Journal of Applied Biological Sciences
E-ISSN: 2146-0108 13 (3): 166-171, 2019

Effect of Cooking Methods on Fatty Acids Profile and Health Lipid Indices of Be-Ni-Guil Lamb Meat from Eastern Morocco

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Received: October 02, 2019
Accepted: November 25, 2019

ABSTRACT

This work focuses on the effects of two common cooking methods "Wet" and "Dry" on intramuscular fat content, fatty acid profile and health lipid indices of the Beni-Guil lamb meat labeled protected geographical indication. Cooking tests performed on *Longissimus Lumborum* muscle show that meat cooking loss is significantly lower for grilling (21.14%) compared to the boiling cooking method (27.06%). Intramuscular fat content, which represents 6.14% of raw meat, decreases after Grilling and boiling to 5.31% and 4.83%, respectively. Saturated (SFA), unsaturated (UFA), particularly polyunsaturated (PUFA) fatty acids, percentages were affected by cooking methods and therefore its affect health lipid indices. Thus, the PUFA/SFA ratio increases after meat cooking from 0.21 for raw meat to 0.37 and 0.29 respectively according to boiling and grilling cooking method. Likewise, the hypocholesterolemic/Hypercholesterolemic ratio, evaluated to 1.62 for raw meat, increases significantly after cooking (1.78 and 1.77 respectively for boiling and grilling). Also, we notice that meat's cooking decreases significantly ($p < 0.001$) the thrombogenicity index from 1.54 for raw meat to 1.42 and 1.26 respectively for cooked meat by boiling and grill mode, however, cooking doesn't have a significant effect on desirable fatty acids ($p > 0.05$). Finally, compared to the wet cooking method, the dry cooking method appeared to be the best cooking mode recommended for Beni-Guil lamb meat, especially from a nutritional standpoint.

Keywords: Lamb meat; Boiling; Grilling; Fatty acids; Lipid indices.

INTRODUCTION

The Beni-Guil (BG) sheep meat labeled protected geographical indication (PGI) is one of the main sheep meats produced and consumed in the Eastern region of Morocco. It is well known for its organoleptic properties (tenderness, color, and juiciness), and nutritive value, due to its high content of long-chain unsaturated fatty acids and essential amino acids [1]. Heat treatments improve the meat microbiological and sanitary quality in such a manner as to minimize the risk of growth of pathogenic microorganisms or the formation of toxins. So, they increase their shelf life and improve their organoleptic qualities [2, 3]. Furthermore, cooking modifies the meat's chemical composition by juice losses and several other biochemical reactions such as lipid oxidation and consequently modifies its nutritional and sensory quality. Of course, the final quality of the cooked meat depends on its quality as raw material but it also depends on the mode and conditions of cooking, particularly time and temperature [4]. Meat's fatty acid profile, health lipid indices (Saturated fatty acids (SFA), mono and polyunsaturated fatty acids (MUFA, PUFA), PUFA/SFA ratio, thrombogenicity index (IT), atherogenicity index (IA), desirable fatty acids etc.…) are necessary parameters to assess meat lipid quality, which is important as a criterion or indicator for healthy good meat [5]. An imbalanced PUFA/ SFA and a high n-6/n-3 ratio may cause serious health problems such as cardiovascular disease, obesity and certain cancers [6, 7]. Those parameters are very influenced by cooking methods (Domínguez, Borrajo [8]. Thus, in some countries, it is common to consume rare, medium rare or medium meat to maintain its nutritional quality, especially to preserve the long-chain unsaturated fatty acids. Unfortunately, this is not the case in Morocco, where often meats are well cooked or even overcooked, with a core temperature of about 70 °C. Also, cooking, as the last stage of meat preparation, is a crucial step for developing flavor and improving meat taste and its palatability [9]. Thus, the culinary practices diversity makes impossible the exhaustive and representative of the compositional analysis carried out on cooked meats, piece by piece and recipe by recipe because the results reproducibility is too randomly [10].

