Anti- and pro-oxidative effect of fresh and freeze-dried vegetables during storage of mayonnaise

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Abstract Mayonnaise was supplemented with vegetables (5 % w/w) and the effect of storage time at 4 °C on the oxidative stability of the dispersed phase was investigated. Results indicated that mayonnaise is prone to lipid oxidation during storage under refrigerator conditions. The type of vegetable used for mayonnaise reformulation was critical in inhibiting oxidation and followed the order beetroot > carrot ≈ onion with respect to antioxidant capacity. Broccoli induced a pro-oxidant effect and the rate of oxidation by the end of the storage period was 42 times higher compared with the control. The addition of beetroot, either fresh or freeze-dried, improved the oxidative stability of mayonnaise significantly. The process of freeze-drying affected adversely the ability of vegetables to decrease oil oxidation of the emulsions. This may reflect loss of important natural antioxidants during the drying procedure.

Keywords Mayonnaise · Vegetable · Freeze-drying · Storage · Antioxidant · Carotenoids

Introduction

Mayonnaise is a popular food product across the world. It is an oil-in-water emulsion which according to most traditional recipes contains 70–80 % fat (Dupree and Savage 2001). Vegetable oils are frequently used for emulsion formulation because of their contribution to the sensory perception of the

final food product. Vegetable oils contain a considerable proportion of unsaturated fatty acids and as a result are particularly prone to lipid oxidation (Mosca et al. 2013). Lipid oxidation in foods is associated with the generation of lipid free radicals which can interact with various macromolecules present and results in the formation of off-flavour and toxic components (Aruoma 1998; Kiokias and Gordon 2004; Kiokias et al. 2010). This cascade of chemical reactions affects adversely the organoleptic and nutritional properties of the product and eventually leads to food deterioration.

A common strategy to retard or decrease the extent of oxidative damage employed by the food industry is the inclusion of a certain class of compounds known as antioxidants in the recipe. Antioxidants used for food manufacturing purposes should be cost-effective, safe for human consumption, efficient and should not have an impact on the organoleptic properties (Schuler 1990). Antioxidants are able to interact either directly with free radicals or with compounds involved in the process of radical generation (Choe and Min 2009). Choosing the appropriate antioxidant(s) to control lipid oxidation in a complex food system such as mayonnaise is a challenging task. Oil-in-water emulsions can be structurally divided in three phases; the aqueous phase, the oil phase and the interface between oil and water. Antioxidants depending on their polarity may partition and fulfil their functional role in any of the three phases (Schwarz et al. 1996). For instance, surface-active lipophilic compounds are considered effective antioxidants for emulsion systems because of their ability to adsorb at the oil–water interface and confer protection against oxidation (Kiokias et al. 2008). However, apart from polarity other factors may determine the ability of an antioxidant to control and reduce oxidative damage. These include antioxidant concentration, interactions with other food components as well as the properties of the microenvironment of the reaction media (Liu and Yang 2008; Mosca et al. 2008). Synthetic antioxidants

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able to act as metal chelators such as ethylene diamine tetraacetic acid (EDTA) are commonly used to control lipid oxidation in mayonnaise. These types of antioxidants are preferred because studies indicate that transition metal-based catalysis is the dominant mechanism of lipid oxidations in food emulsions (McClements and Decker 2000).

Consumers' propensity toward a healthy lifestyle is the main driving force behind food industry's intention to replace synthetic additives in foods with natural products having similar or even better functionality. Herbs and vegetables are desired ingredients for food formulations because they are good sources of antioxidants such as carotenoids, tocopherols and polyphenols (Brewer 2011). Vegetables rich in phytochemicals (broccoli, beetroot, onion, carrot) were selected as potential sources of antioxidants and were used to reformulate the traditional recipe of mayonnaise. The effectiveness of each vegetable was monitored by determining the extent of oxidation with storage time. Furthermore, freeze-drying is a common processing method used to prolong the shelf-life of vegetables. The effect of freeze-drying on the ability of vegetables to retard lipid oxidation in mayonnaise was also evaluated. Results of this study may provide alternative strategies to control lipid oxidation in food emulsions in order to respond to consumer needs and preferences.

