Food Chemistry 115 (2009) 163–168

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant activity of lettuce extract (*Lactuca sativa*) and synergism with added phenolic antioxidants

Arzu Altunkaya [a], Eleonora Miquel Becker [b], Vural Gökmen [a], Leif H. Skibsted [b,*]

[a] Faculty of Engineering, Food Engineering Department, Hacettepe University, Beytepe Campus TR-06800 Ankara, Turkey
[b] Faculty of Life Sciences, Department of Food Science, Food Chemistry, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 15 October 2008
Accepted 26 November 2008

Keywords:
Liposome oxidation
AAPH
AMVN
Lettuce
Synergy

ABSTRACT

Combined antioxidative effects of lettuce extract and α-tocopherol, quercetin or ascorbic acid (AA) were investigated for peroxidation of L-α–phosphatidylcholine liposomes with oxidation initiated by lipophilic or by hydrophilic azo-initiators. Lettuce extract had a clear antioxidative effect as evidenced by a lag phase for formation of conjugated dienes, and α-tocopherol and especially quercetin acted synergistically in prolongation of the lag phase both following initiation in the lipid phase and in the aqueous phase. Combination of AA with lettuce extract showed in contrast a lag phase that was similar to that observed for AA alone. Storage of lettuce extract for 24 h at refrigerator or room temperatures resulted in a decreasing lag phase with increasing storage time for both storage temperatures, an effect not counteracted by addition of quercetin or α-tocopherol. Heating of lettuce extract for 10 min at 80 °C did not affect the lag phase and heating of lettuce extract resulted in an increasing synergism for added quercetin and α-tocopherol indicating that thermal inhibition of polyphenoloxidase (PPO) increases antioxidant potential and interaction.

1. Introduction

Lettuce is an important leafy vegetable mainly consumed fresh in salads and a good dietary source of natural antioxidants. The antioxidant properties of lettuce and similar plants have been attributed to their polyphenol contents as reported for ten genotypes of lettuce belonging to *Lactuca sativa*, *Cicorium intybus*, *Plantago coronopus*, *Eruca sativa* and *Diplotaxis tenuifolia*. Quercetin, kaempferol, luteolin, apigenin and crysoeriol have been identified in the investigated cultivars (Heimler, Isolani, Vignolini, Tombelli, & Romani, 2007). Another antioxidant compound detected in lettuce is ascorbic acid (AA) which also contributes to the total antioxidant capacity of leafy vegetables (Martín-Diana et al., 2007).

Antioxidants are present in vegetables in various combinations, and antioxidant interaction seems important for their effect. For some combinations of antioxidants, a larger overall effect has been found compared to the effect expected from a simple addition of the effects of the individual antioxidants entailing what has been termed antioxidant synergism (Uri, 1961). Several studies have shown that plant polyphenols have a synergistic effect with other antioxidants present in plant material (Graversen, Becker, Skibsted, & Andersen, 2008; Miller & Rice-Evans, 1996; Roberts & Gordon, 2003). Processing, storage and heating during cooking of vegetables affect the phenolic compounds present in vegetables but differently depending on the type of vegetable. Phenolic compounds in yacon leaf and tuberous root were thus completely lost following heating at 80 °C, while antioxidant activity of tomatoes increased with thermal processing despite a non-significant change in total phenolic and flavonoid content (Dewanto, Wu, Kafui, & Liu, 2002; Takenaka, Nanayama, Isobe, & Murata, 2006). These different pattern observed for various vegetables reflects a balance between post harvest oxidation of plant phenols and thermal inactivation of polyphenol oxidases.

The purpose of the present study was to determine whether the antioxidants present in fresh lettuce interact with added quercetin, α-tocopherol or AA following storage at different temperatures and heating leading to thermal inactivation of enzymes. Lipid oxidation was followed by the formation of conjugated dienes in a liposome system using two types of azo-initiators: the water-soluble AAPH and the lipid soluble AMVN. The liposome system was chosen because it allows the investigation of water and lipid soluble antioxidants in the same system and is an appropriate model for biological membranes.

