IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 14, Issue 3 Ser. II (March. 2020), PP 55-58
www.iosrjournals.org

Quantification of malonaldehydes in cured meat products

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Abstract
Background*: Meats and meat products are consumed worldwide, attract taste of most consumers. Many of these products are consumed at meals at home but are also present in snack bars by children and adolescents. However, excessive consumption of these products is associated with several health problems such as increased blood pressure, obesity, several types of cancer and other non-contagious diseases more associated with excess free radicals such as malonaldehyde. Aim of this work was to quantify lipid oxidation through malonaldehyde content in meat sausages during refrigerated storage.* ***Materials and Methods:*** *Nineteen different types of meat sausages from different commercial brands were analyzed during 15-day storage period through lipid oxidation tests measured by number of 2-thiobarbituric acid, whose most important constituent is malonaldehyde.* ***Results****: All products analyzed showed 2-thiobarbituric acid / malonaldehydes levels within limits established by Brazilian legislation.* ***Conclusion:*** *However, it is worth noting that many people consume several meat sausages, in a quantity higher than that used in tests, which exposes them to increased risks associated with lipid oxidation.*

Key words*: legislation; malonaldehydes (MDA); meat sausages; 2-thiobarbituric acid (TBARS), toxicity.*

Date of Submission: 12-03-2020 Date of Acceptance: 27-03-2020

I. Introduction

Meat and meat products are susceptible to several changes that contribute to loss of quality during storage period. Composition of these products that have high water activity, in addition to having several types of saturated and unsaturated lipids, proteins, carbohydrates, contribute to microbial deterioration and biochemical changes.[1,2] However, while deteriorative, microbiological and / or enzymatic reactions can be inhibited with use of low temperatures, lipid oxidation, also called oxidative rancification or self-oxidation, usually occurs at freezing temperature, albeit at reduced rate.[3]

Reactions of lipid oxidation are among main causes associated with deterioration of meat quality[4], which can result in formation of toxic compounds, such as malonaldehyde, as well as accumulation of volatile substances such carbonyls, alcohols and acids. Lipid oxidation is of great concern for meat industry, because it can not only lead to development of undesirable compounds, but can also create precursors or catalysts for formation of reactive oxygen species (ROS) responsible for other deleterious changes in meat.[5, 6]

Lipid oxidation in meats can be monitored by TBARS value, since primary lipid oxidation products are mainly hydroperoxides, which are rapidly decomposed into various reactive substances such as 2-thiobarbituric acid (TBARS), particularly carbonyls, with malonaldehyde (MDA) being most important element.[3] Malonaldehyde is very reactive compound that can interact through cross-links with DNA and proteins, promoting chromosomal aberrations, reducing the capacity for protein synthesis. Aim of this work was to quantify lipid oxidation through malonaldehyde content in meat sausages during refrigerated storage.

II. Materials And Methods

Study location*:* Samples of meat derivatives were purchased in cities of Petrolina-PE and Juazeiro-BA. Petrolina is municipality in the state of Pernambuco, located in São Francisco Valley region, neighboring municipalities of Juazeiro and Sobradinho. Located at 380 meters of altitude, latitude 9° 23' 39" South, longitude 40° 30' 35" West. Juazeiro is city in the state of Bahia. Neighboring municipalities of Petrolina and Sobradinho, Juazeiro is located 5 km south-east of Petrolina. Situated at altitude of 369 meters, latitude: 9° 26' 18" South, longitude: 40° 30' 19" West.

Samples*:* Nineteen types of meat products from different commercial brands were purchased with 15 days of storage. There were six brands of hot dog sausages, six of hams, three Lower ham, three bologna and two turkey breasts (smoked and light). Conservation during this time was carried out in refrigerated environment, at temperature of 4 °C. Samples were ground individually in a meat grinder at time of analysis.

