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Breakdown Products of Adenosine Triphosphate in Heated Fishery Products as an Indicator of Raw Material Freshness and of Storage Quality

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The breakdown products of adenosine triphosphate (ATP) and the K-value were determined as a means of evaluating the quality of some heated fishery products. During hot smoking, the K-value usually increased by 6 to 30%. During sous vide *treatment of whitefish, the K-value increased from 4 to 48%. Nucleotide breakdown did not occur to a marked extent in hot smoked or* sous vide*-treated products during subsequent storage. The denaturation of water-soluble proteins was monitored by electrophoretic methods. The enzymes that degrade nucleotides as well as soluble proteins of rainbow trout were denatured at 65 to 70 °C. The method could be tentatively used for the evaluation of what the level of freshness of the rainbow trout was before hot smoking. The K-value of rainbow trout did not increase during cold smoking but did increase afterwards during subsequent cold storage, in a fashion similar to that occurring with raw fish. Differences in the contents of nucleotide breakdown products between individual fish samples decrease the suitability of this method.*

Introduction

Smoking is widely used as a method of preparing fish for consumption. In Finland, salmon and rainbow trout are often cold smoked, i.e. the fish remains uncooked and the smoking kiln temperature does not exceed 30 °C. Fish that are hot smoked are heated during the smoking process; the kiln temperature may be as high as 80 °C. During the *sous vide* process (1), the flesh of fish is also heated.

The K-value calculated from the breakdown products of adenosine triphosphate (ATP) has been used as a freshness indicator of raw fish (2–4). There is not very much information available concerning the use of K-value as a freshness indicator for heated fish products.

The object of this study was to investigate the applicability of the method in the quality control of certain heated fish products. In particular, it was considered necessary to determine to what extent nucleotides decompose during hot smoking and *sous vide* treatment, and whether the K-value could be used as an indicator of quality during the storage of smoked fish or in determining what the level of freshness was before hot smoking process.

Rehbein (5) has used protein chemical methods for determination of the necessary heating temperature for fish products. In this work, isoelectric focusing was used to test the denaturation of soluble proteins. The method could be applied to distinguish between cold-smoked and hot-smoked fish, as well as to provide information concerning the denaturation of nucleotide-decomposing enzymes.

Materials and Methods

Fish material

Baltic herring (*Clupea harengus membras*), whitefish (*Coregonus lavaretus*), rainbow trout (*Oncorhynchus mykiss*) and mackerel (*Scomber scombrus*) were obtained from local retail shops in southern Finland. The mackerel was kept frozen until smoking. Herring, whitefish and rainbow trout were stored at 5°C and salted before smoking.

Denaturation of water-soluble proteins and nucleotide-degrading enzymes

Rainbow trout (1.2 kg) was filleted. One fillet was cut into five pieces; three pieces were frozen at –20 °C and two pieces were kept at 5 °C overnight. Another fillet was cut into seven pieces, excluding the tail. One piece was left to stand at room temperature overnight, and the other pieces were heat-treated. A thermocouple was placed into the core of each piece, and the piece was wrapped in aluminium foil. The pieces were placed in an oven at 100 °C and removed from the oven when the temperature inside each piece had reached 40, 50, 60, 65, 70 and 75 °C, respectively. The times required to reach these temperatures were, respectively, 23, 31, 39, 43, 48 and 56 min. One half of each piece was frozen at

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–20 °C, and the other was left to stand at room temperature overnight. The samples were frozen until chemical analysis and isoelectric focusing (IEF).

Sous vide *treatment*

Whitefish was caught by trawling, gutted and placed in ice immediately after catching. Half of each fish was dipped in a 100 g/L ascorbic acid solution. After this, the fish was vacuum-packed in laminated polyethene–polyamide bags and immersed in a waterbath, the temperature of which was 98 °C. Heating was continued until the innermost temperature of the fish was 73 °C for at least 3 min. The heat treatment took place 1 day after catching the fish. The packages were cooled and stored in ice water before testing nucleotide breakdown. As a reference, whitefish from the same catch was stored in ice.

