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Prevalence of *Arcobacter butzleri* in fecal samples, on carcasses and in retail meat of cattle, pig and poultry in Switzerland

Prävalenz von Arcobacter butzleri in Kotproben, auf Schlachtkörpern und in Fleisch aus dem Handel bei Rind, Schwein und Geflügel in der Schweiz

S. Keller, S. Räber, T. Tasara, R. Stephan

Summary

The aim of this work was to assess the prevalence of *A. butzleri* in poultry, pig and cattle production lines. A total of 1783 poultry, 568 cattle and 602 pig samples were examined. The samples were enriched in CAT broth, followed by an *A. butzleri* specific PCR. For poultry we found a prevalence of 1.4 % (15/1090) for the fecal swabs, 53.6 % (133/248) for the neck skin, 64.7 % (134/207) for the skin and 15.1 % (36/238) for the meat samples. For cattle and pigs, the prevalence for the feces was 0 % (0/210) and 21.6 % (54/250), for the surface of carcasses 19.7 % (59/300) and 19.7 % (59/300), and for retail meat 0 % (0/150) and 0 % (0/52), respectively. Whilst the role of arcobacter in human disease awaits further evaluation, a precautionary approach is advisable. More data on human isolates are needed.

Keywords: *A. butzleri*, prevalence, cattle, pig, poultry

Zusammenfassung

Ziel dieser Arbeit war es, die Häufigkeit von *A. butzleri* bei Rind, Schwein und Geflügel im Sinne eines Beitrages zum risk assessment aufzuzeigen. Dafür wurden 1783 Proben aus der Geflügelproduktionskette, 568 Proben aus der Rinder- und 602 Proben aus der Schweineproduktionskette untersucht. Die Proben wurden in CAT-Bouillon angereichert. Der Nachweis erfolgte über eine *A. butzleri* spezifische PCR. Beim Geflügel waren 1,4 % (15/1090) der bei der Anlieferung am Schlachthof erhobenen Kloakentupferproben, 53,6 % (133/248) der am Ende des Schlachtprozesses erhobenen Halshautproben sowie 64,7 % (134/207) der Hautproben bzw. 15,1 % (36/238) der Muskelproben von Fleisch aus Handelspackungen PCR-positiv. Beim Rind und Schwein waren 0 % (0/210) bzw. 21,6 % (54/250) der Kotproben, 19,7 % (59/300) bzw. 19,7 % (59/300) der Proben von Schlachttierkörpern, hingegen keine der untersuchten Fleischproben aus dem Handel PCR-positiv.

Die Bedeutung und die Rolle von *Arcobacter* spp. im Hinblick auf Durchfallserkrankungen des Menschen wird weiter diskutiert. Mehr Daten zu humanen Stämmen sind zwingend notwendig.

Schlüsselwörter: *A. butzleri*, Häufigkeit, Rind, Schwein, Geflügel

Introduction

Experts estimate that 76 million cases of human diseases, 325,000 hospital admissions and 5,000 deaths occur annually caused in the USA from the consumption of contaminated food (Mead et al., 1999). The importance of latent zoonoses has increased in recent years in view of food borne diseases: (i) the "healthy" animal represents a reservoir for specific pathogens; (ii) no pathological-anatomical changes in the carcass and its organs show the presence of these pathogens; and (iii) these pathogens may enter the food chain via weak points in the slaughtering or milking process.

Strict maintenance of good practices of slaughter hygiene in meat production is of central importance because microbiological hazards are not eliminated in the slaughtering process. Furthermore, to estimate the risks involved and to take appropriate measures, analysis of the slaughtering process should be complemented by collecting data related to the carriage of latent zoonotic pathogens by the animals.

There is evidence that livestock animals may be significant reservoirs of *Arcobacter* spp., potential latent zoonotic pathogens. Over the last few years the presence of these organisms in raw meat products as well as in surface and ground water has received increasing attention (for review see Lehner et al., 2005).

Arcobacter species are Gram-negative spiral-shaped organisms belonging to the family Campylobacteraceae that can grow microaerobically or aerobically (Vandamme and De Ley, 1991). The arcobacter organisms also have the ability to grow at 25 °C, which is a distinctive feature that differentiates *Arcobacter* species from *Campylobacter* species. Recent evidence suggests that arcobacters, especially *A. butzleri*, may be involved in human enteric diseases (Vandamme et al., 1992; Lerner et al., 1994; Engberget al., 2000; Prouzet-Mauleon et al., 2006). Moreover *A. butzleri* has also occasionally been found in cases of human extraintestinal diseases (Lau et al., 2002). However, up to now little is known about the mechanisms of pathogenicity or potential virulence factors of *Arcobacter* spp. Whilst the role of arcobacter in human diseases awaits further evaluation, a precautionary approach is advisable. In view of control measures to be used to prevent or to eliminate the hazard of *Arcobacter* spp. in food, several treatments have been evaluated for their effectiveness (Phillips, 1999). Measures aimed at reduction or eradication of arcobacter from the human food chain should be encouraged.

But at present, with the available data from literature it is not possible to assess the significance of *Arcobacter* spp. as a human pathogen or as a food- and waterborne pathogen. Besides a standard isolation procedure for *Arcobacter* spp. detection, further studies would be needed to estimate the prevalence of *Arcobacter* spp. in healthy humans and patients with diarrhoea and to illuminate the pathogenic role and potential virulence factors of *Arcobacter* spp. Furthermore, additional information on the epidemiology of these microorganisms is also necessary. Therefore, the aim of this work was to assess the situation in Switzerland in view of the prevalence of *A. butzleri* in poultry, pig and cattle production lines.

Material and methods

This study was based on sampling carried out within nine months (from October 2004 to June 2005).

Samples

Chicken samples

The chicken samples originated from a big slaughterhouse for poultry in Switzerland. Samples were collected on Monday, Tuesday and Wednesday of each week during the sampling period, and sent in a cool box to the laboratory. Microbiological examinations were carried out the next day.

Samples consisted of 1090 fecal swabs taken from the transport crates, 248 neck skins after plucking, as well as 207 skins and 238 skinless chicken breasts collected along the slaughter lines. At each sampling 30 fecal swabs were taken straight after arrival at the slaughterhouse from three different flocks. The neck skin samples were later collected from these three flocks. The skin and chicken breast samples were collected three times during a working day as pooled samples from ten workplaces.

Cattle and pig samples

The fecal and carcass samples of cattle and pigs were collected in a EU-approved slaughterhouse, where cattle and pigs were slaughtered on separate mechanized lines. Sampling was done on Mondays, Tuesdays and Wednesdays. An average of about 15 samples was taken each day. These samples were taken from as many different herds as possible. They were chilled and transported to the laboratory.

460 fecal samples were collected from the caecum immediately after slaughter and placed in sterile plastic test tubes.

508 samples originated from the surface of carcasses stored in the cooling room. They were obtained by the wet-dry double swab technique from four different sites (4 x 100 cm²) stipulated by the EU Decision 2073/2005 (cattle: neck, brisket, flank and rump; pig: back, neck, ham and belly). At each sampling site, a moistened swab (0.1 % buffered peptone water + 0.85 % sodium chloride solution) was rubbed vertically, horizontally and diagonally across the sampling site delineated by a template. Then the same sampling procedure was repeated using a dry swab. Microbiological examinations were carried out about 2 h after sampling.

Furthermore, 150 samples of minced beef meat and 52 samples from pork meat were taken at retail level.

Arcobacter detection

Enrichment step

Five g skin or meat were transferred into a sterile stomacher bag. Next, 45 ml sterile *Arcobacter* enrichment broth CAT (CM 965, Oxoid Ltd., Hampshire, UK) with supplement (SR 174, Oxoid Ltd., Hampshire, UK) was added. The mixtures were homogenized for 30 s with a stomacher blender at normal speed. After homogenization they were incubated for 24 h under aerobic conditions at +37 °C. The fecal swabs were put into sterile glass test tubes filled with 9 ml CAT broth and incubated as described above.

All surface swabs were transferred together into a sterile bag and CAT broth was added until the swabs were totally covered. After homogenization with the blender they were enriched the same way as the other samples.

PCR screening for the presence of *A. butzleri*

The enrichments were screened by PCR for the presence of *A. butzleri*. All PCR assays applied in this study were performed in a T3 thermocycler (Biometra, Göttingen, Germany). PCR reagents were purchased from

PROMEGA (Madison, Wisconsin, USA), and primers were synthesized by MICROSYNTH (Balgach, Switzerland). The 50 µl PCR mixtures contained 2 µl of template (enrichment sample suspension boiled for 11 min), 1.5 mM MgCl₂, 200 µM each dNTP, 30 pmol each primer (primer ARCO 5'-CGTATTCACCGTAGCATAGC-3' and primer BUTZ 5'-CCTGGACTTGA-CATAGTAAGAATGA-3' (target 16S rDNA; Houf et al., 2000), and 2.5 U of *Taq* DNA polymerase.

PCR involved an initial denaturation step (94 °C, 3 min) followed by 32 cycles of denaturation (94 °C, 45 s), primer annealing (61 °C, 45 s) and chain extension (72 °C, 30 s). For each run positive (ATCC 49616) and negative controls were included.

PCR products were analyzed by electrophoresis at 76 V for 30 min through a 1.8 % standard agarose (Eurobio) gel. The amplicons (401-bp fragments) were visualized by staining with ethidium bromide under UV light. A 100-bp DNA ladder was used as molecular weight marker.

For confirmation, the sequence of some amplification products purified with a Min Elute Gel Extraction Kit (Qiagen) was determined by the dideoxynucleotide triphosphate chain termination method of Sanger, with the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3100 Genetic Analyzer (Applied Bio-Systems, Foster City, USA).

Results

The *A. butzleri* prevalence data in the various samples are comparatively shown in Table 1.

Arcobacter prevalence data for poultry

A total of 1.4 % (15/1090) of the fecal samples obtained from the crates were found to be PCR positive for *A. butzleri*, as well as 53.6 % (133/248) of the neck skin samples. For the skin samples, taken from further processing stages, 64.7 % (134/207) were positive. Furthermore, 15.1 % (36/238) of the meat samples were also *A. butzleri* positive by PCR.

Arcobacter prevalence data for cattle

None (0/210) of the fecal samples from caecum were positive by PCR testing. Amongst the carcass samples obtained by the wet-dry double swap technique, 19.7 % (41/208) were PCR positive for *A. butzleri* and none of the meat samples (0/150) were detected as positive.

Arcobacter prevalence data for pigs

Of the fecal samples from the caecum, 21.6 % (54/250) were *A. butzleri* positive by PCR. Meanwhile 19.7 % (59/300) of the carcass surface samples obtained by the wet-dry double swap technique also tested PCR positive. Strikingly, there was a single day during our study with a prevalence of *A. butzleri* in the carcass samples of 68.2 % (15/22). That day, more than 1000 pigs were slaughtered prior to sampling.

Amongst the meat samples no (0/52) *A. butzleri* presence was detected.

Discussion

In poultry the low prevalence (1.4 %; 15/1090) for *A. butzleri* in feces found in our study is comparable with previous findings (Atabay and Corry, 1997; Houf et al., 2002) that arcobacters do not seem to colonize the poul-

try intestinal tract, and with a study of Van Driessche et al. (2003) who isolated arcobacters from feces of other livestock but not from poultry feces. A recent study from the UK also confirms these results by finding no *Arcobacter* spp. in any of the fecal samples examined (Gude et al., 2005).

In our study, the prevalence for *A. butzleri* on neck skin was 53.6 % (133/248). Houf et al. (2001) described a prevalence of 36 % for *A. butzleri* (16/46) on neck skin after plucking. Moreover, in our study 134 out of 207 samples (64.7 %) from carcasses at further stages of processing tested positive for *A. butzleri*. Recently, Atabay et al. (2003) also found a prevalence of 65 % by testing 75 chicken carcasses (44 fresh and 31 frozen) sold in retail markets in Turkey. Two further studies, carried out in different countries, confirm the high prevalence for *A. butzleri* on poultry carcasses (Atabay et al., 1998; Lammerding et al., 1996). The rather high prevalence for *A. butzleri* in our study in samples taken from carcasses at the slaughterhouse compared with the low prevalence found in feces, indicates that a contamination with arcobacter in the abattoir is more likely than a direct fecal contamination. This has also been described in a study by Wesley and Baetz (1999), who found that despite its distribution on poultry carcasses *Arcobacter* spp. are infrequently recovered from the caecum. The occurrence of *A. butzleri* during the slaughter process might occur by two possible means. Either, arcobacters may colonize the environment of a slaughterhouse and bring a constant level of contamination on carcasses or arcobacters may be carried into the slaughterhouse through an arcobacter positive flock in the morning and distributed on all following carcasses through contamination of the plucker or other processing machines used. Gude et al. (2005) suggest that arcobacter colonizes the abattoir environment and chicken carcasses during processing, but that the numbers appear to be low. Houf et al. (2002) supported this finding. They examined samples taken from slaughter equipment and processing machines before slaughter in the morning and after a 3-day period with no slaughter activity and revealed that *A. butzleri* was commonly present. The consistently high presence of *A. butzleri* on carcasses during processing indicates that contamination takes place at an early stage of processing and cannot be eliminated. Houf et al. (2002) found that arcobacters persist in the abattoir even after cleaning and disinfection. So, the similarly high contamination levels throughout all stages of processing might be an indication for inadequate disinfection of the abattoir or for the capacity of arcobacter to survive in an abattoir environment. Nevertheless, it appears unlikely that the high prevalence of *A. butzleri* on carcasses during slaughter is only due to the contamination of slaughter equipment. This question remains to be investigated in the future.

In poultry meat we found an arcobacter prevalence of 15.1 % (36/238), which is comparable with other findings. Kabeya et al. (2004) also found 15 % (15/100) of the meat samples to be positive for *A. butzleri* by isolating the strains after enrichment and finally confirming the isolates by PCR. A study in Belgium describes a prevalence of 24 % (6/25) (Houf et al., 2001). De Boer et al. (1996) examined 224 meat samples and isolated 54 *A. butzleri* strains (24 %) after enrichment. Three other studies carried out in different countries show higher prevalences (48 %–100 %) for *A. butzleri* in poultry meat. However, in each study mentioned, a limited number

of samples was examined. Moreover, it is difficult to directly compare results from different studies, due to varying sample sizes and investigation procedures.

In feces of cattle, no *A. butzleri* was found during our study. In a study carried out in Belgium, Van Driessche et al. (2005) isolated *Arcobacter* species from feces of healthy cattle on three unrelated farms. The *Arcobacter* prevalence on the three farms ranged from 7.5 % to 15 %. It has to be considered, though, that in this study, positive results included *A. cryaerophilus* and *A. skirrowii*, additional to *A. butzleri*. Öngör et al. (2004) found 7.0 % (14/200) of their examined fecal samples to be positive for *A. butzleri* in Turkey. Kabeya et al. (2003) describe a prevalence of 3.0 % (10/332) for *A. butzleri* in feces of cattle in Japan. However, the difficulty in direct comparison of results from different studies, due to varying sample sizes and investigation procedures is pointed out in the study of Golla et al. (2002). The objectives of that study were to determine the prevalence of *A. butzleri* in cattle from Texas and to compare the effectiveness levels of various methods in the isolation of this organism. The different methods resulted in a diversity in results.

