

The antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in dressing stored exposed to light or in darkness

Helle Lindberg Madsen, Bente Sørensen, Leif H. Skibsted & Grete Bertelsen*

Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

(Received 28 November 1997; accepted 5 January 1998)

For an oil-in-water emulsion dressing, addition of 0.15% of dried leaves of summer savory (*Satureja hortensis* L.) or more significantly of rosemary (*Rosmarinus officinalis* L.), resulted in a significantly better antioxidative protection than addition of 80 ppm propyl gallate (standard concentration for this type of product) during dark storage at 19°C for up to 24 weeks, as determined by development of conjugated dienes, peroxide value, head space hexanal and thiobarbituric acid-reactive substances. Addition of freeze-dried methanol extract of the two spices, in an equivalent concentration, had less antioxidative effect, but was comparable to the effect of propyl gallate. Exposure to fluorescent light (850 lux) during storage had a clear pro-oxidative effect for dressing with or without spice or spice extract added, when compared to dark storage. For dressing with extract of savory added, the antioxidative effect found for dark storage was during light exposure, changed to a pro-oxidative effect, and a hexanal level as high as 600 mg kg⁻¹ oil was detected after 8 weeks of storage, while the net antioxidative effect of rosemary was maintained for storage exposed to light.
© 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The use of spices and herbs as antioxidants in processed foods is a promising alternative to the use of synthetic antioxidants. Chipault *et al.* (1952, 1955, 1956) made the first systematic investigation and found, screening some 32 different spices, an antioxidative effect for most of the commonly-used spices. Since then, numerous reports of antioxidative activity of spices have appeared, strongly inspired by an increasing consumer interest in 'natural' food additives. Most investigations have been performed using different model systems, and the spices have been evaluated either as whole spices or as extract of spices (Madsen and Bertelsen, 1995). Already in the studies of Chipault *et al.* (1955), the importance of the substrate was demonstrated, as a very high antioxidative activity of rosemary and sage was found in lard, while clove was the most effective spice in oil-in-water emulsion. In French dressing and mayonnaise

(oil-in-water emulsions), oregano was found to be the most potent spice in retarding oxidative deterioration (Chipault *et al.*, 1955). Prior to practical use in the food industry, any spices or spice extract should accordingly be tested in the actual food under realistic storage conditions, and a three-step procedure has recently been suggested (Madsen *et al.*, 1997).

Most studies have so far concentrated on the antioxidative effects of rosemary and rosemary extract. However, the strong and characteristic flavour of rosemary might limit the use of this spice despite the well-established and very high antioxidative capacity. Compared with other spices belonging to the family Labiatae, rosemary has the more intense flavour, which could be recognized at a lower level in meat balls compared to the flavours of the spices summer savory, Chilean oregano and sage (Madsen *et al.*, 1996a). This observation increases the interest in other spices, since they remain sensory-acceptable when added in a larger amount than rosemary.

A number of foods, such as vegetable oil and dressings, are exposed to light during retail display or in the kitchen of consumers. Light is harmful to many foods

*To whom correspondence should be addressed. Fax: 0045 3528 3344; e-mail: grb@kvl.dk

as it initiates oxidation and, as the level of free radicals increases through photo-oxidation, the induction period for oxidation may decrease due to depletion of radical-scavenging antioxidants (Namiki, 1990). The mechanism of photo-oxidation is different from autoxidation, since it depends on activation of ground state oxygen (triplet state) to singlet oxygen, which may react directly with unsaturated lipid by addition to the double bond. Light is initially absorbed by a photosensitizer, which allows the energy of the photon to be transferred to form singlet oxygen (Baniyas *et al.*, 1992). Chlorophyll is an important photosensitizer in plant-derived foods (Kaur and Perkins, 1991), although the presence of carotenoids in the chloroplast normally yields protection by quenching of singlet oxygen. This protection may, however, be impaired by drying and extraction of herbs, and the influence of light on the antioxidative effect of spices has only been sparsely investigated (Herrmann, 1981; Hall and Cuppett, 1993; Botsoglou *et al.*, 1997). Accordingly we have studied the effect of exposure to light of a dressing as an example of a food rather vulnerable to oxidation, and compared the protection yielded by rosemary and summer savory and extracts of these spices using a number of methods for measurement of different stages of oxidative change.

