



## Detection of some Meat Adulterants in a Raw Minced Meat Product Model using Frozen Tissue Microarray Technique

Kassem Gehan M. Abdel-Aziz<sup>1</sup>, Khalid A. El-Nesr<sup>2</sup> and Heba H.S. Abdel-Naeem<sup>1\*</sup>

<sup>1</sup>Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

<sup>2</sup>Department of Veterinary pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt.

\*Corresponding author: h.hussein@cu.edu.eg; dr\_hoba.h106@yahoo.com

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### ABSTRACT

In recent years, interest in meat authenticity has been increased. Therefore, twelve groups of beef kofta product were formulated as soya bean, skin, skin emulsion and mechanically deboned poultry meat (MDPM); each of these substances was added with three different concentrations (40, 60 and 80%) in addition to control one. All group samples were subjected to two types of histological examination, conventional paraffin embedding method and frozen tissue microarray. The results of histological examination revealed that both techniques were accurate methods for identification of the separate components of the formulated product and considered extremely useful tools to judge its quality. Moreover, frozen tissue microarray was very helpful in obtaining the result in a short time and allows for examination of a large number of samples at the same time comparing to the conventional method. In addition, no hazardous materials like formalin is used, also no ethyl alcohol or xylene is required in this technique. Therefore, microarray technique is reliable and practicable technique that could be used in food quality control laboratory to assess the quality and safety of meat products.

**Key words:** Beef kofta, Soya bean, Skin emulsion, MDPM, Frozen tissue microarray.

### INTRODUCTION

Detection of meat adulterants is a great challenge for food safety agency where falsification of meat products is considered as a main issue facing all the countries. It can be defined as debasing meat product quality intentionally by either mixing or replacement of inferior substances or by the removal of some valuable ingredient (Frank and Habn, 2003; Orlova *et al.*, 2020). Adulteration of meat products with undeclared species, variety meats, and non-meat ingredients such as chicken skin, Mechanically Deboned Poultry Meat (MDPM) and plant protein at the expense of skeletal muscle is a fraudulent act in the industry and in addition, breaking trust in the meat industry (Flores-Munguia *et al.*, 2000). Based on E.S. (1973/2005) the use of undesirable organs of slaughter animals, including the visceral organs, hyaline cartilage and bone instead of meat in production of beef kofta is prohibited. However, no regulations are present in the E.S. for addition of MDPM to meat and poultry products. Several reasons give chance for meat processor to incorporate such non meat ingredients into meat product. The higher demand for meat products accompanied by

their escalating cost as well as the relative shortage in raw meat makes them prone to its adulteration (Emara and Nouman, 2002). Moreover, it is more difficult to detect adulterant in ground or cooked meat than in intact or fresh meat as the grinding and heating processes cause modification of the sensory attributes of meat including appearance, color, texture and flavor (Ayaz *et al.*, 2006). However, addition of such non meat ingredients to meat product is considered as fraud and does not meet the standard and hygiene food regulation.

The determination of meat products authenticity in the meat industry is attracting increasing amount of attention and the quality control of such meat products could be evaluated by some standard histological methods (Branscheid *et al.*, 2009). Histological examination is a specialized technique enabling direct differentiation and identification of individual components of animal and plant origins. Moreover, it could be used as an accurate tool to analyze meat products for presence of bone fragments and evaluate the suitability or acceptability of the individual tissues for the given product (Ghisleni *et al.*, 2010). Therefore, the aim of the present study is to identify and assess the presence of muscular tissue and the normal

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and abnormal tissues in meat product model (beef kofta) histologically using both the conventional paraffin embedding method and the frozen tissue microarray technique. Moreover, to prove that frozen microarray provides an inexpensive method where the results can be delivered within a short time frame and large numbers of samples can be tested.

