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Original scientific paper

Reduction of microbiota in marinated vacuum-packaged poultry breast fillets

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A b s t r a c t: The aim of this study was to determine the effect of different marinade solution on the microbiome of chicken breast fillets packaged under vacuum and stored at 4°C. Three types of marinade were tested. A total of 120 chicken breast fillets were marinated in control (6% NaCl) or three different marinades: 6% NaCl and 2% sodium tripolyphosphate; 6% NaCl and 2% sodium citrate, and; 6% NaCl, 1% sodium tripolyphosphate and 1% sodium citrate. Microorganisms were enumerated on the first day of testing (day 0) and on days 7, 14, 21 and 28 of chilled storage. Marination resulted in significant differences ($p<0.05$) in the numbers of total viable counts, Enterobacteriaceae, lactic acid bacteria and anaerobic bacteria counts. The combination of 6% NaCl and 2% sodium citrate is the most appropriate marinade option for reducing the growth of the examined bacterial groups in vacuum-packaged marinated chicken breast fillets during chilled storage.

Keywords: *poultry meat, shelf life, spoilage bacteria, storage conditions.*

Introduction

Spoilage of meat occurs as a consequence of the growth and metabolic activities of spoilage bacteria. During meat storage, the dominant microbiota can cause product deterioration and release of volatile compounds or formation of slime, resulting in a product unacceptable for human consumption The presence and growth of bacterial contaminants occurring in poultry meat depend on different practices that are using for ensuring microbial quality, such as duration and temperature of storage, composition of marinade and gas composition used for storage under modified atmosphere packaging (MAP) or vacuum packaging (*Kreyenschmidt et al.*, 2010; *Baltic et al.,* 2015; *Rouger et al.,* 2017).

Many studies show the influence of marination on tenderness, texture, moisture, water-holding capacity, oxidative stability and yields of poultry breast. Due to the increasing need of consumers to maintain the freshness of chicken for as long a period of time, both in store and in households, it is necessary to control the bacterial microbiota in chicken meat products (*Petracci et al.*, 2014; *Kim et al.*, 2015; *Mathew et al.*, 2016).

The need for fresh food suitable for supply to distant markets has increased the interest in procedures for extending the shelf-life of meat and meat products. Obviously, this time should include not only the time needed to reach the markets but an additional period encompassing retail refrigerated storage and then storage at the consumer's home, as product could be used some days after purchase. Therefore, this issue has become a great challenge to chicken producers. Chicken is a highly perishable food, and the time it takes to deteriorate varies from 4 to about 10 days after slaughter, in spite of it being stored under chill systems (*Marenzi*, 1986). Deterioration depends on the microbiological quality of the poultry carcasses, which is a direct reflection of sanitation during slaughtering and handling practices. Chicken and other types of poultry have higher pathogenic and spoilage bacterial counts than almost any other food (*Snyder,* 1998). However, marinade treatments and vacuum packaging can have benefits with respect to the shelf-life, sensory

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characteristics and quality attributes of chicken meat (*Buses and Thompson*, 2003; *Piñon et al.*, 2015). Storage temperature and type of packaging are selective for different bacterial populations.

The aim of this study was to determine the effect of different marinades on the microbiome of skinless chicken breast fillets packaged under vacuum and stored at 4°C.

Materials and Methods

Chicken breast fillets and marinades

A total of 120 chicken breasts fillets, without skin, approximately 0.1 kg each, were obtained from a local slaughterhouse. They were taken from the production line and transported under refrigeration to the laboratory within a few hours.

Skinless breasts fillets were divided into four groups. Control, (C) fillets were marinated in a 6% NaCl solution. E1 fillets were marinated in 6% NaCl + 2% sodium tripolyphosphate (STP) (Merck). E2 fillets were marinated in 6% NaCl + 2% sodium citrate (Merck). E3 fillets were marinated in 6% NaCl, 1% STP and 1% sodium citrate. The chicken meat weight-to-marinade volume ratio was 1:2. After five hours of marinating, fillets were individually vacuum-packaged in plastic bags. The air was removed from the bags and they were then heat-sealed. Vacuum-packaged chicken breast fillets were stored at 4°C. On each sampling day (days 0, 7, 14, 21 and 28 of storage), three packages from each treatment were randomly selected analysed for total viable counts (TVCs), *Enterobacteriaceae*, lactic acid bacteria (LAB) and anaerobic bacteria counts. Production of strong off-odours and unacceptable general aspects determined when to stop analysis.

Microbiological analysis

Chicken breasts were aseptically sampled on each sampling day by removing 10 g of fillet meat. The 10 g amounts were homogenised, subjected to tenfold serial dilution in buffered peptone water (BPW) and analysed by surface plating. TVCs were determined using plate count agar (PCA, Merck) after incubation at 30°C for 3 days. For counting the number of *Enterobacteriaceae*, the pour-plate method on violet red bile glucose (VRBG) agar (Merck) was used. Plates were incubated at 37°C for 24±2 hours. After plating on a suitable substrate, MRS Agar (Merck) and PCA (Merck), LAB and anaerobic bacteria, respectively, were incubated at 25°C for 3 days in an anaerobic jar (Merck) with an anaerobic generating gas pack (Merck). The colony forming units per gram (CFU/g) on duplicate countable plates were averaged to determine bacterial counts for each fillet and expressed as logarithms.

Statistical analysis

For statistical analysis, all logarithms of bacterial counts were expressed as mean±standard deviation (SD). Statistical analysis of the results obtained was conducted using Microsoft Office Excel 2010 and GraphPad Prism software, version 7.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). The effects of marination treatment were compared between days, and also different marinade treatments were compared on the same testing day, using one-factor analysis of variance (ANOVA). Statistical significance was at the level of $p<0.05$. Bacterial count trends for TVC, *Enterobacteriaceae*, LAB and anaerobic bacteria during the storage period are presented graphically (Microsoft Office, Excel, 2010).

Results and Discussion

TVCs on the chicken fillets increased during the storage time in all marinade treatments, except E2. The highest TVCs were in C and E1 fillets (7.03 log CFU/g, 6.94 log CFU/g, $P > 0.05$, respectively) (Fig. 1). However, the number of TVC was significantly lower ($P > 0.05$) in E2 fillets than in the other marinade treatments on all days (0, 7, 14, 21, and 28) (Table 1). The highest TVC (7.03±0.23 log CFU/g) was on day 28 in control fillets (Table 1). Meat spoilage results in the development of off-odours and slime formation, making the meat unacceptable for human consumption (*Iulietto et al.*, 2015; *Ercolini et al.*, 2006; *Jay,* 2000). According to many studies (*Nychas et al.*, 2008; *Buses and Thompson*, 2003; *Hollingsworth*, 2000), off-odours in chicken meat develop when TVCs approach 7.2 to 8.0 log CFU/g, so our TVCs were slightly lower than this on day 28, when we decided the off-odour and appearance of the chicken fillets were unacceptable.

The type of marinade and storage conditions affected the decrease in the number of *Enterobacteriaceae* during storage. Specifically, significantly lower numbers of *Enterobacteriaceae* (2.70 log CFU/g, 2.64 log CFU/g, $p<0.05$, respectively) were found in fillets marinated with 1% and 2% sodium citrate than in the other two marinades,

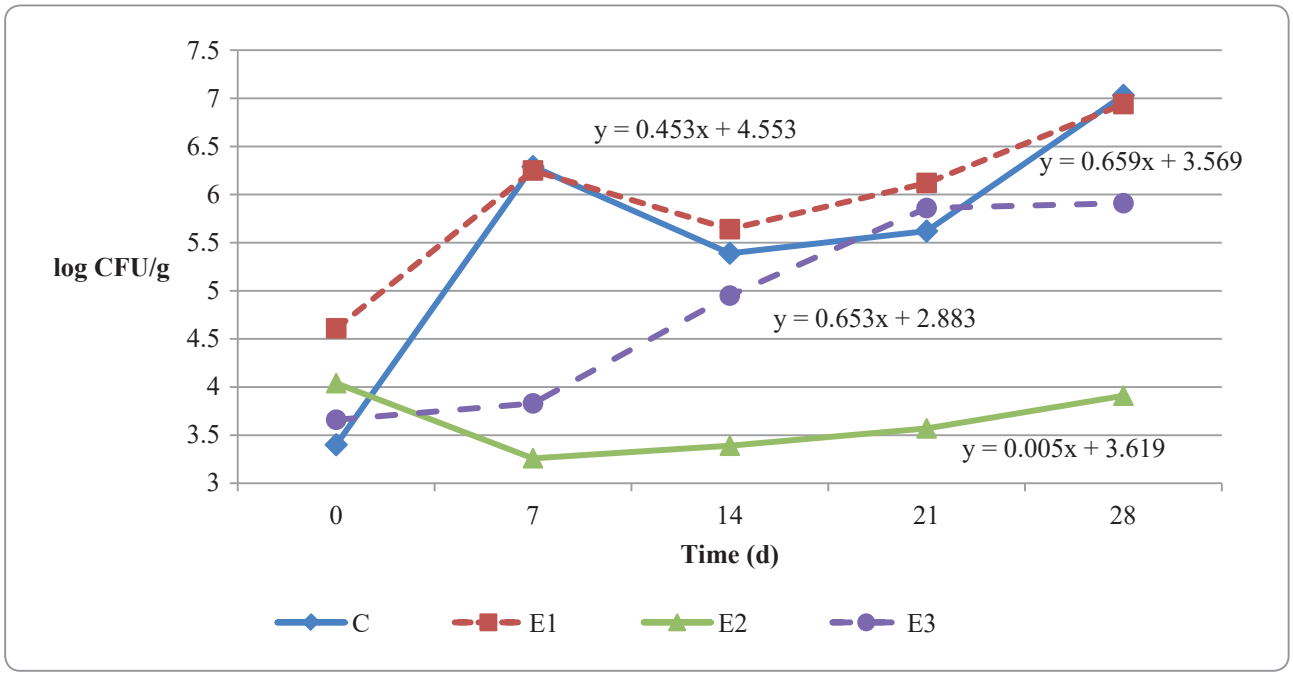

Figure 1. Total viable counts (log CFU/g) in control (C) and marinated (E1, E2 and E3) vacuum-packaged skinless chicken breast fillets (n=120)

while the addition of 2% sodium citrate decreased the *Enterobacteriaceae* count by 0.85 log CFU/g by day 28 (Fig. 2, Table 1). Due to the inconsistency of these results, further tests should be conducted to determine which marinade ingredients improve the reduction of *Enterobacteriaceae* counts. *Enterobacteriaceae* are one of the potential bacterial spoilage groups of poultry meat. However, the involvement of these bacteria and their role in poultry meat spoilage is not completely clarified. Some marinade treatments effectively inhibited coliform growth (*Buses and Thompson*, 2003). Nonetheless, different

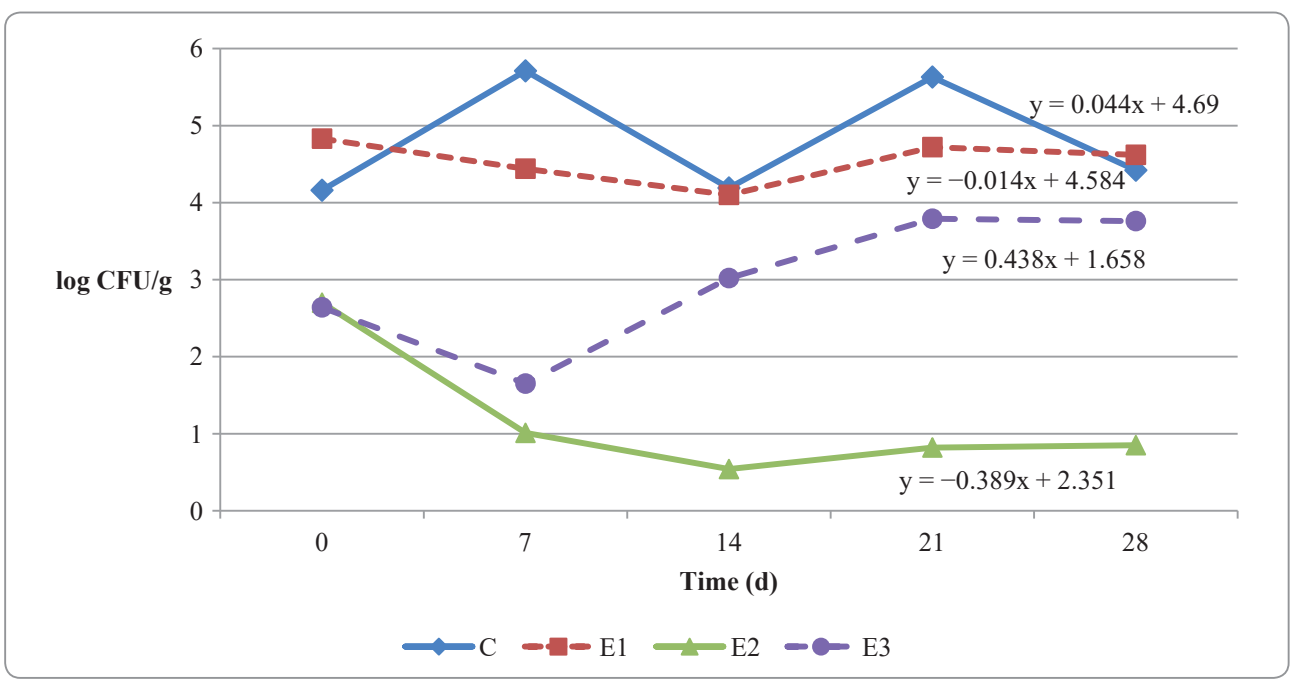

Figure 2. *Enterobacteriaceae* counts (log CFU/g) in control (C) and marinated (E1, E2 and E3) vacuum-packaged skinless chicken breast fillets (n=120)

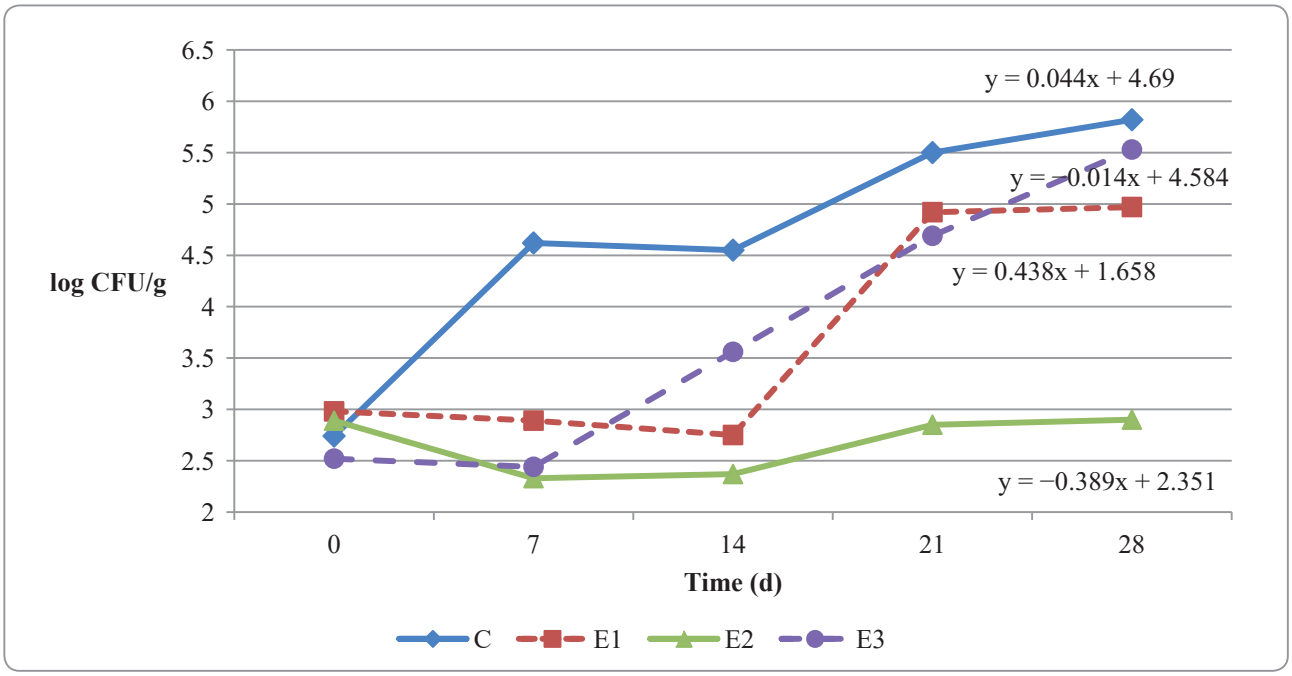

Figure 3. Lactic acid bacteria counts (log CFU/g) in control (C) and marinated (E1, E2 and E3) vacuum-packaged skinless chicken breast fillets (n=120)

packaging types did not affect *Enterobacteriaceae* counts (*Rouger et al.*, 2017). The number of *Enterobacteriaceae* on spoiled chicken meat varies (*Balamatsia et al.*, 2007; *Doulgeraki et al.*, 2012; *Zhang et al.*, 2012). *Enterobacteriaceae* numbers on marinated poultry ranged from 6.0 log CFU/g (stored at 4°C, 15 days) to 8.36 log CFU/g (stored at 4 to 10°C, 4 days). Also, *Enterobacteriaceae* were not detected in spoiled poultry meat in some studies, regardless of the duration and temperature of storage (*Al-Nehlawi et al.*, 2013; *Chouliara et al.*, 2007; *Capita et al.*, 2013).

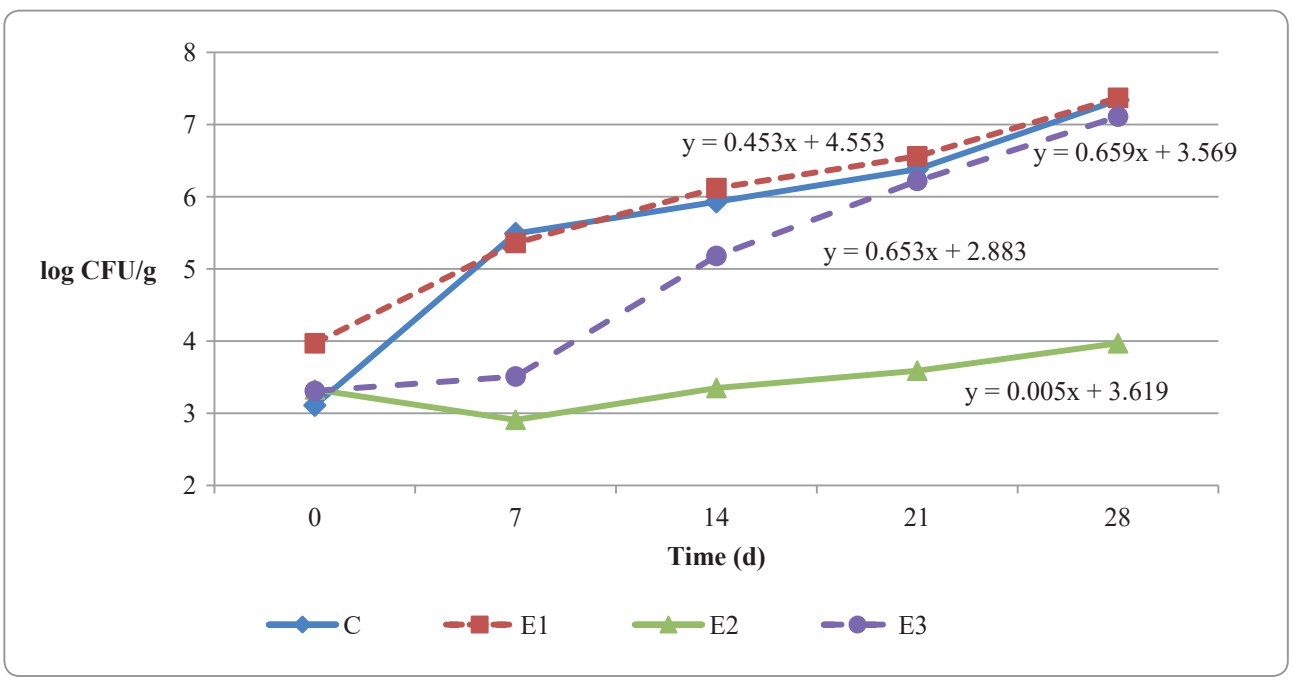

Figure 4. Anaerobic bacterial counts (log CFU/g) in control (C) and marinated (E1, E2 and E3) vacuum-packaged skinless chicken breast fillets (n=120)

The LAB count increased during the storage of chicken breast fillets in all marinade treatments, except in E2 fillets. The addition of 2% sodium citrate inhibited LAB growth, and numbers ranged from 2.33 to 2.90 log CFU/g during the storage (Fig. 3, Table 1). Many previous studies show that any type of marination treatment, either alone or in combination with other treatments such as vacuum packaging, influences the decrease of LAB (*Piñon et al.*, 2015; *Oral et al.*, 2009; *Skandamis et al.*, 2002; *Nieminen et al.*, 2012). *Rouger at al.* (2017) stated that in different studies on poultry meat spoilage, the number of LAB varied in a very wide range (from not detected to 9.04 log CFU/g). Temperature of storage and duration of study did not affect LAB numbers in numerous studies conducted on marinated chicken (*Doulgeraki et al.*, 2012; *Zhang et al.*, 2012; *Capita et al.*, 2013; *Krockel*, 2013; *Kalschne et al.*, 2014). Despite their positive effects, some species of LAB are the major spoilage bacteria in vacuum- and modified atmosphere-packaged poultry meat.

Among the studied bacterial groups, the most significant increase detected in our chicken breast fillets was in the anaerobic bacteria, counts of which were higher than 7 log CFU/g at the end of storage, with the notable exception of E2 fillets (3.97

Table 1. Total viable count, *Enterobacteriaceae* count, lactic acid bacteria count and anaerobic bacterial count (log CFU/g) ($\overline{X}$±Sd) on marinated, vacuum-packaged chicken breast fillets during chilled storage

	C	E1	E2	E3
Total viable counts (TVC)				
day 0	3.40±0.19 ^{ABCDa}	4.61±0.76 ^{ABCDab}	4.04±0.18 ^{ABC}	3.66±0.33 ^{ABCb}
day 7	6.29±0.50 ^{AEFGab}	6.25±0.53 ^{Acd}	3.26±0.42 ^{ADac}	3.83±0.43 ^{DEFbd}
day 14	5.39±0.25 ^{BEHa}	5.64±0.24 ^{BEbc}	3.39±0.13 ^{BEabd}	4.95±0.54 ^{ADGHcd}
day 21	5.62±0.32 ^{CFI}	6.12±0.10 ^{CF}	3.57±0.25 ^{C}	5.86±0.27 ^{BEG}
day 28	7.03±0.23 ^{DGHI}	6.94±0.33 ^{DEF}	3.91±0.28 ^{DE}	5.91±0.20 ^{CFH}
Enterobacteriaceae				
day 0	4.16±0.43 ^{ABabc}	4.83±0.50 ^{Aade}	2.70±0.26 ^{ABCDbd}	2.64±0.13 ^{ABce}
day 7	5.71±0.13 ^{Aabc}	4.44±0.45 ^{ade}	1.01±0.04 ^{AEbd}	1.65±0.63 ^{CDEce}
day 14	4.19±0.32 ^{ab}	4.10±0.05 ^{Acd}	0.54±0.18 ^{BEace}	3.02±1.00 ^{Cbde}
day 21	5.63±0.50 ^{Babc}	4.72±0.42 ^{ade}	0.82±0.40 ^{Cbdf}	3.79±0.29 ^{ADcef}
day 28	4.42±0.22^{a}	4.62±0.43^{bc}	0.85±0.32 ^{Dabd}	3.76±0.69 ^{BEcd}
Lactic acid bacteria (LAB)				
day 0	2.74±0.23 ^{ABCD}	2.98±0.55 ^{AB}	2.89±0.21 ^{AB}	2.52±0.15 ^{ABC}
day 7	4.62±0.46 ^{AEFabc}	2.89±0.18 ^{CDad}	2.33±0.30 ^{ACDbd}	2.44±0.21 ^{DEFc}
day 14	4.55±0.18 ^{BGHabc}	2.75±0.35 ^{EFad}	2.37±0.26 ^{BEbe}	3.56±0.56 ^{ADGHcde}
day 21	5.50±0.10 ^{CEGabc}	4.92±0.31 ^{ACEad}	2.85±0.43 ^{Cbde}	4.69±0.37 ^{BEGIce}
day 28	5.82±0.31 ^{DFHab}	4.97±0.39 ^{BDFacd}	2.90±0.17 ^{DEbce}	5.53±0.19 ^{CFHIde}
Anaerobic bacterial counts				
day 0	3.11±0.19 ^{ABCDa}	3.97±0.55 ^{ABCDabc}	3.33±0.07 ^{Ab}	3.31±0.17 ^{ABCc}
day 7	5.49±0.38 ^{AEFab}	5.36±0.47 ^{AEFGcd}	2.91±0.32 ^{BCDace}	3.51±0.27 ^{DEFbde}
day 14	5.93±0.21 ^{BGab}	6.12±0.18 ^{BEHcd}	3.35±0.11 ^{BEace}	5.18±0.63 ^{ADGHbde}
day 21	6.38±0.65 ^{CEHa}	6.56±0.23 ^{CFIb}	3.59±0.32 ^{Cabc}	6.22±0.24 ^{BEGIc}
day 28	7.34±0.15 ^{DFGHa}	7.37±0.19 ^{DGHIb}	3.97±0.30 ^{ADEabc}	7.11±0.26 ^{CFHIc}

$^{a, b, c}$: Means in the same row with the same superscripts are different at $p<0.05$

$^{A, B, C}$: Means in the same column with the same superscripts are different at $p<0.05$

C – control fillets marinated in a 6% NaCl solution; E1 – fillets marinated in 6% NaCl + 2% sodium tripolyphosphate; E2 – fillets marinated in 6% NaCl + 2% sodium citrate; E3 – fillets marinated in 6% NaCl, 1% sodium tripolyphosphate and 1% sodium citrate.

log CFU/g on day 28) (Fig. 4, Table 1). On day 7 in all marinades, the anaerobic bacterial count was significantly lower ($p<0.05$) than on other days. These results fully coincide with *Piñon et al.* (2015), who used ultrasound treatment combined with oregano oil marinade to study the microbiota of poultry breast meat. The anaerobic bacteria present in poultry meat are responsible for the production of large quantities of gases (H_2 and CO_2), which can cause deformation of the vacuum packaged meat due to their accumulation, putrid odours, the presence of exudates, extensive proteolysis and changes in pH and colour (*Yang et al.*, 2014; *Iulietto et al.*, 2015).

