

On the volatile flavour compounds of
cooked trassi,
a cured shrimp paste condiment of the
Far East



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**On the volatile flavour compounds of cooked trassi,
a cured shrimp paste condiment of the Far East**

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Abstract

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The volatile compounds of cooked trassi, a cured shrimp paste condiment of the Far East have been studied. The techniques of volatiles isolation, concentration, fractionation as well as methods of identification have been described. 138 volatile compounds, which included 16 hydrocarbons, 7 alcohols, 46 carbonyls, 7 fatty acids, 3 esters, 15 sulfur compounds, 34 nitrogenous compounds, and 10 miscellaneous compounds, were identified. Carbonyls represented the most preponderant group and pyrazines were the second. The development of certain volatile compounds in autolyzed shrimp was studied. The experiment indicated that dimethyl-disulfide developed enzymatically.

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Theorems

1

The use of water or odourless oil only as a diluter for the study of isolated/concentrated food aromas in their organoleptic evaluation may give a misleading information.

2

Although freeze concentration is recognized as one of the best methods to separate and concentrate aromas its application has still to be encouraged.

C. Weurman: J. Agric. Fd Chem. 17: 1969.

3

The general belief that the taste acuity in man is inferior to that in woman is in contradiction with so many taste panel practices, where the majority of panel members are males.

R. W. Moncrieff: The chemical senses. Leonard Hill Ltd. London, 1967.

4

Except for the concentration of the odourous compounds, there is actually no fundamental difference between repulsive and enjoyable odours.

5

The most appropriate way to study the odour of a product is to investigate the composition of the odourous vapour over the product.

C. Weurman: J. Agric. Fd Chem. 17: 1969.

6

At least as applied to human beings, the use of the term 'lower senses' for the sense of taste and the sense of odour is misleading and incorrect, and should be replaced by the term 'higher chemical senses'.

M. A. Amerine et al.: Principles of sensory evaluation of food. Acad. Press, 1965.

H. D. Renner: The origin of food habits. Faber and Faber London, 1944.

7

The test of the carcinogenicity of food additives by parenteral administration only of the compounds to experimental animals, e.g. by repeated subcutaneous injection

tions at the same site and at dose levels much exceeding the concentration for intended use, is not appropriate.

P. Grasso & L. Golberg: *Fd Cosmet.Toxicol.* 4: 1966.

S. D. Gangolli et al.: *ibid* 5: 1967.

P. Grasso et al.: *Brit. J. Cancer* 23: 1969.

P. Grasso et al.: *Fd Cosmet.Toxicol.* 9: 1971.

J. Hooson et al.: *Brit. J. Cancer* 25: 1971.

J. C. Roe: *Sci. J.* May 1966.

8

If tobacco were not one of the most important resources of revenue income in many countries, its marketing and consumption as a smoking article would have been much restricted since the moment that survey studies have established a close association between cigarette smoking and the occurrence of lung cancer.

Smoking & health. Report of the Advisory Committee to the Surgeon General, Public Health Service, U.S.Dept.Health, Educ. & welfare, 1964.

Smoking & health now. Report of the Royal college of Physicians of London. Pitman Med. Sci. Publ. Co. London, 1971.

9

Warning notes for possible dangers on packages and containers of imported articles should be printed clearly and in the language of the importing countries concerned.

10

The lowered lethal action of salt to bacteria at low temperature is due to the lower overall activities of the microbes and of the solid phase of the medium at that temperature.

M. Ingram & A. G. Kitchell: *J. Fd Tech.* 2: 1967.

M. Ingram: *Symp. Soc. Gen. Microbiol.* VII, 1957.

11

For the preparation of dried salted fish the role of microbes can be neglected; however, in partially dried fish products and fish pastes certain halophiles/osmophiles/xerophiles might be very important.

J. Westenberg: *Visscherijproducten van Indochina. Mededelingen Onderafd. Zeevischerij*, 1941.

A. G. van Veen: *ibid.*

A. G. van Veen: *Adv. Fd Res.* 1953.

J. Scott: *Adv. Fd Res.* 1957.

12

As all attention was given to the nutritiousness of foods served in schools, no efforts were done to make them attractive; this was evidenced by the abstinence of the majority of schoolchildren from schoolfeeding.

C. den Hartog: *Moderne Voedingsleer* (door B. C. P. Jansen). WYT Rotterdam, 1959 p. 730.

13

'Snoepen' belongs to the normal feeding pattern of the majority of healthy children; nutrition consultants and dietists should utilize this fact beneficially in their efforts to combat caries.

C. den Hartog: Voeding XXXI no. 10, p. 487, 1970.

14

The amount of cholesterol consumed with food and its concentration in blood do not explain the development of atheromatosis and arteriosclerosis.

15

From a biological point of view the milk drinking habit in adults can be considered as abnormal. No member of the mammalian kingdom drinks milk after weaning.

Preface

The realization of this thesis has been made possible by Dr C. Engel, who has sponsored this work at his Institute and who has shown much interest in this investigation. The exceptional opportunity given to me to do all parts of the research at CIVO-TNO in Zeist is highly appreciated.

Honorable promotor, Prof. Dr W. Pilnik, I extend herewith my deepest appreciation for your confidence in my ability to do and to complete this study. Your advices and critics on practical problems concerning odour research are very valuable.

I am very much indebted to Dr C. Weurman, Head of the Department of Aroma CIVO-TNO, where this study had been conducted. He actively followed every progress of this work. His advices and critics on the experimental part of the investigation, from designing to experimentation, have been very valuable.

My thanks are due also:

to Drs S. van Straten of the Department of Aroma for his technical advices concerning the most important aspects of this investigation; volatiles isolation, extraction, and gas chromatographic techniques in odour research,

to Ir M. C. ten Noever de Brauw, Head of the Instrumental Analysis Section CIVO-TNO, and to his staff, who all helped me with the identifications of trassi aroma compounds,

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to all friends, with whom I have been in contact in relation with this study, for their cooperation and technical aids.

To some extent this study has been unique: although the work did not actually concern 'off-flavours', the odour spread in the laboratory during the isolation and

concentration of the trassi aroma surpassed many off-flavours in alliaceousness. My highest appreciation to all who were willing to cheerfully accept the unpleasantness and to take part in the sensory panel testing in this work.

After the termination of my fellowship in April 1972, my extension of stay in Holland has been made possible by a grant from the Curatorium of the State University of Utrecht, through the mediation of Prof. Dr J. F. Arens, and a grant from Stichting Fonds Harald Quintus Bosz.

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Finally my feeling of deep thankfulness is addressed to my dear wife, who gladly joined me during my study in Holland, and whose moral support was essential to the completion of this work.

Curriculum vitae

The author was born in Malang, Indonesia, on May 27, 1928. He is married to Mrs. S. K. Djoko Soedarmo in 1956 and has 4 daughters and 1 son.

After graduating from the high school in Semarang, Indonesia, in 1950 he enrolled in the Universitas Indonesia branch Bogor, where he studied Veterinary Science. He received his degree in 1955 and one year later, in 1956, passed his qualification examination for Veterinary Surgeon. Since his graduation from the Faculty of Veterinary Science he was appointed graduate assistant at the Department of Biochemistry of the Agricultural University in Bogor, and is now a teaching staff member of the university.

In 1958 he was granted a Rockefeller Foundation Fellowship and studied radioisotope techniques and physiological chemistry at the Graduate School of Cornell University Ithaca, New York, where a Master of Science degree was conferred to him in September 1959.

Samenvatting

Een onderzoek werd verricht op gekookte trasi, een door rijpingsproces verkregen garnalenpasta uit Zuid-Oost Azië, met het doel de vluchtige verbindingen te analyseren en te indentificeren. Trasi is één van de vele ingrediënten die in ieder huis in het Verre Oosten praktisch altijd aanwezig zijn. Het wordt benut om sausen te bereiden (vermengd met zout, suiker, specerijen enz.) voor het toebereiden van vele diverse Oosterse gerechten.

De methoden van isolatie van de vluchtige verbindingen, het concentreren, en het scheiden van het concentraat in diverse fracties zijn in dit proefschrift uitvoerig beschreven. Verschillende extracten werden vervaardigd om de vluchtige zure, neutrale en basische verbindingen afzonderlijk te analyseren. Een directe dampfase-analyse van gekookte trasi werd uitgevoerd in een gaschromatograaf voorzien van een zwavelgevoelige vlamfotometerdetector om de zeer vluchtige zwavelverbindingen te onderzoeken. Voor de identificatie werden de retentietijden van de onbekende zwavelverbindingen met die van referentiestoffen vergeleken. Dunne-laag-chromatografie werd toegepast om de carbonylverbindingen van trasi te analyseren door ze eerst om te zetten in hun 2,4-dinitrofenylhydrazonederivaten.

Zoveel mogelijk werd een combinatie van verscheidene identificatiemethoden in dit onderzoek toegepast.

138 vluchtige verbindingen werden in een afgescheiden trasi-destillaat geïdentificeerd, terwijl 5 zwavelverbindingen, waarvan de diverse massaspectra verkregen waren, onbekend zijn gebleven. Doordat niet steeds over referentie-massaspectra beschikt kon worden en ook de synthese van referentiestoffen niet steeds tot een oplossing leidde kan op de identificatie van de zwavelverbindingen niet volledig vertrouwd worden. Tot de geïdentificeerde vluchtige verbindingen in gekookte trasi behoren:

16 koolwaterstofverbindingen	3 esters
7 alkoholen	15 zwavelverbindingen
46 carbonylverbindingen	34 stikstofverbindingen en
7 vetzuren	10 verbindingen van andere dan bovenstaande aard.

Welke bijdrage iedere groep van verbindingen apart kan geven tot het resulterende typische trasi-aroma wordt in de discussie besproken. Geen enkele geïdentificeerde component in dit onderzoek vertoont op zichzelf de karakteristieke trasi-geur. Het cacao-achtige aspect van de geur van trasi is zeer vermoedelijk toe

te schrijven aan de aanwezigheid van diverse pyrazine-verbindingen. Zuren, aminen en ammonia veroorzaken respectievelijk de zurige, visachtige en ammoniakale nuances in de trasi-geur. De zeer vluchtige zwavelverbindingen zijn verantwoordelijk voor het penetrerende onaangename aspect van trasi. Eliminatie van deze laatste verbindingen door aan trasi-destillaten een sublimaatoplossing toe te voegen, had het ontstaan van een heel ander type geur, welke niet penetrerend en niet onaangenaam was, ten gevolge. Deze geur is te vergelijken met die van gebakken garnalen. Carbonylen, voornamelijk bepaalde aldehyden en methylketonen, spelen zeer vermoedelijk een belangrijke rol in de typische rijpingsgeur van gerijpte visprodukten.

Hoewel in dit onderzoek de aminozuursamenstelling van trasi in detail werd geanalyseerd, konden geen verdere onderzoeken verricht worden omtrent de rol van aminozuren als 'precursors' van vluchtige verbindingen in trasi. Wel zijn enige literatuurgegevens vermeld, die voor deze aspecten van de geurontwikkeling bij verhitte produkten en bij modelsystemen van belang zijn. De rol van aminozuren in het te weeg brengen van bepaalde typische roast- of braadaromas (via Strecker degradaties en/of Maillardreacties) bij de bereiding van verschillende voedingsprodukten is bekend. 30 % van de in totaal gevonden aminozuren in trasi bleek in ongebonden staat aanwezig te zijn. Verwacht kan worden dat deze fractie een belangrijke rol speelt bij de ontwikkeling van trasi-geurcomponenten tijdens het koken.

De vorming van bepaalde vluchtige verbindingen die tijdens de autolyse van garnalen ontstaan werd nagegaan, om enig idee te krijgen welke verbinding(en) in trasi van dit proces zijn af te leiden. Hiervoor werd een met merthiolaat behandelde brij van Hollandse garnalen gebruikt. De ontwikkeling van vluchtige verbindingen werd hierbij nagegaan door gaschromatografische analyse van de dampfase van de garnalenbrij na bepaalde incubatieperioden. Het gebruik van merthiolaat is bij deze experimenten aan te bevelen, omdat het binnen een bepaalde tijdsperiode bacteriostatisch werkt zonder dat te veel van de aanwezige enzymen geïnactiveerd worden. Resultaten van dit onderzoek wezen er op, dat dimethyldisulfide enzymatisch werd gevormd.

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1 Introduction

The name trassi denotes a pasty fishery product of Indonesia and Malaysia, which is prepared either from planktonous shrimp or fish. This food commodity belongs to the category of various fish pastes known in South East Asia, which are all prepared following more or less the same traditional fermentation and maturation procedures. Each product is given a local name, usually derived from the raw material exploited for its preparation. The following are some popular fish pastes peculiar to some South East Asian countries:

- Burma: ngapi (fish paste)
- Cambodia: pra-hoc (fish paste)
 - pha-ak (fish paste with fermented gluten rice)
 - mam-ca-sat (fish paste with roasted rice)
 - mam-ca-loc (fish paste with sugar and roasted rice)
- Laos: pa-dec (fish paste with rice bran)
- Vietnam: mam-tom (shrimp paste)
- Malaysia: terasi belachan (shrimp paste)
- Philippines: bagoong (primarily shrimp paste; when shrimp is scarce fish or a mixture of shrimp and fish is used)
- Indonesia: trasi udang (shrimp paste)
 - trasi ikan (fish paste).

Trassi udang is in Indonesia much more popular than trassi ikan and therefore produced in much larger quantities than the latter. Bagansiapiapi (East coast of Sumatra), the most important centre of fishery industry in Indonesia, produces the largest quantity of trassi to supply the whole archipelago. Smaller production centres of trassi are found on the island of Java, mostly spread along the North coast: e.g. Sidohardjo (East Java), Rembang, Juana, Lasem and Pati (Central Java), Indramaju (West Java) and one place in the South coastal region of West Java, Pelabuhanratu. These small industry centres produce trassi more or less typical for each region, but in quantities too small to meet the demand of the population of Java. Much larger quantities have therefore to be imported from Bagansiapiapi. On the other hand Sidohardjo exports trassi to abroad.

Besides rebon (planktonous shrimp *Schizopodes* or *Mytis* sp.) caught in the sea for large scale trassi production (Bagansiapiapi), the brackish water fish ponds along the North coast of Java, where bandeng (milkfish = *Chanos chanos*) are cultivated, give substantial side catches of rebon for small-scale operation. Accordingly two general procedures of trassi preparation have to be followed.

The large scale trassi preparation in Bagansiapiapi has been described by van Veen (1953 & 1965) as follows: Aboard the fishing boats the shrimps are mixed with 10 % salt to retard decay on board. At the time of arrival at the harbour another 5 % salt is then added. The shrimps are spread on drying mats, at the same time graded, and partially dried in the sun for 1 to 3 days. When the moisture content has sufficiently decreased (from 80 to about 50 %) the shrimps are kneaded and mixed quite strongly, dried in the sun and kneaded again. At the same time a synthetic dye carthamine DD or rhodamine B is often added as a colouring. The paste is then pressed in a cylindrical twisted work of nipah leaves and matured until the desired trassi aroma has developed. On the average 3 parts of shrimp yield 2 to 2.5 parts of trassi.

Trassi ikan is prepared in Bangansiapiapi from *teri* (*Stolephorus* or *Engraulis* sp.) following the same procedure as for trassi udang. Trassi ikan is more often coloured with a synthetic dye. Its smell is in general more repulsive than that of trassi udang to the majority of its consumers, a reason why this product is much less popular.

On the island of Java trassi is often prepared from precooked shrimps e.g. in Sidohardjo and Pelabuhanratu. The shrimps, raw or precooked, are mixed with 15 % salt and partially dried in the sun for 1 day only. The shrimps are minced, mixed, and kneaded thoroughly to prepare a paste called brabon (literally means mother paste). The brabon is dried in the sun, kneaded again firmly until a fine homogenous paste is obtained. At the same time the saltiness is judged and some salt is added as desired, and subsequently mixed well. The thick paste is then pressed in cylindrical forms (such as bambu) and matured until the desired trassi aroma has developed (van den Burg, 1904). Rembang is known for its product 'trasi hitam' (black trassi), which is prepared from ikan selar (*Scomber kanagurta*). When the fish are not sufficiently fresh to be salted-dried, pickled, or for fish sauce (petis ikan) preparation purposes, preservation as trassi will then be one method to avoid total spoilage of the fish (Anonymous, 1936). Trassi prepared from ikan selar has a brownish dark grey colour.

