Automated Gas Chromatographic Method for the Determination of Ethanol in Canned Salmon

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A method has been developed for the determination of ethanol in canned salmon using automated headspace sampling in conjunction with analysis by gas chromatography. The thermal process for the commercial sterilization of canned salmon is shown to provide an effective extraction of the ethanol so that the fluid removed from the can may be used as the analytical sample with minimal preparation prior to analysis. Ethanol content is measured directly, without the need for an internal standard, by either GC/MS or GC/FID. The headspace autoanalyzer allows for a rapid determination of ethanol with greater reproducibility than could be obtained with manual injection systems. The GC/MS technique can also provide an advantage in that simultaneous single ion monitoring of the two major ethanol ions provides additional protection from interferences. To assess the applicability of this technique to other substrates, Atlantic sea scallop meats were also successfully analyzed by this technique.

Keywords: *Decomposition; ethanol; salmon*

INTRODUCTION

Previous investigators have shown that ethanol is produced during bacterial decomposition in various fish species (Holaday, 1939; Hillig, 1958; Lerke and Huck, 1977; Hollingworth and Throm, 1982; Kelleher and Zall, 1983; Ahamed and Matches, 1983; Iida et al., 1986). The findings of these researchers have further shown that ethanol can be a useful indicator of the extent of decomposition in a variety of fish species ranging from cod, flounder, and pollock to tuna and salmon. Specific works such as those by Lerke and Huck (1977), Hollingworth and Throm (1982), and Iida et al. (1986) have also shown ethanol to be a useful chemical indicator of quality in processed products such as canned tuna and canned salmon.

Early workers in this field were limited to the use of tedious steam distillation methods. In the 1970s significant improvements arrived with the development of gas chromatographic (GC) methods (Lerke and Huck, 1977; Cosgrove, 1978). The most recently reported advancement in this methodology was the introduction of headspace sampling by Hollingworth and Throm (1983) and Hollingworth et al. (1986). That method forms the basis for the current work.

PROCEDURES

Canned salmon samples representing controlled spoilage increments of Pacific pink salmon (*Oncorhynchus gorbuscha*), prepared under the supervision of Department of Fisheries officials, were used for most of the work reported in this study. Pink salmon were obtained fresh from a single harvest, within 2 days of catch. Although most of the fish were still in rigor, each fish to be used in the study was individually assessed by trained Department of Fisheries inspectors in accordance with the current Department of Fisheries and Oceans *Standard/Grading Guide for Pacific Salmon* (1991) in order to ensure the batch was as homogeneous as possible prior to the controlled spoilage study. The fish were then held under carefully controlled conditions for a period extending well beyond their normal expected shelf life. One batch of fish was held in slush ice, and another was held at ambient temperatures (~14 °C). At periodic intervals a number of fish were removed, evaluated for quality by Department of Fisheries inspectors, and then processed into a canned product. The fish were packed raw, in two-piece tin-free steel 307 × 115 cans, with only the addition of a salt tablet, as is the customary industry practice. Sterilization was effected in horizontal steam retorts using an approved process (based on the National Food Processors Association, Bulletin 26L, 12th ed.) of 72 min at 244 °F. Samples from these controlled spoilage increments were then utilized to provide a series containing a range of ethanol concentrations that had developed from normal decomposition patterns. To average out the natural variability between fish, a minimum of five cans per increment was analyzed.

To assess the applicability of this technique to other substrates, several increments of controlled spoilage samples that had been prepared from fresh Atlantic sea scallops (*Placopecten magellanicus*) were also analyzed according to this technique. Similar to the first batch of salmon, the scallops were held on ice for 19 days, a period well beyond their normal shelf life, with incremental samples being removed at predetermined intervals over the spoilage period and immediately vacuum packed and frozen in a plate freezer.

Sampling and Sample Preparation. The liquid phase from the canned salmon was drained into a clean beaker or into a 20 mL screw cap vial with Teflon liner if interim storage was necessary. Samples were prepared for analysis by pipetting 5 mL of the aqueous layer into a 20 mL hypovial, being careful to avoid getting any of the lipid material into the hypovial. To this was added 3 g of NaCl, and the hypovial was crimp-sealed with a Teflon/silicon septum. For solid salmon flesh samples and for the scallop meats, an analytical sample was prepared by homogenizing the salmon flesh or scallop meats in a food processor to a uniform paste. Five grams of

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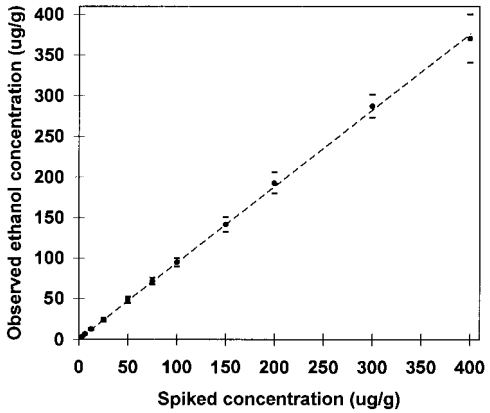

Figure 1. Calibration curve for ethanol spiked in distilled water.

the paste was combined with 3 g of NaCl in a 20 mL hypovial that was then crimp-sealed as above.

