Progress in Nutrition 2016; Vol. 18, N. 3: 276-282

Original article

Histological and immunohistochemical analysis of meat-based food preparations

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Summary. This study aimed to verify the possibility to use standard morphologically-based techniques to assess the tissue composition of typical meat-based food preparations. Twenty-six different types of meat-based products were randomly selected and histologically analyzed. A variety of tissues were identified including connective tissue, blood vessels, peripheral nerve, adipose tissue, glandular tissue, skin, bone and cartilage and plant material. Glial fibrillary acidic protein staining was also used to evaluate the presence of brain tissue. The results obtained suggest that most of the analyzed products display a more complex composition than the one suggested by package label. These findings confirms the suitability of standard morphological techniques to examine the histology of meat-based food preparations and suggest their use for the control and quality certification of these types of products.

Keywords: meat-based food, tissue composition, morphology

Introduction

Recent data have extensively indicated a relationship between red meat and cardiovascular disease and cancer (1, 2). However meat-based food preparations are very popular in Italy and, although with regional differences, they are part of every-day meals in various forms such as tortellini, ravioli, wurstel and mortadella. Most of these foods but few limited exceptions (such as home-made ravioli and tortellini) are industrial products whose manufacturing is regulated by national or European community laws according with health institutions. These regulations impose that package labels provide information on the types of meat and ingredients used in the preparation. However, this information is usually limited only to the type of meat used as the primary ingredient without indicate in full details all the different components of animal origin present in the food.

Purpose of this study was to assess the meat content of several meat-based food preparations and to verify how much accurate package labels are. It was not in the intentions of this work to give indications about the goodness of the aliments nor about their adherence to standard guidelines in terms of preparation and/or packaging procedures. The main purpose was to use standard morphologically based techniques that are commonly used for tissues evaluation in the pathology practice (eg, routine light microscopy with hematoxylin-eosin–stained sections, special staining and immunohistochemistry) to examine the histology of several meat-based food preparations from different brands in order to identify the type of components of animal origin assumed while consuming these aliments.

Indeed, it is well documented that a majority of so-called multifactorial, chronic and life-threatening diseases (such as cardiovascular diseases and cancer) are influenced by diet and especially by animal foods (1, 3).

Thus, an accurate knowledge of the types of aliments consumed is essential for a risk estimation and standard pathology procedures are rapid, easy, inexpensive, accurate and reproducible techniques which can provide clear cut information with no interpretation bias.

Materials and Method

Twenty-six different samples, including different types of products and brands, were randomly purchased from local grocery stores and supermarkets in a medium-size village in Northern Italy. Samples were anonymously designated to prevent any bias during their evaluation. They are indicated in details in Table 1 and include: 9 different brands of meat-based tortellini; 1 package of a variety of tortellini known as "cappelletti"; 3 different preparations of meat-based ravioli; 1 package of so-called "quadretti"; 1 package of so-called "girandole"; 6 different brands of mortadella (Bologna sausage); 2 different brands each of pure pork and mixed chicken/turkey wurstels and 1 package of so-called "pressatella" (meat pressed). Products belonged to 15 different brands and were blindly analyzed using two different techniques depending on the type of aliments: stuffed (eg., ravioli and tortellini) or non-stuffed (eg., mortadella and wurstel). In the former case, for each product the stuffed mixture (refill) was collected from 7 samples and analyzed in toto. Non-stuffed foods were macroscopically examined and, after recording their dimensions and weight, were cut in slices of about 1.5 cm (for big size products, > 500gr/weight) or 0.7-0.8 cm (for small size products, 100-125 gr/weight) thickness. Samples to be analyzed for both types of foods were prepared all at the same time for each product and were weighted in order to determine the amount (in %) of the product analyzed. For non-stuffed foods the slices analyzed were consecutive. Using these procedures, analyses were performed on about 9.0 % of each product (n = 15) (SD = 3.93; range 3.86 –17.50 %) for stuffed foods, such as tortellini, and on about 12.53 % (SD = 2.51; range 9.33 –17.0%) for non-stuffed foods (n = 11).

