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ANTIOXIDANT ACTIVITY OF PUMPKIN SEED OIL AND ITS EFFECT ON OXIDATIVE STABILITY OF SUNFLOWER OIL MONITORED BY FTIR SPECTROSCOPY TECHNIQUE

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Abstract

The aim of this research was to evaluate the antioxidant activity of pumpkin seed oil obtained by cold pressed after roasting under specific conditions, as well as the study of its effect on the oxidative stability of sunflower oil. The first step was obtaining the pumpkin seed oil, then determining the total polyphenols, the antioxidant activity, density and the burning point. Investigation of its effect was checked by using pure sunflower oil, mixture of sunflower oil with 1% pumpkin seed oil and pure corn oil. All three prepared samples were exposed thermally at three serial cycles at the burning point for 8 hours, followed by cooling at room temperature for 16 hours. After each cycle their density and burning point were monitored, finally followed by FTIR Spectroscopy using intensity ratio as an excellent indicator of monitoring oxidative stability for edible oils. Oxidative stability results showed that the mixture of sunflower oil and pumpkin seed oil have higher stability than the two others: pure corn and sunflower oil. Higher thermal resistance of sunflower and pumpkin seed oil mixture is a result from pumpkin seed oil which has high antioxidant activity and this could be used as a natural antioxidant by the food industry.

Keywords*: FTIR-Spectroscopy, pumpkin seed oil, antioxidant activity, oxidative stability, sunflower oil*

Introduction

The quality of most edible oils depends the stability of their constituents under thermal treatment. Edible oils can be under exposure of thermal treatment called frying, which can be applied in industry or domestic cases of food preparations [1]. During frying of edible oil their quality decreases because of the occurrence of the chemical reactions, such as oxidation, hydrolysis, and polymerization. Products of these reactions can be of different types of compounds such as, nonvolatile or insoluble compounds which will change their color. As s a result, they will increase their density and decrease the burning point or change other physicochemical properties [2].

Thermal degradation of edible oils is very well-known, but the challenge is finding a way to prevent degradation of edible oils using natural materials while increasing their health value at the same time.

The focus of this research is to increase knowledge for pumpkin seed oil pressed from pumpkin seed *Cucurbita pepo L.* as a natural source of vegetable oil with high level of α- and γ-tocopherol [3]. Based on literature survey, both tocopherols and other bioactive compounds increase nutritional and medicinal values of pumpkin seed oil as antioxidant, anticancer and anti-inflammatory effects and consequently preventing prostate disease [4]. The report of the

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importance of roasted pumpkin seed suggested temperature from 90 °C to 110 °C as the best thermal condition to increase tocopherol level and other bioactive compounds [5].

FTIR Spectroscopy can be applied as a tool for monitoring degradation degree of edible oils by useful intensity ratio of frequencies such as 3008 cm^{-1} double bond (=C-H) and 2922 cm^{-1}, 2852 cm^{-1} CH_2 aliphatic vibration group [6]. Our research group also reported a strong correlation between physicochemical parameters and FTIR Spectroscopy for oil samples based on their interpretations.

The aim of this research was to test the antioxidant effect of pumpkin seed oil and its preventive effect from the decomposition of edible oils such as sunflower oil. It was conducted by comparing pure sunflower oil and mixture of sunflower oil with low level of pumpkin seed oil during thermal treatment. Monitoring of stability of sunflower oil was performed by FTIR Spectroscopy and some physicochemical parameters.

Materials and Methods

Reagents and Samples

Pumkin seed samples were provided from Peja city in Kosovo in 2019 from varieties *Cucurbita pepo L*. Reagents used in the study were sodium hydroxide, hydrochloric acid, sodium chloride, anhydrous sodium sulfate, aluminum oxide, isooctane, and n-hexane, Folin-Ciocalteu (FC), DPPH (1,1-diphenyl-2-picrylhydrazyl). All these chemical reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Methanol (Merck, Darmstadt, Germany).

Sample Preparation

Our aim of research interest was the study of the antioxidant activity of pumpkin seed oil based on its effect in increasing the stability of other vegetable oils, such as sunflower oil. For this purpose we conducted experiments with pure sunflower oil, pure refined corn oil and the third prepared sample was sunflower oil with 1 % extracted pumpkin seed oil. The sunflower and corn oil were purchased from local markets in Kosovo, except for the pumpkin seed oil, which was extract by press seed equipment.

Pumpkin seed oil Extraction

Before extraction of oil pumpkin seed, Cucurbita Pepo L. were heated in oven at 90°C for roasting according to the procedure described on [5]. Extraction of oil from pumpkin seed was made in low temperature by using a screw press (Koçmaksan, KMS10, Izmir, Turkey). Oil was kept at the -18°C till analyses.

