Ätherische Öle: Einfluss auf die Mast von Broilern, Anteil der Grundteile im Rumpf und sensorische Fleischeigenschaften

Zusammenfassung

Das Ziel der Untersuchung war, den Einfluss der Komponentenkombination von ätherischen Ölen (Carvacrol, Capsaicin und Cinemaldehid) und den Einfluss der Kombination von ätherischen Ölen (Citrus und Fenchel) auf die Herstellungscharakteristiken der Masthähnchen, auf die Charakteristiken des Hähnchenrumpfes zu bestimmen. Es sollte festgestellt werden, ob diese Bestandteile einen Einfluss auf Saftigkeit, Geruch und Geschmack von Hähnhächenkeulen und Hähnchenbrust haben. Das Experiment fand in drei Gruppen je 48 Hähnchen Art Ross 308 in der Zeit von 42 Tagen statt. Die Resultate aus dem Experiment wurden durch die Analyse Variance (ANOVA) bearbeitet, wobei das GLM Modell und Tuckey post hoctest angewendet wurden. Die Analyse der Resultate bestätigte, dass die Zufügung von ätherischen Ölen einen positiven Einfluss auf die Körpermasse von Hähnchen im ersten Mastteil hat. Dasselbe gilt für Rumpfmasse, Anteil von wertvollen Teilen und alle zu beurteilenden sensorischen Charakteristiken

***Schlüsselwörter:** Ätherische Öle, Hähnchen in Mast, Körpermasse, Rumpfmasse, sensorische Eigenschaften von Hähnchenfleisch*

Oli eterici: influenza sull'allevamento dei broiler, percentuale di pezzi fondamentali nell'addome e caratteristiche sensoriche della carne

Sommario

Lo scopo di quest'esame era determinare l'effetto del misto di componenti di oli eterici (carvacrolo, capsaicina e cinnamaldeide) e l'influenza del misto di oli eterici (agrume e finocchio) sulle caratteristiche produttive di pollame durante l'allevamento, sulle caratteristiche dell'addome di pollo e determinare se questi additivi influiscono sulla succosità, odore e sapore della carne di coscie e del petto di pollo. L'esame è stato fatto in tre gruppi a 48 polli del genere Ross 48 ciascuno, nell'ambito di 42 giorni. I risultati ottenuti durante l'esame sono stati analizzati mediante l'analisi della varianza (ANOVA), usando il modello GLM e l'analisi a posteriori o post-hoc di Tuckey. Durante l'analisi dei risultati è stata determinata un'influenza positiva di oli eterici sul peso corporeo, aggiunti al pollame nella prima fase d'allevamento, sul peso di addomi, sulla percentuale di pezzi di valore e su tutte le caratteristiche prese in considerazione durante l'esame.

***Parole chiave:** oli eterici, pollame in allevamento, peso corporeo, caratteristiche sensoriche della carne di pollo*

pends on internal and external factors like: nutritive status of animals, exposure to infections, compatibility with other components of feed and environmental influences (Lee, 2002). Using the combination of essential oil components, most of these negative effects can be excluded. This might explain better live weight and carcass weight in groups feed with EO components: capsaicin, carvacrol and *cinnamaldehyde* and a bit lower for citrus and fennel.

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Control of malachite green in aquaculture products

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review

Summary

Malachite green (MG) is traditionally used as a triphenylmethane dye in the textile industry, as a pigment and a food additive. In fish breeding, it is used as a very effective fungicide, parasiticide, antiprotozoic and bacteriocide. In fish, MG is metabolised to leucomalachite green (LMG) which, due to its lipophilic properties, is retained in fat tissues over longer periods of time. Numerous in vitro and in vivo studies have indicated the cytotoxic, carcinogenic, mutagenic and teratogenic properties of both MG and LMG. For this reason, the use of MG is prohibited in animal species intended for human consumption in the US and EU Member States. Despite this ban, MG is still in use in intensive fish farming, and residues of MG and LMG are the most frequently prohibited substances found in aquaculture products. For that reason, the European Union has prescribed a minimum required performance limit (MRPL) of 2 µg/kg for the methods used for determination of MG and LMG. MG and LMG residues in fish tissue are quantified using liquid chromatography and liquid chromatography with tandem mass spectrometry. Despite the ban in EU Member States, increased concentrations of MG and LMG are systematically found in all species of fish and fish products. In the period from 2002 to 2011, the Rapid Alert System for Food and Feed (RASFF) confirmed increased MG and LMG concentrations in 123 samples of fish and fish products. The highest number, 50 samples, was reported in 2005. Of the total number of positive samples, 27 samples originated from Vietnam, 12 from Indonesia, 10 from China and 3 from Thailand, i.e. 58.5% of samples with residues originated in Asia. Therefore, controls of MG and LMG are important to protect consumer health.