In Morocco, there are few scientific studies on influence of cooking methods on the quality of ovine meat, particularly on its lipid composition and nutritional quality. The aim of this research is to evaluate the effects of boiling (moist heat) and "grilling" (dry heat) cooking methods, commonly used in Morocco, on the nutritional quality of fatty acids of Beni-Guil lamb meat reared extensively in eastern Morocco.

MATERIAL AND METHODS

Animal material and sample preparation

The present study was carried out on the *Longissimus Lumborum* muscle (LLM) of the Beni-Guil female sheep meat obtained 24h *post mortem,* excised from carcass between the 12th and 13th ribs. Lambs aged between 5 and 8 months, weaned at the age of 3 to 4 months, of the L category according to the regulation of the European Commission N° 823/98. The animals have been slaughtered at 33-37 Kg of lively weight, with an average fattening state, which corresponds to the notation 4 according to the Community Scale of Grading Sheep Carcasses EUROP, and the conformation of the R class according to the SEUROP grid. The selection of lambs was carried out with the assistance of an official of the National Association of breed producer (*Association National des Ovins et Caprins*: ANOC, Oujda, Morocco). These lambs belong to herds raised in the rural commune of Ain Beni-Methar, northeastern Morocco (Located at longitude: -2.02, latitude: 34.01, altitude: 921m), The sheep farmer is a member of ANOC, who adopts a rhythm of lambing per year and practice a semi-extensive breeding system (70-80 % Natural pasture and 20-30% supplementation with barley and alfalfa hay depending on the season and forage resource). This area is considered among the major places of Beni-Guil breeding where the main forage resource is rangeland grazing [1]. The ultimate pH (pHu) of each carcass was measured 24 hours post-slaughter, using a pH meter equipped with a penetration electrode (pH/Cond 340i WTW, Weilheim, Germany).

Sample preparation and cooking methods

The *Longissimus Lumborum* muscles of ten carcasses (n = 10) were sliced into 2 cm thick steaks (86 ± 13.21g). A total of 90 samples (10 LLM × 3 groups of meat samples × 3 replicates) were used in the present study. one first group was used as control (raw meat), and the other two groups were cooked using the following method: (i) Boiling method where the samples were dipped into boiling water (wet cooking) using a Moroccan tajine made of terracotta, (ii) and grilling type (dry cooking) using an electric grill (230V - 50 Hz, 2000W).

Internal meat temperature was monitored using handheld instant-read food thermometers (ISOLAB -50 + 300 °C/ -58+572°F), and removed from the cooking surface when an internal core temperature of 70 °C degrees was attained. After cooking, each sample was sponged with a paper towel to remove surface moisture and the cooking loss was calculated according the following formula:

Cooking loss = [(Raw meat weight - cooked meat weight) / Raw meat weight)] * 100

Finally, the samples were frozen, lyophilized, crushed and stored at -20 ° C for subsequent analyzes

Intramuscular fat extraction

The intramuscular fat (IMF) was extracted according to Bligh and Dyer [11], using a chloroform/methanol/water mixture (2/1/1; v/v/v). Then, it was methylated, converted to fatty acid methyl esters (FAME) and analyzed in GC-FID according to the method described by [12]. The FAME standard, containing 37 components (Supelco, Bellefonte, PA, USA), was used to identify the different peaks. The lipid indices were calculated from fatty acid profiles and results were expressed as a percent of the total FAME. SFA were the sum of C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C24:0. MUFA were the sum of C14:1, C16:1, C17:1, *cis*/*trans*-C18:1, and C20:1. PUFA were the sum of n-3 (C18:3n3, C20:5n3 and C22:6n3) and n-6 (*cis*/*trans*-C18:2n6, C20:3n6, and C20:4n6) fatty acids. Unsaturated fatty acids were the sum of MUFA and PUFA. Desirable fatty acids (DFA) were C18:0 and UFA. Odd Fatty Acids (OFA) were the sum of C15:0, C17:0, C17:1. Thrombogenic Index (IT) = [C14:0+C16:0+C18:0] / [(0.5*MUFA) +(0.5*∑n−6) +(3*∑n−3) +(n–3/n−6)]. Atherogenic Index (IA) = [(4*14:0) + 16:0] / [(PUFA)+(MUFA)] [13]. Hypocholesterolemic (h) were the sum of C18:1, C18:2n6, C20:4n6, C18:3n3, C20:5n3, and C22:6-n3. Hypercholesteremic (H) were the sum of C14:0 and C16:0. The h/H was calculated according to [14].

