

**THE EFFECT OF TWO REHEATING METHODS AND
STORAGE ON THE DEVELOPMENT OF WARMED-OVER FLAVOR
IN PRECOOKED CHICKEN PARTS**

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(ABSTRACT)

This study was conducted to determine the effect of heating methods (conventional and microwave), heating temperatures, and refrigerated storage on the flavor deterioration in precooked chicken parts. Chicken breasts and legs were evaluated by chemical analyses and sensory evaluation. Data was analyzed by Fisher's least significant difference (LSD) and Duncan's test.

Results of the thiobarbituric acid test (TBA) indicated that heating temperature, regardless of heating method had a significant influence on oxidative deterioration. When the legs were heated for a longer time at a lower temperature, the TBA values significantly increased indicating heightened warmed-over flavor (WOF). Two-day refrigerated storage had no measurable influence on the TBA numbers. The legs generally had a higher degree of lipid oxidation reflected by higher TBA values.

Heating method and 2-day refrigerated storage did not have a significant effect on the nonheme iron content of breasts or legs. The effects of heating temperature were inconsistent for the legs and breasts which was attributed to the different initial

iron content of the legs, and the release of nonheme iron during the initial processing of the legs. The nonheme iron values of the legs were greater relative to the breasts.

Heating and storage in general increased the area of peak 3 in legs, but had no effect on the breasts. A significant increase in the hexanal (peak 8) content of legs occurred upon 2-day storage, but not for breasts. Peak 7 significantly decreased when the breasts were heated, stored, and reheated. This was not the case for the legs. Sensory panelists could not differentiate between heating methods or temperatures for either part.

This award would not have been awarded if it weren't for Kathleen M. who purchased and delivered the better of the two parts. Thanks to the snowstorm, Matt's Lentner and James Riley, whose statistical brilliance was only surpassed by their statistical brilliance, and Max Taylor, manager of the store, who served as Work-Perfect mentors, sensory panelists, and all of the friends.

Most importantly, I would like to thank my family, who love and support me, given me the strength to endure all of life's challenges. Kirsten Friesen and Mike Pistrer for their endless ability to be lateral thinkers, ability to interrogate and willingly discuss chicken, and for being two of the greatest friends a person could have. Carter Turner for enabling me to take my ongoing career outside of the office and for the great conversations, and lastly, Bill Stewart, Barbara Cohen, and Lisa and Lori Zahradka for their long-distance support, humor, and friendship.

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CHAPTER I

INTRODUCTION

In recent years, the consumer demand for convenient food products has increased. Consumers want food that is easily prepared, nutritious, and palatable. To meet these needs, meat and food industries have made available a variety of ready-to eat foods including precooked meat products. Despite the benefits of convenience, the problem of deteriorative flavor in precooked, stored, then reheated meat products has presented itself as a stumbling block in the market place.

Warmed-over flavor (WOF) is the term used to describe the flavor deterioration of precooked meat characterized by the rapid development of oxidized flavor within a few hours of refrigerated storage (Tims and Watts, 1958). WOF can be contrasted with the slow progression of rancidity which occurs in raw or frozen meat after several weeks or months have elapsed (Cross et al., 1987). In any case, both types of off flavor are related to the autoxidation of lipid fractions, particularly the phospholipid fraction for the former case (Lyon, 1987).

The meat-eating public is becoming increasingly aware of this flavor deterioration. In food service industries as well as various institutions, leftover large cuts of meat are rarely served, and if they are, flavor disguises such as gravies or sauces are used as a camouflage for WOF. The food service on airlines has chosen to offer more nontraditional foods such as fruit plates, cheese, etc. as opposed to main precooked meat entrees (St. Angelo and Bailey, 1987).

because of their high degree of unsaturated fatty acids (Ang, 1988). It has been reported that the oxidative changes, as measured by the thiobarbituric acid test (TBA), existed to the greatest extent in turkey meat followed by chicken, pork, beef, and mutton (Wilson et al., 1976). Further, within the species of poultry, dark meat was more susceptible to oxidation than light meat, presumably due to the phospholipid fraction.

Other factors, in addition to the type of meat, are responsible for oxidation of the lipid fraction in precooked meats. The factors that play instrumental roles in flavor deterioration are: lipid composition, storage time, total time and intensity of heat exposure upon reheating, availability of oxygen, and nonheme iron content (Igene et al., 1979; Lyon and Ang, 1990; Cross et al., 1987). Industry has attempted to control lipid oxidation with the use of antioxidants such as nitrites, ascorbates, and phosphates as well as vacuum packaging. Despite these efforts, WOF in precooked meats continues to remain a problem (Cross et al., 1987).

Lipid oxidation has biological and nutritional significance in addition to these flavor effects. The products of lipid oxidation, including peroxides, aldehydes, free radicals, etc., interact with cell constituents such as protein and vitamins to destroy their biological properties (Buttkus, 1967). It was found by Schricker and Miller (1983) that severe cooking treatments (i.e., microwaving) had a substantial effect upon the amount of absorbable iron existing in meat. Further, Igene et al. (1979) showed that heat released a significant amount of nonheme iron from meat extract

pigments, due to the release of iron from the heme complex. This conversion of heme to nonheme could potentially result in a decrease in absorbable iron, depending on the other enhancing or inhibiting components in the meal.

Consumer awareness of storage and heating methods would be an advancement toward minimizing the effects of lipid oxidation on flavor and nutrition. The purpose of this study was to determine the effect of reheating methods (conventional and microwave) and temperatures on the flavor deterioration in selected precooked chicken parts (legs and breasts); to determine the effect of refrigerated storage on off-flavor development; and to evaluate the products organoleptically in order to assess sensory and objective data.

2.2. Lipid Oxidation

The flavor or quality deterioration of various meats such as poultry, beef, and fish is often caused by lipid oxidation, or "autoxidation." Researchers have recognized the unsaturated lipid fraction as the initial substrate perpetrating the oxidative reaction. This reaction is auto-catalytic in that once the various reactions involved in oxidation begin, the oxidative products proceed to catalyze further oxidative reactions (Lillard, 1987).

CHAPTER II

REVIEW OF LITERATURE

2.1. Development of Warmed-Over Flavor

The short-time storage of precooked meat at refrigerated temperatures results in off-flavor development that was first recognized and labeled by Tims and Watts (1958) as warmed-over flavor (WOF). This flavor deterioration has been attributed to oxidative changes in the unsaturated lipid fraction within the muscle tissue, specifically the phospholipid fraction. Flavor deterioration upon cooking, refrigerated storage, and further reheating, is not limited to meats containing a relatively high percentage of unsaturated fatty acids such as chicken or turkey, but rather, to a whole array of meats including beef, lamb, and veal (Tims and Watts, 1958).

2.2. Lipid Oxidation

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The first step in lipid oxidation is the initial formation of free radicals. This reaction is thermodynamically not favored, and therefore, necessitates some type of catalytic agent. Upon the formation of the free radicals, the reaction is propagated with the abstraction of hydrogen from the unsaturated lipid as well as the addition of oxygen. The primary products resulting from the initial autoxidation are hydroperoxides. Hydroperoxides are unstable by nature, and begin to break down into secondary products once they are formed (Nawar, 1985). These secondary products contribute to warmed-over flavor in foods (Lillard, 1987).

The oxidative potential of a muscle food is dependent upon factors such as lipid composition, available oxygen, heating temperature and duration, and the presence of pro-oxidants and antioxidants (Nawar, 1985).

2.2.1. Lipid Composition

2.2.1.1 Chicken versus Other Meats

Fat content and composition is an important determinant of the oxidative potential of meats. It is generally thought that animal lipids contain a great deal of saturated fat, however, sufficient amounts of unsaturated fatty acids, primarily in the phospholipid fraction, are present as well, and these allow for undesirable oxidative changes to occur (Lillard, 1987).

A number of researchers have compared the lipid composition of meat obtained from different species in order to explain oxidative changes. Wilson et al. (1976)

analyzed several species to determine the effect of lipid composition on the development of WOF. The total lipid (TL) content in red muscles from mutton, pork, and chicken were quite comparable, ranging from 4.74% to 5.58% (% tissue). Beef had an atypically high TL content of 14.79%, while turkey red muscle had a low 1.86%.

In this study, oxidation upon cooking and 2-day refrigerated storage of the various meats was measured with the thiobarbituric acid test (TBA), and correlations were made with lipid composition. The authors found that for red and white pork muscle, as the TL level increased, there was a significantly correlated increase in the TBA value, meaning greater oxidation. Correlation coefficients between TBA and TL for all other species were not significant which means that with the exception of pork, susceptibility to oxidation could feasibly decrease with increasing TL levels.

In the same study, the mean phospholipid (PL) content as a percentage of muscle tissue was determined for various species. The values varied from a low value of 0.50% for beef to a high 1.60% for chicken red muscle. When TBA values were correlated with PL as a percentage of TL, the correlation coefficients were positive and statistically significant for all species. This means that WOF development in cooked meats of various species is dependent upon the proportion of PL relative to TL. This is in agreement with research studies that have reported the PL fraction rather than the neutral lipid fraction as the major contributor to oxidation in chicken, beef, pork, lamb, and seafood (Love and Pearson, 1971; Igene

et al., 1980; Khayat and Schwall, 1983; Melton, 1983).

Similarly, Fooladi et al. (1979) determined the lipid composition of beef, chicken, and pork. Beef was found to be considerably higher in TL and neutral lipids than pork or chicken. PL values, when reported as a percentage of muscle tissue, were constant among the three species; however, when PL content was expressed as a percentage of TL, mean values ranged from 10 to 40% for beef to a high 25 to 57% for chicken. This is in agreement with Wilson et al. (1976).

With regard to the fatty acid composition among species, it has been the general consensus by a number of researchers that poultry lipids as opposed to red meat lipids, contain the highest content of unsaturated fatty acids, and thus, are more susceptible to oxidation (Ang, 1988; Igene et al., 1979, 1980; Melton, 1983). In addition, Chang and Watts (1952) found that poultry lipids were more unsaturated than beef, lamb, and pork. Hilditch et al. (1934) reported that 65% of the fatty acids existing in poultry were unsaturated.

This is not to say that the oxidative process is limited to meats containing a relatively high content of unsaturated fatty acids. Tims and Watts (1958) showed that oxidative changes were apparent upon the cooking and refrigerated storage of beef, chicken, lamb, and veal. Although meats such as beef and lamb, contain smaller amounts of unsaturated fatty acids, oxidation still occurs. Wilson et al. (1976) determined the oxidative changes in a variety of species with the TBA test, and found that oxidation was most apt to occur in turkey followed by chicken, pork,

beef, and mutton.

2.2.1.2 Lipid Composition of Chicken

In the past, whole birds as opposed to chicken parts were stored, and thus, oxidative changes took place primarily in the skin and depot fat because it was more exposed to the oxygen in the atmosphere. In recent years, chicken parts have been sold individually; therefore, more surface area of chicken is exposed, and in danger of oxidation. The lipid composition of the individual parts is of particular concern when considering the stability and quality of the poultry (Katz et al., 1966).

The lipid composition of different poultry tissues varies considerably thereby affecting the oxidative potential of the muscle (Dawson and Schierholz, 1976). A number of investigators have looked at the composition of white versus dark meat in chicken, and have found the TL content of white meat, such as a chicken breast, to be approximately half of the TL content of dark meat, such as a chicken leg (Katz et al., 1966; Peng and Dugan, 1965; Pikul and Kummerow, 1990; Pikul et al., 1985b). Pikul et al. (1985b) determined that chicken breast had 1.10% TL while leg meat had 2.35% TL. Similarly, breast muscle in turkey was found to contain about half as much TL as thigh (Wilson, 1974).

Lipid composition research continued to investigate the composition of the TL within each type of meat. Ang (1988) determined that the percentage of PL relative to TL was much greater in chicken breast meat than leg meat; however, leg meat

contained a greater amount of TL, and therefore, the actual PL content in the leg meat was higher. It is understandable that the percentage of PL relative to TL increases as the percentage of TL decreases.

Pikul et al. (1985a,b) expressed the PL content of chicken breast versus leg as a percentage of the fat extracted from the tissue. Fat from the breast was determined to have 58.4 and 62.6% PL while fat from leg had a PL level of 32.1 and 32.6% for the respective studies. When PL content was determined relative to the weight of the tissue, % PL for white meat and dark meat was comparable at about 40% (Katz et al., 1966; Peng and Dugan, 1965). Peng and Dugan (1965) explained that the difference in fat composition of white and dark meat was due to the amount of exercise a chicken thigh or leg would get as opposed to a chicken breast.

In regard to the neutral lipid content or triglyceride (TG) component of dark versus light chicken meat, once again, Pikul et al. (1985a,b) found that extracted fat from breast muscle was composed of 35.5 and 32.2% TG whereas extracted fat from leg muscle was composed of 62.9 and 62.5% TG. This is consistent with Pikul and Kummerow (1990) who found the percentage of TG in the lipid from chicken drumsticks and wings to be significantly higher than that from breasts.

bird may in fact not accurately represent the fatty acid composition. According to

2.2.1.3. Variation in Lipid Composition within a Species

and Fat content and composition within a species are dependent upon a multitude of factors that must be taken into account upon evaluation of the species. Absolute

values, whether they be results of a TBA test determining the degree of oxidation, or composition values, vary with the age, sex, diet, etc. of the bird (Ang, 1988). This creates difficulty in comparing values obtained in different research laboratories from different chickens.

The age factor may be especially important in female birds due to the extreme changes that accompany the lipid metabolism associated with laying eggs (Pikul et al., 1984a). Marion (1969) showed a decrease in TBA values in breasts and thighs of chickens that were 4 to 6 weeks old. TBA values of chicken parts from both sexes increased from 6 to 8 weeks old. Age influences were statistically significant only for 12 to 20-week old chickens. It was concluded that age is not a major contributing factor in determining oxidative potential, however, there is some decrease in oxidation associated with maturity.

