Protection of Dehydrated Chicken Meat by Natural Antioxidants as Evaluated by Electron Spin Resonance Spectrometry

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Dehydrated chicken meat ($a_w = 0.20-0.35$) made from mechanically deboned chicken necks can be protected against oxidative deterioration during storage by rosemary extract (at a sensory acceptable level of 1000 ppm, incorporated prior to drying). The efficiency of the rosemary extract was similar to that obtained by synthetic antioxidants in a reference product (70 ppm butylated hydroxyanisole and 70 ppm octyl gallate). Tea extract and coffee extract were less efficient than rosemary and synthetic antioxidants. Among the natural antioxidants tested, grape skin extract provided the least protection against oxidative changes in dehydrated chicken meat. Radicals in the product, quantified by direct measurement by electron spin resonance (ESR) spectrometry, developed similarly to headspace ethane, pentane, and hexanal, and to oxygen depletion both in unprotected and protected products. The ESR signal intensity and headspace hexanal both correlated with the sensory descriptor "rancidity" as evaluated by a trained sensory panel. Hexanal, as a secondary lipid oxidation product, showed an exponential dependence on the level of radicals in the product in agreement with a chain reaction mechanism for autoxidation, and direct ESR measurement may be used in quality control of dehydrated food products.

Keywords: *Dehydrated chicken meat; natural antioxidants; storage experiment; ESR; ethane; pentane; hexanal; TBARS; conjugated dienes; sensory profiling*

INTRODUCTION

Dehydrated poultry meat is highly vulnerable to oxidative changes which result in the development of off-flavor and formation of cholesterol oxides (Li et al., 1996). During processing the chicken meat is heated and salt is often added, both factors which have been identified as critical in relation to oxidative deterioration of meat and meat products (Skibsted et al., 1998). In addition, production of dehydrated chicken meat using mechanically deboned meat makes it even more prone to oxidative changes due to the severe physical and chemical stress placed on the material during the deboning process (McNeil et al., 1973; Dimick et al., 1972).

To stabilize dehydrated chicken meat, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are often added. BHA and BHT have both been shown to be effective in reducing lipid oxidation in chill-stored deboned chicken meat (Pikul et al., 1983; MacNeil et al., 1973). However, pressure from the consumer has increased the interest in natural antioxidants, including those present in spices. Most investigations on the antioxidative activity of spices or spice extracts have been performed in model systems (Madsen and Bertelsen, 1995). However, for deboned chicken meat, MacNeil et al. (1973) showed that a rosemary spice extract was as effective as synthetic antioxidants (BHA and citric acid) in retarding oxidation. In sausages containing mechanically deboned chicken meat rosemary extracts or natural tocopherols were found to yield protection of the sausages against oxidation (Lee et al., 1997; Resurreccion and Reynolds, 1990). Another "natural" approach is to improve the inherent antioxidative capacity of the meat by dietary supplementation. It has been demonstrated that increasing the amounts of dietary tocopherol increased lipid and cholesterol stability in chicken meat after freeze-drying (Li et al., 1996).

Because each radical formed during the deboning and drying process is trapped in the rigid structure of the low water-activity product, and on subsequent hydration holds the potential of initiating chain reactions leading to lipid autoxidation, quantification of radicals in the product should provide information on the oxidative status of such dried meat products. By the use of electron spin resonance (ESR) spectrometry, the level of radicals can be measured directly; and for milk powder, another low water-activity product, the intensity of the ESR signal has been found to correlate with a sensory evaluation of oxidized flavor (Stapelfeldt et al., 1997).

The objective of the present study was to determine the effect of four natural extracts (rosemary, green tea, coffee, and grape skin) on the oxidative stability of dehydrated chicken meat. A range of methods for measurement of different stages of oxidative changes has been used and compared with sensory evaluation.

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Table 1. Scheme of Analysis for Storage Experiment with Dehydrated Chicken Meat Packed in Aluminized Sachets

	storage time (weeks)						
analysis	0	0.5	1	1.5	2	5	8
hexanal	+	+	+	+	+	+	+
TBARS	+	+	+	+	+	+	+
residual oxygen in sachets	+	+	+	+	+	+	+
sensory evaluation	+	+	+	+	+	+	+
ESR spectrometry	+	+	+	+	+	+	+
conjugated dienes	+				+	+	+
fat content	+						
fatty acid composition in total fats	+						

Special emphasis has been put on ESR as a method for detection of early events in lipid oxidation.

MATERIALS AND METHODS

Chemicals. All chemicals used were of analytical grade, and water was purified through a Millipore Q-plus purification train (Millipore, Bedford, MA). 2-Heptanone was obtained from Aldrich Chemical Co. (Milwaukee, WI). Propylgallate (PG), ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), and malondialdehyde bis-(diethyl acetal) (TEP) were obtained from Merck (Darmstadt, Germany). Extract of rosemary (Herbor P31), extract of green tea (Licosa-P/Thé Chinois), and freeze-dried coffee extract (made by aqueous extraction of a roasted coffee blend) were provided by Nestlé Research Centre (Lausanne, Switzerland), whereas the grape skin extract (grape polyphenol powder P2157) was obtained from Unilever (Vlaadingen, Holland). In previous work (Schwarz et al., 2000), the extracts have been characterized in detail and the content of polyphenols (mmol g^{-1}) was reported: rosemary, 0.92; tea, 0.57; coffee, 1.23; and grape skin extract, 1.60.

Production and Storage of Dehydrated Chicken Meat. Dehydrated chicken meat was produced from skinless chicken necks by Nestlé R & D Center (Beauvais, France). After the chicken necks were cooked, the bones were separated from the meat, and salt and extract were added prior to mixing. The product was dried under hot air to reach a water activity of 0.20−0.35. The sensorically acceptable level of all extracts in dehydrated chicken meat was found by a trained panel to be 1000 ppm relative to the final dry product. A batch containing a solution of food-grade synthetic antioxidants (10% BHA, 10% octyl gallate, and 10% citric acid in propylene glycol) in a concentration of 700 ppm and a reference batch without antioxidants were also produced. After processing, the chicken meat (100 g), in the form of pellets, was packed under nitrogen in aluminized sachets and stored at −30 °C. At the beginning of the storage period (week 0), the sachets were opened and left for equilibration with atmospheric air. The sachets were then sealed again and stored at 22 ± 2 °C. The storage period lasted for eight weeks and samples were taken for sensory evaluation as well as chemical analyses according to the schedule shown in Table 1. Results reported are averages of four determinations with duplicates for dehydrated chicken meat from two different sachets. Samples were randomized before analysis. To study the oxygen consumption and the development of ethane and pentane during storage, 40 g of freshly processed dehydrated chicken meat was also packed under air in 425-mL aluminized cans coated with resin and tin oxide. The cans were stored at 20 °C, and the headspace was analyzed after 0, 3, 5, and 8 weeks of storage.