MATERIALS AND METHODS

Spices and spice extracts

Summer savory (dried leaves of *Satureja hortensis* L.) and rosemary (dried leaves of *Rosmarinus officinalis* L.) were obtained from Paul Muggenburg GmbH and Co., Alveslohe, Germany. The authenticity and purity of the spices were confirmed by microscopy at the Department of Pharmacognosy, Royal Danish School of Pharmacy, Copenhagen. Extract of the spices was prepared by comminution of 6.0 g of summer savory or rosemary in 35 ml neat methanol (Merck pro analysis, Darmstadt, Germany) using an Ultra Turrax homogenizer (13 500 rpm, 1 min). The suspension was left in darkness with stirring for 30 min. After centrifugation, the supernatant was removed and the precipitate was resuspended in 15 ml of methanol. The procedure with stirring, centrifugation and removal of the supernatant was repeated twice, first with 15 ml methanol and second with 10 ml methanol. The supernatants were combined to yield approximately 60 ml, and 22 ml of water was added. The methanol was removed in a rotary evaporator at 40°C to a final volume of approximately 20 ml. The extract was freeze-dried and the resulting dry material (0.871 g for rosemary and 0.904 g for summer savory) was stored under nitrogen at -18°C until use. During the extraction procedure the extracts were protected against oxygen by flushing all solutions with nitrogen.

Production of dressing

Propyl gallate, available as the commercial antioxidant Grindox[®] 122 (which contains 20% propyl gallate together with 80% of a chelating carrier (citric ester of mono- and diglycerides of fatty acids), Danisco Ingredients, Brabrand, Denmark) was used as an antioxidant reference. The ingredients in the dressing were 50.0% rape seed oil, 3.0% vinegar, 2.0% sugar, 1.0% mustard, 0.7% salt, 0.1% citric acid, 0.1% potassium sorbate and 41.1% water, all of food grade. A hydrocolloid blend MAYODAN[®] M635 (Danisco Ingredients, Brabrand, Denmark) was used as stabiliser in an amount of 2.0%, and 0.010% Grindsted Flavouring 2552 (Danisco Ingredients, Brabrand, Denmark) provided a taste of egg yolk. The dry ingredients were mixed in oil in a ratio one to two. Potassium sorbate and flavouring were dissolved in water and, together with the mix of oil and dry material, the water was poured into a Koruma mixer (Maschinenbau, type DH V 60/10, Germany). The rest of the oil was added continuously under vacuum. The last third of the oil was blended with the vinegar and added to the dressing. For the storage experiment seven batches of 4.0 kg each were produced containing either (1) no antioxidant (control); (2) 6.0 g finely crushed rosemary (0.15%); (3) 6.0 g finely crushed summer savory (0.15%); (4) extract of 6.0 g rosemary (218 ppm); (5) extract of 6.0 g summer savory (226 ppm); (6) 400 ppm Grindox[®] 122 (corresponding to 80 ppm propyl gallate) and (7) 320 ppm citric acid monoglycerol ester, the carrier in Grindox 122 (the carrier constitutes 80%).

Sensory evaluation

One batch of dressing was made prior to the storage experiment. Finely crushed summer savory and rosemary were added separately in different concentrations (0.05, 0.1, 0.15 and 0.20%). The eight different samples were evaluated by a trained sensory panel with 12 members. The judges were told to mark whether the level of spice was too low, too high or suitable in the actual sample. Judges were informed that the product was a dressing, which was supposed to be added to salad, potatoes or meat in a small amount. The samples were served in red light and evaluated at a temperature of 18°C. Between every serving the judges rinsed their mouths with sparkling water, plain water (both room temperature) and crackers.

Storage

Samples of dressing (30.0 ± 0.5 g) in Petri dishes with lids were stored in darkness or exposed to light (850 ± 270 lx. at the surface of the lids) in a thermostatted room at 19 ± 1°C, as measured regularly. A total number of 40 samples were used for each of the seven different dressings and half of the samples were protected against light and the other half were exposed to

light. Throughout the storage period the samples stored exposed to light were randomly interchanged once a week to minimize unequal light exposure.

Chemical analysis

Oxidation was measured at Week 0, 1, 2, 4, 6, 8, 10, 14, 18 and 22. For samples stored in light the changes were only followed until Week 14 and the samples were discarded. Analysis of hexanal content was not performed at Week 1. Four different methods for determination of oxidative changes in the dressing were used and, prior to the analysis, oil was separated from the dressing by freezing of 5.0 g in a centrifuge tube for approximately 24 hours at -18°C followed by centrifugation for 10 min at 20 000 *g* of the thawed samples (Ole Dich microcentrifuge 154, Hvidovre, Denmark).

Peroxide value

1.500 g oil was accurately weighted and used for analysis of peroxide value (AOCS, 1973). Chloroform was substituted by isoctan. The results presented are mean values of analyses in duplicate.

Conjugated dienes

Prior to measurement of conjugated dienes, oil was dissolved in cyclohexane (0.200 mg ml^{-1}). A negative value of the second derivative of the absorption spectra at 238 nm has been demonstrated to indicate an increase in the level of conjugated dienes (Corongiu and Banni, 1995). The second derivative of the absorption spectra was obtained using standard software in the spectrophotometer (HP 8452A diode array spectrophotometer, Hewlett-Packard, Palo Alto, CA) and, for each sample, mean values of measurements in duplicate are reported.