2. Materials and method

2.1. Chemicals

L-α–phosphatidyl choline (PC) from soybean, quercetin dihydrate (QC) and gallic acid were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All-rac-α-tocopherol (TOH;

* Corresponding author. Tel.: +45 35333221; fax: +45 35333344.
E-mail address: ls@life.ku.dk (L.H. Skibsted).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.11.082

synthetic mixture of optical isomers) and AA were from Fluka (Zurich, Switzerland) and Fluka Chemie GmbH (Steinheim, Germany), respectively. 2,2'-Azobis(2-amidiopropane) dihydrochloride (AAPH) and 2-2'azodi(2, 4-dimethylvaleronitrile) (AMVN) were from Wako Chemicals Inc. (Richmond, VA, USA). Other chemicals were of analytical grade. Water was purified through a millipore Q-plus purification train (Millipore Corp., Bedford, MA, USA). Iceberg lettuce (*L. sativa*) grown in Denmark was purchased from a local market in Copenhagen.

2.2. Preparation of lettuce extracts (LE)

Three grams of lettuce were homogenised in 6 mL of water. Aliquots were taken from the slurry at 0 and 24 h. In the heating procedure, the homogenised lettuce extract was heated with stirring for 10 min at 80 °C in a water bath. The slurry was centrifuged at 15,000*g* for 30 min, and the supernatant used for the antioxidant studies. Fresh, heated or stored LE was combined with TOH, QC or AA in the liposome system.

2.3. Total phenol concentration

The total amount of phenols was determined spectrophotometrically using Folin–Ciocalteu's phenol reagent (Amerina & Ough, 1980). The lettuce extract was diluted ten times with water. One hundred microlitre of the diluted extract was mixed with 30 mL of water, and 2.5 mL Folin–Ciocalteu's phenol reagent was subsequently added followed by 7.5 mL of a 20% aqueous sodium carbonate solution, and the total volume was adjusted to 50 mL with water. The reaction mixture was stored at room temperature for 2 h before measuring the absorbance at 765 nm on a Cintra 40, UV–Visible spectrophotometer (GBC scientific equipment, Arlington Heigts, IL, USA). A standard curve based on gallic acid was used for conversion of the absorbance to phenol concentration in gallic acid equivalent (GAE).

2.4. Solutions of antioxidants

The antioxidants were dissolved in different solvents, depending on their solubility. TOH was dissolved in hexane and QC was dissolved in methanol, while AA was dissolved in 0.010 M phosphate buffer (pH 7.4). The concentration of TOH, QC and AA was calculated as mol% of the lipid fraction using a molecular mass of soybean PC equal to 900 g/mol.

2.5. Preparation of liposomes

Liposomes were prepared according to the method described by Roberts and Gordon (2003) with minor modifications. A solution (2 ml) containing soybean PC (1.5 μmoL) dissolved in chloroform was mixed with hexane (1.0 mL), TOH solution (1.0 mL) or QC solution (1.0 mL). About 43 mM AMVN (86 μL) in absolute ethanol was added to the samples for initiation into the lipid phase. Unilamellar liposomes were prepared by passing the suspension 21 times through a double layer of polycarbonate membranes with a pore size of 200 nm. AA and LE with a phenol content of 1.84×10^{-5} g GAE/L was added to liposomes dissolved in a 0.040 M phosphate buffer with pH 7.4. For the experiments where oxidation was initiated in the aqueous phase, 25 μL of 75 mM AAPH was added to the liposomes.

2.6. Determination of lag phase

Peroxidation of liposomes was followed spectrophotometrically. The lag phase before onset of oxidation was defined as the time corresponding to the intercept between the tangent of absorption at 234 nm versus time for the propagation phase and the tangent of the absorption at 234 nm versus time for the lag phase as shown in Fig. 1.

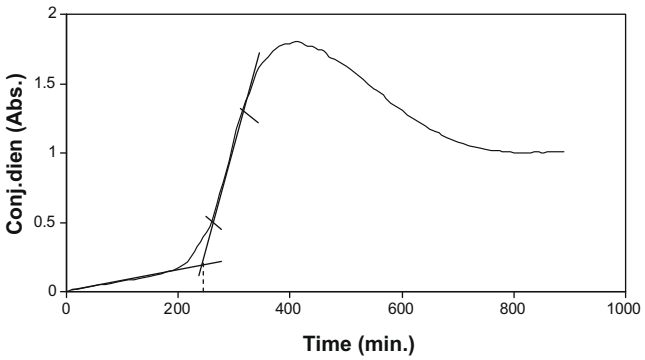

Fig. 1. Calculation of length of lag phase from the spectrophotometric determination of conjugated dienes in phosphatidyl choline liposomes for a combination of QC (1 mol% relative to phosphatidyl choline) and LE (with 1.84×10^{-5} g GAE/L) following initiation of 75 mM AAPH at 37 °C.