Sampling from an industrial smoking kiln

Trial 1. Baltic herring and mackerel were hot smoked according to traditional practices. The rainbow trout that was used for cold smoking had been filleted and pickled in salt solution some days before smoking. The smoked fish and the raw material, which was stored in a cold room at 5 °C, were both frozen for chemical analyses 2 h after the smoking process. Part of both the smoked fish and the raw material were also stored for 9 days at 5 °C to analyse for ATP breakdown compounds (**Table 1**).

Trial 2. Frozen rainbow trout and mackerel fillets were defrosted before hot smoking, whereas Baltic herring and whitefish were delivered fresh. Baltic herring was caught on the previous day, and whitefish 2 days before smoking. Mackerel fillets and whitefish were dipped in salt solution before smoking.

Thermocouples were placed in the middle of the fish or fish fillets, and data were logged with a Grant Squirrel logger during the process. The temperature profiles are shown in **Fig. 1**. Different individual fish were taken for chemical analysis every half an hour by freezing them with dry ice. Hot smoking lasted 2 h 15 min, and cold smoking 2 h 30 min.

Trial 3. Rainbow trout was salted overnight at 5 °C and hot smoked for 4 h 15 min. The inner temperature of the fish increased from 8 to 63 °C. Samples from four marked fish were taken before and after hot smoking.

Methods

Determination of nucleotides and related compounds was performed by the reverse phase HPLC procedure of Ryder (6). The HPLC system and the calculation of K-values have been previously described (7).

The K-value was defined as:

$$K\,(\%) = 100 \times ([Ino] + [Hx])/([ATP] + [ADP] + [AMP] = [IMP] + [Ino] + [Hx]) \quad \text{Eqn [1]}$$

Table 1 K-values and amounts of breakdown products of ATP of smoked fish and the raw fish material used for smoking during chilled storage at 5°C (Trial 1)

Sample	Days at 5°C	K-value (%)	Concentration (mmol/kg wet weight) IMP	Ino	Hx
Rainbow trout					
Raw (material)	0	47	4.74	3.88	0.54
	4	56	3.93	4.66	0.90
	9	70	2.35	4.32	1.84
Rainbow trout					
Cold smoked	0	40	5.50	3.28	0.66
	4	55	4.53	5.00	1.16
	9	69	2.78	5.44	1.61
Baltic herring					
Raw (material)	0	65	2.58	4.62	0.87
Hot smoked	0	87	0.69	4.70	1.79
	4	90	0.43	4.59	2.21
	9	91	0.52	5.15	2.53
Mackerel					
Raw (material)	0	49	4.45	4.06	0.71
Hot smoked	0	67	2.44	4.29	1.76
	4	67	2.44	4.17	1.90
	9	70	2.50	5.22	2.48

