Vitamin loss during cooking in LTLT-meat

Practical Semester

submitted by

Robert Werner

Tutor:

Jette Jakobsen

Department of Food

Danish Technological University

Mari Ann Tørngren

Danish Meat Research Institute

Danish Technological Institute

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Introduction

Vitamins

"Vitamins are defined as a small group of complex organic micronutrients present in small amounts in food or nutritional supplements that the body requires for its normal metabolism and function. They cannot be synthesized by humans in sufficient quantity for normal metabolic requirements (Finglas 2003)." Water soluble vitamins, most acting as a cofactor, take a huge part in the metabolism of carbohydrates, fatty acids, proteins and many more biomolecules. To determine the vitamin loss we use the heat sensitive vitamin thiamin and the UV-light sensitive riboflavin for detection with HPLC.

Function: thiamin& riboflavin

Thiamin or vitamin B_1 occurs in the body in various forms: free thiamin and phosphorylated forms like thiamin monophosphate (TMP), thiamin pyrophosphate (TPP) and thiamin triphosphate (TTP). Especially TPP has an important role in the metabolism. This coenzyme helps decarboxylating pyruvate to Acetyl-Coenzyme A (Acetyl-CoA) via pyruvate dehydrogenase at the entry point of the citric acid cycle and converting α-ketoglutarate to succinyl CoA via α-ketoglutarate dehydrogenase. Thiamin is often used as a marker for vitamin loss due to its liability to heat treatment.

A metabolite of thiaminphosphates, 2-(1-hydroxyethyl)thiamin (HET) was indentified performing a post-column HPLC-method for thiamin (Ujiie *et al.* 1990). A comparison between a manual thiochrom method and the post-column HPLC-method revealed that the sum of thiamin and HET were quantitated by the manual thiochrom method. Furthermore is the relative bioactivity of HET similar to the bioactivity of thiamin (Jakobsen 2007). Therefore it is recommended for a quantitation of vitamin B_1 in food to include HET as well. Due to heat treatment HET is transformed into free thiamin. The HET content in fish and meat products is relatively high (Ujiie *et al.* 1991).

Riboflavin also known as vitamin B_2 is an integral compound of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These two coenzymes are needed for redox reactions like the conversion of succinate to fumarate in the citric acid cycle via succinate dehydrogenase or threonine to pyruvate via monoamine oxidase.

Vitamin deficiency and RDA

As you can see, these two vitamins are vital for generating energy for the body. Suffering of a riboflavin deficiency is medically called ariboflavinosis and for thiamin it is beriberi. Alcoholics are at higher risk due to their poor diet and alcohol affects the absorption of thiamin. To avoid these diseases a daily intake is recommended, because the body can't store the vitamins for a long time. The Recommended Dietary Allowance or RDA is an acknowledged amount of vitamins or trace elements for the daily intake. For riboflavin it's 1,7mg/day and 1,3mg/day for male and female, respectively. The RDA for thiamin is 1,51mg/day for male and 1,1mg/day for female, respectively (Food and Nutrition Board).

Occurrence in food esp. meat

Table1: Thiamin and riboflavin content of different food sources

food source	thiamin content per 100g	riboflavin content per 100g
pork, loin, lean, raw [Sus scrofa]	0,81mg	0,20mg
beef, sirloin, raw [Bos taurus]	0,035mg	0,15mg
Salmon, raw [Salmo salar]	0,23mg	0,10mg
Potato, raw [Solanum tuberosum L.]	0,059mg	0,067mg
Broccoli, raw [Brassica oleracea L. convar. botyris]	0,10mg	0,30mg

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Table 1 shows the content of thiamin and riboflavin in different food sources. Especially pork meat has a very high amount of thiamin compared to other meat. Therefore pork is a very good thiamin source. Also vegetables are not rich in thiamin however broccoli contains much of riboflavin.

Processing of meat

As in many other publications mentioned, during processing meat losses the most percentage of vitamins (Lassen *et al.* 2002, Awonorin and Rotimi 1991, McIntire *et al.* 1942). "Approximately 40-45% of thiamin in the beef was destroyed by cooking (Cooksey 1990)."
There are many different ways to cook, but for our project we used a method, which is accepted in the industry and catering services. The cooking method we worked with, known as sous-vide, is defined as: "raw materials or raw materials with intermediate foods that are cooked under controlled conditions of temperature and time inside heat-stable vacuumized pouches (Schellekens and Martens 1992)." Due to this method the sensory and nutritional quality of sous-vide cooked foods are higher than conventionally cooked (Creed 1995).

One interesting point is the low cooking temperature (53°C) we used. Most people are concerned about the microbiological safety of the meat when cooked at such low temperature but latest projects of the Danish Meat Research Institute (DMRI) shown that the meat is safe when heated for a long time (Tørngren, personal communication, 2010). This is the essence of LTLT-meat, cooking at a *Low Temperature* for *Long Time* to preserve juiciness and tenderness, but still keep the meat safe for the consumer.

We conduct the reheating process to simulate a sous vide cook-chill (SVCC) meal like in a common catering service. Reheating with microwaves and ovens are wide spread (Church and Parsons 1993), so we used these two methods to work as close as possible to the procedure of the catering services.

Analytical methods

The high performance liquid chromatography (HPLC) is used to analyze and separate a complex mixture of chemicals using chromatography. It can also detect, identify, quantify and purify the components of the chemical mixture. The HPLC is mainly divided into three components: a pump system generating the pressure, a column with a stationary phase and in the end a detector to measure the absorbance of the released analyte. In our case we run a reversed-phase HPLC, that means the stationary phase is unpolar and the mobile phase is polar.
To determine the thiamin amount of the samples we used the thiochrom method. Hereby the thiamin is transformed to thiochrom through a post-column derivatization (see appendix). Due to the post-column derivatization you are able to detect HET as well and to quantify vitamin B_1 in food it is recommended to have a separated quantitation of thiamin and HET (Jakobsen 2007). In a pre-column transformation the HET and thiamin would agglomerate and a separation in the column wouldn't occur.
Thiochrom is a fluorophore that means compounds of the molecule causes it fluorescent. Thiochrom can be detected at a wavelength of 368nm; thereby it emits then at wavelength of 420nm.

Aim of the project

Our aim of this project is to assess the effect of cooking temperature on vitamin retention of thiamin but also about changes due to heating methods and storage time. We want to figure out if the LTLT-method could minimize the vitamin loss during cooking. If that turns out, this might be a procedure to a healthier meal especially for elderly or enfeebled subjects.
We are about to determine the vitamin retention of thiamin and riboflavin in loin muscles after sous-vide cooking with two different temperatures (53°C and 80°C), after reheating by microwave and common oven and without subsequent reheating. We also measure the vitamin amount in the juice and the meat separately. With this list of data we can compare the different stages and could comprehend the effects to the vitamin retention.

Material & methods

Experimental design

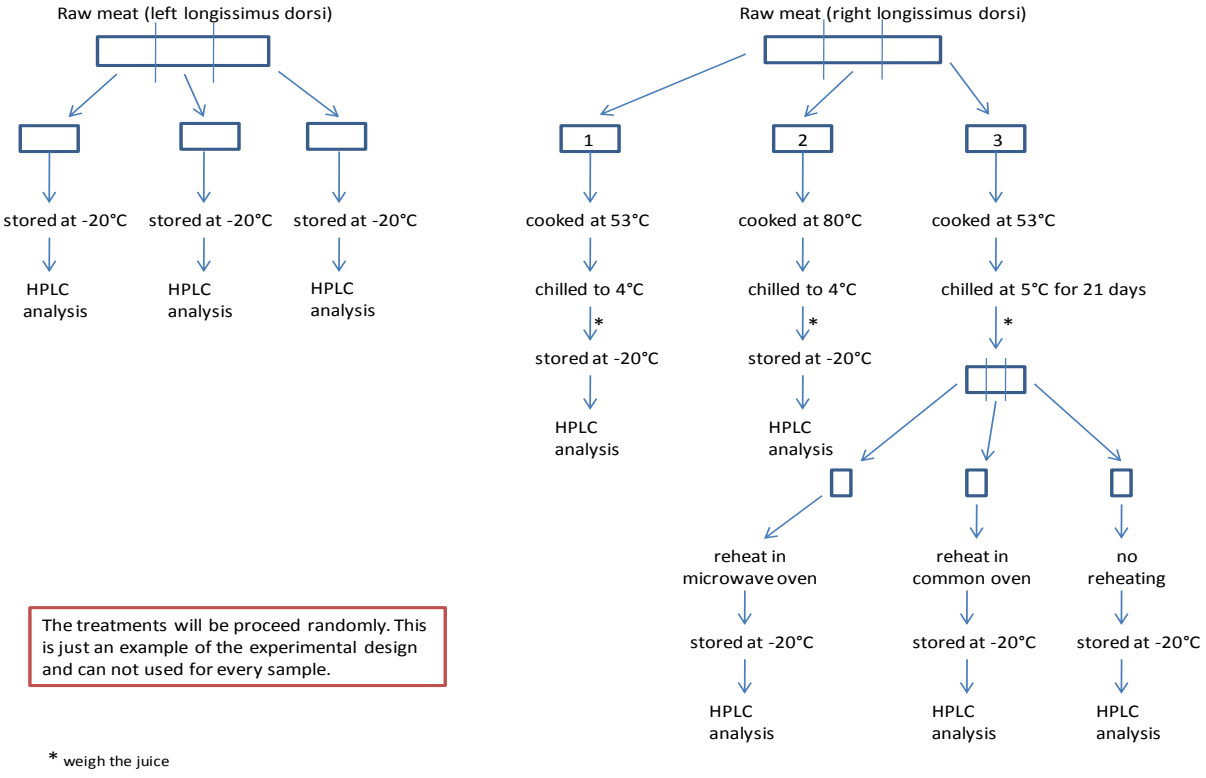

Fig. 1 Experimental design. Raw meat (longissimus dorsi) is cut into three samples and randomly proceeds different treatments (1,2,3) according to the experimental design. HPLC analysis means detection of thiamin and riboflavin. Four replicate experiments were conducted and all HPLC analyses were carried out twice.