Conclusion

Based on the microbiological data obtained, the combination of 6% NaCl and 2% sodium citrate is the most appropriate marinade option for reducing the growth of the examined bacterial groups in vacuum-packaged marinated chicken breast fillets during chilled storage. Further studies should be conducted to determine the best composition of marinade to reduce the microbiota present in poultry meat. Also it is important to establish what type of packaging can improve shelf-life and sensory attributes of poultry meat.

Redukcija mikroflore u mariniranim filetima pilećih grudi pakovanih u vakuum

Jelena Janjić, Jelena Ćirić, Slaven Grbić, Marija Bošković, Milica Glišić, Radmila Mitrović, Adriana Radosavac, Milan Ž. Baltić

A p s t r a k t: Cilj ovog rada bio je da se utvrdi uticaj različitog načina mariniranja na mikrobiotu fileta grudi brojlera pakovanih u vakuum i čuvanih pri 4°C. Ispitivane su tri vrste marinade. Ukupno 120 uzoraka (korišćenih za dva ponavljanja) marinirano je u kontrolnom (6% NaCl) i tri različita tretmana: 6% NaCl i 2% natrijum tripolifosfat (E1), 6% NaCl i 2% natrijum citrat (E2) i rastvor sa 6% NaCl, 1% natrijum tripolifosfata i 1% natrijum citrata (E3). Brojanje mikroorganizama vršeno je prvog dana (0 dan), 7., 14., 21. i 28. dana skladištenja. Utvrđene su statistički značajne razlike ($P<0,05$) između mariniranih uzoraka u ukupnom broju mezofilnih bakterija, Enterobacteriaceae, bakterijama mlečne kiseline i anaerobnim bakterijama. Utvrđeno je da je kombinacija 6% NaCl i 2% natrijum citrata najprikladnija za redukciju rasta ispitivanih grupa bakterija u mariniranim filetima grudi brojlera pakovanih u vakuum i skladištenih pri 4°C.

Ključne reči: *meso živine, rok trajanja, bakterije kvara, uslovi skladištenja.*

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Original scientific paper

Histamine in canned and smoked fishery products sold in Serbia

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A b s t r a c t: An increase in seafood-related illnesses as a consequence of an increase in seafood consumption has been documented recently. The United States Food and Drug Administration (FDA) has identified histamine as the major chemical hazard of seafood. The aim of this study was to determine histamine levels in 227 production lots (2043 subsamples) of canned tuna, canned sardines, canned mackerel and smoked salmon obtained from Serbian retail stores in 2018. Levels of histamine were determined using high performance liquid chromatography tandem mass-spectrometry. In addition, we compared histamine levels found in analysed products with levels established by the European Commission (EC) and FDA. Mean levels of histamine in compliant lots, according to EC Regulation 2073/2005, were 9.21, 3.16, 3.34 and 5.22 mg/kg in canned tuna, canned sardines, canned mackerel and smoked salmon, respectively. The highest histamine levels were found in canned tuna and canned mackerel, 1112 mg/kg and 412 mg/kg, respectively. Histamine was detected in 468 (22.91%) of all analysed subsamples. Levels above 100 mg/kg, which is the maximum histamine level allowed in canned fish in the EU, were found in 38 (1.86%) of analysed subsamples. According to EC Regulation 2073/2005, six (2.64%) of all analysed lots were found non-compliant, while 21 (9.25%) of analysed lots should be rejected according to the FDA limit (50 mg/kg).

Keywords: *histamine, canned tuna, mackerel, sardine, food safety.*

Introduction

Globally, more than 63.5 million tons of seafood is caught and eaten each year, representing a steady global increase in seafood consumption (*Silva et al.*, 2011). Some authors have indicated the health benefits of marine fish consumption, mostly due to the role of n-3 fatty acids in alleviating cardiovascular diseases, lowering blood pressure and in preventing the development of hypertension (*Narayan et al.*, 2006). This is probably the reason why consumer awareness of the health benefits of incorporating fish in diets is constantly growing. However, an increase in seafood-related illnesses as a consequence of this increase in seafood consumption has been documented (*Butt et al.*, 2004). The United States Food and Drug Administration (FDA) has identified histamine as the major chemical hazard of seafood. Recently, greater interest can be observed in the area of seafood safety, especially when it comes to histamine, both in Serbia and worldwide (*Karmi*, 2014; *Dimitrijevic et al.*, 2016; *Petrovic et al.*, 2016; *Ryok Kang et al.*, 2018; *Zhang et al.*, 2018).

Histamine fish poisoning (HFP), also known as scombrotoxin fish poisoning (SFP), is a foodborne chemical intoxication mostly caused by the ingestion of spoiled or bacterially contaminated scombroid fish that are characterised by high levels of the amino acid histidine in muscle tissue. Scombroid fish belong to the families Scombridae (mackerel, tuna, bonito) and Scomberesocidae (saury). High levels of histidine are also found in other fish families such as Clupeidae (sardine, herring), Coryphaenidae (mahi-mahi), Gempylidae (escolar), Istiophoridae (marlin, sailfish) and Carangidae (amberjack or yellowtail), which were implicated in histamine intoxication outbreaks (*Schulze et al.*, 1979; *Muller et al.*, 1992; *Feldman et al.*, 2005; *Chen et al.*, 2010; *Chen et al.*, 2011).

Histamine is a biogenic amine with a heterocyclic structure and it can be found in foods such as meat, cheese, wine and other fermented foods as well as in fish. Histamine is derived from the decarboxylation of histidine by the enzyme histidine decarboxylase (HDC). HDC is produced by bacteria which are part of natural microbiota of fish.

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According to the European Food Safety Authority (*EFSA*, 2011), bacteria species that are the strongest histamine producers are *Hafnia alvei*, *Morganella morganii*, *Klebsiella pneumonia*, *Morganella psychrotolerans*, *Photobacterium phosphoreum* and *Photobacterium psychrotolerans*. Some bacteria that belong to the family *Enterobacteriaceae*, such as *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Citrobacter amalonaticus* and *Cronobacter sakazakii*, are also capable of producing histamine (*Zhang et al.*, 2018). Although the main means to prevent the occurrence of histamine is maintenance of low temperatures during fish processing, *Emborg et al.* (2005) found that psychrotolerant bacteria are capable of producing toxic concentrations of histamine in tuna at 2°C.

In contrast to bacterial pathogens, histamine is heat stable, and it cannot be eliminated or reduced by heat treatment; therefore, maintenance of the cold chain along all stages of fish processing is essential. Heating processes can only be used to eliminate histamine-producing bacteria and their HDC enzymes from the product (*FAO/WHO*, 2013). However, some authors reported different methods that can degrade histamine and other biogenic amines such as gamma irradiation and application of diamine oxidase bacteria (*Dapkevicius et al.*, 2000; *Kim et al.*, 2004).

The symptoms associated with HFP are similar to those of an allergic reaction and include hypertension, flushing, headache, urticaria, nausea, vomiting, diarrhoea and abdominal cramps (*Maintz and Novak*, 2007). The presence of certain histamine potentiators in fish, such as cadaverine and putrescine, could decrease the dose of histamine needed to provoke an adverse reaction in humans (*FAO/WHO*, 2013). The symptoms typically occur rapidly, from 5 mins to 2 h after ingestion, with a usual duration of 8 h. Many incidents go unreported because of the mildness of the disease, lack of mandatory reporting and misdiagnosis, because symptoms can be confused with *Salmonella* infection and food allergy (*Silva et al.*, 2011).

According to reports from the Rapid Alert System for Food and Feed, in 2018, 29 products were reported in which histamine levels were above the European Commission (EC) regulated maximum limit (*European Commission, 2015*). Well designed good hygienic practice (GHP), good manufacturing practice (GMP) and hazard analysis (and) critical control point (HACCP) plans are essential steps in preventing histamine formation above the safety limits. Rapid chilling of the fish soon as possible after harvesting and maintenance of the cold chain through all stages of fish processing are the most important factors in preventing histamine production above the safety limit. EFSA indicate that the food categories showing the highest mean histamine levels are dried anchovies, fish sauce, fermented vegetables, cheese, other fish and fish products and fermented sausages (*EFSA, 2011*). According to *Zhai et al.* (2012), the main fish species used in canned products are scombroid fish (Spanish mackerel, tuna) and non-scombroid fish (anchovy, sardine) that are commonly associated with HFP. Sensory evaluation of the fish is not sufficient to detect the absence or presence of histamine, and it cannot be taken as final assurance of low histamine level; therefore chemical testing is required.

The aim of this study was to provide information regarding the presence of histamine in canned tuna, canned sardines, canned mackerel and smoked salmon present in Serbian retail stores in 2018 and to evaluate the safety of these products in regard to histamine levels established by the European Commission (*European Commission, 2015*) and FDA (*FDA, 1995*).

Materials and Methods

Standards and reagents

Histamine standard was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and water of HPLC grade were from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate and formic acid were purchased from Merck (Darmstadt, Germany). Trichloroacetic acid (TCA) was from Fisher Chemical (Hampton, NH, USA).

Fish samples and extraction

A total of 227 lots of canned fish and smoked salmon samples were analysed. Every lot consisted of 9 subsamples, making a total of 2043 subsamples. All subsamples were collected from Serbian retail stores during 2018 and they were all imported from other countries. The fish samples analysed included canned tuna (91), canned sardines (54), canned mackerel (13) and smoked salmon (69). All samples were homogenised using a bowl chopper (Blixer 2, Robot Coupe, France). A 1 g amount of each sample was transferred into a polypropylene tube, followed by addition of 10 mL of 6% (w/v) TCA. The mixture was vortexed for 20 min and then centrifuged at 4000 rpm for 5 min.

The supernatants were filtered through 0.45 μm pore-size nylon syringe filters (Amtast, Lakeland, FL, USA) into HPLC vials.

LC-MS/MS

The quantification of histamine was carried out using a Shimadzu (Shimadzu Corporation, Kyoto, Japan) system consisting of two LC-30AD UPLC pumps connected in binary gradient mode, DGU-20A degassing unit, SIL-30AC autosampler, CTO-20AC column oven, CBM-20A system controller and LCMS 8040 triple quadrupole MS detector. Heat block, interface and desolvation line temperatures were 400°C, 350°C and 250°C respectively. Instrument control, data acquisition and evaluation were accomplished with the LabSolutions software (Shimadzu Corporation, Kyoto, Japan). Three transitions of histamine were monitored (m/z 112>54, m/z 112>68 and 112>95). Chromatographic separation of histamine was carried out on a Kinetex® (Phenomenex, Torrance, CA, USA) column (100×2.1 mm, 2 μm) at 50°C with an isocratic flow rate of 0.35 mL/min. The mobile phase consisted of 10 mM ammonium acetate in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) in the ratio A:B = 20:80.

Method validation

The analytical method was validated according to *Thompson et al.,* (2002). The experimentally determined limit of detection (LoD) was 2 mg/kg, while the limit of quantification (LoQ) was 5 mg/kg. Linearity was acceptable within the concentration range of 5–250 mg/kg, and the relative standard deviation of reproducibility was between 1.69% (at 250 mg/kg) and 13.93% (at 5 mg/kg). Measurement uncertainty of the method was calculated according to the NORDTEST concept (*Magnusson et al., 2012*) and was 4.42% at 100 mg/kg.

Statistical analysis

The percentage of samples containing histamine among groups based on different histamine level, mean histamine levels and standard deviation were determined using SPSS package (SPSS 23.0, Chicago, IL, USA). To distinguish statistical differences between the data, T-tests were performed with statistical significance being set at $p<0.001$, using the above-mentioned software package.

Results and Discussion

Histamine levels in 2043 subsamples of canned tuna, canned sardines, canned mackerel and smoked salmon are presented in Table 1. Large variations in histamine level in different cans with same lot number were previously reported by *Petrovic et al.* (2016) while investigating a histamine intoxication outbreak in Serbia. When considering product safety, it is essential to present results of all analysed subsamples, rather then mean levels. According to EFSA, fish containing less than 50 mg/kg of histamine seems to be safe for human consumption, whereas concentrations between 50 and 200 mg/kg may cause adverse health effects and levels above 200 mg/kg histamine are reported to cause toxic effects in humans (*EFSA*, 2011). Based on these limits, 78 (3.82%) of 2043 analysed subsamples could have caused adverse health effects when eaten. In the EU, for simple fish products, the critical concentration of histamine is 100 mg/kg, and for the enzyme matured products, the critical histamine concentration is 200 mg/kg. One sample consists of nine units, two of which can contain levels between 100 and 200 mg/kg, i.e. 200–400 mg/kg for enzyme-treated products (*Commission Regulation (EC) No.* 2073/2005). In Serbia, the regulation that governs histamine in fish (*Official Gazette RS, 2010*) is in accordance with EC Regulation 2073/2005. In 78 (3.82%) of all our analysed subsamples, histamine levels were above the *FDA* (1995) action level (50 mg/kg), making a total of 21 rejected lots, while 6 lots were non-compliant according to EC Regulation 2073/2005. Figure 1 shows a chromatogram of three transitional products of histamine at 50 mg/kg in one fish sample.

Histamine levels above 200 mg/kg, which is the upper limit in the EU, were found in 12 subsamples of canned tuna and in 8 subsamples of canned mackerel. *Suyama and Yoshizawa* (1973) investigated the free amino acid composition of some fish species and found histidine levels above 12000 mg/kg and 7400 mg/kg in *Thunnus albacares* (yellowfin) and *Thunnus obesus* (big-eye tuna), respectively. High levels of histidine (8020 mg/kg) were also found in *Scomber japonicus* (mackerel) (*Hibiki and Simidu*, 1959). According to the FAO, the highest histidine level reported for *Sardina pilchardus* (sardines) was 2888 mg/kg, which is much lower than those found in tuna and mackerel (*FAO/WHO*, 2013). In the current study, the mean histamine level found in canned sardines was lower than those of canned tuna and canned mackerel, which

Table 1. Histamine content in all subsamples (n=2043) analysed in this study and number of non-compliant lots according to EC and FDA regulations

Product	No. of subsamples (lots)	No. (%) of subsamples with a histamine content (mg/kg) of: <LoQ[a]	5–50	50–100	100–150	150–200	>200[b]	No. (%) of non-compliant lots (EC)[c]	No. (%) of non-compliant lots (FDA)[d]
Canned tuna	819 (91)	480 (58.91)	286 (34.92)	27 (3.30)	3 (0.37)	11 (1.34)	12 (1.46)	4 (4.40)	13 (14.29)
Canned sardines	486 (54)	440 (90.53)	44 (9.05)	2 (0.41)	0	0	0	0	2 (3.70)
Canned mackerel	117 (13)	92 (78.63)	14 (11.97)	2 (1.71)	1 (0.85)	0	8 (6.84)	2 (15.38)	2 (15.38)
Smoked salmon	621 (69)	563 (90.66)	45 (7.25)	10 (1.61)	2 (0.32)	1 (0.16)	0	0	4 (5.80)

[a] Subsamples in which histamine levels were below limit of quantification (LoQ) (5 mg/kg).
[b] Subsamples in which histamine levels were above 200 mg/kg.
[c] Non-compliant lots according to EC Regulations
[d] Non-compliant lots according to FDA Regulations

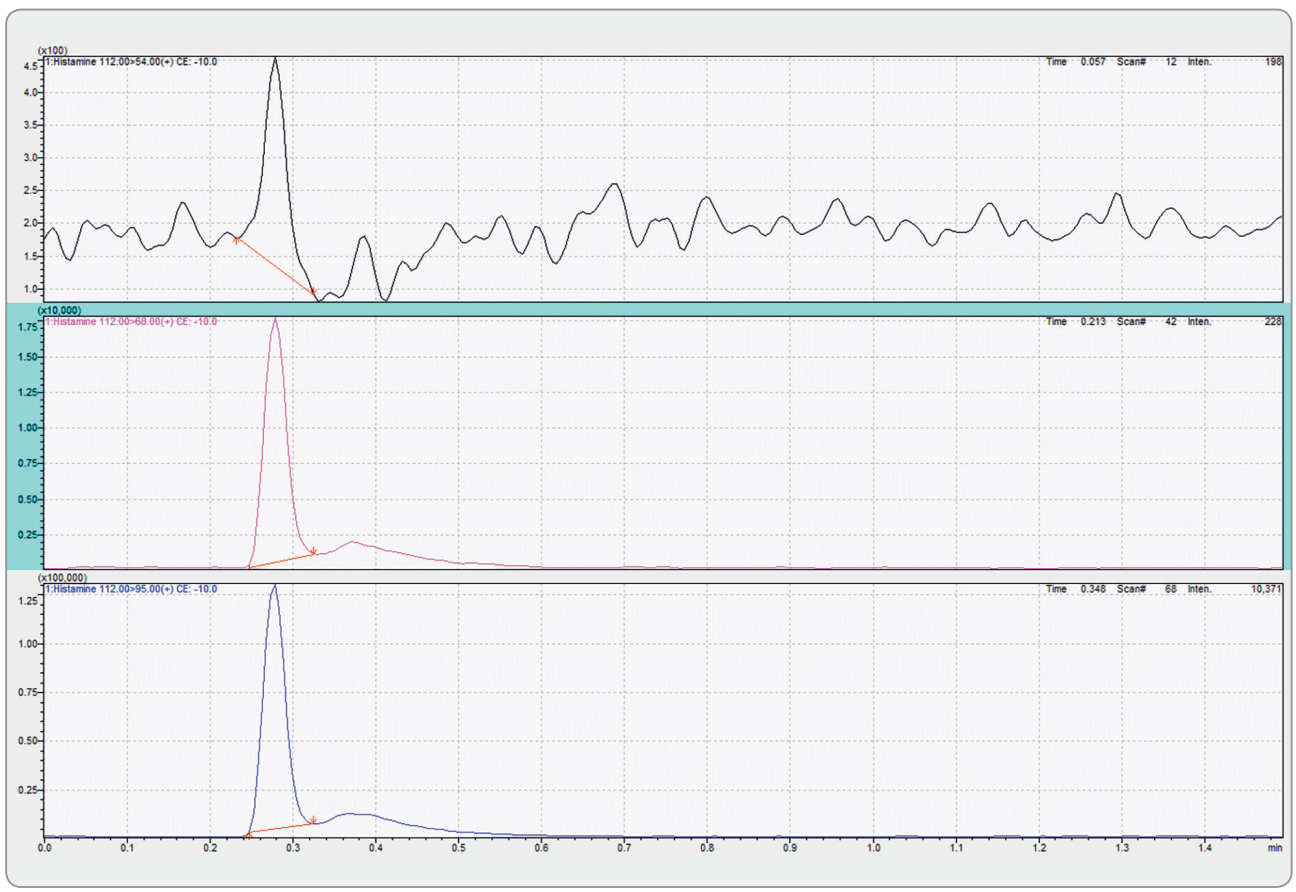

Figure 1. MRM chromatograms of histamine at a concentration of 50 mg/kg. Chromatograms depict transitional products 112>54 Da, 112>68 Da, 112>95.

indicates the strong relationship between histidine level in muscle tissue and high histamine content in the product.

The quality of the analytical process was confirmed by the analysis of the certified reference material T27229QC (Fera Science Ltd., York, United Kingdom) within each batch of analysed samples. The certified histamine content was 45 mg/kg. Obtained histamine contents for this certified product were within ±15% of the certified content. Chromatograms of three transitional products of histamine in the certified reference material is presented in Figure 2.

According to Commission Regulation 2073/2005, all subsamples of our canned sardines and smoked salmon showed histamine levels below the safe level of 100 mg/kg, with the exception of three subsamples of smoked salmon from three different production lots. Histamine levels below the laboratory LoQ were found in 1601 (76.68%) of all analysed samples, while histamine levels in 58.91% samples of canned tuna were below the LoQ. This percentage was slightly higher than that reported by *Dimitrijevic et al.* (2016) who found histamine levels in 50.37% of canned tuna samples to be below the LoQ. In a similar study conducted in Serbia, histamine levels in canned tuna samples were found to be below the LoQ in 54.40%, between 10 and 50 mg/kg in 34.40% and between 50 and 100 mg/kg in 4% of analysed lots (*Petrovic et al.*, 2016), which is in accordance with results found in this study. Levels above 100 mg/kg were found in 25 (3.04%) of our canned tuna samples. In contrast to this, *Karmi* (2014) found histamine levels in 33.3% of canned tuna samples were above 100 mg/kg. High histamine levels in these canned tuna indicate poor temperature treatment and a lack of hygiene during processing. Canned tuna is often used in the preparation of different types of salads, where recontamination and time-temperature abuse can lead to formation of histamine in levels that can cause HFP (*Predy et al.*, 2003; *McCarthy et al.*, 2015).

The highest histamine level found in our canned sardines was 78 mg/kg, which is in accordance with the results reported by *Karmi* (2014) and *Pacheco-Aguilar et al.* (1998), who found maximum levels of 62 and 70.3 mg/kg, respectively. These results differ from data presented by *Petrovic et al.* (2016), who reported a maximum histamine level of 19.1 mg/kg in canned sardines.

Kim et al. (2002) investigated histamine production by *Morganella morganii* in four different fish families at 37, 25, 15 and 4°C for 36, 36, 60 and 14 h, respectively. They found levels below 50 mg/kg in all salmon samples, while levels in mackerel, albacore and mahi-mahi exceeded 50 mg/kg in a few hours at different temperatures, with the exception of fish held at 4°C. Similar result were reported by *Crapo and Himelbloom* (1999), who found no histamine production in pink salmon during 14 days of storage at 10°C. These results are comparable with results found in present study, in which we found 97.91% of smoked salmon samples had histamine levels below 50 mg/kg, while 90.66% were below the LoQ. This is attributed to low histidine contents in muscle tissue of salmon and probably due to good hygiene and low storage temperatures during fish processing. Although salmon is not histidine rich, *Bartholomew et al.* (1987) reported 12 incidents of salmon being involved in HFP from 1976 to 1986 in Britain.

Mean histamine contents were calculated for all analysed subsamples of each product and for all subsamples of compliant production lots according to the Commission Regulation 2073/2005 in order to avoid outliers. Histamine levels varied significantly ($p<0.001$) among the analysed fish products (Table 2). These results are in accordance with those reported by *Yesudhason et al.* (2013), who found a significant difference ($p<0.05$) between mean histamine levels in canned tuna and canned sardines. Our mean histamine level (9.21 mg/kg) in canned tuna calculated for compliant production lots was similar to those found by *Er et al.* (2014), lower than that reported by *Tsai et al.* (2005) but higher then those found by *Silva et al.* (2011), who reported very low levels of histamine for different canned tuna products in Brazil. In the current study, the mean histamine level in canned mackerel samples for compliant lots (3.34 mg/kg) was in accordance with that reported by *Tsai et al.* (2005) but much lower then that reported by *Karmi* (2014), who found histamine at 68 mg/kg in canned mackerel, with a maximum level of 82 kg/kg.

In several histamine intoxication outbreak studies, reported levels of histamine in leftovers of different fish and fish products ranged from 293 mg/kg to 5200 mg/kg (*Feldman et al.*, 2005; *Tsai et al.*, 2005; *Chen et al.*, 2008; *Chen et al.*, 2010; *Ryok Kang et al.*, 2018). Reported symptoms were flushing, headache, rash, nausea, diarrhoea, sweating, peppery taste and dizziness but no cases of death. In the current study, maximum levels of histamine found in canned tuna, canned mackerel and smoked salmon were 1112, 412 and 122 mg/kg, respectively. Based on histamine intoxication studies,

we can conclude that levels of histamine we found in tuna and mackerel could have caused HFP in healthy individuals. However, the usual portion size of canned tuna per consumer is about 130 g, which corresponds to a total of 144.5 mg of histamine in a portion of canned fish containing 1112 mg/kg of histamine. The high levels of histamine we measured in these canned fish products leads to the conclusion that poor quality raw fish has been used for their production. *Motil and Scrimshaw* (1979) reported that ingestion of 50, 100, 150 and 180 mg of histamine *via* tuna caused typical symptoms such as mild headache and flush in 1, 3, 4 and 7 of 8 healthy individuals, respectively. However, this dosage levels will not apply to individuals with a specific sensitivity to histamine and would not apply to children, particularly because they consume more food per unit body weight than adults (*FAO/WHO*, 2013). Storage temperature is the most important factor contributing to biogenic amine

Table 2. Histamine levels (mg/kg) for different fish products analysed in this study and levels for compliant lots according to EC Regulations (mean±SD)

	Compliant lots[a]	Median	Max	All analysed lots[b]	Median	Max
Canned tuna	9.21±14.8[c]	2.5	1112	21.70±89.1[c]	2.5	169
Canned sardines	3.16±3.8[d]	2.5	78	3.16±3.8[d]	2.5	78
Canned mackerel	3.34±3.5[d]	2.5	26	26.45±77.2[c]	2.5	412
Smoked salmon	5.22±13.7[e]	2.5	171	5.22±13.7[e]	2.5	171

[a] Subsamples of compliant lots. For levels below LoQ, the level used was 2.5 mg/kg.

[b] Subsamples of all analysed lots. For levels below LoQ, the level used was 2.5 mg/kg.

[c-e] Values in the same column followed by different letters are significantly different ($p<0.001$).

Figure 2. MRM chromatograms of histamine in certified reference material used for quality control of analytical process. Chromatograms depict transitional products 112>54 Da, 112>68 Da, 112>95.

formation, although a variety of techniques can be combined together to control the microbial growth and enzyme activity during processing and storage for better shelf life extension and food safety (*Chong et al.*, 2011).

Conclusion

In this study, four (4.40%) production lots of canned tuna and two (15.38%) lots of canned mackerel were non-compliant for histamine levels according to Commission Regulation (EC) No. 2073/2005. In contrast, in 13 (14.29%) lots of canned tuna and two (15.38%) lots of canned mackerel, histamine levels were above the FDA action limit of 50 mg/kg (*FDA, 1995*). Although the histamine content in all our canned sardines and smoked salmon samples were compliant according to EC Regulation 2073/2005, two (3.70%) production lots of canned sardines and four (5.80%) production lots of smoked salmon would be non-compliant if we had applied the FDA action limit, due to the much stringent requirements. Canned tuna had the highest mean histamine levels, followed by smoked salmon and canned mackerel, while canned sardines contained the lowest mean histamine level. Histamine was detected in 468 (22.91%) subsamples of all analysed fish products.

