DETERMINATION OF CONTENT, COOKING LOSS, AND BIOACCESSIBILITY OF THIAMINE IN RED MEATS USING *IN VITRO* SIMULATED HUMAN GASTROINTESTINAL DIGESTION SYSTEM

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***Abstract*— Meats are considered a valuable part of the human diet and a best source of B-group vitamins. Thus, investigations that measure the losses by cooking and bioaccessibilities of micronutrients are crucial to knowing the vitamin amounts in meats. The aim of this research was to investigate the content and loss of thiamine in meats by four different cooking practices, boiling, roasting, grilling, and frying, and to determine the bioaccessibility of thiamine in cooked meats by a simulated *in vitro* human gastrointestinal digestion system. High-performance liquid chromatography was used to detect the contents of thiamine in each raw and cooked meat. In raw meats, the measured thiamine amounts varied from 58.67 to 159 µg/100 g. After cooking, the amounts of thiamine ranged between 21 – 93 µg/100 g. While the lowest thiamine loss was determined in the boiled mutton thigh (8%), the highest loss was found in roasted veal thigh and mutton sleeve with 64%. The bioaccessibilities of thiamine in meats after digestion ranged from 12 to 83 µg/100 g. The highest thiamine bioaccessibility was in the grilled veal chop with 91%, while the lowest bioaccessibility was found in the roasted mutton sleeve with 12%. The bioaccessibility of vitamins may be remarkably influenced by factors such as stability, pH of the gastrointestinal tract, and temperature. Determining the bioaccessibility of thiamine in meats after cooking and digestion is important for the correct evaluation of the daily intake values of these vitamins.**

***Keywords*—Meats, Thiamine, Cooking loss, *In vitro* gastrointestinal digestion, HPLC**

I. INTRODUCTION

Red meats are an essential part of the human diet. They are an important source of various vitamins and minerals, especially most water-soluble vitamins. It is well known that vitamins have health-promoting potency (Wood, 2017) that are important organic substances required for normal function, growth, and maintenance of the body (Smith and Lindeman, 2012).

Thiamine is important for carbohydrate metabolism and neural function (Combs and McClung, 2017) and is involved in chemical reactions that ensure the formation of RNA, DNA, and neurotransmitters (Smith *et al*., 2019). It is essential for acetylcholine synthesis, a neurotransmitter that influences several brain functions like memory. It is critical for normal growth and development. It takes part in reproduction, healthy skin, hair, blood production, and immune function significantly (Guo, 2009). Thiamine founds in high amounts in meats, legumes, cereals, and grain products. Other thiamine sources are nuts, seeds, yeast, wheat germ, oat bran, milk, eggs, vegetables, and fruits (Gropper *et al*., 2020; Yaman *et al*., 2021). Plant-based foods comprise mainly free form of thiamine and thiamine monophosphate (TMP) form. On the other hand, in animal tissues, thiamine is found mostly in TPP form (Combs and McClung, 2017). Significant losses occur in vitamin content during the processing of foods (Çatak *et al*., 2020). Water-soluble vitamins as thiamine, can be destroyed with improper storage and overcooking. The factors that can destroy vitamins are heat, light, exposure to the air, cooking in water, and alkalinity (Smith and Lindeman, 2012; Çatak and Çaman, 2020). Considering the effect of cooking techniques on vitamin contents in foods, it has been shown in some studies that cooking causes significant thiamine losses. A few data show that thiamine is one of the most affected B vitamins. Since it is water-soluble, some cooking methods, such as boiling, may cause higher losses (Çatak, 2022); however, since the thiamine is thermally unstable, shorter cooking times may reduce these losses (Castro Cardozo Pereira and dos Reis Baltazar Vicente, 2013; Çatak, 2022). Recently, Yaman *et al*. (2022) studied the nanofiber application for thiamine stability and enhancement of bioaccessibility of raw and cooked red meat samples stored at 4 °C. With the increase in storage period, the thiamine content of cooked red meats ($p < 0.05$) samples decreased.

