Improvement of Gas Chromatographic Determination Methods of Volatile Amines in Fish and Fishery Products

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Summary
Gas chromatographic determination methods of DMA (dimethylamine) and TMA (trimethylamine) in fish and fishery products were improved. Analysis was based on solvent extraction of amines from perchloric acid extract and subsequent gas chromatographic determination. In the first part possible formation of TMA during analysis using n-amyl alcohol as extractant was discussed. In the second part a new developed determination method based on a capillary column in combination with tert.-butyl methyl ether and isooctane, respectively, as solvents was presented. Peaks were very well separated and had nearly no tailing. Limits of determination varied for MMA (monomethylamine), DMA and TMA between 0.01 and 0.9 mg amine-N/100g. Recovery rates varied between 91 and 106% and repeatabilities (RSD, n=6) between 1.5 and 7%. Using isooctane as solvent additional volatile amines could be separated and determined.

Zusammenfassung
Gaschromatografische Bestimmungsmethoden von DMA (Dimethylamin) und TMA (Trimethylamin) in Fisch und Fischprodukten wurden verbessert. Die Analytik basierte auf der Extraktion der Amine aus dem Perchlorsäure-Extrakt mit einem organischen Lösemittel und anschließender gaschromatografischer Bestimmung. Im ersten Teil wurde die mögliche Bildung von TMA während der Analyse bei Verwendung von n-Amylalkohol als Lösemittel diskutiert. Im zweiten Teil wurde eine neue Bestimmungsmethode vorgestellt, die auf der Verwendung einer Kapillarsäule in Kombination mit tert.-Butylmethylether bzw. iso-Oktan als Lösemittel basiert. Die Amine wurden gut getrennt und die Peaks zeigten kaum Tailing. Die Bestimmungsgrenzen für MMA (Monomethylamin), DMA und TMA lagen zwischen 0,01 und 0,9 mg Amin-N/100g. Die Wiederfindungsraten lagen zwischen 91 und 106% und die Reproduzierbarkeit (RSD, n=6) lag zwischen 1,5 und 7%. Bei Extraktion mit iso-Oktan war es möglich, weitere flüchtige Amine zu bestimmen.

Introduction

Formation of trimethylamine (TMA) and dimethylamine (DMA) from trimethylamine oxide (TMAO) during storage has been used for decades to assess quality of fish and fishery products [1]. TMA is producted by bacterial reduction of TMAO and is used as one chemical indicator for quality assessment of iced fresh fish. Cleavage of TMAO by the endogenous enzyme trimethylamine oxide demethylase results in the formation of formaldehyde and DMA [2]. This reaction takes also place during frozen storage. Thus, DMA content can be used to evaluate quality of frozen fish [3].
Various colorimetric and gas chromatographic (GC) methods for determination of DMA and TMA have been reported [4,5,6,7].
Most GC procedures involve as first step the extraction of volatile amines from fish with trichloroacetic acid (TCA) or perchloric acid (PCA). The fish tissue is denaturated and the amines are fixed by the acids. Filtrated extracts can be stored for several weeks before analysis.
Further work up include alkalinization of the extracts followed either by organic extraction of the alkaline fish extract and determination of the amines in the organic layer [8] or by head space technique [9,10]. In Germany an official method for determination of TMA is based on headspace GC of the alkalinized perchloric acid extracts [11]. However, a headspace autosampler is not at every laboratories disposal. Thus, a modified GC method based on solvent extraction was established at our laboratory. The method includes extraction of amines from an aliquot of the perchloric acid extract after alkalinization by n-amyl alcohol followed by direct injection of the organic layer on a packed column and NPD (Nitrogen Phosphorus Detector) detection [12].
The procedure has been successfully applied for years especially for determination of DMA in various sea food.
More recently several ice storage experiments of different fish species were conducted at our institute attended by analysis of changes in TMA and DMA content.
For red fish (*Sebastes spp.*) unusually high contents of 5–7 mg TMA-N/100 g edible part were found in very fresh fish (1–3 d on ice), whereas the DMA content of 0.1 mg DMA-N/100 g corresponded well with known data [13]. The TMA content of freshly caught red fish was reported to be well below 2 mg TMA-N/100g [14]. A contamination of the GC system or the samples could be excluded after careful checking, thus additional TMA must have been formed during analysis.

