Applying Light, Histochemical and Scanning Histological Methods for the Detection of Unauthorized Animal and Herbal Content in Street Meat Sandwich: What is in the Sandwich We Eat?

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Abstract

Samples of the total of 105 different meat sandwiches products were examined (Kofta, Hawawshi, and shawerma sandwich), 35 sandwiches from each type of product were collected from New Valley City from different restaurants during the year 2016 and analyzed by light and scanning electron microscope for detection of meat adulteration. Select half samples from each group for light and histochemical microscopic examination and the rest of the same group for scanning electron microscopic examination. The sections were stained using hematoxylin and eosin, PAS, Wigier's and Crossman's trichrome, bromophenol blue and ATPase. The histological examination revealed that a variety of tissue types besides skeletal muscle were observed including connective tissue fibers, Lung, ruminant stomach, Large elastic blood vessels, heart muscle, adipose tissue, cartilage (hyaline and white fibrocartilage) and spongy bone, lymphatic tissue (spleen), plant materials, in addition to sand particles. With use ATPase enzyme staining can suspect fetal tissue in Hawawshi meat with abundant dark (slow contracted) muscle fiber than light (fast contracting) muscle fibers. The findings of the present research suggest the histological technique as an effective method for qualitative evaluations of street meat sandwich adulteration.

Keywords: Street meat sandwich, Meat adulteration; Histochemical microscopic examination

Introduction

From a socioeconomic point of view, street food plays a great role in conflicting food and nutritional provisions consumed in different cities at cheap payment to the lower and middle livelihood people chiefly meat products which are well known to complement at least half of marketable meat that enclose heat cooked or processed meat [1]. Intentional or economically-motivated adulteration of food has recently been defined as “the fraudulent addition of nonauthentic substances or removal or replacement of authentic substances without the purchaser’s knowledge for economic gain of the seller” [2]. Food adulteration is considered by the USA are conceivable food safety issues on the authority that adulteration of food is simulated that people have narrow or no awareness of any possible food safety indications of their actions. In order to present higher conservation to society, strategic regulations must be established on the hypothesis that food safety may be settled by adulteration is deserved [2]. Alongside from the adulteration form, some animal tissues such as central nervous tissues could be a way to infect human beings [3] inform of bovine spongiform encephalopathy (BSE) which is known to be set by feeding cattle with scrapie infected sheep tissues [4]. It has been recorded that both human new variant Creutzfeldt-Jakob disease (vCJD) and BSE belong to the family of fatal TSE diseases, both contribute the same way of infection, namely the abnormal prion protein (PrPsc), that bundles in cytoplasmic vesicles in the infected individuals’ and animals’ brains and is very resistant to heat [5]. The meat of great economic value so the usage of fraudulent tissues in meat products is unwanted but probable. Formerly, many research works have reported that histological examination as an efficient technique to detect unauthorized tissues in some meat products [6]. This study aimed to use the histological technique as a simple and inexpensive method for determination of unauthorized and herbal content in street meat sandwich. This study aimed to use histological techniques as simple and inexpensive methods for determination of unauthorized animal and herbal content in street meat sandwich.

Materials and Methods

Collected samples

Samples of the total of 105 different meat sandwiches products were examined (Kofta, Hawawshi, and shawerma sandwich, 35 sandwiches from each type of product were collected from New Valley City from different restaurants during the year 2016 and analyzed for detection of meat adulteration.

Sample selection

Select half samples from each group for light microscopic examination and the rest of the same group for scanning electron microscopic examination. In addition to unfixed selected frozen sections (10 μm) were obtained.