Many food composition databases include the nutritional content of raw and cooked foodstuffs. Dietitians can compute daily nutritional intake amounts by employing these given data. It is critical to estimate the daily dietary intake values to guide people correctly. Therefore, *in vivo* bioavailability has been the gold standard for evaluating the availability of nutrients and bioactive compounds in foods (Beceren *et al*., 2022; Demir *et al*., 2023). However, the bioavailabilities of these vitamins are not fully estimated because of their unknown bioaccessibilities in the gastrointestinal tract when vitamin intakes are calculated in daily diets. Since *in vivo* investigations are time-consuming and expensive, bioaccessibility studies have been used to predict bioavailability

(Yaman *et al*., 2021). Therefore, recently a large variety of *in vitro* methods have been developed. Bioaccessibility is the fraction of an ingested biocomponent that becomes accessible for absorption through the epithelial layer of the digestive tract (Çatak and Gizlici, 2022; Andac-Ozturk *et al*., 2022).

All vitamins show certain biochemical properties and different action mechanisms in humans, so each type of vitamin should be discussed separately regarding bioavailability and bioaccessibility (Khaneghah *et al*., 2019). *In vitro* methods simulate the chemical, biochemical, and mechanical conditions of the mouth, stomach, small intestine, and colon (Dima *et al*., 2020; Yaman et al., 2021). There are limited studies on the bioaccessibility of thiamine in red meats. Therefore, knowing the bioaccessibility of thiamine in foods is crucial to determine daily intake and guide more accurate and precise recommendations. Because of all the reasons mentioned above, this study aimed to determine the loss of thiamine in red meats by different cooking methods and investigate the bioaccessibility of thiamine in meats using a simulated *in vitro* gastrointestinal digestive model system.

II. METHODS

A. Materials

The thiamine standard and acid phosphatase from potatoes (0.5-3.0 U/mg, lyophilized powder), taka diastase from *Aspergillus oryzae* (100 U/mg, powder), alpha-amylase from Aspergillus oryzae (1.5 U/mg, powder), beta-glucosidase from almonds (10-30 U/mg solid, lyophilized powder), pepsin from porcine gastric mucosa (≥250 U/mg solid, lyophilized powder), pancreatin (from porcine pancreas 8xUSP specifications), lipase (100–500 U/mg protein, from porcine pancreas Type II), hydrochloric acid (HCl, 37%), NaCl, $CaCl_2.2H_2O$, KCl, $NaHCO_3$, mucin, bovine serum albumin, urea, uric acid, bile salts mixture, methanol (MeOH), ortho-phosphoric acid, potassium ferricyanide (III) ($K_3Fe\ (CN)_6$), potassium dihydrogen phosphate (KH_2PO_4), acetonitrile (ACN), trichloroacetic acid (TCA), 1-heptane sulfonic acid and a sodium salt were supplied from Sigma-Aldrich (St. Louis, Missouri, USA).

B. Sampling and cooking methods

In the study, 3 mutton samples (mutton sleeve, mutton thigh, and mutton back) and 5 veal meat samples (veal sleeve, veal thigh, veal chop, veal steak, veal tenderloin) were purchased from eight different markets in Istanbul, Turkey, and were cooked by boiling, roasting, frying, and grilling methods. Meat samples purchased and cooked ranged in weight from 60 to 75 grams. Three sample replicates were prepared for the cooking. Each sample was divided into 3 groups with three meat parts each, except for veal chop, veal steak, and veal tenderloin. These samples were cut into cubes. The first group (raw) was uncooked, while the other two groups were cooked by boiling and roasting methods. The veal chop, veal steak, and veal tenderloin were divided into 2 groups with three meat parts each. The first group (raw) was uncooked, while the other group was cooked by frying for veal steak and veal tenderloin and by grilling for veal chop. The samples were prepared and analyzed on the same day. The sample weights used for each cooking are as follows: mutton sleeve in boiling: 64.5 g, mutton sleeve in roasting: 64.6 g, mutton back in boiling: 70 g, mutton back in roasting: 68 g, mutton thigh in boiling: 68.4 g, mutton thigh in roasting: 69.3 g, veal chop in grilling: 62.5 g, veal steak in frying: 75 g, veal sleeve in boiling: 60.7 g, veal sleeve in roasting: 60.4 g, veal tenderloin in frying: 59.9 g, veal thigh in boiling: 70 g, veal thigh in roasting: 60.3 g.

