Freshness Quality of Harp Seal (*Phoca groenlandica*) Meat

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Freshness quality of seal meat during the post-mortem storage period was determined by monitoring the profile of nucleotides and their breakdown products. Analytical conditions for quantitative determination of adenosine 5′-triphosphate, adenosine 5′-diphosphate, adenosine 5′-monophosphate, inosine 5′-monophosphate, hypoxanthine, xanthine, and inosine in harp seal meat extracts were examined by reversed-phase high-performance liquid chromatography (HPLC). Freshness indicator K, K_0, K_i, G, and P values were calculated. Linear increases in K, K_i, and K_0 values with increasing storage periods of up to 19 days were observed. Comparison of K_0 values of seal with those from beef indicated that seal meat behaves similarly to beef. Comparison of the K_i values of seal meat with those of cod and pollock indicated that seal meat has a better keeping quality than fish species. Comparison of the G and P values of seal meat with those of cod indicated a similar trend. Thus, HPLC for nucleotide determination lends itself to the evaluation of seal freshness quality. The K, K_i, K_0, and P values offered a better indication for seal meat freshness during the early stages of storage than the G value.

INTRODUCTION

Seal meat has a high protein content with a well-balanced amino acid composition (Shahidi *et al.*, 1990). It is low in cholesterol (approximately 66 mg/100 g) and nucleic acids (approximately 2 mg/g) and is a rich source of B vitamins, iron, and long-chain ω-3 fatty acids. Interest in seal meat as a highly nutritional source of muscle food is well documented (Shahidi *et al.*, 1990). Freshness quality of muscle foods is related to biochemical changes taking place during the post-mortem period. In particular, autolytic and biochemical deteriorations become more important when proper chilling and handling procedures are not used.

Immediately after death, adenosine triphosphate (ATP) begins to degrade to uric acid through the pathway (Terasaki *et al.*, 1965)

$$\mathrm{ATP} \xrightarrow{1} \mathrm{ADP} \xrightarrow{2} \mathrm{AMP} \xrightarrow{3} \mathrm{IMP} \xrightarrow{4} \mathrm{INO} \xrightarrow{5} \mathrm{Hx} \xrightarrow{6} \mathrm{X} \xrightarrow{7} \mathrm{U} \quad (1)$$

where ADP is adenosine diphosphate, AMP is adenosine monophosphate, IMP is inosine monophosphate, INO is inosine, Hx is hypoxanthine, X is xanthine, and U is uric acid. In most cases, the rate-determining step is (5) or (6), depending on the species of animal being examined (Karube *et al.*, 1984; Karube and Sode, 1988). Consequently, the concentration of INO and Hx increases with the length of the storage period. Contents of INO or Hx in fish muscles have been used as indicators of freshness (Jones and Murray, 1964; Burt, 1977).

Molecules of ATP are rapidly degraded after death to AMP and subsequently to IMP by partial dephosphorylation. The dephosphorylation of IMP is primarily autolytic and occurs during the early stages of chilled storage. The disappearance of IMP has been correlated with the loss of fresh fish flavor in some species (Jones and Murray, 1961; Fraser, 1965; Spinelli *et al.*, 1969). The effect of Hx accumulation in fish tissues reflects the initial phase of autolytic deterioration and later includes contributions through bacterial spoilage.

While useful indices of freshness have been based on individual nucleotides or their breakdown products, indicators that incorporate the measurement of several of these nucleotides are advantageous. Thus, the concept of the K value (eq 2) was first introduced by Saito *et al.* (1959).

$$K\,(\%) = \frac{[\mathrm{INO}] + [\mathrm{Hx}]}{[\mathrm{ATP}] + [\mathrm{ADP}] + [\mathrm{AMP}] + [\mathrm{IMP}] + [\mathrm{INO}] + [\mathrm{Hx}]} \times 100 \quad (2)$$

ATP, ADP, and AMP disappear approximately 24 h post-mortem. In practice, muscle foods obtained from the market are at least 24 h after death. Hence, the K value can be reduced to

$$K_i\,(\%) = \frac{[\mathrm{INO}] + [\mathrm{Hx}]}{[\mathrm{IMP}] + [\mathrm{INO}] + [\mathrm{Hx}]} \times 100 \quad (3)$$

Assays monitoring IMP, INO, and Hx are therefore sufficient for determination of fish freshness. However, when nucleotides are degraded fully, the usefulness of these indicators is questionable (Jones and Murray, 1964; Kassemsarn *et al.*, 1973).

