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Effect of *Maclura pomifera* total acetonic extract, pomiferin and osajin on the autooxidation of purified sunflower triacylglycerols

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RESUMEN

Efecto sobre la oxidación de triglicéridos purificados del aceite de girasol, del extracto de acetona del fruto de *Maclura pomifera*, de la pomiferina y de la osajina.

Se han aislado la pomiferina y la osajina del extracto de hexano del fruto de *M. pomifera.* Se han estudiado los efectos sobre la oxidación de triglicéridos purificados del aceite de girasol, del extracto de acetona, de la pomiferina y de la osajina. La pomiferina mostró una actividad antioxidante elevada en cambio, el extracto de acetona mostró una actividad moderada y la osajina baja.

PALABRAS-CLAVE: Aceite de girasol - Antioxidante - Autoxidación - Maclura pomifera - Osajina - Pomiferina -Triglicérido.

SUMMARY

Effect of *Maclura pomifera* total acetonic extract, pomiferin and osajin on the autooxidation of purified sunflower triacylglycerols.

Pomiferin and osajin have been isolated from the acetonic extract of *M. pomifera* fruits. Effects of total acetonic extract, pomiferin and osajin on the autooxidation of purified sunflower triacylglycerol were studied. Pomiferin showed a high antioxidant activity whereas total acetonic extract showed moderate and osajin revealed a low activity.

KEY-WORDS: Antioxidant - Autooxidation - Maclura pomifera - Osajin - Pomiferin - Sunflower oil - Triacylglycerols.

1. INTRODUCTION

The least stable macro-components in foods are the lipids. Depending on the degree of unsaturation, lipids are highly susceptible to oxidation resulting in the development of rancidity (Eskin and Przyblyski, 2001). When this occurs, the food becomes unacceptable and is rejected by the consumer. So, it should be noted that it is not the total fat content that is important, but the amount of unsaturated moieties in the triacylglycerol. For example although beef contains a lot more fat than potatoes, the later have a higher polyunsaturated fat composition and are much more susceptible to oxidation when in the dehydrated state (Labuza and Dugan, 1971; Weiss, 1970; Buttery *et al.*., 1961). As already settled the formation of free radical in lipid oxidation causes a rapid conversion to peroxides and hydroperoxides which are unstable and can breakdown to produce rancid off flavors. What makes this reaction so important, especially in its early stages, is that many of the small molecular weight compounds formed have very low detectable odor threshold values (the lowest concentration of a compound that can be recognized by its odor).

Differences in odor threshold values of several lipid oxidation products (e.g. aldehydes) have been reported using different media (air, milk, vegetable oil, mineral oil, and gelatin) (Fazzalari *et.al*, 1978; Lillard *et al.*., 1962; Lillard and Powers, 1975; Salo, 1970; Vega and Brewer, 1994).

To prevent or retard the oxidation, various protective measures may be used, such as gassing with inert gases, addition of antioxidants, appropriate packaging, special formulation, etc. Most of these methods can only be used for a limited range of products. Therefore the most common procedure is the addition of antioxidants mainly of fat soluble nature (Löliger and Wille). Various chemicals have been designed to function as antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and tert-butyl hydroquinone (TBHQ).

During the past two decades, both consumers and legislation officers have become suspicious about synthetic antioxidants, even chemicals that have been proven by experiment to be save, while natural alternatives are considered not to present significant risks.

The most readily acceptable oxidation inhibitors are common food ingredients, as their use is not limited by legislation. Many plants have been proven to posses considerable amounts of phenolic compounds that act as effective antioxidants either in the form of total extract or after fractionation of their major effective constituents.

Different metabolites have been isolated from the fruits and other parts of *Maclura pomifera* tree (Monache *et al.*, 1994 and Lee *et al.*, 1998). Osajin and pomiferin show antimicrobial activity and fruit extracts are also used in animal immunological studies (Ismail *et al.,* 2001). The higher percentage

of osajin and pomiferin in the fruits suggests the opportunity of examining their antioxidant activity.

So the objective of this study was to investigate the antioxidant activity of the total acetonic extract of *Maclura pomifera* and its two major constituents pomiferin and osajin.

2. MATERIALS AND METHODS

Sunflower oil (SFO) was obtained from local market. Alumina used for column chromatography (AL_2O_3, 70-120 mesh, Art. 15496) was purchased from Merck (Darmstadt, Germany). Sea sand was from Sigma Chemical Co. (St. Louis, MO). α,α-Biphenyl-β-picrylhydrazyl (DPPH) was obtained as a gift from Dr. Khaled Farouk (National Research Centre, Egypt). All other solvents and chemicals were either of BDH grade or Analar.

2.1. Oil purification

SFO was purified from antioxidants, trace metals and other prooxidants via adsorption column chromatography, as described by Fuster *et al*. (1998), to yield purified SFO triacylglycerol (p-SFO).

