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Linseed oil presents different patterns of oxidation in electrospun TA fibrous mats and TA aging assays

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RESEARCH ARTICLE

Abstract

Oxidative deterioration is a major issue limiting the utilisation of linseed oil. This study investigated the effect of electrospun guar gum (GG) fibrous mats with addition of natural antioxidants on the oxidative stability of linseed oil. As a new technology, electrospinning was chosen to prepare nanofibrous mats. Besides, tannic acid (TA) was used as natural antioxidants and water was used as a solvent. The synthesised fibrous were smooth under the electron microscope, which had no defect structure and relatively uniform diameter. Electrospun GG fibrous mats containing TA were added to the linseed oil and the same amount of TA powder was used for comparison, then stored at 60 °C for 30 days to monitor the oxidation of oil. Conjugated dienes, peroxide value, p-anisidine value, thiobarbituric acid and fatty acid composition were used as indicators of oxidative stability. It was shown that TA can delay primary and secondary oxidation and improve oxidation stability. In conclusion, electrospun TA fibrous mats can play a more effective role than adding TA alone in antioxidation of linseed oil, which had potential applications in oil preservation.

Keywords: electrospinning, oxidation, tannic acid, antioxidant, linseed oil

1. Introduction

Linseed oil contains a large amount of essential polyunsaturated fatty acids (PUFAs), of which α-linolenic acid (ALA) is particularly high, accounting for 45-55% (Yadav *et al.*, 2018). Evidence suggested that consumption of PUFAs is necessary and has been associated with a lower incidence of many diseases, including inflammation, cardiovascular disease and cancers (D'Eliseo and Velotti, 2016; Michotte *et al.*, 2011; Sun *et al.*, 2015). Unfortunately, PUFAs make linseed oil extremely sensitive to oxidation. The presence of double bonds in the backbone of the linseed oil fatty acid molecule is susceptible to oxidative properties, resulting in low molecular weight aldehydes (Piornos *et al.*, 2017). The products produced by the oxidation of linseed oil have an adverse effect on flavour, colour and texture, so as to shorten the shelf life and reduce the nutritional quality of linseed oil (Menin *et al.*, 2018).

Recently, there are several important ways to prolong the shelf life by adding effective oxidation inhibitors in food packaging, such as direct addition, film coating, electrospinning. The direct addition of antioxidants to the linseed oil has poor stability and food safety hazards (Fayaz Dastgerdi *et al.*, 2016; Wen *et al.*, 2017). Coating materials containing oxidation inhibitors have small contact area with linseed oil, which affect the efficiency of labile ingredients.

Electrospinning is a powerful and straightforward method for fabricating nanofibers with controllable compositions and thickness (Zhan *et al.*, 2015; Zhou *et al.*, 2015). Surface morphology and size distribution of the nanofibers are modulated by process parameters, such as polymer concentration, applied voltage and solution flow rate (Agarwal *et al.*, 2013; Yao *et al.*, 2016). Among the various modification techniques, polymers and biologically active molecules assembly by hydrogen bonds has been widely adopted to fabricate nanofibrous mats. Electrospun fibrous mat has its advantages over other approaches, such as high

porosity and large surface-to-volume ratios, which can greatly increase the contact area between materials and bioactive molecules (Aytac and Uyar, 2016). The porous reaction interface of electrospun fibrous mats combining with the small size effect of the bioactive molecules, results in efficient composite active packaging. This method also has its advantages of no limit to the complex structure of substrate, and easy control of film thickness and composition (Zhou *et al.*, 2013), which has been largely employed in areas like wound dressings, drug delivery, tissue engineering scaffolds and other fields (Lubambo *et al.*, 2015).

In order to retard, reduce and prevent oxidative deterioration, natural antioxidants were added to the electrospun fibrous mats. It was reported that the preparation of hydroxypropyl methylcellulose electrospun membranes from clove buds, sage and oregano can effectively improve the antioxidant capacity of soybean oil and prolong shelf life (Ghadermazi *et al.*, 2016). Altan *et al.* (2018) found that carvacrol was loaded into electrospun fibres synthesised from zein and polylactic acid, which enhanced the antioxidant activity of the active packaging and prolonged the shelf life of whole wheat bread. At present, most of the antioxidative nanofibers are formed by using chemically synthesised polymers such as polyacrylonitrile and polylactic acid which embedded citric acid, rosemary and other antioxidants. The antioxidative nanofibers prepared by natural polymer materials still have toxic solvents, presence of beads on the fibres and other issues.

As a natural polyphenol, tannic acid (TA) has been shown to have biofunctional properties such as antibacterial and antioxidant activities, which makes it suitable for food packaging applications (Maqsood and Benjakul, 2010; Xu *et al.*, 2015). In addition, TA has been used for electrospinning with high polymers such as polycaprolactone, gelatine and cellulose, and for extensive use in the fabrication of multilayer films, capsules, and cell modification coatings of biomedical relevance (Zhou *et al.*, 2016).

Guar gum (GG) is a natural galactomannan with high solubility in water (Prajapat *et al.*, 2016). In its natural state, the molecule is in entangled network structure. GG is one of the most effective natural polymers known, which is widely used in industry as thickener, stabiliser and emulsifier. As a substrate for fibres formation, GG is hard to electrospin because not all parameters are fundamental or independent (Lubambo *et al.*, 2013). In some instances, more than one parameter controls an apparent feature or several apparent features are controlled by one parameter.

