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INFLUENCE OF MEAT COMPONENTS ON ANTIOXIDANT ACTIVITY OF BEEF SARCOPLASMIC PROTEINS/MALONDIALDEHYDE REACTION PRODUCTS IN MODEL EMULSIONS

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ABSTRACT

The objective of this preliminary study was to determine the optimum concentration of antioxidants in the soluble fractions of Maillard Reaction Products (MRPs similar) (formed by sarcoplasmic proteins of beef and malondialdehyde) and to evaluate the influence of different meat components (individual and combined sarcoplasmic proteins, glucose and Fe^{2+}) on the antioxidant effect of this fraction in a model system. The results showed that the optimum concentration of MRPs similar that should be used to achieve an acceptable antioxidant activity in this model system is 3% (w/v). When the interactions of all the components of meat with MRPs similar were evaluated, the largest concentration of protein and glucose exerted an increase in the antioxidant effect of MRP for the concentrations of iron assayed.

KEYWORDS

Lipid peroxidation - myoglobin - iron catalysis - reducing sugar - oxidative stability.

INTRODUCTION

Researches on various model systems have been carried out to extrapolate their results to food substances; however, due to the complexity in the cmposition of food this is not always possible. Therefore, studies on more complex systems need to be carried out to competely understand the physiology of food substances.

Food emulsions physiologically contain a wide variety of components, including proteins, sugars, salts, surfactants and buffers; therefore, studies of the influence of these components on the oxidative stability of emulsions are required before obtaining a more complete picture of the importance of the various ingredients and their interactions.

These ingredients may act as either pro-oxidants or antioxidants depending on their chemical properties, the prevailing environmental conditions and their interactions with other molecular species involved in the lipid peroxidation reaction.

Reducing sugars have been shown to promote lipid peroxidation in aqueous colloidal dispersions. The origin of this pro-oxidative effect is the ability of reducing sugars to reduce transition metal ions to their most active states (for example, reduction of Fe^{3+} to Fe^{2+}).

Proteins may also inhibit or promote lipid peroxidation through nonenzymatic mechanisms. Casein and lactoferrin have been shown to be strongly antioxidative because of their ability to chelate iron ions.

However, condensation reactions between amino acids and products of lipid peroxidation may yield compounds similar to Maillard reaction products (MRP), the role of oxidized lipids being similar to the role of reducing sugars in the Maillard reaction. The final products of this reaction include high molecular weight melanoidins, which are brown compounds containig furan rings and nitrogen, and also possess antioxidant activity [1].

In a previous work, the authors of this study have reported that the soluble products, Maillard – similar reaction products (MRPs – similar), formed by sarcoplasmic proteins of beef and malondialdehyde (one of the secondary products of lipid peroxidation) have the two following important properties: high reducing power, with a good scavenging ability toward 2,2-diphenyl-1-picrylhyrazol (DPPH) and superoxide radicals, and very effective in the inhibition of the hydroperoxides formation.

Although these reaction products are pale brown in colour, probably because they are produced in the early stages of Maillard reaction, they have an excellent antioxidant activity in fused lard and show a good performance in linoleic acid/water emulsion systems [2], the reacion being concentration- dependent in the latter model. However this effect can not be observed when similar MRPs are added at cooked meat products [3] probably due to the interference by some of the components present in meat.

This preliminary study was undertaken to establish the interactions between MRPs similar and meat components using model systems that simulate raw- meat emulsions, before proceeding to further investigations through systems that simulate cooked meat, where in the major complexity of these interactions is expected to be unraveled.

This work has been designed to determine the optimum antioxidant concentration of the soluble fraction of MRPs-similar, and the influence of different meat components (individual and combined sarcoplasmic proteins, glucose and Fe^{2+}) on its antioxidant effect.

MATERIALS AND METHODS

Preparation of Soluble MRPs-Similar

Sarcoplasmic proteins were prepared according to the procedure of Wagner and Añón [4] from 20 g local - market beef. The proteins obtained were suspended in a solution containig 0,25 M sucrose, 1 mM EDTA; phosphate buffer pH 7.6. Protein concentration was determined by a modified Biuret method [5].

