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Research Article

A COMPARATIVE STUDY OF MALONDIALDEHYDE CONTENTS OF SOME MEAT AND FISH SAMPLES PROCESSED BY DIFFERENT METHODS

Okolie Ngozi Paulinus[1]* and Okugbo, Osarhieme Tinuade[2]
[1]Department of Biochemistry, Faculty of Life Sciences, University of Benin
[2]Department of Biochemistry, Benson Idahosa University, Benin City, Nigeria
*Corresponding Author Email: Okolie_ngozi@yahoo.co.uk
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ABSTRACT
The effect of different processing methods on the malondialdehyde, MDA contents of 7 popular samples of fresh meat and fish eaten in Nigeria, was investigated. The MDA levels were assayed in a colorimetric reaction with thiobarbituric acid in fresh samples of beef, pork, turkey, chicken, snail, catfish and goat meat processed by boiling, frying, roasting and freezing. Results obtained showed that all processing methods led to significant increases in MDA contents of the samples studied relative to their fresh, unprocessed counterparts ($p < 0.05$). For most samples, boiling, roasting and freezing yielded comparable levels of MDA. However, significantly higher levels of MDA were seen in samples fried after boiling, relative to boiled samples ($p < 0.05$). For the boiled samples, beef and goat meat had the least MDA. The order of MDA content in the boiled samples was beef > goat meat > catfish > turkey > chicken > snail > pork. The MDA content of frozen mackerel was significantly increased by boiling and frying and by further freezing ($p < 0.05$). These results suggest that while all the processing methods may be unsafe, the frying of boiled meat and fish may be particularly deleterious to consumer health, in view of the well-known mutagenic and carcinogenic effects of MDA.
Keywords: Malondialdehyde Content, Meat, Fish, Snail, Boiling, Roasting, Frying.

INTRODUCTION

Lipid peroxidation is regarded as a major source of quality deterioration, not only in meat and meat products but also in fish and fish products. Aside from the producing undesirable changes in flavor and loss of food quality, lipid peroxidation increases the level of malondialdehyde, MDA in foods. Studies have revealed that MDA is one of the most abundant lipid peroxidation cytotoxins formed in foods, especially in meat[1,2]. Earlier reports on the mutagenic and carcinogenic potentials of MDA[3,4] have since led to increased interest in its implication for human health. Following ingestion of peroxidised foods, humans and lower animals have been shown to excrete increased amounts of MDA in the urine[5]. Indeed it is now known that MDA can be absorbed from tainted foods when these foods are injested[6,7]. MDA is genotoxic, reacting with DNA to form highly mutagenic adducts in human cells[8-10]. It has been demonstrated that the level of MDA-DNA adducts in human colorectal tissue correlates with diet and incidence of adenoma[11]. Thus the presence of MDA in foods may have deleterious consequences for human health. In Nigeria as is the case in most other countries of the world, meat and fish are the major sources of dietary protein. Both are usually consumed after thermal processing which may entail boiling, frying or roasting/grilling. From toxicological perspectives, fresh meat and fish are safer than frozen ones, because it has been demonstrated that freezing results in increased accumulation of MDA in meat and fish[12]. Thus thermal treatment of fresh samples may be safer. However prolonged heating is known to also increase the level of lipid peroxidation by a combination of several processes including disruption of muscle cell structure, inactivation of anti-oxidant enzymes and release of oxygen and iron from myoglobin[15]. The present study attempts to compare the effects of different thermal processing methods on the MDA contents of popular meat and fish samples consumed in Nigeria, with a view to determining the safest procedure, in view of the toxic implications of MDA.

MATERIALS AND METHODS

Sample Collection and Processing

Lean muscle samples of beef, pork and goat meat were purchased from freshly sacrificed cow, pig and goats respectively, in an abattoir in Benin City, Edo State, Nigeria. Live African giant snail, live chicken and live turkey were procured from Oba Market, Benin City, while live catfish was obtained from Benson Idahosa University Farms, Benin City. Frozen fish (mackerel) was purchased from a retail outlet in a cold room at Oba market, Benin City. The live samples were sacrificed and muscle parts were dissected out. Muscle was also collected from frozen mackerel. All samples were washed clean with tap water and divided into two portions. One portion was used immediately for analysis of MDA contents, while MDA was analysed in the other portion after processing by boiling, frying + roasting, and freezing. The samples were sliced into small sizes similar to those routinely used in cooking, roasting and frying. For processing by boiling, the samples were immersed in boiling water in clean aluminum pots and allowed to cook for exactly 15 minutes before removal. A part of each boiled sample was further processed by deep-frying for 5 minutes in Gino^R brand vegetable oil. For processing by roasting, the sliced fresh samples were wrapped in clean aluminum foil and divided into two parts. One part was roasted for 1 h at 170^o C in an oven, while the other part was stored in a deep freezer at -10^0 C for 5 days. All processed samples were subsequently analysed for their MDA contents.

