The Effect of Superchilling and Rapid Freezing on the HADH Assay for Chicken and Turkey

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Summary

The EC Poultrymeat Marketing Standards Regulation (1906/90)[1], which is now incorporated into Council Regulation 1234/2007[2], requires that poultrymeat (whole birds and portions) is only marketed in certain conditions – 'fresh', 'frozen' or 'quick frozen' and defines the temperature conditions for each category. Newer refrigeration technologies are however, now available which may not comply with the specified conditions and could result in mis-labelling of poultrymeat. A method which measures the activity of an enzyme (HADH) in meat and poultry has previously been used to distinguish between chilled poultry and that which has been frozen and then thawed. In this study, the HADH method has been applied to chilled and frozen chicken and turkey using the new refrigeration technologies, to assess the extent to which the method can distinguish between these and poultry which has been conventionally chilled or frozen. The study showed that the HADH assay was able distinguish between poultry that had been frozen, either conventionally or using a new rapid-freezing technique, and fresh or chilled poultry but was unable to distinguish between normally chilled poultry and a new superchilling process. In addition, it is proposed that the procedure used to press juice from the meat should be modified to reduce variability, and that a new cut-off limit (R_1 = 0.50) should be adopted for chicken.

Introduction

Poultry is a highly perishable food, and spoils rapidly unless kept under refrigerated conditions. Even under good refrigerated storage at 0°C, the shelf life of fresh chicken is around 10 days. Consumers perceive fresh poultry to be a superior product to its frozen equivalent, and it can therefore attract a higher retail price. At present, because of the limited shelf-life, the marketing of fresh/chilled poultry can only be achieved in the UK by home or European production. Third country poultry enters the EU or UK as frozen poultry usually for further processing.

Protection of consumers in the sale of poultry either as whole birds or portions was achieved under the EC Poultrymeat Marketing Regulation 1906/90[1] which has been incorporated into a combined EC Marketing Regulation 1234/2007[2]. This Regulation defines poultrymeat and only allows poultry to be marketed as fresh, frozen or quick-frozen. Fresh poultry is defined as that which has been kept at temperatures between -2°C and +4°C. It is not permitted to market chilled/fresh poultry which has been previously frozen and then thawed.

Newer refrigeration technology is available to help extend the shelf life of poultry. One treatment is called "superchilling", where foods are kept at less than 0°C (usually between -2ºC and -5ºC). At these temperatures, the liquid water in the food is mainly in a supercooled state, and the food is only partially frozen where freezing may have taken place between the cells. After storage at "superchilled" temperatures, the temperature of the food is carefully raised up to commercial chill temperatures at or above 0ºC. This process requires very careful temperature control, but it permits the storage life of some foods to be extended considerably by months. The existing definition of fresh poultry in the EC Regulation would prohibit any "superchilled" poultry stored below -2ºC, to be marketed as fresh poultry.

An improved freezing procedure, based on immersion of poultry in a bath of ethylene glycol at -20°C, considerably reduces freezing time and damage to tissues when compared to conventional freezing in a blast freezer. The frozen poultry is then usually stored at around -18°C. The process was developed in the USA and is called the Arkansas process with a commercial name 'Supachill'. The quality of the thawed product is claimed to be superior to thawed product that has been frozen conventionally. Although this product could lawfully be marketed as quick-frozen poultry, there could be a financial incentive to market the thawed product as fresh, which would not be permitted.

A method to distinguish previously frozen meat and poultry from fresh product was developed in earlier research and applied to the UK market[3]. This was based on measuring the activity of a muscle mitochondrial enzyme, ß-hydroxyacyl-CoA-dehydrogenase (HADH), which is released when the mitochondrial membranes are damaged during freezing and thawing. Measurement of the relative HADH activity in juice expressed from a meat sample before and after a laboratory freezing process gives an indication as to whether the meat has been previously frozen.

The principal aim of the current study was to determine whether the HADH method could distinguish between fresh poultry and that processed using either of the new techniques.

