Study of Tastes and Odors Produced by Chloridation Of Simple Nitrogenous Compounds

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Of 25 amino acids and related compounds studied, alanine, phenylalanine, arginine, and proline produced taste upon reaction with hypochlorous acid and monochloramine, and proline and phenylalanine produced taste with chlorine dioxide. Proline and phenylalanine have taste threshold concentrations of a few parts per billion. All four amino acids are found in common proteins. Other amino acids have been found in lake water in these concentrations. Attempts to find or produce any of the four amino acids in waters about Atlanta during the summer of 1952 were unsuccessful.

TASTES AND ODORS may delight or annoy each of us many times a day, yet we know very little about the mechanism of the biological detection of odor and only a little more about the detection of unusual compounds by taste. Generally, the consumer wants his tap water free of anything that might be classed as taste or odor. However, water as the "universal" solvent carries many contaminants as well as pollutants having their characteristic tastes or acquiring tastes during chlorination. Thus, the water-plant operator must see that the material pumped from his plant has the best, or the least possible, taste.

While the list of contaminants and pollutants which may lead to taste is large, this study has been limited to simple nitrogenous contaminants of biological origin. Pollution may also contribute amino nitrogen, causing trouble with odor control as shown by Williams (12), but probably the most common source of the nitrogen compounds studied will be the algae or other microorganisms found in surface waters. These compounds can be liberated by the autolysis of the microorganisms or by the action of *actinomycetes* as discussed by Silvey and Reach (10). An excellent review of the taste characteristics and natural concentrations of amino acids in lake water has been published by Rohlich and Sarles (8) who (9) have also reviewed the basic mechanisms of the detection and causes of taste and odor. Feben and Taras (2, 3, 11) reported in a series of articles their studies on reactions between chlorine and amino acids for determining most accurately the quantity of chlorine required by the Detroit drinking water. However, they did not refer to the tastes or odors of reaction products. Many authors (2, 3, 5, 11–13) have shown that chlorine may first react

to form an organic monochloramine or may deaminate the amine as more chlorine is added to the amine group, at pH 8.0.

Because chlorine as hypochlorous acid produces tastes in surface waters, numerous techniques have been studied and applied in reducing these tastes by altering the chlorine while retaining its bactericidal powers. Thus monochloramine has been used to reduce the rate of reaction between the hypochlorous acid and the organic matter without reducing the oxidizing capacity of the hypochlorous acid. As monochloramine is widely used in water treatment, this study includes the reactions of monochloramine with amino acids. Chlorine dioxide is a newer chlorine derivative used for controlling tastes, according to Aston (1). Although this gas has been particularly successful in controlling tastes caused by phenol and chlorophenol, as discussed by Ingols and Ridenour (4), it has not been as useful in controlling or eliminating algal tastes. However, chlorine dioxide has been tested with many of the amino acids, and the results of these studies are presented here; a part of these results has been published earlier (5).

Methods

In this study, minimum concentrations of amino acids were used—enough to give adequate energy absorption spectra and proper transmittance curves in the ultraviolet region of the Beckman DU. It was found that a concentration of 10^{-4} mole of amino nitrogen gave adequate energy absorption data, and the concentration was high enough so that a higher concentration in natural water might be excluded. All dilution water was prepared as chlorine-free, chlorine demand–

free water by dosing distilled water with 200 p.p.m. of sodium bicarbonate and then with 20 p.p.m. of chlorine. After the dosed water had stood for several days, the excess hydrochlorous acid was destroyed with ultraviolet light.

The pH-adjusted chlorine solutions were added in increments of 10^{-4} equivalent to separate aliquots of the amino acid solutions prepared from the chlorine-free, chlorine demand-free water. Thus, the smallest dose was 3.5 p.p.m.; the 7.1 p.p.m., 10.6 p.p.m., etc., were added to different aliquots until the highest concentration gave a residual of free chlorine after several hours. Each dose of chlorine was analyzed both for taste and for residual chlorine by the techniques of Palin (7) and of Marks, Williams, and Glasgow (6). Changes in the ultraviolet spectrum were also measured for each sample. These analytical techniques have been described by the authors (5), except for the tasting of the chlorinated amino acids. The minimum concentration causing tastes was determined by diluting amino acid and chlorine mixtures in chlorine-free chlorine demand–free water and arranging them in an increasing order of concentration for testing. The tasters sampled the blank (water plus buffer), and then the lowest concentration, then the blank and the next higher concentration until the amino acid derivatives were detected. Because the amino acid reactions were conducted in buffered solutions, the blank, made from the chlorine-free, chlorine demand–free water contained buffer in the same concentration.

Results of Investigations

Table I lists the amino acids and other simple amino nitrogen compound studies. Only four of these produced an

intensified taste as a result of chlorination: alanine, phenylalanine, arginine, and proline.

