The Influence of Lycopene and Other Natural Antioxidants on Refined Sunflower Oil Stability

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Abstract— **The effects of lycopene (1000 ppm) and rosemary extract concentrate (R), as (R0.02), (R0.1) alone and in combination with 0.01 % citric acid (CA), 0.01% ascorbyl palmitate (AP), and 0.01% combined (AP+CA) on oxidative stability of refined sunflower oil and lycopene degradation were studied. Oxidative stability was assessed on oil heated in the absence of light and presence of nitrogen at 80°C as measured by peroxide value (PV). (R0.1 AP) showed the highest ability to enhance PV. Rancimate test was used to measure the oxidative stability index (OSI). The synergistic effect was observed in the following order (RAPCA), (RCA), (RAP).**

Lycopene was found to reduce the OSI compared to the addition of other antioxidant systems with no lycopene. However, the OSI of containing lycopene systems in comparison to control (sunflower oil) was exceedingly high. Lycopene degradation was determined by HPLC. R0.1AP system showed the highest lycopene protective as total lycopene was 10.9 % higher than that of the control sample as the sunflower oil was heated in the absence of light, and in the presence of nitrogen at 80°C for 72 hours. Natural antioxidants such as Lycopene and rosemary extract are useful to retard lipid oxidation in a variety of food products which can act as functional food to protect biological systems in the human body.

Keywords— Natural antioxidants, lycopene, rosemary extract, refined sunflower oil, oil oxidation.

I. INTRODUCTION

Lipids contribute very significantly to the quality of almost all kinds of foods. They have a direct influence to the quality attributes of various raw materials and affect the nutritional value, safety, and the sensory properties of foods, in addition to performing as a carrier of fat- soluble vitamins and other lipophilic substances needed in nutrition [3]. The oxidation reaction of unsaturated lipids is the most important factor causes of food quality deterioration initiated by free radicals. When lipids are exposed to many factors such as air, light, temperature, moisture and heavy metals, oxidation reactions start to produce undesirable flavours, rancid odours, discoloration and other forms of spoilage. The primary autoxidation products are hydroperoxides, that have no taste and flavour, but their degradation products (aldehydes, ketones…) are very potent taste and flavour modifiers [2], [21]. These products might also initiate oxidative chain processes in the human body, which are implicated in the progress of carcinogenesis, atherosclerosis, Myocardial infarction, allergies, inflammatory bowel and other diseases, Therefore added antioxidant into lipids and food-containing lipids to delay the deterioration of food due to oxidation is needed [21]; [22].

Antioxidants have defined as the compounds serving to inhibit/minimize free radical formation, scavenging them and promoting their decomposition during oxidation processes that deteriorate the quality of lipids or food-containing lipids "food antioxidant" [11]; [26], or protect biological systems against the potentially harmful effects of processes that cause extensive oxidations.''biological antioxidant " [11]. Antioxidant can be of synthetic or natural origin, synthetic antioxidants are particularly widespread use in foods as BHA, BHT, PG (propyl gallate), and TBHQ tertbutyl hydroquinone. Recent reports reveal that these compounds may be implicated in many health risks, Hence, there is more attention has been paid to the possibility that "natural" antioxidants may replace them for at least some food applications [6]; [2]; [1].

Natural antioxidants are compounds from plant or animal sources that retard oxidative rancidity of oils, fats, and fat-soluble components, thus protecting them while delaying the development of unpleasant flavours and odours resulting from oxidation [25]. During the past two decades, a lot of researches about the use of natural plant extracts with antioxidant activities in various edible oils have been carried out. The main goal of these researches was to reduce the application of synthetic compounds as antioxidants because of their potential negative health effects and as a result of consumer demand [25]; [21].

The technologically most important natural antioxidants today are: ascorbic and citric acid and their salts, tocopherols and spice extracts. Citric acid is very effective in retarding the oxidative deterioration of lipids in foods and is commonly added to vegetable oils after deodorization [2].

Rosemary extract provides a natural mixture of bioactive components that may turn out to be the greatest antioxidant activity of all spices and herbs in the range between (200 to 1000) ppm, especially when delivered in fat and products

with a high fat content [8]. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as carnosol and carnosic acid, inhibit lipid peroxidation and deoxyribose damage and scavenge hydrogen peroxide, hypochlorous acid, peroxynitrite, peroxyl, and hydroxyl radicals in lipid and non-lipid systems by hydrogen atom donation, which break free radical chain reactions [23].
Carotenoids are known as natural pigments at first. The antioxidant activities of lycopene and other carotenoids are highlighted by their singlet oxygen quenching properties and their ability to trap peroxyl radicals, The extended system of eleven conjugated and two non-conjugated double bonds make this molecule the most efficient singlet oxygen quencher among thousands of natural carotenoids [27]; [10]; [16]; [9].

