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Maillard reaction products as antioxidants in pre-cooked ground beef

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The antioxidative activity of Maillard reaction products (MRPs) obtained by autoclaving glucose with acid or enzymatic protein hydrolysates of egg albumin or soy protein isolate was evaluated in cooked ground beef by using TBA values and headspace gas chromatographic analysis over 8 days of refrigerated storage. The antioxidative activity of MRPs was found to be influenced by the heating time of protein hydrolysates and glucose solution and by the level added. MRPs formed by heating for 1 h and added to the beef at the 1% level completely inhibited ($P < 0.05$) rancid flavor as determined by TBA values and hexanal and pentanal levels. With 0·5% added MRPs formed for 1 h, TBA values and hexanal and pentanal contents increased slightly over time, but were still different ($P < 0.05$) compared to those in the control meat. The antioxidative activity of MRPs formed by heating for 10 min did not cause significant decreases ($P < 0.05$) in TBA values until after day 2, and in hexanal and pentanal contents until after day 4 of storage.

INTRODUCTION

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products and can directly affect many characteristics such as flavor, color, texture, and nutritive value (Pearson *et al.*, 1983). Cooked meat is more susceptible to lipid oxidation than uncooked meat. Oxidation of the highly unsaturated membrane phospholipids is the primary cause of lipid oxidation in cooked meat (Igene *et al.*, 191; Asghar *et al.*, 1988). Grinding disrupts muscle cell membranes of meat and thereby exposes the phospholipids to oxygen and light (Gray & Pearson, 1987), and heating denatures meat protein and enhances the release of non-heme iron and polyunsaturated fatty acids (Schricker & Miller, 1983; Chen *et al.*, 1984). The development of oxidative rancidity in cooked meat products has become a major issue in restaurants, fast food chains airline food industries, and marketplaces (Cross *et al.*, 1987). Methods that are effective and safe for preventing lipid oxidation in cooked meat products during storage are extremely important to the muscle food industry.

A number of antioxidants both artificial and natural, have been examined in attempts to control lipid oxidation in meat products (Rhee, 1987; St Angelo *et al.*, 1990). However, the use of artificial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in food is undesirable, because consumers are concerned about the safety of all synthetic food additives. This has resulted in increased demand for natural antioxidants. Some natural antioxidants currently available, such as tocopherol and herb extracts, have limited uses because of high costs, and color and flavor attributes.

Maillard reaction products (MRPs) formed from the reaction of reducing sugars and amino acids have been found to have antioxidative activities in meat products (Lingnert & Lundgren, 1980; Bailey *et al.*, 1987). MRPs can be considered natural antioxidants because they exist in food when heated. Bailey (1988) and Bailey *et al.* (1987) reviewed the inhibition of lipid oxidation by MRPs. The predominant antioxidants appeared to be the low molecular weight fraction of MRPs containing compounds such as reductones and maltol (Eichner, 1981; Yamaguchi *et al.*, 1981). There is strong support for the concept that the antioxidative effect of MRPs is due to the formation of free radicals during heating of sugars and amines (Lingnert *et al.*, 1983; Namiki & Hayashi, 1983). Another possible mechanism is the binding of heavy metals (Johnson *et al.*, 1983).

The objective of this study was to evaluate the effect of MRPs prepared by autoclaving enzymatic or acid protein hydrolysates with glucose on lipid oxidation in cooked ground beef. The relationship between heating time of protein hydrolysates with glucose and antioxidative activity of MRPs also was examined.

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METHODS

Preparation of protein hydrolysate

Egg albumin (80·2% protein, ICN Biomedicals, Cleveland, OH) and soy protein isolate (92·0% protein, ICN Biomedicals, Cleveland, OH) were enzymatically hydrolyzed in an aqueous system containing 10% protein and 2% papain (Sigma Chemical, St Louis, MO) (wt enzyme/wt substrate) as described by Vallejo-Cordoba *et al.* (1986). Acid hydrolysates of the two protein sources were prepared by suspending them in 1·0 N hydrochloric acid and refluxing for 7 h. The extent of hydrolysis by the enzymatic or acid treatment was monitored using the trinitrobenzenesulfonic acid (TNBS) method (Adler-Nissen, 1979). The protein hydrolysates were lyophilized by using a freeze-dryer (Model 75050, Labconco Corporation, Kansas City, MO) at –40°C for 30 h. The dried protein hydrolysates were flushed with nitrogen and stored at –13°C.

Preparation of Maillard reaction products (MRPs)

Glucose (2 g) and enzymatic hydrolysates (2 g) of egg albumin (1·02 amino equiv/g protein) or soy protein isolate (0·97 amino equiv/g protein), or acid hydrolysates (2 g) of egg albumin (2·03 amino equiv/g protein) or soy protein isolate (2·18 amino equiv/g protein), were mixed with 25 ml of distilled water. The mixture was autoclaved at 121°C and 15 psi for 10 min or 1 h. The initial pH of the reaction mixture was adjusted to 9·0 with 1 N NaOH. After dilution (20 times) with distilled water, the browning intensity of the MRP solutions was measured at 420 nm with a Perkin-Elmer Spectrophotometer (Model 552, Coleman Instruments Division, Oak Brook, IL). The MRPs were then lyophilized at –40°C for 30 h and flushed with nitrogen prior to storage at –13°C until used.

