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DIFFERENTIATION BETWEEN FRESH MEAT AND FROZEN–THAWED MEAT IN EGYPTIAN MARKET

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ABSTRACT

In order to prevent meat retailers offering thawed – imported frozen meat as fresh domestic meat, β-hydroxyacyl-CoA-dehydrogenase (HOADH) activity was determined. Two methods; i. e., color test and photometric assay, were used in this study. Twenty fresh meat samples were obtained from butcher shops. Each sample was divided into two portions, the first portion was analyzed immediately, while the second one was frozen at – 30°C and stored at – 18°C for 10 days and analyzed after thawing. Concerning color test, the press juice obtained from all fresh meat samples had a purple–red color which retained for several days, while the meat juice obtained from thawed samples had a blue color. Regarding photometric method, the obtained results indicated that activity of HOADH was greater than 3.0 U/ml in the muscle press juice and this indicates the presence of frozen meat samples. A limited survey was also performed, where twenty meat samples were collected randomly from butcher's in poor districts at Cairo markets and analyzed by the two methods. The obtained data revealed that 10% of the collected samples were frozen and thawed before handling.

INTRODUCTION

The food hygienists are expected to protect the health and welfare of the consumers, as well as the interests of the fair and honest food producers, processors, or marketers against dishonest and unfair competition.This function is performed through governmental regulations of the foods and food products to be sure that they are free from contamination, adulteration, and decomposition (Shalaby, 1996).

Fresh meat adulteration may be occurred in Egyptian markets, where the widespread of imported frozen meat in some private butcher's in poor districts without any control may give the chance for substituting fresh meat by frozen – thawed one, since the latter is the cheapest. The imported frozen meat, on the other hand, constitutes a more or less public health hazards concerning the mycological status (Nassar and Ismail, 1995) or the bacteriological status (Nassar and Fathi, 1997). For sanitary and economic purposes, frozen food may not be offered for sale without declaration. This is certainly so, since acceptability in this type of food is related to both the quality and the storability after the food is thawed. Therefore, a simple, rapid and accurate analytical method for differentiating between fresh meat and frozen – thawed one is urgently needed.

To achieve such method, the biochemical changes occurred in muscle tissues as a result of freezing and thawing should be considered. In this concern, Gantner and Hamm (1964) observed that freezing and thawing of skeletal muscles of pigs increase the activity of Glutamic Oxaloacetic

Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) in extracts. Furthermore, Kormendy *et al*. (1965) and Hamm *et al*. (1969) demonstrated the presence of mitochondrial enzyme of GOT (GOTm) in the skeletal muscles of pigs and cattles that were freezed and thawed. So, Hamm and Kormendy (1969) developed a reliable electrophoratic method for differentiating between unfrozen and thawed–frozen meat. However, this electrophoretical technique needs time, equipments and some experience to be a routine method (Gottesmann and Hamm, 1982).

Freezing and thawing the meat lead to release of another mitochondrial enzyme namely β-hydroxyacyl-CoA-dehyrogenase (HOADH) into the sarcoplasm "meat press juice" (Gottesmann and Hamm, 1987). Therefore, the determination of HOADH activity in meat press juice could be differentiated between fresh meat and frozen-thawed one. In this concern, Kobayashi *et al.* (1996) found that mitochondrial β-hydroxyacyl-CoA-dehyrogenases are rich in several animal tissues. As well as, Vallejo-Cordoba *et al.* (2003) stated that β-hydroxyacyl-CoA-dehyrogenase (HOADH) is a significant mitochondrial enzyme in food muscles; thus, the determination of its activity is very important in food analysis. Moreover, β-hydroxyacyl-CoA-dehyrogenase (HOADH) was used as marker of oxidative capacity occurred in pig skeletal muscles (Lefaucheur *et al.*, 2004).