3. Result and discussion

Lipid oxidation in heterogeneous systems has been found to occur mainly at the interface between the lipid and the aqueous phase, and the composition and structure of the interfacial area is therefore highly important for their oxidative stability (Schwarz et al., 2000). Water-soluble azo-initiators such as AAPH added to these types of systems generate peroxyl radicals outside the membranes in the aqueous phase, whereas lipid soluble azo-initiators such as AMVN generate radicals in the lipid phase and accordingly induce oxidative stress within the membrane structure. While radicals formed by lipid soluble initiators have easier access to lipids, radicals formed by the water-soluble initiators are expected to initiate oxidation at the lipid–water interface which may propagate into the lipid substrate (Tsuchiya et al., 2001).

Interactions of the water-soluble lettuce extract with antioxidants of different polarity (QC, TOH and AA) were investigated by studying the ability of the antioxidant to inhibit lipid peroxidation of liposomes made of soybean PC initiated by azo-initiators (AAPH and AMVN). The extent of oxidation was monitored by following the formation of conjugated dienes absorbing at 234 nm by UV-spectroscopy. The formation of conjugated dienes began upon addition of AAPH and AMVN for liposomes without antioxidants or lettuce extract added (Table 1).

3.1. Effect of lettuce extract storage and concentration

LE with different phenol content was added to the liposome system and oxidation was initiated by either AAPH or AMVN (Fig. 2). The total amount of radicals formed in liposomes within 30 min was 1.8×10^{-6} mol/L liposome suspension for AAPH and 2.6×10^{-6} mol/L liposome suspension for AMVN using the equations: R_i (mol/L/s) = 1.36×10^{-6} [AAPH] (Niki, 1990) and R_i (mol/L/s) = 3.88×10^{-6} [AMVN] (Shi, Noguchi, & Niki, 1999) valid for conditions of 37 °C and neutral water (AAPH) or 37 °C and acetonitrile (AMVN), respectively. Thus, the concentration of radicals formed by AMVN is approximately 50% higher than the concentration of radicals formed by AAPH. This is in agreement with the observation that the lag phases were shorter when the lipid soluble azo-initiator AMVN was used compared to the water-soluble AAPH. The better antioxidative effect on lipid oxidation observed for lettuce extract when oxidation was initiated by AAPH can be explained by the localisation of the polyphenols in the homoge-

Table 1
Lag phase found by spectrophotometric measurement of formation of conjugated dienes in soybean phosphatidyl choline liposomes in aqueous solution with pH 7.4 at 37 °C with free radical initiation of oxidation in the aqueous phase using 75 mM AAPH or in the lipid phase using 43 mM AMVN with pH 7.4 at 37 °C (α-tocopherol: 1 mol% TOH relative to phosphatidyl choline, lettuce extract: LE corresponding to a concentration of 1.84×10^{-5} g GAE/L).

Sample	Storage conditions		Lag phase time (min)			
	Time	Temperature	AAPH		AMVN	
			Experimental	*Calculated*	*Experimental*	*Calculated*
Control	0	–	3 ± 1	–	4 ± 1	–
LE	0	–	32 ± 1	–	16 ± 2	–
LE	24 h	4 °C	14 ± 1	–	9 ± 2	–
LE	24 h	25 °C	8 ± 2	–	5 ± 1	–
LE	10 min	80 °C	31 ± 1	–	15 ± 1	–
TOH	0	–	90 ± 1	–	42 ± 1	–
TOH + LE	0	–	132 ± 1	122 ± 1	68 ± 2	58 ± 2
TOH + LE	24 h	4 °C	108 ± 2	104 ± 1	58 ± 1	51 ± 2
TOH + LE	24 h	25 °C	96 ± 1	98 ± 2	45 ± 2	47 ± 1
TOH + LE	10 min	80 °C	141 ± 1	121 ± 1	64 ± 2	57 ± 1