Histamin u konzerviranim i dimljenim proizvodima od ribe koji se prodaju na tržištu u Srbiji

Stefan Simunović, Saša Janković, Tatjana Baltić, Dragica Nikolić, Jasna Đinović-Stojanović, Mirjana Lukić, Nenad Parunović

A p s t r a k t: Poslednjih godina primećeno je povećanje broja bolesti povezanih sa konzumacijom plodova mora kao posledica povećanja konzumacije ovih proizvoda. Uprava za hranu i lekove Sjedinjenih Američkih Država (eng. FDA) označila je histamin kao glavnu hemijsku opasnost plodova mora. Cilj rada bio je da se odredi sadržaj histamina u 227 proizvodnih lotova konzervirane tune, konzervirane sardele, konzervirane skuše i dimljenog lososa. Svi uzorci su prikupljeni iz maloprodajnih objekata u Republici Srbiji tokom 2018. godine. Za ispitivanje histamina korišćena je tečna hromatografija visokih performansi sa masenom detekcijom. Dobijene rezultate smo uporedili sa nivoima koje su uspostavili Evropska komisija i FDA. Histamin je detektovan u 468 (22.91%) jedinica analiziranih proizvoda. U skladu sa propisom Evropske komisije 2073/2005, šest (2.64%) ispitivanih proizvodnih lotova su ocenjeni kao nezadovoljavajući, dok su čak 21 proizvodnih lotova ocenjeni kao nezadovoljavajući u skladu sa propisom FDA.

Ključne reči: *histamin, tuna u konzervi, skuša, sardela, bezbednost hrane.*

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Original scientific paper

Effect of broiler slaughter weight on meat yield and quality

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A b s t r a c t: Broiler meat quality depends on the interaction of several factors, including genotype, slaughter age/body weight, pre-slaughter handling, and slaughter method. The aim of this study was to determine the effect of slaughter weight on meat yield and quality of broilers. The material consisted of 42 broilers, classified into three groups: lighter (<2500 g), medium (2500–3000 g) and heavier (>3000 g). The following meat yield parameters were measured: cold carcass weight, breast weight before and after deboning, breast skin and bone weights, thigh weight before and after deboning, thigh skin and bone weights, drumstick weight before and after deboning, and drumstick skin and bone weights. Meat pH (M. pectoralis major) and instrumental colour (breast and drumstick) were measured 24 h post-mortem. Meat quality classes (pale, soft and exudative and normal meat) were determined based on breast muscle L value. Heavier broilers had higher (P<0.05) cold carcass weight, breast, thigh and drumstick weights both before and after deboning compared to medium and lighter broilers. In contrast, meat quality traits were not significantly (P>0.05) affected by slaughter weight. In conclusion, production of heavy broilers had a beneficial effect on meat quantity, while the effect of slaughter weight on meat quality was negligible.*

Keywords: *heavier broiler, meat yield, meat quality, instrumental color.*

Introduction

Modern livestock production aims to produce animals with high meatiness and good meat quality traits at the same time with minimum production cost (*Adzitey and Nurul*, 2011). These have been achieved through genetic manipulation of animals and careful breeding, which has led to a considerable increase in stress susceptibility, decrease in resistance to diseases and impaired meat quality (*Adzitey and Nurul*, 2011; *Cobanovic et al.*, 2019). In recent years, the worldwide, high demand for broiler meat has resulted in pressure on breeders and nutritionists to increase the growth rate, feed efficiency, and breast muscle size and to reduce the amount of abdominal fat (*Toldrá and Reig*, 2011; *Glamoclija et al.*, 2017). It is believed that the genetic progress of broiler growth leads to stress, resulting in histological and biochemical modifications of the muscle tissue, which have negative effects on meat quality. The latest concerns about broiler meat quality are associated with deep pectoral muscle disease and white striping, which disrupt the appearance of the product and increases the problem of the meat's poor ability to retain water during processing and storage as a result of the occurrence of pale, soft and exudative (PSE) meat (*Barbut et al*., 2008). This meat appearance and loss of protein quality are attributed to the denaturation of proteins caused by an excessively fast pH drop in combination with high muscle temperature within 30 minutes after slaughter (*Massimiliano and Claudio*, 2012). Broiler meat quality depends on the interaction of several factors, including genotype, slaughter age/body weight, pre-slaughter handling, and slaughter method (*Warris*, 2010; *Raseta et al.*, 2017). The high demand for raw cuts and processed products has led to the production of very heavy broilers (*Baéza et al*., 2012). Some authors found that broiler slaughter weight affects meat quality traits (*Bianchi et al.*, 2007; *Janisch et al*., 2011; *Baéza et al*., 2012; *Yalçin and Güler*, 2012; *Yalçin et al.*, 2014). Therefore, the aim of this study was to determine the effect of slaughter weight on meat yield and quality of broilers.

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Materials and methods

The study was conducted during summer of 2018 on 42 male Cobb broilers (about 42 days old, with an average live weight of 2805 ± 558.1 g) slaughtered at the same abattoir. All the broilers were reared on the same commercial farm under identical conditions. The treatment conditions, both before and after slaughter, were identical for all broilers and in accordance with the conventional industrial practice.

Meat yield parameters

Each carcass was weighed immediately after chilling to obtain cold carcass weight. Thereafter, each carcass (including skin and bone) was manually cut using a knife into different commercial cuts, which were breast, thighs, and drumsticks. The different commercial cuts were first weighed to determine breast, thigh and drumstick weights before deboning. Then, breast, thighs and drumsticks were further deskinned and deboned into meat, bone and skin. The breast, thighs and drumsticks were re-weighed to obtain breast, thigh and drumstick weights after deboning. Afterwards, the bones and skin of the three commercial cuts were weighted separately to determine breast skin and bone weights, thigh skin and bone weights, and drumstick skin and bone weights. All cutting, deskinning and deboning were performed by the same personnel. An electronic scale with a high sensitivity was used for weighing (Ard 110 Adventurer, Ohaus-USA).

Meat quality parameters

At 24 h post-mortem, breast muscle pH was directly measured using a pH meter (Cyber Scan pH 510, EUTECH-Netherlands) with a thin penetrating needle inserted in the centre of the M. *pectoralis major*, 0.5 to 1.0 cm below the muscle surface. The pH meter was calibrated with pH 4.00 and 7.00 phosphate buffer before each series of measurements and the electrode was rinsed with distilled water between each measurement. The pH values were measured in triplicate, and the average of the three measurements was taken as the final result.

Instrumental colour (Commission Internationale de l'Eclairage [CIE] L*a*b*) (CIE, 1976) was measured on the carcass surface over the breast and drumstick muscles using a Konica-Minolta portable Chroma Meter (CR 410, Minolta, Osaka, Japan) equipped with a 25 mm aperture, 0° viewing angle, and D65 illuminant. Before each series of measurements, the instrument was calibrated using a white ceramic tile. Breasts and drumsticks were exposed to air for at least 30 min at 15ºC before colour measurement. Lightness (L*), redness (a*), and yellowness (b*) values were determined on the each meat cut at three sites free of any discoloration: the proximal extremity of the muscle, the distal extremity, and between the proximal and distal extremities, and the average value for each meat cut was calculated. Meat quality classes were determined based on breast muscle L* value and placed into one of two categories: meat cuts were classified as PSE when their L* value was equal or higher than 58, while meat cuts were classified as normal meat when their L* value was lower than 58 (*Karunanayaka et al.,* 2016).

Statistical analysis

Statistical analyses of the results were conducted using software GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego CA, USA, www.graphpad.com). Broilers were divided into three weight groups: broilers with a live weight lower than 2500 g (lighter broilers) (n = 20); broilers with a live weight between 2500 and 3000 g (medium broilers) (n = 15); and broilers with a live weight higher than 3000 g (heavier broilers) (n = 7). One-way ANOVA with Tukey's post-hoc test was performed to test the effect of slaughter weight on meat yield and quality of broilers. Data were described by descriptive statistical parameters as the mean value and standard error of the mean (SEM). Also, Pearson correlation analysis was run between the slaughter weight and meat yield and quality parameters to numerically summarise the degree of association between any two variables. The Chi-squared test was used to determine the incidence of meat quality classes with respect to the slaughter weight. Values of $P<0.05$ were considered significant.

Results and discussion

Effects of slaughter weight on meat yield parameters of broilers are reported in Table 1. From this table, it is evident that heavier broilers had higher ($P<0.05$) cold carcass weight, and breast, thigh and drumstick weights both before and after deboning in comparison with medium and lighter broilers. These results are supported because in this investigation the increase in slaughter weight resulted in increased ($P<0.05$) cold carcass weight (r=1.000), breast weight before (r=0.8716) and after deboning (r=0.9406), thigh weight before (r=0.7805) and after deboning (r=0.8199), and drumstick weight before (r=0.7830) and after deboning (r=0.8196) (Table 2).

Similar results were reported by other authors (*Cahaner et al*., 1986; *Brake et al.*, 1993; *Renema et al.*, 1999; *Omojola et al*., 2004), who found direct proportion between the weights of primal cuts (breast, thigh and drumstick) and broiler slaughter weight.

In contrast, there were no significant differences between broilers of different slaughter weights with respect to breast skin and bone weights, thigh skin weight, and drumstick skin weight ($P>0.05$; Table 2). The characteristics of superior carcasses are the maximum proportion of most valuable muscles (i.e. breast and thigh muscles), the minimum proportion of bone and the optimum proportion of fat (*Eltrafi*, 2006), which were the properties of carcasses obtained from heavier broilers. These results indicate that heavier broilers have better meat/bone ratio than medium and lighter broilers. Accordingly,

Table 1. Effects of slaughter weight on meat yield parameters of broilers (n=42)

Parameters	**Heavier**	**Medium**	**Lighter**	**SEM**	***P*-value**	**Significance**
Number of animals	7	15	20			
Cold carcass weight (g)	2414.00[a]	1934.00[b]	1628.00[c]	70.822	<0.0001	*
Breast weight before deboning (g)	916.50[a]	733.60[b]	629.10[c]	43.431	<0.0001	*
Breast weight after deboning (g)	735.20[a]	583.30[b]	474.50[c]	35.835	<0.0001	*
Breast skin weight (g)	57.59	57.00	48.20	7.187	0.1321	ns
Breast bone weight (g)	122.00	94.72	97.70	14.396	0.1006	ns
Thigh weight before deboning (g)	360.30[a]	295.00[b]	251.20[c]	23.046	<0.0001	*
Thigh weight after deboning (g)	249.10[a]	207.30[b]	169.90[c]	15.048	<0.0001	*
Thigh skin weight (g)	36.34	30.11	30.75	4.651	0.3040	ns
Thigh bone weight (g)	74.46[a]	60.59[b]	51.09[b]	6.046	<0.0002	*
Drumstick weight before deboning (g)	306.50[a]	238.80[b]	216.30[b]	15.913	<0.0001	*
Drumstick weight after deboning (g)	190.70[a]	145.00[b]	128.90[c]	9.092	<0.0001	*
Drumstick skin weight (g)	25.94	24.06	20.65	2.851	0.0633	ns
Drumstick bone weight (g)	93.40[a]	73.87[b]	71.03[b]	6.962	0.0022	*

Legend: * Statistical significance at ($P<0.05$); ns: not significant ($P>0.05$); – Different letters in the same row indicate a significant difference at $P<0.05$ ([a-c])

Table 2. Correlations (r) between slaughter weight and meat yield parameters.

Parameters	**Slaughter weight**
Meat yield parameters	
Cold carcass weight (g)	1.0000*
Breast weight before deboning (g)	0.8716*
Breast weight after deboning (g)	0.9406*
Breast skin weight (g)	0.4489*
Breast bone weight (g)	0.2723*
Thigh weight before deboning (g)	0.7805*
Thigh weight after deboning (g)	0.8199*
Thigh skin weight (g)	0.3213*
Thigh bone weight (g)	0.6511*
Drumstick weight before deboning (g)	0.7830*
Drumstick weight after deboning (g)	0.8196*
Drumstick skin weight (g)	0.4400*
Drumstick bone weight (g)	0.5400*

*Statistical significance at ($P<0.05$).

the highest carcass quality was obtained from broilers slaughtered at heavier weights.

In this study, breast bone weight (r=0.2723), thigh bone weight (r=0.6511) and drumstick bone weight (r=0.5400) increased linearly ($P<0.05$) as the slaughter weight of the broiler increased (Table 2). This could be attributed to the fact that the increase in the slaughter weight, as a result of increase in breast and leg muscles, must also be accompanied by an increase in bone strength in the legs to ensure good health of the broilers (*Eltrafi*, 2006).

Effects of slaughter weight on meat quality parameters of broilers are reported in Table 3. None of the examined meat quality parameters was significantly (P>0.05) affected by slaughter weight. The high prevalence of PSE meat in each slaughter weight group recorded in this study (Table 3) could be explained by the fact that the experiment was conducted during the summer. It is well known that broilers, independent of slaughter weight, are extremely sensitive to high environmental temperature and relative humidity (*Oba et al.*, 2009; *Simoes et al*., 2009; *Langer et al*., 2010; *Carvalho et al*.,

Table 3. Effects of slaughter weight on meat quality parameters in broilers (n=42)

Parameters	**Heavier**	**Medium**	**Lighter**	**SEM**	***P*-value**	**Significance**
Number of animals	7	15	20			
pH value	6.19	6.27	6.24	0.098	0.6624	ns
Breast muscle L* value	58.47	56.47	57.97	1.819	0.2660	ns
Breast muscle a* value	2.96	2.20	2.03	0.673	0.2810	ns
Breast muscle b* value	7.34	7.60	7.01	1.486	0.8375	ns
Thigh muscle L* value	54.31	56.42	56.97	1.618	0.1748	ns
Thigh muscle a* value	7.71	6.05	4.90	1.729	0.1700	ns
Thigh muscle b* value	8.79	8.71	8.19	1.464	0.8232	ns
Drumstick muscle L* value	56.58	57.41	58.82	1.830	0.2943	ns
Drumstick muscle a* value	5.88	7.15	6.85	1.434	0.6173	ns
Drumstick muscle b* value	8.96	9.22	8.04	1.230	0.9743	ns
Meat quality classes						
Normal meat (%)	42.86	66.67	55.00		0.5553	ns
Pale, soft, exudative meat (%)	57.14	33.33	45.00		0.5553	ns

Legend: Normal meat: L* ≤ 58; Pale, soft, exudative: L* > 58; * Statistical significance at ($P<0.05$); ns: not significant ($P>0.05$); – Different letters in the same row indicate a significant difference at $P<0.05$

Table 4. Correlations (r) between slaughter weight and meat quality parameters.

Parameters	**Slaughter weight**
Meat quality parameters	
pH value	−0.007[1]
Breast muscle L* value	0.0626
Breast muscle a* value	0.2360
Breast muscle b* value	0.1204
Thigh muscle L* value	−0.229[1]
Thigh muscle a* value	0.3201*
Thigh muscle b* value	0.0248
Drumstick muscle L* value	−0.180[7]
Drumstick muscle a* value	0.0051
Drumstick muscle b* value	0.1798

*Statistical significance at ($P<0.05$).

2014; *Deshani et al*., 2016). Therefore, the high occurrence of PSE meat in broilers recorded in this investigation is most likely the result of the combined effects of high environmental temperatures and relative humidity, which resulted in a greater degree of post-mortem glycolytic metabolism and low breast muscle pH in broilers (*Fraqueza et al.,* 2006; *Bianchi et al.,* 2007; *Yalçin and Güler*, 2012; *Yalçin et al.*, 2014). Contrary to the results of this study, several studies (*Lu et al.*, 2007; *Simoes et al.,* 2009) have shown that heavier broilers are more susceptible to the deleterious effects of higher environmental temperatures and relative humidity. This could be explained by the fact that both heart and lung size decreases as a percentage of body weight with increasing growth rate, which likely affects the ability of heavier broilers to meet the respiratory demands of their bodies under high environmental temperature and relative humidity, and could contribute to the increased occurrence of panting, PSE meat and mortality (*Baéza et al*., 2012).

In this study, the increase in slaughter weight resulted in increased thigh muscle a* value (r=0.3201; $P<0.05$). Meat colour depends on the haem pigment (myoglobin and haemoglobin) concentration, chemical states associated with the myoglobin oxygenation and oxidation processes and the amount of light reflected from the meat (*Abdullah and Matarneh*, 2010). Earlier studies reported that slaughter weight and age of broilers are the main factors affecting the meat colour, because myoglobin concentration increases with increasing age and body weight, shifting the meat colour toward a darker and redder colour, so heavier broilers produce darker breast meat (*Abdullah and Matarneh*, 2010; *Baéza et al*., 2012).

Conclusions

The results of this study showed that production of heavy broilers had a beneficial effect on meat quantity, because such animals provide large amounts of meat presenting with characteristics appropriate for further processing. On the other hand, the effect of slaughter weight on meat quality was negligible. Therefore, further investigation taking into account different pre-slaughter conditions is required to clarify the main factors which affect the meat quality of broilers.

Uticaj telesne mase na prinos i kvalitet mesa brojlera

Aleksandra Nikolić, Milijana Babić, Jelena Jovanović, Nikola Čobanović, Ivana Branković Lazić, Lazar Milojević, Nenad Parunović

A p s t r a k t: Kvalitet mesa brojlera zavisi od interakcije velikog broja faktora, uključujući genotip, starost/telesnu masu, postupaka pre i posle klanja. Cilj ovog ispitivanja bio je da se ispita uticaj telesne mase na prinos i kvalitet mesa brojlera. Istraživanje je sprovedeno na 42 brojlera, grupisanih u tri grupe: brojleri telesne mase manje od 2500 g, brojleri telesne mase između 2500 i 3000 g i brojleri telesne mase veće od 3000 g. Ispitivani su sledeći pokazatelji prinosa mesa: masa hladnog trupa, masa grudi pre i posle otkoštavanja, masa kože i kostiju grudi, masa karabataka pre i posle otkoštavanja, masa kože i kostiju karabataka, masa bataka pre i posle otkoštavanja i masa kože i kostiju bataka. Od pokazatelja kvaliteta mesa određivani su pH vrednost grudne muskulature, instrumentalno (L, a* i b*) boja grudne muskulature, karabataka i bataka 24 časa nakon klanja. Meso brojlera je razvrstavano u klase kvaliteta (bledo, meko, vodenasto – BMV i meso normalnog kvaliteta) na osnovu L* vrednosti instrumentalno određene boje u M. pectoralis major. Brojleri velike telesne mase imali su veću masu hladnog trupa, kao i veću masu grudi, karabataka i bataka u poređenju sa brojlerima iz druge dve grupe. Nije utvrđen uticaj telesne mase na pokazatelje kvaliteta mesa brojlera. Stoga se može zaključiti da se klanjem brojlera velike telesne mase dobija najveći prinos mesa što predstavlja dobru sirovinu za dalju preradu. Sa druge strane, utvrđen je zanemarljiv uticaj telesne mase na kvalitet mesa brojlera.*

Ključne reči: *teži brojleri, prinos mesa, kvalitet mesa, boja mesa.*

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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

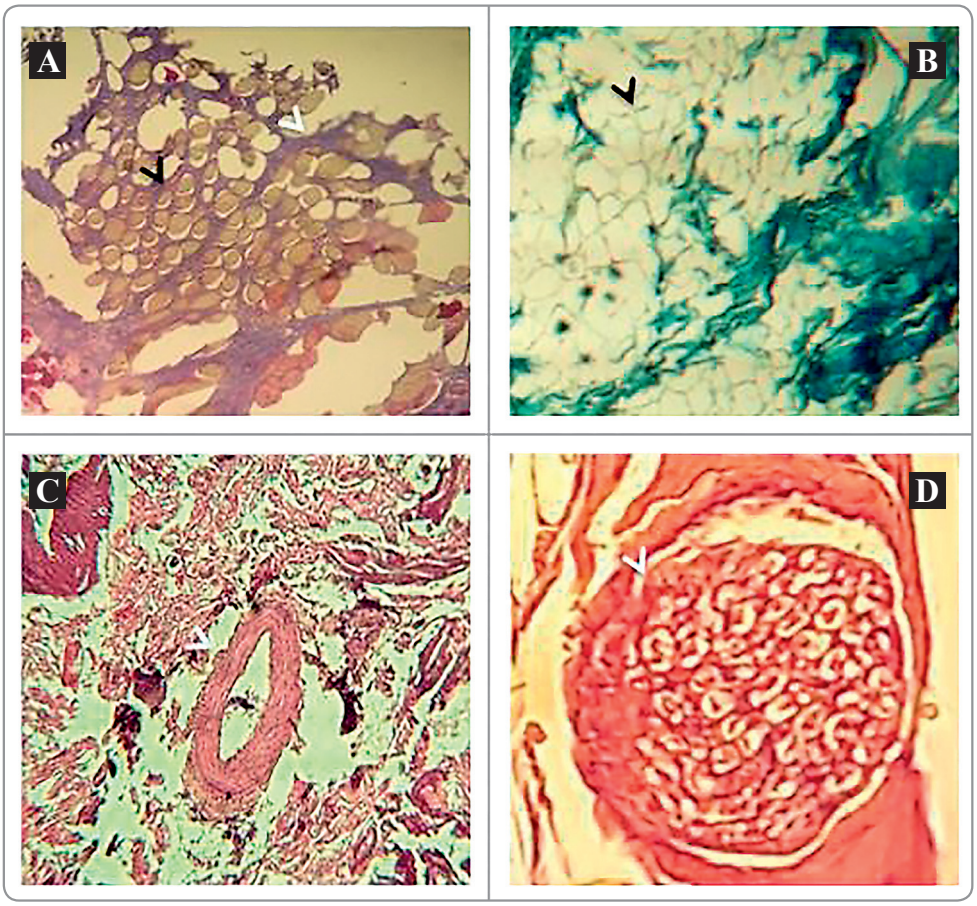

Figure 1. Authorized Tissues. (A) Cachir with striated muscular tissue (black arrow) and connective tissue (white arrow), (B) adipose tissue (white arrow) (PAS-Calleja, ×100). (C) blood vessels (white arrow), (D) Peripheral nerve tissue (white arrow) (hematoxylin eosin, ×100).

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

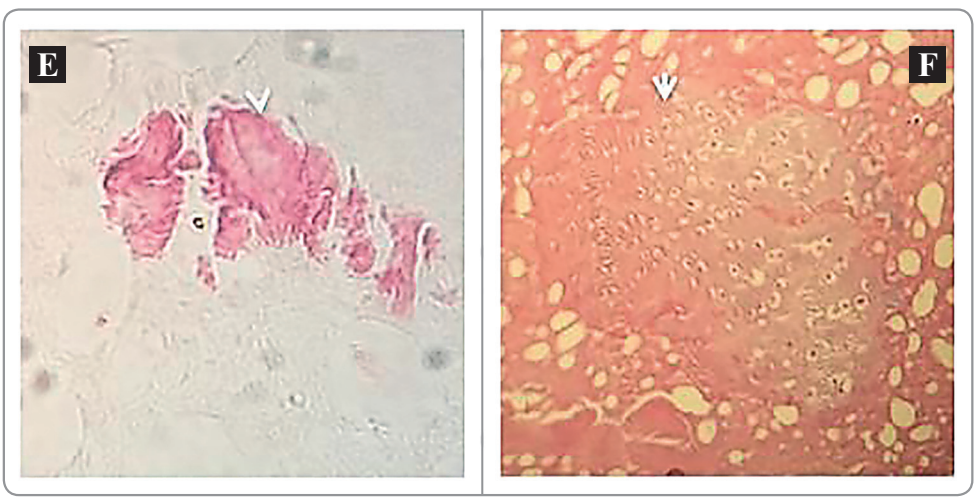

Figure 2. *Unauthorised tissues.* (E) Cortical bone white arrow) (red Alizarin S×100), (F) Cartilaginous tissue (white arrow), (hematoxylin eosin ×100).