Standards. Primary standard grade ethanol (obtained from the National Standards Institute) was used for the preparation of the reference standard. Secondary standard and working standards prepared from 85% ethanol were calibrated against the reference standard. Standard solutions at 5, 10, 20, 30, 40, and 50 μg/g ethanol were prepared in glass-distilled water, and the standard curve was established by using 5 mL of each standard with 3 g of NaCl in hypovials as described above.

Autosampler. An HP-7694 static headspace autosampler with a 44 sample capacity was employed with the following conditions: oven temperature, 65 °C; loop temperature, 75 °C; transfer line temperature, 75 °C; GC cycle time, 15.0 min; vial equilibration, 15.0 min; pressurization, 1.0 min; loop fill time, 0.15 min; loop equilibration time, 0.05 min; injection time, 0.10 min.

Gas Chromatograph. An HP model GCD gas chromatograph interfaced with a mass spectrometer detector and equipped with an HP Innowax, 30 m × 0.25 mm, capillary column, 0.25 μm film thickness, was used. Helium carrier was set at 0.5 mL/min and 1.1 psi at 50 °C. The temperature program was as follows; initial temperature, 32 °C; hold for 4 min; increase temperature at 65 °C/min to 140 °C maximum; hold for 1.3 min to give a 7 min run time. Reset to 32 °C to begin next run.

RESULTS AND DISCUSSION

The calibration curve along with standard deviation (SD) bars is shown in Figure 1. Recoveries of ethanol prepared by spiking known amounts of ethanol standard into distilled water are shown. The results demonstrate a mean recovery of 98.5% (±6.5%) from distilled water, with an r^2 value of 0.9995. The limit of detection was 0.1 μg/g, and the system was seen to remain linear up to the maximum concentration tested (400 μg/g), which is well beyond the area of interest for this application. A smaller scale calibration curve prepared by spiking known amounts of ethanol into a sample of fluid removed from canned pink salmon gave a mean recovery of 96.9% (±7.8%) over the concentration range of 1−120 μg/g.

Reproducibility of results was evaluated from 10 replicate determinations of ethanol in fluids from pink salmon, conducted at three levels of ethanol concentrations. The results shown in Table 1 demonstrate a percent SD of 4.62−4.93 over the ethanol range of 30−90 μg/g, which includes the range where "definitely identifiable decomposition" was identified by Hollingworth and Throm (1982).

To evaluate the usefulness of using the fluid portion of the product for this analysis, both the fluid and solid portions of samples from each spoilage increment were analyzed. The results in Figure 2 show that the ethanol is predominantly partitioned into the fluid phase at all increments. The concentration of ethanol in the fluid phase was on average 73.5% of the combined total (range = 68−83%).

Table 1. Reproducibility Data from 10 Replicate Analyses Conducted at Three Ethanol Concentrations

spike (μg/g)	av found (μg/g)	range (μg/g)	SD	% SD
34	35.4	32.3−38.2	1.74	4.93
51	50.8	47.4−54.4	2.35	4.62
85	83.2	79.3−90.1	3.98	4.79

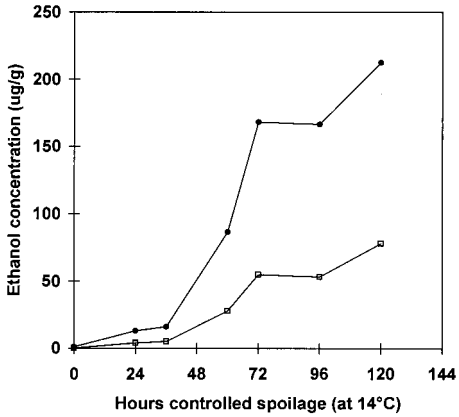

Figure 2. Headspace ethanol concentrations from equal weights of canned pink salmon liquid (●) and meat (□).

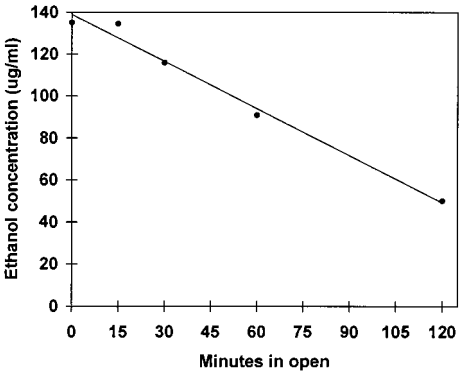

Figure 3. Loss of ethanol from canned salmon liquid upon exposure to room air at various time periods.