In this study, electrospun fibrous mats were prepared by combining GG with TA and using water as a solvent to study the effect of composite electrospun fibrous mats on the oxidation of linseed oil. Electrospun fibrous mats with different concentration of TA (5, 10, 20 wt%) were selected as experimental groups, comparing with different concentration of TA powder (5, 10, 20 wt%). The oxidation of linseed oil was accelerated by oven for 30 days and the changes in conjugated dienes (CD), peroxide value (PV), p-anisidine value (p-AV), thiobarbituric acid (TBA) and fatty acid composition were monitored to determine effectiveness of electrospun GG/TA fibrous mats for preserving linseed oil.

2. Materials and methods

Linseed oil was provided by Xingling Grain & Oil Co. Ltd (Lingwu, Ningxia, China). Commercial GG, TA and p-anisidine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (ACS grade) was obtained from Tianjin Sixth Chemical Ltd (Tianjin, China). Trichloroacetic acid, methanol, 2,2,4-trimethylpentane, chloroform, hexane, ferric chloride ($FeCl_3 \cdot 6H_2O$), green vitriol ($Fe_2SO_4 \cdot 7H_2O$), barium chloride ($BaCl_2 \cdot 2H_2O$), sodium sulphate (Na_2SO_4), ammonium rhodanate (NH_4SCN), thiobarbituric acid (TBA), potassium acid carbonate ($KHCO_3$) and other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Preparation of pure guar gum

Commercial GG was purified by using an ethanol extraction method with slight modification (Hussain *et al.*, 2018). Food grade GG was dissolved in ultrapure water at room temperature and stirred at 1000 r/min overnight using a magnetic stirrer to obtain 1.3 wt% GG solution. Then, it was centrifuged at 40 °C at 8,000 r/min for 40 min. The supernatant was transferred to a 500 ml separating funnel and precipitated with 500 ml of pure ethanol. After that, the precipitated GG was filtered by suction filtration and washed with pure ethanol for three times. The obtained GG was collected and dried at room temperature in vacuum oven for 48 h. The dried purified GG was milled to fine powder for further use.

Preparation of GG/TA spinning solution

Firstly, the purified GG was dissolved in ultrapure water and stirred at 700 r/min 50 °C overnight to obtain a homogeneous GG solution (2 wt%). A certain amount of TA powder (5, 10, 20 wt%, based on the weight of dry GG) was fully dissolved in 1 ml of ultrapure water then blended with GG solution because the direct addition of TA powder to the GG solution cause uneven dissolution. The blended solution was constantly stirred at 800 r/min for 3 h to obtain homogeneously mixed GG/TA spinning solution.

Fabrication of GG/TA electrospun fibres mats

Prior to electrospinning, the GG/TA solution was preheated on a magnetic stirrer at 75 °C for 20 min in order to prevent gelation, then the heated colloidal solution was electrospun using a YF SP-T apparatus (Yunfan Technologies Co., Limited, Tianjin, China). The solution was aspirated into a 5 ml plastic syringe, which attached with a metal needle (outer diameter × length = 0.7×63 mm and inner diameter = 0.40 mm), the flow rate was 0.5 ml/h and the applied voltage was 18 kV. The distance between the needle and the collector was 10 cm. Aluminium foil was used to cover the collector to collect the fibres, the rotational speed was set to 40 r/min. The experiment was carried out at 40 °C, relative humidity was controlled at 20-25% and each of the fibrous mat was electrospun for 6 h.

Characterisation of nanofibers

Scanning electron microscopy (SEM, 0182-S Phenom-World, Thermo Fisher Scientific, Waltham, MA, USA) was used to characterise the surface morphology, fibre diameter size (FDS) and size distribution of electrospining fibres. The samples were fixed on the metal stubs by double-sided tape and coating by an ultrathin layer of platinum. The images were observed under SEM at an accelerating voltage of 10 kV and a working distance of 10.1-11.4 mm. The average fibre diameter was measured using ImagineJ Software v1.45 (National Institutes of Health, Bethesda, MD, USA).

Accelerated oxidation of linseed oil

In this study, the oxidation stability of linseed oil was monitored under accelerated oxidation storage. Each sample contained 10 g of linseed oil and was placed in an amber vial. Then, GG/TA fibrous mats with different TA concentration (5, 10, 20 wt%, based on the weight of dry GG) were separately weighted 20 mg and put into the samples of oil. Meanwhile, TA powder (10, 20, 40 mg) was separately measured and then loaded into another oil sample to obtain 5, 10, 20 wt% TA powder samples. A control sample without antioxidants was also prepared. The samples were strongly mixed by an oscillator for 10 min to made sure completely immersion and placed into a WGL-30B incubator (Taisete Instrument Co., Ltd., Tianjin, China) at 60 °C for 30 days.

Conjugated dienes

The CD value was determined according to the previous method (Belingheri *et al.*, 2015). In brief, linseed oil (0.1 g) was dissolved in 10 ml pure iso-octane. The values of conjugated dienes were measured by an Alpha-1506 UV-vis spectrometer (Shanghai Puyuan Instrument Co., Ltd., Shanghai, China) at 232 nm and read against HPLC grade iso-octane as blank. Each test was performed in triplicate. The CD values were calculated according to Equation 1.

$$K_\lambda = \frac{\varepsilon_\lambda}{c} \times s \quad (1)$$

where ε_λ = absorbance of the sample; c = concentration of sample in g/100 ml; s = cuvette width in cm.

