Dill Is an Efficient Antioxidant Against ROS Specially Singlet Oxygen in the Oleic Acid Media

Mahdi Hajimohammadi* and Maryam Khalaji Verjani

Faculty of Chemistry, Kharazmi University, G. C, Mofateh, Tehran, 14911-15719, Iran

*Corresponding author: Mahdi Hajimohammadi, Faculty of Chemistry, Iran

Abstract

Scavenging of DPPH free radical is the basis of a common antioxidant assay and most often an overall antioxidant effect was measured. However, singlet oxygen (1O₂) has not radical nature and 1O₂ scavenging properties of natural antioxidants oppressed and less have been investigated. In this work effect of dill (Anethum graveolens L) as a natural antioxidant on fatty acid safety was investigated in the presence of OH∙, H₂O₂, O₂ and specially 1O₂. In order to evaluate antioxidant activities of dill extract, oleic acid oxidation was monitored by 1H NMR spectroscopy, peroxide value (PV (meqO₂/kg)) and UV-Vis spectroscopy. The rate of oleic acid oxidation by 1O₂ as a very reactive ROS reduced about 42.5% in the presence of 2 mL methanolic extract of dill (contains 2.24 mg flavonoid compounds) as a natural antioxidant. This result reveals that dill has an efficient role on preservation of unsaturated fatty acids from photooxygenation. Also, UV-Vis spectroscopy as a reliable method to determine oleic acid oxidation showed in the oleic acid oxidation with OH∙ and H₂O₂, bandgaps of oleic acid as a result of oxidation was compacted in the presence of dill which demonstrated dill is effective on control of fatty acid against these types of Reactive Oxygen Species (ROS).

Keywords: Reactive oxygen species; Oleic acid; Singlet oxygen; Dill antioxidant; Porphyrin sensitizers

Introduction

ROS such as Hydroxyl Radicals (OH), Superoxide Anion (O₂⁻), Hydrogen Peroxide (H₂O₂) and singlet oxygen (1O₂) are inevitable results of aerobic metabolism [1]. Because of high activity of ROS lipids, DNA and proteins can be their target [2] that caused many illnesses including cancer, cardiovascular disease, cataracts, Alzheimer's and aging [3,4]. Antioxidants are compounds that can delay, inhibit or prevent the oxidation by scavenging free radicals and diminish oxidative stress [5]. A trend toward the use of natural additives in foods has been apparent for quite some time as a result of consumer demand because safety of synthetic antioxidants such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), Tert-Butyl Hydroquinone (TBHQ) and Propyl Gallate (PG) [6,7] has been questioned [8]. Recent research has focused on isolation and characterization of effective natural antioxidants [9-12]. Natural antioxidants act (a) as reducing agents, (b) as free radical scavengers, and (c) as quenchers of the formation of singlet oxygen. They can be used in the food industry and there is evidence that they may exert their antioxidant effects within the human body [13,14]. People receive antioxidant supplements directly from fresh fruits and vegetables. The World Health Organization estimated that <80% of the earth's inhabitants rely on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts or their active phenolic components [15] which have efficient antioxidant capacity. According to the published papers, the research on OH-, H₂O₂ and O₂ as reactive oxygen species are widely carried out [16-18] whereas 1O₂ oppressed and less have been investigated because scavenging of DPPH free radical is the basis of a common antioxidant assay and most often an overall antioxidant effect was measured [19].

However, singlet oxygen has not radical nature. Oxygen molecule in its ground state has two unpaired electrons and when oxygen molecule has excess energy, these unpaired electrons in the external orbital can be pair and generate singlet oxygen [20]. One of the physical methods for producing singlet oxygen is applied photosensitizer. Great photosensitizers have received attention, due in part to their direct relevance to many biological systems.
The photosensitized production of singlet oxygen has significance in the areas of the photooxidation of organic compounds, DNA damage and Photodynamic therapy [21-26]. Electrophilic tendency of singlet oxygen causes lipids, amino acids, nucleic acids and electron rich molecular can be its target [27]. This project was designed to characterize antioxidant potential of dill as a natural antioxidant due to its phenolic and flavonoids compositions and how its effect on toxic properties of different ROS specially singlet oxygen [28]. Oleic acid oxidation process was monitored by UV-Vis and PV (meq O₂/kg) methods in the presence and absence of dill as an antioxidant and the results showed dill has efficient role to control oxidation process.