***Key words:** malachite green, leucomalahite green, fish, aquaculture*

Introduction

Malachite green (MG) is traditionally and extensively used as a triphenylmethane dye in the textile industry, a colouring agent and a food additive (Singh et al., 2011). Traditionally, it was used as a dye for materials such as silk, leather and paper. Millions of kilograms of MG and related triphenylmethane dyes are produced for this purpose annually. Malachite green has been determined in a large number of various food types in India, with a greater presence in rural areas than in urban food shops (Tripathi et al., 2007).

In intensive fish production, malachite green is used as a very efficacious fungicide, parasiticide, antiprotozic and bacteriocide (Cha et al.; Van de Riet et al., 2005; Yang et al., 2007). Due to its effectiveness and relatively low cost, it is an attractive agent for treating fish in closed farm systems such as fish ponds and lakes, and for fresh, brackish and salt water aquaria. It is lethal for all marine and freshwater invertebrates, algae and plants.

Due to its teratogenic and carcinogenic properties, MG was prohibited for use in animals intended for human consumption in the United States in 1991 (Marking et al., 1994) and in the European Union in 1997 (EC, 1990). Despite the ban, MG is still used in food production, and residues of MG and its metabolite, lecuomalachite green (LMG) are the most common prohibited compounds found in aquaculture products (VRC 2001-2010; Olesen, 2007).

The residues found in farmed fish products may also originate from environmental pollution due to dyestuff discharged into streams without pretreatment (Pourreza & Elhami, 2007). Therefore, surveillance of malachite green and leucomalachite green in aquaculture products is necessary for the purpose of human health protection.

Structure and mechanism of activity of malachite green

The MG molecule (Figure 1), 4-[(4-dimethylaminophenyl)phenyl-methyl]-*N*,*N*-dimethylaniline, is active in its oxidated form and inactive in the form of the non-chromophorous molecule LMG (Figuer 2).

In fish tissue, malachite green is rapidly metabolized to leucomalachite green and it is primarily in this form that it is retained in fish tissues (Henderson et al., 1997). Due to its lipophilic nature, LMG is retained in fatty tissues over long time periods (Stammati et al., 2005; Mitrowska et al., 2008).

In a study on catfish (*Ictalurus punctatus*), malachite green was added in a water tank in a concentration of 0.8 mg kg^{-1}. Fish were exposed for 1 hour and then rinsed and relocated to a tank with water flow. MG concentrations were determined in all tissues, and were found to be highest in fatty

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tissue and lowest in muscle tissue and in plasma. MG concentrations were no longer measurable after 14 days, though leucomalachite green was detected for more than 42 days (Plakas et al., 1996). In a study on eels (*Anguilla anguilla),* malachite green was added in a concentration of 0.1 mg L^{-1} for 24 hours and MG was detectable even 80 days after exposure (Bergwerff et al., 2004). The concentration of LMG on the first day after treatment was 831 µg kg^{-1} but continually declined until the end of treatment, when 15 µg kg^{-1} was measured on day 100 after treatment. LMG can even be measured in fish raised from eggs treated with MG as a fungicide (Meinertz et al., 1995).

The mechanism of activity of MG on bacterial cells is still not known. However, it is assumed that it acts as a respiratory poison, inhibiting the production of energy necessary for vital metabolic processes. The second assumption is based on the disturbance of replicatory processes of the DNA molecule due to the ability to intercalate in the intramolecular DNA space and its interaction with the phosphate backbone and nucleotides (Renwick et al., 2010).