Lycopene can reduce the risks of cardiovascular diseases and cancers, inhibits oxidative DNA damage, and stimulates gap junction communication also satisfy the consumer demands of colourful lycopene-containing food, so it certainly attracts much attention of many scholars. Studies have reported that Z-isomers of lycopene are the predominant proportion of lycopene in human serum and tissues [27]; [15]; [19].

Ascorbic and citric acid and their salts are the most important natural antioxidants nowadays, Ascorbyl palmitate is a synthetically-derived oil-soluble ester of ascorbic acid, Citric Acid is water soluble but has exhibited solubility in lipids to inactivate metals in the lipid phase, its the most common metal chelators used in foods. Its ability to chelate metal ions is by forming bonds between the metal and the multiple carboxyl or hydroxyl [11]; [2]; [20].

In this study, we have determined the antioxidant properties of certain binary blends, ternary blends and quaternary blends of lycopene, Rosemary extract, ascorbyl palmitate, and citric acid in sunflower oil as by peroxide value and oxidative stability index, on the other hand lycopene degradation was measured to test the antioxidant effect on total Lycopene content. We have chosen lycopene for its oxygen quenching ability and the functional properties for human consumption, ascorbyl palmitate in preference to ascorbic acid because of its greater solubility in oil [24]; [14]; [2], citric acid for its high ability to chelate metal and rosemary extraction because of its high oxidative retarding stability, In addition, the synergistic effect of these different antioxidant systems on inhibition of lycopene degradation during heating process was also investigated, in order to determine the possibility of these systems to act as lycopene carrier.

II. MATERIALS AND METHODS

A. *Materials*

Refined Sunflower oil antioxidant-free (Yi Hai Oils Company, Shanghai, China) was purchased from a local supermarket (Wuxi, Jiangsu, China).
This oil had a peroxide value of 5.1, an acid value of 0.035. The fatty acid composition of sunflower oil was determined by gas chromatography (GC) as follows: 6.38%, 4.02%, 25.70%, 62.36%, 0.22%, 0.38%, 0.68% and 0.26% of following fatty acids 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1 and 22:0 respectively.
Lycopene (90% purity) was supplied by North China Pharmaceutical Co. Ltd., Shijiazhuang, China.
Rosemary extract (50% Carnosic acid) was supplied by Wuhu Tianyuan SCI & TECH, Hunan Bio MED & TECH. Ascorbyl palmitate and citric acid supplied by Tianming food additive Co. Ltd, Zhengzhou, China. All other chemicals and solvents used were of analytical grade.

B. *Methods:*

- *Sample preparation:*

The natural antioxidants and their mixtures were added to sunflower oil (wt/wt) containing 1000 ppm lycopene in the following quantities: 0.02 % and 0.1% of rosemary extract with 50% carnosic acid (R0.02), (R0.1), respectively, 0.01% citric acid dissolved in 0.5 ml of propylene glycol (CA), 0.01% of ascorbyl palmitate (AP), 0.01% of combined citric acid with ascorbyl palmitate (APCA).
The established systems were (R0.02), (R0.1), (R0.02AP), (R0.1AP), (R0.02CA), (R0.1CA), (R0.02APCA), (R0.1APCA). All these systems contained 1000 ppm lycopene. The control system for the peroxide test and the total lycopene determination was sunflower oil with 1000 lycopene system (STD). Lycopene was dissolved in tetrathydrofuran (THF) at 45°C under running nitrogen and was stirred for 10 minutes using Vortex. After careful mixing, the antioxidant systems were divided to into 100 g in glass tubes covered with aluminium foil. The tubes were transferred to an oven maintained at 80°C for an accelerated storage study for 3 days. Oil stability is usually determined under accelerated oxidation conditions (60 °C or more) because ambient conditions demand an excessively long period [1]. Three replicate samples were stored.

- *Peroxide value:*

Lipids primary oxidation products (hydroperoxides) concentrations were used as an indicator of oxidation. Lipid hydroperoxide concentrations were determined according to peroxide value measurements (POV). 5±0.1 g of oil each sample was weighed and was subjected to iodometric determination of peroxide value according to AOCS official method [5].