The experimental design is presented in Figure 1. We used pig loins (left and right), which were cut into three samples. The samples had to be free of fat and bones and should only contain muscle meat. To compare the cooked meat, the initial vitamin content was determined in the raw loin from the same cut to grant reproducibility. The loin was divided into three different cuts, because the vitamin content within the loin varies (McIntire *et al.* 1942, Lassen *et al.* 2002). The left loins are reference material for the initial vitamin content and were homogenized raw and stored in a freezer at -20°C till analysis.

Heating

The right loins were put to a heat treatment in a hot water bath by a sous-vide machine. They were cooked with an internal temperature of 53°C and 80°C, respectively. The 53°C meat was heated for seven hours. Following to heat treatment the meat was cooled down to 3-4°C by the sous-vide machine.

Storage

The third treatment, heated to a temperature of 53°C, was chill-stored at 5°C for 21 days for further reheating. To extend the shelf-life to its maximum was a way to check on vitamin changes during storage. So we can simulate a sous-vide cook-chill (SVCC) preparation like it is used in the catering service.

Reheating

After chill-storage the roasts were weighted and divided into thirds. Each new sample got varied reheating treatment. They were reheated in a microwave (800W, 190s) or common oven (160°C) to an internal temperature of 77± 3°C or proceed no reheating, respectively. These samples were weighted (before and after reheating), homogenized and stored in a freezer at -20°C till analysis.

Meat

Four female pig carcasses were selected at the Danish Crown Slaughterhouse in Ringsted at the same day with a weight of 77,8 -79,4kg and a lean meat percentage of 60- 60,8%. The lean meat percentage was measured by a machine using ultrasound during the process-line at the slaughterhouse.

The loin was cut out from the 3rd vertebra of the hip to the 4th rip by a trained technician.

At first each loin was divided into three identical sized roasts of 12cm length (front, middle, rear). The right and left loins were prepared to be free of fat and bones. Only the muscle longissimus dorsi (LD) was used for our samples. They were randomly processed according to the experimental design.

No salt or seasoning was used.

Heat treatment/ cooking

Heat treatment was carried out in a water bath of a sous-vide machine (SousVide 40 Kg Compact No 02774 from Classic Gastro A/S, DK-5500 Langeskov, Denmark). The meat was heated by a hot water bath to 53°C or 80°C and then cooled down to 3-4°C by the machine. To monitor the core temperature an extra sample was added with a thermometer associated to the sous-vide machine. This meat sample was cut off the fifth loin from the slaughterhouse and had the same size as the other samples.

Fig.2 The sous vide machine

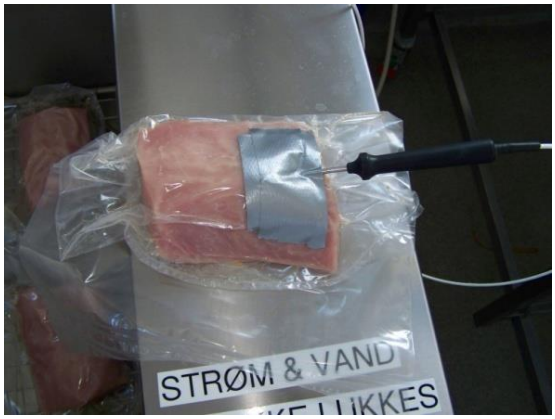

Fig.3 The temperature dummy to monitor the core temperature

80°C

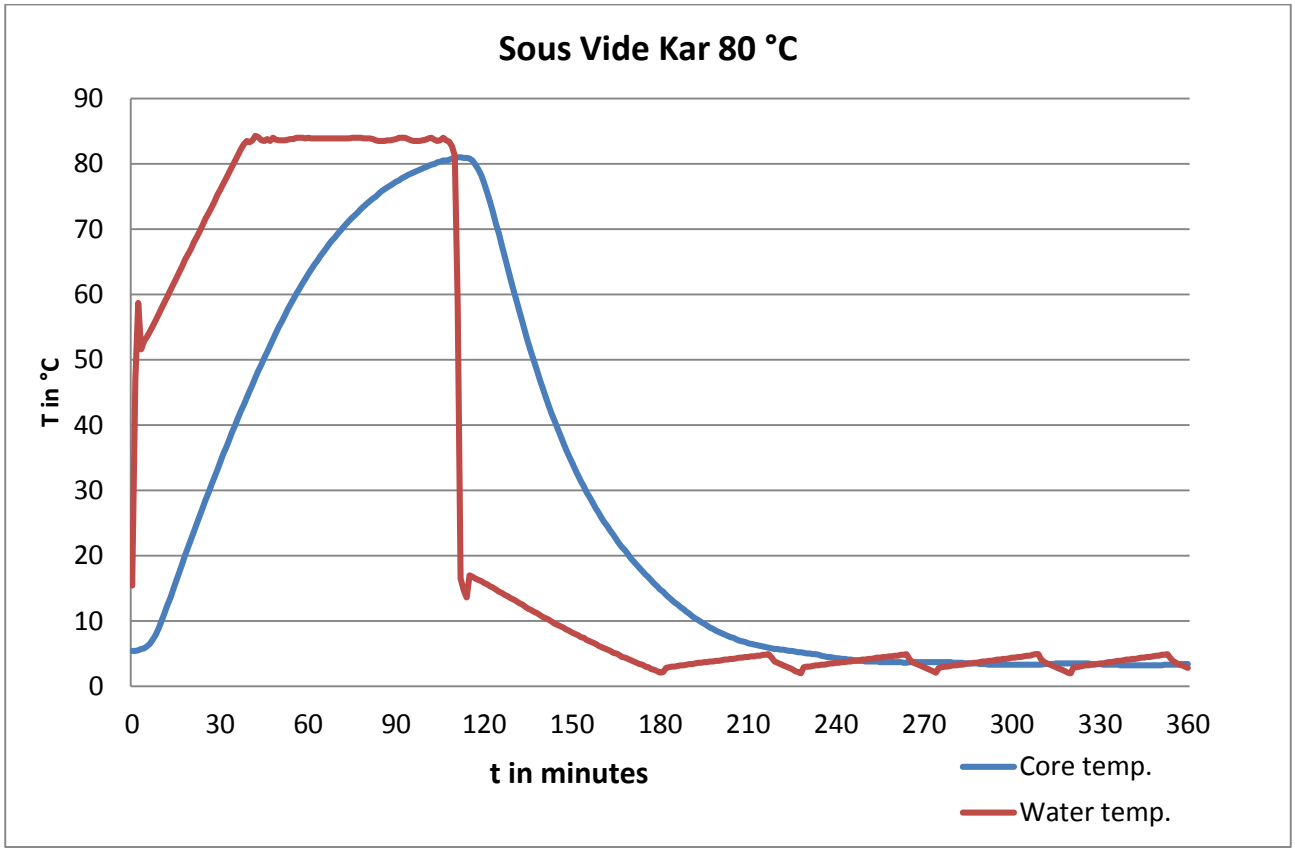

Fig.4 The core temperature and water temperature profiles for 80°C cooking.

As shown in Figure 4, an internal temperature of 80,5°C was reached after 106min with subsequent cooling to a temperature under 5°C within 125min. Till removal the meat was cooled between 3,2 and 3,5°C.
For cooking at 80°C, longer heat treatment is not required after reaching a core temperature of 80°C. There is no microbiological harm after the meat reached a core temperature of 80°C (Tørngren, personal communication, 2010). The temperature was set to 80,5°C and the cooking time to 1min.

