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Accomplishments

Investigating mechanisms of lipid oxidation in muscle food systems using site-directed mutagenesis of sperm whale myoglobin and human hemoglobin. The problem in understanding the pathway by which heme proteins promote lipid oxidation is that several processes such as metHb formation, ferryl Hb radical formation, heme dissociation, heme destruction, and iron release can all occur in a very short time sequence and simultaneously so that the most relevant step related to lipid oxidation is obscured. Our approach is to use site-directed mutagenesis of sperm whale myoglobin to tease out the relevant pathways. Site-directed mutagenesis involves altering the myoglobin gene so that alternative amino acids result in the expressed mutant proteins compared to the native protein. Chemical and physical properties such as oxygen affinity, met formation rate, heme affinity, heme destruction, and Hb subunit

formation can be modulated by this amino acid substitution approach. The native and mutant proteins are examined in lipid substrates (e.g. microsomes and washed fish muscle). The major findings have been that mutants with high heme affinity were found to be especially weak promoters of lipid oxidation while mutants with lower heme affinity effectively oxidize the lipid substrates. Mutants especially prone to heme destruction were weak promoters of lipid oxidation which suggested liberating iron atoms decreased rates of lipid oxidation. We have also uncovered the ability of Hb subunits to accelerate lipid oxidation and the relative roles of met formation compared to heme dissociation. The overwhelming evidence is that released heme is the critical entity that promotes lipid oxidation in the model lipid substrates.

Partitioning and inhibition of lipid oxidation in mechanically separated turkey by components in cranberry press cake. Extracts from cranberry press cake were prepared either using ethanol or an ethyl acetate-acetone mixture. The press cake extracts were compared with extracts from cranberry juice powder (CJP) prepared using chloroform: methanol (1:1), for their ability to inhibit lipid oxidation in mechanically separated turkey (MST). Due to the susceptibility of muscle membrane lipids to oxidation, the ability of quercetin in the extracts to partition between the aqueous and membrane phases was studied. Membrane suspensions were prepared from MST. Partitioning of quercetin was quantified using HPLC. Oxidation was studied by measuring thiobarbituric acid reactive substances and lipid peroxides. The effectiveness of the extracts to inhibit lipid oxidation was, CJP extract > ethyl acetate extract of press cake \geq ethanol extract of press cake. The amount of quercetin in the extracts and the amount of quercetin that partitioned into the membranes followed the same order. However, the total phenolic content of the extracts did not follow the order as that of inhibitory power. The phenolic content of the extracts decreased, ethyl acetate extract > ethanol extract of press cake \geq chloroform extract of CJP. Irrespective of the extraction method, around 78% quercetin from the extracts partitioned into the membranes. It could be concluded that increasing the amount of quercetin in the press cake extracts increases the ability of the extracts to inhibit lipid oxidation in MST. Hence, a proper choice of solvents and extraction method which would increase the amount of quercetin in the press cake extracts might increase the antioxidant potential of the extracts and hence their economic value.

Comparison of Solvent and Microwave Extracts of Cranberry Press Cake on the Inhibition of Lipid Oxidation in Mechanically Separated Turkey. Cranberry press cake, an underutilized by-product of the cranberry processing industry is a potential source of food antioxidants. The objective of this research two fold 1) To prepare extracts from cranberry press cake using solvent extraction (SE) and microwave assisted solvent extraction (MASE) 2) To test the ability of these extracts to inhibit lipid oxidation in mechanically separated turkey (MST). Water, ethanol and acetone were used as extraction solvents. Heating press cake prior to extraction with 70% ethanol increased antioxidant efficacy compared to extracting unheated press cake. Water extracts were least effective in inhibiting lipid oxidation. The most effective extracts were obtained by SE with 100% acetone or MASE with 100% ethanol. There was a poor correlation ($r = 0.69$) between the quercetin equivalent of the extracts and their ability to inhibit TBARS formation in MST. The correlation coefficient between the total amount of quercetin in the extracts and the number of days of TBARS inhibition in MST was 0.87 indicating that there are polyphenols other than quercetin may play a role in the antioxidant activity of the extracts.

Ability of various polyphenolic classes from cranberry to inhibit lipid oxidation in mechanically separated turkey and cooked ground pork. The ability of components from cranberry powder to inhibit lipid oxidation processes in mechanically separated turkey (MST) and cooked ground pork was assessed. Fractions enriched in phenolic acids (Fr. 1), anthocyanins (Fr. 2), flavonols (Fr. 3 and 4), and proanthocyanidins (Fr. 5 and 6) were prepared. Fraction 4 showed the greatest inhibitory effect on lipid oxidation in cooked pork (81% inhibition), followed by fraction 3 (56%), 2 (45%), 5 (46%), 1 (26%) and 6 (22%) during storage at 2°C. Fraction 4 was also the most effective fraction in inhibiting TBARS formation in MST. Fraction 4 was found to partition into lipid phases more readily than the other fractions. Ethanol was the most effective carrier solvent of polyphenolics compared to propylene glycol and water as carrier. Quercetin, a non-glycosylated flavonol present in cranberry powder, inhibited lipid oxidation in MST at low concentrations. These results suggest that flavonol aglycones were the most effective in inhibiting lipid oxidation compared to the other classes of polyphenolics.

Inhibition of hemoglobin-mediated lipid oxidation in washed fish muscle by cranberry components. Fractions enriched in phenolic acids (Fraction 1), anthocyanins (Fraction 2), flavonols (Fraction 3 and 4) and proanthocyanidins (Fraction 5 and 6) were prepared from cranberry powder using Sephadex LH-20 chromatography. Fraction 2, 3, 4, and 5 had nearly equivalent reactivity in the total phenolate assay employed per mg dry weight of each fraction while fraction 1 and 6 were less reactive. The ability of cranberry fractions to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals as well as their inhibitory effects on hemoglobin (Hb)-mediated lipid oxidation in washed cod muscle were assessed. Addition of cranberry fractions at a level of 74 µmol quercetin equivalents per kg of washed cod muscle extended the induction time of thiobarbituric acid reactive substances (TBARS) formation in the order: Fraction 1, Fraction 3, Fraction 4 > Fraction 2 > Fraction 5 > Fraction 6. This suggests that oligomeric polyphenols (e.g. proanthocyanidins) were least effective at inhibiting Hb-mediated lipid oxidation in washed cod muscle compared to the other classes of polyphenolics in cranberry. The ability of the different cranberry fractions to scavenge DPPH radicals did not reflect their relative ability to inhibit lipid oxidation in the washed cod muscle system. Quercetin was tentatively identified as a component in cranberry that was especially effective at inhibiting Hb-mediated lipid oxidation. The ability of flavonol and proanthocyanidin-enriched fractions to inhibit Hb-mediated lipid oxidation in spite of efforts to wash away the added polyphenolics prior to Hb addition indicated these classes of polyphenolics had binding affinities for insoluble components of washed cod muscle.

Impact Statements

1. Mechanisms of lipid oxidation mediated by heme proteins have been elucidated
2. Methods to inhibit lipid oxidation in muscle foods are described.

Publications

Li, R.; Richards, M.P. and Undeland, I. 2005. Characterization of aqueous components in chicken breast muscle as inhibitors of hemoglobin-mediated lipid oxidation.

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