Preparation of cooked ground beef

Top round beef (Kansas State University, Meat Laboratory) was trimmed of all visible fat, and ground through a 0·5 cm plate with a food processor (Rival Manufacturing Co., Model 2250, Kansas City, MO). The MRPs (0·5 and 1%) dissolved in distilled water (10 ml of distilled water/100 g ground meat) were added prior to cooking to each of the treatments except for the control, which contained only distilled water. The ground beef was placed in a 500 ml beaker and cooked in a water bath at 80°C to a final internal temperature of 70°C. The cooked ground beef was cooled in an ice bath and evaluated for oxidation at 0, 2, 4, and 8 days of storage at 4°C. A description of the treatments and the codes used are provided in Table 1.

Thiobarbituric acid (TBA) test

The development of oxidative rancidity was measured by the distillation method of Tarladgis *et al.* (1960). The assay was modified in that the TBA reagent was prepared in distilled water instead of an acidic medium.

Table 1. Description of treatment codes.

Code	Treatment
CONT	Control, no MRPs
RAW	Uncooked ground beef
EAhr	MRPs formed by autoclaving glucose and egg albumin acid hydrolysates for 1 h
EAmn	MRPs formed by autoclaving glucose and egg albumin acid hydrolysates for 10 min
EEhr	MRPs formed by autoclaving glucose and egg albumin enzymatic hydrolysates for 1 h
EEmn	MRPs formed by autoclaving glucose and egg albumin enzymatic hydrolysates for 10 min
SAhr	MRPs formed by autoclaving glucose and soy isolate acid hydrolysates for 1 h
SAmn	MRPs formed by autoclaving glucose and soy isolate acid hydrolysates for 10 min
SEhr	MRPs formed by autoclaving glucose and soy isolate enzymatic hydrolysates for 1 h
SEmn	MRPs formed by autoclaving glucose and soy isolate enzymatic hydrolysates for 10 min

The distillation procedure was performed using a Kemmerer-Hallet type microKjeldahl steam distillation unit (Fisher Scientific, Fair Lawn, NJ). TBA values (mg malondialdehyde/kg meat) were calculated by multiplying the absorbance values at 532 nm by a constant coefficient K (6·87). The constant coefficient value was calculated from standard curves of 1,1,3,3-tetraethoxypropane (TEP) (Sigma Chemical, St Louis, MO) according to the procedure of Pikul *et al.* (1989).

Capillary gas chromatography (GC) of volatile compounds

The steam distillation and headspace sampling method of Lin and Jeon (1985) was used to prepare volatile compounds. A sample (10 g) of cooked ground meat was placed into the 250 ml distillation flask of the steam distillation unit with 50 ml of permanganate distilled water. 2-Butanol (10 μg/g) was present in the ground meat as the internal standard. The mixture was steam-distilled into 10 ml graduated centrifuge tubes submerged in an ice bath. After 2 min of introduction of live steam into the sample, 5 ml of distillate was collected in 3 min. Two millilitres of the distillate were transferred to a 5 ml serum vial containing 1·2 g of sodium sulfate and capped with a Teflon septum cap (Fisher Scientific, Fair Lawn, NJ). The samples were heated in a water bath at 60°C for 2 min, shaken on an automatic shaker at 30 rpm for 5 min, and then heated for another 8 min at 60°C.

One millilitre of the headspace gas was withdrawn using a gas-tight syringe and analysed by gas chromatography. The gas chromatograph used in this study was a Hewlett-Packard Model 5880A equipped with a flame ionization detector. Compounds were separated on an HP-5 capillary column (25 m × 0·32 mm i.d. × 0·52 μm film thickness of cross-linked 5% phenyl methyl silicone,

Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed to start at 40°C, held for 5 min, then elevated to 120°C at 5°C/min and held for 5 min at the final temperature. The injection port and detector temperatures were both 250°C. The carrier gas was helium with a flow rate at 2·0 ml/min. Volatile compounds were identified by comparing their retention times with authentic standards (Aldrich Chemical, Milwaukee, WI) and by gas chromatography–mass spectrometry.

To quantify compounds found in the cooked ground beef, a stock solution (1000 g/ml) of pentanal, hexanal, heptanal, and octanal was prepared. The cooked ground beef was spiked with the stock solution to reach a concentration of 2 μg/g. The meat sample was steam-distilled and analyzed as described in the previous section. The peak areas of volatile compounds were normalized against the area of 10 μg/g of 2-butanol.

Quantification of added compounds was calculated by subtracting the peak areas of spiked samples from the areas of control samples. These analyses were performed in triplicate.