Boiling, roasting, frying, and grilling of the meats were performed in a stainless steel saucepan, pan, domestic frying pan, and a local griller, respectively. The boiling process was carried out in 45 min and the roasting process was carried out in 30 min (mutton sleeve), and 25 min (other samples) until the internal temperature was 75 ℃. The veal steak and veal tenderloin were fried by turning them upside down in a pan until the internal temperature was 75 ℃ for 10 minutes. In our study, the meats were cooked with the lid closed with a very small amount of water over medium heat for the boiling method, and they were cooked in oil at medium heat by stirring constantly for the roasting method. The veal chop was put on the grill and cooked by turning it upside down in the griller until the internal temperature was 75 ℃ for 15 minutes. The internal temperature was measured with a probe thermometer in all samples. Each red meat sample was examined three times in each cooking technique. The meats were grounded by a kitchen blender to ensure homogeneity. The cooked red meats were cooled down to room temperature. In the study, each type of meat was cooked with the methods most preferred by society when consuming that meat.

C. Extraction of thiamine from red meats

The extraction process and HPLC determination method for thiamine described by Tang *et al.* (2006) and Çatak *et al*. (2022b) were used with some modifications. First, a five g of homogenized red meat sample was taken into a 100 mL Erlenmeyer. After adding the 60 mL HCl solution (0.1 N), the sample was autoclaved at 121 °Cf or 30 min. Next, the sample taken from the autoclave was cooled to room temperature. After this stage, an enzymatic procedure was achieved to liberate the phosphorylated forms of thiamine (TMP, TDP, and TTP). Next, using a sodium acetate solution (2.5 mM), the pH was adjusted to 4.5. The enzymatic extraction step was as follows; 100 mg taka diastase and 10 mg acid phosphatase enzymes were added and allowed to incubate for three hours at 37 °C in a shaking water bath. In the latest part of *in vitro digestion*, only the acid phosphatase enzyme was employed in test samples. Next, after cooling the samples, they were filtered through a 0.45 μm filter paper. For thiamine determination, derivatization of thiamine to trichrome is required. Thus, a 20 mL filtrate was received from the earlier solution mixed with a potassium ferricyanide solution (1.5 mL). Following, the pH was

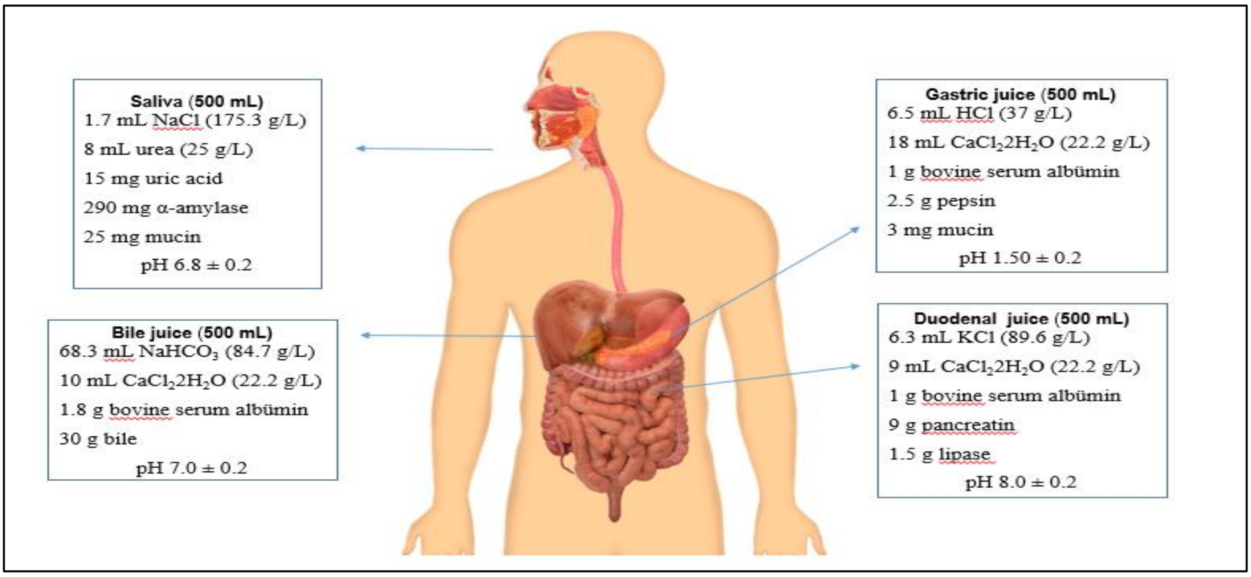

Fig. 1. Concentrations and constituents of the *in vitro* human gastrointestinal model.

adjusted to 7.1 ± 0.1 by ortho-phosphoric acid. Next, through a 0.45 μm CA filter (ISOLAB Laborgerate GmbH, Wertheim, Germany), the derivatized solution was filtered. This solution was injected into the HPLC for thiamine determination. The injection volume was 20 μL.

