USE OF α-HYDROXYACYL-COA-DEHYDROGENASE (HADH) ACTIVITY TO DIFFERENTIATE BETWEEN FRESH AND THAWED CHICKEN MEAT

Eduardo M. RAMOS, Paulo R. FONTES, Alcinéia L.S. RAMOS, Lúcio A.M. GOMIDE
Universidade Federal de Viçosa, UFV; Viçosa-MG, Brazil, CEP 36571-000; E-mail: lagomide@ufv.br

Background

Freezing is one of the most effective methods for extending the shelf life of foods. Low temperature inhibits microbial growth and retards physical and biochemical deteriorative reactions. The freezing storage of meat can have outstanding effects on muscle chemical and structural properties, which may significantly influence quality attributes of meat and meat products (MILLER et al., 1980). Therefore, many consumers prefer fresh meat despite its higher cost than the frozen ones. In addition, as fresh meat has a lower shelf life, the sale of thawed meat, in special poultry and fish, as if they were unfrozen products has become a usual commercial practice. Thus, various methods to differentiate between unfrozen and thawed meat have been proposed to identify, reduce and control this fraud.

Enzyme-based methods has been used efficiently in this purpose and are based on three basic requirements: the enzyme must be release due to the freezing and thawing processes but not due to refrigeration; its total activity should not decrease markedly during meat storage, either frozen or refrigerated; and it should be easily detectable in the muscle juice (GOTTESMANN & HAMM, 1983). Therefore, lysosomes, erythrocytes and mitochondrial enzymes (GOTTESMANN & HAMM, 1983; BARBAGLI & CRESZENCI, 1988; KATAMIKADO, 1990), released to sarcoplasm during freezing and thawing processes have been investigated. Among all mitochondrial enzymes (fumarase, aconitase, lipoamide dehydrogenase, citrate synthase) studied by GOTTESMANN & HAMM (1983), the β-hydroxyacyl-CoA-dehydrogenase (HADH, EC 1.1.1.35), extracted from sarcoplasm by meat pressing, has showed the best characteristics to allow the differentiation of unfrozen from thawed meat. This method has been used for pork, beef, mutton and poultry (GOTTESMANN & HAMM, 1983; CHEN et al., 1988; TOLDRÁ et al., 1991), as well as for game meats, such as venison and hare (GOTTESMANN & HAMM, 1983).

Though efficient in segregating warm-blood meat animals, HADH extraction by the pressing method (GOTTESMANN & HAMM, 1983) has been shown unsatisfactory for fish meat. However, GARCIA de FERNANDO et al. (1992) modified this former enzyme extraction procedure by immersing a piece of sample in phosphate buffer, instead of directly pressing the meat, and found that the determination of HADH activity was useful to distinguish between unfrozen and frozen-thawed trout (*Salmo gairdneri*). HADH extraction by buffer immersion was further assayed and approved for application in others fish and shellfish species (HOZ et al., 1992 and 1993; PAVLOV et al., 1994; FERNANDEZ et al.,1999). The method of HADH extraction by immersion in phosphate buffer (GARCIA de FERNANDO et al., 1992) is much easier to be conduced and showed a better differentiation of fresh from thawed frog meat as compared to the pressing method (PAVLOV et al., 1994). However, the buffer immersion method of HADH extraction has not yet been tested in segregating unfrozen and freeze-thawed conventional meats.

Objectives

The aim of this study was to evaluate the efficiency of the HADH extraction method by phosphate buffer immersion in differentiating fresh from freeze-thawed poultry meat (breast and thigh).