Statistical analysis

The analysis of the cooking method's effect (grilling and Tajine) on the nutritional value of intramuscular fat was carried out on triplicate for each sample and each parameter. All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS for Windows, version 20, SPSS Inc., Chicago, IL, USA). The normal distribution was verified according to the Shapiro Wilk test. One-way ANOVA statistical analysis and Tukey's post-hoc test was used for means comparison; the difference was considered significant (at $p < 0.05$).

RESULT AND DISCUSSION

Intramuscular fat variation and meat's cooking loss

Whatever the cooking mode based on heat treatment of the meat, it always gives rise to a decrease of weight, which is due to water and soluble matter losses (Table 1). The juice loss is due in particular to the temperature effect, which causes water syneresis from the protein network. Indeed, several authors reported these losses of meat juice during cooking which are related to denaturation of proteins during meat's heat treatment [15-19]. The extent of this is dependent upon the meat's surface temperature, the method of heat transfer, and the internal temperature profile, but also on the characteristics of the meat (size, composition, and pH) [20, 21]. Among the meat characteristics most involved in juice loss, are pHu and maturation degree. Table 1 shows that meat pHu used in this study at a value of 5.66 complies with the standards guaranteeing the quality of non-exudative meat. Juice loss is essentially accompanied by matter hydro-soluble losses such as heme iron, vitamins B3 and B6, but may also be due to degradations of other vitamins, lipid and protein denaturation [19, 22]. Compared to raw meat, the wet cooking method (boiling) causes more meat's juice loss and a significant IMF loss than the grilling method (Table 1). This result is in concordance with the fact already reported by several authors showing a greater loss of juice in wet cooking [23-25]. The lowest cooking loss recorded in grilled meat samples could be due to cooking temperature and heat transfer mode, by conduction in case of grilling mode and conduction and convection in case of boiling mode. This could also be due to the crust formed on the meat surface by the grilling method which traps the flow of intramuscular juice [26]. Both cooking modes cause intramuscular fat loss, but the grilling method allows significantly better preservation of IMF (Table 1) and consequently better juiciness. Our results show that estimated IMF content in raw meat (6.14%), decreases to 5.31% for grilled meat and to 4.83% for meat cooked by boiling method. These results are different from those obtained by several authors who, on the contrary, observed an increase in the lipid content after cooking [8, 10, 17, 24]. However, the same authors and others, explain that this fact would be due more to the juice loss than a real increase in the fat content, which would be concentrated in the meat after its dehydration. Traditionally, in Morocco, people prefer well-cooked meats regardless of cooking methods. As a result, the fat loss of meat is mainly due to the longer time required for cooking meat to satisfy the taste of the Moroccan consumer. Thus, variation of fat content and fatty acid composition after the two cooking methods (Table 2) confirms this hypothesis.

Table 1. Effect of wet (Boling) and dry (Grilling) cooking method on cooking loss (CL) and intramuscular fat (IMF) loss of Beni-Guil lamb meat

Parameters	Raw meat	Cooking method	
		Grilling	Boiling (Tagine)
CL %	---	21.14ᵃ±2.3	27.06±3.1ᵇ
IMF %	6.14ᵃ ±1.3	5.31ᵇ ±0.9	4.83±1.1ᶜ
Absolut IMF loss (g)	-	1.95ᵃ±0.3	2.66ᵇ±0.43

pHu = 5.66: Ultimate pH at 24h *post mortem* of the raw meat (*Longissimus Lumborum* muscle) of Beni-Guil lamb
Letters a, b, and c show significant differences.