- *Evaluation of oil oxidative stability conditions:*

The refined sunflower oil oxidative stability index (OSI) was measured by rancimat apparatus, model 743 (Metrohm Ω, Basel, Switzerland) according to (Farhoosh, 2007) [12]. Stability was expressed as the oxidation induction time (h), using an oil sample of 3.0 g heated up to 120 ˚C under constant air flow of 20 L/h. Official and Recommended Analysis Manual (AOCS Cd 12b-92), [4].

- *HPLC analysis of total lycopene content:*

Lycopene in oil samples was dissolved in ethyl acetate and filtered with a 0.22-µm membrane, prior to analysis with a Waters 2695 HPLC system connected to Waters UV-VIS detector and Cosmosil® Cholester column (Nacalai Tesque Inc., Japan). The mobile phase was a mixture of acetonitrile and tetrahydrofuran (95:5, v/v). And, 0.05% of TEA and 250 ppm of BTH were added to the mobile phase to avoid lycopene modification during the analysis process. The flow rate was 1.0 ml/min and detecting wavelength was 472 nm [27]; [13]. To remove the polar oil constituents which are adsorbed on the column during the analyses, methanol was pumped through the HPLC column for 30 mins at the end of the each analysis.

C. Statistical analysis:

All analyses were carried out in triplicate ($n = 3$) for each replicate which were reported as mean ± SD. The variance analysis was performed using ANOVA test. SPSS 16.0 (statistical software; SPSS Inc., Chicago, IL) was used to compare the mean values of each treatment. Significant differences between the means of the parameters were determined by using the Duncan test ($p < 0.05$).

III. RESULT AND DISCUSSION

A. Oil stability index:

Table1 shows the effect of natural antioxidant systems on the OSI time (h) of sunflower oil compared to the control.

Table 1 Oxidative stability index times (OSI) followed by the same superscript letter a–h, significantly different (P < 0.05). The values reflect the means of two replicates for each treatment ± standard deviation. Triplicates of each treatment represent one replicate, OSI explains the percentage of increasing in OSI compared with the sunflower oil sample without additive.

Antioxidant System	OSI for the systems With added 1000 ppm of Lycopene	OSI %	OSI for the systems Without Lycopene	OSI %
Refined sunflower oil	1.48±0.22[a]	73.27	2.02± 0.11[a]	100
R0.02	2.06±0.03[b]	101.73	2.48± 0.05[b]	122.77
R0.1	2.44±0.01[c]	120.54	3.07±0.0[c]	151.98
R0.02AP	2.7±0.05[d]	133.66	3.07±0.03[c]	151.98
R0.1AP	2.84±0.02[d e]	140.59	3.28±0.09[d]	162.13
R0.02CA	3.00±0.03[e]	148.514	3.13±0.03[c]	154.70
R0.1CA	3.27±0.01[f]	161.63	4.92±0.14[e]	243.32
R0.02APCA	3.70±0.23[g]	183.17	5.12±0.11[f]	253.47
R0.1APCA	4.16±0.18[h]	205.94	5.30±0.05[g]	262.37

The OSI value of refined oil with 1000ppm lycopene with and without various antioxidant systems were measured using 0.02% and 0.1% of rosemary extract in each of the system. On the basis of OSI index obtained, all the antioxidative system was found to have increased oil stability. However, the protection of refined sunflower oil, as defined by (OSI), was decreased on adding 1000 ppm of lycopene to all the antioxidant systems compared with the same systems with no lycopene. As the tests were performed under high temperature of 120°C and in the presence of light, thus, the lycopene antioxidant efficiency was decreased by increasing the reactivity of the carotene resonance-stabilized radical adducts [26]. Offord reported that β-carotene is acting as a very effective chain-breaking antioxidant, but as oxygen pressure increased the effectiveness of β-carotene as an antioxidant is reduced [23], therefore lycopene also can be affected by the oxygen pressure. On the other hand, at 100°C and 150°C lycopene degradation proceeded faster than isomerization [7]. Hence, the exposure to the running oxygen during oil stability testing affected the stability of antioxidant systems containing lycopene. The OSI for systems containing and non-containing lycopene was obtained according to the following order (RAPCA), (RCA), (RAP), (R).

The best synergistic effect was obtained from the ternary blends of citric acid, ascorbyle palmitate with rosemary extract, whereas the combination of this system with lycopene was found to decrease the OSI. Yet still the OSI results were adequate for these systems since it could play a great role as lycopene fortification functional food systems.

