MICROBIAL SPOILAGE IN FOODS

INSTRUMENTAL HEADSPACE ANALYSIS FOR ASSESSMENT OF SPOILAGE IN PACKAGED AND STORED RAW SALMON

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Over the years, a great number of methods have been proposed for assessing fish spoilage. Despite the intensive effort to find a rapid and reliable microbiological, chemical, or instrumental method, assessment of freshness or spoilage in the seafood industry has traditionally relied on trained panels or trained inspectors to judge the quality of fish during refrigerated storage. While trained panels have previously been employed to measure spoilage of inoculated *Clostridium botulinum* fish samples, these panels were limited in the number of panelists (2-5 members) due to the constraints that individuals be immunized. More recently, the FDA Institutional Review Board, who reviews studies involving human participants as subjects, has ruled that even immunized trained panelists should not be permitted to smell botulinum inoculated fish samples due to the potential risks of inhaling aerosolized spores or toxin. These imposed constraints require that spoilage studies of *C. botulinum* inoculated fish samples be monitored by chemical or instrumental analyses. Currently within our lab, studies are addressing the potential for toxin development to precede spoilage in a high-fat fish product, salmon, when packaged using films of varying permeabilities (oxygen transmission rates (OTR) of 3, 3,000, 10,000, and 15,000 cc/m^2/24 h at 24°C), different atmospheres (air, vacuum, and modified atmosphere (MA)), and different storage temperatures (4, 8, 12, and 16°C). Consequently, a critical component to these *C. botulinum*-inoculated studies has been to use an ultra-fast gas chromatography instrument called the Znose®, to collect, separate, and quantify individual headspace volatiles, in an effort to identify one or more chemical markers that could be associated with spoilage. In a follow-up study, uninoculated raw salmon was stored for varying periods and presented to a consumer panel to correlate quantities of each volatile peak with their acceptance or rejection of the product.

Several volatiles were generated during storage of *C. botulinum*-inoculated and uninoculated raw salmon, but the dominant volatiles present varied with the package atmosphere, storage temperature, storage time, and presence of *C. botulinum*. The dominant volatile produced under aerobic conditions for uninoculated salmon samples stored at 4, 8, and 12°C had a Kovats Index (KI) of 753 and was tentatively identified as 2- or 3-methyl 1-butanol. No difference in quantity of this volatile occurred during 4 or 16°C aerobic storage of salmon packaged with the 4 different OTR films. Film permeability did significantly affect the size of the KI-753 volatile peak during vacuum and MA storage at 4, 8, and 16°C with the smallest quantities in each atmosphere being produced in salmon packaged with the non-permeable film (OTR of 3). Interestingly, *C. botulinum*-inoculated vacuum samples at both 4 and 8°C had reduced generation of the KI-753 volatile.

Two other volatile peaks were also generated under aerobic conditions and had KIs of 640 and 720. The latter peak was tentatively identified as 3-hydroxy-2-butanone and appeared to become more dominant when samples were stored at higher temperatures. Several peaks (KIs of 806, 975, 1200 and 1465) were evident only when salmon was stored at 12 or 16°C with greater quantities of KI-975, KI-1200, & KI-1465 volatiles being generated during storage for samples packaged in lower OTR films. For inoculated samples stored at 12 and 16°C, a peak with a KI of 688 dominated for most film types under either vacuum or MA.

In the follow-up storage study, 53 elderly consumers (60-85 years of age) participated in a consumer panel by evaluating 20 uninoculated raw salmon samples stored for varying periods of time. Using a hedonic scale, overall, appearance, and aroma acceptance of each product was judged by the panelists. In addition, panelists were asked whether they would prepare the product in their kitchen given they had already purchased the product. Following evaluation by the panelists, the headspace of these samples was analyzed using the zNose and areas of 12 peaks were compared to acceptance ratings. A regression model incorporating both the KI-753 and KI-640 volatiles was significant and accounted for 56% of the variability in the aroma scores. Aroma and overall scores of 2-3 (dislike very much – dislike moderately) did not translate into unwillingness to prepare the sample for up to 7 panelists. Based on their household practices, however, only one of those panelists would ever store the fish for more than one day and hence could likely encounter extremely spoiled fish. In summary, two key volatiles were related to consumer perception. Very high levels of those volatiles were necessary before all panelists would reject the sample.

COMBINATIONS OF ANTIMYCOTICS TO INHIBIT THE GROWTH OF MOLDS CAPABLE OF PRODUCING 1,3-PENTADIENE

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Raw and pasteurized foods and beverages as well as products subjected to more rigorous thermal processes, e.g., hot-fill beverages, can contain a wide range of molds. These molds may grow during the expected shelf life of these products to cause visual spoilage or result in the production of volatile compounds that are offensive to the consumer. Some of the molds known to grow in the presence of potassium sorbate or survive thermal processes commercially applied to foods and beverages can also produce mycotoxins, thereby posing a public health concern. Degradation of sorbate through decarboxylation by some strains of penicillia can result in the accumulation of 1,3-pentadiene, a volatile compound having an odor described as being similar to that of kerosene, acrylic paint, or petroleum products. Other molds that may also degrade sorbate include *Aspergillus, Fusarium, Mucor, Geotrichum*, and *Trichoderma* species. Strains of yeasts belonging to *Zygosaccharomyces rouxii* and *Debaryomyces hansenii* are also capable of spoiling sorbate-containing high-sugar foods by producing 1,3-pentadiene. While the control of sorbate-resistant molds and yeasts in some types of foods and beverages may be achievable through the addition of high concentrations of the preservative, the adverse effect of off aromas and off flavors that may result make this approach impractical. Instead, the use of antimycotics other than sorbate, e.g., natamycin, ethylenediaminetetracetic acid, and propionate, or a low concentration of sorbate in combination with other antimycotics may be an alternative to prevent or retard the growth of 1,3-pentadiene-producing molds. We did a study to evaluate potassium sorbate, sodium benzoate, calcium propionate, disodium ethylenediaminetetraacetic acid (EDTA), and natamycin, alone and in combination, for their effectiveness in preventing the growth of five molds isolated from Parmesan cheese and a lemon-flavored drink subjectively judged to contain 1,3-pentadiene. Growth of *Penicillium brevicompactum, Penicillium roqueforti, Paecilomyces variotii, Aspergillus niger*, and *Cephaloascus fragrans* on model agar media containing Parmesan cheese (PRM agar) (pH 5.5) and lemon-flavored drink (LD agar) (pH 2.6) supplemented with antimycotics was studied. All molds grew well at 21ºC on PRM agar containing potassium sorbate (3,500 µg/ml), calcium propionate (3,000 µg/ml), or natamycin (20 µg/ml). Combinations of potassium sorbate (250 - 1,000 µg/ml), calcium propionate (250 - 1,000 µg/ml), and/or natamycin (10 - 18 µg/ml) greatly inhibited or prevented growth of molds on PRM agar, indicating their potential as preservative systems at pH values resulting in large percentages of the acids in dissociated forms. Three of the five molds grew on LD agar containing potassium sorbate or sodium benzoate at a concentration of 200 µg/ml. Growth did not occur within 70 days on LD agar containing EDTA (30 µg/ml) in combination with potassium sorbate and sodium benzoate at 50 and 175 µg/ml, respectively, or 175 and 50 µg/ml, respectively. Results of this study show that preservative systems containing a reduced concentration of potassium sorbate, in combination with other antimycotics, particularly natamycin, have potential for controlling the growth of molds thought to be capable of producing 1,3-pentadiene.