Monitoring the water distribution in meat upon freezing with X-ray computed tomography

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Abstract
The freezing speed influences the growth and spatial distribution of ice in frozen meat. The formation of ice crystals dehydrates the fibres and can damage the cell structure. This influences the drip loss after thawing and reduces tenderness, which is an important eating characteristic of red meat. A better understanding of ice formation and an efficient method for analysing ice distribution can lead to improved freezing processes in food production. This work demonstrates X-ray computed tomography as a novel method suitable for determining and visualising the ice volume fraction in frozen meat, which is important because ice formation has consequences for meat quality (drip loss on thawing, loss of juiciness and tenderness after cooking). The results obtained are in agreement with the phenomenological predictions of the literature and extend the known microscopy methods with a non-destructive and time efficient analysis technique that provides information about the amount of ice formed and its spatial distribution in frozen meat.

KEYWORDS: freezing behaviour meat; ice distribution; volumetric image analysis; ice volume fraction; high-resolution X-ray computed tomography (XCT)

1. Introduction
Freezing is an important means of preserving food. It is important to freeze foods such as meat at an optimal rate so that ice crystals of suitable size and distribution form to avoid excessive drip loss of water on thawing and thereby loss of tenderness and juiciness after cooking. Studies of the influence of freezing rate on the quality of meat are reported in [1]-[2].

The X-ray computed tomography (XCT) technology is already being used in the food sector, for example for the quality assessment of agricultural products [3]. Thus, if the
formation and distribution of ice can be used as a quality indicator, it seems obvious to consider whether XCT could also be a suitable method for the quantification of ice. The advances of X-ray instrumentation namely in the field of detector sensitivity enabled the resolution of increasingly smaller density differences. In recent studies, XCT has been employed to analyse melting and solidification processes of phase change materials [4] or to track the ice formation in pebbles [5]. The aim of this work is to evaluate XCT as a novel method suitable for monitoring ice crystal structure in frozen meat. To our knowledge, XCT is applied for the first time for the analysis of frozen meat.

2. Material and Methods

2.1 Sample material

Three hours after slaughter, when the carcass was cooled to 15°C, the top side of a bull was excised. A piece with a low fat content was preferred. Using a meat knife, 6 cube-shaped samples were cut by hand to fit into the cube-shaped cavity of the sample holders (cf. Sec 2.2). The temperature increase of the samples during preparation ranged from 0.8°C to 3.7°C.

The samples were frozen in a freezer (Bauknecht, KVC 2433 WS). The temperature changes were measured from the initial temperature of about 17°C down to -25°C. Sheathed Type K thermocouples (TC) with a sheath diameter of 0.5 mm were used to monitor the temperature during freezing. The positions of the thermocouples are shown in Figure 1b). The samples No. 1 and 4 had two thermocouples.

The absolute accuracy of the measuring section, consisting of the TC, the NI CompactDAQ and the LabView programme with a sampling rate of 1 Hz, was ±0.2°C. The mean freezing rate is calculated from the freezing point and the point at which the final temperature is reached for the first time.

In accordance with [2], the time difference between the start of freezing and the time when -7°C is reached is defined as a comparative value. Deviating from [2], the sample surface temperature is used instead of the temperature in the centre of the sample. Due to the low Biot number this is considered acceptable.

The remaining meat was used to determine the macronutrients. The material was first homogenised with a Büchi Mixer B-400 and then measured with the Büchi NIRMaster (FT-NIR spectroscopy). Table 1 shows the results of the triple determination. The protein content is the sum of the proteins from the muscle fibres (20.4%) and the connective tissue (0.68%), which were measured separately with the spectrometer.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mass fraction (%)</th>
<th>Std. dev. (%)</th>
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<tbody>
<tr>
<td>Water</td>
<td>76.1</td>
<td>0.39</td>
</tr>
<tr>
<td>Proteins</td>
<td>21.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Fat</td>
<td>2.3</td>
<td>0.45</td>
</tr>
<tr>
<td>Total</td>
<td>99.5</td>
<td>0.61</td>
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Table 1. Macronutrients of the beef sample

<table>
<thead>
<tr>
<th>Table 2. Labelling of the samples</th>
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<tr>
<td>Label</td>
</tr>
<tr>
<td>-------</td>
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<tr>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>A, B</td>
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Table 2. Labelling of the samples
2.2 Sample holders

To enable a controllable freezing of the meat samples, novel sample holders from extruded polystyrene panels (XPS 300 GE, Swisspor, Boswil, Switzerland) were made. XPS is a convenient material for making a cubical thermally insulating enclosure for the meat sample. To obtain two different freezing rates in the same freezer, the thickness of the walls of the holder was varied. The samples with a wall thickness of 1 cm are labelled A or B, those with 2 cm wall thickness are numbered from 1 to 4, see also Table 2. Figure 1a) displays a holder with a wall thickness of 1 cm. It consists of a base plate that was extended with a T-piece that enabled the thermocouples to be attached. The cube-shaped cavity with a volume of 8 cm³ was cut out of the intermediate layer. This layer was glued to the base plate with an adhesive suitable for XPS. The bottom thermocouple was inserted through a gap created by the cutting. This gap was sealed with black adhesive tape. Figure 1b) shows the attachment of the top thermocouple before the cover plate to close the cavity was attached and fixed with Velcro tape, as shown in Figure 1c).