Samples used in the study

The study was carried out in two parts. The first examined whether the HADH method could distinguish between the new chilling and freezing technologies and conventional technologies. The second part was to increase the number of samples of chicken and turkey to enable the limit values for chicken and turkey to be re-examined.

In the first stage, whole carcasses were subjected to conventional and new refrigeration processes by an industrial poultry producer according to Table 1:

Table 1: Samples used for the First Stage of the Study

Process/Condition	No of Chicken Samples*	No of Turkey Samples*
Fresh/chilled	3	3
Frozen (blast)	3	3
Rapid Frozen ('Supachill')	3	2
Superchilled (-2°C to -3°C)	3	3
Superchilled (-2°C to -3°C) stored for 3 weeks	3	3
Superchilled (-4°C to -5°C)	3	3
Superchilled (-4°C to -5°C) stored for 3 weeks	3	3

** Two breast fillets were taken from each carcase, giving six samples for each technology (four samples for rapid-frozen turkey).*

The breast fillets were removed from each carcase for subsequent analysis. To provide additional data, further samples of fresh/chilled chicken breasts and of rapid-frozen turkey breasts were obtained from the same supplier for the second stage of the study. The samples obtained are shown in Table 2:

Table 2: Samples used in the Second Stage of the Study.

Process/Condition	No of Boxes of Chicken Breasts*	No of Turkey Samples
Fresh/chilled	20	-
Rapid Frozen	-	14

** Three chicken breasts were sampled from each box giving 60 samples of fresh chicken.*

The data from both stages have been combined for the purposes of evaluation and the total number of breast fillet samples sampled for each processing condition is shown in Table 3.

Table 3: Number of Breast Fillet Samples used for the Study

Process/Condition	No of Chicken Samples	No of Turkey Samples
Fresh/chilled	66	6
Frozen	6	6
Rapid Frozen	6	18
Superchilled (-2°C to -3°C)	6	6
Superchilled (-2°C to -3°C) stored for 3 weeks	6	6
Superchilled (-4°C to -5°C)	6	6
Superchilled (-4°C to -5°C) stored for 3 weeks	6	6

Analytical Method

The analytical method used was that described by Hargin[3]. Some modifications to the original method were required to overcome difficulties in extracting sufficient juice from the fresh chicken samples as discussed later.

ß-hydroxyacyl-CoA dehydrogenase (HADH) is an enzyme which is naturally present in muscle mitochondria, the energy-producing centres within cells. The method relies on the fact that when meat freezes ice crystals form within the cells, eventually rupturing the membranes and releasing the soluble contents into the intracellular fluid. The juice pressed from meat that has been frozen and thawed will therefore exhibit higher HADH activity than meat which has not been previously frozen. Since some HADH may be released when the meat is cut during sample preparation the method is a comparative one, where the HADH activity is determined on the sample as received (X_0), and then determined for a second time after a freezing and thawing cycle (X_1). The ratio of the HADH activities (X_0/X_1) is called the R_1 value. If the R_1 value is close to 1, then the meat or poultry is regarded as having been previously frozen, since there is little or no change in HADH activity with subsequent freezing.

In practice, the HADH activity varies between samples, and factors such as analytical uncertainty come into play and cause a spread of values. During the initial method development[3], a large number of samples of different meats were analysed and a statistical model was developed to show the spread of data for authentic samples. Cut-off limits (based on 2.8 times standard deviation plus mean value of R_1) were established for each meat type, which represented the upper 99% confidence limit obtained for authentic chilled samples. Samples having R_1 values exceeding these cut-off values were deemed to have been previously frozen. For poultry, the cut-off limits obtained are shown in Table 4.

Table 4: R_1 Cut-off Values for Poultry

Poultry Type	R_1 (99%) Cut-off Value
Chicken	0.90
Turkey	0.62

The HADH activity was measured spectrometrically by determining the decrease in the absorption obtained from the following reaction:

$$\text{Acetoacetyl-coenzyme A} + \text{NADH} + \text{H}^+ \xrightarrow{\text{HADH}} \text{ß-hydroxybutyryl-coenzyme A} + \text{NAD}^+.$$

The pressed juice from each sample was diluted with phosphate buffer. Two aliquots of the diluted juice were analysed for HADH activity for all samples. The results presented are the means of two determinations.