Materials and Methods

Materials

Oleic acid, ethanol, DMSO, hydrogen peroxide, acetonitrile and KO₂ were purchased from Fluka and Merck without further purification. Tetraphenyl porphyrin (H₄TPP), ZnTPP and FeTPP and were synthesized according to the literatures [29]. Preparation of dill extract: Cold solvent extraction method was applied to separate the dill extract. Dill plant was dried, milled and then strained through sieve No. 40. Powdered form was mixed with solvent at 1:10 ratio on a shaker at room temperature for 24h and then the mixture was filtered using filter paper and vacuum pump. The solvent was removed on a rotary evaporator under vacuum in order to minimize the loss. The remaining solvent was removed using nitrogen [30].

Methods

Sample preparation to oleic acid photooxygenation: 0.2 cc photosensitizers (0.001M), 1 cc oleic acid were added to 5 cc acetonitrile in a test tube. Reactions were irradiated with the sun simulator light (288 power LED lamps, 1W, 2.3 V (59660 LUX)) for 6 hours at room temperature under 1 atm of bubbling of air in the solution. Sample preparation to oleic acid oxidation with H₂O₂ and OH• for monitoring with UV-Vis method: 0.1 cc hydrogen peroxide 30% and 0.1 cc antioxidants (contains 0.4095 mg polyphenolic compounds) were added to 5 cc oleic acid 0.001M. The reactions were irradiated by UV light from a high pressure 30W mercury lamp (Philips, λ= 200–280 nm) for OH• generation. Sample preparation to oleic acid oxidation with H₂O₂ and OH• by iodometric titration: 0.1 cc hydrogen peroxide 30%, 2 cc oleic acid and 2 cc antioxidant (contains 8.19 mg polyphenolic compounds) added to 6 cc ethanol. By irradiation of UV light from a high pressure 30W mercury lamp (Philips, λ= 200–280 nm) in the reactions OH• is generated. In order to avoid interference of hydrogen peroxide in the PV (meq O₂/kg) measurement, organic media which involves oleic acid oxidation products was extracted and work up by water and chloroform. Superoxide anion radical preparation for oleic acid oxidation: 2 cc oleic acid and 0.44 gr KO₂ added to 10 cc DMSO in the presence of 3 cc antioxidant (contains 12.285 mg polyphenolic compounds). A 3.1 × 10⁻³ mol oleic acid, 0.5 cc antioxidant (contains 0.56 mg flavonoid), 5 cc solvent, 0.2 cc (0.001M) sensitizer, air (1 atm) and 288 power LED lamps, 1 W, 2.3 V (59660 LUX).

Analytical methods

PV (meq O₂/kg) of the samples was determined according to the literature [31]. Oleic acid oxidation process was monitored by UV-Vis (Shimadzu 2100 spectrophotometer). 1H NMR spectra were obtained on a Bruker AMX 300 MHz spectrometer using TMS as internal standard.

Results and Discussion

In this work the oxidative alterations of oleic acid as a result of oxidation with singlet oxygen, superoxide radical, hydrogen peroxide and radical hydroxyl were analyzed in the presence and absence of dill as a natural antioxidant due to its phenolic compounds. Our target was fatty acid oxidation by different ROS with focus on singlet oxygen as a noble species which has worked few studies on it [19]. Photooxygenation of oleic acid with H₄TPP was investigated as a typical standard sample to evaluate singlet oxygen production (Scheme 1) and fatty acid oxidation monitored by iodometric method as a popular method. (Table 1) confirmed that singlet oxygen produced by applying different kind of photosensitizers. It is important to note that 1H NMR (see supporting information) and peroxide value the oxidation of oleic acid to peroxide product stopped in the absence of porphyrin (Table 1 entry 1) or when the irradiation was interrupted (Table 1 entry 2). Accordingly, the presence of a porphyrin, light and O₂ are essential for the conversion oleic acid to corresponding products (Table 1 entry 3).

Table 1: PV number of oleic acid oxidation by singlet oxygen in different conditions.

| Entry | Condition | PV  |
|-------|-----------|-----|
| 1     | oleic acid +CH₂CN+air + light | trace |
| 2     | oleic acid +CH₂CN+H₄TPP + air | trace |
| 3     | oleic acid +CH₂CN+H₄TPP + air | 283.14 |
| 4     | oleic acid +CH₂CN+H₄TPP + light + air + dill | 232.58 |
| 5b    | oleic acid +CH₂CN+H₄TPP + NaNO₂ + air | 49.43 |
| 6     | oleic acid +DMSO + H₂O₂ + light | 64.44 |
| 7     | oleic acid +CH₂CN+H₄TPP + light | 258.42 |
| 8     | oleic acid +CH₂CN+H₂O₂ + light + air + dill | 126.96 |
| 9     | oleic acid +CH₂CN+ZnTPP + light | 37.07 |
| 10    | oleic acid +CH₂CN+FeTPP + light | 35.95 |
| 11c   | oleic acid +CH₂CN+H₂O₂ + light | 202.2 |
| 12d   | oleic acid +CH₂CN+H₂O₂ + light + air + dill | 162.92 |