53°C

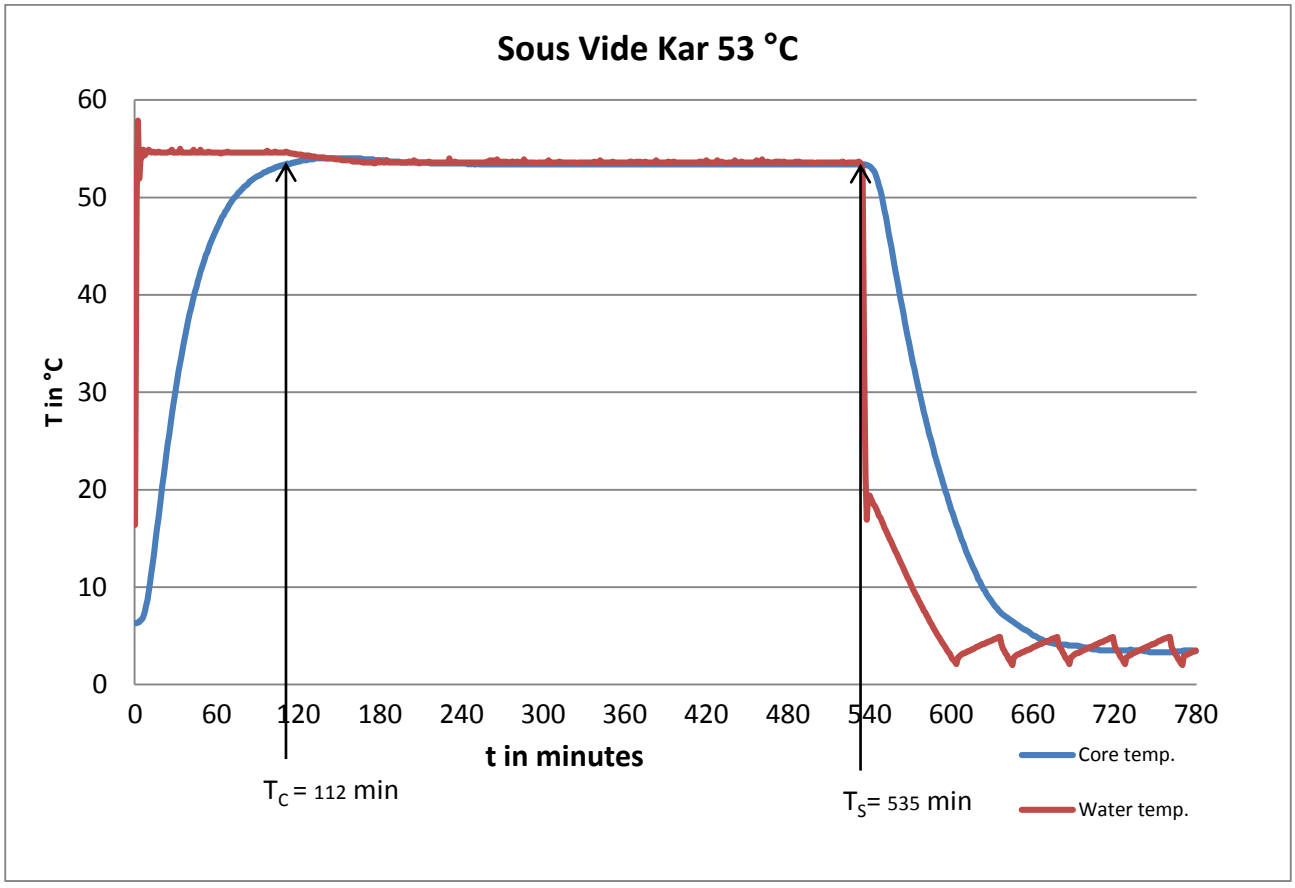

Fig.5 The core temperature and water temperature profiles for 53°C cooking. Time for reaching the core temperature (T_C), time for reaching the safety point (T_S).

The cooking time accounts for reaching the desired core temperature is about 2 hours, but to achieve the safety point T_S, where most of the microorganisms are killed, it takes seven more hours. This time period was determined by using the microorganism *Listeria monocytogenes*. *L. monocytogenes* belongs to the *Clostridium* sub-branch and is non-spore forming. Due to its relatively high heat stability it acts as a marker for food safety and the meat was hold at 53°C till a log 4 reduction occurred. That means the number of germs is 10.000 times smaller. After 112min the temperature dummy reached the core temperature T_C of 53,5°C and was held there for another 7 hours. In about 125min the samples were cooled down and kept at 3,2- 3,5°C till abort. The temperature was set to 53,5°C and cooking time to 420min.

Chill-storage

The samples which proceed the treatment 3 were stored in a cooling room for 21 days to max out the shelf-life. The cooling was controlled by a temperature logger. 21 days later the weight of the meat and the juice of the treatment 3 samples were measured. The loin was cut into thirds and the new samples were weighted again. According to the experimental design the three samples were treated as follows: microwave reheating, oven reheating and no reheating, respectively. The roasts were reheated at least to an internal temperature of 75°C. They were heated without any packaging.
To determine the cooking loss during reheating the meat was weighted. Subsequent homogenization was proceeded for these samples. They were stored with the rest of the cooked samples in identical plastic cups at -20°C.

No heating

One third of every sample was taken and homogenized without any further heat treatment. These samples were used to determine the vitamin content without the reheating process.

Reheating: microwave oven

The slice was put on a plate and reheated with a plastic microwave lid on the top. The microwave reheating was performed by a LG Wavedom with 800W for 190s. Subsequent to heating the core temperature was measured with a mobile thermometer.

Table 2: Core temperature of microwave reheated samples

Meat sample	Core temperature in °C
14232	77,3
24212	75,5
34222	78,0
44232	80,2
Mean temperature	77,8 ±1,9

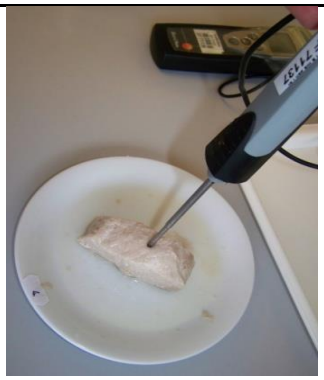

Fig.6 Measuring the core temperature after reheating.

Reheating: common oven

The samples were placed at aluminiumboxes and put into an Elektrolux Air-o-steam oven. The thickest was spiked with a thermometer which was connected to the oven. The oven was set to 160°C and after three-quarters of an hour the core temperature reached the 75°C.

Measurement

pH-measurement

By measuring the pH in the loins we get an insight of the meat quality. A pH between 5,50 - 5,60 was aimed to get good meat quality. A Knick pH-meter Model 913 (x) pH with an Ingold LOT glass electrode ø 6mm (Kaliumchloride electrolyte) type 3120 was used to determine the pH. According to the DMRI measuring method (DMRI /2002) the pH was ascertained by repeat determination. The pH differs between the chosen loins (pH 5,36- 5,50), but we wanted pigs from the same supplier. That should grant a high similarity of the pigs concerning the feeding and deriving from a single source.

Weighting

Before meat samples were packed into the cooking bags at the pilot plant the weight was defined by Delta Range SG16101 from Mettler Toledo.

The cooked meat samples and the juice were weighted with a Sartorius BP 3100S. The released juice was filled to a beaker glass and also the meats weight was determined to calculate the cooking loss.

The weight of the samples for the vitamin extraction was determined on a Sartorius 1702 MP8-1.

Packaging

The meat samples were labeled with plastic charts and put in Cryovac CN 300 (size 300x150mm) plastic cooking bags. The generation of vacuum in the bags was proceeded by a vacuum machine from Röschermatic A/G, Germany.

Homogenization

The meat has to be homogenized for further HPLC analysis. The cooking bags were opened and the meat was taken out. Each sample was cut into slices. Every second slice was cut into smaller pieces and homogenized by a Grindomix GM200 from Retsch for 10 second with 6500 rpm and stored in 50 ml clear standard containers (Nunc A/S, Roskilde, Denmark) at -20°C. Riboflavin is very unstable to sunlight therefore it was minded not expose the samples to sunlight during the preparation.