Toxicology of malachite green and leucomalachite green

Over the past three decades, a series of *in vitro* and *in vivo* studies of MG have been carried out to determine its cytotoxicity and potential carcinogenic, mutagenic and teratogenic properties. A number of toxicological studies have been carried out, applying MG to mice and rats (Meyer and Jorgenson, 1983; Clemmensen et al., 1984; Rao and Fernandes, 1996). Acute oral toxicity of MG has been established with an LD_{50} in two species of rats of 275 and 520 mg/kg B.W. (Meyer and Jorgenson, 1983).

In vitro studies confirmed that MG shows strong cytotoxicity for bacterial cells and mammal cells (Clemmensen et al., 1984; Fessard et al., 1999). Malachite green reduces the capacity for cell proliferation and reduces mitochondrial activity, though this was not detected for LMG (Stammati et al., 2005; Olesen, 2007).

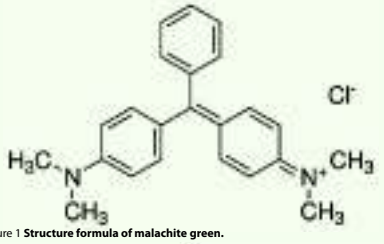

Figure 1 **Structure formula of malachite green.**

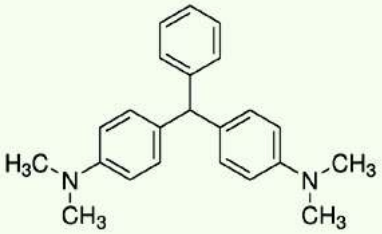

Figure 2. **Structure formula of leucomalachite green.**

Several studies have indicated that MG has both mutagenic and teratogenic properties (Culp et al., 2002; Mittelstaedt et al., 2004). *In vitro* testing on hamster cells showed that MG incited chromosome damage and can cause errors in the regulation mechanism that controls cell development (Rao et al., 2001). The application of MG to rat drinking water in doses of 1.88, 3.75 and 7.5 mg/kg B.W./day caused an increase in N-nitrozodiethylamine induced preneoplastic lesions in the liver at the lowest applied concentration (Rao and Fernandes, 1996). Administering MG and LMG to mice caused changes to DNA that increased proportionally with the dose (Culp et al., 1999). Also, the mutagenic impact was established with the administration of LMG to mice at a maximum dose of 61.2 mg/kg B.W./day over 16 weeks (Mittelstaedt et al., 2004). Recent studies indicate that LMG has greater mutagenic and carcinogenic properties (Culp et al., 2002; Mittelstaedt et al., 2004). The administration of MG and LMG to rats led to the creation of adenoma cells of the follicles of the thyroid gland, hepatocellular adenoma, mammary gland adenoma and adenoma of the interstitial testicular cells (Culp et al., 2006).

MG also proved to have teratogenic effects in gravid rabbits, with an increased incidence of foetal anomalies (Meyer et al., 1983). An impact was also established on the reduced growth, i.e. loss of rabbit body mass.

It has also been determined that both LMG and MG inhibit the homeostasis of thyroid gland hormones. In cases of chronic inhibition of hormone synthesis can cause the emergence of follicular thyroid tumours (Doerge et al., 1998).

Methods of controlling malachite green and leucomalachite green residues

Due to the above described potential effects, the European Union has prescribed the a minimum required performance limit for the determination of MG and LMG of 2 µg/kg, which represents the minimum concentration of MG and LMG that the applied method is required to quantify in the sample (EC, 2004). In the literature, only several procedures for the determination of MG and LMG in animal tissues have been published. Liquid chromatography with detection in the visible spectrum is traditionally used to determine these compounds, and ensuring a limit of detection below 2 µg/kg for each substance (Plakas et al., 1995; Tarbin et al., 1998; Bergwerff and Scherpenisse, 2003; Mitrowska et al., 2005). Today, more complex methods have been developed with greater sensitivity and a greater confirmation character so as to meet the strict legal requirements. Therefore, MG and LMG are quantified in fish tissue using the method of liquid chromatography and liquid chromatography with tandem mass spectrometry (LC-MS/MS). These two methods achieve limits of detection (LOD) of 1.0 and 0.1 µg/kg (Van de Riet et al., 2005; Andersen et al., 2006; Tao et al., 2011). For the purpose of monitoring, fast, specific and sensitive immunoenzyme analyses are used (Yang et al., 2007; Xing et al., 2009).