Materials and methods

Materials

Pasteurised spray dried whole egg powder was purchased from International Egg Products (Essex, UK). Rapeseed organic oil, vinegar, table salt and fresh vegetables were obtained from the local supermarket (Asda, UK). 2-Thiobarbituric acid and 1,1,3,3-Tetramethoxypropane (TEP) were supplied by Sigma-Aldrich. L-ascorbic acid was purchased from BDH. All reagents used were of analytical grade.

Methods

Preparation and storage of o/w emulsions

A mayonnaise recipe was developed that contained the following ingredients in weight ratio (w/w): rapeseed oil (600 g/kg), egg powder (60 g/kg), water (300 g/kg), vinegar (30 g/kg) and salt (10 g/kg). The recipe containing fresh vegetables was adjusted as follows: rapeseed oil (600 g/kg), egg powder (60 g/kg), water (250 g/kg), vegetable (50 g/kg), vinegar (30 g/kg) and salt (10 g/kg). For the recipe containing freeze-dried vegetables the moisture loss was calculated for each vegetable and the corresponding amounts of powder and water were added to equal the amount of the fresh sample. Vegetables were chopped and freeze-dried for 72 h using an

HS⁻¹ freeze drier (Frozen in Time Ltd., UK) and were ground into fine powder with a Wahl James Martin spice grinder (Argos, UK). A coarse emulsion was initially formed by adding the oil at a steady rate and mixing the ingredients using a Morphy Richards hand blender at speed 3 (Argos, UK). Samples were then processed in a single stage valve homogenizer (APV-1000, Denmark) working at an operating pressure of 200 MPa. Each sample was passed twice (recycling) through the homogenizer to ensure complete emulsification. After preparation the mayonnaise was stored at 4 °C for 4 weeks and sampling was performed at timed intervals.

Lipid extraction from mayonnaise

The oil phase was extracted using the method of Lagunes-Galvez et al. (2002) with modifications. Mayonnaise was gently mixed and approximately 50 g were poured into polypropylene centrifuge tubes. Samples were frozen at 70 °C for 24 h and thawed at room temperature to break the emulsion. The oil phase was collected, centrifuged at 10,000 rpm for 5 min and stored at 70 °C for further analysis.

Emulsion microstructure

Light micrographs of the emulsion samples were taken using a Leica DM IL LED inverted laboratory microscope equipped with a Leica DFC295 digital colour camera (Leica microsystems Ltd., UK). Samples (about 1–2 drops) were diluted and mixed with an equal volume of distilled water. A droplet was placed on a microscope slide (76 × 26 mm) and were left uncovered (without cover slip) to avoid destructing the native fat globules structure. The samples were observed with a 40 × dry objective lens. Pictures were taken using the in-built 3 MP digital camera and picture analysis was performed by Leica application suite software (V.3.6.0).

Rancimat analysis

The oxidative stability of the emulsion samples was determined according to Läubli and Bruttel (1986) by using a 743 Rancimat device equipped with the 743 Rancimat control and evaluation program (Metrohm Ltd., Herisau, Switzerland). Three grams of samples (extracted oil) were added to the reaction tubes. Oxidative stability was determined by calculating the induction time of the samples exposed to an air flow of 20 L/h at constant temperature (100 °C). The default value 1 µS/cm was used for evaluation sensitivity of the induction time.

