meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.523.2(65)
ID: 278323212
https://doi.org/10.18485/meattech.2019.60.1.4

Original scientific paper

Application of histological and physico-chemical analyses for evaluating the meat product – Cachir

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A b s t r a c t: Cachir is very representative of Algerian charcuterie tradition, and now is a product manufactured under hygienic conditions imposed by the authorities on meat processors. This meat product is made from beef or chicken and is often seasoned with spices and olives during recent years, probably due to a significant change in eating habits. There has been an increase in the consumption of Cachir in Algeria. This study was carried out to determine the tissue and physico-chemical composition of Cachir on sale. Five different types of Cachir were bought at random from different local grocery stores in the Batna region (Algeria). From a physicochemical point of view, the cachir had an average moisture content of 64.85±0.36%, water activity of 0.920±0.002, and pH of 6.61±0.08. Histological evaluation showed the percentage of meat (defined as the skeletal muscle content) was 0.76±0.21%, and the percentage of connective tissue was 0.16±0.14%. The quality of this meat product is closely related to the ratio of skeletal muscle and connective tissue, which was, on average, 21.05. We found these meat products were of poor quality and badly preserved.

Keywords: *Cachir, evaluation histology, physicochemical analysis.*

Introduction

The agrifood industry sector is booming, experiencing a spectacular extension of manufacturing and processing of food products in recent years. Among the meat products most commonly consumed in Algeria, Cachir represents Algerian charcuterie tradition. It is also a product manufactured under the hygiene conditions imposed by the authorities on the meat processing industry. Cachir is made from beef or chicken and is often seasoned with spices and olives. The strengthening of control and verification measures for foodstuffs is necessary or even indispensable, especially in the face of the upsurge of cases of food fraud and the search for easy profits. In effect, histological techniques are widely used in the United States and the European Union to detect some types of fraud and the incorporation of unauthorised substances (*Prayson et al.,* 2008a; Rodríguez et al., 2014; *Avinee et al,* 2010). These techniques are mainly used in the food manufacturing sector and mainly for meat and meat products (*Latorre et al*., 2015). However, in a number of European countries (Austria, Germany, the Netherlands), they are part of the reviewed food hygiene legislation and are included in the methods for food assessment (*Tremlová and Starha* 2003).

Histological techniques consist of taking samples and making paraffin sections followed by appropriate staining and careful microscopic observation to verify the exact composition of food products and identify the presence of unauthorised substances or even types of parasites (*Kalab et al*., 1995). Other more elaborate techniques such as histochemical and histomorphometric techniques allow the identification of substances more accurately and even estimate the percentage of incorporation of these substances using different types of image analysis software (*Pospiech et al*., 2014).

In Algeria, according to the available references, studies carried out on the histological analysis of foodstuffs are non-existent; however, we are interested in evaluating the quality of the meat products produced locally. This study aimed to use histological techniques as simple and inexpensive methods for determination of unauthorised animal content in Algerian meat products.

Materials and Methods

Five different kinds of Cachir were studied. All of them were packed in meat product factories. The Cachir were taken from five different local grocery stores.

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The moisture composition of the various Cachir was evaluated by desiccation, after weighing fresh product and then dehydration in an oven at 37 °C for three days according to *Avinee et al*. (2010). Water activity (aw) was estimated by Hygroscope BT-RS1 Rotronic. The sample mass of Cachir was cut to small pieces and put into a sample cup that was filled to ¾ volume. The probe was immediately put into the sample cup. The result was read as soon as the humidity and temperature values became stable. To measure pH of a mixture resulting from grinding 10 g of meat product in 90 ml of distilled water, according to *Lorenzo et al.* (2008), a Professional pH Meter INOLAB was used.

To determine the tissue composition of each Cachir, 5 to 10 tissue blocks of each product were randomly selected and were fixed in 10% buffered formaldehyde for at least 24 h. The material was treated by common histological standards and embedded in paraffin. From each of the defined blocks, sections of 4 microns thick were obtained in a microtome.

