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Alaska Pink Salmon (*Oncorhynchus gorbuscha*) Spoilage and Ethanol Incidence in the Canned Product

JIRAPORN CHANTARACHOTI,† ALEXANDRA C. M. OLIVEIRA,*,†
BRIAN H. HIMELBLOOM,† CHARLES A. CRAPO,† AND DAVID G. MCLACHLAN§

Fishery Industrial Technology Center, University of Alaska—Fairbanks, 118 Trident Way, Kodiak, Alaska 99615, and Canadian Food Inspection Agency, P.O. Box 1060, Dartmouth, Nova Scotia, Canada B3B 1Y9

Ethanol was quantified in canned salmon produced from whole fish showing different stages of decomposition due to storage at 1 and 14 °C for up to 3 and 16 days, respectively. Ethanol incidence in the canned salmon was correlated to results from skin aerobic plate counts and sensory evaluations of the whole fish and with sensory evaluations of the canned product. Panelists rejected whole salmon after 3 and 12 days of storage at 14 and 1 °C, respectively. Skin aerobic plate counts reached 4.8 log CFU/cm^2 when fish were rejected, regardless of storage temperature. Panelists rejected canned salmon produced with fish stored for a maximum of 2 and 16 days at 14 and 1 °C, respectively. Ethanol concentrations in the cans produced with fish stored at 14 °C correlated well with sensory evaluation results; however, ethanol concentrations in the cans produced with salmon stored at 1 °C did not agree with sensory results. A correlation could not be established between ethanol concentration in the canned product and microbial content of whole salmon.

KEYWORDS: Canned salmon; fish spoilage; ethanol in fish; headspace analysis

INTRODUCTION

Early stages of fish decomposition are frequently undetected by sensory inspections at seafood processing plants. This may result in lower quality salmon being thermally processed into cans. For canned salmon, researchers at the U.S. Food and Drug Administration and the Canadian Food Inspection Agency (CFIA) have suggested ethanol as a spoilage indicator for substandard raw fish (*1*, *2*). Additionally, researchers have shown that the rate of ethanol production during fish spoilage is temperature dependent (*2*, *3*). This is likely caused by different types of spoilage bacteria in fish held at different temperatures (*4*).

Gas chromatography (GC) is the standard method to quantify ethanol in canned salmon. An early technique was the direct injection of the filtered liquor from canned salmon (*1*, *5*) onto a GC coupled to a flame ionization detector. Hollingworth and Throm (*6*) developed a technique that manually injected the headspace volatiles from a sealed vial containing the filtered liquor from the salmon can, overcoming problematic GC column contamination, reducing maintenance and the cost of analysis. After a collaborative study (*7*), this method was incorporated in the Official Methods of Analysis of the Association of Official Analytical Chemists (*8*). McLachlan et al. (*2*) adapted this technique to an automated static headspace sampler coupled to gas chromatography−mass spectrometry (SHGCMS), improving the accuracy and reproducibility of the method. Chan et al. (*3*) used SHGCMS to quantify ethanol concentrations in the headspace of salmon liquor samples and correlated the results with sensory scores based on accept/reject decisions for the canned salmon. As a result, the authors suggested a cutoff value of 50 ppm of ethanol for rejecting the product (*3*). It is noteworthy to mention that this cutoff value does not correspond to the concentration of ethanol found in the canned product as a whole. Instead, it reflects the concentration of this indicator only in the headspace of the salmon liquor. McLachlan et al. (*2*) also demonstrated that spiked liquor from canned salmon did not differ from the aqueous solutions of ethanol but that the same weight of drained meat had significantly reduced ethanol levels in relation to the liquor from the same can. To examine the relationship of ethanol in the whole contents of a can compared to the ethanol found in the drained liquor of the canned product using SHGCMS, the packed raw salmon samples, in our study, were spiked with known ethanol concentrations prior to the cans being vacuum sealed and retorted.

The specific objectives of this study were to (1) conduct controlled spoilage trials using wild Alaska whole pink salmon and process fish into cans at various sampling intervals; (2) quantify the overall ethanol content in the whole contents of the can using SHGCMS analysis of the drained liquor from a series of canned salmon, where the raw salmon steaks were spiked with various known concentrations of ethanol prior to

* Corresponding author [telephone (907) 486-1530; fax (907) 486-1540; e-mail ffamo@uaf.edu].
† University of Alaska—Fairbanks.
§ Canadian Food Inspection Agecy.

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Table 1. Volumes of Ethanol and Deionized Water Used To Prepare 10 mL Ethanol Aqueous Solutions To Spike Salmon Cans Prior to Commercial Sterilization of Fresh Grade A Pink Salmon at Day 0

	ethanol concentration in the cans[a]						
substance	0 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm	200 ppm	400 ppm
absolute ETOH (mL)	0	0.23	0.45	0.91	1.82	3.63	7.27
DI water (mL)	10	9.77	9.55	9.09	8.18	6.37	2.73

[a] A constant volume of 150 μL of ethanol aqueous solution was added to cans containing 215 g of fish, yielding the above ethanol concentrations in the cans (ppm).

commercial sterilization; and (3) correlate ethanol concentrations in the cans with microbial analysis of whole fish and sensory analyses of whole fish and salmon cans.

MATERIALS AND METHODS

Controlled Fish Spoilage Trials and Canning Process. One hundred and seventy post-rigor and <24 h post-mortem wild fresh whole grade A pink salmon (*Oncorhynchus gorbuscha*) were obtained from a seafood processing plant on Kodiak Island (Alaska) during the summer of 2004 and immediately transported on ice to the Fisheries Industrial Technology Center (FITC) pilot plant. The fish were all seine caught on the same day from the same fishing vessel and were delivered to the seafood processor in a conventional holding tank that used a standard recirculation chilled seawater system. Whole fish weight ranged from 1.5 to 2.5 kg. Salmon were graded according to the Alaska Seafood Marketing Institute skin color evaluation guides (*9*) and showed no skin watermarking (*10*). Fish were separated into two groups; the first group ($n = 60$) was kept submerged in seawater in a temperature-controlled chamber model 9030 (VWR Scientific, West Chester, PA) operated at 14 °C. The second group ($n = 90$) was kept in a slurry of ice water averaging 1 °C held in a chill room set to 1 °C. Temperatures were monitored using an iButton temperature data logger model DS1921G (Semiconductor/Maxim Corp., Dallas, TX). Approximately seven fish were randomly selected for canning after 0, 1, 2, and 3 days of storage at 14 °C and after 0, 4, 6, 9, 12, and 16 days of storage in slush ice as described by McLachlan et al. (*2*). Accordingly, fish were eviscerated, cleaned, cut into steaks of approximately 215 g portions, and placed into 307 × 200.25 (8.73 cm width × 5.12 cm height) metal cans with 3 g of NaCl (VWR Scientific Products, West Chester, PA). Cans were immediately vacuum sealed and retorted at 117 °C for 76 min and finally placed in a water-cooled system (*11*). Approximately 700 cans were produced during the study. Twenty additional fish were also canned at day 0, which resulted in the production of 210 cans. These cans were spiked, shortly before sealing, with 150 μL of aqueous ethanol solutions that contained adjusted volumes of absolute ethanol (200 proof ACS, Spectrum Chemical Manufacturing Corp., Gardena, CA) and deionized water (**Table 1**) to yield the following concentrations of ethanol in the cans: 0, 12.5, 25, 50, 100, 200, and 400 ppm.