## MATERIALS AND METHODS

### Preparation of beef kofta ingredients

Frozen beef meat (Grade II) of first 3<sup>rd</sup> of its shelf life was purchased from local market. Chicken skin was collected immediately from local poultry processing plant after slaughtering and inspection. The skin was frozen at -18°C for 2 days, ground at 2 mm plate grinder (Seydelmann NW 114 E; Stuttgart, Germany) then divided into 2 parts; the first part was used as it is while the second part was used for preparation of skin emulsion.

To prepare skin emulsion, 750 g of frozen minced chicken skin was chopped separately in a bowel chopper with 100 ml water, 3 g sodium tripolyphosphate, 3 g common salt and 150 g corn starch till development of emulsion. The obtained emulsion was kept frozen at -18°C till the next day. Both soya bean and MDPM were ground at 4 mm plate grinder before their use in the processing. Sodium chloride was obtained from a local market while, the sodium tripolyphosphate and seasonings mix were obtained from Loba Chemie, Mumbai, India.

### Beef kofta formulation

For performing the study, five main groups of beef kofta were formulated. The first group was formulated with 100% pure frozen beef meat, 1.8% common salt, 0.3% sodium tripolyphosphate, 0.5% seasonings, and water. This formulation used as control group (pure meat). The rest of 5<sup>th</sup> groups were formulated as control group plus addition of soya bean (SB, 2<sup>nd</sup> group), skin (S, 3<sup>rd</sup> group), skin emulsion (SE, 4<sup>th</sup> group) and mechanically deboned poultry meat (MDPM, 5<sup>th</sup> group). The substitution of pure meat in control group by the above mentioned substances was performed by 3 different concentrations (40, 60 and 80%) for each substance. Therefore, twelve groups were conducted in addition to control group.

### Beef kofta processing and storage

For processing of control group, frozen beef meat (grade II) was partially defrosted and ground with a commercial food processor through a 4mm plate grinder (Seydelmann NW 114 E; Stuttgart, Germany) then transferred to a paddle mixer, where the dry ingredients (common salt, polyphosphates and seasonings) were added as powders slowly while mixing then cold water was incorporated. The addition of ingredients took less than 5 minutes and the final temperature of batters varied between 5 to 10°C. The batter was formed into beef kofta and kept frozen at -18°C. The processing of other groups are the same as control plus replacement of minced meat with 40, 60 and 80% from each soya bean, skin, skin emulsion and MDPM.

### Product investigation

**Physical examination:** All group samples were examined physically for their appearance, color and textures.

**Histological examination:** All group samples were subjected to two types of histological examination as follows.

### Conventional method

Beef kofta samples (1 × 1 cm) from all groups were fixed in 100 ml/L formalin for 24 h, followed washing with running water for 1h. Fixed samples were dehydrated in a chain of upgrading concentration of ethyl alcohol, cleaned in xylene, and embedded in paraffin wax at 70°C in hot air oven for 6 h. Paraffin blocks were sectioned at 4–6 µm thickness, and stained with Haematoxylin and Eosin using the technique recommended by Bancroft and Gamble (2008).

### Frozen microarray

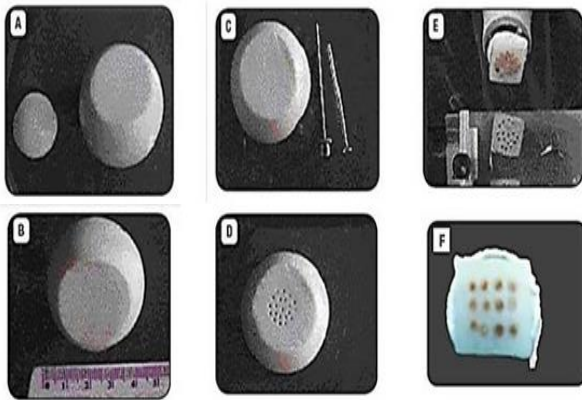
Tissue microarrays from beef kofta samples were done according to Deeb *et al.* (2004). The technique was based on the use of coagulated egg albumin as a mold for the recipient block as follow:

Ten-minute boiled egg was prepared to obtain coagulated egg white, carefully peeled off egg after cooling and used as the recipient block mold. The pointed angle of the boiled egg was removed, to form area of 2 to 3 cm in diameter (Fig.1a: A, B) then the whole egg was cut equality into two parts. The coagulated egg white surface prepared before was punching manually by using needle of 2 mm in diameter (Fig.1a: C, D). Followed by removal of egg yolk and trimming of coagulated egg albumin surface to fit the specimen holder area in the cryostat (Fig.1a: E, F). Cylindrical sample of beef kofta (at 4°C) were gained with fine needle as that used for punching; the obtained tissue cylinders were dipped in egg albumin (fresh) before being inserted into the holes prepared of the recipient block. The array of about 1 mm spaces between punches was created. Using cryostation, sections of 5-8 µm were cut and mounted on glass slides. Heamatoxyalin and Eosin (Fig.1b) was applied to stain the obtained sections according to Bancroft and Gamble (2008).

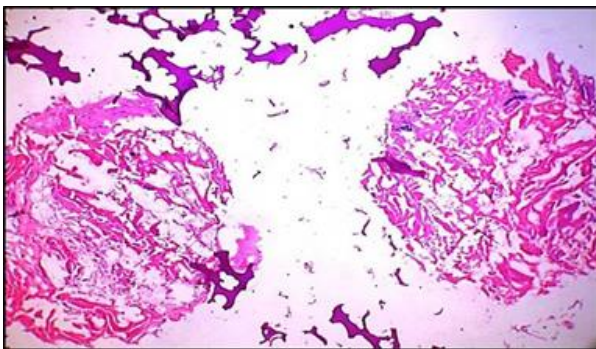
## RESULTS AND DISCUSSION

### Physical examination

Product quality and safety is probably the most important aspect of manufacturing meat products because it addresses the question of consumer acceptance and public health safety. While a processor may produce a wonderful product, it must ultimately satisfy the consumer in terms of appearance, color and texture (Jayasena *et al.*, 2013). In this concern AMSA (1983) pointed out that physical examination of meat and meat products are widely considered to be the most important determinant factor of consumer acceptability. Color is considered a fundamental physical property of meat and meat products, and commonly used as an indicator for composition, quality, formulations and processing conditions. It also facilitates the detection of certain defects in meat and meat product (Hatcher *et al.*, 2004). In addition to, color is the first sensation that influences the consumer purchase decisions and commonly used as an indicator for the acceptance or rejection of the meat products. On the other hand, texture is also an important factor in the process of



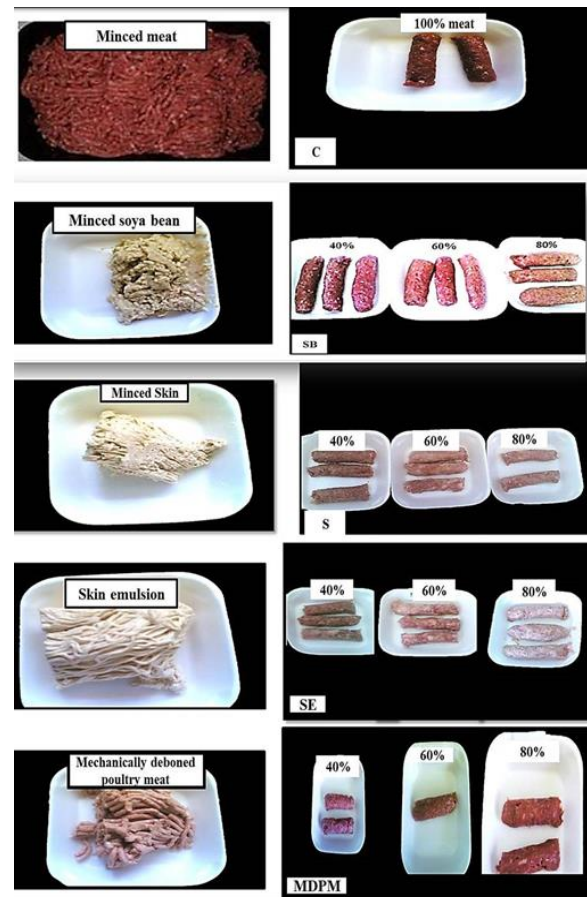
**Fig. 1a:** Construction of recipient block from coagulated egg albumin.