Analytical procedure

Vitamin extraction

The vitamin extraction was conducted as described in Jakobsen (2007) with a slight modification of the enzymatic treatment. The incubating time was reduced from 18h to 1 h since ultrasonication is used.
The homogenized samples were thawed and 1,5g were put in brown 100ml conical flasks (Schott Duran). 25ml of a 0,1M hydrochlorid acid (HCl) solution was added and aluminium foil was placed on the top of the bottle necks. The solution was shaken thoroughly to dispense the meat and autoclaved for 30min at 121°C with subsequently cooling of the samples. The acid hydrolysis and the autoclaving had the purpose to "denature the proteins and release the vitamins from their association with the proteins" (Ndaw *et al.* 1999). The pH of the cooled solutions were adjusted with 4M sodium acetate to a pH of 4,0 - 4,2. Before this, the pH-meter (PHM 210 Standard pH Meter, Radiometer Copenhagen) was calibrated with Radiometers Analytical IUPAC buffers. Adjusting the pH is needed to optimize the conditions for the enzymatic hydrolysis. A 5ml aliquot of a 20mg/ml takadiastase solution was added to each sample. Takadiastase is a α-Amylase produced by *Aspergillus oryzae* which also has phosphatase and proteinase activity; therefore it dephosphorylated the vitamins during the enzymatic hydrolysis (Ndaw *et al.* 1999). After being shaken the conical flasks were incubated for one hour in an ultrasonic bath. As the enzyme may be insufficient to dephosphorylate all TMP to thiamin, the content of thiamin is determined as the sum of thiamin and TMP.
The solutions were transferred quantitatively to 50ml volumetric flasks with 0,01M HCl and centrifuged subsequently. They were centrifuged in 50ml tubes from Sarstedt for 10min and 5000rpm by a Hearaeus Varifuge 3.0R. 15 ml were stored at -80°C for further measurement of the riboflavin content.
The solutions with meat samples were diluted 1:1 in 10ml volumetric flasks with 0,01M HCl in which juice samples were diluted 1:4 in 10ml flasks. That was necessary to get a vitamin concentration of approximately 0,1mg vitamin/ml. Otherwise the vitamin amount couldn't be determined when laying out of range for the calibration curve. These aliquots were filtered through a 0,2µm filter into 1,5ml HPLC vials (Sun Sri, Clear Snap) and close-lipped with snap caps. The vials were placed in the HPLC in a sequence where six standard solutions were at the beginning and in the end of the sample set.

High performance liquid chromatography- HPLC

The HPLC-system (Waters Milford, MA, USA) was set up with a separations module (2695), a scanning fluorescence detector (474) and a reagent manager for the derivatization pump.
A small amount (50µl) of the sample to be analyzed is injected to the mobile phase solution. The mobile phase consists of an aqueous solution and methanol in a 65:35 proportion. The aqueous phase contains water with salts like tetraethylammonium chloride which prevents tailing and sodium heptanesulfonate which is needed for ionpairing. The concentration of the aqueous phase were 0.1% tetraethylammonium chloride,

12,7 mM sodium 1-heptanesulfonate monohydrate and 50 mM potassium dihydrogen phosphate. After dissolving the pH was adjusted to 3.3 with phosphorous acid and the aliquot was filtered through a 0,45µm filter. The aqueous phase and methanol were mixed and degassed in an ultrasonic bath to avoid air bubbles during HPLC processing.
The mobile phase was pumped under high pressure through the HPLC column (Supelcosil LC-18-DB, 5 µm particle size, 250 x 4.6 mm, Supelco, Bellefonte, PA, USA) with a flow of 1ml/min. The matrix of the stationary phase in the column was silica gel in a spherical particle platform. On the silica gel long hydrophobic chains of alkyl groups are attached. That makes the stationary phase hydrophobic. Molecules in the mobile phase interacting with the stationary phase needed more time to pass the column and therefore had a higher retention time. After eluting the column a derivatization solution was now added to the analyte transforming thiamin, TMP and HET via alkaline oxidation to thiochrom, thiochrom-monophosphate and hydroxyethylthiochrom, respectively. Therefore thiamin, TMP and HET were measured indirectly. The derivatization solution is composed of 125mg potassium hexacyanoferrate (III) dissolved in 250ml 3,5M potassium hydroxide solution filtered through a 0,2µm nylon filter. The flow of the derivatization solution was 0,3ml/min. Entering the fluorescence detector with different retention times the absorbance of the separated analytes was measured.
The detector generated energy from a light source and directed it towards the excitation monochromator where the broadband source light was diffracted. Via mirrors the light was aimed to the flow cell and a beam splitter diverted part of the excitation beam towards a photodiode, which converted the light to an electrical signal used as a reference. The emitted light of the sample in the flow cell was collected by emission collector optics and directed to the emission monochromator which lead the diffracted emission spectrum to the photomultiplier tube, where it was amplified and converted into an electrical signal.

To quantify the amount a calibration curve had to be made every time. Calibration standards were always running additionally in the sample set with the samples. A known concentration of free thiamin, TMP and HET was used. The concentrations were 0,1µg/ml, 0,1µg/ml and 0,01µg/ml respectively. Six different injection volumes from 5µl -100µl were run.

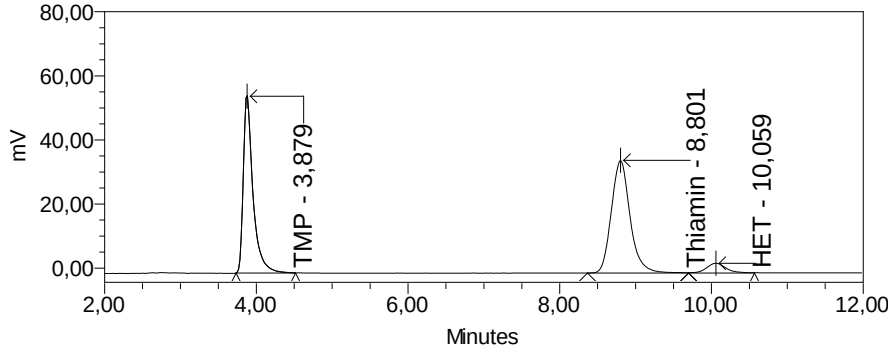

Fig.7 Chromatogram of the calibration standard solution containing TMP, HET and thiamin. Injection volume 100µl.

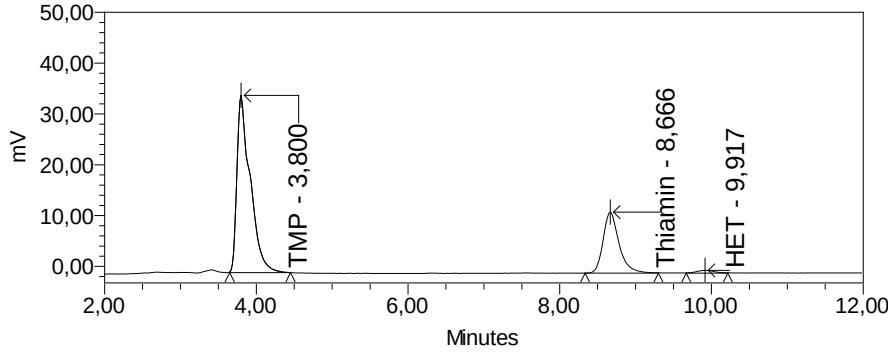

Fig.8 Chromatogram of a raw sample. Injection volume 50µl.

Dry weight

In order to determine the dry matter of the meat the vacuum method (NMKL method No 169, 2002) was used. This method is accepted by the Nordic Committee On Food Analysis and was validated in a collaborative study 1999. The sample is dried at 70°C under vacuum to constant weight.

At first the dishes with their lids underneath were dried in a drying oven (Thermocenter from Salvis) at 103,5°C for at least one hour. After heating they were allowed to cool in a desiccator for another hour with their lids on top. On an analytical balance (Sartorius LA 230S) the dishes were weighted to an accuracy of 0.1 mg. The dishes were always touched with special cotton gloves to pretend them of contamination from the hands.

About 5g of the homogenized meat samples were weighed in with the lid on top and placed with the lid underneath in the vacuum oven at 70°C (Vacutherm from Heraeus Instruments). A vacuum was created and a current of dry air was admitted by passing it through sulfuric acid. The samples were dried over night, e.g. 18-20 hours.

The vacuum was released and the lids were put on top of the dishes again. In the desiccator the dishes cooled down to room temperature and were subsequently weighed on the analytical balance.

The drying process was repeated again for 4 hours at a time, until the weight loss didn’t exceed 2mg per g of the remaining dry matter.
For every meat sample the dry matter was measured twice and the mean of these calculated values was taken as the result.

Quality control

Accuracy

The HPLC results of the certified reference material (lyophilized pigs liver, CRM 487 from IRMM) were as follows with the certification values in brackets (Finglas *et al.* 1998).

The determined amount of thiamin in pigs liver was 0,95 ±0,02 mg/100g (0,86 ±0,11 mg/100g).

A recovery test with TDP results in a percentage range of 86,7-93,4% with three determinations at different days.

Precision

The X-chart of the house reference material presents a standard deviation of 3,4% (n= 17) within 16 were in the 95% and one was in the 99% control range.

A R-chart for the results was designed to determine the deviation between two results. The samples whose deviation were above the limit for 95% range control (7,8) were measured a third time.

Calculation

Cooking loss

To determine the cooking loss due heating, the weight of the samples were measured before and after each heat treatment. The cooking loss was calculated as follows:

$$\text{cooking loss in } \% = \frac{\text{weight of sample before cooking} - \text{weight of sample after cooking}}{\text{weight of sample before cooking}} * 100$$

Dry matter

The dry matter was determined by the vacuum method and was calculated as follows:

$$\%\ dry\ matter = \frac{(\text{weight of drying dish and sample after drying} - \text{weight of drying dish})}{(\text{weight of drying dish and sample before drying} - \text{weight of drying dish})} * 100$$

Vitamin amount

Every sample was at least determined twice with the HPLC. In case the deviation of two results was higher than the value from the calculated R-chart a third measurement was conducted. For further calculations the mean value of the vitamin amounts was used. The determined amount of thiamin was composed of the sum of TMP, HET and thiamin. The amount mg/100g of HET and TMP was adjusted by the analyze program Empower since the molecular weights differ from thiamin.