Malondialdehyde (MAD) was prepared by the acid hydrolysis of 10 µl of 1,1,3,3-tetrametoxypropane (TMP) according to the procedure described by Kakuda, Stanley, and Van de Voort [6]. The stock solution was adjusted to pH 7.6. MDA concentration was quantified by the absorbance at 245 nm using the expression $\varepsilon = 13700\ M^{-1}\ cm^{-1}$.

Sarcoplasmic proteins were incubated with MDA in a ratio 3.5:1 at 80 ºC for 4 h (under moderated agitation), to obtain the MRPs – similar. After incubation the soluble fraction was dried under vacuum (at 50 ºC and 4000 Pa) to get the dry extract.

Antioxidant Activity

Model Systems

Linoleic acid emulsions were prepared by mixing 0.285 g of linoleic acid (ICN biomedicals Inc, Ohio, USA), 0.289 g of Tween 20 as emulsifier and 50 ml of phosphate buffer (pH 7.17), and homogeneizing the mixture for 5 min.

To determine the optimum antioxidant activity, MRPs - similar were added to this emulsion at final concentrations of 1%, 3%, 5% and 10 % of the dry extract. Butylated hydroxyanisole (BHA) was used as the control (0.1%).

Sarcoplasmic proteins, glucose and Fe^{2+} (from $FeSO_4 .7\ H_2O$ 0.018 M) were added to the model systems, each at two final concentrations of 0.3% and 0.6%; 0.1% and 1%; 5 and 25 ppm respectively.

Model systems obtained from the individual and combined treatments described below were incubated at 37 ºC for 24 h. The course of oxidation was monitored by measuring the peroxide value (PV).

The antioxidant activity at the end of the assay time was expressed as the reduction in the percentage (RP%) of PV, with a control containing no antioxidant being considered as 0%.

RP % = [(PV without antioxidant) - (PV with antioxidant) / (PV without antioxidant)] . 100. A higher percentage indicates a higher antioxidant activity.

Determination of Peroxide Values

The ferric thiocyanate (FCT) method was adapted from the method FIL-IDF 74A:1991. Samples (0.02 g) dissolved in 9.8 ml of methanol:chloroform (70:30) solution were mixwd with 0.1 ml of 30% ammonium thiocyanate. Subsequently, 0.1 ml of ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture and precisely 5 minutes later, the absorbance of the resulting red colour was measured at 501 nm against the solvent solution as blank. PV were expressed in terms of millimoles oxygen produced per kilogram of sample.

Statistical Analysis

All analyses were carried out in triplicate. The data were recorded as means ± standard deviations and analyzed using the software package Statgraphics Plus for Windows 4.0. Analysis of variance (ANOVA) was carried out to test for any significant differences at ($p<0.01$).

RESULTS AND DISCUSSION

Determination of Optimum Concentration MRPs - Similar

Although many parameters are available to monitor the extent of lipid peroxidation, PV is the parameter generally chosen to analyze the antioxidant activity [7]. Furthermore, hydroperoxides formation has been reported to be the most sensitive indicator for the evaluation of antioxidant activity of products from MRPs - similar when the course of peroxidation was monitored by measuring the levels of conjugated dienes, peroxide values and thiobarbituric acid - reactive substances [8].

The range of concentrations used in the reported article was wide (1% and 10 % w/v). Consequently, to optimize the optimun quantity of antioxidant that should be added, two intermediate concentrations have also been assayed in this study.

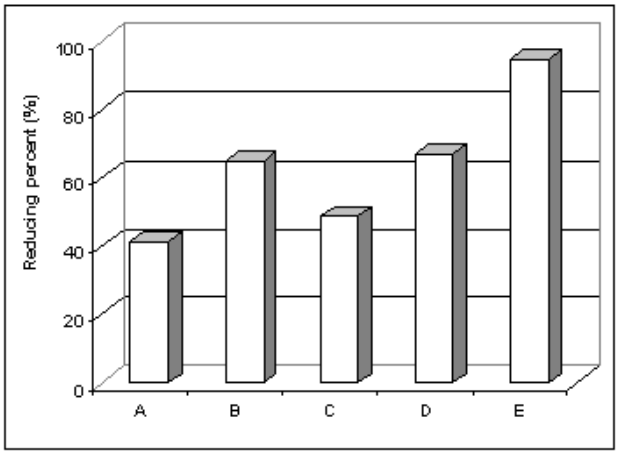

Figure 1: Reduction in percentage of hydroperoxide formation of MRPs- similar and control. (A) 1% MRP; (B) 3% MRP; (C) 5% MRP; (D) 10% MRP and (E) 0.01% BHA.

