The use of aconitase for the detection of frozen/thawed poultry meat

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Abstract

Ice crystals formed during the freezing process damage mitochondria in muscle tissues from which enzymes such as aconitase are released, and their activity may therefore serve as an indicator of previous meat freezing. Aconitase activity was investigated spectrophotometrically in the exudate released from meat samples during refrigerator and freezer storage. This method proved to be appropriate for the detection of previously frozen muscle tissue. An increase in aconitase activity also occurred during refrigerator storage, though to a lesser extent than during freezer storage.

Chicken meat, aconitase, fresh, thawed

Introduction

The freezing of meat causes the loss of its fresh appearance, drip loss, and other changes that have negative effects on the sensory characteristics of the meat; such changes are greater than in meat stored at refrigeration temperatures (Leygonie et al. 2012). Fast and reliable methods that would differentiate between fresh meat and frozen/thawed meat are being sought because customers are sometimes deceived, when buying frozen and thawed meat as fresh (meat). Ice crystals that are formed during the freezing process cause damage to intracellular organelles (mitochondria and lysosomes) and the release of enzymes these organelles contain, which can be detected by a suitable method (Hamm 1979). However, not all enzymes present in cells are suitable for the detection of freezing, as they may not be exclusively intracellular and, therefore, specific (Hamm 1979). Citrate synthase and aconitase are typical intracellular enzymes. They are released after freezing and satisfy the conditions for use as specific markers (Hamm and Gottesmann 1984). Other intracellular enzymes include β-hydroxyacyl-CoA-dehydrogenase (HADH) and N-acetyl-β-glucosaminidase (Ellebroek 1995). In an earlier study (Šimoniová et al. 2013), citrate synthase was used as a marker for the detection of freezing. The same experiments were performed in parallel with aconitase. Aconitase catalyses the reversible isomerisation of citric acid to isocitric acid via the intermediate *cis*-aconitate in the Krebs cycle: citrate → *cis*-aconitate → isocitrate.

The next reaction is the oxidative decarboxylation of the isocitric acid into ketoglutaric acid. In the course of this oxidation process, NADP is reduced to NADPH:
isocitrate + NADP → α-ketoglutarate NADPH + H^+.

The change in NADPH concentration is detected spectrophotometrically at 340 nm, and is directly proportional to aconitase activity. The advantage of this method is that aconitase is a specific enzyme (OxisResearch™ 2012).

The aim of the work was to find out whether the method of aconitase activity determination can be used for chicken meat, and whether it is possible to determine the extent of damage to meat muscle tissues caused by the freezing process and a period of storage. The results were obtained by measuring the absorbance of meat exudate with an admixture of specific reagents from the OxisResearch™ (BIOXYTECH®) commercial enzyme kit.

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Materials and Methods

Four different anatomical parts of chicken carcasses were used to measure aconitase activity, i.e. the right and left breast and the right and left thigh, from which the released exudate was collected. Chicken carcasses were cut and the thighs and breasts wrapped in LDPE foil and stored in a refrigerator and freezer to determine the effects of different storage conditions. The samples were divided in two halves; one half was stored at 4 °C and the other at -22 °C.

The samples stored at a freezer temperature were transferred to a refrigeration temperature environment about 24 hours before the analysis, so that the thawing operation proceeded slowly, similarly to freezing. Aconitase activity in these samples was measured during the following 45 days. Aconitase activity in refrigerated samples was monitored for 10 days. There was no reason to monitor meat freshness after this period, since the samples had already begun to decay.

Aconitase activity was measured by the Spekol® 1300 spectrophotometer (Analytik Jena AG, Germany) on the basis of absorbance changes induced by the production of NADPH, which is detected at the wavelength of 340 nm. NADPH is produced during a reaction in which the isocitric acid is oxidised by decarboxylation and α-ketoglutaric acid is formed. This reaction is preceded by the reversible isomerisation of citric acid via *cis*-aconitic acid as an intermediate.