a) 3.1 × 10⁻³ mol oleic acid, 0.5 cc antioxidant (contains 0.56 mg flavonoid), 5 cc solvent, 0.2 cc (0.001M) sensitizer, air (1 atm) and 288 power LED lamps, 1 W, 2.3 V (59660 LUX).
b) 0.01 gr sodium azide applied as singlet oxygen scavenger.
c) 1 cc antioxidant (contains 1.12 mg flavonoid).
d) 2 cc antioxidant (contains 2.24 mg flavonoid).
Also, in the presence of N\textsuperscript{3}-, which is a well-known (Figure 2) antioxidant with photosensitizers (A). Structure of different applied photosensitizers (B). Singlet oxygen scavenger [32] conversion was inhibited (Table 1, entry 5). In the presence of NaN\textsubscript{3} degradation of the porphyrin sensitizers was also inhibited. Table1 entry 6 indicates that in the presence of DMSO conversion of oleic acid considerably diminished. Singlet oxygen lifetime is the important issue for gaining efficient yield during photooxygenation. According to the literature singlet oxygen lifetime in DMSO is 19μs, 65μs in acetonitrile and 38μs in ethanol which was corresponded with the results in Table 1 (entry 3,6,7) [33] (Scheme 1). One of the key issues to efficient photooxygenation is photosensitizer. Singlet oxygen generation by different photosensitizer and their reactions with the oleic acid obey the order of H\textsubscript{2}TPP > FeTPP > ZnTPP. Paramagnetic metals are claimed to quench singlet oxygen by energy transfer mechanism from oxygen to the low-lying d electron levels and have very short triplet lifetimes (Table 1, entry 9,10). Oleic acid photooxygenation in the presence of dill as an natural antioxidant has the finest effect on limiting or preventing of oxidation due to its flavonoids compounds which is the most important family of exogenous antioxidants (Table 1 entry 4,8) singlet oxygen scavenger. c1 cc antioxidant (contains 1.12 mg flavonoid). d 2 cc antioxidant (contains 2.24 mg flavonoid Scheme 2).
Scheme 2: The mechanism of flavonoids barricade against singlet oxygen.

Figure 2: Oleic acid oxidation by superoxide anion radical in the absence of antioxidant (a), oleic acid oxidation in the presence of dill (b) and oleic acid oxidation in the presence of vitamin E (c).

As a result of daily life diet and the effect of different amount of natural antioxidant such as fruit, vegetable and herbal plants, our experience on dill showed how different amounts of dill as a natural antioxidant has efficient effect on restrict to produce peroxide as primary products. Results showed by increasing dill concentration the oleic acid oxidation rate or PV (meq O₂/kg) numbers were decreased (Table 1 entry 11, 12). In the case of oxidation by radical hydroxyl OH⦁ and hydrogen peroxide H₂O₂, applied Uv-Vis method and idometric titration. Idometric as a popular method has limited and it is not an accurate method to measurable these ROS because of their peroxide agent. Although by extracting this oxidizing specie PV (meq O₂/kg) were calculated and dill showed that it is finest natural antioxidant to face with H₂O₂ after 1h and OH⦁ after 3h (Table 2 entry 1 and 2 for H₂O₂, entry 3 and 4 for OH⦁). Also, with UV-Vis method antioxidant property of dill was proved (Figure 2).

According to the literature oxidation of polyunsaturated fatty acids is accompanied by an increase of absorption in the ultraviolet range (200-380nm). Lipids containing dienes or polyenes show a shift in their Double bond position during oxidation due to isomerization and conjugation formation. In the presence of dill as an antioxidant the oxidation process by OH⦁ and H₂O₂ changed (Figure 1 column b and c). Column a and b represent oleic acid absorption gap spaces at λ=312nm by OH⦁ and column cand d represent oleic acid oxidation absorption gap spaces at λ=230nm by H₂O₂ in the presence and absence of antioxidant after oxidation.