Trassi is solely consumed as a condiment, the flavour of which together with other ingredients and the dressed food material, is relished by its consumers. The flavour of trassi contributes quite importantly to the overall flavour of the dish.

Although the quality of trassi is judged mainly by the smell of the raw material, one has to realize that this type of odour is disliked by the Indonesian as much as by the European, with the difference that the Indonesian can tolerate the smell of trassi better. Vorderman (1904) has noticed that the Europeans disliked the smell of trassi very much, but they would prefer and enjoy a rice table, where sambal gorèng (a dish always prepared with trassi) was presented.

It is quite remarkable that in every home of the Indonesians, rich or poor, trassi is always found as one of their condiments in smaller or bigger quantities. The rural population of Indonesia consumes regularly trassi in the form of sambal (ground mixture of trassi, steamed or roasted, with chilli pepper and other ingredients)

together with raw or blanched vegetables, salted fish, vegetable dishes, and plain steamed rice. The daily intake of trassi is estimated to vary from 5 to 10 gram per person in the well to do families and 1 to 5 gram in the poor (Jansen & Aussen, 1955).

Since the total production of trassi on the island of Java is too small to meet the demand of its population, larger quantities have to be imported from Bagansiapiapi. In 1928 17,500,000 kg of trassi were exported from Bagansiapiapi, mainly to Java (van Veen, 1953).

It is worth to notice that, except for standard chemical data, including of some minerals and vitamins, not much is known about the flavour composition of many fish sauces and fish pastes. Intensive investigations on nuoc-mam, a Vietnamese fish sauce, were conducted mainly to study its content in utilizable nitrogenous compounds; certain regulations have even been instituted for all kinds of nuoc-mam to meet a fixed nutritional requirement (Rosé, 1918; Guillerm, 1928; van Veen, 1941a). It has not been realized that flavour is the most important attribute of the sauce. Trials done by the former French regime to introduce an aseptically manufactured nuoc-mam in Vietnam had failed completely. Despite the nutritiousness of the aseptic product, it was rejected by the population, solely because of the absence of the typical nuoc-mam aroma (Krempf, 1924).

Nutritional data of trassi are found in old publications of the former Netherlands Indies (van den Burg, 1904; Pannekoek et al., 1948) and in Indonesian Food Tables (Oey Kam Nio & Lie Goan Hong, 1967). Intensive investigations on trassi were conducted to analyze the vitamin B₁₂ content of the product (Aussen, 1954 and Jansen & Aussen, 1955). However its most important attribute, aroma, has never been investigated. The same is true for so many other fish condiments.

Trassi is never consumed in the raw state. It is used in sambal following some form of heat treatment, either steaming, roasting, or frying. It is used raw for the preparation of dressings for various dishes, but the dressings and the raw food material have then to be mixed and heated together by cooking or frying to prepare the dish.

In this work all investigations are aimed to investigate the aroma of cooked trassi.

To finally enable visualization of the odour of trassi against a somewhat better known background, the determinations of its chemical as well as its amino acid composition are nearly as essential as the analysis of its aroma.

The author hopes, that following this work many other studies will be initiated to investigate other Far Eastern fishery products, in particular with regards to the aroma of the various fish sauces and fish pastes.

2 General aspects of trassi

Trassi used throughout this investigation was bought locally from a food shop, specializing in Oriental food commodities. It was a 'MINAH BRAND' import product from Sidohardjo, East Java, Indonesia, and was rated as of medium quality by the author. As the quality varies widely, depending on the place of origin, the raw material exploited for its preparation, the season when it was prepared, and the length of storage time after maturation, a sufficient quantity was bought from a single batch for this investigation. It consisted of blocks, weighing 600 gram each, and wrapped quite tightly in 2 layers of absorbing paper, 1 layer of plastic sheet, and 1 outer layer of ordinary wrapping paper. The trassi blocks were put in plastic bags, sealed tightly, and stored at -10°C .

2.1 Chemical composition of trassi

Preliminary to the actual investigation, the trassi was subjected to routine chemical analysis to determine its chemical composition. 100 gram of each 6 blocks of trassi were cut, disintegrated, and mixed well in a glass jar. The mixed trassi was stored at 0°C in a screw-capped glass container. Aliquots of the mixed sample were analyzed for:

water. The water content of trassi was determined indirectly by the method of van de Kamer & Jansen (1949)

total ash. Total ash was analyzed following the method described in 'De Nederlandse Norm NEN 3329'

crude protein. The modified Kjeldahl method (see Bradsheet, 1965) was followed to determine total nitrogen; the factor 6.25 was used to calculate the crude protein content

Table 1. Chemical composition (%) of trassi.

	This research	Oey & Lie (1967)	Pannekoek <i>et al.</i> (1948)
Water	37.95	40.0	30 - 50
Total ash	32.90	.	10 - 40
NaCl	22.98	.	.
Crude protein	27.50	30.0	20 - 40
Carbohydrate	.	3.5	5
Crude fat	2.55	3.5	2 - 5

crude fat. The determination of crude fat was done following the method of van de Kamer *et al.* (1949)

salt. The salt content of trassi was determined in the ash by the Volhardt method.

As the total of water, ash, crude protein, and crude fat was 100 % the determination of carbohydrate was considered of less importance.

Table 1 presents results of the determinations and the data are compared with results of earlier investigators.

2.2 Amino-acid composition of trassi

Heat treatments of raw food material always result in changes in the product such as colour, texture, and most important of all are taste and aroma. These two aspects of changes make the product in general more palatable for the human consumers. In such changes volatile compound precursors, primarily amino acids and minor constituents in the case of products of animal origin, play a major role. Analysis of the amino-acid composition of the product will be very useful in understanding the development of some important aroma compounds.

For this purpose hydrolysates of freeze-dried trassi were used. 100 gram of the mixed sample was stored in petri dishes at -40°C for 1 day. The sample was then freeze dried for 15 hours at -50°C , and pulverized in a cooled disintegrator. The powder was stored in deep freeze in a screw-capped glass container. An acid hydrolysate was prepared to analyze acidic, neutral, and basic amino acids; an alkaline hydrolysate to determine tryptophan; a hydrolysate of the oxidized trassi powder was used to determine cystine and methionine; available lysine was analyzed in the hydrolysate of the sample after treating with dinitrofluorobenzene. Free amino acids were determined in the alcoholic extract of trassi, which was obtained by simple cold ethanol extraction. Free NH_3 and amide- NH_3 were also analyzed to obtain a more correct figure of the amino acid ratios.

2.2.1 Preparation of acid hydrolysate of trassi

An aliquot of dried trassi powder to contain 40 to 50 mg of protein was weighed in a 500 ml round-bottom flask; 200 ml of 6 N HCl (analytical reagent grade) and some pieces of carborundum were added and the sample refluxed continuously for 22 hours at 110°C . The hydrolysate was transferred quantitatively to a 250 ml volumetric flask. 5 ml of a norleucine solution, corresponding to 3 mg of the amino acid, was added as an internal reference standard. The volume was brought to the mark with distilled water. An aliquot of the diluted sample to contain 5 to 7 mg of protein was transferred to a 250 ml round-bottom flask with a volumetric pipette and dried at 45°C in a vacuum rotary evaporator. Traces of HCl, possibly remaining in the dry hydrolysate, were removed by adding a small volume of distilled water and redrying the sample. The dried residue was dissolved in a few ml of citrate buffer (pH 2.2) and quantitatively transferred to a 10 ml volume-

tric flask and using the same buffer for rinsing. The solution was filtered through a filter paper and stored in a refrigerator in a glass-stoppered tube until analysis.

2.2.2 Preparation of alkaline trassi hydrolysate

The method described by Slump & Schreuder (1969) was followed. 8.4 gram of $\text{Ba}(\text{OH})_2$ was weighed in a 100 ml Erlenmeyer flask and suspended in 16 ml of distilled water. The suspension was boiled for a while to remove oxygen present. A weighed aliquot of the dried trassi powder to contain about 750 mg of protein was added to the $\text{Ba}(\text{OH})_2$ suspension and mixed well. Sample weights in excess of 2.5 gram should not be recommended. The mouth of the Erlenmeyer flask was stoppered with a nicely fitting glass marble, and a small beaker was placed on top of it to prevent condense water dripping into the flask. The hydrolysis was carried out at 120 °C for 8 hours in a pressure cooker. The hydrolysate was brought to a pH of 3 to 4 with concentrated HCl (analytical reagent grade) and transferred quantitatively to a 50 ml volumetric flask, and the volume brought to the mark with distilled water. Thymol crystal was added as a preservative.

2.2.3 Preparation of trassi hydrolysate for cystine and methionine determination

The procedure employed for this purpose was that as described by Schram (1952) for cystine with some modifications (Moore, 1963). This method was successfully applied by Slump (1969) to analyze cystine as well as methionine as their oxidation derivatives. To prevent destruction of cystine and methionine during acid hydrolysis of the sample it should first be treated with performic acid, which oxidizes cystine and methionine to cysteic acid and methionine sulfone respectively; the oxidation products of these two amino acids are more resistant to destruction during acid hydrolysis. A weighed aliquot of freeze-dried trassi to contain 40 to 50 mg of protein was placed in a 500 ml round-bottom flask. 30 ml of ice-cooled fresh performic acid reagent was added and the reaction mixture was stored in a glass jar in a refrigerator, surrounded with ice cubes. The oxidation reaction was allowed to proceed during 16 hours' storage in the refrigerator. The excess performic acid was removed by adding HBr. This step of operation was carried out as follows: while the flask was being cooled in ice water, 4.5 ml of a HBr solution was added dropwise to the reaction mixture; 1 drop of octanol was added as an antifoam agent and the flask was immediately connected with a special vacuum rotary evaporator. The liberated bromine vapour was trapped in the bulbtrap of the evaporator, which was filled with 10 ml of a concentrated NaOH solution. When bromine vapour has ceased to evolve the flask was put down in the waterbath and the sample was evaporated at 40 °C until a volume of about 0.5 ml has remained. The oxidized concentrated sample was further subjected to acid hydrolysis as described in section 2.2.1.

2.2.4 Preparation of trassi hydrolysate for available lysine determination

The method used in this work is based on the procedure described by Blom *et al.* (1967). The free ϵ -amino group of lysine in the protein of the sample is reacted with dinitrofluorobenzene (DNFB) and the excess reagent together with ether-soluble reaction products are removed by extraction with peroxide-free ether. The sample is then hydrolyzed with HCl and the free dinitrophenyllysine (DNP-lysine) is separated by passing the hydrolysate through an Amberlite column (Beckman Spinco IR 120 type 15A or 50A) and determined colorimetrically. Carbohydrates will interfere with by decomposing the DNP-lysine during acid hydrolysis. This can be prevented by adding dinitrophenol (DNP) or DNFB.

100 mg of dried trassi powder was weighed in a 250 ml short-neck round-bottom flask. 8 ml of 8 % NaHCO_3 solution was added, mixed well, and allowed to stand for 1 minute. 0.3 ml of an ethanolic DNFB solution was added, and the mixture shaken continuously for 2.5 hours in a waterbath at 40 °C with a shaking machine. At the end of the reaction the sample was evaporated to dryness in a vacuum rotary evaporator and the dried residue extracted successively 3 times with 100 ml of peroxide-free ether. The ether layer was each time discarded by decantation. The last ether extractant remaining in the residue was blown out with a flow of nitrogen. 1.6 gram of DNP and 150 ml of 6.3 N HCl were added and the sample hydrolyzed for 17 hours continuously. The cold hydrolysate was transferred to a glass-stoppered graduated cylinder and extracted successively 3 times with 100 ml peroxide-free ether. The ether layer was each time removed by careful suction. The remaining DNP-lysine was quantitatively transferred to a long-neck round-bottom flask and evaporated to dryness at 40 °C. A sufficient volume of 1 N CH_3COOH was added to dissolve the sample and to obtain a concentration which corresponded to 1.5 to 2.0 mg protein per ml of the solution. The dissolved sample was then filtered through a filter paper. The clear filtrate was stored in a glass-stoppered tube in a refrigerator until analysis. Direct light should be avoided throughout the whole operation.

2.2.5 Determination of free NH_3 and amide- NH_3 of trassi

In the chromatographic analysis of amino acids with ninhydrin as a colour reagent, NH_3 will react also with the reagent to form a blue-violet coloured complex, which will be detected also in the chromatogram. However, this peak represents both free NH_3 and NH_3 derived from glutamine and asparagine and for a small part also from the α -amino-group of other amino acids. On the other hand glutamine and asparagine will be detected as glutamic respectively aspartic acid.

In this study free NH_3 and amide- NH_3 were determined titrimetrically with potassiumbiodate and methyl-red/methylene blue as indicator. In the determination of free NH_3 the CIVO method was followed, while for amide- NH_3 the method of Chibnal *et al.* (1958) was used.

2.2.6 Chromatographic analysis of trassi hydrolysates

A Beckman-Spinco Model 120 and a CIVO automatic amino-acid analyzer were used. While the Beckman-Spinco Model 120 can analyze one sample only for each run, the CIVO analyzer can analyze 6 samples in a single programmed run.

Hydrolysates analyzed, buffers and columns used in this part of experiment were:

acidic and neutral amino acids

column: 55 cm Aminex A4 spherical granules

buffer : citrate buffer pH 3.25 with 4 % methanol and citrate buffer pH 4.25

Table 2. The amino acid composition of trassi.

Amino acids	Freeze dried	Original trassi (calculated)		
	mg % (determ.)	mg %	mg/16 g N	mg N/16 g N
<i>Essential</i>				
iso-leucine	1801	1120	4100	435
leucine	2967	1850	6700	716
lysine	2864	1780	6500	1240
methionine	1046	650	2400	222
cystine	467	290	1050	122
phenylalanine	1546	960	3500	296
tyrosine	1597	990	3600	279
threonine	1572	970	3600	418
tryptophan	360	220	810	118
valine	2008	1250	4500	543
<i>Semi essential</i>				
arginine	1169	730	2600	850
histidine	533	330	1200	326
<i>Non essential</i>				
alanine	2511	1560	5700	892
aspartic acid	3876	2410	8800	921
glutamic acid	6362	3950	14400	1369
glycine	2300	1430	5200	970
proline	1483	920	3400	408
serine	1172	730	2600	353
ornithine	599	370	1350	287
taurine	657	410	1500	166
available lysine	914	570	2070	.
<i>Non amino acids</i>				
glucosamine	925	570	2100	161
amide N	222	140	502	502
amino N	5350	3320		
Kjeldahl N	7090	4400		

Table 3. Peptide bound and free amino acids of trassi, krill¹ and shrimp (mg %).

Amino acids	Peptide bound + free			Free (determ.)	
	trassi ²	krill ³	shrimp ⁴	trassi ²	shrimp ⁵
<i>Essential</i>					
iso-leucine	1120	580	950	390	8.9
leucine	1850	820	1410	810	17.5
lysine	1780	840	1640	940	48.2
methionine	650	330	550	210	4.8
cystine	290	150	250	90	.
phenylalanine	960	470	690	270	7.6
tyrosine	990	440	510	390	19.4
threonine	970	460	810	320	52.6
tryptophan	220	110	190	.	18.4
valine	1250	600	1000	480	8.7
<i>Semi essential</i>					
arginine	730	650	1050	830	219.4
histidine	330	220	.	50	34.9
<i>Non essential</i>					
alanine	1560	620	.	870	15.7
aspartic acid	2410	300	.	580	35.4
glycine	1430	620	.	480	88.2
glutamic acid	3950	1620	.	1180	117.5
proline	920	570	.	920 ⁶	2.2
serine	730	460	.	150	60.5
taurine	657	.	.	.	237.9
citrulline	550	.	.	. ⁶	.
glucosamine	570
sarcosine	29.1

1. The term krill denotes planktonous crustaceae (*Euphasia superba*) which form the main food of whales.

2. This research.

3. Slump *et al.* (1971).

4. Schormüller (1968).

5. Rangaswamy *et al.* (1970).

6. This data include citrulline.

basic amino acids

column: 10 cm Beckman-Spinco PA 35 spherical granules

buffer : citrate buffer pH 5.28

tryptophan

column: 85 cm Sephadex G 25 fine irregular granules

buffer : citrate buffer pH 3.25 with 4 % methanol

available lysine

column: 10 cm Beckman IR 120 15A irregular particles

buffer : pyridine-acetate buffer pH 5.28

separation of ornithine from lysine

column: 15 cm Aminex Q 15 S spherical granules

buffer : citrate buffer pH 4.46.

In tables 2 and 3 amino acid data of trassi are presented and compared with data of shrimp and krill.