D. HPLC analysis of thiamine

For the separation of thiamine, the HPLC (Shimadzu Nexera-i) with a fluorescence detector (Shimadzu RF-20A) was employed (Shimadzu Corporation, Kyoto, Japan). The mobile phase was prepared in a 75% buffer solution (0.033M KH_2PO_4) and 25% methanol. Next, the pH was adjusted to 7.1 ± 0.1 using ortho-phosphoric acid and filtered under vacuum through a 0.22 μm CA filter (ISOLAB Laborgerate GmbH, Wertheim, Germany). The excitation and emission wavelengths were 366 and 445 nm, respectively. The separation of thiamine was handled with an Eclipse X08-C18, 5 μm, 4.6 × 150 mm column (Agilent Technologies, USA). The column temperature was set at 25 °C (flow rate: 1 mL/min).

E. *In vitro* gastrointestinal procedure and bioaccessibility of thiamine

In vitro analysis was achieved via the methodology described by Lee *et al.* (2016) with some modifications. Saliva solution, gastric, duodenal, and bile juices were prepared using organic and inorganic chemicals and enzymes (Fig. 1). In this *in vitro* human digestion model, organic and inorganic constituents were prepared with 500 mL of distilled water for each digestive enzyme. Next, each enzyme was mixed into that solution. Then, using 1M HCl or 0.2M NaOH, the pH was adjusted to the proper value for each solution (given in Fig. 1).

In the mouth stage, a 5 g test sample was mixed with 5 mL saliva solution in a 50 mL falcon tube for 20 s with a vortex. This mixture was incubated for 5 min at 37 °C in a shaking water bath. After this stage, in the gastric step, 12 mL of gastric juice was added to the test sample provided from the mouth stage, and allowed to incubate once more in a shaking water bath at 37 °C for two hours. Next, 10 mL of duodenal juice and 5 mL of bile juice were added to the test sample provided from the gastric stage and incubated for two hours at 37 °C in a shaking water bath. Using trichloroacetic acid, the pH of the solution was adjusted to 4.5 after the digestion procedure was accomplished, finishing volume was diluted to 50 mL by deionized water and centrifuged for 10 minutes at 8000 rpm. The obtained solution was used in thiamine determination analysis.

F. Quantification and Quality Control

Quantification was calculated by dividing the area under the sample in the HPLC chromatogram by the area of the appropriate standard concentration.

G. Statistical analysis

Each sample was analyzed at least three times in each distinct experiment, and the mean value was used. The results were expressed as mean ± standard deviation. Significant differences within groups were statistically identified by ANOVA ($p < 0.05$, Tukey's test).

III. RESULTS AND DISCUSSION

This research was implemented in three stages. At first, thiamine contents were detected in raw red meats. Next, the cooking losses of thiamine between raw and cooked meats were determined using four different cooking methods: boiling, roasting, grilling, and frying. Then the bioaccessible amounts and bioaccessibilities (%) of thiamine were investigated by a simulated *in vitro* human gastrointestinal system. After cooking, the vitamin values of the cooked samples were re-calculated based on water losses. Results are presented as mean ± SD.

A. Thiamine content in raw and cooked meat

The measured thiamine amounts in raw and cooked red meats using four different techniques and cooking losses of thiamine in meats are given in Table 1. The HPLC chromatogram of thiamine in the meat sample is shown in Fig. 2. The amount of thiamine in raw meats varied between 58.67±2.34 μg/100 g and 159±6.36 μg/100 g. In raw red meats, the highest thiamine level was seen in the mutton back, while the lowest level was in the veal thigh. In the American Food Composition Database, in veal, the total thiamine amount is listed as 113 µg/100 g (USDA, 2022). The total amounts of thiamine in veal, in the Turkish National Food Composition Database, ranges between 68 – 369 µg/100 g, similar to our results (TURCOMP, 2022). In the literature, consistent with the findings of our study, thiamine content in beef (40 µg/100 g), veal (60 µg/100 g), lamb (120 µg/100 g), and mutton (160 µg/100 g) are reported as the richest source of thiamin (Williams, 2007). As well known, thiamine defined as water-soluble can be lost in cooking water.