Methods

Twenty-eight recently slaughtered poultry were used. Immediately after slaughtering the thighs were removed from the carcasses and individually packaged in identified plastic bags. The breasts were separated from the carcasses and longitudinally sectioned in half before packaging. One sample of each thigh and breast half was stored under refrigeration (4°C) and the remaining sample of each cut was frozen (-18°C) in a domestic freezer and stored for two days before overnight refrigerated thawing (4°C). Samples HADH activity were immediately determined thereafter and after one, two and three days of refrigerated storage. **HADH Assay:** The HADH activity was measured according to the method of GARCIA de FERNANDO et al. (1992), with some modifications. Portions of sample (about 2 g) were immersed in two volumes (4 mL) of 0.1 M phosphate buffer (pH 6.0) and maintained in a water bath at 25°C for 15 min. The extract was filtered through Whatman paper No. 2. The filtrate was assayed for HADH activity by mixing, in a quartz cuvette, 34 µL of filtrate with 70 µL of EDTA (34.4 mM) and 860 µL of 0.1 M phosphate buffer (pH 6.0). The cuvette was maintained at room temperature for 3 minutes before adding 40 µL of NADH (1.5 mM) and 20 µL of acetoacetyl-CoA (5.9 mM). Immediately thereafter, the absorbance at 340 nm was measured every 0.5 minute up to 3 minutes. The HADH value was calculated by multiplying the slope of the linear regression obtained by a factor of -10^4. **Statistical Analysis:** The 95% confidence limits for the means were used to establish the limits that segregate the unfrozen from freeze-thawed samples under the work conditions. Data were submitted to the analysis of variance in a randomized split-plot scheme, with treatment (frozen and unfrozen) on the plot and the refrigerated storage time on the sub-plot. Variance and regression analysis were conducted with the statistical programming language SAS® System for Windows™ (version 8.0) considering a significance level of 5%.

Results and Discussion

Storage time did not affect ($P > 0.05$) the HADH activity of both fresh and freeze-thawed poultry thighs or of fresh breast. However, the HADH activity of freeze-thawed poultry breasts decreased ($P < 0.05$) with increasing storage time (**Figure 1**). As time did not significantly influence the HADH activity value of both fresh and thawed thighs, the overall mean of the thighs HADH activity value was used to establish the limits leading to the segregation of fresh from frozen thighs. Unfrozen thighs presented average (mean ± standard deviations) HADH values of 137.8 ± 52.5 while frozen-thawed thighs presented average HADH values of 327.7 ± 92.7. Considering a 5% significance level, and that the samples were randomly distributed, it is suggested that poultry thighs may be sorted by HADH values as follows: those with an HADH value bellow 176 are to be considered unfrozen while those with an HADH value over 224 should be considered as frozen-thawed. Samples having an HADH value between these limits are considered uncertain. Using these criteria 87% of the experimental thighs were correctly classified, 2% were wrongly classified and 11% were uncertain. As refrigerated storage time had an effect ($P < 0.05$) on the HADH values of frozen-thawed breasts, the upper and lower HADH values to sort breast samples according to their previous freezing history are time dependent. This makes breasts HADH activity determinations difficult or impossible to be used for routine control/fiscalization purposes, as it would be necessary to establish HADH upper and lower values for each storage time. However, in the experimental conditions used, it can be observed (**Figure 1**) that, even with a decrease in breast HADH values in the evaluated storage time, the HADH values of frozen-thawed breast samples are always

higher than those of unfrozen ones. As refrigerated (4°C) poultry shelf life is at most of 4 days, as long as it had not been previously submitted to irradiation or high-pressure treatment, it is possible to establish limits for HADH values in sorting breast samples. Unfrozen breasts presented average HADH values of 96.3 ± 23.0 while frozen-thawed breasts presented average HADH values of 344.3 ± 101.9. Using the same considerations applied to the thighs, it is suggested that poultry breasts may be sorted by HADH values as follows: those with an HADH value bellow 134 are to be considered unfrozen while those with an HADH value over 177 should be considered as frozen-thawed. Samples having an HADH value between these limits are considered uncertain. Using these criteria 96% of the experimental thighs was correctly classified and 4% were considered uncertain, which is very satisfactory for fiscalization purposes. The correct classification of poultry of different previous freezing history obtained with the HADH limits proposed in this study is very similar to those reported with other meats. In fish meat correct classification of 95% (HOZ et al., 1992) and 84% (HOZ et al., 1993) was obtained, while PAVLOV et al. (1994) established HADH limits that allowed 100% correct classification of frog meats. Using the pressing extraction method GOTTESMANN & HAMM (1983) have also established different HADH limits in poultry breasts and thighs. They proposed an HADH upper limit of 5.0 U/mL for poultry breast extracts and of 15.0 U/mL for poultry thigh extracts. Samples with higher HADH concentrations were considered frozen-thawed.

