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Lipid Deterioration Initiated by Phagocytic Cells in Muscle Foods: β-Carotene Destruction by a Myeloperoxidase-Hydrogen Peroxide-Halide System

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In fresh muscle foods, phagocytic cells may conceivably initiate and accelerate lipid oxidation. Fish leukocytes were obtained by density gradient centrifugation. The isolated neutrophils were rich in myeloperoxidase which was extracted from the leukocytes at pH 4.0 in the presence of 0.3 M sucrose. The crude enzyme showed a peroxidase activity of about 160 nmol of purpurogallin (formed from pyrogallol) (mg of protein)$^{-1}$ min^{-1}. An oxidation system using discoloration of β-carotene as an index of lipid peroxidation was developed. Myeloperoxidase from fish leukocytes caused rapid discoloration of β-carotene in the presence of H_2O_2 and iodide or bromide ions. Purified myeloperixidase cooxidized β-carotene in the presence of chloride ions. No destruction of β-carotene occurred when halogen ions, H_2O_2, or the enzyme was omitted from the system. The data indicate that leukocytes may be a focus for initiation of lipid peroxidation in biological tissues.

Oxidative degradation of lipids is a common and in some cases a major cause of deterioration of a wide range of foods, especially muscle foods, following harvesting or processing (Pearson et al., 1977). A critical question in the control of lipid oxidation concerns the source of the primordial free radicals that initiate the oxidative deterioration of lipids in muscle foods. Many reactions occurring routinely in vivo involve potent prooxidative states which are normally controlled by appropriate enzymes (Flohé, 1982). However, following slaughter, cutting, or exposure of meat tissues to air, such transient prooxidant states may no longer be controlled. We suggest that in fresh muscle foods, phagocytic cells may conceivably initiate and accelerate lipid oxidation.

The process of phagocytosis is associated with a dramatic burst of oxidative metabolism by phagocytic cells (neutrophils, eosinophils, monocytes, and macrophages) (Sbarra and Karnovsky, 1959). Thus, during ingestion of particulate materials (microorganisms, dead cells, oil drops, etc.) the consumption of oxygen is enhanced 10–15-fold within a few seconds after contact with the stimulating substances (Sabarra and Karnovsky, 1959; Takanaka and O'Brien, 1979). Hydrogen peroxide is concurrently produced in large amounts and accumulates in the medium surrounding the phagocytes (Iyer et al., 1961; Root et al., 1975), especially when its enzymatic degradiation is inhibited (Nakagawara et al., 1981). Phagocytic leukocytes also generate large amounts of superoxide anion radical during particle ingestion, and this is released into the medium surrounding the phagocytes (Babior et al., 1973; Johnston et al., 1975). The superoxide is most likely an intermediate in the formation of hydrogen peroxide by phagocytic cells (Root and Metcalf, 1977).

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Lipid peroxidation occurs during phagocytosis as indicated by a considerable loss of arachidonic acid (Shohet et al., 1974). These authors suggested that hydroxyl radicals (HO·) were the possible initiating factors in this oxidation. The initiation of lipid peroxidation in model systems by oxygen free radicals has been described by several researchers (Fong et al., 1973; Kellogg and Fridovich, 1975; Svingen et al., 1978; Cohen and Sinet, 1980; Fridovich and Porter, 1980) and reviewed by Tien et al. (1981). It is conceivable that granulocytes and monocytes in the blood of muscle tissues of animals, avian, and fish species can contribute to lipid oxidation and subsequent deterioration.

The highly reactive products of oxygen, namely, superoxide anion (O_2^{-}·), hydrogen peroxide (H_2O_2), the hydroxyl radical (HO·), and singlet oxygen ($^1O_2$) have been implicated as microbiocidal agents in the phagocytic cells (Gabig and Babior, 1981). The microbiocidal activity of H_2O_2 is considerably enhanced by myeloperoxidase (MPO) in the presence of a halide ion (I^- or Cl^-) (Klebanoff, 1975; Babior, 1978; Klebanoff and Clark, 1978). During the reaction of MPO with chloride or other halides, chlorine, hypochlorite, and other halide equivalents are increased (Harrison and Schultz, 1976).

The blood of fish contain three main groups of leukocytes—the granulocytes (neutrophils, eosinophils, and basophils), monocytes, and lymphocytes (Stachell, 1971). The granulocytes contain myeloperoxidase which is involved in phagocytic and other protective functions.

In the current study we examined the activity of a myeloperoxidase-like enzyme isolated from fish leukocytes and assessed its capacity to initiate lipid deterioration in the presence of H_2O_2 and halides.

EXPERIMENTAL SECTION

Materials. Hydrogen peroxide (30%), sodium chloride, potassium bromide, sodium citrate, and the potassium mono- and diphosphate were purchased from Mallinckrodt, St. Louis, MO. The β-carotene, pyrogallol, purpu-

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rogallin, percoll, and sodium iodide were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium fluoride was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). Purified canine myeloperoxidase was a gift from Dr. J. Schultz (Papanicolaou Cancer Research Institute, Miami, FL).