In samples of cattle carcasses the prevalence for *A. butzleri* was 19.7 % (41/208) in our study. It is not possible to find any comparable data in literature about the prevalence of *A. butzleri* on cattle carcasses. With cattle we found the same situation as with poultry: a low prevalence in feces and a significantly higher prevalence on carcasses. It is possible that the main contamination takes place at different stages of meat processing in the abattoir.

In minced beef no samples were positive for *A. butzleri*. Kabeya et al. (2004) described a prevalence of 1.1 % (1/90) for *A. butzleri* in beef meat in Japan and Öngör et al. (2004) found 5.2 % (5/97) of the beef meat in Turkey to be positive for *A. butzleri*. Significantly higher prevalence (22.0 %) is described in beef meat in Australia (Rivas et al., 2004).

In pig feces the prevalence of *A. butzleri* was 21.6 % (54/250) in our study. Van Driessche et al. (2004) designed a study in which *Arcobacter* species were isolated from clinically healthy pigs from four unrelated farms in Belgium. From the 294 animals examined in total, 31 tested positive for *A. butzleri* after direct isolation. This is a prevalence of 10.5 % for *A. butzleri*. There were also seven samples that tested positive for *A. cryaerophilus* and three for *A. skirrowii*. In a separate study, Van Driessche et al. (2003) described the prevalence for *A. butzleri* in pig feces. In that study a prevalence of 22.0% (18/82) was found. In Japan, Kabeya et al. (2003) determined a prevalence of 6.0 % (15/250) for *A. butzleri* in pig feces.

Amongst the pig carcasses 19.7 % (59/300) tested positive for *A. butzleri* in our study. It is not possible to find any comparable data in literature on prevalence for *A. butzleri* on pig carcasses.

In pork meat, comparable to cattle meat, there were no samples testing positive for *A. butzleri* in our study. In Japan, 7 % (7/100) of pork meat was contaminated with *Arcobacter* spp. (Kabeya et al., 2004). 4 % (4/100) of the samples were positive for *A. butzleri*, 3 % (3/100) for *A. cryaerophilus* and 0 % (0/100) for *A. skirrowii*. Rivas et al. (2004) not only investigated beef, they also investigated pork in the study mentioned above. The prevalence for *A. butzleri* in pork was 29.0 % (6/21).

In Switzerland, this is the first study reporting prevalence data for *A. butzleri* in poultry, cattle and pig meat production lines. The prevalence is higher in poultry meat than in red meat production lines. This is comparable to data from literature.

Further studies are needed to estimate the prevalence of *Arcobacter* spp. in healthy humans and patients with diarrhoea and to illuminate the pathogenic role and potential virulence factors of *Arcobacter* spp. Whilst the role of *Arcobacter* in human disease awaits further evaluation, a precautionary approach is advisable.

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TABLE 1: Prevalence of *A. butzleri* in poultry, cattle and pig samples.

	feces	carcass	meat
Poultry	1.4 % (15/1090)	53.6 % (133/248) ¹ 64.7 % (134/207) ²	15.1 % (36/238)
Cattle	0.0 % (0/210)	19.7 % (41/208)	0.0 % (0/150)
Pig	21.6 % (54/250)	19.7 % (59/300)	0.0 % (0/52)

¹ neck skin samples taken after plucking

² skin samples taken from further processing stages of carcasses

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Effect of different packing methods on the shelf life of marinated rainbow trout

Einfluss verschiedener Verpackungsverfahren auf die Haltbarkeit von marinierten Regenbogenforellen

Özkan Özden, Nuray Erkan

Summary

The effects of packing in oil and under vacuum on sensory, chemical and microbiological changes in marinated rainbow trout stored at chill temperatures ($+4 \pm 1$ °C) were studied. The quality assessment of marinated trout stored in oil and under vacuum was performed by monitoring sensory quality, total volatile basic nitrogen (TVB-N), peroxide value (PV) and thiobarbituric acid (TBA), psychrotrophic bacteria count and mesophilic bacteria count during storage. The observed organoleptic shelf-life of rainbow trout was found to be 105 days in oil and 90 days in vacuum packed samples. At the beginning of storage, the TVB-N value was 6.78 mg/100 g for marinated trout packed under oil and 7.35 mg/100 g for the vacuum packed samples. The TVB-N values increased to 12.08 mg/100 g and 11.98 mg/100 g by the end of the storage period for marinated rainbow trout packed in vacuum and in oil, respectively. No significant differences ($p > 0.05$) between the two groups of samples were found in TVB-N concentrations during the storage period. The fat degradation pattern (peroxide value and thiobarbituric acid content) was found to be similar for all storage conditions, showing a continuous increase reaching critical levels after ca. 60 days of storage for both packing types. Psychrotrophic bacteria count and mesophilic bacteria count of marinated trout were < 10 log CFU/g, throughout the storage period. The results of this study indicate that the shelf-life of marinated rainbow trout stored in cold storage ($+4$ °C), as determined by overall acceptability sensory scores, is 105 days for oil and 90 days for vacuum packaged samples.

Keywords: rainbow trout, marination, shelf life, quality changes, packing type, cold storage

Zusammenfassung

In der vorliegenden Studie wurde der Einfluss verschiedener Verpackungsarten (in Öl bzw. unter Vakuum) auf die Veränderung sensorischer, chemischer und mikrobiologischer Eigenschaften marinierter Regenbogenforellen während der Kühlung ($+4$ °C) untersucht. Die Qualität der marinierten Forellen wurde im Verlauf der Lagerung anhand folgender Parameter beurteilt: sensorische Untersuchung, Gesamtgehalt an flüchtigem Basenstickstoff (TVB-N), Peroxidzahl, Thiobarbitursäure (TBA), Keimzahl psychrotropher Bakterien, mesophile Gesamtkeimzahl. Als organoleptisch feststellbare Haltbarkeit wurden 105 Tage für die in Öl gelagerten Proben und 90 Tage für die unter Vakuum gelagerten Proben ermittelt. Zu Lagerungsbeginn betrug der TVB-N-Wert 6,78 mg/100 g bei den in Öl verpackten marinierten Forellen und 7,35 mg/100 g bei den vakuumverpackten Proben. Die TVB-N-Werte stiegen bis zum Ende der Lagerung auf 12,08 mg/100 g bzw. 11,98 mg/100 g bei den vakuumverpackten bzw. in Öl gelagerten Proben an. Im Verlauf der Lagerung wurden in Bezug auf TVB-N keine signifikanten Unterschiede ($p > 0,05$) zwischen den beiden Lagerungsarten festgestellt. Ebenso zeigte sich ein ähnlicher Verlauf der Indikatoren für den Fettabbau (Peroxidzahl, Thiobarbitursäure) bei beiden Lagerungsbedingungen, wobei die Werte kontinuierlich anstiegen und unabhängig von der Verpackungsart nach ca. 60 Tagen kritische Werte erreichten. Sowohl die Keimzahlen der psychrotrophen Bakterien

als auch die mesophile Gesamtkeimzahl der marinierten Forellen lagen während der gesamten Lagerung unterhalb von $10 \log \text{KbE/g}$. Die Ergebnisse dieser Studie zeigten, dass die anhand der sensorischen Gesamtbewertung ermittelte Haltbarkeit von marinierten, bei $+4^\circ\text{C}$ gelagerten Regenbogenforellen 105 Tage für in Öl gelagerte Forellen und 90 Tage für vakuumverpackte Forellen beträgt.

Schlüsselwörter: Regenbogenforellen, Marinieren, Haltbarkeit, Qualität, Verpackung, Kühlung

Introduction

Marinades are fish products consisting of fresh, frozen or salted fish or portions of fish processed by treatment with edible acids and salt in brines, sauces, creams or oil. Marinating is preserving fish by means of sodium chloride and acetic acid solutions. Even today the herring is the basic raw material for marinades, although they are also made from sprat, sardine and gadoids. The highest quality product is obtained from fresh fish (McLay, 1972). Aquaculture of rainbow trout (*Oncorhynchus mykiss*) has major economic importance in Turkey and farming of rainbow trout is a fast growing aquaculture venture in Turkey and many European countries. Rainbow trout is one of the most widely farmed species with a total production in Europe of 320074 tons in 2001 (FAO, 2003). Rainbow trout has an important commercial value both for consumption in Turkey and for exportation. This fish has white flesh and a medium fat content. These attributes have made several trout popular and high-valued around the world. In Turkey, they are generally marketed fresh, chilled, frozen or smoked. Dressing or splitting the fish, and processing by curing, burger, döner production and/or marinating will enable the fish industry to put a number of desirable, new products on the market. The shelf life of fish products can be extended, depending on raw materials, temperature and packaging materials (Erkan, 2003). In this study, the effects of different packing technologies on sensory, chemical and microbiological changes and shelf life in marinated rainbow trout stored at chill temperature ($+4 \pm 1^\circ\text{C}$) were studied.

Materials and Methods

Materials

Fresh rainbow trout used in this study were obtained directly from a fish market in Istanbul. A total of 16 kg commercial size fish (mean weight 260–295 g, mean length 25–28 cm) was transported to the laboratory in polystyrene boxes with ice within 1/2 h. The fishes were individually beheaded, gutted, filleted and washed. The fillets were dipped in 3 % acetic acid, 10 % salt marination solutions at a ratio of 1 : 1.5 (fish: marination solution, w/w) for 48 h. This marination process was performed at $+4 \pm 1^\circ\text{C}$. After marination, the fish were drained at ambient temperature ($+18 \pm 1^\circ\text{C}$) for 15 min. The marinated fishes were divided into two lots. Two hundred grams of marinated rainbow trout fillets were packed into glass jars of 400 ml capacity with 125 ml sunflower oil. Half of the marinated rainbow trout fillets (200 g) were placed in high-barrier plastic film bags (O_2 transmission rate 6.89 ml/m^2 ; CO_2 5.42 ml/m^2 ;

N_2 2.48 ml/m^2 at $+4^\circ\text{C}$; vapour permeability 7.86 ml/m^2 at $37.8 \pm 1^\circ\text{C}$, $90 \pm 2\% \text{ RH g/m}^2 \cdot \text{days} \cdot \text{atm}$) and packaged under vacuum for 20 s at 760 mm Hg. Plastic film bags were obtained from UPM-Kymmene, Walki-Pack, Plastic Films Factory (Valkeakoski, Finland). A Röscher-matic model vacuum packaging machine (Röscher-matic AG, Germany) was used. All analyses were performed with fresh fish samples prior to and immediately after marination at the beginning of storage. Samples were stored at $+4 \pm 1^\circ\text{C}$, all analytical determinations were done in triplicate at days 30, 60, 90 and 120 of storage.

Methods

Sensory Analyses

Five judges assessed the sensory properties of samples and a hedonic scale (Filsinger, 1987) was used for sensory analyses. General appearance, odour, colour, taste and texture were used as criteria for acceptability. The sensory assessment scale runs from 0 to 100. Scores 86 or greater are for the best quality, 66–85 for very good quality, 46–65 for good quality, 26–45 for regular quality and a score of 25 or less corresponds to spoiled or overmatured products. Marinated rainbow trout was considered unacceptable for consumption when the organoleptic score was below 35.

Chemical Analyses

pH: pH was determined at room temperature on homogenates of marinated rainbow trout muscle in distilled water (5 g/5 ml). The pH value was measured using a Microprocessor-pH-Meter (Type No: WTW 537 pH-Meter, Weilheim, Germany) (Manthey et al., 1988).

Total volatile basic nitrogen (TVB-N): Total volatile basic nitrogen was determined according to the method described by Antonacopoulos and Vyncke (1989). For total volatile basic nitrogen (TVB-N), fish muscle (10 g) was homogenized with 6 % perchloric acid (90 ml) for 1 min in an Ultra-Turrax (IKA T 25 Basic, Staufen, Germany). The homogenates were filtered through a filter paper (Whatman no 1) and filtrates were alkalized with NaOH (20 %) before distillation. Duplicate filtrates were then distilled in a Velp Marka model UDK 140 (Milano, Italy) apparatus. The distillates were titrated with 0.01 N HCl.

Determination of lipid oxidation: The distillation method of Tarladgis et al. (1960) as modified by Witte et al. (1970) was used to determine the degree of lipid oxidation in meat samples. Lipid oxidation was measured using thiobarbituric acid (TBA) values, which are expressed as mg malonaldehyde/kg fish lipid. Malonaldehyde is a breakdown product of lipid oxidation. Peroxide value (PV) was determined as mmol oxygen/kg lipid and by a

titrimetric Wheeler method (Matissek et al., 1992) after fat extraction (Özden et al., 2001).

Microbiological analyses

Samples (25 g) were mixed with 225 ml peptone water (Merck, Kat No: 107228) in a Stomacher (Stomacher, IUL Instruments, Barcelona, Spain). Further serial dilutions were made in tubes before plating. The following media and incubations were used: for mesophilic aerobic plate count PCA agar (PCA, Merck, Kat No: 105463), +37 °C, 1–2 days; for psychrotrophic bacteria PCA agar (PCA, Merck, Kat No: 105463), +7 °C, 10 days (Baumgart, 1986). The results are expressed as a logarithm of colony forming units (log CFU) per gram of sample.

Statistical Analysis

Results are expressed as means and standard deviations. Data were subjected to analyses of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences between means at the 5 % significance level. Correlation coefficients between sensory scores and oxidation products were determined by Pearson test. Statistical analyses of data were calculated by Microsoft Excel XP 2003 (Sümbüloğlu and Sümbüloğlu, 2002).

Results and Discussion

The results of the sensory analyses are presented in Figure 1. The initial sensory scores of fresh rainbow trout of 75 points before and 82 points after marination indicate good quality at the beginning of the storage period. The limit of acceptability of sensory data was reached after 90 days for the vacuum packed rainbow trout samples and after ca. 105 days for the oil packed samples. These results are in agreement with the findings of Özden and Baygar (2003) for marinated chub mackerel, horse mackerel, sardine and anchovy packaged in jars with vegetable oil and vacuum packed in polyethylene bags, then stored at $+4 \pm 1$ °C. The shelf life of marinated salmon in oil stored in refrigerator was found to be 22 weeks at $+2/+8$ °C (Kietzmann and Priebe, 1969). Karl and Schreiber (1990) reported that marinated fish stored in low concentration acetic acid solution was still acceptable after 35 days of storage. The shelf life of chilled marinated anchovy packed in oil was found to be 120 days at +4 °C (Aksu et al., 1997). Similar results (90 days storage at +4 °C) have been reported by Erkan et al. (2000) for marinated rainbow trout under modified atmosphere packing conditions. Arik et al. (2001) showed that marinated fish was fit for consumption for three to four weeks when stored at +7 °C and for four to six weeks at +2 °C. Gökoğlu et al. (2004) reported a shelf life of 120 days for marinated sardine stored at +4 °C. Similar results have been obtained for sardine marinades in tomato sauce by Kiliç and Çakli (2005).