Conclusions

The determination of HADH in samples obtained by the buffer immersion method of extraction allow an efficient sorting of poultry meat of differing freezing history and may be useful for fiscalization purposes due to the fact that this is an easier procedure, allowing between 87 (thighs) and 96% (breasts) accurate classification of the samples. As a higher accuracy was obtained with breast samples it is also suggested that this cut should be used for identifying unfrozen whole poultry carcasses. However, this only applies to poultry that had not been previously irradiated or otherwise treated, in which case the experiment should be repeated in order to establish new HADH limits.

References

BARBAGLI, C., CRESCENZI, S. Influence of Freezing and Thawing on the Released of Cytochrome Oxidase from Chicken Liver and from Beef and Trout Muscle. **Journal of Food Science**, v.46, p.491-493, 1981.

CHEN, M.T., YANG, W.D., GUO, S.L. Differentiation between Fresh Beef and Thawed Frozen Beef. **Meat Science**, v.24, p.223-226, 1988.

GARCIA de FERNANDO, G.D., FERNANDEZ, M., DIAZ, O., ORDOÑEZ, J.A., HOZ, L. An Objective Method to Differentiate Between Fresh and Thawed Trout Meat (*Salmo gairdneri*). **Archiv. Für Lebensmittelhygiene**, v.43, p.13-14, 1992.

GOTTESMANN, P., HAMM, R. New Biochemical Methods of Differentiating between Fresh meat and Thawed, Frozen Meat. **Fleischwirtschaft**, v.63, n.2, p.219-221, 1983.

FERNÁNDEZ, M., MANO, S., GARCÍA de FERNANDO, G.D., ORDÓÑEZ, J.A., HOZ, L. Use of β-Hydroxyacyl-CoA-Dehydrogenase (HADH) Activity to Differentiate Frozen from Unfrozen Fish and Shellfish. **European Food Research and Technology**, v.209, p.205-208, 1999.

HOZ, L., FERNÁNDEZ, M., DIÁZ, O., ORDÓÑEZ, J.A., PAVLOV, A., GARCIA de FERNANDO, G.D. Differentiation of Unfrozen and Frozem-thawed Kuruma Prawn (*Penaeus japonicus*) from the activity of β-Hydroxyacyl-CoA-Dehydrogenase (HADH) in Aqueous Extracts. **Food Chemistry**, v.48, p.127-129, 1993.

HOZ, L.,YUSTES, C., CAMARA, J.M., RAMOS, M.A., GARCIA de FERNANDO, G.D. β-Hydroxyacyl-CoA-Dehydrogenase (HADH) Differentiates Unfrozen from Frozem-thawed Crawfish (*Procambarus clarkii*) and Trout (*Salmo gairdneri*) meat. **International Journal of Food Science and Technology**., v.27, p.133-136, 1992.

KATAMIKADO, M., YUAN, C.S., UENO, R. An Enzymatic Method Designed to Differentiate Between Fresh and Frozen-Thawed Fish. **Journal of Food Science**, v.55, p.74-76, 1990.

MILLER, A.J., ACKERMAN, S.A., PALUMBO, S.A. Effect of Frozen Storage on Functionality of Meat for Processing. **Journal of Food Science**, v.45, p.1466-1471, 1980.

PAVLOV, A., GARCIA de FERNANDO, G.D., ORDONEZ, J.A., HOZ, L. β-hydroxyacyl-CoA-dehydrogenase (HADH) Activity of Unfrozen and Frozen-Thawed Frog (*Rana esculenta*) Legs. **Journal of the Science of Food and Agriculture**, v.64, p.141-143, 1994.

