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Summary

#### Zusammenfassung

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# Changes in health beneficial components during ice storage of African catfish *(Clarias gariepinus)*

Änderungen von gesundheitlich vorteilhaften Bestandteilen während der Eislagerung von Afrikanischem Wels (Clarias gariepinus)

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Ice-storage is the most common method of preserving fresh fish. The aim of this work was to study whether ice storage had an effect on contents of selenium, taurine and fatty acid composition in farmed African catfish (*Clarias gariepinus*). Gutted fish (n = 40) were stored in melting ice for 21 days, and 5 fish were at regular time intervals randomly drawn from the pool, filleted and freeze-dried. The samples were analyzed for contents of selenium, taurine and fatty acids. During ice storage, water content of fillets increased due to influx of water from melted ice. Only concentrations of water soluble taurine were found to decrease significantly, approximately 25 %, whereas concentration of selenium and the fatty acid profile did not substantially change during storage.

Keywords: selenium; taurine; fatty acids; nutritional losses

Die Lagerung in schmelzendem Wassereis ist die Methode der Wahl zur Lagerung von Frischfisch. Ziel dieser Arbeit war, zu untersuchen, ob Eislagerung einen Effekt auf die Gehalte an Selen, Taurin, anderen löslichen freien Aminosäuren und Dipeptiden und die Fettsäurezusammensetzung von Afrikanischem Wels *(Clarius gariepinus)* hat. Ausgenommene Fische (n=40) wurden 21 Tage in schmelzendem Eis gelagert. 5 Fische wurden in regelmäßigem Abstand nach dem Zufallsprinzip entnommen, filetiert und gefriergetrocknet. Die Proben wurden nach Probenvorbereitung auf die Gehalte an Selen, Taurin und Fettsäuren analysiert. Während der Eislagerung nahm der Wassergehalt der Filets wegen des Eindringens von Wasser aus dem schmelzenden Eis zu. Nur die Konzentrationen an wasserlöslichem Taurin nahmen während der Lagerung signifikant um etwa 25 % ab. Die Gehalte an Selen und das Fettsäureprofil änderten sich während der Lagerung nicht substantiell.

Schlüsselwörter: Selen, Taurin, Fettsäuren, Ernährungsverluste

#### Introduction

An increased consumption of seafood is generally recommended by nutritionists and public health authorities, and numerous reports conclude that regular seafood consumption has many beneficial health effects (Committee on Toxicity and Scientific Advisory Committee on Nutrition, 2004; Yaktine et al., 2007). The benefits of eating seafood have, until recently, been linked to the high levels of polyunsaturated fatty acids (PUFA) and their effects on reducing the risk of cardiovascular diseases (CVD) (He et al., 2004; Schmidt et al., 2005a; Schmidt et al., 2005b). However, other components in seafood, beyond the n-3 PUFAs, may contribute to its associated health effects (Undeland et al., 2009). Fish is an excellent protein source because of its high digestibility (low content of connective tissue) and a favourable amino acid composition (Sikorski et al., 1990). Additionally, fish contain relatively high levels of selenium and taurine which are suggested to have beneficial health effects with increased dietary intake.

Selenium is an essential micronutrient with a recommended dietary intake for the majority of the population of 55 µg/day as established by the Institute of Medicine in USA (Institute of Medicine, 2000). A review by Rayman (2004) shows that some European populations have a dietary intake below the recommended level due to low selenium levels in the soil. Thus, increased fish consumption is an approach to achieve the level of recommended intake, partially because fish is a source for highly bioavailable selenium (Fox et al., 2004). Selenium is a constituent of selenoproteins such as glutathione peroxidases, deiodinases and selenoprotein P. These enzyme systems are involved in the protection of tissues and membranes against oxidative stress, as well as being important for proper functioning of the immune system, and as catalysts for production of active thyroid hormone (Rayman, 2000). It has also been proposed that increased selenium intake may be protective against certain forms of cancer (Rayman, 2005).