Burns *et al.* (1985) have proposed another freshness indicator for fish, namely the G value (eq 4), which is

$$G = \frac{[\mathrm{Hx}] + [\mathrm{INO}]}{[\mathrm{INO}] + [\mathrm{IMP}] + [\mathrm{AMP}]} \quad (4)$$

based on accumulation of Hx and disappearance of IMP, AMP, and INO in the muscle after slaughter. The index is useful over the entire iced shelf life of lean fish.

A second quality indicator, namely the P value (eq 5), serves as an indicator of spoilage during the early stages of chilled storage:

$$P = \frac{[\mathrm{Hx}] + [\mathrm{INO}]}{[\mathrm{INO}] + [\mathrm{IMP}] + [\mathrm{Hx}] + [\mathrm{AMP}]} \quad (5)$$

Values of G and P can provide a basis for the establishment of a grading system in fish, and these have been compared with other fish freshness tests and physical evaluations for several fish species. These values are applicable to most fish samples, although species variations can be expected. Values of G and P are most useful with lean fish. In fatty fish, however, factors such as devel-

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opment of rancidity may render the product undesirable before meaningful G and P values can be obtained.

As an indication of freshness of meat, Nakatani *et al.* (1986) proposed the use of the K_0 value (eq 6) in which

$$K_0(\%) = [([INO] + [Hx])/([ATP] + [ADP] + [AMP] + [AD] + [IMP] + [INO] + [Hx] + [X])] \times 100 \quad (6)$$

adenosine (AD) and X were also included. A well-established specific and fast ion-pair reversed-phase high-performance liquid chromatographic (HPLC) method may be employed. This method allows most of the nucleotides to be simultaneously determined on the basis of the property of the molecular electrical charges (Murray and Thomson, 1983; Stocchi *et al.*, 1987; Lang and Rizzi, 1986; Watanabe *et al.*, 1989).

The objective of this study was to evaluate the freshness quality of harp seal meat using nucleotide analyses. Conditions suitable for separation of ATP-related compounds in seal meat were examined. Results of nucleotide analyses were used to calculate relevant indicators of seal meat freshness.

MATERIALS AND METHODS

Materials. Nucleotides and nucleoside standards were obtained from Sigma Chemical Co. (St. Louis, MO). HPLC grade methanol was supplied by Fisher Scientific Ltd. (Dartmouth, NS). Other chemicals used were of commercial ACS grade. A 0.1 M potassium phosphate buffer, pH 6.0, containing 8 mM tetrabutylammonium hydrogen sulfate (buffer B for pump B) and a 0.1 M potassium phosphate buffer, pH 6.0, containing 8 mM tetrabutylammonium hydrogen sulfate and 30% (v/v) methanol (buffer A for pump A) were used.

Instruments. A Shimadzu HPLC system consisting of two Model LC-6A pumps with a mixing chamber, a Model SPD-6AV UV–visible detector, a Model SIL-6B autoinjector, a Model SCL-6B system controller, and a Model CR501 Chromatopac (data processor) were used. A 10-μm particle size LC-18-T reversed-phase analytical column (4.5 mm × 24 cm) from Supelco (Oakville, ON) and a guard column (4.5 mm × 5 cm) which was coupled with the analytical column were used.

Sample Preparation. Seven harp seal carcasses hunted in the waters adjacent to St. Anthony, NF, during April 1991 were used in this study. All seals were bled well and eviscerated, 30 min post-mortem, at the collection site. A known amount of longissimus dorsi muscle was cut from each seal at different time intervals, as indicated in the figures, and nucleotides were extracted into perchloric acid as described below. The remaining carcasses were placed inside plastic bags and stored on ice in a refrigerated room at 0–4 °C during the course of the experiments which lasted 23 days.

Nucleotide Extraction. Ten grams of finely chopped seal muscle was weighed and homogenized in 20 mL of 0.6 N perchloric acid for 30 s using a Brinkmann Polytron homogenizer (Brinkmann Polytron, Westbury, NY) at maximum speed. The homogenate was filtered through a Whatman No. 1 filter paper, and the resultant filtrate containing the nucleotides extract was kept for up to 3 weeks at −60 °C until use.