**Fig. 1b:** Histological section of frozen microarray showing more than one sample could be examined at the same time (H & E X40).

selection of meat products and essential in consumer quality perception (Szczeniak, 1990); and being decisive element influencing products acceptability. Therefore, in the retail sector, the buying decision and consumer acceptability for meat and meat products are not only dependent on the attractive and stable color, but also on the variations and distribution of this color in addition to the amount of other morphological features such as defects and marbling or fat-connective tissue (Du and Sun, 2006).

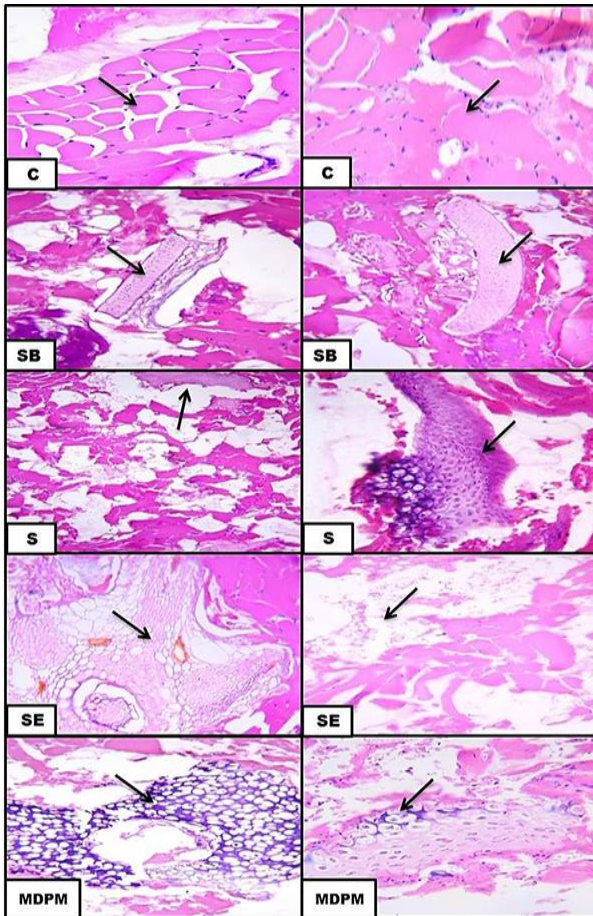
The photographs of experimentally produced beef kofta (Fig. 2) showed varieties of abnormal appearance and colors in soya bean (SB), skin (S) and skin emulsion (SE) formulated groups as compared with control one (C). The color of control group was brick red in color and homogenous in appearance due to pure meat. While, some color changes of other groups ranged from scattered yellow spots in SB formulated beef kofta and scattered whitish spots in S and SE formulated beef kofta groups were detected. These appearance and color deterioration were more intensified in 80% and less in 40% produced beef kofta. While, MDPM improve the color of produced beef kofta and no marked changes were established in the appearance or the color of control and MDPM groups in all concentrations. However, this marked color deterioration (reddish color) is easy to be detected in MDPM incorporated chicken products due to the characteristic whitish color of such products. The aforementioned results clearly indicated that most of meat processors incorporate high levels (80%) of non-meat



**Fig. 2:** Raw meat materials (left column) and experimentally produced beef kofta (right column), control (C), soya bean (SB), skin (S), skin emulsion (SE), mechanically deboned poultry meat (MDPM).

tissues such as soya bean, skin and MDPM at the expense of skeletal muscle in the formulation of different meat products which have negative impact on their quality and safety.