The first part of this paper discusses possible formation of TMA during analysis, using n-amyl alcohol as extractant which can lead to adulterate results.
Consequently a new determination method was developed, which is presented in the second part of this paper.

Problems, encountering the GC determination of TMA using n-amyl alcohol extraction

Within the first extraction step of volatile amines from fish, a range of other N-containing compounds [15] are also coextracted into perchloric acid of which trimethylamine oxide (TMAO) and several betaines are able to form TMA. TMAO is the dominating compound and can reach high concentrations in the extract, depending on the fish species. Red fish is known to contain considerable amounts of TMAO and the red fish samples, which resulted in un-

Tab. 1 Content of TMA-N and TMAO-N in spiked trout samples, using n-amyl alcohol as organic extractant

No.	Unspiked sample	Spiked sample	Spiked sample	Recovery rate TMAO
	TMA-N [mg N/100 g]	TMA-N [mg N/100 g]	TMAO-N [mg N/100 g]	%
1	0.6	4.9	114.0	92.4
2	0.6	3.1	107.6	87.2
3	0.5	5.4	111.1	90.0
4	0.5	6.8	110.8	89.7
$\bar{x}$		5.1		89.8

usually high TMA values contained on an average about 120 mg TMAO-N/100 g edible part.

To study a possible bias of results by additional formation of TMA from extracted TMAO during gas chromatographic determination, a homogenized trout muscle known to contain no TMAO was spiked with TMAO at a level of 123 mg TMAO-N/100 g muscle. Both, spiked and unspiked trout samples were extracted with perchloric acid and analysed for TMA and TMAO, respectively, using n-amyl alcohol as organic extractant. Table 1 gives the results of four determinations.

The original trout sample contained only small amounts of TMA (0.5 mg TMA-N/100 g).

After spiking, 5.1 mg TMA-N/100 g was found on an average, which proved that some TMAO was degraded to TMA during analysis.

Within the analytical procedure, the degradation of TMAO most likely occurs during gas chromatographic determination. This implies that beside of TMA and DMA some TMAO must have been coextracted into n-amyl alcohol which is then decomposed either in the injector or on the column. It is well known, that TMAO can undergo thermal decomposition, forming TMA[16].

To overcome the problem of coextraction of TMAO, the polarity of the organic solvent was changed to a mixture of amyl alcohol/cyclohexane (82 : 18). The repeated work up of spiked trout samples (addition of 120 mg TMAO-N/100 g muscle) showed that a formation of TMA was now almost completely suppressed (see table 2).

The results indicated, that some TMAO from the perchloric acid phase was extracted by n-amyl alcohol and partially decomposed in the gas chromatographic system to yield additional TMA.

Tab. 2 Influence of polarity of the organic extractant on the formation of TMA in trout samples, spiked with TMAO [120 mg N/100 g], n=2

Extractant	Unspiked Trout sample	Spiked Trout sample
	TMA-N [mg/100 g]	TMA-N [mg/100 g]
n-amyl alcohol	<1.0	3.2
n-amyl alcohol/ cyclohexane (82 : 18)	<1.0	<1.0

Although the change of polarity of the organic extractant to n-amyl alcohol/cyclohexane solved the problem nearly, a possible formation of a small amount of TMA in TMAO rich fish samples could not be completely excluded. Thus, the GC procedure had to be adopted to a new extraction solvent to maintain the demands of GLP. It was also decided to change the method to a capillary column.

Development of a modified method including use of a capillary column instead of a packed column and the alteration of the organic solvent to prevent any coextraction of TMAO

Two new solvents were tested with the capillary column: *tert.*-butyl methyl ether and isooctane. The demands of GLP obliged the use of an internal standard for the procedure. n-Propylamine (PA), often used as an internal standard for the GC determination of DMA and TMA[6,9], was used as internal standard for the isooctane variant here, too. However, in case of *tert.*-butyl methyl ether the solvent peak coeluted with n-propylamine. Thus, in this variant the more volatile monoethylamine (MEA) was used as internal standard.

Materials and method

Chemicals

Perchloric acid, 60%, extra pure or p.a. (*Merck*), sodium hydroxide (*Riedel-de-Haën*), *tert.*-butyl methyl ether, supraSolv (*Merck*), isooctane for HPLC or for spectroscopy (*Promochem* or *Merck*).