True retention

The true retention was ascertained by means of the vitamin amount of the raw sample, vitamin amount of the cooked sample and both weights before and after cooking.

$$true\ retention = \frac{\text{nutrient content of cooked meat} * \text{weight of cooked meat}}{\text{nutrient content of raw meat} * \text{weight of raw meat}} * 100$$

For the nutrient content of raw meat we used the mean vitamin amount of the raw samples.

We also calculated the retention including the released juice:

$$retention = \frac{(\text{nutrient content of juice} * \text{weight of juice}) + (\text{nutrient content of cooked meat} * \text{weight of cooked meat})}{\text{nutrient content of raw meat} * \text{weight of raw meat}} * 100$$

Statistical tests

To prove the statement of McIntire (1942) and Lassen (2002) that the vitamin amount within the loin differs, we run a two way Anova test in Microsoft Excel with the determined results of the raw samples. The statistic revealed no difference between the cuts of the loin. There was no difference in the vitamin amount within the loin however the pigs were different. Due to this data we calculated the retention with the mean value of the three raw cuts.

A two way-Anova test was conducted for the calculated vitamin retentions. The results are showing that there is still a significant difference between the four pigs since the F-value is higher than F_{crit}. The p-value was 0,01127. As assumed, there is a significant difference between the heat treatments concerning the retention. The F-value is much higher than the F_{crit} and the p-value is $7{,}5*10^{-18}$.

A one factor Anova was run with the Statistical Analysis System (SAS) program. The hypothesis that all retentions are equal was rejected and a least significant difference (LSD) test was realized. It showed a least significant difference of 3,4613 and ranked the retentions.

Results

Table3: Mean cooking loss, juice weight and dry matter of different treatments.

	80°C	53°C	53°C + storage	oven reheated	microwave reheated
mean cooking loss in %	31,6 ±1,6	10,9 ±1,2	12,1 ±1,8	22,6 ±2	25,7 ±1,1
mean juice weight in g	140,3 ±20	35,8 ±7,2	39,9 ±11,4	32,7 ±4,5	36,6 ±3,6
mean dry matter in %	33,6	27,3	27,4	37,1	37,4

The cooking loss is shown in Table 3. The cooking loss of the 80°C cooked meat is much higher than the 53°C. Due to the high temperature the meat released nearly four times more juice than the samples cooked at only 53°C. During the storage time of 21 days the meat was losing some juice too. The amount of released juice is important, because water soluble vitamins will leak into the juice.

The color of the juice was also different between the temperatures. Cooked at a high temperature the juice color had changed from red to beige. The red color generated by hemoglobin was preserved in the lower heating process (see appendix pictures).

The dry matter results also underline the measured cooking loss. Higher heating temperatures and heating processes increase the dry matter due to the loss of water.

The juice weight of the reheated meat couldn't be used for HPLC analysis, because during microwave and oven reheating the juice was evaporating. It was calculated as the weight deviation between the meat before and after reheating.

Table4: True vitamin retention (thiamin) of different treatments with (*) and without juice in %

pig nr	53°C	80°C	53°C+storage	53°C+CO	53°C+MO	53°C*	80°C*	53°C+storage*
1	91,8	60,4	88,9	88	70,3	103,3	95,9	101,4
2	87,7	59,0	87,3	86,2	73,4	96,5	96,4	96,8
3	90,4	61,4	92,4	84,6	70,4	101,2	94,7	102,3
4	91,5	57,9	87,2	83,9	67,5	99,3	92,2	94,6
mean value	90^{c}	60^{f}	89^{c}	86^{d}	70^{e}	100^{a}	95^{b}	99^{a}

N= 4. Where letters differ means differ significantly (P< 0,05) from each other.

Table 4 shows the true vitamin retention of thiamin in our meat samples. The marked (*) treatments are calculated additionally with the vitamin amount of the juice.

More than one third of the vitamins was lost during the 80°C treatment and therefore remained the lowest retention. The 53°C and the stored samples have high retentions and are not significantly different to each other.

With a significant difference to common oven, the microwave reheated samples retentions are very low.

The results of meat and juice combined cooked at 53°C show the highest retention with nearly 100%. The 80°C* is significantly different to the 53°C* and 53°C+storage*.

Discussion

The study of Brady *et al*. (1944) detected a slight but significant difference between parts of the loin. The difference of the vitamin amount within the raw loin was not validated by our data. Since there is no thiamin detection explained we don't know if he included HET as thiamin as well. HET is not present in huge amounts but the difference between the two determined parts in his publication was also not very large.

The determined riboflavin results couldn't be used based on not comprehensible data and missing quality control for these measurements, but since riboflavin isn't affected by heat like thiamin there is no riboflavin destroyed due to processing.

Effect cooking temperature on vitamin retention

As shown in Table 4 the vitamin retention of thiamin differs between the treatments. The lowest retention by far has the 80°C treatment with a mean value of 60%. More than one third of the vitamins are no longer in the meat. Compared to the mild heat treatment at 53°C, where most of the vitamins are still in the meat, the 80°C has lost four times more vitamins. The weight of the released juice is also four times higher in these samples (see Table 3). These retentions are comparable to the data of Lassen *et al.* (2002) where pork meat was cooked sous-vide to 72°C and 93°C in a former study and retentions of 72% and 27% respectively were determined. Comparison of the temperature and the resulting retention shows a correlation between internal temperature and vitamin loss, but due to the small amount of data it's difficult to assess a linear or exponential correlation.

The high retentions of the vitamin amount of meat and juice combined heated to 53°C demonstrate that there is no difference to the raw meat so there are no vitamins destroyed during processing. They just leaked into the juice. This was also reported by Lassen *et al.* (2002). That's why it is important to reduce the cooking loss and try to minimize the water loss. Cooking at high temperatures also increases the cooking loss and water soluble vitamins are leaving with the juice.

Effect reheating methods on vitamin retention

The microwave reheating did poorly compared to the oven heating. The vitamin retention of the microwave heated meat is nearly as low as the 80°C cooked samples. Many other studies about microwave reheating even showed that there is no difference of the retention between conventional reheating and microwave reheating (Hoffman and Zabik 1992, Kylen *et al*. 1964, Payton and Baldwin 1985). Since both sample groups were heated to the same core temperature and had the same cooking loss, the difference in the retention has to be caused by the heating method. Former microwave studies reported that heating with a low energy level lowers also the vitamin retention (Baldwin *et al*. 1976). This could be the reason for the lower retention since we used 800W. Retentions above 85% were presented for microwave heating with an even higher core

temperature in Uherová *et al.* (1992). The retentions of the oven reheated samples reveal that the heating process was gentle to the vitamins.

Effect storage time on vitamin retention

During storage of 21 days at 5°C no significant changes of the retention occurred. Between the stored and the non stored samples was no significantly difference in the retention. Both treatments show a very high retention since they lost only 10% water during heating. Lassen *et al.* (2002) also determined a not significant decrease of the vitamin retention by 4% during 14 days in sous-vide cooked meat. The stored meat lost a little bit more juice compared to the non stored samples, but without affecting the vitamin retention.

Are the vitamins destroyed or just released into the juice

Calculations of vitamin retentions with meat and juice present that there is no difference to the initial vitamin amount at mild temperatures. Since there is no difference in raw meat and the whole cooked meat vitamins weren't destroyed during heat treatment at 53°C. They are leaking out with the juice.

During the 80°C cooking process, it could be that a small amount of vitamins had a thermal breakdown since there is a significantly difference between 80°C* and 53°C*/53°C+storage*.

Outlook on further studies

The determined microwave retentions and the conclusions of former studies about microwave processing are inconsistent. In our study there is a difference between the two reheating methods. To validate our results a new project about reheating methods should be designed. It should consider the different power levels of the microwave, core temperature and different meat products.

Also a study to assess the linearity or exponential correlation between vitamin retention and cooking temperature could be designed.

Conclusion

The vitamin content of thiamin and riboflavin in pork loin muscles (Longissimus dorsi) was determined before and after cooking. The vitamin amount was determined in 53°C and 80°C cooked meat samples, 21 days stored samples and microwave or oven reheated samples. In addition the released juice was determined too. The vitamin retention of the 80°C cooked meat was only 60% after processing. Meat heated to 53°C still remains with 90% of its initial vitamin content. Also during storage of 21 days the meat still contained 89% of its pre-cooked vitamin amount. Oven and microwave reheated samples had 86% and 70% retention, respectively. No vitamins were destroyed cooked at 53°C since the combination of juice and meat retention is equal to the initial vitamin content of the raw meat.