Hexanal Content in Samples Packed in Aluminized Sachets. Samples of approximately 20 g of chicken meat were homogenized in a household coffee mill for 8 s. Samples of 2.0 ± 0.5 g of the homogenized chicken meat were accurately weighed and transferred into 20-mL vials, and 2-heptanone (to yield 100 ppm w/w) was added as internal standard. The vials were sealed with Teflon-faced septa and stored at −3 °C until analysis. Hexanal in chicken meat was determined by headspace GC according to the method of Shahidi & Pegg (1994) with minor modifications. The samples were preheated in a HP 7694 Headspace Sampler (Hewlett-Packard, Palo Alto, CA) for 45 min at 90 °C before the vapor phase was transferred to a 3-cm^3 loop under the following conditions: carrier gas, helium; vial pressure, 0.90 bar; pressurization time on vials, 0.13 min; loop fill time, 0.04 min; loop temperature, 100 °C; transfer line temperature, 110 °C; and loop equilibration time, 0.02 min. Chromatographic separation was performed by use of a HP 6890 GC−Headspace (Hewlett-Packard, Palo Alto, CA). From the loop, the vapor phase was injected (injection time, 0.40 min; injection temperature, 200 °C) to a high-polarity HP−wax bonded polyethylene glycol column (30.0 m × 530 μm × 1 μm). Helium was the carrier gas and it was employed at an inlet pressure of 0.66 bar with a split ratio of 7:1. The oven temperature programming comprised three steps: 50 °C (5 min), 115 °C (1 min) after heating at 10 °C/minute, followed by 200 °C (1 min) after heating at 30 °C/minute. The flame ionization detector (FID) temperature was 250 °C. Quantification of hexanal content (ppm) in samples was accomplished by calculating the peak area of hexanal relative to that of the internal standard.

2-Thiobarbituric Acid-Reactive Substances (TBARS) in Samples Packed in Aluminized Sachets. The method used for determination of TBARS in meat was according to Vyncke (1970, 1975) with the modifications suggested by Sørensen and Jørgensen (1996). Samples of 2.50 ± 0.05 g of chicken meat were used for TBARS extraction. The concentration of TBARS was calculated from the difference in absorbance, measured as $A_{532\ nm} - A_{600\ nm}$ (HP 8452A Diode Array Spectrophotometer; Hewlett-Packard, Palo Alto, CA), and expressed as μmol malondialdehyde (MDA) per kg of meat.

Residual Oxygen in Sachets. Before the sachets were opened for analyses, the concentration of oxygen was determined using a calibrated Gaspace 2 gas analyzer (Sytech Instruments Ltd., Thame, UK) equipped with a needle for penetration of the sachets through a membrane.

Sensory Evaluation of Samples Packed in Aluminized Sachets. For quantitative sensory profiling, a trained sensory panel chose the following vocabulary of odor characteristics to describe the changes in dehydrated chicken meat during storage: Hot wash, boiled chicken, subcutaneous fat, and rancidity. At the sensory evaluations, the assessors were placed in separate evaluation rooms. Dehydrated chicken meat (40 g) was vigorously mixed in boiling water (1000 mL), and 20 mL portions were placed in lidded plastic cups. The cups were labeled with randomized three-digit codes and served (55 °C) one at a time, in a randomized order, to each of the individual assessors. All odor characteristics were evaluated immediately after opening the cups. Two standards (a reference sample which was stored under nitrogen at −21 °C and a reference sample stored under atmospheric air at 22 °C for 10 days) were included at each evaluation in order to recognize the difference in smell between a fresh and a stored product. The sensory profiling was performed as a Latin Square with crossover design. Scores for the intensity of each descriptor were given as a mark on an unstructured scale (15 cm) by use of a computer program (Fizz; BioSystem, France) and automatically transformed into the distance of centimeters between no recognition (0 cm) and the actual mark given.

Electron Spin Resonance (ESR) Spectroscopy on Samples Packed in Aluminized Sachets. Chicken meat (20 g) was homogenized for 8 s in a household coffee mill. Approximately 0.60 g of the homogenized sample (accurately weighed) was transferred to a cylindrical, thin-walled 702-PQ-7 clear-fused quartz (CFQ) tube (o.d., 5 mm; Wilmad Glass Company Inc., Buena, NJ) which was gently tapped against the table in order to establish a dense and uniform packing (column height, approximately 10 cm; density, approximately 60 mg/cm, calculated for the individual sample). The ESR measurements were performed by use of an ESC 106 spectrometer (Bruker Analytiche Messtechnick GmbH, Karlsruhe, Germany) equipped with an ER 4103TM cavity: sweep width, 60 G; receiver gain, $8.0 \cdot 10^4$; microwave power, 4.0 mW; modulation frequency, 100 kHz; modulation amplitude, 7 G; conversion time, 81.92 ms; time constant, 1311 ms; total scan

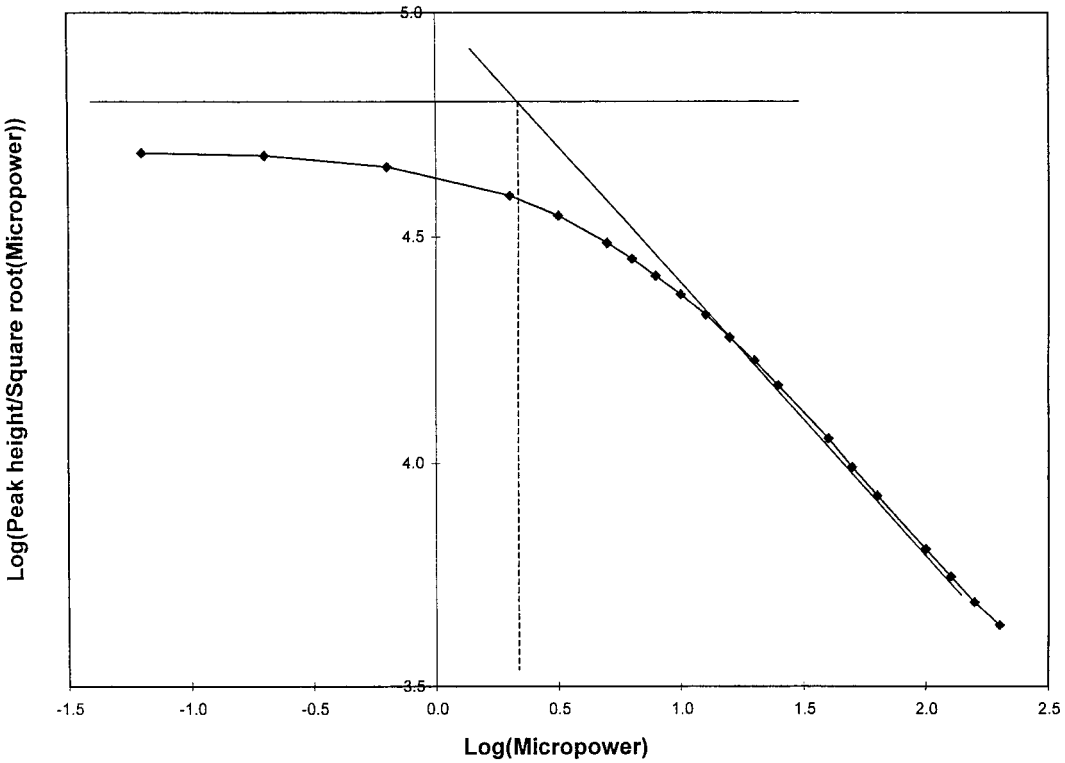

Figure 1. Saturation curve for microwave power obtained by ESR spectrometry on dehydrated chicken meat packed in aluminized sachets.

time, 84 s. ESR signals were converted into areas of peaks by integration and expressed as area of peak relative to the density of meat in the tube. As part of the optimization of the method, the micropower saturation and the pretreatment of samples were evaluated.