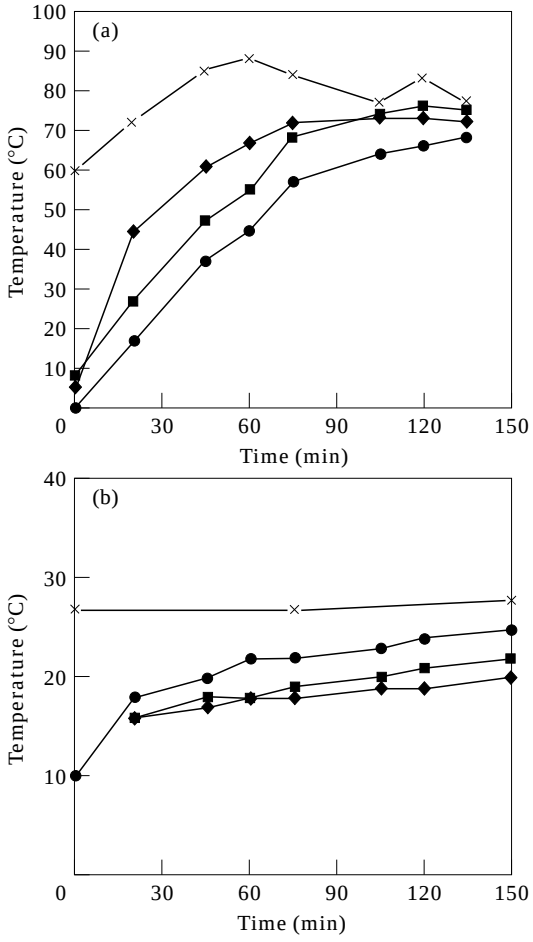

Fig. 1 Temperature profiles of fish during hot (a) (x = air in the kiln; ◆ = mackerel; ■ = whitefish; ● = rainbow trout) and cold (b) (x = air in the kiln; ● = lowest shelf; ◆ = upper shelf; ■ = middle shelf) smoking (Trial 2)

where [Ino], [Hx], [ATP], [ADP], [AMP] and [IMP] are molar concentrations of inosine, hypoxanthine, adenosine triphosphate, adenosine monophosphate and inosine monophosphate, respectively.

IEF and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were carried out on thin layer polyacrylamide gels using LKB 2217 Ultrophor system (LKB, Produkter AB, Brommer, Sweden). The procedure for IEF was described in the LKB Application Note 320 (8), and the pH range used was 3.5 to 10.0. For IEF, fish samples were extracted with water (1,3).

Results and Discussion

Denaturation of water-soluble proteins and nucleotide-degrading enzymes in rainbow trout during heat treatment

Separation patterns obtained in the IEF of aqueous extracts of heated rainbow trout are presented in **Fig. 2**. The patterns of unheated rainbow trout are similar to those of that heated to 40 °C. The number of zones gradually decreased with an increase in the heating temperature. Several extra zones having low pI were observed in the patterns of fish heated at 50 and 60 °C. Rehbein (5) found that the proteins of saithe, haddock and herring were denatured sequentially from high to low pI proteins but that there were no extra zones in the pattern of water-soluble proteins of saithe.

In the SDS-PAGE patterns, the zones also disappeared gradually when the fish was heated above 50 °C, and there was only one zone in the pattern of the samples heated to 65, 70 and 75 °C. The last remaining proteins had a low molecular weight (MW approximately 10,000), which could indicate that these proteins did not coagulate easily. Some degradation products of ATP after heat treatment are presented in **Fig. 3**. The content of IMP decreased from 5.1 to 2.7 mmol/kg when the fish was heated to 65 °C. At the same time, the inosine content increased from 1.3 to 3.2 mmol/kg and the content of hypoxanthine from 0.09 to 0.45 mmol/kg. The content of ATP degradation products did not change in the fish heated at 70 and 75 °C during subsequent incubation at room temperature, indicating total denaturation of the nucleotide-degrading enzymes. The enzymes were in fact denaturated to some extent even at temperatures lower than 70 °C. Furthermore, the K-value did not change during the storage in the samples heated at 70 and 75 °C (**Fig. 4**). Nedachi and Hirota purified IMP degrading enzyme 5'-nucleotidase from snapper muscle and found that the activity diminished a little at 40 °C (9).

ATP breakdown products in sous vide*-treated whitefish*

The K-value and the content of IMP in *sous vide*-treated and untreated whitefish during storage in ice are presented in **Fig. 5**. The K-value increased from a level of 4% to the level of 48% during the *sous vide* treatment. At the same time, the content of IMP decreased from 8.7 to 5 mmol/kg. The K-value and the content of IMP before heating indicated that the raw material was very fresh. In our earlier studies (4), the K-value of whitefish increased very rapidly up to 40% within half a day of catching. The K-value and IMP content did not change during storage in ice, indicating heat denaturation of the degradative enzymes. Ascorbic acid was used as antioxidant and had no effect on nucleotide catabolism.