Table 2: The peroxide number (meq O₂/kg) of oleic acid oxidation by H₂O₂ and OH.

| Entry | Conditions         | Time= 1h | Time= 2h | Time= 3h |
|-------|--------------------|----------|----------|----------|
| 1     | oleic acid + H₂O₂  | 50.56    | 28.09    | Trace    |
| 2     | oleic acid + H₂O₂ + dill | 11.24    | 11.24    | 11.24    |
| 3     | oleic acid + H0.    | 21.86    | 5.62     | 16.86    |
| 4     | oleic acid + OH. + dill | 22.47    | 11.24    | Trace    |

These results showed that in the presence of dill as an antioxidant absorption gap spaces per 5min is less than absorption gap spaces in the absence of dill for 1hour oxidation which demonstrated dill has good effect on control of oxidation because of its polyphenol composition and antioxidant activity. Also comparative of UV-vis and iodometric data have been good agreement in the oxidation process. Our investigation of superoxide anion radical was based on PV (meqO₂/kg) (Figure 2). Lack of willingness oleic acid reaction by superoxide anion radical caused monitoring of products at longer period. Results showed dill had the best effect on limitation of oleic acid oxidation during the 12h oxidation and its antioxidant effect on O₂⦁ is more efficient than vitamin E as one of the best well known lipid soluble antioxidant.
Conclusion

Increase of diseases such as cancer, Alzheimer’s disease, skin disorders, etc. because of human bad lifestyle and their incorrect eating habit turns broaden our view on using new, safe and none side effect medicine such as herbal and plant source. In this study it was showed dill has an efficient effect as a natural antioxidant on restricting or limitation oxidation fatty acid by different toxic ROS. In fact, dill had high antioxidant capacity for inhibition of $1O_2$ and the other ROS.

Acknowledgement

We gratefully acknowledge support from the Kharazmi University.