Microbial Analysis of Whole Salmon. After 0, 1, 2, and 3 days at 14 °C and 0, 4, 6, 9, 12, and 16 days of storage in slush ice, three fish from each storage temperature were randomly selected and sampled for microbial analysis. A 10 cm^2 sterile template cutout and swab were used to sample the area of skin posterior to the gills and pectoral fin of each fish. The swabs were serially diluted in 0.1% sterile peptone water, spread-plated in duplicate on plate count agar (Difco Laboratories, Detroit, MI), supplemented with 0.5% NaCl, and incubated at 25 °C for 48−72 h (*4*, *12*). Colonies were enumerated, and the log colony-forming units (CFU/cm^2) for aerobic plate counts (APC) were calculated.

Sensory Evaluation of Whole Salmon. Five fish were randomly selected for sensory evaluation after 0, 1, 2, and 3 days and 0, 4, 6, 9, 12, and 16 days of storage at 14 °C and in slush ice, respectively. The seven trained panelists, three males and four females ranging in age from 19 to 60 years old, had at least 5 years of experience with the handling and quality attributes of wild Pacific pink salmon through sport, subsistence, or commercial fishing. Each sampling day, participants rated a number of external and internal quality attributes using five fish from each storage regimen in replicate. The attributes for evaluation were as follows: appearance of eyes, gill color, gill odor, body texture, belly cavity appearance, and belly cavity odor. Sensory evaluation was based on the grading guide for whole raw Pacific salmon, which separates fish into grade A, grade B, and reject (*13*). Prior to the evaluation, training sessions were conducted using grade A fresh whole pink salmon (*9*). During training, panelists had a chance to discuss each attribute together with the expected changes through spoilage. During evaluations each panelist was required to wear disposable latex gloves to allow handling of the fish as needed. Five replicate fish were placed in individual metal trays with a random three-digit code assigned. The trays were placed on a large table, and panelists were asked to rate each attribute, one at a time, for each coded fish in a randomized order to avoid bias due to order of sample presentation. Each panelist assigned either grade A, grade B, or reject to each attribute. Sensory scores were translated into numerical numbers, with grade A receiving one point, grade B receiving two points, and grade reject assigned three points. Computation of scores was conducted using unequal weights for the different attributes; thus, higher factors were applied to some of the attributes (*14*, *15*). A factor of 1.25 was used to multiply scores assigned for gill odor and belly cavity appearance, whereas a factor of 1.5 was applied to belly cavity odor and body texture scores (*14*, *15*). At each session a group of five replicate fish was then assigned an overall grade for each attribute. This was accomplished by summing the scores of each individual panelist for every fish within a group for any given attribute. The grade assigned to each attribute was categorized as the following: grade A was <70, grade B was 70−105, and reject was ≥105. Finally, the fish from each sampling period at both storage temperatures received an overall grade that encompassed the scores of all attributes. The final sensory grade for each sampling increment was categorized as follows: grade A was <420, grade B was 420−630, and reject was ≥630.

Sensory Evaluation of Canned Salmon. All canned samples were analyzed at either the Canadian Food Inspection Agency (CFIA) national sensory workshop held from January 31 to February 4, 2005, or at a CFIA regional sensory workshop held October 24−28, 2005 (*16*, *17*). Evaluations were conducted at the CFIA Burnaby Laboratory located in Burnaby, BC, Canada. The workshops were dedicated to salmon species and consisted of 28 sets of 15 samples each, which included initial snapshot, calibration, practice, and assessment sessions. The canned samples from this study were analyzed alongside commercial cans and other spoilage runs produced by CFIA. The cans were inspected by workshop participants and five CFIA calibrated sensory expert analysts. Each CFIA calibrated sensory expert analyst had over 10 years of experience in sensory inspection of fish products, primarily canned salmon. Each canned sample was opened, the liquid was drained, and the meat was transferred into white plastic round salad servers with covers. Each sample was coded with a unique three-digit random number. Samples were randomly presented to each panelist and individually assessed in plastic booths where no talking or sharing of information was allowed. Each panelist recorded their decisions on a prescribed ballot sheet assigned to each set of 15 samples. The ballot sheet was arranged with the sample blind codes printed on the left, followed by two positions to record either an accept or reject decision, followed by a 10 cm line scale with a 5 cm center mark indicating the transition between accept or reject, used to record the overall assessment of the sample, which is referred to as a "line score", and a comment section for the analyst to write descriptive sensory terms associated with the odor/flavor characteristics of the sample (i.e., late, fecal, characteristic, etc.). Each analyst recorded their decisions on the ballot

after performing an assessment of the overall quality of the sample based on CFIA standards, which follow ISO standards for minimum acceptable quality for human consumption (*18*, *19*). Water and unsalted crackers were available to rinse the mouth between each sample as required. The overall official results of all canned samples were calculated on the basis of the average line scores and accept and reject decisions from five calibrated panels.