Histological and histochemical examination of Cachir

From selected Cachir samples, four sections from each block were stained with haematoxylin and eosin (*Luna*, 1968), 15 sections by special microscopic staining (five sections with Lugol-Calleja (*Hildebrandt and Hirst*, 1985), five sections with PAS-Calleja (*Hildebrandt and Hirst*, 1985) and five sections with Alizarin S (*Luna*, 1968).

Results and Discussion

Results showing the physico-chemical and histochemical values of the tested Cachir meat products are presented in Table 1.

The different Cachir were mainly composed of moisture, which made up 64.85% of the products, on average. Lower moisture contents have been reported for meat products in the United States and France (*Prayson et al.,* 2008a; *Avinee et al.,* 2010). According to OJAR (2000), meat products must not contain more than 60% moisture. The high moisture content in the present study could also include other liquids that are part of the emulsifying agents used in the manufacture of meat products (*Prayson et al.*, 2008b).

Aw indicates the availability of water for microbial, enzymatic and chemical reactions that determine the stability of meat products (*Fellows*, 2000). The results of our study revealed the aw values of our Cachir were ≤0.92, and the average pH was 6.61. *Perez-Alvarez et al*. (1999) reported the pH and aw of a meat product obviously depend on the initial pH of the meat used initially and the rate and the nature of the incorporated ingredients. According to the model of *Leistner and Rodel* (1975), the correlation between pH and aw classifies Cachir in the group of perishable meat products with a pH≥5.2 and 0.91≤aw≤0.95; meat products in this group must be stored at ≤10°C.

The results of this study showed this meat product contains several types of tissue. This diversity of the types of tissues observed is not vastly different from what was found in the analysis of meat products in the United States (*Prayson et al*., 2008a; *Prayson et al*., 2008b; *Richard et al.,* 2013).

Histological evaluation of the Cachir revealed that skeletal muscle constituted only a small percentage of striated muscle tissue. In view of the relatively low estimates of skeletal muscle content in this study, the impression that meat is the main component of these Cachir meat products seems misleading, since most of the tissues identified in this microscopic study were connective tissue associated with skeletal muscle (Figure 1A), adipose tissue (Figure 1B), blood vessels (Figure 1C) and peripheral nerve tissue (Figure 1D).

The staining according to Calleja was selected due to its suitability for meat products (*Sifre et al.*, 2009). This stain also enables histochemical quantification of collagen ligaments. The Cachir contained 0.16% connective tissue, on average. The quality of these meat products is closely related to the ratio of skeletal muscle and connective tissue. Our results show this ratio was relatively high for

Table 1. Physico-chemical properties of Cachir meat products

Moisture content (%)	pH	aw	Muscle tissue* (M)	Connective tissue* (C)	C/M
64.85±0.36	6.61±0.08	0.920±0.002	0.76±0.21	0.16±0.14	21.05

Legend: *Percentage area density of skeletal muscles and connective tissues of five different quality of Cachir based on analysis of four digitised images of each histological section