The LTLT-method is in reference to the vitamin retention a good choice for catering services. Not only increases the sous-vide method the sensorial properties of the meat like juiciness and tenderness but also are vitamins treated gentle during processing at low temperatures. Even after reheating an already LTLT-cooked sample the vitamin retention is still higher than cooked at 80°C. In addition during storage there are no changes occurring so a long shelf-life of the vitamins is granted. Furthermore the released juice should always be processed too i.e. to a sauce since the juice contains the "lost" water soluble vitamins.

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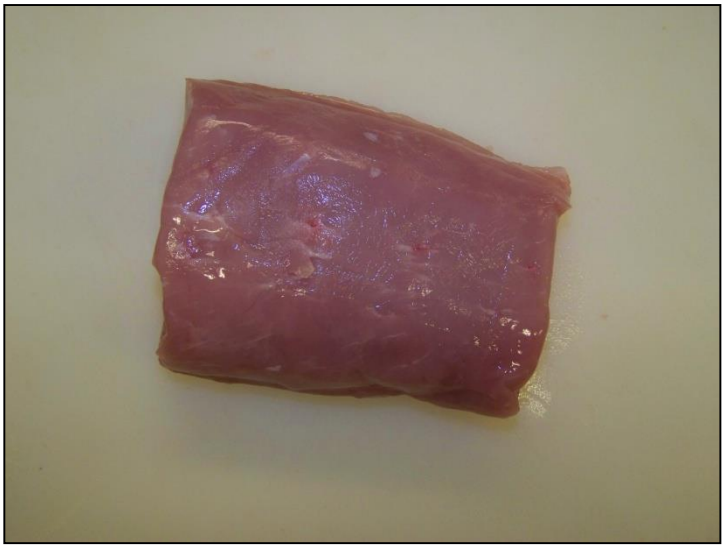
Appendix

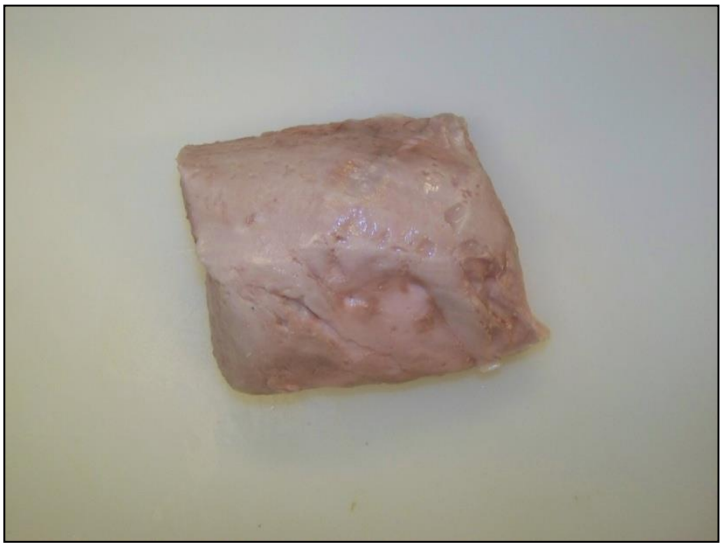


Picture of raw meat sample

A picture of raw muscle meat (longissimus dorsi). It still has its natural color given by the red hemoglobin.

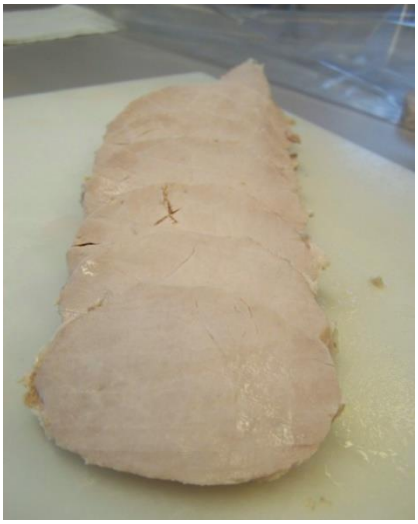
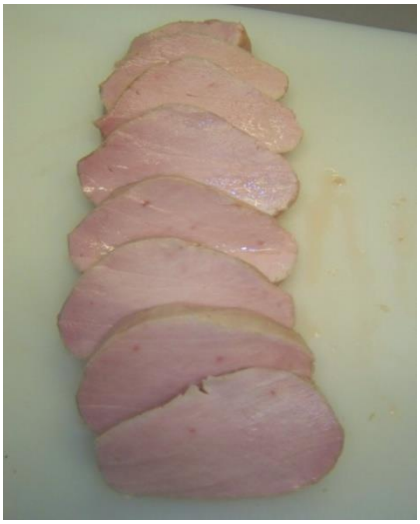
Picture of 80°C cooked sample

One of the 80°C cooked samples. The color changed from light red to light grey.



Picture of 53°C cooked sample

The 53°C cooked samples changed their color too, but still contained some hemoglobin to appear light pink.

raw	80°C	53°C
		
Raw meat sliced	80°C meat sliced	53°C meat sliced
		
Raw meat prepared for homogenization	80°C meat prepared for homogenization	53°C meat prepared for homogenization
		
Raw meat homogenized	80°C meat homogenized	53°C meat homogenized

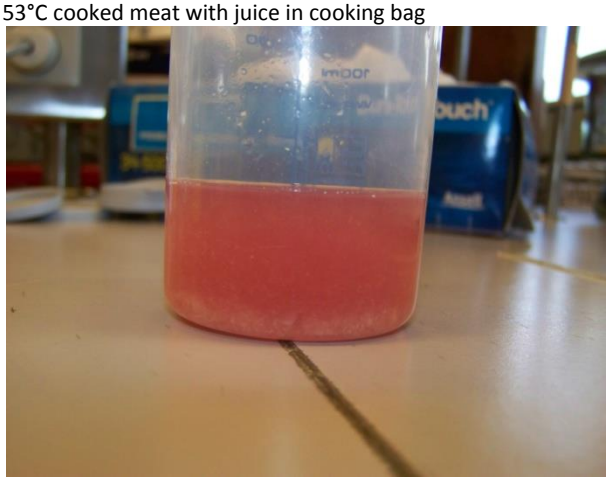
Pictures of the different stages of homogenization. The columns show the meat cut into slices prepared for homogenization. As you can see between the three samples are differences in color and water contend. The raw sample on the left had the highest water contend clearly visible by the shimmering surface. Also the homogenized meat agglomerated to a big cluster.

The middle column presents the 80°C cooked sample. Its appearance is like normal cooked meat: grey and dry. It's forming flakes by homogenization due to high dry matter contend.

The color of the 53°C heated samples indicates that there was still hemoglobin in the meat. During heating the meat just lose 10% of its weight, so its water content was still high. The surface was shimmering and the homogenate also agglomerated. It seemed like a mix of raw and 80°C cooked meat.

53°C cooked meat with juice in cooking bag

80°C cooked meat with juice in cooking bag



Released juice of 53°C cooked meat

Released juice of 80°C cooked meat

The 53°C juice is still red like the droppings of raw meat containing apparently hemoglobin. Obviously the mild heat treatment didn't affect hemoglobin intense in its molecular structure.

Compared to the 53°C the juice of the 80°C has changed its color. The hemoglobin in the blood was destroyed and heme was released. The heme was formed to biliverdin via heme oxidase and subsequently transformed to bilirubin (Kapitulnik 2004). The bilirubin has an orange color that's why the juice appears beige.

Measuring the pH in the loins

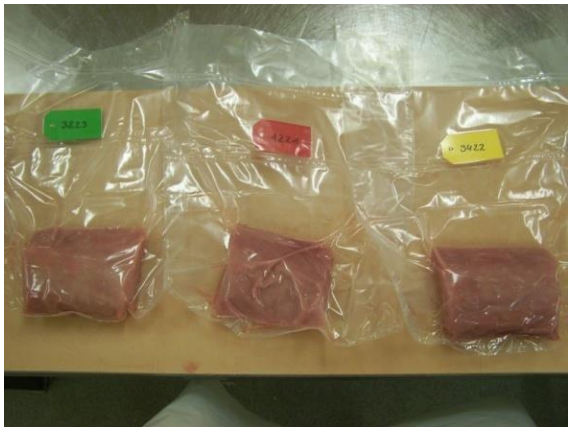
The microwave where the meat was reheated

The oven where the meat was reheated

The blender which homogenized the samples

Loin divided into three cuts; red (front), yellow (middle), green (rear)


Generating vacuum in the cooking bags


Meat samples vacuumized ready for heat treatment

Example: $$true\ retention\ in\ \% = \frac{1{,}376\frac{\mathrm{mg}}{100\mathrm{g}} * 373{,}39\mathrm{g}}{1{,}263\frac{\mathrm{mg}}{100\mathrm{g}} * 425\mathrm{g}} * 100 = 95{,}7\%$$

Example: $$dry\ matter\ in\ \% = \frac{(103{,}9859\mathrm{g} - 102{,}1254\mathrm{g})}{(107{,}2619\mathrm{g} - 102{,}1254\mathrm{g})} * 100 = 36{,}2\%$$