Monomethylamine hydrochloride (MMA*HCl) (*Fluka*), dimethylamine hydrochloride (DMA*HCl) (*Fluka*), trimethylamine hydrochloride (TMA*HCl) (*Fluka* or *Riedel-de-Haën*), monoethylamine hydrochloride (MEA*HCl) (*Fluka*), n-propylamine (PA) (*Merck*).

Amine hydrochlorides were stored in a desiccator over silica gel.

Perchloric acid (6%):	65 ml perchloric acid (60%) were diluted with 900 ml H_2O (desalted)
Sodium hydroxide solution (50%):	50 g sodium hydroxide were solved in 50 ml H_2O (desalted)

All standard materials were solved in perchloric acid (6%). Stock solutions were diluted to following solution concentrations *):

- amine standard solution: 58.0 µg MMA-N/ml, 49.4 µg DMA-N/ml and 49.2 µg TMA-N/ml
- internal standard solution A: 155 µg MEA-N/ml and
- internal standard solution B: 132 µg PA-N/ml

*) The amine standard solution should contain about 50 µg N/ml of each amine to get calibration solutions with amine concentrations in the relevant range of fish samples (see "Sample preparation")

Devices

Normal laboratory equipment including a high speed homogenizer and a shaker for test tubes (*Vortex*), and a capillary gas chromatograph *Carlo ERBA* HRGC 5160 MEGA with Nitrogen Phosphorus Detector (NPD 80 FL, *FISONS Instruments*), autosampler (AS 800, *FISONS Instruments*) and computer based data acquisition (Baseline 810, Version 3.30).

Sample preparation

a) Extraction with perchloric acid:

20 g of the homogenized fish sample were mixed with 180 ml perchloric acid (6%) for about 60 s using a high speed homogenizer. After filtration the perchloric acid extract was either kept frozen at -18°C or further cleaned up according to step b).

b) Solvent extraction:

2 ml of the perchloric acid extract were transferred with a pipette into a lockable test tube. If amine concentration of a sample was expected to exceed the highest calibration level (i.e. if the sample was expected to contain more than 12.1 mg TMA-N/100 g, 12.2 mg DMA-N/100 g or 14.2 mg MMA-N/100 g) less than 2 ml of the perchloric acid extract were used and perchloric acid (6%) was added to get a total volume of 2 ml. 0.2 ml internal standard solution A in combination with *tert.*-butyl methyl ether or 0.2 ml internal standard solution B in combination with isooctane, 5 ml solvent (*tert.*-butyl methyl ether or isooctane) and 1.5 ml sodium hydroxide solution (50%) were added. The test tube was immediately closed and thoroughly vortexed two times for 30 seconds. After separation of the two phases (about 10 minutes) a portion of the upper phase (*tert.*-butyl methyl ether or isooctane) was transferred into a GC vial and analysed by GC.

c) Calibration

For calibration purposes aliquots of 0.1 ml, 0.3 ml and 0.5 ml of the amine standard solution were transferred with a pipette into test tubes and treated in the same way as the perchloric acid extract of the sample (see b)). The calibration solutions corresponded to amine content of fish flesh between 2.8 and 14.2 mg MMA-N/100 g, 2.5 and 12.2 DMA-N/100 g and 2.5 and 12.1 mg TMA-N/100 g.

Gaschromatography

Device:	*Carlo Erba* HRGC 5160 Mega
Injector temperature:	250°C
Detector temperature	(base): 300°C
Temperature program:	130°C (1 min.) –3°C/min. –160°C (0 min.) –49.9°C/min. –220°C (30min.)
Carrier gas:	nitrogen (3 ml/min.)
Injection volume:	1 µl
Split:	1 : 30
Liner:	*Carlo-Erba* Laminar Cup Liner (Restek)
Column type:	*Chrompack* PLOT Fused Silica Capillary column
Stationary phase:	Pora PLOT amines
Column length:	27.5 m (incl. 2.5 m Particle Trap)
Inside diameter:	0.32 mm
Outside diameter:	0.45 mm
Film thickness:	10 µm
Detector:	NPD 80 FL (*FISONS Instruments*) make up gas: nitrogen (20 ml/min.) hydrogen (2 ml/min.) synthetic air (94 ml/min.)