TOLDRÁ, F., TORRERO, Y., FLORES, J. Simple Test for Differentiation between Fresh Pork and Frozen/Thawed Pork. **Meat Science**, v.29, p.177-181, 1991.

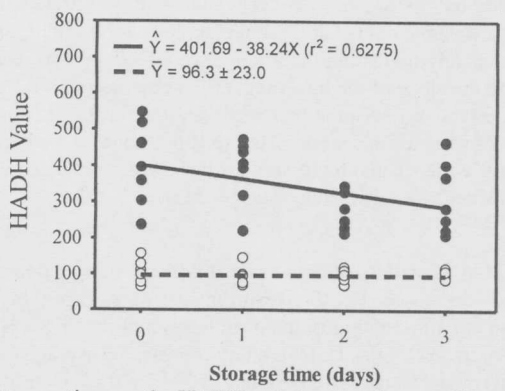

Figure 1. Effect of the refrigerated (4°C) storage time on the HADH activity of unfrozen (○) and thawed (●) breast chicken meat.

USE OF SMOKED DRIED FERMENTED MEAT IN PRODUCTION OF DRY SAUSAGE AND ITS INFLUENCE ON STORAGE STABILITY

Kathrin Ulmer and Albert Fischer

University of Hohenheim, Institute of Food Technology, Department of Meat Technology, D-70599 Stuttgart, Germany

Background

Besides salting and drying, smoking is the oldest procedure to preserve food. Today one uses the sensory properties of smoke such as odor, flavor and color, but its conservative effect should not be neglected. This effect is caused by smoke ingredients precipitating on the surface of food. As a result of antioxidative smoke ingredients the oxidation of fat is slowed down [1]. These insights have been applied to the surface of dry sausages for a long time. If this matter of fact could also be transferred into the dry sausage, autoxidation could be successfully reduced.

The concept of adding a certain amount of smoked dried fermented meat in production of dry sausage would not only improve the storage stability of the product, but also shorten the ripening time.

Objectives

The objective of this study was to develop a novel method to shorten the ripening time of dry sausage by adding a certain amount of dried fermented meat and the application of friction smoke in production of dried fermented meat, which should demonstrate a positive influence on the storage stability of sliced frozen dry sausages, mainly used as pizza topping.

Methods

Production of smoked dried fermented meat: Preparation of meat:

Refrigerated lean pork (estimated fat content of 10%) was coarsely cut into pieces. 2.8% nitrite curing salt, 1.2% mixture of spices, 0.3% dextrose and a 0.05% starter cultures compound (containing *L. sakei* + *Staph. carnosus*) were added. All ingredients were mixed in a blender and minced through an 8 mm plate. Fermentation: The meat was fermented in vacuum-packed bags at 24°C for at least 40 hours depending on the change of the pH value (below pH 5.0). Smoking: The fermented meat was chopped with a cutter on low speed and spread on perforated sheet metal on a trolley. For batch 3 the meat was smoked for 2 minutes at 28°C, then the friction smoke was accumulated for 4 minutes at 28°C and finally, the chamber was ventilated for 5 minutes. In comparison to batch 3 the meat in batch 4 was smoked for 4 minutes, the following treatment was the same as in batch 3. Drying: The meat was dried at 50°C (batch 2 without smoking). The drying process was completed when the weight was reduced by 60%. The dried fermented meat was chopped again on high speed in the cutter, packed in bags and stored frozen at –18°C.

Production of dry sausage (batch 1, control):

Traditional dry sausages were prepared according to the following weight based formula: 30% lean frozen pork, 30% 3 mm minced beef (estimated fat content of 8%), 30% frozen pork back fat (estimated fat content of 90%) and 10% 3 mm minced lean pork. Additives and spices were added per kilogram: 28 g nitrite curing salt, 12 g mixture of spices and 0.5 g of a starter cultures compound (containing *L. sakei* + *Staph. carnosus*). The mass was stuffed into 65 mm diameter regenerated collagen casings (R2, Naturin, Germany).

