Roux S., Petit M., Baeza E., Bastianelli D., Tillard E. & Arnaud E., (2011). Effect of a flax seeds diet on lipid oxidation of raw and cooked poultry meat. *XXth European Symposium on the Quality of Poultry Meat*. Leipzig, Germany, 4-8 september 2011. Proceedings c-067.

Effect of a flax seeds diet on lipid oxidation of raw and cooked chicken meat

Stéphanie ROUX[1], Matthieu PETIT[2], Elisabeth BAEZA[3], Denis BASTIANELLI[4], Emmanuel TILLARD[2], Elodie ARNAUD[1]

[1] CIRAD, UMR Qualisud, BP 20, F-97408 Saint Denis Messagerie cedex 9, La Réunion, France, [2] CIRAD, UMR SELMET, Ligne Paradis, F-97410 Saint Pierre, La Réunion, France, [3] INRA, UR 83 Unité de Recherches Avicoles, F-37380 Nouzilly, France, [4] CIRAD, UMR SELMET, Campus international de Baillarguet, F-34398 Montpellier, France
e-mail: elodie.arnaud@cirad.fr

Short title: Chicken meat and lipid oxidation

Introduction

Improving the nutritional quality of food is one of the current major research areas. Concerning poultry meat, one of the major fields consists in enriching the lipid fraction with unsaturated fatty acids, in order to increase the omega 3 content. Flax seeds are rich in omega 3 (Peiretti & Meineri, 2008). They are commonly used to be the omega 3 supplier in animals' diets with good meat enriching performances (Zuidhof *et al.*, 2009). The negative effect of enriching meat with "good for health" unsaturated fatty acids is that these compounds are highly susceptible to oxidation which generates "bad for health" degradation molecules such as pro-oxidant free radicals. To avoid these reactions, omega 3 are generally coupled with an antioxidant. The classical antioxidant in animals' diet is vitamin E and previous studies have shown its efficacy in preventing lipid oxidation in poultry muscles (Lauridsen *et al.*, 1997, Ryu *et al.*, 2005). Lipid oxidation is classically quantified by measuring the TBARS index which corresponds to every substances reacting with thiobarbituric acid. Many methods exist (with or without an induction of the oxidation by adding iron and heating, by a spectrophotometric or a fluorimetric detection, using kinetics…) whether the objective is to determine the lipid oxidation (direct methods without induction) or the susceptibility to oxidation (complex methods with induction) of meats. For a direct quantification of the level of lipid oxidation, the method of Lynch & Frei (1993) is simple and well adapted. Among methods including an oxidation induction step, the method of Kornbrust & Mavis (1980) coupled with the detection method of Beuge & Aust (1978) is the most

frequently found in literature. In each case, values are expressed as equivalent malondialdehyde (MDA), because this compound is considered as the major lipid oxidation product but TBARS index remains a global measure. That is why, specific MDA measurement by liquid chromatography are now developed and used (Agarwal & Chase, 2002; Gladine *et al.*, 2007).

This work aims to compare the direct TBARS index (Lynch & Frei, 1993) with the MDA content measured by HPLC (Agarwal & Chase, 2002) on meat muscles of chicken fed with diets supplemented or not in omega 3 and vitamin E. Lipid oxidation is then discussed according to the piece of the chicken (breast or thigh) and its further processing (storage at 4 °C with or without vacuum cooking).

Materials and Methods

A total of 800 chickens (JA757 strain) were raised in an experimental poultryhouse (Lycée Agricole, Saint Joseph, La Réunion). They were fed with the same diet for 36 days. Then, they were divided into 3 groups exhibiting equivalent live body weights and fed with different diets for the last 20 days. All diets were formulated (Sanders, Pontivy, France) and provided (Proval, Le Port, La Réunion) specially for the project. The first group was the “control” and had no diet supplementation with omega 3 or vitamin E (ω3 - / vit E -). The second one was the “oxidation control” supplemented with omega 3 but not in vitamin E (ω3 + / vit E -). Finally, the third one was the “enriched diet” supplemented with omega 3 and vitamin E (ω3 + / vit E +). The omega 3 were provided in diet by adding flax seeds, which corresponds to an increase in lipid content from 3.4% in ω3 - diet to 5.3% in ω3 + diets and to an increase in omega 3 content from 0.13% to 1.5%. Concerning vitamin E, contents were 45 ppm in vit E - diets and 160 ppm in vit E + diet.