3 The selection of methods for investigation of trassi aroma

In studying food odours one has to realize that there are three basic aspects of food odours, the accurate knowledge of which is essential (Weurman, 1969):

First is the qualitative and quantitative composition of the odourous volatiles in the food itself. To study this aspect the application of total volatile analysis is required.

Second is the composition of the odourous vapours over the food, which are in equilibrium with the food. This aspect is studied by the application of direct vapour and organoleptic analysis.

In the third place the physical state and the distribution of the odourous volatiles in the food, which could be considered to cause the effectual odour of the food. It relates the composition of the odorous vapours over the food to the overall composition of the volatiles in the product. Two different foods may have the same basic complex mixture of volatiles, qualitatively as well as quantitatively, but the effect may be two different odours. The cause can be sought in the different degrees of solubility of the volatiles in the lipoidic and the aqueous phases of the foods; also their effective distributions in either of the two phases can be altered by adsorption on the solids in the product. As a consequence the relative vapour pressures of some volatiles will be different, which will be observed in the effective different odours and also different chromatograms. This means that for a true understanding of a food odour as an intricate phenomenon neither the knowledge of total volatiles in the product nor the composition of its vapour can be sufficient.

One important property, which all odourous compounds have in common, is volatility. According to this fact distillation will be the most proper way to separate and isolate volatile compounds of foods. All other methods are better judged as concentration procedures, which are as a rule best applied following some form of distillation. Extraction as a first-stage isolation procedure of food aroma is well justified for products which do not contain too much non-volatile material soluble in organic solvents, such as fruit juices, wines, and in particular cognac, whisky, arrak, rum, and other products obtained from distillation.

Each type of product requires a specific distillation procedure to obtain the best results concordant to its nature. Artifacts formation will then be kept minimal. The isolation of the aroma of fruits and wines for example, should not involve any heat treatment, because this may lead to undesirable artifact formation. Often in traditional industrial production of some essential oils e.g. cayuputih oil, clove oil, and cananga oil a distillation procedure is applied without any

consideration to minimize artefact formation. Another group of products on the other hand should undergo one or other form of heat treatment in order to obtain the typical desirable flavour. In this last instance artefact formation is needed to produce the so-called process flavour in the final product. Some good examples are cacao, coffee, potato chips, and bouillon. The isolation of the aroma from these products permits heating to a certain degree.

Trassi has a strong smell of mixed amine- and cacaolike odours, in which faint acidic, but somewhat controversial also ammoniacal tones are observed.

The intense smell of trassi is repulsive to many people, its consumers included, which might be one of the reasons why trassi is never consumed raw. The smell of heat treated (cooked, roasted, or fried) trassi is somewhat neutral, and used in small quantities in a number of food dishes is appreciated by very many consumers.

In this study the aroma of cooked trassi in particular will be considered.

3.1 The isolation of trassi aroma

In analytical chemistry those methods are preferred which yield the correct data within the shortest time possible. As one or more problems are always encountered with every step of the whole operation procedure, those methods with the least steps would be chosen. Hence in the present method steam distillation at atmospheric pressure was considered best to isolate trassi aroma. The procedure is least complicated and can be expected to yield an aroma distillate possessing the same odour as cooked trassi. In this method heat treatment of the product

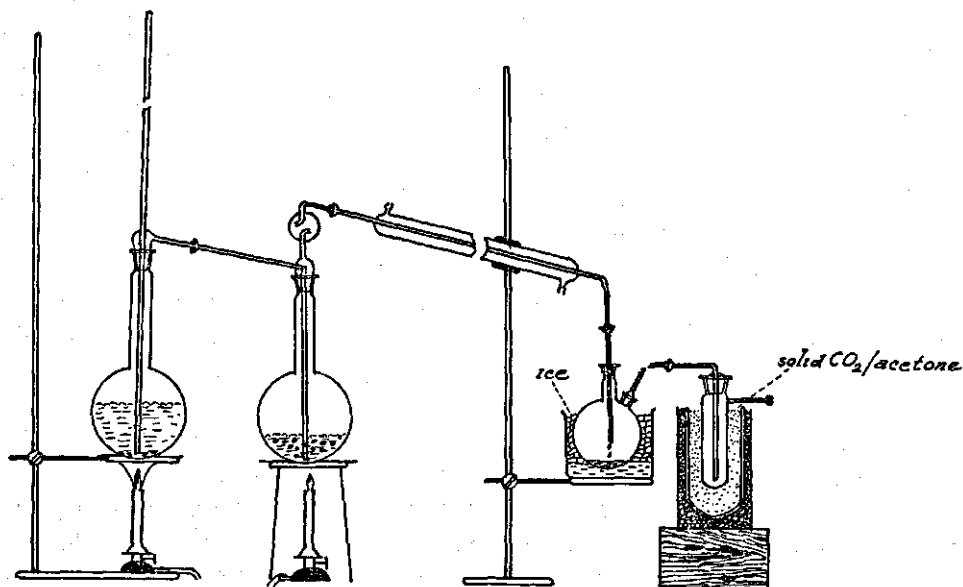


Fig. 1. All-glass steam distillation apparatus.

and sweeping of the volatile components are accomplished in one single operation step. Moreover in this study artifacts formed during distillation is no problem. On the contrary, compounds resulting from the wet heat treatment of trassi are of intrinsic importance to the study.

An all glass steam distillation apparatus (Fig. 1) was used. The steam generator of 2 litres capacity was filled with 1 litre demineralized water; 200 gram of trassi for each run was suspended in 500 ml demineralized water in the distillation flask. Some drops of antifoam silicone emulsion (Dow Corning Foam Preventive – Antifoam) were added to prevent heavy foaming during the distillation. The rate of distillation was adjusted by regulating the flame under the distillation flask to deliver 500 ml distillate within 45 minutes to 1 hour (3 to 4 drops per second). The distillate receiver was cooled in ice water and connected with a volatile trap cooled in a dry-ice/acetone mixture to condense highly volatile material. During the distillation no charring of trassi occurred at the spot directly exposed to the flame. At a higher rate of distillation charring was inevitable. At the end of each distillation the condensed volatiles in the volatile trap were rinsed with a small amount of steam distillate and mixed with it.

3.2 Test on the correct recovery of trassi aroma during isolation

In all aroma isolation procedures it is most important that the isolated fraction contains as much of the aroma components as possible and that the isolate possesses an aroma which equals the odour of the original product. In food odour isolation, however, the aroma of the isolated fraction often exhibits appreciable deviations from the original due to changes in vapour pressures of volatiles over the distillate as compared to those over the original material. The absence of lipoidic material in the aqueous distillate can completely alter the equilibrium of some vapourous components over the distillate as compared to that over the product. This may offer a serious problem in the organoleptic evaluation of the isolated fraction.

3.2.1 Test procedure

Data of volatile constituents of a food aroma are of little value in present day aroma research unless supplemented by an organoleptic evaluation of the odourous fraction obtained at all stages of the isolation and concentration procedures. The test applied in this instance was executed by a panel, consisting of 12 panel members, all unfamiliar with trassi. Samples were tested and their odours compared with that of a reference standard, which was prepared as follows: 200 gram of trassi was suspended in 500 ml of demineralized water, and refluxed for 1 hour in the distillation flask of the all glass steam distillation apparatus. The suspension was allowed to cool to room temperature, and centrifuged in tightly sealed containers (tubes) at 3000 rpm in a refrigerated centrifuge for 30 minutes.

The clear supernatant was collected in a glass-stoppered Erlenmeyer flask and stored in a refrigerator. A reference standard and a synthetic mixture were included in the samples to be tested. Results are presented in table 4.

The sensory test consisted of sniffing the odour of the samples and was carried out in a room specially designed for the sensory testing of food. Equal amounts of samples and reference standard were placed in equal containers. Since the reference standard was red-orange coloured, dark red glasses with rough outer surface were used to compensate for colour differences. Random different code numbers were given to each sample, and samples in each series for each panelist were arranged in different orders. The following samples A to E were tested and their odours compared with the reference standard:

- A. trassi steam distillate pH 8.8
- B. trassi steam distillate pH 1.0
- C. trassi steam distillate pH 13.0
- D. clear supernatant of cooked trassi suspension pH 8.6
- E. synthetic mixture (3 ml NH_4OH + 3 ml isobutyric acid + demineralized water ad 300 ml)

reference standard = clear supernatant of cooked trassi suspension = sample D.

The following scores were used in the sensory odour evaluation:

- 1 = no similarity with standard
- 2 = slight similarity with standard
- 3 = reasonable similarity with standard
- 4 = close similarity with standard
- 5 = the odour of reference standard.

Table 4. Ordered results of the sensory odour evaluation of trassi steam distillate.

Panelists	Samples and scores				
	A	B	C	D	E
I	4	2	3	3	1
II	3	2	3	5	1
III	4	5	2	4	1
IV	2	3	2	5	1
V	4	3	2	5	1
VI	4	3	2	5	1
VII	5	2	4	3	1
VIII	2	2	4	5	1
IX	4	2	3	4	1
X	3	3	2	4	1
XI	5	3	2	4	1
XII	4	3	2	5	1
Total score	44	33	31	52	12
Average	3.7	2.8	2.6	4.3	1

3.2.2. Statistical analysis

The t-test is of primary importance to check the similarity or dissimilarity between trassi steam distillate (sample A) and the clear supernatant of cooked trassi (sample D). Table 5 presents data calculations for the t-test.

The t-value is calculated by the following equation:

$$t = \frac{\overline{\text{diff}}}{\sqrt{\sum (\text{diff} - \overline{\text{diff}})^2}} \cdot \sqrt{N(N-1)} = 1.53$$

This value indicates that there is no significant difference between samples A and D.

From the data obtained by the sensory evaluation test the following information and conclusion can be drawn:

- a. Trassi steam distillate (A) obtained by the procedure employed in this study was with respect to its odour not different from the reference standard (D), and therefore the isolation procedure is considered appropriate
- b. The addition of acid or alkali did not remove the typical character of the trassi aroma, though some change was observed
- b'. After the addition of acid a pungent smell was observed, but the typical trassi character was still retained
- b''. The addition of alkali did not remove the typical character of trassi aroma, however, ammonia and amines were liberated, which caused a more intense tone of these two odour components.

Table 5. Statistical analysis of data in Table 4.

Panel memb.	Scores X _A	X _D	difference (X _A - X _D)	diff - $\overline{\text{diff}}$ (x)	(diff - $\overline{\text{diff}}$) ² (x ²)
I	4	4	0	+ 0.58	0.34
II	3	5	-2	- 1.42	2.01
III	4	4	0	+ 0.58	0.34
IV	2	3	-1	- 0.42	0.18
V	4	5	-1	- 0.42	0.18
VI	4	5	-1	- 0.42	0.18
VII	5	3	2	+ 2.58	6.66
VIII	2	5	-3	- 2.42	5.86
IX	4	4	0	+ 0.58	0.34
X	3	4	-1	- 0.42	0.18
XI	5	4	1	+ 1.58	2.50
XII	4	5	-1	- 0.42	0.18
			$\sum \text{diff} = -7$	$\sum (\text{diff} - \overline{\text{diff}})^2 = 18.95$	
			$\overline{\text{diff}} = -0.58$		

3.3. Extraction and concentration of trassi aroma

The volatile compounds of food obtained by steam distillation is recovered as a very dilute dispersion in the aqueous distillate. It is therefore necessary to concentrate and separate them from the aqueous medium prior to the analysis of individual constituents by gaschromatographic, mass and infrared spectrometric methods. Freeze concentration visualizes a first stage concentration by eliminating most of the water in a solid state (ice) and the remaining water can then be removed by extraction with a non-miscable extractant. A direct extraction on the other hand of the distillate with a fixed volume of a non-polar extractant separates the aroma components from the aqueous phase and at the same time effects concentration of the aroma to a certain degree. As far as steam distillates at atmospheric pressure are concerned freeze concentration offers no specific advantage and a direct continuous extraction of the trassi steam distillate has been applied in the investigation.

In this study trassi steam distillates were extracted with a mixture of pentane: ether 2 : 1 in a continuous all glass liquid/liquid extractor of the type Kutscher & Steudel perforator with some modifications (see fig. 2). The apparatus used in this work permitted the extraction of 1500 ml of fluid for each run. Three successive trassi steam distillates, 500 ml each, were mixed and directly extracted with 450 ml of pentane : ether mixture continuously for 8 hours at 45 °C. Sodium sulfate was added to the distillate to saturation to increase the extraction efficiency.

The ether used for extraction was of an analytical reagent grade, made peroxide free and redistilled. The pentane was of 'Shell' technical grade, purified and redistilled through a 3 m long Vigreux column at a preselected temperature of 40 °C. Pentane obtained in this way appeared to contain less impurities (mainly isopentane) than analytical reagent grade pentane.

300 ml trassi steam distillate extract was obtained from every 1500 ml of trassi steam distillate.

The following trassi steam distillates were prepared for subsequent extraction or to be otherwise treated for further analysis:

A. Trassi steam distillate directly absorbed in H_2SO_4 to produce a pH of 1.0; this distillate was extracted with pentane : ether

B. The aqueous residue of A after extraction with pentane : ether was made alkaline with NaOH to a pH value of 13.0 and re-extracted with pentane : ether to isolate all remaining volatiles not extratable at pH 1.0; this fraction is called the acidfree fraction

C. Trassi steam distillate directly absorbed in NaOH. This distillate was extracted with pentane : ether to remove all volatiles except trassi acids. The aqueous residue was concentrated in a vacuum rotary evaporator, acidified, and extracted with ether to isolate the trassi acids

D. Trassi steam distillate absorbed in H_2SO_4 as in A. This distillate was eva-

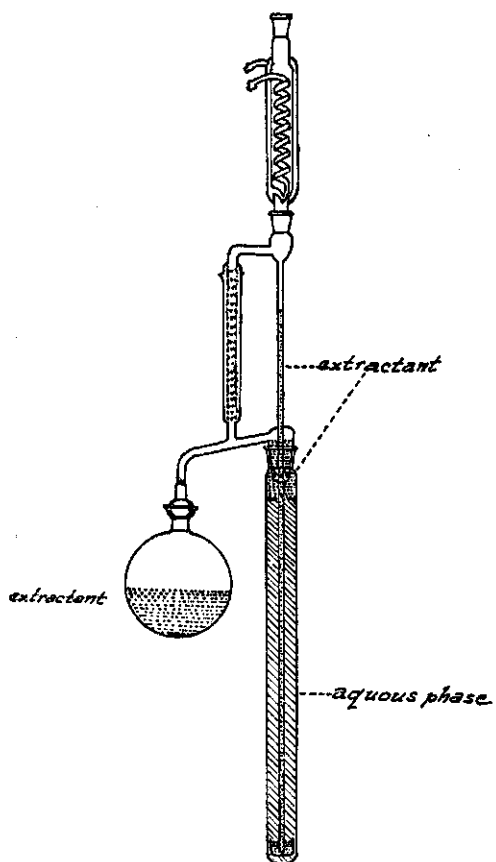


Fig. 2. A modification of Kutscher & Steudel perforator.

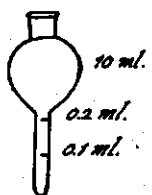
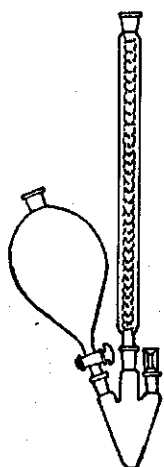


Fig. 3. Pear-shaped flask with reservoir and Vigreux column for initial concentration of extract (left) and concentration flask for final concentration (right).

porated in a vacuum rotary evaporator until a residue of about 5 ml had remained. Further concentration was feared to cause carbonization of the compounds present in the concentrate due to the high concentration of sulfuric acid at the end of the operation. This concentrate was directly subjected to head space analysis after the addition of a sufficient amount of alkali, to analyze the basic compounds.

All extracts were concentrated by evaporation of the extractant in a waterbath at 45 °C using a pear-shaped flask with a reservoir and a Vigreux column (see fig. 3). Further concentration to the final concentrate was accomplished using a concentration flask as designed by Maarse & Kepner (1970) (see fig. 3), with a Vigreux column until a volume of 200 µl was left. It means that a concentration factor of 5 x 1500 was attained.

Blank experiment Blank experiments were run in duplicate to check the possible introduction of artifacts with the apparatus, water, extractants, and silicone emulsion used during the actual experiments.

