep-frying. LPP-NPSE might be fruitfully applied as an antioxidant for acrylamide reduction in sunflower oil and other vegetable oils during deep frying or thermal processing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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