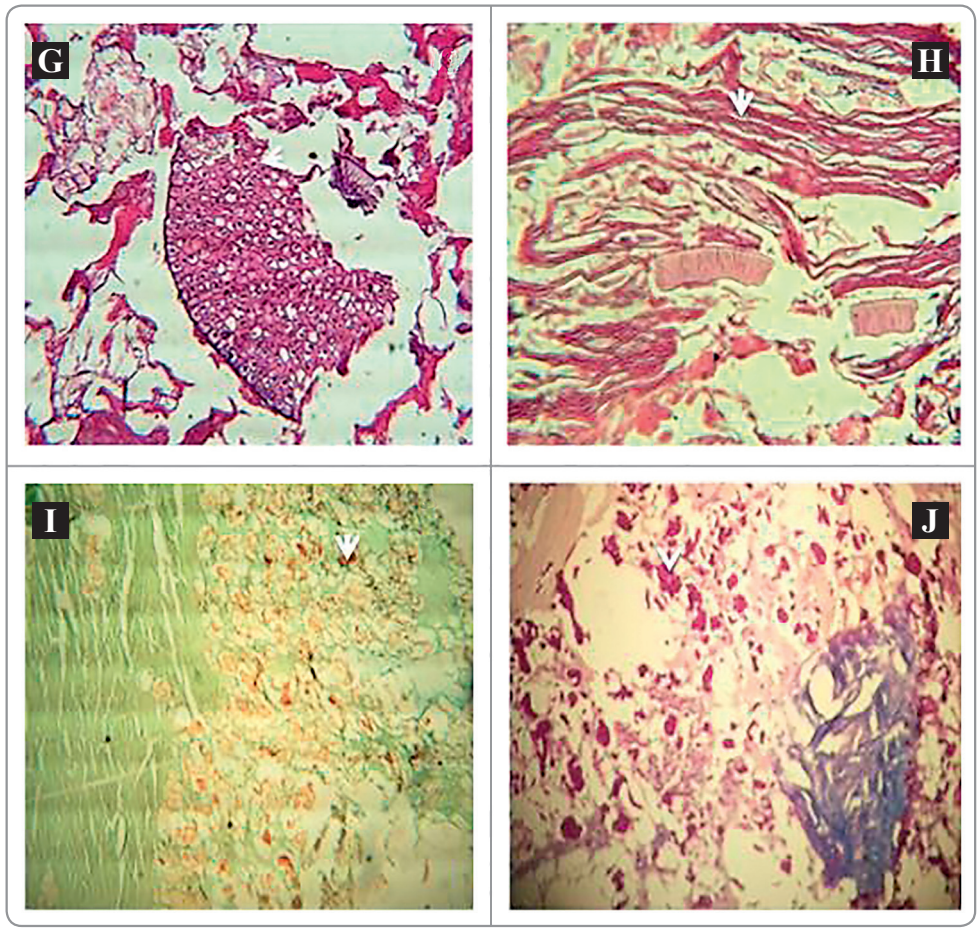

Figure 3. Vegetal tissues. (G) Aromatic herb tissue, (white arrow), (hematoxylin eosin ×100), (H), soybean debris (white arrow) (hematoxylin eosin ×100), (I) starch (white arrow) with Lugol Calleja ×100), (J) polysaccharide (white arrow) with (PAS-Calleja ×100)

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.*

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Original scientific paper

Effect of frying on the fatty acid composition of silver carp and common carp

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A b s t r a c t: The effects of deep frying in sunflower oil or pork fat on the fatty acids (FAs) of silver carp (Hypophthalmichthys molitrix) and common carp (Cyprinus carpio) fillets were determined. The use of sunflower oil and pork fat for frying increased the proportion of polyunsaturated (PUFAs) and saturated fatty acids (SFA), respectively, in the fish fillets. In the fried fish fillets, the linoleic acid content (C18:2n-6) increased after frying in sunflower oil, while n-3 PUFAs, in particular C20:5n-3 (EPA) and C22:6n-3 (DHA), decreased compared with their contents in raw fillets. The apparent retention values of n-3 PUFAs were higher in silver carp (69–78%) than in common carp (21–43%) when the fillets were fried in sunflower oil. After frying the fish fillets in pork fat, contents of SFAs, especially palmitic (C16:0) and stearic (C18:0) acids, were increased compared with the raw fillets. From the public health point of view, it is important to highlight that the ratio of n-6/n-3 in fillets of silver carp and common carp increased after deep frying in sunflower oil (2.61; 28.50), as opposed to frying in pork fat (1.20 7.99). Deep frying the fish fillets in sunflower oil increased the total fat content of silver carp fillets by about 85% and common carp fillets by about 343%, while frying in pork fat increased the total fat content of silver carp fillets by about 78% and common carp fillets by about 191%. The choice of culinary oils/fats affects the total FA contents and the fat composition of prepared, fried fish fillets.

Keywords: *frying, sunflower oil, pork fat, cooking, fish, fat content*

Introduction

Fish species from different ecosystems are known to differ in their fatty acid (FA) composition; therefore, studies of polyunsaturated fatty acid (PUFA) contents of diverse fish from various locations are of great importance for revealing their potential value as sources of the essential n-3 acids in human nutrition (Cirkovic et al., 2011; *Trbovic et al.*, 2013; *Ahlgren et al.*, 1994). Meanwhile, information about PUFA contents of raw fish may have limited value for any conclusion as to their actual food quality. Several studies were undertaken to determine the effects of different cooking methods on the FAs of fish species, in particular boiling, pan-frying, deep fat-frying and oven-baking (*Candela et al.*, 1998; *Al-Saghir et al.*, 2004; *Sioen et al.*, 2006; *Gladyshev et al.*, 2007). The quality of the heating medium is of great concern, since most oils used for this purpose are now vegetable oils containing linoleic (18:2n-6) acid but only low amounts of linolenic (18:3n-3) acid. Such PUFA are susceptible to oxidation and to thermal damage from local excess heating (*Sebedio et al.,* 1993; *Sioen et al.,* 2006). Less attention has been paid to the transfer of lipids between the food item and the frying medium (*Peers and Swoboda*, 1982; *Thompson and Aust*, 1983; *Sebedio et al.,* 1990). Frying is a frequently applied cooking method and gives flavour characteristics to the food that are highly appreciated by consumers. In Serbia, carp is the most commonly consumed species of medium fatty fish (*Markovic and Poleksic,* 2011; Cirkovic et al., 2011). In this study, the effects of frying in sunflower oil or pork fat on the FA composition of silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*) were determined.

Materials and methods

Fish sampling, storage and cooking

Commercially available fish were collected at the same time from the Ecka fish farm (Zrenjanin, Serbia). The fish species were silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*). Upon arrival to the laboratory, fish were held in frozen storage below −20°C. The

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raw fish were thawed at room temperature for about one hour prior to analyses. Muscle tissues (fillets) below the dorsal fin were removed from the thawed fish. Three fishes of each species were used in each analysis, i.e., were studied for each treatment: control (raw) and fried. Therefore, the muscle tissues of six fishes were analyzed. Frying was conducted at 150–170°C for 15–20 min using either sunflower oil, the most common cooking oil in Serbia, or pork fat. The fried fish were analyzed within one hour after frying.

Chemical analysis and FA analysis

To measure moisture content, about 10–15 g of tissue wet weight were taken from the studied fillets and dried to a constant weight at 105°C according to ISO (1997). Lipid extraction after acid hydrolysis of fish tissue was conducted using petroleum ether according to ISO (1973). Fatty acid methyl esters (FAMEs) were analyzed using conditions that have been previously reported (*Spiric et al.,* 2010). Briefly, total lipids for FA determination were extracted from fish muscle tissues by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) with a mixture of n-hexane and iso-propanol (60:40 v/v) in a 33 ml extraction cell at 100°C and nitrogen pressure of 10.3 MPa. The solvent was removed under a stream of nitrogen in a solvent cabinet (Dionex SE 500, Sunnyvale, CA) at 50°C until dryness. The fat extract was further used for FA determination.

FAMES were prepared by transesterification using trimethylsulfonium hydroxide, according to ISO (2000). The gas chromatograph Shimadzu 2010 (Kyoto, Japan) used for FAME determination was equipped with a fused silica cianopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 µm, J&W Scientific, USA), and flame ionization detector. The column temperature was programmed. Injector temperature was 250°C and detector temperature was 280°C. The carrier gas was nitrogen at a flow rate of 1.33 ml/min and injector split ratio 1:50. Injected volume was 1 µl and total analysis time was 50.50 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

Calculation of apparent retention values for fried carp fillets

The apparent retention value (ARV) of fried fish was calculated using the formula:

% ARV = (nutrient content per g of fried food) / (nutrient content per g of raw food) × 100 (*Flakemore et al.*, 2017).

Results and Discussion

The FA content of the sunflower oil and pork fat used is presented in Table 1. Sunflower oil was characterized by a high content of linoleic acid (C18:2n-6) and high total PUFA content. The contents of groups of FAs in sunflower oil decreased in the order PUFA > MUFA > SFA. Pork fat was characterized by high contents of palmitic (C16:0), stearic (C18:0) and oleic (C18:1n-9) acids. The contents of groups of FAs in the pork fat decreased as follows: MUFA > SFA > PUFA.

Table 1. Fatty acid composition (% of total fatty acids) of sunflower oil and pork fat (mean ± SEM)

Fatty acids	Sunflower oil	Pork fat
C16:0	6.21±0.19	24.54±1.52
C16:1	0.11±0.00	2.09±0.19
C17:0	0.03±0.00	0.38±0.03
C18:0	2.50±0.04	12.81±0.55
C18:1n-9	28.47±0.87	42.32±0.05
C18:2n-6	54.71±2.10	13.84±0.41
C20:0	0.21±0.01	0.23±0.02
C18:3n-3	0.06±0.00	0.63±0.08
C20:1	0.29±0.11	0.93±0.10
C22:0	0.67±0.02	0.00±0.00
C24:0	0.14±0.06	0.00±0.00
SFA	9.75±0.14	39.07±0.92
MUFA	28.87±0.76	45.33±0.33
PUFA	54.77±2.10	15.32±0.64
n-3	0.06±0.00	0.62±0.13
n-6	54.71±2.10	13.80±0.50

Legend: SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids;

The FA contents of raw silver carp and silver carp after deep fat-frying in sunflower oil or pork fat and the calculated ARVs are shown in Table 2.

The most abundant FAs in the raw silver carp fillets in this study were MUFAs (51.66%), with oleic acid (C18:1n-9) being the most common MUFA. n-3 PUFAs were the next most common group of FAs (12.78%), with α-linolenic acid (C18:3n-3), eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) being the most common n-3 PUFAs. Linoleic acid (C18:2n-6) was the most abundant n-6 PUFA in raw silver carp

Table 2. Fatty acid composition (% of total fatty acids; means) of silver carp (n = 3) before and after deep fat-frying in sunflower oil or pork fat and mean apparent retention values of the fried fish

Fatty acid or ratio	Raw fish	Fish fried in sunflower oil	Fish fried in pork fat	ARV (%) Fried in sunflower oil	ARV (%) Fried in pork fat
C14:0	2.25	1.43	1.75	64	78
C15:0	0.67	0.41	0.39	61	58
C16:0	21.94	16.95	23.96	77	109
C16:1	14.15	8.97	10.36	63	73
C17:0	0.84	0.59	0.64	70	76
C18:0	3.73	3.72	7.74	100	208
C18:1n-9	34.95	31.82	38.45	91	110
C18:2n-6	2.45	22.87	6.93	933	283
C20:0	0.16	0.16	0.10	100	63
C18:3n-6	0.11	0.08	0	73	0
C18:3n-3	3.49	2.17	1.77	62	51
C20:1	2.56	1.67	1.69	65	66
C20:2	0.16	0.07	0.21	44	131
C20:3n-6	0.40	0.32	0.24	80	60
C20:3n-3	0.25	0.15	0.13	60	52
C22:1+C20:4	2.85	2.01	1.39	71	49
C20:5n-3	3.53	2.77	1.82	78	52
C22:5n-3	1.37	0.94	0.71	69	52
C22:6n-3	4.13	2.9	1.72	70	42
SFA	29.59	23.26	34.57	79	117
MUFA	51.66	42.46	50.51	82	98
PUFA	15.9	32.27	13.53	203	85
n-3	12.78	8.93	6.15	70	48
n-6	3.12	23.34	7.39	748	237
n-3/n-6	4.10	0.38	0.83	9	20
n-6/n-3	0.24	2.62	1.20	1092	500

Legend: SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, ARV – apparent retention value (%)

fillets, comprising 2.45% of the total FAs, but the amount of linoleic acid was much greater after frying in sunflower oil (22.87%), while frying in pork fat also increased the linoleic acid content to 6.93%. ARVs for linoleic acid were 933% and 283% in sunflower-fried and pork fat-fried silver carp, respectively. Among the n-3 PUFAs in raw silver carp fillets, EPA (C20:5n-3) and DHA (C22:6n-3) were the most abundant, 3.53% and 4.13%, respectively. Their contents decreased in silver carp after frying in sunflower oil to 2.77% and 2.90%, respectively. The ARVs of EPA and DHA after frying silver carp in sunflower oil were 78% and 70% respectively. The content of EPA and DHA decreased in silver carp fillets after frying in pork fat to 1.82% and 1.72%, respectively, and ARVs after frying in pork fat were 52% and 42% respectively. As a rule of thumb, the greater the ARV, the worse the impact of frying.

Pan-frying in margarine also changed the FA profile of cod (*Sioen et al.* 2006). While PUFA accounted for 56.0% and SFA for 25.9% of the total FAs in raw cod, frying in margarine resulted in decreased PUFA and increased SFA contents of 16.7% and 50.7%, respectively. The n-6/n-3 ratio altered from 0.10 before frying to 0.73 after pan-frying. In addition, whereas EPA and DHA accounted for 49.7% of

the total FAs in the raw cod, they only accounted for 9.8% after pan-frying. The content of EPA and DHA after pan-frying cod in margarine amounted only to, respectively 66.5% and 74.7% of the original content (*Sioen et al.* 2006). After pan-frying, SFA accounted for 27.2% of the total FAs, whereas 22.8% of the total FAs were SFA before pan-frying. Frying in margarine did not affect the PUFA content of cod. The n-6/n-3 ratio altered from 0.30 before to 0.32 after pan-frying. In the case of cod fillets, the PUFA content decreased from 11.40% to 9.46% whereas it increased from 11.43% to 16.54% in salmon fillets after pan-frying. Frying salmon fillets in margarine also caused notable decreases in the percentages of SFA and MUFA (*Sioen et al.* 2006).

The FA contents of raw common carp and common carp after deep fat-frying in sunflower oil or pork fat and the calculated ARVs are shown in Table 3.

The most abundant FAs in the raw common carp fillets were MUFAs (49.01%), with oleic acid being the most common one. Linoleic acid (C18:2n-6) was the most abundant n-6 PUFA in common carp, comprising 17.67% of total FAs, but it comprised even more of the total FAs after frying in sunflower oil (34.05%), while frying in pork fat

Table 3. Fatty acid composition (% of total fatty acids; means) of common carp (n = 3) before and after deep fat-frying in sunflower oil or pork fat and mean apparent retention values of the fried fish

Fatty acid or ratio	Raw fish	Fish fried in sunflower oil	Fish fried in pork fat	ARV (%) fried in sunflower oil	ARV (%) fried in pork fat
C14:0	0.70	0.43	0.88	61	126
C15:0	0.19	0.06	0.15	32	79
C16:0	18.48	11.99	22.15	65	120
C16:1	6.44	4.52	7.67	70	119
C17:0	0.27	0.10	0.28	37	104
C18:0	5.71	4.19	8.32	73	146
C18:1n-9	40.09	40.98	45.39	102	113
C18:2n-6	17.67	34.05	10.45	193	59
C20:0	0.14	0.13	0.11	93	79
C18:3n-6	0.31	0.11	0.08	35	26
C18:3n-3	1.34	0.39	0.65	29	49
C20:1	2.48	1.32	1.81	53	73
C20:2	0.56	0.12	0.36	21	64
C20:3n-6	0.75	0.21	0.22	28	29
C20:3n-3	0.11	0	0.05	0	45
C22:1+C20:4	2.34	0.58	0.73	25	31
C20:5n-3	0.70	0.19	0.23	27	33
C22:5n-3	0.52	0.11	0.15	21	29
C22:6n-3	1.22	0.52	0.31	43	25
SFA	25.48	16.90	31.88	66	125
MUFA	49.01	46.81	54.88	96	112
PUFA	23.16	35.71	12.51	154	54
n-3	3.87	1.21	1.39	31	36
n-6	19.29	34.49	11.11	179	58
n-3/n-6	0.20	0.04	0.13	20	65
n-6/n-3	4.98	28.46	7.99	571	160

Legend: SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, ARV – apparent retention value (%)

reduce the linoleic acid content to 10.45%. ARVs for linoleic acid were 193% and 53% in sunflower-fried and pork fat-fried common carp fillets, respectively. α-linolenic acid (C18:3n-3) was most abundant n-3 PUFA in common carp fillets, comprising 1.34%, but there was even less of this fatty acid after fish fillets were fried in sunflower oil (0.39%) or pork fat (0.65%). ARVs for α-linolenic acid were 29% and 49% in sunflower-fried and pork fat-fried common carp, respectively.

Using sunflower oil to fry silver carp and common carp fillets resulted in decreased amounts of SFAs (ARVs were from 66 to 79%), decreased MUFAs (ARVs were from 82 to 96%), but increased PUFAs (ARVs were from 154 to 203%). n-6 PUFA contents increased in fish fried in sunflower oil (179 to 748%), and thus, decreased n-3 PUFAs (31 to 70%) were measured. The lowest ARVs for n-6 and n-3 FAs were calculated for common carp. Hence, from a public health point of view, it is important to stress that the n-6/n-3 ratios in carp fillets (silver carp and common carp) altered after frying the fish in sunflower oil (2.62 and 28.46, respectively).

Sánchez-Muniz et al. (1992) showed the FA composition of raw and fried sardines. Frying involves an exchange of FAs between the fat in the sardines and the culinary fat used, which caused significant changes in the FA composition of the oily fish. Pan-frying cod fillets in olive oil resulted in decreased amounts of SFA and PUFA (from 23.9% to 18.6% and from 53.2% to 21.6%, respectively) and increased MUFAs (from 22.9% to 59.8%) (*Sioen et al.,* 2006). The n-6/n-3 ratio altered from 0.10 before to 0.40 after pan-frying (*Sioen et al.,* 2006). Pan-frying in olive oil did not affect the FA profile in a significant way, and the n-6/n-3 ratio altered only from 0.34 before to 0.32 after pan-frying. In the case of cod fillets, the PUFA content decreased from 8.51% to 6.49%, whereas it increased from 8.51% to 12.2% after pan-frying salmon fillets. Over the whole study, the mean recoveries varied between 41.9% and 102% for cod and between 59.0% and 87.0% for salmon (*Sioen et al.,* 2006).

PUFA degradation occurs more readily in combination with oxygen and PUFA undergo pronounced oxidative effects (*Little et al.,* 2000). In the case of oxidation, it is important to note that different FAs respond in different ways to heat treatment. SFA are fairly heat stable in the range of temperatures commonly encountered during cooking. However, above 150°C, and when oxygen is present, a variety of oxidation products can be detected. Possible mechanisms for the changes occurring in the culinary process are absorption of culinary fat in the fish, moisture loss of the food, leaching of fat soluble molecules out of the food and oxidation reactions with free radicals generated in the hot culinary fat (*Little et al.,* 2000).

Frying silver carp fillets in pork fat resulted in significant increases in the total SFAs and n-6 PUFAs (ARVs were 117 and 237, respectively). Frying in pork fat also changed the FA profile of common carp fillets. While n-6 PUFA accounted for 19.29% of the total FAs in raw common carp, frying in pork fat increased the total SFAs and MUFAs (ARVs were 125 and 112%, respectively) but decreased n-6 and n-3 PUFAs (ARVs were 58 and 36%, respectively). In common carp, the n-6/n-3 ratio altered from 4.98 in raw fillets to 7.99 after frying in pork fat.

Table 4. Mean moisture content (MC %), fat content (FC %) and apparent retention values (ARV %) of raw and fried silver carp (n = 3)

Nutrient	Raw	Fried in sunflower oil	Fried in pork fat	ARV% fried in sunflower oil	ARV% fried in pork fat
MC %	74.39	71.46	69.96	96	94
FC %	4.16	7.68	7.39	85	78

Table 5. Mean moisture content (MC %), fat content (FC %) and apparent retention values (ARV %) of raw and fried common carp (n = 3)

Nutrient	Raw	Fried in sunflower oil	Fried in pork fat	ARV% fried in sunflower oil	ARV% Fried in pork fat
MC %	77.98	67.41	71.07	86	91
FC %	2.58	11.42	7.50	343	191

The silver carp and common carp in our study belong to the medium fatty fish species, containing less than 4 g fat per 100 g fish (*Huss,* 1995). Frying in sunflower oil increased the fat content of silver carp from 4.16 to 7.68 g/100 g (i.e. by ~85%), whereas for common carp, the fat content increase was greater (from 2.58 to 11.42 g/100 g, i.e. by ~343%, Tables 4 and 5). Using pork fat to fry silver carp increased the fat content from 4.16 to 7.39 g/ 100 g (~78%) while in common carp, the fat content increase was greater (from 2.58 to 7.50 g/100 g, i.e. by ~191%, Tables 4 and 5). Fish that have a low fat content absorb more fat (*Sánchez-Muniz et al.,* 1992). The moisture content varied in both species of raw fish from about 74% to 78% (Tables 4 and 5). Higher moisture contents were characteristic of common carp, and lower contents were found for silver carp. When fillets from both carp species were fried, they lost water and so an explicit trend of decreased moisture contents after frying occurred (Tables 4 and 5). This is in accordance with the study of *Sánchez-Muniz et al.* (1992).

Conclusion

Comparing the use of culinary fats with different FA profiles is relevant to investigate their influence on the FA profile of the food after preparation, and as would be consumed. Using sunflower oil significantly increased the total PUFA content in fried fillets from both carp species, whereas using pork fat resulted in increased SFAs in the fried silver carp and common carp fillets. Hence, control over the FA composition of the consumed fried fish can be achieved to some extent by the selection of culinary fat. The fish fillets that contained lower levels of total FAs tended to uptake more FA from the culinary fat (data not shown). Consequently, the culinary oil/fat selection affects the total FA content and composition of fried carp fillet.

Uticaj prženja na sastav masnih kiselina mesa tolstolobika i šarana

Vesna Đorđević, Dejana Trbović, Ivan Nastasijević, Dragana Ljubojević, Brankica Lakićević, Aleksandra Nikolić

A p s t r a k t: Ispitan je uticaj dubokog prženja u suncokretovom ulju i svinjskoj masti na sastav masnih kiselina (MK) tolstolobika (Hypophthalmichthys molitrix) i šarana (Cyprinus carpio). Upotreba suncokretovog ulja i svinjske masti povećao je udeo polinezasićenih MK i zasićenih MK, respektivno. Rezultati su pokazali povećanje sadržaja linolne kiseline (C18:2n-6) u ribi koja je pržena u suncokretovom ulju i smanjenje n-3 polinezasićenih MK, naročito u prženoj ribi C20:5n-3 (EPA) i C22:6n-3 (DHA). Očigledna vrednost zadržavanja n-3 polinezasićenih MK u tolstolobiku (69–78%) je bila veća nego u šaranu (21–43%). Rezultati su pokazali, da riba koja je pržena u svinjskoj masti, pokazala je povećanje zasićenih MK, naročito palmitinske kiseline (C16:0) i stearinske kiseline (C18:0). Sa stanovišta javnog zdravlja, važno je napomenuti da se odnos n-6/n-3 u filetima tolstolobika i šarana povećao nakon prženja u suncokretovom ulju (2,61; 28,50), za razliku od prženja u svinjskoj masti (1.20 7.99). Duboko prženje ribe u suncokretovom ulju povećalo je ukupni sadržaj masti u tolstolobiku za oko 85%, a šarana za oko 343%, dok je prženje u svinjskoj masti povećalo ukupni sadržaj masti u tolstolobiku za oko 78%, a šarana za 191 %. Izbor kulinarskih ulja/masti utiče na ukupan sadržaj FA i sastav pripremljenog ribljeg filea

Ključne reči: *prženje, suncokretovo ulje, svinjska mast, tolstolobik, šaran.*

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Review paper

Smoke and smoked fish production

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A b s t r a c t: Smoking is a method for preserving fishes that, after previous salting, are processed with organic components obtained from smoke. Smoke contains volatile aromatic substances that give specific features to fish flesh (attractive appearance, colour, flavour and aroma) and have bactericidal effects. Smoked fish is a flavourful and nutritious product, ready for use with or without further cooking or processing. Besides useful compounds, smoke contains harmful substances that have carcinogenic properties. The quality of smoked fish depends on many factors including the species and size of fish, fish diet, condition and treatment before smoking (fresh, frozen), and the methods of salting, heat treatment and smoking. Depending on the temperature of the smoking chamber, smoking can be cold, warm or hot, and depending on the environment in which smoking is conducted, different smoking techniques are employed: smoking with natural smoke, smoking without smoke and mixed smoking. When smoking is with natural smoke, fish is processed in a smoke-air mixture, which is formed during the direct incomplete combustion (pyrolysis) of wood. Smoking without smoke utilises smoke preparations obtained from smoke or its components. Fish smoked with smoke preparations does not contain harmful components, because smoke preparations are previously purified to remove these substances. Liquid smoke (smoke aqueous solution), which is sufficiently studied, available and has minimal potential toxicity, is attracting more attention today.

Keywords: *smoke, smoked fish, polycyclic aromatic hydrocarbon, nitrosamine.*

Introduction

Smoking as a method of preserving food with high amounts of added salt has been applied for centuries for domestic needs. As a result of development of analytical techniques, in recent decades, the notion of traditional smoked products has been overturned. Whole groups of carcinogenic polycyclic aromatic hydrocarbons (PAHs), mutagenic nitrosamines and other harmful substances such as methanol and formaldehyde have been detected in smoke and smoked products. This has imposed the need to find environmentally friendly methods to smoke foods based on effectively cleaning the smoke from the harmful substances and using the smoke preparations thereby produced. Also, the harmful role of excess sodium in the body has served as a stimulus for development of technology to produce low salt smoked foods. Investigation of the effects of smoking on colour, flavour and aroma became the basis for the construction of modern smoking devices, by which process control over smoking is possible. Most attention today is focused on liquid smoke (smoke aqueous solution), which is sufficiently studied and available and has minimal potential for toxicity (*Мезенова et al*., 2001).

Smoked fish is certainly one of the most attractive fish products. This accounts for the fact that nearly 20% of the total fish supply on the French market is smoked fish. Cold smoked fish is particularly interesting, and which, depending on the fish species and size, the amount of salt and the thermal processing used, can be consumed with or without additional heat treatment (*Baltic et al*., 2009).

Production of smoke

Smoke is a source of heat and of the basic components responsible for the effects of smoking on fish. The composition and properties of smoke depend on the type of wood, its chemical composition, physical properties and burning conditions. Wood from deciduous trees, mostly beech, oak, birch (without bark), linden, maple or aspen are used for quality smoke production. These tree woods burn gently and produce aromatic smoke. Coniferous trees are not recommended, because their wood contains significant quantities of resins that give an unpleasant, bitter taste and aroma and a dark colour to the fish. Wood, shavings or sawdust are usually used for burning. Sawdust in

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the form of granules is commonly used in practice (Быков, 1980; Мезенова *et al*., 2001). The humidity of wood should not exceed 25%, and that of sawdust should be 40–50%. Large amounts of steam and low molecular weight acids (e.g. formic, propionic) are created in the smoking chamber at higher humidities, which is undesirable (*Мезенова et al*., 2001). Smoked fish with an unattractive colour and a bitter taste of resin are obtained if the humidity of raw wood smoke is more than 50%. Moreover, the necessary extraction of water from the fish practically does not occur if the humidity of the smoke is 75–80% (*Быков*, 1980).

Wood dry matter consists of cellulose, lignin and hemicellulose. Technological smoke is produced at the optimum temperature for combustion of these substances, i.e. 300–400°C and when access of oxygen to the pyrolysis zone of the wood is limited. During pyrolysis of wood materials, the following stages are differentiated:

1) intensive evaporation of water at 100–170°C;
2) thermal decomposition of hemicellulose at 200–260ºC;
3) thermal decomposition of cellulose at 260–310ºC;
4) thermal decomposition of lignin at 310–500ºC.

