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RESEARCH ARTICLE

Vitamin B12 Content in Lamb Meat: Effect of Cooking and Freezing Temperatures

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ABSTRACT

The meat industry has taken several measures to expand its shelf life, such as frozen storage and thermal treatments. Hence, the valuable content in red meat, such as vitamin B12, could be affected by this handling. This study aimed to assess the vitamin B12 losses in Algerian Hamra lamb meat caused by frozen storage (06 months at -18 °C) and boiling for 45 minutes at 80 °C with regard to modifications in water-holding capacity (WHC). Samples of *Longissimus lumborum* (LL) and *Longissimus thoracis* (LT) were utilized to assess this micronutrient using microbiological assay (VitaFast®). In comparison with Fresh Raw Meat (FeRM), Frozen Raw Meat (FoRM) was marked by a reduced difference in B12 content (1.36 ± 0.14 vs. 1.19 ± 0.12 µg/100 g, respectively). However, the values of B12 decreased significantly in Fresh Boiled Meat (FeBM) (0.78 ± 0.40) and Frozen Boiled Meat (FoBM) (1.00 ± 0.07) compared to FeRM. The recorded values of cooking loss (CL) and forced drip loss (FDP) appeared identical. The B12 vitamin content in Hamra lamb meat is generally affected by cooking processes (longer time and higher temperature treatments) than short freezing storage (less than 06 months at – 18 °C).

Keywords: B12 vitamin; Cooking; Freezing storage; Lamb meat; Microbiological assay

INTRODUCTION

The meat industry significantly contributed to the diet of humans millions of years ago (Klurfeld, 2015); due to their nutritional richness, consumers are more aware of the dietary contents of this food. The meat supplied a valuable source of vital micro and macronutrients, particularly of bioavailable iron (Fe) and zinc (Zn) (Laskowski et al., 2018), proteins, trace elements and B vitamins, especially vitamin B12 (Nicklas et al., 2012). For our nutrition, meat and offal from ruminants are potential vitamin sources of B12 (Williams, 2007). These species have a microbial origin of vitamin B12 (Obeid et al., 2019); Vitamin B12 is made by the rumen and accumulated in the liver. This is why the vitamin B12 levels in ruminant products are more elevated than in monogastric meat, which is why ruminants are not deficient in B12 despite the lack of food. However, the synthesis of B12 by the intestinal microbiota depends on the nutritional intake of cobalt (Co) (Gonzalez-Montana et al., 2020). Being one of the essential vitamin B groups, this micronutrient is a consisting complex and cofactors essential to the metabolisms of almost all types of organisms (Banerjee and Ragsdale, 2003, Fedosov, 2012), that is required for DNA production, neurotransmitters, proteins and fats metabolism (Gille and Schmid, 2015).

Vitamin B12 deficiency causes severe human health problems, and it is a known fact that vitamin B12 deficiency can cause several neurological and hematological disorders and syndromes in humans (Yang and Cook, 2012).

Fresh meats spoil easily during storage, decay quickly, and have a limited storage period (Djenane et al., 2000, Luño et al., 2000), possibly becoming unsafe for consumers. Over the last decades, numerous practices have been applied to preserve and keep them safe for longer, even during the supply chain (Djenane et al., 2020, Djenane and Roncales, 2018).

Currently, the storage of lamb meat by freezing is commonly used to enhance its preservation time beyond

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a year (Muela et al., 2015, 2016). Nevertheless, limited data in literature reporting freezer storage temperatures (-18 °C), though based on sensory quality (Coombs et al., 2017). In addition, most forenamed investigations have not considered the influence of freezing storage on the variation of nutrient levels in lamb meat. However, nutrient preservation is a significant concern for consumers.

Furthermore, consumer handling plays a crucial role in food quality. Cooking is a generally convenient heating operation for meat, and before consumption, cooking increases safety, improves taste and enhances the bioavailability and nutrients digestibility (Oz et al., 2017, Sobral et al., 2018). The juice expulsion during cooking increases its dry matter content and determines its overall performance. It is also at the origin of water-soluble micronutrient expulsion (iron, vitamins in particular). Although vitamin B12 is among the most thermostable of the other B-group vitamins. Several investigations have shown the link between direct heat contact with the surface of meat during cooking and the presence of high levels of B12 in water loss (Ortigues-Marty et al., 2006, Pereira and Vicente, 2013), in which a level of vitamin B12 range of meat was seriously influenced by heat treatment. More studies have shown that B12 and B1 are lost during cooking, compared with B2 and B3 (D'Evoli et al., 2009, Riccio et al., 2006).

