Research Article

Using Histological and Chemical Methods for Detection of Unauthorized Tissues Addition in Emulsion Type Meat Product

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ABSTRACT

Total twenty different processed meat plant producing emulsion type sausage were histologically and chemically examined for detection of adulteration with unauthorized tissues. Results revealed that samples were adulterated with different types of animal tissues included; hyaline cartilage, tendon, spongy bone, peripheral nerve trunk, basophilic matrix, lymphatic tissue, fascia, fibrocartilage and vascular tissue. Moreover, these samples were adulterated Also, adulterated with plant tissue included; plant stem, leaves and root. Chemical analysis showed a significant difference in their chemical composition (moisture, fat, protein, ash and calcium) content. Moisture and fat content varied around the permissible limit of E.S.S. while low protein, high ash and calcium content was detected in the examined samples. Therefore, Histological and chemical examinations can be used as reliable methods to detect adultration using unauthorized addition of both animal and plant tissues in processed meat product samples which revealed a high level of falsification.

Key words: Bone content, MDM, Processed meat, Plant fiber, Unauthorized tissues.

INTRODUCTION

Meat considered one of the high cost food item especially with high poverty percentage in developing countries (Hagen-Zanker *et al.*, 2011), therefore increasing the world population raised the need for a lower cost meat alternatives, usually meat products provides a satisfactory alternative with delicious variable taste, longer shelf life and mainly lower prices (Delgado, 2003 and Rohrmann, 2016). Unfortunately, most meat products especially emulsion types subjected to various forms of mincing and chopping leaving a chance for being adulterated either through using a low quality meat or by substituting (complete/partial) raw meat materials by offal's, skin, mechanically recovered meat and plant based additives. However, these items are allowed to be used in meat products for many technical aspects i.e. binding, texture, water holding, flavoring etc. under certain regulations within the permissible limits. Many processors abuse their use which poses a form of consumer adulteration as well as they may cause a serious health hazards (Vaňha *et al.*, 2009; Ballin, 2010 and Esslinger *et al*., 2014). Food adulteration defined internationally as "the fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller". According to the US Code "if any substance has been added to, increasing its bulk or weight, reducing its quality or strength, or making it appear better or of greater value than it is" especially when people have low or no awareness of the possible food safety consequences (Moore, *et al.* 2012 and U.S. law [21 U.S.C. § 342]). Although emulsion type products cooking may affect the different structures the histological methods provide a quite powerful tools to identify such adulteration (ISIR, 2016). The aim of this work was to use histological techniques as well as chemical analysis to investigate one of emulsion type sausages (Traditional Egyptian Luncheon) adulteration of twenty different meat processing plants located in Egypt.

MATERIALS AND METHODS

Sample preparation for light microscopic examination

Samples of traditional Egyptian luncheon from twenty different meat processing factory (at least three samples from each factory) were collected and examined.

Samples from four different areas of each product were collected. Then were fixed in 10% neutral-buffered formalin for 48 hrs. The fixed samples were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax, then 3 – 4 μm thick sections were obtained by rotatory microtome and stained with Hematoxylin and Eosin for general tissue structure examination

Histochemical investigations

The following staining methods were used: Masson Trichrome stain for collagenous fibers and muscle; Weigert stain for staining of elastic tissue and Bromophenol blue stain for detection of proteins. All staining were outlined by (Bancroft *et al.*, 2013). Stained sections were examined using light microscope with full HD microscopic camera (Leica Microsystems, Germany).

Chemical examinations

Five pieces from each individual luncheon sample were rendered into a uniform mass by passing three times through a meat mincer and mixed thoroughly after each mincing time then sample was taken form this uniform mass for futher analysis. The method of AOAC (2000) was used to measure the moisture, protein, fat and ash contents of luncheon samples after processing. For determination of moisture contents (g% sample), 10 g of sample were dried at 100°C until constant weight was obtained. Protein content (g% sample) was determined according to the Kjeldahl method of analysis. Fat (g% sample) was determined by 6-cycle extraction with petroleum ether in a soxhlet apparatus and calculating the weight loss. Ash was determined by ignition at 500°C for 5 h (g% sample). The calcium percentage was determined using EDTA titration method described by George *et al.* 2013.

