OFF—FLAVORS IN FOODS & BEVERAGES

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OFF-FLAVORS IN FOODS AND BEVERAGES

Edited by

GEORGE CHARALAMBOUS

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PREFACE

Human well-being depends on the availability of a diet that meets not only all nutritional needs, but also appropriate requirements in flavor - taste and aroma - quality. Therefore, a detailed and critical discussion of recent developments governing taste and odor, flavor and off-flavors, is a most appropriate and valuable contribution in this area.

In a rapidly changing world of food production and processing, quality control and analysis, the present treatise reviews the latest information and up-to-date concepts concerning very many aspects of flavor quality. Much effort was expended in achieving a balance between food and beverage chemistry, biochemistry, microbiology, nutritional, processing, packaging, storage, computer applications, and chemometrics.

Twenty six specialists, all recognized experts, were invited to discuss the present state of knowledge in their particular fields. Along with their co-workers (a total of sixty one well known researchers) the authors were drawn from the international spectrum of academia, government institutes and industry: they have presented in this book original research results, background reviews, and comprehensive bibliographies.

The information collected in this book will improve our knowledge in a complex area and will increase our understanding of high quality foods and beverages.

Jürg Solms
Eidgenössische Technische
Hochschule Zürich

LIST OF CONTRIBUTORS

Numbers in parentheses indicate where contributions begin

P. ADAMEK (37) SIK, The Swedish Institute for Food Research, P.O. Box 5401, S-400 23 Göteborg, Sweden

H. AKIYAMA (473) Kyowa Hakko Kogyo Co. Ltd, 1-6-1 Ohtemachi, Chiyoda-ku, Tokyo 100, Japan

R.H. ALBERT (669) Division of Mathematics, OTS, CFSAN, Food and Drug Administration, Washington, DC 20204, U.S.A.

G.C. ARGANOSA (103) Department of Food Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

S. ARAI (547) Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

C.D. AZZARA (329) Hershey Foods Corporation, Corporate Technical Center, Hershey, PA 17033, U.S.A.

M.E. BAILEY (127) Food Science and Nutrition Department, University of Missouri, 21 Agriculture Building, Columbia, MO 65211, U.S.A.

T. BÖRJESSON (37) Department of Microbiology, The Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

L.B. CAMPBELL (329) Hershey Foods Corporation, Corporate Technical Center, Hershey, PA 17033, U.S.A.

T.S. CHAMBLEE (229) The Coca-Cola Company, Corporate Research and Development, P.O. Drawer 1734, Atlanta, GA 30301, U.S.A.

A.O. CHEN (375) Department of Food Science, National Chung Hsing University, 250 Kuokuang Road, Taichung, Taiwan, Republic of China

W.T.F. CHIU (375) Taiwan Tea Experiment Station, Taoyuan, Yangmei, Taiwan, Republic of China

B.C. CLARK, Jr. (229) The Coca-Cola Company, Corporate Research and Development, P.O. Drawer 1734, Atlanta, GA 30301, U.S.A.

K.L. CRIPPEN (57) Food Flavor Quality Research, USDA, ARS, SRRC, P.O. Box 19687, New Orleans, LA 70179, U.S.A.

L. DEBRAUWER (567) Laboratoire de Chimie Organique, Chimie des Arômes-Oenologie, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille, Ave Escadrille Normandie-Niemen, F-13397 Marseille Cédex 13, France

R. ENTZ (1) Department of Chemistry, Kansas State University, Shellenberger Hall, Manhattan, KS 66506, U.S.A.

C.E. ERIKSSON (37) SIK, The Swedish Institute for Food Research, P.O. Box 5401, S-400 23 Göteborg, Sweden

G.J. FLICK Jr. (77, 103) Department of Food Science and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, U.S.A.

R.A. GUTHEIL (127) Food Science and Human Nutrition Department, University of Missouri, 21 Agriculture Building, Columbia, MO 65211, U.S.A.