Sample preparations for light microscopic examinations

Six different areas were obtained from each sample from different parts (size of the sample about 1 cm long and 0.5 cm thickness) and finally, each sample has six blocks represented different parts. During trimming of samples found hard structure and very difficult in cutting, then selected sample were put in decalcifying agent (neutral buffer formalin-nitric acid) composed of the following constituents for 20
days: 20 ml nitric acid-80% 20 ml-10% neutral buffer foramalin, 160 ml distilled water [7]. Then Specimens were washed by 0.1 M Na-phosphate buffer (pH 7.2-7.4), then immediately fixed in Bouin’s fluid for 2 h. Bouin’s fixed samples were extensively washed in 70% ethanol (3 x 24 hours) to get rid of the fixative before the subsequent steps of tissue processing for preparation of paraffin blocks. Fixed samples were dehydrated in ascending grades of alcohols at 80%, 90% for 3 hours at each concentration and 100% for two hours. The samples were cleared using methyl benzoate. Dehydrated samples were then impregnated and embedded in Paraplast (sigma Aldrich). Serial sections of 5-7 µm were cut using a Richert Leica RM 2125 Microtome, Germany and mounted on glass slides. Sections were kept in an incubator at 40°C for dryness and stained with Hematoxylin and eosin stain [8] and used for general histological examination.

### Histochemical investigations

The following staining methods were used: Crossmon’s trichrome for collagenous fibers and to differentiate between the different tissue constituents [9]; Weigert [10] for staining of elastic tissue; the periodic acid-Schiff reaction (PAS) according to McManus [11] and Representative sections were stained with bromophenol blue stain for detection of proteins [12]. All staining were cited by Bancroft et al. [7]. Stained sections were then; examined using DMLS light microscope (Leica, Germany) outfitted with MC120 HD camera (Leica, Germany).

Adenosinetriphosphatase (ATPase) histochemical staining: The adenosine triphosphates (ATPase) stain is to use as a histochemical stain for to distinguish between muscle fiber types. Individual muscle sections were stained with either acidic myofibrillar ATPase, alkaline myofibrillar ATPase, for assessment of primary fibers and secondary fibers [13]. ATPase is method based on contractile type alone, as revealed by myofibrillar [14]. Different researchers have used this method for classifying muscle fiber types in different species, Peter et al. [15] in Pork, Picard et al. [14] and Crosier et al. [16] in bovine, De Freitas et al. [17] in ovine, Francisco et al. [18] in buffalo. Histochemical analysis of ATPase was performed on unfixed selected frozen sections (10 µm) were obtained in Leica cryostat CM 1900-6-1 (Richert, Germany) and stained with varying pH (acidic pH 4.2 and alkaline pH 9.4). The procedure was done as the description in Bancroft et al. [7]. During the staining procedure, different muscle fiber, type I (FOG, fast-twitch-oxidative-glycolytic, dark fiber) and type II (Light fiber, SO, slow-twitch oxidative) may be differentially stained [7]. Histochemically, slow fibers display high mATPase activity under alkaline conditions and low activity under acid conditions (alkali-stable, acid-la-bile), whereas slow fibers exhibit the inverse (alkali-labile, acid-stable) [19].

Morphometrical and statistical analysis of ATPase enzyme: Dark and light muscle fibers were counted using Image J in the different meat product. Fiber counts were performed to estimate fiber number of dark and light muscle cells /500 mm² using 20x objective in each product. All the data are expressed as the mean ± SE.

Sample preparations for scanning electron microscopic (SEM) examinations: Small specimens were Selected from different areas from the rest of Half samples from each group, then washed with 0.1 M Na-phosphate buffer fixed in Karnovsky fixative (10 ml paraformaldehyde 25%, 10 ml glutaraldehyde 50%, 50 ml Phosphate buffer and 30 ml DW) [20] for 4 hours at 4°C, then was used for SEM examination. Thereafter, they were washed in the same buffer used in fixation 5 minutes x 4 times and post-fixed in 1% osmic acid in 0.1 M Na-phosphate buffer for further 2 hours at room temperature. They were washed by 0.1 M Na-phosphate buffer 15 minutes x 4 times. The samples were dehydrated by alcohol 50%, 70%, 90% for 30 min in each concentration and 100% for 2 days with changes many times followed by isomyl acetate for 2 days and then subjected to critical point drying method with a polaron apparatus. Finally, they were coated with gold using JEOL -1100 E-ion sputtering Device and observed with JEOL scanning electron microscope (JSM – 5400 LV) at KV10 at the Electron Microscopy Unit of Assiut University.