Chemical tests (TVB-N, TBA, PV, and pH) were used for quality assessment of fish products. Decomposition of fish is a progressive proteolysis of muscle tissue brought about primarily by the action of microorganisms and, to a lesser extent, by autolytic enzymes. Sensory evaluation has long been recognized as important in determining the quality of sea food, but the establishment of effective organoleptic panels can be costly and the results are ultimately limited by the evaluator's subjectivity. Various chemical tests are also available to measure seafood wholesomeness. Chemical markers such as indole in shrimp, histamines in tuna and other fish belonging to the family *Scombridae*, and trimethylamine-nitrogen for various marine fish such as cod and mackerel are useful and specific indicators of seafood quality. Among the more general chemical markers of seafood spoilage are: total volatile bases, indicative of protein breakdown, volatile amines (trimethylamine) and thiobarbituric acid as indicators of lipid breakdown and volatile acid number, a measure of volatile acids resulting from bacterial proteolysis (McCarthy et al., 1989; Sikorski et al., 1990; Tülsner, 1994; Erkan, 2003).

The changes in the pH of the samples are shown in Figure 2. The pH of very fresh raw fish was initially approximately 6.31 and then changed during the maturation process to 4.23 after 2 days. At the borderline of acceptability the pH level was 4.29 (90 days) and 4.44 (90 days), respectively, in the vacuum and oil packaged samples. Samples packaged in oil always showed higher pH values than vacuum packaged samples. According to statistical analysis the differences between vacuum and oil packaged trout marinades were found to be statistically significant ($p < 0.05$) for pH value. For vacuum packaged fish it is well known that carbon dioxide can be absorbed into the fish muscle surface, acidifying it via the formation of carbonic acid. Aksu et al. (1997) reported that pH values in anchovy marinated with 2 % and 4 % acetic acid increased from

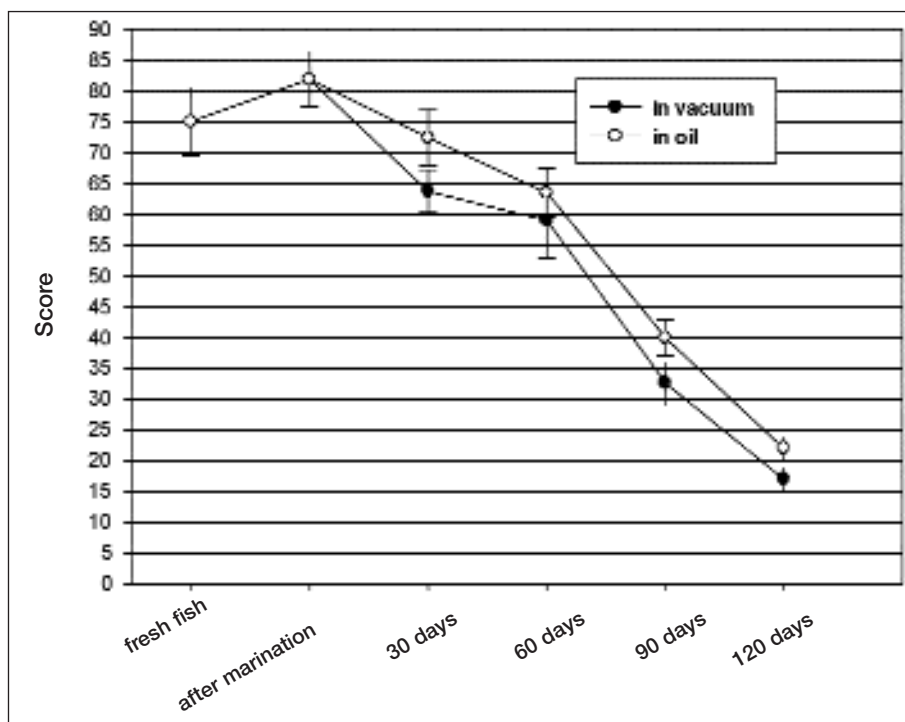


FIGURE 1: Sensory score changes of marinated rainbow trout stored in oil and vacuum packaged at $+4 \pm 1$ °C.

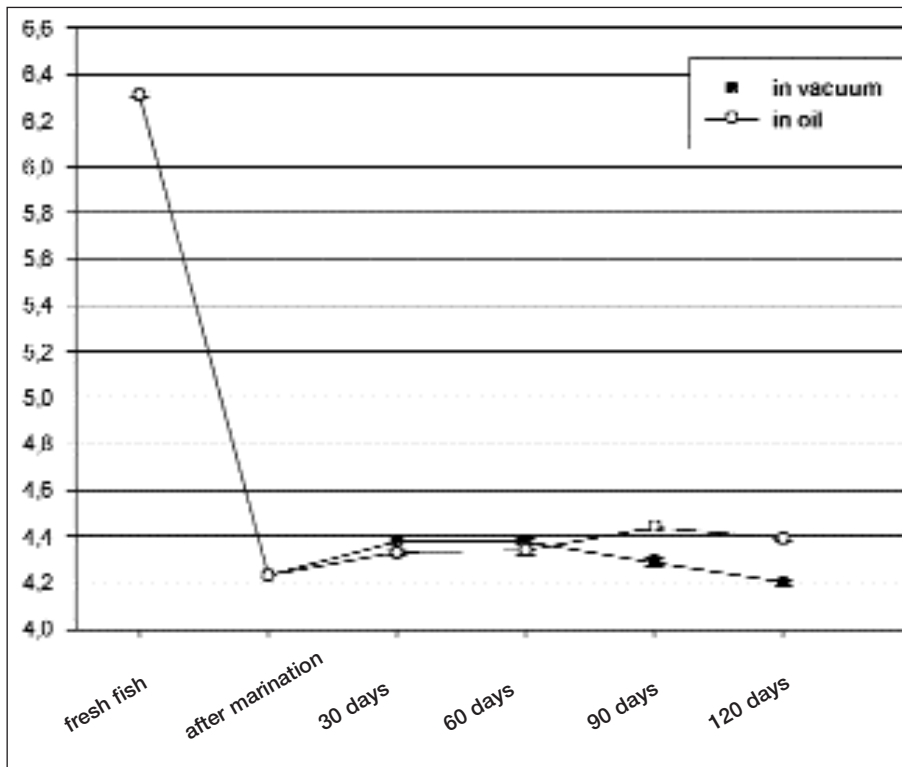


FIGURE 2: Changes in pH value of marinated rainbow trout stored in oil and vacuum packaged at $+4 \pm 1$ °C.

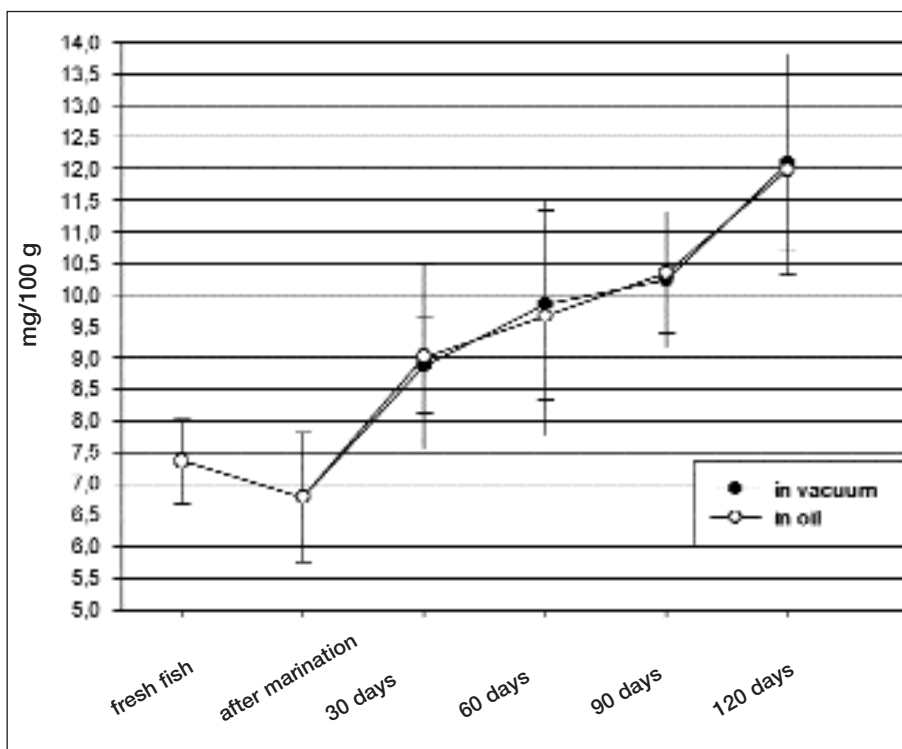


FIGURE 3: TVB-N value changes of marinated rainbow trout stored in oil and vacuum packaged at $+4 \pm 1$ °C.

4.25 and 4.18 to 4.53 and 4.31, respectively. Poligne and Colignan (2000) found that the pH levels of anchovies pickled with acetic acid increased from 3.90 to 4.21 after 20 days of storage and then remained constant until the end of the storage. During the storage period pH values increased according to storage time, but the pH value is not a criterion of spoilage. It has to be supported by other chemical and sensory analyses (Ludorff and Meyer, 1973; Schormüller, 1968). Marinades have a low pH due to acetic acid content. During the storage of marinades, heterofermentative lactic acid bacteria can grow and cause the amino acids to degrade. Thus, the formation of carbon dioxide and other decarboxylation products is observed. Due to these degradation products the pH of marinade increases (Shenderyuk and Bykowski, 1990). Similar results have been obtained in marinated fish packaged in vacuum (4.42) and oil (4.31) (Arik et al., 2001).

Erkan et al. (2000) reported similar pH values of 4.33 and 4.50–4.40 for fatty fish marinated species, rainbow trout packed in air and under modified atmosphere (MAP). Final muscle pH of marinated fish ranged from 4.0 to 4.5 (Ludorff and Meyer, 1973). Tülsner (1994) reported pH 4.5 to be a critical value for safety (especially *Clostridium botulinum*) of marinated fish.

The changes in TVB-N levels throughout the storage of refrigerated marinated rainbow trout packaged in vacuum and oil are presented in Figure 3. The TVB-N contents in fresh fish and marinated trout were 7.35 mg/100 g and 6.78 mg/100 g, which increased during cold storage to 12.08 mg/100 g and 11.98 mg/100 g at the end of storage in vacuum and oil packed samples, respectively. Ludorff and Meyer (1973) reported an acceptability level of 35 mg TVB-N per 100 g in sea fish. A rejection limit of 25 mg/100 g has been proposed for fresh water fish (Giménez et al., 2002). Total volatile basic nitrogen content of fish is in general an indicator of the freshness. Yet, based on the results obtained in this study, TVB-N is a poor indicator of marinated fish freshness, as also proposed by

Arik et al. (2001). In one report the TVB-N value of 8.31 mg/100 g in anchovy marinated using 2 % acetic acid increased to 15.18 mg/100 g, the TVB-N value of 7.79 mg/100 g in anchovy marinated using 4 % acetic acid increased to 13.48 mg/100 g and the TVB-N value of 7.41 mg/100 g in anchovy marinated using 6 % acetic acid increased to 12.34 mg/100 g after storage of 150 days (Aksu et al., 1997). Metin et al. (2000) reported that modified atmosphere packaged marinated rainbow trout reached 9.33 and 10.18 mg TVB-N/100 g muscle after 90 days cold storage. These findings are very similar to our observations concerning trout marinade.

Lipid oxidation is a major quality problem. It leads to the development of off-odours and off-flavours in edible oils and fat-containing foods, called oxidative rancidity (Ashie et al., 1996). Fatty fish muscles are rich in polyunsaturated fatty acids which are susceptible to peroxidation. The fatty acid peroxides are free radicals which can attack another lipid molecule, resulting in peroxide and a new free radical (Hamre et al., 2003). The primary product of lipid oxidation is fatty acid hydroperoxide, measured as peroxide value. Peroxides are instable compounds and they break down to aldehydes, ketones and alcohols which are volatile products causing off-flavour in products. Peroxide and thio-barbituric acid values are the major chemical indices of oxidative rancidity (Kolokowska and Deutry, 1983; Lubis and Buckle, 1990; Pérez-Villarreal and Howgate, 1991; Ashie et al., 1996; Lie, 2001). Peroxide values increased with time (Fig. 4) in the present study. At the start of the experiments the levels in fresh fish were low (2.19 mmol O₂/kg) and similar to the ones reported in other species (Özden and Gököglü, 1997). At the beginning of storage, peroxide values of marinated trout were determined as 3.9 mmol O₂/kg. In both types of packing, peroxide values increased according to the duration of storage. At the storage period of 90 days, peroxide values reached 10.85 mmol O₂/kg and 10.23 mmol O₂/kg for trout marinades packed in vacuum and oil, respectively. A highly significant correlation was found between sensory data and peroxide value (in vacuum, $r = -0.9273$; in oil, $r = -0.9040$). Peroxide levels of vacuum and oil

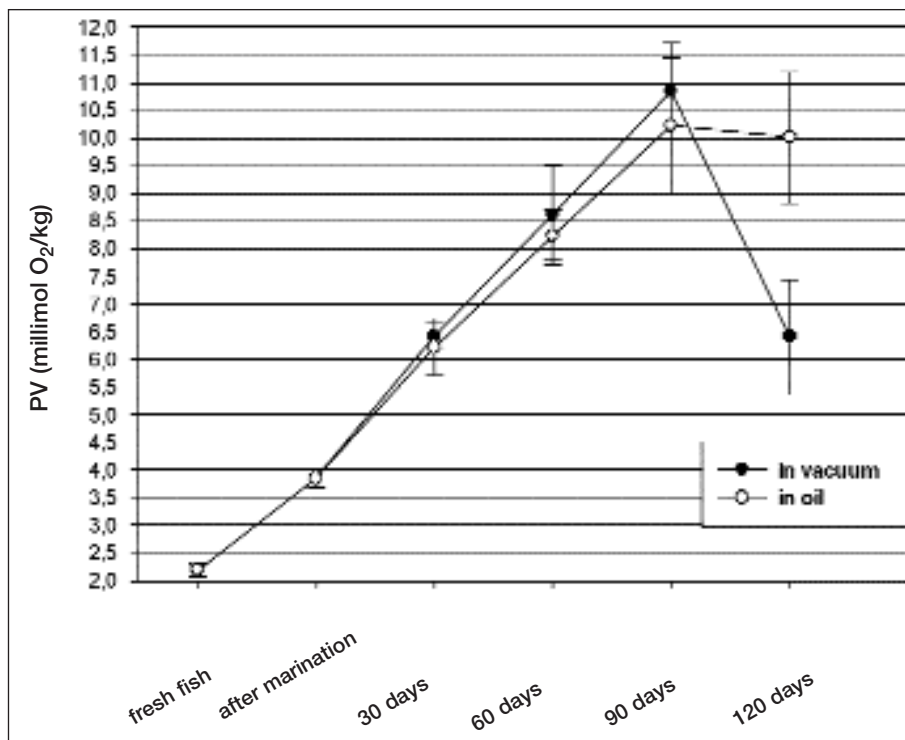


FIGURE 4: Peroxide value changes of marinated rainbow trout stored in oil and vacuum packaged at $+4 \pm 1$ °C.