Peroxide value

PV of the linseed oil and its mixtures were evaluated using an International Dairy Federation method with slight modification (Shantha and Decker, 1994). A standard curve of Fe^{3+} was constructed by measuring the absorbance of Fe^{3+} solution reacted with SCN^-. Initially, a solution of SCN^- was prepared by dissolving 30 g of ammonium thiocyanate in 100 g distilled water. $FeCl_3 \cdot 6H_2O$ (0.0483 g) was accurately weighed and dissolved in 100 ml of distilled water to obtain 100 µg/ml of Fe^{3+} solution. The solution was diluted in different multiples to obtain 1, 2, 3, 5, 10 and 20 µg/ml Fe^{3+} solution, then 10 ml Fe^{3+} solution of different concentrations was separately measured into amber vials and 50 µl of SCN^- solution was added. The mixtures were measured at 500 nm by using above mentioned spectrometer. A curve was obtained with an R^2 value of 0.9902 and the slope was 0.0042.

The standard Fe^{2+} solution was prepared by adding 50 ml of $BaCl_2 \cdot 2H_2O$ (0.8 wt%) and 50 ml of $FeSO_4 \cdot 7H_2O$ (1.48 wt%) respectively. The solution was homogeneous mixed in a 250 ml beaker under constant agitation (200 r/min, 5 min), then 2 ml of 10 N HCl solution were added into the beaker. The mixture was centrifuged at 6,000 r/min for 20 min to filter off the precipitate of barium sulphate and obtain a clear Fe^{2+} solution. Finally, the Fe^{2+} solution was stored in a brown bottle covered with aluminium foil and kept away from light.

Approximately 0.02 g of each sample was dissolved in 9.8 ml mixture of chloroform: methanol solution (2:1 v/v). Then 50 µl of SCN^- solution was added and mixed with a vortex mixer for 10-20 s. Fe^{2+} solution (50 µl) was subsequently added into the mixture and the vial was mixed with the vortex mixer again. The resulting mixture was kept at room temperature for 10 min and measured at 500 nm. A blank that contained all reagents except oil was used to evaluate the stability of the Fe^{2+} solution. All samples were record three times. PVs were calculated according to Equation 2:

$$PV(meqO_2 / kg) = \frac{(A_s - A_b)}{2 \times 55.84 \times m_0 \times m} \quad (2)$$

where A_s = absorbance of the sample, A_b = absorbance of the blank, m = slope of the Fe^{3+} calibration curve, 55.84 = atomic weight of iron, m_o = mass of oil in sample in gram.

p-anisidine value

The p-AV was measured according to the method AOCS Official Method Cd 18-90 (AOCS, 1994). Linseed oil (0.5 g) was fully dissolved in 25 ml n-hexane and followed by vortexing for 10-20 s. The absorbances (A_1) of the mixtures were measured at 350 nm by above mentioned spectrometer, where pure n-hexane was used as a blank. Aliquots (5 ml) of the solutions were then mixed with 1 ml of p-anisidine solution (0.25 wt%) and kept for 10 min before measured the absorbances (A_2) at 350 nm. The n-hexane added with p-anisidine was used as a control. Each test was performed in triplicate. The p-AVs were calculated according to the following Equation 3:

$$\text{p-AV} = \frac{25 \times (1.2 \times A_2 - A_1)}{W} \quad (3)$$

where W = weight of sample in grams.

Thiobarbituric acid

The TBA value was measured according to AOCS Method Cd 19-90 (AOCS, 2009). A calibration curve was obtained using 1,1,3,3-tetramethoxypropane and the TBA value was expressed as the malondialdehyde equivalent of mmol in the oil. Initially, the oil sample (0.1 g) was mixed with 25 ml of 7.5 wt% trichloroacetic acid in a 150 ml flask and reacted in a water bath at 30 °C for 30 min. The resultant mixture was filtered twice by using 0.45 μm filters, the obtained aliquots (5 ml) of filtrate were mixed with 5 ml of 2.88 wt% thiobarbituric acid and heated in a water bath at 90 °C for 40 min. After cooling down to ambient temperature, the mixture was added with 5 ml of chloroform followed by vortexing for 10 s. The resulting solution was transferred into a 50 ml capped centrifuge tube and centrifuged at 6,000 r/min for 20 min. The absorbance of the supernatant was recorded at a wavelength of 538 nm. Each sample was measured in triplicate. TBA values were calculated according to Equation 4:

$$\text{TBA} = \frac{c}{m} \quad (4)$$

where TBA =thiobarbituric acid value in mg/kg; c = micrograms of malondialdehyde from the standard curve in μg; m = mass of linseed oil sample in g.

Fatty acid composition analysis

Samples of linseed oil (20 mg) was dissolved in 4 ml of methanol-sulphuric acid (2% v/v). The mixture was incubated in a water bath at 80 °C for at least 60 min until no oil droplet was visible. Distilled water (2 ml) and n-hexane (2 ml) were subsequently added after the solution was cooled down to the ambient temperature. The flask was shaken vigorously for 10 s before adding 1 ml of $KHCO_3$ (2 wt%) to neutralise excess vitriol. The extracted solution was then transferred to a clean tube and added approximately 0.5 g of anhydrous sodium sulphate for dehydration.

The fatty acid methyl esters were separated and quantified by gas chromatography (Shimadzu GC-2010, Shimadzu Inc., Kyoto, Japan) coupled with a flame ionisation detector (FID). A HP-88 capillary column (Agilent Technologies, Inc., La Jolla, CA, USA; 100 m × 0.250×0.20 mm) was used with splitless injection. The initial temperature of oven was programmed at 120 °C for 4 min, then ramped at 10 °C/min to 175 °C which was held for 6 min, after that, ramped at 5 °C/min to 210 °C for 5 min until achieving a final column temperature of 230 °C which was held for 30 min with 99.99% nitrogen as carrier gas. Hydrogen (40 ml/min), nitrogen (30 ml/min), and air (400 ml/min) were used for the FID detector. Identification and calculation for fatty acids were performed by comparing the retention time with that of the internal standard and with retention times reported in the literature. Each sample was measured in triplicate. The fatty acids present in the samples were quantified by area percentage calculation. Results were expressed by following Equation 5.

$$\text{Fatty acid component content (mass fraction)} = \frac{A_i}{A_i} \times 100\% \quad (5)$$

Statistical analysis

Statistical analysis was performed using Spass Statistics 22 software (IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) and Tukey's multiple comparisons test were performed to determine the significance of differences between each mean value of CD, PV, p-AV, TBA and fatty acid composition defined for $P<0.05$.