Statistical analysis

Statistical data analysis for the three independent replicates was carried out using SPSS statistics 17.0 for windows (SPSS Inc, Chicago, IL, USA). The difference between means values of proximate composition analysis using one-way analysis of variance (ANOVA) were considered significant at the $P<0.05$ level.

RESULTS

Results of histological examinations showed that in paraffin sections, luncheon samples were adulterated with different types of animal tissues (Fig. 1) included; hyaline cartilage, tendon, spongy bone, peripheral nerve trunk, basophilic matrix, lymphatic tissue, fascia, fibrocartilage and vascular tissue. Hyaline cartilage was distinguished by presence of chondrocytes in their lacunae embedded in basophilic matrix (Fig. 1A and B). Tendon was characterized by parallel collagen bundles and squeezed fibroblasts in between (Fig. 1C). Spongy bone composed of bony trabeculae separated by bone marrow (Fig. 1D and E). Peripheral nerve trunk which composed of myelinated axons was noted within the luncheon samples (Fig. 1 F and G). Basophilic matrix was observed in the samples that may attribute to the reminants of the cartilaginous or bony tissues (Fig. 1H). Lymphatic tissue was marked by presence of lymphoreticular network (Fig. 1I). Skeletal muscle fibers were recognized under degenerative changes

(Fig. 1J). Fascia consisted of irregular arranged collagen fibers (Fig. 1K). Fibrocartilage was represented by parallel collagen fibers and chondrocytes in between (Fig. 1L). The vascular tissue was identified by prominent elastic fibers which accompanied by large sized arteries (Fig. 1M) and muscular artery that characterized by thick smooth muscular layer in the tunica media (Fig. 1N). By histological examination, paraffin sections of luncheon samples were adulterated with plant tissue (Fig. 2) included; plant stem, leaves and root. Plant stem was identified by collenchyma (Fig. 2F and H) while, plant leaves were determined by mesophyll (Fig. 2D and G). Dicot leaf (Fig. 2E), cotyledon (Fig. 2A), palisade of soya (Fig. 2B) and plant root (Fig. 2 C) were observed in the examined samples). The results of chemical examinations was adressed clearly in Table 1.

DISCUSSION

In recent years the quality of meat products became a global concern especially with the growing public awareness of the adverse health effects caused by eating low quality meat products. Adulteration of meat products is the major cause for such adverse health effects. So adequate investigations to identify adulterant addition in several meat products are required to assess its value as well as to protect consumers against falsified activities (Bansal *et al.*, 2017). Detection of food fraud increased significantly over the last few years including false labeling and unstated usage of food additives or fillers to substitute skeletal muscles in the product to reach economical profits (Everstine *et al.*, 2013; Ruiz Orduna *et al.*, 2015). Histological method and chemical analysis are the most functional techniques in identifying unauthorized tissues in meat products (Abbasy-Fasarani *et al.*, 2012). Using chemical methods only cannot determine the quality of meat products however, using those methods side by side with histological investigation techniques can be applied to identify the features of non-meat tissues and morphological variations in the meat structure (Izadi *et al.*, 2016). Moreover, quality control sector can depend on these methods based on several studies which revealed the ability of these methods to detect food fraud and adulteration in meat products (Damez *et al.*, 2008; Ghislen *et al.*, 2010; Sezer *et al.*, 2013 and Latorre *et al.*, 2015). In the current study chemical analysis (Table 1) for twenty different traditional Egyptian luncheon processing planets showed a significant difference $(P<0.05)$ in their chemical composition (moisture, fat, protein, ash and calcium) content. Comparing these results with the limits described by the Egyptian standards specification (E.S.S.) for traditional Egyptian luncheon (E.S.S. no. 1114-2005) it was found that moisture content (Table 1) varied around the permissible limit. This difference may be attributed to amount of lean meat added (Lotfi and Youssef, 1966) or may be linked to using of sodium chloride and/or addition of water which is added to facilitate the chopping of meat and the mixing of the ingredients (Pearson and Tauber 1984). Meanwhile, the percentage of the samples fat content (Table 1) ranged from 1.07 to 10.55 although the E.S.S. described a limit of about 35% for this product. The variation in the fat content among the examined processing plants may be attributed to the differences of used meat cuts