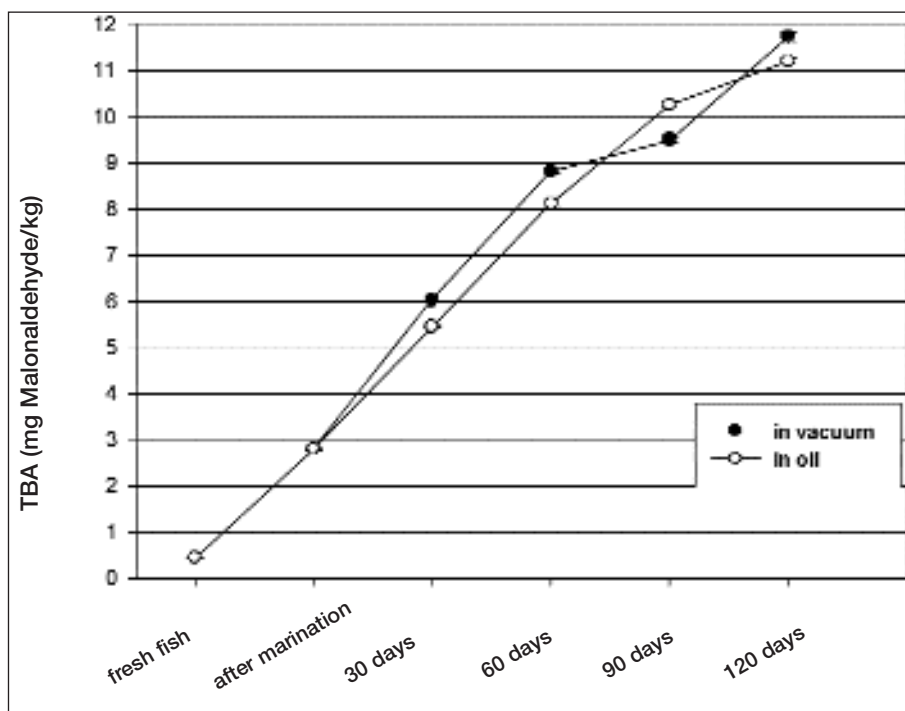


FIGURE 5: TBA value changes of marinated rainbow trout stored in oil and vacuum packaged at $+4 \pm 1$ °C.

packed trout marinades decreased from 10.85 mmol O₂/kg to 6.4 mmol O₂/kg and from 10.23 mmol O₂/kg to 10 mmol O₂/kg, respectively at the end of the storage period. The decrease in per-oxide values of marinated trout in vacuum and oil after 90 days of storage may represent the breakdown of peroxide to tertiary degradation products. Ludorff and Meyer (1973) proposed the following peroxide value scale as a basis for determining

the freshness of fish oil: PV = 0–2 very good, PV = 2–5 good, PV = 5–8 acceptable, PV = 8–10 spoiled. Özden and Gökoğlu (1997) and Verma et al. (1995) reported 8 mmol O₂/kg of fish oil as the maximum permissible limit of the peroxide value.

TBA values of marinated fish are shown in Figure 5. At the beginning of the storage period, TBA values of fresh rainbow trout and marinated fish were determined as 0.45 mg malonaldehyde/kg and 2.8 mg malonaldehyde/kg. TBA values in chilled samples increased gradually with time. After 90 days, TBA levels were determined as 9.5 mg malonaldehyde/kg and 10.26 mg malonaldehyde/kg in vacuum and oil packaged samples, respectively. A good correlation was found to exist with the sensory score (in vacuum, $r = -0.9365$; in oil, $r = -0.9444$). The initial TBA values in sardine marinades (4.47 mg malonaldehyde/kg) and changes up to 8.21 mg malonaldehyde/kg during storage are comparable to the ones observed in the marinated fish of the present study (Kilinc and Çakli, 2005). The concentration of TBA in good quality frozen and chilled fish or fish stored on ice is typically between 5 and 8 mg malonaldehyde/kg, whereas levels of 8 mg malonaldehyde/kg flesh are generally regarded as the limit of acceptability for most fish species, such as sardine and salmon (Schormüller, 1968; Mendenhall, 1972; Andersen et al., 1990; Beltran and Moral, 1990; Nunes et al., 1992). Based on the the limits of acceptability of the peroxide and thiobarbituric acid values, the shelf life of both kinds of marinated trout was approximately 60 days at $+4 \pm 1$ °C.

The high peroxide and thiobarbituric values showed that, although the fatty fish species (rainbow trout) were vacuum packed, they were oxidized during the preliminary processes and marination. The peroxide and thiobarbituric values may be used as chemical quality indices because oxidation is inevitable during processing.

The initial values of psychrotrophic bacteria count and mesophilic bacteria count were 3 log CFU/g and 3.4 log CFU/g. In vacuum and in oil packed trout marinades < 10 CFU/g (1 log CFU/g) psychrotrophic bacteria and < 10 CFU/g mesophilic bacteria were detected during the storage period of 4 months. Aksu et al. (1997) determined the effect of different salt and acetic acid concentrations on the shelf life of marinated anchovy. Dokuzlu (1997) studied the influence of various salt/acetic acid concentrations on microbiological and sensory properties of marinated anchovy stored at +4 °C. Arik et al. (2002) determined the shelf life of marinated fresh water fish, also finding < 3 log CFU/g psychrotrophic bacteria and mesophilic bacteria in marinated samples.

Conclusions

The effects of oil and vacuum packing on sensory, chemical and microbiological changes in marinated rainbow trout stored at chill temperature (+4 °C) were studied. A quality assessment was performed by monitoring sensory quality, total volatile basic nitrogen, peroxide value and thiobarbituric acid, psychrotrophic bacteria count and mesophilic bacteria count. After storage of 2 months both types of samples were found consumable according to the results of peroxide value and thiobarbituric values. The results of this study indicate that the shelf life of vacuum and oil packaged marinated trout stored at +4 °C as determined by sensory data is 90 and 105 days, respectively. This clearly shows that packing in oil affects the shelf life of trout marinades.

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Prevalence of *Escherichia coli* O157:H7 in various foods in Turkey: a study on the use of the IMS technique

Prävalenz von Escherichia coli O157:H7 in verschiedenen Lebensmitteln in der Türkei: Studie zum Einsatz der IMS-Methode

B. Mercanoglu, S. A. Aytac

Summary

In this study, the specificity and the sensitivity of the immunomagnetic separation (IMS) technique were determined in a model system. The efficiency of the conventional cultural method (USDA) with and without IMS was compared both in spiked and naturally contaminated foods. Both methods were used to isolate *Escherichia coli* O157:H7 from 111 food samples sold in markets of Ankara, Turkey. In an evaluation using 8 spiked food samples, the conventional cultural method with IMS showed no difference in performance. The result of the analysis of 103 naturally contaminated foods showed that the conventional cultural method with IMS enabled the detection of 3 (2.9%) EHEC positive samples whereas the conventional cultural method without IMS detected only one (0.9%). Although poultry products are not believed to be a potential source of EHEC, we did isolate this pathogen in 2 of 57 poultry products by conventional cultural method with IMS.

Keywords: *Escherichia coli* O157:H7, EHEC, immunomagnetic separation

Zusammenfassung

In dieser Studie wurden die Spezifität und die Sensitivität der Methode der Immunomagnetischen Separation (IMS) in einem Modellsystem untersucht. Es wurde ein Vergleich der Leistungsfähigkeit der konventionellen kulturellen Methode (USDA) mit und ohne IMS angestellt, sowohl in beimpften als auch in natürlich kontaminierten Lebensmitteln. Beide Verfahren wurden eingesetzt, um *Escherichia coli* O157:H7 aus 111 Lebensmittelproben, die von Märkten in Ankara stammten, zu isolieren. Bei der Untersuchung von 8 beimpften Proben zeigte die konventionelle kulturelle Methode keine Unterschiede in der Leistungsfähigkeit. Als Ergebnis der Untersuchung von 103 natürlich kontaminierten Proben ergab sich, dass die Methode mit IMS den Nachweis von 3 (2,9%) EHEC-positiven Proben ermöglichte, während die konventionelle kulturelle Methode ohne IMS lediglich eine positive Probe (0,9%) nachwies. Obwohl Geflügelprodukte nicht als potentielle Quelle für EHEC angesehen werden, konnten wir diesen pathogenen Erreger aus 2 von 57 untersuchten Geflügelerzeugnissen mittels der kulturellen Methode mit IMS nachweisen.

Keywords: *Escherichia coli* O157:H7, EHEC, Immunomagnetische Separation

Introduction

Escherichia (E.) coli, member of the family *Enterobacteriaceae*, is a normal inhabitant of the gut of many animals and human beings, and it is therefore used as an indicator of faecal contamination. The importance of *E. coli* as a cause of diarrheal disease has been recognized over the past 25 years. Detailed comparisons of the properties of diarrheagenic strains have resulted in the recognition of several categories of *E. coli* associated with disease. According to this classification of *E. coli*, enterohaemorrhagic *E. coli* (*E. coli* O157:H7) is a subset of verotoxin producing *E. coli* (VTEC) (Levine, 1987; Willshaw et al., 2000).

E. coli O157:H7 has been identified as the cause of numerous outbreaks and sporadic cases of foodborne illness since its recognition as a pathogen in 1982. Infection is initiated by consumption of raw or undercooked contaminated food of animal origin or contaminated water (Feng, 1995). In most cases, the illnesses have been self-limiting; but complications such as haemorrhagic colitis (HC), haemolytic-uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) have occurred, sometimes leading to death (Fratamico and Crawford, 1999).

In food microbiology, various conventional cultural methods have been improved and applied for the isolation of *E. coli* O157:H7 from foods. These methods have four major stages: (1) pre-enrichment stage, (2) selective enrichment stage, (3) isolation stage and (4) confirmation stage (Patel, 1994). Since conventional cultural methods are labour-intensive and require 4 days to presumptively identify *E. coli* O157:H7 and up to 7 days to confirm the identity of the isolate, rapid isolation and identification procedures of *E. coli* O157:H7 in food enable to increase the chances of preventing diseases caused by this pathogen (Fratamico and Crawford, 1999). One such improvement involves the use of polystyrene particles called Dynabeads®, that are uniform, superparamagnetic, monodisperse polymer particles with a diameter of 4.5 µm (Anonymous, 2000). These beads consist of fine granules of iron oxide making them superparamagnetic (Molla et al., 1996). The use of these beads together with specific antibodies enables the sequestration of target bacteria from the contaminating microflora and concentration of the bacteria into smaller volumes for further testing (Safarik et al., 1995; Fratamico and Crawford, 1999). Thus, the term immunomagnetic separation (IMS) is used to describe this technique which is based on immunology, molecular biology and microbiology (Patel, 1994; Fratamico and Crawford, 1999).

Due to these properties, IMS has been used in many studies (Safarikova and Safarik, 2001; Mercanoglu and Aytac, 2002). By using IMS, not only the analysis time can be reduced, but also both sensitivity and specificity of the cultural method can be increased.

In this study, the specificity and the sensitivity of the IMS technique were determined in a model system. The efficiency of the conventional cultural method, which is recommended by United States Department of Agriculture (USDA), with and without IMS was compared using spiked food. We also believed it would be useful to test retail meat and meat products, milk and milk products, poultry and poultry products, vegetables and delicatessen in Ankara, Turkey for the presence of *E. coli* O157:H7 by using the conventional cultural isolation method with and without IMS in order to establish

if there were significant levels of contamination that could threaten the public health.

Materials and Method

Bacterial strains

E. coli O157:H7 was obtained from Prof. M.P. Doyle, Georgia University, USA and *E. coli* was provided by Refik Saydam Hygiene Center, Turkey.

Food samples

One hundred and eleven (111) foodstuffs were purchased randomly from the markets in Ankara, Turkey. Eight of these were spiked with *E. coli* O157:H7 and used in an artificial inoculation study and the rest were analysed to detect the natural contamination with *E. coli* O157.

Conventional cultural method

The method recommended by United States Department of Agriculture (USDA) was used as the conventional cultural method. In this method, homogenized samples were inoculated in mTS (modified Tryptic Soy) + novobiocin broth (Merck, Germany) and incubated at +37 °C for 24 hours. After incubation, enriched broths and their appropriate dilutions (10⁻¹ to 10⁻⁹) were streaked onto CT-SMAC (Cefixime Tellurite-Sorbitol MacConkey) agar (Merck, Germany). Plates were incubated at +37 °C for 18–24 hours. For confirmation, 5 typical colonies were picked from each selective medium and inoculated onto TSA (Tryptic Soy Agar) (Merck, Germany) in order to use in the biochemical and serological tests.

Immunomagnetic separation (IMS) technique

In the IMS technique, Dynabeads® anti-*E. coli* O157 (Dynal, Norway) were used to concentrate *E. coli* O157 from enriched broths. 20 µl of bead suspension was incubated with 1 ml of enriched broth at room temperature for 30 minutes with continuous mixing (Dynal MX3; Dynal, Norway) so that the specific antibodies coated onto the beads would bind the target bacteria. The bead-bacteria complexes were subsequently separated using a magnetic particle concentrator (Dynal MPC-M®; Dynal, Norway). Afterwards, 1 ml of washing buffer (0.15 M NaCl, 0.01 M Na-phosphate buffer, 0.05 % Tween 20; pH 7.4) was added to resuspend the bead-bacteria complexes. The washing step was repeated twice. Then, 100 µl of washing buffer was added to resuspend the beads again. Next, the resuspended bead-bacteria complexes were streaked onto CT-SMAC agar and the plates were incubated at +37 °C for 18–24 hours. At the end of the incubation period, presumptive colonies were examined both biochemically and serologically as described in the conventional cultural isolation method.

Determinatin of specificity

In order to determine the specificity of Dynabeads® anti-*E. coli* O157, *E. coli* O157:H7, *E. coli* and *E. coli* O157:H7 + *E. coli* cultures were inoculated into 10 ml of TSB (Tryptic Soy Broth) (Merck, Germany) and incubated at +37 °C for 24 hours. After the incubation, the IMS technique was applied.

Determination of recovery sensitivity

In order to determine the recovery sensitivity of Dynabeads® anti-*E. coli* O157, *E. coli* O157:H7 and *E. coli* cultures were inoculated into 10 ml of TSB and incubated at +37 °C for 24 hours. Then, the appropriate dilutions (10^{-1} to 10^{-9}) of these overnight enrichment cultures were directly plated with and without an IMS step on both TSA and CT-SMAC agar. Finally, bacterial counts from each dilution were determined.