HPLC Analyses. Before HPLC analyses, the nucleotide extracts were thawed at 0–4 °C and the pH was adjusted to 6.5 by diluting it 1/10 with 0.1 M K_2HPO_4 to avoid crystallization of perchloric acid during the HPLC analyses. The pH-adjusted sample was then filtered through a 0.45-μm nylon filter (Cameo II, MSI, Westboro, MA) into the HPLC sampling vial and was then used directly for analysis. Nucleotides in the samples were determined by a reversed-phase HPLC. Twenty microliters of a standard or filtered solution was injected into the column automatically using the autoinjector. The detector response for each nucleotide and nucleoside was calibrated daily by injecting a known mixture of the reference compounds. Initially, a four-level calibration run was performed, giving a linear response (standard curve) for each reference compound. A modified Stocchi method was used for the analytical separation conditions (Stocchi *et al.*, 1987): 0.01–3.8 min at 100% buffer B, 7.8 min at up to 20% buffer A, 15 min at up to 40% buffer A, 19.5 min at up to 100% buffer A, and hold until 27 min. The gradient was then immediately returned to 100% buffer B and was held there until 31 min, when the analysis was terminated. The flow rate was 1 mL/min, and detection was measured at 254 nm. The analyses were performed at room temperature, but the samples were kept at 0 °C until the time of the injection. Quantification of the endogenous nucleotide and nucleoside concentrations was carried out using the data module in the external standardization mode. Results are expressed as micromoles per gram of muscle. The K, K_0, K_i, G, and P values were then calculated. Mean values of, at least, triplicate determinations ± standard determinations are reported in each case.

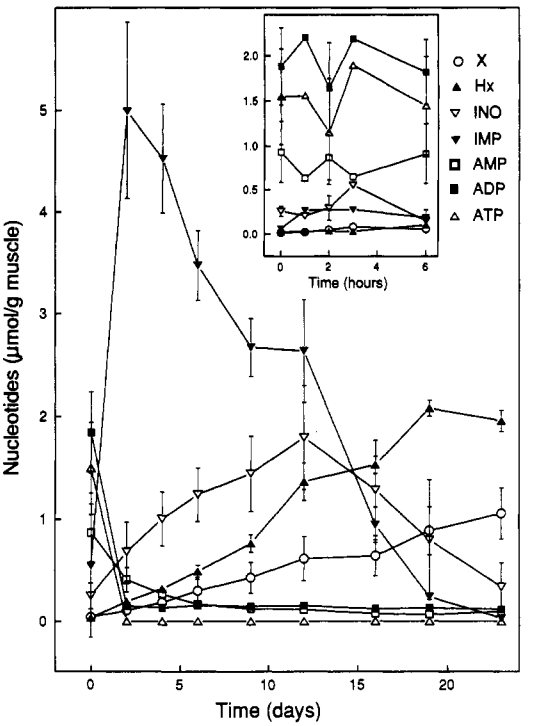

Figure 1. Changes in the content of nucleotides and nucleosides of seal meat during post-mortem storage at 0 °C. Each point represents the mean value of triplicate determinations of samples from seven seals ± standard deviation.

RESULTS AND DISCUSSION

Figure 1 shows changes in the concentration of nucleotides and nucleosides during a 3-week storage of seal meat in a refrigerated room at 0–4 °C. Concentrations of ATP, ADP, and AMP decreased and concentrations of IMP, INO, Hx, and X increased during the first 48 h. After 48 h, ATP was no longer detected, but the concentrations of ADP and AMP remained unchanged at a very low level. IMP concentration increased extremely quickly, reaching a maximum after 48 h, and then started a decline to almost zero at day 23. However, the level of INO increased during the first 12 days before starting to decline. The steady increase in the concentration of Hx was noted up to 19 days. During the 23-day post-mortem storage, there was a steady increase in the concentration of X with time. These observations are in line with the reported results on fish freshness (Burt, 1977; Jones and Murray, 1964). The disappearance of IMP has been correlated with the loss of fresh fish flavor in some species (Fraser, 1965; Spinelli *et al.*, 1969; Jones and Murray, 1961). In addition, Hx has been reported to be a good indicator of freshness (Jahns *et al.*, 1976); the present data on seal agree with this suggestion and show that xanthine may serve as a good indicator for seal meat freshness.