ATP breakdown products of smoked fish

The results of Trial 1 are presented in **Table 1**. The K-value was about 20% greater in hot smoked Baltic herring and mackerel than in raw fish kept in ice. This was expected because the catabolism of nucleotides is known to increase at higher temperatures (4,8). Hara and Uda found an increase in K-value parallel with temperature increase, but they studied fish only between 0 and 25 °C (10).

During cold storage, the K-value of cold smoked rainbow trout increased as rapidly as that of the freshly stored rainbow trout, indicating that catabolic enzymes

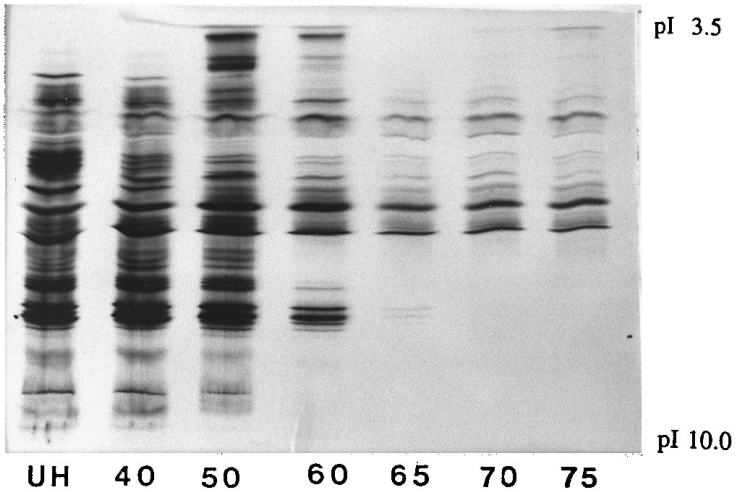

Fig. 2 Isoelectric focusing patterns of aqueous extracts of heated rainbow trout. UH = unheated; 40, 50 etc. indicate the temperatures of the fish samples