The system with added citric acid showed high OSI than the system containing ascorbyl palmitate. Increasing the rosemary extract concentration from (0.02 to 0.1) % in different systems significantly increased the OSI of the antioxidant systems.

3.2. Peroxide value:

The effect of different natural antioxidant alone on edible oil primary and secondary oxidation products formation have been investigated in detail by a large number of researchers [8].

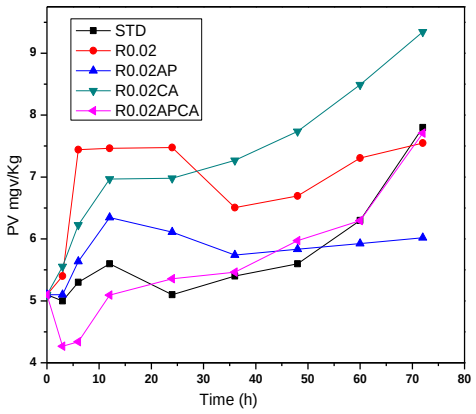

Figure 1 Effect of different antioxidant systems with 0.02 % rosemary extract on the peroxide value of sunflower containing 1000 ppm Lycopene in presence of nitrogen, absence of light at 80 °C.

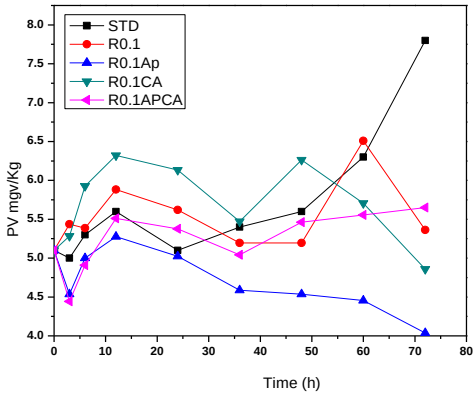

Figure 2 Effect of different antioxidant systems with 0.1 % rosemary extract on the peroxide value of sunflower containing 1000 ppm Lycopene in presence of nitrogen, absence of light at 80 °C

Whereas R.J, reported that ascorbyl palmitate gave only small improvements in overall oxidative stability when used unaided at the 0.1 and 0.5% level [24]. T and Donald, observed that Lycopene showed higher antioxidant effectiveness to inhibit hydroperoxide formation in lipids as compared to α- and β-Carotene consistent with increased reactivity of lycopene towards singlet oxygen quenching and free radical scavenging [26]; [9].

Fig 1, 2 illustrate the changes in peroxide values of refined sunflower oil with added natural antioxidant systems, including lycopene, rosemary extract (50% Carnosic Acid), ascorbyl palmitate and citric acid, determined at 80 C° in the presence of nitrogen and in the absence of the light. The antioxidant systems were (R0.02), (R0.1), (R0.02AP), (R0.1AP), (R0.02CA), (R0.1CA), (R0.02APCA), (R0.1APCA) and sunflower containing 1000 ppm lycopene as standard (STD).

The results demonstrated the rapid increase of the formation of peroxide from hour 0 to hour12 during the heating procedure. This observation may be due to the fact that, the degraded lycopene products did not have enough conjugated double bonds to efficiently absorb energy from singlet oxygen and lower it to its ground-state triplet form. Therefore the singlet oxygen was able to abstract electrons from the unsaturated fatty acyl chains found in oil. The result, was increased peroxy-radical and peroxide formation [9], then the peroxide value dropped over the next hours of heating. It can be seen that during the first hours of heating the systems contained (AP) the peroxide value decreased considerably and then raised continuously at the next hours of heating, because the ascorbyle palmitate reacted with the free radical species [20]. After about 36 hours of heating, the peroxide value demonstrated relatively the lowest value among almost all of the antioxidant systems. This might refer to the lycopene isomers ability to react with hydroperoxides, or the interaction between lycopene radicals, ascorbyle palmitate and rosemary extract. [10].