Gas chromatography–mass spectrometry (GC–MS)

Twenty steam distillates were collected as described for GC analysis. The 100 ml of distillate was extracted twice with 100 ml redistilled diethyl ether. Volatiles in the organic phase were concentrated to 1 ml by evaporating the solvent under a gentle stream of nitrogen gas. A Hewlett-Packard Model 5890A GC interfaced with an HP 5970 mass-selective detector and an HP 59970C ChemStation was used for the GC–MS analysis. The volatile compounds were resolved on an HP-5 capillary column (12 m × 0·20 mm i.d. × 0·33 μm film thickness of cross-linked 5% phenyl methyl silicone). The oven temperature program and the injection port temperature were the same as described for GC analysis. The carrier gas was helium with a flow rate at 1·0 ml/min, and the temperature of the transfer line was maintained at 280°C. The mass spectrometer was operated in the electron ionization mode at 70 eV, mass/charge 30–500, and electron multiplier of 2200 eV.

Statistical analysis

Analysis of variance was conducted for 18 treatments × 4 storage times × 3 replications, using repeated measure with treatment as the whole plot and storage time as the subplot. The Statistical Analysis System (release 6.07; SAS Institute, 1989) was used to perform statistical computations. The General Linear Model (GLM) procedure was used to test main factors (treatment, storage time, and interactions) and significant differences were accepted at the 5% level of probability. Comparisons among days for each treatment were tested using a *t*-test of the residual mean squared errors from the analysis of variance. Comparisons between treatments for each day were conducted using a *t*-test of a pooled error, combining the whole plot and residual mean squared errors from the analysis of variance and a satterwaite adjusted degree of freedom.

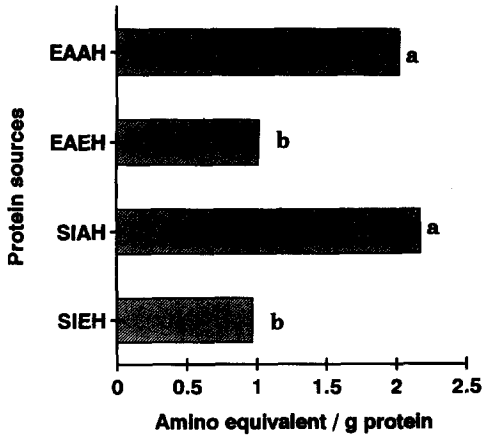

Fig. 1. The extent of acid or enzymatic hydrolysis of egg albumin or soy protein isolate expressed as amino equivalents/g protein. EAAH, egg albumin acid hydrolysate; EAEH, egg albumin enzymatic hydrolysate; SIAH, soy isolate acid hydrolysate; SIEH, soy isolate enzymatic hydrolysate. a,b, Value with different letters are significantly different ($P < 0·05$).

RESULTS AND DISCUSSION

The extent of protein hydrolysates and intensity of MRPs

The extent of acid or enzymatic hydrolysis of egg albumin or soy protein isolate used as antioxidant precursors is shown in Fig. 1. Higher values indicate a more complete hydrolysis of the protein sources. The extent of hydrolysis was lower ($P < 0·05$) for enzymatic treatment of egg albumin or soy protein isolate than for acid treatment. No differences ($P > 0·06$) occurred between the extent of hydrolysis for enzymatic egg albumin hydrolysates or soy protein isolate hydrolysates. Also, no differences ($P > 0·05$) occurred between the extent of hydrolysis for acid egg albumin hydrolysates or soy protein isolate hydrolysates.

The color intensity of MRPs was measured at 420 nm (Fig. 2). In general, MRPs prepared by heating for 1 h had higher ($P < 0·05$) color intensity than MRPs prepared by heating for 10 min. Although the color in-

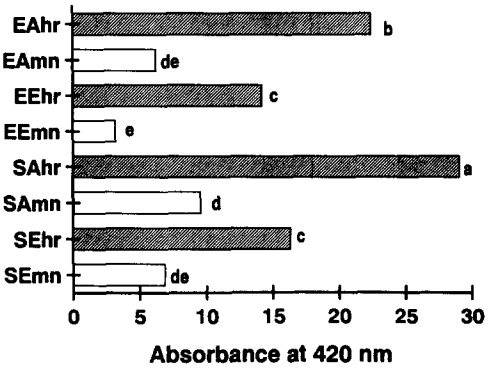

Fig. 2. The browning intensity of MRPs prepared from various protein hydrolysates and glucose for 1 h or 10 min as measured at 420 nm (see Table 1 for a description of the codes). a–e, Values with different letters are significantly different ($P < 0·05$).

tensities of MRPs made with acid hydrolysates of egg albumin and soy protein isolate and glucose for 1 h (SAhr and EAhr) were different ($P < 0.05$), the color intensities of those made from enzymatic hydrolysates of the two proteins and glucose for 1 h (EEhr and SEhr) were not different ($P > 0.05$). However, the color intensities of MRPs formed from either acid or enzymatic hydrolysates of egg albumin and soy protein isolate and glucose for 10 min were slightly different.