Example: $$retention\ in\ \% = \frac{\left(1{,}644\frac{\mathrm{mg}}{100\mathrm{g}} * 39{,}32\mathrm{g}\right) + (1{,}376\frac{\mathrm{mg}}{100\mathrm{g}} * 373{,}39\mathrm{g})}{1{,}263\frac{\mathrm{mg}}{100\mathrm{g}} * 425\mathrm{g}} * 100 = 107{,}7\%$$

Example: $$cooking\ loss\ in\ \% = \frac{517{,}7\mathrm{g} - 385{,}81\mathrm{g}}{517{,}7\mathrm{g}} * 100 = 32\%$$

Table5: Weight of the samples before and after heat treatment, calculated cooking loss and weight of the juice

label number	weight in g before	weight in g after	weight in g before- after	cooking loss in %	weight in g juice
1111	384,4				
1112	489,9				
1113	562,4				
1221	425	373,39	51,61	12,1	39,32
1322	517,7	351,85	165,85	32,0	149,37
1423	537,2	457,9	79,3	14,8	56,48
14231	167,6	133,3	34,3	20,5	34,3
14232	154,4	113,6	40,8	26,4	40,8
14233	127,3				
2111	458,3				
2112	488,5				
2113	582,4				
2222	521,9	468,79	53,11	10,2	37,32
2323	537	359,27	177,73	33,1	163,99
2421	460,8	408,07	52,73	11,4	33,72
24211	129,1	96,5	32,6	25,3	32,60
24212	148,8	110,5	38,3	25,7	38,30
24213	121,6				
3111	433,5				
3112	481,5				
3113	505,9				
3223	470,6	416,5	54,1	11,5	41,28
3321	446,3	314,93	131,37	29,4	121,35
3422	473,7	419,36	54,34	11,5	38,09
34221	122	95,4	26,6	21,8	26,60
34222	139,8	106,1	33,7	24,1	33,70
34223	152,3				
4111	389,7				
4112	441,1				
4113	494,8				
4221	395,2	357,2	38	9,6	25,29
4322	440,7	299,99	140,71	31,9	126,33
4423	485,1	433,33	51,77	10,7	31,38
44231	164	126,8	37,2	22,7	37,20
44232	126,3	92,9	33,4	26,4	33,40
44233	135,2				

Table6: Datasheet from the pigs at the slaughterhouse. The pH and pig sample number were listed in person aditionally .

pig number	Time of classification	Supplier number	Id number	Lean Meat Percentage	Sex	Slaughter Weight in kg	pH left	pH right	pig sample nr
1	063430	0025068	076384	59,3	0	78,5	5,43	5,43	
2	063714	0025068	071961	60,5	0	79,0	5,63	5,69	
3	063734	0025068	084775	60,4	0	78,9	5,92	5,95	
4	063609	0067866	085952	60,1	0	79,4			
5	064237	0025068	061957	59,3	0	78,4	5,36	5,36	
6	064405	0087212	026887	60,0	0	77,8	5,50	5,50	1
7	064839	0040083	048284	60,5	0	78,5	5,53	5,52	
8	065010	0040083	062156	60,7	0	79,3			
9	065400	0014846	062081	60,7	0	79,4	5,60	5,56	
10	065303	0025068	076570	60,0	0	79,5	5,48	5,48	
11	065324	0025068	088224	59,4	0	79,7			
12	070132	0043690	054156	59,3	0	78,8	5,50	5,50	
13	070415	0040083	078482	60,5	0	79,4	5,56	5,54	
14	070501	0082102	026617	60,6	0	79,4			
15	070404	0059120	026827	59,6	0	79,2	5,70	5,75	
16	070751	0087212	084184	60,1	0	79,1	5,40	5,40	2
17	070805	0087212	065121	60,1	0	79,4	5,36	5,36	3
18	070816	0087212	053145	60,8	0	78,2	5,39	5,41	4
19	071104	0059120	057959	59,9	0	78,8	5,38	5,38	
20	071500	0082102	087338	60,7	0	79,3	5,39	5,41	5

This datasheet is a personel adapted form send us by the slaughterhouse in Ringsted. The 0 equates with the female gender in the sex column. The pH and the chosen pigs were added to complete the data of the pigs. The fifth loin was used as temperature dummy during cooking.

Table7: Register of sample numbers and its fragmentation

label number	pig nr	treatment	site	cut
1111	1	1 (raw)	1 (left)	1 (F)
1112	1	1 (raw)	1 (left)	2 (M)
1113	1	1 (raw)	1 (left)	3 (R)
1221	1	2 (53°C)	2 (right)	1 (F)
1322	1	3 (80°C)	2 (right)	2 (M)
1423	1	4 (53°C reheat)	2 (right)	3 (R)
14231	1	4 (53°C reheat oven)	2 (right)	3 (R)
14232	1	4 (53°C reheat mw)	2 (right)	3 (R)
14233	1	4 (53°C no reheat)	2 (right)	3 (R)
2111	2	1 (raw)	1 (left)	1 (F)
2112	2	1 (raw)	1 (left)	2 (M)
2113	2	1 (raw)	1 (left)	3 (R)
2222	2	2 (53°C)	2 (right)	2 (M)
2323	2	3 (80°C)	2 (right)	3 (R)
2421	2	4 (53°C reheat)	2 (right)	1 (F)
24211	2	4 (53°C reheat oven)	2 (right)	1 (F)
24212	2	4 (53°C reheat mw)	2 (right)	1 (F)
24213	2	4 (53°C no reheat)	2 (right)	1 (F)
3111	3	1 (raw)	1 (left)	1 (F)
3112	3	1 (raw)	1 (left)	2 (M)
3113	3	1 (raw)	1 (left)	3 (R)
3223	3	2 (53°C)	2 (right)	3 (R)
3321	3	3 (80°C)	2 (right)	1 (F)
3422	3	4 (53°C reheat)	2 (right)	2 (M)
34221	3	4 (53°C reheat oven)	2 (right)	2 (M)
34222	3	4 (53°C reheat mw)	2 (right)	2 (M)
34223	3	4 (53°C no reheat)	2 (right)	2 (M)
4111	4	1 (raw)	1 (left)	1 (F)
4112	4	1 (raw)	1 (left)	2 (M)
4113	4	1 (raw)	1 (left)	3 (R)
4221	4	2 (53°C)	2 (right)	1 (F)
4322	4	3 (80°C)	2 (right)	2 (M)
4423	4	4 (53°C reheat)	2 (right)	3 (R)
44231	4	4 (53°C reheat oven)	2 (right)	3 (R)
44232	4	4 (53°C reheat mw)	2 (right)	3 (R)
44233	4	4 (53°C no reheat)	2 (right)	3 (R)

Table8: HPLC results of thiamin analysis

Date	Sample nr.	TMP as thiamin mg/100g	Thiamin mg/100g	Het as thiamin mg/100g	TMP, thiamin,HET mg/100g
171110	1111	0,8662	0,5105	0,0203	1,397
181110	1111	0,6635	0,5715	0,0425	1,2775
291110	1111	0,5801	0,5984	0,0491	1,2276
231110	1112	0,932	0,3941	0,0199	1,346
241110	1112	0,773	0,4812	0,0462	1,3004
241110	1113	0,762	0,4794	0,0513	1,2927
301110	1113	0,7942	0,5323	0,0529	1,3794
191110	2111	0,8232	0,4757	0,002	1,3009
221110	2111	0,4867	0,6277	0,0752	1,1896
291110	2111	0,5345	0,5934	0,0583	1,1862
151110	2112	0,8962	0,4146	0,0094	1,3202
291110	2112	0,6051	0,6093	0,0538	1,2682
171110	2113	0,6707	0,5458	0,0547	0,8075
301110	2113	0,7065	0,5513	0,0525	1,2712
251110	3111	1,1209	0,4554	0,024	1,3103
261110	3111	0,9854	0,5766	0,004	1,6003
171110	3112	0,9089	0,5564	0,0681	1,566
191110	3112	0,6191	0,9801	0,1512	1,0158
301110	3112	0,7672	0,5096	0,0571	1,5334
251110	3113	1,0858	0,4216	0,0285	1,7504
261110	3113	1,0085	0,5298	0,0653	1,3339
251110	4111	0,9892	0,4618	0,0213	1,5934
261110	4111	0,8316	0,5934	0,0592	1,5359
191110	4112	0,9984	0,4249	0,0227	1,6036
221110	4112	0,5948	0,582	0,0892	1,4723
301110	4112	0,7534	0,5954	0,0673	1,4842
251110	4113	0,9367	0,3988	0,0212	1,3567
261110	4113	0,8439	0,4993	0,0559	1,3991
231110	1221	0,9575	0,3849	0,0032	1,3456
241110	1221	1,0238	0,3853	0,0024	1,4115
171110	2222	0,9342	0,3255	0,0031	1,2628
181110	2222	0,851	0,2781	0	1,1291
161110	3223	1,1867	0,4014	0,0035	1,5916
171110	3223	1,2175	0,384	0,0035	1,605
151110	4221	1,0571	0,3672	0	1,4243
161110	4221	1,037	0,3921	0,0023	1,4314
291110	1221 Juice	1,2653	0,3458	0	1,6111
301110	1221 Juice	1,325	0,3914	0	1,7164
251110	2222 juice	1,2058	0,325	0	1,5308
261110	2222 Juice	1,2563	0,3364	0	1,5927

