Effect of Frozen Storage Time, Cooking and Holding Temperature upon Extractable Lipids and TBA Values of Beef and Chicken

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J ANIM SCI 1979, 49:701-707.

EFFECT OF FROZEN STORAGE TIME, COOKING AND HOLDING TEMPERATURE UPON EXTRACTABLE LIPIDS AND TBA VALUES OF BEEF AND CHICKEN[1,2]

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Summary

Fresh cuts of beef, chicken dark meat and chicken white meat were frozen and stored at -18C for up to 13 months. At 0, 8 and 13 months of frozen storage, the lipid composition of fresh frozen raw meat and its TBA numbers were measured. A portion of raw frozen meat was also cooked after each storage period and subsequently held at either 4C or -18C for 48 hr, after which it was analyzed for constituent lipids and malonaldehyde (TBA numbers). Changes in total lipids of raw meat during frozen storage were largely due to losses in the triglyceride fraction. The phospholipid content of frozen raw meat was relatively constant, irrespective of length of time in freezer storage. Cooking elevated ($P<.01$) the percentage of phospholipids in relation to total lipids and accounted for a significant increase in rate of lipid oxidation. Cooked meat subsequently held at 4C for 48 hr was more susceptible to development of off-flavor than similar samples held at -18C for 48 hours. The stability of different types of meat, either raw, frozen or cooked was in order of: beef> chicken white meat> chicken dark meat.

(Key Words: Lipids, Phospholipids, Oxidation, Freezing, Beef, Chicken.)

Introduction

According to deFremery *et al.* (1977) extensive consumer surveys have indicated that three-fourths of all consumers prefer fresh to frozen meat, yet two-thirds of consumers freeze meat after purchasing it. These authors cited another study showing that 63% of consumers freeze meat after purchase, even when holding it for only a few days. This practice is not uncommon in commercial meat processing establishments, yet the stability of such meat is unknown.

The quality of raw frozen or cooked meat depends essentially on the composition and stability of the constituent lipids (Watts, 1954; 1962). Wilson *et al.* (1976) observed a relationship between phospholipid levels and the development of warmed-over flavor (WOF) from different species of meat. Igene and Pearson (1978) presented evidence that phospholipids are the major contributors to development of WOF in cooked meat model systems while triglycerides enhance development of WOF only when combined with phospholipids. Fragmentary evidence (Pearson *et al.*, 1977) suggests that susceptibility to WOF differs between red meat and poultry, yet little is known about the variation among and within species.

This study was designed first to determine the importance of triglycerides, total lipids and phospholipids to the oxidative stability of beef, chicken white meat and chicken dark meat during frozen storage. The second objective was to examine the effects of frozen storage upon TBA values following cooking during subsequent holding at either 4C or -18C for 48 hours.

Materials and Methods

Source of Meat. Beef and chicken were obtained from the Michigan State University Meat and Poultry Processing Laboratories. Portions of *longissimus* (LD) muscle were excised from beef carcasses at 24 hr postmortem. Thigh (dark meat) and breast (white meat)

[1] Presented at the 39th Annual Meeting of Food Technologists, St. Louis, MO, June 10 to 13, 1979.

[2] Michigan Agr. Exp. Sta. Journal Article 8869. The data presented herein is based upon work supported by the National Science Foundation under Grant No. ENG 76-04591. The authors acknowledge that the retortable pouches were furnished gratis by Continental Diversified Industries of Chicago, IL.

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meat were removed from old hen carcasses 24 hr postmortem.

Storage of Meat Samples. In order to establish a basis for changes during frozen storage, fresh bone-in samples of beef muscle were cut identical thicknesses (5 cm) and sizes (1 kg), wrapped in freezer paper, numbered and frozen at -18C. Likewise, fresh chicken unskinned thighs and breast meat were wrapped in packages of 1.8 kg and frozen at -18C. The meat was held at -18C for periods up to 13 months. At designated storage periods, samples were removed from frozen storage, thawed overnight at room temperature, cooked and stored at either 4C or -18C for 24 hours. The meat was evaluated by TBA numbers.

