

Evaluation of the Morphologic Method for the Detection of Animal and Herbal Content in Minced Meat

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Abstract

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The quantitative and qualitative accuracy of the routine histological method for the determination of unauthorised animal and herbal content in minced meat was evaluated. Laboratory adulterated minced beef meat; each containing 5, 10, 15 and 20% of soya and chicken gizzard was prepared. Then each sample was divided into three parts and four paraffin embedded blocks were prepared from each part. The sections were stained using haematoxylin and eosin, toluidine blue and Masson's trichrome. The histological examination revealed the soya and gizzard tissues clearly in all the samples. The histometrical analysis showed that there was no significant difference between the estimated percentages of both additive tissues and the real related percentages. Overall, neither was there any significant difference between the data of the three parts of each sample and the real percentages. The findings of the present research suggest the histological technique as an effective method for qualitative and quantitative evaluations of minced meat.

Keywords: histology; meat; adulteration; soya; chicken gizzard

Producing meat products with ingredients which are not consistent with the label is considered fraud (BALLIN 2010). There are several reports available regarding the presence of plant materials and animal tissues alongside skeletal muscle, collagen and adipose tissue as standard ingredients in meat products (DING & XU 2000; PRAYSON *et al.* 2008a,b; BOTKA-PETRAK *et al.* 2011).

Plant additives in meat products are important not only in meat product quality as adulterations but also for the food safety as allergens (POSPIECH *et al.* 2009). Therefore various methods such as immunochemical (MORIYAMA *et al.* 2005; RENČOVÁ & TREMLOVÁ 2009) and molecular biological (MEYER *et al.* 1996; HERNÁNDEZ *et al.* 2006) as well as histological methods have been developed to detect the plant materials in meat products. Nevertheless, in most cases these methods are time consuming or

they do not qualify for the quantitative analysis of the plant materials (CASTRO *et al.* 2007).

Several methods such as electrophoresis, chromatography and DNA-based assays are available for the detection of all animal species present in the meat products (HSIEH *et al.* 1995; MACEDO-SILVA *et al.* 2000; RODRÍGUEZ *et al.* 2004; KESMEN *et al.* 2007; RAO & HSIEH 2007). Evaluation of collagen content is the major method for the tissue differentiation in the meat products using spectroscopic and chromatographic techniques (COLGRAVE *et al.* 2008). Aside from the adulteration aspect, it is important to note that some animal tissues like the brain and the spinal cord can bear infective agents which are transmissible to humans (HERDE *et al.* 2005).

There are some reports regarding the detection of herbal content (BOUTTEN *et al.* 1999; POSPIECH *et al.* 2009) and animal tissues (WENISCH *et al.* 1999;

TREMLOVÁ & STARHA 2003; GOUT *et al.* 2004; PRAYSON *et al.* 2008a,b) in the cooked meat products by a histological technique.

On the basis of all that has been said above and considering that the previous studies mainly focused on cooked meat products, this experimental study was designed to evaluate the accuracy of the routine histological technique as a simple and inexpensive method for the determination of unauthorised animal and herbal content in raw minced meat. Furthermore, the findings presented in this article suggest a rapid and economical procedure for a quantitative analysis of the unauthorised tissues in meat products.

MATERIAL AND METHODS

Preparation of meat samples. Samples of minced beef meat, each containing 5, 10, 15, and 20% textured soya protein (as a plant material) and chicken gizzard (as an animal material) were prepared. All the samples with different percentages were evaluated for flavour, tenderness, juiciness, and overall acceptability by 10 different persons. Then each sample was divided into three equal parts, four pieces of each part were collected, and fixed in 10% neutral-buffered formalin.

Histological and histometrical study. The tissues were routinely processed for light microscopy and embedded in paraffin. Each paraffin-embedded block was cut into 6 µm sections and one slide from each block was taken and stained using haematoxylin and eosin (H&E). Toluidine blue and Masson's trichrome were used to detect more details of added soya and chicken gizzard, respectively. After that, the tissue sections were dehydrated in alcohol, cleared in xylene, and mounted in a resinous mountant (Entellan New; Merck, Darmstadt, Germany). The slides were observed by a histologist under a microscope (N-180 M; NOVEL, Beijing, China) equipped with electronic eyepiece (MD130; OME-TOP System, New Taipei City, Taiwan) for the detection of additive tissues. Then five photographs were taken from random points of each slide. The area surface of the additive tissues (soya and gizzard) was measured using the Axiovision software (Carl Zeiss, Oberkochen, Germany).