TBARS

Thiobarbituric acid reactive substances (TBARS) were determined according to a method developed in situ by using reversed phase HPLC (RP-HPLC). Triplicate samples were

allowed to defrost at room temperature and 150 μ l of extracted oil were weighed and added to 4 ml distilled H_2O . 1 ml of thiobarbituric acid (0.67 % w/v) was added to the reaction mixture before heating the samples for 30 min in a boiling water bath. Samples were allowed to cool and were centrifuged at 3500 rpm for 15 min. HPLC analysis was performed using a Waters 2695 Separations Module (Waters Corporation, Milford, USA) equipped with a Waters 2475 fluorescence detector and a Luna® 5 μ m C18 (2) 100 Å, 100 $\times$ 4.6 mm column. TBARS was determined with isocratic elution at a flow rate of 0.8 ml/min, sample run was 15 min, injection volume was 20 μ l, and fluorescence detector wavelengths were set to 515 nm (excitation) and 546 nm (emission). The mobile phase consisted of 60 % (v/v) KH_2PO_4 (50 mM, pH 7.0) and 40 % (v/v) methanol and results are expressed in nmoles of malondialdehyde (MDA) equivalents/g of sample.

Determination of carotenoids and tocopherols

A reverse phase HPLC method which allows the simultaneous measurement of retinol, six carotenoids and α and γ -tocopherol in vegetable powders using fluorescence and visible detection was employed (Hess et al. 1991). Carotenoids and tocopherols were extracted from the oil phase as follows: a pre-weighed amount of oil was mixed with 200 μ l H_2O , 100 μ l ascorbic acid (25 % w/v) and 250 μ l ethanol. Each tube was vortexed and heated at 60 °C for 5 min. 250 μ l of KOH (10 M) were then added to each tube and the samples were vortexed and heated at 60 °C for 30 min. After cooling on ice, 100 μ l of echinone and 700 μ l hexane were added and the samples were mixed thoroughly before centrifugation at 14,000 rpm for 5 min. The supernatant hexane layer (600 μ l) was removed and dried down on the speed vacuum for 10 min. Each sample was then dissolved in 200 μ l of DEA (20 % (v/v) 1,4 dioxan, 20 % (v/v) ethanol, 60 % (v/v) acetonitrile) and was shaken for 5–10 min before injected for HPLC analysis. HPLC analysis was performed using a Waters 717plus Autosampler Module (Waters Corporation, Milford, USA) equipped with a Waters 2475 scanning fluorescence detector, a 2487 UV/VIS absorbance detector and a C-18 silica (Beckman Ultrasphere ODS) analytical column (250 $\times$ 4.6 mm ID 5 μ m particle size). Elution flow rate was 1.2 ml/min, sample run was 30 min and injection volume was 150 μ l. Measurements were determined with mixed standards containing carotenoids, retinol and tocopherol at appropriate concentrations and results were expressed in μ g/g of vegetable powder. Echinone was used as an internal standard.

Statistical analysis

Measurements were carried out in triplicate and results are given as the mean of 3 measurements $\pm$ standard deviation. Statistical analyses were performed using the t-test and one-

way ANOVA to detect significant differences between samples (IBM SPSS statistics 22). A P value <0.05 was considered significant. Oxidation rates were based on TBARS and were calculated from the slopes of the respective concentration vs. storage time (4 weeks) experimental curves.

Results and discussion

Oxidative stability of mayonnaise enriched with fresh vegetables as affected by storage

Fine emulsions were produced with the vegetable supplementation which differed in colour but were similar in terms of consistency both visually and under the microscope (Fig. 1). Oxidative lipid deterioration was monitored by the formation

Fig. 1 Bright field optical microscopy images and photographs of mayonnaise **a** and mayonnaise supplemented with **b** broccoli, **c** beetroot, **d** onion and **e** carrot. Bar equals to 20 μ m