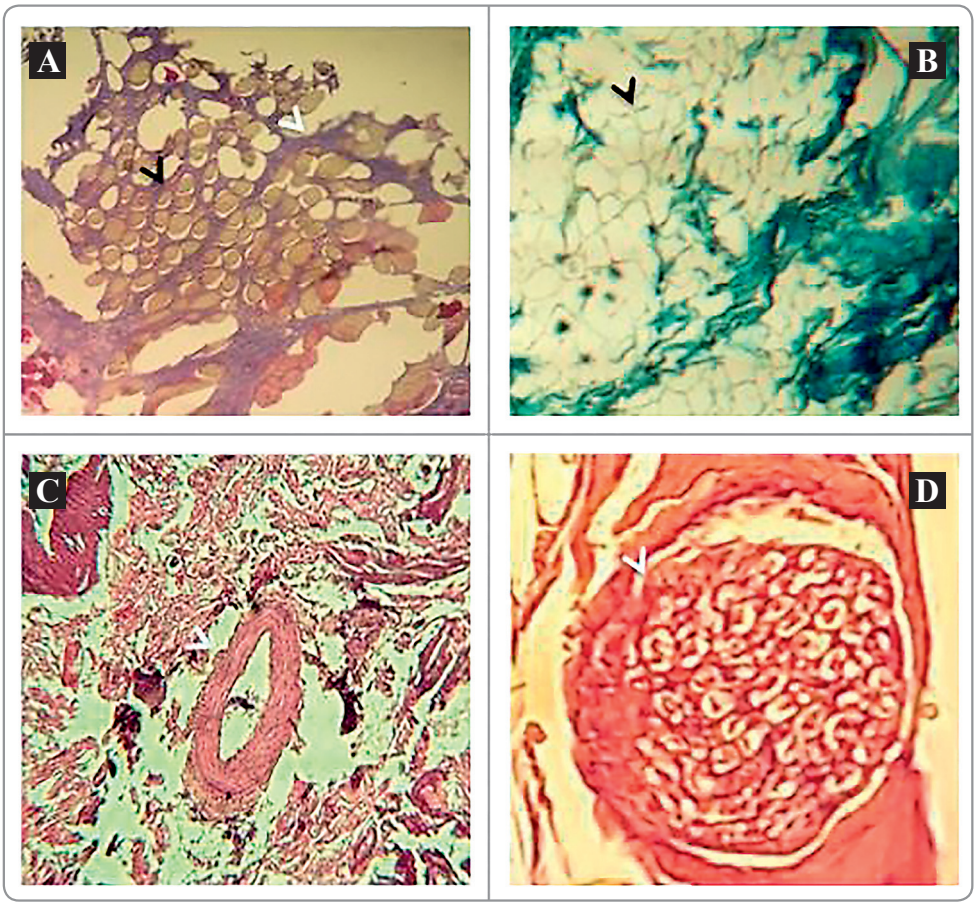

Figure 1. Authorized Tissues. (A) Cachir with striated muscular tissue (black arrow) and connective tissue (white arrow), (B) adipose tissue (white arrow) (PAS-Calleja, ×100). (C) blood vessels (white arrow), (D) Peripheral nerve tissue (white arrow) (hematoxylin eosin, ×100).

the Cachir studied, being 21.05. This result meets the standard allowed (≤35%) (OJAR, 2000).

The Cachir all contained a significant amount of adipose tissue, composed of mature adipocytes. Adipose tissue is usually dissolved by the solvents (xylene) used during paraffin embedding, so a narrow band of cytoplasm surrounding a central space is observed optically. Fragments of cartilage (Figure

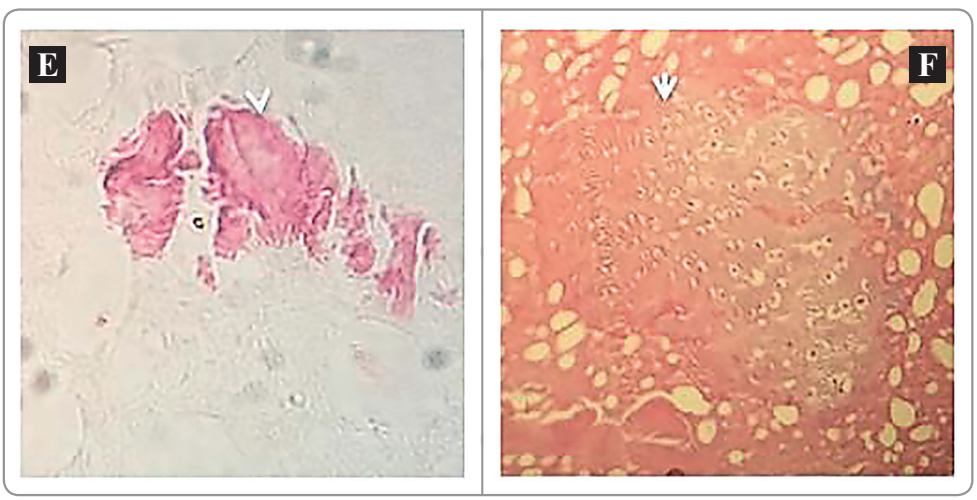

Figure 2. *Unauthorised tissues.* (E) Cortical bone white arrow) (red Alizarin S×100), (F) Cartilaginous tissue (white arrow), (hematoxylin eosin ×100).