Figure 1 shows the reduction percent in peroxidation (expressed as hydroperoxide formation) at different concentrations of antioxidants and BHA (0.01%) in model emulsion systems at 37º C. A significant difference ($p<0.001$) in RP was found between A, C and B, D and BHA samples.

As RP obtained with 10 % antioxidant was similar to that obtained with 3% (73 % of RP), this last has been chosen for the subsequent interaction assays.

Sarcoplasmic Protein Influence on Antioxidant Activity of MRPs - similar

A variety of physico-chemical mechanisms have been identified as contributing to the pro-oxidant or antioxidant activity of proteins, including catalysis of specific reactions, chelation of transition metals or other reactive species, preferential peroxidation, and free radical scavenging [9]. Sarcoplasmic proteins such as metmyoglobine and other endogenous food catalyzers could accelerate lipid peroxidation at low concentrations, but at high concentrations, they inhibit lipid peroxidation [10]. Heme proteins have also been established to serve as sources of free iron during their denaturation, thus catalyzing lipid autooxidation [11].

The reductions in the percentage of hydroperoxide formation by MRPs in model systems, both with and without proteins, and with only BHA as control are presented in the Figure 2.

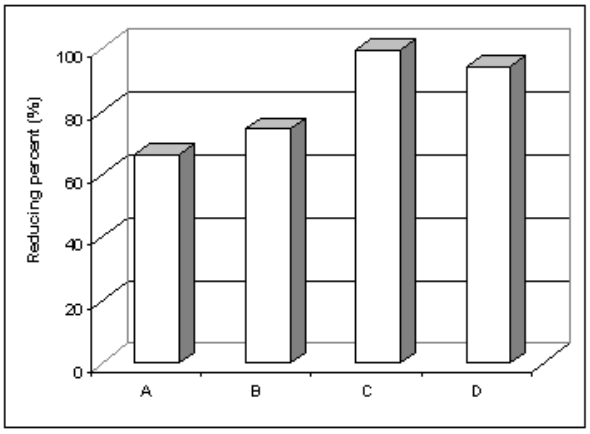

Figure 2: Reduction in percentage of hydroperoxide formation of MRPs- similar when it is added to model systems without and with sarcoplasmic proteins (SP) and control. *(A): 3% MRP; (B) 3% MRP + 0.3% SP; (C) 3% MRP + 0.6% SP and (C) 0.01% BHA.*

The percentages of reduction in model systems containig both protein concentrations are higher than in emulsions with only MRPs - similar, the antioxidant effect being

dependent on protein concentration. Furthermore, the protein concentration of 0.6% plus MRPs increased significantly ($p>0.001$) the RP, exerting a higher antioxidant effect than BHA alone. This suggests that the use of proteins enhances the inhibitory effect of MRPs - similar on the lipid peroxidation.

Influence of Glucose on Antioxidant Activity of MRPs Similar

Shimada, Fujikawa & Nakamura, [12] have reported that reducing sugars promote lipid peroxidation in aqueous colloidal dispersions due to their ability to reduce transition metal ions to their most active state.

Nevertheless, the hydroxyl groups of sugars and sugar analogs may inhibit lipid peroxidation by the formation of hydrogen bonds between the hydroxyl groups and hydroperoxides, rather than by scavenging lipid radicals [13].