### Digital coloring scanning electron microscopic images:

To increase the visual contrast between several structures on the same electron micrograph, we digitally colored specific structures either of animal or plant origin (bone, lung tissue, heart, ruminant stomach, blood vessels, fascia and different parts of plants, stem, root, leaves, etc.) to make them more visible to the untrained eye. All the elements were carefully hand colored using the Photo Filtre 6.3.2 program. Coloring images required to change the color balance using the stamp tool to color the objective structures.

### Results

In paraffin sections, hawawshi samples were adulterated with lung, hyaline cartilage, Fascia, white fibrocartilage, bone, vascular tissue, tubular organ, spleen, adipose tissue. Lung tissue was distinguished by the alveoli and basophilic cartilage (Figures 1A and 1B). Hyaline cartilage was marked by the presence of chondrocytes embedded in the basophilic cartilage matrix (Figures 1C and 1D). Fascia composed of dense regular collagenous connective tissue (Figure 1E). The white fibrocartilage was characterized by the presence of row aligned chondrocytes parallel to collagen fibers (Figure 1F). The bone tissue could be differentiated by osteocytes (embedded in the bone matrix (Figures 1G and 1H). Vascular tissue was represented by the elastic and muscular artery. The elastic artery was determined by the regularly arranged elastic fibers (Figure 1I). While the muscular artery was identified by the muscular layer in the tunica media (Figures 1J and 1K). The tubular organ was identified by the muscular coat which comprised of smooth muscle fibers (Figure 1M). The spleen was marked by red and white pulps (Figures 1N and 1O). The adipose tissue contained white fat cells (Figure 1P).

Scanned samples of Hawawshi were adulterated with different organs of animal origin. Lung tissues were identified by the presence of alveoli (Figures 2A and 2C). Samples were also contained different organs of the gastrointestinal tract including ruminant stomach, glandular stomach, fascia, bone and vascular tissue. The reticulum was characterized by honey-comb shaped reticular folds (Figures 2D and 2E) and the omasum was recognized by the omasal lamina (Figure 2F). The Glandular stomach was marked by the presence of gastric pits (Figure 2G). Fascia appeared as regularly arranged collagenous fibers (Figures 2H and 2I). Spongy bone was recognized by osteocytes embedded in the bone matrix (Figure 2J). Vascular tissue contained wide blood vessels which filled with blood (Figure 2K). By light microscopy, hawawshi samples were adulterated with plant tissues including leaves, stem, and root. plant stem was determined by the epidermis (Figures 3B, 3P and 3Q), parenchyma cells (Figures 3B, 3C, 3H, 3I, 3F and 3W), sclerenchyma (Figures 3A-3D , 3G, 3V and 3X), vascular tissue (Figures 3E, 3J and 3L), and collenchyma (Figures 3F, 3V and 3W). Parenchyma cells of the stem of some plant were rich in starch granules (Figure 3O). Plant leaf was identified by mesophyll (Figures 3K-3R and 3S). The root of some plant was recognized in...
Note: Paraffin sections of samples of Hawawshi stained with H & E (A, E, F, G, I, J, K, L, M, N, O, P) and bromophenol blue (B, C, D, H) showed adulterations with different animal tissues and organs. A, B: lung tissue was identified by Alveoli (a), basophilic cartilage remnants (C). Note blood vessels (Bv). C, D: hyaline cartilage (h) contained chondrocytes (arrow). E: Fascia composed of dense regular collagenous connective tissue. Note, regularly oriented collagen bundles (c), arrow refers to the fibroblasts which had oval to the flattened nucleus. F: white fibrocartilage contained chondrocytes with rounded nuclei (arrow) arrange in rows which aligned parallel to collagen fibres (c). G, H: A part of bone tissue contained osteocytes (arrows) which embedded in the bone matrix (b). I, J, K: muscular arteries identified by the muscular layer (m) in the tunica media. L: a part of the wall of the elastic artery contained regularly arranged elastic fibres (e). M: A part of the muscular coat of the tubular organ which contained smooth muscle fibres (m). N, O: spleen (s) contained red (r) and white (w) pulp. P: a part of the adipose tissue contained white fat cells (F).