Vitamin B12 losses during meat cooking are directly proportional to those of juice. However, 100 g of cooked meats can cover between 50% and 100% of the recommended nutritional intake of B12 (Duchène and Gandemer, 2017, Gandemer G, 2015). Protein denaturation by heat treatments is an important phenomenon shown during meat cooking. (Sayd et al., 2016), which might induce fluid loss showing an immediate effect on the nutrient amount of prepared meats. Most studies of the nutritional values of cooked meats have been carried out under experimental conditions close to consumer practice. This leads to poorly reproducible and disparate results due to the difficulty of controlling all the factors involved. Duchène and Gandemer (2017) carried out modeling work on cooking losses under extreme cooking conditions (long times, high temperatures) by integrating several factors (geometry of the parts, specialized treatments of the meat: freezing, maturation, the direction of cutting of the fibers). Vitamins, being the most sensible nutrients after cooking in particular, B-group vitamins, few studies have assessed them in cooked meat prior to and after frozen storage. For human nutritional purposes, various foods and beverages enriched with vitamin B12 are now manufactured in developed countries to meet the growing public demand. The vitamin content in these foods must be controlled for product development, quality control, and compliance.

Several approaches are recommended to quantify vitamin B12 in foods. These included spectral, chromatography, polarographic, biosensors techniques, and microbiological tests that use various microorganisms. However, the results of these methods differ depending on the sample preparation and the form of vitamin B12. Typically three sample treatments have been used individually or in combination: thermal, enzymatic, or acid (Perez-Fernandez et al., 2016). *Lactobacillus delbrueckii* subsp. *lactis*, was used in the microbiological test to assess the vitamin B12 in foods (Watanabe & Bito, 2018). Furthermore, an excellent standard curve can be obtained, varying from approximately 10 to 80 pg (Bito et al., 2016). Therefore, Vitamin B12 detection in nutritional supplements and fortified foods by sensitive, rapid, and efficient immunoassays has resulted in recoveries following the fortified sample assay and the microbiological assay method (Kong et al., 2017). Numerous studies described the use of vitafast in quantifying vitamin B12 in child formulations (Zhang et al., 2014), in drink food, cereals, fruit mix, juice, and meats (Weber et al., 2011; Guggisberg et al., 2012). Due to the ever-increasing number of people suffering from vitamin deficiencies B12 and the easy degradation of this micronutrient when subjected to unfavorable conditions, we aimed, in the current investigation, to analyze the effect of heat (boiling at 80°C for 45 minutes), and cold (frozen storage at -18 °C during 6 months) temperatures on content of vitamin B12 in Algerian Hamra lamb meat. In addition, the choice of boiling as a cooking parameter of this study is justified by the fact that it could guarantee several qualities to consumers, such as organoleptic, microbiological, and probably nutritional qualities.

MATERIAL AND METHODS

Reagents and chemicals

Vitamin B12 (Cyanocobalamin) was valued using VitaFast® Kits bought from R-Biopharm AG (Darmstadt, Germany). The kit of microbiological assay contains all the reagents necessary for the microbiological analysis (microtiter plate pre-coated with microorganisms, adhesive sheet, additional support, vitamin standard, sterile water, and the analysis medium). Sigma Aldrich, Merck supplied the Takadiastase from *Aspergillus oryzae* and NaCN.

Animals

Details of animals, diets, and slaughter procedures are given by Ziani and Khaled (2016). In brief, 10 male Algerian Hamra lambs (age: 141.15±1.07 days, weight: 24.35 ± 0.64 kg) were selected. The animals were adapted to the diet and facilities for 10 days before the formal trial. Afterward, lambs were fed with a diet consisting of dry matter 90.4%, organic

matter 84.37%, crude protein 14.92%, and ether extract 27.8%. The animals were slaughtered according to the standards of Algerian regulations (JORADP, 2014) in the municipal slaughterhouse of Saida city (North-west of Algeria). The chilled carcasses were divided longitudinally into two equal parts.

Samples' collection

The samples used in this study came from *Longissimus thoracis* (LT), and *Longissimus lumborum* (LL) dissected from each animal. After that, the muscles were sliced into two portions. In the first analysis, we assessed the fresh meat before (Fresh Raw Meat "FeRM") and after boiling (Fresh Boiled Meat "FeBM"). However, the frozen samples were analyzed in the second analysis (over 6 months of storage at -18 °C) before (Frozen Raw Meat "FoRM") and after boiling (Frozen Boiled Meat "FoBM"). Both juices obtained after freezing/defrosting and boiling were collected and mixed into the appropriate extract (Fig.1).