Using avian skin as a cheap ingredient in formulation of meat products in order to decrease the cost is recently practiced into the field of meat processing in Egypt (Emara and Nouman, 2002). Furthermore, with increasing awareness of health and obesity hazards associated with excess dietary fat and consumer demand enforce poultry processors to innovate poultry cuts without skin. Therefore, there are surpluses of poultry skin produced which could be added in the formulation of different meat and poultry products. Its main value is in boosting the typical chicken flavor, juiciness, and for cost reduction. On the other hand, it has the tendency to impart a slightly off-white color to these products and contributes to high microbial risks, especially when the product is not fully cooked and promotes oxidative rancidity (Ukabam, 1998). Concerning the color deterioration caused by addition of skin or skin emulsion, this result was in agreement with Heba *et al.* (2010) who reported that using of chicken skin (comminuted or skin emulsion) resulted in appearance of white spots scattered on the surface of the product. Moreover, it was noticed that the incorporation of chicken fat and skin significantly affects overall acceptability of chicken sausage (Biswas *et al.*, 2007).



**Fig. 3:** Light micrographs of frozen tissue microarray stained with H&E of control (C) untreated beef kofta showing muscle fiber (arrow); 80 % soya bean (SB, arrow) treated beef kofta; 80 % Skin (S, arrow) treated beef kofta; 80% skin emulsion (SE, arrow) treated beef kofta and 80% mechanically deboned poultry meat (MDPM) treated beef kofta showing cartilage (arrow). The magnification power of left column is x20 and right column is x40.

MDPM is commonly used as a major ingredient in producing of emulsified and comminuted meat products either as a binding agent or as an inexpensive source of protein to decrease the cost of the products. It offers the food industry with a raw material with excellent nutritional, good technological and functional properties (Püssa *et al.*, 2009). However, it has some disadvantages, such as rapid onset of oxidative rancidity resulting in off-flavours, colour and texture problems. Moreover, it contains high microbial load which makes it a highly perishable raw material and decrease the shelf life of MDPM incorporated meat products (Tintchev, 2013). The observed color improvement in MDPM formulated beef kofta was disagreement with Trindade *et al.* (2005) who found that MDPM significantly deteriorated appearance and color of mortadella. Moreover, Ch'ng *et al.* (2014) reported that addition of MDPM into emulsion sausage resulted in darker and redder color due to the high content of hemoglobin and ferrous. The color of MDPM significantly affected the color of MDPM incorporated products especially if pigment oxidation occurred (Serdaroğlu, 2005). Presence of hemoglobin in MDPM as a result of bone crushing and release of bone marrow makes it more subject to abnormal color problems since it

is more easily oxidized and more liable for heat denaturation during processing and storage (Hrynets *et al.*, 2011).

Vegetable protein like soya protein could be added to meat products for technological and economic reasons. It could be used as a method of adulteration of meat products to reduce their cost by replacing the more expensive beef (Flores-Munguia *et al.*, 2000). Moreover, Soybean is widely used in processed foods and represents a particularly insidious source of hidden allergens and threat health of consumer due to this allergic reaction (Yang *et al.*, 2011). The detected yellow color of soya bean formulated beef kofta was in agreement with (Ng'ong'ola-Manani *et al.*, 2014) who attributed the yellow color of naturally fermented products to soybeans added.

Concerning texture, SB formulated beef kofta showed granular and hard in texture while S and SE formulated beef kofta is soft in texture and in case of MDPM formulated beef kofta is pasty in texture as compared to control group which is firm and tender. The obtained data were in agreement with the previous observations which proved that increasing rate of MDPM addition makes luncheon sausage pasty (Bodner and Sieg, 2009) and in disagreement with Babji *et al.* (1998) who reported that the addition of chicken skin increased hardness of the frankfurters.