500 ml of demineralized water with 5 drops of antifoam silicone emulsion was steam-distilled as described in section 3.1. Three blank steam distillates were mixed and extracted with 450 ml of pentane/ether 2/1 extractant continuously for 8 hours after the addition of sodium sulfate to saturation. The extract was concentrated to a volume of 200 µl and subjected to a gaschromatographic analysis using the same isolative gaschromatograph as for the actual experiments.

The results of the blank experiment have indicated no evidence of the presence of extraneous compounds.

3.4 The isolation of trassi carbonyl compounds

Carbonyl compounds are conveniently analyzed as their dinitrophenylhydrazone derivatives for the following reasons:

1. the volatile carbonyls are converted into solids and as such are easier to handle
2. they are relatively stable
3. carbonyl-dinitrophenylhydrazones are readily to isolate as a group with virtually no impurities
4. it is relatively easy to separate their mixtures into their constituents by TLC (thin-layer chromatography)
5. for further identification of components only a small amount is needed, 5 to 10 µg in KBr disc or 10 to 20 µg dissolved in carbon tetrachloride for infrared spectrometric analysis.

The volatile compounds of trassi were first isolated by continuous extraction of 400 gram of material in a modified Clevenger distillation-extraction apparatus (see fig. 4). The trassi was suspended in 1 litre of demineralized water in the boiling flask and extracted continuously for 2 hours with 75 ml of hexane (analytical reagent grade). The hexane extract was made free of water with dry sodium sulfate powder and directly passed through a reaction column according to

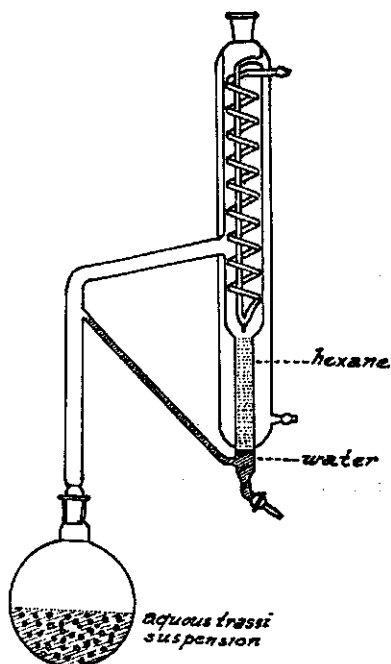


Fig. 4. A modification of Clevenger distillation extraction apparatus.

Schwartz & Parks (1961). 0.5 gram of 2,4-DNPH was dissolved in 6 ml 85 % H_3PO_4 by grinding in a mortar. Some drops of water were added and mixed until all DNPH dissolved completely. 10 gram of Celite was then added and ground until a damp powder mass was formed. The impregnated Celite was then transferred into a chromatographic column tube (2.5 inner diameter and 30 cm long) with sintered-glass disk. All chemicals used were of the highest purity grade. While passing through the reaction column all carbonyls present in the extract were converted into their DNPH derivatives, which left the column in the hexane eluate. The approximate amount of DNPH-carbonyls was determined by spectrophotometry of their ethyl acetate solution at 430 nm, after removal of the hexane by evaporating on a waterbath. An ethyl acetate solution of acetaldehyde-DNPH was used as a reference standard. The reading of 1 mg of standard in 50 ml of solution at 430 nm was $E = 0.247$. The trassi carbonyl-DNPH in 250 ml solution and diluted 5 times had $E = 0.262$. The trassi distillate obtained from 400 gram of trassi thus contained: $0.247/0.262 \times 250/50 \times 5 = 23.5$ mg carbonyl-DNPH, calculated as acetaldehyde-DNPH.

3.4.1 Group separation of carbonyl-2,4-DNPH of trassi

The carbonyl-2,4-DNPH of trassi in ethyl acetate solution were separated into groups according to Dhont & Dijkman (1967) on 20×20 cm MgO-plates. The solution was applied as a band on the MgO plates using the apparatus described

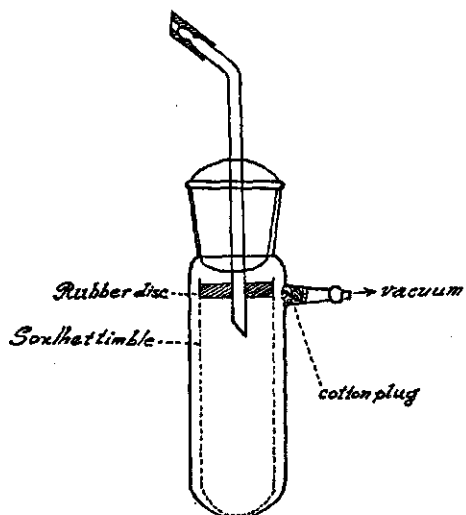


Fig. 5. Suction flask for the collection of TLC scrapings.

by Ritter & Mayer (1962) and developed 1 time in a hexane/chloroform 7/3 mixture.

The following groups of 2,4-dinitrophenylhydrazones, with decreasing R_f values can be separated on the MgO plate:

2-alkanones	diënones and diënals
alkanals	aromatic carbonyls
alkenones	osazones
alkenals	

Keto acids, if present in the solution, can be easily removed by extraction with an aqueous carbonate solution before applying the sample on the MgO plate.

The various groups of carbonyl-2,4-DNPH have each their characteristic colour on the MgO plate:

brown	: alkanals
brick red	: alkenals
violet	: diënals
deep blue	: osazones

Five bands were separated in this way which were then each scraped and collected in a Soxhlet thimble using an all glass 'micro vacuum cleaner' connected with a vacuum line (see fig. 5). The scrapings were each extracted with ethyl acetate and dioxane and evaporated to dryness in vacuo. The residues were redissolved in a minimum volume of ethyl acetate.

3.4.2 Separation of the trassi MgO-fractions into components

The five fractions obtained from the group separation on MgO-plates were subjected to further separation. The chromatoplates and mobile phases employed for this step of operation are listed in table 6.

Table 6. Thin-layer chromatographic systems for further separation of trassi MgO-fractions.

System	Stationary phase	Mobile phase	Irrigations	Literature
I	Equal parts of carbowax 400 and 2 phenoxy-ethanol on Kieselguhr	Hexane	2 ×	
II	Silicagel G	Hexane/benzene 1/1	5 ×	Dhont & Dijkman (1967)
III	Nitromethane adsorbed on Silicagel G	Hexane	3 ×	Dhont & Dijkman (1969)
IV	Acetonitril adsorbed on Silicagel G	Hexane	3 ×	ibid.
V	Polyvinylacetate adsorbed on Kieselguhr G	Hexane/dibutyl ether/ethyl acetate/methanol 85/5/5/5	1 ×	Dhont <i>et al.</i> (1970)

Table 7. R_f values of carbonyl-2,4-DNPH components of trassi for systems I to V.

Systems	Trassi MgO-plate fractions and R_f values of components				
	A	B	C	D	E
I	0.456 (2) 0.548 (1) 0.695 (4) 0.751 (?) 0.798 (?) 0.845 (?) 0.918 (5)	0.291 (?) 0.401 (2) 0.458 (2) 0.556 (1) 0.918 (5+mix)	0.164 (6, 9) 0.283 (8)	0.168 (6) 0.262 (11) 0.352 (?) 0.417 (10)	0.062 (8, 9, 12) 0.173 (9) 0.274 (8)
II	0.342 (1) 0.408 (2) 0.460 (3, 4)	0.262 (6) 0.408 (2, 7)	0.245 (9) 0.332 (8)	0.451 (10, 11)	0.408 (12)
III	0.138 (1, 2) 0.221 (3, 4)	0.073 (6) 0.149 (2)	0.037 (?) 0.111 (8)	0.041 (?) 0.234 (10)	0.023 (12)
IV	0.309 (1, 2) 0.405 (3, 4) 0.895 (?)	0.157 (62) 0.312 (2, 7) 0.895 (?)	0.111 (6, 9) 0.220 (8)	0.048 (?) 0.120 (?) 0.390 (10)	0.073 (12)
V	0.547 (1, 2) 0.653 (3, 4) 1.013 (5)	0.334 (?) 0.454 (2) 0.538 (2, 7) 1.008 (5)	0.270 (6, 9) 0.357 (8)	0.270 (6, 9) 0.357 (8) 0.478 (?) 0.547 (10)	0.205 (12) 0.344 (?) 0.094 (13)

Numbers in parenthesis correspond to numbers of identified carbonyls in table 8.

Table 8. Trassi carbonyls identified as their 2,4-DNPH derivatives.

	UV-max	MS-spectra
<i>fraction A</i>		
1. butan-2-one	365	-
2. pentanal	359	-
3. hexanal	359	-
4. hexan-2-one	-	-
5. nonan-2-one	-	-
0.751	-	-
0.798	-	-
0.845	-	-
<i>fraction B</i>		
2. pentanal	360	n-pentanal & iso-pentanal
5. nonan-2-one	-	-
6. phenylpropanal	356	-
7. pentan-2-one	366	-
0.291	-	-
0.918	-	mixture of saturated aliphatic aldehydes C ₁₄ , C ₁₆ and C ₁₈
<i>fraction C</i>		
8. 5-methylfurfural	-	-
9. furfural	385	furfural m.w. 96
6. phenylpropanal	-	-
<i>fraction D</i>		
10. hept-2 enal	372	-
11. pent-2-enal	373	-
6. phenylpropanal	-	-
8. 5 methylfurfural	-	-
9. furfural	-	-
0.352	-	-
<i>fraction E</i>		
12. benzaldehyde	378	benzaldehyde m.w. 106
13. p-hydroxybenzaldehyde	-	-

R_f values calculations were done following the method of Galanos & Kapoulas (1964, see also Dhont & Mulders-Dijkman, 1969). Table 7 presents R_f values of all components of trassi carbonyl-DNPH calculated by this method and by the application of systems I to V. The identities of carbonyls characterized as their 2,4-DNPH derivatives are presented in table 8.

3.5. Fractionation of trassi aroma and isolation of the aroma components

The use of sensitive detectors and the application of more adequate methods in the study of food odours in the past decennium have brought about the identifi-

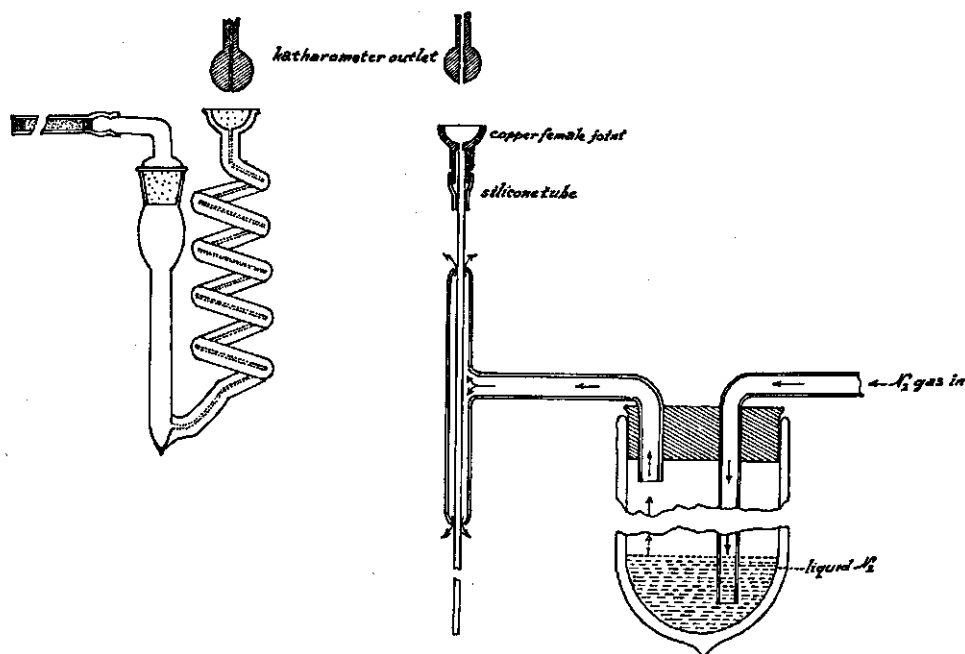
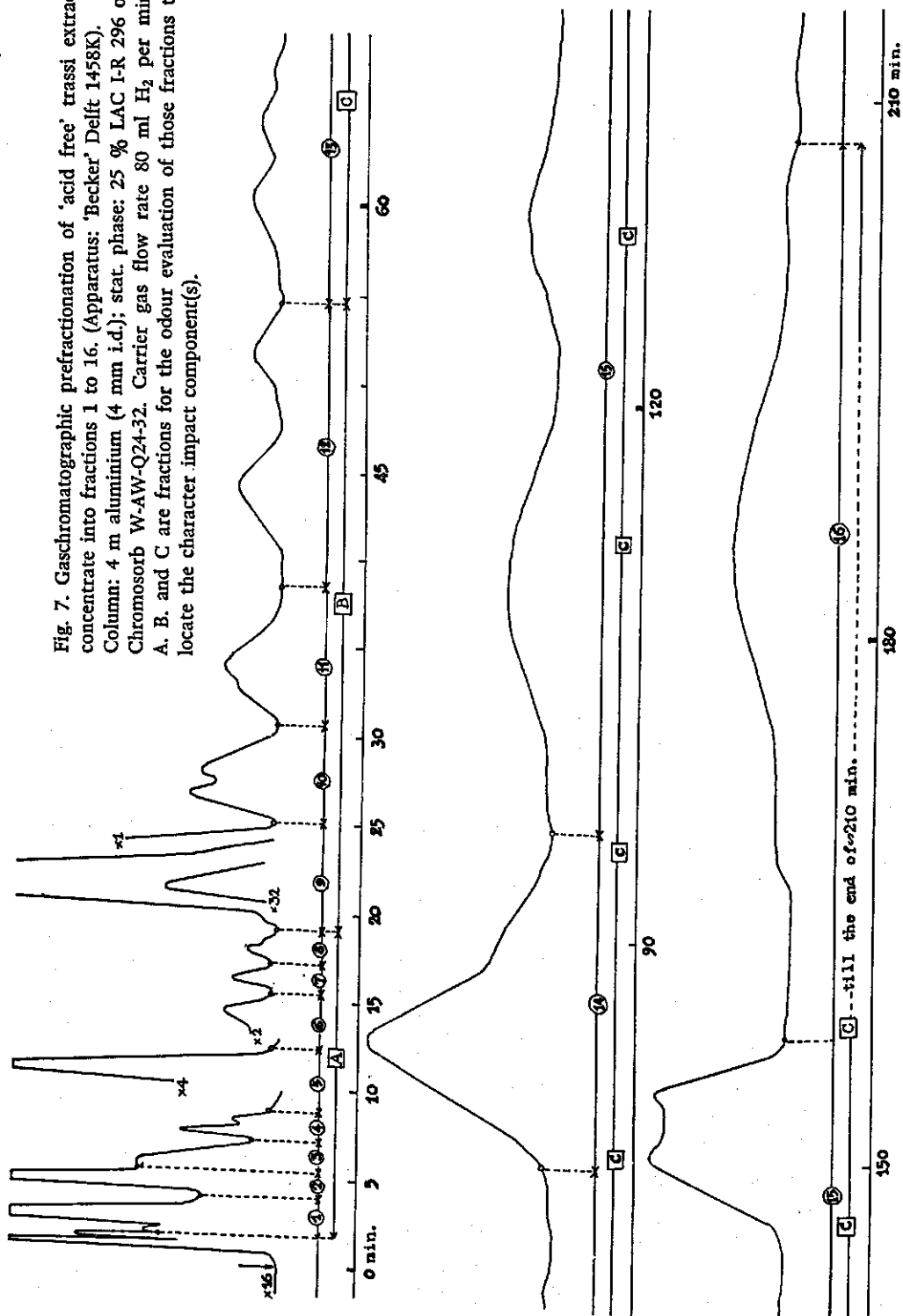


Fig. 6. Volatile-trap according to Badings & Wassink (1965) (left) and capillary volatile-trapping device according to Burson & Kenner (1969) (right).

cation of ever increasing numbers of aroma compounds of many food items. To mention just a single example, in early studies of white bread aroma 36 compounds were reported, while in later studies of the same product by Mulders & Weurman (1970) nearly the triple of this number of white bread aroma compounds were identified. In their studies efforts were made and those methods employed, which were expected to keep artefacts formation at a minimum level.