3. Results and discussion

Characterisation of nanofibers

The SEM photographs and mean diameters of the fibres spun with TA concentrations of 5, 10, 20 wt% are respectively displayed in Figure 1. The fibres containing 5 wt% and 10 wt% TA were round and smooth. It can be seen that the fibres became nonuniform as the TA concentration increased from 10 wt% to 20 wt% (Figure 1C), which could be due to increases in conductivity from 63.95 μs/cm to 88.25 μs/cm and surface tension from 68.62 mN/m to 72.11 mN/m (not shown). It indicated that the spinnability of the solution depends on the TA concentrations (Whittaker *et al.*, 2016).

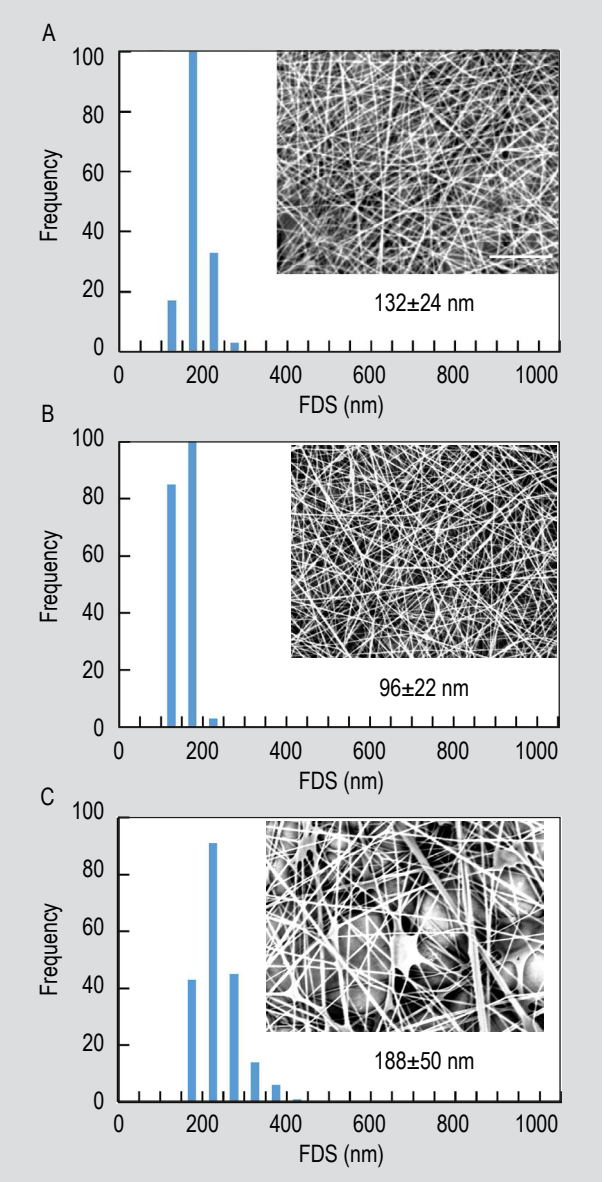

Figure 1. Scanning electron microscopy images and fibre diameter size (FDS) of nanofibers electrospun from 5 wt% tannic acid (TA) (A), 10 wt% TA (B), 20 wt% TA (C) based on the dry weight of guar gum (GG), GG/TA blended solution with 2 wt% concentration of GG.

FDS of the fibres ranged between 96 and 188 nm and followed a non-normal distribution (P<0.05). The average diameters of nanofibrous mats containing 5, 10, 20 wt% TA were 132±24 nm, 96±22 nm and 188±50 nm. The results showed that the TA concentration played an important role in the morphology and average diameters of the fibres. After observing the microstructure of the fibrous mats, loading efficiency of TA is listed in Table. 1. It shows that the loading efficiency of 5 wt% TA fibres was 3.32±0.92%, while that of 10 wt% TA fibres was 9.98±1.19% and 20 wt% TA fibres was 19.99±3.06%. The results indicated that there was almost no loss of TA during the electrospinning process, which TA showed high cross-linking ability (Tavassoli-Kafrani *et al.*, 2017). It meant that the TA can be efficiently encapsulated into GG by electrospinning technique.

Table 1. Loading efficiency of tannic acid (TA) in electrospun guar gum fibrous mats, 5 wt% TA, 10 wt% TA and 20 wt% TA. Mean ± standard deviation of three independent experiments.

Concentration (wt%)	Loading efficiency (%)
5	3.32±0.92
10	9.98±1.19
20	19.99±3.06

Primary oxidation products

Conjugated dienes

The conjugated dienes are rearrangement of a fatty acid position which are formed by oxidation of unsaturated fatty acids containing two or more double bonds (Akhtar *et al.*, 2010; Smith *et al.*, 2007). For the dienes, the absorbance value of the control was 4.34±0.03 on day 0 and increased rapidly to 9.51±0.03 on day 30 (Figure 2A). On day 30, the mean CD value of oil samples added with 20 wt% TA powder was significantly reduced by 3.34 compared to the control (P<0.05), indicating that the effectiveness of TA was evident. TA has a good inhibitory effect on CD because its high free radical scavenging activity led to subsequent production of low subsequent generation of reactive lipid radicals (Maqsood and Benjakul, 2010).