Ethanol Quantification in Canned Salmon Using SHGCMS. *Sample Preparation*. After 9 months of storage at room temperature, liquor from canned samples was analyzed using SHGCMS. Sample preparation and analysis was adapted from AOAC method 986.12 following McLachlan et al. modifications (*2*, *8*). Accordingly, each can was opened and the liquid phase was drained into a 40 mL screw cap vial. Lipids and suspended solids were discarded after 5 min of standing time, and then the tube was sealed with Teflon-lined screw cap. A quantity of 5 mL of the canned salmon liquor was transferred into a 20 mL crimp-top headspace vial using a standard calibrated electronic pipet to which approximately 3 g of NaCl (VWR Scientific Products) was added using a calibrated powder measure, and the vial was sealed with a thermally resistant and chemically inert Teflon/butyl septum (*2*, *8*).

Preparation of Standards. Aqueous solutions of ethanol were prepared (*2*, *8*) by adding deionized water and absolute ethanol (200 proof ACS, Spectrum Chemical Manufacturing Corp.) to achieve the following concentrations: 0, 12.5, 25, 50, 100, 200, and 400 ppm. A quantity of 5 mL of prepared standard was transferred into a 20 mL crimp-top headspace vial with 3 g of NaCl added, and the vial was sealed as described above. Another set of standards was prepared using the liquors of the spiked canned salmon samples. Two calibration curves were determined, one based on the response of the pure aqueous solutions of ethanol (EW) and another based on the response of the aqueous solutions of ethanol when added to the salmon cans prior to thermally processing fresh grade A salmon at day 0 (EC).

SHGCMS Analysis. The SHGCMS methodology was adapted from that of McLachlan et al. (*2*), which followed that of Hollingworth and Throm (*6*) and Hollingworth et al. (*7*). A headspace autosampler model 7694 (Agilent Technologies, Wilmington, DE) with a 44 sample capacity was operated under the following conditions: oven temperature, 58 °C; loop temperature, 120 °C; transfer line temperature, 140 °C; vial equilibrium time, 1.5 min; vial pressurization time, 0.10 min; loop capacity, 1 mL; loop fill time, 0.10 min; loop equilibrium time, 0.05 min; injection time, 0.15 min; vial pressure, 10 psi; shake mode, fast; GC cycle time, 20 min. A gas chromatograph model 6890 (Agilent) interfaced with a mass spectrometer detector model 5973 (Agilent) and equipped with a HP-Innowax capillary column of 0.25 mm × 30 m × 0.25 μm model 19091N-133 (Agilent) was operated under the following conditions: helium as a carrier gas at 0.9 mL/min with the average velocity of 35 cm/s in constant flow mode; front inlet initial temperature, 140 °C; pressure, 6.19 psi; split ratio, 20:1. The oven was programmed as follows: initial temperature, 38 °C; held for 4.5 min; temperature raised at 40 °C/min to 140 °C; and held for 1.95 min to give total run time of 9 min. The mass spectrometer detector conditions were as follows: solvent delay, 1.45 min; acquisition mode, scan with mass ranging from 33 to 300 amu; scan rate, 5.24 scans/s; source temperature, 230 °C; quadrupole temperature, 150 °C. Ethanol peak identification was based on comparison of GC retention time and mass spectra of absolute ethanol, and quantification of ethanol in the salmon cans was carried out using calibration curves EW and EC.

Statistical Analysis. Factorial analysis of variance followed by Tukey's honest significant difference test ($P < 0.05$) was used to determine significant differences in ethanol concentrations between calibration curves and storage days for each storage temperature using Statistica version 6.1 (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Microbial Analysis of Whole Salmon. Figure 1 represents the skin aerobic plate count of whole fish for both storage temperatures. The skin APC of whole fish stored at 14 °C ranged from 3.4 to 4.8 log CFU/cm^2. For fish stored in slush ice, the skin APC decreased from day 0 (3.4 log CFU/cm^2) to day 4 (2.4 log CFU/cm^2) and then continually increased from day 4 (2.4 log CFU/cm^2) to day 16 (5.5 log CFU/cm^2). The reduction of skin APC at the initial storage day was likely due to the washing effect when unwashed fish (day 0) were transferred to slush ice (1 °C). Our finding was similar to that of Rodriguez et al. (*20*), who observed a decrease of APC between days 0 and 2 in horse mackerel muscle stored in slurry ice. It has been shown that low temperature suppresses microbial growth (*4*). Temperatures below 10 °C inhibit the growth of most bacteria as well as retard the growth of psychotropic bacteria and extend their lag phase as they become accustomed to the environment (*21*). The lower skin APC of fish stored in slush ice (1 °C) as compared to fish stored at 14 °C was therefore not unexpected.

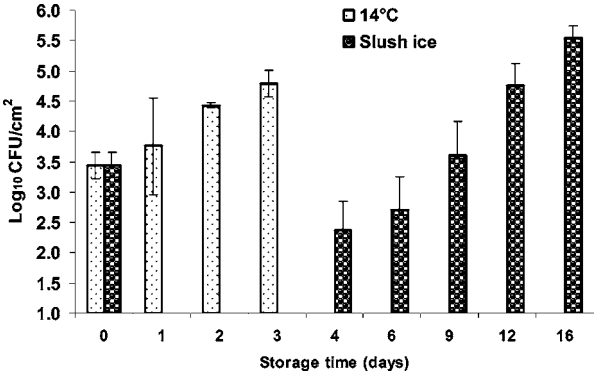

Figure 1. Skin aerobic plate counts (colony-forming units) for whole pink salmon stored at 14 °C and in slush ice (1 °C).

At the time fish were considered to be spoiled the skin APC was 4.8 log CFU/cm^2, regardless of storage temperature. Skin APC of fish spoiled in slush ice (1 °C) in our study was similar to the skin APC of whole pink salmon spoiled in chilled seawater (−0.5 °C) reported by Himelbloom et al. (*4*). Moreover, the skin APC measured for fresh fish in our study was in agreement with Himelbloom et al. (*12*) values of <4 log CFU/cm^2 for fresh whole pink salmon. The onset of microbial spoilage is initiated as nutrients become available in fish muscle due to a variety of post-mortem autolytic processes. As autolysis continues over the storage time, the available nutrients in the muscle sustain the growth and increasing abundance of microorganisms. As expected, bacterial metabolism and growth cause the formation of a number of important chemical compounds associated with fish spoilage such as amines, sulfur compounds, short-chain alcohols, and carbonyls, which include aldehydes, ketones, and ethanol (*22*−*25*). It must be kept in mind that in addition to external skin APC, internal spoilage processes are also proceeding due to autolytic and microbial breakdown of organs once the fish dies, which eventually leads to membrane disruption and general microbial contamination and breakdown of musculature and associated chemical compounds associated with spoilage, irrespective of the external APC.