Samples' treatment

The cooking procedure was performed by boiling (80°C/45 min) the raw and frozen meat in a water bath (Memmert, Germany). Each sample was weighed before and after heating.

Water-holding capacity

WHC is the quantity of water that meat can maintain during handling. The water losses can be called drip, cook loss, purge, weep, or exudate.

The measurement of WHC was evaluated in this study by three methods: Natural Drip Loss (NDL), Forced Drip Loss (FDP), and Cooking Loss (CL). The application of mechanical pressure was measured using Grau and Hamm method modified by Beriain et al. (2000), and Warner (2014) was used at 72 h *post-mortem*. The forced drip loss was expressed as the weight loss percentage of 5 g meat samples after being kept under a pressure of 2,250 kg for 5 min. The results were described as the percentage of liquid expelled (Dhanda et al., 1999).

The second group involved WHC methods with the application of thermal force. The samples were boiling in a water bath (1 h at 75 °C). The loss of weight due to cooking (LWC) was estimated using the change in initial and final cooked weight, and expressed as the percentage (%) (Hoffman et al., 2003).

$$\text{Cooking loss} = \frac{\text{Initial raw meat weight} - \text{Final cooked meat weight}}{\text{Initial raw meat weight}} 100$$

Contrary to the previously mentioned methods, the evaluation of the WHC of fresh and uncooked meat by determining the natural drip losses through passive exudation, is the method where no external force is applied, only the "free" drip due to gravity was measured (Honikel, 2004). The meat sample was suspended in a bag without touching any edges. Then, it closed and left for 7 days at 5 ± 2 °C. Tray (DL) was measured as the difference between the first and the last weight (Honikel, 1998).

In addition, to evaluate technological properties of Algerian Hamra lamb meat, we measured the different pH values (24 h *post-mortem*) using an Italian model pH meter (HANNA instruments Hi 8519N, Italy).

The electrode is inserted into the test socket, and the pH meter's temperature correction system at the test socket's

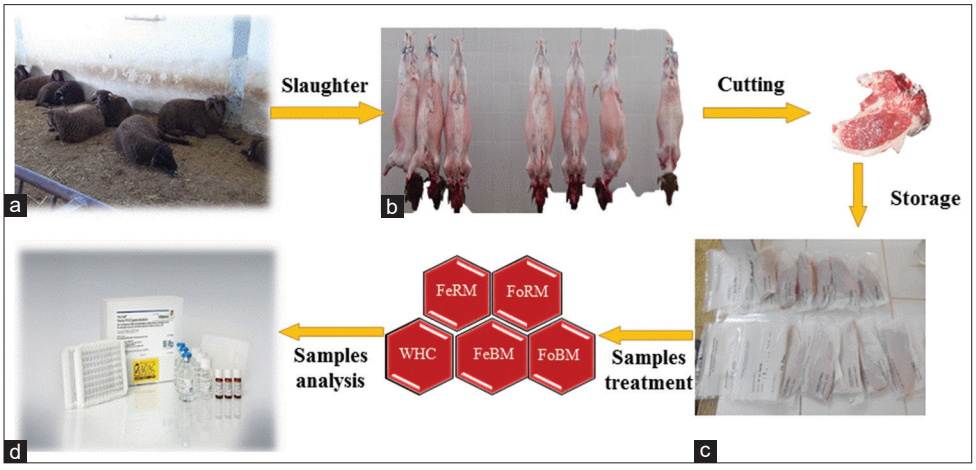

Fig 1. Illustration of the flow chart for the B12 vitamin analysis of "Hamra" sheep meat samples. (a): Hamra lambs; (b): Carcass of slaughtered lambs; (c): Samples of meat; (d):Samples allocation (Fresh Raw Meat "FeRM", Fresh Boiled Meat "FeBM", Frozen Raw Meat "FoRM", Frozen Boiled Meat "FoBM", and samples to measure Water-holding capacity "WHC" and pH) ; (e): VitaFast® kit (Cyanocobalamin)

temperature is set to 20 °C. 2 to 3 determinations are made on the same sample. Then the result is expressed as the arithmetic mean of the measured values.