At the beginning of pyrolysis of wood material, undesirable substances, primarily non-aromatic gases and liquids, charcoal and resin, appear in the smoke. Very important substances are formed in the second stage of pyrolysis, when a complex chemical mixture forms consisting of approximately 10,000 solid, liquid and gaseous organic components, of which about 1,000 participate in creating the properties of the smoked fish. These smoke components are deposited onto the surface of the fish. The intensity of precipitation is proportional to the concentration of smoke, the speed of its movement, the degree of its dispersion, and the temperature and humidity of the product to be smoked. In the primary drip-liquid phase, the smoke is precipitated on dry surfaces, and the gaseous phase onto wet surfaces, due to vapour condensation. The components of the smoke penetrate the fish, with the concentration gradient being the driving force of the process (*Мезенова et al*., 2001).

Composition of smoke

The chemical composition of smoke and its condensates is not fully elucidated. So far, 300 compounds have been identified in smoke, 288 in condensate, and about 68 in smoked foodstuffs. This testifies to the high reactive ability of the basic components of smoke (alcohols, aldehydes, ketones, keto acids, esters) with smoked foods like fish. The most common compounds are phenols, which lead to the formation of all the effects of smoking (*Мезенова et al*., 2001).

Forty-seven PAHs were identified in smoke, and about 20 PAHs were detected in smoked meat or fish. However, there are about 200 PAHs, some of which have carcinogenic or mutagenic properties. Studies have shown that not all PAHs have carcinogenic or mutagenic properties, but one of the most common cancer-inducing PAHs is 3,4-benzopyrene, which is abundant in smoke (*Мезенова et al*., 2001). According to other sources, 660 different PAHs have been described so far, and about 100 PAHs and their alkaline derivatives were identified in smoked fish. It has been proven that 15 PAHs had mutagenic and genotoxic effect on somatic cells of experimental animals in *in vivo* conditions (*Stolyhwo and Sikorski*, 2005). These genotoxic PAHs can be considered as potentially genotoxic and carcinogenic to humans. It is considered that PAHs with molecular weights below 216 Da have no carcinogenic effect (*Sander and Wise*, 1997).

PAHs in smoke are formed during wood combustion from practically all organic compounds in conditions of low oxygen supply and temperatures higher than 400ºC. The likelihood of PAH formation is especially high when the temperature reaches more than 1000ºC (*Мезенова et al*., 2001). The content of PAH in smoked fish and meats depends on several factors, the most important being the smoking method (artisanal or industrial) and the temperature at which wood pyrolysis takes place. Temperature plays a significant role in PAH formation, since the concentration of PAH increases proportionally as the pyrolysis temperature increases from 400ºC to 1000ºC. The optimal temperatures for wood pyrolysis, 300 to 600ºC, are also those at which more useful and fewer harmful, especially potentially carcinogenic PAH compounds are created (*Vukovic*, 1998).

However, the optimal temperature for wood pyrolysis cannot be produced when fish is traditionally smoked, and nor can the smoke be cleaned of carcinogens. Therefore, there is a risk of the occurrence of harmful compounds in higher concentrations in such traditionally smoked fish products. In modern industrial facilities, smoke is produced in a generator that is separated from the fish. In such conditions, the temperature of sawdust combustion, the presence of air (oxygen) and the air circulation can be controlled. Also, the smoke is channelled from the smoke generator to the smoking chamber, when it can be purified using a variety of techniques (*Kilibarda et al.*, 2009).

Reduction of PAHs in smoked fish and other foods is possible by regulating the process in the smoke generator, maintaining the sawdust temperature at low levels (<400ºC, so the sawdust smoulders), purifying the smoke before it enters the smoking chamber (mechanical filtration, sedimentation or condensation in water or electrostatic filtration) and using smoke preparations previously cleaned of tar and PAH fractions instead of raw wood smoke. To date, utilisation of smoke preparations appears to be the most effective way to limit the occurrence of PAHs in smoked foods like fish (*Мезенова et al*., 2001).

3,4-Benzopyrene is the best-studied PAH carcinogenic compound in smoke, and it is also the most abundant (it constitutes about 50% of all the carcinogenic compounds present in smoke). Therefore, the amount of 3,4-benzopyrene is used as an indicator of all PAH compounds present in smoked foods, and the maximum allowable level is restricted by European Union law in foods of different origins, including smoked meats and foods intended for feeding infants and young children (*Anon*, 2005). The content of 3,4-benzopyrene in smoked foods ranges from 0 to 500 ppb. According to some standards, the maximum content of 3,4-benzopyrene in smoked products should not exceed 1 μg/kg (*Mezanova et al*., 2001). Certainly the intake of these carcinogenic compounds is a matter of concern in countries and communities in which fish smoking in artisanal conditions is a local tradition (*Baltic et al*., 2006).

Basic effects of smoking

The major beneficial effects of fish smoking are the formation of the typical colour, aroma and flavour of smoked fish and the fish preservation effect. Toxic compounds (PAH, methanol, formaldehyde, some phenols and others) have negative effects, because they precipitate onto the fish surface and reduce the nutrient and biological value of the fish. Therefore it is necessary to control the chemical composition of the smoke, the process of cleaning the smoke (if this is used) and the quality of the finished product. Also, the decreased content (10–20% decrease) of available amino acids in the fish flesh, since some amino acids react with the smoke components, is a negative effect. Despite this causing the reduced nutritional value of fish after smoking, the digestibility of fish is increased by smoking. Therefore, digestibility of the same kind of fish is in the following order: smoked > boiled > raw > dried > salted. This can be explained by secretory gland activation in digestive organs during digestion of smoked fish (*Мезенова et al*., 2001).

The intensities of colour, aroma and flavour of smoked fish depend largely on the phenol content in the fish flesh. The actual concentration of smoke affects the sensory properties of smoked fish. High density smoke contains large amounts of resinous substances and acids, and so gives the fish a sour-bitter taste. Low density smoke does not form the desirable golden colour, and the odour of smoke in the finished fish is weak (*Быков*, 1980). The hue and colour of smoked fish depends on the type of wood used to produce the smoke. Beech, maple and linden give golden-yellow hues, acacia produces a lemon shade, oak produces a cinnamon colour, pear a reddish hue and so on. Smoke from pine (soft wood) colours the smoked fish more intensively than smoke from deciduous trees (*Мезенова et al*., 2001).

Fish dehydrates during smoking, losing weight and changing its structural-mechanical properties. Organic compounds from the smoke that are deposited on the surface of the fish gradually diffuse into the muscle tissue. The diffusion of various smoke compounds into the fish tissue is uneven; some of them penetrate into the fish flesh, but others remain on the skin or muscle surface, forming a golden brown-coloured membrane (*Быков*, 1980).

The preservative effect of smoking is due to the antioxidant actions of smoke components (phenols, polycarboxylic acids), bactericidal effects (the combined effect of antiseptic components of smoke, largely some phenols and acids, dehydration, salt, reducing the pH and high temperature during hot smoking) and antiproteolytic action (slowing down the autolytic processes, due to the effect of smoke on tissue enzymes) (*Мезенова et al*., 2001). Meat preservation by smoking is based on the action of heat and smoke components on the microorganisms present and changes in the basic components of the fish muscle. In addition, the amount of water in the flesh decreases, and this is reflected in the quality of the finished smoked fish product (*Kolodziejska et al*., 2002). Numerous smoke components, i.e. organic acids, alcohols, aldehydes, ketones and particularly phenols, have bacteriostatic and/or fungistatic effects on some types of bacteria and/or fungi (*Doe et al*., 1998; *Leroi et al*., 1998; *Guillén and Errecalde*, 2002). The most important cause of smoked fish spoilage is the growth of microorganisms accompanied by build-up of their metabolic products during storage of smoked fish, which lead to undesirable odours and flavours. Deterioration is also a consequence of enzymes that cause autolytic changes in the texture of the smoked fish during storage (*Hansen et al*., 1996).

Fish smoking methods

Depending on the temperature of the smoking chamber, smoking can be cold, warm or hot. In cold smoking, the temperature in the smoking chamber should not exceed 40ºC. Cold smoked fish is a product of the complex action of NaCl, smoke components, dehydration, and proteolytic and lipolytic enzymes (*Мезенова et al*., 2001). Cold smoked fish has a delicate aroma of smoke, and it has a longer shelf life than hot smoked fish because it contains significantly less water and more salt (*Быков*, 1980; *Витченко et al.*, 1981).

In warm smoking, the temperature ranges from 40ºC to 80ºC. Proteins in the fish are partially denatured, and enzymes are completely inactivated (*Мезенова et al*., 2001).

In hot smoking, the temperature ranges from 80ºC to 170ºC. Proteins in the fish are completely denatured, and enzymes are inactivated. The product has low salinity and high water content, it is slightly smoked, soft, and juicy, the mild aroma of smoke is noticeable, and it cannot be stored for a long time (*Быков*, 1980).

According to *Stolyhwo and Sikorski* (2005), temperatures during cold smoking are between 12ºC and 25ºC, in warm smoking are 25ºC to 45ºC and in hot smoking are 40ºC to 100ºC, reaching temperatures up to 80ºC in depth of the product. Hot smoking can be performed at 60ºC to 80ºC and even higher, so heat processing of the fish occurs simultaneously (*Vasiliadou et al*., 2005; *Salán et al*., 2006; *Duyar et al*., 2008).

Depending on the environment where fish smoking is conducted, there are different smoking methods: smoking with smoke, smoking without smoke and mixed smoking. When smoking with smoke, fish is processed in a smoke-air mixture that is formed by the direct combustion of wood. This fish has a unique flavour and aroma due to the rich chemical composition of the smoke. At the same time, harmful substances like PAHs, formaldehyde, methanol and nitrosamines precipitate on the product.

Smokeless smoking is processing the fish with smoke preparations obtained from smoke or with smoke components. Fish treated with smoke preparations do not contain harmful components such as PAHs and nitrosamines, because these preparations are previously purified. Due to the difficulties in obtaining perfect smoke preparations (with no carcinogens) and suitable processing equipment, smokeless smoking is only slowly being accepted for smoked fish production.

In mixed smoking, processing with and without smoke is combined, which simplifies and expedites the process. Smoking without smoke is preferable from an environmental and sanitary-hygiene aspect (*Мезенова et al*., 2001). Electrostatic smoking results in the smoking duration being 8 to 10 times shorter than that of ordinary smoking (*Быков*, 1980).

The process of smoked fish production (method of salting, amount of salt, method of smoking) should ensure production of smoked fish that meet the demands of customers and bring economic benefits to the producer (*Cardinal et al*., 2001; *Espe et al*., 2001; *Røra et al*. 2004; *Gallart-Jornet et al*., 2007). Regardless of the method of smoking to be used, the fish product should be safe for consumption in spite of smoke containing carcinogenic compounds. Chilled smoked fish must be kept in appropriate conditions and its shelf life monitored. Shelf life determination must be accompanied by sensory evaluation and chemical and bacteriological analyses.

Hot smoked fish

Many commercial fish species are suitable for production of hot smoked fish. Technologically, hot smoking consists of the following operations: receipt of raw material, defrosting, cutting, washing, salting, rinsing, smoking with or without smoke, packaging and storing.

Initially, fish are sorted and defrosted according to weight. Large fish are defrosted in air at <20ºC for 20–30 h depending on the size of the fish. Small and medium-sized fish are defrosted in water at 15°C for 1.5–6 h. Defrosting can also be performed in brine (3–4% NaCl) at 20–25°C. Fish weighing up to 1.5 kg are directed to hot smoking without cutting. Large fish should be cut into pieces and washed in clean running water at 15–20°C. Fish are salted to a final salt content in the fish meat of 1.8–2.0% to enhance the flavour of the finished smoked fish. Dry salting and brining are used. In dry salting, fish are placed in a salting tub and dry salt (NaCl) is poured and rubbed over them; salt is poured into the abdominal cavity and gills. Dry salting requires 7–15% of the fish weight in salt, and it lasts 6–12 h. Brining is conducted using brine (i.e. a saturated solution of NaCl in water), a brine:fish ratio of 1:2, and it lasts 2–6 h. Simultaneous defrosting and salting can also be applied (*Быков*, 1980). Salting has a preservative effect and is a critical production stage that contributes to the smoked fish having adequate shelf life, good flavour and being of suitable quality. The preservative effect of salt is based on the fact that it reduces the water activity of the fish flesh, reducing the amount of water available for microorganisms (*Baltic and Teodorovic*, 1997). Furthermore, the chloride ions are toxic to some microorganisms

(*Goulas and Kontominas*, 2005). Excessive salt is removed from the fish after brining by immersing the fish in water, either running water or showering.

Desalted fish are loaded onto trolleys that enter an industrial smoking chamber with parameters that are automatically managed by a microprocessor. The temperature can be adjusted from 10°C to 200°C and humidity from 10% to 100%. Industrial chambers can be adjusted to smoking without smoke (i.e. using liquid smoke). In hot smoked fish production, smokeless smoking is very promising. Special smoking chambers disperse liquid smoke into the atmosphere around the fish or disperse the smoke in the form of steam (*Мезенова et al*., 2001).

The following operations can be differentiated in hot smoking processes: light drying, light steaming and smoking. Light drying is conducted in warm air at 60–80°C and 40–60% humidity for 20–30 minutes (*Мезенова et al*., 2001). The purpose of this operation is to dry the fish surface. Dry surfaces better absorb the smoke and prevent the accumulation of soot. The result is a smooth, solid membrane which gives a desirable appearance to the finished smoked fish (*Doe et al*., 1998). Light steaming is conducted in hot smoke, air or steam at 100–170°C for from 40 minutes to 3 hours. Lower temperatures are used for fatty fish. Smoking is the final operation, and it is performed at 100–120°C for from 30 minutes to 3 hours. The finished, hot smoked fish is cooled to 8–12°C, sorted by size and quality and packed. Packaged hot smoked fish is shelf stable for three days at –2 to 2°C (*Мезенова et al*., 2001).

Cold smoked fish

Cold smoked fish has a unique flavour and aroma and it is frequently consumed without further cooking. It is prepared from fresh, frozen or salted fish. Frozen fish is defrosted in air, by immersion in water or by water spraying using special equipment. Depending on the species and size of the fish, various fish cuts are produced, but small fish are left entire. Some large fish are just eviscerated, while others are eviscerated, deheaded and cut, depending on the purpose of the finished product (Быков, 1980). Recently, there are trends towards maximum possible cutting of all fish species to increase the yield of edible parts, complex utilisation of the fish raw material, shortening the duration of basic operations (salting, smoking) and application of small packages to increase/improve the marketing possibilities (*Мезенова et al*., 2001).

Fish intended for cold smoking are salted in one of the ways described earlier. Salted fish containing more than 6% salt are desalted. Desalted or defrosted fish, and also chilled or freshly harvested fish are rinsed in clean water. Fish are then loaded onto trolleys and continue to cold smoking. Cold smoking is performed in different types of smoking plants (chambers, tunnels, towers). Unlike hot smoking, cold smoking gives a partial curing effect to the fish through the anti-oxidative and antimicrobial actions of smoke and water deprivation. Due to the absence of perfect smoke preparations (i.e. in their production) and to technological weaknesses in their application, cold smoking more often includes the traditional steps of slight air drying and smoking with wood smoke.

Slight air drying is necessary to create a desirable colour on the surface of the cold smoked fish. Cold smoking is conducted at 18–24°C and 40–60% humidity. The more fatty the fish, the lower the temperature. Slight air drying depends on the size of the fish and takes 1–12 h; weight loss is 7–20%. Cold smoking is conducted in a smoke-air mixture at 20–30°C and 40–60% humidity. Fatty fish are cold smoked at 20–24ºC and leaner fish at 26–30°C, whereby the temperature should be gradually increased to the maximum (*Мезенова et al*., 2001). According to other recommendations, the temperature maximum can be up to 32ºC and for fatty fish up to 29°C, with ~45% relative air humidity. Drying and smoking last 24–72 h. During this time, drying shrinkage can be up to 30%. In that case, cold smoked fish is shelf stable for a longer duration, has a more gentle smoke aroma, firmer texture, and contains less water and more salt than hot smoked fish (*Sosa*, 1989). Cold smoked fish should be cooled to 10–12ºC and then packaged for storage at 8–10°C. The shelf life duration depends on the type of packaging, the degree of salinity and smoking and storage conditions, and it ranges between 3 days and 3 months (*Мезенова et al*., 2001).

Warm smoked fish

The technology for producing warm smoked fish differs from that for producing cold and hot smoked fish. The main differences are temperature, the duration of processing and fish salinity. Application of warm smoking technology to fish is limited and is used primarily for small fish with salt content of 5–8%. Smoking is conducted in two stages: firstly, smoking is at 18–20ºC for 1.5–2 h to complete the process of slight drying and secondly, dense smoke is added, the temperature is increased to 80°C and the fish is smoked for 6–8 h. Warm smoked fish can be stored for up to 7 days (*Быков*, 1980).

Conclusion

Further research is required on the individual components of smoke, their qualitative and quantitative determination in smoked fish products, and on limiting the content of harmful smoke components in finished smoked fish products. To date, smoking fish using smoke preparations is considered to be safer than using wood smoke.

Dim i proizvodnja dimljene ribe

Katerina Beličovska, Daniela Beličovska, Zlatko Pejkovski

A p s t r a k t : Dimljenje je način očuvanja ribe koja se, nakon prethodnog soljenja, obrađuje organskim komponentama koje se nalaze u dimu tokom nepotpunog sagorevanja (pirolize) drveta. Dim sadrži isparljive aromatske supstance koje daju specifične osobine mesu (atraktivan izgled, boja, ukus, miris) i imaju baktericidno dejstvo. Dimljena riba je ukusan i hranljiv proizvod, spreman za upotrebu bez ili sa dodatnim kuvanjem ili preradom. Osim korisnog, dim sadrži i štetne supstance koje imaju karcinogena svojstva. Kvalitet dimljene ribe zavisi od mnogih faktora kao što su: vrsta ribe, ishrana, stanje i tretman pre dimljenja (sveži, zamrznuti), način soljenja, termička obrada, način dimljenja, itd. U zavisnosti od temperature komore za dimljenje, dimljenje može biti: hladno, toplo i vruće, a u zavisnosti od sredine u kojoj se dimljenje odvija, postoje različiti načini dimljenja: dimljenje prirodnim dimom, dimljenje bez dima i mešano/kombinovano dimljenje. U slučaju dimljenja sa prirodnim dimom, riba se prerađuje u mešavini dima i vazduha, koja se formira tokom direktnog sagorevanja drveta. Dimljenje bez dima je način prerade ribe korišćenjem preparata dima dobijenih dimom ili njegovim komponentama. Ribe dimljene sa preparatima dima ne sadrže štetne sastojke, jer su preparati dima prethodno prečišćeni od ovih supstanci. Tečni dim (vodeni rastvor dima), koji je dovoljno proučen, dostupan i ima minimalnu potencijalnu toksičnost, danas privlači više pažnje.

Ključne reči: *dim, proizvodnja, sastav, dimljena riba.*

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Original Scientific Paper

Effect of incorporating orange maize flour into beef sausage and its associated quality attributes

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A b s t r a c t: The aim of this study was to incorporate different percentages (0%, 10%, 20%, 30%, and 50%) of orange maize flour (OMF) into beef sausage, and to study the bioactive qualities of the OMF. Also, the physicochemical, microbiological and sensory characteristics of the OMF beef sausages were explored. Results from the bioactive analyses of the OMF indicated it had total carotenoid content of 14.31 µg/g, while the tannin content was 1.52 mg/g. The moisture content of the beef sausages decreased when OMF levels in the sausages were increased. The fat contents of the OMF beef sausages were significantly lower ($p<0.05$) than that of the control, while the emulsion stability of the 30% OMF beef sausage was not statistically different to that of the control sausages. The iodine values of the OMF beef sausages tended to increase with an increased content of OMF. Salmonella spp. and Staphylococcus aureus were not detected in the sausages. Escherichia coli detected were within the range of 1.0 to 2.0 CFU/g. A consumer preference study showed the most acceptable sausage among the OMF beef sausages contained 20% OMF. This study showed that OMF has the potential to be used as a fat replacer, can maintain the sensory properties of beef sausages and prevent or limit microbial growth on beef sausages formulated with added OMF.

Keywords: *orange maize flour, bioactive, total carotenoid, iodine value, fat replacer.*

Introduction

Orange maize (OM) is a new crop that is bio-fortified with vitamin A. This crop is a staple food that provides substantial amounts of energy, vitamin A, antioxidants (carotene, xanthophylls, polyphenols), and vitamins C, E, and D (*Alamu et al.,* 2014). It is widely grown commercially in

Zambia, Nigeria, Zimbabwe, Tanzania and Ghana (*Alamu et al., 2015*; *Pixley et al.,* 2013; *Smale et al.,* 2015; *Hwang et al.,* 2015). The polyphenols and the carotenoids present in OM flour (OMF) are known to be antioxidants, which play a vital functions as anti-carcinogenic, antioxidant, antiviral, antimicrobial, and anti-inflammatory molecules. The potency of this bio-fortified maize is to enhance vitamin A status in children (*Gannon et al.,* 2014; *Palmer et al.,* 2016). The presence of vitamins C and K, β-carotene and selenium in OMF could improve the functions of the human thyroid gland and immune system (*Shah et al.,* 2016).

Maize is used to produce products including corn flour, cornstarch, corn gluten, corn syrup, tortillas, tortilla chips, polenta cornmeal, corn oil, popcorn, and cornflakes, but the usage of OM in food production is limited, despite its nutritional attributes. However, the intake of OM-based products could contribute to phenolic acid ingestion (*Alamu,* 2014) and other vital nutrients.

Thus, consumption of sausage with incorporated OMF could improve the vitamin A status in children, pregnant woman and lactating mothers and could provide the potential to scavenge generated free radicals, prevent protein energy malnutrition, reduce the risks of coronary heart disease, tumour incidence, and cancer, decrease blood cholesterol level and fat absorption and could have the potential to lower blood pressure (*Jo et al.,* 2015). Also, vitamin A is noted to play a very key role in good vision and cell differentiation, and therefore, OMF intake could prevent vitamin A deficiency that can result in blindness, increased child and maternal mortality, immune system function impairment and abnormal foetal development (WHO, 2009).

In this paper, some bioactive qualities of OMF, and the physicochemical properties, the sensory attributes and the microbiological quality of sausages with incorporated OMF were studied.

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Materials and methods

The solar dried OM was provided by the Department of Food Science and Technology, University for Development Studies, Tamale, Ghana. Adobo, white pepper, black pepper, chilli pepper, polyphosphate, iodised salt and purified water were purchased from a supermarket in Tamale, the northern region of Ghana. Lean meat (boneless beef) used for the sausage was obtained from the meat unit of the Animal Science Department, University for Development Studies. The boneless beef was acquired from a healthy adult cow slaughtered based on the approved code of ethics for slaughtering animals in Ghana. The lean meat was trimmed of subcutaneous fat and of connective tissues. The meat was stored in a refrigerator at 4±1°C for about 6–8 h and frozen at −18°C until it was processed.

Orange maize flour preparation

Five (5) kilograms of solar dried OM was milled into flour using a plate mill (Quaker City Grinding Co, Model 4-E, Phoenixville, PA, USA). It was then sieved through a 0.2 mm screen. The OMF obtained was kept in a container with fitted lid and was stored at room temperature until it was utilised.

Determination of bioactive compounds in solar dried OMF

Carotenoid extraction and its total content

To determine the total content of carotenoids, the method described by *Lucia et al. (*2012) was employed. In brief, 5 g of the OMF was weighed using a digital balance (Bel Engineering, model MA0434/05). The carotenoid was extracted by continuously adding 15 mL of acetone until a paste was obtained. This was transferred into a sintered-funnel (5 μm), which was fixed to a 150 mL Buchner flask. This was filtered under vacuum. This technique was repeated three times. The extract acquired was conveyed to a 200 mL sieve funnel containing 20 mL petroleum ether. The acetone removal was conducted by adding slowly ultrapure-water (Milli-Q-Millipore). The extract was then conveyed through a funnel to a 50 mL volumetric flask containing 10 g of anhydrous sodium sulphate. The volume was made up using petroleum ether, and the absorbance was read at 450 nm. The total carotenoid content was calculated using the following equation:

$$\text{Caroteniod content}\left(\mu\frac{g}{g}\right)=\frac{A\times V(mL)\times 10^4}{\lambda\,{}^{1\%}_{1\,cm}\times Z(g)} \quad (1)$$

Where: A = absorbance; V = total extract volume; Z = sample weight; $\lambda\,{}^{1\%}_{1\,cm}$ = 2592 (β-carotene extinction coefficient in petroleum ether).

Determination of ascorbic acid

Ascorbic acid was determined using the dyestuff titration method, which was expatiated according to *AOAC* (2006). OMF (5 g) was digested with oxalic acid (0.4 g/100 g). The aliquot was titrated against dyestuff that was formerly standardised using ascorbic acid solution. The ascorbic acid content was calculated using the following equation.

$$\text{Vitamin C (mg/100g)} = \text{titre value}\times 0.606\times\frac{100}{\text{weight of sample}}$$

Determination of phytic acid content

The phytic acid content of the OMF was determined using the method of *Alamu et al.* (2014), wherein the atomic ratio of 4:6 Fe:P was used to calculate the phytic acid content.