Fatty acids profile

As shown in Table 2, the lipid profile of the Beni-Guil sheep meat is 86.71% composed by *cis*/*trans* C18:1, C16:0, C18:0 and *cis*/*trans* C18:2n6. These findings are consistent with our results published on the same sheep meat (Belhaj, *et al*., 2018). This lipid profile seems to be favorable than the one found by Flakemore, Malau-Aduli [27] in Australian lamb meat. This difference can be explained by extensive feeding (grazing system) adopted in eastern Morocco breeders. The quantitative and qualitative analysis of the LLM's fatty acid profile shows that cooking has a significant effect on the lipid quality of the Béni-Guil lamb meat. Most of the fatty acids, sums, and indices were modified by the cooking methods ($p < 0.001$). Ramamurti [28], Rodriguez-Estrada, Penazzi [29] and Jiang, Busboom [30] have reported similar results.

Table 2 shows that the SFA and MUFA rates decreased while that of PUFA increased after cooking. The decrease in SFA mainly concerns C16:0 and C18:0, whereas that of MUFA is mainly related to a decrease in C18:1. These two decreases are due to a loss of the main molecular species of triglycerides (OOP, SOP, POP, OOS) consisting mainly of these fatty acids [31]. It can be seen that this decrease in SFA is slightly but significantly greater (p <0.001) in the case of grilling cooking method (from 47.82% in the raw meat (RM) to 44.36 % and 45.43% in meat cooked by "grilling" and boiling methods, respectively). Meanwhile, the proportion of monounsaturated fatty acids decreased from 42.26% in RM to 39.14% in meat cooked in grill and to 41.53% in meat cooked by boiling mode. Librelotto, Bastida [32], have already reported that cooking in the water allows better preservation of lamb meat components because of the low temperature of the liquid surrounding the meat during cooking. Significantly higher than boiling the grilling mode causes an increase in the PUFA fraction resulting in an increase of PUFA/SFA, UFA/SFA, and h/H ratios. Thus, PUFA have evolved to 13.04% and 16.05% of TFA, respectively for meats cooked in boiling and grilling mode (Table 2). Our result is in agreement with those reported by Juárez, Failla [17] and Jiang, Busboom [30] in beef meat. The increase in PUFA would not be due to an increase in their quantity, but only to an increase in their relative percentage to TFA, due to the fusion during cooking of triacylglycerols rich in SFA and MUFA previously suggested by Ramamurti [28]. This increase mainly affects C18:2 (increase of 84.86% and 46.18% respectively for grilling and boiling) and C20:4 (increase of 108.37% and 63.54% respectively for grilling and boiling). Our results are in agreement with those of Juárez, Failla [17] in Buffalo Beef Meat, and Domínguez, Borrajo [8] in foal meat, but different from the results of most works on the cooking effect, which note either a non-significant change [24, 27, 33, 34] or a decrease in PUFA [35, 36]. In fact, normally an increase in temperature leads to lipid oxidation phenomena which mainly affect PUFA and cooking should, therefore, lead to oxidative degradation of PUFAs, and consequently a reduction of these fatty acids. Several studies have shown a decrease in PUFA concomitant with an increase in fat oxidative degradation products especially malondialdehyde and secondary products responsible for the particular flavor of cooked meats. However, our results could be explained by the fact that fatty acids are distributed differently on triglycerides and phospholipids, which results in a different effect during cooking. PUFA are more related to membrane phospholipids and therefore would be relatively more stable than triacylglycerols [37]. In addition, the extensive feeding of Beni-Guil sheep in the eastern region of Morocco would be beneficial for enriching the meat with omega 3 and increasing the antioxidant content. Indeed, several studies have shown a decrease in the level of malondialdehyde in the case of grass-fed lamb [38] or supplementary feeding by vitamin E [39]. In fact, eastern Morocco's highlands are rich in aromatic plants like white Artemisia (*white wormwood* "*Artemisia herba-alba*") and *alfalfa* [40], that would promote an increase in antioxidant component in meat. Others results (data not shown) demonstrated that Beni-Guil meat is rich in tocopherols. Ripoll, González-Calvo [39] have reported that feeding concentrate supplemented with vitamin E for 10 days or grazing *alfalfa* greatly diminished lipid oxidation of meat and therefore increased the shelf life.