Thiobarbituric acid-reactive substances (TBARS)

1.000 g dressing was dissolved in 3.5 ml cyclohexane, and 4.5 ml of 7.5% trichloroacetic acid (TCA)/ 0.34% thiobarbituric acid (TBA) was subsequently added, and the resulting mixture was shaken for 5 min. After centrifuging for 15 min at 2870 *g* the TCA-TBA phase was removed and heated in a water bath at 100°C for 10 min. The absorption at 532 nm was measured. Samples were analysed in duplicate and results expressed as equivalent μmole of malondialdehyde per kilogram dressing using a standard curve (concentration range 1 mM to 20 mM) based on tetraethoxypropane (pro analysis, Merck, Darmstadt, Germany).

Hexanal

The hexanal content was analysed by a GC (Chrompack CP 9001, Middelburg, Netherlands) static head space method according to Shahidi and Pegg (1994) with minor modifications. A CPSIL 88 column (25 m \times 0.32 mm D, 0.2 μm film, Chrompack, Cat No 6174, Middelburg, Netherlands) was used; the oven

temperature was raised linearly from 50°C to 200°C . Helium was used as the carrier gas with a flow of 1 ml min^{-1} , a pressure of 0.7 bar and a split flow of 10 ml/min. 2-Heptanone (approx. 100 ppm w/w) was used as internal standard. The injector was adjusted to 230°C and the flame ionization detector to a temperature of 230°C . 2.0 g portions of dressing were transferred to 5 ml glass head space vials. Prior to analysis, the vials were placed in a heating block (90°C) for 45 min. Vapour phase (1.00 ml) was injected into the GC. Hexanal values were each expressed as means of two analyses.

Statistical analysis

Effect of the experimental factors

Treatment (addition of antioxidants) and storage time (Week 0, 1, 2, 4, 6, 8, 10 and 14 for samples stored in light, Week 0, 1, 2, 4, 6, 8, 10, 14, 18 and 22 for samples stored in darkness) were evaluated by General Linear Model. Using SAS version 6.08 software (SAS, 1990). The analyses were performed for the response variables: peroxide value, conjugated dienes (the second derivative at 238 nm), TBARS and hexanal.

RESULTS

Sensory evaluation

The sensory panel evaluating the dressing found a level of 0.15% spice suitable for the product. For rosemary, four out of the 12 panel members selected the 0.15% level, while two members preferred a lower level and six members a higher. For summer savory, 11 panel members selected the 0.15% level while one member preferred a lower level and no members a higher. Based on these results, the two spices were added in an amount of 0.15% to the dressing prepared for the storage experiment and the extract addition matched this level (218 ppm rosemary extract and 226 ppm summer savory extract each correspond to 0.15% spice).

Effect of exposure to light

After 1 week of storage a pronounced effect of light was observed. The peroxide value reached a level of 30–60 meq kg^{-1} , except for the dressing with extract of summer savory added, this sample had the extremely high peroxide value of 156 meq kg^{-1} (Fig. 1B). The samples stored in darkness had, after 1 week of storage, peroxide values in the range 2–4 meq kg^{-1} , which is very similar to the values in the freshly prepared dressing (Fig. 1A).

Secondary oxidation products, determined as TBARS, showed a similar development. However, while peroxide concentration increased rapidly on exposure to light, secondary oxidation products in dressing stored in light developed after a lag phase of 4–6 weeks, as clearly