Isolation of *E. coli* O157:H7 from spiked food samples

Eight foodstuffs were spiked with approximately 102 cfu/ml or g of *E. coli* O157:H7 culture prior to incubation. The inoculated culture was prepared by allowing the growth of this pathogen in TSB at +37 °C for 24 hours and appropriately diluting this culture in a sterile saline solution. 25 gram of each spiked foodstuff was weighted into a filtered sterile Stomacher plastic bag with 225 ml mTS+novobiocin broth. Next, these suspensions were mechanically shaken for 1 minute in a Stomacher (Seeward 400, England). All suspensions were incubated at +37 °C for 24 hours and used in the conventional cultural isolation method with and without IMS as described previously. The same procedure was also applied without using the enrichment stage. Finally, confirmation of the suspected *E. coli* O157:H7 colonies was done.

Isolation of *E. coli* O157:H7 from naturally contaminated food samples

Of each of the 103 food samples, 25 g was put into a filtered sterile Stomacher plastic bag with 225 ml mTS+novobiocin broth and mechanically shaken for 1 minute in a Stomacher. These foodstuffs consisted of 27 meat and meat products, 57 poultry and poultry products, 10 milk and dairy products, 4 vegetables and 5 remaining samples from different food groups including delicatessen. All suspensions were incubated at +37 °C for 24 hours and studied by conventional cultural isolation methods with and without IMS as described previously. Finally, confirmation of the suspected *E. coli* O157:H7 colonies was done.

Statistical analysis

Wilcoxon test in the SPSS analysis computer program was used in order to analyse the data.

Results and discussion

The specificity of Dynabeads® anti-*E. coli* O157 is shown in Table 1 as positive or negative result according to the presence or absence of *E. coli* O157:H7 colonies on CT-SMAC agar. Table 1 shows that, although *E. coli* O157:H7 cells were captured by the beads, *E. coli* cells were not captured. The same result was obtained when the culture mixture was used. So, it can be said that these beads are highly specific to *E. coli* O157:H7 in case of being in the same media together with *E. coli*. There have been several studies on the specificity of these beads and their use in detection of *E. coli* O157 from several food samples (Wright et al., 1994; Lukasik et al., 2001).

The recovery sensitivity of Dynabeads® anti-*E. coli* O157 is shown in Table 2. In this part of the study, CT-SMAC agar was used as a selective medium whereas TSA was used as a non-selective medium. Table 2 indicates that the results of cultural plating with and without IMS show no significant rate of recovery difference

when TSA was used. The same result was also obtained when CT-SMAC agar was used. So, it can be said that recovery sensitivity using anti-*E. coli* O157 beads is as effective as the cultural plating without using the beads.

Table 3 gives an overview of the detection of *E. coli* O157:H7 from spiked food samples. Since *E. coli* O157:H7 was isolated from all foods without an enrichment step, it might be suspected that there was no difference if IMS was used or not before culturing. Yet, the conventional cultural method with IMS enabled the detection of 6 (75 %) *E. coli* O157:H7 positive samples of the 8 samples examined whereas the conventional cultural method without IMS detected 7 (87.5 %) *E. coli* O157:H7 positive samples when an enrichment step was done. Data presented indicate that, due to the low pH level of apple juice during the enrichment step, growth of *E. coli* O157:H7 was inhibited. This data also shows that the competitive background flora of parsley non-specifically bound to the beads and hindered the binding of *E. coli* O157:H7 to the beads.

Table 4 gives a comparison between the conventional cultural method with and without IMS in detecting *E. coli* O157:H7 from naturally contaminated food samples. The food samples covered five different groups: milk and milk products, meat and meat products, poultry and poultry products, vegetables and delicatessen. As presented in Table 4, *E. coli* O157:H7 could not be isolated from milk and milk products, vegetables and delicatessen. The conventional cultural method with IMS enabled to detect 3 (2.9 %) *E. coli* O157:H7 positive samples whereas the conventional cultural method without IMS detected one (0.9 %) *E. coli* O157:H7 positive sample. As a result, it is proposed that since meat and meat products (Willshaw et al., 1994; Chapman et al., 1996) and milk and milk products (Chapman et al., 1993; Heuvelink et al., 1998) are the most commonly implicated foods in *E. coli* O157:H7 food poisoning, the recovery rate of this organism will be higher when IMS

TABLE 1: Specificity of Dynabeads® anti-*E. coli* O157.

Strain	Recovery of <i>E. coli</i> O157:H7
<i>E. coli</i> O157:H7	+
<i>E. coli</i>	ND
<i>E. coli</i> O157:H7 + <i>E. coli</i>	+ / ND

+ : positive; ND : not detected

TABLE 2: Recovery sensitivity of Dynabeads® anti-*E. coli* O157.

Plating media	Cultural plating (log cfu/ml)	
	Without IMS	With IMS
TSA	10	9.3
CT-SMAC Agar	9.1	8.8

TABLE 3: Isolation of *E. coli* O157:H7 from spiked food samples.

Food samples	Conventional cultural method			
	Without IMS*	With IMS*	Without IMS**	With IMS**
Pasteurized milk	+	+	+	+
Pasteurized apple juice	ND	ND	+	+
Parsley	+	ND	+	+
Spinach	+	+	+	+
White cheese	+	+	+	+
Minced raw meat	+	+	+	+
Sausage	+	+	+	+
Yogurt	+	+	+	+

* With enrichment stage; ** Without enrichment stage

TABLE 4: Isolation of *E. coli* O157:H7 from naturally contaminated food samples.

Food Group	Conventional cultural method (a/b)	
	Without IMS	With IMS
Milk and milk products	ND/10	ND/10
Pasteurized milk	ND/2	ND/2
Raw milk	ND/1	ND/1
White cheese	ND/3	ND/3
Soft cheese	ND/1	ND/1
Traditional cheese (lor, tulum)	ND/2	ND/2
Otantic cheese (Urfa)	ND/1	ND/1
Meat and meat products	ND/27	1/27
Bacon	ND/1	ND/1
Salami	ND/1	ND/1
Sausage	ND/5	ND/5
Fermented sausage	ND/1	ND/1
Ground meat	ND/3	ND/3
Traditional meatball	ND/11	1/11
Hamburger raw meat	ND/3	ND/3
Hamburger meat	ND/2	ND/2
Poultry and poultry products*	1/57	2/57
Chicken steak and neck	1/57	2/57
Vegetables	ND/4	ND/4
Parsley	ND/1	ND/1
Lettuce	ND/1	ND/1
Mushroom	ND/1	ND/1
Soy sprouts	ND/1	ND/1
Delicatessen	ND/5	ND/5
Pepper paste	ND/1	ND/1
Otantic red pepper (Isot)	ND/1	ND/1
Fruit cake with cream	ND/1	ND/1
Traditional salad	ND/1	ND/1
Traditional seafood (mussels)	ND/1	ND/1
TOTAL	1/103	3/103

a/b: number of *E. coli* O157:H7 positive foodstuff / total number of foodstuff

ND: not detected

*The same sample has been found *E. coli* O157:H7 positive.

is used. Also, little is known about the prevalence of *E. coli* O157:H7 in poultry and poultry products because there have not been any reports since 1987, when *E. coli* O157:H7 was determined in chicken meat purchased from Wisconsin, Alberta and other Canadian supermarkets (Carter et al., 1987; Doyle and Schoeni, 1987). After this report, investigators have focused attention on the need for isolation of this pathogen also from poultry and poultry products.

In the present study, the statistical analysis showed no significant difference ($p > 0.05$) between the conventional cultural method with and without IMS technique. In other words, the conventional cultural method with IMS is as specific and sensitive as without IMS.

This study indicates that the IMS technique enhances the sensitivity of the detection of *E. coli* O157:H7 from various foodstuff only slightly. IMS is easy to perform and can easily be applied to isolation and detection procedures for *E. coli* O157:H7. This study has also shown that contamination of the foodstuffs with *E. coli* O157:H7 occurs at a very low level. This should provide some reassurance both to producers, retailers and consumers of these items in Ankara. The absence of any outbreaks and reports of laboratory-confirmed human infections in Turkey might be attributed to both dietary habits of Turkish people, who prefer to eat well-cooked meat and meat products, poultry and poultry products and well-pasteurized milk and milk products.

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Nutrient content comparison of the new culture species brown meagre (*Sciaena umbra*)

Nährstoffgehalt der neuen Aquakultur-Spezies Adlerfisch (*Sciaena umbra*)

Şükran Cakli¹, Tolga Dincer¹, Asli Cadun¹, Şahin Saka², Kürşat Firat²

Summary

The Mediterranean fishery and aquaculture industry is looking for alternative species to culture in sea cages. Different kinds of species can be chosen as a potential aquaculture candidate because of their eating qualities, their nature and ability to be held in captivity at high densities. In this regard, brown meagre (*Sciaena umbra*) is one of the candidate species, which offers good possibilities. No studies about the fish quality of this species exist, neither for wild nor for cultured fishes. In the current study, the proximate and fatty acid composition of the flesh of cultured and wild brown meagre (*Sciaena umbra*) were evaluated monthly during 8 months. Major quality parameters, such as muscle composition, fat deposition, muscle fatty acid composition and sensorial quality were studied in wild and cultured brown meagre. Lipid content of cultured meagre was much higher than that of wild fish. Differences were also observed in fatty acid profiles. Cultured fish were characterized by higher levels of monoenes, and wild fish by higher levels of saturates and n-3/n-6 ratios. Differences were also noted in the sensorial appearance of fish.

Keywords: *Sciaena umbra*, meagre, aquaculture, fatty acids, color, proximate composition

Zusammenfassung

Die mediterrane Fisch- und Aquakultur-Industrie sucht nach alternativen Möglichkeiten der Fischzucht in Käfigen im Meer. Verschiedene Arten von Spezies kämen hierzu für die Aquakultur in Frage aufgrund ihrer Verzehreignung, ihrer Eigenschaften und Eignung für die Zucht in Gefangenschaft und auf engstem Raum. In dieser Hinsicht ist der Adlerfisch (*Sciaena umbra*) einer der Kandidaten, der hierfür gute Möglichkeiten bietet. Es gibt keine Studien über die Qualität dieser Spezies als Lebensmittel, weder für wildlebende noch für in Aquakultur gezüchtete Fische. In der vorliegenden Studie wurden während eines Zeitraums von 8 Monaten monatlich sowohl die Ergebnisse chemischer Vollanalysen als auch die Fettsäuremuster gezüchteter und wilder Adlerfische (*Sciaena umbra*) ermittelt.

Wichtige Qualitätsparameter wie Muskelzusammensetzung, Fettablagerung, Fettsäuremuster der Muskulatur und sensorische Qualität wurden bei wildlebenden und in Aquakultur gehaltenen Adlerfischen untersucht. Der Fettgehalt von kultivierten Adlerfischen war bedeutend höher als der von Wildfischen. Unterschiede wurden außerdem im Fettsäuremuster beobachtet. In Aquakultur aufgezogene Fische wiesen einen höheren Gehalt an gesättigten Fettsäuren auf als wildlebende Fische, die einen größeren Anteil ungesättigter Fettsäuren zeigten mit einem höheren Verhältnis von Omega-3- zu Omega-6-ungesättigten Fettsäuren. Unterschiede wurden außerdem im Geschmack und Aussehen der Fische ermittelt.

Schlüsselwörter: *Sciaena umbra*, Adlerfisch, Aquakultur, Fettsäuren, Farbmessung, Vollanalyse

Introduction

The quality difference of fish caught in the wild and fish from aquaculture continues to be an important issue in all of the countries of the world. Many criteria have been established and many studies have been executed on the quality of fish flesh (Iwamoto and Yamanaka, 1986; Khalil et al., 1986; Aoki et al., 1991; Nettleton and Exler, 1992; Cakli and Alpaz, 1997; Jonsson, 1997; Gallagher et al., 1998; Jeong et al., 2002; Alam et al., 2002; Fåhræus-Van Ree and Spurrell, 2003). Fish culture in the Mediterranean is essentially based on two species, sea bream and sea bass, with productions which have increased in a spectacular way in recent years, from 37,179 mt in 1994 to 76,000 mt in 1998. This increase in production has led to market saturation and a fall in price. One of the forms in which market supply may be increased and a contribution be made to development and expansion of aquaculture is to diversify the species being cultured.

Nowadays, the search for new species suitable for farming is becoming a research priority. As there is a large number of candidate species, an effort has been made to identify the most promising (Quémener et al., 2002). Among others, brown meagre (*Sciaena umbra* Linnaeus 1758) could be a candidate species for Mediterranean aquaculture. Total annual catches of brown meagre in the Mediterranean have reached 1000 t (CBD, 2004). Although it is a rare species and its existence is usually threatened by fishing, brown meagre populations have recovered in protected areas in the Mediterranean Sea. Brown meagre is a demersal species living at depths of 0 to 200 m with a wide distribution in the Mediterranean Sea, Black Sea and eastern Atlantic Ocean (Chao, 1986; Reshetnikov et al., 1997). It exhibits nocturnal behavior and occupies bottom caves and sandy seabeds covered with vegetation; therefore, it exhibits motor activity during night and day. Growth is considerably repressed during low temperatures in winter but accelerated from spring until autumn when the water temperature rises (Karmanova et al., 1976). Females grow faster than males. The sexually mature fish spawn from May to July producing pelagic eggs. Feeding is reduced during gonad maturation and brown meagre may use as an energy source hepatic lipid reserves accumulated during the sexual resting period. Knowledge of the nutritional requirements of brown meagre is scarce and the only available information refers to its dietary habits in the wild (Chakroun-Marzouk and Ktari, 2003; Froglija and Gramitto, 1998).

Turkey is the second largest producer of farmed fish in the Mediterranean region. Fishery production of Turkey is approximately 627,847 tons/year. Aquaculture production is 61,165 tons/year (FAO, 2002). Aquaculture of sea bass and sea bream has been carried out successfully in Turkey since 1990. Besides, the aquaculture of new commercial species, common dentex, sharpnose sea-bream, brown meagre and red sea bream, began in 2000 with few farms and is still a successful activity. Continuous fishing pressure and pollution in some coastal areas were expected to result in a serious depletion of several fish stocks over the past three decades, at that time culture activities of these species became important. In this regard, cultured meagre is one of the candidate economical species, which offers good financial possibilities to the fishermen and good nutriment quality for the consumers.

The species *Sciaena umbra* has great potential for aquaculture in Turkey where the Aegean aquaculture industry is looking for alternative species to culture in sea cages. According to fish sellers brown meagre obtains high prices because it is one of the preferred fish in Turkey. The species *Sciaena umbra* is distributed throughout Turkey from the western to the southern part, from Izmir to Antalya. Understanding and controlling reproduction in brown meagre is of fundamental importance to its use as a new species for aquaculture. The culture of brown meagre, which is being done in small-size operations, is a new pilot activity. For that reason, no studies about the quality of wild of cultured forms of this species exist in Turkey.

In this study, a comparison of the substantial quality of cultured and wild meagre was to be obtained. To achieve this aim, determinations of proximate composition (protein, fat, moisture) and fatty acid composition, color measurements and sensory analyses have been performed monthly for a period of 8 months.