Taurine (2-aminoethanesulfonic acid) is an exclusively free amino sulfonic acid, which may be regarded as conditionally essential (Nittynen et al., 1999). It is important in membrane stabilization and in the development of the central nervous system (O'Flaherty et al., 1997). Seafood, especially invertebrates such as mollusks and crustaceans, are high in taurine (Roe and Weston, 1965; Spitze et al., 2003; Dragnes et al., 2009). The taurine content of fish flesh was in the order plaice (146 mg/100 g), cod (108 mg/100 g), mackerel (78 mg/100 g) and farmed salmon (60 mg/100 g fresh weight) (Gormley et al., 2007). Suggestions have been put forward of reduced risk of CVD through the effects of both taurine alone and combined with n-3 PUFA (Mizushima et al., 1997; Yamori et al., 2001; Elvevoll and Østerud, 2003). Indeed, beneficial effects of increased dietary taurine intake on risk of CVD have been observed in both animal and human studies (Militante and Lombardini, 2002; Chen et al., 2004; Oudit et al., 2004; Yamori et al., 2004; Yamori et al., 2006; Elvevoll et al., 2008). Adults can produce the sulfurcontaining taurine from cysteine with the help of pyridoxine, vitamin B6. However, also for adultsIt is possible that if not enough taurine is built in the body (if cysteine or B6 is deficient) it might be further required in the diet.

Taurine has been shown to be essential in certain aspects of mammalian development, and *in vitro* studies in various species could show that low levels of taurine are associated with various pathological lesions, including cardiomyopathy, retinal degeneration, and growth retardation, especially if deficiency occurs during development of the fetus or the young child. Metabolic functions of taurine include: bile acid conjugation, detoxification, membrane stabilization, osmoregulation, and modulation of cellular calcium levels. Taurine has been used clinically in the treatment of a wide variety of diseases or disorders: cardiovascular diseases, hypercholesterolemia, epilepsy and other seizure disorders, macular degeneration, Alzheimer's disease, hepatic disorders, alcoholism, and cystic fibrosis.

Of the total world fishery production of species intended for human consumption, 51.6 % is marketed as fresh (Food and Agricultural Organization, 2005), and ice storage (storage in melting water ice at a room temperature of approx +2 °C) is the most common method of preserving the fish. Changes in fish muscle composition start immediately after the fish is caught, and since most of these changes are temperature dependent, ice storage is an efficient method to extend the shelf-life. The autolytic reactions and microbial activity that follow post mortem may influence the content of components. Alterations in fatty acid profile may be caused by lipid oxidation, and water soluble components may be lost via drip or due to diffusion from the fish meat to the ice-water phase. Thus, ice storage may alter the nutritional properties of the fish and affect the contents of health beneficial components.

The current trial was part of a large integrated study with an objective to produce farmed fish enriched with selenium via feed, and thereby create seafood with potential anti-carcinogenic properties. The aim of this study was to investigate whether selenium enriched African catfish retained the selenium during ice storage. In addition, changes in concentrations of free amino acids including taurine, and the fatty acid profile were investigated.

This study was part of the project "Seafood from source to Consumer Products" a project in the integrated project (IP) SEAFOODplus. The title of this sub-project was: Consumer driven development of innovative tailor-made seafood products with functional components of plant or marine origin to improve the health of the consumer (Careche et al., 2009).

#### Materials and methods

#### Raw material and experimental design

African catfish were reared at the Institute of Marine Resources and Ecosystem Studies (IMARES, IJmuiden, The Netherlands) for 43 days with a diet supplemented with selenium-enriched garlic as described by Schram et al. (Schram et al., 2008). The fish (n = 40, length  $36 \pm 2$  cm, weight  $394 \pm 77$ ) was killed, gutted and stored in melting ice for a period of 21 days. The ice: fish ratio during storage was approximately 2:1, and the fish were stored inside self-draining polystyrene boxes at temperature  $2 \pm 1$  °C. The ice was replenished every second day or when necessary. At regular time intervals after slaughter (day 1, 5, 8, 11, 13, 15, 18 and 21), five fish were collected, filleted and skinned. Both fillets from the same fish were pooled to one sample, and the samples were freeze-dried.

