قسم : الصحة والطب الوقائي +
كلية : الطب البيطرى - جامعة الزقازيق (فرع بنها)
رئيس القسم : أ.د / جمال الدين محمد العليمي.

بعض الطرق الكيميائية الجديدة للكشف عن اللحوم المجمدة والمتداولة في الاسواق

سعد محمود

تم في هذا البحث فحص ثلاثون عينة من لحوم الابقار مأخوذة من بعض محلات الجزارة في المناطق الفقيرة والشعبية في القاهرة.

بعد أخذ عصارة العينات تم قياس نشاط انزيم الـ HADH باستخدام طريقة طيفية وأخرى لونية. وقد اتضح من النتائج أن السوق المصرى وعلى وجه الخصوص في المناطق الشعبية يوجد به بعض اللحوم السابق تجميدها وتباع للمستهلك على أنها طازجة. فقد وجد أن حوالي ١٠٪ من عينات اللحوم المفحوصة كانت سابقة التجميد قبل تداولها.

وعلاوة على ذلك اهتمت الدراسة بمناقشة أهمية وخطورة تداول مثل هذه اللحوم المجمدة في الاسواق بدون رقابة صارمة.

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NEW BIOCHEMICAL METHODS FOR DETECTION OF MARKETED FROZEN-THAWED MEAT

(With 2 Tables)

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(Received at 25/6/1985)

SUMMARY

Random samples of beef meat (30 samples) were collected from different butcher shops in poor districts. After the preparation of press juice, samples were assayed (measurement of HADH activity in the press juice) using a spectrophotometric and colour test techniques. Both techniques gave similar results.

The presence of frozen and thawed beef marketed as fresh food is a fact in some unhygienic Egyptian markets. About 0% of the beef samples were frozen and thawed, then marketed as fresh meat.

The importance and danger of marketing the imported frozen meat without any control have been discussed.

INTRODUCTION

There are two kinds of beef meat in the Egyptian market, namely, the local fresh meat, and the imported frozen one. For sanitary and economic purposes, frozen and thawed food may not be offered for sale without declaration that the food was frozen and that it has to be used as soon as possible. This is certainly so, since acceptability in this type of food is related both to its quality and to the storage life after the food is thawed.

Undoubtedly, the widespread frozen meat in some private butcher shops in poor districts, without any control, may give the chance for adulteration and substitution of other fresh meat by the frozen/thawed one since the latter is considerably cheaper than the former. A keen interest has been felt in discovering this type of adulteration by the food hygienists and Veterinary Services in Egypt.

On the whole, earlier work on this topic was published by GANTNER et al. (1964) who observed that freezing and thawing of skeletal muscle tissue of pigs increase the activity of Glutamic-Oxaloacetic-Transaminase (GOT) and Glutamic-Pyruvic-Transaminase (GPT) in extracts. Moreover, KÖRMENDY et al. (1965) and HAMM et al. (1969) demonstrated the presence of mitochondrial isozyme (GOT_m) of GOT in the skeletal muscles of pigs and cattle. Freezing and thawing of muscles cause a remarkable release of the GOT from the mitochondrial structures resulting in an increase of GOT_m activity in muscle press juice (HAMM and KÖRMENDY, 1969). Thus, the latter authors reported a reliable electrophoratic method for differentiation between unfrozen and frozen/thawed meat. This electrophoretical technique

(GOT_m method time, equipments and some experience to be a reliable routine method (GOTTESMANN and HAMM, 1982). Thereby, two new rapid methods based on the release of another mitochondrial enzyme (B-hydroxyacyl-Co A- dehydrogenase- HADH) into the sarcoplasm (meat press juice) were developed (GOTTESMANN and HAMM, 1982; HAMM and GOTTESMANN, 1982).

The present work has been done to apply these two new biochemical methods to check up or discover the possible adulteration of the marketed fresh by the frozen/thawed one, and also to compare between these two techniques.