Quantification of ESR signals thus requires that the spectra have been recorded under nonsaturating conditions to ensure that the intensity of the ESR signal is directly proportional to the concentration of spins. Provided that the ESR spectra are recorded under the same conditions (temperature, sample volume, magnetic field modulation frequency and amplitude, microwave power, etc.) the ratio of the double-integrated areas of samples will be equal to their concentrations (Brudvig, 1995). To establish microwave power saturation curves, ESR spectra were obtained for various microwave powers using the same sample. The reason for saturation, which will lead to inaccurate results, is that unpaired electrons are excited faster from the spin down- to the spin up-state than they can relax back to the spin down-state. From a plot of the saturation behavior of the spin system in dehydrated chicken meat (Figure 1), it was found that the microwave power in this system was optimal (i.e., highest sensitivity without interference from saturation) at 4.0 mW (attenuation, 17 dB).

To ensure homogeneous samples of dehydrated chicken meat, which could be packed with high density and uniformly in the test tubes, a standard procedure for grinding of the product had to be established. Grinding might, however, increase the level of free radicals in the product. The effect of pretreatment of samples was, therefore, investigated by measuring the ESR signals for identical samples subjected to three different grinding procedures: (A) manual grinding, (B) grinding for 5 s in a household coffee mill, and (C) grinding for 8 s in a household coffee mill. ESR spectroscopy was performed four times throughout a period of 24 h of storage for each sample (density approximately 60 mg/cm), and mean values and standard deviation were calculated (A, 1.55 ± 0.06; B, 1.64 ± 0.06; and C, 1.73 ± 0.04). As might be expected, the ESR signal tended to increase with increasing intensity of grinding. However, as some variation in size of particles after grinding procedures A and B was observed, it was decided to employ grinding procedure C for the storage experiment.

The relative standard deviation of the ESR method was found to be 8.0% for 10 independent measurements of the same sample.

Fat Content and Fatty Acid Composition of Samples Packed in Aluminized Sachets. Chicken meat (1.5 g) was homogenized by a Ultra-Turrax (13 500 rpm; 1 min) in 100 mL of chloroform/methanol (2:1, v/v) before 25 mL of aqueous $CaCl_2$ (1.0 mM) was added. After the samples were mixed in the Ultra-Tarrax (13 500 rpm; 15 s), each sample was centrifuged (1000 rpm; 20 min). The chloroform phase was separated from the aqueous phase and evaporated to dryness (45 °C) (Nielsen et al., 1997). A second extraction of the aqueous phase was performed using the same procedure, and the combined residuals were dissolved in 2×2 mL chloroform. Aqueous $CaCl_2$ (2.0 mL; 1.0 mM) was added, and after centrifugation (2500 rpm; 20 min) the chloroform phase was separated from the aqueous phase, and evaporated to dryness (45 °C), and the weight of the lipid was determined.

Transformation of the lipid into methyl-ester was performed on 10 mg of lipid dissolved in 1.00 mL of sodium methylate solution (1.35 mg of sodium/mL of dry methanol) by heating (60 °C; approximately 40 min; Jart, 1997). A 4.00-mL portion of a saturated sodium chloride solution and 1.00 mL of pentane were subsequently added, and followed by vigorously mixing for 30 s. After separation of the phases, the pentane phase was concentrated by evaporation to 0.5 mL and 1 μL was subjected to gas chromatography using a HP 5890 series II (Hewlett-Packard, Palo Alto, CA) equipped with a FID detector. The injection port temperature was 250 °C and a split ratio of 1:8 was used. A high-polarity HP19091F-102 HP−FFAP column ($25.0 \text{ m} \times 0.20 \text{ mm} \times 0.33 \mu\text{m}$) was used, with helium as the carrier gas (Jart, 1997). The oven temperature programming comprised three steps; 50 °C (1 min), 180 °C at 5 °C/minute, and 200 °C (10 min) at 5 °C/minute. The flame ionization detector (FID) temperature was 300 °C.

Conjugated Dienes in Samples Packed in Aluminized Sachets. The relative concentration of conjugated dienes in the meat lipid was determined according to Corongiu and Banni (1994) using a HP 8425A Diode Array Spectrophotometer (Hewlett-Packard, Palo Alto, CA). A 10-mg aliquot of lipid (accurately weighed) was dissolved in cyclohexane (6.00 mL)

Table 2. Fat Content (%), Fatty Acid Composition (%), and Oxidative Status Measured by ESR Spectrometry, Headspace Ethane, Pentane and Hexanal, TBARS, Conjugated Dienes, and by Evaluation of the Sensory Characteristics "Hot Wash", "Boiled Chicken", "Subcutaneous Fat", and "Rancidity" at the Beginning of the Storage Period (week 0) for Dehydrated Chicken Meat Containing Various Natural Antioxidant Extracts and Synthetic Antioxidants