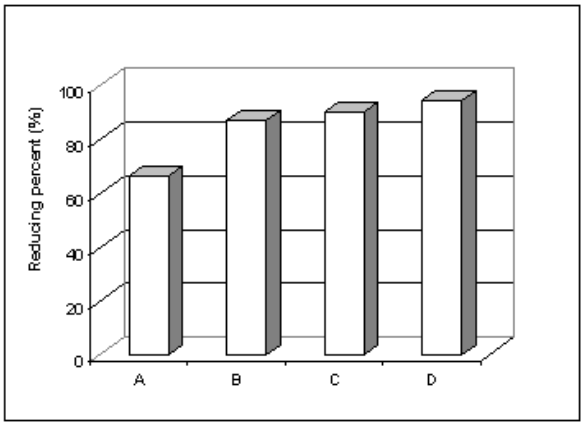

Figure 3: Reduction in percentage of hydroperoxide formation in model systems containing MRPs-similar without and with glucose (Glu) and control. *(A): 3% MRP; (B): 3% MRP + 0.1 g Glu; (C): 3% MRP + 1 g Glu.*

As seen in Figure 3, two different concentrations of glucose added into MRPs emulsions show an increase in the percentage of reduction of lipid peroxidation, without any significant difference ($p<0.001$) either between them or in comparison with BHA.

This increase in the antioxidant activity may be related to the reducing action of sugars on the carbonyl groups of MRPs, which are formed during their synthesis process, thus improving their ability to donate hydrogen and trap free radicals.

Influence of Iron ions on Antioxidant Activity of MRPs - Similar

Cations, such as iron and copper, are capable of directly breaking down unsaturated lipids into alkyl radicals, but this reaction occurs extremely slowly and is therefore not believed to be important in promoting lipid peroxidation [14]. The most likely mechanism for the acceleration of lipid peroxidation in emulsion is the decomposition of lipid hydroperoxides (ROOH) into highly reactive peroxyl (ROO) or alkoxyl (RO) radicals by the transition metals or other pro-oxidants.

Moreover many substances can retard metal - catalyzed lipid peroxidation reaction, through a variety of mechanisms involving chelation of transition metals, including prevention of metal redox cycling, formation of insoluble metal complexes, occupation of metal - coordination sites and steric hindrance of interactions between metals and lipid substrates, among others.

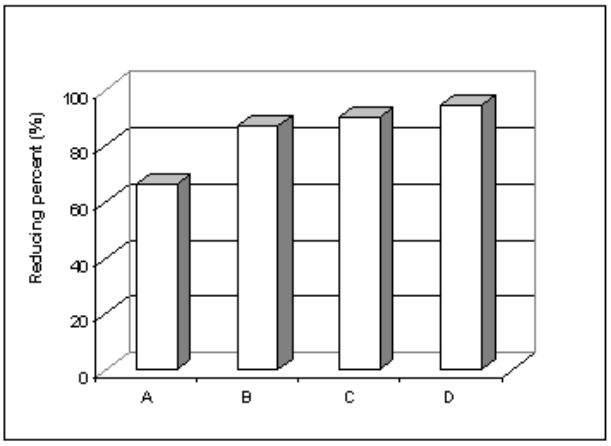

Figure 4: Reduction in percentage of hydroperoxide formation in model systems with MRPs–similar, in both absence and presence of iron (Fe^{2+}) and control. (A) *3% MRP; (B), 3% MRP + 5 ppm Fe^{2+}; (C) 3% MRP + 25 ppm Fe^{2+}; and (D) 0.01% BHA.*

Figure 4 shows the reduction in percentage of lipid peroxidation in a model emulsion, both with and without iron plus MRP, at two different concentrations of metal and with BHA alone. From the figure it can be seen that the two concentrations of iron increased ($p< 0.001$) the antioxidant activity of MRPs.

As this effect can not be easily explained, an assay of the emulsions with only iron has been carried out, and a large pro-oxidant effect of the metal is observed (data no shown). A possible explanation for this phenomenon (Increased antioxidant effect) might be that reaction between Fe and MRPs - similar, results in a spatial change in the structure of MRPs

- similar, which increases the exposure of the reactive groups towards the lipid peroxy radicals, thereby improving the antioxidant effect.

Influence of Interaction between all the meat components on the Antioxidant Activity of MRPs Similar

When the interactions of all the meat components with MRPs - similar are evaluated, the greatest concentrations of proteins and glucose exert a significant increase ($p< 0.001$) on the antioxidant effect of MRP for both iron concentrations assayed (Figure 5a and 5b). This effect may be due to the reducing power of glucose, which increases the inhibitory effect exerted by proteins and MRPs. This effect is much higher for the lowest iron concentration, possibly because the reducing environment created by glucose is not enough to maintain the oxidation status of Fe^{2+} when the iron concentration is higher.