Production of dry sausage with dried fermented meat (batch 2 - 4):

Dry sausages were prepared according to the following weight based formula: 30% 3 mm minced beef, 30% frozen pork back fat, 12.5% lean frozen pork, 10% 3 mm minced pork and 7.5% dried fermented meat. Additives and spices were added per kilogram (except dried fermented meat): 28 g nitrite curing salt, 12 g mixture of spices, 1 g sodium diphosphate and 0.5 g of a starter cultures compound (containing *L. sakei* + *Staph. carnosus*). The lack of freezing capacity during chopping makes it necessary to add liquid N_2 periodically. The mass was stuffed into 60 mm diameter regenerated collagen casings (R2, Naturin, Germany).

Ripening: The sausages were placed in a drying chamber under the following conditions: 2 days at 24°C, 88 – 92% relative humidity (RH); 2 days at 20°C, 85 – 88% RH; 3 days at 18°C, 82 – 86% RH and finally the dry sausages were ripened another 4 or 5 days (control 10 – 12 days) at 14°C, 75 – 85% RH until a weight loss of 26% was reached. The sausages were smoked after 2, 3 and 5 days under friction smoke conditions for 30 minutes each time.

pH measurement: The course of pH while ripening was measured using a spear tip electrode (Schott, Germany). The electrode was calibrated with two buffer solutions of pH 4.000 and 7.000.

Weight loss: The sausages were weighed once a day (Sartorius Universal pro 32/34 F, Germany) until a weight loss of 26% was reached.

Storage conditions: The sausages were cut in 2 mm slices; 15 slices were put fan-shaped into vacuum foil and were aerated with synthetic air. At –18°C the slices were stored; after a certain storage time they were analyzed.

TBARS: TBARS [2], [3] of sliced frozen dry sausage were determined on stored dry sausages (4, 6 and 8 weeks).

Hexanal: Hexanal [4] of sliced frozen dry sausage was determined on stored dry sausages (4 and 6 weeks).

Sensory evaluation: A taste panel consisting of 10 members determined the rancidity on a 10-point scale after 3, 4 and 6 weeks as follows: not perceived (0-1), slightly perceived (2-3), moderately perceived (4-5), strongly perceived (6-7) and very strongly perceived (8-9).

Results and discussion

In the following, different characteristics such as weight loss and pH between the batches were investigated. A fundamental advantage of the batches 2 - 4 is the shortening of ripening time. Figure 1 shows the weight loss during ripening. In comparison with the control, the batches with dried fermented meat reach a weight loss of 26% after 12 days. As can be seen, the dry sausage already has a 10% weight loss at the beginning of the ripening process. This can be explained by the fact that moisture has been removed from the fermented meat during drying.

Differences in TBARS and Hexanal between the batches after 4, 6 and 8 weeks of storage are presented in Figures 3 and 4. With increasing smoking time of the fermented meat, the rancidity of the samples is decreased. TBARS and Hexanal values indicate that without smoking the dried fermented meat, the sausages lead to a worse result as regards rancidity (batches 1 and 2). The slightly higher values of batch 2 can be explained by the fact that the drying procedure during processing of dried fermented meat, induces accelerated oxidation during storage. Also

the time factor of smoking plays an important role here, but it should be mentioned that too much smoke makes the sausages inedible. The results shown in Figure 5 represent averages of the ratings reported by panelists. As can be seen, the analytical data correlate well with the sensory results after the storage period

Conclusions

The aim of this study was to show that the use of dried fermented meat in the production of dry sausage can shorten the ripening time by about one week. Furthermore, the investigations have shown that smoking of dried fermented meat can improve the storage stability. Regarding the smoked flavor and the rancidity, a compromise must be made since there is an inverse relationship.