Analysis of Samples for MDA

Fresh and processed samples were analysed for MDA using the colorimetric reaction with thiobarbituric acid, TBA[14]. In essence, 0.5 g of sample was thoroughly ground with some

grains of acid-washed sand in a hand mortar and extracted with 10 ml of 15 % TCA for 15 minutes. The resultant homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant fraction subjected to MDA assay. The reaction mixture contained 1 ml of extract and 2 ml of TBA reagent. The mixture was heated for 15 minutes in a boiling water bath, and on cooling; the resultant precipitate was removed by centrifugation at 3000 g for 10 minutes. Then the absorbance of the pink-colored solution was read at 535 nm and converted to moles of MDA by using its molar extinction coefficient. MDA levels were expressed as mole/g fresh wt.

Statistics

The levels of MDA (Mean ± SD) arising from the various processing methods were each compared with corresponding values for unprocessed samples using student's t-test. P values < 0.05 were taken as significant. T-test was also used to compare values obtained for boiled samples and boiled + fried samples; while analysis of variance, ANOVA was used to compare differences between frozen mackerel MDA values arising from different processing methods.

RESULTS

Table 1 shows the levels of MDA in fresh and processed samples of beef, pork, catfish, snail, chicken, turkey and goat meat. MDA was lowest in the fresh samples, but increased with boiling and frying, except in snail where boiling actually led to a decrease in MDA. For most samples, boiling and roasting produced comparable levels of MDA. The highest increases in MDA were seen in boiled and fried samples.

Table 1: MDA Contents of Fresh and Processed Samples of Catfish, Goat meat, Beef, Chicken, Turkey, Pork and Snail

	MDA Content (mole/g fresh wt x 10^{-3})				
Sample / Processing method	**Fresh**	**Boiled**	**Boiled and deep-fried**	**Roasted**	**Frozen**
Catfish	1.71 ± 0.20^{a}	2.30 ± 0.26^{b}	3.33 ± 0.46^{c}	2.91 ± 0.20^{c}	2.65 ± 0.2^{c}
Beef	0.13 ± 0.04^{a}	1.62 ± 0.20^{b}	4.19 ± 0.39^{c}	2.26 ± 0.27^{d}	2.44 ± 0.13^{d}
Snail	4.74 ± 1.11^{a}	3.72 ± 0.97^{b}	8.99 ± 1.32^{c}	4.55 ± 0.64^{a}	5.81 ± 0.65^{d}
Turkey	0.38 ± 0.20^{a}	2.38 ± 0.35^{b}	5.00 ± 0.46^{c}	3.02 ± 0.42^{d}	2.69 ± 0.34^{b}
Chicken	0.60 ± 0.15^{a}	2.46 ± 1.02^{b}	6.48 ± 0.35^{c}	3.12 ± 0.20^{d}	3.95 ± 0.34^{e}
Pork	1.41 ± 0.13^{a}	3.80 ± 0.58^{b}	7.31 ± 0.68^{c}	4.74 ± 0.68^{d}	2.91 ± 0.49^{e}
Goat meat	0.26 ± 0.07^{a}	1.76 ± 0.26^{b}	4.49 ± 0.13^{c}	2.31 ± 0.13^{d}	1.92 ± 0.26^{b}

Results are expressed as Mean ± SD (n = 3). For each sample, values across bearing superscripts different from the corresponding value for fresh sample differ significantly from it ($p < 0.5$).

Samples subjected to dual heat treatment of boiling and frying had at least twice as much % increase in MDA over fresh samples as boiled samples (Table 2). This trend was also seen in snail, where boiling and frying brought about significant increases in MDA levels in spite of the fact that boiling alone actually decreased its MDA content (Table 1).

Table 2: Increase in Mean MDA Levels of Boiled Samples and Boiled + Fried Samples Relative to Unprocessed Samples (%)

Sample	Boiled	Boiled + fried
Catfish	34	130
Beef	1146	3123
Snail	-22	89
Turkey	526	1216
Chicken	310	980
Pork	170	418
Goat meat	577	1627

Boiling significantly reduced the MDA content of raw, frozen mackerel, but with boiling and frying, the MDA level was significantly increased relative to the value for the raw sample ($p < 0.05$) (Table 3). Roasting and further freezing also led to significant increases in the MDA content of the raw, frozen mackerel ($p < 0.05$).