### Histological examination

Meat filling quality parameters such as meat density, size of meat fragments, uniformity of batches, and presence of tissues different from skeletal muscle can be effectively evaluated and predicted using histology and image analysis (Ghisleni *et al.*, 2010). A variety of routine techniques including routine light microscopy with heamatoxyaline-eosin stained sections, special staining, immunohistochemistry, and electron microscopy were used to asses for meat content and for other recognizable components (Prayson *et al.*, 2008). Histological methods especially frozen tissue microarray facilitated the detection of the abnormal contents found in the processed meat (Deeb *et al.*, 2010). Also, they proved that the morphological techniques based on microscopic examination of processed meat, have the advantage of being able to determine the grade of meat depending on how much meat is incorporated during meat processing.

The histological examination of control group formulated with pure beef meat (Fig. 3C) showed that skeletal muscle was the predominant findings in cross and longitudinal sections with few fat cells in between. Plant materials were observed in soya bean (Fig. 3SB) formulated product, however massive aggregation of fatty cells as well as the presence of skin tissue were evident in case of the product formulated with comminuted skin (Fig. 3S) and skin emulsion (Fig. 3SE). Cartilage was the characteristic material in mechanically deboned poultry meat (Fig. 3 MDPM) formulated product. All these structures were observed in all concentration (40, 60 and 80%) formulated product with variable degree. However increasing the amount of additives was associated with uneven distribution of muscular tissues and remarkable reduction in the amount of meat (Ghisleni *et al.*, 2010). Moreover, the quality of meat product was conversely

proportional to amount of additive or the other sources of protein (Prayson *et al.*, 2008).

The incorporation of any undesirable organs of slaughter animals, including the visceral organs, hyaline cartilage and bone in formulation of beef kofta is not allowed (E.S. 1973/2005). Presence of cartilage particles is one of the most important physical hazards in meat products and many gastroenterological side effects may result from frequent ingestion of products containing bone. Potential hazards in terms of impact of bone particles on gums and teeth or between teeth have also been hypothesized. Standard histological methods are ideal methods for the detection of MDPM in meat products as bone and cartilage particles may be indicators for detection of MDPM (Groves, 2011). Moreover, Sephiri (2008) assessed different types of sausages from a histological point of view and observed presence of non meat tissue like chicken skin and hyaline cartilage in heated sausage. At the same time, Latorre *et al.* (2015) concluded that the histological evaluation of meat products markedly showed that the formulation used in the preparation of these products do not respect the standard and hygiene food regulation and the products are not very high quality overall.

The result of the conventional method was similar to that obtained from frozen tissue microarray method. However, the application of frozen tissue microarray method facilitated the examination of different samples at the same time as compared to the conventional method. Where, more than one sample could be examined on the same slide (Fig. 1b), It may be reached to 12 samples according to the size of egg albumen used as shown in Fig1a. Moreover, it introduces an inexpensive, simple and rapid technique for the histological examination of meat products as it reduces the time of sample preparation from 3 days of conventional method to less than hour in microarray technique. Furthermore, no hazard materials like formalin is used, also no ethyl alcohol or xylene is required in this technique. Therefore, microarray technique is reliable and practicable technique that could be used in food quality control laboratory for assessment of the quality and safety of meat products.

### Conclusions

This study substantiated that most of meat processors adulterate meat products with several non meat tissues such as soya bean, skin, skin emulsion and MDPM and use them as major ingredients in the products formulation. These adulterants could be detected using histological technique by both conventional paraffin embedding method and frozen tissue microarray which are extremely useful and reliable to judge the products quality. Moreover, frozen tissue microarrays have several advantages as compared to the conventional method. Where, it provides an inexpensive, simple, safe and rapid technique as it gives chance for examination of large numbers of samples at the same time and obtaining the result in a short time.

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