Sensory Evaluation of Whole Salmon. Figure 2 represents the overall sensory evaluation for whole fish samples stored at 14 °C and in slush ice, respectively. Whole fish stored at 14 °C maintained grade A for only 1 day. After 2 days of storage, whole fish received grade B mainly due to changes in body cavity and gill odors. After 3 days of storage at 14 °C, panelists rejected the samples with sensory scores indicating that all attributes evaluated showed signs of quality deterioration such as advanced eye dullness, strong stale and sour gill odors, strong sour and putrid belly cavity odors, very soft body texture, pink to buff gill color, and an extensively reddened and ruptured belly cavity. Whole fish stored in slush ice slowly changed from grade A to grade reject. Fish received grade A for up to 4 days

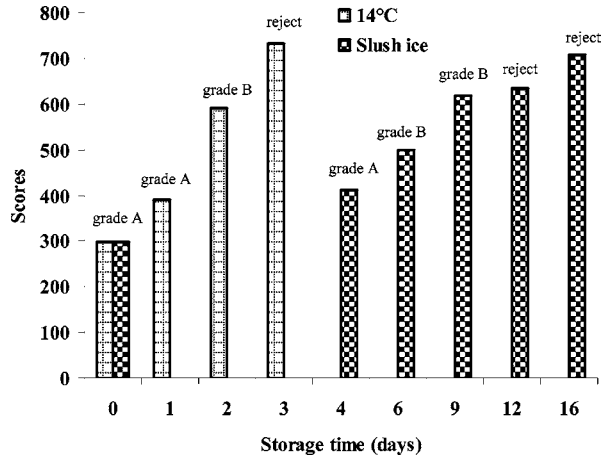

Figure 2. Overall sensory scores for whole pink salmon stored at 14 °C and in slush ice (1 °C).

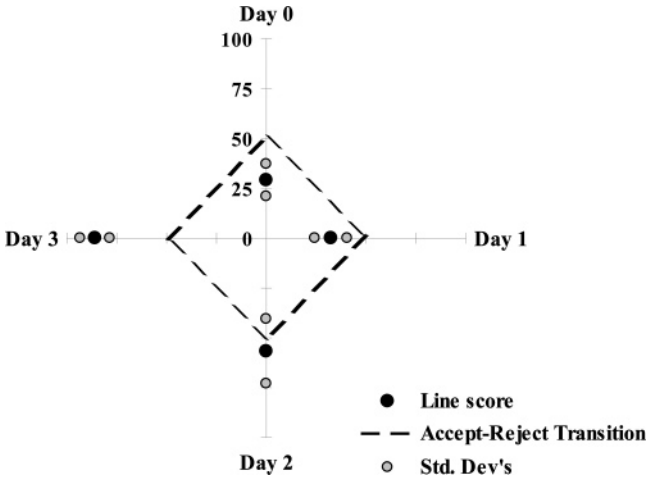

Figure 3. Sensory line score of canned salmon produced from fish stored at 14 °C.

of storage. After 6 days of storage fish received grade B, mainly due to changes in gill odors, body texture, and belly cavity odors. After 9 days of storage, samples maintained grade B with scores being slightly below grade reject. Fish were rejected after 12 and 16 days of storage. Our sensory evaluation results for whole pink salmon are in agreement with results previously reported by Chan et al. (*3*), which indicated that whole pink salmon stored at 14 °C and in ice were rejected by sensory panelists after 3 and 14 days of storage, respectively.

Sensory Evaluation of Canned Salmon. Figures 3 and **4** depict the overall results of sensory evaluations of canned samples produced from fish stored at 14 °C and in slush ice, respectively. Even though the ethanol concentration in the headspace of canned salmon can be increased by the salting-out techniques, the odor threshold of ethanol in water has been reported to be 760 ppm (*26*). Therefore, panelists were most likely not able to smell ethanol at the concentrations present in the cans. Instead, panelists used other odor cues that characterize canned salmon produced from spoiled fish such as the presence of stale, sour, and fecal odors. As expected, the average line score increased as the storage time of whole fish increased (*3*). On the basis of CFIA standards, which follow ISO standards for minimum acceptable quality for human consumption, all cans of fish stored at 14 °C produced from whole fish samples on days 0 and 1 were of acceptable quality, with expert panelist evaluations averaging scores of 29 and 32, respectively (**Figure 3**). The odors of these samples were described as neutral (day

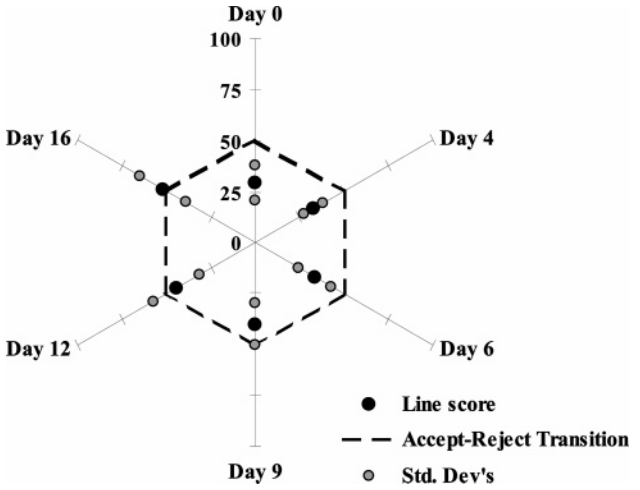

Figure 4. Sensory line score of canned salmon produced from fish stored in slush ice (1 °C).

0) and neutral with slight late-odor notes (day 1). It is important to mention that late-odor notes are often related to canning of salmon with advanced skin watermarking (*10*). In our study, all salmon procured from the cannery were bright grade A with no traces of skin watermarking. Therefore, it is suggested that during salmon spoilage off-odors are produced that resemble the ones found in heavily watermarked fish. Canned samples produced from day 2 fish stored at 14 °C received an average line score of 57 (**Figure 3**), indicating an overlap between accept and reject decisions. The day 2 (14 °C) acceptable quality canned salmon were downgraded close to the reject line score transition due to off-odors described as late and stale. For rejected cans, the most frequently used terms were sour, slightly fermented, fermented, and fecal odors, which led to a line score above 50, beyond the reject transition mark. All canned samples produced from day 3 fish stored at 14 °C were most frequently described as having a fecal odor, and the average line score was 85.9 (**Figure 3**).