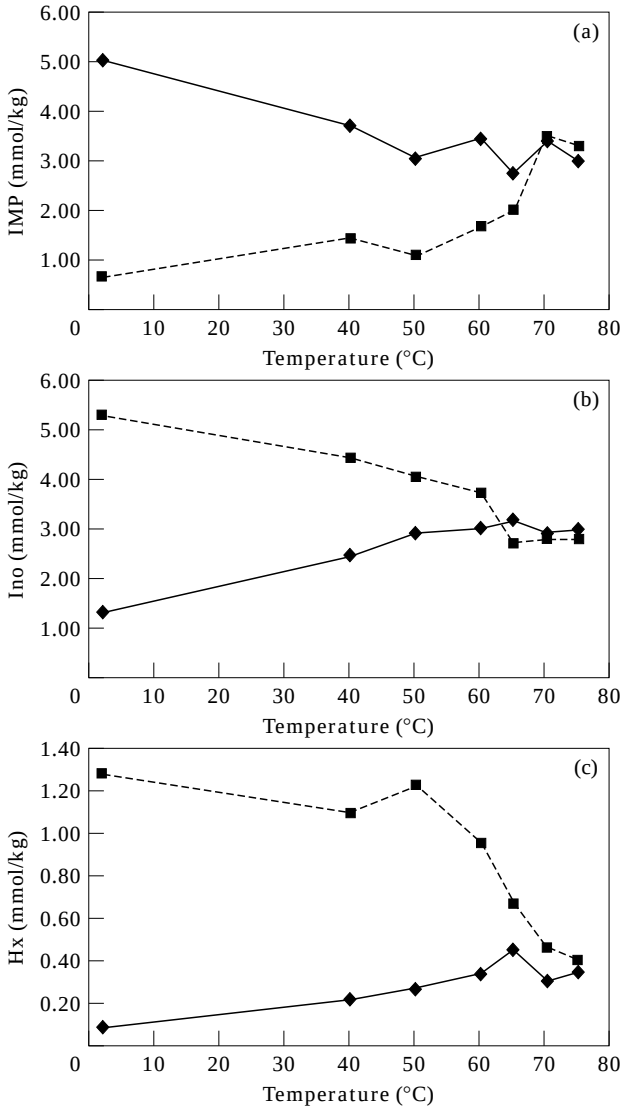

Fig. 3 The amounts of inosine monophosphate (a), inosine (b) and hypoxanthine (c) in rainbow trout after heat treatment (-◆-) and after subsequent overnight storage at room temperature (-■-)

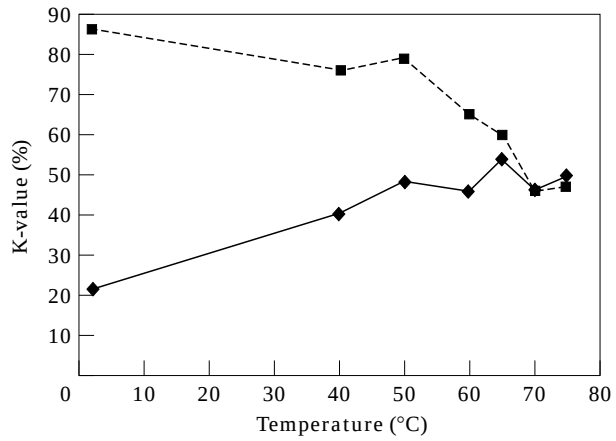

Fig. 4 Changes in K-value after heating of rainbow trout (-◆-) and after subsequent overnight storage at room temperature (-■-)

were active after cold smoking. In hot-smoked fish, the changes in K-values were negligible.

Trial 2 was organized in order to monitor changes in ATP-related compounds during the smoking process. The average increase in the K-value was about 20% in Baltic herring, whitefish and mackerel. The content of IMP decreased considerably in Baltic herring and whitefish and also, to some extent, in mackerel during hot smoking. The amounts of inosine and hypoxanthine increased most dramatically in Baltic herring.

The changes in ATP breakdown products in rainbow trout were rather small during both cold and hot smoking (**Table 2**). The unexpected decrease of the K-value could be explained by individual differences between fish. Samples for chemical analysis were taken from different individuals, and it is known that there may be marked differences in nucleotide composition between different individuals (3,4).

In Trial 3, the samples were taken from the same fish before and after smoking (**Table 3**). The K-value increased by an average of 6% during hot smoking.