Concentrations of internal standard solutions were chosen to be at least 125 µg amine-N/ml to maintain a response as high as the amine response of the highest calibration level.

After samples of high amine content a blank was injected to prevent memory effects.

Results and discussion

Suitability of solvents in combination with the capillary column

MMA, DMA, TMA and MEA were very well separated and the peaks had nearly no tailing using both solvents. With isooctane as solvent also a range of additional other volatile amines including PA could be separated.

None of the new solvents allowed an artificial formation of TMA or DMA from TMAO. Therefore isooctane and *tert.*-butyl methyl ether in combination with the capillary column are suitable for the determination of amines.

The retention times of DMA and TMA are short (4.5 to 5.5 minutes). However, it is important to heat the column at a temperature of 220°C for 30 minutes after every run to maintain stable retention times.

Validation

Limits of detection and determination

Detection and determination limits of MMA, DMA and TMA for the two solvents were calculated on the basis of standard deviations of blanks. The number of blanks varied between 9 and 12. Detection limits were calculated as three times the standard deviation divided by the sensitivity (slope of the calibration). Determination limits were calculated as nine times the standard deviation divided by the sensitivity (slope of the calibration). Although extraction by *tert.*-butyl methyl ether resulted into about 4 times larger peak areas for MMA and about 2 times larger peak areas for DMA limits of detection and determination were quite equal for both solvents. Detection limits were below or equal to 0.3 mg MMA-N/100 g, 0.05 mg DMA-N/100 g and 0.01 mg TMA-N/100 g. Determination limits were below or equal to 0.9 mg MMA-N/100 g, 0.2 mg DMA-N/100 g and 0.03 mg TMA-N/100 g.

Tab. 3 Recovery rates [%] of spiked trout muscle

	Spiking Level [mg N/100 g]	Recovery[a] [%]	Recovery[b] [%]
MMA	2.9	98	100
	14.5	106	100
DMA	2.5	104	99
	12.4	104	104
TMA	2.5	91	99
	12.3	95	95

[a] Solvent: isooctane; Internal Standard: n-propylamine
[b] Solvent: tert.-butyl methyl ether; Internal Standard: monoethylamine

Linearity

For determination of low concentrations (concentrations below the lowest calibration level) it was important to check the validity of the calibration curves especially for DMA extracted by isooctane. Depending on the amine, solvent and concentration a second calibration was sometimes necessary to get accurate results. However, for routine analysis of fish TMA concentrations below 2 mg TMA-N/100 g are not relevant as freshly caught fish was reported to have on an average 2 mg TMA-N/100 g [17]. Depending on the species the TMA content varied between 1.3 and 3.6 mg N/100 g. In the range between 2.5 and 12 mg N/100 g the linearity of calibration curves were good for the three amines and the two solvents with R^2 (coefficient of determination) varying in routine between 0.99 and 0.9997.

Recovery

For determination of recovery rates trout flesh was homogenised and spiked with different levels of MMA, DMA and TMA. No MMA, DMA and TMA could be detected in the trout flesh itself. Every spiking level was analysed twice with good agreement. Within the relevant concentration range of 2.5 to 12 mg N/100 g recovery rates for the three amines varied around 100% (see table 3).

Repeatability

To determine the repeatabilities of the procedures, i.e. the solvent extraction and GC determination, a cod and a shrimp sample (from the fish market) were stored on ice and at 10°C, respectively, to get increased DMA and TMA content. After 9 and 6 days, respectively, the samples were extracted with perchloric acid. The perchloric acid extracts were analysed 6 times and the relative standard deviations were calculated (see table 4). The relative standard deviations were very good for *tert.*-butyl methyl ether and slightly higher for isooctane.