Macroscopic analysis and related description was performed by a single pathologist (MM). All samples were fixed in 10% buffered formalin for 24h and paraffin-embedded. Seven five-micrometer-thick sections were cut from each sample and one was hematoxylin-eosin–stained and used for quantitative and morphological evaluation to identify tissue types present and to quantify the number of fragments of each tissue type per cross section. All slides were microscopically evaluated independently by two pathologists (MM and GR) blind about the brand and the origin of the samples and no discrepancies were recorded between them. The other sections were mounted on pre-cleaned, charged microscopic slides to be used for immunohistochemical analyses. All the cases were analyzed for the immunohistochemical expression of Glial Fibrillary Acidic Protein (GFAP) using two specific anti-GFAP antibodies: a mouse monoclonal antibody (Clone G-A-5, Cell Marque Corporation, California USA) and a polyclonal rabbit antibody (DAKO, Denmark). For both antibodies a microwave treatment for antigen unmasking was used and staining was performed using an automated immunostainer (*Benchmark*®, Ventana, Tuscon, AZ, USA). The immunostaining results were evaluated independently by two pathologists (MM and GR) blind about the brand and the origin of the samples. Comparable results were obtained with the two antibodies.

Statistical analysis

All data were recorded and collected in a database and successively analyzed by SPSS (Statistical Package for Social Science rel. 13) software (Chicago, IL, USA).

Results

Macroscopic description of tested products

Samples were all processed between September and December 2008. The length, weight, major features and expiration date of each food preparation were recorded before sampling.

Product **Alfa**: pure pork wurstel (brand 12) in form of three cylinders 15.5 x 2.5Ø cm, 250g weight, pale-pink colour and tough at touch; expiration October 2008.

Product **A:** pure pork mortadella (brand 11) in form of a single cylinder 14cm long, 430g weight, pale-pink colour and tough at touch. The fat component was macroscopically well evident in all the slices; expiration November 2008.

Product **N:** mixed chicken/turkey wurstels (brand 12) in form of three cylinders 17.5 x 2.5Ø cm, 250g weight, pale-pink colour and tough at touch; expiration October 2008.

Product **X:** mixed chicken/turkey wurstels (brand 13) in form of four cylinders 10.0 x 2.0Ø cm, 100g weight, pale-pink colour and tough at touch; expiration September 2008.

Product **V**: pure pork wurstels (brand 13) in form of four cylinders 10.0 x 2.0Ø cm, 250 g weight, pale-pink colour and tough at touch; expiration October 2008.

Product **B:** pure pork mortadella (brand 10) in form of a single cylinder 9cm long, 500g weight, pale-pink colour and tough at touch. The non-fat component was macroscopically well evident in all the slices; expiration December 2008.

Product **C:** pure pork mortadella (brand 10) in form of a single cylinder 7cm long, 150g weight, pale-pink colour and tough at touch. Both the fat and non-fat components were macroscopically equally represented in all the slices; expiration December 2008.

Product **BJ:** pure pork mortadella (brand 9) in form of a single cylinder 16 cm long, 900g weight, pale-pink colour and tough at touch. The non-fat component was macroscopically prevalent over the fat one in all the slices; expiration November 2008.

Product **E:** pure pork mortadella (brand 7) in form of a single cylinder 8.5cm long, 500g weight, pale-pink colour and tough at touch. The non-fat component was macroscopically prevalent over the fat one in all the slices; expiration September 2008.

Product **BO:** Meat "pressed" (pressatella) (brand 14) in form of a single cylinder 5,5 cm long, 210 g weight, homogeneously pink colour and tough at touch. Muscle component was macroscopically prevalent over the fat one in all the slices; expiration end of 2010.

Product **D:** pure pork mortadella (brand 8) in form of a single cone-cylinder 8.5cm long, 500g weight, pale-pink colour and tough at touch. Both the fat and non-fat components were macroscopically equally represented in all the slices; expiration November 2008.

Product **R:** meat-based tortellini (brand 2), 118 pieces, 250g weight, exp. November 2008.

Product **Z:** Parma ham-based tortellini (brand 3), 92 pieces, 250g weight, exp. October 2008.

Product **P:** meat-based girandole (brand 4), 85 pieces, 250g weight, expiration October 2008.

Product **Q:** fresh meat-based ravioli (brand 4), 55 pieces, 250g weight, exp. September 2008.

Product **H:** fresh meat-based ravioli (brand 1), 40 pieces, 250g weight, exp. September 2008.

Product **W:** fresh meat-based tortellini (brand 15), 86 pieces, 250g weight, exp. October 2008.