Determination of tannin content

Tannin content determination was carried out using the *AOAC* (2006) method. OMF (5 g) was added to 50 mL of distilled water and agitated. The mixture was held for 30 min at room temperature and was filtered using Whatman no. 4 grade filter paper. The extract (2 mL) was put into a 50 mL volumetric flask. Similarly, 2 mL standard tannic solution (0.1 mg/mL tannic acid) and 2 mL distilled water were put in separate volumetric flasks and used as standards. A saturated sodium carbonate (Na_2CO_3; 2.5 mL) solution and Folin-C (1 mL) reagent were added to a separate flask and volume was made up to 50 mL and vortexed. Afterward 1.5 h, the OMF was filtered using Whatman no.4 grade filter paper. The absorbance was read at 760 nm against a reagent blank. Tannin was calculated according to equation (2) and is expressed as mg of tannic acid equivalence (TAE) per 100 g.

$$\text{Tanin} = \frac{(\text{standard concentration}\times\text{sample absorbance})}{(\text{standard absorbance}\times\text{weight of sample})} \quad (2)$$

Sausage preparation and processing

An adapted method of *Afoakwah et al.* (2015) with modification was employed for the preparation of the OMF beef sausages. Before the sausages were prepared, the lean meat and fat were stored at −18ºC. A mixture of lean meat, fat, salt, ice, adobo, white pepper, black pepper, chilli pepper, polyphosphate, and water was considered as a control (OMF 0%). Different quantities (10%, 20%, 30% and 50%) of OMF, adobo, white pepper, black pepper, chilli pepper, polyphosphate, were added to the sausage, which constituted the experimental groups. The preparation of the ingredient mixtures using the correct ratio of fat and lean meat and different concentrations of OMF was carried out with caution. The mixture was minced at an emulsion temperature of 10ºC for 10 min. The raw sausage emulsion was stuffed into cellulose casing using a sausage stuffer. Then, the sausages were tied manually into 10–13 cm lengths that were weighed and smoked for 45 min at 70°C in an electric sausage smoker. The sausages were kept in a refrigerator at 4°C.

Component analyses of the sausages

Moisture determination

The moisture content in the sausages was determined using *AOAC* (2006). In brief, 5 g of sausage sample was transferred into a previously dried and weighed dish. The dish was placed in an oven thermostatically controlled at 105°C for 5 h until a constant weight was obtained. The sausage sample was again weighed after cooling in a desiccator. The percentage of moisture was then calculated using the equation:

$$\text{Moisture} = \frac{(\text{weight of wet sample} \times \text{weight of dry sample})}{\text{weight of wet sample}} \times 100\,[\%] \quad (3)$$

Protein determination

Protein content of the sausages was determined by employing the Kjeldahl method with KELPLUS nitrogen estimation system employing the method of *AOAC* (2006). Sausage (2 g) was placed into the digestion flask. A selenium tablet utilised as Kjeldahl catalyst was added to the sausage sample. Concentrated sulphuric acid (10 mL) was mixed with the sample and was fixed to the digester for 8 h until the solution obtained was clear. The cooled digest was placed into a 100 mL volumetric flask and made up to the mark with distilled water. The distillation apparatus was set and rinsed for 10 min after boiling. Twenty-five millilitres (25 mL) of 2% boric acid was pipetted into a conical flask. Five (5) drops of methyl red were added to the flask as an indicator and the sample was diluted with 75 mL distilled water. The digest (10 mL) was made alkaline with 20 mL of 40% NaOH and distilled. The steam exit of the distillation apparatus was closed and the change of colour of the boric acid solution to green was timed. The mixture was distilled for 15 min. The filtrate was then titrated against 0.1 N HCl. The percentage total was calculated:

$$\text{Protein} = \%\ \text{nitrogen} \times \text{conversion factor (6.25)}\ \textbf{[\%]} \quad (4).$$

Crude fat determination

Using the *AOAC* (2006) method, the fat content of the sausages was estimated using Soxhlet extraction apparatus. In brief, a previously dried (air oven at 100°C) 250 mL round bottom flask was weighed accurately. About 5.0 g of sausage sample was weighed on to a 22 × 80 mm paper thimble. About 150 mL of petroleum spirit B.P. 40–60°C was added to the round bottom flask and the apparatus assembled. The Soxhlet extractor with a condenser connected was refluxed for 6 h on a heating mantle. After extraction, the thimble was removed and the solvent recovered by distillation. The flask and fat/oil were then heated in an oven at about 103°C to evaporate the solvent. The flask and contents were then transferred into a desiccator to cool. They were then weighed, the values recorded and the % fat was calculated according to equation (5).

$$\text{Fat} = \frac{W_4 - W_3}{W_2 - W_1} \times 100\,[\%] \quad (5)$$

Where: W_1 = weight of oven dried thimble, W_2 = weight of bottom flask with sample, W_3 = weight of round bottom flask, W_4 = weight of round bottom flask with fat.

Ash determination

Ash content in the sausages was estimated by employing the *AOAC* (2006) method. In brief, 5 g of dried sausage was placed in a weighed crucible and ignited until no charred particles remained in the crucible. The crucible was put in muffle furnace for 2 h at 600°C until a white ash was obtained. Thereafter, the crucible was cooled in a desiccator and reweighed. The percent ash was calculated using equation (6)

$$\text{Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100\,[\%] \quad (6)$$

Total carbohydrate determination

The total carbohydrate in the sausages was determined by the differential method, and this was achieved by subtracting percent amounts of crude protein, crude fat, moisture, fiber and ash from 100%.

$$\text{Carbohydrate} = 100 - [\%]\,(\text{protein} + \text{fat} + \text{fiber} + \text{ash} + \frac{\text{moisture}}{\text{content}}) \quad (7)$$

Determination of pH

Using *ISO 2917* (2004) method, the pH of the sausages were measured using Crison pH meter. Prior to the pH reading, the pH meter was standardised using a buffer solution of pH =7.02 and 4.00 to 20°C. In brief, 5 g sausage sample was homogenised with 20 mL distilled water for 10–15 s. The pH meter electrodes were inserted into the slurry, and the pH values recorded in triplicate.

Functional properties of the sausages

Water holding capacity

Employing the method of *Maltin et al*. (2003), empty containers used for this test were labelled and weighed. The sausage samples were pierced all around with a needle and were hung on a stick in the container. The new weight (weight of sample and container together) was measured and recorded, then the samples were kept in a desiccator for 24 h at room temperature. The samples were then placed in a bag and hung in an airtight container using a hook under the lid. After the required storage time, i.e. 24 h at room temperature, samples were weighed again. The water holding capacity (WHC) was calculated using equation (8):

$$\text{WHC } \% = \frac{T3 - T2}{T3 - T1} \times 100\% \quad (8);$$

Where: T1 is weight of empty container, T2 is weight of container and drained liquid, T3 is weight of container and whole sample.

Emulsion stability

The emulsion stability was measured according to the modified method as described by *Jitngarmkusol et al.* (2008). In brief, 1 g of each sample was dispersed in 16 mL of distilled water contained in falcon tube. The mixture was homogenised for 30 s. Approximately 2 mL of petroleum ether was added, and the mixture was homogenised again for 30 s. Each emulsified sample was transferred into a centrifuge tube, heated in a water bath at 85°C for 15 min, cooled down to room temperature, and centrifuged at 1100 g for 5 min.

The emulsion stability (ES) was calculated using the equation:

$$\text{Emulsion Stability} = \frac{V_3}{V_1} \times 100[\%] \quad (9);$$

Where; V_1 is the volume of emulsion before centrifugation, V_3 is the volume of the remaining emulsified layer after centrifugation.

Determination of the iodine value of the sausages

The iodine value was determined by extracting fat from the sausage samples using the Soxhlet method. The fat obtained after extraction was kept in an oven to dry. After cooling in a desiccator, fats were transferred into centrifuge tubes. About 1g of each fat sample was weighed into conical flasks. Approximately 10 mL of chloroform was added and mixed. Hanus iodine solution (25 mL) was added with a pipette. The mixture in the flask was kept in the dark for 30 mins. About 10 mL potassium iodide solution and 100 mL distilled water was added and was the flask was shaken. The iodine liberated in the process was titrated with 0.1N sodium thiosulfate solution. The flask was stoppered and shaken vigorously while titrating until a pale-yellow colour was obtained. Exactly 2 mL of 0.5% starch solution was used and titration was continued until the blue color disappeared. Blank titration was also performed. The entire test was performed in triplicate. Iodine values were calculated according to equation (10).

$$\text{Iodine value} = \frac{(B-S) \times N \text{ of } NaS_2O_3 \times 0.127}{\text{Weight of sample}} \times 100[\%] \quad (10)$$

Where B = blank titre, S = sample titre and N = normality of NaS_2O_3 – 0.1N

Microbiological analyses

The microbial analysis of the sausages was performed using the method as described by *Philip et al.* (2002). Briefly, 25 g of sausage was placed in 225 mL buffered peptone water (Merck, Darmstadt, Germany) and homogenised in a stomacher for 2 min. The homogenate (1 mL) was pipetted into 9 mL of buffered peptone water to obtain a tenfold dilution. The solution was further step-wise diluted up to 10^{-5} dilution. The spread plate method was

used to plate 0.5 mL of the 5 dilution levels. Total Plate Count Agar (Oxoid) was used for total aerobic plate count determination. Some microorganisms were isolated and identified from this agar. *Salmonella-Shigella* Agar (Oxoid) was used for *Salmonella* enumeration, Baird Parker Agar (Oxoid) was used for *Staphylococcus aureus* and MacConkey Agar (Oxoid) was used for *Escherichia coli* detection. Agar plates for *Salmonella* and total aerobic plate count was incubated at 37°C. Baird Parker Agar plates for *S. aureus* were incubated at 35°C, while MacConkey Agar plates for *E. coli* were incubated at 44.5°C. In all, the incubation period was 24 h, after which, suspect colonies of *Salmonella, Shigella*, and *E. coli* were identified prior to counting the colonies. Suspected *Salmonella* colonies on *Salmonella-Shigella* agar plates were recognised as light pink with dark/black centres and *Shigella* were colourless, black colonies were suspect *S. aureus* on Baird Parker agar plates, while pink colonies of *E. coli* were observed on the MacConkey agar plates. The citrate test was used as a confirmatory test for the suspect *E. coli* even though it is a common test for *Salmonella* and *Shigella* as well. In this test, Simmons citrate (Oxoid) agar plates was prepared according to the manufacturer's instructions and all suspected *E. coli* colonies transferred aseptically with further incubation at 37°C for 24 h, after which, results were observed and recorded. Simmons citrate agar after preparation looked green; *Salmonella*, if positive in the citrate test, can change the colour to blue, whereas *E. coli* does not change the colour of the medium. For *S. aureus*, the use of Baird parker together with its supplement (rabbit plasma fibrinogen) was enough and, hence, no further test was needed.

Sensory analysis of the sausages

Sensory analysis was conducted using 50 untrained panellists. Panellists were asked to indicate how much they liked or disliked each of the sausages. Quality attributes (colour, texture, flavour, taste) and overall acceptability were scored based on five-point hedonic scale (1=dislike extremely, 2=dislike moderately, 3=neither like nor dislike 4=like moderately 5=like extremely). All sausages were prepared by grilling for about 10 mins. Sausage pieces (1.5 cm long) were wrapped in aluminium foil and different samples were kept in separate plastic bags in an ice chest. They were then served to the panellists with three digit codes on disposable plates in a randomised order for evaluation. Panellists were asked to rinse their mouth with water between tasting samples.

Statistical analysis

One-way ANOVA was performed to determine differences in characterisation parameters of the sausages with different levels of OMF incorporated. Minitab was used for the analysis of proximate data, XLSTAT-2016 for sensory data and SPSS version 17.0 for microbial data analysis. The significance was determined using the Tukey's pairwise comparison at $p<0.05$.

Results and discussion

Bioactive qualities of the bio-fortified orange maize flour

The total carotenoid in the bio-fortified OMF was 14.31±0.3 µg/g. From a comparative point of view, the values found in this study were higher than those reported for the conventionally bred bio-fortified orange maize varieties (6–9 µg/g) of vac and for white maize (2 µg/g) (*Pixley et al.,* 2013). In this study, ascorbic acid was not detected in the OMF. This could be attributed to the drying method employed, which probably degraded it. Also, it could be attributed to the fact that ascorbic acid is sensitive to heat treatment, and so processing the OMF could have contributed to the ascorbic acid loss (*Odriozola-Serrano et al.,* 2007).

The OMF contained tannin (1.52±0.5 mg/g). The concentration of tannins greatly varies among different cultivars, as well as among plants belonging to the same cultivar, and values of 0.8–70 mg/g, expressed as catechin equivalent, have been reported by *McMillan et al.* (2007), so the amount of tannin in the OMF used in the current study is in this range *(*McMillan *et al.,* 2007). However, the total content of tannin observed in this study is difficult to compare with the total tannin contents of other plants found in the literature, because methods of analysis, maturity stage of the plants, the type of plant cultivars and the plant part used for analysis can be different (*Mamta and Shashi,* 2015). Besides, tannins are a diverse group of compounds present in foods at variable levels. Hence, their consumption is virtually universal. Also, differences at the chemical level among the different tannins have resulted in diverse methods for measuring their concentrations. This could be why it is problematic to quantify the consumption levels of these compounds among dissimilar populations. The effect of food tannins on health is a public health issue (*Mamta and Shashi,*

2015), although tannins have prophylactic effects, noted to be cardio-protective, anti-inflammatory, anti-carcinogenic and anti-mutagenic, amongst others. These attributes may be due to their free radical scavenging abilities and the potential to activate antioxidant enzymes (*Mamta and Shashi,* 2015).

A relatively low content of phytic acid was determined in the OMF (6.34±0.6 mg/100g). Phytic acid is the primary phosphorus storage compound in cereals, legumes, nuts and oil seeds, and it accounts for up to 90% of the total phosphorous content, contributing as much as 1.5% of seed dry weight (*Lan and Lott et al.,* 2005). This suggests that the phytate in the flour has the potential to increase the bio-accessibility and bioavailability of minerals and proteins. However, phytates should be 25 mg or <100 g (*Onomi et al.,* 2004). The phytate content in the current research was in close agreement with *Khan et al.* (1991) and *Nawab Khan and Manzoor* (2006), who indicated a decreased concentration of phytic acid during heat treatment. *Shahidi and Naczk* (1995) showed that polyphenols are not distributed evenly in plant tissues; hence, fractional food processing can lead to loss or enrichment of some phenolic compounds.

Proximate Composition of the Sausage

As depicted in Table 1, the moisture contents of the sausages were significantly different ($p<0.05$), and ranged between 61 to 67%. As the percentage of OMF incorporated increased, the moisture content decreased ($p<0.05$). This might be as a result of OMF's ability, due to its fibre content, to hold most of the water as bound water (*Choi et al.,* 2009). A similar result for moisture was reported by *Afoakwah et al.* (2015). Additionally, the fat contents of the OMF beef sausages (Table 1) were lower ($P < 0.05$) than the control (18.04±2.13) beef sausages. This was expected since the control sausages were formulated with a higher percentage of fat. *Tobin et al*. (2012) obtained similar results in their study where sausages prepared with high-fat levels had a higher fat content.

The ash content in the sausages showed a gradual increase as the OMF concentration increased. A significant difference ($p<0.05$) between the control beef sausage and the 50% OMF and 30% OMF beef sausages was observed. The increasing value for the ash content detected in the OMF incorporated sausages indicates the increasing mineral content in the sausages, which confirms that OMF is a rich source of mineral elements that are vital for biological functions in humans (*Ullah et al*., 2010; *Nascimento et al*., 2014).

The protein content in the OMF sausages increased as the content of OMF increased (Table 1). The protein results obtained were higher than those recorded by *Méndez-Zamora et al.* (2015) who used inulin and pectin in low-fat frankfurter sausages. OMF, which contains a higher quantity of carbohydrate, resulted in increasing carbohydrate content as the OMF content increased. Quinoa flour led to an increase in carbohydrate content and a decrease in moisture in sausages that were prepared with tilapia fillet waste (*Zapata and de la Pava,* 2018).

pH of the Sausages

As the amount of OMF in the sausages increased, the sausage pHs also increased (Figure 1). Sausage containing 50% OMF had a significantly higher pH of 6.03 than did 10% OMF sausage or control sausage, the pH of which was 5.93 ($p<0.05$).

Table 1. Proximate composition of beef sausages with added orange maize flour (g/100g) and statistical differences

Type of Sausage	Moisture Content	Fat Content	Ash Content	Protein Content	Carbohydrate Content
Control	66.77 ±1.81^{a}	18.04±2.1^{3a}	1.49 ±0.26^{b}	12.34±1.26	1.36 ±0.63^{b}
OMF 10%	66.52 ±0.88^{a}	16.94±1.25^{ab}	1.57±0.10^{b}	12.28±0.10	2.69±1.53^{ab}
OMF 20%	63.72 ±0.08^{ab}	14.97±2.13^{ab}	1.95±0.17^{ab}	13.46 ± 2.03	5.90±2.68^{ab}
OMF 30%	63.69 ±1.42^{ab}	12.41±1.57^{ab}	2.23±0.00^{a}	15.50 ±2.88	6.17±0.10^{ab}
OMF 50%	61.03 ±1.17^{b}	11.21±2.30^{b}	2.25±0.10^{a}	16.68 ±0.23	8.83±2.00^{a}
p value	0.024	0.037	0.010	0.165	0.042

Legend: Means with the same superscript letters in the same column are not significantly different at P<0.05; OMF: orange maize flour.

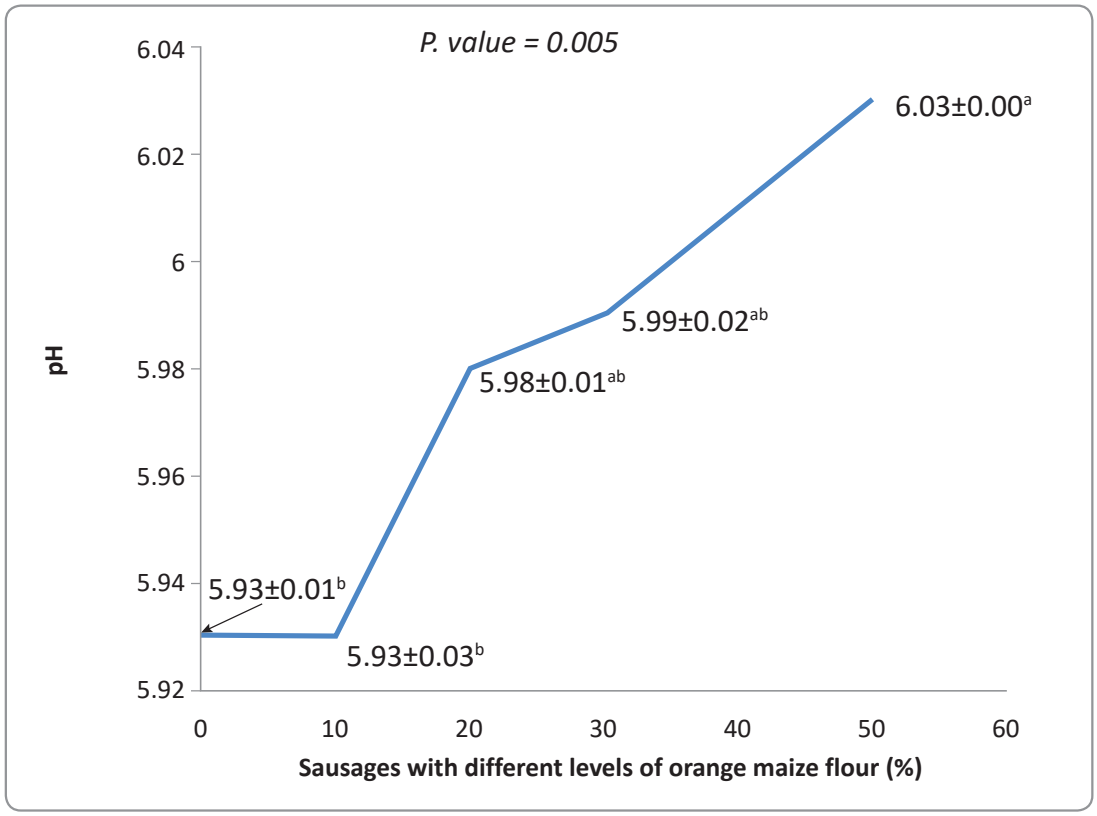

Figure 1. pH of sausages with different levels of incorporated orange maize flour.

In comparison to the results obtained by *Choi et al.* (2009), higher pH (6.47) was observed in sausages formulated with rice bran fibre, which was attributed to the effect of the minerals, such as iron, phosphorus, and calcium, present in the fibre. Higher pH values according to *Kristinsson and Hultin* (2003) are desirable for better water holding capacity, juiciness and other sensory parameters of sausages.

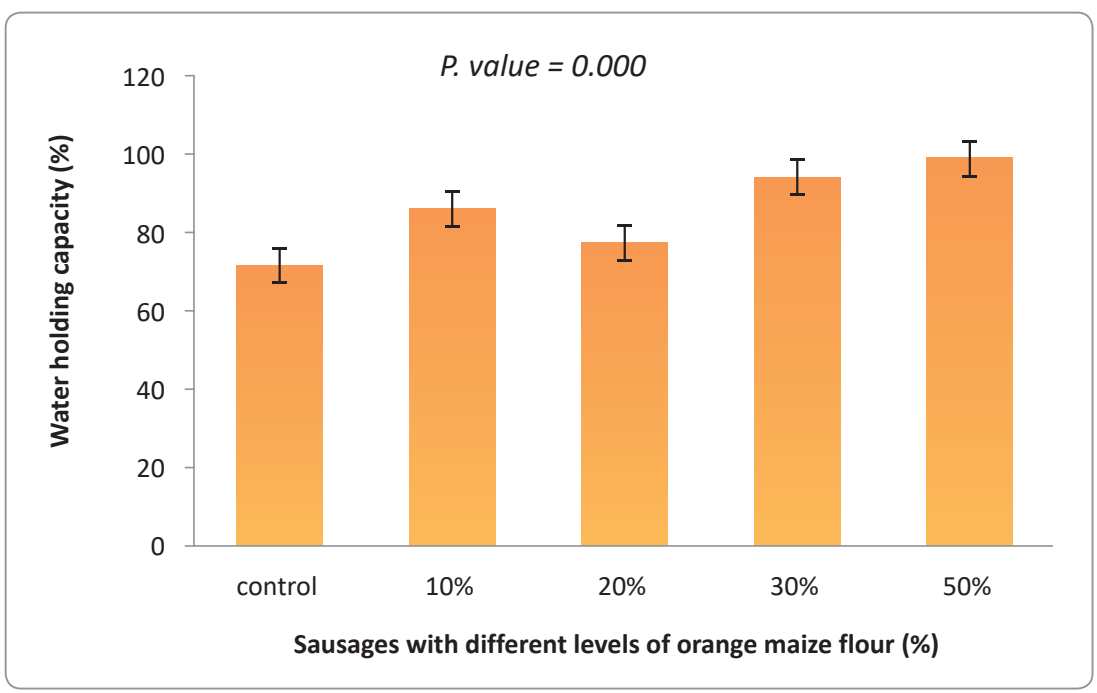

Figure 2. Water holding capacity of sausages with different levels of incorporated orange maize flour.

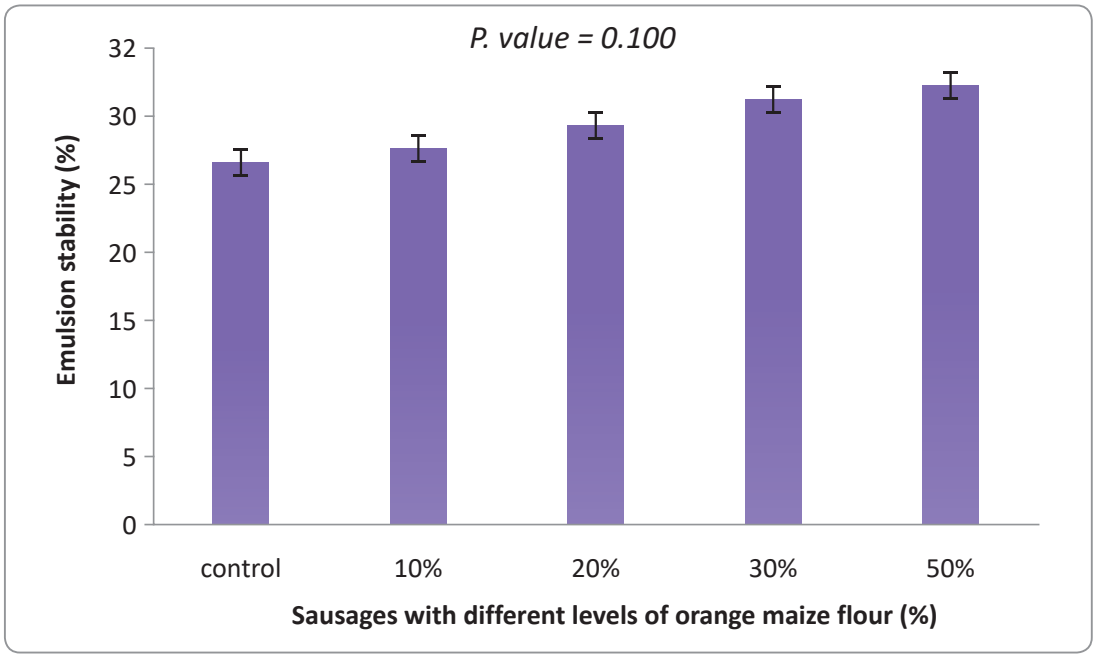

Figure 3. Emulsion stability of sausages with different levels of incorporated orange maize flour.

Water holding capacity of the sausages

Control sausages had the lowest WHC, while the 50% OMF sausages had a significantly higher WHC (Figure 2). This indicates that OMF has good WHC. However, the 20% OMF sausage had lower WHC than that of 10% OMF sausage. *Serdaroglu and Degirmencioglu* (2004) showed that corn flour (2% and 4% level) increased moisture retention in treated meat balls. Also, the increase in WHC caused by the OMF proves OMF has a good ability to form gel (*Rodriguez-Furlan et al.,* 2014; *Petersson et al.,* 2014). This could probably make OMF a suitable candidate for incorporation into sausages. WHC is the ability of meat to retain its water or added water during the use of external forces like cutting, heating, grinding or pressing (Judge *et al*., 1990). WHC is a significant indicator of dietary fibre, suggesting the inclusion of fibre in food product formulation *(Petersson et al.,* 2014).