Results

The additional data for fresh chicken and for rapid-frozen turkey from the second stage of the study were combined with the data from the first stage as the treatments and preparation of the samples were considered essentially the same.

Superchilled samples were sampled at 0 and 3 weeks. Subsequent statistical analysis showed no significant difference between the R_1 values for these samples therefore these data were combined.

The results obtained for chicken and turkey are shown in Tables 5 & 6 respectively:

Table 5: R_1 values for Chicken

Refrigeration Method	Mean R_1 Value	Range	No. of Samples
Fresh	0.18	0.02 - 0.43	66
Superchilled (-2°C to -3°C)	0.21	0.11 - 0.26	12*
Superchilled (-4°C to -5°C)	0.20	0.10 - 0.30	12*
Conventionally Frozen	0.77	0.68 - 0.80	6
Rapidly Frozen ('Supachill')	0.57	0.43 - 0.70	6

* *Data for 0 and 3 week storage samples combined*

Table 6: R_1 values for Turkey

Refrigeration Method	Mean R_1 Value	Range	Number of Samples
Fresh	0.18	0.06 -0.33	6
Superchilled (-2°C to -3°C)	0.20	0.11 – 0.28	12*
Superchilled (-4°C to -5°C)	0.21	0.05 – 0.35	12*
Conventionally Frozen	0.98	0.76 – 1.24	6
Rapidly Frozen ('Supachill')	0.79	0.53 – 1.04	18

* *Data for 0 and 3 week storage samples combined*

Discussion

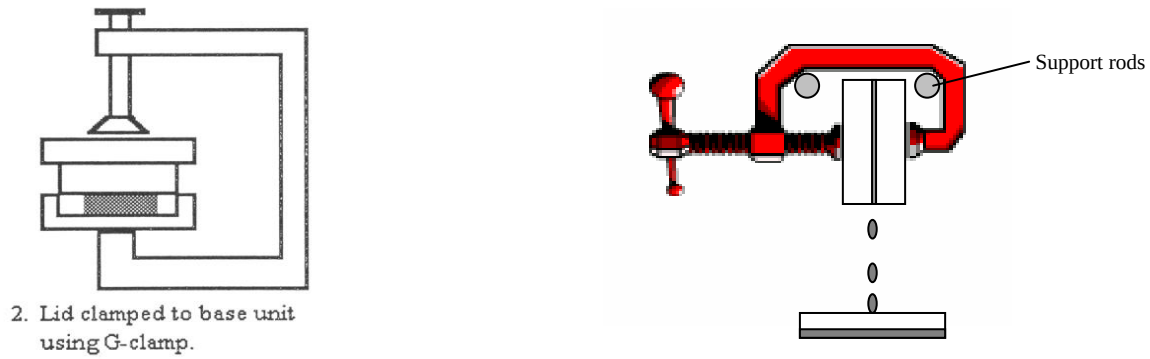
a) Modification of the Pressing Procedure

The press used to express juice consists of a perspex cup and a piston which is held under pressure by a G clamp. In the original protocol, the press was used in a vertical position as shown in Figure 1a. The sample is squeezed between the piston and the cup to a pre-determined and consistent level of compression and when the pressure is released, the piston is removed and the expressed juice is pipetted from the base of the cup. This technique

works well for firm meat samples, but several problems were encountered with softer chicken fillets especially fresh fillets.

It was difficult to cut 30mm cubes of meat from the chicken sample as required by the protocol due to the variable thickness and irregular shape of the breast fillets. It was sometimes necessary to combine two pieces to give the required thickness. Because of the softness of the fresh chicken, it tended to flow around the sides of the piston when compressed and the little juice that was expelled was reabsorbed when the pressure was released. Because of these difficulties, initial fresh chicken HADH results were very variable.