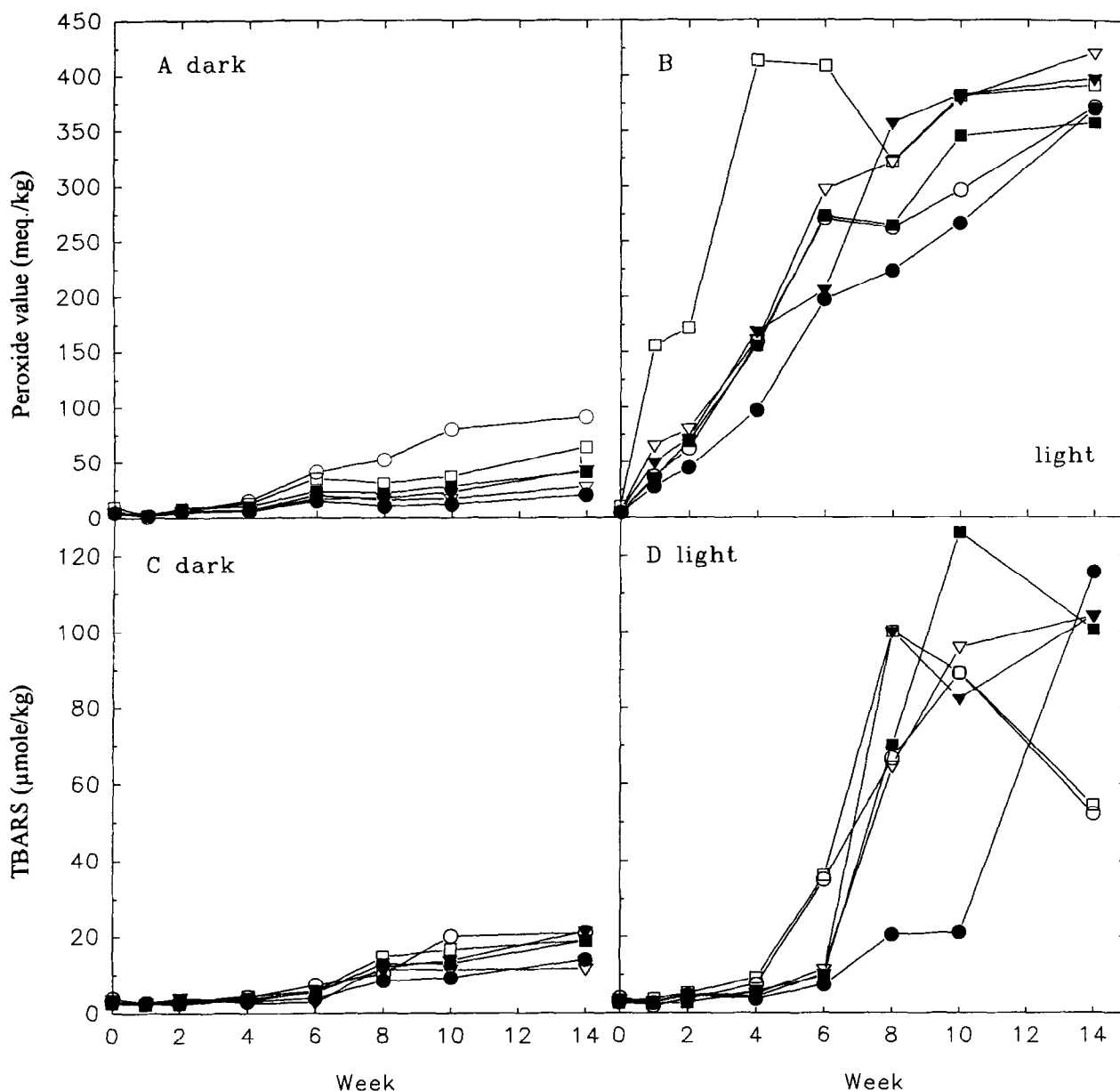


Fig. 1. Lipid oxidation measured as peroxide value (meq kg^{-1}) and TBARS ($\mu\text{mole kg}^{-1}$) during storage (19°C) for up to 14 weeks protected against light or exposed to fluorescent light. The dressing contained either $\circ\circ$, no antioxidant (control samples); $\bullet\bullet$, rosemary (0.15%); $\nabla\nabla$, summer savory (0.15%); $\blacktriangledown\blacktriangledown$, extract of rosemary (approx. 200 ppm); $\square\square$, extract of summer savory (approx. 200 ppm); or $\blacksquare\blacksquare$, propyl gallate (80 ppm as part of 400 ppm Grindox[®] 122).

seen in Fig. 1. After 8 weeks of storage exposed to light, all dressings, except the dressing with rosemary added, had a high value of TBARS (in the range $70\text{--}110 \mu\text{mole kg}^{-1}$), which remained constant until the end of the storage period at Week 14 (Fig. 1D), except for dressing without antioxidant added and dressing with summer savory extract added, in which a smaller decline towards the end of the storage period was noted. For dressings stored in darkness, a certain increase in TBARS was observed after 8 weeks to reach a level in TBARS of $10\text{--}20 \mu\text{mole kg}^{-1}$ after 10 weeks of storage, which was found constant until Week 22 (the end of the storage period).

Conjugated dienes, measured as the second derivative of the absorption spectra and analysis of hexanal by head space, showed the same trend as seen for the analysis of peroxides and TBARS, respectively, confirming the significant effect of exposure to light on oxidative deterioration of dressing.

Effect of addition of antioxidants

Samples stored in darkness

For dressing stored in darkness, a clear antioxidative effect was seen of the added spices, spice extracts and propyl gallate. For the latter synthetic antioxidant, a

control experiment (results not shown) with the carrier confirmed that it was propyl gallate and not any component of the carrier which was the active antioxidant. When stored in the dark, the oxidative changes of the dressing were moderate (Fig. 1A, C) and development of primary oxidative products, measured as conjugated dienes (Fig. 2), will be used for a more detailed comparison between the different antioxidants. The dressing with summer savory or rosemary added had a significant ($p < 0.001$) lower level of conjugated dienes compared to the control samples already after 4 weeks of storage. The activities of rosemary and summer savory were rather similar for up to 14 weeks of storage after which (analysis at Week 18 and 22), the effect of rosemary was significantly ($p < 0.05$) better compared to the effect of summer savory. Both spices were better antioxidants in the dressing than propyl gallate, an effect which proved significant ($p < 0.05$) from Week 8 and throughout the storage period. Peroxide value and TBARS showed a similar picture and confirmed the high antioxidative effect of both rosemary and summer savory, while hexanal analyses were less conclusive due to the low level of hexanal and a corresponding large relative standard deviation.

