From sub-zero to cryo-freezing: detectability of beef quality differences among different freezing temperatures

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I. INTRODUCTION

Food safety agencies in the European Union require previously frozen, unprocessed meat to be labeled as 'defrosted'. While different test methods for authentication of defrosted versus fresh meat have been suggested, no standard method for detection of freeze damage has been established yet [1]. A key challenge is that suitable test methods should reliably detect freeze–-related damage in meat over a wide range of temperatures that are currently used in the meat sector. This includes freezing at higher sub-zero temperatures (around –5°C) that do not fulfill EU regulations for the 'quick-frozen' label. In contrast, most studies on assessing improved freezing by novel technologies have typically focused on lower temperature ranges covered by the 'quick-frozen' label (–18°C and below). We could previously show that different spectroscopic methods only could resolve larger freezing temperature differences among ca. –25°C and cryo-freezing (below –196°C), while cryo-electron-microscopy also detected differences between –25°C and –35°C freezing. Using beef samples, we here assess the suitability of two additional test assays and ask if they allow detecting differences among four freezing temperatures between –4°C and –80°C. To this end we use an established enzyme assay that analyses the activity of a mitochondrial enzyme (HADH) that is released by freeze–related damage. In addition, we have adopted a protocol that allows preserving the 3-D matrix, including ice-crystal cavities, of frozen meat. Lastly, using the HADH-based enzyme assay, we also ask if higher temperature sub-zero freezing at –4°C can be distinguished from refrigerated only beef.

II. MATERIALS AND METHODS

We obtained beef (*Bos taurus*) samples 1d postmortem (semimembranosus, N=24 individuals). Five replicate samples (5x5x4cm^3) from each individual were subjected to the different treatments: 2°C (chilled only), –4°C, –14°C, –20°C, –80°C. Post-treatment samples were collected after 21d, including two days of defrosting for enzyme activity analyzes. Samples for 'frozen state' analyses were 'cryo-fixated' and stored at –80°C. Preserving the frozen state, i.e., cavities from ice crystallization, was done by adopting common freeze-dry protocols for microscopy. Briefly, cryo-stored samples were initially fixated with a 1% glutaraldehyde in acetone solution (also –80°C), then temperature equilibrated at (–20°C), and finally dried at room temperature using a drying chamber to evaporate acetone. With microscopy we observed that different freezing caused marked differences in cavity size, causing samples to appear darker respectively lighter. To assess such differences in lightness we used a Konica Minolta Chroma Meter CR-400 (Konica Minolta Sensing INC, Japan) to record the *L** parameter. For HADH testing, the drip loss was collected and diluted 1:5 in phosphate buffer (0.1M, pH6.0). The measurement of HADH enzyme activity followed Gottesmann et al. [2]. In brief, to one volume of diluted drip loss we added 2V EDTA solution (34,4mM), 2V NADH solution (7,5mM) and 22V of the phosphate buffer. Addition of 3V of acetoacetyl-CoA solution (5,9mM) started the reaction.

The reduction of NADH was measured at 340nm with a photospectrometer, and activity was calculated in U/ml using the molar extinction coefficient of NADH of 6.3 [l×mmol–1×cm–1]. Statistics were calculated using a one-way ANOVA for overall treatment effects and Tukey's test for pairwise testing.

III. RESULTS AND DISCUSSION

Both, L* values of the 'frozen-state' samples (Fig. 1A) and HADH activity (Fig. 1B) indicate marked effects of freezing temperatures, with –80°C showing significant differences compared to other temperatures. However, while L* values at –4°C were not distinguishable from –14°C and –20°C, the HADH assay showed such difference. The HADH assay also allowed a direct comparison with chilled controls (2°C). We found that the HADH assay could separate all lower temperature freeze treatments (–80/–20/–14°C), but not –4°C sub-zero freezing, from the chilled group (2°C, Fig. 1B)

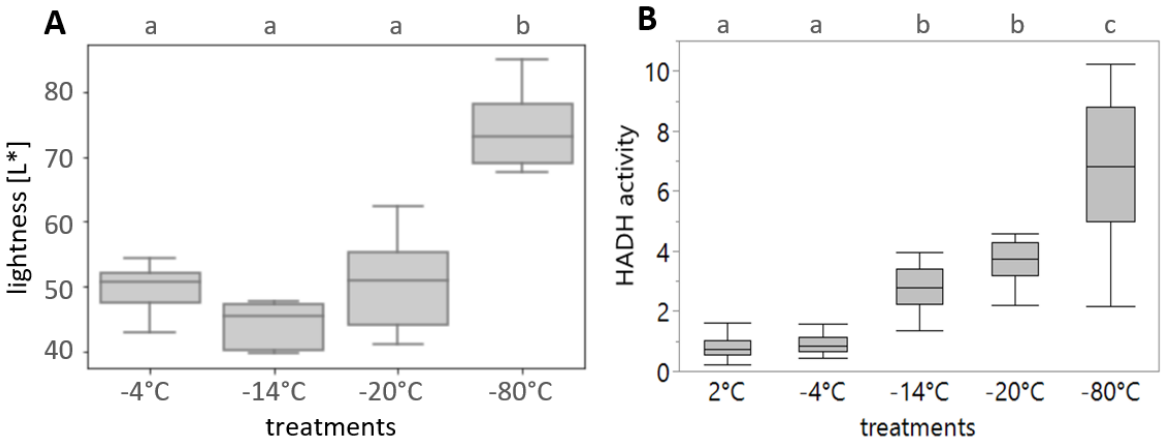

Fig. 1. Comparison of beef samples frozen at temperatures ranging from –4°C to –80°C. (A) Data for samples for which the frozen structure was preserved and assessed using the L* parameter. (B) HADH-activity for the same freeze treatments and for a refrigerated control. Letters indicate significant differences at P≤0.005 (Tukey's). Overall treatments were significant for both test assays (one–way ANOVA).

IV. CONCLUSION

Both methods allowed to separate very quick freezing at –80°C from freezing at higher temperatures. Similar to our previous study using bioimpedance–based testing [3], sub-zero frozen (–4°C) samples were not distinguishable from refrigerated only beef (2°C). This can have implications for understanding the extent of freeze damage at temperatures, where meat is only incompletely frozen.

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