Marion (1969) showed that sex had no significant impact on the TBA values of chicken. Slightly higher values were found, however, in 8-week old male birds.

Fingenbaum and Fisher (1959) and Marion and Woodroof (1962) demonstrated that the dietary fat of the bird does in fact affect its body fat composition. PUFA are synthesized within the chicken body, and therefore, the dietary fat intake of the bird may in fact not accurately represent the fatty acid composition. According to Igene et al. (1979), old hens contain a greater amount of intramuscular depot fat, TL and TG fraction than younger birds.

Further, the researchers went on to show that the neutral fat has the

2.2.1.4. Role of TL, PL, and TG in the Development of WOF

The roles of the individual fractions as well as TL have been extensively researched to relate lipid composition to oxidative flavor deterioration. The PL fraction has been implicated as playing a major role in the autoxidative processes that occur in cooked meats that have been refrigerated for a short period (Pearson and Gray, 1983; Younathan and Watts, 1960; Wilson et al., 1976; Chen et al., 1984). It is generally known that the PL fraction contains a greater amount of polyunsaturated fatty acids (PUFA) than the TG fraction, and thereby, oxidizes more rapidly than the neutral lipids (El-Gharbawi, 1964; Wilson et al., 1976; Igene and Pearson, 1979). Pikul et al. (1985a) determined that the PL fraction of chicken was composed of an average of 37.0% PUFA (an average of dark and light meat) while the TG fraction contained an average of 24.1% PUFA. In addition, PL may be in closer contact with catalysts of oxidation within the tissue, thus, increasing their susceptibility to oxidize (Love and Pearson, 1971).

In regard to the roles of these lipid fractions in the development of WOF, many researchers have attempted to quantify the roles of the fractions. Younathan and Watts (1960) demonstrated that less oxidation had occurred in the neutral lipid fraction extracted from cooked pork than in the TL or the PL extract. Hornstein et al. (1961) found that off-flavor development was less likely in the neutral lipids, and this led the researchers to the conclusion that the PL was a major contributor to oxidation. Further, the researchers went on to state that the neutral fat has the

capacity to trap the volatiles released upon the decomposition of the PL, and hence, reduce their effect on flavor.

Once again, Pikul et al. (1984a), in an attempt to quantify the contributions of the lipid fractions, calculated the TBA numbers for each lipid class. The PL component, relative to the TBA number of the TL, was calculated to be 97 and 94% for chicken breast and leg, respectively. Thin layer chromatography (TLC) was used to identify PL, and was thought to allow for sample autoxidation. The researchers, therefore, subtracted TBA values of all other fractions from the total TBA value to obtain a 94 and 85% PL contribution from the breast and leg meat, respectively. This study also determined the contributions of cholesterol, and cholesterol esters, but these fractions, in addition to free fatty acids, were found to have minimal to no TBA reactivity. The researchers concluded that approximately 90% of the TBA value of TL in raw chicken meat was due to PL.

Igene and Pearson (1979) looked at the contribution of lipid fractions to WOF development in model systems. After cooking the meats, researchers extracted lipid fractions from both light and dark chicken meat, and beef, and added known amounts of each fraction back to the muscle residue. Neither the extracted muscle residue nor the TG significantly affected the TBA values; however, the TL and PL levels significantly increased the TBA values of the model systems. In this study, the TL model systems yielded the highest TBA values, meaning that the TG did have an additive impact when combined with PL. The findings of this study agreed with the

results of Acosta et al. (1966) who found the PL fraction as opposed to the TG fraction to be responsible for the oxidative deterioration in cooked turkey meat.

Fooladi et al. (1979) found that the TBA values had a low and nonsignificant correlation with TL. This implied that the contribution of TL to TBA values is minimal. The relationship between TBA values and PL as a percentage of total tissue was much greater than the relationship between TBA values and PL as a percentage of total lipid. This further emphasized the contention that the level of PL is closely related to TBA values, and thus, the degree of oxidative deterioration.

MA to the extent that it which the lipids were exposed to oxygen in addition to an increased volatility of the MA. Likewise, they attributed the latter case to a greater

2.2.2. The Effect of Heat on Lipid Oxidation

It has been agreed upon by a number of investigators that the mechanisms involved in the flavor deterioration of precooked reheated meats are associated with the autoxidation of lipid fractions of muscle tissues brought about by heat (Tims and Watts, 1958; Younathan and Watts, 1959; Igene and Pearson, 1979). Specifically, heating acts to rupture the lipoprotein complex allowing the lipid fraction to disperse throughout the tissue, and become more prone to participating in chemical reactions (Dawson and Gartner, 1983; Wasserman, 1972).

Heat exerts an impact on off-flavor development in several ways. Cooking methods (Arafa and Chen, 1976), cooking temperatures (Einerson and Reineccius, 1977, 1978), cooking times (Schricker and Miller, 1983), and end point temperature

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resulted in greater TBA values than in the turkey samples cooked for a short time at high temperature (4.75 hours at 177°C).

Newburg and Concon (1980) cooked hamburgers at different temperatures to the same degree of doneness, and found that the hamburgers cooked for a short time at an increased temperature had a decreased rate of oxidation. The authors evaluated oxidation by measuring malonaldehyde (MA), which is a secondary product of lipid oxidation. Hamburgers which were cooked for a long time at a decreased temperature had an elevated MA content. The authors attributed the decrease in MA to the shorter time in which the lipids were exposed to oxygen in addition to an increased volatility of the MA. Likewise, they attributed the latter case to a greater rate of MA formation than rate of loss.

Contrary to this, Su et al. (1991) found that TBA values for chicken patties cooked within the same medium at different temperatures were not affected. Patties exposed to a longer period of heating at lower temperatures had the same MA content as patties cooked for shorter periods at higher temperatures.

Lower temperature roasting is often recommended to obtain ideal juiciness and tenderness in cooked turkey. If turkey is going to be cooked, stored, and reheated, these quality attributes as well as off-flavor development must be considered. High-temperature roasting could possibly minimize flavor deterioration (Dawson and Schierholz, 1976).

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Schricker et al. (1982) demonstrated that heating ground beef and red blood cells to a temperature of 100°C resulted in a substantial increase in the level of nonheme Fe. They postulated that this increase was due to the oxidative cleavage of the porphyrin followed by the release of nonheme Fe. These transformations of heme to nonheme caused by the heating could feasibly affect the availability of the Fe in the food.

Chen et al. (1984) noticed that when a fresh beef pigment extract was heated for 20 min., there was a greater rate of increase in the content of nonheme Fe between 5 and 10 min. than there was between 10 and 20 min. Since the final temperatures were different at each time interval, the researchers postulated that the optimum temperature for nonheme Fe was more dependent upon the rate of heating rather than temperature.

In a study by Schricker and Miller (1983), conventional and microwave cooking increased the level of nonheme Fe in the meat compared to the unheated control. This study also showed that the surface samples of meat as opposed to the center samples, consisted of a higher percentage of nonheme Fe. The authors suggested that this was due to the heightened exposure of the surface samples to higher temperatures as well as more atmospheric oxygen.

2.2.2.3. Microwave versus Conventional

It has been a controversial issue in literature whether or not meats reheated in

a microwave oven have minimal off-flavor development as opposed to meats reheated conventionally. One factor that distinguishes these two forms of heating, and is usually involved in WOF development is the total time that the meat is exposed to the heat (Lyon and Ang, 1990). Microwaves heat at a faster rate than conventional sources of heating, and thus, there is a limited total time of heat exposure (Cipra and Bowers, 1971). Several studies have reported that the flavor of poultry products reheated by a microwave as opposed to a conventional oven, was more comparable to the flavor of freshly cooked poultry products (Cipra and Bowers, 1971; Bowers, 1972; Steiner et al., 1985).

Cipra and Bowers (1971) compared the flavor deterioration of precooked frozen turkey reheated by a microwave (MW) and conventional oven (CO). According to sensory evaluation, a meaty/brothy aroma and flavor (characteristic of freshly cooked poultry) was more intense for the turkey reheated in the MW as opposed to the CO. Although the conventionally-reheated turkey was juicier, stale aroma was more apparent.

Similarly, Steiner et al. (1985) evaluated the WOF development of chicken breasts reheated by a MW and a CO. The sensory panelists in this study were able to distinguish the degree of WOF between the two reheating methods at the 99% level of significance for flavor and aroma. Panelists detected less WOF development in chicken breasts reheated in a MW after 24 and 48 hours of storage than the conventionally-reheated counterparts.

Other researchers have demonstrated that for various meats, there are no differences in off flavor between the two reheating methods; however, samples reheated by either method were different from freshly cooked products (Penner and Bowers, 1973; Johnston and Baldwin, 1980; Albrecht and Baldwin, 1982). Lyon and Ang (1990) evaluated MW versus CO reheating for precooked chicken patties, and found that there were no differences in TBA values between the two reheating methods; however, there were significant TBA changes due to 2-day storage. The authors hypothesized that perhaps the initial TBA values influence the reheating effects of MW versus CO. They also postulated that perhaps other factors are involved that may be sensitive to heating duration, and affect the chemical and sensory changes occurring in the tissue.

Pikul et al. (1985a) studied the oxidative behavior of precooked and stored chicken legs and breasts that were reheated in a MW and CO. Through TBA analysis, they found no difference between the two reheating methods in terms of the promotion of oxidation. Their results did demonstrate that when the concentration of MA in the raw meat was originally high (such as in meat that has been frozen for 6 months), the CO-reheated parts had higher TBA values than the MW-reheated parts. In addition, through fluorescence analysis, which analyzes oxidative products beyond lipid oxidation, they concluded that the CO resulted in greater decomposition of fractions such as proteins and amino acids that could account for this difference.

Lyon and Ang (1990) evaluated chicken patties that were precooked, stored up

to 3 days, and reheated by MW or CO. Contrary to the past study, TBA values were increased for the MW-reheated patties after 2 and 3 days of storage. Sensory data, however, showed no difference in the reheating methods in terms of WOF development.

2.2.3. The Effect of Storage Time on Lipid Oxidation

The storage time, characterized as the time the meat was cooked to the time it was reheated for consumption, plays a large role in the development of off flavors. Lyon and Ang (1990) as well as Lyon (1988) emphasized in their studies the need for researchers to focus on storage times of 1 day or less to discover possible initial causal factors in flavor changes. The researchers felt that it would be advantageous for studies to focus on desirable flavor notes that are present early in storage in order to maintain flavor and quality.

Katz et al. (1966) stated that the rate and extent of oxidative deterioration during storage is dependent upon the degree of unsaturation of the fatty acids. Therefore, determining lipid composition throughout storage is of value. Numerous studies have reported TBA values as well as sensory data, but none have traced the lipid composition of the muscle tissue throughout the storage period. Studies of cooked poultry have generally shown that as the refrigerated-storage time increased, the TBA values increased, and the sensory flavor notes associated with freshly cooked poultry (i.e. chickeny, meaty, brothy) decreased (Igene et al., 1985; Jacobson and

Koehler, 1970; Lyon, 1988; Lyon et al., 1988; Su et al., 1991; Pikul and Kummerow, 1990).

2.2.4. Role of Pro-oxidants

Another influential factor involved in the oxidative flavor changes of precooked meats is the presence of metal catalysts that have the capacity to influence the rate of oxidation. Included in the list of catalysts is heme and nonheme Fe.

2.2.4.1 Role of Heme versus Nonheme as Pro-oxidants

Originally, it was generally accepted by researchers that the heme compounds of meat were somehow involved in lipid oxidation (Tappel, 1952; Younathan and Watts, 1959). Watts (1954) assessed earlier works which showed that lipid oxidation destroyed meat pigments. Keskinel et al. (1964) demonstrated that the TBA values of cooked dark meat were greater than the TBA values of cooked light meat. This was thought to be due to the catalytic effect of the heme pigments which are present in greater amounts in dark meat (Lew and Tappel, 1956; Younathan and Watts, 1959; Liu, 1970; Liu and Watts, 1970).

Sato and Hegarty (1971) extracted heme pigment from beef, and noticed that when they cooked this extract to 70°C, the WOF development was accelerated. They then decided to add hemoglobin (Hb) and myoglobin (Mb) back to the extracted muscle residue, and observed no WOF development upon the heating of the

extracted beef muscle. It seemed as if the catalyst of oxidation had been water extracted, but it was neither the Hb nor the Mb. At this point, the researchers noticed that the grinding of meat with an Fe blade resulted in increased TBA values, so they tested Fe powder (cross between ferric and ferrous Fe), and noted that the ferrous Fe affected TBA values. It was thus concluded that ferrous Fe played a role in WOF development.

Later, Love and Pearson (1974) found that when they dialyzed beef muscle extract, and added the diffusate back to the residue, that higher TBA values were observed upon heating. This confirmed the water solubility of the catalyst, and thus, heme protein was discounted. When metmyoglobin (MetMb) or ferrous Fe were added to muscle residue, increased oxidation shown by increased TBA numbers occurred only with the ferrous Fe confirming that it had pro-oxidant activity.

Igene et al. (1979) determined that the removal of heme pigment from beef and chicken decreased the TBA value of chicken white meat by twofold, and decreased the TBA of chicken dark meat by threefold. With the use of peroxide and heat to destroy heme pigment and release nonheme Fe, as well as the use of EDTA, which is able to chelate nonheme Fe, the researchers confirmed the role of nonheme Fe as a major pro-oxidant in cooked meat. Schricker and Miller (1983) did a similar experiment with ground beef and arrived at similar results.

The mechanism behind this acceleration of lipid oxidation with the addition of ferrous Fe involves the two valency states of the Fe. The Fe donates an electron to

the hydroperoxide to introduce a free radical into the system which then initiates lipid oxidation (Ingold, 1962). Liu (1970) observed that the pro-oxidant activity of Fe was at its optimal in the pH range of 5.0 to 5.5.