Figure 1: Adulterations of Hawawshi samples with animal tissues.

Note: Digitally coloured scanning electron micrographs of animal tissue in Hawawshi samples. A, B, C: lung tissues (blue coloured) contained of alveoli D: low magnification showed ruminant stomach (blue coloured). E: higher magnification of ruminant stomach showed the mucosa of the reticulum which was characterized by honey-comb shaped reticular folds (blue coloured). F: higher magnification of ruminant stomach showed the mucosa of the omasum which was characterized by omasal lamina (blue coloured). G: showed the mucosa of the glandular stomach which was marked by presence of gastric pits (blue coloured). H, I: showed fibrous tissue (fascia) which was composed of regularly arranged collagenous fibres (blue coloured). J: showed bone tissue of spongy type K: showed vascular rich tissue. Note, the wall of the blood vessels (red coloured).

Figure 2: Adulterations of Hawawshi samples with animal tissues.

meat samples (Figures 3T and 3U). The onion was recognized by the epidermal cells (Figure 3P and 3Q).

Scanned samples of Hawawshi were adulterated with leaf and stem of some plants. The stem of the plant was distinguished by the parenchymatous cells (Figures 4A-4C and 4O), Xylem (Figures 4F and 4G), and phloem vessels (Figure 4H). Plant leaf was recognized by outer epidermal and the covering cuticle and vascular tissue such as...
Note: Paraffin sections of samples of kofta stained with H&E (A, B, D, E, G, H, K, M, P, Q, T, U), bromophenol blue (C, I, J, L, O, R, S, V, W, X), PAS stain (F) showed adulterations with the plant. A: Apart of plant stem (s) inside meat sample. B: higher magnification of the plant stem in (fig A) showed epidermis (e), parenchyma cells (P), sclerenchyma (sc). C: plant stem showed parenchyma cells (P), sclerenchyma (sc). D: Plants stem (s). E: A part of plant stem with vascular tissue (v). F: A part of plant stem with collenchyma (co). G: A part of a plant stem contained sclerenchyma (sc). H: parenchyma cells (P) of the plant stem. I: Parts of plant stem with parenchyma cells (P) rich in starch granules. J: Vascular tissue (v) of the plant. K: mat sample adulterated with plant leaf which was identified by mesophyll (m). L: Vascular tissue (v) of the plant. M, N: plant leaf contained mesophyll (m). P, Q: epidermal cells (ep) of the onion. R, S: plant leaf contained mesophyll (m). T, U: meat sample contained the root of the plant (r). V, W: plant stem showed Xylem composed of tracheids (T) and larger vessels (L). I, J: a particle of plant wood (yellow coloured) inside the muscles (red coloured). K, L: showed the surface of the onion epidermal cells (E) which had a characteristic rectangular shape. M-O: showed the part of plant stem (light blue coloured) note xylem (X) and phloem (P).

**Figure 3:** Adulterations of Hawawshi samples with plant tissues.

Note: Digitally coloured scanning electron micrographs of plant tissue in Hawawshi samples. A: low magnification showed the stem of the plant. Longitudinal section in the parenchymatous cells (light blue). Xylem vessels (pink). B, C: high magnification of the plant stem showed Xylem vessels (pink) and parenchymatous cells (p). D, E: Cross section in disintegrated plant leaf showed outer epidermal layer (blue coloured) and the covering cuticle (deep blue coloured). F: low magnification of plant leaf showed vascular tissue. Note, longitudinal section in Xylem vessels (pink coloured) and cross section of Xylem vessels (light blue coloured). G, H: higher magnification of the plant leaf showed the vascular tissue of the plant. Note, longitudinal section in Xylem vessels (pink coloured) and cross section of Xylem vessels (light blue coloured). Xylem composed of tracheids (T) and larger vessels (L). I, J: a particle of plant wood (yellow coloured) inside the muscles (red coloured). K, L: showed the surface of the onion epidermal cells (E) which had a characteristic rectangular shape. M-O: showed the part of plant stem (light blue coloured) note xylem (X) and phloem (P).