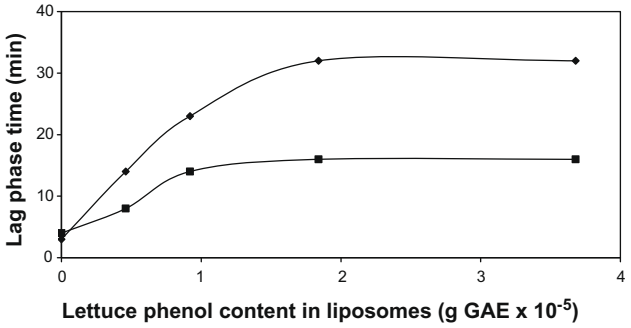

Fig. 2. Lag phase found by spectrophotometric measurement of conjugated dienes in soybean phosphatidyl choline liposomes in aqueous solution with pH 7.4 at 37 °C with free radical initiation of oxidation in aqueous phase with 75 mM AAPH (♦) or in lipid phase with 43 mM AMVN (■) for different lettuce phenol content.

neous water phase or at the interface protecting the lipids against radicals generated in the water phase. The lag phase increased with lettuce phenol content and reached saturation at a concentration of 1.84×10^{-5} g GAE/L (Fig. 2).

The effect of lettuce extract was investigated immediately after preparation (time = 0), after storage for 24 h at refrigeration temperature, or at room temperature. Additionally, the effect of heating at 80 °C for 10 min was investigated. LE showed an antioxidative effect which decreased after storage and decreased with increasing storage temperature (Table 1). The decrease in antioxidative effect may be explained by antioxidant degradation, observed as a decrease in phenol content during storage (results not shown). One reason for the loss of antioxidant activity during storage could be the activity of polyphenoloxidase (PPO). In order to investigate whether enzymes are involved in the loss of polyphenols and antioxdative capacity, LE was heated for 10 min at 80 °C. The heated LE showed the same lag phase as fresh prepared LE, indicating that high temperature had no effect on lag phase. The lack of temperature effect could be explained by the compensation of two opposite effects on phenol content: the thermal degradation of the phenols, and the inactivation of PPO, which otherwise oxidises the phenols. It has also been observed that during thermal processing of vegetables, some phenols are degraded, while at the same time bound phenols are released from cellular structures (Dewanto et al., 2002). Moreover, it has been suggested that oxidation of phenols could decrease due to removal of oxygen from tissue as a result of heating (Kaufman, 1955). Yamaguchi et al. (2003) studied the changes in total phenol content and radical scavenging activity of heated and unheated lettuce samples. While the content remained constant in heated lettuce, a drastic decrease was observed in unheated samples during storage. This finding supports our interpretation of the results obtained in the present study, where no decrease was observed in the lag phase for lipid oxidation in the liposomes added LE or in the phenolic content of heated LE compared to non-heated. The heated LE stored for 24 h at room temperature showed no significant change in lag phase compared to the non-heated, indicating that high temperature retained antioxidant activity (results not shown). Inactivation of the enzymes was also observed due to the lack of browning of the heated LE during the 24 h of storage.

3.2. Interaction of lettuce extract with α-tocopherol

In the presence of AAPH and AMVN, the observed lag phase for 1 mol% TOH was 90 ± 1 and 42 ± 1 min, respectively, for the actual conditions, while LE showed a lag phase of 32 min and 16 min, respectively. Combinations of LE with TOH had lag phases of 132 ± 1 and 68 ± 2 min, respectively, and clearly showed a lag phase that was longer than the sum of lag phases of each component (calculated lag phase, Table 1). Thus, a small synergistic effect between LE and TOH was demonstrated. This finding is in agreement with other studies where synergistic effects were observed for α-tocopherol and polyphenols in heterogeneous systems (Graversen et al., 2008; Zhu, Huang, Tsang, & Chen, 1999). These synergistic effects may be understood by different solubilities and localisations of the antioxidants in different phases in the heterogeneous systems.

When TOH was combined with LE following different storage conditions for LE, the observed lag phases decreased with increasing storage temperature. A synergistic effect between TOH and LE was not observed after 24 h of storage (Table 1). Heating of LE (80 °C for 10 min) increased the lag phase for liposomes with added TOH compared to a fresh sample indicating that enzyme inactivation and/or phenol release from the lettuce matrix increased the antioxidant capacity. The extract heated to 80 °C and combined with TOH showed the highest synergistic effect, which can be explained by a better interaction between released phenols and TOH or between antioxidants which otherwise are affected by the enzymes (Dewanto et al., 2002; Yamaguchi et al., 2003).