The ionic nature of MG contributes to the detection by mass spectrometry and improves sensitivity in the use of electrospray ionization (ESI). The technique of atmospheric pressure photoionization (APPI) is considered to be more suitable for the analysis of LMG that electrospray ionization (ESI) and achieved twice the sensitivity for LMG in fish extracts and greater robustness for the matrix components and ion suppression in comparison to methods using the ESI technique (Bergwerff and Scherpenisse, 2003). In order to improve the sensitivity of the method in the use of ESI ion sources, it is necessary to ionize the LMG molecule in the parent molecule after the chromatographic separation. This is most often conducted with the addition of lead (IV) oxide (PbO_2) in the eluent, usually in an oxidation reactor that is serially connected to the entire system. Regardless of whether LMG is oxidized off-line or serially, the sensitivity of the signal for LMG must be less than or equal to the signal for MG.

Extracting MG from homogenised fish tissue is carried out using a mixture of McIlvaine buffer (pH 3) and acetonitrile, after which solid phase extraction is carried out using cationic exchange (columns filled with aromatic sulphuric acid), which separates the MG from the weakly polar tissue components (Bergwerff and Scherpenisse, 2003). For the purpose of reducing MG molecule demethylation, ascorbic acid with N,N,N′,N′-tetramethyl-1,4-phenylendiamine-2HCl is added. After post-column oxidation with PbO_2, samples are analysed using LC-UV_{620} and LC-MS/MS (ESI in positive ionization mode) and limits of determination are achieved for MG and LMG of 1 µg/kg for the LC-UV_{620} and 0.2 µg/kg for LC-MS/MS (Bergwerff and Scherpenisse 2003). Oxidation of LMG into MG can also be achieved by adding 2,3-dichlor-5,6-dicyano-1,4-benzoquinone, which has the advantage of not requiring the use of an oxidation reactor (Andersen et al., 2006). The achieved limit of detection for the liquid chromatography LC-UV_{618} is 1 µg/kg.

Residues in samples of fish and fish products

In considering the potential intended use of MG and the likelihood of pollution of water courses, control of MG and LMG is essential in aquaculture products. Despite the ban on their use in the European Union Member States, increased concentrations of MG and LMG are systematically found in all types of fish and fish products. In Great Britain, increased concentrations of MG and LMG were established in samples of farmed fish from 2001 to 2010 (VRC, 2001–2010). The most samples with increased concentrations were determined in 2001, 2002 and 2003, i.e. 16 of 99, 14 of 141 and 7 of 168, respectively. The highest concentrations determined were: 35 µg/kg MZ in salmon muscle tissue and 500 µg/kg LMG in trout muscle tissue. In the period 2004–2010, increased concentrations were detected in only seven samples. The results were achieved by taking strict control measures in fish farms where high concentrations of these two compounds were determined.

In Denmark, the following concentrations of LMG were determined: 4 samples < 4 ug/kg in 2000, 1 sample of 28 ug/kg in 2003 and 1 sample of 2.7 ug/kg in 2005 (Olesen, 2007). Also, concentrations of LMG greater than 100 µg/kg were measured in 2003 in 19 samples of eel originating from China. In 2005, 2 imported samples had LMG concentrations of 5.6 and 6.1 µg/kg.

Introduction of the Rapid Alert System for Food and Feed (RASFF) in the European Union Member States confirmed increased concentrations of MG and LMG in 123 different types of fish and fish products in the period from 2002 to 2011 (RASFF, 2011). The RASFF system is used to report positive findings of products reaching the European Union market. The highest number of samples (50) with increased concentrations of MG and LMG was recorded in 2005 (Table 1). Of these, the majority (21) of positive samples were of nursehounds originating from Vietnam. The high-

est reported concentrations of MG and LMG in individual types of fish and fish products are shown in Table 2. Of the total number of samples, 47 samples originated from Vietnam, 12 from Indonesia, 10 from China and 3 from Thailand, i.e. 58.5% of all positive samples originated from Asia. By species, the largest number of positive samples was as follows: 37 iridescent shark (*Pangasius hypophthalmus*), 23 eel (*Anguilla anguilla*), 15 tilapia (*Oreochromis niloticus*) and 15 salmon (*Salmo salar*) samples.