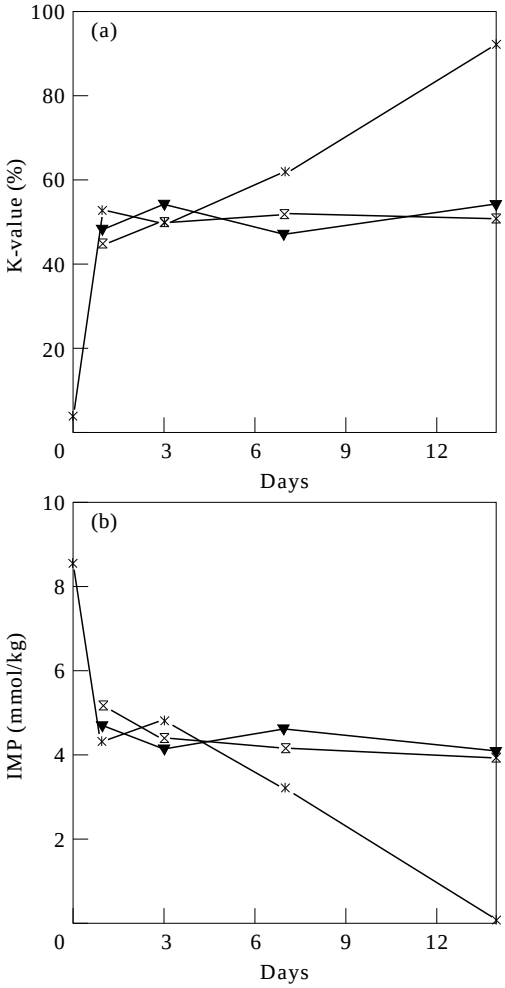

Fig. 5 Changes in K-value (a) and content of inosine monophosphate (b) in raw and two different *sous vide*-treated whitefish during storage in ice. (x) 0 °C, raw; (-▼-) 0 °C, *sous vide*; (-⧖-) 0 °C, *sous vide*, pretreated with 10% ascorbic acid solution

When examining the denaturation of nucleotide-degrading enzymes, the K-value of rainbow trout increased during heating from 20 to 50% (**Fig. 4**). Apparently, when the K-value before smoking amounts to 50%, the K-value of rainbow trout increases much more slowly.

The results concerning the denaturation studies were obtained with much smaller samples than that of fish fillet or whole fish, which could have accounted for the different behaviour of the enzyme system.

Table 2 K-values and amounts of breakdown products of ATP of fish during smoking (Trial 2)

Sample	Time (h)	K-value (%)	Concentration (mmol/kg wet weight) IMP	Ino	Hx
Cold smoking					
Rainbow trout	0.0	58	3.04	3.28	1.76
	0.5	65	2.04	3.30	1.55
	1.0	49	3.39	3.08	0.75
	1.5	52	3.00	2.86	0.85
	2.0	53	3.37	3.68	0.98
	2.5	53	3.38	3.38	1.20
Hot smoking					
Rainbow trout	0.0	60	2.84	3.59	1.20
	0.5	54	3.26	3.50	0.95
	1.0	48	3.34	2.92	0.74
	1.5	53	3.31	3.35	1.03
	2.25	52	3.30	3.09	0.96
Baltic herring	0.0	63	2.32	3.05	1.49
	0.5	93	0.17	3.59	2.78
	1.0	86	0.60	3.85	1.20
	1.5	95	0.16	4.43	3.15
	2.25	89	0.57	4.43	2.23
Whitefish	0.0	73	1.45	2.76	1.99
	0.5	90	0.06	2.73	2.57
	1.0	83	0.65	3.98	2.76
	1.5	92	0.17	2.61	3.77
	2.25	93	0.13	3.33	2.95
Mackerel	0.0	60	2.23	2.31	1.36
	0.5	55	2.33	2.39	0.80
	1.0	75	1.06	2.78	1.02
	1.5	71	1.27	2.75	0.93
	2.25	78	0.87	3.01	0.72

Table 3 K-values and contents of ATP breakdown products of four individual rainbow trout before and after hot smoking (Trial 3)

	K-value (%)	Concentration (mmol/kg wet weight) IMP	Ino	Hx
Before smoking				
Mean	47.8	4.39	3.50	0.59
Standard deviation	5.0	0.77	0.24	0.07
After smoking				
Mean	54.0	4.18	3.71	1.46
Standard deviation	2.2	0.31	0.17	0.24

Conclusion

The K-value is a relevant method for monitoring the freshness of cold smoked rainbow trout during cold storage because nucleotide breakdown during the cold smoking process is very limited and the enzymes are not denatured at the temperatures used in the process. The enzymes that degrade nucleotides are denaturated during hot smoking, and the ATP breakdown products do not change during subsequent cold storage. Determination of the K-value could be used as a tentative method for determining that raw material was very fresh before heat treatment, as in our study of rainbow trout before hot smoking or whitefish before *sous vide* treatment. If the K-value of fish raw material is more than 70% before heat treatment, other methods, such as sensory tests, volatiles and amines, for evaluating the quality of heated fish products should be used.

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