Preparation of Meat for Chemical Analysis. After removal of external fat, the meat was cut into pieces and ground twice through a .47 cm plate with a Hobart meat grinder. Portions of the raw ground meat were analyzed for triglycerides, total lipids and phospholipids, and TBA numbers. At the same time, about 400 g of meat were packed into two 16 x 21.5 cm retortable pouches (Continental Diversified Industries, Chicago, IL) and heat sealed with a Multi-Vac sealing machine (Busch, W. Germany). The bags were cooked in boiling water to an internal temperature of 70C. Immediately following cooking, the bags were opened, drippings collected and the meat and drippings were thoroughly mixed. Prior to storing the cooked meat at 4C or -18C, TBA values of freshly cooked meat were measured. Equal amounts of the cooked meat were held at 4C and at -18C for 48 hr in unsealed retortable pouches. At the end of 48 hr storage, TBA values as well as total lipids, triglycerides and phospholipids of the cooked meat were determined.

Extraction of Lipid from Muscle Tissue. Total lipid was extracted from fresh or cooked meat by the procedure of Folch *et al.* (1957). Separation of total lipids into triglycerides and phospholipids was achieved by the method of Choudhury *et al.* (1960).

TBA Test. The distillation method of Tarlidgis *et al.* (1960) was utilized to follow development of oxidative rancidity by the TBA test.

Statistical Methods. Analysis of variance for mean TBA values, total lipids, triglycerides and phospholipids were calculated on a Control Data Corporation (CDC) 6500 computer. Significance of treatment means was determined using Tukey's test for multiple comparisons (Steel and Torrie, 1960).

Results and Discussion

Levels of Extracted Lipids from Muscle Tissue

Beef Lipids. Amounts of total lipids, triglycerides and phospholipids are presented in table 1. Prior to frozen storage, fresh raw beef contained 13.72% total lipid, but it decreased ($P<.05$) to 9.52 and 9.82% at 8 and 13 months of frozen storage, respectively. Levels of total lipids and triglycerides in the cooked beef held at 4C and -18C without frozen storage were not significantly different than that in fresh uncooked meat. After 8 and 13 months of frozen storage, however, the concentrations of both triglycerides and total lipids were higher in cooked than in fresh meat. Results showed that both total lipids and triglycerides decreased ($P<.01$) during frozen storage, regardless of whether the meat was fresh or cooked. The decline in total lipids and triglycerides during frozen storage is consistent with the report of Braddock and Dugan (1972), who reported a decline in most of the constituent fatty acids of the triglycerides during frozen storage of Coho salmon. The decrease in percentage of triglycerides during frozen storage may be due to lipolysis (Awad *et al.*, 1969) or to a decrease in extractability due to formation of insoluble lipid complexes (Buttkus, 1967).

The content of total phospholipids in raw meat was constant during frozen storage, being .71, .71 and .70% at 0, 8 and 13 months, respectively. Results verify the report by Pearson *et al.* (1977) that the phospholipid fraction of meat is relatively constant although fat content is highly variable. Results are also in close agreement with the report by Dugan (1971) that the level of phospholipids in meat ranges from .5 to 1.0%.

Results also showed that cooking significantly increased the amount of phospholipids and are supported by the data presented by Campbell and Turkki (1967) and Fooladi (1977). However, the differences in the phospholipid content of cooked meat held at 4C and -18C were not statistically significant.

Chicken Dark Meat Lipids. Levels of total lipids, triglycerides and phospholipids in chicken dark meat are presented in table 1. The amount of total lipids in raw meat was 9.12,