Samples' extraction

The samples of each piece were sliced and blended using a laboratory homogenizer (Moulinex, France). Each one (01) g of samples was homogenized in both 20 and 40 mL redist, respectively. Then, 250 µL of NaCN was added, and the mix was shaken. The pH was adjusted to 4.5. After that, the resulting mixture was treated with 300mg of Takadiastase (α-Amylase from *Aspergillus oryzae*, Sigma Aldrich, Merck) and subjected to a temperature of 37°C/1h in the dark. Afterward, the assay tubes were loaded up to 40 mL with redist, and the sample was heated at 95°C/30 min; after that, immediately chilled to below 30 °C and centrifuged at 8000×g for 5min. As a final step, the clear supernatant was diluted with sterile water in 1.5mL of sterile Eppendorf to correct the sample concentration to interpolate from the standard calibration.

Samples' analysis

After the previous step, the assay implementation step was conducted as described in the kit manual, which is per international norms and is adapted by official methods AOAC 960.46 (AOAC, 2017). Briefly, each 150 µL of the diluted extract or standard and the assay medium are pipetted into wells previously coated with *Lactobacillus delbrueckii*. This microorganism development depends on the sample's vitamin content. The intensity of development was measured by the difference in turbidity when compared to a standard curve. The absorbance was measured using a microtiter plate reader at 610-630 nm (alternatively at 540 – 550 nm) after an incubation period (at 37 °C for 44-48 h in the dark)., The amount of vitamin B12 was calculated as described below:

$$\text{Vitamin B12 in g / 100 g} = \frac{\text{concentration standard curve} \quad \text{dilution factor}}{\text{sample weightin (g)}}$$

Data statistical analysis

The calculations were accomplished using Prism 6.00 for Windows (GraphPad Software, California, USA). Statistical calculations included the effects of freezing and cooking and their interaction. The normality and variance homogeneity of all variables were verified by Pearson "omnibus K2" and D'Agostino test. One-way repeated-measure ANOVA analyzed data was used to control the variances in the vitamin B12 amounts between the meats samples and heat treatments by comparing the treatments and the initial and final values for each sets followed by post-hoc comparison of means using two-tailed Dunnett's multiple comparisons test with a single pooled variance ($\alpha = 0.05$). The values in the text are mean ±S.E, and the level of statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSION

To determine the amount of vitamin B12 boiling losses in Tepic Algerian Hamra lamb meat. Fresh and frozen samples were processed at 80 °C for 45 minutes. It is widely believed that the B12 amount in the meat of ruminants can be affected by several factors as well as the analytical methods, dietary strategies, the pieces of meat (i.e., type of muscle) selected, cooking procedures, and their manufacturing process. Although, in the literature review, the vitamin B12 contents in meat reported a wide variation between samples and amid detection methods used (microbiological assay, HPLC, ELISA, Etc.).These techniques presented varying sensibilities to inactive and active natural vitamin forms.

Methods of analysis

It is increasingly common to fortify foods and pharmaceutical products with vitamin B12 to provide a good supply of vitamin B12 to consumers. The food industry requires accurate and sensitive techniques to detect vitamin B12. The analysis is based on the verification of compliance with the legal provisions. Nevertheless, the fluctuation and low concentration of vitamin B12 create difficulties in the analysis. The VitaFast® (AOAC performance tested SM, certificate N° 101002) test, based on the microbiological assay, was required to analyze vitamin B12 in a large number of foods. Numerous studies have compared various methods used to measure vitamin B12 in meat and found that they are similar. In particular, the investigation of Guggisberg et al. (2012) revealed that vitamin B12 amounts in 50 meat products tested by UV detection and RP-HPLC were comparable to or more inferior to those found by the microbiological assay, the same authors observed that certain technological factors, as well as the components of the meat samples (especially fat content), might also affect the analysis methods. Gauch et al. (1992) used different HPLC methods to determine vitamin B-groups in milk. Albala-Hurtado et al. (1997) determined B12 in a one analysis with UV-detection and ion-pair chromatography developed at different wavelengths. Lebiedzinska et al. (2007) determined vitamin B12 in fruit juices and seafood by coulometric electrochemical and UV detection. Scientists may prefer these multiple methods. They have the advantage of saving time but sometimes lack sensitivity and selectivity under different chemical structures. Luo et al. (2006) suggested an Electrospray Ionization-mass Spectrometry (ESI-MS) method for foods.

Iwase and Ono (1997) revealed the B12 levels in mayonnaise and milk; they have developed methods for extraction, cleaning, and concentrating the analytes before quantification. Fernandez et al. (2008) have been proposed chromatographic method for vitamin B12 (LPLC: low-pressure liquid chromatographic) based on monolithic column separation as a cost-effective instrument for pharmaceutical analysis. Pakin et al. (2005) have adapted a fluorometric detecting method for the detection of low amounts of B12 in fish, milk powder, liver, and egg after immunoaffinity purification. The results of vitamin B12 measurement by immunoaffinity columns were compared to those obtained from MBA (Marley et al., 2009). These authors use a microbiological method to evaluate a B12 in 03 muscles of pigs (*Longissimus dorsi, Triceps brachii,* and *Biceps femoris*). Other techniques were developed to quantify vitamin B12 in foods. These include spectrometric methods, liquid chromatography methods, and MBA (Kumar and Aalbersberg, 2006).