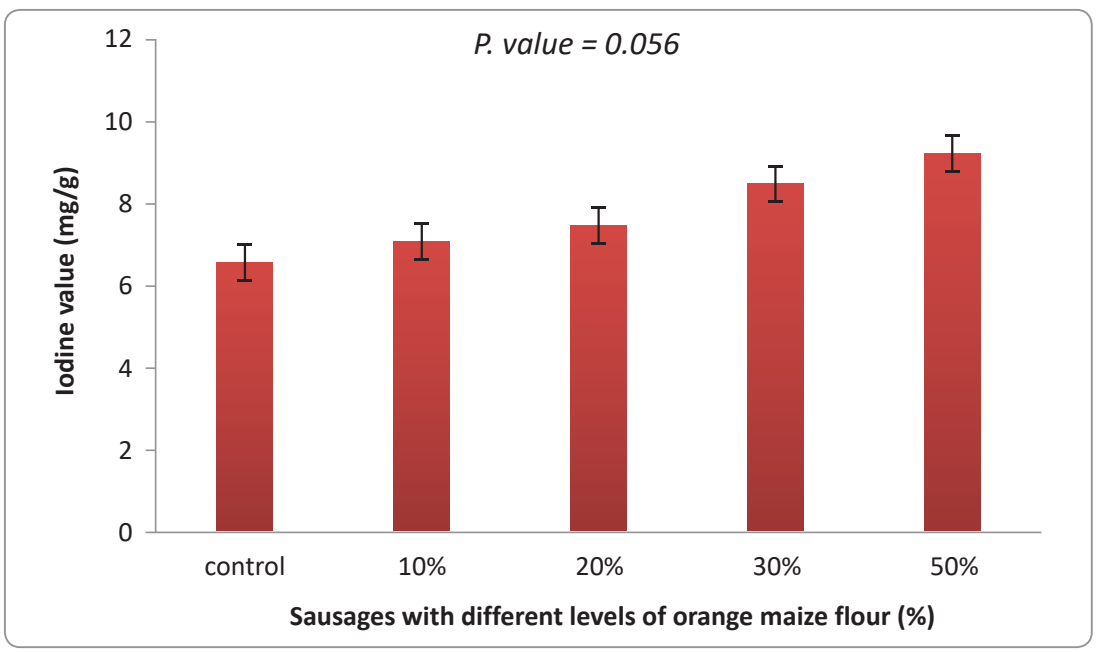

Figure 4. Iodine values of sausages with different levels of incorporated orange maize flour.

Emulsion stability of the sausages

As shown in Figure 3, the emulsion stability, an indicator of unseparated fat and water retained by meat proteins upon heat processing, increased with increasing OMF content in the sausages. However as reported by *Alvarez and Barbut* (2013), a reduction in fat content of meat products leads to a drop in emulsion stability, because fat is needed to stabilise batters. However, in our study, the emulsion stabilities of the OMF beef sausages were comparable with the high fat control sausages, since no significant difference was observed ($p<0.05$). This suggests that OMF exhibited a fat-like property and also acted as an emulsifier.

Iodine value of the sausages

Iodine value was determined (Figure 4) to estimate the unsaturation level of the fat present in the formulated sausage products. The results indicated the unsaturation level improved with the increased addition of OMF, although there was no significant difference between the sausages ($p<0.05$). This result confirms the fact that maize contains oil, which is rich in poly– and monounsaturated fatty acids (*CRA,* 2006). This rise in the unsaturated fat could make the product more desirable for consumption since unsaturated fat is considered as a healthy dietary fat.

Microbiological quality of the formulated sausages

As shown in Table 2, the effects of OMF in the sausages stored at 4°C were studied on days 0, 4, 8 and 12 to determine its microbiological qualities. Furthermore, the presence of *E. coli*, *Salmonella* spp. and *S. aureus* was determined. On day 0 and on day 4 of storage, there were no detectable bacteria in any of the sausages. However, on day 8, a total plate count of 11.50 CFU/g and 5.00 CFU/g were observed for the control and 10% OMF sausages

Table 2. Microbiological quality (CFU/g) of the sausages.

Sausage	Day of Storage			
	Day 0	Day 4	Day 8	Day 12
Total Plate Count				
Control	nd	nd	11.50±2.12^{a}	21.00±2.83^{b}
OMF 10%	nd	nd	5.00±2.83^{c}	18.50±2.12^{d}
OMF 20%	nd	nd	nd	14.50±7.78^{e}
OMF 30%	nd	nd	nd	7.50±2.12^{f}
OMF 50%	nd	nd	nd	7.50±0.71^{f}
***Salmonella* spp.**				
Control	nd	nd	nd	nd
OMF 10%	nd	nd	nd	nd
OMF 20%	nd	nd	nd	nd
OMF 30%	nd	nd	nd	nd
OMF 50%	nd	nd	nd	nd
E. coli				
Control	nd	nd	1.00±0.00^{a}	3.50±2.12^{b}
OMF 10%	nd	nd	1.00±0.00^{a}	2.00±1.01^{a}
OMF 20%	nd	nd	nd	1.00±1.41b^{a}
OMF 30%	nd	nd	nd	1.50±0.71^{c}
OMF 50%	nd	nd	nd	2.00±0.00^{a}
Staphylococcus aureus				
Control	nd	nd	nd	nd
OMF 10%	nd	nd	nd	nd
OMF 20%	nd	nd	nd	nd
OMF 30%	nd	nd	nd	nd
OMF 50%	nd	nd	nd	nd

Legend: Means with the same superscript letters in the same column are not significantly different at $P<0.05$; OMF: orange maize flour; nd: not detected; OMF: orange maize flour

Table 3. Mean ranks of sensory properties of sausages and statistical differences.

Treatment	Colour	Texture	Flavour	Taste	Overall Acceptability
Control	114.00	133.11ab	126.32abc	144.47b	129.16ab
OMF 10%	133.58	130.56ab	152.82c	142.71b	101.17a
OMF 20%	139.41	118.70ab	108.76ab	101.41a	153.71b
OMF 30%	112.05	145.66b	140.76bc	139.5^{6}b	144.94b
OMF 50%	128.46	99.47 a	98.84 a	99.35^{a}	98.52a
p value	0.206	0.012	0.000	0.000	< 0.0001

Legend: Means with the same superscript letters in the same column are not significantly different at p< 0.05; OMF: orange maize flour

respectively, and on day 8, an *E. coli* count of 1.00 CFU/g was observed in both the control and 10% OMF sausages. On day 12, the total plate count increased significantly in almost all sausage formulations, but the highest. *E. coli* counts were measured in control sausage. However, the results were all within acceptable level of microbiological quality for processed meat, since according to Nel *et al.* (2004), the maximum limit of *E. coli* in meat and meat products is 10 CFU/g. The presence of *E. coli* in high numbers indicates the presence of organisms originating from faecal sources (Nel *et al*., 2004). The presence of *S. aureus* can be used as indicator of personal hygiene since the source is usually the skin/hand of food handlers (*Mulder,* 1996). Also, the lower microbial counts in the OMF sausages could be attributed to the microbial inhibition potential of the OMF due to its total carotene concentration (*Arief et al*., 2014).

Sensory evaluation of the sausages

Table 3 shows the mean ratings of sensory attributes for each sausage. There were significant differences ($p<0.05$) in consumer preference among the sensory parameters assessed except for colour, which showed no significant difference. Consumer preference scores based on texture were the lowest for 50% OMF sausages as compared to the other sausages, suggesting inclusion of 50% OMF negatively affected the texture. The consumer preference taste score was higher for control sausage, but this was similar to the taste scores for 10% and 30% OMF sausages. The sausage with the most preferred flavour was 10% OMF sausage. Table 3 shows the mean consumer preference score for overall acceptability was given to sausages with moderate addition of OMF (20% and 30% OMF sausages achieved the highest scores).

Conclusions

In conclusion, the tannin and the phytic acid contents of the OMF were low, while the physicochemical properties of the OMF beef sausages were comparable to those of the control beef sausages. The microbiological results were all satisfactory, showing levels of specific bacteria below the maximum allowable levels for meat and meat products. Thus, OMF can be used to substitute fat in sausages without sausage sensory characteristics being affected.

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Uticaj uključivanja brašna narandžastog kukuruza u proizvodnji kobasica od goveđeg mesa i osobine kvaliteta proizvoda

Arthur Vera, Newlove A. Afoakwah

A p s t r a k t : Cilj ove studije bio je uključivanje, u različitom procentu (0%, 10%, 20%, 30% i 50%), narandžastog kukuruznog brašna (OMF) u kobasice proizvedene od goveđeg mesa i proučavanje bioaktivnih osobina OMF-a. Takođe, ispitivane su fizičko--hemijske, mikrobiološke i senzorne karakteristike OMF kobasica. Rezultati bioaktivnih analiza OMF-a su pokazali da je ukupan sadržaj karotenoida u OMF bio od 14,31 µg/g, dok je zabeležen sadržaj tanina 1,52 mg/g. Sadržaj vlage u kobasicama smanjio se sa povećanjem udela OMF-a. Sadržaj masti kobasica od goveđeg mesa – OMF bio je značajno niži (p<0,05) u poređenju sa kontrolom, a stabilnost emulzije 30% OMF kobasice nije se statistički razlikovala od kontrolnih kobasica. Vrednosti joda OMF goveđih kobasica pokazuju trend porasta sa povećanjem koncentracije OMF-a. Salmonella spp. i Staphilococcus aureus nisu otkriveni u kobasicama. Otkrivena Escherichia coli bila je u opsegu od 1,0 do 2,0 CFU/g. Najprihvatljivija OMF goveđa kobasica je bila ona sa 20% narandžastog kukuruznog brašna. Ova studija je pokazala da narandžasto kukuruzno brašno ima potencijal da se koristi kao zamena za masti, uz očuvanje senzornih svojstava i sprečavanje mikrobnog rasta u goveđim kobasicama.

Ključne reči: *narandžasto kukuruzno brašno, bioaktivnost, ukupni karotenoid, jodna vrednost, goveđa kobasica*

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Review paper

Insects – a promising feed and food protein source?

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A b s t r a c t: Insect use in feed and food in European countries is now an increasingly debated topic, although these animals have traditionally been nutritional components in Asian, African, Central American and South American cultures. This review addresses the issue of eating insects and using them as feed components, especially considering the nutritionally important factors. Safety risks are also discussed, as well as allergies, toxicity, consumer acceptance, legislative, welfare and environmental concerns and possibilities for laboratory control. Insects play and will play important roles in the future in various fields of research and utilisation, but especially and increasingly as feed and food ingredients.

Keywords: *edible insects, entomophagy, insect meal.*

Introduction

It has been estimated that the world population will increase by 25% in the next decades to reach over 9 billion by 2050 (*United Nations*, 2015). Therefore, a demand to increase food production will be unavoidable, while other influences such as climate change will make this situation more difficult (*Nesic,* 2018). Recent predictions foresee necessary food augmentation by up to 70% (*Hunter et al.,* 2017), with a particular need for protein sources. In addition, there has been an approximate fivefold increase in global meat consumption since the 1940s as a result of income growth, increasing urbanisation and changes in lifestyles and food preferences. The Food and Agriculture Organization of the United Nations (FAO) forecasted, in a report from 2003, a worldwide increase in meat consumption from 41.3 to 45.3 kg capita^{-1} between 2015 and 2030 (*FAO,* 2003). Meanwhile, recent statistical sources predict much higher figures of global meat consumption, reaching 51.7 kg capita^{-1} in 2030 (*Statista,* 2018). This has also resulted in increasing pressure on the production of protein sources for animal feed. The main protein feedstuffs currently in use are soya and fishmeal, but European countries depend on the import of these ingredients, which makes the livestock sector vulnerable to price inconsistency and trade deviations.

Further deterioration of the situation is the result of the rise in the consumption of fish, as aquaculture is now the fastest growing food production system in the world. This sector mostly relies on fishmeal as a protein source, but fish farms are increasingly looking to crop proteins as a lower-cost replacement for fishmeal. On the other hand, fishmeal is not only used in aquaculture but is also an excellent source of highly digestible protein ideal for poultry and pig diets. Thus, fish and animal farming are together placing growing demands on protein crops and fishmeal supply. Furthermore, issues regarding the availability of land and water put even more pressure on the world's ability to meet the increasing requirements for the production of animal protein (*PROTEINSECT*, 2016). The response to this escalating problem goes into two directions: to return to previous strategies or to seek new and alternative solutions.

The first option is based on the reintroduction of processed animal proteins (PAP). Besides being from the fish processing industry, these feedstuffs are largely obtained as by-products of dairies and slaughterhouses. Although in principle bearing nutritional value, for safety reasons they have been excluded from the food chain over the years. PAP eradication from the food chain started after the outbreak of bovine spongiform encephalopathy (BSE) in 1986, when it was found that infectious ruminant prions spread by insufficiently processed meat and bone meal (MBM) used as feed. One of the most important measures was to endorse common European rules to avoid entry of these nutrients

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into the food chain (*Nesic and Radosavljevic*, 2014). As a consequence, several regulations were established and have been continuously modified, repealed or amended over time according to the findings of regularly updated risk assessments. Still, the basic European Union (EU) Regulation (EC) No. 999/2001 (*European Union*, 2001) generally prohibits, with a few exemptions, the application of PAP in the human and animal food/feed chains. In addition, regulations (EC) No. 1069/2009 (*European Union*, 2009a) and (EU) No. 142/2011 (*European Union*, 2011), prescribe general guidelines for the safe use of animal by-products and provide definitions for the various types of materials of animal origin. Furthermore, the use of protein originating from the same species in animal nutrition was forbidden. Such rigorous measures were introduced due to the fact that those ingredients have potential to cause prion infections not only in animals, but also in human populations consuming food of animal origin. Due to the strict approach during more than 20 years, the epidemiological situation in Europe has improved greatly (*Paisley et al*., 2008). Even in the United Kingdom, a former hotspot of the BSE crisis, the number of officially reported cases declined to zero in 2016 (*OIE,* 2018), so the possibility of mitigating the ban opened stepwise. Since 2013, the use of non-ruminant PAP, has been approved again in the EU for use in aquaculture (*European Union*, 2013). Accordingly, on 1 April 2016, amendments to Serbian Regulation on determination, diagnostics and prevention of transmissible spongiform encephalopathies (*Serbia,* 2016) were published as a result of harmonisation with EU legislation (*Nesic et al.,* 2016; *Nesic and Nikolic-Stajkovic*, 2016).

In recent years, insects have become increasingly relevant to satisfy the ever growing need for protein in animal feed, and consequently or directly in the food for humans. As a branch of zoology, entomology has a wide spectrum and is a well developed scientific discipline. It includes the biology and control of insects, as well as their ecology and impact on animal, plant and human health. Although they are often considered a nuisance to human beings and mere pests for crops and animals, insects are crucial components of many ecosystems, where they perform very important functions. They aerate the soil, pollinate blossoms and control insect and plant pests. Many insects, especially beetles, are scavengers, feeding on dead corpses and plants, thereby recycling nutrients back into the soil. Based on this role, forensic entomology was developed as an emerging area in forensic science. It has become an important tool in criminal investigations (*Amendt et al.,* 2011). As decomposers, insects help create top nutrient-rich layers of soil that help plants grow and, thus, participate in waste bioconversion. Burrowing bugs, such as ants and beetles, dig tunnels that provide channels for water, benefiting plants. Finally, all insects fertilise the soil with the nutrients from their droppings. Some species produce useful substances, such as honey, wax, lacquer or silk, while some have also been used in medicine. Insects are eaten by many amphibians, reptiles, birds and mammals, making their roles in food chains irreplaceable. Also, it is estimated that they form part of the traditional diets of at least 2 billion people and more than 1,900 species have reportedly been used as food (*FAO,* 2013).

Insects as feed

The deficit of protein ingredients, especially for animal feed due to the limited availability of natural resources, ongoing climatic changes, food-feed-fuel competition, recent high demand and consequent high prices for fishmeal and soya, increasing production pressure on aquaculture and restrictions on other animal protein usage have led to growing need to include insect protein in diets for aquaculture and livestock. The search for alternative and sustainable proteins is an issue of major importance that requires viable solutions in the short term, making insects an increasingly attractive feed option. Therefore, in mid-2017, the European Commission adopted the amendment EU Regulation No. 2017/893 (*European Union*, 2017), allowing seven species to be reared and used in feeding aquaculture. This closed list of authorised insects includes: black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis*). The conditions for the production of insect PAP are strictly regulated. They must be fed only with material of category 3 (material which in principle would still be appropriate for human consumption), not allowing, for example, manure or heavy metal contaminated debris as a feed source. Furthermore, insect PAP has to be treated at least according to method no. 7, following Regulation (EU) No. 142/2011 (*European Union*, 2011), which means that bacterial contamination must be reduced in order to make a safe product.

The number of new scientific publications on the topic of insects as feed illustrates the growing academic interest, which is also a consequence of the high costs of producing meat, fish and soybean

meal, i.e., around 70% of costs in animal production. Therefore, insects, as good sources of nutrients, having a low environmental impact, requiring less space for production and being already part of the natural diets of pigs, poultry and fish, are an ideal feed alternative (*Rumpold and Schluter,* 2013). The feed conversion efficiency of insects is good, like crickets for example, which require only 2 kg of feed for every 1 kg of body weight gain (*Collavo et al.,* 2005). Apart from good quality protein, we can also obtain fat as a by-product of protein production, and which can be considered for making biodiesel (*Wang et al.,* 2017).

In the trial of *Newton et al*. (2005), when a choice was given, pigs did not discriminate against a diet containing ground soldier fly larvae (*Hermetia illucens*) compared with soybean meal. Also, recent experiments on piglets (*Spranghers et al.,* 2018) showed that a substantial amount of soybean products (meal and/or toasted beans) can be replaced with black soldier fly without adverse effects on performance. Furthermore, grasshoppers (*Acrida cinerea*) and Mormon crickets (*Anabrus simplex*) were also able to replace fishmeal and soybean meal in poultry diets (*Wang et al*., 2007). In several investigations, it was shown that larvae of the common housefly (*Musca domestica*), containing 54% crude protein in dry matter (DM), could successfully replace fishmeal in broiler diets (*Teguia et al*., 2002; *Awoniyi et al*., 2004; *Hwangbo et al*., 2009). When *Ijaiya and Eko* (2009) replaced 25, 50, 75 or 100 % of fishmeal with silkworm meal in the diet for broilers, they did not find any significant effect on body weight gain, feed intake, feed conversion, slaughtering results or meat quality. In the review of *Makkar et al.* (2014), 24 feeding experiments (17 from Africa, 4 from Asia and 3 from the USA) with broilers fed various portions of house fly larvae meal (maggot meal) are mentioned. They also included 12 broiler studies (9 from India) describing results with silkworm meal. The authors concluded that fishmeal, soybean meal and groundnut cake could be successfully replaced up to 100 % with insect protein.

Insect protein is a suitable replacement for fishmeal in diets for juvenile fish and crustaceans (*Riddick,* 2014; *van Huis et al.,* 2015). Insects in the form of meal or pellets can provide adequate protein to replace standard fishmeal in feed for omnivorous fish such as carp and catfish. However, there is evidence that in carnivorous fish such as salmon and trout, only a portion of fishmeal can be replaced with insect products (*Riddick,* 2014). In spite of that, *Lock et al.* (2016) found an uneven outcome of fish meal replacement in amounts up to 100% in the diet with two differently processed *H. illucens* meals in feed for Atlantic Salmon (*Salmo salar*) fed for 15 weeks. The group of fish fed insect meal higher in energy, lipids and carbohydrates performed as well as the fish raised on a fishmeal based diet, whereas fish fed the other insect meal did not. *Makkar et al.* (2014) reviewed studies with catfish, tilapia, rainbow trout, Atlantic salmon, turbot and crustaceans, where fishmeal was replaced with dried black soldier fly larvae meal, housefly maggots, dried mealworms, locust meal, grasshoppers or silkworm pupae meal. Most studies demonstrated that about 50% replacement of fishmeal had no adverse effect on animal performance. The research of *Devic et al.* (2018) concluded that inclusions of up to 80 g kg^{-1} of black soldier fly larvae meal did not affect the feed quality for advanced nursing tilapia. They emphasised that, as the market price of this ingredient is competitive, feed production costs would be alleviated by reducing use of fish meal, fish oil and soyabean meal. More broadly, inclusions of cheaper, sustainable and locally available feedstuffs in juvenile tilapia commercial feed could support the sustainable intensification of aquaculture and contribute more widely to food security.

Regarding other animal species, insects are used as pet food (e.g. crickets for many insectivorous reptiles and amphibians), and are fed alive or prepared in different ways. The recent opinion of the European Food Safety Authority (*EFSA,* 2015) noted that some insects are low in calcium content, but they are still suitable in such diets as a natural component for these animal groups.

Anyhow, the policy debate recommends focusing primarily on the use of insects in feed for fish, poultry, pigs and pets, while the use of insects in feed for farmed ruminants like cattle is currently not an issue, although the risk for prion transfection by insects is regarded negligible (*EFSA*, 2015). The overall positive atmosphere surrounding the idea of using insects in animal feed indicates the situation is favourable for moving forward with this development and taking advantage of this opportunity to improve the real and perceived sustainability of animal feed and livestock production (*Verbeke et al.,* 2015).

Insects as food

The consumption of insects, or entomophagy**,** is heavily influenced by cultural and religious practices. These animals, as they belong to the kingdom *Animalia*, are commonly a food source in many regions of the world, especially in Asia, Africa, Central America and South America. In European countries, however, people usually view entomophagy

with disgust and associate eating insects with rather exotic ethnic habits. This attitude has resulted in the neglect of insects in agricultural research for a long time. Despite historical references to the use of insects for food, the topic of entomophagy has only very recently started to attract public attention worldwide (*FAO*, 2013). Globally, the most commonly consumed insects are beetles (*Coleoptera*) (31 percent), caterpillars (*Lepidoptera*) (18 percent) and bees, wasps and ants (*Hymenoptera*) (14 percent). Following these are grasshoppers, locusts and crickets (*Orthoptera*) (13 percent), cicadas, leafhoppers, planthoppers, scale insects and true bugs (*Hemiptera*) (10 percent), termites (*Isoptera*) (3 percent), dragonflies (*Odonata*) (3 percent), flies (*Diptera*) (2 percent) and other orders (5 percent).

Starting in 2018, Regulation (EU) No. 2015/2283 entered into force, laying down provisions for the approval of novel foods in Europe (*European Union*, 2015). This new regulation establishes the requirements that enable food business operators to bring new food items onto the EU market, while ensuring high levels of food safety for European consumers. Insects, for which no traditional use in the EU is evident, are explicitly mentioned. Thus, for insects, companies have to make an application which is examined and finally approved or rejected by EFSA. However, in certain cases a simpler notification procedure is possible for traditional food from third countries if it is proven that such foods have been part of human nutrition for at least 25 years without any safety concerns. Accordingly, it is reasonable to expect that more insects and insect products will enter the European food market. Interestingly, in Switzerland, an EU-associated partner state, since 1 May 2017, three insect species (*Acheta domesticus, Locusta migratoria and Tenebrio molitor*) have already been allowed for human consumption. Nevertheless, up to now, there are no systematically collected data available on insect consumption in Europe, although it is known they are used in some elite cuisines, but not comparable with their use in North-Eastern India, South-East Asian countries and parts of Australia. *Premalatha et al.* (2011) gave some examples: wasps, bamboo caterpillars, cricket and locusts are sold as delicacies in the finest restaurants and food shops in Thailand; annual sales of ant food in China reach $100 million; the rice field grasshopper, called inago, is a luxury food item in Japan; canned wasps, as a 65 g can, sell for over $10, while hornets are even more expensive, selling at over $20 per 100 g. There is an explosion of tourist interest in the native Australian "bush tucker" foods, which include insects such as wichety grubs (*Cossidae*), bogong moth and bardee larva. In Mexico, upmarket restaurants charge upwards of $25 per plate of escamoles (pupae of an ant species) and gusanos (butterfly larvae), and when exported to Canada, escamoles muster a fantastic price of $50 for a 30 g can (almost $2 per gram).

Previously, insects were eaten alive and later, in some cultures, they were also served cooked, roasted or boiled (either insects *per se* or insect additives to food), or prepared using other culinary techniques. Many people prefer incorporating insects into the food in a manner that renders them not visible – only accepting the idea that insects have beneficial nutritional value. This shows that people, especially in North America and Europe, would eat insects if they do not know what they are eating, with the exception of individuals who have allergic reactions (and are aware of possible cross reactions) (*Mlcek et al.,* 2014). These factors suggest that insect transformation, specifically their mode of processing, most certainly would facilitate consumption in the future. In practice, dried insects can be crushed or pulverised, and raw or boiled insects ground or mashed, making their form unrecognisable. Extract of insect protein and fat can also be used. If insects are prepared as masses of protein and lipids, these can be mixed with other foodstuffs, such as grain, ground meat or mashed potatoes to make a variety of dishes and become more acceptable to most people. Insects can also be a serious alternative for vegetarians and – though at present rather hypothetical science fiction – their nutritional potential for a relatively long-term future human settlement in space has been discussed (*Mlcek et al.,* 2014).

Among different possibilities for preservation and storage of insects, the most appropriate have to be chosen. Live insects, after washing, are typically transported in ice coolers shortly after collection. Refrigeration is also recommended for fried and boiled insects. Some are preserved and traded after sun-drying. Other simple preservation methods such as acidifying insects with vinegar have been successful. Another example is the use of insects for protein enrichment in fermented food products. This is a viable processing option with mutual benefits, since the decreased pH in lactic acid-fermented products prevents the growth of potentially harmful microorganisms (*Klunder et al.*, 2012). Sometimes freeze-drying is implemented. However, other contemporary preservation methods should be explored, such as the application of ultraviolet light and high-pressure technologies, as well as adequate packaging methods. For this entire topic, further research on the best, most suitable solutions is necessary.

Nutritional value

Insects are usually considered as valuable sources of protein. Because of their high protein content, well balanced amino acid composition and the other nutrients they contain, beside their use as animal feed, they could become an attractive alternative to traditional foods of animal origin, such as milk, meat, fish and eggs in human nutrition. Depending on the insect species, protein levels can be up to 63% on a DM basis, as is the case with the larvae of black soldier fly (*Hermetia illucens*) and house fly (*Musca domestica*), which contain up to around 63% protein and 36% fat (*Makkar et al.,* 2014). They contain high levels of key amino acids (e.g. lysine, tryptophan) when compared to most crop plants. A recent study comparing nutritive characteristics of a range of insects showed that the amino acid profile of dipteran insects is superior to soybean meal and more similar to fishmeal (*Barroso et al.,* 2014).

Lipids are a large component of fly larvae with crude fat content in meal produced from house fly larvae reported to range from 14 to 27% (*Fasakin et al.,* 2003; *Aniebo et al.,* 2008; *Pretorius,* 2011). The principal fatty acids found in *M. domestica* larvae and pupae are palmitic, palmitoleic, oleic and linoleic (*St-Hilaire et al.,* 2007; *Hwangbo et al.,* 2009).