Methods. *Leukocyte Preparation.* Blood samples were obtained from rainbow trout by vein puncture from the area of the caudal sinus. The blood was diluted 1:1 with citrated buffer phosphate, pH 7.2. The citrated buffer contained KH_2PO_4 (50 mM), K_2HPO_4 (65 mM), and sodium citrate (34 mM) which was adjusted to pH 7.2 by KOH (1.0 M). The leukocytes were separated from the citrated blood by density centrifugation in a 33% v/v of percoll in the presence of sucrose (0.25 M). The density gradient was formed by centrifuging the percoll–sucrose solution at 48000 *g* for 35 min at room temperature. Blood suspensions (4 mL) were loaded onto percoll (25 mL) and centrifuged at 400*g* for 10 min. The leukocytes were collected from the gradient by removal of the visible white fraction with a pipette. The entire procedure was carried out at 4 °C (Simon, 1981).

The fractions collected from the percoll were diluted 1:1 with 0.15 M KCl and centrifuged at 1100*g* for 10 min. The pellet containing the leukocytes was stored at −20 °C as raw material for myeloperoxidase extraction.

Staining Identification. Portions of the leukocyte pellet were taken for staining before freezing. Smears of the pellet were air-dried and fixed in absolute methanol and stained with Wright's stain (Christensen et al., 1978). The peroxidase granules in neutrophils were stained after the interaction of the pellet material (diluted 1:1 with 0.15 M KCl) with H_2O_2 (1 mM) and pyrogallol (3 mM) for 30 min. Photographs of the stained cells were taken using a Zeiss photomicroscope.

Isolation of Myeloperoxidase. The crude myeloperoxidase was prepared from the pelleted fish leukocytes dispersed in sucrose (0.34 M) at pH 4 according to the method developed for human myeloperoxidase by Olsson et al. (1972) and Matheson et al. (1981). From 16 mL of citrated blood we obtained 2 mL of crude myeloperoxidase active extract with a protein concentration of 20–30 μg/0.1 mL. The partially purified myeloperoxidase concentration was calculated from an extinction coefficient of $E^{1\%}_{430nm}$ = 120, as reported by Agner (1958).

Myeloperoxidase Assay. Myeloperoxidase activity was determined spectrophotometrically by a method based on the peroxidation of pyrogallol to purpurogallin. The absorbance changes at 420 and 30 nm were recorded by a Cary DB (Model 214) spectrophotometer. In preliminary studies it was found that the purpurogallin formed by oxidation of pyrogallol has an absorbance at 300 nm that is 9.3-fold higher than that at 420 nm. Therefore, we routinely monitored the changes in absorbance at 300 nm to measure myeloperoxidase activity (Figure 1). A typical assay contained 0.1 mL of enzyme homogenate (15–30 μg of protein), 0.1 mL of H_2O_2 (4 mM), and 0.1 mL of pyrogallol (4 mM) in 1.7 mL of sodium acetate buffer (0.05 M), pH 6.0, at 25 °C. One unit of myeloperoxidase activity formed 1 nmol of purpurogallin/min at 25 °C at pH 6.0. We used an extinction coefficient of 2470 at 420 nm for purpurogallin (Dawson et. al., 1969).

Carotene Destruction. The discoloration of β-carotene was used to determine the cooxidation reaction resulting from the oxidation of halides ions by the myeloperoxidase–H_2O_2 complex. The oxidative equivalents derived from the halides could cooxidize other molecules. We developed a method based on the enzymatic cooxidation of β-carotene. The technique consists of monitoring the decrease in absorbance at 460 nm in a cuvette containing the enzyme system. The test sample contained 1.5 mL or buffered carotene solubilized by Tween 20 (Kanner et al., 1976), 0.1–0.4 mL of active reagents (enzyme, H_2O_2, and halides), and distilled water to a final volume of 2.0 mL. The concentrations of the reactants were β-carotene, 14 μM, Tween 20, 0.05%, and sodium acetate buffer, 0.1 M. The sample in the control cuvette contained all the reagents except β-carotene. The initial linear rate of decrease in absorbance was computed from a recorder tracing and converted into the rate of carotene destruction in nanomoles per minute.

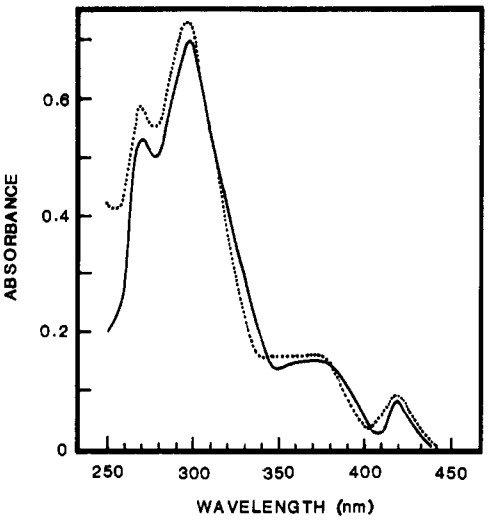


Figure 1. The absorbance of purpurogallin (—) and a mixture (---) of crude myeloperoxidase (12 μg of protein) extracted from fish leukocytes in the presence of H_2O_2 (4 mM), pyrogallol (4 mM), and acetate buffer (0.05 M), pH 6.0, at 25 °C.