Due to the volatile properties of ethanol, a series of determinations was made to evaluate the potential dissipation rate of ethanol after the canned salmon had been opened. A can of salmon from the spoilage run with an initial ethanol concentration of 135 μg/g was opened, and the fluids were analyzed at intervals of up to 2 h of standing uncovered at room temperature (20−22 °C). The results of these measurements, shown in Figure 3, indicate that the ethanol dissipates very quickly once the can is opened, and it is therefore critical to ensure that the sample is transferred to a vial and sealed as quickly as possible. The results showed a loss of ~30% in just 1 h and >50% in 2 h. In a similar test, 15 mL aliquots of ethanol-spiked distilled water (45 μg/g) were placed in 3 in. diameter watch glasses. Ethanol concentrations were measured immediately and then at various intervals, giving results very similar to those obtained with the canned salmon.

The correlation of ethanol concentration with the degree of time/temperature abuse in spoilage incre-

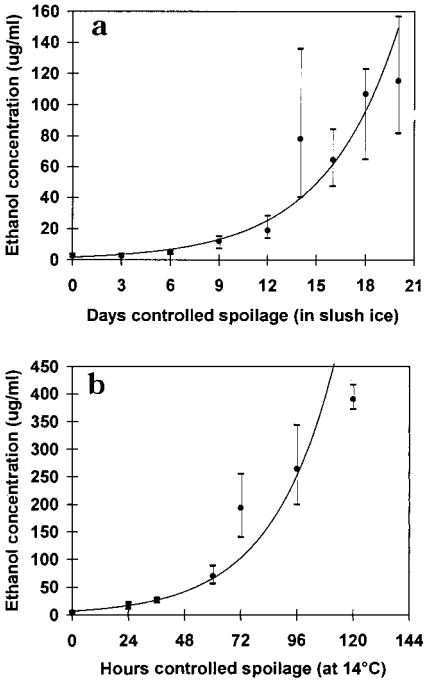

Figure 4. Maximum, minimum, and mean ethanol concentrations for controlled spoilage increments of pink salmon held (a) under slush ice conditions and (b) under ambient conditions (14 °C).

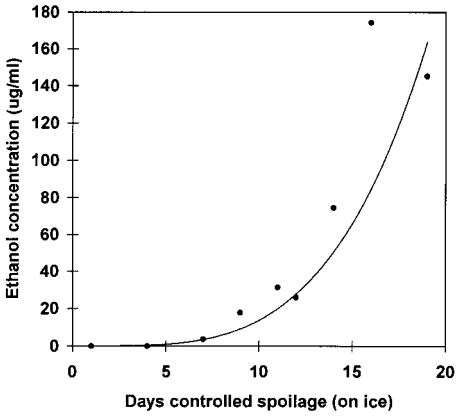

Figure 5. Ethanol concentration for controlled spoilage increments of scallop meats held on ice.

ments of canned pink salmon is shown in Figure 4. The r^2 value for the fish held in slush ice was 0.9517, and for fish held at ambient temperature (14 °C) the r^2 value was 0.9527. The first increments at which significant decomposition would be expected (i.e., 12−14 days in ice and 36−48 h at 14 °C) showed ethanol contents in the 30−50 μg/g range, which fits very well with the previous findings of Hollingworth and Throm (1983). Under both conditions of storage abuse, the development of ethanol showed excellent correlation with the extent of time−temperature abuse of the fish.

To assess whether this method could be employed for solid samples such as fillets or meats, samples from controlled spoilage increments of sea scallops were also analyzed for ethanol content. The results shown in Figure 5 demonstrate that this technique is also applicable to the analysis of such substrates, and the correlation of ethanol concentrations with duration of spoilage had an r^2 value of 0.8471. These results also show the ethanol levels increasing from 20 to 80 μg/g over the increments of 9−14 days of storage on ice. These increases correspond with the period when decomposition should become evident in this type of product.

CONCLUSIONS

This automated technique for the determination of ethanol was found to be rapid, accurate, reproducible, and sufficiently sensitive for this type of application. The automated system provides more accurate control of sample temperature and equilibration times, more accurate injections, and a higher throughput than a manual system. A major advantage of this method using MS detection is the selective advantage afforded by single ion monitoring for the two major ions of ethanol. Monitoring the abundance of both ions at mass 31 and 45 greatly increases the probability that, in the case of coeluting compounds, only the contributions of ethanol would be assessed. This method of detection greatly reduces the potential for interferences from other compounds. Other advantages of this method include the minimal sample preparation time as no extraction or cleanup step is required, an internal standard is not necessary (due to the inherent accuracy of the autosampler), and up to 44 samples (depending upon the autosampler) can be automatically analyzed while the equipment functions unattended. Either a flame ionization detector or a mass spectrometer detector can be used, depending upon availability and preference of the analyst. However, it should be noted that the use of an flame ionization detector will result in a loss of selectivity as well as an increased possibility of interferences relative to the reported mass spectrometer procedure.

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