The amounts of thiamine in red meats after boiling varied from 28.67±1.14 to 93±3.72 µg/100 g. After boiling, the highest thiamine level was found in the mutton back, while the lowest level in the veal thigh. The levels of thiamine in meats after roasting ranged between 21±0.84 and 74.67±2.98 µg/100 g. The highest level after roasting was found in the mutton back, while the lowest level was found in the veal thigh. After frying, the amount of thiamine was found as 89±3,56 and 57.33±2.29 µg/100 g, in veal tenderloin and veal steak, respectively. Cooking is responsible for vitamin loss in foods. Factors affecting the thiamine stability of cooked meats include moisture content, cooking time, and cooking temperature. Ureta *et al.* (2019) stated that during cooking, both temperature and time have a large effect on the physical properties of meat, mainly due to protein denaturation. Kondjoyan *et al*. (2018) stated that cooking losses of vitamins may be due to expulsions in juice from food and thermal denaturation. In our study, thiamine was more stable with boiling than roasting in all cooked red meat samples. For example, mutton thigh was boiled for 45 min and roasted for 25 min (loss by boiling: 8%; loss by roasting: 31.1%). Higher cooking durations not resulted in a higher loss of thiamine in samples. Although the red meats were boiled for a longer cooking time, there was no effect of cooking time on thiamine loss in this study.

In the literature, losses of thiamine change according to cooking methods; 14 – 66% of thiamine loss occurs by boiling vegetables, 14 – 35% by pressure cooking, and 11 – 27% by steam cooking. In a study performed with

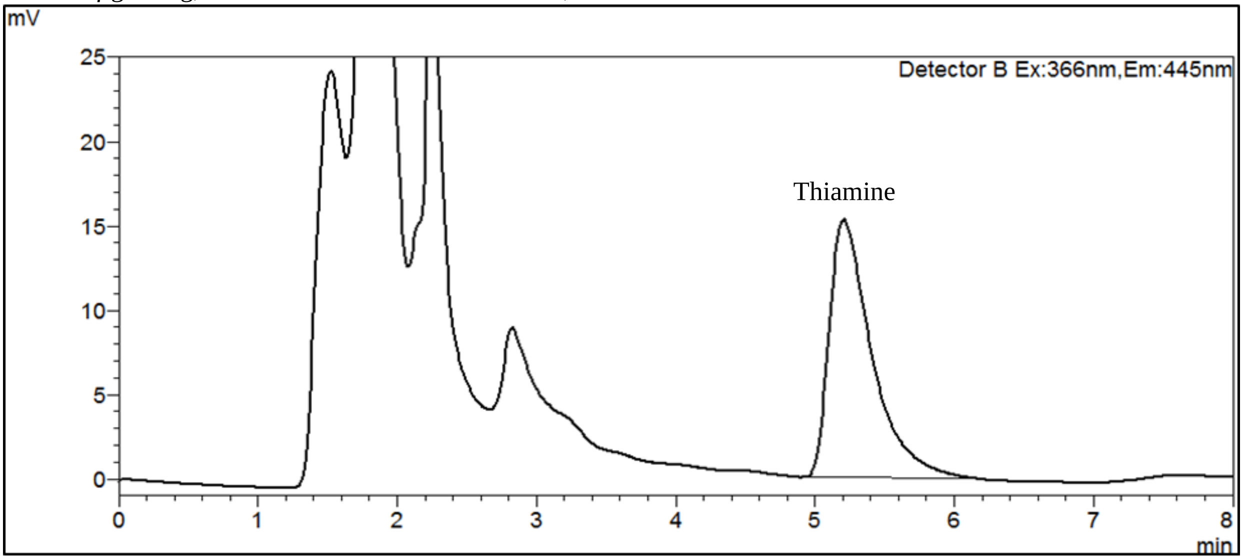

Fig. 2. HPLC chromatogram of thiamine in red meat sample.

Table 1. The amount of thiamine in raw and cooked meats with four different methods and cooking losses.