Protein concentration was determined by the Lowry procedure (Lowry et al., 1951) using bovine serum albumin as the standard.

RESULTS

The separation of leukocytes from citrated fish blood by low-speed centrifugation or sedimentation in the presence of 6% dextran (M_r 100 000–200 000) (Skoog and Beck, 1956) was not satisfactory because of contamination by red blood cells. However, fish leukocytes free from erythrocytes were obtained by density gradient centrifugation using the procedure of Simon (1981). The isolated neutrophils were rich in MPO. Staining with pyrogallol–H_2O_2 revealed the dark spots of purpurogallin, corresponding to intracellular vacuoles loaded with myeloperoxidase (Figure 2).

The isolation of MPO from the whole blood by acetone precipitation following solubilization and fractionation by ammonium sulfate failed to give a fraction with high myeloperoxidase activity. However, separation of the leukocytes from other blood components (as described under Methods) and homogenization at pH 4.0 in the presence of 0.34 M sucrose followed by centrifugation yielded a crude preparation high in myeloperoxidase activity.

The crude enzyme showed a peroxidase activity of 160 pupurogallin units (produced from pyrogallol)/mg of protein. The optimal concentration of H_2O_2 for the peroxidation of pyrogallol was 4 mM, and the activity remained high even at a concentration of more than 15 mM H_2O_2 (Figure 3). No oxidation occurred in the absence of H_2O_2. The activity increased with increased concentration of pyrogallol, and no inhibition was observed even

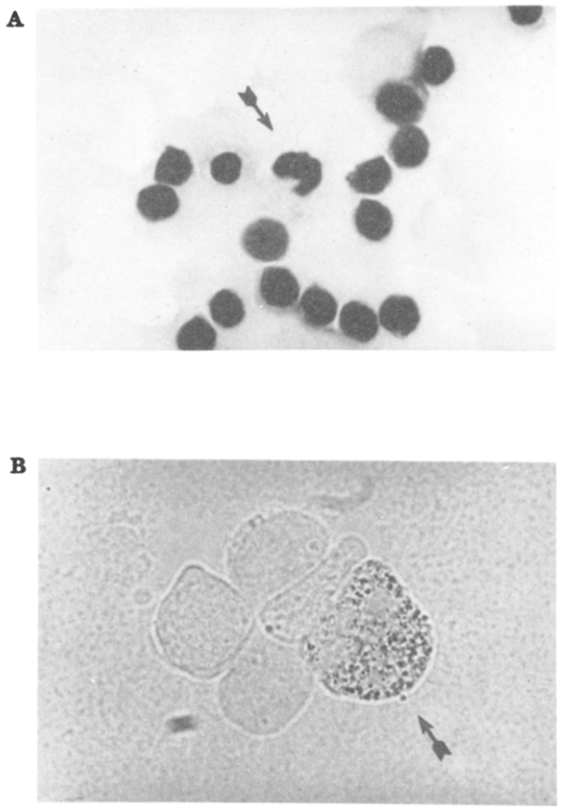


Figure 2. Leukocytes from rainbow trout blood stained with Wright's stain (A) and with pyrogallol (3 mM) and H_2O_2 (1 mM) (B). The dark areas, spots, correspond to vacuoles containing myeloperoxidase.

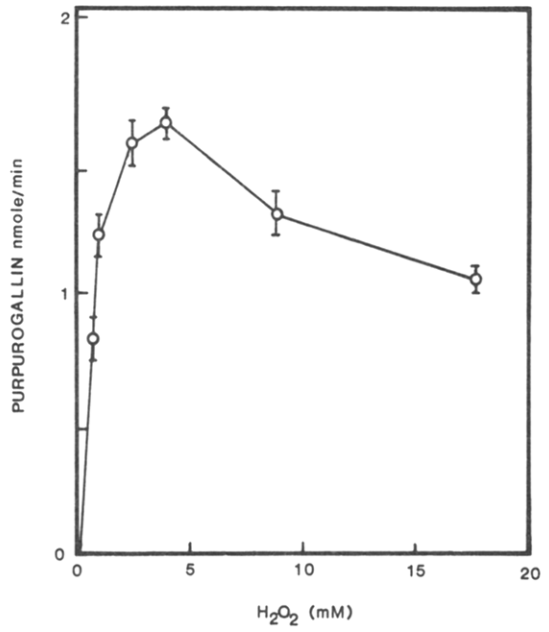


Figure 3. Pyrogallol peroxidation by myeloperoxidase-like enzyme as affected by H_2O_2 concentration. The reaction mixture contained crude enzyme from (9 μg) fish leukocytes and pyrogallol (4 mM) in 2 mL of acetate buffer (0.05 M) pH 6.0, at 25 °C. Error bars denote standard deviation (n = 3).

with a concentration of 8 mM (Figure 4). The enzymatic peroxidation of pyrogallol was optimal at pH 6.5. At higher pH, pyrogallol was oxidized very rapidly by an nonenzymatic process (Figure 5). The increase in the peroxidation of pyrogallol was almost linear with respect to the protein concentration in the range used (Figure 6).