Table 2. Effect of wet (Boling) and dry (Grilling) cooking methods on fatty acids profile and lipid quality indices of Beni-Guil lamb meat from eastern Morocco

Fatty acids (%)	Raw Meat	Cooking method		Signification
		Grilling	Boiling	
C14:0	4.33±1.02	4.39±0.89	4.28±1.32	NS
C15:0	0.54^{a}±0.11	0.47^{b}±0.09	0.48^{ab}±0.07	*
C16:0	26.16±1.17	25.57±3.48	25.39±1.7	NS
C17:0	1.22^{a}±0.13	0.94^{c}±0.15	1.09^{b}±0.14	***
C18:0	15.46^{a}±1.95	12.96^{b}±1.42	14.17^{ab}±1.97	***
C20:0	0.09^{a}±0.02	0.03^{b}±0.03	ND	***
SFA	**47.82a±0.96**	**44.36c±4.13**	**45.43b±2.21**	***
C14:1	0.29^{ab}±0.02	0.48^{a}±0.07	0.24^{b}±0.01	*
C16:1	1.89^{a}±0.34	1.72^{ab}±0.44	1.52^{b}±0.43	**
C17:1	0.83±0.1	0.8±0.08	0.85±0.08	NS
cis/trans-C18:1n9	39.14^{a}±3.34	36.09^{b}±1.83	38.86^{a}±2.89	**
C20:1n9	0.10^{a}±0.03	0.09^{a}±0.01	0.06^{b}±0.02	***
MUFA	**42.26^{a}**±3.49	**39.14^{c}**±1.74	**41.53^{b}**±2.69	**
cis/trans-C18:2n6	5.95^{c}±1.93	8.94^{a}±0.7	7.75^{b}±1.09	***
C18:3n3	0.8^{b}±0.29	1.12^{a}±0.33	0.82^{b}±0.19	**
C20:2	0.23^{b}±0.12	0.35^{a}±0.13	0.26^{b}±0.07	***
C20:3n6	0.18^{c}±0.07	0.36^{a}±0.05	0.27^{b}±0.05	***
C20:4n6	2.03^{c}±0.93	4.23^{a}±0.58	3.32^{b}±0.62	***
C20:5n3	0.34^{b}±0.1	0.86^{a}±0.22	0.48^{b}±0.16	***
C22:6n3	0.39^{b}±0.14	0.63^{a}±0.23	0.13^{ab}±0.07	***
PUFA	**8.92^{c}**±3.5	**16.5^{a}**±1.87	**13.04^{b}**±1.98	***
UFA	52.18^{c}±1.06	55.64^{a}±1.00	54.57^{b}±2.00	***
DFA	67.63±2.29	68.6±2.36	68.76±3.54	NS
OFA	2.6^{a}±0.23	2.21^{c}±0.2	2.43^{b}±0.2	NS
UFA/SFA	1.09^{b}±0.04	1.26^{a}±0.05	1.2^{a}±0.10	***
PUFA/SFA	0.21^{c}±0.05	0.37^{a}±0.04	0.29^{b}±0.04	***
PUFA n-6	8.16^{c}±2.19	13.58^{a}±1.23	11.34^{b}±1.65	***
PUFA n-3	1.53^{b}±0.6	2.6^{a}±0.95	1.43^{b}±0.78	***
n-6/n-3	5.7^{b}±1.38	6.35^{b}±1.33	7.93^{a}±1.95	***
h/H	1.62^{b}±0.16	1.77^{a}±0.19	1.78^{a}±0.25	*
IT	1.54^{a}±0.08	1.26^{c}±0.09	1.42^{b}±0.08	***
IA	0.84^{a}±0.09	0.78^{ba}±0.1	0.78^{ab}±0.14	NS
Nutritive value	2.1^{ab}±0.19	1.93^{b}±0.21	2.1^{a}±0.29	*