The chlorination of alanine produces pyruvic acid or acetaldehyde, as shown by Ingols and coworkers (5). Pyruvic acid has a strong burnt taste; it can be produced by either chlorination or bacterial deamination, as pointed out by Rohlich and Sarles (8). The decarboxylation of pyruvic acid yields acetaldehyde, which has a pleasant aromatic taste and aroma. This taste does not occur frequently as a result of chlorinating alanine, since acetaldehyde will not persist in solutions containing excess chlorine. Acetaldehyde would probably not be found in the chlorination of natural water because of the usual high ratio of chlorine to amine. The use of monochloramine with alanine also yields pyruvic acid, but more slowly than with hydrochlorous acid. Chlorine dioxide apparently does not react with alanine.

The chlorination of α-aminobutyric acid (which does not occur naturally) or of the amino acids with the five-membered aliphatic chain fails to produce any taste at 0.1 millimolar concentration. However, chlorination of the phenyl-substituted alanine yields a stewed-tomato taste with a rather low threshold concentration. The chemical data indicate that the odor-bearing compound is essentially phenylpyruvic acid as it is produced in the largest amount of the quantity of chlorine required for de-amination and it remains or persists in the presence of free chlorine. A very small percentage of the organic nitrogen can be recovered from these phenylpyruvic acid solutions which have tastes. Phenylpyruvic acid is also formed by monochloramine, but at a much slower rate than by hypochlorous acid. The

effects of chlorine dioxide on several simple amino acids and on tyrosine have given no indication of deamination. However, chlorine dioxide does react with a portion of the phenylalanine to produce a taste compound which appears to be identical to the reaction product of phenylalanine and hypochlorous acid, both in taste and in ultraviolet spectrum.

Upon chlorination with hypochlorous acid, proline produces a carnation or floral taste at the very low concentration of 10 to 30 p.p.b. Monochloramine and chlorine dioxide also react with proline to produce the same taste, but at a slower rate than hypochlorous acid. Approximately 4 moles of hypochlorous acid are consumed by the proline before an excess is present. This is the amount that would be needed to break the ring, oxidize the nitrogen, and form α-ketoglutaric acid:

$$2 \begin{matrix} H & H \\ | & | \\ H-C-C-H \ O \\ | & | \quad \| \\ H-C \quad C-C-OH \\ \diagup \ \diagdown \diagup \ \diagdown \\ H \ \ N \ \ H \\ | \\ H \end{matrix} + 9HOCl \rightarrow 2 \left[HO\overset{O}{\overset{\|}{C}}\overset{H}{\overset{|}{C}}\overset{H}{\overset{|}{C}}\overset{O}{\overset{O}{C}}\overset{O}{\overset{\|}{C}}OH \right] + N_2 + 9HCl + 3H_2O$$

Proline   α-Ketoglutaric acid
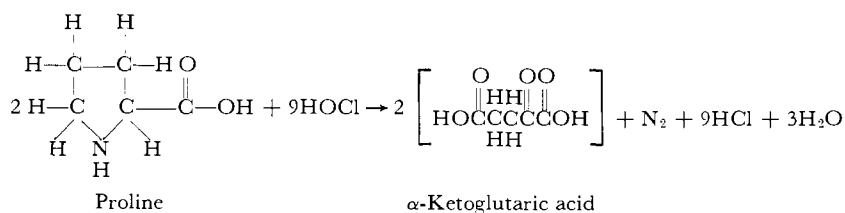
A comparison of the spectrophotometric curves in the low ultraviolet portion of the spectrum fails to indicate any similarity between α-ketoglutaric acid and the chlorine-treated proline. Furthermore, hydroxyproline yields no taste and odor upon chlorination at the concentration of 10^{-4} mole. Apparently the compound which causes the taste is very specific, as is pyruvic acid against α-ketobutyric acid. There may be some oxidation and substitution on the proline ring when hypochlorous acid is used, but it is not understood how chlorine dioxide can produce the taste compound if substitution on the ring is also necessary.

Arginine was studied at one fourth the concentration used for most other amino acids because of the four nitrogen atoms in the arginine molecule. However, it would seem that at low ratios of chlorine to total nitrogen the chlorine reacts rapidly and predominantly with the one nitrogen atom in the imine configuration to produce trichloramine. When there is a high ratio of chlorine to nitrogen, a small amount of trichloramine can be produced by the chlorination of almost all of the amino acids with free amino groups. The trichloramine is formed from arginine at a low ratio of chlorine to total nitrogen.

After four common amino acids had been found to produce tastes in chlorinated water at very low amino acid concentrations, an attempt was made to find microorganisms that might liberate these amino acids in natural waters. Many heavily populated stream and pond water

samples from the Atlanta area were brought into the laboratory, chlorinated, and tasted. The samples were tested immediately, and again after standing for various periods of time, as well as after chemical treatments with copper ions which might cause autolysis of the algae and protozoa. Some were seeded with soil to try to favor actinomycetes (10) or other antagonistic organisms (but no count was taken of the samples). Some samples were placed under artificial light, and necessary mineral nutrients were added for the growth of algae. Heavy growths of protococci were obtained under the artificial conditions and these cultures were tested under many conditions. A heavy inoculum of Volvox was purchased and cultured for several days to provide an organism whose presence is known to be correlated with taste in natural water. There was no indication at any time that chlorinating the samples produced any tastes in a sample.