Samples	Raw (µg/100 g)	Boiling (µg/100 g)	Roasting (µg/100 g)	Frying (µg/100 g)	Grilling (µg/100 g)	Loss by boiling (%)	Loss by roasting (%)	Loss by frying (%)	Loss by grilling (%)
Mutton sleeve	104.33±4.16[a]	73±2.92[b]	33.67±1.33[c]	-	-	29.8	64.4	-	-
Mutton back	159±6.36[a]	93±3.72[b]	74.67±2.98[c]	-	-	41.5	52.8	-	-
Mutton thigh	87.33±3.49[a]	79.67±3.18[a]	60.33±2.41[b]	-	-	8	31.1	-	-
Veal chop	60±2.40[a]	-	-	-	36.33±1.45[b]	-	-	-	40
Veal steak	73.67±2.94[a]	-	-	57.33±2.29[b]	-	-	-	21	-
Veal sleeve	80.67±3.22[a]	54.67±2.18[b]	45.67±1.82[b]	-	-	32.1	43.2	-	-
Veal tenderloin	100.33±4.01[a]	-	-	89±3.56[a]	-	-	-	11	-
Veal thigh	58.67±2.34[a]	28.67±1.14[b]	21±0.84[b]	-	-	50.8	64.4	-	-

Different letters in the same row demonstrate statistical differences between the applications ($p < 0.05$)

-: Not applicated, Limit of detection (LOD): 0.006 µg/100 g, Limit of quantification (LOQ): 0.019 µg/100 g.

Table 2. Bioaccessible amounts and bioaccessibilities of thiamine in red meats after *in vitro* digestion

Samples	Boiled digested (µg/100 g)	Roasted digested (µg/100 g)	Fried digested (µg/100 g)	Grilled digested (µg/100 g)	Boiling Bioaccessibility (%)	Roasting Bioaccessibility (%)	Frying Bioaccessibility (%)	Grilling Bioaccessibility (%)
Mutton sleeve	24±0.75[a]	12±0.75[b]	-	-	33±3	12±1	-	-
Mutton back	83±2.94[a]	63±2.94[b]	-	-	89±7	84±7	-	-
Mutton thigh	23±0.94[a]	23±0.94[a]	-	-	29±2	38±3	-	-
Veal chop	-	-	-	33	-	-	-	91±7
Veal steak	-	-	33	-	-	-	58±5	-
Veal sleeve	47±1.62[a]	33±1.62[b]	-	-	86±7	72±6	-	-
Veal tenderloin	-	-	58	-	-	-	65±5	-
Veal thigh	19±0.70[a]	16±0.70[b]	-	-	66±5	76±6	-	-

Different letters in the same row demonstrate statistical differences between the applications ($p < 0.05$)
-: Not applicated, Limit of detection (LOD): 0.006 µg/100 g, Limit of quantification (LOQ): 0.019 µg/100 g.

meat products, the results showed that cooking processes caused a reduction in vitamin B content in both mild (70 – 90 °C) and severe (120 °C) conditions (Chandra-Hioe *et al*., 2015). As seen in our results, the cooking loss of thiamine in red meats ranged from 8 to 64.4%. The highest loss was determined in the mutton sleeve and veal thigh by roasting method (64.4%), while the lowest thiamine loss was in the mutton thigh by boiling. By boiling, the cooking loss of thiamine was between 8 – 50.8%, the highest thiamine loss was detected in the veal thigh, while the lowest loss was in the mutton thigh. However, by roasting, the thiamine loss ranged from 31.1 to 64.4%. In our study, by frying, the cooking loss of thiamine in veal tenderloin was determined at 11%, and in veal steak was found with 21%. Çatak *et al*. (2022b) reported that the thiamine level of grilled fish and chicken meats was higher than those of baked meats. In the present study, by grilling, the loss of thiamine in veal chop was determined at 40%.

Ozunlu *et al*. (2021) reported that the effective moisture diffusivity values of chicken meat were increased as the water rehydration temperature increased. In a study, it has been reported that cooking has a negative effect on B vitamins concentration in boiled ham, after 5 min of cooking at 100 °C, all vitamins were not detectable. In the same study, after cooking on the grill, thiamine was reduced by 40% in boiled ham and fortified cow meat (Riccio *et al*., 2006). In another study by Lombardi-Boccia *et al.* (2005), vitamin B concentrations were measured before and after cooking in different types of meats, and a significant decrease of thiamine with a loss of 85% in pork and a loss of 98% in veal was reported.

In a study conducted by Kimura *et al*. (1990), thiamine loss was reported by boiling, steaming, roasting, and frying in pork meat was 70%, 40%, 35%, and 30%, respectively. This was explained by the water-soluble nature of the thiamine that leaches into the water (Chandra-Hioe *et al*., 2015). However, the loss was found to be lower in the boiling method in our study, since the meat was cooked by absorbing its water and the water was not spilled.