of TBARS and Rancimat analysis which is an accelerated oxidation test. The results obtained from both methods are in agreement and indicate that the oil phase of mayonnaise is prone to oxidation during storage at 4 °C. As illustrated in Fig. 2, in all cases TBARS were significantly increased between week 1 and 4 ($P < 0.05$). The concentration of TBARS at the end of storage period is 3.5 times higher for the control sample ($P = 0.0006$). The incorporation of fresh vegetables in the recipe had different effects on the oxidative stability of mayonnaise, depending on the type of vegetable. Broccoli showed a pro-oxidant effect which was most marked after the first week of storage. Thereafter, oil oxidation still continued at high rates for the whole period of storage ($P = 0.004$). The formation of TBARS for the samples containing onion and carrot followed the same pattern as the control ($P = 0.002$ and $P = 0.006$ respectively). There was a steady increase in TBARS throughout the study period and levels were even higher compared to the control. Beetroot was the only vegetable which seemed to offer a protective effect against lipid oxidation of mayonnaise ($P = 0.02$). The TBARS concentration of the sample containing beetroot is significantly lower compared to the control at the end of storage period ($P = 0.003$). The beneficial effect of beetroot to the oxidation progress of the oil fraction of mayonnaise is also evidenced by

Fig. 2 TBARS of mayonnaise samples supplemented with fresh vegetables as affected by storage time at 4 °C. Different letters denote significant differences between weeks of storage ($P < 0.05$)

Fig. 3 Effect of storage time at 4 °C on the oxidative stability of mayonnaise samples supplemented with fresh vegetables as determined by the Rancimat accelerated oxidation test

the oxidation rates of the samples. The oxidation rate (κ) of mayonnaise supplemented with beetroot after four weeks of storage is half (0.27 ± 0.06) that of the control sample (0.54 ± 0.17).

A similar trend with respect to lipid oxidation of the mayonnaise samples during storage was observed with Rancimat analysis (Fig.3). This accelerated oxidation test provides a good estimate of the oxidation process of fats and oils (Méndez et al. 1997). Although relatively nonspecific it

Table 1 Tocopherol and carotenoid content of the oil phase of mayonnaise and mayonnaise supplemented with fresh vegetables. Values are the mean $\pm$ SD of at least three determinations in $\mu\text{g/g}$ oil

	Week 1						Week 2					
	Co	Br	Be	On	Ca	Co	Co	Br	Be	On	Ca	Ca
α -tocopherol	1233.2 $\pm$ 39.2*	1001.4 $\pm$ 41.2	1110.6 $\pm$ 40.7*	1054.2 $\pm$ 38.7	1079.4 $\pm$ 25.0e	1150.2 $\pm$ 23.5*	1150.2 $\pm$ 23.5*	272.1 $\pm$ 25.2	1176.1 $\pm$ 30.9*	1045.1 $\pm$ 18.2*	1134.1 $\pm$ 48.6*	1134.1 $\pm$ 48.6*
γ -tocopherol	1142.1 $\pm$ 57.9*	875.1 $\pm$ 39.7	983.0 $\pm$ 46.2	995.5 $\pm$ 40.5	889.7 $\pm$ 15.9	1064.2 $\pm$ 46.0*	1064.2 $\pm$ 46.0*	267.1 $\pm$ 36.4	1024.6 $\pm$ 27.1*	985.3 $\pm$ 40.7*	937.2 $\pm$ 49.3*	937.2 $\pm$ 49.3*
Lutein/Zeaxanthin	2.1 $\pm$ 0.2	4.1 $\pm$ 0.2	1.7 $\pm$ 0.1	3.9 $\pm$ 0.2	2.2 $\pm$ 0.1	1.9 $\pm$ 0.1	1.9 $\pm$ 0.1	2.9 $\pm$ 0.1	1.8 $\pm$ 0.1	3.7 $\pm$ 0.5	2.4 $\pm$ 0.1	2.4 $\pm$ 0.1
β -cryptoxanthin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lycopene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
α -carotene	tr	tr	tr	tr	14.0 $\pm$ 0.4	tr	tr	tr	tr	tr	12.7 $\pm$ 0.9	12.7 $\pm$ 0.9
β -carotene	tr	2.32 $\pm$ 0.2	tr	tr	27.7 $\pm$ 0.6	tr	tr	1.8 $\pm$ 0.2	tr	tr	25.4 $\pm$ 1.9	25.4 $\pm$ 1.9
	Week 3						Week 4					
	Co	Br	Be	On	Ca	Co	Co	Br	Be	On	Ca	Ca
α -tocopherol	1092.3 $\pm$ 41.7*	382.7 $\pm$ 15.4	1093.4 $\pm$ 78.6*	1001.2 $\pm$ 25.2*	1128.2 $\pm$ 60.7*	1073.4 $\pm$ 38.8*	1073.4 $\pm$ 38.8*	263.3 $\pm$ 19.2	1003.1 $\pm$ 37.1*	974.0 $\pm$ 49.2*	1186.5 $\pm$ 2.61*	1186.5 $\pm$ 2.61*
γ -tocopherol	983.6 $\pm$ 78.3*	360.3 $\pm$ 2.0	1015.2 $\pm$ 33.1*	949.9 $\pm$ 29.8*	974.6 $\pm$ 39.9*	999.8 $\pm$ 41.0*	999.8 $\pm$ 41.0*	239.1 $\pm$ 2.5	945.2 $\pm$ 55.6*	941.9 $\pm$ 28.5*	981.2 $\pm$ 6.4*	981.2 $\pm$ 6.4*
Lutein/Zeaxanthin	2.1 $\pm$ 0.1	3.1 $\pm$ 0.3	1.8 $\pm$ 0.1	3.4 $\pm$ 0.3	2.4 $\pm$ 0.3	2.1 $\pm$ 0.0	2.1 $\pm$ 0.0	2.8 $\pm$ 0.3	1.7 $\pm$ 0.1	3.2 $\pm$ 0.2	2.4 $\pm$ 0.1	2.4 $\pm$ 0.1
β -cryptoxanthin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lycopene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
α -carotene	tr	tr	tr	tr	12.2 $\pm$ 1.0	tr	tr	tr	tr	tr	13.0 $\pm$ 1.6	13.0 $\pm$ 1.6
β -carotene	tr	1.9 $\pm$ 0.2	tr	tr	24.2 $\pm$ 1.9	tr	tr	1.8 $\pm$ 0.3	tr	tr	26.0 $\pm$ 3.4	26.0 $\pm$ 3.4