Vitamin B12 levels in Hamra lamb meat

The mean contents of B12 in meat Hamra lamb varied from 0.78 to 1.36μg/100 g, as summarized in Table 1. The highest values were shown in fresh raw meat, frozen raw meat, frozen and boiled meat, respectively. In a comparison of this study, some investigations on the same subject have considered the amount of B12 in muscles of lamb and mutton varied between 1.2 to 5.0 μg/100 g (Gille and Schmid, 2015).

The values we found in (FeRM) are upper than the values described by Ortigues-Marty et al. (2005). In a previously cited work, the authors revealed that the B12 concentrations in meat differs depending on the anatomical location of the muscle. The oxidative-type muscles which have been most metabolically active contain more vitamin B12 in comparison with glycolytic type muscles (Kerry and Ledward, 2009, Chikuni et al., 2010). The LT and LL, as intermediate muscles, can contain an amount of vitamin B12 ranging between 0.55 to 0.99 μg/100 g (Ortigues-Marty et al., 2005). In this experiment, the mean average of vitamin B12 amount in fresh Hamra lamb meat was 1.36 μg/100 g. The results obtained in Hamra lamb meat are lower by 12.26–62.64% than those available for beef (Indyk et al., 2002).

In summary, our results show that 100 g of both FeBM and FoBM provided an average of 25 to 55.5% of the vitamin B12 recommended daily intake (RDA) for adults (European food safety authority (EFSA), 2015). In contrast, 100 g of cooked meat of ruminants could cover 63% to 74%, respectively, of the RDA for vitamin B12 of men in New Zealand and Australia (Purchas et al., 2014). Unfortunately, in human nutrition, the RDA of B12 for adults and elderly reviewed in prior studies is widely varied among international organizations: 2 μg/d(day) (EFSA, 2015, Nordic Council of Ministers (NCM), 2014), 2,4 μg/d (AFSSA, 2001, WHO/UNU, 2004), 3 to 4 μg/d (Strohle et al., 2019). On the other hand, van Heerden and Strydom (2017) show that the animals' age considerably influences the retention of the nutrients in the meat. In the case of vitamins, the lamb retained the least B12 compared to the mutton.

Regarding the impact of the feed regime, the variation in the B12 concentrations in red meat was not considerably affected by an animal's diet. Although, the differences reported in the literature mostly correlated with the cobalt (Co) content in animal feeds (Ortigues-Marty et al., 2005, Stangl et al., 2000).

Agreeing with Ortigues-Marty et al. (2006), the cooking method of beef and the increase in core temperature significantly modify its vitamin B12 content. In comparison, B12 amounts in milk are noticeably inferior to meat (Matte et al., 2012). Heat processing can have a negative effect. An appraised 50% of vitamin B12 was decrease by 5 min microwave cooking, 30% and 50% by boiling for 2-5 and 30 min, respectively, while 5-10% was lost by pasteurization (Watanabe, 2007). Riccio et al. (2006) enhanced ham with 25 μg/g of vitamin B12. After a boiling (100 °C for 5 to 15 minutes), the results showed degradation of this micronutrient in the enriched product for the first 5 minutes, After that, no further degradation was observed. However, heating at 120 °C during 20 min allowed an 84% loss of this vitamin. Ortigues-Marty et al. (2006) described the impact of meat maturation and cooking conditions (deep fryer, roast, pan-fried, grilled, braised) on water losses and consequently on vitamin B12 amounts in different muscles (*Triceps brachii Longissimus lumborum*,

Table 1: Means of vitamin B12 concentration (μg/100 g) in meat of Hamra lamb.

Parameters	FeRM	FeBM	FoRM	FoBM
Mean	1.36	0.78	1.19	1.00
SD	0.14	0.40	0.12	0.07

FeRM: Fresh Raw Meat; FeBM: Fresh Boiled Meat; FoRM: Frozen Raw Meat; FoBM: Frozen boiled Meat. SD: Standard Deviation.

Table 2: Dunnett's multiple comparisons test (α = 0.05).