Extraction of Phenolic Compounds from Pumpkin seed oil

Total Phenolic compounds of pumpkin seed oil were extracted according to described reports with minor modifications [7]. Approximately 1 g of oil fresh pressed sample was added to 20 ml solvent mixture of methanol and water (1:1). Mixture was treated in ultrasonic bath for 1 hour continued by centrifugation at 6000 rpm for 15 minutes

Total phenolic determination

The determination of total phenolic compounds was done by Folin-Ciocalteu (FC) reagent in concordance with the subscribed procedure published from authors with minor modifications [8]. From 1 ml of FC reagent was added in oil extract of phenolic compounds, then 10 mL of 7.5% Na_2CO_3 was added in mixture and residue volume of 25 ml was added with distilled water. After 1h, the sample's absorbance intensity was measured using UV-Spectrophotometer-(Yasco-Japan 630V) at 750 nm selected wavelength. As a reference, standard compound was used. Gallic acid was utilized for calibration curve creation and as a result total phenolic compounds were measured and calculated as GAEs. Results were reported as mg of Gallic acid equivalents (mg GAE/g).

Antioxidant activity

Antioxidant activity was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) [9] with slight modifications. Phenolic extract and 2 mL methanol solvent of DPPH was shaken under room temperature for 30 min and absorbance was measured at 517 nm using a UV-VIS spectrophotometer. The capability to scavenge the DPPH radicals was calculated as a percentage of DPPH discoloration, on the basis of the following equation (1):

$$\text{DPPH inhibition (\%)} = \frac{Ac - As}{Ac} \text{x}100 \qquad (1)$$

Where: *Ac* is the absorbance of the DPPH solution (control) and *As* is the absorbance in the presence of oil sample.

FTIR Measurements

FTIR spectra of samples were obtained using Shimadzu FTIR-Iraffinity-1 equipped with DLATGS as detector and calcium fluoride CaF_2 as transparent window and managed by the IR software. All thermal heated samples were monitored by FTIR –Spectroscopy and pure pumpkin seed oil was included. Approximately ∼20 μL of the sample was deposited between two well-polished CaF_2 disks, and analyzed in IR region of 4000–650 cm−1, by accumulating 32 scans with the resolution of 4 cm^{-1}. At the end of every scan, the surface of CaF_2 disk was cleaned with acetone dried with special soft tissue.

Density Measurements and burning point of oils

Density was measured by pycnometer using 10 ml of sample at 25°C. Burning points were determined by full automatic equipment based on standard methods [10].

Results and Discussion

Our results from measurements of total polyphenols compounds, antioxidant activity, burning point and density in pumpkin seed oil are presented in Table 1. The published report about total phenolic compounds measured the lipid fraction from pumpkin seed oil of several samples with origin of Brazil. Based on their results, polyphenols varied between 1.35 and 3.62 mg GAE/g [11].

Table 1. Physicochemical parameters of extracted pumpkin seed oil

Physicochemical parameters	Pumpkin seed oil
Density kg/m^3	912.7
Burning point °C	164
Polyphenols mg GAE/g	2.5±0.1
Antioxidant activity	59±2.5

If we compare our results and reported variance of polyphenols, then we are able to conclude the high level of polyphenols and also high antioxidant activity in our sample with origin from Kosovo. Density and burning points are in accordance with other pumpkin seed oil originating from other countries and we can confirm the purity of extracted pumpkin seed oil. FTIR characterization of extracted pumpkin seed oil shows basic peak for most, which is characteristic for the most vegetable oils Figure 1. Basic functional group we can see from 1600-3100 cm^{-1} all peaks are presented in Table 2 and all these peaks are common for the majority of vegetable oils, especially intensity of peak 3007 cm^{-1} which can be interpreted by the high unsaturation level. Also, from FTIR spectra we can confirm triglycerides which are mayor compounds in oil based on carbonyl group 1745 cm^{-1}.

Characterization of pumpkin seed oil confirms high content of polyphenols compounds and high antioxidant activity. Density and burning point confirm high quality of pumpkin seed oil while the vibrational spectroscopy shows basic functional groups common for most edible oils. High intensity of several crucial peaks such as 3007 cm^{-1}, 2924 cm^{-1}, 2852 cm^{-1} and 1745 cm^{-1} shows highest lipid content and other organic compounds which are encouraging results for extracted pumpkin seed oil.