The lyophilized samples were shipped to participating laboratories and submitted to analysis of taurine and free amino acids (FAA), selenium and fatty acid composition. Water content was indirectly determined by measuring the weight loss of samples during freeze drying.

# Determination of Selenium content

The determination of selenium was performed as previously described by Mierke-Klemeyer et al. (2008). In short, samples of 0.30–0.35 g were digested with 4 mL 65 % nitric acid and 1 mL 30 % hydrogen peroxide in a microwave high pressure system (ultraCLAVE III, MLS GmbH, Leutkirch, Germany). The digested samples were analyzed by a Perkin Elmer ZL 4100 graphite furnace atomic absorption spectrometer with an electrode-less discharge lamp for selenium analysis.

# Analysis of free amino acids

The determination of taurine and FAA was performed as previously described by Mierke-Klemeyer et al. (2008). In short, the FAA were extracted by homogenizing 1 g sample with 9 mL milli-Q H<sub>2</sub>O, 1 mL of 20 mmol/L norleucine and 1 mL 35 % sulphosalicylic acid. The suspension was centrifuged and an aliquot of the supernatant was diluted with sample buffer. The concentration of FAA was determined using a Biochrom B30 amino acid analyzer (Biochrom, Cambridge, UK) equipped with a lithium citrate equilibrated column.

# Determination of fatty acid profile

Fatty acid methyl esters (FAMEs) were prepared according to the procedure described by Bandarra et al. (1997). The analyses were performed in a Varian CP-3800 gas chromatograph (Walnut Creek, CA, USA) equipped with an auto sampler and fitted with a flame ionization detector at 250 °C. The separation was achieved using a capillary

column DB-WAX (30 m length, 0.25  $\mu$ m i. d. and 0.25 mm film thickness) from Hewlett Packard (Albertville, MN, USA). After holding at 180 °C for 5 min the temperature was ramped at 4 °C/min to 220 °C, maintained at 220 °C for 25 min with the injector at 250 °C. The split ratio was 100:1. The identification of the sample peaks was made by comparison of the retention times with the standards from Sigma. The fatty acid profile was obtained by calculating the relative area percent of the chromatographic peaks.

#### **Statistical analysis**

Statistical analysis was performed using SPSS 15.0 (SPSS inc., Chicago, IL, USA). Changes in component concentration were evaluated with linear regression of component concentration as an effect of storage time. To confirm trends from the linear regression, data was treated as repeated measurements and analyzed with repeated measures analysis of variance (ANOVA) using the Greenhouse-

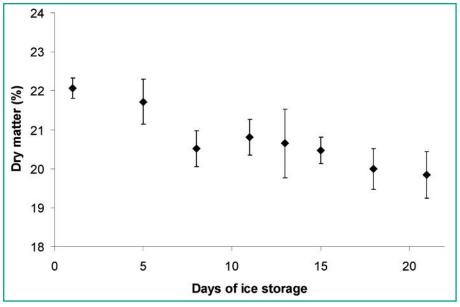
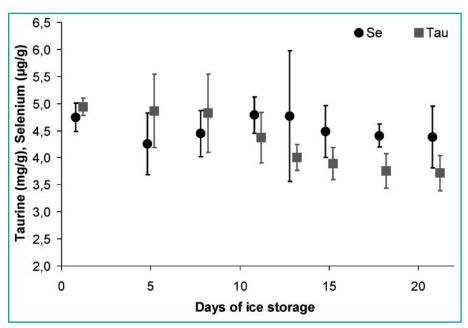


FIGURE 1: Dry matter (%) in fillets of African catfish stored in ice for 21 days.



**FIGURE 2:** Concentrations of selenium ( $\mu g/g$ ) and taurine (mg/g) on a dry weight basis in fillets of African catfish stored in ice for 21 days. Arithmetic mean  $\pm$  SD.

Geisser test of within subjects effects. A significant change in concentration was assumed if P < 0.01.

