Alkylpyrazines in Processed American Cheese

The volatile flavor components of a processed American cheese were analyzed with an adsorption–desorption gas chromatographic (GC) technique, and identified with GC–MS and GC retention time. 2,5-Dimethylpyrazine and 2,3,5-trimethylpyrazine are identified in addition to the alcohols, methyl ketones, esters, alkylbenzenes, lactones, aldehydes, and volatile acids which are commonly present in cheese. The pyrazines are believed to be the main contributor to the weak nutty notes detected in processed American cheese.

Processed American cheese is one of the most popular cheese products in the U.S.; however, its flavor is one of the subjects least studied. Although processed American cheese is manufactured from cheddar cheese, it has a flavor quite distinctive from cheddar. In addition to the delicate mild butteriness and cheesiness, a weak nuttiness can also be detected.

The objective of this research was to identify the volatile flavor composition of processed American cheese and to formulate a synthetic flavor system for our imitation cheese products. The analysis of cheese volatiles was carried out with an adsorption–desorption technique very similar to those reported by Sirikulvadhana et al. (1975) and Bertsch and Zlatkis (1974). Tenax-GC and Porapak-Q were used as the adsorbents. The details of this flavor analysis technique and the identification of alkylpyrazines and their relationship to processed American cheese flavor are described and discussed.

EXPERIMENTAL SECTION

Materials. Processed American cheese in a 2-lb block was purchased from local supermarkets. Tenax-GC (60/80) and Porapak-Q (80/100) were purchased from Applied Science, State College, Pa.

Adsorbing Tube. The adsorbing tube was constructed by connecting pieces of a 5 cm long × 0.635 cm (0.25 in.) o.d. Pyrex glass tube to the ends of a 12 cm × 1 cm Pyrex glass tube. The porous polymer was packed in the larger section between plugs of glass wool. The tube was conditioned at 200 °C and 40 ml/min of helium for 2 days before being used.

Flavor Isolation. The cheese was kept in a freezer overnight and ground into a powder with a Waring Blendor. The frozen cheese enabled one to obtain a finer powder than the refrigerated sample. A 400-g sample of cheese powder was packed into 4 × 60 cm Pyrex glass chromatographic columns (Scientific Glass Apparatus, Bloomfield, N.J.). The tip of the cheese column was connected to a Tenax-GC tube and a Porapak-Q tube and the head to a nitrogen source. An additional pressure line regulator (The Devilbiss Co., Toledo, Ohio) was used for fine control of the head pressure. Nitrogen gas was passed through the cheese column and the adsorbing tubes at a flow rate of approximately 120 ml/min and a head pressure of less than 3 psi for approximately 20 h. The nitrogen gas was used directly from the tank without further purification.

GC Analysis. The instrument used in this study is a Hewlett-Packard GC Model 5750 equipped with FID and stream splitter. The GC column was a 0.25 in. × 6 ft Pyrex glass column with some modification. The front end of the column was connected to a U loop of glass tube (same size) with its front arm extending into the injection port. The column was packed only to the front neck of the U loop, with the portion inside the injector empty. The U loop was positioned inside a 100-ml Pyrex glass beaker, which was wrapped with insulation tape. Two Toggle valves were installed for instantaneous control of helium flow, one for the column and another one for an outside line used in flavor desorption.

The flavor sample was transferred from the adsorbing tubes to the GC with the following technique. The adsorbing tube was removed from the cheese column and its front end was connected to the outside helium source mentioned above through a Teflon tubing. The other end of the adsorbing tube was connected to a specially made luer lock and a needle. The luer lock was silver soldered onto a 0.25–0.125 in. stainless steel reducer union with the 0.125 in. end cut off. The helium flow through the column was turned off with the Toggle valve. The needle was punched through the injector septum and the outside helium flow was turned on. The oven fan was turned off and liquid nitrogen was poured into the beaker to cool the U loop. The adsorbing tube was heated with a heating tube which was a 14 cm × 2 cm Pyrex glass tube wrapped with a heating tape. The temperature was controlled with a mini Variac (Un-A-Watt, Kontes Glass Co., Vineland, N.J.) and set at 230 °C for Tenax-GC and 200 °C for Porapak-Q. The helium flow was set at 40 ml/min. The desorption time was 30 min for both adsorbing tubes, which were desorbed consecutively for a single analysis.

At the end of desorption, the needle was pulled out of the injector and the oven fan was turned on to accelerate the evaporation of excess liquid nitrogen left in the beaker. Once the liquid nitrogen was evaporated completely, the oven cover was closed and the helium flow to the column was turned on with the Toggle valve. The GC analysis was carried out with temperature programming.