Analysis of the purified oil by thin layer chromatography (Merck precoated silica gel 60, 0.25 mm layer thickness and chloroform/ diethyl ether 90:10, v/v) showed that it is composed mainly of triacylglycerols together with negligible amounts of sterol esters (results not shown). The purified oil was found to contain undetectable amounts of tocopherols (HPLC, <0.5 ppm, cf. *2.2.*), peroxides (ferric thiocyanate method, <0.7 meq O_2 / kg oil) and iron and copper (atomic adsorption spectroscopy, <0.015 and 0.001ppm, respectively, cf. *2.3.*).

2.2. Determination of tocopherols content by high performance liquid chromatography (HPLC)

Without pretreatment of the purified sunflower oil triacylglycerols, the tocopherol isomers were determined by HPLC (Gertz and Hermann, 1982) using fluorescence detector. α-, and γ-Tocopherols were separated on a Lichrosorb Si60, 5 μm column (250 x 4 mm, Knauer, Berlin, Germany) in isocratic elution using Isooctane / tert-methylbutylether (96:4, v/v) as solvent during 50 minutes at a flow rate of 1.3 ml / min. A fluorescence detector (Kratos) monitored the eluates at 295 nm absorption and at 340 nm emission.

One gram water-free oil was weighed in a 10-ml measuring flask and completed to the mark with n-hexane. The content of the flask was dried over anhydrous sodium sulfate and then filtered and the filtrate was degassed in an ultrasonic-water-bath for 5 minutes. 20 μl of this solution was injected and duplicates were run for each sample.

2.3. Determination of copper and iron

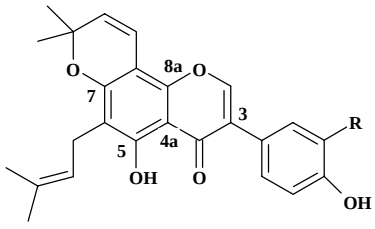
The copper and iron residues in p-SFO were determined by AOAC Official Method 990.05 (AOAC, 1995) using atomic absorption spectrophotometer Perkin – Elmer 2380.

2.4. Extraction and isolation of plant materil

150 gm of *Maclura pomifera* fruits was extracted with acetone (500 ml X 2). After filtration, the combined extract was concentrated under reduced pressure to yield a semisolid residue. The residue was subjected to silica gel column (Merck no. 7734), deactivated with 10% H_2O, eluted with a mixture of hexane – EtOAc, and finally with 10% MeOH in EtOAc. The fractions containing osajin and pomiferin (fig.1) among other flavonoids were further purified using silica gel column and Sephadex LH-20 eluted with MeOH, and finally the two compounds were isolated separately by crystallization from EtOAc. Purity of the two compounds (osajin and pomiferin) were monitored by TLC and H^1NMR. The data obtained were identical with those reported in the literature (Monache *et al.,* 1994; Lee *et al.*, 1998).

2.5. Evaluation of Antioxidant activity by Radical Scavenging Assay DPPH

The antioxidant activity of the tested samples was measured using α,α-biphenyl-β-picrylhydrazyl (DPPH) as a radical scavenging compound. The deep violet color of DPPH vanishes stoichiometrically with the number of electrons taken up (Blois, 1958). 2 mg of the solid extract was dissolved into 2 ml methanol and then added to one ml DPPH standard solution (0.3 mmol) and mixed thoroughly. The absorbance of the solution was read spectrophotometrically at 516 nm against pure methanol. Exactly after 10 min the solution was read at 516 nm against pure methanol. A standard curve was made by plotting the absorbance for a series of dilutions of the standard DPPH solution against their concentrations (in mmol).



R

1 H

2 OH

Figure 1
General Structure of Osajin (1) and Pomiferin(2).

Calculation:

Concentrations of total antioxidants present in the sample could be obtained directly from the standard curve by subtracting the absorbance of the sample from that of the standard solution and reading the corresponding concentration from the curve.

2.6. Autooxidation Experiment

The oxidative stability of p-SFO was first compared with that of the unpurified oil without adding any antioxidants. Thereafter study of antioxidant effects of *Maclura pomifera* total extract, osajin and pomiferin at concentrations of 200 ppm were carried out in p-SFO sample (10 g) as a substrate for the autooxidation experiment. p-SFO samples containing these antioxidant were oxidized in brown glass bottles (to prevent light exposure) in a thermostated oven maintained at 55 ^{o}C $\pm$ 1 in the dark and p-SFO sample without any antioxidant was taken as a control for the experiment. The oxidative stability of the samples was followed up by measuring peroxide value (PV) by the modified ferric thiocyanate method (Shantha and Decker, 1994). The hydroperoxide inhibition percentage as a measure of the protective value was calculated as follows:

$$\text{Inhibition \%} = (PV_{control} - PV_{sample}) / PV_{control} \times 100$$

Where: $PV_{control}$ was the amount of hydroperoxides formed in the control sample and PV_{sample} was the amount of hydroperoxides formed in the sample containing *Maclura pomifera* total extract, osajin or pomiferin.