XPS as insulation and the removable attachment of the cover plate have two advantages. The holder material can be easily cut to size for measurement using a hot wire saw and by removing the cover plate, a reference material can be measured at the same time. The use of both options can be seen in Figure 2.

Figure 1. Sample preparation. Positioning of the samples in the cavity of the holder. Attaching the thermocouples at the bottom and, in the case of two samples, also at the top. By closing the cavity with the cover plate and a Velcro fastener, the top thermocouple was pressed onto the sample.

2.2 XCT measurements and image processing

To assess the ice distribution, the meat samples were scanned in an industrial XCT device (Diondo D2, Hattingen, Germany) with spatial resolution of 10 µm and 60 keV acceleration voltage of the source. The source detector distance (FDD) and the source object distance (FOD) were chosen to be 386 mm and 25.8 mm, respectively. A total of 1000 angle positions were recorded equally spread over 360°.

During the measurement, the samples could not be cooled. With a long measurement time, the samples would thaw, and the resulting structural changes would in turn cause jitter effects in the images. An optimum had to be found between the measurement time and the resolution. This was achieved for the samples A (1 cm XPS) and 1 (2 cm XPS) with the parameters mentioned above and a measurement time of 34.5 minutes.
To distinguish ice from the residual tissue in the data analysis, a measurement was carried out to verify the ice phase. Pure ice was placed on the meat sample and scanned at the same time. This set-up is shown in Figure 2.

The recorded transmission images were reconstructed with X-Aid, from Mitos, Germany. The acquisition protocol was finetuned to enable optimum contrast between water and the meat matrix.

The data analysis was carried out in Volume Graphics Studio Max. After surface detection, the ice and fibre volumes were separated in a region of interest (ROI) with a porosity/inclusion analysis.

### 3. Results and discussion

The temperature changes on the sample surfaces are shown in Figure 3. The samples supercool until the onset of ice formation. Then the temperatures rise to the initial freezing temperature of the solution and continue to fall as the concentration of the dissolved substances in the not-yet-frozen water increases [2].

The time from the initial freezing point to -25°C is 4.75 h for the samples A and B with 1 cm thick XPS walls and 5.5 h for the samples 1 to 4 with 2 cm thick walls. This leads to average freezing rates of -0.16 K/min and -0.12 K/min. At the time of 5 h, when the final temperature is almost reached, the disadvantage of the on-off control of the freezer becomes visible. The oscillating temperature is the reason why the characteristic time is used as a comparative value.

The characteristic freezing times (timespan from -1°C to reach -7°C) for sample A and B are 139 min and 138 min. For the samples 1 to 4 the shortest characteristic times correspond to the temperature point on top of sample 1 and 4 with 157 min and the longest times correspond to the bottom temperatures with 178 min. The characteristic times for sample 2 and 3 are 167 min and 173 min. On average the characteristic times are 138.6 min for A and B and 168.5 min for the samples 1 to 4.

In meat freezing with these characteristic times, the ice crystals grow between the fibres (intercellular ice) [3].

![Figure 2. Ice reference. The sample holder was cut to an appropriate size.](image)

![Figure 3. Sample surface temperatures. Wall thickness of the XPS is 2 cm for 1 to 4 and 1 cm for A and B.](image)
The goal of different freezing rates and the formation of ice in the intercellular space could be achieved by the manufactured sample holders.

Figure 4 shows representative cross-sectional images for samples A and 1. The fibres are aligned in vertical direction. The separation of ice (light blue) and fibrous tissue (red) was performed with the porosity/inclusion analysis. One can see the influence of the freezing rate when comparing 4a) with 4b). For the cooling rate of -0.16 K/min the structures are finer than those obtained with -0.12 K/min. This observation agrees with the explanations in [2] and the observations in [3]. Even though the structures in 4a) and 4b) show similarities to the cell biological structure of unfrozen muscle tissue, the freezing pattern deviates from this due to shifts in the tissue and damage to structures. In addition, slight mechanical damage to the samples during preparation could occur.

The volume fraction of ice was determined for the ROI in Figure 4. The analysis resulted in mean values of 60.4±0.33 % (95%-CI) and 52.8±0.37 % (95%-CI) for the freezing rates -0.16 K/min and -0.12 K/min. These very first results suggest that the freezing rate, in addition to its effect on the size of the ice crystals, could also influence the volume fraction of the ice and thus also the osmotic flow that leads to the dehydration of the fibre.

![Figure 4. ROI cross sections. Cross sections of the cube with 4 mm edge length for evaluation of the ice volume fraction. In a) sample A with a freezing rate of -0.16 K/min is shown and in b) sample 1 with a freezing rate of -0.12 K/min. The top views (above pictures) show the cross-section through the fibres. The front views (lower pictures) show cut planes along the direction of the fibres.](image)

4. Conclusions

The present study confirms that XCT can provide useful information on the tissue structure and the quantity of ice formed in frozen meat as a function of freezing rate. The XCT measurement principle and post-processing algorithms enable new possibilities for the analysis of anisotropic tissue structure and ice distribution. Besides the obvious advantage of the XCT analysis that the structural changes due to freezing and temperature rates can be analysed non-destructively a further advantage is
that this method is time efficient for sample preparation and measurement. If the method is used for repetitive analysis in product development, the reconstruction and image processing algorithms can largely be automated. Thus, our study shows that further development of this method for frozen meat can help improve the quality of the freezing process for meat preservation, which in turn can lead to less food waste. Eventually, the measurement technique and method for image analysis can be directly transferred to other frozen food products.

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