Table 1: Chemical analysis for twenty different traditional Egyptian luncheon processing planets

Factory no.	Moisture %	Fat %	Protein %	Ash %	Calcium %
1	62.37±0.143[Aa]	4.59±0.017[A,B]	16.14±0.037[A]	3.13±0.013[A,B]	1.293±0.003[A]
2	58.04±0.145[B]	8.55±0.027[C]	18.89±0.012[B]	3.74±0.024[C,D]	0.933±0.004[B]
3	57.42±0.174[C,D]	3.14±0.026[D]	4.36±0.031[C]	3.44±0.028[E,F]	1.920±0.001[C]
4	58.45±0.126[E]	5.02±0.058[E,J]	7.88±0.020[D]	3.25±0.024[G,J]	2.122±0.003[D]
5	57.80±0.154[B,C]	10.55±0.017[F]	15.20±0.042[E]	3.33±0.022[G,H]	2.453±0.004[E]
6	58.59±0.174[E]	8.03±0.019[G]	18.35±0.023[F]	3.10±0.009[A]	2.309±0.010[F]
7	55.73±0.163[F]	3.93±0.009[H]	3.99±0.053[G]	3.50±0.012[E]	2.783±0.007[G]
8	60.55±0.144[G]	4.35±0.023[A]	17.85±0.040[H]	2.96±0.031[I]	1.927±0.009[C]
9	57.16±0.145[D]	1.07±0.016[I]	3.50±0.021[I]	3.68±0.020[C]	1.873±0.012[H]
10	59.53±0.166[H]	4.85±0.027[B,E]	6.99±0.051[J]	2.96±0.022[I]	2.300±0.006[F]
11	56.30±0.152[I]	4.52±0.015[A,B]	3.51±0.012[I]	3.74±0.023[C,D]	2.252±0.004[G]
12	60.37±0.089[G]	8.23±0.027[C,G]	14.70±0.020[K]	3.45±0.022[E,F]	2.493±0.003[H]
13	53.86±0.087[J]	5.28± 0.019[J]	14.30±0.065[L]	3.16±0.026[A,J]	2.007±0.003[I]
14	65.18±0.117[K]	3.42±0.016[D]	26.93±0.042[M]	3.23±0.014[B,G]	1.250±0.006[J]
15	68.90±0.098[L]	4.32±0.019[A]	23.86±0.058[N]	2.85±0.018[K]	1.093±0.004[K]
16	64.26±0.179[M]	7.03±0.020[K]	25.25±0.162[O]	3.38±0.009[F,H]	3.212±0.017[L]
17	65.01±0.059[K]	4.60±0.006[A,B]	25.47±0.042[P]	3.84±0.012[D]	1.262±0.004[J]
18	55.15±0.180[N]	5.93±0.014[L]	28.72±0.082[Q]	0.92±0.001[L]	1.030±0.001[M]
19	65.43±0.148[K]	9.93±0.591[M]	22.33±0.146[R]	2.26±0.139[M]	2.737±0.009[H]
20	63.20±0.115[O]	9.90±0.018[M]	23.40±0.065[S]	3.43±0.032[E,H]	0.902±0.004[O]