Y. HORIE (313) Meidi-Ya Food Factory Co. Ltd, 1-13, Nishigawara 3-chome, Ibaraki-shi, Osaka 567, Japan

N. INFANTI-PAPATRAGIANNI (411) Department of Food Chemistry, University of Athens, Athens, Greece

M. KAMIMURA (433) Sapporo Breweries Ltd, Shizuoka Brewery, Hamatohme, Yaizu-shi, Shizuoka 425, Japan

E. KAMINSKI (37) Institute of Food Technology, The University of Agriculture, Wojska Polskiego 31, 60-624 Poznan, Poland

H. KANEDA (433) Sapporo Breweries Ltd, Shizuoka Brewery, Hamatohme, Yaizu-shi, Shizuoka 425, Japan

M.E. KOMAITIS (411, 417) Department of Agricultural Industries, Agricultural University of Athens, Athens, Greece

K. KOTANI (523) Takeda Chemical Industries Ltd, Osaka, Japan

D. KUGLER (485) Bundesanstalt für Züchtungsforschung im Wein-und Gartenbau, Geilweilerhof, D-6741 Siebeldingen, Germany

N.V. LOVEGREN (57) Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, U.S.A.

J.L. LARICE (567) Laboratoire de Chimie Organique, Faculté des Sciences, F-84000 Avignon, France

E. MELISSARI-PANAGIOTOU (411) Department of Food Chemistry, University of Athens, Athens, Greece

C.E. MELOAN (1) Department of Chemistry, Kansas State University, Shellenberger Hall, Manhattan, KS 66506, U.S.A.

J. METZGER (567) Laboratoire de Chimie Organique, Chimie des Arômes-Oenologie, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille, Ave Escadrille Normandie-Niemen, F-13397 Marseille Cédex 13, France

D.B. MIN (171) Department of Food Science and Technology, The Ohio State University, Vivian Hall, Columbus, OH 43210, U.S.A.

B.S. MISTRY (171) Coca-Cola Foods, Citrus Research and Development, Plymouth, FL 32768, U.S.A.

S. NAGY (211) IFAS, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, U.S.A.

M. NAIM (211) IFAS, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, U.S.A.

K.H. NEY (419, 665) Heydewisch 27, D-2000 Hamburg 56, Germany

N. NUNOMURA (287) Kikkoman Foods Inc., P.O. Box 69, Walworth, WI 53184, U.S.A.

R.L. ORY (57, 77) Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, U.S.A.

T. OHBA (473) National Research Institute of Brewing (Jozo Shikenjo), 2-6-30 Takinogawa, Kita-ku, Tokyo 114, Japan

C. PÁRKÁNYI (567) Department of Chemistry, Florida Atlantic University, P.O. Box 3091, Boca Raton, FL 33431, U.S.A.

P. PRETORIUS (485) Viticultural and Oenological Research Institute Nietvoorbij, Stellenbosch 7600, Republic of South Africa

A. RAPP (485) Bundesanstalt für Züchtungsforschung im Wein- und Gartenbau, Geilweilerhof, D-6741 Siebeldingen, Germany

T.J. ROURKE (127) Food Science and Nutrition Department, University of Missouri, 21 Agriculture Building, Columbia, MO 65211, U.S.A.

R.L. ROUSEFF (211) IFAS, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, U.S.A.

M. SASAKI (287) Kikkoman Foods Inc., P.O. Box 69, Walworth, WI 53184, U.S.A.

D.B. SAUER (17) U.S. Grain Marketing Research Laboratory, Grain Quality and Structure Research Unit, 1515 College Avenue, Manhattan, KS 66502, U.S.A.

L.M. SEITZ (17) U.S. Grain Marketing Research Laboratory, Grain Quality and Structure Research Unit, 1515 College Avenue, Manhattan, KS 66502, U.S.A.