All analyses were done in duplicate.

3. RESULTS AND DISCUSSION

Purified sunflower triacylglycerols was chosen to study the oxidation of vegetable oils for many reasons: (i) it provides the natural complexity of fatty acid distribution of the mother oil, (ii) to exhibit a similar viscosity to the original oil, (iii) it contains a considerable amount of linoleic acid (ca. 64 %, Table I) so it is readily susceptible to oxidation, and (iv) to eliminate the anti- and prooxidant constituents normally present in the oils (Fuster *et al.*, 1998).

Table I
Fatty Acid Composition and Tocopherol Concentration of Sunflower oil

Fatty Acid Composition	Percentage (%)
Palmitic Acid	7.1
Stearic Acid	3.7
Oleic Acid	24.0
Linoleic Acid	64.3
Linolenic Acid	0.2
Eicosa-11-enoic acid	0.7
Tocopherol Concentration (ppm)	
α- Tocopherol	523
γ- Tocopherol	9

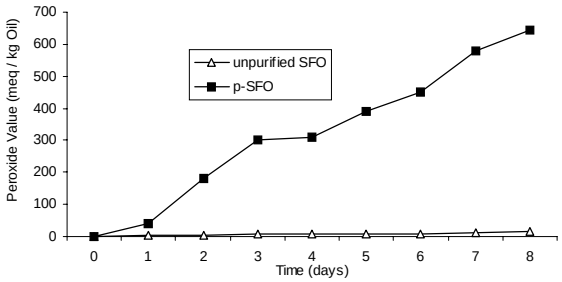


Figure 2
Oxidative Stability of Purified and Nonpurified Sunflower Oil Triacylglycerol unpurified SFO= unpurified sunflower oil; p-SFO=purified sunflower oil.

The oxidative stability of purified and unpurified sunflower oil were compared before carrying out the main experiment to indicate that the non-glyceride components play an important role in oil stability as shown in fig. 2.

There was a large difference in the stability between them which reflects the importance of the non-glyceride minor constituents including the naturally occurring antioxidants (see Table I).

Table II
Antioxidant Activity as Measured By DPPH

Sample	Absorbance	Activity %*
standard DPPH	1.05	0.00
Total extract	0.50	52.86
Osajin	0.88	16.38
Pomiferin	0.15	85.52

$$\text{*Activity \%} = \frac{(\text{Absorbance of standard DPPH} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of standard DPPH}}$$

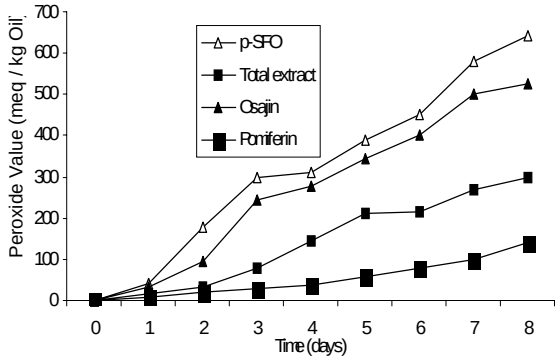


Figure 3
Hydroperoxide Formation of Sunflower oil Samples containing Total Extract,Osajin, Pomiferin and purified sunflower oil (p-SFO).

Table II indicates that the antioxidant activity measured by the radical scavenger,α-biphenyl-β-picrylhydrazyl (DPPH) increased in the order osajin< total< extract <pomiferin.

This was also emphasized by the autooxidation experiment in which the hydroperoxide value of sample containing pomiferin gave the lowest value and osajin gave the highest (Fig. 3).

So, pomiferin is considered to be more active as oxidation inhibitor than its original total extract or osajin as clearly shown by calculating hydroperoxide inhibition (Fig. 4).

4. CONCLUSION

In conclusion, pomiferin was found to be more active as antioxidant in purified sunflower oil triacylglycerols than its original total extract of *Maclura Pomifera* fruit or osajin . From the current experimental data it is clear that, ortho-dihydroxy structure of the ring B shown to be essential for radical scavenging properties of many flavonoids (Dugas, 2000). Such system does not exist in osajin.

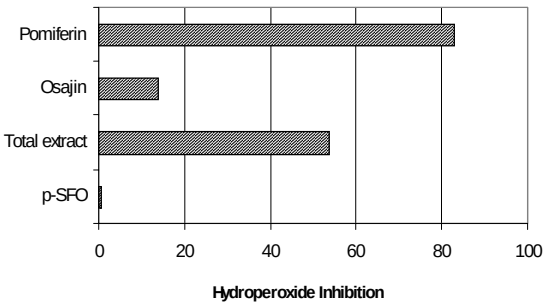


Figure 4
Inhibition Percentage of Hydroperoxide Formation of purified sunflower oil (p-SFO) Samples containing Total Extract, Osajin and Pomiferin. p-SFO = purified Sunflower triacylglycerol without addition (control)

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