[A-S] Values with different superscripts within the same column are significantly ($P < 0.05$) different. [a] Data represent average of three independent repeats plus standard Error
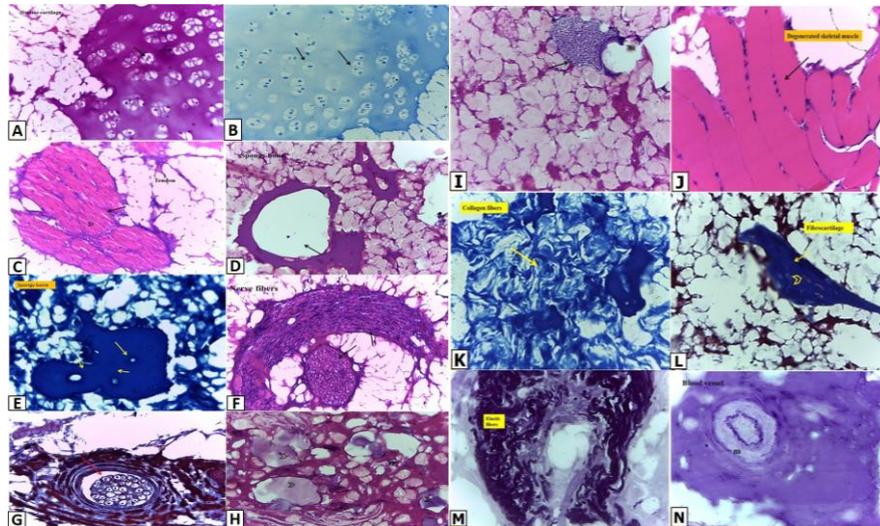

Fig. 1: photomicrograph of luncheon samples stained with H & E (A, C, D, F, H, I, J); Bromophenol blue (B, E); Masson′s trichrome (G, K, L) and Weighert stain (M, N) showed adulteration with different animal tissues. A, B: hyaline cartilage contained chondrocytes (arrows) x1000. C: Tendon consisted of parallel collagen bundles (arrow) and squeezed fibroblasts (arrow head) x400. D, E: spongy bone composed of bony trabeculae separated by bone marrow (arrows) x400 & x1000. F, G: nerve trunk contained myelinated axons (arrows) x400 & x1000. H: basophilic matrix (arrow head) as a reminants of bony or cartilaginous tissue x400. I: lymphatic tissue (arrow) x400. J: skeletal muscle fibers showed degenerative changes (arrow) x400. K: fascia composed of irregular collagen fibers (arrow) x400. L: fibrocartilage identified by parallel collagen bundles (arrow) and chondrocytes (arrow head) x400. M: elastic fibers that associated with large arteries x1000. N: muscular artery that identified by thick layer of smooth muscles (m) in tunica media.

(Mousa, 1996) or using mechanically deboned meat (MDM) in the formulation. On the other hand, the recovered protein percentage (Table 1) of some samples were around ≈ 15 which agreed with the E.S.S. limit while other processing plants (III, IV, VII and IX) showed a very low protein percentage which were 4.36, 7.88, 3.99 and 6.99 respectively. The significant differences (p<0.05) between mean values of protein content of samples collected from different meat processing plants may be attributed to the variation in type and level of extenders and fillers used (Kdous *et al.*, 2016). Moreover, it may be due to meat processors are usually use improper meat cuts, meat trimmings or substite meat material with non-meat components which are relatively less expensive than meat