For fish stored in slurry ice, all canned salmon samples of day 0 and 4 fish were accepted by the CFIA sensory panel experts. These samples received average line scores of 29 and 33, respectively (**Figure 4**). The odors of these samples were most frequently described as neutral and slight late-odor. Canned samples of day 6 and 9 fish were also of acceptable quality, and their odors were described as slight to late-odors. Canned salmon from fish that had been stored for up to 12 days in slurry ice were of acceptable quality, with sensory panel line scores ranging from 29 to 44 (day 12; **Figure 4**). There was an obvious overlap between accept and reject decisions in the sensory evaluation of canned salmon samples produced from day 16 fish. These samples were rejected on the basis of the majority of the decisions by the expert panelists, who described their odor as sour. Canned salmon samples from day 16 fish received an average line score of 52 (**Figure 4**).

Our sensory evaluation results for fish stored at either temperature corroborate the findings of Chan et al. (*3*). Their results indicated that canned Pacific pink salmon produced from fish stored at 14 °C over 36 h were of marginal to unacceptable quality, whereas canned salmon produced from fish stored in slush ice for up to 12 days were of acceptable quality. Furthermore, Chan et al. (*3*) also observed variability and overlap between accept and reject panelists' decisions in sensory evaluations of canned pink salmon samples from fish stored in slush ice for 14 and 16 days (*3*).

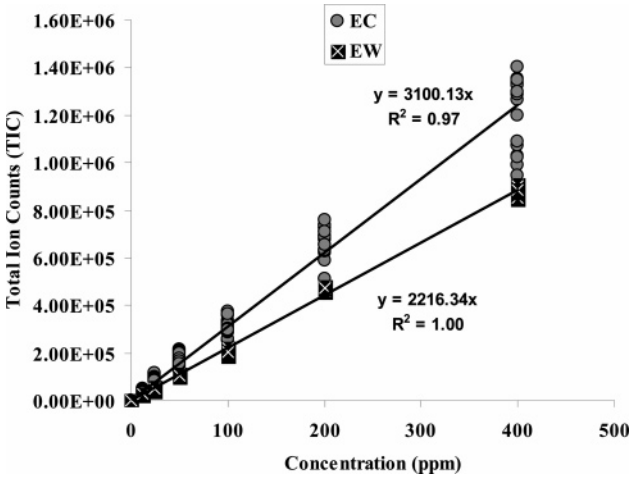

Figure 5. Calibration curves based on the response of the aqueous solutions of ethanol when added to the can before thermal processing of fresh grade A salmon at day 0 (EC) and based on the response of the pure aqueous solutions of ethanol (EW) of 0, 12.5, 25, 50, 100, 200, and 400 ppm.

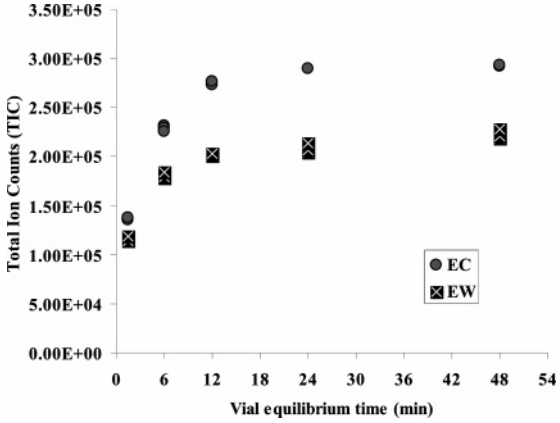

Figure 6. Equilibrium curve at 58 °C based on the response of the aqueous solutions of ethanol when added to the can before thermal processing of fresh grade A salmon at day 0 (EC) and based on the response of the pure aqueous solutions of ethanol (EW) of 50 ppm depicting results from triplicate analysis for each point in time.

In conclusion, despite the intrinsic difficulties of correlating the quality of raw fish with the quality of the canned product, the sensory evaluation results of whole pink salmon and canned pink salmon were mostly consistent with each other. A few differences were recorded in the ice storage study, where raw fish were rejected after 12 days, whereas the majority of canned samples produced from those fish were of acceptable quality. This difference may due to a number of reasons such as the sensory assessment method used for evaluating the quality of raw and canned salmon, the use of different participants for the raw and the canned panels using different criteria (the CFIA panel assessed the canned product on the basis of minimum acceptable quality for human consumption), panelist variability, and the changes that occur in food products during thermal processing (*27*). The latter includes changes in color and texture that are largely due to protein denaturation at high temperatures (*28*). The color and texture of the canned product are similar regardless of the degree of spoilage of the raw material, whereas changes in color and texture of whole fish more obviously indicate spoilage (*13*−*15*, *18*, *19*). In addition, other compounds that are formed by thermal degradation during commercial sterilization may alter or mask the odor of chemical compounds associated with the spoilage of raw material. Therefore, the odor spoilage cues used to judge raw fish quality are not useful for judging canned products, noticeable by the different criteria typically used to judge the quality of raw and canned fishery products (*13*−*15*, *18*, *19*).

Ethanol Quantification in Canned Salmon Using SHGC-MS. A linear relationship between ethanol concentration and the total ion count (TIC) response with R^2 values of 0.97 and 1.00 were calculated for EC and EW (**Figure 5**). EC had a higher TIC response than EW; this is in large part attributed to the fact that the spike amounts for EC were based on the total weight of the raw fish in the can. The ethanol preferentially partitions into the water phase of the flesh, which typically varies between 70 and 78% of the total flesh weight (*2*, *3*); hence, the liquor derived from the flesh would have a higher TIC than would be expected for the spike amount. McLachlan et al. (*2*) found that the liquor had on average 73.5% of the combined total ethanol from both the meat and liquor from a limited set of cans, which is consistent with the difference between the slopes for EW and EC. McLachlan et al. (*2*) also showed that spiking known amounts of ethanol into the liquor removed from canned pink salmon resulted in a mean recovery of 96.9% over the concentration range of 1−120 μg/g, when compared to standard ethanol and water solutions of identical concentrations. This observation further substantiates that the difference between EC and EW is principally the partitioning of ethanol into the water phase of the retorted meat and the can liquor.