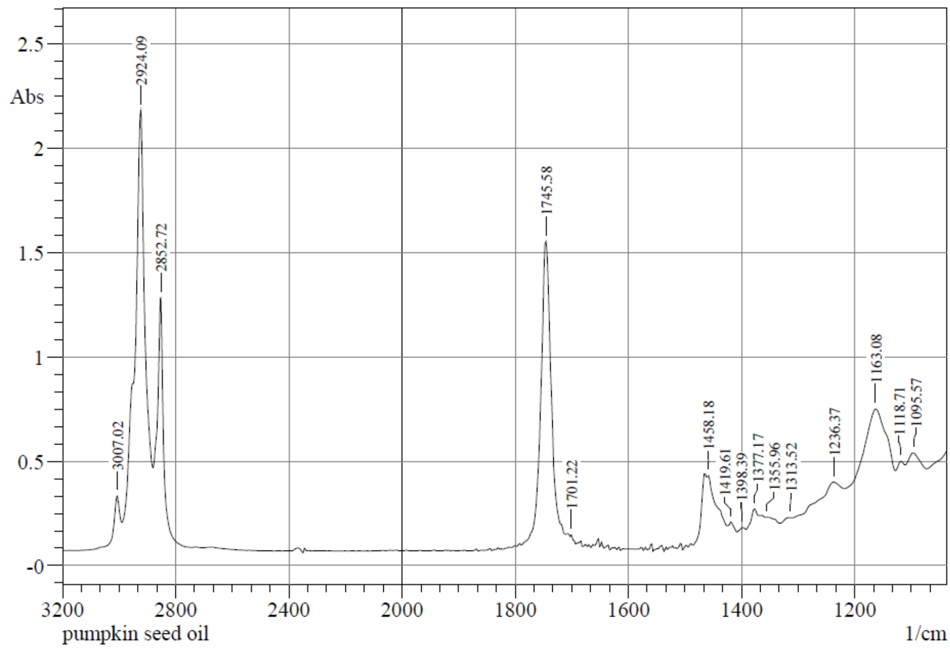

Fig. 1. FTIR Spectra of fresh extracted sample of pumpkin seed oil

Can low percentage of pumpkin seed oil present in other vegetable oil increase their stability? Can low percentage of pumpkin seed oil present in other edible oils prevent decomposition of edible oils? Answers of these questions can start if we make comparative experiments with pure sunflower oil, mixture of same sunflower oil with 1 % pumpkin seed oil and the third sample with pure corn oil which is known as the oil with the highest stability.

The three samples examined for their stability were monitored by their physicochemical parameters exactly by density and burning point and at the same time by FTIR Spectroscopy using intensity ratio of selected wavenumbers.

Density changes during heating for three samples are presented in Figure 2. Differences in density are very clear between the samples of (SFO) and mixture of (SFO-PO) where the effect of pumpkin seed oil is highly rated. This sample mixture rapidly changes its level of density, as a result the density increases much slower. Density changes of corn oil are in completely other ranges where increased density value is lower compared with both sunflower oils. This is probably due to higher thermal stability of corn oil.

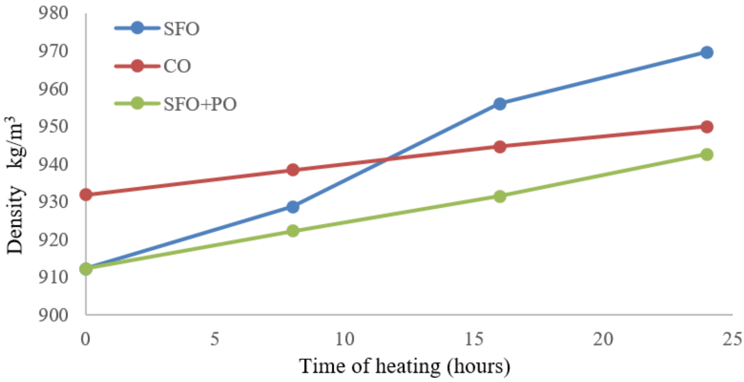

Fig. 2. Density [kg/m^3] measured for three type of heated oil samples sunflower oil, corn oil and sunflower oil mixed with 1% pumpkin seed oil.

Another parameter used as an indicator for chemical changes of oil is burning point, which monitored three samples under thermal treatment. This is presented in Figure 3. Fresh samples of pure sunflower oil and mixture of the same oil with 1% pumpkin seed oil have same burning point before thermal treatment but after their heating there are changes between them. Both of them decreased their burning point but the mixture of (SFO-PO) decreases more slowly compared with pure (SFO). Burning point of corn oil sample under thermal treatment also decreases but it happens more slowly and this is probably from higher thermal stability of this oil.

Both physicochemical parameters observed for three type of samples are in correlation with chemical changes of edible oils which can happen during thermal treatment. Thus, density increasing and burning point decreasing but (SFO) and (SFO-PO) have strong differences between them due to the pumpkin seed oil presence with high total polyphenol content and high antioxidant level.