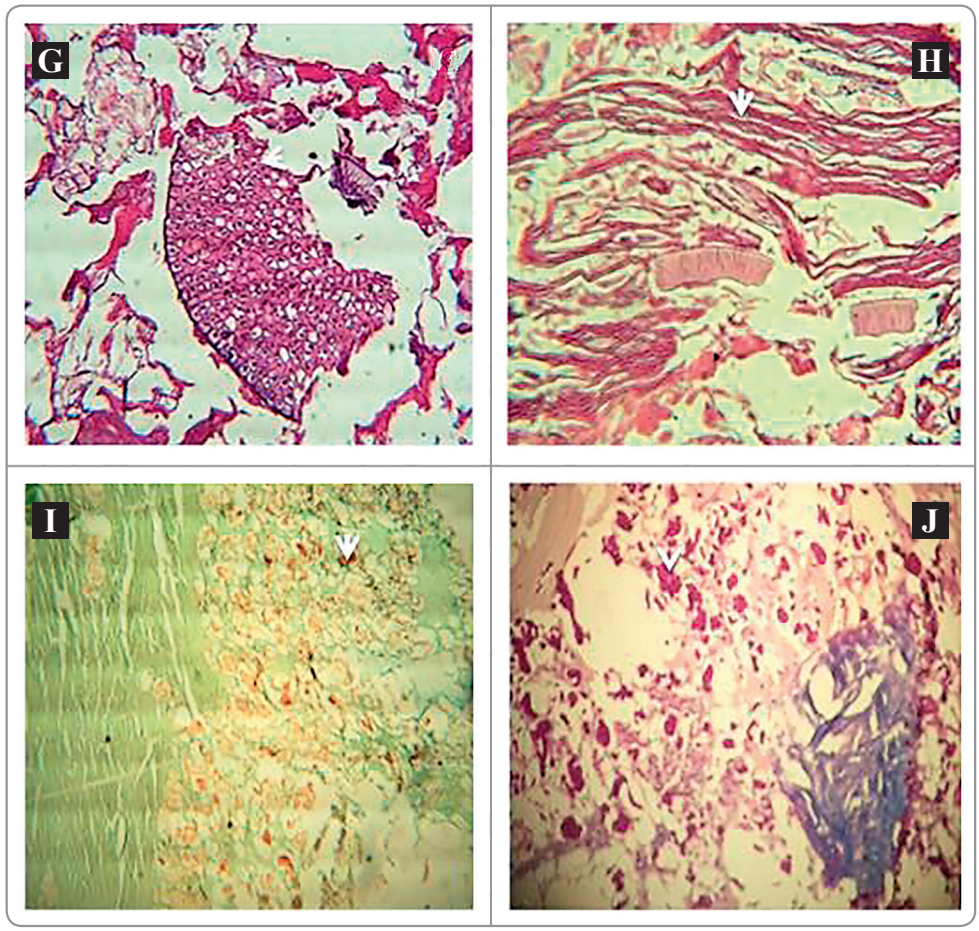

Figure 3. Vegetal tissues. (G) Aromatic herb tissue, (white arrow), (hematoxylin eosin ×100), (H), soybean debris (white arrow) (hematoxylin eosin ×100), (I) starch (white arrow) with Lugol Calleja ×100), (J) polysaccharide (white arrow) with (PAS-Calleja ×100)

2E) and bony (Figure 2F) tissues were observed in some samples of Cachir. Their presence implies a mechanical process was used to separate the meat and attached tissues from the bones (*Prayson et al.,* 2016).