In this study trassi aroma concentrates prepared as described earlier were first subjected to a prefractionation by the application of gaschromatography using an isolative gaschromatograph. A Becker type 1458 K gaschromatograph equipped with a katharometer detector was used for this purpose. The prefractionation was accomplished on a 4 m aluminum column (4 mm i.d.) filled with 25 % LAC I-R 196 adsorbed on Chromosorb W-AW, 60-80 mesh solid support, and gaschromatographic runs were done at 170 °C. Fractions were collected in volatile-traps as designed by Badings & Wassink (1965) (see fig. 6) cooled in solid CO₂. Successive gaschromatographic prefractionations of 50 µl samples of trassi aroma concentrate were run, and identical fractions were collected in one and the same volatile-trap to obtain sufficient material for further separation or for identification purposes. All collected samples were directly stored in deep freeze until needed for further separation (see 3.5.3). 16 fractions were separated from extract 3.3.A, 16 others



from extract 3.3.B, and 7 from extract 3.3.C. The gaschromatographic pre-fractionation of extract 3.3.B is illustrated in fig. 7.

3.5.1 Evaluation of the odour characteristic of trassi aroma fractions

Along with the prefractionation of trassi aroma extract 3.3.B into 16 fractions, three crude cuts A, B, and C (see fig. 7) were collected for the organoleptic evaluation of their odours to find out which crude fraction (A, B, or C) might or might not exhibit odour similarities with the trassi extract. Fraction A contained components with the lowest retention times, fraction B comprising the intermediate range, and all components with relatively high retention times were contained in fraction C. A total condensate of the trassi extract was also collected in a single trap.

The samples thus collected were tested for similarity and dissimilarity. It appeared that none of the separate fractions A, B, and C were similar to trassi extract, while the total condensate showed odour similarities with the trassi to a high degree. Fraction A exhibited least difference from the trassi extract if compared with fraction B or C.

3.5.2 Separation of trassi aroma fractions into components

Further separation of all fractions obtained was accomplished following basically the same procedure as in Section 3.5 and using the same gaschromatographic column, operated at lower temperatures to effect better separation of the components. This was of particular profit for the low-boiling fractions. Since almost all fractions were recovered in minute amounts and just visible as very small droplets adhering to the capillary wall of the Badings & Wassink's trap, an eluent was added to facilitate transfer of the fractions into a gaschromatographic micro syringe. A maximum of 50 μ l of acetone (tested to be gaschromatographically pure) was added at the capillary side of the trap. The droplet was forced hence and forth in the capillary by applying a thumb pressure at both ends of the trap to secure maximum absorption of the volatiles by the eluent. The eluent was finally collected in the pointed bottom of the trap. The eluted sample was drawn into a micro syringe and then injected into the isolative gaschromatographic column. Components of individual peaks were trapped in a glass capillary cooled by a flow of cold nitrogen according to Burson & Kenner (1969) (see fig. 6). Nitrogen gas was allowed to flow through liquid nitrogen in a Dewar flask and cold nitrogen was thus swept with it along the outer wall of the capillary trap. 75 fractions were collected in this way from extract 3.3.B and 69 from extract 3.3.A. All capillaries were directly fused over a microburner and stored in deep freeze prior to further analysis. The odours of the peak components of fraction 1 were judged by smelling at the outlet of the katharometer to locate the component(s) possessing more or less similar odour characteristics as trassi (see fig. 8).

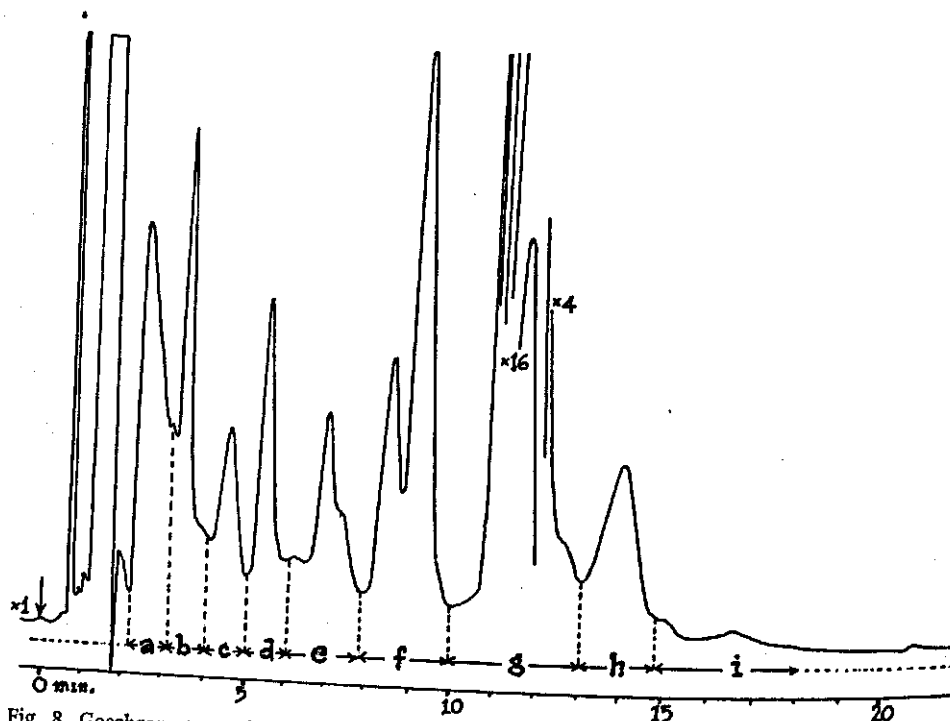


Fig. 8. Gaschromatographic separation of fraction 1 into 9 subfractions a to i (see also fig. 7) to locate the place of peak components possessing odour similarities to trassi. Apparatus and column see fig. 7. Operating temperature 120 °C.

3.6 Identification of trassi aroma compounds

In this study combined gas-liquid partition chromatography and mass spectrometry in a coupled GLC-MS arrangement has been the primary method employed to identify most of the aroma components of cooked trassi. In addition infrared spectrometry, ultraviolet spectrophotometry, and gaschromatographic retention parameters were utilized whenever possible to supplement MS data or to separately identify certain compounds recovered in trassi aroma. The combined GLC-MS identification procedure has in the past years gained increasing importance for the analysis of multicomponent mixtures because of the following reasons:

- both analytical methods complement each other: the high separating power typical for gaschromatography is the prerequisite for the subsequent analysis with a mass spectrometer, which in many cases allows identification of the gaschromatographic peak component(s),
- both instruments are quite compatible: both have about the same sensitivities, and in many cases samples as small as 0.0001 µg will suffice for a complete analysis (ten Noever de Brauw & Brunnée, 1967); the response times of both instruments are also quite comparable,
- further more it is note worthy that the separating power of the GLC column,

whether packed or capillary, is practically unaffected by the instrumental connection with the MS; on the other hand the function of the MS is not disturbed by the carrier gas coming from the GLC together with the separated components when helium is used,

d. for both instruments compounds to be analyzed should be volatilizable without decomposition in the range between ambient temperature and about 400 °C.

Heins *et al.* (1966) have even employed this method to analyze directly vapours over dry food products such as tea and coffee. Optimal operating conditions of the coupled GLC-MS device have been investigated by ten Noever de Brauw & Brunnée (1967) and many others.

3.6.1. Instruments used and procedures of identification

This investigation has the objective to simply identify compounds, which were separated and isolated from the trassi aroma fractions. Informations obtained by all methods employed in this study were benefitted to determine the identities of compounds. In most cases one method was applied supplementary to the other to confirm data for compound identification.

Gaschromatography Besides for component separation purposes gaschromatography was used in this study as an identification procedure to supplement mass spectrometry or infrared spectrometry. The same apparatus and column as described in section 3.5 were used, and in addition to these other gaschromatographs equipped with different columns were employed to specially identify special groups of compounds. Retention data of compounds identified by mass spectrometry were compared with data of reference chemicals, using 2 different columns.

The volatile fatty acids of trassi separated from extract 3.3.C were identified gaschromatographically on a SP-1000 stationary phase adsorbed on Chromosorb W-AW, 60-80 mesh support. Peak components were isolated and their identities confirmed by infrared spectrometry.

The highly volatile sulfur compounds of trassi were analyzed with a Micro Tek MT 160 gaschromatograph equipped with a Melpar flamephotometric detector, which was made selective for the detection of sulfur compounds only by inserting a narrow band-width filter of 394 nm wavelength between the emission source and the photomultiplier tube. A 5.5 m coiled glass column (3.6 mm i.d.) filled with a doubly coated (3 % Igepal CA 630 and didecylphthalate) solid support of Chromosorb G-AW, 80-100 mesh was used at an operating temperature of 40 °C. The column packing and solid support coating procedure have been described in detail by Jansen *et al.* (1971). 50 gram of trassi was suspended in 150 ml demineralized water and refluxed for 1 hour. A special condenser cooled with liquid nitrogen was connected on top of the ordinary water condenser to reduce loss of highly volatile sulfur compounds as much as possible. The cold suspension was transferred into an infusion bottle. 2 ml headspace samples were injected

into the gaschromatograph using a gas-tight Hamilton syringe. Retention data of the unknowns were compared with data of reference compounds (Fluka AG, purum grade).

Trassi amines were analyzed with a Hewlett Packard type 7620 A Research gaschromatograph equipped with a flame ionization detector. A 1.5 m aluminium column (4 mm i.d.) packed with uncoated Chromosorb 103 (polystyrene resin granules), 80-100 mesh grains was used. The analysis was carried out at a programmed temperature from 150 to 200 °C (1 °C/min). The trassi extract 3.3.D (see Section 3.3.) was transferred into an infusion bottle and made alkaline by adding 6 N NaOH in excess. 6.5 ml headspace sample was introduced into the gaschromatograph using a gas-tight syringe and the retention data of the unknowns were compared with data of reference amines (Baker Organic Chemicals, pure grade).

Infrared spectrometry In this investigation infrared spectrometry was of great use in the identification of volatile fatty acids. Compounds emerging at high retention times from the column of the isolative gaschromatograph and producing well formed symmetrical peaks in the chromatographs of the other extracts were also analyzed by infrared spectrometry. The identities of substituted pyrazines were confirmed by a combination of GLC, MS, and IR data. Recordings of infrared spectra were made with a Perkin Elmer Model 13 and Model 257 infrared spectrometer, depending on the size of sample to be analyzed. Large sized samples were analyzed with the model 13 in semimicro cuvettes ($2 \times 0.2 \times 0.2 \text{ cm}^3$), while for small samples the model 257 with micro cuvettes ($0.5 \times 0.1 \times 0.02 \text{ cm}^3$) was used. All samples were analyzed in their carbon tetrachloride solutions (BDH, special for spectrometry).

Ultraviolet spectrophotometry Ultraviolet spectral analysis was done for carbonyl-2,4-DNPH's of trassi. A Beckman DK 2A double beam recording spectrophoto-

Table 9. UV maxima of dinitrophenylhydrazones of some aliphatic carbonyls¹.

Compounds	λ max in chloroform
saturated aldehydes	358 - 361
saturated ketones	365 - 368
2-enals	373
2-enones	380
2,4-dienals	388 - 407
2,4-dienones	388 - 407
2,4,6-trienals	400 - 415
2,4,6-trienones	400 - 415

1. After Braude & Jones (1945).

meter (Hydrogen and Wolfram light source) equipped with 1 cm optical path quartz cuvettes was used. Samples were analyzed in chloroform solutions. Groups of carbonyl-2,4-DNPH's obtained in step 3.4.1 have been characterized by their UV maxima (see table 9).

The identities of some of the trassi carbonyl-dinitrophenylhydrazones, which were recovered in sufficient quantities were determined by their specific UV maxima and/or mass spectra.

Mass spectrometry Mass spectrometry was applied to characterize the identities of most of the volatile compounds found in trassi. Except for the dinitrophenylhydrazones of a few carbonyls, mass spectral analysis was done with the coupled GLC-MS device (see diagram in fig. 9).

An Atlas-CH 4 mass spectrometer with a double ion source (20 and 70 eV) coupled with a Varian Aerograph Type 550 gaschromatograph via a dual stage Bieman-Watson type molecular separator was used. The operating temperatures of the ion chamber and the molecular separator were 200 °C and 170 °C res-

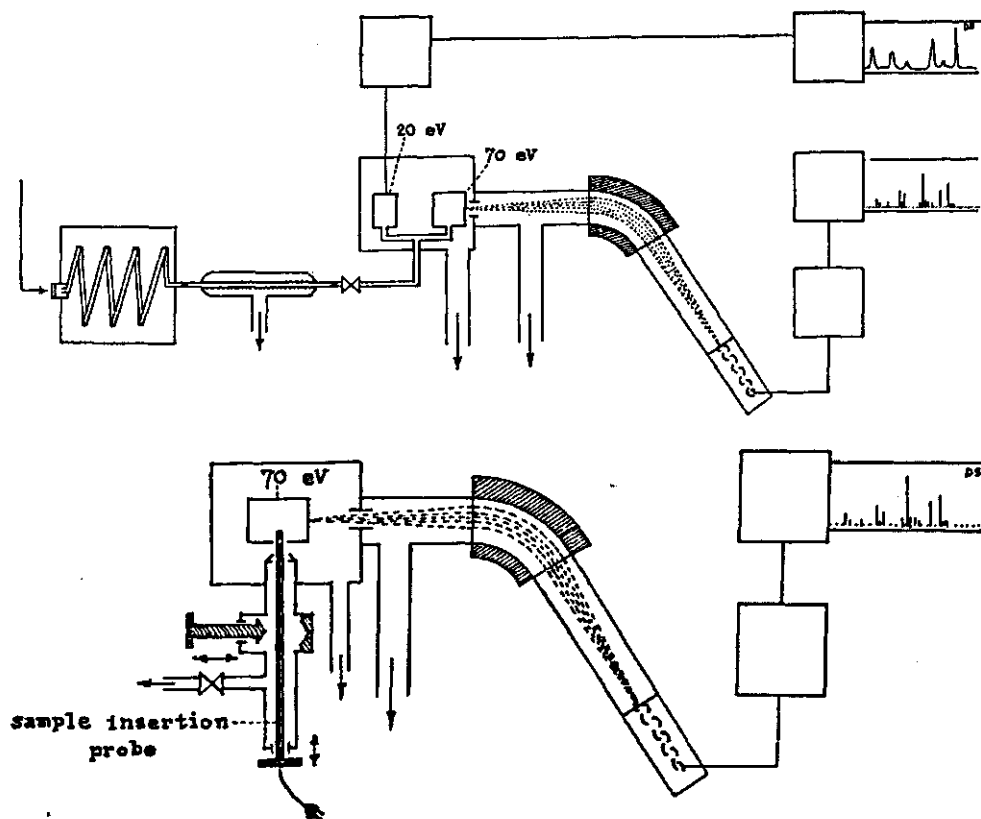


Fig. 9. Schematic diagram of a coupled GLC-MS apparatus (above) and of a CH-5 mass spectrometer equipped with a thermo-controlled direct sample insertion probe (below).

pectively. The column temperature was adjusted to the boiling range of the fractions to be analyzed. In most instances a 150 m stainless steel column (0.03 inch i.d.), wall-coated with Carbowax 20 M was employed.

Trassi aroma fractions obtained by gaschromatographic separation were eluted in methanol (Merck, chromatographic grade) and 0.1 to 0.2 μ l samples were introduced into the column of the GLC-MS device.

The dinitrophenylhydrazones of trassi carbonyls were analyzed using the CH 5 mass spectrometer. The 2,4-DNPH samples were dissolved in a few μ l of hexane and the solutions were applied onto a small piece of glass paper (3 \times 5 mm Glasfaserpapier No. 6, Carl Schleicher & Schull). Prior to use the glass paper was made free from extraneous material by heating in a small oven at 600 °C for about 2 hours. The 2,4-DNPH samples adsorbed on the glass paper were introduced into the sample tip of the direct sample insertion probe of the mass spectrometer. Fig. 9 illustrates a schematic diagram of the CH 5 mass spectrometer equipped with a thermocontrolled direct sample insertion probe. The CH 5 apparatus differs from the CH 4 in its higher mass range and extremely high resolving power. While the CH 4 has a mass range of 1-500 at 3 kV accelerating voltage, the CH 5 is suitable to analyze compounds up to 1200 molecular weights at the same accelerating potential. Three ranges can be chosen by adjusting the accelerating potential: mass range 1-1200 at 3 kV, 1-1800 at 2 kV, and 2-3600 at 1 kV. The resolving power of the CH 5 is extremely high, >10,000 (50 % peak width) or 7500 (10 % valley), while the CH 4 has a maximum of only 800 (Varian MAT information booklet).