	treatment					
analysis	reference	rosemary	tea	coffee	grape skin	synthetic antioxidants
fat content (%w/w)	29.3^{a}	28.0^{a}	28.2^{a}	27.5^{a}	26.5^{a}	25.1^{a}
saturated/unsaturated fatty acid ratio	0.50^{c}	0.52^{b}	0.53^{b}	0.53^{b}	0.53^{b}	0.54^{a}
myristic acid (%)	0.71^{a}	0.69^{a}	0.70^{a}	0.35^{a}	0.69^{a}	0.70^{a}
palmitic acid (%)	24.8^{c}	25.7^{b}	25.8^{b}	26.3^{a,b}	26.1^{a,b}	26.5^{a}
stearic acid (%)	7.61^{a}	7.68^{a}	7.69^{a}	7.69^{a}	7.62^{a}	7.74^{a}
oleic acid (%)	39.6^{a}	37.5^{c}	38.0^{b}	38.0^{b}	37.3^{c}	36.6^{d}
linoleic acid (%)	24.5^{b}	25.1^{a}	25.0^{a}	25.3^{a}	25.1^{a}	25.0^{a}
linolenic acid (%)	1.84^{a}	1.80^{a}	1.77^{a}	1.76^{a}	1.75^{a}	1.72^{a}
arachidonic acid (%)	0.90^{a}	1.53^{a}	1.04^{a}	0.67^{a}	1.43^{a}	1.75^{a}
conjugated dienes ((A254-A234)/g lipid)	0.76^{a}	0.16^{c}	0.26^{b}	0.25^{b}	0.32^{b}	0.12^{c}
ESR (relative signal height)	1.24^{a}	0.72^{c,d}	1.08^{a,b}	0.95^{b,c}	1.10^{a,b}	0.59^{d}
ethane (ppb)	455^{a}	84^{c}	184^{b}	196^{b}	195^{b}	87^{c}
pentane (ppb)	261^{a}	82^{e}	106^{d}	133^{c}	175^{b}	40^{f}
hexanal (ppm)	7.62^{a}	1.24^{c}	3.17^{b,c}	2.97^{b,c}	5.58^{a,b}	1.96^{c}
TBARS (mg TEP/kg product)	77.1^{a}	17.8^{d}	34.3^{b}	29.8^{c}	25.9^{c}	16.7^{d}
hot wash (arbitrary units 0−15)	5.70^{a}	6.98^{a}	5.63^{a}	5.38^{a}	6.21^{a}	6.73^{a}
boiled chicken (arbitrary units 0−15)	6.43^{c}	8.14^{a}	6.92^{bc}	8.13^{a}	7.53^{a,b}	8.51^{a}
subcutaneous fat (arbitrary units 0−15)	6.72^{a}	5.48^{c}	7.14^{a}	6.47^{a,b}	6.54^{a,b}	5.79^{b,c}
rancidity (arbitrary units 0−15)	4.24^{a}	1.77^{a}	3.24^{a}	2.58^{a}	3.03^{a}	1.89^{a}

$^{a-f}$Classification obtained by analysis of variance. Different letters in the same row indicate significant difference at $p<0.05$ level.

and used for analysis. The content of conjugated dienes (per g of lipid) was calculated from the second derivative of the absorption spectrum as the difference between the maximum (A_{254}) and the minimum (A_{234}).

Ethane, Pentane, and Residual Oxygen in Cans. Ethane, pentane, and residual oxygen were determined directly on the can headspace gas above the food sample. The experimental setup consisted of a vacuum pump, manometer, gas sampling valve, oxygen concentration device, and gas chromatograph. Any two-stage oil vacuum pump could be used. The manometer was a Micro Manometer (Thommen, Waldenburg), 0 to 2000 mbar. The oxygen and CO_2 analyzer was a Servomex, Model 14050B1 (Dr. Marino Müller AG, Esslingen, CH). The gas sampling valve (automatic Vici 6-port gas sampling with 2 mL sample loop) was mounted on the gas chromatograph. All the connections were made with ($^1/_8$ in.) stainless steel tubing, plunger for puncturing the tins, and a rubber seal. For normal operation, the system was evacuated to about 0 to 1 mbar, then the can was punctured and the gas was allowed to reach equilibrium. The manometer reading was used to calculate the normalized integration values for determination of the volatile products of oxidation and the residual oxygen content at normal pressure. Volatile products of oxidation in the headspace gas were quantitatively determined by gas chromatography. A 30-m GSQ Megabore (i.d., 0.53 mm) column on a Fisons Instruments GC 8000 series (8130) chromatograph fitted with a flame ionization detector (FID) was used. The temperature was programmed at 25 °C for 2 min, from 25 °C to 150 °C at 40 °C min^{-1}, 150 °C for 3 min, from 150 °C to 200 °C at 40 °C min^{-1}, and then held isothermally at 200 °C for 10 min. Nitrogen was used as carrier gas at a flow rate of 12 mL min^{-1}. Quantitative results were obtained using a PE-Nelson data acquisition system (Turbochrom 4.1). The individual responses for the hydrocarbons methane, ethane, propane, butane, pentane, and hexane were calibrated using a tailor-made standard containing 1 μL liter^{-1} of each in nitrogen (Matheson Gas Products Oevel, BE). Analyses were performed at least in duplicates.

Statistical Analyses. To evaluate the effect of incorporated antioxidants on the initial oxidative status of dehydrated chicken meat, initial values of the response variables (ESR, hexanal, TBARS, conjugated dienes, sensory variables, fatty acids, and fat content) were subjected to analysis of variance in the procedure ANOVA in SAS 6.12 (SAS Institute, Inc., Cary, NC), and significant treatment effects were further classified by LSD ($p < 0.05$).

RESULTS

Characterization of Chicken Meat Before Storage. At the beginning of the storage period, the oxidative status and the composition of the lipid fraction of the dehydrated chicken meat were evaluated. Mean values of these results are presented in Table 2. The lipid content of dehydrated chicken pellets was found to be 27.4% w/w (SD = 1.6, n = 12) regardless of the incorporation of any of the antioxidants. The lipid fraction of the chicken meat was characterized by an almost equal amount of saturated (S) and unsaturated (U) fatty acids (S/U ratio = 0.50−0.54) of which the unsaturated fatty acids were dominated by oleic and linoleic acid with minor contents of linolenic and arachidonic acid. The saturated fatty acids comprised a high content of palmitic acid, however, stearic and myristic acid were also present. Analysis of variance showed minor but significant differences in the fatty acid composition of the six batches of chicken meat, which was supposed to be due to biological variation.

Evaluation of the initial levels of radicals, and primary and secondary oxidation products showed that the oxidative status of chicken meat already at the beginning at the storage period was highly dependent on the type of antioxidant added to the product. For the six batches, the level of oxidation for the reference batch with no incorporation of antioxidants was significantly higher than that with either rosemary or synthetic antioxidants added ($p < 0.05$). The addition of tea, coffee, or grape skin extracts resulted in an intermediate oxidative status. Similar findings were observed for the sensory attributes "boiled chicken" and " subcutaneous fat". However, "hot wash" and "rancidity" did not reveal any significant differences at the beginning of the storage period.