# **Results and discussion**

The percentage dry weight of the fillets significantly decreased (P = 0.002) during ice storage as can be observed in figure 1. The ice melted during storage, and although the integument is a rather effective barrier against water fluxes, the water content in muscle increased by soaking up water von the melting ice. The fish was stored as gutted fish, and thus the open abdominal cavity was an area were water uptake was likely to occur.

#### Selenium and FAA

Simultaneously with water fluxes from the melted ice into the muscle, soluble components may diffuse from the

TABLE 1:	<i>Concentrations in dry weight of the most abundant free</i>
	amino acids (FAA) and dipeptides anserine and sarco-
	sine in fillets of African catfish at day 1, and statistical
	evaluation of changes in concentration during storage.

	Concentration (mg/g)	R <sup>2</sup>	Slope	Р
Glycine	1.8 ± 0.4	0.29	-0.03	NS
Alanine	1.0 ± 0.3	0.22	0.02	NS
Anserine	1.5 ± 0.2	0.25	-0.02	NS
Lysine	0.8 ± 0.3	0.08	0.01	NS
Glutamic acid	0.4 ± 0.3	0.04	0.01	NS
Sarcosine	1.0 ± 0.4	0.02	-0.01	NS

 $R^2$ : Explained variance, linear model fit. Slope: +/– Describes a trend of decreasing or increasing concentrations. NS = not significant

muscle to the ice-water fraction. Additionally, biochemical reactions post mortem may convert compounds both enzymatically and non-enzymatically. Microbiological activity may also convert components, but this occurs primarily at the surface of the fish.

The concentrations of selenium  $(\mu g/g)$  and taurine  $(\mu g/g)$  in freeze-dried muscle samples at each sampling day are displayed in figure 2. Mean concentration of selenium was 4.7 µg/g dry weight at day 1, and at day 21 the concentration was 4.3 µg/g dry weight. The measured decrease in concentration during ice storage was not significant, thus selenium content in fillets of African catfish seemed little affected by ice storage. The selenium concentration was relatively high compared to African catfish fillets not fed a selenium enriched feed (Schram et al., 2008). Nearly all the selenium in animal tissues is associated with protein, with selenocysteine and selenomethionine being the most prevalent protein bound forms (Burk and Hill, 1993). As most selenium is bound to proteins, instead of being in free form, this may explain why retention of selenium was high during ice storage. Akesson and Srikumar (1994) found that cold storage of cod and herring for one week did not significantly affect the proportion of low-molecular-weight selenocompounds, indicating that there was little degradation of selenoproteins.

On the other hand, concentrations of taurine significantly decreased during ice storage (P = 0.008). At day 1, mean taurine concentration in fillets amounted to 4.9 mg/g dry weight. Taurine remained on this high level until day 8 (4.7 mg/g dry weight), then the concentration started to decrease and reached a minimum of 3.7 mg/g dry weight on day 21. Losses of taurine from fish muscle during ice storage have been found for both codling (Jones, 1954) and cod (Shewan and Jones, 1957), and the losses were primarily attributed to leaching rather than degradation due to microbiological activity. Initial taurine concentration in this freshwater species amounted only to ca. 10 % of taurine content reported for marine fish species (Gormley et al., 2007).

Concentrations of the other most abundant FAA and dipeptides at day 1 are shown in table 1. In general, there were little changes in concentrations of FAA during ice storage. In addition to taurine, only glycine, and the dipeptides anserine and sarcosine were found to have a trend of decreasing concentration. The other FAA showed no change or a slight increase in concentration during storage.

Changes in FAA during storage may be a combined result of increasing concentrations due to autolysis of proteins, and decreasing concentrations due to leaching. Only taurine was found to decrease significantly with an estimated apparent loss of 25 %. However, the taurine content in the fish flesh was stable until day 10; only after day 10 the losses could be observed. Taurine has osmoregulatory functions within cells, and there exist specific taurine channels in cell membranes for rapid release of taurine in extracellular spaces if osmotic environment in the cell is altered (Lambert et al., 2001). Release of taurine have been found both during exercise (Cuisinier et al., 2002) and anoxia (Ortenblad et al., 2003), and is thus likely to occur during development of rigor mortis which is characterised by anoxia and muscle contraction. This may explain why taurine seems more susceptible to leaching than other amino acids. Also, since taurine is not incorporated into proteins, it can not be formed from autolysis of proteins as the other amino acids can.