Product **F:** fresh meat-based tortellini (brand 1), 107 pieces, 250g weight, exp. November 2008.

Product **O:** fresh Parma ham-based tortellini (brand 1), 68 pieces, 250g weight, exp. October 2008.

Product **T:** fresh meat-based tortellini (brand 12), 81 pieces, 250g weight, exp. September 2008.

Product **J:** meat-based quadrucci (brand 6), 103 pieces, 250g weight, exp. October 2008.

Product **I:** fresh meat-based raviolini (brand 5), 42 pieces, 250g weight, exp. September 2008.

Product **CS:** Parma ham-based tortellini (brand 15), 120 pieces, 250g weight, exp. November, 2008.

Product **BZ:** Parma ham-based cappelletti (brand 4), 92 pieces, 230g weight, exp. November, 2008.

Product **TU:** Parma ham-based tortellini (brand 5), 111 pieces, 250g weight, exp. December, 2008.

Product **ET:** Parma ham-based tortellini (brand 13), 86 pieces, 250g weight, exp. December, 2008.

A summary of the types of foods included in the study with indication of the amount analyzed is reported in Table 1.

Microscopic description of tested products

The major features of the twenty-six products analyzed are summarized in Table 2. Eight different types of animal tissues were identified: fat, muscle, cartilage, bone, gland and nervous tissue, blood-vessels and skin (Fig. 1). Gland tissues were further differentiated in serous and mucinous. A vegetable component

Table 1. Summary of package information and fraction (%) of product analyzed for each tested meat-based food preparation.

Identification Letter	BRAND	Type of product	Amount analyzed	%
NON-STUFFED FOODS			Weight (gr) Total/examined	
A	11	Mortadella Bologna PP^	430/40	9,3
alfa	12	Pure pork wurstel	250/25	10,0
B	10	Mortadella Bologna PP^	500/70	14,0
BJ	9	Mortadella Bologna PP	900/110	12,2
BO	14	Pressatella meat mixed stuff	210/30	14,3
C	9	Mortadella Bologna PP^	125/15	12,0
D	8	Mortadella Bologna PP^	500/85	17,0
E	7	Mortadella Bologna PP^	500/80	16,0
N	12	Chicken/turkey wurstel	250/25	10,0
V	13	Pure pork wurstel	100/12	12,0
X	13	Chicken/turkey wurstel	100/11	11,0
STUFFED FOODS			Numbers Total/examined	
BZ	4	Cappelletti Parma ham **	92/7	7,6
CS	15	Parma ham-based Tortellini *	120/7	5,8
F	1	Meat-based fresh tortellini*	107/7	6,5
H	1	Meat-based fresh ravioli*	40/7	17,5
I	5	Meat-based raviolini *	181/7	3,9
J	6	Meat-based quadrucci *	103/7	6,8
ET	13	Parma ham-based tortellini *	86/7	8,1
O	1	Tortellini Parma ham *	68/7	10,3
P	4	Meat-based girandole with carrots *	85/7	8,2
Q	4	Meat (brasato)-based ravioli **	55/7	12,7
R	2	Meat-based tortellini *	118/7	5,9
T	12	Meat-based tortellini *	61/7	11,5
TU	5	Parma ham-based tortellini *	111/7	6,3
W	13	Meat-based fresh tortellini *	43/7	16,3
Z	3	Parma ham-based tortellini *	92/7	7,6

^ *PP = pure pork* *(* 250 gr - ** 230 gr.)*

was often detected in most of the tested products. The eight types of tissues were differentially represented in the various products (Tab. 2). Thus, all eight tissues were identified in 5 products (B, A, Alfa, V, X); seven tissues were identified in 3 products (E, C, N); six tissues were identified in 3 products (B, J, BZ); five tissues were identified in 5 products (F, O, R, T, BO); four tissues were identified in six products (CS, ET, H, P, Q, Z) and, finally, three tissues were identified in 4 products (I, J, T, W). Thus, all products analyzed displayed at least three different types of tissues and at least 5 different types were observed in 61% of cases.