Tab. 4 Repeatabilities of the procedures, i.e. the solvent extraction and GC determination (RSD: Relative standard deviations, n= 6)

		Concentrations found [mg N/100 g]	RSD[a] [%]	RSD[b] [%]
DMA	cod	2.6	2	2.5
TMA	cod	5	5	1.9
	shrimps	21	7	1.5

[a] Solvent: isooctane; Internal Standard: n-propylamine
[b] Solvent: tert.-butyl methyl ether; Internal Standard: monoethylamine

Tab. 5 Boiling points and vapour pressures of isooctane and tert.-butyl methyl ether

	Isooctane	*tert.*-Butyl Methyl Ether
Boiling point	99°C	55°C
Vapour pressure (20°C)	51 mbar	417 mbar

[a] Solvent: isooctane; Internal Standard: n-propylamine
[b] Solvent: tert.-butyl methyl ether; Internal Standard: monoethylamine

Further possibilities of application

Additional volatile amines may be of importance in other protein rich food. Using isooctane as organic extractant a range of other volatile amines like ethylmethylamine (EMA), ethyldimethylamine (EDMA), n-propylamine (PA), diethylamine (DEA) and diethylmethylamine (DEMA) could be successfully separated and determined with the described GC procedure. With PA as internal standard linearities for EMA, EDMA, DEA and DEMA were very good (R^2 = 0.999–0.9999) and it was possible to determine concentrations of 0.1 µg amine-N/ml (or less) in the perchloric acid solution. Recovery rates were determined by adding different levels of these amines to homogenized trout flesh. Within the concentration range of 2–16 mg amine-N/100 g recovery was between 86 and 105% (mean value of two determinations). Triethylamine was also separated from the other amines, but coeluted with isooctane and could not be quantified.

Safety and handling aspects

tert.-Butyl methyl ether as well as isooctane are considered to be of relatively low toxicity. However, as they are organic solvents precautions such as prevention of inhalation or skin contact have to be taken. Comparison of their boiling points and vapour pressure shows that *tert.*-butyl methyl ether is more volatile than isooctane (see table 5). Therefore, handling of isooctane might be easier and safer compared to *tert.*-butyl ether at least if temperatures rise in summer.

Conclusion

All results show that the new developed method works very well using a capillary column in combination with isooctane or *tert.*-butyl methyl ether as solvents. Repeatability of TMA and extraction of MMA and DMA was slightly better by *tert.*-butyl methyl ether. On the other hand, the handling of isooctane in routine analysis might be easier as it is less volatile than *tert.*-butyl methyl ether.

The column is able to separate a variety of volatile amines. Using isooctane and PA as internal standard also EMA, EDMA, DEA and DEMA could be determined.

Literature

1) *Ludorff, W.* und *V. Meyer:* Fische und Fischerzeugnisse, Verlag Paul Parey, Berlin, Hamburg (1973).
2) *Rehbein, H.:* Relevance of Trimethylamine Oxide Demethylase Activity and Haemoglobin Content on Formaldehyde Production and Texture Detoriation in Frozen Stored Minced Fish Muscle. J. Sci. Food Agric. **43**, 261–276 (1988).
3) *Mackie, I. M.* et al.: Acceptability of frozen stored consumer fish products. Rev. Int. Froid **9**, 169–173 (1986).
4) *Garcia-Garrido, J. A. and Lugue de Castro, M. D.:* Determination of Trimethylamine in Fish by Perevaporation and Photometric Detection. Analyst **122**, 663–666 (1997).
5) *Dyer, W. J.* and *Y. A. Mounsey:* Amines in fish muscle. II. Development of trimethylamine and other amines. J. Fish. Board Canada **6**, 359–367 (1945).
6) *Veciana-Nogues, M. T., M. S. Albala-Hurtado, M. Izquierdo-Pulido* and *M. C. Vidal-Carou:* Validation of a Gas Chromatographic method for volatile amine determination in fish samples. Fd . Chem. **57**, 569–573 (1996).
7) *Gruger Jr., E.H.:* Chromatographic Analysis of Volatile Amines in Marine Fish. J. Agr. Food Chem. **20**, 781–785 (1972).
8) *Tokunaga, T. H. Iida* and *K. Miwa:* The Gas Chromatographic Analysis of Amines in Fish. Bull. Jap.Soc.Sci. Fish. **43**, 219–227 (1977).
9) *Kruse, R.* und *J. Stockemer:* Einsatzmöglichkeiten der Dampfraum-Gaschromatographie bei der Untersuchung von Fischen und Garnelen: Bestimmung von Mono-, Di- und Trimethylamin. Arch. Lebensmittelhyg. **40**, 87–89 (1989).
10) *Fiddler, W., R. C. Doerr* and *R. A. Gates:* Gas chromatographic method for the determination of dimethylamine, trimethylamine, and trimethylamine oxide in fish-meat Frankfurters. J. Assoc. Off. Anal. Chem. **74**, 400–403 (1991).
11) Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG (1988): Bestimmung des Gehaltes von Trimethylamin-Stickstoff (TMA-N) in Fischen und Fischerzeugnissen, L 10.004
12) *Manthey, M.:* Gaschromatographische Bestimmung von Dimethyl- und Trimethylamin in Fisch und Fischerzeugnissen. Infn. Fischw. **35**, 131–135 (1988).
13) *Oehlenschläger, J.:* Die Gehalte an flüchtigen Aminen und Trimethylaminoxid in fangfrischen Rotbarschen (*Sebastes marinus* und *Sebastes mentella*) aus verschiedenen Fanggebieten des Nordatlantiks. Arch. Lebensmittelhgy. **40**, 55–58 (1989).
14) *Rehbein, H., E. Martinsdottir, F. Blomsterberg, G. Valdimarson* and *J. Oehlenschläger.* Shelf life of ice-stored redfish, *Sebastes marinus* and *S. mentella.* Int. J. Fd.Sci.Technol. **29**, 303–313 (1994).
15) *Konosu, S., K. Watanabe* and *T. Shimizu:* Distribution of nitrogenous constituents in the muscle extracts of eight species of fish. Bull. Jap. Soc. Sci. Fish. **40**, 909–915 (1974).
16) *Tokunaga, T.:* Biochemical and food scientific study on trimethylamine oxide and its related substances in marine fishes. Bull. Tokai Reg. Fish. Res. Lab. **101**, 1–129 (1980).
17) *Oehlenschläger J.*: Volatile Amines as Freshness / Spoilage Indicators. A Literature Review. In: J.B. Luten, T. Boerresen, J. Oehlenschläger: Seafood from Producer to Consumer, Integrated Approach to Quality, Elsevier Science B.V., Amsterdam (1997).