	Mean Difference	Significant?	Summary	Adjusted *P* Value
FeRM *vs.* FoRM	0.16	No	Ns	0.2806
FeRM *vs.* FoBM	0.36	Yes	**	0.0060
FeRM *vs.* FeBM	0.57	Yes	***	0.0009
FoRM *vs.* FoBM	0.19	Yes	**	0.0095

Ns: No significant ($p > 0.05$). * One-way ANOVA followed by Dunnett's multiple comparison test ($p > 0.05$). ** One-way ANOVA followed by Dunnett's multiple comparison test ($p > 0.01$). *** One-way ANOVA followed by Dunnett's multiple comparison test ($p > 0.001$).

and *Longissimus thoracis*). They concluded that the post-mortem period does not influence these vitamin levels, even in a raw state, then cooked. However, the initial amount of vitamin B12 in raw wet muscle was considerably more increased in Triceps brachii than in the other two muscles (20.86, 11.53, and 9.21 ng/g, respectively). To examine the impact of cooking procedures on vitamin B12 losses, the authors performed measurements based on: wet weight, in which all B12 levels were considerably improved by cooking; or on a dry weight basis without fat. In this latest analysis, important losses (-25%) of B12 were observed in *Triceps brachii* cooked by braising and in *Longissimus lumborum* cooked by frying (-5.5%), probably due to a long cooking time and temperature, respectively.

Influence of the boiling on vitamin B12 content

The current study found that the concentrations of B12 in muscles after cooking varied from (Min-Max) 0.50 to 1.11 μg/100 g, with average values of 0.78 ± 0.40 and 1.00 ± 0.07 μg/100g in fresh and frozen meat respectively. The modification was higher significant ($P < 0.05$) between the raw and boiled in both fresh and frozen meat (Table 1 and 2).

The literature cites various retention values for the B group's hydrophilic vitamins. Nonetheless, countless research studies recognize that the leaching of vitamin B12 during cooking is responsible for most of the losses. In addition, this retention could be influenced by many parameters, such as the shape of the cut and the size, method and time of cooking, thermal gradients, Etc.

Following the present results, similar studies have demonstrated that vitamin B12 losses changed between 10 and 40% in cooked meat procedures (Bennink and Ono, 1982, Ortigues-Marty et al., 2006). The destruction of this component could explain this reduction as a consequence of heat temperature (thermal denaturation) and vitamins leaching (expulsion in juice) into water (boiling) or the drip (grilling) (Ersoy and Özeren, 2009, Gerber et al., 2009, Kondjoyan et al., 2018). The previous track described that B-vitamin drops increase more during submerged cooking than during microwave cooking compared to expulsion into the juice (Kumar and Aalbersberg, 2006).

Therefore, as indicated in the literature review, several authors have grouped fat-soluble vitamins as relatively thermostable, whereas water-soluble vitamins are thermosensitive (Mehta, 2015). Furthermore, vitamin B12 is relatively heat-resistance compared to other Vitamins B. Nevertheless, like all water-soluble vitamins, it undergoes substantial losses by leaching during its expulsion in the juice (Lešková et al., 2006). These losses were clearly connected to the heating time temperature, meat cut dimensions, boundary conditions in the equipment, and the cooking process (Kondjoyan et al., 2014). The leading cause of this change depended on the degree of the destruction of vitamin B12 under the influence of high temperature and long cooking duration. In other cases, these losses could also be affected by the drop in moisture and fat (Molonon et al., 1980).

Many studies on the effects of types of cooking of ruminant meats on vitamin B12 have shown that culinary procedures that do not use water, such as microwaving and grilling, lead to better retention of vitamins in the meats compared to cooking methods involving water, such as boiling and steaming (Alfaia et al., 2013). In beef, Czerwonka et al. (2014) showed similar amounts in raw, grilled, and roasted meat. However, the authors observed less, about 32% and 49%, after the poorly frying process compared to the initial amount of vitamin B12 in raw meat after frying the meat.

Generally, the nutrients (proteins, lipids, zinc, and selenium) which are not lost (retention coefficient = 100%) during the cooking of the different muscles (lamb, beef, horse, and veal), do are not expelled in the juice and remain insensitive to thermal degradation (Duchène and Gandemer, 2017). The soluble and heat-resistant vitamin B12 suffers proportional losses only by expelling in the cooking juice. Depending on the technique and the degree of cooking, the retention coefficient of vitamin B12 varies from 70 to 95% for short and intermediate cooking and from 50 to 70% for long cooking.

Influence of frozen storage on the vitamin B12

Identifying the technological factors that influence the B12 levels in meat is essential. Few studies are published on the impact of both industrial and domestic processes, such as meat freezing and cooking time. B12 could also be easily degraded when subjected to light during prolonged freezing storage. In the case of freezing storage (06 months at -18°C), the values of vitamin B12 obtained from frozen meat were lower than those observed from fresh meat, although not significantly (Fig. 3).