Co: control, Br: broccoli, Be: beetroot, On: onion, Ca: carrot, nd: none detected, tr: traces

*denotes samples with significant differences in tocopherol levels compared to broccoli

detects changes in the concentration of secondary oxidation products such as alkanes, alcohols, aldehydes and carboxylic acids and is therefore closely related to flavour deterioration (Osborn and Akoh 2003). The induction time of the control sample (10.63 h) was reduced by 25 % during the 4 weeks of storage whereas the corresponding reduction for broccoli was 40.5 %. The oxidative stability of the samples containing onion (10.64 h) and carrot (10.49 h) at the end of the storage period were similar to the control sample. The beetroot-containing sample was the least affected by the accelerated oxidation test and had a significantly higher induction time (11.98 h) compared with the control.

Carotenoids are known to act as radical scavengers owing their chemical structure and are thus able to enhance the oxidative stability of oils (Mordi 1993). The antioxidant activity of carotenoids in edible oils depends on the carotenoid structure, the oxygen concentration and the presence of other oxidants (Kiritsakis and Dugan 1985). The carotenoid (lutein/zeaxanthin, β -cryptoxanthin, lycopene, α -carotene and β -carotene) and tocopherol (γ - and α -tocopherol) concentration of the oil phase during the storage period of the mayonnaise was determined and results are presented in Table 1. Carotenoids did not seem to offer any protection against lipid oxidation in the present study, although significant amounts of α - and β -carotene and lutein/zeaxanthin were detected in the mayonnaise samples containing carrot, broccoli and onion. Similar