At 56 days of age, all birds were slaughtered under industrial conditions (Crête d’Or Entreprise, Etang Salé, La Réunion). At the end of the slaughter procedure (D+0), the two breasts and one thigh were removed from 12 chickens per diet and immediately stored at 4 °C. After 2 days of storage, one breast was cut into two parts. The first part was directly used (D+2 raw) and the second one was cooked (D+2 cooked). After 3 days of storage, a piece of thigh (about 5 g) was removed (D+3 raw). After 9 days of storage the second breast was submitted to the same treatment as the first one (D+9 raw and D+9 cooked). After 10 days of storage, another piece of thigh was removed (D+10 raw). After 13 days of storage, the rest of thighs were cooked (D+13 cooked). Meat pieces were wrapped under vacuum and

cooked in boiling water till the inside temperature reached 72 °C (5 min for breasts and 20 min for thighs). For every storage time, each sample of raw or cooked meat was frozen in liquid nitrogen before grinding and stored at -20 °C before analysis.

TBARS index was measured according to the method of Lynch & Frei (1993) adapted to meat. Briefly, 1 g of meat powder was put in 100 µL of BHT (butylhydroxytoluene) 0.1 mM in ethanol and 9.9 mL of KCl 0.15 M in water and ground for 1 min at 15,000 rpm with a Polytron grinder (Kinematica AG, Switzerland). 0.5 mL of the solution was then mixed with 0.25 mL of TBA (thiobarbituric acid) 1% in NaOH 1 mM and 0.25 mL of TCA (trichloroacetic acid) 2.8% in water and incubated 10 min at 80 °C. After cooling in ice for 15 min, 2 mL of butanol were added for the extraction of lipid oxidation compounds. After centrifugation, the butanol phase was read at 535 nm using a spectrophotometer against a butanol blank. Absorbance values were converted in µg equivalent MDA per g of meat thanks to a calibration curve using 1,1,3,3-tetramethoxypropane (MDA precursor) treated in the same way as the samples.

MDA content was measured according to the method of Agarwal & Chase (2002), slightly modified. Briefly, 500 mg of powdered meat was mixed with 4 mL of BHT 0.8% in hexane and 6 mL of TCA 5% in water and ground for 30 s at 30,000 rpm with the Polytron grinder. The lower phase was then filtered and 1.4 mL was mixed with 0.7 mL of TCA and 1.5 mL of TBA 0.8% in NaOH 1 mM. Tubes were then incubated for 30 min at 70 °C. After cooling at ambient temperature, 1.5 mL of butanol was added for the MDA extraction. After centrifugation, the butanol phase was injected in a HPLC system equipped with a RP C18 column with a mobile phase being a mixture of potassium phosphate 0.05 M pH 6.8 and methanol (60/40, v/v). Detection was done in fluorescence (excitation at 515 nm and emission at 553 nm). Areas were converted in µg MDA per g of meat using a calibration curve of 1,1,3,3-tetramethoxypropane, treated in the same way as the samples.

ANOVA analyses were done using Statistica 7.1 (Statsoft, United States). Fisher least significant difference test was applied to determine if the difference between means were statistically different or not, with a statistical risk of 5%.