References

1. Luis A, Sandalo LM, Corpus FJ, Palma JM, Barroso JB, et al. (2006) Reactive Oxygen Species and Reactive Nitrogen Species in Peroxisomes. Production, Scavenging, and Role in Cell Signaling. Plant Physiol 141(2): 330-335.
2. Mates JM (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 153(1-3): 83-104.
3. Kinnula VL, Crapo JD, Free Radical Bio Med (2004) Kinnula VL, Crapo JDSuperoxide dismutases in malignant cells and human tumors 36: 718-744.
4. Singh I, Jialal I (2006) Pathophysiology 13: 139-142.
5. Ani V, Varadaraj MC, Naidu KA (2006) Antioxidant and antibacterial activities of polyphenolic compounds from bitter Cumin. Eur Food Res Technol 224: 109-115.
6. Kobayashi Y, Nakano Y, Inayama K, Sakai A, Kamiya T (2003) Dietary intake of the flower extracts of German Chamomile (Matricaria recutita L) inhibited compound 48/80-induced itch-scratch responses in mice. Phytomedicine 10(8): 657-664.
7. Matos FJA, Machado MIL, Alencar JW, Craveiro AA (1993) Constituents of Brazilian chamomile oil. J Essent Oil Res 5: 337-339.
8. Duh PD, Yeh DB, Yen GC (1992) Pharmacological Potential of Matricaria recutita-A Review. J Am Oil Chem Soc 69: 814-818.
9. Cho ML, Ko SB, Kim JM (2016) Appl Biol Chem 59: 329-336.
10. Shrestha S, Boo KH, Cho SK (2015) Evaluation of antioxidant potential of ethyl acetate fraction of Rosmarinus officinalis L. and major components. Appl Biol Chem 58(1): 715-722.
11. Grice HC (1988) Safety evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. Food Chem Toxicol 26(8): 717-723.
12. Wichi HC (1986) Enhanced tumour development by butylated hydroxytoluene (BHT) in the liver, lung and gastro-intestinal tract. Food Chem Toxicol 24(11): 1127-1130.
13. Koski A, Psmiadou E, Tsimidou M, Hlopia A, Kefalas P, et al. (2002) Oil Eur Food Res 214: 294-298.
14. Hamid AA, Ayelagb BeOO, Usmon LA, Ameen OM, Lawal A (2010) Pure Appl Chem 4: 142-151.
15. Bruneton J (1995) Process for the Preparation of Chromones, Isoflavones and Homoisoflavones Using Vilsmeier Reactant Generated from Phthaloyl Dichloride and DMF. Pharmacognosy, phytochemistry medicinal plants. Lavoisier publishing.
16. Choi MH, Lee IK, Kim GW, Kim BU, Han YH, et al. (2005) Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. Nature 435(7040): 347-353.
17. Forman HJ, Torres M (2002) Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. Am J Respir Crit Care Med 166(2): 4-8.
18. Morrell CN (2008) Reactive Oxygen Species Circ Res 103(1): 571-572.
19. Terao J, Minami Y, Bando N (2011) Singlet molecular oxygen-quenching activity of carotenoids: relevance to protection of the skin from photoaging. J Clin Biochem Nutr 48(1): 57-62.
20. Min DB, Boff JM (2002) Chemistry and Reaction of Singlet Oxygen in Foods. Compr Rev Food Sci Food Saf 1: 58-72.
21. De Rosa MC, Crutchley RJ (2002) Photosensitized singlet oxygen and its applications. Coord Chem Rev 233: 351-371.
22. Greer A (2006) Christopher Foote’s Discovery of the Role of Singlet Oxygen [102 (1Ag)] in Photosensitized Oxidation Reactions. Acc Chem Res 39(1): 797-804.
23. Hajimohammadi M, Vaziri Sereshk A, Schwarzenegger C and Knör G (2018) Suppressing effect of 2-nitrobenzaldehyde on singlet oxygen generation, Fatty Acid Photooxidation, and Dye-Sensitizer Degradation. Antioxidants 7: 194.
24. Hajimohammadi M, Nosrati P (2018) Scavenging effect of pasiphae (passiflora incarnata L.) on singlet oxygen generation and fatty acid photooxygogenation. Food Science & Nutrition 6: 1670-1675.
25. Hajimohammadi M, Safari N, Mofakhah H, Deyhimif F (2011) Highly efficient, green and solvent-free photooxygenation of alkenes by air and visible light or sunlight in the presence of porphyrin sensitizers. Green Chem 13: 991-997.
26. Hajimohammadi M, Ahamadi Khamesi Z, Nosrati P (2019) Efficient aerobic photooxygenation of aldehydes to carboxylic acids using cobalt (II) phthalocyanine sulfonate as a photosensitizer in organic-water biphasic media. Transition Metal Chemistry 44: 167.
27. Kramarenko GG, Hummel SG, Martin SM, Buettner GR (2007) J Photochem Photobiol B: 86:134-1367.
28. Decker EA (1995) The role of phenolics, conjugated linoleic acid, carnosine, and pyrroloquinoline quinone as nonessential dietary antioxidants. Nutr Rev 53(3): 49-58.
29. Lindsay J, Wagner RW (1989) Investigation of the synthesis of ortho-substituted tetraphenylporphyrins. J Org Chem 54(1): 828-836.
30. Su L, Yin J, Charles D, Zhou K, Moore J, Yu L (2007) Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, moship, cinnamon and oregano leaf [2007]. Food Chemistry 100: 990-997.
31. Barthel G, Grosch W (1974) Peroxide value determination-Comparison of some methods | Am Oil Chem Soc 51(6): 540-54.
32. Harbour JR, Issler SL (1982) J Am Oil Chem Soc 10: 903-905.
33. Chen Y, Xu, S, Li L, Zhang M, Shen J, Shen T (2001) Dyes and Pigments 51: 63-69.
34. Bressan M, Morvillo A (1989) Alkene epoxidation by ruthenium (II) phthalocyanine sulfonate as a photosensitizer in organic-water biphasic media. Coord Chem Rev 233: 351-371.
35. Tofidi DJ, Gomes LR, Junior NDV, Courrol LC, Wetter NU, et al. (2008) Aip Conf Proc 992: 1207-1212.
36. Bonnett R, Martinez G (2001) Tetrahedron Lett 57: 9513.
37. Shyu YS, Lin JT, Chang YT, Chiang CJ, Yang DJ (2009) Food Chem 115: 515-521.
38. Vieira TM (1999) Identification of Character Impact Odorants of Different Soybean Lecithins | Agr Food Chem 47(7): 2203-2206.
39. Reby RB, Zakhama N, Karoui, Hj, Marzouk B (2012) Development and Application of a Database of Food Ingredient Fraud and Economically Motivated Adulteration from 1980 to 2010. J Food Sci 77: 34-39.
40. Augusto O, Miyamoto S (2011) Oxygen radicals and related species. Nova Science Publishers, New York, USA.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2019.21.003650

Mahdi Hajimohammadi. Biomed J Sci & Tech Res

Submission Link: https://biomedres.us/submit-manuscript.php

This work is licensed under Creative Commons Attribution 4.0 License

Assets of Publishing with us

• Global archiving of articles
• Immediate, unrestricted online access
• Rigorous Peer Review Process
• Authors Retain Copyrights
• Unique DOI for all articles

https://biomedres.us/