The measured concentrations of MG were in the range from 0.3 µg/kg to the maximum value of 4872 µg/kg measured in 2006 in a sample of eel originating from The Netherlands. LMG concentrations ranged from 1.08 µg/kg to the maximum 5680 µg/kg determined in 2006 in an eel sample in Poland that originated from Indonesia. In Great Britain in 2010, a LMG concentration of 20 µg/kg was measured in frozen tilapia sticks originating from China (RASFF, 2011). The following number of samples with increased concentrations of MG and LMG were determined in these EU Member States: 37 in Great Britain, 19 in Germany, 14 in Poland, 12 in Estonia, 11 in The Netherlands, 9 in Belgium, 6 in Greece and 4 in Czech Republic and Denmark.

In the Republic of Croatia, carp and trout are farmed in freshwater, while sea bass, sea bream and tuna are farmed in the sea. The total annual production of fish products is over 16 thousand tonnes. Control of MG residues is carried out in Croatia, and the results to date do not indicate a cause for concern for consumers (Bilandžić et al., 2012).

The results shown indicate that controlling MG and LMG concentrations is of primary importance for protecting consumer health.

Conclusions

Due to its effectiveness as a fungicide, parasiticide, antiprotozoic and bacteriocide and its relatively low cost, malachite green is considered to be an attractive agent in the breeding of fish in closed systems, such as fish ponds and lakes and in fresh, brackish and salt water aquaria. However, due to its confirmed cytotoxicity, teratogenic and carcinogenic properties, its use has been prohibited in the European Union on animal species intended for human consumption. Despite the ban, malachite green is still used in food production, and residues of this compound and its metabolite, leucomalachite green, are the most commonly detected prohibited substances in aquaculture products.

Due to the above described effects, a minimum required detection limit of the method of 2 µg/kg has been prescribed in the European Union. Today, controls of residues of these two compounds in fish tissues are conducted using the methods of liquid chromatography and high sensitivity liquid chromatography tandem mass spectrometry.

Over the past decade, increased concentrations of these two compounds have been systematically detected in the European Union Member States in all types of fish and fish products. Introduction of the Rapid Alert System for Food and Feed (RASFF) confirmed increased concentrations of malachite green and leucomalachite green in 123 samples. Of the total number of positive samples, 58.5% originated from Asian countries. The highest number of positive samples was found in iridescent shark, eel, tilapia and salmon. In accordance with the above, controlling residues of malachite green and leucomalachite green in aquaculture products is essential in order to ensure food safety.

Table 1. **Number and samples with elevated concentrations of malachite green and leucomalachite green reported by RASFF in period 2002 to 2011.**

	Number of samples with elevated MG and LMG concentration										
Fish and fish products	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	Total
eel	1		1	12	7	2					23
catfish				21	8	5		1	1	1	37
tilapia			6	5	1	2			1		15
rainbow trout	1		2	3	1	1	1	2		3	14
salmon		11	2	1					1		15
cultered caviar										1	1
shrimp tails									1		1
mudfish								1			1
yellow catfish				1			1				12
black cat fish			1								1
milkfish			1	3							4
red tail tinfoil barb			1	1							2
barramundi				1							1
yellowtail kingfish			3								3
trout eggs			1							1	2
other				2							2
Total	2	11	18	50	17	10	2	4	4	5	123

Table 2. **Highest concentrations of MG and LMG reported by RASFF in period from 2002 to 2011.**

Year	Determined in EU country	Country of product origin	Fish and fish products	Concentration of malachite green (MG) and leucomalachite green (LMG) (µg/kg)
2010	Great Britain	China	tilapia (*Oreochromis niloticus*)	LMG 20
2007	Denmark	China	eel (*Anguilla anguilla*)	LMG 330
2006	Poland	Indonezija	eel (*Anguilla anguilla*)	MG 8,4 LMG 409,4
2006	Poland	Indonezija	eel (*Anguilla anguilla*)	LMG 38.5; 111,2; 5680
2005	Great Britan	Malaysia	barramundi (*Lates calcarifer*)	MG 12 LMG 416
2005	Germany	Netherlands	eel (*Anguilla anguilla*)	MG 2035; 4872
2005	Germany	Sweden	trout eggs	MG 579; 619
2004	Germany	Germany	eel (*Anguilla anguilla*)	MG 5 – 70
2004	Great Britan	Great Britan	tilapia (*Oreochromis mossambicus*)	MG 12 LMG 86
2002	Germany	Germany	eel (*Anguilla anguilla*)	MG 384; 39,4; 524