The summer savory extract was the less efficient antioxidant in the dressing stored in the dark. However, after 6 weeks of storage, a significant ($p < 0.01$) protection of the dressing was found as measured by the level of conjugated dienes in comparison with dressing without antioxidant added. During the early stages of oxidation, the antioxidative activity of savory extract was similar

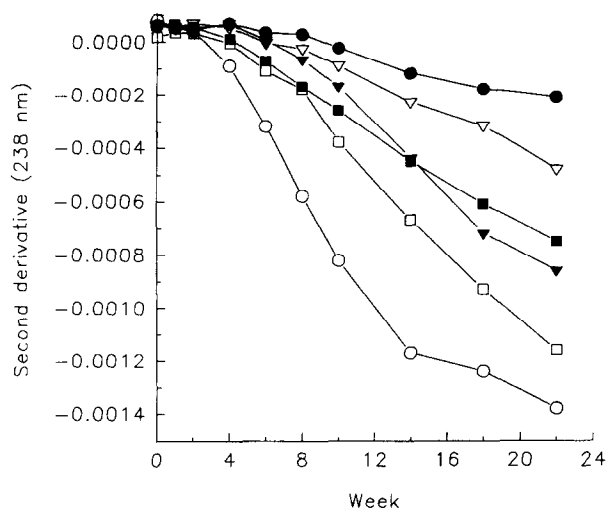


Fig. 2. Effect of addition of spices, spice extract and propyl gallate to dressing on lipid oxidation during storage in darkness (19°C) for up to 22 weeks. Oxidation was measured spectrophotometrically as conjugated dienes (the second derivative of the absorption spectrum at 238 nm). The dressing contained either ○○, no antioxidant (control samples); ●●, rosemary (0.15%); △△, summer savory (0.15%); ▼▼, extract of rosemary (approx. 200 ppm); □□, extract of summer savory (approx. 200 ppm); or ■■, propyl gallate (80 ppm as part of 400 ppm Grindox[®] 122).

to the activity seen for both propyl gallate and rosemary extract (up to 10 weeks of storage), while on longer storage the savory extract was less effective. A similar pattern was found for the peroxide value, while again the results from measurement of the secondary oxidation products such as TBARS were less clear, due to the relatively low level developed in the dark. Comparing propyl gallate and extract of rosemary, the antioxidant with intermediate effect in dressing, development of conjugated dienes during the first weeks of storage was most effectively retarded by the addition of rosemary extract. However, for longer storage, the propyl gallate appeared marginally better, although the development in peroxide value was nearly identical.

Samples stored exposed to light

The effect of exposure to light was most significant for the dressing with savory extract added. After only 1 week of storage a significantly higher level of conjugated dienes was seen (Fig. 3A) in samples containing extract of summer savory compared to both the control dressing ($p < 0.001$) and the dressing with the other antioxidants added ($p < 0.001$). The very high level of conjugated dienes continued until Week 8 and, similarly, the peroxide value clearly indicated a strong pro-oxidative activity of summer savory extract for dressing stored exposed to light (Fig. 1B). The sudden development of primary oxidation products on exposure to light in dressing with savory extract added was followed by a marked increase in hexanal evolution starting at Week 4 and it was significantly more rapid than the hexanal evolution in the dressing without an antioxidant added or with any of the other antioxidants added. Notably, using the TBARS method, this pro-oxidative effect of summer savory extract was not evident (Fig. 1D), indicating that photo-oxidation selectively leads to formation of hexanal. In summer savory, apparently some protection against this efficient photocatalyst exists and only on prolonged storage, a significant higher level ($p < 0.001$) of conjugated dienes was seen in samples with whole summer savory added, compared to dressing with no antioxidant added, an observation confirmed by the measurement of peroxide value. Secondary oxidation products showed an antioxidative activity of summer savory, but the spice was significantly less effective than rosemary, when the dressing was exposed to light (Figs 1D and 3B), in contrast to what was seen for dressing stored in darkness (Fig. 1C). After 8 weeks of storage, the level of hexanal increased rapidly in dressing containing summer savory, while samples with rosemary added remained at a low level for at least 14 weeks of storage.