(Lawrie, 1998). Comparing the obtained ash content results with E.S.S. limits it was found that some samples were higher than acceptable limit (3.5%) which indicated addition of high carbohydrate (Mohamed *et al.*, 2016) and/or high bone content which could be correlated to using high amount of mechanically recovered meat (Field, 1988). These findings strongly agreed with Elbazidy *et al.* 2017. To ascertain all the previous hypothesizes in the present study, histological examination were carried out using microscopic examination to reveal the presence of unauthorized tissue, animal and plant tissue. These unauthorized tissues were reducing the quality and nutritional value of the product. Luncheon samples of current investigation were adulterated with different types
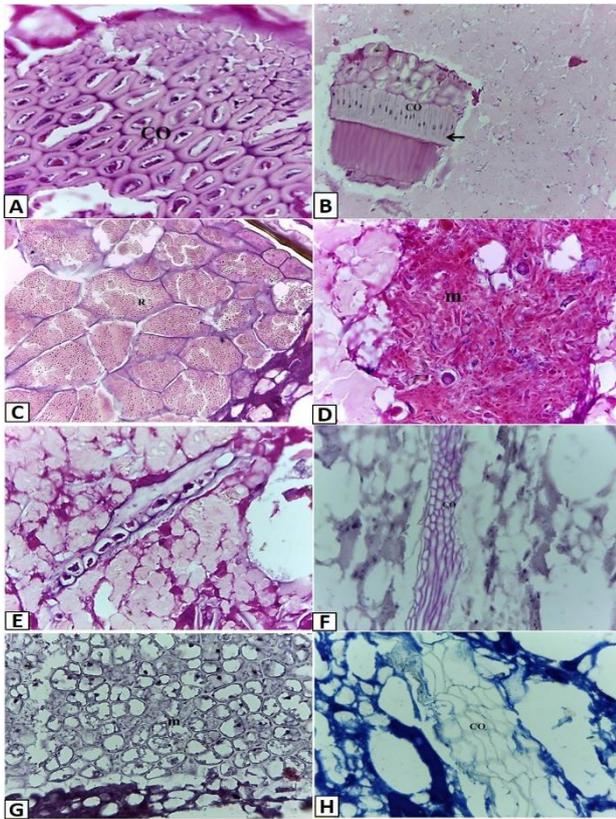
Fig. 2: photomicrograph of luncheon samples stained with H&E x1000 (A, B, C, D, E); Weighert stain x1000 (F); Masson′s trichrome x1000 (G) and Bromophenol blue x1000 (H). A: cotyledon cells of soya plant (CO). B: Palisade of soya (arrow) x400. C: plant root (R). D: a part of plant leaf contained mesophyll (m). E: dicot leaf was recognized in luncheon sample. F: Plant stem identified by collenchyma (CO). G: Plant leaf with mesophyll (m).H: Plant stem with collenchyma (CO).

of animal tissue included hyaline cartilage, tendon, spongy bone, peripheral nerve trunk, lymphatic tissue, fascia, fibrocartilage and vascular tissue (Figure 1). The presence of hyaline cartilage, spongy bone and tendon in our luncheon samples was in line with Mokhtar *et al.* 2018. Also, our current investigation supports the opinion of Latorre *et al.* 2015 who reported adulteration of sausage with cartilage and this agrees with the obtained results of high ash and calcium contents of the current study which noticed in most marketed samples (Table 1). Ash content exceeded the permissible limit stated by the Egyptian Standard Specification (E.S.S. no. 1114-2005) which indicated high mineral content and it was strongly linked to high Ash content. This was referred to the addition of high amount of mechanically deboned poultry meat MDPM in the formulation of luncheon sausage. This added MDPM could be correlated with high bone content in the examined samples which may have a detrimental effect on human health leading to mineral imbalance (Field, 1988). Also presence of peripheral nerve trunk and blood vessels in current study (Fig. 1 F&G) was in coincidence with Rokni *et al.* 1999 and Malakauskienė *et al.* 2016 who reported that nerve fiber and blood vessels were detectable in sausage samples.

Using light microscopy revealed that luncheon samples were adulterated with plant tissue included plant stem, root and leaves. In our study, cotyledon and palisade

of soya was detected which was in a good agreement with the finding of Rokni *et al.*, 1999 and Latorre *et al.*, 2015 who reported the presence of soya in sausage samples as unpermitted tissue. Also plant root observed in our samples was similar to finding of Mokhtar *et al.*, 2018.

Conclusions

Histological and chemical examinations can be used as a reliable methods to investigate using of unauthorized addition of both animal and plant tissues in traditional Egyptian luncheon (emulsion type meat product) samples which revealed a high level of falsification.

Authors contribution

Nermeen Makram Louis Malak and Hamdy Mohamed Bakry Abdelhady Zaki shared the same effort in designing the work plan, conducting and collecting the market samples, chemical examination of the samples reporting and disscussing it as well as drafting and revising of the final manuscript in addition to performing the required statical analysis. Yasmine Hamdy Ahmed Awad Allah, examined samples histologically and reported their results and figures interpretation.

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