Quantitative analysis. The proportions of the area surface of the additive tissues and the total area surface were reported for each photograph as mean ± standard deviation. The *t*-test was used to determine significant differences between the real and the estimated percentages. A value of $P < 0.05$ was

considered significant. In addition, the values of the estimated percentages were compared with those of the real percentages using the correlation coefficient.

RESULTS AND DISCUSSION

Meat adulteration is a worldwide problem which violates the diet, health and religious concerns. The prevalence of meat fraud is difficult to measure and thus various methods have been used for this issue (BALLIN 2010). The findings obtained in this study show in the organoleptic evaluation that neither soya nor gizzard was detectable in the prepared minced meat with different percentages. The striated skeletal muscles which accompanied the soya or gizzard tissues were seen in all the slides under the light microscope using H&E staining. The skeletal muscle fibres were observed in the longitudinal and transverse sections. The stained acidophilic cytoplasm and the multiple nuclei were displaced to the periphery of the cells (Figure 1). The gizzard was apparently recognisable by its epithelium lining surface and simple tubular glands (Figure 1a). The *tunica muscularis* was composed of smooth muscle cells, in which the dense connective tissue was very well detectable by Masson's trichrome (Figure 1b). Toluidine blue staining allowed the fine details of soya histology, i.e. palisade, extrudate tissue and cotyledon cells, to be detected (Figure 1c and d). The histological technique was used in this study and found to be an efficient method for the identification of soya and chicken gizzard in minced meat. Considering that routine H&E staining was able to detect the additive vegetable and animal tissues in all the different percentages, it defines the histological method as a simple procedure in this field. PRAYSON *et al.* (2008a) used H&E and also special staining, i.e. oil red O stain and glial fibrillary acidic protein (GFAP) immunostaining, to examine the histology of hotdogs and identified bone and cartilaginous tissues. They implied that in most cases the skeletal muscle nuclei do not appear to stain intensely with H&E. In minced meat, despite the mechanical manipulation of the meat, the nuclei are strongly labelled due to the lack of cooking in this type of product. As the collagen tissue is indicative of the quality of the meat, the examination can be helped by Masson's trichrome. Our personal observations indicate that other selective histological staining like oil red O for fat tissue and alizarin red for bone tissue can be useful (data has not yet been published). Due to their high specificity, the immunohistochemical