Date	Sample nr.	TMP as thiamin mg/100g	Thiamin mg/100g	Het as thiamin mg/100g	TMP, thiamin, HET mg/100g
191110	3223 Juice	1,5105	0,4349	0,0052	1,9506
221110	3223 Juice	1,4058	0,4594	0,0057	1,8709
191110	4221 Juice	1,2635	0,4262	0,005	1,6947
221110	4221 Juice	1,2682	0,4693	0,0062	1,7437
191110	1322	0,764	0,4284	0	1,1924
221110	1322	0,7096	0,4451	0	1,1547
161110	2323	0,6685	0,4537	0	1,1222
171110	2323	0,678	0,4402	0	1,1182
151110	3321	0,9151	0,4464	0	1,3615
161110	3321	0,8842	0,4719	0,0026	1,3587
171110	4322	0,8281	0,3817	0	1,2098
181110	4322	0,8291	0,3609	0	1,19
191110	1322 Juice	1,0897	0,5488	0,0018	1,6403
221110	1322 Juice	1,039	0,5625	0	1,6015
251110	2323 Juice	1,0201	0,5435	0	1,5636
261110	2323 Juice	1,0103	0,5345	0	1,5448
241110	3321 Juice	1,3834	0,5566	0	1,94
231110	3321 Juice	1,329	0,5618	0,0048	1,8956
251110	4322 Juice	1,2148	0,4585	0	1,6733
261110	4322 Juice	1,2387	0,46	0	1,6987
171110	14231	1,0297	0,4356	0,0036	1,4689
181110	14231	1,0352	0,4184	0	1,4536
151110	14232	0,9077	0,3545	0	1,2622
161110	14232	0,8837	0,3773	0	1,261
251110	14233	1,0163	0,3492	0,0034	1,3689
261110	14233	1,0358	0,3502	0	1,386
291110	24211	1,1133	0,3482	0	1,4615
301110	24211	1,1115	0,3571	0	1,4686
151110	24212	0,8927	0,36	0	1,2527
161110	24212	0,8724	0,3816	0,0033	1,2573
191110	24213	0,9029	0,3685	0,0028	1,2742
221110	24213	0,8426	0,3852	0,0031	1,2309
231110	34221	1,2928	0,3857	0,0043	1,6828
241110	34221	1,3039	0,3919	0,0042	1,7
231110	34222	1,0826	0,3388	0,0045	1,4259
241110	34222	1,1117	0,3587	0,0039	1,4743
291110	34223	1,3099	0,3355	0	1,6454
301110	34223	1,2756	0,3423	0	1,6179
231110	44231	1,1144	0,4035	0,0028	1,5207
241110	44231	1,1442	0,3937	0,0035	1,5414
161110	44232	0,9107	0,3879	0,0027	1,3013

Date	Sample nr.	TMP as thiamin mg/100g	Thiamin mg/100g	Het as thiamin mg/100g	TMP, thiamin, HET mg/100g
171110	44232	0,9156	0,3668	0,0036	1,286
251110	44233	1,0824	0,3159	0	1,3983
261110	44233	0,9867	0,2851	0	1,2718
301110	44233	1,1321	0,3311	0	1,4632
191110	1423 Juice	1,0923	0,4299	0,0041	1,5263
221110	1423 Juice	1,1204	0,4718	0	1,5922
291110	2421 Juice	1,2769	0,3468	0	1,6237
301110	2421 Juice	1,2691	0,401	0	1,6701
291110	3422 Juice	1,6045	0,3605	0	1,965
301110	3422 Juice	1,5036	0,3814	0	1,885
191110	4423 Juice	1,1687	0,403	0,0034	1,5751
221110	4423 Juice	1,1525	0,461	0	1,6135

Table9: The results of the two factor Anova test without repetition in Microsoft Excel concerning the thiamin retention

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Row	55,741	3	18,58033185	4,7316421	0,01127	3,07247
Column	5607,252	7	801,0360313	203,99075	7,5E-18	2,48758
Error	82,46333	21	3,92682527			
Total	5745,457	31				

$F>F_{crit}$ = difference between these results, $F<F_{crit}$ = no difference between these results

Row = pigs, Column = treatments

Table10: Two way ANOVA results of the vitamin amount of raw meet concerning the different cuts

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Row	0,149928	3	0,049976	27,08137	0,000693	4,757063
Column	0,000412	2	0,000206	0,111663	0,896161	5,143253
Error	0,011072	6	0,001845			
Total	0,161413	11				

$F>F_{crit}$ = difference between these results, $F<F_{crit}$ = no difference between these results

Row = pigs, Column = different cuts (front, middle, rear)

Table11: Results of the one way Anova test in SAS

Means with the same letter are not significantly different.					
t	Grouping	Mean	N	proc	treatment
A		99,50	4	6	53°C*
A		98,00	4	8	53°C+storage*
B		94,25	4	7	80°C*
C		89,75	4	1	53°C
C		88,50	4	3	53°C+ storage
D		85,00	4	4	53°C+CO
E		69,75	4	5	53°C+MO
F		59,25	4	2	80°C

chemicals:

takadiastase: Pfalz& Bauer T00040, EC 3.2.1.1

takadiastase solution 20mg/ml: 2,0g dissolved in 100ml deionized water

hydrochlorid acid 2M: Bie& Berntsen A/S , 1l

hydrochlorid acid 0,1M: 50ml 2M HCl and ad 1l deionized water

hydrochlorid acid 0,01M: 5ml 2M HCl and ad 1l deionized water

sodium acetate pellets: Merck 1.06268.1000

sodium acetate 4M: 328g sodium acetate pellets dissolved in 1000ml deionized water

deionized water: conditioned by Milli-Q Intergral Water Purification System from Millipore

Methanol: Rathburn

Tetraethylammonium chloride: Merck 8.22148.0100

Sodium 1-heptanesulfonate monohydrate: Fluka EC: 2453105

Potassium dihydrogen phosphate: Merck 1.04873.1000

Phosphorous acid 85%: Merck 1.00573

Potassium hexacyanoferrate (III): Merck

Potassium hydroxide (pellets): Merck 1.05033.1000

Potassium hydroxide solution 3,5M: 196,4g potassium hydroxide pellets dissolved in 1l deionized water

Standards

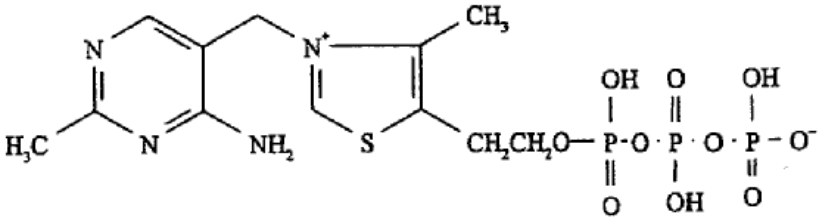
Thiaminchlorid, chloride, USP Rockville, nr. 65600, MD (M_W= 337,28)

Thiaminmonophosphatchlorid chloride (TMP), Sigma-Aldrich T-8637, MD (M_W= 416,82)

Thiamin pyrophosphatchlorid, thiamindiphosphatchlorid (TDP), ICN Biochemicals, cat. No. 194643, MD (M_W= 460,76)

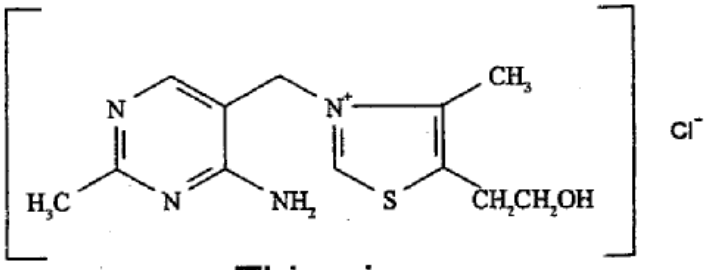
2-(1-Hydroxyethyl)thiamin (HET), Wako Chemicals GmbH, Nr. 085-07111, MD (M_W= 381,33)

B_1-VITAMIN.



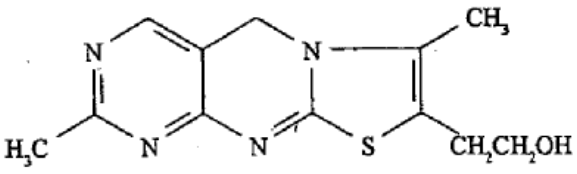
Thiamintriphosphat

ENZYMATISK HYDROLYSE



Thiamin

BASISK OXIDATION



Thiochrom