There are contradictory reports in the literature concerning the losses of water-soluble vitamins in meat during and after frozen storage. Whereas freezing is less destructive than other preserving procedures (Zaritzky, 2010). Nevertheless, most of the published research has essentially been focused towards determining the effects on sensory quality rather than on nutrient retention in meat. In addition, few studies have discriminated between the loss of nutrients caused by the chemical breakdown and the physical loss of nutrients during the thawing process (Mark Berry et al., 2008). However, the present trial's outcomes support the previous research of Chan et al. (1996), when

they have not observed a difference between frozen and chilled meat in vitamin B12 content.

Overall, several factors will influence these conflicting results, such as the freezing process (the rate of freezing, range of temperature fluctuation, Etc.), type of meat product, size of the cut, method of packaging and thawing, length of storage (Adams and Erdman, 1988). Although, our results illustrated in Fig. 2 & 3 differ from published studies of Pinheiro et al. (2019), in which they described that the losses of vitamins in meat could be more significant if it froze then cooked compared to the loss of fresh meat after cooking. However, these controversial results should be linked to the long-time freezing storage (up to 12 months). However, it is essential to note that comparing our results with the literature is difficult because the B12 was present in diverse forms, and the specificity and accuracy of the analytical techniques utilized are dissimilar.

pH measurements

The results of the pH measurements of the studied meat showed an overall average equal to 5.54 ± 0.03. These pH values recorded are considered optimal (Martinez-Cerezo et al., 2005). Horcada et al. (2010) obtained similar findings for the Lacha and Aragonesa breeds, with higher ultimate pH in the carcasses of 24 kg lambs compared to those of 12 kg. Also, Díaz et al. (2003), working on Manchego breed lambs with live weights of 10, 12, and 14 kg, showed that the *Longissimus dorsi* of lighter lambs had the lowest ultimate pH.

Table 3: Water losses measurement (% of expelled liquid).

	Cooking Loss (CL)	Forced Drip Loss (FDP)	Natural Drip Loss (NDL)
Mean±SD*	27.50±3.92^{b}	27.10±5.15^{b}	5.25±1.34^{a}
Min-Max	17.8–31.4	20–36	3.82–8.53
CV%	14.26	19.03	25.60

*Means of the same row (between three methods of the measurement of WHC: FDP), CL and NDL) with different letters differ significantly ($p > 0.05$).

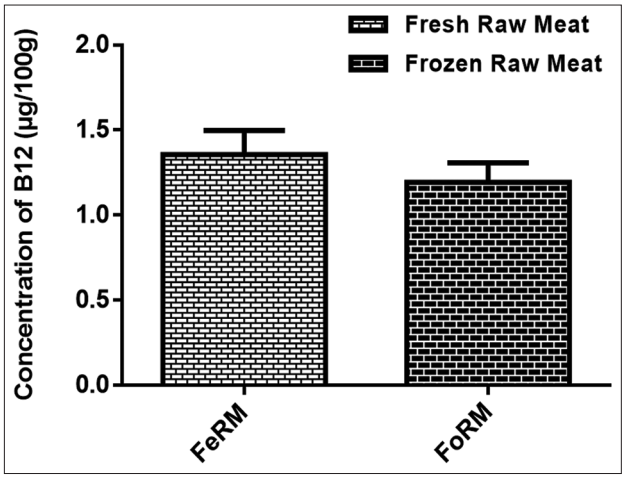

Fig 2. Influence of frozen storage (6 months at -18 °C) in B12 content

Water-Holding Capacity

WHC does not determine only the water loss during storage, transport, or cooking but also the visual acceptability of the product. Fresh muscle contains about 70% water, linked principally by forces of capillary within the myofibrillar structure and in the sarcoplasm. The pH value of the muscle largely determines its water retention capacity. Indeed, during the post-mortem period, myofibrils retract, and water is expelled to the extracellular area, which is determined by cooking loss, drip loss, and purging.

The average variation in WHC with or without the application of mechanical and thermal force are summarized in Table 3. The highest losses were recorded after the application of external force than in its absence. Likewise, the results of (CL), and (FDP) appeared similar ($p > 0.05$), and their respective water losses were higher than by exudation (NDL). It has been reported, through the literature, that the WHC measurement could be generally affected by either the animal species itself (breed, sex, age, feeding system, pre-slaughter and slaughter conditions, etc.), or the part of a muscle (anatomical location, geometry, weight, fiber direction) and also by the methodology applied (the type of force applied, *post-mortem* period, duration of treatment, etc.) (M. Font-i-Furnols, 2015). Poor WHC results from low cook yields and often-dry meat.