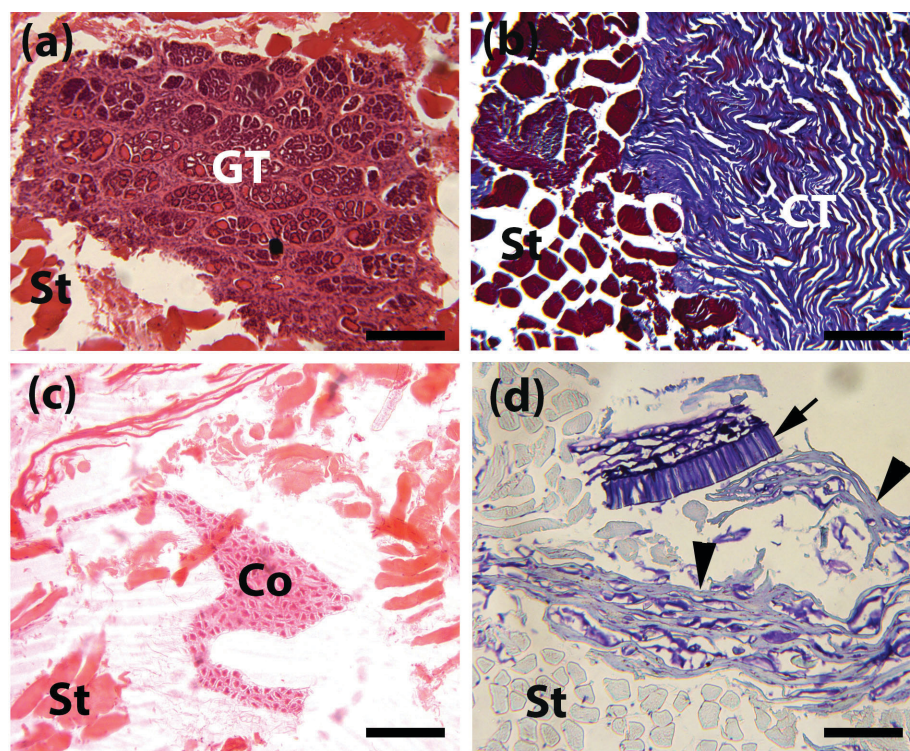


Figure 1. Photomicrograph of additive tissues in minced meat: (a) The histological section shows the glandular tissue (GT) of the gizzard alongside striated skeletal muscle (St) (H&E); (b) The photomicrograph shows dense connective tissue and smooth muscles of the gizzard. The striated skeletal muscle (St) has been specified (Masson's trichrome staining); (c) Photomicrograph of the cotyledon cells of soya (Co). Some transverse sections of the striated skeletal muscle (St) are labelled (H&E); (d) Photomicrograph of the palisade (arrow) and extrudate (arrowheads) tissue of the soya among the striated skeletal muscle (St) in the minced meat (toluidine blue staining) (scale bars 100 μ m)

(IHC) methods have mostly been used for the detection of central nervous tissue as a hazardous material in the transmission of the infective agent of bovine spongiform encephalopathy (ZIJDERVELD & KOOLMEES 1990; TERSTEEG *et al.* 2002; HERDE *et al.* 2005). The problem with using IHC is the heating process of the cooked-meat products in which the antigens became altered. Beyond that, neuron-specific enolase (NSE) immunoreaction was suggested as a reliable marker of CNT in meat products as a consequence of the

extraordinary resistance of the enzyme (LÜCKER *et al.* 1999; WENISCH *et al.* 1999). It has recently been reported that the effect of heating can be minimised by antigen retrieval (BEDNÁŘOVÁ *et al.* 2015). A normal histological staining can easily identify the presence of plant materials based on their typical morphology. By using basic staining with toluidine blue, the vegetable proteins become different shades of blue, in contrast to other components in the product, for example soya was labelled dark blue in this study. It

Table 1. The estimated percentages of additive soya and chicken gizzard with different percentages

Samples	5%	10%	15%	20%	Correlation coefficient
Soya	4.1 $\pm$ 2.6 (<i>P</i> = 0.2)	8.06 $\pm$ 6.24 (<i>P</i> = 0.3)	13.65 $\pm$ 6.78 (<i>P</i> = 0.5)	19.06 $\pm$ 6.39 (<i>P</i> = 0.62)	0/71*
Chicken gizzard	10.28 $\pm$ 9.79 (<i>P</i> = 0.08)	14.91 $\pm$ 12.49 (<i>P</i> = 0.2)	18.03 $\pm$ 9.1 (<i>P</i> = 0.27)	20.07 $\pm$ 7.36 (<i>P</i> = 0.97)	0/35**

Measurements are expressed as mean $\pm$ standard deviation; significance level (*P*-value) calculated for the comparison of the estimated percentages with the real percentages is given within parenthesis; correlation between various estimated and real percentages is significant at the 0.05 level (*) and at the 0.01 level (**)

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Table 2. The estimated percentages of the first, second and third parts of the soya and chicken gizzard samples with different percentages