The assessment of the HOADH activity could be achieved either by color test or by photometric assay. In the color test, Gottesmann and Hamm (1987) used meldolablue as electron–transmitter, while Chen *et al.* (1988) used resazurine as an electron–transmitter which resulted in much color stability after reaction with fresh meat extracts. Color development depends on the following reaction :-

1. Acetoacetyl-CoA + NADH + H^+ $\xrightarrow{\text{HOADH}}$ β-hydroxybutyryl-CoA + NAD^+

2. NADH + H^+ + resazurine (oxidized form, blue) $\xrightarrow{\text{HOADH}}$ NAD^+ + resorufin (reduced form, purplish red)

If the meat has not been frozen, the solution color changes from blue to pink after 1 hr, and after 2 hrs it becomes red. After 3 hrs the resazurine transforms into the purple-red hue of resorufin, this color being retained for several days. On the other hand, with meat juice obtained from frozen and thawed beef, the color remains blue Chen *et al.* (1988).

Concerning photometric assay of HOADH activity, the method of Gottesmann and Hamm (1987) depending on the following reactions:-

1. Acetoacetyl-CoA + NADH + H^+ $\xrightarrow{\text{HOADH}}$ L-3-hydroxybutyryl-CoA+ NAD^+

The NADH is removed by iodonitrotetrazoliumchloride (INT) and diaphorase

2. NADH + H^+ + INT (oxidized form, blue) $\xrightarrow{\text{Diaphorase}}$ NAD^+ + formazane (reduced form, purplish red)

The formazane released by this reaction ensure the quantitative reaction of L-3-hyroxybutryl-CoA and could be measured at 492 nm (Hg). The change in absorbance is proportional to the HOADH activity in the tested samples.

This study was planned to apply the previously mentioned methods; i. e., color test and photometric assay, for differentiating between fresh meat and frozen – thawed one at Egyptian markets.

MATERIALS AND METHODS

Materials

Samples

Twenty fresh meat samples, each weighing one kg, were obtained from private butcher shops at Assiut city. Each sample was divided into two portions, the first portion was analyzed immediately, while the second one was frozen at –30°C and stored at –18°C for 10 days, then analyzed after the thawing. Another twenty samples, each weighing one kg, were collected randomly from private butcher shops in poor districts at Cairo markets.

Chemicals

All the chemicals were obtained from Boehringer Mannheim GmbH Biochemica, Germany.

Preparation of meat juice :

The meat juice was prepared by putting 20g of ungrounded meat sample between two thick glass plates, using hydraulic press (about 1 kg/cm^2 pressure), the meat juice, which separated, was collected in a clean dry tubes.

Methods

1- Color test technique:

The method of Chen *et al.* (1988) was used. Accurately, 2.4ml of 0.1 M phosphate buffer (pH 6.0), 0.2ml of 34.4 mM EDTA, 0.2ml of 1.5 mM NADH, 0.12ml of 5.9 mM Acetoacetyl-CoA and 0.1ml of diluted meat juice (diluted 1 : 100 in phosphate buffer) were pipette in a test tube, mixed and stored in darkness at a room temperature. The reaction time for beef is 60 min. Resazurin solution (0.2ml), which is prepared by dissolving of resazurin in 200 ml of hot distilled water, is then added and the mixture shaken for 30s. The color was visually estimated and recorded after 1, 2 and 3 hrs.

2- Spectrophotometric technique:

The method mentioned by Gottesmann and Hamm (1987) was used as follows :

Reagents:

- Tris-HCl buffer solution, 250 mmol/l; EDTA 2.5mmol/l.
- NAD/diophrase solution, 0.65 mmol/diophrase, 150 u/l.
- Iodonitrotetrazoliuum (INT) solution, 0.12 mmol/l.
- Hydroxyacyl-CoA-dehydrogenase (HADOH) suspension, up to 6.2U/l.

Standard solution was prepared by diluting 0.05ml HADOH in 5ml ammonium sulphate, 3.2 mol/l and mix. Then, 0.1ml of this was mixtured with 1.9 ml of ammonium sulphate, 3.2 mol/l.