To overcome these problems, the press was rotated through 90°(Fig 1b) and mounted on two supporting rods. 30 x 30mm squares of meat were placed in the press using two pieces if required to reach the specified 30mm thickness. The press was then clamped into position and the juice was allowed to drip from the press for a set time of 30mins. Using this technique, it was possible to standardise the pressure applied and the collection time, which allowed the expressed juice to be collected more reproducibly. This in turn gave less variability in the HADH results, in particular for chilled chicken compared to the original 1997 study, and was used to determine all the chicken results.



1a) Original position of press 1b) Modified press position

Figure 1: Press used in HADH determination

b) Effect of Superchilling Time and Temperature

Superchilled poultry was tested after storage for 0 and 3 weeks at each of two refrigeration temperatures (-2°C to -3°C and -4°C to -5°C). The results were statistically evaluated to assess whether the storage temperature or time affected the subsequent determination of the HADH activity. For both chicken and turkey, there were no significant differences in relation to either storage temperature or storage time. Results are presented for each storage temperature, but the data for 0 and 3 weeks storage time have been combined.

c) Effect of Processing on HADH Activity

All of the results were statistically analysed to determine whether the differences in R_1 values between the refrigeration methods were significant, and thus could be used to determine how a sample had been treated. Figures 2 and 3 show box plots of the results obtained for chicken and turkey respectively. The combined results are shown in Figure 4.

The cut-off limits from the original research and suggested by the current study are shown and are discussed in the next section.

Examining the combined results for turkey, it can be seen that there was good separation between the R_1 values for either the fresh or superchilled turkey breast and the conventionally or rapid-frozen samples. This separation also exists for chicken although differences between the chilled/superchilled and frozen groups were smaller.

The datasets for fresh chicken and superchilled chicken and for fresh turkey and superchilled turkey overlapped, therefore it was not possible to use this technique to distinguish between fresh samples and those that had been superchilled.

There were significant differences between the conventionally frozen and rapidly frozen samples for both chicken and turkey which suggests that it may be possible to distinguish between these techniques if sufficient reference data can be compiled however it was not possible to distinguish between these techniques using the established R_1 cut-off values. Samples from either procedure were correctly identified as frozen.