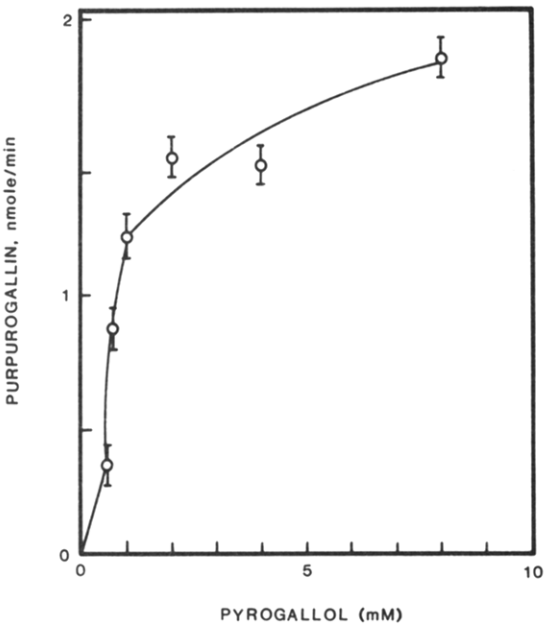


Figure 4. Peroxidation of pyrogallol by myeloperoxidase-like enzyme as affected by pyrogallol concentration. The reaction mixture contained crude enzyme (9 μg of protein from fish leukocytes) and H_2O_2, 4 mM, in 2 mL of acetate buffer, 0.05 M, pH 6.0, at 25 °C. Error bars denote standard deviation (n = 3).

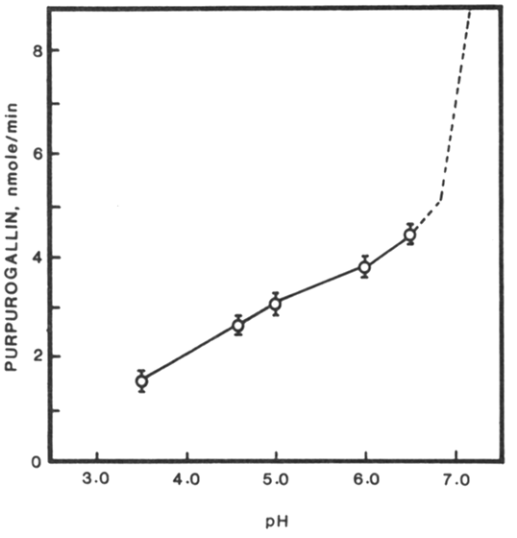


Figure 5. Effect of pH on pyrogallol peroxidation by myeloperoxidase-like enzyme. The reaction mixture contained crude enzyme (32 μg of protein) from fish leukocytes, pyrogallol (4 mM), and H_2O_2 (4 mM) in 2.0 mL of acetate and phosphate buffer (0.05 M) at 25 °C.

These data indicated that fish leukocytes had a significant MPO activity. To determine if MPO from fish could conceivably initiate degradation of lipids, we developed a complete cooxidation system using discoloration of β-carotene as an index of lipid oxidation. Myeloperoxidase from fish leukocytes caused rapid discoloration of β-carotene in the presence of iodide and bromide ions (Figure 7). An induction period was observed during the first minutes of the reaction when low amounts of iodide or bromide ions were present. No destruction was observed when chloride ions replaced the other halides or when iodide, bromide, H_2O_2, or the enzyme was omitted from the system (results not shown).

The destruction of β-carotene was found to be highly senstive to H_2O_2 concentration (Figure 8). In the presence of Br^- ions, myeloperoxidase showed an optimum activity

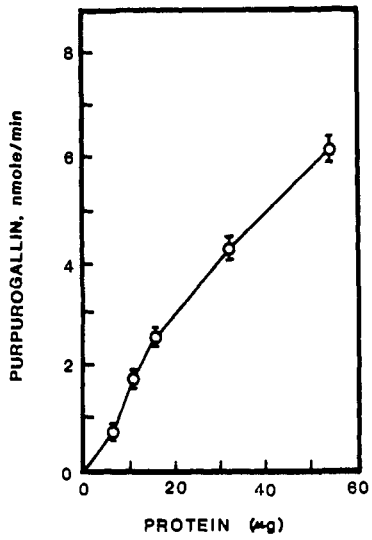


Figure 6. Effect of enzyme concentration on pyrogallol peroxidation. Reaction mixture contained pyrogallol (4 mM) and H_2O_2 (4 mM), in 2 mL of acetate buffer (0.05 M), pH 6.0, and various concentration of myeloperoxidase-like enzyme from fish leukocytes.