Chitin comprises the main carbohydrate in insects. As reviewed by *Mlcek et al.* (2014), the carbohydrate content of edible insects ranged from 6.71% to 15.98%. Studies dealing with the vitamin content in insects are insufficient, although it is known that edible insects contain mainly carotene and vitamins B1, B2, B6, D, E, K and C. Analysis of mineral elements showed that edible insects are rich in nutritious elements such as potassium and sodium (e.g. cricket nymph), calcium (e.g. cricket adult), copper (e.g. *Usta terpsichore*, mealworm adult), iron (e.g. axayacatl – a mixture of several species of aquatic Hemiptera, giant mealworm), zinc (e.g. cricket nymph), manganese (e.g. cricket adult) and phosphorus (e.g. cricket adult). Summarised proximate analysis data by *Makkar et al.* (2014) and *EFSA* (2015) on crude nutrients in different insect species are shown in Table 1.

Tables 2 and 3 show some collected data on nutritional composition of insects in comparison to other food protein sources.

As *Premalatha et al.* (2011) noted, it is a supreme irony that all over the world billions are spent every year to save crops that contain no more than

Table 1. Crude nutrients of different insect species measured by Weende analysis, in % of dry matter (Adapted from *Makkar et al.* (2011) and *EFSA* (2015))

Insect species	Crude protein %	Crude fat %	Carbohydrates %	Crude ash %
Black soldier fly larvae	41.1–43.6	15.0–34.8	7.0	14.6–26.8
Housefly maggot meal	42.3–60.4	9.0–26.0	1.6–8.6	6.2–17.3
Tenebrio molitor	47.2–60.3	31.1–43.1	7.4–15.0	1.0–4.5
Locust or grasshopper meal	29.2–65.9	4.2–14.1	2.4–14.0	4.4–10.0
House cricket	55.0–67.2	9.8–22.4	15.7–22.1	3.6–9.1
Silkworm pupae meal	51.6–70.6	6.2–37.1	2.5–5.8	3.3–10.6

Table 2. B-vitamins in 100 g servings of chicken and bean dishes in comparison to the content of some insects (Adapted from *Premalatha et al.* (2011)).

	Thiamine	Riboflavin	Niacin
Daily human requirement	1.5 mg	1.7 mg	20 mg
Portion from roasted chicken	5.4%	–	45%
Portion from backed beans	10.8%	–	3%
Portion from termites	8.7%	67.4%	47.7%
Portion from silkmoth larvae	224.7%	112.2%	26%
Portion from palm weevil	201.3%	131.7%	38.9%

Table 3. Protein and iron in 100 g servings of beef and of two insects (Adopted from *Premalatha et al.* (2011)).

Food	Protein, g	Iron, mg
Beef (boiled)	22.3	2.9
Silkmoth larvae (boiled)	28.2	35.5
Grasshoppers (fried)	61.1	–

14% of plant protein by killing another food source (insects) that can contain up to 75% of high quality animal protein.

What bugs us about edible bugs?

A major consideration in the use or applicability of any novel food product is to demonstrate its safety. Studies on the microbiological and chemical safety of insects reared for feed and food are limited, but some authors have published review papers on this issue (*Belluco et al.,* 2013; *Van der Spiegel et al.,* 2013), FAO discussed it in its booklet (*FAO,* 2013), EFSA gave summarised data in its scientific opinion (*EFSA,* 2015) and project PROTEINSECTS reported their final viewpoint (*PROTEINSECTS,* 2016). Finally, all of them agree that insects as a feed and food category, in principle, could be consumed with no additional hazards in comparison with usually eaten animal products. Levels of more than 500 potentially toxic chemical contaminants were all below recommended maximum amounts suggested by bodies such as the European Commission, World Health Organisation and Codex. However, elevated levels of the toxic heavy metal cadmium in some insects indicated that pre-screening of rearing substrates would be necessary to mitigate risk. Tests found no evidence of the presence of viable biological contaminants such as *Salmonella* and *Campylobacter* (*PROTEINSECTS,* 2016). On the basis of these facts, insects can be regarded as safe, if properly managed and consumed.

Some toxicological and allergy threats were also considered. Relatively heat-resistant thiaminase was detected and characterised from the silkworm, so it needs thorough heat treatment for detoxification (*Nishimune et al*., 2000). Pesticide applications against locusts and grasshoppers can cause problems because of their toxic residues (*Van Huis,* 2003; *Yen,* 2009). In most cases, allergies to insects are associated with a job where employees deal with insects. In clinical practice in relation to insects, the most commonly reported allergic reactions are to chitin, which is the second most abundant biopolymer in nature (*Rop et al.,* 2009). Crickets can trigger allergic reactions in sensitive consumers (*Fernandez-Cassi et al.,* 2018). Homologue proteins shared between different species can cause such responses. Tropomyosin, arginine-kinase and glyceraldehyde-3-phosphate dehydrogenase have been identified as highly allergenic. Hexamerin B1, with allergenic potential requiring more research, has been described as a specific cricket allergen. Pilot results of *Broekman et al.* (2016) suggest that shrimp-allergic patients might be at risk for mealworm allergy because IgE binding to tropomyosin and arginine kinase (major shellfish allergens) and arcoplasmic calcium-binding protein and myosin light chain (minor shellfish allergens) were detected. For safety reasons, insect food products should be labelled to raise awareness in susceptible consumers.

Consumer acceptance is the key to the successful adoption of insects as a source of protein for feed and food. Recent studies have shown that willingness to eat insect based foods is determined by a diversity of personal attitudes and interests (food neophobia i.e., fear of trying new foods; interest in the environmental impact of personal food choices and; openness to change dietary habits), as well as cultural exposure, familiarity or past experience and knowledge (*Verbeke*, 2015; *Tan et al.,* 2015). One of the most common refusals is based on hygienic reasons, but most insects, especially edible insects such as grasshoppers and lepidopteran or coleopteran larvae mostly eat fresh plant leaves or wood and are, therefore, cleaner and more hygienic than crabs or lobsters, which eat carrion. In general, the application of insects for food and feed purposes is environmentally more beneficial than traditional sources of proteins (*Smetana et al.,* 2016). Insects can be reared on organic side-streams (including human and animal waste), emit fewer greenhouse gases and less ammonia than cattle or pigs and require significantly less land and water than cattle rearing. As public attention could gradually evolve over time, willingness to adopt the use of insects as nutritive dietary components can modify. Especially, their implementation in animal feed can be expected to evolve.

Other issues such as the welfare of insects raised for use in feed and food also need to be considered. Harvesting insects as food from the wild,

like any other hunting and collecting activities, has the potential to become a threat to both the target species and the environment. *Ramos-Elorduy* (2006), for example, reported that the populations of some of the 30 edible insect species in the Mexican town of Tulancalo have declined because of overexploitation, and this situation has led to a call for regulation of edible insects' exploitation in Mexico to ensure better management, production and conservation. Semi-cultivation of edible insects is often used, as well as rearing and farming in various degrees. To ensure animal welfare, farmed insects should be provided with adequate space to minimise mortality and increase productivity. Little is known about the extent to which insects experience pain and discomfort (*Erens et al.*, 2012), but insect-killing methods that would reduce suffering include freezing or instantaneous techniques such as shredding.

Further regulatory aspects have to be solved in the future. Western legislation is greatly focused on safety concerns about new food or new ingredients (*Belluco et al.,* 2013). In addition, there are merely technical difficulties, e.g., regarding slaughterhouse registration, which is not yet applicable to insects, and in relation to the substrate on which the insects are reared. Insects produced for feed would be classed as farmed animals, for which only category 3 materials are allowed as a feed source. Manure is a category 2 material and, therefore, not permitted to be fed to farmed animals; neither can insects be fed on catering waste or former foodstuffs containing meat and/or fish.

As for any other ingredient, adequate monitoring and control of feed and food has to be implemented. However, no laboratory test for their detection in feed/food has been officially validated and standardised yet.

Laboratory control

Enforcement of any legislation requires adequate analytical tools and implementation of an appropriate control system (*Nesic and Pavlovic,* 2012). Hence, the design of laboratory methods should serve for monitoring authenticity, control of labels/declarations and detection of fraud (*van Raamsdonk et al.,* 2017). When it comes to insects that belong to animal protein and are subject to PAP regulations, the only methods authorised for feed testing are light microscopy and polymerase chain reaction (PCR) (*European Union*, 2013). There is also a corresponding harmonised regulation in Serbia (*Serbia,* 2016). These two methods are used for aquaculture feed control individually or in combination (*EURL-AP,* 2015), but usually microscopy gives the first information on the presence of PAP material. Light microscopy can distinguish between fish and terrestrial particles, as it relies principally on the categorisation of bone fragments into those two groups. The team of the EU Reference Laboratory for animal proteins in feedstuffs recently proposed an improvement of Annex VI of EU regulation (EC) No. 152/2009 (*European Union*, 2009b), publishing a modified and adapted microscopy protocol for detection of insects. Furthermore, the implementation of a third category of animal material in addition to terrestrial as "terrestrial invertebrates" was proposed (*Veys et al.,* 2018).

However, microscopic discrimination between authorised species and undesired ones is not possible. Hence, the use of other methods, such as DNA targeting of authorised species, would offer additional information. Polymerase chain reaction (PCR) is the most frequently applied method for reliable detection of species-specific DNA. It has already been officially accepted for ruminant DNA detection in feed (*EURL-AP,* 2017), and two new protocols for poultry and pig DNA have been validated by the European Union Reference Laboratory for Animal Proteins in Feedingstuffs, but not yet included in the official catalogue of methods. Regarding other animal species detection, PCR has also found a role especially in forensic investigations (*Davitkov et al.,* 2017), while forensic entomology as a scientific discipline developed the application of insects in criminal investigation (*Joseph et al.,* 2011). To unravel food fraud, PCR is the most promising method of choice (*Nesic et al.,* 2017).

To fill the existing analytical gaps and to detect the species of interest rapidly, the most promising approach today is real-time PCR. Recently, three fully validated methods were published. One protocol is to detect in food and feed the beetle *Tenebrio molitor* (*Debode et al.,* 2017), the larvae of which are allowed for aquaculture feed, but are also a popular pet food for reptiles and birds. Two papers were published on the detection of black soldier fly (*Hermetia illucens*) in feedstuffs by newly developed real-time PCR methods (*Marien et al.,* 2018; *Zagon et al.,* 2018). However, five other insect species are allowed in feed and for which proper detection and control methods remain to be ascertained, while more than one million species of insects are unable to be discriminated. There is also a challenge in relation to food matrices and, as mentioned by *Zagon et al.* (2018), it might be expected that sooner or later, insects will be added to the list of commodities in the frame of the European allergen labelling

directives. To cope with the huge diversity of insects belonging to the big phylum of arthropods, the possibility of multiplexing solutions with other insect-targeted PCR methods could be an option. Alternatively, the application of next-generation sequencing (NGS) based on metabarcoding of arthropods (*Deiner et al.,* 2017; *Richardson et al.,* 2018; *Toju and Baba;* 2018) can be considered. This powerful technique enables the simultaneous detection of many different species in one and the same DNA extract, as demonstrated for native DNA isolated from environmental samples or faeces from insectivorous animals to evaluate their spectra of insect prey (*Sint et al.,*2014; *Galan et al.,* 2018). A major challenge in terms of processed materials will be to identify universal primers targeting DNA fragments of suitable size (≤ 150 bp), but at the same time covering all major groups of insects which are at least *Diptera*, *Orthoptera*, *Lepidoptera* and *Coleoptera*, accounting for the most important cultured insects. Apart from this, sensitivity in the case of only trace amounts of one particular species might be an issue, as could be economic aspects, since NGS machines, consumables and operator's knowledge are significant costs for these tests. The future will show how far and how rapidly this new sequencing method will find its way into routine application.

In any case, DNA-based methods and possibly some other laboratory tests used in feed control, like immunoassays (*Nesic et al.,* 2012), near-infrared spectrometry or mass spectrometry, at present, should probably be used as complementary tests once insect fragments have been microscopically confirmed in a non-specific screening approach (*Nesic et al.,* 2014). This would be a continuation of the present strategy for the disclosure of PAPs in feed (*Veys et al.,* 2018; *EFSA,* 2018). Nevertheless, different teams worldwide are rapidly pursuing research work and results are expected soon, as feed regulations in this area certainly will reflect on and push forward the science. Surely, food control, in response to future regulative tendencies, will require analytical approaches at the highest level.

Although up to now, no thresholds for PAPs have been implemented, it has to be critically remarked that still no reliable quantitative method exists. DNA-based methods (qPCR) are well suited for relative quantitation (e.g. to express the percent DNA of a given species in relation to DNA of another matrix component), as, for example, in genetically modified organism (GMO) detection. This is easily done if single copy genes are chosen for quantitation, and standard matrices or plasmids are available. Absolute results can be expressed as copy numbers of the DNA fragment under investigation. Further, digital PCR techniques, determining exact copy numbers by separation of DNA targets in multifold reactions, are ideally suited for copy number-based relative quantitation, e.g., as proven for GMO (*Koeppel and Bucher,* 2015). However, if absolute amounts (gram) of an analyte are to be calculated, the true value is difficult to deduce merely from copy numbers, since the amount of DNA in different tissue types can vary considerably. The bias is worse if, to enhance sensitivity, multi-copy genes (e.g. mitochondrial genes) are targeted by the PCR method. Nonetheless, encouraging results were achieved in estimating the prey-predator relation for sea lions using multi-copy targets in qPCR for species detection in scats (*Tollit et al.,* 2009). This principle might also be an option for discriminating allowed insect species against the background of unknown insect species. However, such quantitative approaches require well-characterised, universal group-specific primers and possibly numerical and empirically evaluated correction factors. Therefore, intensive research in this field is urgently required.

Conclusion

Based on novel data, insects represent a promising feed and food protein source, but future research needs to provide some solutions before they can be widely utilised in food and/or feed. Clarification is required to determine how the nutritional value of insects can be managed systematically, establish clear processing and storage methodologies, define rearing practices and implement regulations and adequate laboratory control with regard to food and feed safety. Overall, entomophagy can be promoted for several reasons: insects, if reared properly, are in principle, healthy, sustainable and nutritious alternatives to other animal food/feed sources, while insects' low negative environmental influence favours them, and economic factors are also on their side. Last but not least, the decision should be up to the informed consumer, accompanied by thorough risk assessment and correct labelling and controls, whether to accept this kind of novel food and feed in the future.

Insekti – potencijalni izvor proteina?

Ksenija Nešić Jutta Zagon

A b s t r a k t: Mogućnost upotrebe insekata kao izvora proteina u hrani i hrani za životinje tema je o kojoj se sve više raspravlja u evropskim zemljama, mada su ove životinje tradicionalno korišćene u ishrani u azijskim, afričkim, srednjeameričkim i južnoameričkim kulturama. U radu je predstavljan pregled primene insekata u nutritivne svrhe, a takođe su navedeni potencijalni rizici po bezbednost, alergijski aspekti, toksičnost, prihvatljivost za potrošače, zakonodavstvo, elementi dobrobiti, uticaj na životnu sredinu i mogućnosti za laboratorijsku kontrolu. Insekti imaju mnogobrojne važne uloge u različitim oblastima istraživanja i upotrebe, ali se sve više pominju kao hraniva i prehrambeni sastojci budućnosti.

Ključne reči: *entomofagija, jestivi insekti, hrana i hrana za životinje.*

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In memoriam – prof. dr Isidor Savić

Prof. dr Isidor Savić, rođen je 16. 06. 1919. godine u Gradišci danas u Republici Srpskoj, Bosna i Hercegovina. Osnovnu školu završio je u rodnom mestu, a realnu gimnaziju u Novoj Gradišci (Hrvatska). Na Veterinarski fakultet u Beogradu upisao se 1937. godine (kao student se izdržavao radeći fizičke poslove i podučavajući decu).

Po završetku rata vraća se na fakultet i odmah posle diplomiranja 1946. godine upućen je u Sovjetski Savez na jednogodišnje usavršavanje. Tu je došao u kontakt sa novim dostignućima u proizvodnji i preradi mesa i taj boravak tokom koga se upoznao sa konceptom klanica industrijskog tipa (koje tada u Evropi nisu bile zastupljene – prihvaćene su tek desetak godina kasnije), bitno je odredio daljnju profesionalnu sudbinu prof. Savića.

Nakon 1948. godine i političkog raskida Jugoslavije i Sovjetskog Saveza, naša zemlja je dobila značajnu tzv. tripartitnu pomoć (američko-britansko-francusku) u hrani i razvijanju novih pogona prehrambene industrije. Prof. Savić je od američkih stručnjaka i rukovodstva zemlje prepoznat kao mladi ekspert koji je shvatio prednosti industrijskog tipa klanica i dobija zadatak da navedeni koncept uvede u klaničnu industriju Jugoslavije koja je bila u izgradnji.

Posle povratka u Beograd izabran je za asistenta na predmetu Higijena namirnica životinjskog porekla, a 1948. godine za predavača na predmetu Higijena mesa.

Doktorsku disertaciju pod nazivom „Prilog poznavanju fermentacije goveđeg mesa“, odbranio je na Veterinarskom fakultetu u Beogradu, 1952. godine.

Boravio je i u SAD radi upoznavanja sa radom naučnih instituta, inspekcije i vodećih pogona industrije mesa kao i novih principa u industrijskoj proizvodnji i preradi mesa. 1956. i 1957. godini boravio je, radi usavršavanja u Nemačkom saveznom institutu za nauku o mesu.

Na inicijativu članica „Udruženja konzervne industrije Jugoslavije“ početkom 1955. godine osnovao je Institut za tehnologiju mesa FNRJ, sa zadatkom unapređenja saradnje nauke i privrede, projektovanja novih industrijskih objekata industrije mesa i bržeg transfera novih tehnologija u rastuću industrijsku proizvodnju. Dužnost stručnog rukovodioca Instituta obavljao je sve do 1964. godine.

Neumoran rad profesora i njegovih saradnika dali su velike rezultate koji su omogućili proizvodnju domaće industrije mesa za najzahtevnija svetska tržišta. Takav je slučaj bio pre svega sa šunkom u limenci koju je prihvatilo i američko tržište.

Tokom rukovođenja Institutom prof. Savić je inicirao uspostavljanje značajne međunarodne saradnje, kao i posete najznačajnih svetskih imena iz oblasti proizvodnje i prerade mesa koji su pomagali domaćim stručnjacima u rešavanju raznih problema u proizvodnji. Istovremeno se zalagao za kontinuirane obuke i usavršavanja domaćih kadrova od kojih su mnogi prvo usavršavani u Institutu, kasnije preuzeli naviše funkcije u pogonima klanične industrije Jugoslavije.

Oslonac isključivo na svoj sopstveni rad i zalaganje a ne na pomoć države, kao i uvek otvorena vrata i nesebična pomoć za industriju mesa naše zemlje ostali su do danas osnovni postulati rada u Institutu.

Ovakav pristup je omogućio opstanak Instituta i u najtežim vremenima rata i sankcija, a posredni uticaj se prenosio i na celu industriju mesa u Srbiji, koja se za razliku od mnogih industrijskih grana održala i danas uspešno odoleva konkurenciji iz celog sveta.

1958.godine godine prof.Savić je organizovao prvo savetovanje industrije mesa, koje se i ove godine održava po 60. jubilarni put, a 1960. godine osnovao je stručni i naučni časopis „Tehnologija mesa“, čiji je glavni i odgovorni urednik bio prve četiri godine njegovog izlaženja, Tehnologija mesa u kontinuitetu izlazi već 60 godina.

Prof. Savić je osnivač katedre za tehnologije mesa i riba na Prehrambeno-tehnološkom fakultetu Univerziteta u Zagrebu, kao i na Tehnološkom i Poljoprivrednom fakultetu u Beogradu.

Obavljao je i dužnost dekana Fakulteta veterinarske medicine u Beogradu.

Od 1967. do 1977. godine, kao ekspert FAO/UNU boravio je četiri godine u Senegalu, tri godine u Maleziji i tri godine u Brazilu (i u tim zemljama je osnovao Institute za tehnologiju mesa). Radio je na unapređenju proizvodnje i prerade mesa u ovim zemljama i širim područjima Afrike, Azije i Južne Amerike.

Kao savetnik za proizvodnju hrane i ishranu FAO/UNU i UNIDO od 1977. do 1989. godine boravio je kraće ili duže vreme u čitavom nizu zemalja u razvoju. Osnovni zadaci ovih misija bili su unapređenje proizvodnje, prerade, prometa i kontrole mesa i proizvoda od mesa, kao i usklađivanje planova i programa školovanja kadrova iz oblasti higijene i tehnologije mesa.

Pored velikog angažovanja na mnogim poljima nauke o mesu i na primeni novih dostignuća u praksi, prof. dr Isidor Savić nije stavljao u drugi plan stalno usavršavanje mladih kadrova kroz izradu specijalističkih, magistarskih i doktorskih disertacija. Bio je mentor u izradi i uspešnoj odbrani 29 doktorskih disertacija, više desetina specijalističkih i magistarskih radova.

Objavio je preko 300 naučnih radova, od kojih je više od polovine štampano u vodećim svetskim časopisima. Autor je 11 monografija i 9 knjiga i udžbenika.

Prof. dr Isidor Savić je bio među osnivačima godišnjih evropskih sastanaka naučnih radnika iz oblasti mesa, koji su od 1987. godine prerasli u Međunarodni kongres nauke o mesu i tehnologije (ICoMST). Na brojnim zasedanjima ovih evropskih sastanaka i međunarodnih kongresa on je predsedavao ili podnosio uvodna izlaganja. Tokom svog radnog veka održao je veliki broj predavanja po pozivu, na više univerziteta i istraživačkih instituta u zemlji i inostranstvu. Tvorac je većine naših propisa o veterinarsko-sanitarnoj kontroli i kontroli kvaliteta mesa i proizvoda od mesa. Kao predstavnik Jugoslavije bio je član ISO komiteta i FAO/SZO (Svetska zdravstvena organizacija) komisija, koje su kreirale preporuke za izradu međunarodnih standarda za kontrolu higijenske ispravnosti i kvaliteta mesa i proizvoda od mesa.

Bio je veoma aktivan u radu stručnih i naučnih profesionalnih organizacija. Dugogodišnji je generalni sekretar (1948–1956) Saveza društava veterinara i veterinarskih tehničara Jugoslavije, predsednik (1962–1966) i član predsedništva Saveza društava za unapređenje ishrane naroda Jugoslavije, član Izvršnog odbora Savezne sekcije za higijenu i tehnologiju namirnica životinjskog porekla, član Svetskog društva za higijenu namirnica životinjskog porekla, član predsedništva Jugoslovenskog centra za nauku o mesu itd.

Stalni saradnik naučnog časopisa „Die Fleischwirtschaft“ postao je 1965. godine, a član je Institute of Food Technologiest iz SAD od 1971. godine gde je dobio status počasnog člana.

Prof. dr Isidor Savić je 1983. godine otišao u penziju, ali je nastavio svoju stvaralačku aktivnost nesmanjenim intenzitetom. O tome govore njegovi objavljeni radovi, knjige, monografije, učešća na domaćim i međunarodnim savetovanjima, simpozijumima i seminarima.

Lično sam se divio njegovom radnom elanu i interesovanjima za dešavanja u Institutu i u industriji mesa naše zemlje. Upoređivao ih je sa iskustvima iz inostranstva i najnovijim sazanjima do kojih je svakodnevno dolazio proučavajući naučne i stručne časopise i literaturu. Do poslednjeg dana profesor se trudio da najnovija saznanja iz tehnologije nesebično prenese svima koji su imali sa njim neposredan kontakt.

Imajući u vidu ovako bogatu profesionalnu biografiju nameće s zaključak da je prof.dr Isidor Savić tokom svog dugog života i izuzetno plodne profesionalne karijere postavio temelje nove naučne discipline – Tehnologije mesa u našoj zemlji i u velikom broju zemalja u razvoju. Profesor je uložio ogroman trud da se rezultati naučnih istraživanja i nova saznanja neposredno primenjuju u industriji mesa, ali je što je možda još značajnije, tokom svog stručnog i pedagoškog rada iza sebe je ostavio brojne stručnjake koji su učestvovali u stvaranju i vođenju industrije mesa, inspekcijskih službi, stručnih i naučnih instituta, ili su postali istaknuti profesori na domaćim i stranim fakultetima.

Što se tiče zaposlenih u Institutu, ostajemo večno zahvalni profesoru Saviću koji je formirao ovu instituciju, jedinstvenu u regionu, a 64 godine uspešnog rada dokazalo je dalekovidost jedne takve odluke. Nama kao svojim naslednicima ostavio je jedinstven lični primer posvećenosti nauci o mesu i njenoj praktičnoj primeni koju je shvatao kao oruđe u stalnoj borbi za unapređenje ishrane rastućeg stanovništva naše planete.

Neka je večna slava i hvala osnivaču našeg Instituta profesoru Isidoru Saviću!

Autor teksta
Saša Prečanica,
Pomoćnik direktora za opšte i pravne poslove
Instituta za higijenu i tehnologiju mesa

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Journals:

Givens, D. I., Kliem, K. E., Gibbs, R. A. (2006). The role of meat as a source of n3 polyunsaturated fatty acids in the human diet. Meat Science, 74 (1), 209218.

Books:

Bao, Y., Fenwick, R. (2004). Phytochemicals in Health and Disease, CRC Press, Los Angeles.

Books with more chapters:

Marasas, W. F. O. (1996). Fumonisins: History, worldwide occurrence and impact. In Fumonisins in food, advances in experimental medicine and biology.Eds. L. S. Jackson, J. W. DeVries, L. B. Bullerman, Plenum Press, New York, pp. 118.

PhD and MSc thesis:

Radeka, S. (2005). Grape mash maceration and varietal aroma of Malvazija istarska wine, PhD Thesis, Faculty of Agriculture, University of Zagreb, Croatia.

Symposiums, Congresses:

Harvey, J. (1992). Changing waste protein from a waste disposal problem to a valuable feed protein source: a role for enzymes in processing offal, feathers and dead birds. Alltech's 8th Annual Symposium, Nichdasville, Kentucky, Proceedings, 109−119.

Software:

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