TABLE 1. EFFECT OF LENGTH OF FROZEN STORAGE, COOKING AND STORAGE TEMPERATURE UPON LIPIDS FROM DIFFERENT KINDS OF MEAT

Lipids[a]	Storage temp.	Storage time (months)[d] 0	8	13
		Beef lipids		
Total lipids	Fresh/frozen	13.72 ± .06[e]	9.52 ± .60[f]	9.82 ± .93[f]
	Cooked/4 C[b]	13.01 ± .04[e]	10.01 ± .17[f]	10.45 ± .21[f]
	Cooked/-18 C[c]	13.26 ± .20[e]	10.01 ± .22[f]	10.95 ± .42[f]
Triglycerides	Fresh/frozen	12.89 ± .12[e]	8.70 ± .42[g]	9.09 ± .83[g]
	Cooked/4 C[b]	11.98 ± .05[f]	8.97 ± .11[g]	9.33 ± .04[g]
	Cooked/-18 C[c]	12.18 ± .23[f]	9.01 ± .19[g]	9.85 ± .43[g]
Phospholipids	Fresh/frozen	.71 ± .03[e]	.71 ± .09[e]	.70 ± .13[e]
	Cooked/4 C[b]	.93 ± .02[f]	.99 ± .02[f]	.94 ± .01[f]
	Cooked/-18 C[c]	.95 ± .01[f]	.95 ± .01[f]	.98 ± .01[f]
		Chicken dark meat lipids		
Total lipids	Fresh/frozen	9.12 ± .47[e]	6.06 ± .21[g]	7.27 ± .06[h]
	Cooked/4 C[b]	10.66 ± .20[f]	7.00 ± .19[h]	8.61 ± .05[i]
	Cooked/-18 C[c]	10.73 ± .42[f]	7.18 ± .07[h]	9.17 ± .04[j]
Triglycerides	Fresh/frozen	8.20 ± .43[e]	5.15 ± .21[g]	6.42 ± .06[i]
	Cooked/4 C[b]	9.53 ± .16[f]	5.89 ± .02[h]	7.55 ± .07[j]
	Cooked/-18 C[c]	9.64 ± .35[f]	6.03 ± .11[h]	7.99 ± .01[k]
Phospholipids	Fresh/frozen	.82 ± .03[e]	.83 ± .02[e]	.85 ± .01[e]
	Cooked/4 C[b]	.96 ± .01[f]	1.01 ± .13[f]	.98 ± .00[f]
	Cooked/-18 C[c]	.99 ± .01[f]	.98 ± .02[f]	.99 ± .01[f]
		Chicken white meat lipids		
Total lipids	Fresh/frozen	2.58 ± .21[e]	1.93 ± .00[h]	1.85 ± .09[h]
	Cooked/4 C[b]	2.94 ± .22[f]	2.25 ± .15[i]	2.39 ± .13[i]
	Cooked/-18 C[c]	3.24 ± .14[g]	2.31 ± .01[i]	2.30 ± .14[i]
Triglycerides	Fresh/frozen	2.00 ± .14[e]	1.34 ± .01[g]	1.31 ± .06[g]
	Cooked/4 C[b]	2.66 ± .25[f]	1.45 ± .13[g]	1.54 ± .06[g]
	Cooked/-18 C[c]	2.48 ± .11[f]	1.56 ± .04[g,h]	1.61 ± .09[g]
Phospholipids	Fresh/frozen	.54 ± .03[e]	.53 ± .01[e]	.52 ± .04[e]
	Cooked/4 C[b]	.64 ± .01[f]	.67 ± .06[f]	.62 ± .02[f]
	Cooked/-18 C[c]	.66 ± .01[f]	.67 ± .02[f]	.60 ± .01[g]

[a]Expressed as percentage of total tissue. Each value represents the mean of four determinations.

[b]Meat was cooked and held at 4 C for 48 hour.

[c]Meat was cooked and held at -18 C for 48 hour.

[d]Values in the same lipid group and in same column or row within the same species and bearing the same superscript are not significantly different at $P < .05$.

6.06 and 7.27% during storage at 0, 8 and 13 months, respectively. When cooked, the levels of total lipids, triglycerides and phospholipids were significantly higher than the corresponding amounts of uncooked meat, regardless of length of freezer storage. Similar to beef, total lipids and triglycerides decreased ($P<.01$) during frozen storage, while phospholipids remained relatively constant. The amount of phospholipids in raw chicken dark meat was .82, .83 and .85% at 0, 8 and 13 months, respectively. Hence, levels of phospholipids in dark meat were higher than those found in beef as reported earlier by Watts (1954) and Acosta *et al.* (1966).

Chicken White Meat Lipids. In raw chicken white meat, the amounts of total lipids were 2.58, 1.93 and 1.85% at 0, 8 and 13 months of frozen storage, respectively (table 1). Levels of total lipids and triglycerides in raw chicken white meat also decreased during frozen storage as was found in beef and chicken dark meat. Similar to beef and chicken dark meat, the percentage of total lipids, triglycerides and

phospholipids increased (P<.05) during cooking, regardless of the length of freezer storage.

The phospholipids in raw chicken white meat amounted to .54, .53 and .52% at 0, 8 and 13 months of frozen storage, respectively. Phospholipids were higher in both raw and cooked chicken dark meat than in similarly treated chicken white meat (table 1). This is in agreement with Peng and Dugan (1965) and Acosta *et al.* (1966), who reported that chicken dark meat contains higher levels of phospholipids than chicken white meat.