B. *In vitro* study

Bioaccessible amounts and bioaccessibilities of thiamine in meat samples after *in vitro* digestion are shown in Table 2. After digestion, the amount of thiamine was between 19±0.70 – 83±2.94 µg/100 g in boiled meat and between 12±0.75 – 63±2.94 µg/100 g in roasted meat. In fried meats, after digestion, the amount of thiamine was 58 µg/100 g and 33 µg/100 g in veal tenderloin and veal steak, respectively.

As seen in our results, by boiling, the highest thiamine bioaccessibility was seen in the mutton back with 89±7%, while the lowest was found in the mutton thigh with 29±2%. By roasting, the highest thiamine bioaccessibility was seen in the mutton back with 84±7%, while the lowest was seen in the mutton sleeve with 12±1%. By frying, the bioaccessibility of thiamine was determined in veal tenderloin with 65±5%, while it was determined in veal steak with 58±5%. By grilling, the thiamine bioaccessibility was 91±7% in veal chop.

In foods, thiamine is found in free or phosphorylated forms. It can also non-covalently bind to proteins (Akça *et al*., 2019). Therefore, the thiamine may not be released depending on the pH of the gastrointestinal tract and the intake level of thiamine in the gastrointestinal tract may be affected (Çatak and Gizlici, 2022). Thiamine is stable at room temperature under slightly acidic conditions while under neutral pH conditions it is sensitive to destruction by heating (Combs and McClung, 2017). In our study, since the small intestine environment was adjusted to pH: 8, it is believed that thiamine was affected by this pH value and its bioaccessibility decreased.

In addition, since the temperature of the *in vitro* environment is 37 °C, this may decrease the bioaccessibility of thiamine. About 50% to 90% of dietary B vitamins are absorbed, expressing they have a relatively high bioavailability (Smith and Lindeman, 2012). In our study, thiamine bioaccessibility ranged from 12 to 91%.

Noteworthy losses occur in vitamin level during the processing of foods. Water-soluble vitamins as thiamine, can be destroyed with overcooking. The factors that can destroy vitamins are heat, light, exposure to the air, cooking in water, and alkalinity (Smith and Lindeman, 2012). Thiamine is stable in dry preparations and under slightly acidic conditions at room temperature, while sensitive to neutral pH and heating (Combs and McClung, 2017). Since the maximum stability in solution is between pH 2.0 and 4.0, thiamine in low acid foods is highly susceptible to losses during the heat process (Eitenmiller *et al*., 2008). Additionally, other substances in foods, called anti thiamine factors, also support the breakdown of thiamine. However, these thiaminases are readily deactivated by heat (Gropper *et al*., 2020). Loss of thiamin in meat also depends on several factors such as the size of the cut, fat content and thermal gradient while cooking.

In our study, another remarkable point is that the thiamine levels in red meat samples differed in the meats belonging to the different regions of the animal. The new rearing systems, the utilization of animal feeding fortified in some nutrients, and the slaughtering practices contribute to the variability of the vitamin composition in meat (Lombardi-Boccia *et al*., 2005).

Primary and secondary food processing may unfavorably affect the thiamine content. Boiling, stewing, roasting, and frying methods lead to thiamine cooking losses in meats. The respective cooking losses from pork were 70%, 38–55%, 18–40%, and 10–50%, while, from beef, the losses were 60–80%, 55–68%, 12–38%, and 55%, respectively (Kimura *et al*., 1990; Bognar, 2002; Hrusba *et al.*, 2022). Thiamine leaches into the water, owing to its solubility, and will be lost in any soaking or cooking water that is thrown away, as well as being destroyed by heating during cooking processes, in dependence on a type of food, a method used, temperature, and duration. Consumption of meat and other food with cooking liquids, such as water added or exuding juices, is hindering the loss of thiamine. For example, thiamine contents decrease by 33–40% in boiled beef with soup, and by 30% in braised pork with gravy, whereas they decrease by 60–80% and 55% solely in beef and pork, (Kimura *et al*., 1990; Bognar, 2002; Hrusba *et al*., 2022). Fried breaded meats, such as pork, chicken, lamb, and beef, contain 15–40% more thiamine than variants with no breading (Bognar, 2002). Meat with a lower initial fat content loses more water during thermal cooking, resulting in more thiamine leaching out, so the fat content might be a factor with a positive influence on vitamin retention in the course of heat treatment of meat (Leskova *et al*., 2006).