H. SINGH (625) Atomic Energy of Canada Limited Research Company, Whiteshell Laboratories, Pinawa, Manitoba, Canada R0E 1L0

S. TANIMOTO (547) Department of Home Economics, Aoyama Gakuin Woman's Junior College, 4-4-25 Shibuya, Shibuya-ku, Tokyo 150, Japan

T. TOYOTA (523) Takeda Chemical Industries Ltd, Vitamin and Food Division, 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

Y.S. TSAI (375) Taiwan Tea Experiment Station, Pushin, 32613 Yangmei, Republic of China

G. VERNIN (567) Laboratoire de Chimie Organique, Chimie des Arômes-Oenologie, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille, Ave Escadrille Normandie-Niemen, F-13397 Marseille Cédex 13, France

G.M.F. VERNIN (567) Laboratoire de Chimie Organique, Chimie des Arômes-Oenologie, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille, Ave Escadrille Normandie-Niemen, F-13397 Marseille Cédex 13, France

C.Y-J. WANG (127) Food Science and Nutrition Department, University of Missouri, 21 Agriculture Building, Columbia, MO 65211, U.S.A.

M. WATANABE (547) Department of Education, Tokyo Kakugei University, 4-1-1 Nukui-kitamachi, Koganei-shi, Tokyo 184, Japan

K. YASUMATSU (523) Takeda Chemical Industries Ltd, 12-10 Nihonbashi, 2-chome, Chuo-ku, Tokyo 103, Japan

R-M. ZAMKOTSIAN (567) Laboratoire de Chimie Organique, Chimie des Arômes-Oenologie, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille, Ave Escadrille Normandie-Niemen, F-13397 Marseille Cédex 13, France

U. ZEHAVI (211) IFAS, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, U.S.A.

C. ZERVOS (669) Department of Health and Human Services, Division of Research and Testing, ORR, CDER, Food and Drug Administration, Washington, DC 20204, U.S.A.

P. ZERVOS (669) Department of Agriculture, Food Safety and Inspection Service, Food and Drug Administration, Washington, DC 20204, U.S.A.

CONTENTS

G. Charalambous (Ed.), Off-Flavors in Foods and Beverages

ANALYSIS OF GRAIN VOLATILES AND DEVELOPMENT OF A SIMPLE CHEMICAL ASSAY FOR FUNGAL INFESTATION OF GRAIN

By

Richard Entz[1]

and

Clifton E. Meloan

Department of Chemistry

Kansas State University

Manhattan, Kansas, 66506

ABSTRACT

A chemical means to quantitatively measure mustiness in stored grains is proposed. It is based on the observation that the fungi Aspergillus flavus, A. glaucus, and A. niger produced ethanol in direct relation to the increase in the mustiness odor produced. A small glass cylinder is filled with silica gel coated with potassium dichromate. When a measured volume of air from the storage bin is passed through the cylinder the ethanol present reduces the orange dichromate to green chromium (III). The length of the green portion is proportional to the amount of ethanol and hence the mustiness present. A portable and inexpensive instrument is proposed.

INTRODUCTION

The problem is that if farmers in the U.S. A. try to sell grain that is musty they are docked as much as 50% on the sale price depending upon the degree of mustiness. This degree of mustiness is currently determined by an organoleptic "sniff" test by federal inspectors (1). This requires human judgment. Consequently, the training and certification of inspectors for odor analysis of grains is a lengthy and painstaking process. A more objective test is needed, particularly for field testing. At present the farmer has no easy method which he can use himself to test his grain in the bin prior to sale to determine if it is becoming musty and to what extent the mustiness is present.

A project was undertaken to develop a physical or chemical test which could be used to supplement the organoleptic testing of grain quality that could be used by the farmer prior to shipment as well as by the federal inspectors in the event these highly trained people were sick or were challenged

[1] Presently at Lancaster Laboratories, Lancaster, PA.

to provide a more quantitative evaluation. The organoleptic testing of grain has a crucial effect upon its grading. At the present time, the United States Department of Agriculture standards for grain quality state that any grain which has a musty or sour odor, as judged by federal inspectors, is given the "sample grade" designation, the lowest possible grade. What is desired is a simple, quick, and reliable test that could be done by farmers or grain storage operators to test their stored grain for mustiness so it could be shipped and sold before it became "sample grade".

Much previous work has been done related to mustiness on food items. A few of those that are pertinent to this work and illustrate the scope is the work of Bullard and Holguin on rice (2), Hougen, Quilliam, and Curran, working with wheat, triticale, and rye (3), Legendre, Dupuy, Ory, and McIlrath, working with rice and corn (4), Flora and Wiley with sweet corn (5), Kaminiski and Stawicke (6-8), Dravnieks, Reilich, and Whitfield on corn (9), and Kaminiski et al. on wheat meal (10).