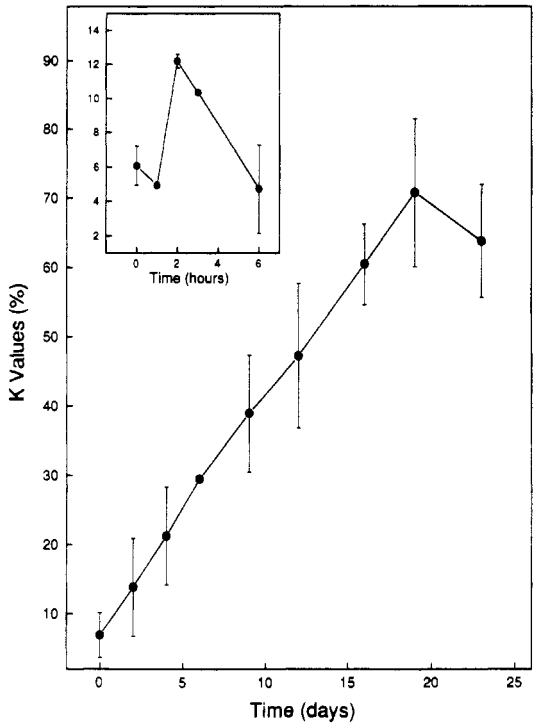

Figure 2. Changes in freshness indicator K values of seal meat during storage at 0 °C. Each point represents the mean value of triplicate determinations of samples from seven seals ± standard deviation.

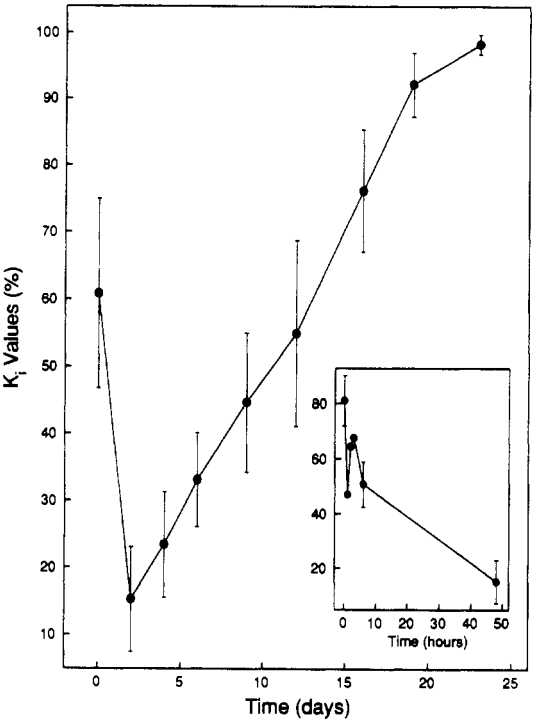

Figure 4. Changes in freshness indicator K_i values of seal meat during storage at 0 °C. Each point represents the mean value of triplicate determinations of samples from seven seals ± standard deviation.

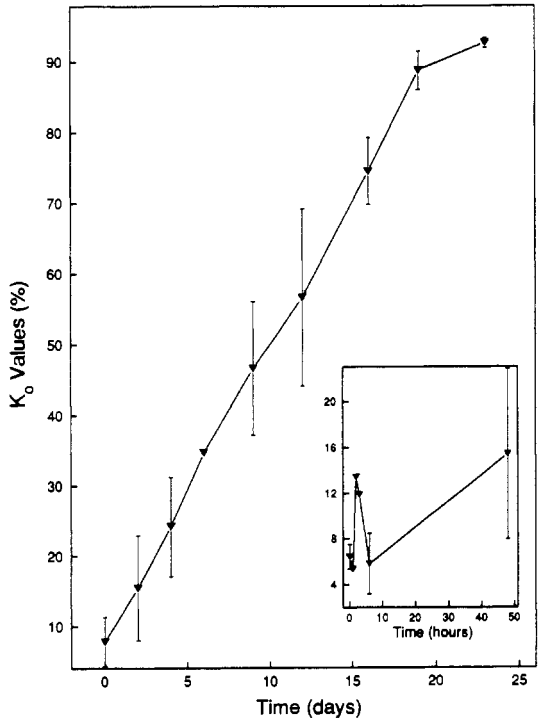

Figure 3. Changes in freshness indicator K_0 values of seal meat during storage at 0 °C. Each point represents the mean value of triplicate determinations of samples from seven seals ± standard deviation.