Letters **a, b,** and **c** show significant differences, **NS:** no significant,

ND: no detected

SFA: Saturated fatty acids; **UFA**: Unsaturated fatty acids; **PUFA**: Polyunsaturated fatty acids;

DFA: Desirable Fatty Acids (C18:0 + UFA); **OFA**: Odd Fatty Acids;

IT = Thrombogenic Index [C14:0+C16:0+C18:0] / [(0.5*MUFA) + (0.5*∑n−6) +(3*∑n−3) +(n–3/n−6)].

IA = Atherogenic Index [(4*C14:0) + C16:0] / [(PUFA)+(MUFA)], calculated without the inclusion of 18:0 which is considered to be neutral on serum cholesterol

Hypocholesterolemic (h) / Hypercholesterolemic (H):

h/H= (18:1n9c + 18:2ω6 + 20:4ω6 + 18:3ω3 + 20:5ω3 + 22:5ω3 + 22:6ω3) / (14:0+16:0).

Nutritive value of intramuscular fat: (C18:0 + C18:1) / C16:0

Thereby, the cooking generates an increase in the C18:2n-6, C20:3n-6 and C20:4n-6 fraction, increasing the n-6/n-3 ratio (5.7% vs 6.35% vs 7.73% for RM, grilling and boiling). Similar results were reported by Juárez, Failla [17].

Concerning the lipid quality indices, the results show that after cooking the IT and IA indices decrease both for grilling and boiling mode and consequently decrease the cardio vascular risks [41, 42]. Indeed, the first index evaluates the risk of atherosclerosis, while the second one, is used as a sign of potential platelet aggregation [41]. IT decreased significantly ($p < 0.001$) from 1.54 in raw meat to 1.26 and 1.42 respectively in grilling and boiling mode cooking while IA decreased significantly ($p < 0.05$) from 0.84 in raw meat to 0.78 in meat cooked.

CONCLUSION

In gastronomic gourmet or diet dish design based on lamb meat, intramuscular fat content (IMF), cover fat quality and cooking methods are important parameters for meat dishes preparation. In addition, although cooking modifies meat's quantitative characteristics such as the levels of essential fatty acids and amino acids, it contributes significantly to the development of aromas and the improvement of the final taste of meat. The effect of cooking methods on the nutritional value of intramuscular fat in lamb meat was the subject of many studies, but it will be difficult to draw an overall and unique conclusion. From this study, it could be concluded that the two common cooking modes (Grilling or Boiling) significantly modifies the lipid quality profile of lamb meat, mainly by increasing the polyunsaturated fatty acids proportion and subsequently increasing the hypocholesterolemic fatty acids which helps reduce cardiovascular disease. Furthermore, it seems that the abundance of aromatic plants rich in antioxidants (tocopherols in particular) in the eastern high-lands of Morocco, which is the pasture zone of Beni-Guil's sheep, helps to preserve the meat's lipid quality during cooking, all the while preserving the meat's nutritional quality and improving its taste.

Acknowledgments

We are grateful to the Moroccan-Belgian bilateral cooperation program for the financial support of this research through a "WBI-Project 1-6 "2015-2017. Our thanks are also due to the ANOC "*Association Nationale Ovine et Caprine*" for its collaboration, particularly to Mr. A. Kacem and A. Mejdoubi.

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