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The Lysosomal Enzyme Activities of Fresh, Cooled, Frozen and Smoked Salmon Fish Species *(Onchorhyncus keta* and *Salmo salar)*

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Abstract: Frozen-thawed fish and fillets are being marketed as fresh fish fillets. One of the methods which helps us to differentiate unfrozen from frozen-thawed fish fillet is the enzymatic method. By means of the freezing and thawing process lysosomal enzymes are released. Lysosomal enzyme activities have the enzymes which decompose protein, carbohydrate, fat and nucleic acids.

In this research, fresh, stored in ice, frozen and smoked salmon fish (*Onchorhyncus keta* and *Salmo salar*) have been used. In press juice and in extracts of these fish a muscle lysosomal enzyme α-Glucosidase (E.C. 3.2.1.20) in activitation quantities was detected. The lysosomal enzyme activities of the press juice of frozen-thawed salmon fish fillets were observed to be higher thanthose of fresh salmon fish fillets. The α-Glucosidase activity ($\Delta E_{405} x 10^{-2} h^{-1}$ x mg protein) was detected as 0.151 (at the beginning) in fresh fillets of salmon fish and that value of the salmon fish which are stored in ice was observed to have increased to 0.309 by the 9th day. The activity in frozen-thawed fillets was detected as 1.185 to 1.503. The α-Glucosidase activity of smoked salmon with different storage expiry periods was observed to be 0.969-4.51. At the end of the research it can be said that the lisosomal enzyme α-Glucosidase (E.C. 3.2.1.20) can be used to differentiate between processed, nonprocessed, fresh and frozen-thawed fish and fillets.

Key Words: Lysosomal Enzyme Activite, α-Glucosidase, Salmon, *Onchorhyncus keta, Salmo salar.*

Taze, Soğutulmuş, Dondurulmuş ve Tütsülenmiş Som Balığı Türlerinde *(Onchorhyncus keta ve Salmo salar)* Lizozomal Enzim Aktiveleri*

Özet: Dondurularak çözündürülmüş balık ve balık filetoları, günümüz balıkcılarında taze balık ve balık filetoları gibi satışa sunulmaktadır. Çözündürülmüş balık filetolarını taze balık filetolarından ayırt etmeye yardımcı olan metotlardan bir tanesi de enzimatik metodlardır. Dondurma ve çözdürme işlemi ile lizozomal enzimler serbest kalır. Lizozomal enzim aktiviteleri, protein, karbonhidrat, yağ ve nükleik asitleri ayıran enzimleri içerir.

Bu çalışmada taze, buzda depolanmış, dondurulmuş ve tütsülenmiş som balık türleri (*Onchorhyncus keta* ve *Salmo salar*) kullanılmıştır. Bu balıkların pres suyu ve ekstraktlarında bir lizozomal enzim olan α-Glukosidaz (E.C. 3.2.1.20) aktivite oranları belirlenmiştir. Dondurarak çözdürülmüş som balıkları filetolarının pres suyundaki lizozomal enzim aktiviteleri, taze som balık filetolarının aktivitelerinden açık olarak yüksek değerlerde bulgulanmıştır. α-Glucosidaz aktivite (ΔE_{405} x $10^{-2} h^{-1}$ x mg protein) som balıklarının taze filetolarında 0.151 (0. gün) olarak bulgulanmış olup buzda depolanmış som balıklarında bu değerin 9. günde 0.309'a yükseldiği saptanmıştır. Dondurarak çözündürülmüş filetolardaki aktivite 1.185-1.503 değerleri arasında ölçülmüştür. Değişik depolama ömrüne sahip tütsülenmiş som balıklarında α-Glukosidaz aktivitesi 0.969-4.512 değerleri arasında bulgulanmıştır. Araştırma sonucunda bir lizozomal enzim olan α-Glukosidaz (E.C. 3.2.1.20) aktivite oranlarının işlenmiş ve işlenmemiş ve de, taze ve dondurarak çözündürülmüş balık ve filetolar arasındaki farkı ayırt etmek için kullanılabileceği söylenebilir.

Anahtar Sözcükler: Lizozomal enzim aktivite, α-Glukosidaz, Som Balığı, *Onchorhyncus keta, Salmo salar*.

Introduction

In many countries, thawed and ice stored fish and fillets are sold as fresh fish and fillets. For the benefit of customers and in order to prevent unjustifiable profit it is necessary to label thawed fish and fillets correctly. Controlling of the labels is possible only if there are rapid and reliable methods by food authorities separating fresh or thawed fish or fillets. Recently many different methods can be seen in published articles. (5, 6, 13, 19, 23).