4 Results

Data obtained from the different identification techniques applied in this investigation, each separate or/and combined, revealed the presence in trassi of many volatile compounds isolated by steam distillation at atmospheric pressure. Table 10 presents and summarizes volatile compounds of trassi so far identified in this study. They are grouped according to their chemical functions for convenience. Five unidentified sulfur compounds are presented as compounds nr. 139-143 together with their mass spectra (figs. 10 to 14).

Table 10. Volatile compounds of trassi identified by different or combined identification techniques.

Techniques.									
No.	Compounds	Identification evidence				Remarks			
A. Hydrocarbons									
1.	tridecene	MS
2.	tetradecene	MS
3.	pentadecene	MS
4.	benzene	MS	.	GLC
5.	toluene	MS	.	GLC
6.	p-xylene	MS	.	GLC
7.	ethylbenzene	MS	.	GLC
8.	styrene	MS
9.	mesitylene	MS
10.	isopropylbenzene	MS	.	GLC
11.	n-butylbenzene	MS	.	GLC
12.	p-cymene	MS
13.	limonene	MS	.	GLC
14.	α -terpinene	MS	.	GLC
15.	naphtalene	MS
16.	α -methylnaphtalene	MS
B. Alcohols									
17.	ethanol	MS	.	GLC
18.	2 methylbutan-1-ol	MS	.	GLC
19.	2-methylbutan-2-ol	MS
20.	n-pentanol	MS	.	GLC
21.	hex-3-en-1-ol	MS	.	GLC
22.	oct-3-en-1-ol	MS
23.	furfurylalcohol	MS	.	GLC

No.	Compounds	Identification evidence				Remarks
C. Carbonyl compounds						
24.	2-methylbutanal	MS	.	.	TLC	.
25.	pentanal	MS	.	.	TLC	UV
26.	2-methylpentanal	MS
27.	hexanal	MS	.	.	TLC	UV
28.	heptanal	MS	.	GLC	.	.
29.	octanal	MS	.	GLC	.	.
30.	tetradecanal	MS	.	.	TLC	.
31.	hexadecanal	MS	.	.	TLC	.
32.	octadecanal	MS	.	.	TLC	.
33.	pent-2-enal	MS	.	.	TLC	UV
34.	pent-3-enal	MS	.	GLC	.	.
35.	hex-2 enal	MS	.	GLC	.	.
36.	hex-3-enal	MS	.	GLC	.	.
37.	hept-2-enal	MS	.	.	TLC	UV
38.	hept-4-cis-enal	MS	.	GLC	.	.
39.	nona-2,6-dienal	MS	.	GLC	.	.
40.	deca-2,4-dienal	MS
41.	benzaldehyde	MS	.	GLC	TLC	UV
42.	p-ethylbenzaldehyde	MS
43.	p-hydroxybenzaldehyde	MS	.	.	TLC	.
44.	phenylacetaldehyde	MS
45.	phenylpropylaldehyde	MS	.	.	TLC	.
46.	furfural	MS	.	GLC	TLC	UV
47.	5-methylfurfural	MS	.	.	TLC	.
48.	butan-2-one	MS	.	GLC	TLC	UV
49.	pentan-2-one	MS	.	GLC	TLC	UV
50.	4 methylpentan-2-one	MS
51.	hexan-2-one	MS	.	GLC	TLC	.
52.	heptan-2-one	MS	.	GLC	.	.
53.	octan-2-one	MS	.	GLC	.	.
54.	octan-3-one	MS	.	GLC	.	.
55.	nonan-2-one	MS	.	GLC	TLC	.
56.	dodecan-2-one	MS
57.	pent-1-en-3-one	MS	.	GLC	.	.
58.	pent-3-en-2-one	MS	.	GLC	.	.
59.	hex-3-en-2-one	MS	.	GLC	.	.
60.	oct-3-en-2-one	MS	.	GLC	.	.
61.	non-3-en-2-one	MS
62.	octa-3,5-dien-2-one	MS
63.	nona-3,5-dien-2-one	MS
64.	acetyl furan	MS
65.	acetophenone	MS	.	GLC	.	.
66.	2-methylcyclopentanone	MS	.	GLC	.	.
67.	phenylpropanone	MS
68.	verbanone	MS
69.	verbenone	MS

No.	Compounds	Identification evidence				Remarks
D. Fatty acids						
70.	acetic acid	.	IR	GLC	.	.
71.	<i>n</i> -butyric acid	.	IR	GLC	.	.
72.	isobutyric acid	.	IR	GLC	.	.
73.	<i>n</i> -pentanoic acid	.	IR	GLC	.	.
74.	2-methylbutyric acid	.	IR	GLC	.	.
75.	3-methylbutyric acid	.	IR	GLC	.	.
76.	dodecanoic acid	.	IR	.	.	.
E. Esters						
77.	ethylacetate	.	IR	GLC	.	.
78.	isoamylpentanoate	MS
79.	ethyl ester of a C ₁₀ acid	MS
F. Sulfur compounds						
80.	H ₂ S	.	.	GLC	.	.
81.	methylmercaptan	.	.	GLC	.	.
82.	ethylmercaptan	.	.	GLC	.	.
83.	phenylethylmercaptan	MS
84.	tertiary heptylmercaptan	MS
85.	dimethylsulfide	MS	.	GLC	.	.
86.	thiodimethylacetal	MS
87.	ethylamylsulfide	MS
88.	methylcyclohexylsulfide	MS
89.	phenylpropylsulfide	MS
90.	2-ethylthiophen	MS
91.	2-acetylthiophen	MS	.	GLC	.	.
92.	2-pentylthiophen	MS
93.	2-hexylthiophen	MS
94.	dimethyldisulfide	MS	IR	GLC	.	.
G. Nitrogen compounds						
95.	ammonia	odour, chem.
96.	trimethylamine	.	.	GLC	.	.
97.	dipropylamine	.	.	GLC	.	.
98.	octylamine	MS
99.	nonylamine	MS
100.	benzonitril	MS
101.	tolunitril	.	.	GLC	.	.
102.	methylthioethanenitril	MS
103.	2,4-dimethylpyrrol	MS
104.	indol	MS	IR	GLC	.	.
105.	skatol	MS	IR	GLC	.	.
106.	5-methylindol	MS	IR	.	.	.
107.	2-ethylpyridine	MS
108.	benzamide	MS
109.	methylpyrazine	MS	IR	GLC	.	.
110.	2,5-dimethylpyrazine	MS	IR	GLC	.	.
111.	2,6-dimethylpyrazine	MS	IR	GLC	.	.
112.	2,3-dimethylpyrazine	MS	IR	GLC	.	.

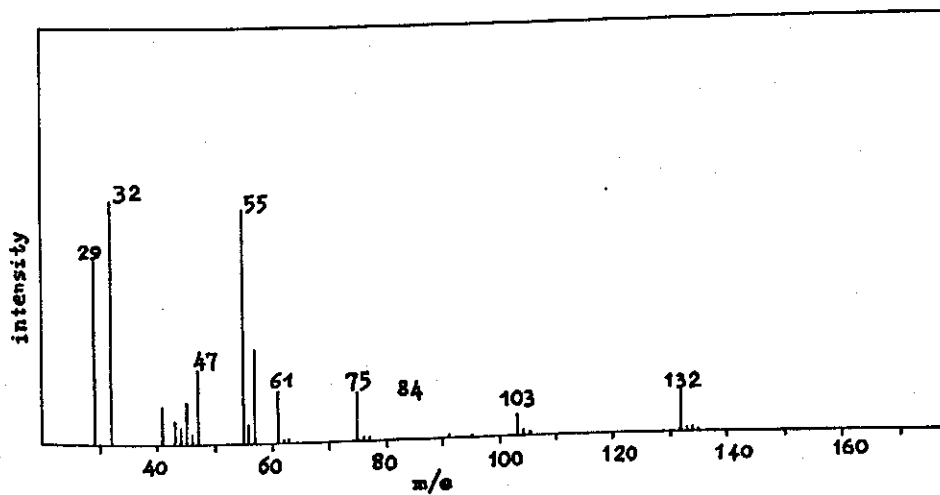
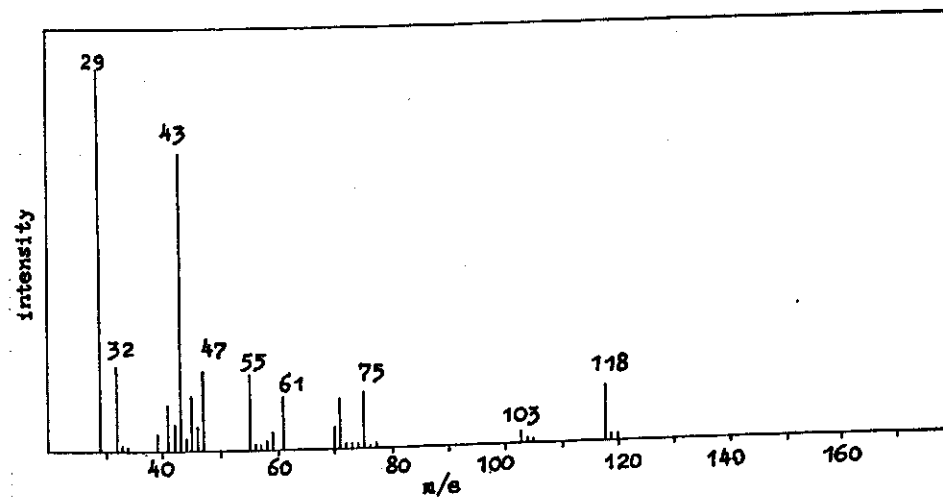
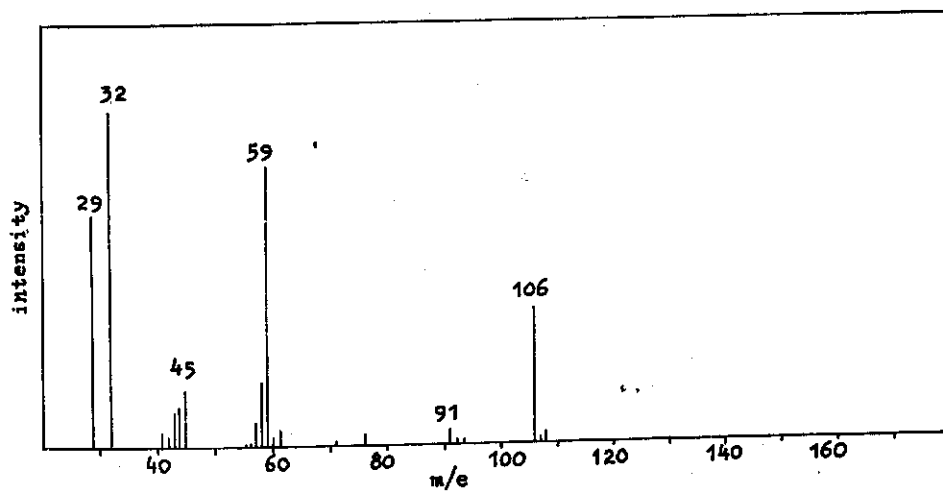
No.	Compounds	Identification evidence					Remarks
113.	ethylpyrazine	MS	.	GLC	.	.	
114.	trimethylpyrazine	MS	IR	GLC	.	.	
115.	2-methyl-6-ethylpyrazine	MS	IR	GLC	.	.	
116.	2-methyl-5-ethylpyrazine	MS	IR	GLC	.	.	
117.	2-methyl-3-ethylpyrazine	MS	IR	GLC	.	.	
118.	2,5-dimethyl-3-ethyl-pyrazine	MS	IR	GLC	.	.	
119.	2,6-dimethyl-3-ethyl-pyrazine	MS	IR	GLC	.	.	
120.	tetramethylpyrazine	MS	IR	GLC	.	.	
121.	2-methyl-5-isopropyl-pyrazine	MS	IR	GLC	.	.	
122.	2-methyl-6-isopropyl-pyrazine	MS	IR	GLC	.	.	
123.	2,6-diethylpyrazine	MS	IR	GLC	.	.	
124.	trimethylethylpyrazine	MS	IR	GLC	.	.	
125.	2-methyl-5,6-diethyl-pyrazine	MS	
126.	2-methyl-5-isopropyl-pyrazine	MS	
127.	trimethylisopropyl-pyrazine	MS	
128.	2,5-dimethyl-3,6-diethylpyrazine	MS	IR	GLC	.	.	
<i>H. Miscellaneous compounds</i>							
129.	phenol	MS	IR	GLC	.	.	
130.	p-dichlorobenzene	MS	.	GLC	.	.	
131.	dibromoethane	MS	
132.	p-cresol	MS	.	GLC	.	.	
133.	1-ethyl-2,3-dihydro-indene	MS	
134.	pentylfuran	MS	
135.	2-methyltetrahydrofuran	MS	
136.	ethylguaicol	MS	
137.	4-hydroxyhexanoic acid lactone	MS	
138.	4-hydroxyheptanoic acid lactone	MS	
<i>I. Unidentified compounds</i>							
139.	m.w. 106 C ₈ H ₈ S ₂ (?)	MS	see fig. 10
140.	m.w. 118 C ₈ H ₁₄ S (?)	MS	see fig. 11
141.	m.w. 132 C ₇ H ₁₀ S (?)	MS	see fig. 12
142.	m.w. 132 C ₆ H ₁₂ SO (?)	MS	see fig. 13
143.	m.w. 164 C ₈ H ₈ S ₃ (?)	MS	see fig. 14

Right, page 37:

Fig. 10. Unknown compound 139.

Fig. 11. Unknown compound 140.

Fig. 12. Unknown compound 141.



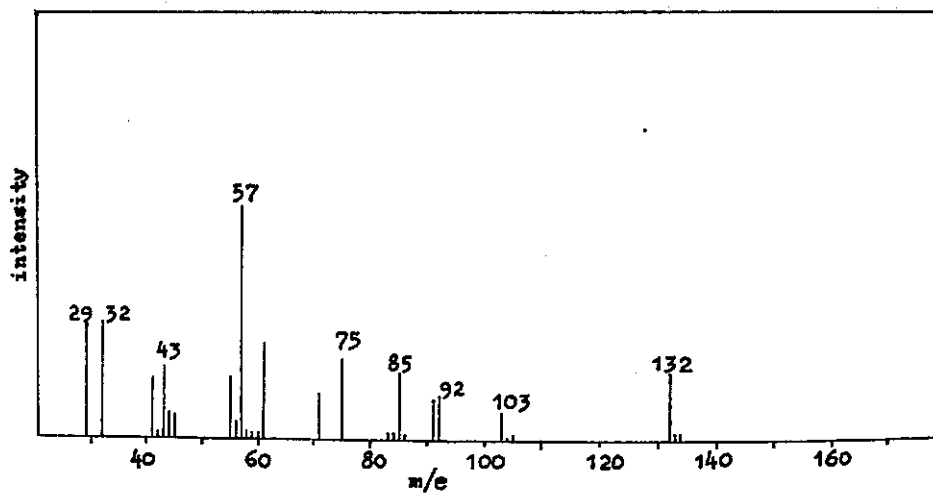


Fig. 13. Unknown compound 142.

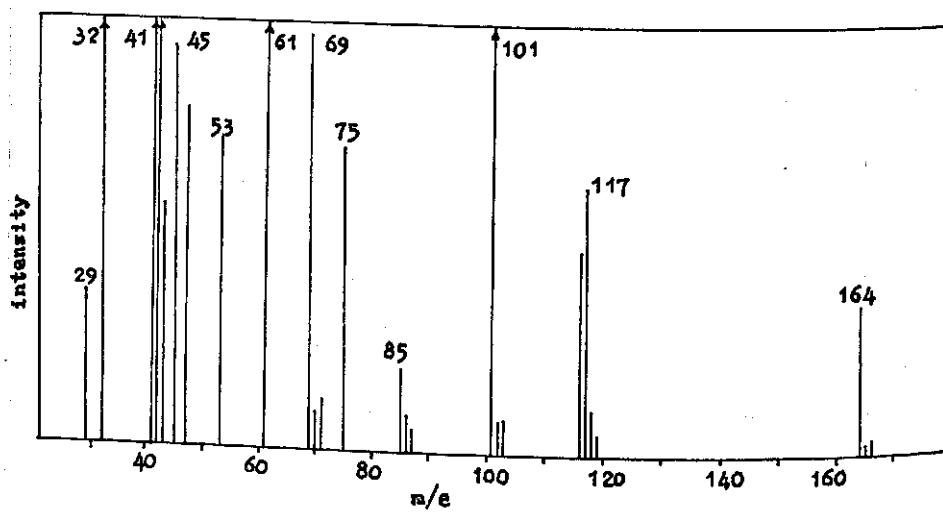


Fig. 14. Unknown compound 143.