Results

Samples were analyzed for TBARS index and for MDA content. Results show that both values were not well correlated (Figure 1). For small MDA values (<0.5 µg/g, corresponding to raw thigh and breasts with the shortest storage times), TBARS indexes were mostly higher

whereas for higher MDA values (corresponding to longer storage times and cooked meats), the tendency was not so clear; TBARS values seemed to become quite equivalent to MDA content. TBARS is a global measure of all the components which are reactive with TBA. It is expressed as equivalent MDA because MDA is considered as the major component. Our results suggest that TBARS index may include MDA precursors in raw meat which turn into MDA when meat becomes more oxidized and explaining why TBARS index over evaluates MDA content in low oxidized meats. Consequently, as the purpose here is to discuss the effect of diets on lipid oxidation through storage and cooking, MDA has been selected as the oxidation marker.

MDA contents were almost two times higher in thigh than in breast muscle of chicken (Figure 2), probably because thigh contains more lipids (about 7% in thigh *vs* 1% in breast, data not shown). Whatever the type of muscle and its processing, meat MDA contents of animals fed with omega 3 enriched diets were higher for the group not supplemented with vitamin E (except for the breast D+9 cooked for which the difference was not significant). The control diet (ω3 - / vit E -) was equivalent to the enriched one (ω3 + / vit E +) but was also sometimes equivalent to the oxidation control (ω3 + / vit E -). As expected, these results confirmed that meat from chicken fed with omega 3 enriched diets is more oxidized when there is no vitamin E supplementation.

Refrigerated storage significantly increased MDA contents in raw breast meat (Figure 2). In thigh, the increase was not significant except for the control diet. Cooking significantly increases MDA values in thigh for each diet. The same was observed in breast only after a short storage (D+2). For a longer storage (D+9), MDA values were quite the same in raw and in cooked breasts and were mostly equivalent to those of D+2 cooked breasts. It seems that MDA content in breast reached its maximum in each diet by cooking at D+2 or storing during 9 days at 4 °C and that no further treatments could increase it.

Discussion

TBARS index has been used as a lipid oxidation marker for a long time (first references during the end of the sixties). Many researchers have developed their own method leading to different results and complicating the comparison between the studies. In fact, depending on the analysis method, more or less compounds are created and quantified. For example, Betti *et al.* (2009) found, in chicken muscles, TBARS values really high compared to other authors, with a method including an induction phase. Difficulties with TBARS index methods

encourage some researchers to develop more specific methods like MDA direct quantification by HPLC. Our results show clearly that there is no good correlation between a direct TBARS quantification and a direct MDA quantification. As methods present almost the same difficulties and the same length, MDA should be privileged, as far as an HPLC device is available.

In this study, MDA contents are different in breast and thigh muscles of poultry, being almost two times higher in thigh (0.13 to 1.0 µg/g µg/g in thigh and 0.09 to 0.5 µg/g in breast). These values are consistent with those found by Ryu *et al.* (2005) who found, in meat of chickens fed with a classical diet, TBARS indexes expressed in MDA equivalent between 0.1 µg/g and 1 µg/g after of 1 and 12 days of refrigerated storage. The increase in lipid oxidation during their storage is also in the same range as that found in this study. However, in their study, no difference was found between breast and thigh TBARS indexes contrary to our results. Another study by Betti *et al.* (2009) found a difference in TBARS indexes between thigh (from 54 µg/g for control to 165 µg/g for the enriched diet) and breast (from 15 µg/g to 27 µg/g). Their study aimed to enrich poultry meat with omega 3 by adding flax seeds in the diet (content and duration of enrichment similar to ours). The diets contained 50 IU vitamin E/kg (around 33 mg/kg of d-alpha-tocopherol) which is equivalent to our control diets (30 ppm of vitamin E). Despite, oxidation seems to be much higher. Such differences might be linked with the quantification method.

In conclusion, this study has shown the interest of using a specific quantification method for lipid oxidation (MDA by HPLC) rather than a global method (TBARS index) mostly when lipid oxidation is investigated through further processing. Oxidation is enhanced by the omega 3 enrichment but vitamin E enables to control the degradation and to keep the same MDA content as in the classical diet. Thigh has shown higher amount of MDA than breast, so it should be considered as a better support to study lipid oxidation.