TBA values of cooked ground beef

Effect of MRPs formed for 1 h

The antioxidative activity of 1 or 0·5% MRPs obtained by reaction of the protein hydrolysates with glucose as measured by TBA values of cooked ground beef during storage at 4°C is shown in Table 2. The TBA values for all treatments increased over time, with the control (no added MRPs) oxidizing most rapidly and to the greatest extent, as expected. The TBA values of raw ground meat were lower than those of the control cooked beef during storage. Generally, cooking is expected to initiate the rapid development of oxidative rancidity by liberating iron from the various heme proteins and by disrupting tissue membranes, thus bringing lipid substrates and catalysts into closer proximity (Asghar *et al.*, 1988). All additives were able to control lipid oxidation during the 8 days of storage at 4°C; however, MRPs at 1% were more effective inhibitors ($P < 0.05$) than MRPs at 0·5%.

Table 2. Antioxidative effect of MRPs formed by heating for 1 h or 10 min as measured by TBA values (mg malonaldehyde/kg meat) in cooked ground beef during storage at 4°C

TRT[1]	CONC[2]	TBA values				% Inhibition[3]
		Day 0	Day 2	Day 4	Day 8	
CONT	0	0·46^{wa}	5·40^{x}	8·31^{y}	12·14^{z}	
RAW	0	0·39^{wa}	2·69^{*xad}	3·71^{*xydef}	4·99^{*ye}	
MRPs formed for 1 h						
EAHr	1	0·15^{wa}	0·79^{*we}	1·46^{*wxh}	2·22^{*wg}	81·71
EAhr	0·5	0·26^{wa}	1·75^{*xcde}	3·18^{*yeg}	4·61^{*ze}	62·03
EEhr	1	0·18^{wa}	0·79^{*wxe}	1·62^{*xyh}	2·78^{*yg}	77·10
EEhr	0·5	0·29^{wa}	2·66^{*xacd}	4·50^{*yeef}	5·93^{*zde}	51·15
SAhr	1	0·21^{wa}	1·20^{*wxde}	2·06^{*xygh}	3·07^{*yfg}	74·71
SAhr	0·5	0·21^{wa}	2·05^{*xcde}	3·45^{*yfg}	4·80^{*ze}	60·46
SEhr	1	0·33^{wa}	1·99^{*xcde}	3·41^{*yfg}	4·58^{*yefe}	62·36
SEhr	0·5	0·31^{wa}	3·06^{*xac}	4·73^{*ybcef}	6·14^{*zde}	49·47
MRPs formed for 10 min						
EAmn	1	0·21^{wa}	2·63^{*xbcd}	4·40^{*yeef}	5·96^{*zde}	50·91
EAmn	0·5	0·35^{wa}	3·80^{*xab}	6·34^{*ya}	8·69^{*zab}	28·42
EEmn	1	0·30^{wa}	2·66^{*xacd}	4·77^{*ybcf}	6·86^{*zcd}	43·49
EEmn	0·5	0·35^{wa}	3·65^{*xab}	6·12^{*yab}	8·19^{*zac}	32·54
SAmn	1	0·28^{wa}	3·01^{*xac}	4·64^{*ubcef}	6·00^{*zde}	50·58
SAmn	0·5	0·33^{wa}	4·16^{xab}	6·73^{*ya}	9·07^{*za}	25·29
SEmn	1	0·34^{wa}	3·12^{*xac}	5·26^{*yacd}	8·12^{*zacd}	33·11
SEmn	0·5	0·28^{wa}	4·21^{xa}	6·38^{*ya}	8·86^{*za}	27·02

[1] Treatment see Table 1 for Description of codes.
[2] CONC: MRPs were added at 0·5 or 1% (w/w).
[3] Calculated as: 1- (TBA value of TRT/TBA value for CONC) × 100.
*Significantly different from the value of the control within the same storage period ($P < 0.05$).
$^{w-z}$Different letters in the same row are significantly different ($P < 0.05$).
$^{a-h}$Different letters in the same column are significantly different ($P < 0.05$).

At day 0, no significant differences ($P > 0.05$) were found between the TBA values of the control and the various treatments. However, MRPs had a significant effect on the rate at which the TBA values of cooked ground beef increased throughout the storage period. Although the TBA values of the control increased rapidly with increasing storage time (1·46 mg MA/kg per day), the TBA values of the best antioxidant MRPs (EAhr, EEhr, and SAhr at 1%) increased by only 0·31 mg MA/kg per day, and the TBA values of the least antioxidant MRPs (SEhr at 0·5%) increased by 0·87 mg MA/kg per day.

The most effective antioxidant MRPs were EAhr at 1% with 81·76%, EEhr at 1% with 77·10%, and SAhr at 1% with 74·73% inhibition of TBA as compared to control cooked ground beef. Although the TBA values with these additives were initially low (0·18 mg MAkg) and increased slightly ($P > 0.05$) during storage, their antioxidative activities were not different ($P < 0.06$) throughout the storage period. The antioxidative activities of the MRPs were not influenced by the choice of protein sources or by method of protein hydrolysis. When protein hydrolysates and glucose were heated for 1 h and the added level of MRPs was kept constant at 1%, the antioxidative effects of MRPs from EAhr, EEhr, and SAhr were not significantly different ($P > 0.05$), but that of SEhr was slightly different ($P < 0.05$) throughout the storage period. Furthermore, at the 0·5% level, the antioxidant effects of MRPs from EAhr, EEhr, SAhr, and SEhr were not different ($P > 0.05$) during storage. When the protein sources and added level were kept constant, no differences ($P > 0.05$) occurred between the antioxidative activities of MRPs from enzymatic hydrolysates and acid hydrolysates of egg albumin (EAhr and EEhr) or soy protein isolate (SAhr and SEhr). Therefore, the choice of protein source or method of protein hydrolysis appears to be of minor importance.