* Bu makale 9-11 Nisan 1997'de İzmir'de yapılan "Akdeniz Balıkçılık Kongresi'nde" bildiri olarak sunulmuştur.

- Measurement of the electric properties of fish tissue.

- Visual inspection of the eye lens.

- Judgement of the integrity of red blood cells by microscopy or estimation of the hematocrit value.

- Determination of the release of enzymes originally bound to mitochondria, lysosomes or red blood cells.

The cells of fish muscle and their organelles are destroyed by freezing and thawing. Enzymes located inside the particles or bound to the membranes are released into thaw drip and press juice (23).

When muscle of fresh fish was carefully extracted with isotonic solution (0.25-0.30 M sucrose) only low activities of lysosomal and mitochondrial enzymes were detectable in the soluble fraction (supernatant after high-speed centrifugation); the same treatment applied to frozen-thawed fish muscle gave considerably enhanced activities of these enzymes in this fraction (5). The appearance of originally partide bound enzymes in drip, press juice or isotonic extract has been used by a number of research groups to differentiate between fresh and frozen-thawed fish fillets.

Compared with mitochondria, lysosomes seem to be less structured. Lysosomes are "single membrane-bounded organelles containing numerous hydrolytic enzymes to digest materials ingested by endocytosis and recycle cellular components" (19).

However, this view has to be differentiated. At least three types of lysosome can be distinguished: primary and secondary lysosomes as well as residual bodies (4). A great number of lysosomal enzymes have been detected in extracts of fish muscle. Examples from recently published studies are:

Aryl sulfatase (EC 3.1.6.1), beta-glucuronidase (EC 3.2.1.31), RNAse (EC 2.7.7.16), acid phosphatase (EC 3.1.3.2) and acid proteinase (EC 3.4.23) in saithe (*Pollaius virens*) (1) cathepsin D (EC 3.4.4.23) in herring (5); beta-N-acetylglucosaminidase (EC 3.2.1.30) in eight species of fish (16); beta-glucuronidase, beta-N-acetylglucosaminidase, acid phosphatase and cathepsin D in Pacific mackerel (*Scomber japonicus*) (17).

These enzymes expressed maximal activity in the acidic pH-range (3-5). This characteristic property can be used to distinguish lysosomal enzymes from their neutral counterpart (2).

Three distinct populations of lysosome were identified in muscle tissue of salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairneri*) by electron microscopy. One population was observed in connective tissue cells tentatively identified as macrophages; a second population was detected within but at the periphery of muscle cells and a third population of lysosomes was localized among myofibrils (15).

Some of the above methioned lysosomal hydrolases have been utilized as a tool to differentiate unfrozen from frozen thawed fish fillet (14).

Alpha-glucosidase activity (EC 3.2.1.20) was measured in press juice from fresh and frozen-thawed fish of cod (*Gadus morhua*), saithe (*Pollachius virens*), redfish (*Sebastes marinus*) and haddock (M*elanogrammus aeglefinus*) (8). The press juice was obtained by high-speed centrifugation of muscle tissue; the enzyme activity was determined using the chromogenic subtrate, p-nitrophenyl-alpha-D-glucopyranoside, at pH 4.5.

The specific activity of alpha-glucosidase was considerably enhanced in press juice from frozen-thawed fillets compared with press juice from fresh ones. This allowed the differentiation of the two categories of fillet. It was difficult, however, to get standard values of the specific alpha-glucosidase activity for both types of press juice, because the activity in fillets of the same fish species varied to some extent. This might be due to biological factors or to enzyme denaturation during processing and storage.

Therefore the ratio of the specific activities in press juice and sediment extract was determined to reduce the variation. The activity ratios were 0.05-0.20 for non-frozen fillets of cod and saithe and 0.50-1.20 for frozen-thawed fillets of these species (10).

During storage of wet fish (cod) in ice the activity ratio for the alpha-glucosidase increased gradually indicating the destruction of lysosomes by fish muscle and bacterial proteases. Spoiled fish had an activity ratio nearly as high as that found for unspoiled frozen-thawed fish (8), but both products could be differentiated without difficulties by the estimation of parameters of spoilage such as total volatile bases-nitrogen or trimethylamine (21).

α-Glucosidase activities of fresh, ice stored, frozen and thawed fish such as saithe, cod, eel and herring fish were determined (8, 9, 11-13).

In this article α-Glucosidase (EC 3.2.1.20) activity was studied as a lysosomal enzyme present in press juice and extracts of fresh, ice stored, frozen and smoked salmos species (*Onchorhynchus keta* and *Salmo salar*).