MATERIALS and METHODS

Samples :

All materials employed in this study were collected from different private butcher shops in poor districts in Cairo.

For the purpose of the investigation, 30 fresh beef meat samples, each weighing about 100 g, were purchased. For the senstitvity of the determination capability of the techniques, two fresh meat samples and other two frozen/thawed samples of known source were employed as controls.

Preparation of meat juice :

About 20 g of unground meat were squeezed between two thick glass plates using a hydraulic press (about 10 kg/cm^2 pressure). The juice was collected in a clean test tube.

Method :

Samples were assayed according to the two techniques outlined by Sigma Chemical Company (1978) and GOTTESMANN and HAMM (1982).

1- Spectrophotometric technique :

The HADH activity was measured in the press juice according to the following reaction:

$$\text{Acetoacetyl-Co A} + \text{NADH} + \text{H} \xrightleftharpoons{\text{HADH}} \text{B-hydroxybutyryl-Co A} + \text{NAD}^+$$

Procedure :

2.6 ml 0.1 M phosphate buffer (pH 6.0) (Merk), 0.2 ml 34.4 mM Ethylenediaminetetraacetic acid (EDTA), disodium salt (Sigma Co., product No. ED2SS), 0.05 ml 7.5 mM Nicotinamide Adenine Dinucleatide, Reduced form (NADH), disodium salt (Sigma Co., product No. N6005), and 0.1 ml diluted press juice (1:200 with phosphate buffer) were pipetted into a quartz cuvette (1 cm light path) and mixed. Then, 0.05 ml 5.9 mM Acetoacetyl-Co A, sodium salt (Sigma Co., product No. A1625) was added to the mixture and mixed. Immediately, the solution was measured in a spectrophotometer (CECIL, UV/visible scanning, mod, CE599, England) at 340 nm (against air). After a three minute reaction at 25 °C, the extinction was measured again at the same wavelength.

The decrease in extinction per minute (Δ E/min) was caloulated. The HADH activity per ml expressed in International Units per ml press juice (U/ml) was calculated by means of the following formula: $U/ml = \frac{V}{\varepsilon \times d \times v} \times \Delta$ E/min

X dilution factor where, V = volume of the test mixture (3 ml), = extinction coeficient for NADH at 340 nm (6.3), d = light path of the ouvette (1 cm), and V = volume of the meat juice (0.1 ml), dilution factor = 200.

In case of freezing and thawing the HADH activity of the press juice exceeded 3.5 U/ml. Results below this value, were considered fresh (not frozen).

2- Colour test technique :

This technique was based on the following two reactions :

a- Acetoacetyl-Co A + NADH + H $\overset{\text{HADH}}{\underset{\longleftarrow}{\longrightarrow}}$ B-hydroxybutyryl-Co A + NAD^+

b- NDAH + H + meldolablue (oxidized)--- NAD + meldolablue (reduced)
(blue) (colourless)

Procedure :

2.4 ml 0.1 M phosphate buffer (pH 6.0), 0.2 ml 34.4 mM EDTA, 0.2 ml 1.5 mM NDAH, 0.12 ml 5.9 mM Acetoacetyl-Co A, and 0.1 ml diluted press juice (1 : 100 with phosphate buffer) were pipetted into a test tube, mixed and stored for 60 min. Then, 0.1 ml Meldolablue solution (28 mg Meldolablue, Sigma Co., product No. D8142, dissolved in 100 ml distilled water) was added and the mixture was shaken for 30 sec.

If the meat sample was fresh, the solution becomes colourless. If it was frozen and thawed, the solution remains blue.

RESULTS

The results obtained are recorded in table 1 & 2. Table 1 demonstrates the HADH activity in the press juice of the beef samples using both spectrophotometric and colour test techniaues. It is clear that among the 30 beef samples examined 3 samples had HADH activity more than 3.5 U/ml press juice using the spectrophotometric technique and the same these 3 samples gave blue colour using the colour test technique. This indicates that these samples were frozen and thawed. It is also proved that both techniques gave similar results.