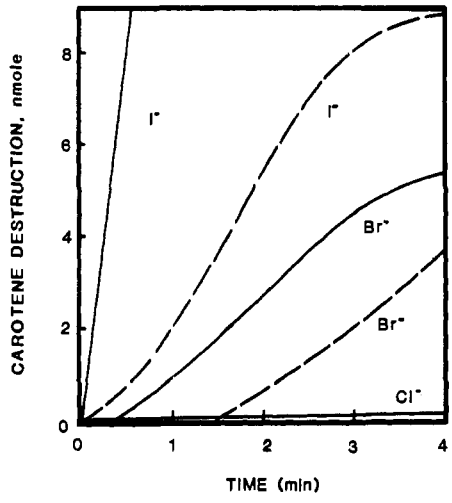


Figure 7. β-Carotene destruction by a myeloperoxidase–H_2O_2–halide system. The reaction mixture contained crude enzyme (24 μg of protein from fish leukocytes), H_2O_2 (50 μM) and sodium iodide [(—) 50 μM; (---) 10 μM] or H_2O_2 (100 μM) and potassium bromide [(—) 50 μM; (---) 10 μM], β-carotene (14 μM), and Tween 20 (0.05%) in a final volume of 2.0 mL of acetate buffer (0.1 M), pH 5.0, at 25 °C.

at 100 μM H_2O_2, and its activity fell sharply at a concentration of 500 μM. In the presence of I^- ions, the optimal activity was found at 2 mM and considerable inhibition (60%) occurred at 10 mM.

Significant β-carotene destruction in the presence of chloride ions also occurred when the system contained a purified mammalian myeloperoxidase. The destruction of β-carotene by the purified myeloperoxidase enzyme, H_2O_2, and chloride was optimal at H_2O_2 concentration of 40 μM (Figure 9). Compared to myeloperoxidase from fish, the concentration of hydrogen peroxide required by the purified enzyme from canine blood for optimal activity was 5–50-fold less in the presence of chloride than in systems containing Br^- or I^- ions, respectively.

DISCUSSION

The leukocyte population from rainbow trout showed a signficant number of peroxidase-positive cells. These cells resembled human neutrophils by their numerous small granules and lobed nucleus (Fey, 1966; Lester and Daniels, 1976). The fish neutrophils were rich in MPO, as evidenced by the prominent staining with pyrogallol–H_2O_2 which revealed the dark spots of purpurogallin, indicating the location of intracellular vacuoles loaded with the enzyme.

The crude myeloperoxidase-like enzyme which was solubilized from the granular particles by the procedure

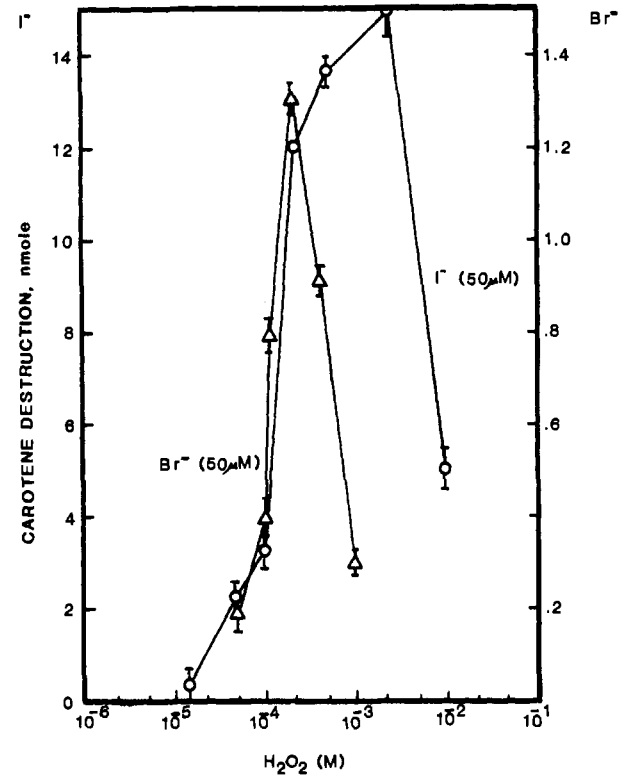


Figure 8. β-Carotene destruction by a myeloperoxidase–H_2O_2–halide system as affected by H_2O_2 concentration. The reaction mixture contained crude myeloperoxidase (24 μg of protein from fish leukocytes), sodium iodide or potassium bromide, 50 μM, β-carotene, 14 μM, and Tween 20, 0.05%, in 2 mL of buffer acetate (0.1 M), pH 5.0, at 25 °C.