2.2.5. Other Factors Affecting Oxidation

There are other factors in addition to the above mentioned that have an influence upon lipid oxidation. The protein content as well as the water content of the meat are important. These components make up the physical environment allowing for the oxidation of meat to occur. Water has an accelerating effect on oxidation presumably due to the fact that it can act as a solvent to dissolve oxygen and metal catalysts (Ang, 1988). If skin is left on the muscle, it acts as a protection against the high temperatures and exposure to the air (Pikul and Kummerow, 1990). If the meat is ground, this will increase the exposure of the lipid fractions to oxygen as well as oxidation catalysts (Sato and Hegarty, 1971). If nitrite is used, it acts to convert the meat pigments to catalytically inactive forms, thereby preventing oxidation (Zipser et al., 1964).

2.3. Lipid Oxidation Measurement

2.3.1. Malonaldehyde

Malonaldehyde (MA) is known as a product of the autoxidation of polyunsaturated fatty acids (PUFA) (Sinnhuber et al., 1958). Although MA is

frequently associated with oxidative deterioration and rancidity, it exists in wholesome meats as well. Shamberger et al. (1977) determined that there was a range of 1 to 14 $\mu\text{g/g}$ and 0.3 to 39 $\mu\text{g/g}$ MA in raw and cooked meats, respectively. Siu and Draper (1978) determined the ranges of 0.35 to 2.9 $\mu\text{g/g}$ and 0.7 to 5.3 $\mu\text{g/g}$ MA in raw and cooked meats, respectively.

Malonaldehyde exists in meat products that have oxidized unsaturated fatty acids, and in the presence of water, it is nonvolatile (Kwon and Watts, 1964; Kwon et al., 1965). It is well known among researchers that MA reacts with TBA reagent to give spectrophotometric readings at 538 nm (Sinnhuber et al., 1958; Yamauchi, 1972; Melton, 1983). The distillation method involved in the TBA procedure extracts MA which is considered to be a primary thiobarbituric reacting substance (TBRS) (Tarladgis et al., 1960, 1964; Yamauchi, 1972).

Malonaldehyde has been shown to be mutagenic to Salmonella typhimurium (Mukai and Goldstein, 1976; Marnett and Tuttle, 1980), and is associated with skin tumors in mice (Shamberger et al., 1974). The authors theorized that this mutagenicity may be from the intermediates that are formed during MA formation, and that MA itself is not a strong mutagen. MA will react with the two nucleic acids, guanine and cytosine, and may modify DNA structures (Reiss et al., 1972; Brooks and Klammerth, 1968). Given the high per capita meat consumption in the U.S. as well as the ease in which cooked and refrigerated meats form MA, these toxicological parameters should be of concern (Cairns, 1975; Newburg and Concon,

1980). The absolute values of MA content were dependent upon initial MA values. They stated that this would make it virtually impossible to compare studies that had

2.3.1.1. TBA Test and Criticisms

The TBA assay (Tarladgis et al., 1960, 1964) is widely used by researchers to measure the extent of lipid oxidation in a whole food product, a distillate of the food, or an extract of the food (Rhee, 1978). It is frequently used as a chemical assessment of WOF, however, TBA values are not specific for WOF (Sato and Hegarty, 1971; Sato et al., 1973; Pearson et al., 1977). The TBA assay measures the amount of MA, which is a secondary product resulting from the oxidation of PUFA's (Sinnhuber and Yu, 1977).

The mechanism behind the method involves heating the sample in an acidic medium to form and release MA. TBA reagent is added to the sample to produce a red pigment which is the result of 2 moles of TBA combining with 1 mole of MA (Sinnhuber and Yu, 1958). The intensity of red pigment that is read spectrophotometrically reflects the MA content of the sample (Tarladgis et al., 1960, 1964). The TBA reagent combines with the MA formed upon heating as well as preformed MA. The change in the MA content from cooking therefore, may reflect a change in precursors, namely hydroperoxides, to MA, or may evaluate already existing MA (Newburg and Concon, 1980).

Researchers have severely criticized the TBA method of assessing lipid oxidation, however, it is still a very useful and common procedure. Pikul et al. (1984b) stated

that the absolute values of MA content were dependent upon initial MA values. They stated that this would make it virtually impossible to compare studies that had used different starting materials. Igene et al. (1985) pointed out that the TBA number reflects the amount of MA in a sample, however, there could be additional TBA reacting substances present, thereby making the value inaccurate.

A major complaint regarding the TBA procedure is the possible sample autoxidation that occurs during preparation, extraction, distillation, and heating steps. These occurrences could lead to extremely high TBA values that are not reflective of the actual oxidation that occurred purely from cooking, and that make it impossible to compare values from different studies (Melton, 1983; Pikul et al., 1983). To combat this problem, Pikul et al. (1983) recommended the addition of butylated hydroxy toluene (BHT) to eliminate autoxidation during the procedure. The researchers demonstrated that the addition of this compound did not interfere with the release of MA nor the binding of MA with TBA, but rather, inhibited the initiation of further lipid oxidation. This was indicated by a decrease in the TBA values.

Although TBA values and WOF sensory evaluation have been shown to correlate (Igene and Pearson, 1979; Igene et al., 1979), it would be untrue to assume that sensory attributes were solely due to MA content. It would therefore, be necessary to identify components beyond MA that possibly would cause sensory changes.

2.3.2. Volatile Analysis-Gas Chromatography

Numerous researchers have agreed that raw meat has little flavor, but rather flavor precursors that are extractable (Crocker, 1948; Hornstein and Crowe, 1960; Kramlich and Pearson, 1958). The characteristic aroma of meat can be created by heating together extracted precursors and the fat fraction of a meat. Hornstein et al. (1961) contended that this characteristic odor brought about by these precursors was similar for all meats, and that the flavor differences between meats was due to volatiles from the lipid fraction. Phippen and Eyring (1957) stated that identifying volatile components, and determining their contribution to flavor was an instrumental part of a flavor study.

Recently, gas chromatography (GC) has been used to identify the volatile compounds contributing to WOF (Ang and Young, 1989; St. Angelo et al., 1987, 1988; Wu and Sheldon, 1988). Crocker (1948) identified hydrogen sulfide as a primary volatile of cooked beef. Bouthilet (1949, 1950, 1951a,b) showed that a sulfur-containing compound was important to the chicken flavor of a chicken broth distillate. This sulfur compound was later found to exist as ammonia (Phippen and Eyring, 1957). Through sensory evaluation, volatile nitrogen was found to be unimportant to chicken flavor.

Cross and Ziegler (1965) found that hexanal and valeraldehyde were major components of uncured ham, beef and chicken, while acetaldehyde, propionaldehyde, and butyraldehyde were present in smaller amounts. The

researchers assumed that the former two major components as well as butyraldehyde, were formed from the oxidation of unsaturated fatty acids. They also found the branched-chain aldehydes were equally present in uncured versus cured ham.

Further, in the same study, when volatiles were rid of carbonyl compounds, the remaining volatiles were odorless. The authors concluded that the variety of aromas from different types of cooked meat were dependent upon the carbonyl compounds.

Lyon and Ang (1990) found that precooked, stored, and reheated chicken patties contained volatiles that were the by-products of oxidation. Three peaks were identified on the gas chromatogram: pentane, pentanal, and hexanal. Hexanal has been used as a standard to show oxidation, but in a study by St. Angelo et al. (1988), the hexanal content did not significantly change over the 2-day storage period or between the reheating methods.

According to Dupuy et al. (1987), the markers indicating WOF are propanal, pentanal, hexanal, 2,3 octanedione and nonanal. Hexanal had the largest increase over the storage period for white and dark chicken meat, and therefore, was suggested to have great importance in the development of WOF. Similarly, Lamikanra and Dupuy (1990) found that in cooked chevon, pentanal, heptanal, 2,3 octanedione, and nonanal were the major peaks.

In a study by Su et al. (1991), precooked stored chicken patties showed 13 volatile compounds that appeared to be secondary products of oxidized PUFA's. Within the same method of cooking and over the storage period, there were few

changes in peak areas of the compounds. Hexanal was repeatedly found to be the predominant peak.

In regard to sensory, some researchers have shown significant correlations between volatiles, TBA numbers, and sensory scores (Fooladi et al., 1979; Igene et al., 1985). Researchers such as Wu and Sheldon (1988) found that the volatiles from cooked and stored turkey rolls changed over a 4-day storage period, but the correlation with sensory was less than 0.80. Researchers Su et al. (1991) found a significant and positive correlation with TBA values and 7 out of 13 peaks that they had found in precooked and stored chicken patties. The highest correlation was for hexanal followed by heptanal. Generally, the results indicated that the readings obtained from the chemical and instrumental testing of lipid oxidation were in agreement.

2.3.3 Sensory Evaluation

Researchers emphasize a great need for sensory evaluation to be correlated with analytical testing in evaluating the WOF of a meat product (Johnson and Civille, 1986; Igene et al., 1985). The sensory evaluation methods used to describe and define the flavor changes in precooked and stored meat vary depending on the study (Poste et al., 1986). Many studies include a list of descriptive terms such as oxidized, brothy, cardboardy, etc. in which the panelists rate each flavor attribute present in the precooked, stored, and reheated meat. Most studies, however, use a WOF scale

in which the panelists give a score of WOF intensity as a single flavor note (Lyon, 1988; Igene and Pearson, 1979).

Poste et al. (1986) noted that the absence of a standard method of sensory analysis, and the lack of a common terminology had made it difficult to assess the WOF problems of cooked meat. The researchers began with a 45-word list of terms to describe the flavor changes in cooked, stored, and reheated chicken patties, and used statistical applications to reduce the list to 12 attributes. The authors contended that the flavor changes occurring were more complicated, and went beyond being simply off-flavored.

In a study by Johnson and Civille (1986), a list of descriptive attributes to describe the flavor changes in precooked, stored, and reheated beef was presented. The panelists were given a variety of meats, cooking procedures, storage times, and reheating procedures to capture the spectrum of flavors or combinations of flavors characteristic of WOF. The results showed that the panelists were able to differentiate between products given the list of attributes.

2.3.3.1. Sensory/TBA Relationship

Numerous studies have reported a correlation between TBA values and sensory scores of oxidized flavor (Jacobson and Koehler, 1970; Fooladi et al., 1979; Melton, 1983; Igene et al., 1985). In results reported by Igene et al. (1985) comparing light and dark chicken meat after 3 days of storage, the TBA values of light meat almost

doubled. This was accompanied by an increase in off-flavor development indicated by a "pronounced WOF" by the sensory panelists. The results for dark meat were similar to this, however, TBA values were higher than white meat after cooking as well as after 3-day storage at 4°C.

Jacobson and Koehler (1970) demonstrated similar results relating sensory and TBA data for light and dark chicken meat. A 0 to 10 numerical scale was used with 10 representing the "best chicken flavor." After 3 days of storage, light meat ratings dropped from 8.6 for freshly cooked chicken to 5.4. Similarly, dark meat ratings went from 8.5 for freshly cooked to 4.1. The authors stated that the dark meat samples were obviously rancid. Corresponding to this, the TBA values for both light and dark meat significantly increased.

Igene et al. (1979) noted a sensory and TBA correlation as well. White and dark chicken meat was evaluated with and without pigment, and on a 1 to 5 scale, 1 being "pronounced WOF" and 5 being "no WOF," was used. The authors noted that the samples containing pigment had the highest TBA value and the lowest sensory rating. The authors contended that this study emphasized the TBA and sensory relationship as well as the importance of meat pigments to the development of WOF.

Steiner et al. (1985) reported that the sensory panelists could not distinguish flavor or aroma changes after the 24 or 48-hour storage of cooked chicken breasts, however, they could significantly differentiate between the MW and CO reheating methods. On the other hand, TBA values did not distinguish between reheating

methods or the storage periods. The authors concluded that since the difference in the reheating methods was perceived by sensory, but not reflected by TBA values, other flavor and aroma compounds contributed to WOF.

For the 12-week study, precooked Holly Farms Tyson Roasted Chicken breasts and legs treated with brown sugar, sodium phosphate, dextrose, and salt were purchased in 2 lots from Kroger's in Blacksburg, VA. One hundred and twenty packages of breast halves, weighing on the average 4.7 lbs and 30 packages of drumsticks (6 drumsticks per package), weighing on the average 4 lbs, were purchased at the beginning of the first week and on the seventh week of the study. The chicken parts were promptly frozen. Thirty legs or breasts (depending on what part was tested on the given week) were thawed overnight in a refrigerator prior to testing.

3.2. Experimental Design

The 4 reheating treatments (2 types of oven, 2 temperatures) were randomly organized over the 12-week period, so that 2 reheating treatments were tested each week (Appendix A). For each chicken part, each reheating method was replicated 3 times throughout the experiment. The same treatments that were used on chicken breasts one week were used on drumsticks the following week.

Figure 1 delineates the heating and handling treatments performed on a daily

CHAPTER III

MATERIALS AND METHODS

3.1. Chicken Samples

For the 12-week study, precooked Holly Farms, Tyson Roasted Chicken breasts and legs treated with brown sugar, sodium phosphate, dextrose, and salt were purchased in 2 lots from Kroger's in Blacksburg, VA. One hundred and twenty packages of breast halves, weighing on the average 0.5 lbs. and 20 packages of drumsticks (6 drumsticks per package), weighing on the average 1 lb., were purchased at the beginning of the first week and on the seventh week of the study. The chicken parts were promptly frozen. Thirty legs or breasts (depending on what part was tested on the given week) were thawed overnight in a refrigerator prior to testing.

3.2. Experimental Design

The 4 reheating treatments (2 types of oven, 2 temperatures) were randomly organized over the 12-week period, so that 2 reheating treatments were tested each week (Appendix A). For each chicken part, each reheating method was replicated 3 times throughout the experiment. The same treatments that were used on chicken breasts one week were used on drumsticks the following week.