References

Bem, Z., H. Hechelmann and Z. Bozidar (1994). Die Fleischerei 45 (12), 67 - 73

Tarladgis, B.G. et al. (1960). J Am Oil Chem Soc 37, 44 - 48

Zipser, M.W. and B.W. Watts (1962). Food Technol 16, 102

Lin, J.C.C. and I.J. Jeon (1985). J Food Sci 50, 843 – 846

Data in the form of tables

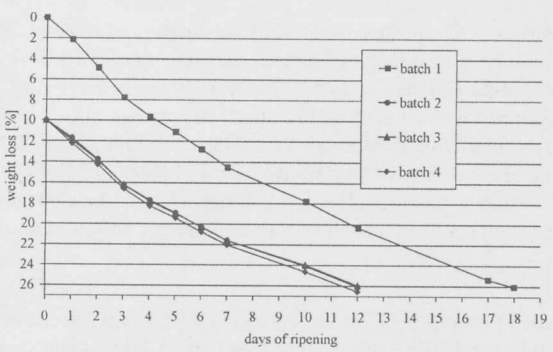

Fig. 1: Plot of weight until 25% loss

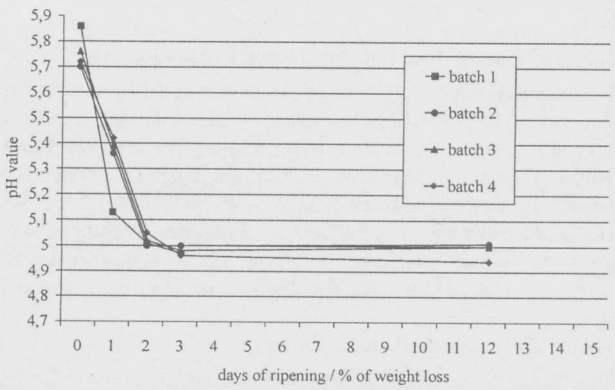

Fig. 2: Plot of pH change during ripening

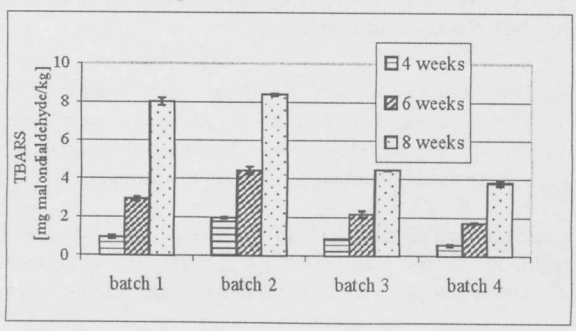

Fig. 3: TBARS of sliced frozen dry sausages after storage

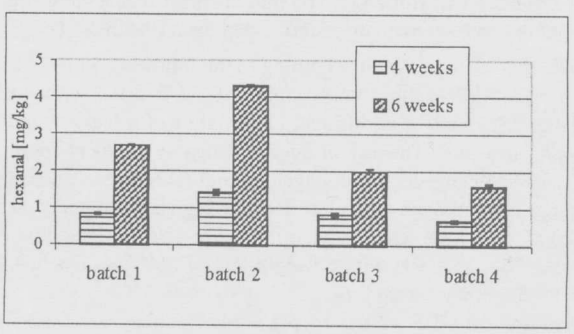

Fig. 4: Hexanal of sliced frozen dry sausages after storage

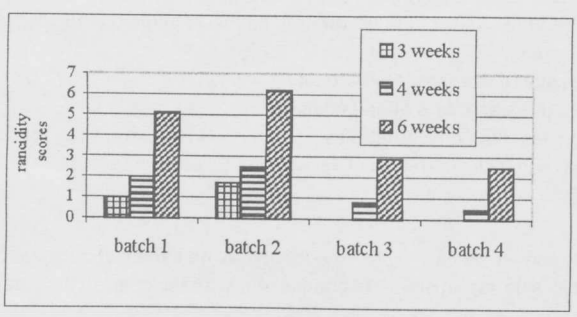

Fig. 5: Sensory tasting