Abstract

Enriching food with omega 3 is a largely explored way of improving nutritional quality of feedstuffs. Concerning fresh meat, supplementing the animal diet with natural omega 3 seems to be the best way. However, omega 3 are fragile compounds with a high susceptibility to lipid oxidation. This study investigated meat lipid oxidation of chicken fed with diet enriched with flax seeds and vitamin E as antioxidant. Lipid oxidation was measured by two different methods (TBARS index and MDA by HPLC) and measured through meat processing (storage

and cooking) on thigh and breast. Results showed that TBARS index and MDA are not well correlated. MDA contents are almost two times higher in thigh than in breast (0.13 µg/g to 1.0 µg/g in thigh against 0.09 µg/g to 0.5 µg/g in breast). Omega 3 enrichment increases MDA content when there is no vitamin E supplementation. However, with vitamin E supplementation, MDA values stays at the same level as in the control diet. MDA content increases during refrigerated storage and cooking for each piece of meat.

Keywords: lipid oxidation, poultry meat, omega 3, vitamin E, malondialdehyde

Acknowledgements

Authors want to thanks the “Fond Unique Interministériel” of France, the “Région Réunion” and the “Département de la Réunion” for financing the NUTRIVOL project. They are also grateful to each partner of the project, Crête d’Or Entreprise, Proval, Sanders and Avi-pôle for their participation in the study and to the Lycée Agricole de Saint Joseph for raising the chickens.

References

AGARWAL, R. and CHASE, S.D., (2002). Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples. Journal of Chromatography B **775**, 121-126.

BETTI, M., SCHNEIDER, B.L., WISMER, W.V., CARNEY, V.L., ZUIDHOF, M.J. and RENEMA, R.A., (2009). Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. Poultry Science **88**, 1085-1095.

BEUGE, J.A. and AUST, S.D., (1978). Microsomal lipid peroxidation. In Methods in enzymology, Vol.52, pp. 302-310, by Fleisher, L. & Packer, L., ed. Academic Press, New-York.

GLADINE, C., MORAND, C., ROCK, E., GRUFFAT, D., BAUCHART, D. and DURAND, D., (2007). The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. Animal Feed Science and Technology **139**, 257-272.

KORNBRUST, D.J. and MAVIS, R.D., (1980). Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correlation with vitamin E content. Lipids **15**, 315-322.

LAURIDSEN, C., BUCKLEY, D.J. and MORRISSEY, P.A., (1997). Influence of dietary fat and vitamin E supplementation on [alpha]-tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. Meat Science **46**, 9-22.

LYNCH, S.M. and FREI, B., (1993). Mechanisms of copper-dependant and iron-dependant oxidative modification of Human low-density-lipoprotein. Journal of Lipid Research **34**, 1745-1753.

PEIRETTI, P.G. and MEINERI, G., (2008). Chemical composition, organic matter digestibility and fatty acid content of linseed (Linum usitatissimum L.) harvested at five stages of growth. Journal of the Science of Food and Agriculture **88**, 1850-1854.

RYU, Y.C., RHEE, M.S., LEE, K.M. and KIM, B.C., (2005). Effects of different levels of dietary supplemental selenium on performance, lipid oxidation, and color stability of broiler chicks. Poultry Science **84**, 809-815.

ZUIDHOF, M.J., BETTI, M., KORVER, D.R., HERNANDEZ, F.I.L., SCHNEIDER, B.L., CARNEY, V.L. and RENEMA, R.A., (2009). Omega-3-enriched broiler meat: 1. Optimization of a production system. Poultry Science **88**, 1108-1120.