In Figure 2B, it can be seen that the CD values of linseed oil with TA fibrous mats showed a slow increase in the first 15 days, and followed by a decrease. It could be due to the initial formation of hydroperoxide in PUFAs (mainly ALA), followed by the breakdown of hydroperoxide molecules, which release volatile compounds (Belingheri *et al.*, 2015). Over the accelerated oxidation storage, electrospun fibrous mats showed much lower CD values than the control and TA powder sample with the same corresponding amounts of TA (P<0.05), demonstrating that electrospinning can effectively exert the antioxidant effect of TA. After 25 days, the samples added with TA powder showed a sharp increase in CD values, whereas the TA fibrous mats samples showed opposite result which was the slow decrease of the CD values (P<0.05). This can be attributed to the transformation of TA into nano-sized particles by electrospinning, which increased the contact area with oil samples and reduced the production of hydroperoxides. The natural electrospun fibrous mats can be used as inner layer to enhance oxidative stability index and to extend the shelf-life of the edible oil.

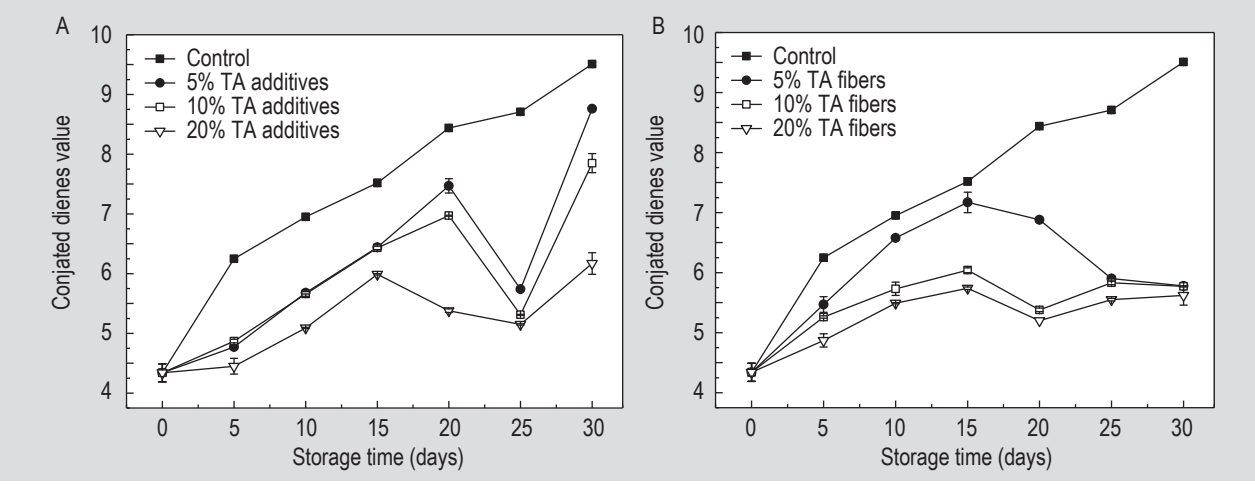

Figure 2. Evolution of the oxidation of linseed oil, expressed as conjugated dienes in refined linseed oil during accelerated real-time aging at 60±1 °C for 30 days. Mean ± standard deviation (SD) of three independent experiments. TA = tannic acid.

Peroxide value

Peroxides are the primary products formed when PUFAs oxidised, which can be broken down into non-volatile and volatile secondary products and reduce the quality of the oil (Gallego *et al.*, 2016). As a technique commonly used for determining hydroperoxides in products, PV can be used to reflect the degree of lipids oxidation. Addition of TA can reduce the PV of linseed oil (Mei *et al.*, 2014).

Figure 3 depicts the changes in PV of linseed oil with varied concentrations of TA powder and TA fibrous mats over 30 days storage at 60 °C. Linseed oil without adding any additive showed a rapid PV increase in the dark, reaching the maximum value of 16.33±0.08 meqO_2/kg oil (P<0.05), which was likely ascribed to the fact of primary lipid oxidation. It can be seen that the PV of linseed oil with TA powder was always lower than that of the control within 30 days, indicating that the formation of hydroperoxide could be successfully reduced by this natural antioxidant. In addition, the PV of oil samples with different TA concentrations were significantly different on day 20 (P<0.05) and the PV was lowest at 20 wt% TA (8.58±0.10 meqO_2/kg oil), among the three TA concentration conditions tested. Mohanan *et al.* (2018) discovered that the effectiveness of TA powder decreased with the TA concentration above 10 wt% in linseed oil over 30 days storage at 60 °C, which was different from our results. The discrepancy can be attributed to the presence of natural antioxidants in linseed oil, which can enhance the effectiveness of TA through synergistic interactions.