5 Discussion

In this investigation 138 volatile compounds were identified in the steam distillate of cooked trassi. These include:

16 hydrocarbons	3 esters
7 alcohols	15 sulfur compounds
46 carbonyl compounds	34 nitrogen compounds
7 fatty acids	10 miscellaneous compounds.

Five other sulfur compounds were not successfully characterized. Mass spectra of the unknown compounds concerned (see figs. 10 to 14) did not fully conform with the available reference spectra as well as spectra of synthesized suspected compounds.

The possible introduction of artifacts in the trassi aroma concentrate produced during steam distillation, extraction and concentration was checked by the blank experiment. No evidence was found of the presence of such extraneous compounds in the gaschromatogram of the blank concentrate, run with the isolative gas-chromatograph. This would suggest that virtually all compounds identified in the volatile fraction of trassi were derived from the steam distillate.

Hydrocarbons were found in substantial number in this study. However, their importance in the odour of products of animal origin, trassi in this instance, is negligible. This may be true also for alcohols and esters, although their presence in products resulting from fermentation is quite normal.

Carbonyl compounds appeared to be most preponderant in the trassi volatiles investigated in this work and represented 33 % of the total volatiles. 24 of the identified carbonyls were aldehydes, 16 were methyl-ketones, and 6 were other ketones. The presence of carbonyls in relatively abundance in the volatile fraction of trassi would clearly indicate their importance as a contributory factor to the odour of trassi. They are derived primarily from fats as a result of oxidation and microbial action.

Carbonyl compounds have been implicated in the off flavour development of lipids (Lea & Swoboda, 1953) and in the browning reaction of fishery products (Nonaka, 1957). The formation of carbonyls in the autoxidation of salmon oil had been studied by Wyatt & Day (1963). Certain carbonyl compounds are very important in imparting the typical aroma of many kinds of cheese, e.g. methyl-ketones from C₃ to C₁₁ were considered to be responsible for the typical aroma of Roquefort and Blue cheese (Liebich *et al.*, 1970). On the other hand the presence of other carbonyls in relatively excessive concentrations causes off-flavour defects

in other dairy products. The evolution of certain carbonyls and their role as the causative factor of the cold-storage defects in butter had been intensively studied by Badings (1970). Langler *et al.* (1967) have identified many aldehydes and methyl-ketones in the Swiss cheese aroma concentrate they investigated.

Van Veen (1941b) studied the typical aroma of some pedah products (salted/partially dried/cured *Scomber Kanagurta* Russel) and found relatively high concentrations of carbonyls in the fish flesh slurry. He considered carbonyls, particularly butanal, being responsible for the typical pedah aroma. On reacting the steam distillate of pedah slurry with 2,4-dinitrophenylhydrazine the typical odour disappeared almost entirely. A pleasant ethereal smell remained after oxidation of the aldehydes with alkaline silver oxide. It appeared that methyl-ketones were the cause of the ethereal odour. His results also indicated that good quality pedah contained higher concentrations of methyl-ketones than pedah of inferior quality. He succeeded in isolating 3 to 9 mg of butanal-2,4-DNPH from each 100 gram of good quality pedah and only a few mg of methyl-ketones-2,4-DNPH from each kg of the same product. In some pedahs an increasing concentration of methyl-ketones was observed with the length of storage time, which meant improvement in quality. In bad quality pedah on the other hand a longer storage time resulted in lower concentration of methyl-ketones accompanied by a more intense ammonia and amine odour. It is quite well understood, that such conditions apply also to other salted/cured fish products, trassi included.

In this study 23.5 mg of total carbonyl-2,4-DNPH was recovered from the steam distillate of 400 gram trassi, obtained by continuous distillation/extraction for 2 hours.

n-Butyric acid, isobutyric acid, *n*-pentanoic acid, 2- and 3-methyl butyric acid, and dodecanoic acid were recovered in sufficient quantities from the isolated fractions of extract 3.3.C, which enabled their identification by infrared spectrometry. This may clearly explain the sour tone in the odour of trassi.

Volatile sulfur compounds have in general relatively very low odour threshold values. Values for lower mercaptans, lower sulfides, and lower disulfides fall in the range of a few parts per billion (10^9) (ppb). The smell of last mentioned compounds has been described as foul or alliaceous, and bad industrial smells are ascribed to these compounds (Moncrieff, 1967).

15 sulfur compounds were identified in this study, and only dimethyldisulfide was recovered in sufficient quantity. This compound has a penetrating garlic odour and together with the other sulfur compounds present in trassi volatiles may have an important effect on the overall odour of trassi. The influence of sulfur compounds to the odour of trassi was studied by treating trassi steam distillate with HgCl_2 . This treatment will eliminate H_2S , sulfides, and disulfides. A quite different odour remained after this treatment; it was more neutral and not penetrating/offensive. It resembled or was even equal to the odour of fried shrimp. It is important to mention that about a similar odour was observed in the aqueous residue of extract 3.3.A after the addition of alkali for the preparation of extract

3.3.B. This would indicate that certain sulfur compounds, extractable with pentane/ether at low pH, were responsible for the penetrating/offensive odour of trassi. On concentration however, both extracts 3.3.A and 3.3.B have the same typical smell of trassi, with a difference in offensive tone. In the odour evaluation of gaschromatographic peak components of extract 3.3.B-fraction 1, the odour of peak 1g (see fig. 8) was to a considerable extent similar to the odour of trassi. The GLC-MS analysis of the isolated peak components revealed the presence of thiodimethylacetal, 2,5- and 2,6-dimethyl pyrazine, and ethyl-pyrazine. The individual pyrazines were not reminiscent of trassi odour; the thioacetal was not commercially available and there was no opportunity for its synthesis.

Extract 3.3.B yielded more species of pyrazines than extract 3.3.A. 20 pyrazines in total were identified. In number they formed the second predominating group of trassi volatiles. Pyrazines are basically tertiary ammonium bases, becoming more polar in acid medium and as such are more soluble in water than in pentane/ether. These compounds are formed primarily as a result of heat treatment of foods and are implicated in the development of typical roasted food aromas and flavours. This group of compounds has drawn much attention of many investigators and food aroma industries. Many pyrazine species have been synthesized (Kleipool, 1966; Dawes & Edwards, 1966; Bondarovich *et al.*, 1967; Koehler *et al.*, 1969) and many spectral data of pyrazines have been published (Kleipool, 1966; Bondarovich *et al.*, 1967; Friedel *et al.*, 1971). Odour threshold values of certain pyrazines and their role in the flavour of roasted foods have been investigated by Koehler *et al.* (1971). Table 11 presents threshold data of certain pyrazines.

Many pyrazine compounds have nutty odours, and all pyrazines synthesized so

Table 11. Odour detection threshold levels in water and in mineral oil of certain substituted pyrazines.¹

Compounds	Concentration in water		Concentration in oil	
	μmolar	ppm	μmolar	ppm
2-methylpyrazine	1,122	105	282	27
2,5-dimethylpyrazine	320	35	159	17
2,6-dimethylpyrazine	501	54	71	8
2-ethylpyrazine	200	22	159	17
trimethylpyrazine	71	9	224	27
monomethylmonoethylpyrazine	4	0.5	7	0.9
tetramethylpyrazine	71	10	282	38
2,5-dimethyl-3-ethylpyrazine	316	43	180	24
2,6-dimethyl-3-ethylpyrazine	112	15	178	24
2-n-pentylpyrazine	7	1	57	9

1. After Koehler *et al.* (1971).

Table 12. Odour descriptions of certain pyrazine species, identified in trassi.

Compounds	Type of odour
methyl pyrazine	floral, raw coffee
2,5-dimethyl pyrazine	floral, raw coffee, sweet, cacao
2,6-dimethyl pyrazine	floral, raw coffee
2,3-dimethyl-pyrazine	higher aldehyde
2-methyl-5-ethylpyrazine	roasted coffee, cacao
2-methyl-6-ethylpyrazine	roasted peanut, hazelnut, cacao
trimethyl-3,6-diethyl pyrazine	roasted coffee, hazelnut, cacao
2,5-dimethyl-3,6-diethylpyrazine	roasted coffee, cacao

far have a vegetable tone of odour (Kleipool, 1966). 2,5- and 2,6-dimethylpyrazines were recovered in relatively high yield from the alkaline trassi aroma extract. Synthesized 2,6-dimethylpyrazine has a floral, raw coffee odour and its 2,5-isomer has in addition a sweet (chloroformlike), cacao, and coffee tone.

The role of pyrazine compounds in imparting the cacaolike odour to trassi is not to be doubted.

Types of odour of certain pyrazines, present in trassi volatiles, are listed in table 12.

In the identification of pyrazine compounds the work of Kleipool (1966), Bondarovich *et al.*, (1967), and Friedel *et al.* (1971) have been very useful. Mass spectra of pyrazines are not quite peculiar for their group, and therefore molecular weights and fragmentation patterns are very valuable to determine their structures. Pyrazines with substituents of 3 C or longer produce a fragmentation pattern with an intense peak (base peak) at even m/e numbers, resulting from β -cleavage of the substituent concerned with rearrangement. The presence of an isopropyl group is evidenced by a rearrangement peak at an even m/e number, but this is in intensity lower than P.P.—15. The absence of an even m/e number would indicate, that all substituents are of the order of C_1 and/or C_2 . Methyl substituents produce an intense peak at P.P.—1 or if it occupies an iso position P.P.—15. In this last instance P.P.—15 should be more intense than P.P.—29.

Infrared spectra give strong evidence of the substitution pattern of pyrazine compounds. Although substituents show by themselves absorption bands within the range of the substituted pyrazines concerned, they will not effect a variation beyond the range of 10 cm^{-1} of the indicated frequencies. Table 13 lists the most important absorption bands of the 6 types substitution patterns of pyrazines.

Last but not least the role of ammonia and amines in the effectual total odour of trassi should not be overlooked. Amines develop easily and rapidly in fish, shortly after death and are responsible for the typical fishy odour. The concentration of free nitrogen bases in fish is used as an indicator of the degree of spoilage in fish and fish products. In living fish a certain equilibrium is established between trimethyl-

Table 13. Absorption frequencies in cm^{-1} of substituted pyrazines¹. Strongest absorption in italics.

mono-	2,3-di	2,5-di	2,6-di	tri-	tetra-
	1656 W				
1575 W					
1526 W	1535 W		1530 S		
		1485 S			
1470 M		1460 M	1464 M	1450 S	1450 MS
		1445 M	1416 S		1413 S
1404 S	1400 S	1390 M		1390 M	
		1325 M		1280 W	
			1263 M	1250 W	1222 M
1150 M	1167 S	1160 M	1165 S	1165 S	1184 M
1056 M		1035 S		1046 M	
1015 S	1011 M		1019 M	1011 W	
	988 M			940 W	990 M
	968 M				
	846 M	881 W		897 W	800 M

1. After Kleipool (1966).

amine oxide and trimethylamine. After death, the alterations in the tissues are accompanied by a considerable increase in the di- and trimethylamine forms as a consequence of the reduction of the oxide into free bases. Trimethylamine is a gas at ordinary temperature. Its odour is pronounced fishy and tends to adhere strongly to clothes or skin. The same behaviour is observed in trassi volatiles. The odour of trassi adhering to the hand, after touching the product, is not easily removed by washing with water.

Microbial activities play undoubtedly a very important role in the development of the typical trassi aroma. However, no experiments have been conducted concerning this aspect of trassi. Van Veen (1941) has isolated a clostridium strain from pedah, which was able to induce the typical pedah flavour on microbial media. Certain clostridia have been implicated to the typical nuoc-mam aroma (van Veen, 1953).

Part of the volatile compounds in trassi steam distillate should have been derived from non-volatile precursors as a result of non-enzymic flavour compounds formation associated with browning reactions.

Amino acids are the most important non-volatile precursors of many volatile compounds. Together with certain carbonyls they may be the starting material in the formation of N-heterocyclic volatile compounds. Pyrazines have been synthesized in model systems containing amino acid-sugar mixtures, amine-sugar and ammonia-sugar mixtures (Dawes & Edwards, 1966; Bondarovich *et al.*, 1967; Koehler *et al.* 1969; Koehler & Odell, 1970), while Kleipool used methyl ketones

and alkyl nitrite as starting material (1966). Temperature, duration of heating, and pH influence the rate of pyrazine formation quite importantly. Below 100 °C essentially no pyrazines were formed (Koehler & Odell, 1970), raising the temperature to 100 °C or higher increased the yield markedly, but at 150 °C variable results were observed. The addition of 0.1 mol of acid to a mixture containing 0.1 mol amino acid and 0.1 mol sugar in 200 ml diethyleneglycol + 20 ml water medium inhibited the reaction, while the addition of the same molar amount of alkali increased the yield about tenfold. As the trassi suspension in this study was at the alkaline side, a reasonable production of pyrazines could be expected.

Amino acids have also been found to participate in the non-enzymic formation of certain aldehydes during baking (Linko, 1963). The concentration of amino acids in the crust of bread was found to be much lower than in the crumb and at the same time certain aldehydes were formed. Leucine, isoleucine, valine, methionine, alanine, and phenylalanine were found to be most reactive, which formed pentanal, 2-methylbutanal, methional, acetaldehyde, and phenylacetaldehyde respectively.

The Strecker degradation of methionine yields methional as an intermediate which decomposes into methylmercaptan, from which dimethylsulfide and dimethyldisulfide may be formed (Ballance, 1961).

A decrease in the concentration of amino acids in aqueous medium on heating had been investigated by Wasserman & Spinelli (1970). The decrease in the concentration of amino acids with the duration of heating was accompanied by the development of certain odours. 30 minutes resulted in broth-like odour, and longer heating induced the aroma of roasted beef to pungent burned odour.

Those findings would indicate the importance of amino acids as precursors of certain volatiles in heated and heat processed products and in this respect free amino acids ($\pm 30\%$ of the total amino acids in trassi was found in the free state, see table 3) should have a very important contribution to the development of many volatile compounds.

Some volatile compounds in trassi must have their origin from enzymatic processes occurring after the shrimp have been caught; some part of these volatiles undergo alteration during curing and maturation and the other part might survive the process. This part of the study is presented in Chapter 6.

6 Development of some volatile compounds in autolyzed shrimp

This part of the study had the objective to investigate the origin of some volatile compounds of trassi, formed during autolysis of shrimp without the interaction of microbial activities. Since the body surface, the gills, and the intestinal tract of the shrimp are invested by microbes it is not easy to study autolysis of whole shrimp, the form of the raw material used for trassi preparation. The use of antibiotic only would not be sufficient to kill all microbes, and besides the antibiotic would not be able to penetrate sufficiently deep into the gills; it will not reach the entrails and cannot penetrate the shrimp shell. As antibiotics are generally used to destroy pathogenic micro-organisms, their use in this experiment would not be very fruitful. A choice was made on the sodium salt of ethyl-mercurisalicylate (Merthiolate = Mersalate = Merthiosal = Thimerosal = Thiomersalate). Hecht & Fredenburgh (1956) have used Merthiolate to prepare a buffered antimicrobial peptone preparation for external application. In addition to merthiolate their preparation contained 0.5-1 % Tween 80. Merthiolate was used at 0.004 to 0.1 % level. The pH of the preparation was kept at 3.5 with a gluconic acid buffer. Aizawa (1955) has studied the influence of Merthiolate and other drugs on the respiration and adaptive oxidation of mannose and galactose in *C. albicans*, and found that both processes were inhibited by Merthiolate. Kato & Mamoru (1953) found Merthiolate to be effective as antiseptic but not as stabilizer for liquid preparations containing pepsin. Jones (1955) observed that Merthiolate was a good preservative for blood-typing sera if kept under sterile conditions and low temperatures; under ordinary conditions and at room temperature the sera deteriorated. Weaver (1956) used Merthiolate to increase the yield of starch-converting enzymes, which were produced by certain microbes; a higher yield was obtained when the micro-organism was inactivated with Merthiolate. This property of Merthiolate in destroying microbes but at the same time leaving enzymes active is very ideal in the study of autolytic processes.

For this experiment the common Dutch shrimp (*Crangon vulgaris*) were used. The shrimp were kept alive during the transportation from the fishing harbour to our laboratory. Practically all shrimp survived the transportation. The animals were directly rinsed with cold tap water and graded. Portions of about 500 gram of live shrimp were put in a cylindrical gauze wire sieve and plunged directly in liquid nitrogen. The quick frozen shrimp were then stored at -40°C in plastic bags.