The identification of other ingredients used in the production of meat products is also important for the evaluation of the quality of the final product (*Pospiech et al.,* 2009). Therefore, various molecular biology (*Doosti et al*., 2011; *Izadpanah et al.*, 2017, *Di Pinto et al.,* 2015) and histological methods (*Sadeghinezhad et al.*, 2015; *Abdel Hafeez et al. al.,* 2016) have been developed to detect plant additives in meat products. The most important additives that can be studied with the use of histological techniques are plant additives such as plant tissues and starches. Plant tissues corresponding to aromatic herbs including leaves (Figure 3G) were observed in most histological sections of our meat products. These structures have been observed in the research of *Abdel Hafeez et al.* (2016) and *Pospiech et al* (2014). On investigating the label data, manufacturers usually included in the composition of food products, at the top of the list, "beef or poultry meat" and additives, but without ever specifying the exact nature of the additives involved in the constitution of the products. In addition, the Algerian regulations do not specify precisely the nature of the vegetal ingredients approved for inclusion in the composition of the products. Comparing the structures found in our Cachir meats with those found by Iranians (*Latorre et al,* 2015; *Sadeghinezhad et al*, 2015), we also found structures corresponding to soybean debris (Figure 3H).

The Lugol Calleja histochemical method was selected for the preliminary analysis of starches in our meat products. This method was selected because of the binding of Lugol solution iodine to starch polymer helices (Figure 3I) (*Saibene and Seetharaman*, 2006). Among other histochemical techniques, PAS-Calleja staining can also be used to detect starches. However, in the case of starch

detection in meat products, PAS-Calleja staining also reacts with other polysaccharides, and so this stain cannot be considered a relevant method for starches exclusively (Figure 3J).

In conclusion, the Cachir meat products, despite their transformation during technological processing, retain their recognisable microstructures, and so some ingredients are easily identifiable with the use of adequate histological stains. In Algeria, these techniques are still far from being applied and require the passing of regulations that ensure the implementation of these techniques. This would reveal some fraudulent practices and show the hidden ingredients in these products.

Primena histološke i fizičko-hemijske analize za procenu proizvoda od mesa: Cachir

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A p s t r a k t: Cachir sažima čitavu tradiciju kobasica Alžirski je proizvod proizveden pod higijenskim uslovima koje su vlasti nametnule grupama prerade mesa. Ovaj mesni proizvod se proizvodi od govedine ili piletine, a često je začinjen začinima i maslinama, Tokom posljednjih godina, vjerovatno zbog značajne rekonstrukcije u prehrambenim navikama u Alžiru je došlo do povećanja potrošnje Cachir. Ova studija je sprovedena radi utvrđivanja tkiva i fizičko-hemijskog sastava Cachira koji je predviđen za prodaju. Pet različitih tipova Cachira kupljeno je nasumično iz različitih lokalnih prodavnica hrane u regiji Batna (Alžir). Sa fizičko-hemijske tačke gledišta, većina uzoraka ima sadržaj vlage od 64,85±0,36%. Mesni proizvodi imali su aw od 0,920±0,002, i pH od 6,61±0,08. Histološka procena daje procenat sadržaja mesa (definisan kao sadržaj skeletnih mišića) sa 0,76±0,21% i procenat vezivnog tkiva sa 0,16±0,14%. Kvalitet ovog proizvoda od mesa usko je povezan sa odnosom skeletnog mišića i vezivnog tkiva u vrednosti od 21,05%. Otkrili smo da su ovi mesni proizvodi lošeg kvaliteta i loše očuvani.

Ključne reči: *Cachir, histološka evaluacija, fizičko-hemijska analiza.*

Disclosure statement: No potential conflict of interest was reported by authors.

References

Abdel Hafeez, H. H., Zaki, R. S. & Abd El-Magiud, D. S. (2016). 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? *Journal of Food Processing and Technology*, *7 (12)*, 3–11.

Avinee, G. Charfi, S., Stocker, A., Gyde, E., Hebert, A., Mabille, M.P., Sevestre, H. & Chatelain, D. (2010). L'anatomie pathologique, une méthode d'étude originale des denrées alimentaires*. Annales de Pathologie, 30, 344*–349.

Doosti, A, Dehkordi, P. G. & Rahimi, E. (2011). Molecular assay to fraud identification of meat products. *Journal of Food Science and Technology, 51(1),* 148–152.

Di Pinto, A., Bottaro, M., Bonerba, E., Bozzo, G., Ceci, E., Marchetti, P., Mottola, A. & Tantillo, G. (2015). Occurrence of mislabeling in meat products using DNA-based assay. *Journal of Food Science and Technology, 52(4),* 2479–2484.