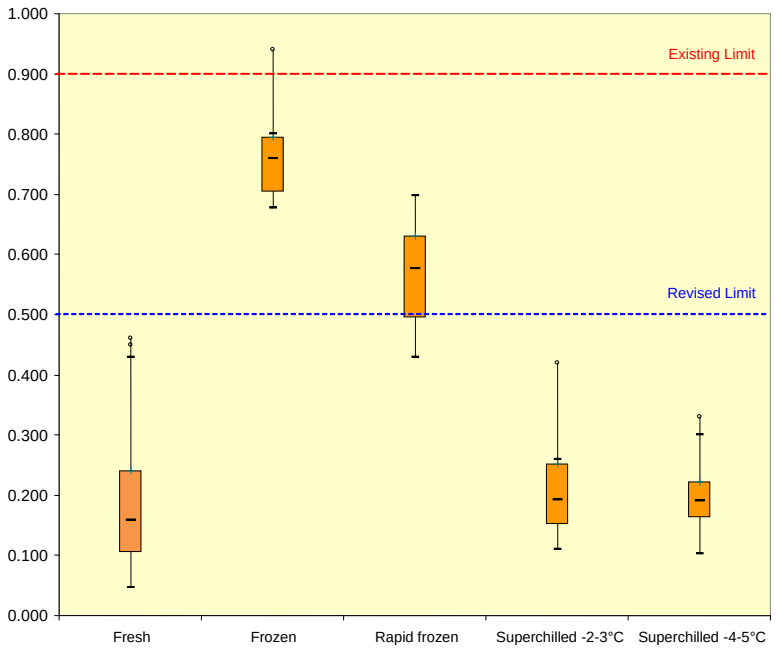


Figure 2: R_1 Values for Chicken Subjected to Different Refrigeration Methods

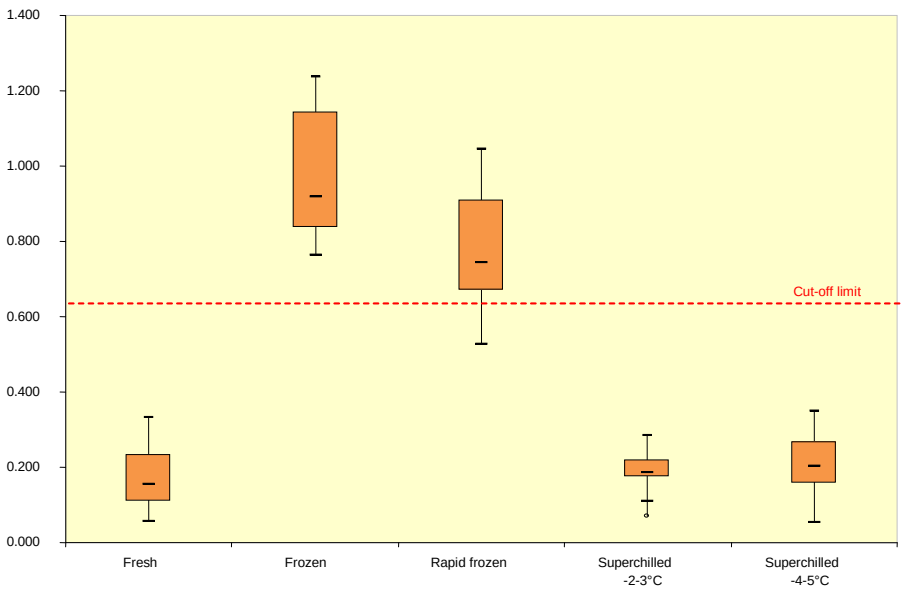


Figure 3: R_1 Values for Turkey Subjected to Different Refrigeration Methods

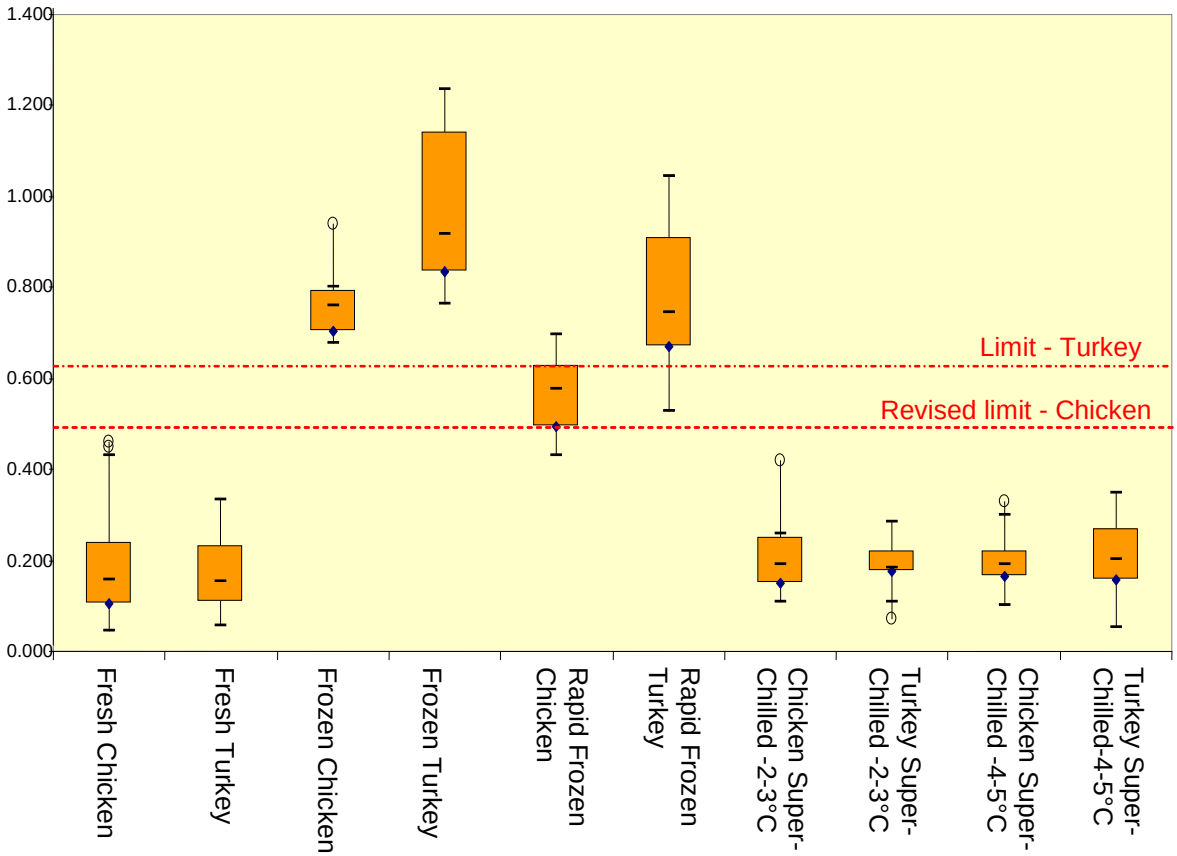


Figure 4: R_1 Values for Chicken and Turkey

d) Proposed Revision of the R_1 Cut-off Limit for Chicken

The results also show a significant difference between fresh or superchilled chicken and previously frozen chicken (regardless of which freezing process is used). However, the very high R_1 cut-off value (0.9) for chicken means that any determination on chicken would result in a large number of false negatives (i.e. previously frozen chicken would have an R_1 value lower than 0.9, and thus could not be distinguished from fresh chicken).

This cut-off value was obtained using the original pressing protocol, which was found to cause highly variable results in the current study. The results from the current study vary considerably from the earlier data. In the original paper, several laboratories reported difficulties in using the pressing protocol, particularly with soft tissue such as offal and fresh chicken. It is suggested that the increased variability resulting from problems in obtaining reproducible samples of meat juice from fresh chicken may have led to the higher R_1 cut-off value obtained.

In the current study, the fresh chicken data for chilled chicken breast, gave a mean R_1 value for fresh chicken of 0.18, with a standard deviation (SD) of 0.109. Using a factor of 2.8*SD to estimate the 99% cut-off limit, the new recommended R_1 limit for fresh chicken should be 0.5. Although this is a within-lab cut-off figure, it should be possible for laboratories to achieve this figure with the improved procedure for expression of juice. Known samples of fresh and frozen chicken should be included with each batch of analysis as reference samples.