Material and Methods

Material

In this study, 40 samples of wild and 40 samples of aquacultured *Sciaena umbra* were included; cultured samples were taken from a single aquaculture farm and wild samples were caught in the Aegean Sea. Over a period of 8 months (October 2003 to May 2004), 5 samples each of wild and cultured meagre were taken and analysed every month. Of each batch of 5 samples 3 were used for the determination of the proximate composition, the analysis of fatty acid composition as well as color measurements. The remaining two samples were subjected to a sensory analysis. Each of the samples was approximately 200 g.

Methods

Analysis of proximate composition

Three of the wild, respectively the cultured meagre samples taken each month were pooled and homogenized. Dry matter was determined by drying the samples at 105 °C to constant weight (AOAC, 1990). Crude protein content was calculated by converting the nitrogen content determined by Kjeldahl's method (6.25 x N). Fat was determined using the method described by Bligh and Dyer (1959). The analyses of the pooled samples were all carried out in triplicate.

Analysis of fatty acid composition

Total lipid (TL) was extracted and purified according to Bligh and Dyer (1959), and TL content was determined gravimetrically. The lipids were saponified and esterified for fatty acid analysis by the method IUPAC II D19. Separation of fatty acid methyl esters was achieved on a SP-2330 Fused Silica Capillary Column (30 m x 0.25 mm i.d., 0.20 µm). The oven temperature was 120 °C for 5 min, programmed to 180 °C at 10°C/min, then programmed to 220 °C at 20 °C/min and then held there for 20 min and maintained at 240 °C and 250 °C, respectively. The carrier gas was helium with a linear flow rate of 0.5 ml/min and a split ratio of 1/150. The fatty acids were identified using marine lipid methyl esters as standards.

Color measurement

The color measurements of the fish samples were carried out with the spectral color meter Spectro-pen® (Dr. Lange, Düsseldorf, Germany). The color was measured on the homogenates prepared each month for the proximate analysis (pool samples of 3 wild and 3 cultured fish, respectively). The homogenate was placed in plastic petri dishes, the color of each of homogenate was measured in ten different locations and the average values of the measurements were determined. In the CIE Lab system L^* denotes lightness on a 0 to 100 scale from black to white, a^* red (+) or green (-), and b^* yellow (+) or blue (-) (Schubring and Meyer, 2002).

Sensory analysis

The sensory panel was prepared according to Carbonell et al. (2002). Dorsal fillets were cut from each meagre. The parts close to the head and the tail were removed as to obtain portions of approximately 6 cm x 3 cm. For sessions, each fillet was divided into two portions of approximately 3 cm x 3 cm. Two cultured and two wild meagres were analysed each month and the fillets (approximately 3 x 3 cm) were presented for sensory analysis. They were wrapped in aluminium foil without any seasoning added and baked in a preheated conventional household oven. Preheating took 30 min at +200 °C, the cooking time was 5 min for 3 x 3 cm portions. Portions from the same sample batch were heated together. The sensory analyses were carried out each month of the study with the main aim of determining differences in the sensory quality of cultured and wild meagre.

Statistical analysis

The SPSS 9.0 program was used to search for significant differences between mean values of the different results. Differences between means were analysed by one-way analysis of variance (ANOVA) followed by Tukey and Duncan tests. The results are presented as means \pm SD.

Results

The changes in the proximate composition of wild and cultured meagre are summarized in Table 1 for the duration of the study. For wild meagre the chemical compo-

sition was determined as follows: moisture values were between 72.96 ± 0.53 % and 76.36 ± 0.66 %, crude fat between 0.75 ± 0.04 % and 1.89 ± 0.27 % and protein between 19.24 ± 1.44 % and 23.53 ± 0.25 % during the 8 months study. The analysis of the cultured meagre resulted in moisture values ranging from 68.11 ± 0.77 % to 76.54 ± 0.19 %, crude fat from 0.55 ± 0.05 % to 4.81 ± 0.01 % and protein from 19.24 ± 1.44 % to 21.82 ± 1.46 %. In some months, there were significant differences between cultured and wild meagre in their moisture, fat and protein contents ($p < 0.05$). The fat and moisture values had inverse correlations, higher fat values resulting in lower moisture values.

The fatty acid profiles of wild and cultured brown meagre are presented in Table 2 and Table 3. Statistically significant differences in fatty acid profiles were found between wild and cultured meagre. Cultured fish had significantly higher values of monoenes, while wild specimen contained higher levels of saturates. Higher levels of monoenes and n-9 resulted from the increased presence of 18:1n-9 in tissues of farmed fish, while both 16:0 and 18:0 were contributing to the higher levels of saturates in wild meagre. Differences of this type have also been observed for a number of other species like Atlantic salmon (Bergstrom, 1989), red sea bream (Morishita et al., 1989), red porgy (Rueda et al., 1997) and turbot (Serot et al., 1998). Another marked difference between cultured and wild fish was the higher level of n-6 that cultured fish contained. Linoleic acid is not a normal constituent of the marine food chain, which is characterized mostly by polyunsaturated fatty acids (Sargent et al., 1999). This fatty acid is contained in plant oils included in the feed of cultured fish and is accumulated largely unchanged in the lipids of marine fish because of their reduced capacity for chain elongation and desaturation (Owen et al., 1975; Yamada et al., 1980). It has been observed in higher concentrations in cultured than in wild populations of other marine species studied (Morishita et al., 1989; Rueda et al., 1997; Serot et al., 1998). Similar observations have also been made for other Mediterranean species, like red porgy.

Cultured meagre contained significantly higher levels of total n-3 Highly Unsaturated Fatty Acids (HUFA) and Polyunsaturated Fatty Acids (PUFA) than wild forms of this fish. The ratio of n-3 PUFA to n-6 PUFA was higher in cultured meagre. Manipulation of nutrient sources of cultured meagre might influence their lipid composition. Yet, consumption of either wild or cultured meagre will contribute to dietary n-3 PUFA intake, with benefits to human health. In Tables 2 and 3 an increase in the values of PUFA and HUFA during the study can be seen. This may be the result of changes in diet and the intake of lipid. In summary, cultured fish had higher levels of monoenes, n-3, n-6, PUFA and HUFA fatty acids, while wild fish had higher levels of saturated fatty acids. These results have some similarities with the study of Peng and Tsai (2004). The values of n-3/n-6 ratios reported for wild compared with cultured Mediterranean fish have been found to be higher in some studies (Renon et al., 1994; Serot et al., 1998) and lower in other studies (Rueda et al., 1997) because of the significant contribution of arachidonic acid to n-6 fatty acids of wild fish. Polyunsaturated fatty acids give, upon oxidation, carbonyl and alcohol compounds which are related to the typical aroma of fish (Kawai, 1996). Different volatile aroma compounds are produced by n-3 or n-6 fatty acids (Josephson et al., 1983). Production of

TABLE 1: Proximate composition of brown meagre.

Month	Moisture %	Fat %	Protein %
Wild Meagre			
October	75.55 \pm 0.44 ^a	1.89 \pm 0.27 ^a	20.35 \pm 0.13 ^a
November	75.32 \pm 0.33 ^a	1.46 \pm 0.07 ^b	20.76 \pm 0.09 ^a
December	76.36 \pm 0.66 ^a	1.23 \pm 0.27 ^b	22.10 \pm 0.04 ^b
January	74.30 \pm 0.26 ^a	1.64 \pm 0.30 ^a	23.53 \pm 0.25 ^c
February	74.78 \pm 0.23 ^a	0.75 \pm 0.04 ^c	23.23 \pm 0.20 ^c
March	71.99 \pm 2.66 ^b	1.22 \pm 0.05 ^b	22.22 \pm 0.91 ^b
April	72.96 \pm 0.53 ^b	1.48 \pm 0.05 ^b	23.24 \pm 0.05 ^c
May	73.66 \pm 1.44 ^b	1.48 \pm 0.05 ^b	23.05 \pm 0.14 ^c
Cultured Meagre			
October	76.54 \pm 0.20 ^a	1.81 \pm 0.01 ^a	19.24 \pm 1.44 ^a
November	76.35 \pm 0.35 ^a	0.55 \pm 0.05 ^c	20.30 \pm 0.35 ^a
December	75.02 \pm 0.73 ^a	3.12 \pm 0.25 ^d	20.40 \pm 0.20 ^a
January	75.20 \pm 1.81 ^a	2.85 \pm 0.73 ^e	20.65 \pm 0.82 ^a
February	68.11 \pm 0.77 ^c	3.86 \pm 0.27 ^f	21.82 \pm 1.46 ^b
March	72.09 \pm 1.15 ^b	4.13 \pm 0.34 ^f	20.55 \pm 0.57 ^b
April	72.21 \pm 1.10 ^b	4.39 \pm 1.08 ^f	19.60 \pm 0.58 ^a
May	76.54 \pm 0.19 ^a	4.81 \pm 0.01 ^g	19.24 \pm 1.24 ^a

Arithmetic means and standard deviation, different superscripts in one column characterize significant differences ($p < 0.05$).

TABLE 2: Fatty acid composition of wild meagre.

Month	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Saturates	50.39 ± 0.21 ^a	48.80 ± 0.05 ^a	49.45 ± 0.21 ^a	50.00 ± 0.34 ^a	56.87 ± 0.24 ^b	55.97 ± 0.21 ^b	50.95 ± 0.41 ^a	50.47 ± 0.25 ^a
Monoenes	34.27 ± 0.25 ^a	35.38 ± 0.10 ^a	36.41 ± 0.02 ^a	38.41 ± 0.13 ^a	25.99 ± 0.18 ^b	31.34 ± 0.16 ^b	35.71 ± 0.27 ^a	30.15 ± 0.13 ^b
n-3	9.62 ± 0.17 ^a	9.60 ± 0.13 ^a	9.86 ± 0.16 ^a	9.79 ± 0.10 ^a	7.52 ± 0.04 ^b	7.52 ± 0.27 ^b	9.65 ± 0.18 ^a	9.84 ± 0.54 ^a
n-6	2.65 ± 0.20 ^a	2.54 ± 0.21 ^a	2.74 ± 0.05 ^a	2.74 ± 0.04 ^a	3.14 ± 0.28 ^b	3.15 ± 0.18 ^b	2.77 ± 0.01 ^a	2.77 ± 0.34 ^a
n-3 HUFA	8.88 ± 0.12 ^a	9.42 ± 0.14 ^b	9.43 ± 0.23 ^b	9.03 ± 0.07 ^a	6.05 ± 0.31 ^c	6.18 ± 0.31 ^c	9.23 ± 0.05 ^b	8.44 ± 0.52 ^a
PUFA	15.34 ± 0.14 ^a	15.82 ± 0.31 ^a	14.14 ± 0.02 ^b	13.20 ± 0.06 ^c	17.14 ± 0.02 ^d	12.68 ± 0.17 ^c	11.52 ± 0.41 ^c	19.38 ± 0.21 ^e

Arithmetic means and standard deviation, different superscripts in one row characterize significant differences ($p < 0.05$).

TABLE 3: Fatty acid composition of cultured meagre.

Month	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Saturates	43.14 ± 0.02 ^a	42.23 ± 0.06 ^a	41.89 ± 0.25 ^b	42.51 ± 0.42 ^a	36.93 ± 0.17 ^c	43.14 ± 0.02 ^a	42.23 ± 0.06 ^a	41.89 ± 0.25 ^b
Monoenes	35.10 ± 0.03 ^a	35.56 ± 0.04 ^a	41.14 ± 0.18 ^b	35.52 ± 0.16 ^a	40.36 ± 0.02 ^b	35.10 ± 0.03 ^a	35.56 ± 0.04 ^a	41.14 ± 0.18 ^b
n-3	13.75 ± 0.16 ^a	14.27 ± 0.54 ^a	9.21 ± 0.32 ^b	13.22 ± 0.06 ^a	11.71 ± 0.07 ^c	13.75 ± 0.16 ^a	14.27 ± 0.54 ^a	9.21 ± 0.32 ^b
n-6	7.92 ± 0.02 ^a	7.65 ± 0.15 ^a	8.29 ± 0.56 ^b	8.82 ± 0.16 ^c	8.35 ± 0.16 ^a	7.92 ± 0.02 ^a	7.65 ± 0.15 ^a	8.29 ± 0.56 ^b
n-3 HUFA	10.65 ± 0.04 ^a	11.18 ± 0.07 ^a	8.85 ± 0.32 ^b	12.64 ± 0.05 ^c	9.65 ± 0.25 ^a	10.65 ± 0.04 ^a	11.18 ± 0.07 ^a	8.85 ± 0.32 ^b
PUFA	21.82 ± 0.51 ^a	22.08 ± 0.35 ^a	16.98 ± 0.01 ^b	22.33 ± 0.02 ^a	19.70 ± 0.12 ^b	21.82 ± 0.51 ^a	22.08 ± 0.35 ^a	16.98 ± 0.01 ^b

Arithmetic means and standard deviation, different superscripts in one row characterize significant differences ($p < 0.05$).

cultured fish with fatty acid profiles similar to those of wild ones might therefore aid in the production of fish of similar taste characteristics.

Table 4 presents the color measurement results for wild and cultured meagre obtained during the 8 months study. For wild meagre L^* values between 51.5 ± 2.08 and 61.8 ± 3.15 were determined, a^* values between -2.8 ± 0.12 and 0.1 ± 0.18 and b^* values between 8.7 ± 0.41 and 11.9 ± 1.17 . On average, higher L^* values were measured in the cultured samples, which ranged from 51.5 ± 2.22 to 60.2 ± 1.10 . The a^* values of the cultured and wild samples were similar. Yet, as in the cultured form b^* values were between 5.0 ± 1.15 and 11.6 ± 1.13 , it was shown that b^* values were generally higher than in wild meagre. Therefore, it can be concluded that cultured brown meagre have a lighter and more yellow color than wild meagre. Similar results were obtained in a study of different fish species (Cakli et al., 2005).

In the sensory analysis, an acceptance test was performed using a scale from 0 to 10 and the descriptors shown in Table 5. In the preliminary session, sample preparation and experimental conditions were established and 13 sensory attributes were selected as descriptors (3 for odor, 2 for appearance, 3 for flavor and 5 for texture). Data obtained from the panel evaluation sessions of the main study were analyzed by ANOVA. Significant differences between samples were detected for seven attributes (odor intensity, fresh odor, color, flavor intensity, fresh flavor, juiciness and fatness). Cooked samples of wild fish were perceived as having less fresh odor and flavor as well as being less fat and juicy.

Conclusion

Different degrees of fat deposition in wild and cultured brown meagre indicate seasonal variation with a minimum of deposition at the end of spring and a maximum at the end of summer. In all cases wild fish had much

TABLE 4: Color values of wild and cultured meagre.