Figure 3B shows the effects of electrospun GG/TA fibrous mats with different TA concentrations on the PV of linseed

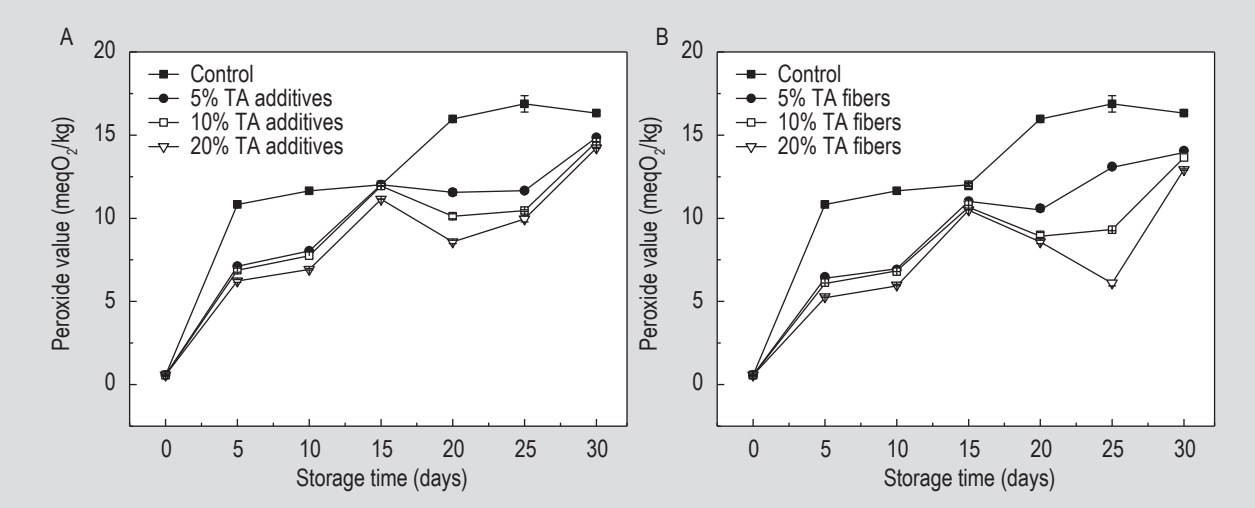

Figure 3. Evolution of the oxidation of linseed oil, expressed as peroxide value (meqO_2/kg oil) in refined linseed oil during accelerated real-time aging at 60±1 °C for 30 days. Mean ± standard deviation (SD) of three independent experiments. TA = tannic acid.

oil. During the storage period of 20 days, the hydroperoxide formation tendency of linseed oil with electrospun TA fibrous mats was consistent with that of linseed oil with TA powder. The PV of oil samples containing 20 wt% TA fibrous mats (12.93±0.03) was slightly lower than that of oil sample containing 20 wt% TA powder (14.23±0.03) at the end of storage period (P>0.05). The results showed that TA was added into natural colloidal materials by electrospinning, and the antioxidant activity of natural polyphenols was retained. At the same time, due to the transformation of TA into nanoparticles by electrospinning, the contact area with oil samples were increased, resulting in the reduction of the hydroperoxides and better antioxidant effects than TA powder. Numerous studies have demonstrated the significance of electrospinning methodologies as effective platforms to increase natural antioxidants stability and bioactivity (Yao *et al.*, 2016).

Secondary oxidation products

p-anisidine value

The p-AV is extensively used to measure secondary oxidation products formed during lipid oxidative degradation. The aldehydes in oil (principally 2-alkenal and 2, 4-dienal) and the p-anisidine reagent react with each other under acidic conditions, resulting in the formation of a yellow pigment. Therefore, an increase in p-AV will reflect a higher concentration of aldehydes and thus reflect a worse oxidative stability of the oil. The p-AV of linseed oil and its mixture with varied concentrations of TA measured as a function of storage time at 60 °C are shown in Figure 4.

As seen from Figure 4A, the TA remained active throughout 30 days at 60 °C, and kept the p-AV always below the control at each sampling day. The significantly large amount of TA (20 wt%) had better antioxidant capacity than the small amount of TA (5 wt%, 10 wt%) during the storage period (P<0.05), which can effectively reduce the oxidation of linseed oil. Zhang et al. have shown that 10 wt% TA displayed minor antioxidant properties during the secondary oxidation process of pecan oil at 60 °C, which was different with our results (Zhang *et al.*, 2018). Probably, this discrepancy was due to different type of oil, shorter storage time and whether to provide the atmospheric air.

It can be seen from Figure 4B that compared with the control, electrospun fibrous mats with different concentrations of TA contributed to low p-AVs within 30 days, which achieved significant effects (P<0.05). After 30 days of accelerated oxidation, the p-AV of linseed oil added with 5 wt% TA electrospun fibrous mats approached 26.53±0.18 which was lower than the 5 wt% TA powder samples (34.95±0.96), demonstrating better ability to inhibit the oxidation of linseed oil. However, there is no significant difference (P>0.05) in p-AV between the two treatments at high TA concentrations (10 and 20 wt%), The results suggested that high porosity, high surface-to-volume ratio, and ultrafine structures of the obtained fibre mats can promote the interaction between TA and oil samples and effectively exert the antioxidant effect of TA under low TA concentration (Wen *et al.*, 2017).

Thiobarbituric acid

During the lipid oxidation process, the degradation of PUFAs formed secondary oxidation products such as malondialdehyde (MDA). In order to get a better understanding of the lipid oxidation of the GG/TA electrospun fibrous mats during the storage period, the determination of MDA is necessary and important. In addition, the formation of MDA may have significant