Table 3: Effect of Various Processing Methods on the MDA Content of Frozen Mackerel

Frozen mackerel	MDA (mole/g fresh wt x 10^{-3})
Raw	9.61 ± 0.38^{a}
Boiled	6.79 ± 0.38^{b}
Boiled and fried	11.97 ± 0.45^{c}
Roasted	11.80 ± 0.71^{c}
Further freezing	10.81 ± 0.51^{c}

Values are Mean ± SD; n=3. Values with different superscripts differ significantly ($p < 0.05$)

DISCUSSION

The results obtained in this study show that heat and cold treatments accentuate levels of MDA in fish and meat. It has been reported that heating increases the level of lipid peroxidation in meat by a number of processes including inactivation of protective antioxidant enzymes, disruption of muscle cell structure and release of oxygen and iron from myoglobin[14]. Iron has been implicated as the most probable catalyst in the promotion of lipid peroxidation through generation of hydroxyl radicals by the Fenton reaction[15,16]. Most muscle foods contain myoglobin and hemoglobin-bound iron, which contribute 74, 47 and 28 % of total iron in beef, pork and chicken thigh respectively[17]. In addition, the oxidative stability of meat is dependent on the degree of unsaturation of its sub-cellular membrane phospholipids: a high degree of unsaturation is associated with higher oxidative potentials[18]. Although snail is low in fat, about 75 % of the fat is composed of polyunsaturated fatty acids, which render it vulnerable to lipid peroxidation[19]. Thus the differences in levels of MDA among the samples studied may

be a reflection of the differences in their susceptibilities to lipid peroxidation. In general it has been demonstrated that the extent of lipid peroxidation in meat depends on the anatomical location of the muscle, muscle type and animal species[20,21]. Freezing significantly increased the MDA contents of all the fresh samples of meat and catfish relative to fresh samples and in most cases relative to boiled samples also. This is in agreement with previous reports that freezing increases MDA contents of fish and meat[12,22,23]. Although the precise mechanism by which refrigeration increases lipid peroxidation is not well understood, it may not be unconnected with disruption of membrane structure and exposure of phospholipids to pro-oxidants through slicing as well as freezing and thawing. Fatty fish such as mackerel have high oxidation potentials, even when stored at sub-zero temperatures[23]. This explains the high level of MDA in frozen mackerel and the fact that the MDA increased significantly with further freezing. The levels of MDA resulting from roasting (grilling) were in most samples comparable with MDA levels from boiling. This is surprising, considering the fact that the samples were grilled for I hour while boiling lasted exactly 15 minutes. It is possible that the higher grilling temperature of 150^{o}C resulted in lower rate of lipid peroxidation due to rapid dehydration of the samples, unlike boiling which took place in a hydrated medium and at a lower temperature. Boiling resulted in the lowest levels of MDA in all samples and thus appears to be the safest of all the processing methods investigated. It also resulted in significant reduction of MDA in frozen mackerel and was the only processing method that was able to achieve this. The reduction of MDA content of frozen mackerel by boiling is considered a crucial finding, because most Nigerian homes and hotels depend on frozen meat and fish, which contain appreciable levels of MDA[12]. It is interesting that samples fried after boiling had significantly higher level of MDA when compared to MDA resulting from any other treatment. This may be explained by the fact that the samples were exposed to two different heat treatments, each of which is known to enhance lipid peroxidation. Frying of boiled meat and fish are rather popular culinary habits in most Nigerian homes and public eating places. From the results obtained in this study, it appears that this practice may be deleterious to consumer health due to rapid build-up of MDA. The presence of MDA in foods is of considerable health concern to the consumer, in view of its well-known carcinogenic and mutagenic effects[24]. MDA is classified as perhaps the most abundant cytotoxin arising from lipid peroxidation in muscle-derived foods, especially meat[1,2]. MDA is readily absorbed from ingested food in the form of N-(2-propenal)-lysine[6]. Indeed N-2-propenal lysine derivative is the major form of MDA in the urine following consumption of peroxidised foods[2]. In this form, MDA still retains its ability to modify DNA. Thus absorbed MDA forms adducts with deoxyadenosine and deoxyguanosine, leading to DNA damage which may pose serious health problems to the consumers[25]. Consequently processing methods that lead to reduction of MDA in muscle foods are highly desirable. Since meat and fish are rarely eaten raw, our results show that boiling of these vital sources of dietary protein may be the safest method of cooking them in homes and public places.

CONCLUSION

The results of this study have shown that the practice of frying meat and fish after boiling, as well as the popular habit of protracted refrigeration should be discouraged, in view of the significant elevation of lipid peroxidation associated with them. Boiling appears to be the safest way to process muscle foods.

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