Rosemary is also concluded to be an efficient antioxidant in dressing exposed to light. The level of primary oxidation products, determined as conjugated dienes, in dressing containing rosemary was thus significantly lower for up to 10 weeks of storage, when it was compared to the control samples (Fig. 3A). Also,

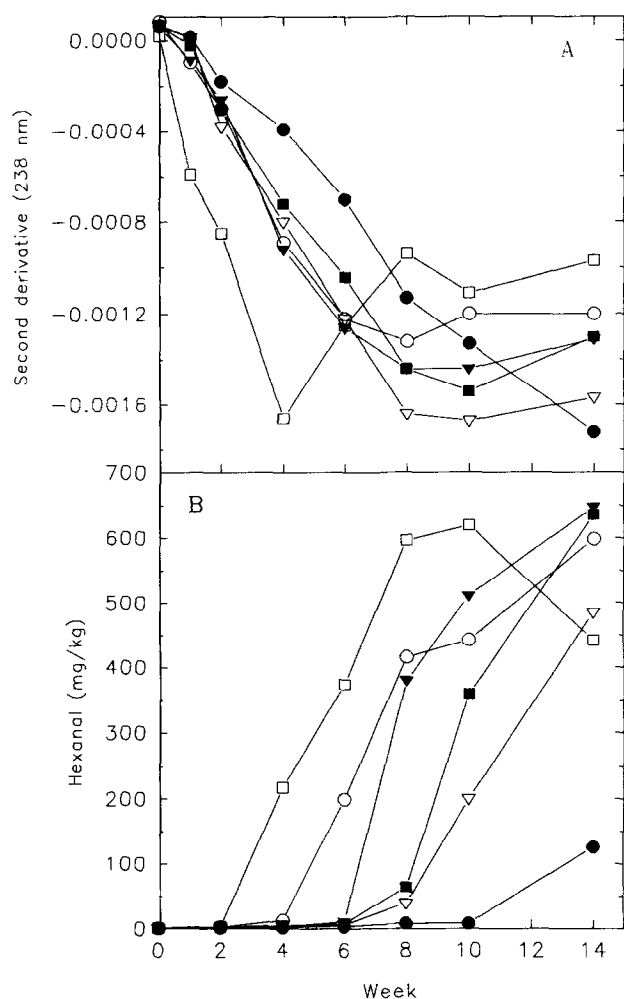


Fig. 3. Effect of addition of spices, spice extract and propyl gallate to dressing on lipid oxidation during storage in light (19°C) for up to 14 weeks. Oxidation was measured by (A) the development of conjugated dienes (the second derivative at 238 nm) and (B) the development of hexanal (mg kg^{-1}). The dressing contained either \circ , no antioxidant (control samples); \bullet , rosemary (0.15%); ∇ , summer savory (0.15%); \blacktriangledown , extract of rosemary (approx. 200 ppm); \square , extract of summer savory (approx. 200 ppm); or \blacksquare , propyl gallate (80 ppm as part of 400 ppm Grindox[®] 122).

the level of the secondary oxidation products was lower when compared to the control dressing ($p < 0.001$) throughout the storage period (hexanal) and TBARS analysis).

The effect of extract of rosemary and of propyl gallate was less clear. Analysis of the primary oxidation product revealed only small effects, while a reduced development of hexanal prevailed until the middle of the storage period. The ranking of the antioxidants other than the spice extract was the same for dressing stored in light as stored in darkness, and propyl gallate showed less efficiency than both rosemary and summer savory for each storage condition.

Statistical analysis of the results from storage of dressing both exposed to light and in darkness showed a significant ($p < 0.001$) interaction, except for the analysis

of hexanal in dressing stored in darkness. This result is, however, to be expected since the figures clearly show a non-additive effect of the two different factor treatments (addition of an antioxidant) and storage time.

DISCUSSION

Both rosemary and summer savory were found to be efficient antioxidants in dressing stored in light or even more significantly in dressing stored in darkness. Rosemary was found to reduce lipid oxidation most effectively both when dressing was stored exposed to light or stored in darkness. The high activity of rosemary as antioxidant compared both to summer savory and to other spices, has been reported previously for other systems (Chipault *et al.*, 1952, 1955; Gerhardt and Blat, 1984; Madsen *et al.*, 1996b), although the antioxidative properties of rosemary and summer savory were very similar in cooked meat balls during chill storage (Madsen *et al.*, 1996a).

The methanol extract of both rosemary and summer savory showed less antioxidative activity than the parent spice, when the extract used quantitatively matched the spice added directly. Although methanol has been shown to be an effective solvent for extraction of compounds with antioxidative properties from spices (Chang *et al.*, 1977), clearly compounds important for the antioxidative effect were not extracted. Also, the level of volatile compounds was reduced in the extract, when compared to the spices, as evidenced by a less intense aroma of the savory and especially of rosemary for the dressings with extracts added. Chipault *et al.* (1952) also found reduced antioxidative activity in extracts prepared from an equivalent amount of spice than in the whole spice, confirming that a wide range of compounds together is important as antioxidants in herbs, which further may act synergistically.