	5%	10%	15%	20%	Correlation coefficient
Soya samples					
First part	4.58 ± 3.3 (<i>P</i> = 0.81)	6.45 ± 3.98 (<i>P</i> = 0.17)	16.17 ± 11.01 (<i>P</i> = 0.84)	21.61 ± 7.56 (<i>P</i> = 0.69)	0.72*
Second part	2.57 ± 2.2 (<i>P</i> = 0.12)	8.98 ± 5.52 (<i>P</i> = 0.73)	13.76 ± 5.17 (<i>P</i> = 0.66)	17.64 ± 8.52 (<i>P</i> = 0.61)	0.74*
Third part	5.16 ± 1.87 (<i>P</i> = 0.87)	8.7 ± 9.56 (<i>P</i> = 0.81)	11.02 ± 1.78 (<i>P</i> = 0.02)	17.93 ± 2.6 (<i>P</i> = 0.21)	0.70*
Chicken gizzard samples					
First part	13.3 ± 11.45 (<i>P</i> = 0.24)	8.12 ± 6.97 (<i>P</i> = 0.62)	16.6 ± 8.12 (<i>P</i> = 0.71)	19.85 ± 8.4 (<i>P</i> = 0.97)	0.35
Second part	4.8 ± 5.91 (<i>P</i> = 0.97)	23.7 ± 15.19 (<i>P</i> = 0.16)	17.93 ± 4.89 (<i>P</i> = 0.31)	9.5 ± 8.68 (<i>P</i> = 0.91)	0.39
Third part	12.69 ± 11.27 (<i>P</i> = 0.26)	12.94 ± 11.11 (<i>P</i> = 0.63)	19.56 ± 14.43 (<i>P</i> = 0.57)	20 ± 7.11 (<i>P</i> = 0.81)	0.33

The measurements are expressed as mean ± standard deviation; significance level (*P*-value) calculated for the comparison of the estimated percentages with the real percentages is given within parenthesis; correlation calculated between various estimated and real percentages is significant at the 0.01 level (*)

has been revealed that some special staining such as the PAS-Calleja stain which targets polysaccharides can indicate soya flour (POSPIECH *et al.* 2011). FLINT (1990) applied a micro-technique which relied on the microscopic appearance of the different hydrocolloid particles when treated with aqueous Toluidine Blue under neutral and acid conditions. Furthermore, the immunohistochemical methods have been used to label the vegetable proteins even in low percentages, but it has limitations due to the low protein content in some vegetables, like wheat (POSPIECH *et al.* 2009; RANDULOVÁ *et al.* 2011).

The mean values of additive soya and chicken gizzard with different percentages are shown in Table 1. Notably, there was no significant difference between the estimated percentages of both additive tissues and the real percentages. The values $4.1 \pm 2.6\%$ (*P* = 0.2) and $10.28 \pm 9.79\%$ (*P* = 0.08), $8.06 \pm 6.24\%$ (*P* = 0.3) and $14.91 \pm 12.49\%$ (*P* = 0.2), $13.65 \pm 6.78\%$ (*P* = 0.5) and $18.03 \pm 9.1\%$ (*P* = 0.27), $19.06 \pm 6.39\%$ (*P* = 0.62), and $20.07 \pm 7.36\%$ (*P* = 0.97) were obtained for the samples, each containing 5, 10, 15 and 20% soya and chicken gizzard, respectively.

The values of the three different parts of each sample with a different percentage of soya and chicken gizzard are summarised in Table 2. The statistical analysis did not indicate any differences in the data of the three parts of each sample. The comparison of the results with the real percentages did not show

any significant difference, except for the third part of the 15% soya sample in which the related difference was significant (11.02 ± 1.78 vs. 15%, *P* = 0.02). Overall, according to the correlation coefficient (*r* = 0.7) there were correlations between the real and the estimated percentages in different parts of the soya samples.

As the estimated percentages of additive tissues in minced meat closely resemble those real percentages, they must be regarded as a sufficient method for the quantitative evaluation of the meat products. The amount of meat in the different brands of hamburgers (PRAYSON *et al.* 2008b) and hotdogs (PRAYSON *et al.* 2008a) were previously measured using grids in histological photographs. TREMLOVÁ and STARHA (2003) established a quantitative examination of the bone tissue content in meat products using image analysis. SIFRE *et al.* (2009) set up a system based on the histology and the image analysis to quantify muscle fibre destruction in meat.

In conclusion, the histological technique is an effective method for qualitative and quantitative evaluations of minced meat. At least four histological sections were offered in this examination.

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