The antioxidative properties of MRPs from protein hydrolysates and glucose were not tested previously in cooked ground beef during refrigerated storage. Lingnert and Lundgren (1980) found that preformed MRPs from enzymatic hemoglobin hydrolysate and glucose were effective antioxidants in sausage during frozen storage as determined by sensory evaluation, gas chromatographic analysis of volatile compounds, and peroxide value. Lingnert and Eriksson (1980*a*) also reported that MRPs from protein hydrolysates and glucose were effective in preventing the oxidation of linoleic acid as evaluated by a polarographic and a gas chromatographic method. Bailey *et al.* (1987) found that the addition of MRPs from histidine and glucose was effective in retarding rancid flavor in cooked ground beef during refrigerated storage as measured by TBA value and volatile compounds analysis. Sato *et al.* (1973) demon-

strated that the addition of MRPs formed from glycine and glucose or lysine and glucose to ground beef before cooking effectively inhibited rancid flavor. Obretenov *et al.* (1986) showed that MRPs prepared from acid bovine blood hydrolysate with hydrolyzed starch provided a strong antioxidative effect in lard.

The formation of antioxidative compounds was not correlated with the color intensity when the protein hydrolysates and glucose were heated for 1 h. Although the color intensities of MRPs from EAhr, EEhr and SAhr were significantly different ($P < 0.05$), their antioxidative activities were not different ($P > 0.05$). This result agreed with Lingnert and Eriksson (1980*b*) and Beckel and Waller (1983), who found no relationship between the color intensity and the antioxidative activity of MRPs.

However, Lee (1992) reported that the antioxidative activity of MRPs from storage-aged orange juice was correlated highly with browning intensity during oxidation of linoleic acid as evaluated by conjugated diene level. Yamaguchi *et al.* (1981) also reported that the antioxidative activity of MRPs increased in proportion to the color intensity.

Effect of MRPs formed for 10 min

The antioxidative effects of various MRPs prepared for 10 min and added at 1 and 0·5% as indicated by TBA values of cooked ground beef are presented in Table 2. In general, the TBA values of the samples treated with MRPs formed for 10 min showed less antioxidative activity than those of samples treated with the MRPs formed for 1 h. The reduction in TBA values ranged between 81·17 and 48·27% for MRPs heated for 1 h and between 50·91 and 25·29% for MRPs heated for 10 min. TBA values of all treatments were significantly different ($P < 0.05$) than the values of the control at 2, 4 and 8 days, except for the TBA values of 0·5% SAmn and SEmn at day 2. Again, 1% MRPs were more effective antioxidants than 0·5% MRPs ($P < 0.05$). The antioxidative activities of MRPs heated for 10 min were not influenced by the choice of the protein sources or by the method of protein hydrolysis. This trend agreed with data for the MRPs heated for 1 h. Again, no correlation occurred between the color intensity and the antioxidative activity of MRPs heated for 10 min.

The purpose of heating protein hydrolysates with glucose for 1 h and 10 min was to encourage the formation of high molecular weight MRPs (melanoidins) in the former and of low molecular weight MRPs (amino-reductones and reductones) in the latter, because these compounds have different antioxidative mechanisms. The antioxidative mechanism of reductones and amino reductones is thought to be due to their reducing activity (Eichner, 1981) and that of melanoidins due to metal binding ability (Johnson *et al.*, 1983). Because iron from heme proteins appears to be the most important catalyst of lipid oxidation in cooked meat products (Schricker & Miller, 1983), any compound with metal-chelating ability should be effective in retarding lipid oxidation. This study indicated that MRPs formed for 1 h were more effective in protection against lipid oxidation in cooked ground beef than MRPs prepared for 10 min. Therefore, we assume that the melanoidin pigment might be produced in higher content in MRPs formed for 1 h than in MRPs prepared for 10 min. Yamaguchi *et al.* (1981) reported that strong antioxidative activity was found in the melanoidin fractions when a mixture of xylose and glycine was heated at 100°C for 2 h.

Effect of MRPs on headspace volatiles

Most of the headspace volatile compounds determined were aldehydes. The formation of these compounds is most likely from oxidation of unsaturated fatty acids (Frankel, 1984). In a recent study, Frankel and Tappel (1991) reported that hexanal and pentanal were oxidation products of linoleic acid. Aldehydes have been used to measure lipid oxidation in foods (Melton, 1983).

Effect of MRPs heated for 1 h

Changes of average concentrations of selected headspace volatiles from cooked ground beef as affected by 1 or 0·5% added MRPs heated for 1 h over storage time are presented in Tables 3–6. The aldehyde concentrations of raw ground beef were very low and did not change ($P > 0.05$) during the storage period. The control sample had a higher ($P < 0.05$) aldehyde content compared to raw ground beef during the storage time.