Results of this study showed that total lipids decreased during frozen storage, which is in agreement with the work of Zipser *et al.* (1962). They reported a 21% decrease in total lipids in oxidizing mullet tissue during 5 days of refrigerated storage. On the contrary, Acosta *et al.* (1966) reported an apparent decrease in phospholipid content of turkey tissues frozen for 180 days at -25C. They also reported increased levels of total lipids in all tissues except liver. Results of the present study showed that losses observed in total lipids during frozen storage were primarily due to changes in the triglycerides, since the phospholipids were relatively constant.

The reason for the decreased concentration of total lipids and of the triglycerides during frozen storage is not known. The procedure of Folch *et al.* (1957) for quantitative removal of total lipids from animal tissues involves extraction with chloroform-methanol and removes phospholipids and proteolipids as well as triglycerides from various fresh animal tissues. However, Zipser *et al.* (1962) have indicated that chloroform-methanol extraction does not remove all TBA reactive material from cooked or oxidizing fish tissue, probably due to peroxidation or oxidative scission of fatty acids, which decreases their solubility in fat solvents. They suggested that changes in solubility of oxidized lipid materials interfere drastically with extraction and estimation. These authors have also speculated that even though oxidation occurs mainly in the polyenes, their oxidation could affect the solubility of the larger triglyceride molecules. This may account for the decreased level of triglycerides observed in the present study with both raw frozen and cooked meat. Acosta *et al.* (1966) suggested that molecular changes, such as the formation of polymers and complexes, may reduce the extent of lipid extractability in solvents such as choloroform and methanol.

TBA Numbers of Frozen and Cooked Meat

Beef. TBA numbers for raw frozen and cooked beef are presented in table 2. In raw frozen beef, TBA values rose from .27 at 0 time to .31 at 8 months and to .41 at 13 months of frozen storage, with the latter value being significantly higher than the other two. Thus, raw beef was very stable during frozen storage, as TBA values were still well below the threshold levels for rancidity of 1 to 2 as outlined by Watts (1962).

When beef was cooked and subsequently held at -18C for 48 hr after cooking, TBA values were 1.63, 2.64 and .79 at 0, 8 and 13 months of frozen storage, respectively. The values were significantly (P<.01) different from each other. Although the reason for the drop in TBA value at 13 months is unknown, Buttkus (1967) has postulated that a reaction between myosin and malonaldehyde may take place during storage and cause a decline in TBA numbers.

When beef was cooked and subsequently held at 4C for 48 hr after cooking, the TBA values were higher (P<.001) than those in other treatments. Variation in TBA values is related to changes in the amount of total lipids (tables 1 and 2), i.e., high TBA values and high lipid content are directly related and *vice versa.* The relationship is not surprising as TBA values are expressed as milligrams of malonaldehyde per 1,000 g of meat (Tarladgis *et al.*, 1960).

Chicken Dark Meat. TBA values for fresh raw frozen and cooked chicken dark meat are presented in table 2. TBA numbers for raw chicken dark meat exceeded the threshold values (1-2) for acceptability after 8 months in freezer storage. Thus, a higher rate of lipid oxidation took place in raw chicken dark meat than in raw beef. Differences in rate of oxidation can be largely explained on the basis of a greater amount of lipid unsaturation in the former than in the latter.

Cooked chicken dark meat held at -18C for 48 hr after cooking was considerably more stable to lipid autoxidation than that held at 4C for 48 hr following cooking (table 2). Thus, variation in TBA values tended to be related to differences in concentration of total lipids (table 2) in cooked dark meat. This observation further emphasizes the relationship between lipid composition and lipid oxidation in cooked meat.

Chicken White Meat. TBA numbers of raw

TABLE 2. EFFECT OF LENGTH OF FROZEN STORAGE AT -18 C AND COOKING ON TBA NUMBERS IN DIFFERENT KINDS OF MEAT[a,b,c]