Materials and Methods

Wet fish or unfrozen fillets

These were obtained either from the local fish market or from fish shops in Hamburg-Altona. Because of their commercial importance salmon (*S. salar* and *O.keta*) were investigated.

Blocks of Sea-frozen fillets

These were received directly from commercial fishing trawlers or from the local cold store and stored at -25 to -30°C in a deep freezing room. For investigations the fillets were thawed.

Smoked salmon species

These were obtained either from the local fish market or from fish shops in Hamburg-Altona. Because of their commercial importance smoking salmon (*S.salar* and *O. keta*) were investigated.

Press juices

These were prepared as follows: white muscle (40 g), taken when possible from the middle, part of the fillet was cut into pieces and centrifuged at 5°C at 18,000 rev min^{-1} (28000 g) for 30 minutes (MSE 25, rotor 43 114-115).

Extracts

These were prepared by homogenising an aliquot of the sediment about 1+3 (w+v) with 0.2% (w/v) Triton x-100, polyethylene glycolmono [*p*-(1.1, 3.3-tetramethylbutyl)-phenyl) ether, for homogenisation with an ultra-Turrax (Janke and Kunkel KG, Staufen/Breisgau), after standing at 4°C for 30 minutes the mixture was centrifuged as described for press juice, yielding a clear or slightly turbid extract.

Enzyme assays

α-Glucosidase activity was determined by the method of Milanesi et al. (7). The assay mixture contained 0.20 ml *p*-nitrophenl-α-glucopyranoside solution (7.53 mgxml^{-1} distilled water) 0-30 ml 0.2 M K-citrate pH 4.5, 0.2 ml 1 M-NaCl, and enzyme solution; it was adjusted with distilled water 1.20 ml test volume. The reaction was started by the addition of enzyme and allowed to incubate

Table 1. Comparison between the specific activity ratio for salmon (*S. salar* and *O. keta*).

FISH SPECIES	α-Glucosidase Specific Activity	
	Muscle Ext. (AV_1)	Sediment Ext. (AV_2)
Salmon (Fresh) (*S. salar*)		
Salmon Fillets (Ice Stored)	0.151	0.018
2 days Ice Stored	0.248	0.019
3 days Ice Stored	0.070	0.0167
5 days Ice Stored	0.080	0.0140
9 days Ice Stored	0.309	0.035
Salmon Fillets (*S. salar*) -25°C Freezing	1.185	0.252
Salmon Fillets (*S. salar*) -25°C Freezing	1.503	0.267
Smoking Salmon Species		
Smoking Salmon (*S. salar*) (Fresh)	1.026	0.063
Frozen-Thawed Smoking Salmon (*O. keta*)	0.969	0.137
Frozen-Thawed Smoking Salmon(*O. keta*)	4.512	0.245
Frozen-Thawed Smoking Salmon (*S. salar*)	2.554	0.267
Frozen-Thawed Smoking Salmon	1.718	0.267
Frozen-Thawed Smoking Salmon	3.096	0.241
Frozen-Thawed Smoking Salmon	3.596	0.334
Frozen-Thawed Smoking Salmon	2.629	0.373
Frozen-Thawed Smoking Salmon	2.078	0.163
Frozen-Thawed Smoking Salmon	1.941	0.190
Frozen-Thawed Smoking Salmon	1.026	0.063
Frozen-Thawed Smoking Salmon	1.803	0.146

a Specific Activty; $\Delta E_{405}x10^{-2}h^{-1}$ xmg protein (Muscle extr.).

for 2h at 37°C. It was stopped by adding 1.0 ml 0.2 M-KOH with vigorous stirring. Immediately after centrifugation for 1.5 min (8000 g, Eppendorf centrifuge 3200) the absorbance was measured against a blank at 405 nm. In the blank enzyme solution was added after KOH. Since the solution was readily hydrolysed in alkaline solution, all steps after addition of the KOH had to be performed rapidly.

