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DIFFERENTIATION OF SCOMBROIDS BY PCR-BASED DNA ANALYSIS OF THE CYTOCHROME B AND PARVALBUMIN GENE

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DNA-based seafood authenticity methods are predominantly performed by PCR analysis. Scombroidae fish family contains several species with high values of commercialization, which are *Thunnus obesus, T. albacares, T. maccoyii, T. tonggol, T. alalunga, Katsuwonus pelamis, Auxis spp.*, and *Scomberomorus spp.* among others. Furthermore, most of world Tuna's supplies are captured in western central Pacific Ocean. However, Indo-west Pacific Ocean part urgently needs more concern in effective tuna fisheries management. On the other hand, an appropriate method to differentiate between closely related Thunnus species is continuously questionable either by genetic or morphologic identification. An accurate method of Thunnus species differentiation will support sustainable fishery and trade of tunas. Our present studies comprise:
(1) fish species identification using mitochondrial cytochrome b gene;
(2) differentiation of Scombroid fish from Indo-west Pacific and Indian Ocean by exon-primed intron-crossing (EPIC) PCR of a parvalbumin gene intron;
(3) new information about applicability of SSCP and RFLP technique to discriminate Thunnus species.
Using cytochrome b gene as an identification marker we could determine a number of SNP characteristics and SSCP electropherogram results within Scombroid group. Species-specific EPIC primers were successfully constructed and amplified from the third and fourth intron of parvalbumin gene. Moreover, SSCP and RFLP electropherogram results from parvalbumin gene intron show reliable differentiation of closely related Thunnus species. Therefore, these results showed potentially reliable species identification methods for high-priced tuna's products as well as other Scombroid fish. Difficulties and limitation of Thunnus spp. reliable identification are discussed.

Keywords: *Parvalbumin gene, EPIC–PCR, cytochrome b gene, Thunnus, species identification.*

A-14
UPLC ANALYSIS OF BIOGENIC AMINES IN DIFFERENT CHEESE VARIETIES

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High concentrations of biogenic amines can be found due to microbial activity intrinsic to typical fermented foods such as wine, fermented meat and especially cheese. During cheese ripening, accumulated free amino acids may act as precursors for the conversion into biogenic amines mostly affected by bacterial decarboxylases of a contaminating microflora. Thus, biogenic amines in foods are of main concern in relation to food spoilage/hygiene and food safety aspects. Considering the toxicological implications of these amines, and the general interest in occurrence data for "risk assessment" of fermented foods, the objective of this study was to analyse the concentration of biogenic amines in various commercial cheese samples (n=151) representing most common cheese varieties. AccQ-Fluor derivatizing reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) was used to analyze primary and secondary biogenic amines by ultra-performance liquid chromatography (UPLC). In general, cumulative levels of biogenic amines varied to a great extent with exceptional samples having amounts up to 150–300 mg/100 g cheese (e.g., Tiroler Graukäse with 313 mg/100g or Tiroler Almkäse with 185 g/100 g), whereas only 5% of the analyzed cheeses showed total concentrations higher than 90 mg/100 g (median 5.7 mg/100 g). Regarding the most relevant biogenic amines, histamine was found in 79% of all samples, with maximum concentrations for Tiroler Almkäse (116 and 82 mg/100 g), but only 5% of the cheeses had a histamine level above 17 mg/100 g (median 0.9 mg/100 g). For tyramine (72% occurrence; 5% > 37 mg/100 g), highest values were found for Tiroler Graukäse (160 mg/100 g), Tiroler Almkäse, French raw milk cheese, Olmützer Quargel or Harzer cheese (each ~50 mg/100 g; median 1.0 mg/100 g). Putrescine was detected in 70% of the cheeses (up to 80 mg/100 g for some acid-curd cheeses; median 0.6 mg/100 g; 5% > 26 mg/100 g). Cadaverine was found in 47% of the samples (5% > 22 mg/100 g), with highest concentrations for Harzer cheese and Olmützer Quargel (126 and 75 mg/100g, median 0.2 mg/100 g). Tryptamine had the lowest occurrence (15%; 5% > 8 mg/100 g) and a median concentration of 0.3 mg/100 g. In conclusion, high (and toxicologically critical) levels of biogenic amines are definitely not associated with a certain type of cheese (as it is sometimes reported in former literature), but may vary depending on a large number of different incalculable factors (e.g., hygiene during the whole cheese production process, number and class of contaminants, degree of proteolysis in cheese, uncontrolled technological aspects). For all analyzed cheeses, both the individual and the total amounts varied greatly, making it virtually impossible/inadequate to pinpoint certain cheese types as more potent sources for intrinsically high biogenic amine levels. Thus, obligatory monitoring of biogenic amines should be considered to ensure high quality and safety of cheese products in future.

Keywords: *Biogenic amines, cheese varieties, UPLC, AQC derivatives*