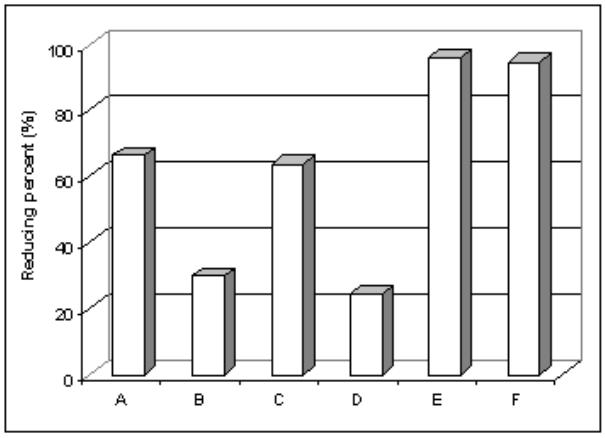

Figure 5 (a): Reduction in percentage of hydroperoxide formation in model systems with MRPs-similar, singly and in combination with all meat components (at 5 ppm Fe^{2+}) and control. *(A): 3 % MRP; (B) 3% MRP + 5 ppm Fe^{2+} + 0.3% SP + 0.1 g Glu; (C)3% MRP + 5 ppm Fe^{2+} + 0.3% SP + 1 g Glu (D); 3% MRP + 5 ppm Fe^{2+} + 0.6% SP + 0.1 g Glu (E) 3% MRP + 5 ppm Fe^{2+} + 0.6% SP + 1 g Glu; and (F) 0.01% BHA.*

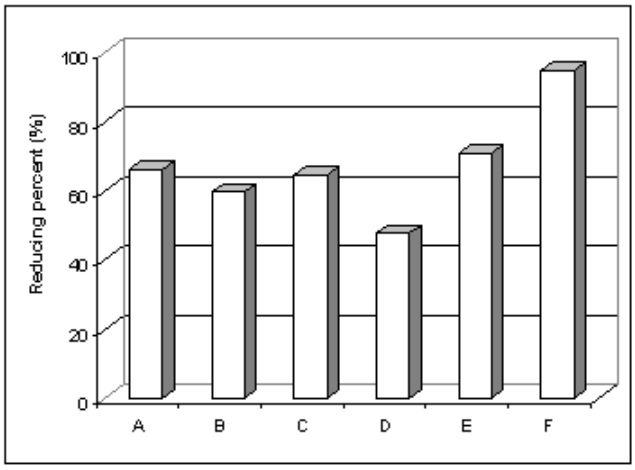

Figure 5 (b): Reduction in percentage of hydroperoxide formation in model systems with MRPs–similar, alone and with all meat components (at 25 ppm Fe^{2+}) and control. *(A): 3 % MRP; (B) 3% MRP + 25 ppm Fe^{2+} + 0.3% SP + 0.1 g Glu; (C) 3% MRP + 25 ppm Fe^{2+} + 0.3% SP + 1 g Glu; (D) 3% MRP + 25 ppm Fe^{2+} + 0.6% SP + 0.1 g Glu; (E) 3% MRP + 25 ppm Fe^{2+} + 0.6% SP + 1 g Glu; and (F)0.01% BHA.*

Some of the assayed combination, as for example, small concentrations of glucose and iron, decreased the inhibitory effect of MRPs - similar, although they have not pro-oxidant behavior.

CONCLUSIONS

This preliminary study showed that the optimum concentration of MRPs - similar that should be used to achieve an acceptable antioxidant activity in this model system is 3%.
Moreover none of the meat components evaluated in the concentrations tested, in both the individual or combination forms, when interacting with the MRPs - similar turned it into a pro-oxidant additive.

Therefore it is necessary to conduct researches on cooking temperatures and other concentrations of proteins, to elucidate the mechanism of the pro-oxidant behavior of this additive in cooked meat products.

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