The Merthiolate solution was prepared by dissolving the compound in buffer-

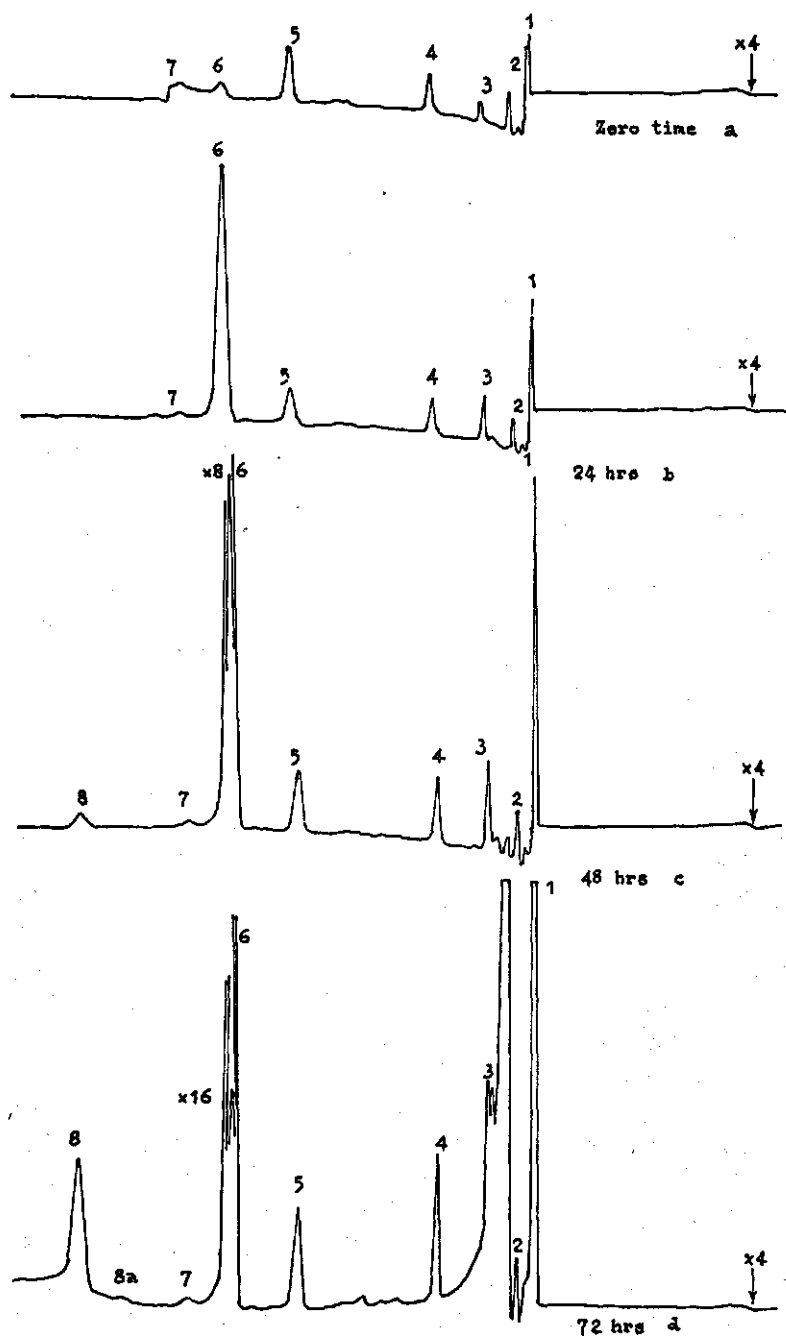


Fig. 15. Course of volatiles development in autolyzed shrimp (a-e) and headspace of cooked trassi (f).

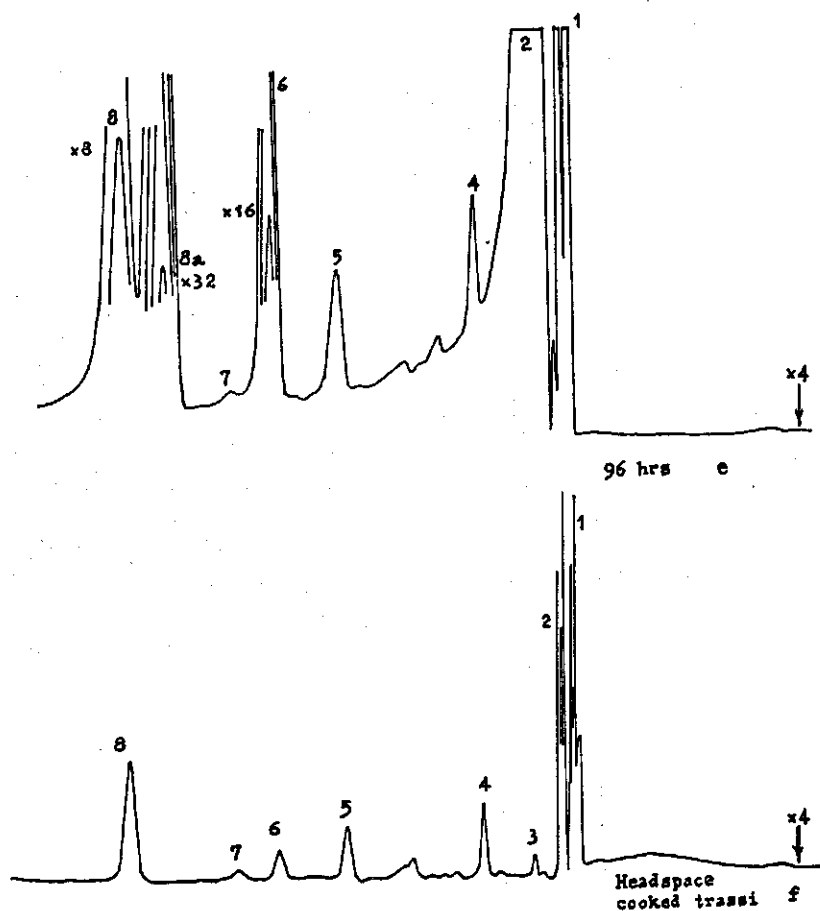


Fig. 15. Continued.

ed physiological saline solution of pH 6.8. Phosphate buffer 1 : 1 was used for buffering. The bacteriostatic action of the Merthiolate was tested, beginning at 1 : 10,000 concentration and higher. 100 gram of shrimp was mixed thoroughly with the buffered Merthiolate solution (100 g : 100 ml) in a Waring blender. An aliquot of the slurry was checked for bacterial growth after 24 hours incubation at 28 °C. It appeared that Merthiolate at 1 : 10,000 concentration was not effective in preventing bacterial growth. A 1 : 2000 concentration was effective for the first 3 days; at the 4th day a considerable increase of bacterial count was observed.

In the actual experiment on autolysis thymol at a concentration of 10 mg % was used in addition to Merthiolate in order to suppress mold growth. A quantity of shrimp and Merthiolate solution with the required amount of thymol were disintegrated in a Waring blender, and the slurry transferred into an infusion bottle. A series of 6 bottles of shrimp slurry were prepared, each containing about 600 gram. The development of certain volatile compounds was followed by analyzing

the headspace samples with a gaschromatograph equipped with a flame ionization detector. The first headspace sample was taken after equilibration of the slurry in a waterbath at 35 °C for 15 minutes. The following analysis were run after 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, and 96 hours incubation at 28 °C. Gaschromatographic runs were made on a 4 m packed aluminium column (4 mm i.d.) containing Carbowax 400 adsorbed on Chromosorb W-AW 60-80 mesh support. The operating temperature was 55 °C. The development of peak components can be followed from the gaschromatograms presented in fig. 15. During the first 3 days' incubation only components 1, 4, and 6 have increased considerably. The identities of the 3 components concerned were done using the extract concentrate of shrimp slurry prepared following the procedure as for trassi, with the difference that the distillation was accomplished at reduced pressure. Only component 6 was successfully identified as being dimethyldisulfide. The recovered quantities of components 1 and 4 were too small and the GLC-MS analysis failed to give any result.

Enzyme induced products have in general typical developmental patterns, which differ clearly from typical microbial induced reactions. For this purpose peak heights of autolyzed shrimp volatiles were plotted against time of incubation. Fig. 16 presents graphically the course of development of the shrimp volatiles.

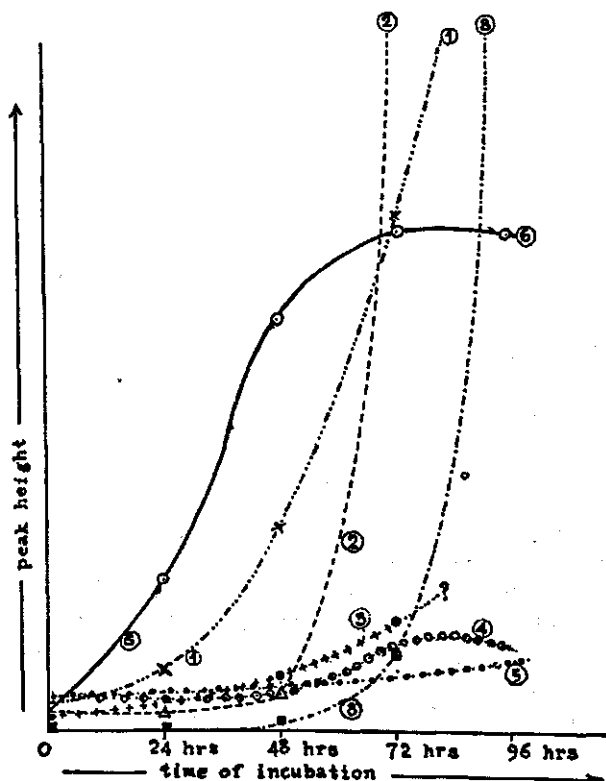


Fig. 16. Course of development of some volatiles in autolyzed shrimp slurry.

Compounds 6 and 4 represents typical enzyme-induced developmental patterns, although less typical for compound 4. Except for compound 5 the other volatiles showed clearly the typical microbial induced development.

This experiment, although far from being perfect, would suggest that dimethyldisulfide was formed enzymatically during autolysis; this compound represents part of dimethyldisulfide found in trassi. Some part of dimethyldisulfide in trassi must have been formed from methionine during the Strecker degradation process.

7 Summary

An investigation was conducted to study the volatile compounds of cooked trassi, a cured shrimp paste condiment of the Far East. The methods of volatiles isolation, concentration, and fractionation of the concentrate into its components have been described. Different extracts were prepared to subsequently analyze volatile fatty acids, neutral volatile compounds, and basic volatiles separately. Highly volatile sulfur compounds were analyzed directly in the vapour over cooked trassi using a flamephotometric detector made selective for sulfur compounds only. A thin-layer chromatographic technique was applied to analyze carbonyl compounds as their 2,4-dinitrophenylhydrazone derivatives. A combination of different identification techniques was applied.

138 volatile compounds were identified in the isolated volatiles of cooked trassi, while 5 sulfur compounds, even though their mass spectra were obtained, remained unidentified. The volatile compounds identified in trassi steam distillate included:

16 hydrocarbons	3 esters
7 alcohols	15 sulfur compounds
46 carbonyls	34 nitrogenous compounds
7 fatty acids	10 miscellaneous compounds

The role of each group of compounds as a contributory factor to the odour of trassi has been discussed. Pyrazines and amines were considered to impart the amine- and cacao-like odour of trassi, while volatile fatty acids and ammonia were considered to be responsible for the acidic and ammoniacal tone of trassi aroma. Highly volatile sulfur compounds were found to give the offensive/penetrating character to the trassi odour. Carbonyl compounds must be responsible for the typical cured fish aroma.

No investigation was conducted to study the role of amino acids in the formation of certain volatiles in trassi. However, some literature references have been cited, particularly those dealing with the development of certain odours accompanied by a decrease in amino acid concentration following heat treatment of foods or model systems. Since about 30 % of the total amino acids in trassi was found in the free state in this study, its importance as direct or indirect precursors of certain cooked trassi volatiles should not be overlooked.

Experiments with autolyzed shrimp indicated that dimethyldisulfide developed enzymatically.

Ringkasan

Dalam karja ilmiah ini telah diuraikan penjelidikan tentang zat2-terbang dalam trasi matang. Untuk mendapat pengertian jang lebih mendalam tentang terbentuknja berbagai zat-terbang jang berperanan dalam aroma trasi sebagai hasil pemanasan, perlu diteliti pula susunan kimia serta susunan asam aminonja.

Pemisahan zat2 aroma trasi dilakukan dengan djalan distilasi beruap pada tekanan udara luar. Tjara ini menghasilkan distilat jang dalam hal aromanja dapat dianggap sama dengan aroma trasi matang (dikukus). Setelah zat2-terbang dibebaskan dari air dengan djalan ekstraksi dengan pentana-eter 2 : 1 jang disusul dengan pemekatan sampai kira2 7500 kali, maka zat2-terbang dipisah2kan dalam fraksi2 melalui kolom chromatografi gas, dan masing2 fraksi dipisah2kan lebih landjut. Hasil2 pemisahan langsung dianalisa dengan alat GLC-MS dan berdasarkan mass spectra jang diperoleh ditentukan rumus kimia/rumus bangunnja. Tjara2 identifikasi jang lain, misalnja spektrometri infra merah, spektrofotometri ultraviolet, chromatografi gas dan chromatografi lapis tipis djuga ditrapkan dalam penjelidikan tersebut.

Telah ditemukan sedikitnja 138 zat-terbang dalam aroma trasi matang, jang terdiri dari kelompok2 senjawa berikut:

16 senjawa hidrokarbon	3 ester
7 alkohol	34 senjawa nitrogen
46 senjawa karbonil	15 senjawa belerang
7 asam lemak	10 senjawa lain2.

Peranan masing2 kelompok senjawa dalam aroma trasi telah dibahas. Asam lemak terbang menjejabbkan bau keasaman, sedangkan amonia dan amine menjejabbkan bau anjir beramonia. Senjawa belerang sederhana (H_2S , merkaptan, sulfida dan disulfida) menjejabbkan bau rangsang pada trasi. Senjawa pyrazina memberikan bau tjoklat kegurihan. Senjawa karbonil besar sekali kemungkinannja memberikan bau khusus jang terdapat pada semua hasil ikan jang diawetkan dengan djalan penggaraman/pengeringan disertai fermentasi oleh mikroba.

Pertjobaan dengan udang jang dibiarkan mengalami autolysis menundjukkan, bahwa dimetildisulfida terbentuk sebagai hasil reaksi enzim.

List of foreign terminologies

bagoong (Philipp.)	shrimp and/or fish paste
bambu (Indon.)	bamboo, <i>Panicum montanum</i>
banděng (Indon.)	milkfish, <i>Chanos chanos</i>
bělachan (Malay.)	shrimp
brabon (Jav.)	paste before maturation sets on
gorèng (Indon., Jav.)	to fry (with oil)
hitam (Indon.)	black
ikan (Indon.)	fish
krill (orig. Norw)	planktonous shrimp belonging to the genus <i>Euphasia superba</i> , which form the main food of whales
mam-ca-loc (Cambod.)	fish paste w. sugar & roasted rice
mam-ca-sat (Cambod.)	fish paste w. roasted rice
mam-tom (Vietn.)	shrimp paste
ngapi (Burm.)	fish paste
nipah (Indon., Jav.)	palm variety growing on marshy areas, <i>Nipa fruticans</i>
nuoc-mam (Vietn.)	fish sauce
pa-dec (Laot.)	fish paste w. rice bran
pědah = pěda (Indon., Jav.)	matured partially dried/salted <i>Scomber Kanagurta</i> Russel
pětis (Indon., Jav.)	black salvy paste, prepared by concentration of fish or shrimp bouillon
pha-ak (Cambod.)	fish paste w. fermented gulten rice
rěbon (Indon., Sudan.)	planktonous shrimp belonging to the genus <i>Schizopodes</i> or <i>Mytis</i>
sambal (Indon., Jav.)	hot chilli sauce prepared by grinding together chilli pepper, cooked trassi and other ingredients
'sambal gorèng' (Indon., Jav.)	name of an Indonesian dish
sělar (Indon., Jav.)	small mackerel <i>S. Kanagurta</i> R.
těri (Indon.)	tiny fish <i>Stolephorus</i> or <i>Engraulis</i>
trasi (Indon.) = těrasi (Malay)	fish or shrimp paste
udang (Indon.)	shrimp

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