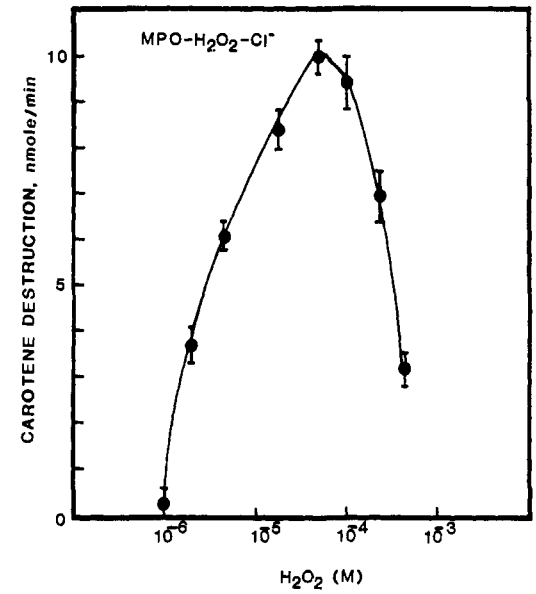


Figure 9. β-Carotene destruction by a purified canine myeloperoxidase–H_2O_2–Cl–system as affected by H_2O_2 concentration. The reaction mixture contained purified myeloperoxidase (22 μg), sodium chloride (125 μM), β-carotene (14 μM), and Tween 20 (0.05%) in 2 mL of acetate buffer (0.1 M), pH 5.0, at 25 °C. Error bars denote standard deviation (n = 3).

used for human myeloperoxidase (Olsson et al., 1972; Matheson et al., 1981) resembled mammalian myeloperoxidase in its properties; i.e., its response to hydrogen peroxide concentration and the optimal pH for peroxidation of phenolic hydrogen donors (Agner, 1958; Saunders et al., 1964).

Peroxidase-catalyzed halogenation reactions have been known for many years and were recently reviewed by Morrison and Schonbaum (1976). The destruction of β-carotene as a rapid and sensitive method to determine cooxidation reactions concomitant with the peroxidation of halides by peroxidases was developed during this study. β-Carotene destruction by the fish MPO–H_2O_2–halide system showed an induction period especially with bromide or with low iodide concentrations. This induction period in our system probably resulted from the interaction of the active halogen compounds with protein sulfhydryl (SH) groups in the enzyme preparation. For example, the addition of cysteine produces very similar effects in a system containing lactoperoxidase–H_2O_2–halides (Kanner and Kinsella, (1983). In our system, chloride ions failed to cause β-carotene discoloration possibly because the rapid interaction of chlorine or hypochlorite with SH groups or amino acids available in the homogenate of the leukocytes. However, β-carotene destruction was found in the presence of chloride ions when the system was incubated with purified myeloperoxidase from mammalian leukocytes.

The discoloration of β-carotene was very sensitive to the H_2O_2 concentration. The fall in the activity at high H_2O_2 concentration may be caused by the inactivation of the enzyme by excess H_2O_2 as observed for perioxidase (Agner, 1963; Rosen and Klebanoff, 1977; Virion et al., 1981).

The rate of H_2O_2 decomposition catalyzed by lactoperoxidase [an enzyme with activities very similar to those of myeloperoxidase (Piatt and O'Brien, 1979)] when stimulated by thiocyanate ions is known to be dependent on the ratio of SCN^- to H_2O_2 (Oram and Reiter, 1966). In our model system not only did the activation of the enzyme depend on the halide to H_2O_2 ratio but also this ratio varied with the different halides.

With regard to bleaching of β-carotene is should be understood that the breakdown of a mere single double bond is sufficient to cause the discoloration of β-carotene by the myeloperoxidase–H_2O_2–halide system. This could be caused by any of three mechanisms, viz., free radical addition, free radical abstraction, or singlet oxygen addition to the double bonds of β-carotene. Free radicals of electron donor molecules are formed in the peroxidase reaction (George, 1952; Chance, 1952; Yamazaki et al., 1960; Yamazaki, 1977). An example of a free radical mechanism involving a halide radical is shown:

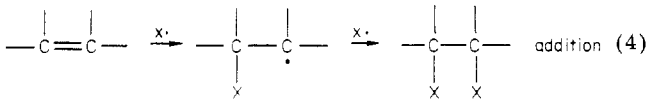
$$\text{myeloperoxidase} + H_2O_2 \rightarrow \text{compound I} \quad (1)$$

$$\text{compound I} + X^- \rightarrow \text{compound II} + X\cdot \quad (2)$$

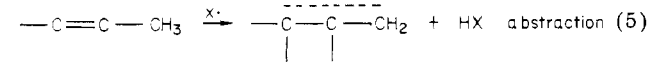
$$\text{compound II} + X^- \rightarrow \text{myeloperoxidase} + X\cdot \quad (3)$$

$$\beta\text{-carotene} + X\cdot \rightarrow \text{discoloration}$$

where X^- = halide ion and $X\cdot$ = halide radical. The radicals derived from this reaction could also interact with the π electrons of the double bonds by the addition mechanism



or it could abstract an allylic hydrogen



Both radical compounds could also interact with oxygen to form a hydroperoxide or with other halide radicals. The activation energy for the addition of chlorine or bromine and especially iodine to a double bond is lower than the hydrogen abstraction step. However, at very low concentrations, substitution is the dominant reaction (Stewart et al., 1937; Sixma et al., 1958; McGrath and Tedder, 1961; Pryor and Lightsey, 1981).