Oxidative Changes in Chicken Meat During Storage. During the eight weeks of storage a consistent pattern in oxidative changes was observed as shown in Figures 2 and 3. For the reference batch, the levels of radicals and hexanal, as well as the intensity of rancid smell, had a pronounced increase after 10 days, suc-

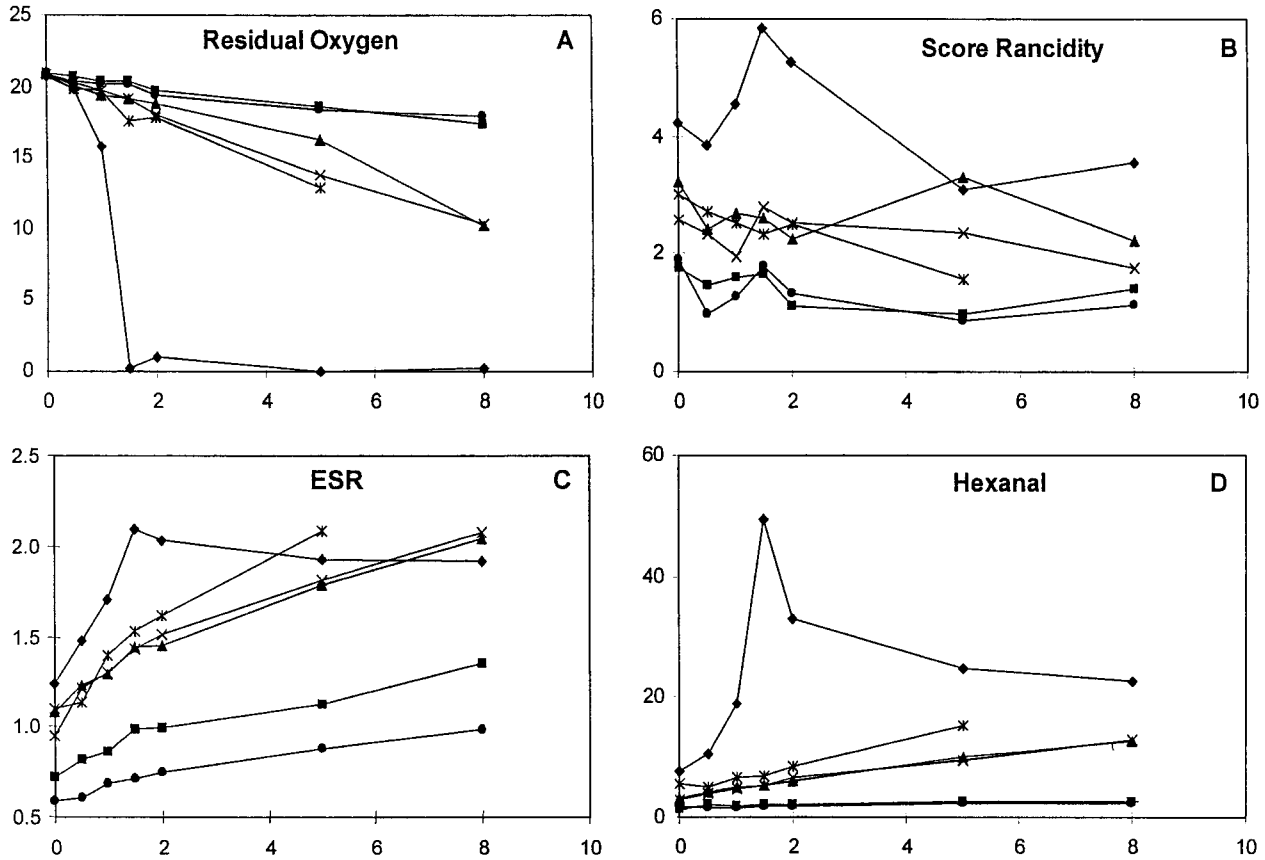

Figure 2. Oxidative changes in dehydrated chicken meat packed in aluminized sachets during eight weeks of storage (22 °C, atmospheric air) measured by (A) residual oxygen concentration (%); (B) sensory score for the descriptor "rancidity" (arbitrary units 0−15); (C) relative peak area/product density in ESR spectra; and (D) headspace hexanal (ppm). Legend for graphs: (♦) nonprotected reference product; (●) synthetic antioxidants; (■) rosemary; (×) coffee; (▲) tea; and (∗) grape skin.

ceeded by a decrease to reach a stable level after two weeks of storage. The need for oxygen when both radicals and oxidation products are formed was clearly demonstrated as the increased levels of these components were accompanied by a concomitant decrease in the oxygen concentration of the packaging sachets. Such a need of oxygen for oxidation was also demonstrated in airtight cans, as the decrease in oxygen paralleled the increase of hydrocarbon oxidation markers, i.e., ethane and pentane (Table 3 and Figure 3). The batches containing either rosemary or synthetic antioxidants showed a high oxidative stability throughout the whole period, whereas the batches containing tea, coffee, and grape skin extracts were less efficient in retarding oxidative changes. The protection of dehydrated chicken meat by natural antioxidants could also be observed by a decrease in the content of the fatty acids linoleic acid and linolenic acid during storage (Figure 4), for which a significant decrease was found for the unprotected meat.

Analysis of Regression. The relationship between chemical and sensory variables of samples packed in aluminized sachets was evaluated using Principal Component Analysis (full cross validation, log-transformations of chemical data and rancidity, all samples weighted with 1/SDev, all data centered, replicates were not averaged). In the loading plot of the PCA, the first 2 PC's described 77% (70+7) of the variance in the data matrix. Furthermore, all chemical and sensory variables were found to correlate; the sensory characteristics "boiled chicken" and "hot wash" being negatively correlated to the other variables (Figure 5).

Despite the findings of the PCA, establishment of simple linear regression of each chemical and sensory variable of samples packed in aluminized sachets showed rather poor correlation as given by values of regression (R^2) in Table 3, which show regression coefficients in the range of 0.31−0.52 for ESR, 0.37−0.63 for log(hexanal), 0.33−0.73 for log(TBARS), and 0.21−0.46 for log(conjugated dienes). For all analytical techniques, the correlation with "subcutaneous fat" had the lowest regression coefficient. For secondary oxidation products (TBARS and hexanal), the highest value of regression was observed when correlated with the descriptors "rancidity" and "boiled chicken", whereas the primary oxidation products (ESR and conjugated dienes) had highest correlation with descriptors "boiled chicken" and "hot wash". Regression between chemical methods revealed that both ESR results and concentration of conjugated dienes were rather correlated to hexanal (R^2 = 0.86 and R^2 = 0.80) than to TBARS (R^2 = 0.48 and R^2 = 0.68). Furthermore, the highest regression coefficient was obtained between ESR and log(hexanal) (Figure 6).

DISCUSSION

Drying of chicken meat from mechanically deboned meat induces significant oxidation, especially in products not protected by antioxidants (Table 2). The added antioxidants protected the product. A comparison between the results obtained by different methods for analysis of lipid oxidation shows that the levels of both the secondary lipid oxidation products (measured as