From **Fig1,** the refined sunflower oil with the addition of antioxidant system (R0.02AP) had the final peroxide value PV that was significantly ($P < 0.05$) lower than those observed from the other antioxidant systems containing (R0.02) including the control. Whereas (R0.02AC) system exhibited the highest (PV under the same condition. **Fig 2** depicts the PV of sunflower oil samples stabilized with higher rosemary extract containing systems (R0.1). A significant difference ($p<0.05$) in PV was observed between the control system and sunflower oil samples contained (R0.1), (R0.1CA), (R0.1AP) and (R0.1APCA), whereas the rate of oxidation was found to be reduced by all of the four tested antioxidative systems. In general, these results demonstrated that the combination of (0.1) % rosemary extract and ascorbyl palmitate in the presence of lycopene had the highest antioxidant activity in reducing formation of peroxide during heating. In agreement with our findings, the positive effects of the mixture of rosemary extract, ascorbyl palmitate and citric acid for the prevention of lipid oxidation are reported by [2].

Judging from the PV test, the oxidative stability was decreased in the order (R0.1AP), (R0.1CA), (R0.1), (R0.1APCA), (R0.02AP), (R0.02), (R0.02APCA) and the control system followed by (R0.02CA) as the lowest oxidative stability compared to the other systems during heating at 80°C in the presence of nitrogen and absence of light.

The stability of carotenoid and phenol radicals formed from the reaction of carotenoid and phenol compounds with free radicals reduced the rate of propagation and further reactions and thus increases the oxidative stability of the systems containing higher rosemary extract [3]. (R0.1AP) remained the most effective and gave the lowest PV compared with the other systems under the same condition.

3.3. Lycopene Degradation:

Most stability studies of lycopene in food systems focused on its degradation [19]. Degradation of lycopene not only affects the attractive colour of final products but also their nutritive value for health benefit [15]. The main cause of lycopene degradation in food systems is isomerization and oxidation [16].

Fig 3 shows the percentage of lycopene remaining over time after heating the antioxidant system containing lycopene at 80°C, in presence of nitrogen for the carotenoids have been shown to exert antioxidant behaviour at low oxygen partial pressure [18]; [10]. After 72 hours (R0.1AP) significantly increased the remaining lycopene content compared to the control samples without the addition of antioxidant systems. All the examined antioxidant systems improved the remained Lycopene content, whereas, (R0.02CA) showed the lower lycopene percentage during the same treatment.

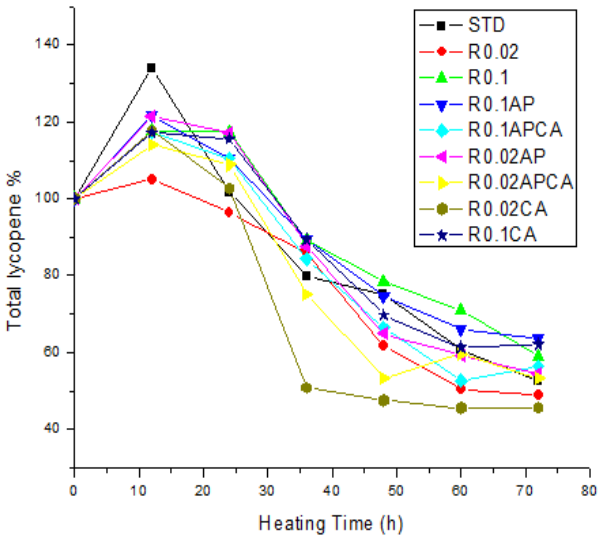

Figure 3 Lycopene degradation during the acceleration of Lycopene containing antioxidant systems in presence of nitrogen, absence of light, at 80 °C under as determined by total Lycopene content.

The highest lycopene remaining percentage obtained with (R0.1AP) was 63.53%, followed by 62.1%, 59.08%, 56.3%, 54.38%, 53.31%, 52.63%, 48.7%, 45.66% for (R0.1CA), (R0.1), (R0.1APCA), (R0.02AP), (R0.02APCA), (STD, (R0.02), (R0.02CA), respectively.

These results concurred the behaviour of hedroperoxide retarding which can be explained by lycopene scavenging radicals in an initial stage of oxidative reaction by transferring electron, hydrogen abstraction, or combined with the free radical to form lycopene radicals [10]; [17].

IV. CONCLUSIONS

The results of the present study demonstrated the positive effects of combined natural antioxidants containing lycopene, rosemary extract, ascorbyl palmitate, citric acid to retard refined sunflower oil oxidation as determined by measuring the peroxide value and the oil oxidative index. The antioxidant systems also played rule on lycopene protection during the heating process under the presence of nitrogen and in the absence of the light, but lycopene showed high sensitivity when exposed to running oxygen and high temperature. Thus, all the systems with lycopene had relatively lower oil stability compared to the same system without lycopene.

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