**Figure 4:** Adulterations of Hawawshi samples with plant tissues.
Xylem vessels which composed of tracheids and larger vessels (Figures 4D, 4C, 4M and 4N). A particle of plant wood could be observed in meat sample (Figures 4I and 4J). The onion could be identified by their characteristic rectangular shape epidermal cells (Figures 4K and 4L).

In Paraffin sections, kofta samples were adulterated with different animal tissues and organs including heart, vascular tissues, lung tissue, adipose tissue, nerve trunk, fascia, hollow organs, and bone. Heart tissue was distinguished by Purkinje cell fibers (Figure 5H), cardiac muscles (Figures 5E and 5F) and the associated large arterial vessels which had prominent elastic fibers (Figures 5A and 5B) and muscular arteries which were predominated by smooth muscle fibers (Figures 5C, 5D, 5Q and 5R). Meat samples contained adipose tissue which consisted of white fat cells (Figures 5G). Lung tissue was determined by the presence of lung alveoli and basophilic stained hyaline cartilage (Figures 5I-5L). Nerve trunk, which composed of myelinated axons, was observed in meat samples (Figures 5M and 5N). The fascia was identified by regularly arranged dense collagen fibers (Figures 5O and 5P). Meat samples also contained hollow or tubular organs which were recognized by the muscular coat; inner circular and outer longitudinal smooth muscle fibers (Figure 5S). Kofta samples were adulterated by bone tissue (Figure 5T). Scanning samples of kofta showed adulteration with different organs of animal including heart, bone, Vascular tissue, lung tissue, ruminant stomach. The heart was identified by the presence of the papillary D (Figure 6A-6C); a bone tissue of spongy type was recognized in meat samples (Figures 6D and 6E). Vascular tissue contained artery (Figure 6F). Lung tissue characterized by alveoli (Figures 6G-6I). The Reticulum of the ruminant stomach was characterized by honeycomb shaped reticular folds (Figures 6J-6L). By paraffin sections, Kofta samples contained different parts of the plant; leaves and stem. Plant leaves were identified by cuticle, epidermis, mesophyll, parenchyma cells, and vascular tissue (Figures 7D, 7E, 7K-7O and 7Y). Some of the mesophyll cells contained starch granules. Plant stem was recognized by the epidermis, sclerenchyma, collenchyma, and a prominent vascular tissue including Xylem and phloem (Figures 1P-1X, 7A-7C and 7F-7J). Scanning kofta samples showed parts of plant tissue; stem and leaves. Plant stem was distinguished by parenchymatous cells vascular tissue; xylem. Plant leaves were identified by cuticle, epidermal cells, and cortical cells (Figures 8A-8I). Parts of onion were recognized by the outer epidermal cells (Figures 8J-8L).

Scanned kofta samples were contaminated by different types of microbes. The surface of the muscle samples was contaminated with cocci-shaped bacteria, rod-shaped bacteria, thread-like fungi (Figures 9 D-9G). A part of heart tissue was contaminated by cocci-shaped bacteria (Figures 9A and 9B). The mucosa of the reticulum was contaminated by cocci-shaped bacteria (Figures 9H and 9I).

In paraffin sections, samples of shawerma were adulterated with animal and plant tissues. Meat samples were adulterated with adipose tissue which contained fat cells and blood vessels (Figures 10A and 10B). The samples also contained nerve trunk (Figure 10C), cartilage tissue which was distinguished by the basophilic matrix and lacuna (Figure 10C). Meat samples had a part of plant leaf which was recognized by mesophyll and plant stem with parenchyma cells (Figures 10E and 10F).