Some of the TIC variability between cans spiked at the same concentrations for the EC calibration in **Figure 5** may in part be due to widely different percent moisture levels found between individual fish, when compared to the much smaller standard deviation for the EW calibration. **Figure 6** demonstrates the relationship between the TIC and vial equilibrium time (minutes) at 58 °C for 50 ppm of ethanol in the liquor of the spiked salmon cans (EC) and in the ethanol aqueous solutions (EW). Regardless of calibration curve, ethanol TIC increased rapidly up to 12 min, followed by a slow rise from 12 to 24 min, and reached a constant value between 24 and 48 min. When ethanol concentration in the headspace is constant, equilibrium has been reached and ethanol TIC is directly proportional to the ethanol concentration in the sample (*29*). **Figure 6** demonstrates that EC showed higher TIC than EW under both equilibrium and nonequilibrium conditions at 58 °C, consistent with results shown in **Figure 5**.

Figures 7 and **8** present ethanol concentrations of canned samples produced from fish held at 14 °C and in slush ice, respectively. As expected, ethanol concentrations increased with storage time (**Figures 7** and **8)** (*1*−*3*). The use of curve EW yielded higher concentrations of ethanol than did EC, with most significant differences occurring toward the end of the spoilage trials (**Figures 7** and **8**). This result is consistent with McLachlan et al.'s (*2*) observation that a fraction of the ethanol present in the fish muscle remains in the retorted meat (range = 17−32%). Ethanol concentrations in day 2 samples (14 °C) were about 5 ppm lower than the cutoff value to reject the product (50 ppm) according to EW, but with EC these samples had values 18 ppm below the cutoff value. Ethanol concentration in canned salmon increased significantly with storage time of raw fish held at 14 °C and reached about 200 ppm at day 3, a 4-fold difference from day 2 samples (**Figure 7**). Ethanol concentrations of EW in this study were similar to those reported by McLachlan et al. (*2*). They found ethanol concentrations in canned salmon produced from fish stored at 14 °C after 36−48 h were in the range of 30−50 ppm and significantly rose to 200 ppm after 3 days of storage.

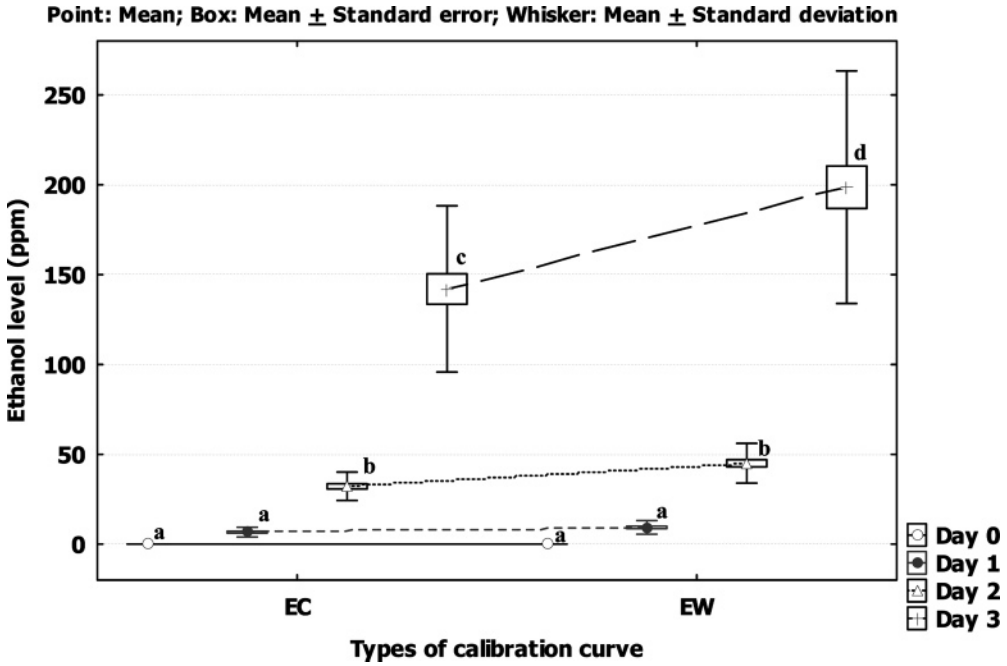

Figure 7. Ethanol concentrations in canned salmon produced from fish stored at 14 °C quantified using EC (calibration curve based on the response of the aqueous solutions of ethanol when added to the can before thermal processing) and EW (calibration curve based on the response of the pure aqueous solutions of ethanol). Significant differences between curves and storage time are expressed as different letters ($P < 0.05$).

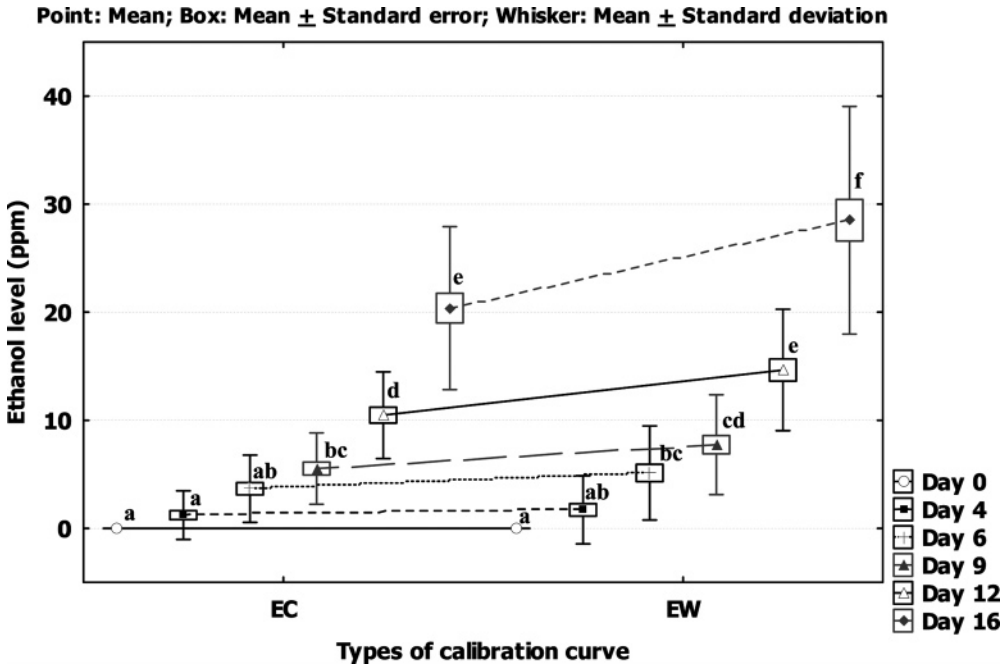

Figure 8. Ethanol concentrations in canned salmon produced from fish stored in slush ice (1 °C) quantified using EC (calibration curve based on the response of the aqueous solutions of ethanol when added to the can before thermal processing) and EW (calibration curve based on the response of the pure aqueous solutions of ethanol). Significant differences between curves and storage time are expressed as different letters ($P < 0.05$).