Fungi invading stored grain or growing on grain while still in the field is a major cause of spoilage, ranking second only to insects as a cause of deterioration (11). The fungi may cause a decrease in germination of the seeds, discoloration of all or part of the kernel, the loss of weight for the grain, biochemical changes, heating and mustiness, or may produce toxins which are hazardous to both man and domesticated animals (12). The fungi that grows on or in the seeds are divided into two groups based on the ecology of the fungi: field fungi and storage fungi (11). Field fungi are generally found in or on crops when they are growing in the fields, though in the case of cob corn they may also be growing in cobs stored in a crib. These fungi generally require a high moisture content, approximately 22% (11). The fungi may "weather" (discolor) seeds or kernels, weaken or kill the embryo, bring on disease in the plant or the next generation, produce toxins, or effect the quality of the grain for various uses. The damage is usually caused before harvest and does not continue to increase during storage (12).

Storage fungi do not to any serious extent invade grains such as wheat, corn, barley, oats, or sorghum before harvest (12). They are found in bulk storage of grain and require lower moisture contents than field fungi, between 13.5 and 19% (11) and storage temperatures $\geq 20^{o}C$. Common fungi are 5 to 6 group species from the Aspergillus genus and Penicillins(11). The most likely group species of Aspergillus are A. restrictus (13.5% moisture), A. glaucus (14%), A. canidus and A.ochraceus (15%), and A. flavus (18%). Temperature is also critical. At $15^{o}C$ only 15% of the grain was attacked after 600 days, while at $20^{o}C$ 20% was attacked in 8-10 days (11,12).

Various attempts have been made to provide an objective means to augment

organoleptic testing. One technique used by Dravenicks (9,13) searched for a statistical correlation between a portion of the gas chromatographic "fingerprint" of the headspace vapors above grains and the organoleptic evaluation of the grain. However, they did not find an acceptable correlation. The food industry has used the same technique to find odor and flavor components in foods or to determine if food deterioration has taken place (14). Another direction commonly used by the food industry to provide objective criteria for organoleptic testing, chemically identifies the particular component or components which provide the particular odor or flavor (15,16).

This Process has been used to identify components of cultures of fungi and bacteria which have a musty odor. The work of Kaminski, Stawicki and others has indicated several strains of fungi associated with a musty odor in cereal grains (6-8, 10).

The solution to the problem is not necessarily to determine what compounds are formed on musty grain, but of those compounds that can be related to mustiness, which can be used as a basis to develop a simple detector.

Particular attention in this project was placed on the compounds present in malodorous grain responsible for the grain having an odor characterized as musty. Previous work (10) had indicated that certain compounds found to have a musty odor were present in grain which had a high fungal invasion. Three groups from the genus Aspergillus were chosen to inoculate grain. These were chosen due to their link with malodorous grain (10). These are also among the most harmful of the storage fungi: A. flavus, A. glaucus, and A. niger. These have been shown to grow under conditions of moderate water content, 14 to 17% by weight in grains, and under moderate temperature condition, 20 to 26°C. Those compounds that increased in concentration with an observed increase in the fungal growth as evidenced by microscopic counts and an increase in mustiness odor were separated and identified. A simple semi-quantitative method was then developed to measure the most prominent compound and relate it to mustiness. The rest of this paper supports the conclusions drawn.

EXPERIMENTAL

What was done in this project was to test both corn and wheat. The samples were first sterilized by soaking in hypochlorite solution, dried, and brought to a controlled moisture content. The volatiles from these reference grains were collected and the components separated by gas-liquid chromatography to determine those volatiles normally given off. Aliquot portions of the grains were then inoculated individually with three species of fungi; Aspergillis Glaucus, Aspergillis Flavus, and Aspergillis Niger. After incubation under controlled humidity and temperature, the volatiles were again collected and the components determined at several day intervals for up to one month. It

was found that of the many compounds produced by each fungus that ethanol was produced in the largest amount and increased more rapidly than any other component. A simple device to obtain a sample from the grain bin and determine the degree of mustiness semi-quantitatively based on the ethanol increase is described.