The parameters for GC analysis were as follows. The packing material was 20% 20M-TPA coated on 60/80 Anarkrom ABS. The helium gas was chromatographic grade purified through a molecular sieve filter. The flow rate was 40 ml/min for helium, 20 ml/min for hydrogen, and 200 ml/min for compressed air. The oven temperature was programmed from 40 to 240 °C at 2 °C/min and held. A single flame detector was used. The attenuation was set at 4 × 10.

GC–MS. A Finnigan GC–MS 3200 with computer data system 6110 was used. The sample was introduced to the instrument similar to the method described above. A specially made glass column (0.25 in. × 6 ft) was used. A vertical U loop was attached to the front end of the column. The packing material was the same as in GC analysis.

RESULTS AND DISCUSSIONS

Flavor Identification. The conventional technique of analyzing cheese flavor generally includes the use of steam distillation or solvent extraction of whole cheese, or vacuum degassing of the cheese oil after it is separated from cheese by melting and centrifugation (Day and Libbey, 1964). The adsorption–desorption technique has

become popular only recently. Dravnieks (1973) used Chromosorb 105 in classifying corn order, Bertsch and Zlatkis (1974) used Tenax-GC in identifying the volatiles in the cabin atmosphere of Skylab 4, Tassan and Russell (1974) used Porapak-Q in studying coffee brew aroma, Bertuccioli and Montedoro (1974) used Porapak-Q in studying wine aroma, and Sirikulvadhana et al. (1975) also used Porapak-Q in studying rose flower aroma. Micketts and Lindsay (1974) compared the efficiency between the retention of beer flavor by Tenax-GC and Porapak-Q. Butler and Burke (1976) compared the efficiency of Tenax-GC, Chromosorb 101 and 102, and Porapak-P, Q, R, and T.

In our study of cheese flavor, it was found that the adsorption–desorption technique was much more convenient than the conventional technique, particularly for the application in flavor formulation. It also gave a more complete profile of the cheese aroma.

In this study, a group of volatiles with a wide range of boiling points were identified. The low boilers include acetaldehyde, acetone, and ethanol; the high boilers include caprylic acid, δ-decalactone, and δ-dodecalactone. The findings of Butler and Burke (1976) were confirmed, that Porapak-Q has a higher capacity for retaining the volatiles, particularly the low boilers, than Tenax-GC but requires a longer desorption time.

When the adsorbility of Tenax-GC and Porapak-Q was compared, each polymer was used as the sole adsorbent under the same conditions. The nitrogen gas after passing through the cheese columns had a typical aroma of processed American cheese, but it became practically odorless after passing through the adsorbing tubes. However, after 20 h of flavor isolation, a weak odor reminiscent of that of acetaldehyde was detectable after the Tenax-GC tube but only a very faint odor after the Porapak-Q tube. By analyzing the composition of volatiles adsorbed in these two tubes, it was found that Porapak-Q had adsorbed more lower boilers than Tenax-GC.

Desorption at 230 °C for 30 min was sufficient to desorb all the volatiles from Tenax-GC, but not long enough for Porapak-Q. An experiment of injecting 0.1 μl of a known flavor mixture into Porapak-Q indicated that δ-dodecalactone was only partially desorbed after 1 h of desorption at 230 °C. The desorption of Porapak-Q at this condition also had the problem of slight bleeding.

Therefore, the best arrangement for studying the volatile composition with a wide range of boiling points was to use two adsorbing tubes in a series, a Tenax-GC tube in front and a Porapak-Q tube behind it. The volatiles with higher boiling points will be totally adsorbed by Tenax-GC, the ones with lower boiling points will be partially retained by Tenax-GC and partially passed into Porapak-Q and retained there. After the isolation, the volatiles in these two tubes can be desorbed together for a single analysis. The conditions for desorption of Porapak-Q can be set at a lower temperature such as 200 °C to avoid bleeding.

20M-TPA was selected as the stationary phase because short-chain fatty acids are not retained and the retention characteristics of the column are not changed. However, it was found that the acids had long tailing and interfered with the GC peaks behind them during GC–MS analysis. They also interfered with the odor characteristics of the peaks behind. The acids can be conveniently removed by packing approximately 0.1 g of potassium carbonate powder in the glass tube between the adsorbing tube and luer lock during flavor desorption. Zinc oxide has been used by Beroza (1970) for acid subtracting. This acid subtracting process enabled one to obtain cleaner mass spectra of and to better determine the odor of peaks behind the acid peaks. It also enabled one to analyze the cheese flavor with different types of stationary phases such as CW-20M or SE-30, which are not suitable for acid separation.