3.3. Interaction of lettuce extract with quercetin

Incorporation of 1 mol% QC in the liposomes after initiation of oxidation with AAPH or AMVN showed, for the actual conditions, a lag phase of 171 ± 1 and 117 ± 1 min, respectively, whereas combination of LE with QC was found to have a lag phase of 212 ± 1 and 143 ± 1 min, respectively (Table 2). The lag phase of the combina-

Table 2
Lag phase found by spectrophotometric measurement of conjugated dienes in soybean phosphatidyl choline liposomes in aqueous solution with pH 7.4 at 37 °C with free radical initiation of oxidation in the aqueous phase using 75 mM AAPH or in lipid phase using 43 mM AMVN with pH 7.4 at 37 °C (quercetin: 1 mol% QC relative to phosphatidyl choline, lettuce extract: LE corresponding to a concentration of 1.84×10^{-5} g GAE/L).

Sample	Storage conditions		Lag phase time (min.)			
	Time	Temperature	AAPH		AMVN	
			Experimental	*Calculated*	*Experimental*	*Calculated*
QC	0	–	171 ± 1	–	117 ± 1	–
QC + LE	0	–	212 ± 1	203 ± 1	143 ± 1	133 ± 1
QC + LE	24 h	4 °C	198 ± 1	185 ± 1	134 ± 2	126 ± 2
QC + LE	24 h	25 °C	184 ± 1	178 ± 2	121 ± 3	123 ± 1.4
QC + LE	10 min	80 °C	224 ± 2	202 ± 1	147 ± 3	132 ± 1

tion of QC and LE was accordingly found longer than the sum of lag phases of each component (Table 2), showing a small synergistic effect. Combination of QC with LE after storage of LE for 24 h at room or refrigerator temperature or after heating of LE for 10 min at 80 °C was investigated as well. A synergistic effect was observed for all samples containing LE and QC except for LE stored at room temperature for 24 h. The highest synergistic effect was again observed for the heated LE. The synergy between QC and LE has been suggested to be due to an optimal localisation of QC at the interface (Becker, Ntouma, & Skibsted, 2007; Beer, Joubert, Gelderblom, & Manley, 2005).

3.4. Interaction of lettuce extract with ascorbic acid

A combination of AA and LE exhibited a lag phase when oxidation was initiated with AAPH that was approximately the same as AA when present alone, while the use of AMVN showed a small increase in the antioxidant effect when LE was present together with AA (Table 3). Synergistic effects could not be demonstrated for LE combined with AA at any of the experimental conditions for which the phenolic antioxidants showed such synergism. In contrast, clear antagonistic effects were observed. Besides being a good radical scavenger, AA is also an effective reducing agent and may reduce PPO enzymes products which are present in lettuce and other leafy vegetables (Dincer, Çolak, Aydin, Kadioglu, & Güner, 2002). In the liposome system, AA can react either with the radicals generated in the water phase by AAPH, or it can regenerate polyphenols from their oxidised forms due to the low oxidation potential of AA. In order to obtain synergy *via* regeneration, the regeneration reaction between AA and the polyphenols must compete efficiently with the other reactions in which AA is oxidised (Beer et al., 2005). Kinetic factors may be responsible for the observed antagonistic effects, as the reaction rates of phenols and AA with lipid radicals may be too different due to a too small effective concentration of AA at the site of oxidation (Peytar-Maillard, Cuvelier, & Berset, 2003). Notably, AA is known to be prooxidative at low concentrations, while being an important oxygen scavenger at higher concentrations (Andersen, Lygren, Maage, & Waagbø, 1998).

Storage conditions of LE had no effect on the length of the lag phase seen for the combination of LE with AA probably due to the reducing ability of AA on the oxidation products of PPO. A combination of AA with heated LE showed an increase in the lag phase compared to fresh LE. The increase in lag phase may partially be explained by the degradation of the endogeneous AA, thus inhibiting its prooxidative effect in combination with the phenols. Additionally, release of new phenols may increase the antioxidative effect as suggested for combinations of TOH or QC with LE. Antioxidant activity may also be enhanced because the reaction of AA with radicals does not have to compete with regeneration of PPO products.