results were reported by previous studies in which carotenoid extracts showed no inhibitory effect against the oxidative deterioration of sunflower oil-in-water emulsions (Kiokias et al. 2009). On the contrary, the sample containing broccoli showed a strong pro-oxidant effect possibly reflecting the presence of pro-oxidising amino acids (Gln, Pro, Val, Arg, Ile, Thr, Leu, Phe, Asp, Ala, His, GABA, Gly, Ser) endogenous to the vegetable (Martínez-Tomé et al. 2001). Carotenoids such as β -carotene may shift to a pro-oxidant character depending on the oxygen and carotenoid concentration (Haila and Heinonen 1994; Henry et al. 1998; Liebler 1993). Kiokias and Varzakas (2014) suggested that β -carotene may promote the formation of hydroperoxides in the emulsion under relatively high oxygen pressure conditions. The pro-oxidant effect of the mayonnaise containing broccoli was manifested by the significant decrease in α - and γ -tocopherols levels. Figure 4 is indicative of the low tocopherol concentration for the emulsion containing broccoli after one week of storage. The significant reduction in tocopherol concentration presumably by oxidation reflects their participation to antioxidant reactions leading to their degradation and consumption (Murkovic et al. 1997). A proposed mechanism for rapid tocopherol degradation is presented in eqs. 1 and 2.

Fig. 4 Overlaid HPLC chromatographic signals of fluorescent absorption spectra of α - and γ -tocopherol detected in the dispersed phase of mayonnaise samples. The chromatograph represents the tocopherol levels of the samples containing fresh vegetables after 2 weeks of storage at 4 °C

On the other hand, the sample with beetroot contained very low amounts of carotenoids. Thus, other classes of antioxidant molecules may be more effective in retarding the oxidation process in this type of oil-in-water emulsions. Recent studies indicate that extracts from purple corn were very effective against oxidation of mayonnaise during storage (Li et al. 2014). This effect was attributed to the high levels of anthocyanins of the husk extract. Polyphenols naturally occurring in beetroot such as betalains could offer a higher degree of protection against lipid oxidation of mayonnaise compared to carotenoids.

Oxidative stability of mayonnaise enriched with freeze-dried vegetables

Vegetables are commonly subjected to processing in order to prolong their self-life. Freeze-drying is a popular method for food processing because the sensory properties are not affected to a great extent by the process (Nawirska et al. 2009). The oxidative stability of the mayonnaise samples containing freeze-dried vegetables was compared with the formulations with the fresh vegetables for a storage period of two weeks. This storage period was adequate in order to assess the effect of freeze drying on the oxidative stability of mayonnaise containing processed vegetables. In agreement with the previous findings of this study the ability of the vegetables to retard lipid oxidation followed the order beetroot > carrot ≈ onion > broccoli. The determination of the carotenoid/tocopherol content of the dispersed phase indicated tocopherol degradation for the sample containing freeze-dried broccoli during storage (Table 2). The TBARS analysis indicated that mayonnaise samples containing freeze dried vegetables were more prone to oxidation compared to the ones with the fresh vegetables (Fig. 5). All types of vegetables showed some loss of functionality after freeze-drying and the effect was more noticeable for beetroot. The TBARS concentration of the mayonnaise with freeze-dried beetroot was significantly higher for the total storage period compared to the emulsion containing fresh beetroot ($P = 0.04$ and $P = 0.03$ respectively for weeks 1 and 2). The results obtained with the complementary method of analysis (Rancimat) also confirmed the effect on the oxidative stability of mayonnaise supplemented with freeze-dried vegetables (Fig. 6). The induction period was reduced for most of the samples containing freeze-dried vegetables, particularly for beetroot (fresh: 16.07 h – freeze-dried: 14.27 h), onion (fresh: 14.68 h – freeze-dried: 12.53 h) and carrot (fresh: 13.82 h – freeze-dried: 11.29 h) during the first week of storage.