As shown in Table 2, none of the fifteen stuffed products displayed more than six different types of animal tissues, while only (BZ) one showed 6 types, four (F, O, R, T) included 5 types, six (H, Z, CS, ET, P, Q) showed four types of tissues and four included three types (I, J, T, W). Thus, all products analyzed displayed

Table 2. Summary of the types of tissue identified in each tested meat-based food preparation

Sample	Type of tissue									
	Fat	Muscle	Cartil	Bone	Gland§	Vessels	Nerv	Skin	Plant	GFAP
A	+*	+	+	+	+ (SM)	+	+	+	+	-
Alfa	+	+	+	+	+ (SM)	+	+	+	+	-
B	+	+	+	+	+ (SM)	+	+	+	+	-
BJ	+	+	+	+	-	+	+	-	-	-
BO	+	+	-	+	-	+	+	-	-	-
BZ	+	+	+	+	-	+	+	-	+	-
C	+	+	+	+	+ (M)	+	+	-	-	-
CS	+	+	-	+	-	+	-	-	+	-
D	+	+	+	+	-	+	+	-	+	-
E	+	+	+	+	+(M)	+	+	-	-	-
ET	+	+	+	-	-	+	-	-	+	-
F	+	+	+	-	+ (S)	+	-	-	+	-
H	-	+	+	-	+ (M)	+	+	-	+	-
I	+	+	-	-	-	+	-	-	+	-
J	-	+	-	-	+ (S)	+	-	-	-	-
N	-	+	+	+	-	+	+	+	-	-
O	-	+	+	-	+ (M)	+	+	-	+	-
P	+	+	+	-	-	+	-	-	+	-
Q	+	+	-	+	-	+	+	-	+	-
R	+	+	+	-	-	+	+	-	+	-
T	+	+	+	-	-	-	-	-	-	-
TU	+	+	-	+	-	+	+	-	-	-
V	+	+	+	+	+ (S)	+	+	+	+	-
W	+	+	+	-	-	-	-	-	-	-
X	+	+	+	+	+ (S)	+	+	+	+	-
Z	+	+	-	-	-	+	+	-	+	-

** (+) indicates the presence and (-) indicates the absence of each tissue type; Plant: refers to vegetable components while GFAP refers to GFAP immunostaining; § For gland tissues, when present (+) the letter S and/or M indicate the type of gland tissue: M= mucinous and S = serous.*

at least three different types of tissues and at least 5 different types were observed in 33% of cases.

In the group of non-stuffed foods (11 cases), the distribution of the different types of tissues was the following: 8 different types in five products (A/alfa/B/V/X); 7 different types in 3 products (C/E/N); six different types in 2 products (BJ/D) and five different types in the remaining product (BO). Thus, this second group of products showed an higher heterogeneity with all the products (100%) displaying at least 5 different types of animal tissues and 45.5 % of them displaying all the eight different tissues.

It is noteworthy, however, that GFAP was not detectable with any of the two antibodies in all the products analyzed thus allowing to exclude the presence of nervous tissue in the foods analyzed (data not shown) (4).

Discussion

The food preparations analyzed in this study are very popular in Italy as demonstrated by the constant increase in their production and sales volumes over