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**Note:** Paraffin sections of samples of kofta stained with H&E (A, E, F, G, N.O, P, Q, T ) and bromophenol blue (D, I, M, S), Weigert stain (B, C, H, R), Crossman’s trichrome (I, JK) showed adulterations with different animal tissues and organs. A: B: large elastic artery note tunica media rich in elastic fibres (e). D: large blood vessels associated with the elastic artery (e). E: the wall of a blood vessel (bv). F: large blood vessel (bv). G: cardiac muscles (m). O: adipose tissue contained white fat cells (f). H: showed heart with Purkinje cell fibres (p). I- L: lung tissue contained alveoli (a) and hyaline cartilage (h), blood vessel (bv). M, N: showed nerve trunk composed of myelinated axons (A). O, P: meat samples contained fascia (F) which composed of regularly arranged dense collagen fibres. Note, blood vessel (bv). Q: muscular artery had smooth muscle rich tunica media (m). R: long thin artery note, elastic fibres rich tunica media (m) and prominent lamina elastic interna in tunica intima (f). S: Apart of the muscular wall of the hollow organ. Note inner circular (c) and outer longitudinal (l) smooth muscle fibres. T: spongy bone. Note bone matrix (b).

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**Figure 5: Adulterations of kofta samples with animal tissues.**
Note: Digitally coloured scanning electron micrographs of animal tissue in kofta samples. A, B, C: Meat samples contained a part of the heart. Note: papillary muscles of the heart (P). Cardiac muscles (cm), D, E: bone tissue (brown coloured) within the muscles (red coloured). Note bone was of spongy type (S). F: An artery (pink coloured) inside the muscular tissue (red coloured). Note peri-arterial connective tissue (violet color). G, H, I: lung tissue (blue coloured) characterized by alveoli (a). J, K, L: Reticulum (r) of the ruminant stomach characterized by honey comb shaped reticular folds.

Figure 6: Adulterations of Kofta samples with animal tissues.

Note: Paraffin sections of samples of kofta stained with H&E (A, F, K, L, S) and bromophenol blue (C, D, E, N, R, T, U, V, X, Y), Weigert stain (G, Q, O), Crossman’s trichrome (B, H, L, J, M, P) showed adulterations with the plant. A, B, C: cross section in a part of plant stems which was identified by epidermis (ep) parenchyma cells (P), collenchyma (co). D, E: transverse section in plant leaf which was composed of the epidermis (ep), mesophyll (m), vascular tissue (v). F: G: Cross sections in plant stem which had a prominent vascular tissue (v) and epidermis (ep). H, I, J: a part of the plant leaf vascular tissue (v) and epidermis (ep), parenchyma cells (P), collenchyma (co). K, L, M, N, O: plant leaf contained outer epidermis which consisted of cuticle (c) and epidermal cells (e), mesophyll cells contained starch granules (s), sieve tube (st). P, Q, R, S, T, U, V, X and Y: A part of plant stem which could be recognized by epidermis (ep), parenchyma cells (P), collenchyma (co), sclerenchyma (sc), vascular tissue (V) including Xylem (X), phloem (p). W, Y: a part of a plant leaf. Note mesophyll (m).

Figure 7: Adulterations of kofta samples with plant tissues.
Scanned samples of shawerma samples contained different organs and tissues of the animal. Meat samples contained fibrous tissue which had few fat cells (Figures 11A and 11B). Meat samples was an adulterated by lung (Figures 11C and 11D), spongy bone (Figures 11E and 11F) and different parts of ruminant stomach particularly omasum and glandular stomach (Figures 11M, 11N, 11O and 11P). A large part of intact stomach filled with ingesta which contained herbal contents and Pollen grains (Figures 11I and 11L). Moreover, Scanned samples

Figure 8: Adulterations of Kofta samples with plant tissues.

Figure 9: Contamination of Kofta samples by microbial cells.
of shawerma samples contaminated by microbial cells which were rod-shaped bacteria (Figures 11G and 11H).