Detection of Malonaldehyde in a UV-VIS spectrophotometer: Malonaldehyde (MDA) in meat products was detected by 2-thiobarbituric acid (TBA) assay using spectrophotometry (Lambda Model 20, Brand Perkin Elmer).[7] Malonaldehyde (MDA) was extracted from samples with 5% trichloroacetic acid (TCA) solution. Sample of meat product (5 g) was homogenized with 18 mL of 5% TCA solution, 0.5 mL of 0.15% hidroxitolueno butilado (BHT) in ethanol, 2 mL of 0.5% sulfanilamide solution using stick glass for 30 seconds. Homogenate was centrifuged at 2090 rpm / g for 15 min, 2 mL of supernatant was removed and transferred in triplicate to test tubes with cap where 2 mL of 0.08M TBA was added. Tubes were vortexed for 10 seconds, heated in water bath at 100 °C for 50 minutes, cooled to room temperature, absorbances of MDA-TBA were read in spectrophotometer at 532 nm. TCA Solution 5% was used as blank to reset device. Number of TBARS, results quantified using calibration curve of 1,1,3,3-tetraethoxypropane (TEP) at concentrations of 0.02-0.09 µg mL^{-1} using aliquots of TEP stock solution of 0, 4; 0.5; 1.0; 1.5; 2.0 µg mL^{-1}. Concentration of MDA in samples was expressed in milligrams of MDA per kilogram of meat product (mg MDA kg^{-1} of meat product) by calculating number of TBA in 2 mL used in tests, converting concentrations to milligram, then correspondence of results to 1 kg of meat product.

Statistical analysis: Statistical analysis was performed by One-way ANOVA, using STATISTICA® 7.0 program, values considered significant with $p > 0.05$. All determinations were performed in triplicate (N = 3), data were expressed as average ± standard deviation. Results were compared using Tukey test to identify existence of significant differences between test results, with significance level of 95% for each evaluated parameter.

III. Results And Discussion

Table 1 shows levels of lipid oxidation measured by number of TBARS in meat products using equation of line $y = 10.34X-0.030$, $R^2 = 0.997$. Most of analyzed products showed significant difference when compared by tukey test. All products analyzed showed levels of lipid oxidation below the maximum values allowed by Brazilian legislation during 15-day storage, however some exceeded maximum recommended daily intake index. TBARS values above 1.59 mg of malonic aldehyde / kg of sample can cause damage to consumer health.[8]

Table 1: Amount of lipid oxidation through levels of MDA / TBARS in cured meat products.

Test	Product	TBARS (mg MDA Kg^{-1} meat)
Braz. Leg	Max.Value	1.59 mg kg^{-1}
	IDA	0-0.7 mg kg^{-1}
1	Hot dog sausages A	$0.12^{h} \pm 0.01$
2	Hot dog sausages B	$0.18^{b} \pm 0.01$
3	Hot dog sausages C	$0.20^{a} \pm 0.00$
4	Hot dog sausages D	$0.13^{g} \pm 0.00$
5	Hot dog sausages E	$0.16^{c} \pm 0.01$
6	Hot dog sausages F	$0.14^{f} \pm 0.00$
7	Ham A	$0.13^{g} \pm 0.00$
8	Ham B	$0.12^{h} \pm 0.00$
9	Ham C	$0.14^{e} \pm 0.00$
10	Ham D	$0.04^{o} \pm 0.00$
11	Ham E	$0.15^{d} \pm 0.00$
12	Lower ham A	$0.60^{m} \pm 0.00$
13	Lower ham B	$0.60^{m} \pm 0.00$
14	Lower ham C	$0.11^{i} \pm 0.00$
15	Mortadella A	$0.05^{n} \pm 0.01$
16	Mortadella B	$0.05^{n} \pm 0.01$
17	Mortadella C	$0.09^{j} \pm 0.01$
18	Turkey breast Light	$0.05^{n} \pm 0.00$
19	Smoked turkey breast	$0.08^{l} \pm 0.00$

* Values expressed as average ± standard deviation followed by same lower-case letters in same columns do not differ statistically at 5% level (Tukey test). TBARS number measured after 15 days of storage. IDA (Daily Intake Index).

In this study, TBARS / MDA value ranging from 0.05 to 0.6 mg kg^{-1} was found. It can be noted that all TBARS / MDA values in 15-day period are below maximum value considered safe, maximum values of daily intake. These values can increase without causing any noticeable alteration to consumer's taste, this means that he can continue consuming product potentially dangerous to his health because sensorially he does not present great noticeable alterations. According to Campo et al. [9] consumer will only detect rancidity in meat based on concentration of 2.3 mg of malonaldehyde kg^{-1} of meat.