e) Classification of Turkey

It was possible to distinguish between fresh or superchilled turkey and previously frozen turkey (regardless of which freezing process is used) using the existing R_1 value (0.62) which continues to be appropriate for survey and enforcement purposes.

Acknowledgements

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References

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2. Council Regulation (EEC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (incorporating Marketing Standards for Poultry) OJ No L299 1-149 of 16.11. 2007.

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Glossary

Fresh poultrymeat – Poultrymeat not stiffened by the cooling process, which is to be kept at a temperature not below -2°C and not higher than 4°C at any time.

Frozen poultrymeat – Poultrymeat which must be frozen as soon as possible within the constraints of normal slaughtering procedures and is to be kept at a temperature no higher than -12°C at any time.

Quick-frozen poultrymeat – Poultrymeat which is to be kept at a temperature no higher than -18°C at any time within the tolerances as provided for in Council Directive 89/108/EEC of

21 December 1988 on the approximation of the laws of the Member States relating to quick-frozen foodstuffs for human consumption.

Superchilling – Refrigeration whereby foodstuffs are chilled to less than 0°C (usually between -2ºC and -5ºC) and are held at that temperature under carefully controlled conditions. At these temperatures, the foodstuff is only partially frozen and the shelf-life may be extended significantly.

Supachill (Arkansas process) – Quick-freezing whereby poultry is rapidly frozen by immersion in an ethylene glycol bath and is then stored at a temperature of -18°C.

HADH – A muscle mitochondrial enzyme, ß-hydroxyacyl-CoA-dehydrogenase, which is released into the intracellular fluid when the mitochondrial membranes are damaged during freezing and thawing.