3.5. Comparison of combinations of antioxidants with lettuce extract

The interaction between LE with TOH, QC or AA in the liposome system has been investigated. When the initiation occurred in the water phase by AAPH, all combinations of LE showed a better antioxidative effect against lipid oxidation than when initiation occurred in lipid phase with AMVN. The better antioxidative effect on lipid oxidation of LE when oxidation was initiated by AAPH can mainly be explained by the localisation of phenolic compounds at the lipid–water interface of the liposomes where oxidation occurs. TOH is more lipid soluble than QC with an *n*-octanol/water partition coefficient of 549 (Liao & Yin, 2000). TOH will accordingly locate in the lipid phase near the interface and may scavenge both AMVN and AAPH-derived peroxyl radicals and lipid radicals. It was observed that the antioxidative effect of TOH was highest when AAPH-derived radicals initiated oxidation suggesting that the phenolic group of TOH is located at the interface to the aqueous phase. QC is one of the most abundant flavonoids (Sarkar & Das, 2006). QC and QC glycosides contain a number of phenolic groups and QC is located essentially in the water phase with a partition coefficient of 3.84 (Liao & Yin, 2000) and may scavenge AAPH-derived radicals as well as lipid peroxyl radicals at the lipid–water interface, as evi-

Table 3
Lag phase found by spectrophotometric measurement of conjugated dienes in soybean phosphatidyl choline liposomes with free radical initiation of oxidation in the aqueous phase using 75 mM AAPH or in lipid phase using 43 mM AMVN with pH 7.4 at 37 °C (ascorbic acid: 1 mol% AA relative to phosphatidyl choline, lettuce extract: LE corresponding to a concentration of 1.84×10^{-5} g GAE/L).

Sample	Storage conditions		Lag phase time (min.)			
	Time	Temperature	AAPH		AMVN	
			Experimental	*Calculated*	*Experimental*	*Calculated*
AA	0	–	72 ± 1	–	34 ± 1	–
AA + LE	0	–	74 ± 1	104 ± 1	45 ± 1	50 ± 2
AA + LE	24 h	4 °C	71 ± 1	86 ± 1	38 ± 2	43 ± 2
AA + LE	24 h	25 °C	72 ± 1	80 ± 2	37 ± 1	39 ± 1
AA + LE	10 min	80 °C	82 ± 3	103 ± 1	41 ± 2	49 ± 1

denced by the better antioxidative effect seen for AAPH-initiated oxidation. AA is water-soluble with an *n*-octanol/water partition coefficient of 0.029 (Nenadis, Lazaridou, & Tsimidou, 2007) and is located in the homogeneous water phase away from the interface with the lipid phase. Its effect was highest against AAPH-derived radicals; however, it also showed some antioxidative effects against AMVN-derived radicals. The best antioxidant was QC, an observation which may be understood by its location at the interface where it is able to scavenge radicals formed in both phases.

Synergism between antioxidants may arise *via* different mechanisms: (i) *via* regeneration reactions, (ii) *via* sacrificial oxidation (where one antioxidant, by radical scavenging, protects the other one) or (iii) by exertion of different mechanisms of action, thus prolonging the antioxidative effect of each other (Becker, Nissen, & Skibsted, 2004). The interaction of TOH with polyphenols depends on subtle differences in thermodynamic properties sensitive to solvent and temperature. TOHs have both been found to be regenerated from their oxidised forms by polyphenols (Graversen et al., 2008; Zhu et al., 1999) and to regenerate polyphenols (Joergensen, Madsen, Thomsen, Dragsted, & Skibsted, 1999). Also synergism *via* sacrificial oxidation of the polyphenols has been suggested, thus protecting the TOHs against radicals generated in the water phase (Becker et al., 2007). Combinations of different mechanisms is also a possible mechanism of synergy between TOH and polyphenols where polyphenols act as chelators of catalytic metal ions and TOHs as radical scavengers, thus protecting each other against reactive oxygen species (Hudson & Lewis, 1983). A synergistic effect was also observed in the liposome systems with AAPH-initiated oxidation for combinations of QC and rutin (Becker et al., 2007). Based on standard reduction potentials, it has been predicted and confirmed by kinetic studies that ascorbate regenerates various plant polyphenols in homogeneous solutions from their oxidised form (Jovanovic, Steenken, Tosic, Marjanovic, & Simic, 1994). Also, in membranal systems, such regeneration has been confirmed (Jan, Takahama, & Kimura, 1991; Negresalvayre, Affany, Hariton, & Salvayre, 1991). However, synergy is often dose dependent as shown for combinations of almond skin polyphenols and AA (Chen, Milbury, Chung, & Blumberg, 2007). In the present study, AA did not show synergistic effects in combination with LE in liposomes but rather antagonistic effects. An antagonistic effect was also shown between malt rootless extract and AA in corn oil, which was explained by the presence of iron in rootless extracts (Peytar-Maillard, Bonnely, Rondini, & Berset, 2001).