Development of both primary and secondary oxidation products was strongly enhanced by exposure of the dressing to light as seen for dressing without an antioxidant added. Photo-oxidation of lipids depends on a photosensitizer (Foote, 1976), which may generate free radicals directly (Type 1 photosensitizer) or generate singlet oxygen (Type 2 photosensitizer). We have not identified the active photosensitizer in the dressing; however, chlorophyll, a well-documented Type 2 photosensitizer will be present in the product after addition of spices or spice extracts to the dressing (Foote, 1985). In dressing with summer savory added as such or as an extract, a significant antioxidative effect was seen for storage in the dark. Exposure to light changed this antioxidative effect to a pro-oxidative effect, especially in the dressing with extract of summer savory added. The chlorophyll present in summer savory is believed to have acted as an efficient sensitizer, since oxidation was higher than the oxidation in the control dressing already after 1 week of storage in moderately intense

light. The decrease in secondary oxidation products seen for both TBARS and hexanal in dressing samples with summer savory extract added, could be indicative of a polymerisation reaction initiated by aldol condensation, since the dressing developed a rather strong 'paint' odour and was far beyond the point of sensory acceptability. In dressing with summer savory added rather than the extract, higher levels of antioxidants extracted were apparently capable of scavenging free radicals in the early stages of the storage period. However, after depletion of the antioxidants, which also may have included carotenoids as singlet oxygen quenchers, the lipids became unprotected against chlorophyll-sensitized oxidation. For storage in light a relatively poor antioxidative effect is seen for summer savory compared to rosemary, while the effect of the two spices is very similar for dressing stored in the dark.

Pro-oxidative effects of spices added to food have previously been found to dominate relative to any antioxidative effect for food stored exposed to light and, in a selection of 23 commonly used spices, only in 6 could the antioxidative effect counteract the sensitized photo-oxidation (Herrmann, 1981). Thus the leafy spices thyme, marjoram, basil and sage all showed pro-oxidative activity for foods exposed to light, while evaluation of the same food stored in the dark confirmed the antioxidative effect of the spices. Pro-oxidative activity of rosemary oleoresin has also been reported under certain conditions, confirming that the use of spices or spice extracts has to be tested for the actual product under the actual storage condition (Hall and Cuppett, 1993).

The progression of photo-oxidation in the dressing investigated follows the pattern normally encountered for autoxidation with an initial increase in lipid peroxides followed by a subsequent increase in secondary oxidation products. For the dressing with extract of summer savory added, which is photo-oxidizing extremely rapidly, the initial level of peroxides decreases concomitantly with development of the secondary lipid oxidation products. Notably, the analysis of conjugated dienes showed the same trend in the development of oxidation as seen from the analysis of the peroxide value. The derivative absorbancy technique is simpler than the classical peroxide determination and provides more detailed information about the nature and transformation of the primary oxidation products. Transformation to the second derivative of the absorption around 234 nm (dienes absorption) thus reveals two minima at 233 nm and 242 nm which are due to the presence of *trans,trans* hydroperoxides and *cis,trans* hydroperoxides, respectively (Corongiu *et al.*, 1986). The two minima in the second derivative of the absorption spectrum in the oil separated from the dressing were found at 236–238 nm and at 244 nm, respectively, confirming that the derivative method is very sensitive to the spectral band width of the spectrophotometer (Owen, 1995). Notably, the minimum at 244 nm was seen early in the storage period, and later the minimum

at 238 nm developed, while the minimum at 244 nm was slowly disappearing. This change is explained by the built up of *cis,trans* hydroperoxides, when the hydrogen donation ability of the environment is high. When the concentration of the antioxidant (the hydrogen donors) decreases, the amount of *trans,trans* hydroperoxides increase and the minima at 238 nm become more clear (Corongiu *et al.*, 1986).

In dressing stored exposed to light, the level of hexanal was as high as 500–700 mg hexanal kg⁻¹ oil while, for dressing stored in the dark, a more moderate level of 10 mg kg⁻¹ was typical. This large difference in secondary oxidation products between dressing stored exposed to light compared to dressing stored in the dark was not evident from determination of TBARS. Besides accelerating the degradation of primary oxidation products, illumination can also change the ratio between specific compounds by favouring different routes of decomposition (Frankel *et al.*, 1981). Thus an enhanced degradation of primary lipid oxidation products could increase the level of 2,4-decadienal with a possible further degradation to hexanal (Grosch, 1987).

In conclusion, the effect of photosensitization of chlorophyll present in spices may be more important than the effect of the antioxidants for foods exposed to light. This has been shown for methanol extract of summer savory added to dressing. However, the balance between photosensitization and antioxidative effect is very delicate and may depend on co-extraction of carotenoids, which may act as singlet oxygen quenchers.

ACKNOWLEDGEMENTS

This research results from a collaboration project between LMC—Centre for Advanced food Studies and Danisco Ingredients A/S, Brabrand, Denmark, supported by the Danish Ministry of Education and Research, the Danish Ministry of Agriculture and Fisheries, and the Danish Ministry of Industry as part of the FØTEK Programme. The assistance given by Product Manager Jens Birk Lauridsen and Senior Applications Manager Annemarie Dalentoft, Danisco Ingredients A/S and by Anne Marie Laustsen, LMC is gratefully acknowledged.