Assay conditions:

A wave length (Hg lampe) is 492nm, half micro l cm light path, at a temperature of 25 ±1ºC, assay volume is 1ml, and measurement is against air.

Procedure:

In a clean dry cuvettes pipette 0.50ml tris buffer, 0.10ml NAD/diophrase sol., 0.10ml INT solution in each of blank and sample tubes, 0.05ml of sample solution in the sample tube and 0.5ml of standard solution in the blank, 0.20ml and 0.15ml redistilled water in blank and sample tube, respectively. Mix, wait for approx. 5min at 25 ±1ºC and then add 0.10ml L-HB CoA 0.52 mmol/l for each blank and sample tube. Mix and read the absorbance after 5min and start the time measurement. The reading was repeated after 1, 2, 3 and 4min.

Calculation:

Absorbance differences per min for the sample and the blank were calculated as follows:

$$\Delta A_{HOADH}/min = \Delta A_{sample} - \Delta A_{blank}/min$$

then, calculate the HOADH activity from the following equation:-

$$U/ml = 1.005 \cdot \Delta A_{HOADH}/min$$

RESULTS AND DISCUSSION

The data given in Table (1) demonstrate HOADH activity in the press juice of the fresh meat samples using both color test and spectrophotometric technique. Concerning color test, it is clear that the solution color of fresh samples changed from blue to purple–red which was retained for several days because the resorufin can not be oxidized to the primary color of resazurin; blue (Chin *et al.*, 1988). Concerning spectrophotometric assay, the obtained data of fresh samples revealed that HOADH had an activity of less than 3.0 U/ml of the press juice.

Table (1):The color test and HOADH activity of fresh meat samples.

Sample No.	Solution color	HOADH activity, U/ml	Sample No.	Solution color	HOADH activity, U/ml
1	Purple - red	1	11	Purple - red	2.8
2	Purple - red	0	12	Purple - red	1.3
3	Purple - red	1.9	13	Purple - red	1.8
4	Purple - red	1.1	14	Purple - red	0
5	Purple - red	2.3	15	Purple - red	2.6
6	Purple - red	3.0	16	Purple - red	2.9
7	Purple - red	1.5	17	Purple - red	2.5
8	Purple - red	2.7	18	Purple - red	1.9
9	Purple - red	1.7	19	Purple - red	1.1
10	Purple - red	2.1	20	Purple - red	2.9

The HOADH activity in the press juice of the frozen–thawed meat samples was determined and the obtained data were given in Table (2). It is clear that the solution color of these samples remained blue when color test was applied indicating that resorufin was oxidized to resazurin as a result of HOADH activity. The data of spectrophotometric assay proven this finding where the HOADH activity was among 5.0 - 20.1 U/ml press juice.

By comparing the data given in the previous two tables (1 and 2), it is easily to determine whether a given meat sample is fresh or has been submitted to one or more freeze – thaw cycles. In this respect, Daskalov and Pavlov (1999) found no significant differences between HOADH activity values of eel meat frozen at –18° and –35°C, whilst, HOADH activity was significant higher in frozen–thawed samples than in unfrozen meat due to release of HOADH during freezing.

Table (2):The color test and HOADH activity of frozen thawed meat samples.

Sample No.	Solution color	HOADH activity, U/ml	Sa mple No.	Solution color	HOADH activity, U/ml
1	Blue	17.1	11	Blue	10.1
2	Blue	7.0	12	Blue	5.3
3	Blue	9.0	13	Blue	11.1
4	Blue	8.0	14	Blue	13.1
5	Blue	15.1	15	Blue	10.0
6	Blue	16.1	16	Blue	13.1
7	Blue	14.1	17	Blue	13.1
8	Blue	17.1	18	Blue	5.0
9	Blue	12.1	19	Blue	5.0
10	Blue	20.1	20	Blue	13.1

A limited survey was done to differentiate unfrozen fresh meat from the frozen–thawed commodity at Cairo markets and the obtained data were presented in Table (3). It is clear that among the 20 meat samples examined 2 samples gave blue color using the color test and the same samples had HOADH activity more than 5.0 U/ml press juice using spectrophotometer technique, whereas the rest of samples had HOADH activity 3.0 U/ml press juice or less. This indicates that these samples were frozen and thawed. It is also proven that both techniques gave similar results.