Cheese Flavor. The volatile flavor compounds identified in this study include alcohols, methyl ketones, aldehydes, diacetyl, acetoin, short-chain fatty acids, alkylbenzene, esters, lactones, and alkylpyrazines. Most of the compounds have been reported previously in the flavor of cheddar cheese and other types of cheese. Alkylpyrazines had never been identified in any cheese until recently. Dumont et al. (1974) identified dimethylpyrazine in grated parmesan cheese. Sloot and Hofman (1975) identified six alkylpyrazines in Emmental cheese and Sloot and Harkes (1975) identified five alkylpyrazines in Gouda cheese.

The alkylpyrazines were identified with the combination of GC–MS, GC retention time, and the odor at GC exit port. The pyrazine peaks were eluted out of the GC column between acetoin and acetic acid, with 2,5-dimethylpyrazine right behind acetoin and 2,3,5-trimethylpyrazine right before acetic acid. Both peaks had the characteristic odor of nuttiness. Their mass spectra matched the published data (Stenhagen et al., 1974) and their GC retention time also matched those of the authentic compounds.

From this author's experience of formulating imitation processed American cheese flavor, the author believes the following relationships exist between the different flavor components and the different notes of processed American cheese. The creamy and buttery notes of processed American cheese are due to diacetyl, acetoin, and the lactones. The alcohols and methyl ketones also contribute to the total flavor of the cheese, but the role of aldehydes and alkylbenzenes is questionable. Acetaldehyde and isovaleraldehyde may be beneficial but not others. The identification of alkylbenzene casts some doubt on their existence. But an experiment of injecting a known flavor mixture into the adsorbing tube and desorbed and analyzed with GC did not result in identification of any alkylbenzenes. A number of alkylbenzenes have also been identified in other types of cheese (Day and Libbey, 1964). The esters are certainly the contributor of the weak fruity notes. The role of short-chain fatty acids is rather questionable. Patton (1963) attributed the acids to the cheesiness of cheddar cheese. But our experiments on the flavoring of imitation processed American cheese indicated that our panelists were unable to tell the difference between the cheesiness of cheeses flavored with and without short-chain fatty acids. Our GC analysis did reveal a small peak possessing a pleasant cheesy note but its retention time indicated that it was not an acid. The alkylpyrazines, 2,5-dimethylpyrazines, and 2,3,5-trimethylpyrazines are very likely the contributors of the weak nutty notes. The size of their GC peaks suggested that they were present in cheese in a very low concentration. Actual flavor formulation indicated that their concentrations in cheese were below the 0.5-ppm level.

ACKNOWLEDGMENT

The GC–MS analysis by Van Gray at Applied Science Institute of North Texas State University is appreciated.

LITERATURE CITED

Beroza, M. (1970), *Chem. Res.* **3**, 33–40.
Bertsch, W., Zlatkis, A. (1974), *J. Chromatogr.* **99**, 673–687.
Bertuccioli, M., Montedoro, G. (1974), *J. Sci. Food Agric.* **25**, 675–687.
Butler, L. D., Burke, M. F. (1976), *J. Chromatogr. Sci.* **14**, 117–122.

Day, E. A., Libbey, L. M. (1964), *J. Food Sci.* **29**, 583–589.
Dravnieks, A. (1973), *J. Food Sci.* **38**, 1024–1027.
Dumont, J. P., Roger, S., Adda, J. (1974), *Lait* **537**, 386–396.
Micketts, R. J., Lindsay, R. C. (1974), MBAA Technical Quarter 11, XIX-XX.
Patton, S. (1963), *J. Dairy Sci.* **46**, 856–858.
Sirikulvadhana, S., Jennings, W. A., Vogel, G. (1975), *Int. Flavor Food Additives* **6**, 126–128.
Sloot, D., Harkes, P. D. (1975), *J. Agric. Food Chem.* **23**, 356–357.
Sloot, D., Hofman, H. J. (1975), *J. Agric. Food Chem.* **23**, 358.
Stenhagen, E., Abrahamson, S., McLafferty, F. W., "Registry of Mass Spectral Data", Vol. 1, Wiley, New York, N.Y., 1974, pp 120 and 189.
Tassan, C. G., Russell, G. F. (1974), *J. Food Sci.* **39**, 64–68.

Sherman S. Lin

Anderson Clayton Foods
Richardson, Texas 75080

Received for review May 7, 1976. Accepted July 6, 1976.