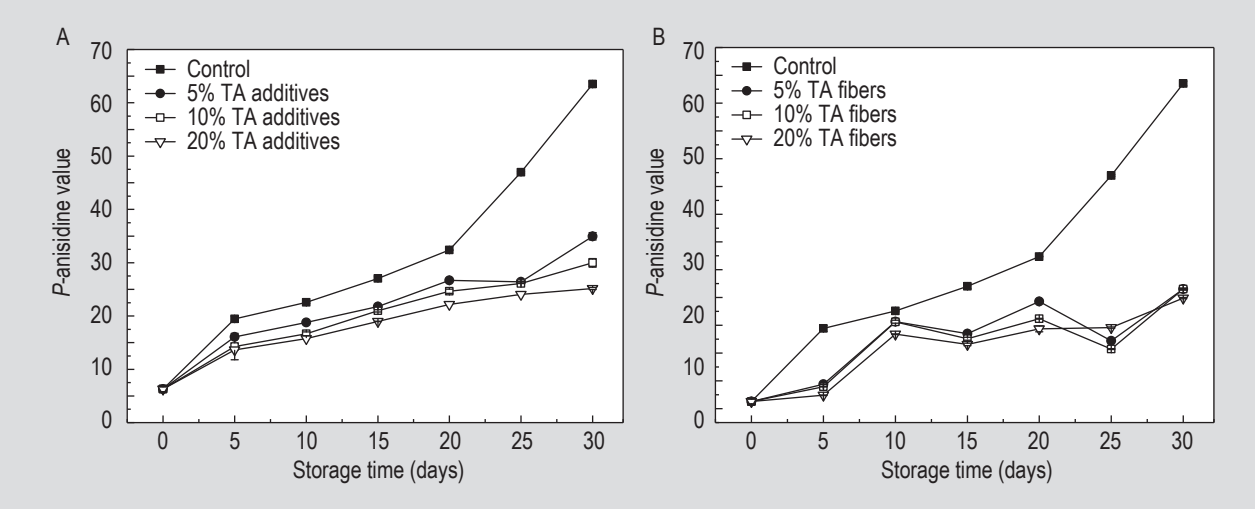

Figure 4. Evolution of the oxidation of linseed oil, expressed as p-anisidine value in refined linseed oil during accelerated real-time aging at 60±1 °C for 30 days. Mean ± standard deviation (SD) of three independent experiments. TA = tannic acid.

sensory effects as it can deliver off-flavours at very low concentrations and short the shelf life (Qiu *et al.*, 2018; Yeşilsu and Özyurt, 2019). As shown in Figure 5A, the TBA values decreased as the concentration of TA increased ($P<0.05$), which was in accordance with the results obtained in the study of rambutan extracts added to sunflower oil (Mei *et al.*, 2014).

In Figure 5B, TBA values of all the samples added with the electrospun fibrous mats increased sharply up to day 10 of storage, followed by a slight increase until the end of storage period. It reported that the volatile oxidation products with low molecular weight could be lost during extended storage, which probably lead to the decrease of the growth rate of TBA values (Maqsood *et al.*, 2012). After 30 days of storage, the TBA values of linseed oil with 5, 10 and 20 wt% TA fibrous mats were 47.21±2.42, 39.77±0.04, 34.21±0.18 mg/kg, respectively. Compared with the TBA of samples added with TA powder (54.65±1.21, 48.09±2.05, 35.36±0.08 mg/kg), they were reduced by 13.6, 17.3 and 3.3%, respectively. Compared with the addition of TA powder, the electrospun TA fibrous mats can make more effect on inhibiting the formation of MDA, then reduce the reaction between MDA and TBA, resulting in lower TBA values. In the prepared electrospun fibre mats, natural polysaccharides and phenolic substances (as spinning materials) ensured that the electrospun fibre mats were safe and edible. When immersed in linseed oil, these TA molecules could be released from the thin fibrous mats to achieve the effect of antioxidant.

Fatty acid composition analysis

Table 2 shows the fatty acid composition of the linseed oil samples before and after 30 days of accelerated oxidation. Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n-9), linoleic acid (18:2 n-6) and ALA (18:3 n-3) were the main fatty acids (FAs) present in the linseed oil. The most abundant FA was ALA, which accounts for 58% of total measured fatty acids. Compared to saturated fatty acids (SFAs), PUFAs were more susceptible to oxidation due to their highly unsaturated fatty acid structure, which in turn led to a relative increase in the total amount of SFAs and a relative decrease in the total amount of unsaturated fatty acids. After 30 days accelerated oxidation at 60 °C, linseed oil samples showed a slight difference in the relative contents of FAs according to the different treatments. ALA, oleic acid along with palmitoleic acid were relatively unstable in accelerated oxidation test compared to other unsaturated fatty acids. Oleic acid in the oil increased from 18.62 to 19.151%, while ALA and palmitoleic acid decreased by 0.41 and 1.16% respectively. It indicated that oleic acid was relatively stable in accelerated oxidation compared with the reduction of other unsaturated fatty acids, showing a small increase in relative contents (Zanqui *et al.*, 2015). The samples containing TA exhibited a slightly higher relative content of unsaturated fatty acids than the control ($P>0.05$). The relative fatty acid content of linseed oil containing electrospun fibrous mats with different concentrations of TA changed little after 30 days of storage, which also had no significant difference with the effect of TA powder ($P>0.05$), indicating that the addition of TA fibrous mats had no significant effect on fatty acid composition.