Table 3. Antioxidative effect of MRPs formed by heating for 1 h or 10 min as measured by hexanal formation (mg hexanal/kg meat in cooked ground beef storage at 4°C

TRT[1]	CONC[2]	Day 0	Day 2	Day 4	Day 8
CONT	0	0.18^{wa}	4.20^{x}	6.80^{y}	10.20^{z}
FRSH	0	0.08^{wa}	0.12^{*wd}	0.66^{*wf}	0.79^{*wg}
MRP formed for 1 h					
EAhr	1	0.02^{wa}	0.61^{*wcd}	0.85^{*wef}	1.70^{*wfg}
EAhr	0·5	0.06^{wa}	1.14^{*wabcd}	$2.03^{*wxbcdef}$	4.02^{*xcdef}
EEhr	1	0.03^{wa}	0.67^{*wcd}	1.55^{*wcdef}	1.98^{*wefg}
EEhr	0·5	0.10^{wa}	2.93^{xabc}	3.42^{*xabcd}	4.62^{*xbcd}
SAhr	1	0.03^{wa}	0.85^{*wxbcd}	1.53^{*wxdef}	2.91^{*xdefg}
SAhr	0·5	0.07^{wa}	1.96^{wxabcd}	$2.29^{*wxbcdef}$	2.74^{*xdefg}
SEhr	1	0.13^{wa}	1.49^{*wabcd}	$2.40^{*wxbcdef}$	4.65^{*xbcd}
Sehr	0·5	0.12^{wa}	3.23^{xab}	3.68^{*xabcd}	4.41^{*xbcde}
MRPs formed for 10 min					
EAmn	1	0.04^{wa}	1.98^{wxabcd}	$3.00^{*xabcdef}$	6.27^{*yabc}
EAmn	0·5	0.13^{wa}	2.52^{xancd}	3.70^{*xabcd}	7.76^{ya}
EEmn	1	0.06^{wa}	2.41^{xabcd}	3.61^{*xabcd}	4.62^{*xbcd}
EEmn	0·5	0.12^{wa}	2.88^{xabc}	4.11^{*xab}	6.85^{*yab}
SAmn	1	0.19^{wa}	2.86^{xabc}	$3.16^{*xabcde}$	4.16^{*xcde}
SAmn	0·5	0.11^{wa}	3.58^{xa}	4.86^{xya}	6.00^{*yabc}
SEmn	1	0.13^{wa}	2.96^{xabc}	4.00^{*abc}	5.08^{*xbcd}
SEmn	0·5	0.11^{wa}	3.00^{xabc}	3.91^{*xabcd}	8.11^{ya}

[1]Treatment see Table 1 for Description of codes.
[2]CONC: MRPs were added at 0·5 or 1% (w/w).
*Significantly different from the value of the control within the same storage period ($P < 0.05$).
[w–z]Different letters in the same row are significantly different ($P < 0.05$).
[a–f]Different letters in the same column are significantly different ($P < 0.05$).

Table 4. Antioxidative effect of MRPs formed by heating for 1 h or 10 min as measured by pentanal formation (mg pentanal/kg meat) in cooked ground beef during storage at 4°C

TRT[1]	CONC[2]	Day 0	Day 2	Day 4	Day 8
CONT	0	0.02^{wa}	0.36^{*x}	0.53^{y}	0.88^{z}
RAW	0	0.00^{wa}	0.04^{*we}	0.03^{*we}	0.06^{*wg}
MRPs formed for 1 h					
EAHr	1	0.00^{wa}	0.05^{*wbc}	0.07^{*wde}	0.16^{*weg}
EAhr	0.5	0.00^{wa}	0.09^{*wabc}	$0.16^{*wxabcde}$	0.32^{*xbcde}
EEhr	1	0.00^{wa}	0.05^{*wbc}	0.12^{*wcde}	0.13^{*wefg}
EEhr	0.5	0.01^{wa}	0.22^{xabc}	0.25^{*xabcd}	0.35^{*xbcde}
SAHr	1	0.00^{wa}	0.07^{*wxabc}	$0.13^{*wxbcde}$	0.23^{*xdefg}
SAhr	0.5	0.00^{wa}	0.15^{*wxabc}	$0.17^{*wxabcde}$	0.21^{*xdefg}
SEhr	1	0.01^{wa}	0.12^{*wabc}	$0.18^{*wxbcde}$	0.34^{*xbcde}
SEhr	0.5	0.01^{wa}	0.23^{xab}	0.26^{*xabcd}	0.33^{*xbcde}
MRPs formed for 10 min					
EAmn	1	0.00^{wa}	0.16^{*wxabc}	0.24^{xyabcd}	0.39^{*yabcd}
EAmn	0.5	0.01^{wa}	0.19^{xabc}	0.31^{*xab}	0.50^{*yab}
EEmn	1	0.00^{wa}	0.17^{wxabc}	0.31^{*xab}	0.31^{*xcdef}
EEmn	0.5	0.01^{wa}	0.19^{xabc}	0.29^{*xdx}	0.50^{*yab}
SAmn	1	0.01^{Wa}	0.22^{xabc}	0.24^{*xabcd}	0.32^{*xbcde}
SAmn	0.5	0.01^{wa}	0.25^{xa}	0.33^{*xya}	0.45^{*yabc}
SEmn	1	0.01^{wa}	0.22^{xabc}	0.24^{*xabcd}	0.31^{*xcdef}
SEmn	0.5	0.01^{wa}	0.18^{*xabc}	0.26^{*abcd}	0.57^{*ya}

[1] Treatment; see Table 1 for Description of codes.
[2] CONC: MRPs were added at 0.5 or 1% (w/w).
*Significantly different from the value of the control within the same storage period ($P < 0.05$).
[w-z]Different letters in the same row are significantly different ($P < 0.05$).
[a-f]Different letters in the same column are significantly different ($P < 0.05$).