#### Fatty acids

The mean concentration of total fatty acids in muscle at day 1 was 81 mg/g dry weight. The predominant fatty acids were 16:0 and 18:0 among the saturated fatty acids (SFA) and 18:1 among the monounsaturated fatty acids (MUFA). Within the PUFAs, the most abundant were docosahexaenoic acid (DHA, 22:6n3) followed by eicosapentaenoic acid (EPA, 20:5n3) and linoleic acid (LA, 18:2n6). Table 2 shows the development in fatty acid profile during ice storage. Within the time span of 21 days the fatty acid profile was relatively stable, and no clear trend of changes in fatty acid profile was observed. The two important polyunsaturated fatty acids DHA and EPA were found to amount to 4.4 % and 10.5 %, respectively, at the start of the experiment and to 5.2 % and 12.3 %, respectively, after 21 days of ice storage.

The stability of fatty acids during ice storage has been investigated for turbot (Aubourg et al., 2005) and yellowtail (Sohn et al., 2007). Although lipid oxidation and hydrolysis is reported to occur during ice storage, changes in the fatty acid profile did not correlate with such changes. Fatty acids in muscle are stored mainly as triacylglycerols or are incorporated into membranes as phospholipids, in both cases, the lipid components are not in a water soluble form. Thus, changes in fatty acid profile due to leaching during ice storage are highly unlikely. As a rule, the main changes in fish constituents during ice storage are usually related to water soluble compounds, such as nucleotides and non protein nitrogen compounds, as well as the formation of volatile and biogenic amines and hypoxanthine (Aubourg et al., 2005).

# Conclusion

Of the investigated components, only taurine decreased during ice storage to a maximum of 25 % of initial content after 3 weeks of storage. The traditional ice storage method did not affect the levels of selenium, nor was there any major change in the fatty acid profile. Thus, health beneficial components have a different susceptibility to be lost during ice storage. Therefore it is recommendable to consume ice stored fish in early stages of ice storage before losses of taurine can be observed and to reduce ice storage time to about 10 days.