Effect of Corn Trypsin Inhibitor on Growth of Rats

Although the opaque-2 gene approximately doubles the lysine and tryptophan contents of corn, it also greatly increases trypsin inhibitor content. To determine the nutritional effect of the inhibitor, it was isolated and added to a 10% casein diet at a level equal to 80% of opaque-2 corn in the diet. The inhibitor did not decrease growth or cause pancreatic hypertrophy. If rats and humans respond similarly, the higher level of inhibitor in opaque-2 corn should not detract from the benefits of the higher lysine and tryptophan contents.

Corn often is the principal component of the diets of people living in rural areas of tropical and subtropical regions (CIMMYT, 1975). However, such diets tend to be of poor nutritional quality because protein of locally grown corn is low in lysine and tryptophan. Following the discovery that the opaque-2 gene approximately doubles lysine and tryptophan levels in the corn endosperm (Mertz et al., 1964), corn strains were developed that contain the gene and are adapted to those regions, thus making available corns which can improve the nutritional status of the people of the regions (Bressani, 1966).

Corn containing the opaque-2 gene also contains about twice as much trypsin inhibitor as does normal corn (Mertz, 1972; Halim et al., 1973). The nutritional effects of the inhibitor are not known. However, soybean trypsin inhibitor depresses rat growth and causes pancreatic hypertrophy (Rackis, 1965; Kakade et al., 1973). Fortunately, the soybean inhibitor is inactivated when soybean products are cooked. Isolated corn trypsin inhibitor in water solution is only slowly inactivated when refluxed for several hours (Chen and Mitchell, 1973), and may survive rapid cooking processes applied to corn products. If corn trypsin inhibitor should possess adverse nutritional properties, the higher level in opaque-2 strains might reduce the nutritional advantages of the higher lysine and tryptophan contents; if so, efforts should be made to reduce inhibitor content genetically without reducing lysine and tryptophan contents. We report a rat growth study designed to assess the nutritional effects of the inhibitor.

METHODS

An opaque-2 strain of corn was finely ground, defatted with acetone, and assayed for trypsin inhibitor content (Erlanger et al., 1961). The inhibitor was removed from 400-g batches of the meal by two 4-h extractions with 0.2 M NaCl. The extract was adjusted to 1 M NaCl, 0.05 M Tris, 0.02 M $CaCl_2$, pH 8.2, and was centrifuged 30 min at 10000*g*. The extract was percolated through a trypsin affinity column (Loeffler and Pierce, 1973), and the column was washed with a buffer composed of the same salts and of the same pH as the extract. Washing was continued until absorbance of the eluate at 280 nm was below 0.05, thus eliminating noninhibitor proteins. The column then was washed with 300 ml of 0.01 M HCl to elute the inhibitor. The inhibitor was concentrated to about 100 ml in dialysis tubing suspended in front of an electric fan, and the concentrate was freeze-dried. The isolate was highly active, 0.2 mg causing 45% inhibition of trypsin in the Erlanger assay procedure.

Table I. Effect of Trypsin Inhibitor from Corn on Growth of Rats

Control			Inhibitor		
Rat no.	4-week gain, g	PER	Rat no.	4-week gain, g	PER
1	166	3.25	6	134	3.11
2	150	3.04	7	129	3.05
3	95	2.39	8	136	3.29
4	127	2.95	9	156	3.33
5	141	3.08	10	134	3.15
Av	136	2.97		138	3.19

It was established from the initial assay that the opaque-2 corn contained 1325 mg of inhibitor/kg, about twice the amount in normal corn. It was assumed the diets of people in the rural areas of developing regions might consist of 80% corn products. Therefore, the isolated inhibitor was added at a rate of 1060 mg/kg to a purified rat diet containing 10% protein (supplied by casein), vitamins, and minerals calculated to meet the requirements (National Academy of Sciences, 1972), and corn starch–glucose (3:1) to complete the diet. Charles River strain male albino rats were weaned at 21 days and fed commercial laboratory diet until 24 days old. Five randomly selected rats per group (initial weight, 58 to 62 g) were fed the diet with or without the trypsin inhibitor for 4 weeks.

Neither average weight gains nor PER's (Table I) were significantly different ($P = 0.05$). No hypertrophy of the pancreas was observed. Thus, we conclude that the higher levels of trypsin inhibitor in corn containing the opaque-2 gene are not deleterious to rats. If humans and rats respond similarly, the higher levels of inhibitor associated with the opaque-2 gene should not detract from the