Figure 1 delineates the heating and handling treatments performed on a daily

Figure 1. Study design.

Chemical Analyses

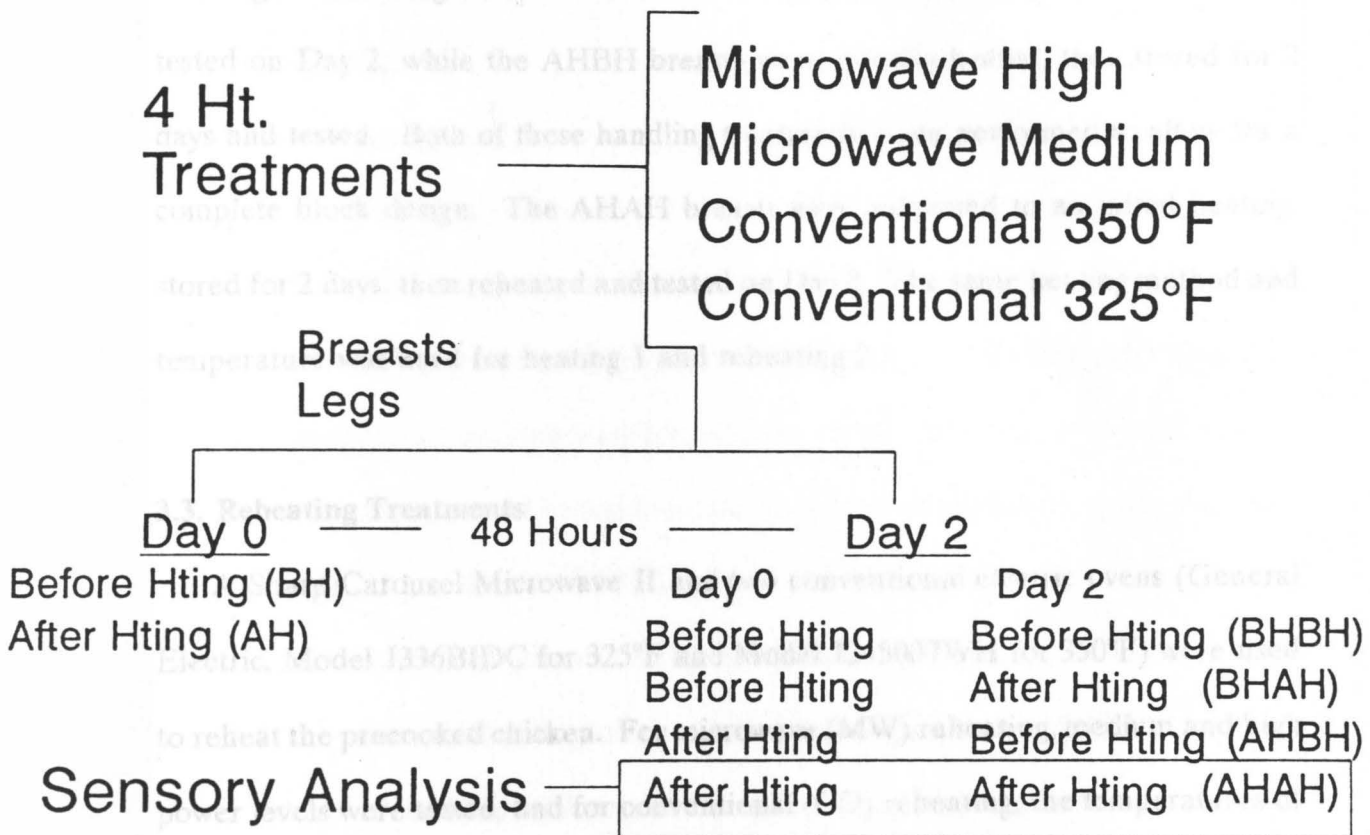


Figure 1. Study design.

basis. Using breasts as an example, on Day 0 (first day of each week), a breast was taken out of the package and tested (BH), and a different breast was subjected to the initial heat treatment and tested (AH). The BHBH breasts were stored for 48 hours with no heat treatment, and tested on Day 2. This was to evaluate the effects of refrigerated storage. The BHAH breasts were stored for 2 days, then heated and tested on Day 2, while the AHBH breasts were initially heated, then stored for 2 days and tested. Both of these handling treatments were performed to allow for a complete block design. The AHAH breasts were subjected to an initial heating, stored for 2 days, then reheated and tested on Day 2. The same heating method and temperature was used for heating 1 and reheating 2.

3.3. Reheating Treatments

A Sharp Carousel Microwave II and two conventional electric ovens (General Electric, Model J336BIDC for 325°F and Model J245007WH for 350°F) were used to reheat the precooked chicken. For microwave (MW) reheating, medium and high power levels were tested, and for conventional (CO) reheating, the temperatures of 325°F and 350°F were used. A pilot study was conducted to establish the heating times necessary to reach an approximate internal temperature of 150°F (Appendix B).

A digital thermometer (Tegam 872A) with two probes was used to determine the internal temperatures. The two probes were placed in the same area of each chicken

part throughout the experiment to minimize temperature variation. Before each MW-heating, a 600 ml. beaker of distilled water was heated for 5 min. on high. For CO-heating, each oven was calibrated before use. The chicken breasts were heated individually while the legs were heated 3 at a time. Chicken parts were MW-heated on polyethylene plates, while cookie trays were used for CO-heated samples.

3.4. Chemical Analyses

3.4.1. Thiobarbituric Acid Test

The thiobarbituric (TBA) method of Tarladgis et al. (1960) was conducted to determine the extent of malonaldehyde (MA) formation in the samples (Appendix C). The antioxidant butylated hydroxytoluene (BHT) was added to each sample (0.03% by weight) before blending to minimize sample autoxidation during blending and distillation (Pikul et al., 1983).

A standard curve was prepared daily by making appropriate dilutions of a 1,1,3,3-tetraethoxypropane (TEP) to give concentrations ranging from 0.1×10^{-8} to 3.0×10^{-8} moles of MA in 10 mls (Ke and Woyewoda, 1979). Absorbance readings were made on a UV spectrophotometer (Milton Roy, 601) at 538nm using distilled water as a blank. TBA numbers were expressed as mg of MA per kg of meat, and were calculated by multiplying each absorbance reading by a constant K value of 7.8 that had been established by Tarladgis et al. (1960).

3.4.2. Nonheme Iron Analysis

The modified procedure of Torrance and Bothwell (1968) given by Schricker et al. (1982) was used to determine the nonheme iron content of the samples (Appendix D). The chicken samples were ground in a micro processor (Handy Shortcut II, Model HMP60, Black & Decker, Shelton, CT) for 30 sec, and allowed to remain in an acid mixture for approximately 20 hours prior to analysis.

Each day a standard curve ranging from 0.5 ppm to 5 ppm of an iron solution was prepared to determine nonheme iron values from absorbance readings. The absorbance readings were determined with a UV spectrophotometer (Milton Roy, 601) at 540nm.

3.5. Gas Chromatographic Analysis

The volatile components of the reheated chicken breasts and legs were determined by the rapid headspace GC method of Ang and Young (1989) using a packed column (Appendix E). One gram of the same chicken blended in the microprocessor (Handy Shortcut II, Model HMP60, Black & Decker, Shelton, CT) for nonheme iron analysis was used in each headspace vial. The vials were flushed with nitrogen for 5 sec prior to sealing.

The chicken samples were conditioned for 30 min each at 80°C for headspace analysis. A 3.2 mm id X 3.05 m Nickle 200 column packed with 8% Poly MPE (poly-metaphenoxylene) on 60-80 mesh Tenax GC was used. A short temperature

program where the column temperature was increased from 50 to 200°C at 10°/min, and held at 200°C for 5 min was used. The injector was at 175°C, and the the flow rate of the carrier gas helium (He) was set at 40 ml/min. Air and hydrogen pressure were at 50 and 30 psi, respectively.

Changes in peak areas for peaks 3, 7, and 8 were qualitatively analyzed for the samples. Peaks with less than 1% were not integrated in the chromatograms. Standards were run in mixture as well as individually to determine retention times.

3.6. Sensory Evaluation

A modified version of the sensory evaluation procedure of Igene and Pearson (1979) was used. Nine panelists were trained in a pilot study by allowing them to differentiate between the flavor of fresh chicken, and the flavor of stored reheated cooked chicken.

Each week the panelists were presented with 2 coded samples that had been heated, stored, and reheated (AHAH). The panelists evaluated the chicken directly after the second reheating, and scored the samples with the following system: 1=very pronounced WOF; 2=pronounced WOF; 3=moderate WOF; 4=slight WOF; and 5=no WOF (Appendix F).

3.7. Statistical Analysis

Statistical analysis was performed using the Statistical Analysis System (SAS Institute Incorporated, SAS Circle, Box 8000, Cary, N.C.27512-8000). Analysis of Variance (ANOVA) was used for analyzing the objective and sensory data. Fisher's Least Significant Difference (LSD) procedure was performed on the overall means, and if significant interaction was found, the individual means were evaluated with the Duncan's test.

4.1.1. The Effect of Heating Temperature on TBA Values

The average TBA number in both breasts and legs was significantly affected by the heating temperature regardless of the heating method used (Table 1). The average TBA number of 0.36 for the breasts heated at the higher temperatures was significantly lower ($p=0.0567$) than the average value of 0.51 obtained from the breasts heated at the lower temperatures. Similarly, the legs heated at higher temperatures had a significantly lower ($p=0.0432$) average TBA value of 0.58 than the legs heated at lower temperatures with a value of 0.69.

These results are consistent with Dawson and Scherholz (1976) who investigated the effect of cooking temperature on the degree of lipid oxidation in turkey. Turkey

CHAPTER IV

RESULTS AND DISCUSSION

4.1. TBA Test

The thiobarbituric acid test (TBA) of Tarladgis et al. (1960) was used in this study to evaluate the extent of lipid oxidation in the precooked chicken parts. The effect of heating temperature, heating method, and handling treatment on the amount of malonaldehyde (MA) produced from the oxidation of unsaturated fatty acids was observed.

4.1.1. The Effect of Heating Temperature on TBA Values

The average TBA number in both breasts and legs was significantly affected by the heating temperature regardless of the heating method used (Table 1). The average TBA number of 0.36 for the breasts heated at the higher temperatures was significantly lower ($p=0.0567$) than the average value of 0.51 obtained from the breasts heated at the lower temperatures. Similarly, the legs heated at higher temperatures had a significantly lower ($p=0.0432$) average TBA value of 0.58 than the legs heated at lower temperatures with a value of 0.69.

These results are consistent with Dawson and Schierholz (1976) who investigated the effect of cooking temperature on the degree of lipid oxidation in turkey. Turkey

Table 1. The effect of heating temperature on the TBA number for breasts and legs heated by either method (CO or MW).

Temperature	Avg. TBA Number (mg MA per kg mt.)	
	Breasts*	Legs ⁺
MW High or CO 350°F	0.36	0.58
MW Med or CO 325°F	0.51	0.69

*Means for breasts are significantly different ($p=0.0567$)

⁺Means for legs are significantly different ($p=0.0432$)

(See Appendix B for Heating Times)

samples that were cooked for a longer time at a lower temperature resulted in greater TBA values than the samples cooked for a shorter time at a higher temperature.

Similarly, Newburg and Concon (1980) investigated the amount of MA produced from hamburger cooked to the same degree of doneness at different temperatures. They found that hamburgers cooked for a short time at an increased temperature had lower MA concentrations than those cooked at a decreased temperature for a long period of time. The authors attributed this disparity to the shorter time in which the lipids were exposed to molecular oxygen as well as increased loss of MA due to volatility or destruction.

4.1.2. The Effect of Heating Method on TBA Values

No effect of heating method on the TBA values of legs and breasts was observed. This is consistent with Lyon and Ang (1990) who investigated MW versus CO reheating methods for precooked chicken patties. They found that the TBA values of the chicken patties were not significantly affected by the reheating method. Likewise, Pikul et al. (1985a) studied the oxidative behavior of precooked chicken legs and breasts reheated by MW and CO. They found no difference in TBA values between the two methods.

In this study, it is quite possible that any effect of heating method was camouflaged by the fact that different precooked breasts and legs were tested each

time. For example, one breast was tested prior to heating and a different breast was tested after reheating. The precooked chicken was obtained from different production lots, and therefore lipid composition, and initial MA content may have varied with starting material. Pikul et al. (1984a) contended that the values of MA obtained from TBA analyses were dependent on the MA content of the starting material. The effect of the heating method may have not been accurately reflected due to the different initial MA content.

4.1.3. The Effect of 2-Day Refrigerated Storage on TBA Values

Two-day refrigerated storage did not have a significant effect on the TBA values of breasts or legs. This is inconsistent with Lyon and Ang (1990) who found significant TBA changes with 2-day refrigerated storage of precooked chicken patties. Tarladgis and Watts (1960) found that there is not necessarily an increase in TBA numbers with the storage of muscle foods. In fact, TBA values have been shown to decline during the storage of cooked meat, possibly due to the reaction of MA with proteins existing in the meat (Buttkus, 1967).

The storage time is generally characterized as the time the meat was cooked to the time that it was reheated for consumption (Lyon and Ang, 1990). In this study the initial point of storage began from the time the precooked chicken part was taken out of the vacuum package. The precooked chicken used in this study was first cooked in the food plant, and perhaps the majority of lipid oxidation that

occurs during initial processing and storage had already taken place prior to testing.

4.1.4. The Effect of Lipid Composition on TBA Value

Although statistical analysis was not performed on the values obtained from breasts versus legs, the precooked legs generally had a higher degree of lipid oxidation reflected by the higher TBA values (Table 1). Ang (1988) determined that the percentage of phospholipids (PL) relative to total lipids (TL) was much greater in chicken breast meat than leg meat; however, given that leg meat had a greater amount of TL, the actual PL content was greater. A number of researchers have found that the PL fraction plays a major role in the autoxidative processes that occur in cooked meats that have been refrigerated for a short period (Pearson and Gray, 1983; Younathan and Watts, 1960; Wilson et al., 1976; Chen et al., 1984).