In the last decade, there are no studies on Hamra meat quality to compare with our results. However, it is sometimes possible to discuss these results referring to the literature review for practical reasons. The differences between the protocols used in each study on the same animal species model, i.e., age, sex, selected muscle, etc., especially with the same temperature and the duration of cooking applied. Furthermore, WHC was not impacted by breed and weight at slaughter (Ekiz et al., 2010). In

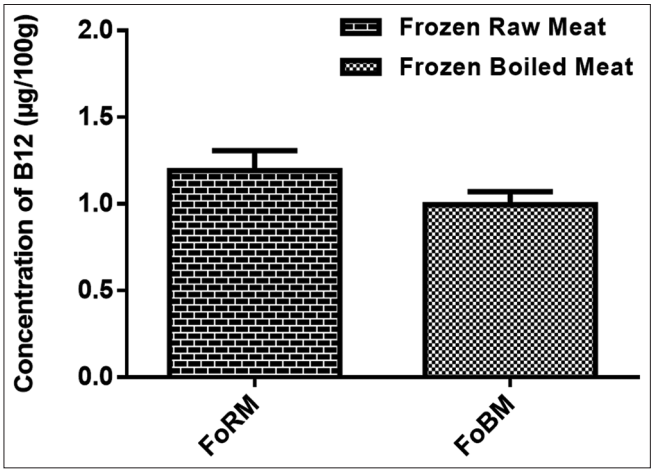

Fig 3. Influence of the boiling (80°C during 45 minutes) of frozen meat on vitamin B12 content

contrast, it was influenced by sex and the feeding system. The WHC was higher in males than females (Díaz et al., 2003). The animals' feeding system is a critical parameter that affects the WHC (Cheng and Sun, 2008). Lambs fed a concentrate diet had a higher water retention capacity than those fed a pasture diet (Santos-Silva et al., 2002). Some studies have shown that the WHC was lower during the frozen shelf-life periods (3, 6, 9, and 12 months) than unfrozen meat (Pinheiro et al., 2019). Ice crystals damage muscle cells when meat is frozen, decreasing WHC and increasing cooking loss (de Paula Paseto Fernandes et al., 2013, Muela et al., 2010).

The meat may drop a considerable amount of its mass and may affect the cooking performance during cooking. Heating processes are responsible for the denaturation of proteins and therefore induce the highest observed water losses (Warner, 2017). On the other hand, van Heerden and Strydom (2017) have shown that the animal's age could have a different effect within the same animal species. Indeed, the same authors have also underlined that lamb recorded lower cooking yield than mutton. The muscle type affected the B12 range of fresh meat. More than, Higher levels are recorded in the oxidative than in the glycolytic muscles (Ortigues-Marty et al., 2005, Ortigues-Marty et al., 2006).

CONCLUSION

Meat is a valuable supply of various healthy nutrients, including vitamin B12. Accurate nutritional composition facts are still needed to meet public health, clinical, practical, regulatory, and scientific needs. The nutritional challenges lie in preserving the original quality and quantity of food and increasing shelf life to ensure food safety for consumers throughout the year. However, the impact of particular technological treatments, such as freezing and cooking, is only sometimes well considered due to the loss of juices and nutrients.

In this study, freezing did not significantly alter the amount of vitamin B12 in Hamra lamb meat, which was qualified as providing a product comparable in nutritional content to fresh meat. Nevertheless, the cooking processes (temperature and time) are the key factors influencing the variable losses of vitamin B12 in Hamra lamb meat. This finding highlights the importance of considering the changes in beef composition due to cooking to assess its contribution to nutritional intake. The meat industry, notably those that manufacture meat preparations, requires analytical methods for quantifying vitamin B12 that are accurate and compassionate. Many ways to determine vitamin B12 in the meat industry have been developed. These include microbiological assays using various vitamin B12-dependent microorganisms.

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Conflicts of Interest

We have no conflicts of interest to disclose.

Authors' Contributions

Kaddour Ziani and Méghit Boumédiène Khaled developed the experiment and drafted the manuscript. Kaddour Ziani, Djallal Eddine Houari Adli, Noureddine Halla, and Abdeldjallil Mansouri experimented. Kahled Kahloula carried out part of the experiments. Djamel Djenane commented on the manuscript critically by making all necessary and relevant changes. All authors read and approved the final manuscript.

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