Irrespective of the economic aspect of this fraudulent substitution, the sanitary viewpoint seems to be absolutely essential. Some investigators have pointed out the foods thawed from the frozen state load more bacteria and spoil faster than similar fresh products (Jay, 1970). Moreover, spores of the pathogenic remain unchanged in frozen meat, while multiplication can be rapid during thawing under warm conditions (Mossel *et al*., 1972). Consequently, thawed meat is more perishable than fresh, unfrozen meat (ICMSF, 1980).

In view of the above, one could therefore expect the danger of marketing the imported frozen meat without any control, especially the

unknown conditions of thawing and subsequent handling, processing or storage. So, it is of importance to differentiate between fresh meat and frozen–thawed one handled in Egyptian makets. Results of the present work revealed that both two methods used could be fulfill such purpose.

Table (3): The color test and HOADH activity of the market meat samples.

Sample No.	Solution color	HOADH activity, U/ml	Sample No.	Solution color	HOADH activity, U/ml
1	Purple - red	2.7	11	Purple - red	1.3
2	Purple - red	1.5	12	Purple - red	1.7
3	Purple - red	1.7	13	Blue	17.1
4	Purple - red	2.3	14	Purple - red	2.3
5	Purple - red	2.3	15	Purple - red	0
6	Purple - red	3.0	16	Purple - red	0
7	Blue	15.1	17	Purple - red	0
8	Purple - red	3.0	18	Purple - red	3.0
9	Purple - red	2.1	19	Purple - red	2.7
10	Purple - red	2.7	20	Purple - red	1.3

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التفريق بين اللحوم الطازجة وتلك السابق تجميدها فى الأسواق المصرية

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*** قسم الصناعات الغذائية – المركز القومى للبحوث – الدقى – الجيزة**

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أجرى هذا البحث لمحاولة التفريق بين اللحوم الطازجة واللحوم السابق تجميدها والمتداولة فى الأسواق المصرية ، وذلك بتقدير نشاط إنزيم HOADH فى عصارة عينات اللحوم باستخدام طريقة طيفية وأخرى لونية . وقد تم فى هذا البحث تجميع عشرون عينة من لحوم الأبقار الطازجة فى مدينة أسيوط ، حيث قسمت كل عينة إلى جزئين : الجزء الأول تم فحصه مباشرة عند وصوله للمعمل ، أما الجزء الثانى فقد تم تجميده على درجة -٣٠° م وخزن لمدة عشرة أيام على درجة -١٨° م ثم فحص بعد تسييحه . كما تم تجميع عشرون عينة من لحوم الأبقار من أسواق المناطق الشعبية فى مدينة القاهرة الكبرى ، وتم فحصها عند وصولها إلى المعمل . بالنسبة للطريقة اللونية ، فقد أعطت جميع عينات اللحوم الطازجة لونا أحمر قرمزى ثابت لعدة أيام ، بينما أعطت عينات اللحوم السابق تجميدها لونا أزرق . بالنسبة للطريقة الطيفية ، فقد دلت النتائج على أنه عندما يكون نشاط إنزيم HOADH أكثر من ٣,٠ وحدة دولية/مل فإن ذلك يدل على أن العينات قد سبق تجميدها . أما بالنسبة للدراسة المسحية المحدودة ، فقد دلت النتائج أن ١٠% من عينات اللحوم المفحوصة كانت سابقة التجميد قبل تداولها فى الأسواق .