Table 5. Antioxidative effect of MRPs formed by heating for 1 h or 10 min as measured by heptanal formation (mg heptanal/kg meat) in cooked ground beef during storage at 4°C

TRT[1]	CONC[2]	Day 0	Day 2	Day 4	Day 8
CONT	0	0.00^{wa}	0.07^{x}	0.09^{x}	0.17^{y}
FRSH	0	0.00^{wa}	0.02^{wab}	0.02^{*wc}	0.01^{*we}
MRPs formed for 1 h					
EAhr	1	0.00^{wa}	0.01^{*wb}	0.02^{*wc}	0.04^{*wef}
EAhr	0.5	0.00^{wa}	0.01^{*wb}	0.03^{*wbc}	0.08^{*xdef}
EEhr	1	0.00^{wa}	0.01^{*wb}	0.03^{*wbc}	0.03^{*wefg}
EEhr	0.5	0.00^{wa}	0.05^{xab}	0.06^{xabc}	0.08^{*xdef}
SAhr	1	0.00^{wa}	0.02^{wxab}	0.03^{*wxbc}	0.06^{*xefg}
SAhr	0.5	0.00^{wa}	0.04^{wxab}	0.05^{*abc}	0.05^{*xefg}
SEhr	1	0.00^{wa}	0.03^{wab}	0.04^{*wabc}	0.09^{*xcde}
SEhr	0.5	0.00^{wa}	0.06^{xa}	0.06^{xabc}	0.08^{*xdef}
MRPs formed for 10 min					
EAmn	1	0.00^{wa}	0.04^{wxab}	0.06^{xabc}	0.12^{*yad}
EAmn	0.5	0.00^{wa}	0.05^{xab}	0.08^{xa}	0.14^{yab}
EEmn	1	0.00^{wa}	0.04^{wxab}	0.07^{xab}	0.08^{*xdef}
EEmn	0.5	0.00^{wa}	0.05^{xab}	0.08^{xa}	0.13^{yac}
SAmn	1	0.00^{wa}	0.05^{xab}	0.05^{*xabc}	0.07^{*xefg}
SAmn	0.5	0.00^{wa}	0.06^{xa}	0.08^{xa}	0.10^{*xbcde}
SEmn	1	0.00^{wa}	0.05^{xab}	0.08^{xa}	0.08^{*xdef}
SEmn	0.5	0.00^{wa}	0.05^{xab}	0.07^{xab}	0.16^{ya}

[1] Treatment see Table 1 for Description of codes.
[2] CONC: MRPs were added at 0.5 or 1% (w/w).
*Significantly different from the value of the control within the same storage period ($P < 0.05$).
[w-z]Different letters in the same row are significantly different ($P < 0.05$).
[a-f]Different letters in the same column are significantly different ($P < 0.05$).

The eight treatments processed with MRPs at 1 or 0.5% showed very little increase in aldehyde contents over 8 days of storage (Tables 3–6). These results agreed with those of Bailey *et al.* (1987), who found MRPs from histidine and glucose to be effective antioxidants in cooked ground beef stored at 4°C. The concentrations of hexanal and pentanal were much lower in cooked ground beef treated with 1 or 0.5% MRPs than in the control samples and were significant ($P < 0.05$) at days 2, 4, and 8 (Tables 3 and 4). Also, heptanal and octanal formation was significantly lower ($P < 0.05$) in the treated beef than in the controls at day 4 for samples with 1% MRPs and at day 8 for samples with 0.5% MRPs (Tables 5 and 6). However, 1% MRPs from EAhr and EEhr provided the best antioxidant activity in cooked ground beef, and the aldehyde concentration did not change significantly ($P < 0.05$) over the storage period. MRPs at the 1% level did not exhibit superior ($P > 0.05$) inhibitor activity to MRPs at 0.5% at any storage time, except for hexanal content with EEhr (Table 3) and octanal content with EAhr and EEhr (Table 6). In general, these results show that 1% MRPs provided strong antioxidative activity, but it was not statistically different ($P > 0.05$) than that of 0.5% MRPs. This indicated that 1% MRPs were equally as effective as 0.5% MRPs in retarding lipid oxidation when measured by aldehyde content.