Table 2. α-Glucosidase Specific Activity in Different Fish Species*

Saithe fillets (*Gadus virens* L.)		
- Fresh	2.64 - (1.69-3.53)	
- Frozen**	15.5 - (13.1-18.7)	(8)
- Seafrozen***	7.95 - (5.30-10.7)	
Cod fillets (*Gadus morhua* L.)		
- Fresh	$0.13^{a}\pm5.49^{b}$ %	(9)
- Frozen-Thawed	$0.72^{a}\pm5.08^{b}$ %	
Cod fillets (*Gadus morhua* L.)		
- Fresh	0.05-0.20	(10)
- Frozen-Thawed	0.50-1.20	
Saithe fillets (*Gadus virens* L.)		
- Fresh	0.06-0.20	
- Frozen-Thawed	0.50-1.20	
Cod fillets (*Gadus morhua* L.)		
- Fresh	1.03 - (0.71-1.51)	
- Frozen**	5.86 - (4.45-7.83)	
- Seafrozen	2.70 - (2.52-2.82)	
Saithe fillets (*Gadus virens* L.)		
-Fresh	15.9	(10)
- Frozen-Thawed	20.1	
Trout (*Onchorhynchus mykiss*)		
- Fresh	$0.078a\pm0.024^{b}$	
- Ice Stored	$0.11a\pm0.045^{b}$	(11)
- Frozen-Thawed	$0.39a\pm0.084^{b}$	
Eel (*Anguilla* spp.)		
- Fresh	$0.25^{a}\pm0.094^{b}$	
- Frozen-Thawed		
- 16 days Stored	$0.75^{a}\pm0.070$	
- 45 days Stored	$1.11^{a}\pm0.160$	(12)
- 180 days Stored	$3.40^{a}\pm1.00$	
- 780 days Stored	$3.24^{a}\pm0.32^{b}$	

a= Mean value. b= Standard deviation

* Specific activity, $\Delta E_{405}x10^{-2}$ h^2 x mg protein.

** The back parts of the fresh fillets were stored for 1 day at -29°C, the figures in the brackets express the fluctuations.

*** The sea-frozen fillets had been produced from saithe caught in November 1976 near the Norwegian coast.

Findings

α-Glucosidase enzyme activities of fresh, ice stored, frozen and smoked salmon species (*Onchorhynchus keta* and *Salmo salar*) are given in Table 1.

For fresh salmon AV1 (muscle extract)=0.151 and AV_2 (sediment extract)=0.018; for ice stored fish, it was found that after 2 days and 9 days values are different such as AV_1=0.248-0.309 and AV_2=0.019-0.035. Salmon stored at -25°C had values in the range of AV_1= 1.185-1.503 and AV_2= 0.252-0.267. It is evident that alpha-glucosidase activity (EC 3.2.1.20) of the press water of frozen/thawed and ice stored fish are higher than those of fresh fish fillet press water. Unstored but smoked salmon (*S. salar*) have AV_1=1.026 and AV_2= 0.063 and these values differ from one company to another within the range of AV_1= 0.969-4.512 and AV_2= 0.063-0.373 for the frozen/thawed salmon (*O. keta* and *S. salar*). According to these results, alpha-Glucosidase activity increases with smoke treatment and unsmoked fish values are lower than frozen/thawed fish activities.

Results and Discussion

There are enzymatic methods which distinguish fresh fish fillets from thawed fish fillets. Lysosomal enzyme activity in the press water of thawed fillet is clearly higher than that of fresh fillet (Table 2). For the cod alpha-Glucosidase activity (EC 3.2.1.20) of fresh fillet is 0.05-0.20, and of thawed fillet is 0.50-1.20. For the saithe fish it was found to be 0.06-0.20 for fresh fillet and 0.50-1.20 for the thawed fillet. Another lysosomal enzyme β-N-Acetylglucosamidase (EC 3.2.1.30) activity was 0.15-0.40 for fresh fillet and 0.50-1.50 for thawed fillet of Rotbarsch; 0.10-0.25 for fresh fillet and 0.60-1.30 for thawed shellfish. These results, tested on 100 fillets by Rehbein (10), agree well with our findings.

Rehbein (10) states that fresh and thawed fish fillets can be differentiated as follows:

- Frozen and thawed fish fillets have higher press water efficiency (for example, cod fillets have 25.1 g per 100 muscles and thawed fillets have 30.5 g per 100 muscles).

- Ice stored and thawed fish fillets have higher enzyme activities than fresh fish fillets.

These two studies demonstrate that enzymes from both cell organelles, mithochondria as well as lysosomes, can be used to differentiate between fresh and thawed salmon fish species (*O. keta* and *S. salar*).

Which enzyme gives the best result may depend on the fish species, the product (round fish, fillets with or without skin) and the storage conditions (cooling without ice, storage in refrigerated sea water or in ice).

For differentiation of fillets, one of the above-mentioned enzymatic methods may be used. Which type of enzyme (located in the mitochondria, lysosomes, or red blood cells) gives the best results depends to some extent on the fish species.

It is not sufficient to compare enzyme activities in press juice or extract from thawed and very fresh fillets. The release of enzymes during ice-storage of "fresh" (unfrozen) fillets must also be determined, because during spoilage of fillets partide bound enzymes are gradually set free (14).

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