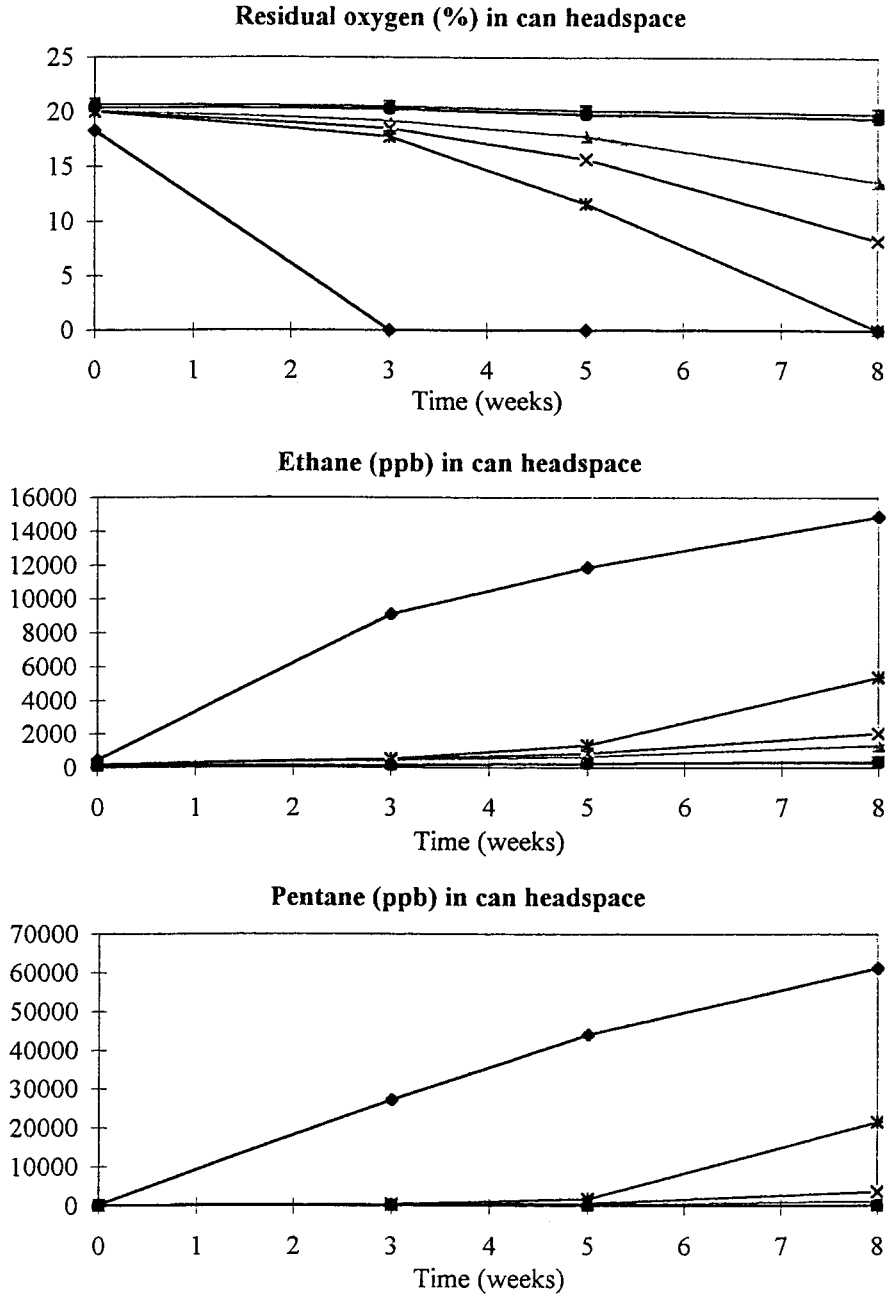

Figure 3. Oxidative changes in the can headspace containing dehydrated chicken meat during storage at 20 °C measured by (A) residual oxygen (%); (B) ethane (ppb); and (C) pentane (ppb). Legends for graph: (♦) nonprotected reference product; (●) synthetic antioxidants; (■) rosemary; (×) coffee; (▲) tea; and (∗) grape skin.

ethane, pentane, hexanal, and TBARS), and of primary oxidation products (such as the hydroperoxides measured as conjugated dienes), are increased in the unprotected product (results not shown). Lipid oxidation can accordingly be concluded to have progressed significantly during the processing as indicated by the level of secondary oxidation products. However, the level of primary oxidation products further indicate that the product will undergo subsequent oxidation during storage and it will be dependent on catalytical cleavage of the peroxides (Skibsted et al., 1998). The antioxidants protect the product during drying, and the rosemary extract added to a sensory-acceptable level is fully as efficient as the combination of BHA and octyl gallate normally used. It is, however, notable that neither rosemary nor the synthetic antioxidants protect the primary oxidation products against further decomposition as have been observed for other combinations of antioxidants in cooked turkey meat in cellulose peel-off casings, in which tocopherols and ascorbyl palmitate resulted in an accumulation of hydroperoxides (Bruun-Jensen et al., 1995). This discrepancy could be explained by the drying process, which seems to decompose the hydroperoxides. An important aspect of the antioxidant activity in the dried product is accordingly a protection against initiation of new chain reactions. The level of radicals measured by ESR in the products is in agreement with this conclusion, as the products protected by antioxidants have the lowest level of radicals trapped in the low-water-activity matrix capable of initiating chain reactions on hydration leading to primary and subsequent secondary lipid oxidation products (Kristensen and Skibsted, 1999). Lipid oxidation prior to storage has, however, not progressed to a degree where it can be recognized as a change in fatty acid distribu-

Table 3. Regression Coefficients (R^2) for Simple Linear Regression of Each Method of Analysis against Each Sensory Descriptor and Each Other Analytical Method, Which Was Performed on Samples Packed in Aluminized Sachets

	ESR	log (ESR)	conj. dien.	log (conj. dien.)	hexanal	log (hexanal)	TBARS	log (TBARS)
hot wash[a]	0.463	0.455	0.327	0.459	0.249	0.488	0.300	0.412
log(hot wash)	0.468	0.453	0.344	0.463	0.260	0.491	0.307	0.414
boiled chicken[a]	0.505	0.518	0.297	0.437	0.496	0.618	0.617	0.704
log(boiled chicken)	0.491	0.496	0.297	0.433	0.542	0.626	0.648	0.717
subcutaneous fat[a]	0.295	0.311	0.096	0.202	0.190	0.365	0.238	0.326
log(subcutaneous fat)	0.291	0.309	0.099	0.206	0.188	0.366	0.237	0.326
rancidity[a]	0.298	0.297	0.228	0.249	0.527	0.524	0.703	0.728
log(rancidity)	0.306	0.322	0.218	0.323	0.381	0.490	0.584	0.672
conjugated dienes	0.416	0.380						
log(conjugated dienes)	0.662	0.657						
hexanal	0.558	0.463	0.682	0.710				
log(hexanal)	0.861	0.821	0.594	0.802				
TBARS	0.349	0.339	0.655	0.636	0.597	0.605		
log(TBARS)	0.465	0.477	0.631	0.682	0.567	0.695		

[a] Sensory smell descriptors.