Keskinel et al. (1964) demonstrated that the TBA values of dark meat were greater than light meat, and this was attributed to the catalytic effect of the heme pigment which is present in a greater amount in dark meat (Lew and Tappel, 1956; Younathan and Watts, 1959; Liu, 1970; Liu and Watts, 1970). The high linoleic acid and heme pigment contents in the legs could be responsible for aldehyde formation from the breakdown of hydroperoxides (deMan, 1992).

It is therefore understandable that despite the similar heat treatments used in this study for both parts, the legs showed a greater degree of lipid oxidation relative to the breasts. Oxidation would occur more rapidly in legs than breasts given the

following criteria: (a) the increased PL content; (b) the greater amount of unsaturated fatty acids present in the PL fraction versus the TG fraction; and (c) the increased heme pigment content (El-Gharbawi, 1964; Wilson et al., 1976; Igene and Pearson, 1979).

4.2. Nonheme Iron Analysis

The nonheme iron analysis was used in this study as another index of lipid oxidation. Heat acts to cleave the heme pigment which releases nonheme Fe, and this nonheme Fe serves as a catalyst for the autoxidation of lipids (Igene and Pearson, 1979). The effects of handling treatment, temperature, and heating method were observed with nonheme Fe analysis.

4.2.1. The Effect of Handling Treatment on the NHFe Content of Breasts

Table 2 shows the effect of handling treatment on the nonheme Fe content of the precooked breasts heated by either method. From the table, it can be noted that the breasts taken out of the package and tested on Day 0 (BH) had an average NHFe content of 1.82 $\mu\text{g/g}$ which was not significantly different from the mean value of 1.90 $\mu\text{g/g}$ obtained from the breasts that were heated on that same day (AH). There was a significant difference in NHFe content between the mean value of 1.82 $\mu\text{g/g}$ for breasts taken out of the package and tested (BH) versus the value of 2.52

Table 2. The effect of handling treatment on the NHFe content ($\mu\text{g/g}$) of breasts heated by either method (CO or MW).

Handling. Trt.	Nonheme Fe ($\mu\text{g/g}$) *
BH	1.82 c
AH	1.90 bc
BHBH	1.94 bc
BHAH	2.21 abc
AHBH	2.27 ab
AHAH	2.52 a

* Means in column with the same letter are not significantly different ($p > 0.0927$)

Day 0

BH = Before Heating
AH = After Heating

Day 2

Day 0 Day 2
BHBH = Before Heating Before Heating
BHAH = Before Heating After Heating
AHBH = After Heating Before Heating
AHAH = After Heating After Heating

As for the effect of refrigerated storage on the NHFe content of the breasts, the data in Table 2 indicate no significant differences existing between the mean

$\mu\text{g/g}$ for breasts that were subjected to two reheatings and refrigerated storage (AHAH). A 2-day refrigerated storage alone did not significantly affect the NHFe content as evidenced by the slight increase from $1.82 \mu\text{g/g}$ to $1.94 \mu\text{g/g}$.

Since the chicken breasts used in this study were precooked, it is quite possible that the initial heat treatment used at the processing plant resulted in a significant increase in NHFe. Therefore, the effects of the initial heating in this study were minimal due to the fact that the NHFe may have already been released. The nonheme Fe content significantly increased in heated, stored, and reheated (AHAH) breasts. It could be proposed that there was an additional release of NHFe that had not been released in the past; however, Smith et al. (1987) made the observation that the NHFe content of light chicken meat was not affected by the heating history of the meat.

Most research has addressed the change in the NHFe content of raw versus cooked meat (Igene et al., 1979; Schricker and Miller, 1983). The few studies that have concentrated on the reheating of cooked and stored meat have not included NHFe analysis in their methods. Igene et al. (1985) extracted heme pigment from beef to determine the roles of heme and NHFe in the lipid oxidation of cooked meat. The NHFe relative to total Fe increased from 8.72% in fresh pigment to 27.0% in the cooked pigment, and this accounted for increased lipid oxidation.

As for the effect of refrigerated storage on the NHFe content of the breasts, the data in Table 2 indicate no significant differences existing between the mean

values of BH breasts versus BHBH breasts. A significant difference did, however, exist between the breasts that were initially heated (AH) and those that were subjected to two heatings and refrigerated storage (AHAH). It would be unwarranted to make assumptions on these findings due to the fact that studies have not addressed the issue of NHFe changes due to storage.

4.2.2. The Effect of Handling Treatment and Heating Temperature on the NHFe Content of Legs

The interactive effect of handling treatment and heating temperature on the NHFe content of legs is shown in Table 3. Results indicated that the effect of the initial heating (AH) was dependent upon the heating temperature regardless of the heating method used. At the higher heating temperatures, the NHFe content significantly increased from $4.69 \pm 0.89 \mu\text{g/g}$ to 5.85 ± 0.94 while at the lower heating temperatures, the NHFe value decreased from $5.71 \pm 1.05 \mu\text{g/g}$ to $4.85 \pm 0.89 \mu\text{g/g}$. Heating temperatures did not have a significant effect on the BH legs compared with the AHAH legs, and there was no significant effect due to refrigerated storage (BH vs. BHBH).

Studies which have investigated the effect of the rate of cooking on the NHFe content have shown the exact opposite results. Chen et al. (1984) heated raw beef extract for 50 sec. and for 9 min. (fast and slow heating, respectively). In the slowly heated extract, the NHFe concentration increased from $1.31 \mu\text{g/g}$ to $2.25 \mu\text{g/g}$ of

meat. On the contrary, the fast heating resulted in the beef extract having a NHFe content of 1.38 $\mu\text{g/g}$. The researchers attributed the former case to the cleavage of the porphyrin ring followed by the release of NHFe. They explained that the latter case was due to the coagulation of the myoglobin molecule, thereby, not allowing for the release of NHFe.

It is difficult to hypothesize the reason for the NHFe increase at the high heating temperatures, and the decrease at the low heating temperatures. Once again, the use of different legs for each treatment could have caused the data to behave this way. Each precooked leg that was tested had a different starting NHFe content, and therefore the effects of the treatment could have been masked. It is also possible that the heating temperature did not have an effect on the NHFe content, if NHFe was released when the raw legs were initially processed in the plant. This would explain the inconsistencies within the leg data as well as relative to the breast data.

The NHFe content was not affected by refrigerated storage. Once again, no studies have addressed the issue of the effect of refrigerated storage on NHFe content, and there is really no reason to believe that a change would occur.

(Pearson, 1974; Igene and Pearson, 1979)

4.2.3. The Effect of Heating Method on the NHFe Content of Breasts and Legs

The heating method did not have a significant effect on neither the precooked breasts nor the legs. This is consistent with Schricker and Miller (1983) who found that there was not a significant difference in the NHFe content of ground beef

cooked by MW and CO methods, but there was a significant increase in NHFe content relative to the raw beef control. In addition, within each method of cooking, the longer time of heat exposure resulted in significantly greater NHFe values.

To the contrary, Su et al. (1991) found that the release of NHFe in chicken patties was significantly affected by the cooking method but not by the temperature within the same cooking method; however, oven-cooked versus water-cooked were investigated rather than MW versus CO.

4.2.4. The Effect of Dark versus Light meat on the NHFe Content

NHFe values shown in Table 3 versus the values given in Table 2, show evidence that the NHFe content of legs is greater relative to the breasts. Chicken dark meat contains a greater amount of Fe than light meat (Ang, 1988), and therefore has a greater amount of potential NHFe to be released. The overall values coincide with the average TBA values provided in Table 1. Being that NHFe catalyzes the rapid oxidation of lipids in cooked meat, it is understandable that the TBA values of legs versus breasts were greater (Sato and Hegarty, 1971; Sato et al., 1973; Love and Pearson, 1974; Igene and Pearson, 1979).

It is difficult to explain why heating temperature had only a significant effect on the NHFe content of legs, and not the NHFe content of breasts. Perhaps the fact that legs have a high amount of Fe, and therefore, a great potential for NHFe to be released, the disparity between the effect of heating temperatures was more apparent

in the legs.

4.3. Gas Chromatographic Analysis

Selected volatile components used as indices for measuring oxidative changes were analyzed by the rapid headspace gas chromatographic method of Ang and Young (1989) using a packed column. Changes in peak areas due to handling treatment, heating temperature, and heating method were qualitatively analyzed for the the precooked breast and leg samples.

4.3.1. Analysis of Peak 3

4.3.1.1. The Effect of Handling Treatment, Heating Method, and Heating Temperature on the Area of Peak 3 in Legs

Table 4 shows the effects of handling treatment and heating temperature on the area of peak 3 in MW-heated legs. The results indicate that for both MW temperatures, there was not a significant increase in the peak area values of legs taken out of the package and tested (BH) versus the values obtained upon initial heating (AH). Upon initial heating on MW high, the legs had an insignificant increase from 24 ± 14 to 38 ± 18 . Similarly, upon initial heating on MW medium, the legs had a insignificant increase from 21 ± 1 to 23 ± 9 . There was, however, a significant increase between the legs taken out of the package and tested (BH) versus the legs that were subjected to two reheatings and refrigerated storage (AHAH)

Table 4. The effect of handling treatment and heating temperature on the area of Peak 3 in MW-heated legs.

Handling Trt.	Peak Area ** in $10^3 \pm SD$ @	
	High	Med.
BH	24 ± 14 c	21 ± 1 c
AH	38 ± 18 bc	23 ± 9 c
BHBH	47 ± 22 ab	67 ± 22 a
BHAH	44 ± 24 b	48 ± 28 b
AHBH	64 ± 24 a	43 ± 29 b
AHAH	45 ± 32 b	48 ± 29 b

* Values represent mean \pm SD

** Means in column with the same letter are not significantly different ($p > 0.0557$)

In comparing Tables 4 and 5, it can be noted that the mean values obtained for the legs taken out of the package and tested on Day 0 (BH) were all quite comparable except for the legs that were heated at 325°F. The value of 21 for the legs heated at 325°F was significantly lower than the value of 24 for the legs heated at 350°F. This increase was not significant. In comparing Tables 4 and 5, it can be noted that the mean values obtained for the legs taken out of the package and tested on Day 0 (BH) were all quite comparable except for the legs that were heated at 325°F. The value of 21 for the legs heated at 325°F was significantly lower than the value of 24 for the legs heated at 350°F. This increase was not significant.

Day 0	Day 0	Day 2
BH = Before Heating	BHBH = Before Heating	Before Heating
AH = After Heating	BHAH = Before Heating	After Heating
	AHBH = After Heating	Before Heating
	AHAH = After Heating	After Heating

From the volatile profiles of cooked dark chicken meat stored at 4°C for 3 days given by Dupuy et al. (1987), it is apparent that there was a substantial increase in the unidentified volatile with the retention time of 4.74 (peak 3 in this study) upon the cooking and refrigerated storage of the dark chicken meat. It is possible that the

regardless of MW-heating temperatures, as illustrated in Figure 2.

Table 5 shows the effect of handling treatment, and heating temperature on the area of peak 3 in CO-heated legs. Legs taken out of the package and tested on Day 0 (BH) had an average peak area of 34 ± 15 which was significantly higher than the mean value of 16 ± 1 that was obtained from the legs that were heated at 350°F on that same day (AH). In contrast, no significant difference was observed between the value of 21 ± 3 obtained from the legs taken out of the package and tested (BH) relative to the value of 26 ± 11 obtained from the legs that were heated at 325°F (AH). Once again, for both CO-heating temperatures, there was an increase in the mean values of the BH legs versus the AHAH legs, but for the legs heated at CO 350°F, this increase was not significant.

In comparing Tables 4 and 5, it can be noted that the mean values obtained for the legs taken out of the package and tested on Day 0 (BH) were all quite comparable except for the legs that were tested prior to heating at CO 350°F. The value of 34 ± 15 seems unusually high for legs that were not subjected to heating, and perhaps this is why a significant difference in BH versus AHAH legs heated at 350°F was not apparent.