For fish stored in slush ice, the ethanol concentrations of canned samples increased over time from 0 to 20.38 ppm for EC and from 0 to 28.51 ppm for EW (**Figure 8**). Ethanol concentrations in salmon cans produced from fish stored for up to 9 days determined with EC and EW were not significantly different ($P > 0.05$), but a significant difference ($P < 0.05$) was determined for salmon cans produced from fish stored after 12 and 16 days, with EW showing higher ethanol concentrations than EC (**Figure 8**). Using either EC or EW, ethanol concentrations in salmon cans produced from fish stored for up to 9 days were significantly different ($P < 0.05$) from salmon cans produced from fish stored for 12 and 16 days (**Figure 8**). Between salmon cans produced from fish stored for up to 9 and 16 days, a significant increase of ethanol concentration was observed. Nonetheless, ethanol concentrations never reached 50 ppm in canned salmon produced from fish stored in slush ice up to 16 days. McLachlan et al. (*2*) found ethanol concentrations (<30 ppm) in canned salmon produced from fish that had been stored in ice for up to 12 days consistent with results from this study. However, these researchers (*2*) observed ethanol concentrations above the cutoff value of 50 ppm in canned salmon produced from whole fish that had been stored for more than

12 days in slurry ice, whereas our results do not agree. This discrepancy may be due to differences in concentrations and species of specific spoilage organisms between the two studies that may be related to environmental factors such as catch location, feeding grounds, and time of harvest.

Correlation between Ethanol Concentrations in Canned Salmon and Microbial Analysis of Whole Salmon. No correlations were observed between ethanol concentrations in canned salmon and skin APC of whole fish, regardless of storage temperature. Whereas microbial analysis indicated that fish rejected by sensory panelists had similar APC values when held at 14 °C or in slush ice (1 °C), ethanol concentrations were significantly higher ($P < 0.05$) in canned salmon produced from fish stored at 14 °C than in cans produced from fish stored in slush ice (1 °C) (**Figures 7** and **8**). The difference in ethanol concentrations could be due to a number of interrelated factors, including substrates, temperatures, and types and ratio of specific spoilage bacteria capable of producing ethanol under each storage condition. Several types of bacteria produce ethanol as a common metabolite, which is derived either from carbohydrate compounds via a glycolytic pathway under anaerobic conditions or from amino acids such as alanine by way of deamination followed by decarboxylation (*1*, *22*). Possible substrates utilized by bacteria include glycogen, glucose, lactic acid, ribose from ATP degradation, and amino acids from fish tissue components (*30*). Although those substrates are present in the fish muscle, it is not known which substrates are present in fish skin and what their concentrations are. Because skin-on fish were processed into cans, the role of fish skin as a source of substrates for metabolism to ethanol remained unknown.

Temperature is an important regulator of the kinetic activity of endogenous enzymes such as phosphorylase, an important enzyme in fish muscle capable of breaking down glycogen into glucose (*31*). Because enzymatic activity is greater at higher temperatures, fish spoiled for a given period of time at 14 °C are expected to contain higher ethanol concentrations than fish spoiled in ice, as demonstrated in this study as well as others [McLachlan et al. (*2*) and Chan et al. (*3*)].

The types and relative abundance of spoilage bacteria likely differed under each temperature regime, and this is another possible cause for the different ethanol concentrations. *Shewanella*, *Pseudomonas*, *Moraxella*, *Acinetobacter*, and *Flavobacterium* are microflora commonly found in and on temperate water fish (*24*). Three genera, *Pseudomonas*, *Moraxella*, and *Flavobacterium*, are known for their ability to produce ethanol (*30*). *Moraxella* and *Pseudomonas* have been found to be the most predominant bacteria found on the skin of iced and chilled seawater-held Alaska pink salmon, respectively (*4*), and *Pseudomonas* was recognized as a significant fish spoilage bacterium at temperatures near 0 °C (*32*). *Pseudomonas* can grow across a wide temperature range from <4 °C to a maximum of 43 °C (*33*). *Pseudomonas* may have been responsible for the spoilage at 1 °C, but at the storage temperature of 14 °C, it was likely that several different bacteria were responsible. The mixed microflora of *Pseudomonas* and other suspected ethanol producers that require higher temperatures for growth and metabolism may produce more ethanol than *Pseudomonas* alone. As there are a number of possible bacterial species present on pink salmon spoiled at two different temperatures, each likely having different favored environments and abilities to produce ethanol, additional studies are needed to verify and thoroughly explain the different ethanol concentrations obtained in cans of fish spoiled at two different temperatures. Ideally, specific spoilage bacteria and their relative abundance should be identified in conjunction with ethanol concentration measurements when Alaska pink salmon are stored at both temperatures prior to thermal processing.

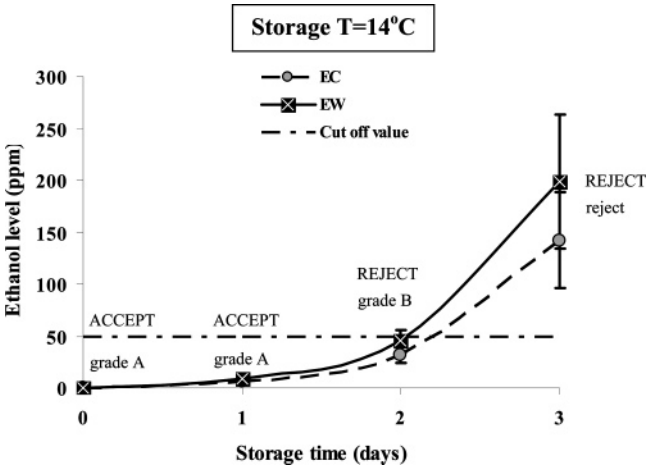

Figure 9. Correlation between sensory evaluation results of whole salmon (grades A, B, and reject) and canned salmon (accept/reject decisions), with ethanol concentrations in the cans quantified using EC (calibration curve based on the response of the aqueous solutions of ethanol when added to the can before thermal processing) and EW (calibration curve based on the response of the pure aqueous solutions of ethanol).