Discussion

By chlorinating proline and phenylalanine, tastes were obtained which are described below in terms typical of those used in the literature to describe the tastes frequently obtained when natural waters are chlorinated. According to Rohlich and Sarles (8), the amino acids tryptophan, tyrosine, histidine, and cystine have been found in lake waters in 2 to 20 p.p.b. concentrations. These amounts would be great enough to explain tastes caused by chlorination of proline and phenylalanine at the same concentrations. However, the present authors were unsuccessful in finding proline or phenylalanine in the waters of the Atlanta area in concentrations high enough to yield tastes upon chlorination.

Oxidative hydrolysis of proteins and polypeptides could readily liberate such compounds as pyruvic acid and phenylpyruvic acid. Research is in progress to assay the amount and rate of hydrolytic degradation by hypochlorous acid on proteins. It is hoped that the hydrolytic data will aid in understanding the role of chlorine in taste production as well as its bactericidal properties.

Conclusion

Hypochlorous acid readily deaminates amino acids with free amine groups, especially arginine, to produce small amounts of trichloramine. The organic

residues of alanine and phenylalanine possess tastes; the phenylalanine residue, phenylpyruvic acid, can be detected at very low concentrations.

Hypochlorous acid, monochloramine, or chlorine dioxide react with proline to yield a compound with a very low threshold taste.

Monochloramine deaminates alanine and phenylalanine at a slower rate than hypochlorous acid. Chlorine dioxide apparently deaminates phenylalanine to form the phenylpyruvic acid, although the mechanism of the reaction is not understood, as chlorine dioxide does not react with alanine.

Acknowledgment

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PLANT STARCH ANALYSIS

Rapid Determination of Starch in Apples

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A rapid colorimetric procedure was developed for determining starch in apples in the maturity range of commercial harvest and storage. The method was found to be accurate for the Jonathan, Golden Delicious, standard Delicious, and Winesap varieties over this maturity range. One fourth of the apple starch in these varieties appears to be amylose.

THE QUANTITATIVE DETERMINATION OF starch in plant material is usually a tedious and time-consuming procedure. Purification by extraction or precipitation and acid or enzymic hydrolysis are frequently required. The determination of starch in apples requires purification to remove sugars and also (when acid hydrolysis is used) polysaccharides such as pectic substances, which are partially hydrolyzed to reducing compounds (*14*).

Rapid colorimetric methods depending on the formation of colored starch-iodine complexes have been proposed by several workers (*6, 11, 12, 15*). Most starch is composed of two fractions, a straight-chained amylose component and a branched-chain amylopectin fraction (*7*). Iodine reacts with amylose to give an intense blue complex with about six times as much color as the less intense blue-violet complex formed with amylopectin (*7*). Methods depending on the formation of these complexes therefore require that the amylose-amylopectin ratio be constant, or that the proportions be determined, if results are to be accurate. Methods involving specific rotation or enzymic hydrolysis are also dependent on a constant ratio. This ratio may vary with plant species (*1, 12*), variety (*4–6, 8, 12*), maturity (*2, 5, 6, 11, 12*), and in the case of sweet corn with growing conditions (*2*). Nielsen and Gleason (*12*) have applied correction factors for iodine color values of starches from different plant sources. The literature thus indicates that the starch-iodine color complex can be used for the quantitative determination of starch in a given plant material only after it is established that the ratio of amylose to amylopectin is constant within the species and at different maturities, and under various growing conditions.

The time-saving possibilities of the colorimetric method justified an investigation of its applicability to several varieties of apples for which numerous starch analyses were required. This report describes the method developed and presents a comparison of results with those obtained by the method of Pucher, Leavenworth, and Vickery (*14*) on four apple varieties of different maturities. The procedure is essentially a modification of the method developed by Pucher and Vickery (*15*) and involves the use of perchloric acid as a starch solvent as proposed by Nielsen (*11*). The method was used in studies of the juice quality of Delicious apples (*9*).

Apparatus and Reagents

Photoelectric colorimeter, 620 mμ filter.
Food disintegrator.
Perchloric acid, 7.8N.
Potassium iodide, 5% solution.
Potassium iodate, 0.01N solution.
Sodium thiosulfate, 0.16N solution.

Analytical Procedure

Wedge-shaped slices cut from stem to calyx on opposite sides of each apple were ground 3 minutes in a food disintegrator to obtain a fine pulp. A 5.0-gram sample of this slurry was weighed into a 50-ml. beaker, and 20 ml. of 7.8N perchloric were added with rapid initial stirring to avoid high acid concentrations. Digestion was allowed to proceed for 5 to 10 minutes at room temperature and the mixture was then diluted with distilled water and transferred to a volumetric flask of a size depending on the approximate amount of starch in the sample.