It is worth remembering that these values are for 5 grams of meat sausage sample, if person consumes larger quantity or several products cured or stored for more than 15 days, they can reach maximum daily intake value in at least two products, consequently increasing reactive free radicals to your body. Presence of high content of free radicals in foods is associated with carcinogenesis, mutagenesis, inflammation, changes in DNA, aging, cardiovascular diseases.[10]

Nieto et al.[11] when analyzing lipid oxidation in cooked and stored lamb meat for 0, 2 and 4 days, under retail conditions they found TBARS values ranging from 0.19 to 4.28 mg MDA / kg meat in 4 days showed an increase in levels of aldehydes, alcohols characteristic of lipid peroxidation process in meat. Limbo et al.[12] analyzed peroxidation process in ruminant meat when subjected to different storage temperatures, observed higher values of TBA in meat stored at 15.5 °C (0.305 mg MDA/ kg of meat) when compared to meat stored at 8.1 °C (0.105 mg MDA / kg of meat) or 4.3 °C (0.081 mg MDA / kg of meat). Proving that refrigeration decreased molecular activity, reduces interactions between oxidative molecules and meat lipids.

Not many articles have been found that aim to quantify malonaldehyde content in meat products, to study all effects that high consumption can cause in humans. It is worth mentioning that production of MDA is not only danger associated with high consumption of cured products, some authors have already reported high content of lipids that can contribute to obesity, increased total cholesterol and LDL[2], high use of additives such as sorbate and sodium nitrite which, although it has technological function of intensifying color, reducing microbial deterioration, overuse is associated with development of several types of cancer [13, 14] excessive use of sodium chloride, which can contribute to increased blood pressure, fluid retention, stomach cancer, especially in people with *H. pylori* bacterium. [15, 16] Therefore, MDA levels are just one of dangers associated with consuming these foods.

Articles related to this theme focus on finding and testing ways to reduce lipid oxidation, either by investing in new packaging that prevents entry of oxygen, incorporating new antioxidants and / or ingredients. Some authors suggest use of vacuum or low gas permeability packaging is recommended to minimize meat's contact with oxygen, protecting them from oxidation, resulting in longer commercial period for meat, O_2 level is reduced to less than 1% by vacuum inside package before sealing.[17, 18, 19]. Santos et al.[20] studied different types of packaging, vacuum and different combinations between gases (N_2, O_2, CO and CO_2), in beef kept at 2 °C, verifying that lipid oxidation increases significantly when in packaging systems in modified atmosphere when compared to vacuum.

Brazilian legislation allows use of synthetic antioxidants such as BHT, BHA, propyl gallate in order to prevent or reduce oxidation in meat products as long as maximum values established by law are observed. [21, 22] Some authors suggest, however, replacement of synthetic antioxidants by natural antioxidants due to consumer's natural concern about side, cumulative effects of their long-term intake. Fact that FDA (Food and Drug Administration) classifies tannic acid, phenolic compound present in wide variety of fruits and cereals, as known safe substance (GRAS) for addition to foods, including meats, has encouraged further research to prove efficiency of other compounds present in foods that have antioxidant activity.[23, 24] Antioxidant effects of pomegranate [25], tomato pomaces [26], annatto [27], pomaces of grape, passion fruit, orange. [28, 25]

Other authors suggest reformulation of meat products incorporating bioactive cereal compounds such as fibers, monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) or replacing the harmful components incorporating proteins of plant origin. [29, 30] All these studies only prove concern of several researchers with harmful effects of consuming these foods, offering alternatives that make this consumption safer.

IV. Conclusions

Lipid oxidation is natural process of lipid degradation that occurs in meat products even during cold storage. Products of this oxidation are reactive substances like MDA-TBARS that can interact with DNA and cause damage. Although all products analyzed have levels of MDA-TBARS below maximum values considered dangerous, one should be aware of excessive consumption of these foods since higher values can be produced in longer storage time. In addition, these foods present other hazards such as excess sodium chloride, lipids, presence of cacyrogenic additives such nitrite and sodium nitrate.

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Josileide Gonçalves Borges, etal. " Quantification of malonaldehydes in cured meat products." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 13(3), (2020): pp 55-58.