Hildebrandt, G. & Hirst, L. (1985). Determination of the collagen, elastin and bone content in meat products using television image analysis. *Journal of Food Science, 50,* 568–570.

Izadpanah, M., Mohebali, N., Elyasigorji, Z.,Farzaneh, P., Vakhshiteh, F. & Fazeli S.A. (2017). Simple and fast multiplex PCR method for detection of species origin in meat products. *Journal of Food Science and Technology, 55(2) ,* 698–703.

Kalab, M., Allan-Wojm, P. & Miller S. (1995). Microscopy and other imaging techniques in food structure analysis. *Trends Food Science Technology ,6,* 177–186.

Latorre, R., Sadeghinezhad, J., Hajimohammadi, B., Izadi, F. & Sheibani M. T. (2015). Application of morphological method for detection of unauthorized tissues in processed meat products*. Journal of Food Quality Hazards Control, 2*, 71–74.

Leistner, L. & Rodel, W. (1975). Water Relations of Foods, RB. Duckworth, Ed. Academic Press, London*.*

Lorenzo, J. M., García Fontán, M. C., Franco, I. & Carballo, J. (2008). Biochemical characteristics of dry-cured *lacón* (a Spanish traditional meat product) throughout the manufacture, and sensorial properties of the final product Effect of some additives. *Food Control, 19 (12), 1148*–1158.

Luna, L.G. (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Edition, McGraw-Hill, New York, USA.

OJAR Official Journal of the Algerian Republic (2000). Relating to the rules applicable to the composition and release for consumption of cooked of meat products

Pospiech, M., Tremlová, B., Petrášová, M. & Bednářová, M. (2014). Identifying the composition of meat preparations. *Maso International Journal Food Science Technology, 2,* 81–98.

Pospiech, M., Tremlová, B., Renčová, E., & Randulová, Z. (2009). Immunohistochemical detection of soya protein: Optimisation and verification of the method. *Czech Journal Food Science*, *27*, 11–19.

Prayson, B., McMahon, J. T. & Prayson R. A. (2008a). Fast food hamburgers: what are we really eating? *Annals of Diagnostic Pathology 2008a, 12*, 406–409.

Prayson, B., McMahon, J. T. & Prayson R. A. (2008b). Applying morphologic techniques to evaluate hotdogs: what is in the hotdogs we eat? *Annals of Diagnostic Pathology 2008b, 12,* 98–102.

Prayson, B., Poulson, W.R. & Prayson, R. A. (2016). Histologic examination of canned beef dog food: what does it really contain? *Journal of Histotechnology, 39,1,* 26–28.

Richard, D., DeShazo, M. D., Bigler, M. D., & Skipworth, L. B. (2013). The Autopsy of Chicken Nuggets Reads "Chicken Little". *The American Journal of Medicine, 126*, 1018–1019.

Paper received: 29.01.2019.
Paper corrected: 26.07.2019.
Paper accepted: 16.07.2019.

Rodríguez, F. A., Picazo, L. G., Pérez, P. C, Moratilla, A. B., Medel, M. P, & Pe˜nasco, M. M. (2014). Somos lo que comemos? Un estudio sobre lacomposición tisular y microbiológica de la carne picadade vacuno. *Revista Española de Patología,47(4),* 235–241.

Sadeghinezhad, J., Hajimohammadi, B., Izadi, F., Yarmahmoud, F, & Latorre, R. (2015). Evaluation of the Morphologic Method for the Detection of Animal and Herbal Content in Minced Meat. *Czech Journal. Food Science, 33(6)*, 564–569.

Saibene, D. & Seetharaman, K. (2006). Segmental mobility of polymers in starch granules at low moisture contents. *Carbohydrate Polymers, 64(4),539*–547.

Sifre, L., André, B., & Coton J. P. (2009). Development of a system to quantify muscle fibre destructuration. *Meat Science, 81*, 515–522.

Tremlová, B., & Štarha, P. (2003). Histometric evaluation of meat products – determination of area and comparison of results obtained by histology and chemistry. *Czech Journal of Food Sciences*, *21,* 101–106.