Halogens facilely add to the double bonds of unsaturated compounds (Thaler, 1969) and compounds like *N*-chlorosuccinimide (Adam et al., 1953) and *N*-bromosuccinimide (Ziegler et al., 1942), which serve as a source of Cl_2 and Br_2 at low concentration, can substitute an allylic hydrogen and the allylic radical formed is not appreciably reversible. Both of these mechanisms could result in breakdown of the double bonds of β-carotene.

Another possible mechanism could involve singlet oxygen and β-carotene. Originally Allen et al. (1972) hypothesized that the antimicrobial effect resulting from the action of myeloperoxidase–H_2O_2–halide was singlet oxygen ($^1O_2$). The mechanism for the formation of $^1O_2$ was based in the interaction of hypochlorite, which is a byproduct of the enzymatic reaction (Harrison et al., 1978; Harrison and Schultz, 1976), with H_2O_2 and halide ions, especially Br^- and Cl^- ions,

$$OCl^- + H_2O_2 \rightarrow Cl^- + H_2O + {}^1O_2 \quad (6)$$

Futhermore, Krinsky (1974) reported that unlike a wild-type strain, containing the singlet oxygen quenching carotenoids, carotenoids-less mutant organism was rapidly killed by polymorphomuclear leukocytes. This finding with carotenoids supported the hypothesis of the involvement of $^1O_2$ in oxidation first reported by Allen et al. (1972).

During recent years many attempts have been made to prove this hypothesis (Rosen and Klebanoff, 1977; Piatt et al., 1977; Piatt and O'Brien, 1979; Tsan and Chen, 1980). The basis of much of this research depended on the use of $^1O_2$ traps. However, recently it has been demonstrated that most of $^1O_2$ traps used are nonspecific and can react with a variety of other oxidants including HOCl (Harrison et al., 1978; Held and Hurst, 1978). This finding has vitiated many of the conclusions made from the research based upon nonspecific $^1O_2$ trapping compounds. The conversion of cholesterol to the 5α-hyperoxide is presently considered the most specific method for detection of $^1O_2$, but attempts to demonstrate this product in cholesterol droplets internalized by neutrophils have been unsuccessful (Foote, 1979). Thus, there is strong evidence against the generation of $^1O_2$ by the MPO–H_2O_2–halide system of intact leukocytes.

In addition, β-carotene in nonaqueous dispersions is known as an efficient singlet oxygen quencher without itself being oxidized (Foote and Denny, 1968); however, β-carotene in micellar dispersion failed to quench $^1O_2$ (Linding and Rodgers, 1981). Therefore, we suggest that β-carotene in our system was discolored only by halides radicals or by the breakdown products from hypohalites.

We do not have enough evidence to conclude which reaction is dominant in the presence of β-carotene; however, because of its highly reactive double bonds, it seems that the addition process is the dominant one. More recently, we have observed that linoleic acid can be degraded in this model system, and the data suggested that the peroxidase–H_2O_2–halide system acts with linoleate via an allylic hydrogen abstraction (Kanner and Kinsella, 1983).

β-Carotene with its polyene structure is known as a radical quencher, and for this reason, it was used as a coupling agent during linoleate autoxidation (Kanner and

Budowski, 1978). This study showed that β-carotene is a very efficient halide radical quencher. Our method is sufficiently sensitive to detect the elimination of one double bond; however, on the basis of the high degree of unsaturation of this molecule (eleven double bonds and eight allylic hydrogens), we hypothesize that more than one site is available for radical attack. This conclusion is consistent with Krinsky's (1974) data concerning the rapid killing of carotenoid-less mutant organisms by neutrophils unlike the wild-type strain containing the carotenoids.

Muscle foods, just after slaughtering, are exposed to processing, cutting, chopping, deboning, etc. During these processes the blood components are spread over the surface of the product and are contaminated by microorganisms, dust particles, and dead cells, in an environment with plenty of oxygen. These conditions could initiate the phagocytic process.

Myeloperoxidase, H_2O_2, and halides form a potent antimicrobial system which is operative in the neutrophils (Klebanoff and Clark, 1978). Under certain conditions (e.g., injury), H_2O_2 and myeloperoxidase can be released from the leukocytes (Klebanoff, 1975), and thus the microbicidal system (enzyme, H_2O_2, and halides) may be present extracellularly while this may permit antimicrobial and cytotoxic activities outside the leukocyte and may also cause peroxidative damage to adjacent tissues. Thus, it is conceivable that blood components could play an important role in initiating reactions resulting in the deterioration of the lipids in muscle products. It is known that the MPO–H_2O_2–halide system which generates HOCl and other oxidizing equivalents (Harrison and Schultz, 1976; Thomas, 1979) can oxidize sulfydryl groups or amino acids to yield aldehydes (Ademyi-Jones and Karnovsky, 1981) which can further intereact with proteins and cause cross-linking. The oxidation of sulfydryl groups could also affect the antioxidative tone of meat products.