	Day 1 FA%	Day 5 FA%	Day 8 FA%	Day 11 FA%	Day 13 FA%	Day 15 FA%	Day 18 FA%	Day 21 FA%	R <sup>2</sup>	Slope	Ρ
14:0	2.8 < 0.1	2.7 ± 0.1	2.9 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.5 ± 0.1	3.1 ± 0.3	2.9 ± 0.1	0.23	+0.02	NS
16:0	20.6 ± 0.1	21.3 ± 0.3	21.4 ± 0.3	22.6 ± 0.3	23.6 ± 0.3	21.0 ± 0.2	22.1 ± 0.5	22.1 ± 0.4	0.21	+0.07	NS
18:0	6.5 ± 0.1	6.2 ± 0.1	6.2 ± 0.2	6.3 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	6.6 ± 0.2	6.3 < 0.1	0.02	-0.01	NS
∑ SAT	31.0 ± 0.1	31.4 ± 0.5	31.8 ± 0.3	33.1 ± 0.3	34.0 ± 0.4	31.6 ± 0.1	33.2 ± 0.7	32.5 ± 0.4	0.31	+0.09	NS
16:1	4.8 ± 0.1	4.5 ± 0.2	4.2 ± 0.8	4.8 ± 0.1	5.6 ± 0.1	4.9 ± 0.2	4.7 ± 0.4	4.7 ± 0.2	0.04	+0.01	NS
18:1	26.1 ± 0.2	20.0 ± 0.6	21.1 ± 0.1	19.7 ± 0.3	21.5 ± 0.3	19.4 ± 0.2	20.1 ± 1.0	20.8 ± 0.4	0.35	-0.20	< 0.01
20:1	5.5 ± 0.1	5.6 ± 0.3	5.5 ± 0.4	5.9 ± 0.1	5.8 ± 0.1	6.2 ± 0.1	6.3 ± 0.4	5.9 ± 0.1	0.42	+0.03	NS
∑mufa	40.0 ± 0.2	33.9 ± 1.3	34.7 ± 1.3	34.4 ± 0.5	36.9 ± 0.3	34.7 ± 0.6	35.8 ± 1.9	35.3 ± 0.8	0.11	-0.11	NS
18:2n6	5.4 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	6.5 ± 0.1	6.0 ± 0.2	6.1 ± 0.1	0.03	+0.03	NS
18:3n3	0.9 < 0.1	0.9 ± 0.1	0.9 < 0.1	0.9 < 0.1	0.9 < 0.1	1.1 < 0.1	0.9 ± 0.1	0.9 < 0.1	0.13	+0.00	NS
18:4n3	0.8 < 0.1	0.8 < 0.1	0.8 < 0.1	0.9 < 0.1	0.9 < 0.1	1.1 < 0.1	0.9 ± 0.1	0.9 < 0.1	0.18	+0.01	NS
20:4n6	0.5 < 0.1	0.6 < 0.1	0.6 < 0.1	0.6 < 0.1	0.5 < 0.1	0.6 < 0.1	0.6 ± 0.1	0.6 < 0.1	0.04	+0.00	NS
20:4n3	0.6 < 0.1	0.7 < 0.1	0.8 < 0.1	0.7 < 0.1	0.7 < 0.1	0.7 < 0.1	0.7 ± 0.1	0.7 < 0.1	0.01	-0.00	NS
20:5n3	4.4 ± 0.1	5.3 < 0.1	5.4 ± 0.2	5.2 ± 0.1	4.5 ± 0.1	5.3 ± 0.1	$4.8 \pm 0.4$	5.2 ± 0.1	0.05	+0.01	NS
22:5n3	1.5 < 0.1	1.8 < 0.1	1.8 ± 0.1	1.8 ± 0.1	1.5 < 0.1	1.7 < 0.1	1.7 ± 0.2	1.7 ± 0.1	0.00	-0.00	NS
22:6n3	10.5 ± 0.3	13.0 ± 0.4	12.9 ± 0.6	12.7 ± 0.2	10.4 ± 0.1	12.9 ± 0.3	11.7 ± 1.7	12.3 ± 0.5	0.02	-0.02	NS
∑ PUFA	26.3 ± 0.5	30.9 ± 0.6	30.8 ± 0.8	30.4 ± 0.2	26.7 ± 0.3	31.5 ± 0.3	29.0 ± 2.4	29.8 ± 0.8	0.05	+0.07	NS
∑(n-3)	19.4 ± 0.5	23.2 ± 0.4	23.4 ± 0.9	22.9 ± 0.3	19.4 ± 0.2	23.3 ± 0.5	21.5 ± 2.4	22.3 ± 0.7	0.03	+0.05	NS
$\Sigma(n-6)$	6.6 ± 0.1	7.4 ± 0.1	7.2 < 0.1	7.3 ± 0.1	7.0 ± 0.1	7.9 ± 0.2	7.3 ± 0.1	7.3 ± 0.1	0.24	+0.03	NS
(n-3)/(n-6)	2.9 + 0.1	3.1 < 0.1	3.3 ± 0.1	3.1 < 0.1	2.8 < 0.1	3.0 ± 0.1	3.0 ± 0.4	3.0 < 0.1	0.02	-0.00	NS
ΣN id	2.7 ± 0.4	3.9 ± 1.6	2.8 ± 0.6	2.1 ± 0.3	2.4 ± 0.2	2.3 ± 0.3	2.0 ± 0.2	2.3 ± 0.4			

TABLE 2:	Changes in	fatty aci	l profile in	fillets o	f Af	<sup>f</sup> rican cat	fish durin	ig ice storag	e t	for 21 da	vs. % e	of total	fatty aci	ds.

R<sup>2</sup>: Explained variance, linear model fit. Slope: +/- Describes a trend of decreasing or increasing concentrations. NS = not significant

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