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Kontrolle von Malachitgrüne in den Erzeugnissen der Aquakultur

Zusammenfassung

Das Malachitgrüne (MG) wird traditionell als Tryphenilmetanfarbe in Textilindustrie, als Pigment und als Nahrungsadditiv benutzt. In der Fischzucht wird es als wirkungsvolles Fungizid, Parisitizid, Antiprotosoan und Bakteriozid benutzt. Im Fischorganismus metabolisiert sich das MG in Leukomalachitgrüne (LMG), das wegen seiner lipophylen Eigenschaften eine längere Zeit im Fettgewebe anhält. Zahlreiche Untersuchungen in vitro und in vivo zeigten zytotoksische, kanzerogene, mutagene und teratogene Eigenschaften von MG und LMG vor. Deshalb ist die Anwendung von MG verboten bei Tierarten, die für die menschliche Nahrung bestimmt sind, u.zw. in den USA und in der EU. Trotzdem wird das MG immer noch in der intensiven Fischerei benutzt, so befinden sich Reste von MG und LMG am häufigsten in Inzidenz der nicht erlaubten Substanzen in den Erzeugnissen der Aquakultur. Die EU hat deshalb die Grenze der mindest erforderlichen Wirksamkeit der Methodendurchführung MRPL (engl. minimum required performance limit) von 2 µg/kg für die Bestimmung von MG und LMG vorgeschrieben.Heutzutage werden für die Quantifikation von MG und LMG im Fischgewebe Methoden der Flüssigkeitschromatographie und Flüssigkeitschromatographie der Tandemspektometrie der Massen verwendet. Trotz des Verbotes in den Ländern der EU werden systematisch erhöhte Konzentrationen von MG und LMG in allen Fischen und Fischerzeugnissen vorgefunden. In der Zeitspanne von 2002 und 2011 wurden durch die schnelle Warnung für Nahrung und Tiernahrung RASFF (engl. Rapid Alert System for Food und Feed) erhöhte Konzentrationen von MG und LMG bei 123 Fischarten und Fischerzeugnissproben vorgefunden. Die höchste Zahl von 50 Proben wurde im Jahr 2005 notiert. In der Gesamtzahl der Muster waren 47 Muster aus Vietnam, 12 aus Indonesien, 10 aus China und 3 aus Thailand, bzw. 58,5 % der Muster stammte aus Asien. Demzufolge ist die Kontrolle der MG und LMG außerordentlich wichtig für den Gesundheitsschutz der Verbraucher.

Schlüsselwörter: *Malachitgrüne, Leukomalachitgrüne, Fische, Aquakultur*

Controllo del verde di malachite nei prodotti di acquacoltura

Sommario

Il verde di malachite (VM) di solito si usa come il colore trefenilmetanico nell'industria tessile, come il pigmento e anche come l'additivo alimentare. Nell'allevamento di pesci viene usato come un fungicido molto efficiente, parasitícide, antiprotosoico e batterocido. Nell'organismo del pesce il VM si metabolizza nel verde leucomalachite (VLM) che, per le sue caratteristiche lipofili, si mantiene nel tessuto grasso per un periodo più lungo. Numerose ricerche in vitro hanno dimostrato le caratteristiche citotossiche, cancerogene, mutagene e taretogene del VM e del VLM. Perciò l'uso del VM è proibito dalle sorte alimentari destinate all'alimentazione umana negli Stati Uniti e nell'Unione europea. Nonostante questa proibizione il VM si usa ancora nell'intensa pesca negli stagni ed i residui di VM e VLM appaiono più spesso nell'incidenza delle materie non permesse nei prodotti dell'acquacoltura. Perciò l'Unione europea ha prescritto il limite della meno richiesta efficienza di esecuzione di metodi (MRPL, in inglese minimum required performance limit) di 2 µg/kg per determinare i VM e VLM. Oggi per quantificare i residui di VM e VLM nei tessuti del pesce si applicano i metodi della cromatografia di liquidi e della cromatografia di liquidi di spettrometria delle masse. Nonostante il divieto dell'uso negli paesi dell'Unione europa si scoprono sistematicamente le percentuali aumentate dei VM e VLM in tutti i tipi del pesce e dei prodotti di pesce. Nel periodo dal 2002 al 2011 tramite il sistema dell'allarme urgente per gli alimentari e gli alimentari per gli animali (RASFF, in inglese Rapid Alert System for Food and Feed) le percentuali aumentate dei VM e VLM sono state determinate in 123 campioni del pesce e dei prodotti di pesce. Il numero più grande di 50 campioni è stato registrato nel 2005. Dal numero totale di campioni positivi 47 di loro sono di Vietnam, 12 di Indonesia, 10 di Cina e 3 di Tailanda, cioè il 58,5% di campioni provengono dall'Asia. Da questo segue la conclusione che il controllo dei VM e VLM è importantissimo per la protezione sanitaria di consumatori.