Charakterisierung pulverisierter Sauerteige und Möglichkeiten ihrer qualitativen Bestimmung im Brot

Teil III: Freie Aminosäuren der Sauerteige, der Sauerteigpulver und der Brote

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Zusammenfassung

In den Teilen I und II der Veröffentlichung wurden die Ergebnisse der Säuregrade, L-Malat-, Citrat-, Acetat-, Lactose-, Hexanal-, Calcium-, Kalium- und Natriumgehalte sowie der Thiobarbitursäure-reaktiven Substanzen von Sauerteigen unterschiedlicher Führungsarten und Starterkulturen im Vergleich zu 15 handelsüblichen Sauerteigpulvern untersucht. Die im ersten Teil beschriebenen hohen Säuregrade der Sauerteigpulver führten zu der Annahme, daß diese infolge proteolytischer Vorgänge eine erhöhte Konzentration freier Aminosäuren aufweisen. Daher wurden die freien Aminosäuren der Sauerteige, Sauerteigpulver und der damit hergestellten Brote analysiert. Die Analysen beziehen sich auf die Aminosäuren Phosphoserin, Asparaginsäure, Threonin, Serin, Glutaminsäure, Glycin, Alanin, Valin, Cystein, Methionin, Isoleucin, Leucin, Tyrosin, Phenylalanin, Histidin, Ornithin, Lysin und Arginin. Zur Charakterisierung pulverisierter Sauerteige und der damit hergestellten Brote beschränkt sich die Ergebnisdarstellung auf die Aminosäuren Leucin, Phenylalanin, Ornithin, Alanin und Serin. Im Vergleich zu den Sauerteigen waren die Leucin- und Phenylalaninkonzentrationen der Sauerteigpulver deutlich erhöht und lassen einen Zusammenhang zu den hohen Säuregraden erkennen. Durch die mit höherem Säuregrad geringere Dosierung der pulverisierten Sauerteige werden die erhöhten Leucin- und Phenylalaninkonzentrationen bei der Herstellung von Broten jedoch weitgehend kompensiert. Deutlicher waren die Unterschiede im Gehalt der nicht proteinogenen Aminosäure Ornithin. Während die Sauerteige, mit Ausnahme eines Wochensauers, kein Ornithin enthielten, wurden in den pulverisierten Sauerteigen bis zu 232,2 mg Ornithin/100 g TS nachgewiesen. Es wird angenommen, daß

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