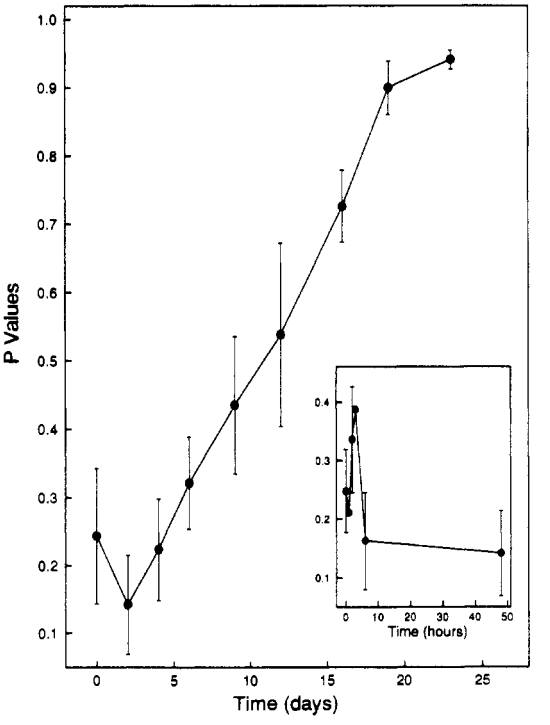

Figure 5. Changes in freshness indicator P values of seal meat during storage at 0 °C.

The commonly used indicators of freshness quality, namely K, G, P, K_0, and K_i values, of seal meat were calculated from the concentrations of the nucleotides. Figures 2–4 show changes of K, K_0, and K_i values of seal meat during a 23-day storage period. During the first 6 h, no clear trends in changes of K, K_0, and K_i values were evident. However, K, K_0, and K_i increased linearly with storage time (19 days). Figure 5 shows a linear increase in P values during the storage period between days 2 and 19. However, no clear trend during the early storage period (up to 13 days) was noticed when G values were considered (Figure 6). However, a linear increase in the G values was noticed during prolonged storage of the meat. Compared with other freshness indicators, the G value is the only parameter without Hx in the denominator. Thus, it is clear that Hx plays an important role in the freshness quality of seal meat and may serve as a good freshness quality indicator by itself.

Comparison of K and K_0 values of seal meat with K_0

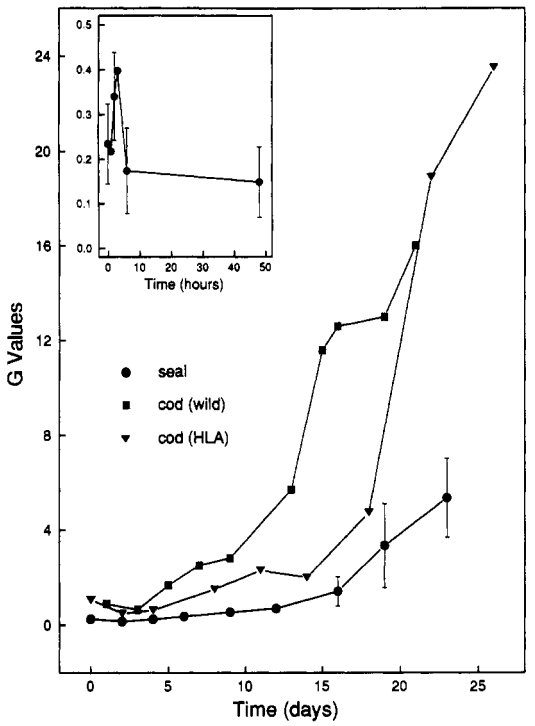

Figure 6. Changes in freshness indicator G values of seal meat as compared with wild Atlantic cod and cod fillets from Halifax Laboratory Aquaria (HLA) (Burns *et al.*, 1985) during storage at 0 °C.

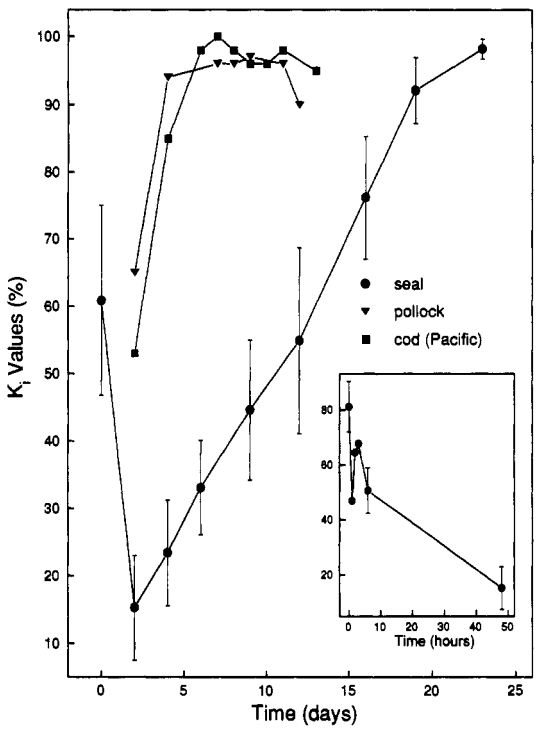

Figure 8. Changes in freshness indicator K_i values of seal meat as compared with Pacific cod and pollock (Green and Bernatt-Byrne, 1990) during storage at 0 °C.