Correlation between Ethanol Concentrations in Canned Salmon and Sensory Evaluation of Whole Salmon. Results of whole fish sensory evaluation were in good agreement with ethanol concentrations in the canned product only at elevated storage temperature. Ethanol concentrations in canned salmon produced from grade A (days 0 and 1) and grade B (day 2) fish held at 14 °C were below 50 ppm, whereas in cans produced from rejected fish ethanol concentrations were 4-fold the cutoff value to reject the product (**Figure 9**). In slush ice, ethanol concentrations determined for the canned salmon and sensory panel grades for whole fish disagreed. Panelists rejected whole pink salmon held in slush ice at 12 and 16 days of storage in this study, but ethanol concentrations did not exceed 30 ppm in canned products (**Figure 10**). This suggests that it is possible for product to develop odors of decomposition under optimum conditions while ethanol concentrations remain below the accepted limit of 50 ppm ethanol in canned salmon.

Correlation between Ethanol Concentrations in Canned Salmon and Sensory Evaluation of Canned Salmon. Ethanol concentrations and sensory grades for canned salmon produced from fish stored in ice and at 14 °C were in fair agreement. For fish that had been stored at 14 °C, ethanol concentrations in the canned product produced from day 0 and 1 fish were below 50 ppm, using either EC or EW, which agrees with panelists results that showed these samples to be of acceptable quality (**Figure 9**). A disagreement between ethanol concentrations and sensory scores was observed for salmon cans produced from fish stored for 2 days at 14 °C. Although the average ethanol concentration was slightly below 50 ppm, using either EC or EW, the average sensory score was above the reject limit of 57 (**Figure 9**). For canned salmon samples containing fish held for 3 days at 14 °C, ethanol concentrations reached about 200 ppm, and sensory evaluations corroborated with this result; the product was unanimously rejected (**Figure 9**). In sum, our results support the findings reported by Hollingworth and Throm (*1*) for fish stored at 14 °C. These researchers (*1*) proposed tentative ranges for ethanol concentrations in canned salmon to reflect sensory evaluation scores, with sensory class I (passable) canned salmon having ethanol concentrations that ranged from 0 to 24

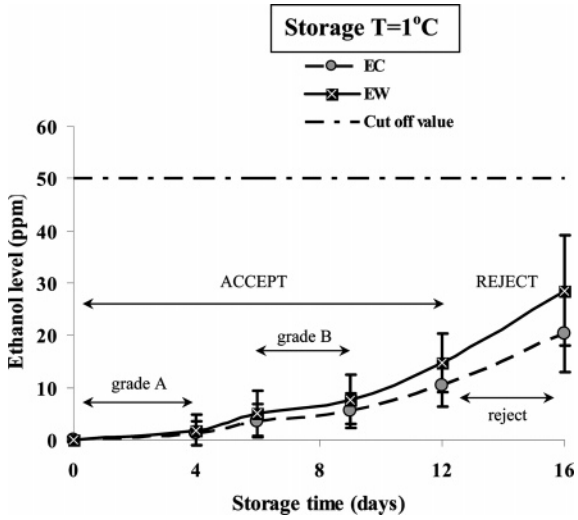

Figure 10. Correlation between sensory evaluation results of whole salmon (grades A, B, and reject) and canned salmon (accept/reject decisions), with ethanol concentrations in the cans quantified using EC (calibration curve based on the response of the aqueous solutions of ethanol when added to the can before the thermal process) and EW (calibration curve based on the response of the pure aqueous solutions of ethanol).

ppm, sensory class II (slightly decomposed) canned salmon having ethanol concentrations that ranged from 25 to 74 ppm, and sensory class III (advanced decomposition) canned salmon having ethanol concentrations above 75 ppm.

For canned salmon produced from fish stored in slush ice, ethanol concentrations never exceeded the cutoff value of 50 ppm, using either curve EC or curve EW. These results were in good agreement with the majority of sensory evaluations in which panelists indicated that canned samples produced from fish stored up to 12 days were of acceptable quality (**Figure 10**). Sensory scores determined for canned salmon produced from fish held in slurry ice for 16 days varied, with some cans being rejected and others being judged to be of acceptable quality. This would indicate that it is possible for some fish to produce odors of decomposition sufficient to reject the product even though the ethanol level does not exceed 50 ppm when held under optimum conditions. Other chilled controlled spoilage samples produced under similar conditions by CFIA, which were examined at the same time, did exceed 50 ppm when they were rejected.

The few inconsistencies observed between ethanol concentrations in canned salmon and their scores from sensory evaluation may have occurred because of the natural variability among fish. Not all fish will spoil equally during a spoilage trial because the types of microflora and their relative abundance originally present in fish skin, gills, and intestine may differ due to a number of environmental factors such as overall health, gender, sexual maturity, and feeding grounds (*21*). Fish with an empty gastrointestinal tract and in good health have a tendency to spoil more slowly than fish that were in poorer condition (*21*).

Conclusion. Results of whole fish sensory evaluation correlated well with ethanol concentrations in the canned product only at elevated storage temperature (14 °C). Conversely, whereas whole fish held in slush ice (1 °C) for 12 and 16 days were rejected by sensory panelists, ethanol concentrations in the corresponding canned products never exceeded the reject cutoff value (50 ppm). The spiked raw salmon used in the EC calibration demonstrated that ethanol produced during spoilage preferentially partitions into the aqueous phase of the fish and that the EC slope compared to the EW slope was consistent with the total ethanol recovered from the liquor and meat as seen in McLachlan et al. (*2*). The actual partitioning of ethanol into the nonaqueous phase of the retorted meat could not be calculated in the present study. Further studies should include determination of fish muscle and retorted meat moisture contents to investigate the influence of these variables in the quantification of ethanol in canned salmon. Additionally, research aiming at the identification of bacteria associated with the spoilage of pink salmon stored at 14 and 1 °C must be conducted for a better understanding of the differences observed in ethanol concentrations between optimal and high-temperature storage conditions.

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