Scanning samples of shawerma adulterated with plant tissue and sand particles. Plant stem was identified by the vascular tissue; xylem (Figures 12A-12E). A part of the onion was observed in the meat sample (Figure 12F). The sand particle was recognized in the meat sample (Figures 12G and 12H).

**ATPase staining result**

Table 1 Reaction for myofibrillar ATPase, in different meat product after preincubation in alkaline medium (pH 9.4) and in acidic media (pH 4.2), to evaluate the contractile ability of muscle fiber types. Slow (dark) and fast (light) fibers. According to the counting of fibers in the different meat product, kofta, shwerma and hwawashi, (Figure 13 and Table 1) found that reactivity in the slow (dark) fiber after...
preincubation in alkaline medium (pH 9.4) was more than fast (light) fiber in hawawshi other product. We suspect that the hawawshi are adulterated with fetal flesh. In addition to the reactivity of ATPase in muscle fiber we are observed the reaction after preincubation in alkaline medium (pH 9.4) and in acidic media (pH 4.2) in different parts of plants (Figure 14).

Discussion

In Egypt, variable meat sandwich sold invariant cities in different governorates as hawawshi (made from minced beef or mutton meat mixed with chopped onion, garlic, black pepper, spices, salt and some fat), Kofta (prepared from cow or sheep meat, salt, black pepper, onion, garlic in addition to amount of fat) and shawerma (made from slices of ground cattle meat or chicken in our study shawerma composed of slices of beef meat, fat tomato, green pepper, onion, black pepper and salts). But our study explores a thunderbolt though you must think before eating. What is the real constituent of street meat sandwich which is a far-reaching maintenance of our health diet?

12 hours of our day life are outdoors subjecting our feed dependence on street meat sandwich at lunch time as quick food and fewer prices especially in developing countries. Fewer prices mean the ability of adulteration as meat is so expensive in developing countries. Evaluation using histological and histochemical methods markedly demonstrated that the constitutions used in preparing these products do not reverence the standard and hygiene food regulation and the products are comprehensively bad quality. Electron microscope and ATPase staining pinpointed the unpermitted tissues. Besides the skeletal muscles, the samples included lung, heart, spleen, bone, cartilage, fibrous connective tissues, hollow tubular organs, plants, sands, fat, pollen grains, suspecting fetal tissues, sand and more illegal tissues illustrated in our results. Skeletal muscle, the normal “meat” of street meat sandwich, virtually composed of one type of cell that is marked in minute fragments, but other tissues or organs detected by some structural arrangement or by the affiliation of certain cell types. The heating and cooking degree does not prevent the histological and histochemical detection of the meat constituent used [21]. Various studies were compiled for disclosure of unauthorized tissues in meat and meat products. The USA conveyed histological examination on eight different brands of hamburgers. Paryson et al. [22] and demonstrated the presence of plant material, adipose tissue, blood vessels, peripheral nerves, bone, and cartilage. Lattore et al. [1] distinguished uncommitted tissues in processed meat products besides the skeletal muscles included gizzard, gland tissue, cartilage, soya, ovary, connective tissue, adipose tissue and lymph node. Histologically smooth muscles and soya tissues identified by Rokni et al. [23]. In Iranian heated sausage salivary gland and nuchal ligament have been investigated that conveys the use of meat achieved from the head of slaughtered animals meat products [24]. Assessment by Sepheri [24] revealed the presence of peritoneal fat, chicken skin, hyaline, cartilage

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Table 1: Reaction for myofibrillar ATPase, in different meat product after pre-incubation in alkaline medium (pH-9.4) and in acidic media (pH-4.2), to evaluate the contractile ability of muscle fiber types. Slow (dark) and fast (light) fibers.
Agreement to the histological estimation of hamburger, minced meat, and kabab loghme demonstrated the residence of fraud tissues such as adipose tissue, plant material, nerves and cartilages, blood vessels that in correspondence with our observation. Sadeghinezhad et al. [25] scoped on qualitative and quantitative accuracy of histological examination for the dedication of herbal content and unauthorized animal in minced beef meat as 5%, 10%, 15% and 20% of soya and chicken gizzard were composed. The presence of ruminant stomach in hawawshi, kofta and shawerma settled with Baskaya et al. [26] as they examined 27 ready to sell minced meat samples in Ankara and found cartilage tissue (11.1%) of the samples, and organs related to alimentary canal in another 3 (11.1%) of the samples tested. Little inedible tissue traces identified by Yildiz et al. [27] of 75 ready to sell meatball samples in Istanbul.