The natural concentration of I^- and Cl^- in fish and other animal blood is high enough (especially if the meat is salted after the slaughter) to facilitate the reaction observed in our studies. Salting animal tissue after slaughtering with sea salt, which is a rich source of Cl^- and I^-, is one of the main Kosher processes. Several researchers have shown that salting (Ellis et al., 1968) of the Kosher process (Powers and Mast, 1980; Shegalowitz and Kanner, 1979) can accelerate lipid oxidation. One of the explanations for this phenomenon could be the activation of the peroxidative reactions associated with leukocytes or leukocytes lysates. This could be exacerbated in damaged or bruised tissues in muscle foods.

It is well-known that activated neutrophils and monocytes produce a large amount of superoxide anion radical, hydrogen peroxide and hydroxyl radical (Gabig and Babior, 1981), and these species can initiate lipid peroxidation. We have shown in a model system with β-carotene that purified lactoperoxidase chloride ions and that LPO–H_2O_2–Cl could initiate lipid oxidation (Kanner and Kinsella, 1983). These data indicate that leukocytes may be a focus of initiation of lipid peroxidation and thereby contribute to lipid deterioration and adversely affect the quality of certain foods. This possibility is being explored in model systems.

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Registry No. Myeloperoxidase, 9003-99-0; β-carotene, 7235-40-7; hydrogen peroxide, 7722-84-1; iodide, 20461-54-5; bromide, 24959-67-9; chloride, 16887-00-6.

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Varietal Influence on the Quantity of Glycinin in Soybeans

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Glycinin content (11S protein) was determined in 10 soybean varieties (all grown in a uniform environment) by analysis of eluted Coomassie blue dye from proteins separated on $NaDodSO_4$-gradient polyacrylamide gels. Significant differences in glycinin content between varieties were identified. Total protein content for the 10 varieties (Coles, Corsoy, Hodgson, Kitamusume, Tokachi-nagaha, Toyosuzu, Vinton, Wase-Kogane, Weber, and Yuuzuru) ranged from 39.4 to 44.1%. The content of glycinin per total protein was observed between 31.4 and 38.3%. The percent glycinin per total weight was found between 13.5 and 17.8%. High concentrations of total protein did not necessarily correlate with high glycinin concentration although on a total glycinin per seed basis, this correlation was much closer. Glycinin has previously been reported to possess gelatination properties vital for the production of some soy foods. The confirmation that varietal variation in glycinin content does exist and application of a method to quantitate these differences might aid in soybean selection for soy food manufacture.

Differences in the quantity of individual soy proteins (namely, glycinin, β-conglycinin, and the 2S fraction) between soybean varieties were reported by Wolf et al. (1961). The source of these differences, genetic or environmental, has not been previously investigated. Recently, several groups have reported that there was heterogeneity in the glycinin fraction both among soybean varieties (Kitamura et al., 1980; Mori et al., 1981) and within single varieties (Utsumi et al., 1981). However, these reports have focused on qualitative data in terms of glycinin. We are interested in the potential quantitative difference in glycinin concentration that might be observed in soybean varieties. In this study, all soybean varieties were exposed to equivalent environmental influences so that the importance of variety alone could be evaluated. Glycinin was selected for study in these soybean varieties because it has been reported to be responsible for most of the hardness, cohesiveness, and springiness in tofu, a gelatinous soy food (Saio et al., 1969). Tofu producers in the United States and Japan have observed varietal differences in the suitability of soybeans for tofu making (Leviton, 1979; Smith et al., 1960).

Polyacrylamide gel electrophoresis is a highly sensitive technique making possible identification of micrograms of protein. It produces better separation of protein mixtures than do other available techniques (Fishbein, 1972). These characteristics make utilization of this technique desirable for quantitation as well as identification of proteins. The method of Fenner et al. (1975), with some modification to accommodate soy protein, has been utilized in the present study.

MATERIALS AND METHODS

Plant Materials. Ten varieties of soybeans, five of Japanese lineage (Kitamusume, Toyosuzu, Yuuzuru, Tokachi-nagaha, and Wase-Kogane) and five of American lineage (Hodgson, Corsoy, Cole, Vinton, and Weber), were used for comparison in this study. The varieties were all grown in Ames, IA, during the summer of 1980. The 10 soybean varieties each were analyzed for protein by a micro-Kjeldahl technique (AOAC, 1970, Method 38.012). The crude lipids were measured by hexane extraction (AACC, 1969). Protein in solution, after extraction from

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