From the volatile profiles of cooked dark chicken meat stored at 4°C for 3 days given by Dupuy et al. (1987), it is apparent that there was a substantial increase in the unidentified volatile with the retention time of 4.74 (peak 3 in this study) upon the cooking and refrigerated storage of the dark chicken meat. It is possible that the

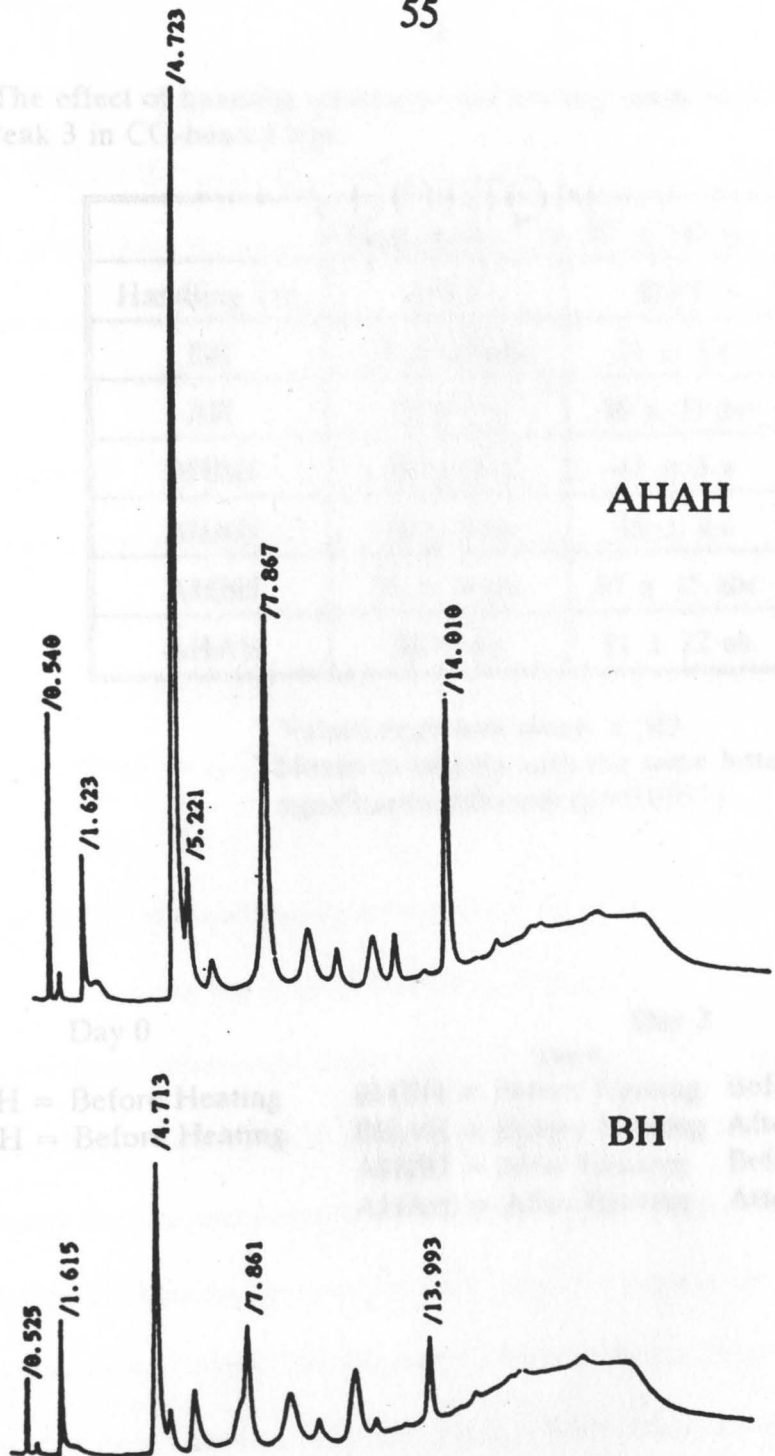


Figure 2. Gas chromatograms showing the effect of two heatings and storage (AHAH) versus before heating (BH) on the area of peak 3 in MW-medium-heated legs.

Table 5. The effect of handling treatment and heating temperature on the area of Peak 3 in CO-heated legs.

Handling Trt.	Peak Area ** in $10^3 \pm SD$ @	
	350°F	325°F
BH	34 ± 15 ab	21 ± 3 c
AH	16 ± 1 c	26 ± 11 bc
BHBH	40 ± 15 a	47 ± 3 a
BHAH	18 ± 6 bc	19 ± 4 c
AHBH	24 ± .9 abc	35 ± 15 abc
AHAH	36 ± 6 a	41 ± 22 ab

* Values represent mean ± SD

** Means in column with the same letter are not significantly different ($p > 0.0557$)

4.3.1.2. The Effect of Handling Treatment, Heating Method, and Heating

Temperature on the Area of Peak 3 in Breasts

Day 0

Day 2

BH = Before Heating

BHBH = Before Heating

Day 2

Before Heating

AH = Before Heating

BHAH = Before Heating

After Heating

AHBH = After Heating

Before Heating

AHAH = After Heating

After Heating

lack of consistency upon initial heating (BH versus AH) for both heating methods was due to the fact that different starting material was used for each test. Lyon and Ang (1990) addressed the fact that the composition of starting material obtained from the processor was a potential source of variation in chemical analyses in their study.

It is apparent from both Tables 4 and 5 that 2-day refrigerated storage (BH versus BHBH) resulted in a significant increase in peak area for all legs except for the BH legs with the value of 34 ± 15 . Once again, these legs had an unusually high mean area for peak 3 relative to the other BH legs, and this could explain why there was not a significant increase due to storage.

4.3.1.2. The Effect of Handling Treatment, Heating Method, and Heating Temperature on the Area of Peak 3 in Breasts

No significant differences in the mean area of peak 3 were observed due to handling treatment, heating method, or heating temperature. Su et al. (1991) found similar results in analyzing selected volatiles for broiler breast patties. In this study, no significant changes in the volatile peak area for unidentified peak 2, with a retention time of 4.23 (peak 3 in this study), were observed. Likewise, in analyzing cooked broiler breast patties, Ang and Young (1989) found no significant changes in the area of peak 3 due to cooking and refrigerated storage. The GC headspace method used in this study was adapted directly from Ang and Young (1989), and

therefore, it is understandable that the results were similar.

4.3 In contrast, from the volatile profiles of cooked white chicken meat stored at 4°C for 3 days given by Dupuy et al. (1987), there was once again, a substantial increase in the unidentified peak with the retention time of 4.78 (peak 3 in this study) upon the cooking and refrigerated storage of the white chicken meat.

4.3.1.3. The Effect of Fatty Acid Composition on the Area of Peak 3

It is difficult to explain the difference in behavior of peak 3 for legs versus breasts given that researchers have not identified the compound. Ang and Young (1989) selected peak 3 as one of the volatiles used as an index for measuring oxidative changes, however, the researchers did not address the source of this volatile or whether the oxidative changes were beneficial or detrimental to the flavor of the chicken.

Due to the short retention time, it is probable that the volatile stemmed from a short-chained fatty acid with a high degree of unsaturation or a highly reactive double bond due to its positioning. If dark meat contains more of this unidentified fatty acid than light meat, this could explain the differences found between the legs and breasts.

Research has focused on the effect of cooking and subsequent storage on the peak area of hexanal. Hexanal is a major oxidative product of PUFA, and has been used as a marker for showing oxidative changes in muscle foods (Ang and Young,

4.3.2. Analysis of Peak 8 (Hexanal)

4.3.2.1. The Effect of Handling Treatment on the Area of Peak 8 in Breasts and Legs

The effect of handling treatment on the area of peak 8 (hexanal) for the precooked breasts and legs regardless of the heating method and temperature is shown in Tables 6 and 7. For both legs and breasts, no significant differences were observed between those that were taken out of the package and tested (BH), and the parts that were heated on that same day (AH). For the breasts, the mean peak area decreased from 20 ± 15 to 11 ± 5 , and for the legs the mean value decreased from 14 ± 7 to 12 ± 5 . Similarly, no significant differences were noted for neither breasts nor legs that were taken out of the package and tested (BH) versus the parts that were subjected to reheating and refrigerated storage (AHAH). The mean value for breasts decreased from 20 ± 15 to 13 ± 10 (Table 6), and for legs decreased from 14 ± 7 to 11 ± 6 (Table 7).

Refrigerated storage caused an insignificant increase in the peak area of hexanal for breasts from a mean value of 20 ± 15 (BH) to 22 ± 17 (BHBH) (Table 6). There was a significant increase in the peak area of hexanal for the precooked legs from a mean value of 14 ± 7 to 39 ± 33 (Table 7).

Research has focussed on the effect of cooking and subsequent storage on the peak area of hexanal. Hexanal is a major oxidative product of PUFA, and has been used as a marker for showing oxidative changes in muscle foods (Ang and Young,

Table 6. The effect of handling treatment on the area of Peak 8 (hexanal) in breasts heated by either method (CO or MW).

Handling Trt.	Peak Area ** in $10^3 \pm SD$
BH	20 ± 15 abc
AH	11 ± 5 c
BHBH	22 ± 17 ab
BHAH	19 ± 13 abc
AHBH	25 ± 21 a
AHAH	13 ± 10 bc

• Values represent mean \pm SD

* Means in column with the same letter are not significantly different ($p > 0.0237$)

Day 0		Day 2
BH = Before Heating	Day 0	Day 2
AH = After Heating	BHBH = Before Heating	Before Heating
	BHAH = Before Heating	After Heating
	AHBH = After Heating	Before Heating
	AHAH = After Heating	After Heating

Table 7. The effect of handling treatment on the area of Peak 8 (hexanal) in legs heated by either method (CO or MW).

Handling Trt.	Peak Area ** in $10^3 \pm SD$
BH	14 ± 7 a
AH	12 ± 5 a
BHBH	39 ± 33 b
BHAH	15 ± 11 a
AHBH	16 ± 7 a
AHAH	11 ± 6 a

• Values represent mean \pm SD

* Means in column with the same letter are not significantly different ($p > 0.0037$)

	Day 0	Day 0	Day 2
BH = Before Heating	BHBH = Before Heating	Day 0	Day 2
AH = After Heating	BHAH = Before Heating	Before Heating	After Heating
	AHBH = After Heating	After Heating	Before Heating
	AHAH = After Heating	Before Heating	After Heating

4.3.2.2. The Effect of Heating Method and Heating Temperature on the Area of Peak 8 in Legs

Table 8 shows the effect of heating method and temperature on the peak area of hexanal for legs. A significant decrease in the peak area existed between the legs

1989; Lyon and Ang, 1990). Lamikanra and Dupuy (1990) suggested that hexanal be considered a primary marker of WOF, since its concentration increased more substantially than other aldehydes.

Dupuy et al. (1987) provided volatile profiles for cooked white and dark chicken meat. Results indicate that there was a substantial increase in the peak area values for both types of meat upon cooking and storage. Su et al. (1991) found similar results when investigating the behavior of hexanal in both oven-cooked and water-cooked chicken patties. The peak area of hexanal significantly increased upon cooking and 3-day refrigerated storage. Similarly, Ang and Young (1989) investigated the volatile profiles of cooked and stored broiler breast patties, and found that the peak area of hexanal increased upon the cooking and refrigerated storage of the patties.

In contrast, Lyon and Ang (1990) investigated the peak area changes in hexanal upon the reheating of cooked chicken patties, and found that there were no significant changes in hexanal content over the 2-day storage period. The data of the present study was consistent with this finding.

4.3.2.2. The Effect of Heating Method and Heating Temperature on the Area of Peak 8 in Legs

Table 8 shows the effect of heating method and temperature on the peak area of hexanal for legs. A significant decrease in the peak area existed between the legs

Table 8. The effect of heating method and heating temperature on the area of Peak 8 in legs.

Ht. Mthd.	Temp.	Peak Area ** in $10^3 \pm SD$
CO	350°F	23 \pm 31 a
	325°F	11 \pm 6 b
MW	High	17 \pm 9 ab
	Med.	19 \pm 13 ab

* Values represent mean \pm SD

* Means in column with the same letter are not significantly different ($p > 0.0944$)

4.3.2.3. The Effect of Dark versus Light Meat on the Area of Peak 8

It is difficult to interpret the legs versus breasts data given that statistical analysis was not performed. Literature addressed the cooking and storing rather than the reheating of white chicken meat. It could be proposed that upon the reheating of both types of meat, the hexanal further decomposed due to heating. Nawar (1985) has stated that aldehydes can easily oxidize to form acids, or participate in further reactions. Perhaps the legs heated at CO 325°F (See Appendix B) were exposed to heat and oxygen for a longer time which allowed for a heightened breakdown of hexanal. Perhaps within the MW medium, this temperature differential was not

heated at 350°F with a value of 23 ± 31 and the legs heated at 325°F with a value of 11 ± 6 . The temperature difference, however, was not seen within the MW heating medium. The effect of heating method and temperature was not observed in breasts.

Su et al. (1991) investigated precooked broiler breast patties, and found that temperature within the same cooking method did not affect the peak area of hexanal. Lyon and Ang (1990) investigated the reheating of precooked and stored chicken patties, and found no significant changes in hexanal content between methods of reheating. However, both of these studies investigated white chicken meat whereas the effect of heating temperature and method in this study was found in dark chicken meat.

4.3.2.3. The Effect of Dark versus Light Meat on the Area of Peak 8

It is difficult to interpret the legs versus breasts data given that statistical analysis was not performed. Literature addressed the cooking and storing rather than the reheating of white chicken meat. It could be proposed that upon the reheating of both types of meat, the hexanal further decomposed due to heating. Nawar (1985) has stated that aldehydes can easily oxidize to form acids, or participate in further reactions. Perhaps the legs heated at CO 325°F (See Appendix B) were exposed to heat and oxygen for a longer time which allowed for a heightened breakdown of hexanal. Perhaps within the MW medium, this temperature differential was not

apparent due to the overall shorter heating times.

Hexanal may have been formed from the oxidation of a saturated fatty acid that is more prominent in dark meat than light meat. Pikul et al. (1985b) looked at the lipid composition of leg versus breast meat, and found that leg meat was 62.9% TG (% of total fat) while breast meat was composed of 35.5% TG. According to Pikul et al. (1984a), there are no apparent differences in fatty acid composition of triglycerides between breast and leg meat. Despite the similar fatty acid composition of TG in white versus dark meat, the absolute content of linoleic acid is greater, and this could be responsible for the disparity in the behavior of peak 8 in the legs versus the breasts.

Decker and Cantor (1992) found that there is 0.82 g of linoleic acid (18:2) per 100 g of dark muscle while there is only 0.22 g per 100 g of light muscle. De Man (1992) has listed hexanal as a principal aldehyde formed from the breakdown of hydroperoxides originating from linoleic acid. Perhaps these oxidative changes due to linoleic acid were responsible for the peak area changes in hexanal predominating in the precooked legs.

4.3.3. Analysis of Peak 7

4.3.3.1. The Effect of Handling Treatment and Heating Method on the Area of Peak 7 in Breasts

Table 9 shows the effect of handling treatment and heating method on the area

Table 9. The effect of handling treatment and heating method on the area of Peak 7 in breasts.