Samples were prepared as follows: the exudate released from meat was diluted with demineralised water and filtered. At this stage, the samples were pipetted to a cuvette and NADP, substrate and isocitrate dehydrogenase were added. The mix was stirred carefully to prevent the formation of air bubbles that might interfere with the measurement. The samples were left to incubate at 37 °C, after which measurements that lasted for 5 minutes were performed. The absorbance monitoring interval was 20 seconds. Each sample was measured 3 times.

Aconitase activity was calculated using the following formula: $mU = ((A_{340}/min) / (\varepsilon.c))\ d$ [mU/ml]; ε…molar absorption coefficient (6,220 $M^{-1}.cm^{-1}$), c…temperature correlation coefficient (2.4435), d…dilution factor (1:9)

Method verification

The experiments were divided into three sets. The first set of measurements was designed to verify the used method to determine whether it is possible to detect aconitase activity in chicken meat and, if it can be detected, how it is affected by the way and length of storage. Aconitase activity in refrigerated samples was measured on days 2 and 7 of storage. Aconitase activity in freezer-stored samples was measured not only on days 2 and 7, but also on day 12 of storage.

Second experiment

The results of the experiment, in which the method of aconitase activity determination was verified, showed that the exudate produced is turbid in some cases and that this turbidity might affect absorbance values. For this reason, the second experiment included exudate filtering using a syringe filter (CHS FilterPure Nylon Syringe Filter, 0.45 µm, 25 mm). The quantity of released exudate was also monitored in this experiment because its quantity differed and it was later used in calculations. Aconitase activity in refrigerated samples was measured on storage days 2, 3, 5, 6, 7, 8, 9, 10 and 13. No measurements were performed on storage days 0 and 1, because there was no exudate released from the samples. Measurements in freezer-stored samples were performed on storage days 3, 17 and 45.

Third experiment

The third experiment was based on the knowledge and findings from the previous experiments and was therefore a review test of the two previous experiments.

Results and Discussion

Alizadeh et al. (2007) pointed out that specific enzymes should only be present in exudates of thawed meat, and should therefore not be present in fresh meat. The results of several other studies have, however, failed to confirm this assumption (Hamm and Gottesmann 1984; Guillaume et al. 2002; Šimoniová et al. 2013). In view of these findings, our objective was to differentiate fresh meat from thawed meat by means of changes in absorbance values of aconitase activity.

The first verification experiment

Aconitase activity values increased as the storage time progressed. Aconitase activity was also detected in fresh meat, nevertheless, the same phenomenon also occurred in a

previous study (Šimoniová et al. 2013) in which citrate synthase was used as a marker. Enzyme activity increased in time under both storage conditions (Table 1). The reason for this, in the case of frozen meat, may be the change in the size of the ice crystals caused by recrystallisation. An increase in aconitase activity in refrigerated meat may be explained by continuing post-mortal changes and a higher number of microorganisms possessing their own aconitase and therefore capable of influencing the results. Chicken breasts released more exudate than thighs, when stored under the same conditions (refrigerated and freezer storage). In contrast to the chicken breasts, the thighs were stored on the bone, which reduced the area measured, and in their skin, which could have absorbed some of the released exudate. This fact was therefore taken into account in the next experiments and the enzyme activity measured was adjusted to the amount of exudate released. Moreover, the exudate appeared turbid, which may have been caused by released proteins; subsequent experiments therefore included exudate filtering in which a syringe filter was used.

Table 1. Relationship between aconitase activity and the time and type of storage

Storage time [days]	U [mU.ml^{-1}] refrigerated	frozen
2	6.198 ± 1.198	10.931 ± 0.462
7	23.508 ± 1.827	19.570 ± 1.300
12	-	20.081 ± 1.274

The second experiment

After the exudate was filtered and converted to the amount of released exudate, the absorbance values showed a smaller measurement deviation, though the differences between fresh and thawed samples were more significant. The measured values differed from the first experiment, though the trend towards an increase in activity was similar. The increase in aconitase activity in freezer-stored samples was much more pronounced than that in refrigerator-stored samples (Fig. 1). More exudate was released from chicken