4. Conclusion

Lettuce was found to be a rich source of antioxidants as was shown for lipid oxidation in a liposome system. The degree of lipid oxidation in the liposomes in the presence of α-tocopherol, quercetin and AA with lettuce extract was significantly lower when oxidation was initiated by free radicals formed in the water phase compared to initiation in the lipid phase. Antioxidants localised at or near the interface of the liposomes such as quercetin and α-tocopherol acted synergistically with lettuce extract as an antioxidant, while the hydrophilic antioxidant AA showed no synergism. Increasing concentration of phenols from lettuce extracts led to a limiting protection against oxidation, and heating of lettuce extract protected the active phenols against enzymatic oxidation, in effect improving their efficiency.

References

Amerina, M. A., & Ough, C. S. (1980). *Methods for analysis of musts and wines*. New York: Wiley.

Andersen, F., Lygren, B., Maage, A., & Waagbø, R. (1998). Interaction between two dietary levels of iron and two forms of ascorbic acid and the effect on growth, antioxidant status and some non-specific immune parameters in Atlantic salmon (*Salmo salar*) smolts. *Aquacultura, 161*(1–4), 437–451.

Becker, E. M., Ntouma, G., & Skibsted, L. H. (2007). Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organization. *Food Chemistry, 103*, 1288–1296.

Becker, E. M., Nissen, N. L., & Skibsted, L. H. (2004). Antioxidant evaluation protocols: Food quality and health effects. *European Food Research and Technology, 219*(6), 561–571.

Beer, D., Joubert, E., Gelderblom, W. C. A., & Manley, M. (2005). Antioxidant activity of South African red and white cultivar wines and selected phenolic compounds: In vitro inhibition of microsomal lipid peroxidation. *Food Chemistry, 90*, 569–577.

Chen, C. Y., Milbury, P. E., Chung, S. K., & Blumberg, J. (2007). Effect of almond skin polyphenolics and quercetin on human LDL and apolipoprotein B-100 and conformation. *Journal of Nutritional Biochemistry, 18*(12), 785–794.

Dewanto, V., Wu, X., Kafui, K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*(10), 3010–3014.

Dincer, B., Çolak, A., Aydin, N., Kadioglu, A., & Güner, S. (2002). Characterization of polyphenoloxidase from medlar fruits (*Mespilus germanica* L., Rosaceae). *Food Chemistry, 77*(1), 1–7.

Graversen, H. B., Becker, E. M., Skibsted, L. H., & Andersen, M. L. (2008). Antioxidant synergism between fruit juice and alpha-tocopherol. A comparison between high phenolic black chokeberry (*Aronia melanocarpa*) and high ascorbic blackcurrant (*Ribes nigrum*). *European Food Research and Technology, 226*(4), 737–743.

Heimler, D., Isolani, L., Vignolini, P., Tombelli, S., & Romani, A. (2007). Polyphenol content and antioxidant activity some species of freshly consumed salads. *Journal of Agricultural and Food Chemistry, 55*(5), 1724–1729.

Hudson, B. J. F., & Lewis, J. I. (1983). Polyhydroxy flavonoid antioxidant for edible oils structural criteria for activity. *Food Chemistry, 10*, 47.

Liao, K. L., & Yin, M. C. (2000). Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. *Journal of Agricultural and Food Chemistry, 48*(6), 2266–2270.

Jan, C. Y., Takahama, U., & Kimura, M. (1991). Inhibition of photooxidation of alpha-tocopherol by quercetin in human blood cell membranes in the presence of hematoporphyrin as a photosentisizer. *Biochimica et Biophsica Acta, 1086*, 7.

Joergensen, L. V., Madsen, H. L., Thomsen, M. K., Dragsted, L. O., & Skibsted, L. H. (1999). Regeneration of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemistry study of antioxidant hierarchy. *Free Radical Research, 30*(3), 207–220.