Storage time (months)	Raw meat stored at -18 C	Cooked meat	
		Held at -18 C for 48 hr	Held at 4 C for 48 hr
	Beef (LD)		
0	.27 ± .02[d]	1.63 ± .08[g]	7.26 ± .20[k]
8	.31 ± .03[d]	2.64 ± .21[h]	6.09 ± .16[i]
13	.41 ± .01[e]	.79 ± .03[f]	6.55 ± .07[j]
	Chicken dark meat (thighs)		
0	.36 ± .06[d]	6.80 ± .15[i]	16.65 ± .36[l]
8	1.78 ± .34[e]	5.42 ± .14[g]	12.22 ± .50[j]
13	2.44 ± .51[f]	5.73 ± .10[h]	13.34 ± .55[k]
	Chicken white meat (breast)		
0	.37 ± .02[d]	5.56 ± .09[i]	12.59 ± .35[l]
8	1.45 ± .05[f]	3.58 ± .19[g]	6.58 ± .11[j]
13	1.09 ± .04[e]	4.15 ± .02[h]	8.89 ± .09[k]

[a]TBA number is expressed as milligram malonaldehyde/kilogram meat.

[b]Values in the same column or row, bearing the same letter are not significantly different $P < .05$.

[c]Each value represents a mean of four replicates.

frozen chicken white meat (table 2) were .37, 1.45 and 1.09 at 0, 8 and 13 months of frozen storage, respectively. Thus, raw chicken white meat was more stable during freezer storage than raw chicken dark meat but less stable than beef. Lipid oxidation was considerably higher in cooked chicken white meat held at 4C for 48 hr in comparison to the same meat held at -18C for 48 hr following cooking.

Table 3 presents TBA values of cooked meat immediately following cooking and prior to holding either at 4C or -18C for 48 hours. Prior to frozen storage of raw meat at 0 time, a portion was cooked and assessed for TBA numbers immediately following cooking. After 8 months of freezer storage, a portion of raw frozen meat was cooked and analyzed for TBA values. The TBA value for freshly cooked beef at 0 time was .60. After 8 months of frozen storage of raw beef, the TBA value following cooking was .26. Lower values were also found in both chicken dark and white meat analyzed after frozen storage.

These results were unexpected, since it is

TABLE 3. INFLUENCE OF FROZEN STORAGE OF MEAT IN THE RAW STATE ON TBA VALUES OF THE COOKED MEAT PRIOR TO HOLDING AT 4 C OR -18 C FOR 48 HOURS[a,b,c]

Time of raw meat in frozen storage	Beef	Chicken dark meat	Chicken white meat
0 month	.60	7.71	5.02
8 months	.26	2.10	1.52

[a]Fresh/frozen raw meat samples were cooked and the TBA number measured immediately after cooking.

[b]TBA number is expressed as milligram malonaldehyde/kilogram meat.

[c]Each value represents a mean of four replicates.

usually believed that frozen raw meat is less stable to autoxidative degradation than fresh unfrozen meat (Bratzler *et al.*, 1977). Even though the TBA test does not fulfill all the requirements of a reproducible technique and has been criticized on several points (Lea, 1962; Gray, 1978), it is more closely related to taste panel scores than other oxidative tests (Watts, 1962). Although malonaldehyde (MA) can be used to follow oxidative deterioration of food and food products, the relationship between oxidation and MA production is not a simple one (Arata and Chen, 1976). This underlines the need for a sensory measurement along with TBA evaluation of test products.

The reason for the decreasing level of malonaldehyde during freezer storage is not actually known. Chang *et al.* (1961) suggested that formation of carbonyl addition products may account for the loss of malonaldehyde during frozen storage. A decline in TBA values was observed during frozen storage of cooked meat and fishery products by Tarladgis and Watts (1960). The decrease in TBA values could be due, at least in part, to the interaction of myosin and malonaldehyde (Buttkus, 1967). Results of the present study indicate that the decrease in TBA numbers for cooked meat previously frozen in the raw state is at least in part due to the differences in the lipid content of the meat. In this study, there was an inconsistent but decreasing concentration of total lipids and triglycerides during frozen storage of the raw meat.

Results of this study suggest that it is not advisable to hold precooked meat at 4C, even for short periods of time. However, precooked meat can be held at -18C on a short term basis without any great loss of quality. Most commercial enterprises freeze precooked meat and meat products at -18C, which would help minimize development of oxidative rancidity. Malonaldehyde measurement is used to determine whether or not foods are stable or rancid. Recently, malonaldehyde has been implicated as a cause of stomach cancer (Shamberger, 1978), especially in countries with high meat consumption. Thus, the fact that lower levels of malonaldehyde were formed in cooked meat that had been previously frozen in the raw state (tables 2 and 3) may be of public health significance. However, further research on this controversial subject will be needed to ascertain if this is true.

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