Handling Trt.	Peak Area ** in $10^3 \pm SD$	
	MW	CO
BH	26 \pm 11 a	22 \pm 9 a
AH	14 \pm .77 b	17 \pm .54 ab
BHBH	15 \pm 5 b	14 \pm 8 b
BHAH	11 \pm 3 b	11 \pm 1 b
AHBH	14 \pm 4 b	16 \pm 6 ab
AHAH	10 \pm 3 b	11 \pm 4 b

* Values represent mean \pm SD

** Means in column with the same letter are not significantly different ($p > 0.0809$)

Day 0

BH = Before Heating
AH = After Heating

Day 0

BHBH = Before Heating
BHAH = Before Heating
AHBH = After Heating
AHAH = After Heating

Day 2

Day 2

Before Heating
After Heating
Before Heating
After Heating

4.3.3.2. The Effect of Dark versus Light Storage on the Area of Peak 7

No significant changes were found in the area of peak 7 in breasts stored in the dark versus the light for 3 days. These results contrasted the findings of researchers Ang and Young (1997), who found that the area of peak 7 in breasts stored in the dark was significantly higher than those stored in the light.

of peak 7 in breasts. For both heating methods, the results indicate that there was a decrease in the peak area values of the breasts taken out of the package and tested (BH) versus the values obtained upon initial heating (AH). Upon initial MW-heating, peak area for breasts significantly decreased from 26 ± 11 to $14 \pm .77$, while the initially CO-heated breasts insignificantly decreased from 22 ± 9 to $17 \pm .54$. There was, however, a significant decrease for both heating methods between the breasts taken out of the package and tested (BH) versus the breasts that were subjected to two reheatings and refrigerated storage (AHAH). Peak areas also decreased significantly when breasts were subjected to 2-day refrigerated storage (BH versus BHBH).

Dupuy et al. (1987) found a substantial increase in the unidentified volatile with a retention time of 10.94 min. (peak 7) when cooked white meat was stored at 4°C for 3 days. These results contradict the findings of this study. However, it is possible that the volatiles in question may be different. The compound responsible for the peak in the study conducted by Dupuy et al. (1987) could be an entirely different compound than peak 7 identified in this study.

4.3.3.2. The Effect of Dark versus Light Meat on the Area of Peak 7

No significant changes were found in the area of peak 7 in the legs, whereas in the breasts, significant changes were observed. Since peak 7 was not identified by researchers Ang and Young (1989) from which the methodology in this study was

adapted, it is unwarranted to make assumptions on the source of this volatile.

The decrease in peak 7 due to initial heating (BH versus AH); refrigerated storage (BH versus BHBH); and two reheatings combined with refrigerated storage (BH versus AHAH) could be due to the oxidation of an unsaturated fatty acid more prevalent in breasts, or the location of a particular fatty acid within the muscle.

4.4. Sensory Evaluation

A modified version of the sensory evaluation procedure of Igene and Pearson (1979) was used. Each week the panelists were presented with breasts or legs that had been subjected to two heatings and refrigerated storage (AHAH), and asked to rate the degree of WOF (see Appendix F). No significant differences were found between heating methods nor heating temperatures within each part.

These findings are inconsistent with Steiner et al. (1985) who found that panelists could significantly differentiate between MW and CO reheating methods. The panelists did detect less WOF development in the MW-reheated chicken breasts that had been stored for 24 hours than in CO-reheated and stored breasts.

Statistical analysis was not performed on the sensory versus TBA data, but some interpretations can be made looking from Table 1. Within each part, there was a significant increase in the TBA value with decreasing temperature. The panelists, however, did not perceive a significant temperature-dependent difference in flavor within each part. The possibility arises that the ingredients (salt, dextrose, sodium

phosphate, and brown sugar) acted as preservatives, and at the same time, camouflaged any existing WOF.

Thiobarbituric acid values are not specific for WOF (Sato and Hegarty, 1971; Sato et al., 1973; Pearson et al., 1977). Igene and Pearson (1979) reported that the use of a trained panel is the best way to evaluate WOF, but even that method is not foolproof. A panel may not be able to differentiate between WOF and other autoxidative changes occurring.

For this study, on one given week breasts were evaluated, and on the next week, legs were evaluated. Statistical analysis, was therefore, not conducted on the degree of WOF in the legs versus the breasts. Data did indicate that the panelists generally rated the breasts with a slightly higher score, suggesting that they found less WOF apparent.

Results of the thiobarbituric acid test indicated that heating temperature, regardless of the heating method, was a significant influence on oxidative deterioration. When the legs or breasts were heated for a longer time at a lower temperature, the TBA values significantly increased, highlighting WOF. On the contrary, 2-day refrigerated storage and heating method had no measurable influence on the TBA number, and therefore, had no apparent effect on WOF development. Although statistical analysis was not performed on the legs versus the

CHAPTER V

SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH RECOMMENDATIONS

As the consumer demand for ready-to-eat meat products continues to increase, the problem of deteriorative flavor due to oxidation will approach the forefront. It is to the consumers' advantage to be aware of what they can do regarding food preparation to maximize the quality of the meat product. This involves having knowledge of reheating temperatures and methods as well as storage techniques in order to maintain the integrity of the product.

This study investigated the effects of handling treatments, heating temperatures, and heating methods on the development of warmed-over flavor in precooked chicken breasts and legs. Chemical analyses as well as a trained sensory panel were used to evaluate oxidative deterioration.

Results of the thiobarbituric acid test (TBA) indicated that heating temperature, regardless of the heating method had a significant influence on oxidative deterioration. When the legs or breasts were heated for a longer time at a lower temperature, the TBA values significantly increased indicating heightened WOF. On the contrary, 2-day refrigerated storage and heating method had no measurable influence on the TBA number, and therefore, had no apparent effect on WOF development. Although statistical analysis was not performed on the legs versus the

breasts, the precooked legs generally had a higher degree of lipid oxidation reflected by the higher TBA values.

Nonheme iron was also used as another index of WOF development due to the fact that metals act as catalysts of oxidation, and could influence WOF. Nonheme iron analysis indicated that heating method and 2-day refrigerated storage did not have a significant influence on the nonheme iron content of the breasts or the legs. When the breasts were subjected to two reheatings and storage (AHAH), however, there was a significant increase in this pro-oxidant. A significant difference between heating temperatures occurred upon initial heating of the legs. The legs that were heated at the higher temperatures, regardless of the method, had an increased nonheme iron content, while those heated at the lower temperatures decreased in nonheme iron content. When legs were subjected to two reheatings and refrigerated storage, no significant effects occurred. The inconsistent findings of the nonheme iron in legs was attributed to the different nonheme iron content in each leg, as well as nonheme iron being released during the initial processing of the legs at the plant.

The nonheme iron values of the legs were greater relative to the breasts. This is consistent with the higher TBA numbers for the legs, given that nonheme iron catalyzes oxidation, and the TBA number measures oxidation.

For gas chromatographic analysis, the changes in peak area occurring for 3 selected volatiles were evaluated. Peak 3, with the retention time of ~ 4.691 min., significantly increased upon the 2-day refrigerated storage of the legs. Similarly,

there was a significant increase in this volatile upon subjecting the legs to two reheatings and refrigerated storage, regardless of the heating method or temperature. No significant changes due to handling treatment, heating method, or heating temperature were found for the breasts. Whether or not the volatile is beneficial to flavor and its specific source have not been elucidated.

The presence of hexanal significantly increased upon 2-day refrigerated storage in the legs, but not in the breasts. This could be due to the legs having a high content of linoleic acid, which produces hexanal upon autoxidation. Heating method had no significant effects on hexanal content in the breasts or the legs. Within the CO-heated legs, however, those heated at the lower temperature significantly decreased in hexanal content. This could be due to the heightened exposure to oxygen and heat at the lower temperature which may have caused further decomposition of the hexanal.

Peak 7, with a retention time of ~ 11.491 min., significantly decreased when the breasts were subjected to two reheatings and refrigerated storage (AHAH). There were no significant changes due to heating method, heating temperature, or handling treatment in the legs. Once again, the source of this volatile as well as its flavor effects are not known.

The chemical tests performed in this study were meant to assess the effects of various treatments on the indices of oxidation, and thus, warmed-over flavor. In making general conclusions, the heating method did not have any substantial effects

on these flavor indices. Lower heating temperatures resulted in a significant increase in the TBA values for both parts. Nonheme iron content of both parts varied upon heating, and was not affected by 2-day refrigerated storage. Heating and storage in general increased the area of peak 3 in legs, but had no effect in the breasts. A significant increase in the hexanal content of the legs occurred upon 2-day refrigerated storage, but not for the breasts. Lastly, peak 7 significantly decreased when the breasts were heated, stored, and reheated. This was not the case for the legs.

These chemical tests would have had little meaning without the accompaniment of sensory evaluation. Despite the results of chemical analyses, a consumer may not be able to perceive an oxidized flavor. That was precisely the case in this study. The panelists found no significant differences due to heating method or heating temperature. Any oxidized flavor in existence may have been masked by ingredients such as salt, dextrose, sodium phosphate and brown sugar that were used in the precooked parts.

Future research in this area might focus on tracing these volatiles that are used as indices of WOF to a source. Analyzing various meat products for their fatty acid composition would then allow researchers to trace the pathway of the respective volatile, and its potential effect on the product. In addition, fatty acid composition changes due to refrigerated storage, heating temperature, and heating methods should be investigated. Once again, this would allow researchers to predict the

potential of a given product for oxidation. Finally, researchers should concentrate on the effects of **reheating** as opposed to cooking techniques. Given that precooked meats are on the upswing, it would be to the researchers benefit as well as the consumers to address this issue.

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Experimental Design

Week #1	MW Med	vs.	MW High	Breasts
Week #2	MW Med	vs.	MW High	Legs
Week #3	MW High	vs.	CO 350°	Breasts
Week #4	MW High	vs.	CO 350°	Legs
Week #5	CO 325°	vs.	CO 330°	Breasts
Week #6	CO 325°	vs.	CO 350°	Legs
Week #7	MW Med	vs.	CO 350°	Breasts
Week #8	MW Med	vs.	CO 350°	Legs
Week #9	MW High	vs.	CO 325°	Breasts
Week #10	MW High	vs.	CO 325°	Legs
Week #11	MW Med	vs.	CO 325°	Breasts
Week #12	MW Med	vs.	CO 325°	Legs

Scale:

APPENDIX A

MW = Microwave
 CO = **EXPERIMENTAL DESIGN**
 Med = Medium

Experimental Design

Week #1	MW Med	vs.	MW High	Breasts
Week #2	MW Med	vs.	MW High	Legs
Week #3	MW High	vs.	CO 350°	Breasts
Week #4	MW High	vs.	CO 350°	Legs
Week #5	CO 325°	vs.	CO 350°	Breasts
Week #6	CO 325°	vs.	CO 350°	Legs
Week #7	MW Med	vs.	CO 350°	Breasts
Week #8	MW Med	vs.	CO 350°	Legs
Week #9	MW High	vs.	CO 325°	Breasts
Week #10	MW High	vs.	CO 325°	Legs
Week #11	MW Med	vs.	CO 325°	Breasts
Week #12	MW Med	vs.	CO 325°	Legs

Scale:

MW = Microwave
CO = Conventional
Med = Medium

Heating Times

Microwave:

	Medium	High
Breast	4min 30sec	3min 15sec
3 Legs	4min	2min 30sec

Conventional:

	325°F	350°F
Breast	30min	20min
3 Legs	17min	15min

Notes:

MW temps were taken 2 minutes after the chicken was out of the oven.

These heating times were used throughout the study. At the fact that internal temps varied.

APPENDIX B

**ESTABLISHED HEATING TIMES TO REACH AN
INTERNAL TEMPERATURE OF 150°F**

Heating Times

Microwave:

	<u>Medium</u>	<u>High</u>
Breast	4min 30sec	3min 15sec
3 Legs	4min	2min 30sec

Conventional:

	<u>325°F</u>	<u>350°F</u>
Breast	30min	20min
3 Legs	17min	15min

Notes:

MW temps were taken 2 minutes after the chicken was out of the oven.

These heating times were used throughout the entire experiment despite the fact that internal temps. varied.

APPENDIX C

THIOBARBITURIC ACID TEST (TBA)

Thioarbituric Acid Test (TBA)

Taniguchi et al. (1960)

Reagents:

TBA Reagent

Make a 90% glacial acetic acid solution by adding 900 mL of glacial acetic acid to 100 mL of DeI water in a 1000 mL volumetric flask.

Add approximately 4.5664 g of 2-thioarbituric acid (Sigma Chemical Co., St. Louis, MO) to about 200 mL of the acetic acid solution, and gently heat and stir the covered mixture until the TBA is dissolved (about 10 min).

After cooling, add this solution to the remaining acetic acid in the volumetric flask, and stir. This makes a 0.02 M 2-thioarbituric acid in 90% glacial acetic acid solution.

1,1,3,3-Tetraethoxypropane (TEP) Standard and Working Solution (Ke and Woyewoda, 1983)

Dissolve 220 μ L of TEP (Aldrich, Milwaukee, WI) in approximately 90 mL of DeI water. This 0.01 M stock solution can be kept for one week if stored in the refrigerator.

For the working solution, pipette 1 mL of the stock solution into a 100 mL volumetric flask, and make to volume with DeI water.

HCL Solution

Add 60 mL of concentrated HCL to 120 mL of DeI water to make 200 mL of 4 N HCL (1 part of concentrated HCL to 2 parts of DeI water).

Procedure:

1. Weigh 0.003 g of the antioxidant, butylated hydroxytoluene (BHT) (Sigma Chemical Co., St. Louis, MO), and put it into an 8 oz Ostraver Blender (Pikul et al., 1983).
2. Weigh 10 g of the fish, and add this to the blender. Add 50 mL of DeI water, and blend for 2 min.
3. Transfer the mixture to a Kjeldahl flask, and rinse the blender with an additional 57.5 mL of DeI water.

4. Pipette **Thiobarbituric Acid Test (TBA)** the flask, and pipette a small amount of the solution into a small test tube. (Tarladgis et al. (1960) Sigma Chemical Co., St. Louis, MO, onto the neck of the flask.