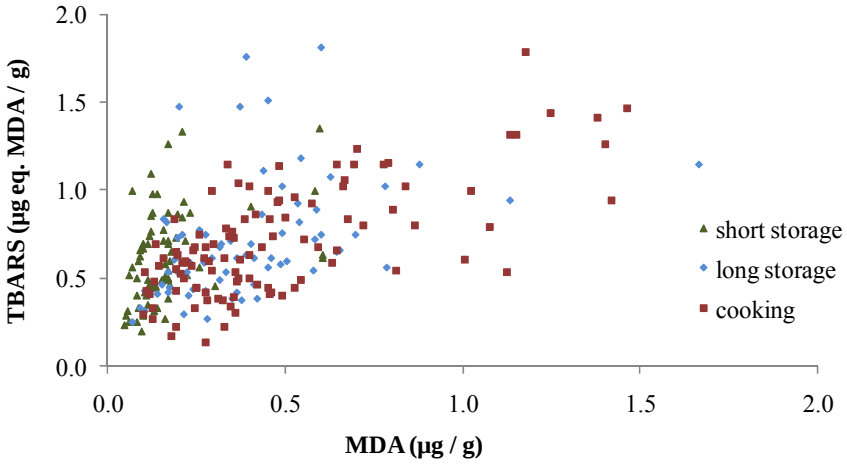

Figure 1. Correlation between MDA and TBARS values obtained on chicken thighs and breasts.

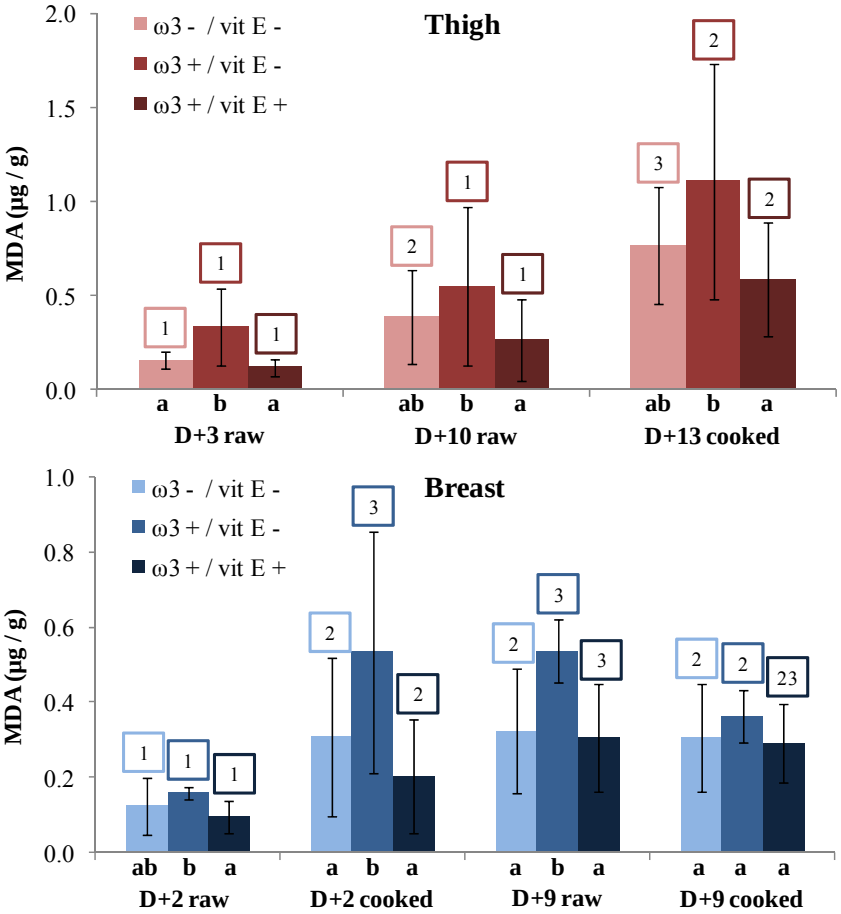

Figure 2. MDA contents determined in chicken thighs (top) and breasts (bottom) after refrigerated storage and with or without cooking (n = 12).

Different letters represent a significant difference between the diets for a same measurement condition and different numbers represent a significant difference between the measurement conditions for a same diet with $P < 0.05$.