Parole chiave: *verde di malachite, verde leucomalachite, pesce, acquacoltura*

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Farming of mussels (*Mytilus galloprovincialis*) as safe food

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review

Summary

Shellfish and crab production is an ancient, traditional trade, which has lately become a profitable industry connected with tourism. In Croatia some 3 000 tons of mussels are produced annually and sold exclusively on the domestic market, as delicious quality food with singular sensory traits and high quality proteins. Mussels harvested for the domestic market meet the requirements of the Regulation on the hygiene of food of animal origin (NN 97/2007) and as such are delivered to dispatch centers, where they are packaged. In addition to monitoring in production areas and relaying areas, a monitoring system which includes laboratory testing has also been set up for food business operators, with the aim of establishing whether they meet the requirements for the final product in all phases of production and distribution. Apart from these control measures, it is also important to maintain good hygiene practices (and the HACCP system) during transportation and storage of shellfish, with the aim of protecting consumer health.

Keywords: *mussels, monitoring*

Introduction

Shellfish and crab production is an ancient, traditional trade, which has lately become a profitable industry connected with tourism. In Croatia some 3 000 tons of mussels are produced annually and sold exclusively on the domestic market. What makes shellfish special is the fact that they are sold live and are the most valued as live, rather than thermally processed, with the meat separated from the shell. Live shellfish are a delicacy, eaten raw or only slightly thermally processed.

Mussel Farming and Harvesting

All shellfish, including mussels, are very good for human nutrition because they contain high quality proteins and have distinctive sensory traits. Due to the constantly increasing exploitation of natural sources of bivalve shellfish, the density in natural habitats is significantly reduced and it is now an imperative to intervene by creating farming areas. The coastal areas of estuaries are the most productive and are being densely populated by shellfish for use in the food industry. Shellfish are usually bred on vertical lines, the so - called pergolari, and in baskets. Collectors may collect and harvest live shellfish only in production areas which have defined location and boundaries, and have been classified by an authorized body into classes A, B or C. Shellfish are harvested when they have reached commercial size, which depends, among other things, on the farming method. The vertical lines ('pergolari') and baskets are drawn out of the sea and shellfish are 'harvested', then washed in pure sea water to remove slime and algae, and sent to dispatch centers. Shellfish living at the bottom of the sea are harvested by special fishing tools (rapido trawls, mussel rakes, dredges) dragged along the sea bottom, or by divers.

Production areas have to be classified in accordance with the Regulation on the official control of food of animal origin (Anon., 2007, c). Shellfish harvested in a class A production area may be directly transported to dispatch centers, whereas those harvested in a class B area may be placed on the market for human consumption only after processing in a depuration facility or a relaying center. Shellfish from a class C harvesting area may be placed on the market only after relaying over a long period.

Dispatch Centers and Depuration Centers

Harvested shellfish to be placed on the market are transported to dispatch centers and packaged there. Dispatch centers may process only shellfish which meet the requirements of the Regulation on the hygiene of food of animal origin (Anon., 2007, b), i.e. shellfish from the class A harvesting area or from a relaying area or a depuration center or another dispatch center.

Shellfish which have to be purifi-

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