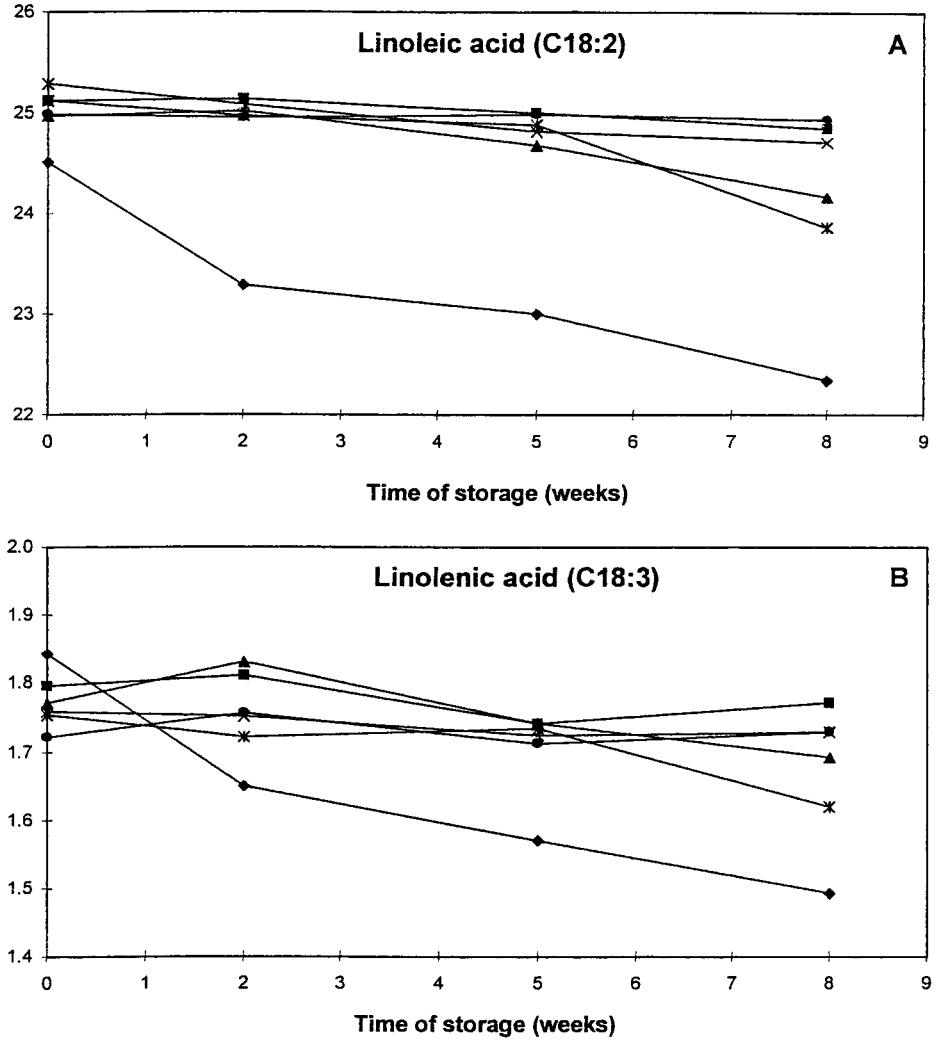

Figure 4. Content of (A) linoleic acid (%) and (B) linolenic acid (%) in dehydrated chicken meat packed in aluminized sachets during eight weeks of storage (22 °C, atmospheric air). Legends for graph: (♦) nonprotected reference product; (●) synthetic antioxidants; (■) rosemary; (×) coffee; (▲) tea; and (∗) grape skin.

tion. Also, the sensory evaluation seems uncertain, although the smell descriptor rancidity shows a clear trend.

During storage in air in closed containers (sachets and cans), oxidation initiated during the drying of the meat shows further progression (Figures 2 and 3). For the unprotected meat, development of rancidity followed oxygen depletion, and the level of radicals shows a maximum for the storage time with a maximal hexanal concentration (Figure 2). The sensory evaluation of rancidity also shows a maximum corresponding to the depletion of oxygen. Carbonyl compounds such as hexanal are responsible for the rancid flavor, and the decrease in rancid smell is most likely caused by condensation reactions of carbonyl with proteins, a process which is not dependent on the presence of

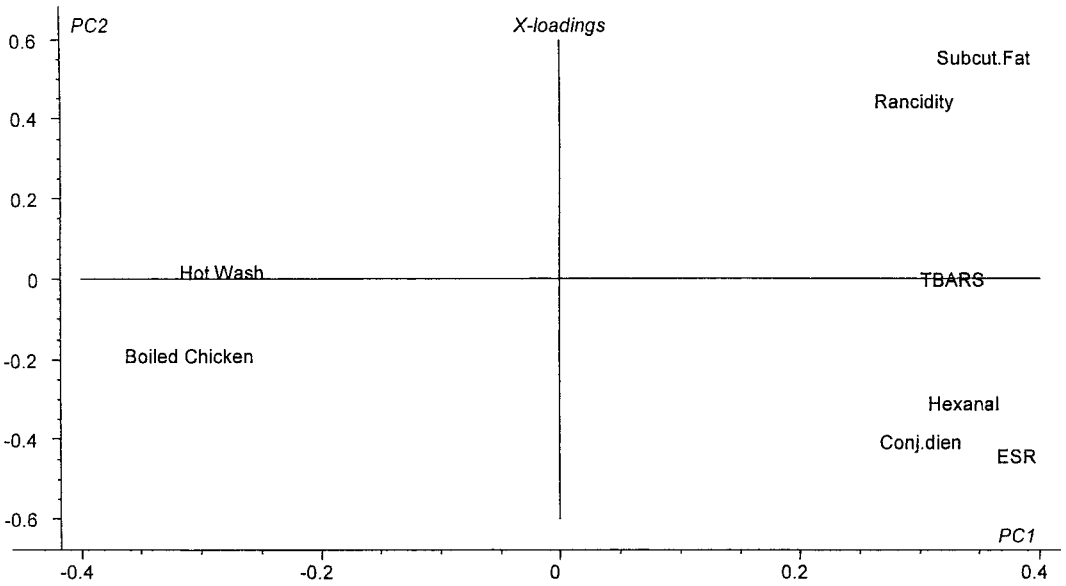

Figure 5. Loading plot from PCA on chemical and sensory data obtained during storage (eight weeks, 22 °C, atmospheric air) of dehydrated chicken meat packed in aluminized sachets.

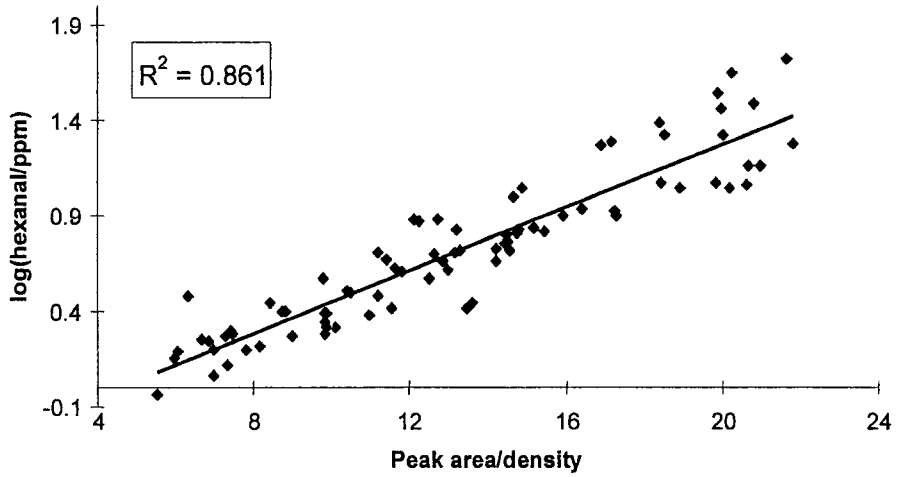

Figure 6. Correlation between ESR (peak area/product density) and log(hexanal/ppm) values obtained during storage (eight weeks, 22 °C, atmospheric air) of dehydrated chicken meat packed in aluminized sachets with or without incorporation of antioxidants.