Jovanovic, S. V., Steenken, S., Tosic, M., Marjanovic, B., & Simic, M. G. (1994). Flavanoids as antioxidants. *Journal of American Chemical Society, 116*, 4846.

Kaufman, V.F. (1955). Food processing. Process for food preservation. United States of America as represented by secretary of agricultura (p 1–3).

Martín-Diana Ana, B., Rico, D., Barry-Ryan, C., Frías, J., Henehan, Gary, T. M., et al. (2007). Efficiency of steamer jet-injection as alternative to chlorine in fresh-cut lettuce. *Postharvest Biology and Technology, 45*(1), 97–107.

Miller, N. J., & Rice-Evans, C. (1996). Antioxidant activities of flavonoids as bioactive components of food. *Biochemical Society Transactions, 24*, 790–794.

Nenadis, N., Lazaridou, O., & Tsimidou, M. Z. (2007). Use of reference compounds in antioxidant activity assessment. *Journal of Agricultural and Food Chemistry, 55*, 5542–5560.

Negresalvayre, A., Affany, A., Hariton, C., & Salvayre, R. (1991). Additional antilipoperoxidant activities of alpha-tocopherol and ascorbic acid on membrane like systems are potentiated by rutin. *Pharmacology, 42*, 262.

Niki, E. (1990). Free-radical initiators as source of water-soluble or lipid-soluble peroxyl radicals. *Methods in Enzymology, 186*, 100–108.

Peytar-Maillard, M. N., Bonnely, S., Rondini, L., & Berset, C. (2001). Effect of vitamin E and vitamin C on the antioxidative activity of malt rootlets extracts. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 34*(3), 176–182.

Peytar-Maillard, M. N., Cuvelier, M. E., & Berset, C. (2003). Antioxidant activity of phenolic componds in 2, 2- azobis(2-amidopropane) dihydrochloride (AAPH)-induced oxidation: synergistic and antagonistic effects. *Journal of American Chemical Society, 80*(10), 1007–1012.

Roberts, W. G., & Gordon, M. H. (2003). Determination of the total antioxidant activity of fruits and vegetables by a liposome assay. *Journal of Agricultural of Food Chemistry, 51*, 1486–1493.

Sarkar, S., & Das, N. (2006). Mannosylated liposomal flavonoid in combating age-related ischemia–reperfusion induced oxidative damage in rat brain. *Mechanisms of Ageing and Development, 127*(4), 391–397.

Schwarz, K., Huang, S. W., German, J. B., Tiersch, B., Hartmann, J., & Frankel, E. N. (2000). Activities of antioxidants are affected by colloidal properties of oil-in-water and water-in-oil emulsions and bulk oils. *Journal of Agricultural and Food Chemistry, 48*(10), 4874–4882.

Shi, H. L., Noguchi, N., & Niki, E. (1999). Comparative study on dynamics of antioxidative action of alpha-tocopheryl hydroquinone, ubiquinol, and alpha-tocopherol, against lipid peroxidation. *Free Radical Biology and Medicine, 27*(3–4), 334–346.

Takenaka, M., Nanayama, K., Isobe, S., & Murata, M. (2006). Changes in concentrations of phenolic compounds during growing, storing and

processing of yacon. *Journal of the Japanese Society for Food Science and Technology, 70*(1), 172–174.

Tsuchiya, M., Asada, A., Maeda, K., Ueda, Y., Sato, E. F., Shindo, M., et al. (2001). Propofol versus midazolam regarding their antioxidant activities. *American Journal of Respiratory and Critical Care Medicine, 163*(1), 26–31.

Uri, N. (1961). Mechanisms of autoxidation. In W. O. Lundberg (Ed.), *Autoxidation and antioxidants*. New York: John Wiley & Sons.

Yamaguchi, T., Katsuda, M., Oda, Y., Terao, J., Kanazawa, K., Oshima, S., et al. (2003). Influence of polyphenoloxidase and ascorbate oxidase during cooking process on the radical scavenging activity of vegetables. *Food Science and Technology Research, 9*(1), 79–83.

Zhu, Q. Y., Huang, Y., Tsang, D., & Chen, Z. Y. (1999). Regeneration of alpha-tocopherol in human low density lipoprotein by green tea catechins. *Journal of Agricultural and Food Chemistry, 47*, 2020–2027.