Reagents:

TBA Reagent

Make a 90% glacial acetic acid solution by adding 900 mL of glacial acetic acid to 100 mL of DeI water in a 1000 mL volumetric flask.

Add approximately 4.5664 g of 2-thiobarbituric acid (Sigma Chemical Co., St. Louis, MO) to about 200 mL of the acetic acid solution, and gently heat and stir the covered mixture until the TBA is dissolved (about 10min).

After cooling, add this solution to the remaining acetic acid in the volumetric flask, and stir. This makes a 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid solution.

1,1,3,3 Tetraethoxypropane (TEP) Standard and Working Solution (Ke and Woyewoda, 1979)

Dissolve 220 μ L of TEP (Aldrich, Milwaukee, WI) in approximately 90 mL of DeI water, and bring to 100 mL with DeI water. This 0.01 M stock solution can be kept for one week if stored in the refrigerator.

For the working solution, pipette 1 mL of the stock solution into a 100 mL volumetric flask, and make to volume with DeI water.

HCL Solution

Add 60 mL of concentrated HCL to 120 mL of DeI water to make 200 mL of 4 N HCL (1 part of concentrated HCL to 2 parts of DeI water).

Procedure:

1. Weigh 0.003 g of the antioxidant, butylated hydroxytoluene (BHT) (Sigma Chemical Co., St. Louis, MO), and put it into an 8 oz Osterizer blender (Pikul et al., 1983).
2. Weigh 10 g of chicken, and add this to the blender. Add 50 mL of DeI water, and liquefy for 2 min.
3. Transfer the mixture to a Kjeldahl flask, and rinse the blender with an additional 47.5 mL of DeI water.

- Pipette 2.5 mL of HCL solution into the flask, and pipette a small amount of Antifoam A (Sigma Chemical Co., St. Louis, MO) onto the neck of the flask.

Procedure for Distillation:

- Rinse the Büchi 322 Distillation unit with a Kjeldahl flask filled a quarter of the way with DeI water. Do this by pushing preheat, and allowing 50 mL of the distillate to be produced.
- Once the apparatus is rinsed, collect 50 mL of distillate from each sample, rinsing in between by the above method.
- For a final rinsing, use a flask filled half of the way with DeI water, and collect about 200 mL of distillate.

Standard Curve Preparation:

- From the 1.0×10^{-4} M working solution of TEP, prepare the following 6 standards for breasts:

Table 1. Standards to prepare for TBA evaluation of breasts.

Standards	TBA Reag.	Water	TEP	Conc. (M)
Blank	5 mL	5 mL	-----	-----
10 μ L	9.99 mL	-----	10 μ L	0.1×10^{-8}
20 μ L	9.98 mL	-----	20 μ L	0.2×10^{-8}
30 μ L	9.97 mL	-----	30 μ L	0.3×10^{-8}
50 μ L	9.95 mL	-----	50 μ L	0.5×10^{-8}
70 μ L	9.93 mL	-----	70 μ L	0.7×10^{-8}
100 μ L	9.90 mL	-----	100 μ L	1.0×10^{-8}

2. From the 1.0×10^{-4} M working solution of TEP, prepare the following 7 standards for legs:

Table 2. Standards to prepare for TBA evaluation of legs.

Standards	TBA Reag.	Water	TEP	Conc. (M)
Blank	5 mL	5 mL	-----	-----
30 μ L	9.97 mL	-----	30 μ L	0.3×10^{-8}
50 μ L	9.95 mL	-----	50 μ L	0.5×10^{-8}
100 μ L	9.90 mL	-----	100 μ L	1.0×10^{-8}
150 μ L	9.85 mL	-----	150 μ L	1.5×10^{-8}
200 μ L	9.80 mL	-----	200 μ L	2.0×10^{-8}
250 μ L	9.75 mL	-----	250 μ L	2.5×10^{-8}
300 μ L	9.70 mL	-----	300 μ L	3.0×10^{-8}

Sample Preparation:

1. For each 50 mL of distillate, pipette 5 mL of distillate and 5 mL of TBA reagent into test tube (2 samples/50 mL).
2. Cap the tubes, invert each, and immerse standards and samples in a boiling water bath for 35 minutes.
3. After heating, cool under tap water for 10 minutes, and read the optical density at 538 nm (2 cuvettes/tube).

Nonheme Iron Analysis
Schricker et al. (1982)

Reagents:

40% Trichloroacetic Acid

Weigh 60 g of trichloroacetic acid in a glass beaker, and slowly add it to DeI water while stirring so that the volume is 150 ml.

Acid Mixture

Mix equal volumes of 6 N hydrochloric acid and 40% trichloroacetic acid. The amount used will vary depending upon the number of samples. For this study, 250 mL of the acid mixture was made weekly.

Saturated Sodium Acetate

Add sodium acetate to a given volume of DeI water (however much is needed for the study), and stir at room temperature until the sodium acetate no longer goes into solution.

Sulphonated Batho **APPENDIX D**

Weigh 0.162 g of sulphonated bathoanthroline (GFS Chemicals, Columbus, OH) with DeI water. Add 1 mL of thioglycolic acid (Sigma Chemical Co., St. Louis, MO), and leave the covered solution underneath the hood.

NONHEME IRON ANALYSIS

Color Reagent

Mix the sulphonated bathoanthroline with the saturated sodium acetate and DeI water in the ratio of 1:20:20. This solution should be prepared daily, and made according to the amount needed.

In this study, when 4 samples were evaluated, 10 mL of saturated sodium acetate, 10 mL of DeI water, and 0.5 mL of sulphonated bathoanthroline was combined.

When 8 samples were evaluated, 15 mL of saturated sodium acetate, 15 mL of DeI water, and 0.75 mL of sulphonated bathoanthroline were combined.

Procedure for the Previous Night:

1. Weigh 5 g of chicken that has been ground for 30 sec in a micro processor while being flushed with nitrogen, and place each sample in a 50 mL centrifuge tube.

Nonheme Iron Analysis

Schricker et al. (1982)

Reagents:

40% Trichloroacetic Acid

Weigh 60 g of trichloroacetic acid in a glass beaker, and slowly add it to DeI water while stirring so that the volume is 150 mL.

Sample Preparation:

Acid Mixture

Mix equal volumes of 6 N hydrochloric acid and 40% trichloroacetic acid. The amount used will vary depending upon the number of samples. For this study, 250 mL of the acid mixture was made weekly.

Saturated Sodium Acetate

Add sodium acetate to a given volume of DeI water (however much is needed for the study), and stir at room temperature until the sodium acetate no longer goes into solution.

Standard Solution

Sulphonated Bathophenanthroline

Weigh 0.162 g of sulphonated bathophenanthroline (GFS Chemicals, Columbus, OH), and fill to 100 mL volume with DeI water. Add 1 mL of thioglycolic acid (Sigma Chemical Co., St. Louis, MO), and leave the covered solution underneath the hood.

Color Reagent

Mix the sulphonated bathophenanthroline with the saturated sodium acetate and DeI water in the ratio of 1:20:20. This solution should be prepared daily, and made according to the amount needed.

In this study, when 4 samples were evaluated, 10 mL of saturated sodium acetate, 10 mL of DeI water, and 0.5 mL of sulphonated bathophenanthroline was combined.

When 8 samples were evaluated, 15 mL of saturated sodium acetate, 15 mL of DeI water, and 0.75 mL of sulphonated bathophenanthroline were combined.

Procedure for the Previous Night:

1. Weigh 5 g of chicken that has been ground for 30 sec in a micro processor while being flushed with nitrogen, and place each sample in a 50 mL centrifuge tube.

Readings:

2. Add 15 mL of acid mixture to each of the loosely capped tubes, and incubate the tubes at 65°C (on a hot plate) for approximately 20 hours.
3. Include a tube with 15 mL of just acid mixture. This will act as a blank.

Sample Preparation:

1. When 20 hours has passed, allow the tubes to cool, and vortex each tube. Allow the sediment to settle prior to pipetting.
2. Pipette 200 μ L of the supernatant into 3.5 polypropylene tubes, and add 1 mL of color reagent to each. Do two samples from each centrifuge tube.
3. For the blank, pipette 200 μ L of acid mixture, and add 1 mL of color reagent.

Standard Curve Preparation:

1. Using Iron Atomic Absorption Standard Solution (Sigma Chemical Co., St. Louis, MO) prepare 10 mL of a 100 μ g/mL solution by adding 1.01 mL of the standard solution to 8.99 mL of DeI water. This solution can be used throughout the study, however, should be vortexed prior to use.
2. From the 100 μ g/mL Fe solution, make the following standards in 500 Micro test tubes (1.5 mL capacity):

Fe Standard	Amt. of 100 μ g/mL soln.	Amt. of Acid Mix
0.5 ppm	4.96 μ L	.996 mL
1.0 ppm	9.9 μ L	.990 mL
2.0 ppm	19.8 μ L	.980 mL
3.0 ppm	29.7 μ L	.970 mL
4.0 ppm	39.6 μ L	.960 mL
5.0 ppm	49.5 μ L	.950 mL

3. After vortexing each of the standards, pipette 200 μ L of each into 3.5 mL polypropylene tubes, and add 1 mL of color reagent to each.

Readings:

1. For standards as well as samples, obtain absorbance readings from a UV spectrophotometer (Milton Roy, 601) (2 cuvettes/polypropylene tube) at 540 nm.
2. To obtain μg of nonheme iron per g of chicken meat, calculate the amount of iron from the standard curve. Multiply this value by 15 mL of acid mixture, and divide by the g of chicken meat in the sample.

APPENDIX E

RAPID HEADSPACE GAS CHROMATOGRAPHIC METHOD

Rapid Headspace Chromatographic Method Ang and Young (1989)

Sample Preparation:

1. Using the same chicken that was ground in the micro processor for nonheme analysis, weigh 1 g of blended sample into a headspace vial.
2. Pack the chicken around the sides of the vial, flush with nitrogen for 5 sec, and seal.

Standard Preparation:

- Inject 0.5 μ L of the following standards into a headspace vial:
- Hexanal Std (aldehyde C-6)
 - Heptanal Std (aldehyde C-7)
 - Octanal Std (aldehyde C-8)
 - Nonanal Std (aldehyde C-9)
 - Decanal Std (aldehyde C-10)
 - Undecanal Std (aldehyde C-11)
 - Dodecanal Std (aldehyde C-12)
- Waters, Milford, MA; Chromatography, Wallingford, CT.

APPENDIX E

RAPID HEADSPACE GAS CHROMATOGRAPHIC METHOD

Procedure:

1. Follow general procedures for turning on the GC-9A with HSS-2A Headspace Sampler (Shimadzu, Columbia, MD). Use the following settings:

Air = 0.3	Make up Gas = 0
Hydrogen 1 = 0.4	Carrier Gas Det. = 25
Hydrogen 2 = 0	Split Pressure = 1

2. Rapid Headspace Chromatographic Method

Ang and Young (1989)

Sample Preparation:

1. Using the same chicken that was ground in the micro processor for nonheme analysis, weigh 1 g of blended sample into a headspace vial.
2. Pack the chicken around the sides of the vial, flush with nitrogen for 5 sec, and seal.

Standard Preparation:

1. Pipette 0.5 μ L of the following standards into a headspace vial:
Hexanal Bri (aldehyde C-6)
Nonanal Bri (aldehyde C-9)
Octanal Bri (aldehyde C-8)
Heptanal Bri (aldehyde C-7)
Trans-2-penten-1-al
Bedoukian Research Inc., Danbury, CT.
2. Fill the vial half full with DeI water, flush with nitrogen, and seal.

Procedure:

1. Follow general procedures for turning on the GC-9A with HSS-2A Headspace Sampler (Shimadzu, Columbia, MD). Use the following settings:

Air= 0.3
Hydrogen 1= 0.4
Hydrogen 2= 0

Make up Gas= 0
Carrier Gas Det.= 25
Split Pressure= 1

2. Set the parameters as follows:
- | | | |
|----|----------------|--------------|
| 1 | Cond. Temp. | 80°C |
| 2 | Cond. Time | 30 min |
| 3 | Final S.No | Varies w/day |
| 4 | Repeat | 1 |
| 5 | Col. Ini. Temp | 50°C |
| 6 | Ini. Time | 0 min |
| 7 | P. Rate | 10°C/min |
| 8 | Fin. Temp | 200°C |
| 9 | Fin. Time | 5 min |
| 10 | Inj. Temp. | 175°C |
| 11 | Syr. Temp. | 85°C |
| 12 | Det. Pol. | 1 |
| 13 | Det. Range | 0 |
| 14 | Anal. Time | 38 min |
3. Set the attenuation at 3 for all samples, and set at 10 for the standards.
4. Run an empty headspace vial as sample number 1 to minimize contamination of the injector needle.

Sensory Scorecard
Igene and Pearson (1979)

Panelist # _____

You have two coded samples in front of you. Please indicate the intensity of warmed-over flavor (WOF) (if any) in each of the samples by using the following scale:

1 = Very Pronounced WOF	Sample	Intensity Rating
2 = Pronounced WOF	_____	_____
3 = Moderate WOF		_____
4 = Slight WOF		
5 = No WOF		

APPENDIX F

SENSORY EVALUATION SCORECARD

Sensory Scorecard
Igene and Pearson (1979)

Michelle Guaffrade was born in Elmira, N.Y. on August 5, 1968. She attended Cornell University in Ithaca, N.Y., and received a Bachelor of Science Degree in Experimental Foods in 1990. The following year she spent in Washington, D.C.

Panelist # _____

You have two coded samples in front of you. Please indicate the intensity of warmed-over flavor (WOF) (if any) in each of the samples by using the following scale:

	Sample	Intensity Rating
1 = Very Pronounced WOF	_____	_____
2 = Pronounced WOF	_____	_____
3 = Moderate WOF	_____	_____
4 = Slight WOF		
5 = No WOF		

Michelle A. Guaffrade

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