DISCUSSION

It is evident from the summarized results indicated in Table (2) that the presence of frozen and thawed beef marketed as fresh food is a fact in some unhygienic Egyptian markets. About 10% of the beef samples were frozen and thawed and marketed as fresh meat. This type of adulteration happened in the poor, public and crowdy localities, and most sources of the samples were taken from small cuts or trimmed meat which seemed to be easy to adulterate.

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 (HARTSELL et al., 1959; HARTSELL, 1962; JAY, 1970). Moreover, there are textural changes associated with freezing that would seem to aid the invasion of surface contaminants into deeper parts of the product and consequently facilitate the spoilage process (JAY, 1970). Much has been speculated about the storage life of thawed as compared to unfrozen meat. It is often stated that thawed meat is more perishable than fresh, unfrozen meat; especially because of the drip exuded from thawed meat, which constitutes a medium favourable for microbial growth (ELLIOTT and MICHENER, 1965; ICMSF, 1980). One effect of freezing and thawing of meat is the release of lysosomal enzymes consisting of cathepsins, nucleases, phosphatases, glycosidases,and other (TAPPEL, 1966; HARPER, 1975). Once released, these enzymes may act to degrade macromolecules (fats, proteins, nucleic acids and others) and thus make available simpler compounds which are more readily utilized by the spoilage flora (JAY, 1970; HARPER, 1975).

Regarding the conditions of thawing, it is undesirable to use temperatures above 10 °C (BAILEY et al., 1974; JAMES et al., 1977). Hence, it is common to observe that bacterial load rises 10 or 100 fold during commercial thawing, leading to a shorter storage life (ICMSF, 1980). Spores of the pathogenic Cl. perfringens remain unchanged in frozen meat, while multiplication can be rapid during thawing under warm conditions. Furthermore, Enterobacter and Salmonella microorganisms could be isolated in large numbers from frozen and thawed beef meat (MOSSEL et al., 1972).

In view of the above, one could therefore expect the danger of marketing the imported frozen meat without any control, especially the unknown conditions o thawing and subsequent handling, processing or storage.

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Table (1)

Results of HADH activity in the press juice using spectrophotometric and colour test techniques for beef samples

No.	Spectrophotometric tech.		Colour-test tech.
	ΔE/min	HADH activity U/ml	
1	0.0027	2.6	colourless
2	0.0026	2.5	colourless
3	0.0019	1.8	colourless
4	0.0024	2.3	colourless
5	0.0018	1.7	colourless
6	0.0048	4.6	blue
7	0.0034	3.2	colourless
8	0.0016	1.5	colourless
9	0.0033	3.1	colourless
10	0.0023	2.2	colourless
11	0.0055	5.2	blue
12	0.0027	2.6	colourless
13	0.0012	1.1	colourless
14	0.0023	2.2	colourless
15	0.0034	3.2	colourless
16	0.0013	1.2	colourless
17	0.0014	1.3	colourless
18	0.0024	2.3	colourless
19	0.0012	1.1	colourless
20	0.0061	5.8	blue
21	0.0025	2.4	colourless
22	0.0024	2.3	colourless
23	0.0013	1.2	colourless
24	0.0023	2.2	colourless
25	0.0022	2.1	colourless
26	0.0015	1.4	colourless
27	0.0023	2.2	colourless
28	0.0016	1.5	colourless
29	0.0017	1.6	colourless
30	0.0024	2.3	colourless

ΔE/min = decrease in extinction per minute at 340 nm

U/ml = International Units per ml press juice

Table (2)

Summarized results indicate the presence of frozen and thawed beef meat marketed as fresh food

State	Spectrophotometric tech.	Colour tech.
Frozen	3 (10 %)	3 (10 %)
Fresh	27 (90 %)	27 (90 %)
Total	30 (100 %)	30 (100 %)