Wild meagre	L^*	a^*	b^*
October 2003	52.5 ± 1.18 ^a	0.1 ± 0.18 ^a	10.6 ± 0.28 ^a
November 2003	51.5 ± 2.08 ^b	-0.5 ± 0.12 ^b	8.7 ± 0.41 ^b
December 2003	55.4 ± 2.13 ^c	-1.3 ± 0.01 ^b	9.8 ± 1.32 ^a
January 2004	52.7 ± 0.85 ^a	-1.4 ± 0.20 ^b	9.1 ± 1.34 ^b
February 2004	59.3 ± 1.12 ^d	-0.2 ± 0.14 ^a	10.0 ± 0.82 ^a
March 2004	61.8 ± 3.15 ^d	-1.3 ± 0.14 ^b	11.9 ± 1.17 ^c
April 2004	59.9 ± 1.12 ^d	-2.8 ± 0.12 ^c	9.3 ± 3.10 ^b
May 2004	56.6 ± 1.02 ^c	-1.5 ± 0.01 ^b	11.4 ± 2.17 ^c
Cultured meagre	L^*	a^*	b^*
October 2003	57.4 ± 1.17 ^a	-1.7 ± 0.12 ^a	9.3 ± 1.21 ^a
November 2003	57.5 ± 1.04 ^a	0.6 ± 0.13 ^b	11.6 ± 1.13 ^b
December 2003	58.8 ± 1.16 ^b	-1.4 ± 0.23 ^a	6.3 ± 2.07 ^c
January 2004	51.5 ± 2.22 ^c	-1.4 ± 0.05 ^a	5.0 ± 1.15 ^c
February 2004	56.0 ± 1.14 ^a	-2.4 ± 0.10 ^c	7.3 ± 1.62 ^d
March 2004	56.5 ± 2.23 ^a	-2.3 ± 0.31 ^c	8.9 ± 0.86 ^e
April 2004	60.2 ± 1.10 ^c	-2.6 ± 0.08 ^c	5.4 ± 1.23 ^c
May 2004	54.8 ± 2.12 ^c	-1.0 ± 0.19 ^a	9.8 ± 0.80 ^a

Arithmetic means and standard deviation, different superscripts in one column characterize significant differences ($p < 0.05$).

lower fat contents than their cultured counterparts. Significant differences were also apparent in the fatty acid contents. Differences in lipid and fatty acid composition of cultured compared with wild fish need specific attention to maximize the nutritional quality of cultured fish and to optimize sensory characteristics. According to the instrumental analysis, higher lightness values and lower yellowness values were determined in cultured brown meagre than in wild meagre.

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TABLE 5: Sensory evaluation of wild and cultured meagre (scale 0–10).

Sample	Odor intensity	Fresh odor	Color	Flakiness	Flavor intensity	Fresh flavor	Firmness	Chewiness	Fibrousness	Juiciness	Fatness
Wild	6.87 ± 2.21 ^a	6.12 ± 2.43 ^a	2.66 ± 2.40 ^a	5.00 ± 2.04 ^a	6.32 ± 1.53 ^a	6.85 ± 1.20 ^a	6.9 ± 2.71 ^a	3.20 ± 1.89 ^a	5.35 ± 2.63 ^a	5.12 ± 2.37 ^a	5.12 ± 2.06 ^a
Cultured	6.17 ± 2.80 ^b	6.94 ± 2.07 ^b	3.24 ± 1.01 ^b	5.04 ± 3.06 ^a	8.97 ± 1.33 ^b	9.02 ± 1.46 ^b	6.91 ± 2.61 ^a	3.22 ± 2.48 ^a	5.40 ± 2.21 ^a	6.95 ± 2.54 ^b	6.74 ± 1.13 ^b

Arithmetic means and standard deviation, different superscripts in one column characterize significant differences ($p < 0.05$).

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Summary

Zusammenfassung

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Histological analysis of different kinds of mechanically recovered meat

Histologische Untersuchung verschiedener Arten von Separatorenfleisch

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Zdenka Randulová¹

Generally speaking, mechanically recovered meat is relatively non-standard raw material. At present, milder production technologies are used, and the product obtained usually has a low calcium (bone) content and its structure is similar to that of minced meat. In the present study, histological examinations (Green Trichrome and Alizarine Red staining) were used to qualitatively and semi-quantitatively compare samples of mechanically recovered meat (MRM) manufactured under different conditions. The examination was focused on the evaluation of muscle, fat, collagenous connective tissue and bone fragment contents by histological as well as chemical methods. The results of the histological analysis were generally in accordance with the results of the chemical analysis. When comparing groups of samples of mechanically recovered meat produced at an interval of several years, it was shown that the quality of the raw material was improved in compliance with the general trend and the application of more refined technologies.

For the quantitative determination of collagen by means of image analysis, a method previously validated for the determination of bone tissue was used. The results of image analysis did not confirm a correlation between histological and chemical determination of collagenous connective tissue. However, taking into consideration statistical results, this hypothesis cannot be finally rejected, and ways to maintain independence of individual observations will further be sought after, e.g. taking into account sufficient numbers and homogenous distribution of samples withdrawn or examination of an adequate number of sections.

Keywords: mechanically recovered meat, histological examination, collagenous connective tissue, image analysis

Derzeit werden schonendere Methoden zur Herstellung von Separatorenfleisch verwendet, und daher hat das gewonnene Produkt in der Regel einen niedrigen Kalziumgehalt (Knochen) und eine dem Hackfleisch ähnliche Struktur. In der vorliegenden Arbeit wurden für den qualitativen und semiquantitativen Vergleich von Separatorenfleisch-Proben, die unter verschiedenen Bedingungen gewonnen wurden, histologische Methoden verwendet (Färbung mit Trichrom-Grün und Alizarinrot). Die Untersuchung basierte auf der Auswertung des Gehaltes an Muskelgewebe, Fett, Kollagenbindegewebe und Knochenpartikeln mittels histologischer sowie chemischer Methoden. Die Ergebnisse der histologischen Analyse entsprachen hierbei im allgemeinen denen der chemischen Untersuchungen. Durch Vergleich von Proben von Separatorenfleisch, das im Abstand einiger Jahre gewonnen wurde, konnte festgestellt werden, dass sich im Einklang mit dem allgemeinen Trend und dem Einsatz verbesserter Technologien die Qualität des Rohstoffes verbessert hat.

Zur quantitativen Bestimmung von Kollagen mit Hilfe einer Bildanalyse wurde ein Verfahren eingesetzt, das sich bereits bei der Bestimmung von Knochengewebe bewährt hatte. Die Resultate der Bildanalyse konnten keine gute Korrelation zwischen der histologischen und der chemischen Bestimmung des Kollagen-gewebes liefern. Diese Hypothese kann jedoch im Hinblick auf die statistischen

Ergebnisse nicht endgültig verworfen werden, vielmehr sollen Möglichkeiten zur Gewährleistung der Unabhängigkeit der jeweiligen Beobachtungen gesucht werden durch Berücksichtigung u.a. einer ausreichenden Probenanzahl und homogener Verteilung der entnommenen Proben bzw. Untersuchung einer angemessenen Zahl von Schnitten.

Schlüsselwörter: Separatorenfleisch, histologische Untersuchung, Kollagenes Bindegewebe, Bildanalyse

Introduction

One of the frequently discussed raw materials is mechanically (machine) recovered meat (MRM), called separated meat as well. Within the European Union, large amounts of this raw material are produced. There are legislative restrictions not only on separated meat recovery, but also on the treatment of this raw material and its use in products, as well as labelling of finished products. In general, MRM is non-standard raw material. Its properties depend not only upon the type of raw material processed, but also on the machines used and various processing parameters. Due to improper selection of raw material and technologies used in the past (high pressure), separated meat received a bad reputation. If a product obtained in such a way was used in meat products, it negatively influenced chemical composition, microbiological quality and texture of these products. At present, milder technologies are used, therefore the material obtained usually has a low calcium (bone) content, and its structure is similar to that of minced meat or the material obtained by so called soft separation, i. e. the removal of fibrous parts from meat (Stiebing, 2002).

In practice, mainly two types of separation machines are used. There are continuous separators that destroy flesh-bearing bones, thus producing pulp first, which is then forced by an auger out to the separator head through a sieve with different size holes, leaving behind a slurry containing bone fragments and other non-processed parts that is ejected into the other part of the separator head (Beehive). Another type are discontinuous separators, with a hydraulic piston forcing flesh-bearing bones to the separation chamber while crushing them and squeezing meat puree through thin slits between concentric rings (Protecon). The efficiency and yield of separation depend on the pressure used. Another way of separation has been reported, which is based on forcing material to a rotating cylinder by the pressure of a moving conveyor belt. This method is used mainly to remove fascia and tendons from meat, so called soft separation (Stiebing, 1998).

Henckel et al. (2004) compared samples of meat recovered by two mechanical methods – manual and machine recovery. The quality of meat recovered by squeezing material through a cylinder by a conveyor belt was the same as in manually recovered meat. The degradation of muscle structure was greater in meat recovered using an auger, and smaller in meat recovered manually. On the basis of the results we have obtained,

we suggest a scale to assess the quality of separated meat, based on biochemical examination and histological determination of the degree of degradation of muscle fibre structure.

Troeger (2002) used a combination of several methods to recover meat by separation from pork bones. He reported results obtained using different amounts of this material in different types of meat products, showing that their quality was not markedly affected.

From the viewpoint of supervision, the legislative definition of this raw material is of importance. According to Regulation (EC) No. 853/2004, mechanically separated meat is a product obtained by machine separation of meat residues that remained attached to bones after deboning, or from processed poultry carcasses, in a way which causes disappearance of muscle fibres or changes in their structure. The text of the Regulation excludes some parts of the skeleton from processing, specifies requirements for production and manipulation of the material recovered and puts limitations on its use. The amendment to Regulation 853/2004 (Commission Regulation 2074/2005) uses the term mechanically separated meat and distinguishes techniques of its recovery according to whether bones are damaged or not. So far, the legislation in force has not specified further quality requirements on machine separated meat itself, mentioning only the maximum calcium content in mechanically separated meat which is not to exceed 0.1 % (Commission Regulation 2074/2005).

Yet, as to the composition and functional properties of separated meat there are several important parameters, in particular content of bone fragments, muscle and connective tissue protein, water and fat (Wenthe et al., 2001). Crosland et al. (1995) analysed the composition of different kinds of mechanically recovered meat. The data obtained demonstrated the variability in composition, which is related to the kind of raw material and the technical conditions of its recovery.

Histological methods belong to less used methods assessing quality of raw materials of animal origin. These techniques are based on simple principles, but the examination and evaluation of results requires experience. The identification of different parts of the microscopic image is based on the knowledge of basic architecture and structure of animal tissues. In case of separated meat these are muscle, adipose and connective tissues, including ligaments and bones. A characteristic feature of separated meat is the presence of bone fragments that do not usually occur in regular meat,

which can also be used when identifying this raw material in products (Schulte-Sutrum and Horn, 2003). Also, changes occurring during technological processing must be taken into account. Therefore, it is suitable to use different staining methods that make the identification of tissues easier. These are usually qualitative methods, but they also enable a quantitative evaluation, particularly in association with image analysis. As early as 1985, Koolmees and Bijker demonstrated that histological and chemical examination methods are of the same value for the determination of collagen in meat. Beside the determination of collagenous tissue, a qualitative histological determination of elastic connective tissue and bone contents in meat products was described in the literature (Hildebrandt and Hirst, 1985). Tremlová and Štarha (2003) measured the amount of bone fragments in a defined area in meat products. Using correlation coefficients the results were compared with the chemical determination of calcium in the products examined.

In the present study, histological examinations were used for the qualitative and semi-quantitative comparison of samples of separated meat manufactured under different conditions (continuous and discontinuous machines). This comparison was based on the evaluation of muscle tissue, adipose tissue, collagenous connective tissue and bone fragments. A group of samples obtained by so called soft separation (Baader) was also evaluated. The study has several objectives. The first objective is to compare results of histological and chemical analyses of separated meat samples of different origin. The study is also aimed at a comparison of samples coming from the same plant, produced in 2001 and 2005, with the objective to assess whether there had been a shift in quality of the raw material produced. The third objective is to determine collagen contents using image analysis. For the quantitative determination of collagen by means of histological examination and

image analysis a similar method was used as in the study of Tremlová and Štarha (2003). The results of the histological examination were compared with values obtained by chemical analyses.

Material and Methods

The samples examined include mechanically recovered pork and poultry meat obtained with different types of machines (Tab. 1). Different groups of samples were included in the qualitative and quantitative examinations (Tab. 2–4).

TABLE 1: Identification and characteristics of the analysed samples.

Sample no.	Kind of raw material	Type of machine
1	Poultry bones	Protecon
2		
3		
4		
5		
6		
7	Poultry bones	Beehive
8		
9		
10		
11		
12		
13	Poultry trimmings	Baader
14		
15		
16		
17		
18		
19	Pork bones	Protecon
20		
21		

TABLE 2: Comparison of results of histological and chemical analyses.

Sample no.	Raw material / conditions			
	Pork bones / Protecon	Poultry bones / Protecon	Poultry bones / Beehive	Poultry trimmings / Baader
	19	6	7	15
Muscle tissue	Considerable amount, muscle fibres partially damaged	Medium amount, muscle fibres partially damaged	Prevailing, muscle fibres partially damaged	Prevailing, muscle fibres little damaged
Net muscle protein g.kg ⁻¹	157.04	128.22	158.15	188.66
Collagenous tissue	Medium amount	Medium amount, thin cartilage connective tissue	Moderate amount, thin cartilage connective tissue	Moderate amount, thin connective tissue
Collagen g.kg ⁻¹	12.19	7.54	6.41	5.29
Adipose cells	Medium amount	Moderate amount	Moderate amount	Negligible amount
Fat g.kg ⁻¹	155.10	190.00	148.83	65.25
Bone fragments	More than 3 fragments in 10 sections, small bone fragments	More than 3 fragments in 10 sections, small bone fragments	More than 3 fragments in 10 sections, both small and very big bone fragments	Less than 3 fragments in 10 sections
Calcium mg.kg ⁻¹	374.50	197.50	254.50	116.50

TABLE 3: Comparison of different kinds of mechanically separated meat by histological examination.

Raw material (machine type) Year of production (sample no.)	Parameters under evaluation			
	Muscle tissue	Collagenous connective tissue	Adipose cells	Bone fragments
Poultry bones (Protecon) 2001 (1,3,4,5)	Medium amount, muscle fibres partially damaged	Prevailing, large fragments	Moderate amount	More than 3 fragments in 10 sections, small fragments
	Medium amount, muscle fibres largely damaged	Prevailing, large fragments	Moderate amount	More than 3 fragments in 10 sections, larger fragments
	Medium amount, muscle fibres largely damaged	Prevailing, large fragments	Moderate amount	More than 3 fragments in 10 sections, larger fragments
	Medium amount, muscle fibres largely damaged	Prevailing, large fragments	Moderate amount	More than 3 fragments in 10 sections, larger fragments
Overall evaluation	Medium amount, muscle fibres largely damaged	Prevailing, large fragments	Moderate amount	More than 3 fragments in 10 sections, larger fragments
Poultry bones (Beehive) 2005 (7,8,9,10)	Medium amount, muscle fibres partially damaged	Medium amount, thin connective tissue of cartilage	Moderate amount	More than 3 fragments in 10 sections, small fragments
	Prevailing, muscle fibres partially damaged	Moderate amount, thin connective tissue of cartilage	Moderate amount	More than 3 fragments in 10 sections, both small and very large fragments
	Prevailing, muscle fibres little damaged	Moderate amount, sporadic occurrence of cartilage	Negligible amount	Less than 3 fragments in 10 sections, small fragments
	Prevailing, muscle fibres little damaged	Negligible amount, sporadic occurrence of cartilage	Moderate to medium amounts	More than 3 fragments in 10 sections, small fragments
Overall evaluation	Prevailing, muscle fibres little damaged	Moderate amount, sporadic occurrence of cartilage	Moderate amount	More than 3 fragments in 10 sections, small fragments prevail, large ones sporadically
Poultry trimmings (Baader) 2005 (13,14,15)	Prevailing, muscle fibres little damaged	Medium amount of cartilage	Negligible amount	Less than 3 fragments in 10 sections
	Prevailing, muscle fibres moderately damaged	Moderate amount of cartilage	Negligible amount	More than 3 fragments in 10 sections
	Prevailing, muscle fibres little damaged	Moderate amount of thin connective tissue	Negligible amount	Less than 3 fragments in 10 sections
Overall evaluation	Prevailing, muscle fibres little damaged	Moderate amount of cartilage	Negligible amount	Less than 3 fragments in 10 sections

TABLE 4: Quantitative determination of collagen by image analysis.