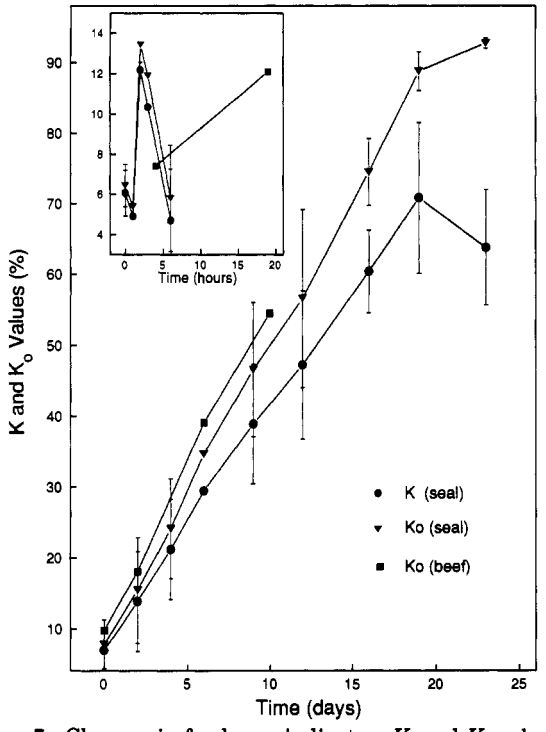

Figure 7. Changes in freshness indicators K_i and K_0 values of seal meat as compared with beef (Watanabe *et al.*, 1989) during storage at 0 °C.

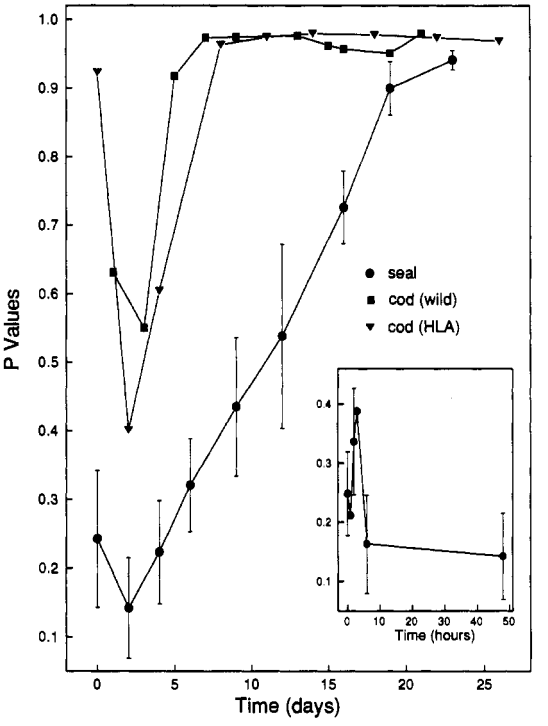

Figure 9. Changes in freshness indicator P values of seal meat as compared with wild Atlantic cod and cod from Halifax Laboratory Aquaria (HLA) (Burns *et al.*, 1985) during storage at 0 °C.

values of beef (Watanabe *et al.*, 1989) is shown in Figure 7. It is apparent that seal meat behaves similarly to beef. Both seal meat and beef are from warm-blooded animals, and this might explain the similarity in their post-mortem behavior. Comparison of the K_i values of seal meat with those of the Pacific cod and pollock (Greene and Bernatt-Byrne, 1990) indicated that seal meat has a better keeping quality than either species of fish considered (Figure 8). Comparison of the G and P values of seal meat with those of Atlantic wild cod and the cod from the Halifax Laboratory Aquaria (HLA) (Burns *et al.*, 1985) indicated a similar trend (Figures 6–9).

In summary, the present study shows that the HPLC method provides an adequate procedure for evaluating freshness quality of harp seal meat. Data presented also indicated that harp seal meat behaves similarly to beef in terms of its storage freshness quality. The K, K_i, K_0, and P values offered a better indication for seal meat freshness

than the G value, as a better linear relationship was obtained between these indicators and the storage of the meat for up to 19 days.

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