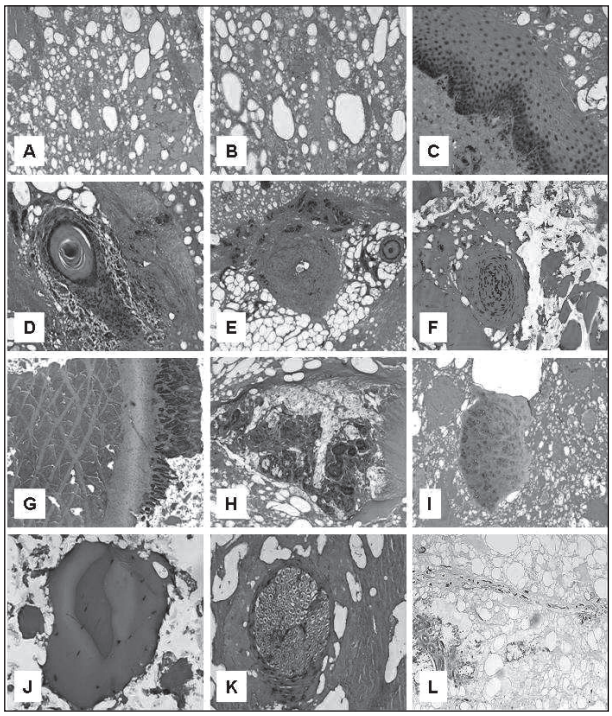

Figure 1. Representative examples of different types of tissues identified by microscopic analysis in meat-based food preparations (HE, 10X).
(A) Sample alpha: Cross section of scattered fragments of skeletal muscle with many vacuoles of lipid and amorphous eosinophilic material. (B) Sample BO: Skeletal muscle (meat) in cross section. (C) Sample C: Fragment of cartilage tissue. (D) Sample W: Fragment of bone tissue. (E) Sample F: Fragment of gastrointestinal mucosa. (F) Sample X: Area of soft tissue; note the massive presence of blood vessels and lipids. (G) Sample B: Cross section of a peripheral nerve fascicle. (H) Sample A: Fragment of mucosal tissue. (I). Fragment of cartilage tissue (J). Fragment of bone tissue. (K). Cross section of a peripheral nerve fascicle. (L). Immunohistochemistry positivity of peripheral nervous (GFAP).

the last years. Thus, production of Mortadella, and especially of the one designated as IGP (IGP products have obtained the official designation of Protected Geographical Indication from the European Union) is continuosly increasing. IGP Mortadella is produced following strict rules in terms of handcraft working, meats selection and row control in order to guarantee the high quality of the product (Fondazione Qualivita, www.qualivita.it) and its production is evaluated to be around 38.000 tons/year with a slight increase (about 1%) each year. Moreover, it is noteworthy that about 18 % of the entire national production (corresponding to 6000 tons) is exported worldwide. The Mortadellas analyzed in this study are produced by big industrial brands, some of which with a long established tradition in the sausage production assuring high quality standards in terms of meat selection, traceability and production processing. However, all of the different Mortadellas analyzed displayed a complex composition with at least six different types of animal tissues. Two (33.3 %) out of six included six different types of tissues with no skin and gland tissue, as expected. Gland tissue was present in two of the remaining while in the last two also skin was detected and it is of interest that the latter two had not obtained the IGP mark. This finding confirms that brands with IGP mark does exert a more accurate attention in the selection of meat and guarantee a better quality of products.

Analysis of wurstels, both pure pork and mixed chicken/turkey-based, gave similar results for all the brands tested. Thus, three of the four products analyzed displayed all the eight different types of tissues while seven of them were detected in the last one (only gland tissue was missing). These findings are in agreement with processing procedures of pork meat with wurstels being obtained by low quality beef and swine fat, thinly minced, cooked and smoked. Thus, the presence of different types of tissues is expected although consumers are not always aware of it (5).

Only one type of pressatella (meat-pressed) was included in this study and the results might not be representative. However, it is noteworthy that five of the eight types of tissues were detected, with skin, bone and gland tissues being not detectable.

Stuffed pasta is also a very popular product in Italy and its production increases about 4% each year with a good worldwide export. A lower complexity was overall detected in stuffed products analyzed in this study. In fact, ten (66.6 %) out of the 15 products analyzed presented only four different types of animal tissues. In only one product (BZ) six different types of animal tissues were detected. Moreover, skin was never detected and in 11 (73.3 %) of them gland tissue was not present. However, it has to be highlighted that the presence of gland tissues in Parma ham-based tortellini, mainly due to gastric mucosa, is often indicated, although in small characters, in the label as “durelli di pollo” (chiken gizzard) whose use is common in the

ancient culinary traditions of the region, Emilia Romagna, whose capital is Bologna.

Overall, it is noteworthy that brain tissue as well as a positive immunostaining for proteins of nervous origin were not detected in any of the products analyzed. This finding confirms a strict adherence to regulation and standards prohibiting the use of nervous tissue as well as other risk materials in human food (6,7). On the other hand, bone and cartilage tissues were frequently detected being present in 15 (57.69 %) and 19 (73 %) of the 26 products analyzed, respectively. This finding, however, might well be due to the presence of small residues which are produced during the mechanical cleaning of animal carcasses.

In conclusion, this study confirms the suitability of standard morphological techniques commonly used in the pathology practice to examine the histology of meat-based food preparations, as previously suggested by other Authors (5, 8, 9, 10) due to their reproducibility, precision and standardization, pathology tecniques might play an important role for the control and quality certification of meet-based aliments with a great marketability.

Acknowledgments

The Authors wish to thank Mr. Mangiacotti Massimiliano for assistance in the preparation of samples.

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