Citizen et al. [28] showed the presence of non-meat materials such as mechanically deboned meat and ground bone fragments to processed meat. The presence of all morphological characters and structure of the plants as a whole with presence of pollen grains in shawerma indicate that all of the contents of these meat sandwich not only minced meat or slices of meat, additives or spices but also the food contents of the stomach of animals in which its meat muscles are used in the sandwich which led to contamination also it may be due to unhygienic measures during preparation as it appeared with presence of different microbial cells whether rods, cocci bacteria which may lead to food spoilage and food poisoning in addition to the presence of thread-like fungi in kofta in spite perfectly an internal temperature of 80°C should be attained to distinct in raw meat mixes. Nevertheless, not only adulteration by unauthorized tissues but also a source of specific species of bacteria likes Staphylococcus aureus, Campylobacter jeguni, Escherichia coli O157H7 and Salmonella. Because of its great level and its liability to detent pathogenic bacteria, minced meat is an important product to make a threat to human health [28]. The existence of hollow organs or tubular organs in kofta and hawawshi suspect the presence of the intestine or uterus, the especially uterus is prohibited and forbidden to be used in according to religious factor. Different researchers were observed the similar result of the unauthorized organ by histological methods. Rezain and Rokni [29] were observed that the udder, salivary gland, lymph node, skin and its accessories are unauthorized organs authenticated by the histological study of heated meat products in Iran. Citizen et al. [28] observed in processed meat products foreign tissues originated from poultry as well as Kidneys, skin, heart, bones, cartilage, gizzards heart and lung. TFC [30] itemized the structural elements required to be added in the composed meat mixtures (Skeletal muscles and a small amount of fat). As the presence of unauthorized tissues as inedible parts with high amounts of fat horns, bones, cartilage and nail are prohibited; the tissues antithetically alter the structural element of processed and manufactured meat products and originate biased antagonism and profit.

The high presence of fascia (dense fibrous connective tissue), cartilage either hyaline or white fibrocartilage, in an excessive amount in all samples in accordance with Citizen et al. [28] as they implicated their addition reduce the quality and nutritional value of meat used. Knowing the differences between skeletal muscle fiber types as type I (dark-slow fiber) and type II (Light-fast fiber) is important for researchers and of particular interest for the food industry because meat tenderness depends in part on the proportion of the different types of fibers [14]. In addition for detection of fetal and adult flesh disease and kidney in sausage. Fraud authentication detected by Sadeghi et al. [25] counting esophagus, heart muscles, cartilage, bone, lymph nodes and spleen in sausage samples as shown in kofta and hawawshi samples.

Note: A, B: Kofta samples, C and D: Hwawashi samples adulterated with plant stained with ATPase at pH 9.4. A and B: a part of plant stem contained ATPase–ve sclerenchyma (sc) and vascular tissue (v). C, D: plant leaf had ATPase–ve epidemis and ATPase +ve mesophyll (m). E, F, G, H: Hwawashi samples adulterated with plant stained with ATPase at pH 4.2 E: plant leaf had ATPase +ve mesophyll (m). F: part of plant stem contained weak and strong ATPase +ve collenchyma (co). G, H: part of plant stems showed weak and strong ATPase +ve parenchyma cells (P)

Figure 13: Thick frozen section (10 μm) section stained by m-ATPase enzyme.

Figure 14: Adulterations of kofta and hawawshi samples with plant tissues and stained with ATPase at pH 9.4 and 4.2.
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