4. Conclusion

Overall, the study shows that electrospinning nanofiber membranes containing natural antioxidants inhibited the oxidation of linseed oil. The nanofibers prepared by natural antioxidants and edible polysaccharides have an uniform overall micromorphology. After 30 days of accelerated oxidation test, electrospun fibrous mats reflected a superior

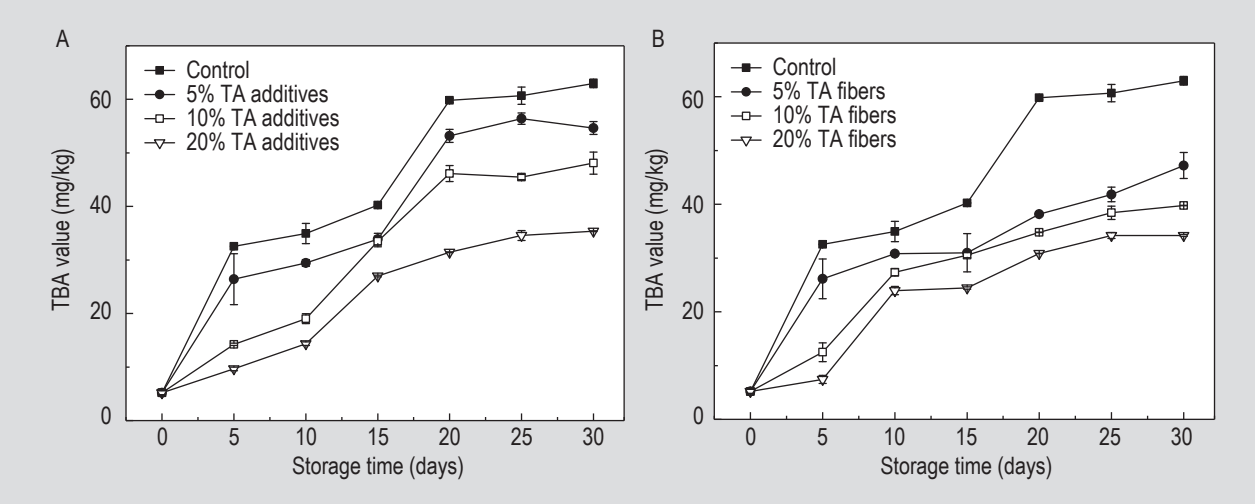

Figure 5. Evolution of the oxidation of linseed oil, expressed as thiobarbituric acid (TBA) (mg/kg oil) in refined linseed oil during accelerated real-time aging at 60±1 °C for 30 days. Mean ± standard deviation (SD) of three independent experiments. TA = tannic acid.

Table 2. Changes (%) of fatty acid compositions before and after 30 days storage at 60 °C. Mean ± standard deviation of three independent experiments.[1,2]

Fatty acid composition	Fresh	Control	5% TA additives	10% TA additives	20% TA additives	5% TA fibrous mats	10% TA fibrous mats	20% TA fibrous mats
Saturated fatty acids	8.4±0.07^{b}	8.66±0.20^{a}	8.44±0.29^{b}	8.41±0.39^{b}	8.45±0.33^{b}	8.43±0.37^{b}	8.4±0.39^{b}	8.39±0.30^{b}
Myristic acid C14:0	0.01±0.00^{b}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}
Palmitic acid C16:0	5.17±0.07^{a}	5.11±0.13^{b}	5.06±0.13^{bc}	5.06±0.20^{bc}	5.08±0.14^{bc}	5.07±0.15^{bc}	5.05±0.17^{c}	5.04±0.12^{c}
Stearic acid C18:0	3.18±0.01^{ab}	3.23±0.10^{a}	3.18±0.14^{ab}	3.12±0.18^{c}	3.14±0.18^{bc}	3.13±0.17^{bc}	3.14±0.19^{bc}	3.12±0.17^{c}
Arachidic acid C20:0	0.02±0.04^{c}	0.22±0.00^{a}	0.13±0.01^{b}	0.15±0.03^{b}	0.14±0.01^{b}	0.14±0.01^{b}	0.13±0.02^{b}	0.14±0.01^{b}
Behenic acid C22:0	0.01±0.04^{e}	0.07±0.02^{a}	0.04±0.01^{d}	0.05±0.01^{c}	0.05±0.01^{c}	0.05±0.01^{c}	0.04±0.01^{d}	0.06±0.00^{b}
Unsaturated fatty acids	91.6±0.07^{a}	91.34±0.20^{c}	91.56±0.29^{ab}	91.59±0.39^{ab}	91.55±0.33^{b}	91.57±0.37^{ab}	91.6±0.39^{a}	91.61±0.30^{a}
Oleic acid C18:1	18.62±0.20^{ef}	19.151±0.31^{a}	18.87±0.41^{b}	18.42±0.50^{ef}	18.71±0.40^{c}	18.59±0.51^{f}	18.63±0.59^{de}	18.66±0.41^{d}
Linoleic acid C18:2	14.71±0.44^{a}	14.65±0.13^{b}	14.59±0.05^{c}	14.55±0.06^{d}	14.55±0.08^{cd}	14.53±0.11^{d}	14.53±0.10^{d}	14.55±0.07^{cd}
Linolenic acid C18:3	58.20±0.67^{b}	57.51±0.53^{d}	58.05±0.72^{c}	58.56±0.97^{a}	58.22±0.82^{b}	58.4±0.92^{a}	58.39±1.08^{a}	58.34±0.80^{a}
Palmitoleic acid C16:1	0.07±0.02^{a}	0.03±0.00^{d}	0.05±0.00^{c}	0.06±0.02^{b}	0.07±0.01^{a}	0.05±0.00^{c}	0.05±0.00^{c}	0.06±0.02^{b}

[1] Superscripts with different letter in the same row are significantly different (P<0.05) from each other for each property.
[2] TA = tannic acid.

inhibitory effect on the oxidation of linseed oil than powder at the same TA content, which was determined by CD, PV and other indicators. Finally, it can be concluded that the electrospun GG/TA membranes have excellent inhibitory effect on the formation of primary and secondary oxidation products of lipids, and the greater the concentration of TA, the better the inhibition effect, which provide a new idea for the preservation of edible oil.

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Conflict of interest statement

The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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