REFERENCES

- AOCS (1973) *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 3rd edn. Method Cd. 8.53. Champaign, American Oil Chemists' Society.
- Banias, C., Oreopoulou, V. and Thomopoulos, C. D. (1992) The effect of primary antioxidants and synergists on the activity of plant extracts in lard. *Journal of American Oil Chemists' Society* **69**, 520–524.
- Botsoglou, N. A., Yannakopoulos, A. L., Fletouris, D. J., Tserveni-Goussi, A. S. and Fortomaris, D. P. (1997) Effect of dietary thyme on the oxidative stability of egg yolk. *Journal of Agricultural and Food Chemistry* **45**, 3711–3716.

- Chang, S. S., Ostric-Matijasevic, B., Hsieh, O. A. L. and Huang, C.-L. (1977) Natural antioxidants from rosemary and sage. *Journal of Food Sciences* **42**, 1102–1106.
- Chipault, J. R., Mizuno, G. R., Hawkins, J. M. and Lundberg, W. O. (1952) The antioxidant properties of natural spices. *Food Research* **17**, 46–55.
- Chipault, J. R., Mizuno, G. R. and Lundberg, W. O. (1955) Antioxidant properties of spices in oil-in-water emulsions. *Food Research* **20**, 443–448.
- Chipault, J. R., Mizuno, G. R. and Lundberg, W. O. (1956) The antioxidant properties of spices in foods. *Food Technology* **10**, 209–211.
- Corongiu, F. P., Poli, G., Dianzani, M. U., Cheeseman, K. H. and Slater, T. F. (1986) Lipid peroxidation and molecular damage to polyunsaturated fatty acids in rat liver. Recognition of two classes of hydroperoxides formed under conditions *in vivo*. *Chemical and Biological Interactions* **59**, 147–155.
- Corongiu, F. P. and Banni, S. (1995) Detection of conjugated dienes by second derivative ultraviolet spectrophotometry. In *Methods in Enzymology. Oxygen Radicals in Biological Systems. Part D* **234**, 303–310.
- Foote, C. S. (1976) Photosensitized oxidation and singlet oxygen: Consequences in biological systems. In *Free Radicals in Biology, Vol II*, ed. W. A. Pryor. Academic Press, New York, pp. 85–133.
- Foote, C. S. (1985) Chemistry of reactive oxygen species. In *Chemical Changes in Food During Processing*, eds T. Richardson and J. W. Finley. Avi Publishing Company, Westport, pp. 17–32.
- Frankel, E. N., Neff, W. E. and Selke, E. (1981) Analysis of Autoxidized fats by gas chromatography-mass spectrometry: VII. Volatile thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate and linolenate. *Lipids* **16**, 279–285.
- Gerhardt, U. and Blat, P. (1984) Dynamische Messmethode zur Ermittlung der Fettstabilität. *Fleischwirtschaft* **64**, 484–486.
- Grosch, W. (1987) Reactions of hydroperoxides—Products of low molecular weight. In *Autoxidation of Unsaturated Lipids*, ed. H. W.-S. Chan. Academic Press, London, pp. 95–139.
- Hall, C. and Cuppett, S. (1993) The effects of bleached and unbleached rosemary oleoresins on light-sensitized oxidation of soybean oil. *Journal of American Oil Chemists' Society* **70**, 477–482.
- Herrmann, K. (1981) Über die antioxidative Wirkung von Gewürzen. *Deutsche Lebensmittel-Rundschau* **77**, 134–139.
- Kaur, H. and Perkins, M. J. (1991) The free-radical chemistry of food additives. In *Free Radicals and Food Additives*, eds O. I. Aruoma and B. Halliwell. Taylor and Francis, London, pp. 17–35.
- Madsen, H. L. and Bertelsen, G. (1995) Spices as antioxidants. *Trends in Food Science and Technology* **6**, 271–277.
- Madsen, H. L., Andersen, L., Kristiansen, L., Brockhoff, P. and Bertelsen, G. (1996a) Antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in minced, cooked pork meat. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* **203**, 333–338.
- Madsen, H. L., Nielsen, B. R., Bertelsen, G. and Skibsted, L. H. (1996b) Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. *Food Chemistry* **57**, 331–337.
- Madsen, H. L., Bertelsen, G. and Skibsted, L. H. (1997) Antioxidative activity of spices and spice extract. In *Spices. Flavor Chemistry and Antioxidant Properties*, eds S. J. Risch and C.-T. Ho. Washington, ACS. ACS Symposium Series 660, pp. 176–187.
- Namiki, M. (1990) Antioxidants/antimutagens in food. *CRC Critical Reviews in Food Science and Nutrition*, **29**, 273–300.
- Owen, A. J. (1995) Uses of derivative spectroscopy. *Application Note*. Hewlett Packard, Waldbronn, Germany.
- SAS Institute (1990) SAS/STAT User's guide, version 6, 4th edn. N.C., Cary, USA.
- Shahidi, F. and Pegg, R. B. (1994) Hexanal as an indicator of meat flavor deterioration. *Journal of Food Lipids* **1**, 177–186.