oxygen. For the protected meat, there is only a moderate oxygen consumption leading to an increased level of radicals and further to an increased concentration of hexanal. The efficiency of the antioxidants in preventing the oxidation processes was in the order of rosemary ~ synthetic antioxidants > coffee ~ tea > grape skin. It is worth noting that the same ranking of antioxidant efficiency was obtained with all the analytical methods used in this study (Figures 2 and 3), except for determination of fatty acid composition (Figure 4) which is a less sensitive measure of lipid oxidation.

The sensory evaluation did not lead to a clear picture, most likely due to the high degree of oxidation in all products. The descriptor rancidity, however, showed a clear correlation with log(TBARS). This exponential dependence is in agreement with Weber-Fechner's law, as has also been found for warmed-over flavor in cooked sliced beef (Stapelfeldt et al., 1992).

ESR spectroscopy is a relatively new method for evaluation of oxidation in foods. For food products with low water-activity, direct measurement is possible in order to monitor the level of radicals as part of quality control. The high correlation found between the level of radicals and headspace hexanal is an important observation (Table 2). Hexanal is a secondary lipid oxidation product and the exponential dependence on the level of radicals in the product is in agreement with a chain reaction mechanism where each radical initiates a cascade of reactions leading to hexanal and many other secondary lipid oxidation products.

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LITERATURE CITED

Brudvig, G. W. Electron Paramagnetic Resonance Spectroscopy. *Methods Enzymol.* **1995**, *246*, 536−554.

Bruun-Jensen, L.; Colarow, L.; Skibsted, L. H. Detection and quantification of phospholipid hydroperoxides in turkey

meat extracts by planar chromatography. *J. Planar Chromatogr.*—Mod. TLC **1995**, *8*, 475−479.

Corongiu, F. P.; Banni, S. Detection of conjugated dienes by second derivatives ultraviolet spectrometry. *Methods Enzymol.* **1994**, *233*, 303−310.

Dimick, P. S.; MacNeil, J. H.; Grunden, L. P. Poultry product quality. Carbonyl composition and organoleptic evaluation of mechanically deboned poultry meat. *J. Food Sci.* **1972**, *37*, 544−546.

Jart, A. The magnetic field as an additional selectivity parameter in fat hydrogenation. *J. Am. Oil Chem. Soc.* **1997**, *74(5)*, 615−617.

Kristensen, D.; Skibsted, L. H. Comparison of three methods based on electron spin resonance spectrometry for evaluation of oxidative stability of processed cheese. *J. Agric. Food Sci.* **1999**, *47*, 3099−3104.

Lee, T. G.; Williams, S. K.; Sloan, D.; Littell, R. Development and evaluation of a chicken breakfast sausage manufactured with mechanically deboned chicken meat. *Poult. Sci.* **1997**, *76*, 415−421.

Li, S. X.; Ahn, D. U.; Cherian, G.; Chung, T. Y.; Sim, J. S. Dietary oils and tocopherol supplementation on cholesterol oxide formation in freeze-dried chicken meat during storage. *J. Food Lipids* **1996**, *3*, 27−42.

MacNeil, J. H.; Dimick, P. S.; Mast, M. G. Use of chemical compounds and a rosemary spice extract in quality maintenance of deboned poultry meat. *J. Food Sci.* **1973**, *38*, 1081−1081.

Madsen, H. L.; Bertelsen, G. Spices as antioxidants. *Trends Food Sci. Technol.* **1995**, *6*, 271−277.

Nielsen, J. H.; Sørensen, B.; Skibsted, L. H.; Bertelsen, G. Oxidation in pre-cooked minced pork as influenced by chill storage of raw muscle. *Meat Sci.* **1997**, *46(2)*, 191−197.

Pikul, J.; Niewiarowicz, A.; Kijowski, J. Einfluss von Antioxidantien auf die Stabilität maschinell gewonnenen und gefrorenen Geflügelfleisches. *Fleischwirtschaft* **1983**, *63(5)*, 960−964.

Resurreccion, A. V. A.; Reynolds, A. E., Jr. Evaluation of natural antioxidants in Frankfurters containing chicken and pork. *J. Food Sci.* **1990**, *55(3)*, 629−631, 654.

Schwarz K.; Bertelsen, G.; Gardener P.; Heinonen M.; Hopia, A.; Huynh-Ba, T.; Lambelet, P.; McPhail, D.; Nissen, L. R.; Skibsted, L. H.; Tijburg, L. Comparing the antioxidant activity of different plant extracts by six different assays and their principal antioxidant compounds. Submitted to *European Food Research and Technology*, 2000.

Shahidi, F.; Pegg, R. Hexanal as an indicator of meat flavor deterioration. *J. Food Lipids* 1994, *1*, 177−186.

Skibsted, L. H.; Mikkelsen, A.; Bertelsen, G. Lipid-derived off-flavours in meat. In: *Flavor of meat, meat products and seafoods*. Shahidi, F., Ed.; Blackie Academic & Professional: London, 1998.

Sørensen, G.; Jørgensen, S. S. A critical examination of some experimental variables in the 2-thiobabituric acid (TBA) test for lipid oxidation in meat products. *Zeitschrifft für Lebensmittel- Untersuchung und −Forschung.* **1996**, *202*, 205−210.

Stapelfeldt, H.; Bjørn, H.; Skovgaard, I. M.; Skibsted, L. H.; Bertelsen, G. Warmed-over flavour in cooked sliced beef. Chemical analysis in relation to sensory evaluation. *Z. Lebensm.-Unters.-Forsch.* **1992**, *195*, 203−208.

Stapelfeldt, H.; Nielsen, B. R.; Skibsted, L. H. Towards use of electron spin resonance spectrometry in quality control of milk powder. Correlation between sensory score of instant whole milk powder and concentration of free radicals and 2-thiobarbituric acid reactive substances. *Milchwissenschaft* **1997**, *52(12)*, 682−685.

Vyncke, W. Direct determination of the thiobarbituric acid value in trichloacetic acid extracts of fish as a measure of oxidative rancidity. *Fette, Seifen, Anstrichm.* **1970**, *72*, 1084−1087.

Vyncke, W. Evaluation of direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (*Scomber scombrus L.*). *Fette, Seifen, Anstrichm.* **1975**, *77*, 239−240.

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