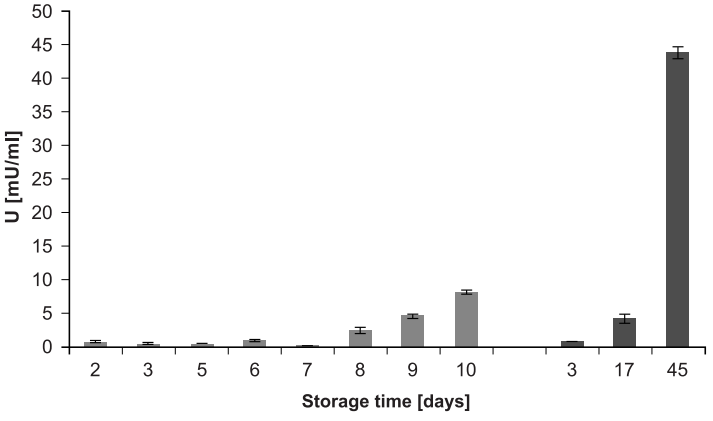

Fig. 1. Relationship between aconitase activity and the time and type of storage (average values). Grey – refrigerated storage, black– freezer storage

breasts than from thighs (adjusted for sample weights) under both ways of storage conditions (Table 2).

Table 2. The relationship between the quantity of exudate released and the time and way of storage

Storage time [days]	breasts m [%]	thighs m [%]
2	0.650	0.726
3	0.943	0.723
5	2.445	2.185
6	1.787	1.127
7	1.043	1.161
8	1.067	1.010
9	1.834	1.488
10	2.641	2.807
13	1.747	1.705
3	2.053	1.354
17	1.131	0.322
45	3.834	2.631

The third experiment

As in the previous experiments, aconitase activity increased under both ways of storage conditions, with the given trends being more clearly after the adjustment for the relevant exudate volume.There was no significant difference between chicken breasts and thighs at the beginning of refrigerated storage. The turning point came after the 10^{th} storage day, when aconitase activity in the chicken breasts was almost three times higher than in the thighs. Aconitase activity in thighs and breasts was 4.411 ± 0.372 mU.ml^{-1} and 11.644 ± 0.256 mU.ml^{-1}, respectively. Such an increase after a long period of refrigerated storage can be explained by the release of mitochondrial enzymes as a result of major post-mortal changes.

Table 3. Relationship between aconitase activity and the time and way of storage in different anatomical parts

Type of storage	Storage time [days]	Anatomical part	
		Thighs U [mU.ml^{-1}]	Breasts U [mU.ml^{-1}]
Refrigeration	2	0.442 ± 0.215	0.958 ± 0.215
	3	0.792 ± 0.248	-
	5	0.761 ± 0.000	-
	6	-	1.722 ± 0.248
	7	-	0.189 ± 0.124
	8	2.949 ± 0.328	1.763 ± 0.700
	9	4.575 ± 0.263	4.374 ± 0.452
	10	4.411 ± 0.372	11.644 ± 0.256
Freezing	3	0.330 ± 0.000	1.080 ± 0.256
	17	1.034 ± 0.200	7.166 ± 1.063
	45	8.948 ± 0.396	78.636 ± 1.352

However, a difference between anatomical parts was found at the very beginning of freezer storage, with higher values again found in chicken breasts. These values were not, however, remarkably different from the aconitase activity values determined on the same day in a refrigerated sample. Nevertheless, a significant difference was observed after the 17th storage day, and the increase of the enzyme activity continued (Table 3).

Conclusions

Aconitase activity was measured in exudate spontaneously released from whole meat samples. Aconitase activity increased during both refrigerator and freezer storage, although its initial values were similar under both storage conditions. Enzyme activity values in the chicken breasts were higher and their increase was more pronounced than in thighs. Aconitase proved to be a suitable enzyme for the detection of frozen/thawed meat. Its activity progressed similarly to that of citrate synthase, though a comparison of the two methods showed that aconitase is more sensitive of these two enzymes. The question remains as to whether the released mitochondrial enzymes could be collected directly from muscle tissue. This will be the subject of further experimental investigations.

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