APPARATUS

Gas chromatograph: Bendix Model 2200 with TC and FID detectors.

Column: 180 cm x 3 mm stainless steel washed with ethyl acetate, acetone, water, and dried with He passing through it.

Packings: 10% SP-1000 on 60-80 mesh Supelcoport.

Tenax G.C. (60-80 mesh)

10% Apiezon L on 30-60 mesh Chromasorb T and on 60-80 mesh Poropak QS, conditioned at 225° for 24 hours.

Conditions: He or N_2 carrier gas, 16 mL/min. Injection port 200°C, FID detector 250°C, Initial column temp. 50°C for 5 minutes, 10°C/min to 230°C and hold for 5 min.

To eliminate the background from the carrier gas contaminants, a dual column system was used. The Bendix 2200 has the capability of dual column operation. However, only one electrometer is used, the bias on the collector jet tip being reversed on one detector.

Thermocouple: Model 74 solid state iron-constantan, Athena Controls Co.

Mass spectrometer: MS 902 and MAT CH-4.

CHEMICALS

N_2, zero grade

He, zero grade

All chemicals were reagent grade and used as received to match GC retention times and to obtain comparison mass spectra.

Ethanol, formaldehyde, acetaldehyde, propanol, butanone, benzene, toluene isobutyraldehyde, acetone, Potassium dichromate, 1-octanol, 3-octanone, 1-octen-3-ol, 3-methyl-1-butanol, 1-hexanol, 1-propanol

Chromasorb W, acid washed (Johns-Manville) calcined with sodium carbonate

Tenax GC: Enka NV, Netherlands

Poropak Q and QP, Waters Associates, Framingham, Massachusetts

Molecular sieves, 5A, as a cleanup trap for the carrier gas.

Silica gel, Davidson Co. Grade 08 (12-28 mesh) grade 12 (28-200 mesh).

GRAIN

Eighteen samples of about 20 kg each of yellow corn and hard red winter wheat (Table 1) were obtained from the U.S. Grain marketing Research Center in

Manhattan Kansas. Each was placed in a plastic bag and then inside of burlap bags. This grain was stored at 5°C until used.

Each sample was stored in a refrigerator to maintain the original condition until used. It was determined that no detectable alteration was observed under these storage conditions. Prior to inoculation, the grain was stored at 30°C and 90% humidity for two days in a constant humidity chamber.

FUNGI

Three groups from the genus Aspergillus were chosen to inoculate grain because of their link with malodorous grain and because they are also the most harmful of the storage fungi, Aspergillus flavus, glaucus, and niger. These have been shown to grow under conditions of moderate water content, 14 to 17% by weight, and under moderate temperature conditions, 20 to 26°C.

Inoculation procedure

Cultures of pure strains of were obtained from the U.S. Grain Marketing Research Center, Manhattan, Kansas. The cultures of the molds were removed from the refrigerator and diluted with about 25 mL of sterile water containing a drop of surfactant. This mixture was shaken in the test tube originally containing the culture to dislodge the spores from the gel support. These were then decanted into plastic bags, each containing about 500 grams of grain, leaving the agar behind. The bags were shaken to ensure the coating of the grain with the spores and then the grain was placed in a monolayer on a tray. The trays were placed in a controlled environment room (Grain Marketing Research Center) set at approximately 90% humidity and 70°C to be sampled at 2 to 3 day intervals.

Grain Volatiles Sampling Procedure

Two methods were used. The first, involved adsorption of the volatiles on either Tenax GC (17,19) or Poropak QS (18,19), and was used to concentrate the volatiles for the initial identification. The second method was a straight headspace analysis of the volatiles.

To a 250 mL filtering flask was added 150 g of the test grain. A small volume recirculating pump was attached to the top of the flask by rubber tubing and the side arm was attached to the inlet of the pump by tubing. Between the side arm and the pump inlet was placed a two piece glass cylinder made from a 24/40 ST joint. Inside of this cylinder was placed a 14 mm diameter glass cylinder filled with the adsorbing compound and held in place with glass wool. Vapors were collected from 4 to 8 hours.