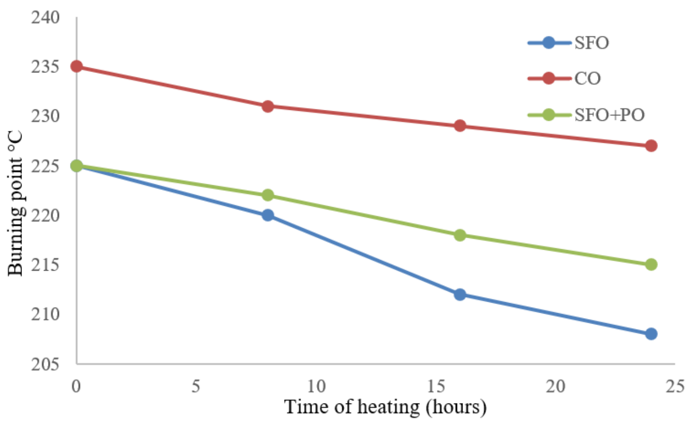

Fig. 3. Burning point of three monitored oil samples. (SFO) sunflower oil, (CO) corn oil, (SFO-PO) Sunflower oil containing 1% pumpkin seed oil

FTIR Spectroscopy was the other observation parameter used to monitor the chemical changes. Based on their ratio intensities of the selected peak, it can be used as an indicator for the chemical changes between the three monitored samples in Figure 4. The crucial functional group used in this research and their characteristic wavenumbers applied in vibrational spectroscopy are presented in Table 2.

Table 2. FTIR absorbance bands and their characteristic functional groups [12]

Wavenumbers (cm^{-1})	Functional group	Responsible vibrational mode
3008	=C-H (cis)	Symmetric stretching vibration of the *cis* double bond
2922 and 2850	-C-H (CH_2)	Symmetric and asymmetric stretching vibration of the aliphatic CH_2 group
1744	C=O (ester)	Stretching of ester Carbonyl (C=O) functional groups from the triglycerides

Considering the previous discussion about the possibility of applying FTIR ratio intensities of chemical vibrations, it is easy to understand vibrations' difference between double bonds (=C-H) and single bonds (-C-H). During oxidation of double bonds and their chemical changes usually they will be converted in single bonds as a first step to hydroperoxides formation. If this ratio of intensities 2850/3008 is increasing, then this means that double bonds are converted in single bonds. As a result, the number of single bonds increases their level and if this ratio decreases, it implies that the reverse reaction has happened and the number of double bonds increase their level [6].

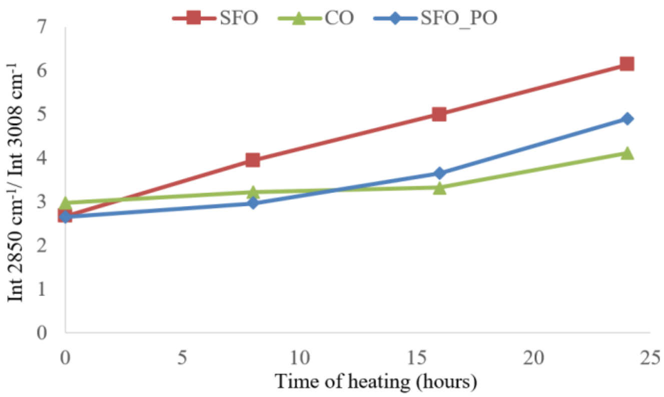

Figure 4. Intensity ratio at 2850 cm^{-1} and 3008 cm^{-1} as function of heating time: (1) sunflower oil, (2) corn oil and (3) mixture of sunflower oil and 1% pumpkin seed oil

After the analysis of three observed samples, it can be seen that ratio intensities are increasing during thermal treatment of samples, especially sunflower oil samples. Nevertheless, they have differences between them because (SFO) is rapidly increasing compared to (SFO-PO) where this ratio is increasing very slowly. If we consider corn oil sample, this ratio intensity is really stable until 16 hours of thermal treatment but after this time of heating intensity ratio increases. This can be explained with time when the oxidative decomposition of pure corn oil starts.

Conclusion

Oxidative resistance of vegetable oils is more than a need for human health in general, yet most of the conventional vegetable oils cannot fulfill this requirement. As a result, they can form other organic compounds as a product of decomposition which can cause serious health problems. An alternative method to increase stability of oil can be applied by blending, so using cold pressed oil from different plants such as pumpkin seed oil, black seed oil, walnut oil etc.

In this research pumpkin seed oil was extracted and characterized with high concentration of polyphenol compounds and high antioxidant activity. This oil with high antioxidative activity added as an ingredient to sunflower oil shows high antioxidative effect and only 1 % of pumpkin seed oil increases thermal stability of sunflower oil. This way it plays the role of protective agents from thermal oxidative decomposition of sunflower oil.

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