During freeze-drying, large amounts of frozen water (in the form of ice crystals) are removed via sublimation into vapour. Typically, the vegetables are thinly sliced in order to expose large surface areas and for enhanced dehydration. This could in turn result in higher degree of oxidation and degradation

Table 2 Tocopherol and carotenoid content of the oil phase of mayonnaise and mayonnaise supplemented with freeze-dried vegetables. Values are the mean ± SD of at least three determinations in µg/oil

	Week 1					Week 2				
	Co	Br	Be	On	Ca	Co	Br	Be	On	Ca
α-tocopherol	1105.9 ± 16.8	1165.0 ± 16.6	1047.7 ± 37.5*	1114.2 ± 24.4	1157.0 ± 63.7	1099.8 ± 68.7*	559.71 ± 74.3	1030.1 ± 21.6*	1125.1 ± 72.4*	1046.4 ± 15.7*
γ-tocopherol	1039.5 ± 32.8	1255.7 ± 56.3	1083.6 ± 67.1	1267.8 ± 6.3	1229.3 ± 149.8	1166.5 ± 64.5*	667.8 ± 74.7	1126.7 ± 26.0*	1274.8 ± 72.2*	1216.8 ± 59.7*
Lutein/Zeaxanthin	2.3 ± 0.3	3.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.2	3.4 ± 0.2	2.2 ± 0.2	3.1 ± 0.1	2.8 ± 0.1	2.3 ± 0.5	3.4 ± 0.3
β-cryptoxanthin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lycopene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
α-carotene	tr	0.7 ± 0.1	tr	tr	21.4 ± 0.8	tr	0.6 ± 0.1	tr	tr	20.4 ± 0.7
β-carotene	tr	3.6 ± 0.1	tr	tr	53.6 ± 1.7	tr	3.5 ± 0.2	tr	tr	51.7 ± 1.6

Co: control, Br: broccoli, Be: beetroot, On: onion, Ca: carrot, nd: none detected, tr: traces

*denotes samples with significant differences in tocopherol levels compared to broccoli

Fig. 5 Comparative TBARS of mayonnaise samples supplemented with freeze-dried and fresh vegetables and stored for a period of 2 weeks at 4 °C. Different letters denote significant differences ($P < 0.05$)

during the freeze-drying process (Leong and Oey 2012). Significant losses of antioxidants including phytochemicals and vitamins as a result of freeze-drying of foods have been previously documented (Topuz et al. 2011). In the present study the oil phase of the mayonnaise samples supplemented with freeze-dried vegetables (e.g. carrot) contained considerable amounts of carotenoids, indicating that this class of antioxidants is not playing a major role against lipid oxidation. Thus,

these findings are not in agreement with the “polar paradox” theory which states that lipophilic antioxidants are more effective in delaying the oxidation process in oil-in-water emulsions because they are readily dispersed in the oil phase (Porter 1993). Although a direct relationship between carotenoid content and anti- or pro-oxidant activity is difficult to ascertain in such a complex food system, recent studies indicate that not all antioxidants behave according to the “polar paradox”

Fig. 6 Induction period of mayonnaise supplemented with fresh and freeze-dried vegetables and stored for a period of 2 weeks at 4 °C. Different letters denote significant differences ($P < 0.05$)

hypothesis (Laguerre et al. 2009). More complex factors in addition to polarity (i.e. antioxidant molecular structure, emulsifier concentration) need to be taken into account when interpreting antioxidant efficacy (Shahidi and Zhong 2011). On the other hand, there seems to be a negative correlation between broccoli and the tocopherol levels of the dispersed phase, which in turn is critical for the process of oxidation in oil-in-water emulsions.

Conclusions

Mayonnaise is susceptible to lipid oxidation during storage at 4 °C. In the present study vegetables were used as natural sources of antioxidants in an attempt to decrease the rate of oxidation of the dispersed phase. Broccoli induced a pro-oxidant effect, which was reflected by a sharp decrease in tocopherol content after one week of storage. The supplementation of mayonnaise with beetroot resulted in increased oxidative stability as evidenced by both TBARS and Rancimat. Lipophilic antioxidants such as carotenoids seem to be less effective in retarding the oxidation process under the specified experimental conditions. Freeze-dried vegetables underwent significant loss of “antioxidant” functionality compared to the fresh ones. Further investigations are required in order to assess whether selected vegetables could be used to replace synthetic antioxidants in mayonnaise formulations.

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