No.	Sample		Collagen content (%)		
	Kind	Machine type	Relative	Chemical	Weighted relative
6		Protecon	6.957249275	0.75	1.726185508
7			6.739352443	0.64	1.050480937
10	Poultry bones	Beehive	2.153112345	0.77	0.469170116
11			4.323136397	0.83	0.729912759
12			2.553981437	1.01	0.467911081
15			6.298503123	0.53	1.102656946
16	Poultry trimmings	Baader	1.803731932	0.64	0.586911378
17			13.15306919	0.86	1.314768132
18			3.264965295	0.85	0.712742788
19			3.613654454	1.22	1.051665118
20	Pork bones	Protecon	1.431522183	1.07	0.392590455
21			27.0741628	0.72	12.17632848
	Mean value		3.913438785	0.81	0.756668232
	Correlation		-0.254364773		-0.103836729
Hypothesis test H: p = 0					
	Test criterion value t		-0.822426292		-0.329548396
	Significance level		0.05		
	Interval of practically possible values		< -1.960 ; 1.960 >		

Chemical analyses of samples included the determination of selected basic parameters (net muscle protein, fat, collagen and calcium) and were conducted in compliance with approved methods in a certified laboratory (food testing laboratory no. 1051). The results of the chemical examination were used for comparison with the results of the histological analysis.

The histological examination was carried out at the Department of Vegetable Foodstuffs and Plant Production, VFU. The aims of the qualitative and semi-quantitative histological examinations were to determine the nature of the sample, to diagnose the presence of different tissue types and to assess the amount and the degree of impairment of the original structure of the tissue types. For this purpose, microscopic slides stained with Alizarine Red (Fig. 1 and 2; Manual, 1994) and Green Trichrome (Fig. 3 and 4; Official Standard, 1989) were prepared. For each stain, 10 sections of each sample (prepared

from 4 paraffin blocks) were examined by microscope. Semi-quantitative evaluation is expressed verbally using the following scale: prevailing, considerable amount, medium amount, moderate amount, negligible amount, and sporadic occurrence (Official Standard, 1989).

The quantitative histological determination of the collagen content was performed by image analysis (ACC, v. 6.0, SOFO CZ), using slides stained with Trichrome Green. For each sample, 10 sections were examined. After scanning the sections, results were obtained as follows: a photometric calibration of the slide was carried out to adjust contrast, brightness and colour. Then, the area of the whole slide and image segmentation were calculated, based on the stain used to identify analysed objects, i.e. the area of collagen stained green was measured. This method was previously used to

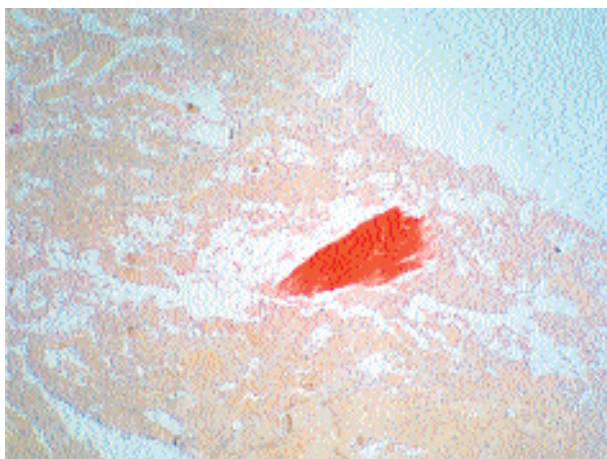


FIGURE 1: *Pork MSM, Alizarine Red stain (bone tissue – red).*

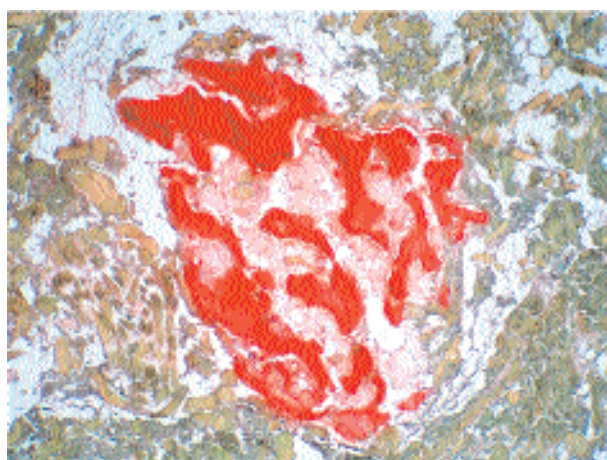


FIGURE 2: *Poultry MSM, Alizarine Red stain (bone tissue – red).*

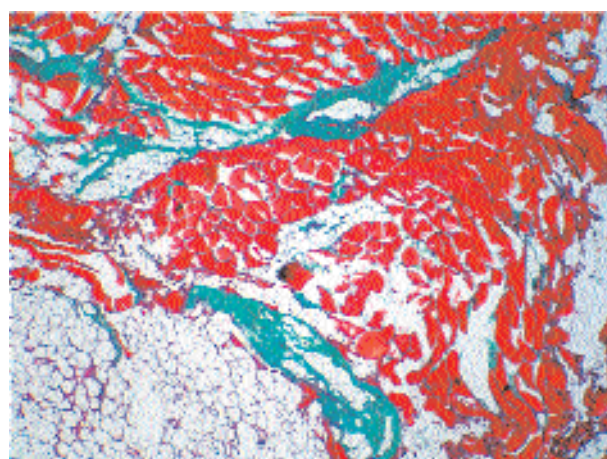


FIGURE 3: *Pork MSM, Trichrome Green stain (collagen tissue – green, muscle fibre – red).*

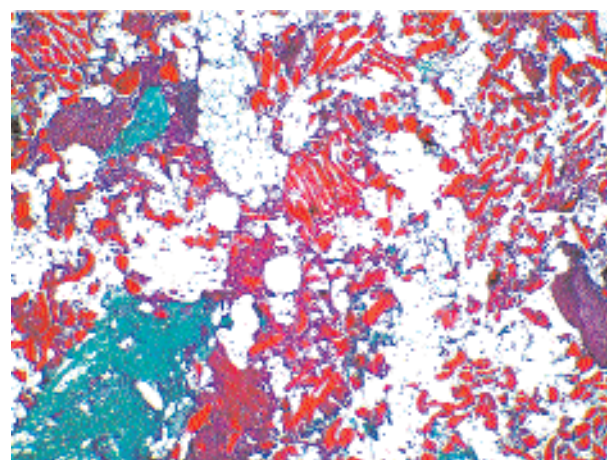


FIGURE 4: *Poultry MSM, Trichrome Green stain (collagen tissue – green, muscle fibre – red).*

determine bone tissue as described by Tremlová and Štarha (2003). Statistical analyses included calculations of the relative amount of analysed objects and weighted relative amount of analysed objects where weight is proportionate to the colour intensity of an analysed object. The sizes of the sections under study were different, the area of each section examined was always precisely measured during analysis and then values converted to area units were used. The results were compared with the results of the chemical analysis and correlations were calculated.

Results and Discussion

Quality requirements for separated meat are not specified in the legislation, with the exception of calcium content (Commission Regulation 2074/ 2005). The results of the comparison of the histological and the chemical analyses of selected samples are listed in Table 2. These are always mean values of 3 examinations per sample. Separated pork (sample no. 18) showed muscle and fat contents similar to separated poultry meat obtained with a Beehive separator (sample no. 7). The higher collagen content in separated pork is due to the properties of the raw material. This difference could not be shown very clearly in the histological image. Differences in the results of chemical analysis of separated

meat (poultry and pork), obtained with different machines, were also reported by Crosland et al. (1995). The sample of mechanically separated meat obtained by a Baader separator (sample no. 15) gave the best results in histological and chemical analyses, which is similarly due to the raw material used (poultry trimmings). Chemically determined calcium concentrations and bone contents obtained by histological examination can be correlated with the type of machine and separation pressure used. Calcium contents ranged from 0.01 to 0.03 % and were clearly beneath the set limit of 0.1 %. The results of histological analysis were in accordance with the results of chemical analysis. Moreover, the histological examination enables to determine contents of different kinds of tissue, as well as tissue character (kind of connective tissue, cartilage) or size (cartilage and bone fragments).

The results shown in Table 3 were evaluated with respect to the type of machine used to obtain separated meat. The way of recovery (separation principle, machine alignment, pressure) influences mainly the amount of bone tissue in the resulting material, the size of fragments of different tissue types, and the degree of damage to the muscle fibres. The amount of collagen, muscle tissue and fat is also associated with the kind of raw material (bones, trimmings).

The comparison of groups of samples of machine separated poultry meat produced in one plant at a four year interval (2001 and 2005) shows that, in accordance with a general trend, the quality of raw material was improved. This development can be explained by the change of the way of recovery of machine separated meat used in the above-mentioned plant. Nowadays (Beehive), there is less damage to muscle tissue, as reported by Stiebing (2002). An interesting finding was the detection of bone fragments in raw material obtained, according to the producer's statement, from trimmings by so-called soft separation, which should not contain bone tissue. The comparison with data on chemical examination of various kinds of separated meat shows that all samples of poultry material examined had lower contents of fat, collagen and calcium than those reported in the study of Crosland et al. (1995), regardless of the conditions under which they were obtained. In the raw material from pork bones, the values ascertained were either the same as the data reported (fat) or slightly higher (collagen), respectively markedly higher (calcium).

The quantitative histological examination was carried out according to the method reported in the study of Tremlová and Štarha (2003) comparing chemical and histological determinations of bone tissue in meat products. In this study, a statistically significant correlation of results in 24 samples examined was found (correlation coefficient 0.78, significance level $\alpha = 0.05$). Hildebrandt and Hirst (1985) monitored contents of bone and collagenous as well as elastic connective tissue in meat products. They examined 51 samples and compared the results obtained from image analysis with chemical results. Image analysis was considered an adequate alternative to chemical analysis (correlation coefficient

> 0.9). Also, the correlation between results obtained by histological and chemical analyses for determination of collagen in meat, reported in the study of Koolmees and Bijker (1985) was high (0.88). Results obtained in the present study, however, do not confirm the conclusions reported as far as the quantitative determination of collagen is considered (Tab. 4).

However, the statistical evaluation of the results of this study does not completely exclude the possibility of a reasonable correlation between collagen values determined by histological and chemical analyses. The colour contrast is sufficient when using the above-mentioned staining method for processing with the image analyser. There can be several reasons for the statistical independence in case of collagen determination. It is possible that the independence of individual observations was not observed because the samples were not withdrawn evenly and in sufficient numbers. Unlike the bone fragments that were assessed in the previous study (Tremlová and Štarha, 2003), the objects determined (collagen areas) in the histological sections are so large that the size of the section examined is not suitable. In this case, the results of the histological observation were random and not representative for the sample.

Conclusion

The quality of food raw material is most often expressed by chemical composition, but in the case of raw materials of animal origin, the chemical characteristics do not always cover all quality aspects. The evaluation of the structure and the degree of damage of different tissues contained in production raw materials is possible only by means of histological examination. In the present study, the results of the semi-quantitative evaluation of histological analyses of samples of different kinds of machine recovered meat were in accordance with contents of muscle protein, collagenous connective tissue, adipose cells and bone tissue determined by chemical analysis. The results of this part of the study imply that histological examination can be used as a tool of orientative evaluation of quality parameters of separated meat.

By comparing the samples of poultry meat separated from poultry bones using different types of machines, no marked differences in the parameters under study were found. Still, the results received using Beehive seem to be better, particularly in terms of content in and degree of damage of muscle and connective tissue. Undoubtedly, the best results were obtained in machine recovered meat the raw material of which were not poultry bones but poultry trimmings.

Using digital image analysis quantitative results can also be obtained. Although the literature data and our own experience confirm that there can be a good correlation between results of histological and chemical analyses, the results of this study show that these methods should be further verified and standardized.

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Nachrichten

Handwerkliches Hygienekonzept fertiggestellt

Das DFV-Konzept "Gute Hygienepaxis – Eigenkontrollen – Dokumentation" ist fertiggestellt. Im Rahmen der Internationalen Handwerksmesse in München trafen Prof. Dr. Dr. h.c. Andreas Stolle vom Institut für Hygiene und Technologie der Ludwig-Maximilians-Universität München, DFV-Präsident Manfred Rycken und DFV-Referent Dr. Wolfgang Lutz zu einem Abschlussgespräch zusammen. Prof. Stolle und seine Mitarbeiter waren an der Entwicklung des DFV-Konzeptes beteiligt und haben die Arbeiten wissenschaftlich betreut.

Das DFV-Konzept "Gute Hygienepaxis – Eigenkontrollen – Dokumentation" konkretisiert die Anforderungen der neuen EU-Hygienevorschriften im Hinblick auf die Verhältnisse in handwerklich arbeitenden Fleischereibetrieben. Das Konzept soll die handwerklichen Fleischereien bei der Umsetzung der neuen Vorschriften des EU-Hygienepaketes unterstützen und bei dessen Umsetzung Hilfestellung leisten. Damit verfügen die fleischerhandwerklichen Betriebe über klare Vorgaben für die tägliche Hygienepaxis sowie über einfache und pragmatische Anleitungen und Unterlagen zur Durchführung von Eigenkontrollen und Schulungen sowie zu deren Dokumentation.



Das Kernstück des DFV-Konzeptes, die Leitlinie für eine gute Hygienepaxis in fleischerhandwerklichen Betrieben, wurde Anfang Mai an alle zuständigen Ministerien, Regierungspräsidien und Veterinärämter versandt. Derzeit werden die handwerklichen Fleischereibetriebe flächendeckend über das DFV-Konzept "Gute Hygienepaxis – Eigenkontrollen – Dokumentation" mit Leitlinie, Eigenkontrollkonzept und betrieblichen Umsetzungshilfen informiert und geschult.

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