The present results concerning the loss of thiamine in red meats after cooking were in agreement with some of the previous data of other researchers. However, the data on thiamine bioaccessibility in cooked red meats was limited. Williams (2007) studied the nutritional composition of red meat and revealed that of the four types of meat (beef, veal, lamb, mutton), mutton was particularly nutrient-dense, and the richest source of thiamine.

The fundamental purpose to study food composition is to take advantage of its potential health benefits. Knowledge of the composition of the food ingested is crucial for evaluating diet quality and developing and employing dietary guidelines. In addition, the nutrient composition of processed foods is a critical input in determining average daily nutrient intake and constructing more precise recommendations. Nutritionists require comprehensive data on their biochemical composition for advice on dietary intake and the formulation of food products. Due to all the reasons declared, directly or indirectly, the stability or preservation of vitamins in foods is a major subject for food manufacturers, particularly consumers.

The laws of thermodynamics manage the flow of energy in ecological systems. The transfer of energy throughout the ecosystem is vital for all living (Çatak *et al*., 2018; Çatak *et al*., 2022a). Nevertheless, each energy transfer along the food chain causes the loss of usable metabolic energy (Yildiz *et al*., 2020; Dutta *et al*., 2022). Consumers acquire substantial quantities of ATP and thermal energy in mitochondria from the energy stored in the food's chemical bonds due to cellular metabolism (Çatak *et al*., 2021). Mitochondria are called "the cell powerhouses" that regulate ATP production and cellular metabolism (Putti *et al*., 2015). Recently, researchers have focused on the functionality of mitochondria in the regulation of cellular energy production (Forbes and Thorburn, 2018). Recent studies show that the deficiency of some B group vitamins decreases mitochondrial function and disrupts energy metabolism (Mkrtchyan *et al*., 2018; Udhayabanu *et al*., 2017). The deficiency of B group vitamins, vital for energy metabolism, threatens cellular growth, function, and survival. Red meats are the main dietary source of vitamin B_{12} and they provide other B-vitamins, such as pyridoxine, biotin, niacin, pantothenic acid, riboflavin, and thiamine (Lofgren, 2012; Castro Cardozo Pereira and dos Reis Baltazar Vicente, 2013; Çatak, 2019). Hence, in humans, thiamine is an essential vitamin that needs to be continuously supplied by the diet. The deficiency of thiamine results in malaise, loss of weight, irritability, and confusion, but if the deficiency is severe, results in nerve disease, heart disease, and beriberi (Combs and McClung, 2017). The recommended daily thiamine intake is about 1.1 mg for adult women and 1.2 mg for men (Food and Nutrition Board, 1998).

Vitamins perform specific biochemical properties and different mechanisms of action in the body, so each type of vitamin should be discussed separately in terms of bioavailability and bioaccessibility.

IV. CONCLUSIONS

In this study, two different red meats (mutton and veal meats) were cooked by boiling, roasting, grilling, and frying, and then they were digested in the gastrointestinal tract *in vitro*, and the thiamine levels were measured in each raw, cooked and digested red meats. The limitations of our study include meat-specific cooking methods. All the meats in the study were not cooked by all methods. The cooking methods applied for the meat samples were chosen according to the methods used by society while consuming these meats. The results of this study revealed that the thiamine content in red meats was mainly affected by cooking and *in vitro* digestion. It is believed that the bioaccessibilities of vitamins were significantly affected by the stability, temperature, and pH of the gastrointestinal tract. This study contributes to updating the literature data about the thiamine profile of the red meats mostly consumed in Turkey. Our findings showed differences in thiamine content not only among red meats of different species but also among cooking of the same species with different techniques. Knowing the content of thiamine in cooked red meats after digestion is required for the healthy nutrition of the population. Our study showed that thiamine had different bioaccessibilities in both different red meat species and different cooking methods. By choosing the right cooking method, the bioaccessibility of thiamine may be increased. Boiling with little water, not being exposed to heat for too long, and cooking with the lid closed may reduce vitamin losses. There are limited studies on the bioaccessibility of thiamine in cooked red meats. Considering the effects of thiamine on health, more research is needed on thiamine bioaccessibility.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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