Of all the volatile compounds observed, hexanal was the most predominant in the cooked beef samples throughout storage (Table 3). Pentanal was the second most predominant volatile compound (Table 4). From previous studies, hexanal was shown to be the predominant volatile compound in cooked beef (Dupuy *et al.*, 1987; St. Angelo *et al.*, 1987) and in cooked chicken (Dupuy *et al.*, 1987; Ang & Lyon, 1990; Ajuyah *et al.*, 1993). Hexanal and pentanal have been reported as primary markers to monitor lipid oxidation during storage (Melton, 1983; Dupuy *et al.*, 1987).

Effect of MRPs heated for 10 min

The antioxidative effects of MRPs prepared for 10 min and added at 0.5 or 1% as measured by aldehyde formation in cooked ground beef stored at 4°C are presented in Tables 3–6. Addition of MRPs formed for 10 min decreased the hexanal contents in the eight treatments, and the reduction was significant ($P < 0.05$) at days 4 and 8. However, hexanal contents of samples treated with 0.5% MRPs from SAmn were not significantly lower ($P > 0.05$) at day 4, and those of samples treated with 0.5% MRPs from EAmn and SEmn were not significantly lower ($P > 0.05$) at day 8 (Table 3). Pentanal formation with added MRPs was also lower ($P < 0.05$) than that of the control at 4 and 8 days, but was not lower ($P > 0.05$) at 0 and 2 days (Table 4). Heptanal and octanal content with added MRPs were not lower ($P > 0.05$) than those of the control at days 0, 2, and 4, but were slightly lower ($P < 0.05$) at day 8 (Tables 5 and 6). These results indicate that MRPs prepared for 10 min had lower antioxidant activity than

Table 6. Antioxidative effect of MRPs formed by heating for 1 h or 10 min as measured by octanal formation (mg octanal/kg meat) in cooked ground beef during storage at 4°C

TRT[1]	CONC[2]	Day 0	Day 2	Day 4	Day 8
CONT	0	0.01^{wa}	0.09^{x}	0.13^{x}	$0,24^{y}$
FRSH	0	0.02^{wa}	0.03^{*wbc}	0.04^{*wd}	0.05^{*wf}
MRPs formed for 1 h					
EAHr	1	0.00^{wa}	0.04^{wxbc}	0.04^{*wxd}	0.07^{*xef}
EAhr	0.5	0.00^{wa}	0.05^{xabc}	0.06^{*xbd}	0.12^{*ybd}
EEhr	1	0.01^{wa}	0.02^{*wxc}	0.05^{*wxcd}	0.06^{*xef}
EEhr	0.5	0.00^{wa}	0.08^{xab}	0.09^{xabc}	0.12^{*xbd}
SAhr	1	0.00^{wa}	0.03^{*wbc}	0.03^{*wd}	0.10^{*xdce}
SAhr	0.5	0.01^{wa}	0.06^{wxabc}	0.07^{xabd}	0.08^{*xdef}
SEhr	1	0.00^{wa}	0.04^{wxbc}	0.06^{*xbd}	0.13^{*ybd}
SEhr	0.5	0.00^{wa}	0.09^{xa}	0.09^{xabc}	0.12^{*xbd}
MRPs formed for 10 min					
EAmn	1	0.00^{wa}	0.07^{xab}	0.09^{xabc}	0.17^{*yabc}
EAmn	0.5	0.00^{wa}	0.08^{xab}	0.11^{xab}	0.16^{*yabc}
EEmn	1	0.00^{wa}	0.07^{xab}	0.12^{ya}	0.14^{*ybc}
EEmn	0.5	0.00^{wa}	0.07^{xab}	0.11^{xyab}	0.15^{*yab}
SAmn	1	0.00^{wa}	0.08^{xab}	0.10^{xab}	0.12^{*xbd}
SAmn	0.5	0.00^{wa}	0.09^{xa}	0.12^{xya}	0.15^{*yab}
SEmn	1	0.00^{wa}	0.07^{xab}	0.12^{ya}	0.12^{*ybd}
SEmn	0.5	0.01^{wa}	0.08^{xab}	0.10^{xab}	0.20^{ya}

[1]Treatment see Table 1 for Description of codes.
[2]CONC: MRPs were added at 0·5 or 1% (w/w).
*Significantly different from the value of the control within the same storage period ($P < 0.05$).
[w z]Different letters in the same row are significantly different ($P < 0.05$).
[a f]Different letters in the same column are significantly different ($P < 0.05$).

MRPs prepared for 1 h. This trend was in agreement with the TBA values.

The present investigation evaluated the antioxidative activity of MRPs prepared from protein hydrolysates and glucose in cooked ground beef during 8 days of refrigerated storage, as measured by both TBA values and aldehyde content. The results demonstrated that the pattern of antioxidative activity of MRPs was parallel to the formation of browning pigment when MRPs prepared for 1 h were compared with those formed for 10 min. Thus, heating protein hydrolysates and glucose for 1 h resulted in MRPs with greater antioxidative activity. Also, MRPs used at 1% were more effective inhibitors of lipid oxidation than MRPs added at the 0·5% level. MRPs formed for 1 h and added at the 1% level completely inhibited lipid oxidation in cooked ground beef over 8 days of refrigerated storage. These data suggest that synthetic antioxidants could be replaced by MRPs to increase shelf stability of cooked beef during refrigerated storage.

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