Furaneol: Odor Threshold and Importance to Tomato Aroma

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The determination of the odor thresholds of Furaneol and norfuraneol in water and in buffered solutions showed that their thresholds varied depending on the pH of the solution, being lower the more acid the solution. Calculations of concentration/threshold ratios showed that Furaneol occurs at a concentration well above its threshold and is among the 10 compounds with the highest probability of contributing to both fresh and processed tomato aroma and flavor. Norfuraneol, however, occurs below its threshold concentration in fresh tomato and only slightly above in the paste and has only a low probability of contributing.

Keywords: *Furaneol; norfuraneol; tomato; quantitative analysis; odor threshold*

INTRODUCTION

Furaneol [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone] is a well-known important aroma component of a number of fruits, particularly pineapple (Rodin et al., 1965), strawberry (Re et al., 1973; Pickenhagen et al., 1981), and raspberry (Honkanen, 1980). The presence of Furaneol glucoside (Krammer et al., 1994) and free Furaneol and 5-methyl-4-hydroxy-3(2*H*)-furanone (referred to as norfuraneol) in tomatoes was recently reported (Buttery et al., 1994). The odor threshold of Furaneol in water solution has been reported by several different authors (Pittet et al., 1970; Honkanen et al., 1980; Wilson et al., 1990). There was considerable variation in the figures reported, which prompted the authors to carry out further studies on the odor threshold determination of Furaneol and of the related compound norfuraneol to assess the importance of these compounds to both fresh and processed tomato aroma and flavor.

EXPERIMENTAL PROCEDURES

Materials. Tomatoes (Ace cultivar) were grown in El Cerrito, CA, in the summer of 1994 and were at the deep red vine ripe stage. Tomato paste (major retail brands, 23–25% solids) was obtained from local supermarkets. Tomato paste in California is usually produced from FM785, GS-12, or related tomato cultivars.

Diethyl ether was freshly distilled and contained ca. 0.001% Antioxidant 330, Ethyl Corp., Baton Rouge, LA. NaCl and Na_2SO_4 were heated at 150 °C for several hours to remove any volatiles and then stored in clean glass jars. Celite 545 was graded by allowing to settle in water to remove fines and washed well.

Isolation of Furaneol from Tomato. This was similar to that previously described by the authors (Buttery et al., 1994). Fresh tomatoes (150 g) were blended at room temperature (ca. 25 °C) with the addition of 30 mL of water. The blended material was held for 3 min for normal flavor producing enzyme action to occur. It was then saturated with excess NaCl (75 g) by blending, cooled to close to 0 °C externally with ice, and filtered through a 1–2 cm thick layer of Celite 545. An additional 30 mL of NaCl saturated water was used to wash the Celite filter. An internal standard (2.00 mL of a 50.0 ppm water solution of 2-octanone) was added at this point. The clear mixture was then placed in a liquid–liquid continuous extractor and extracted for 8 h with purified diethyl ether. The blended tomato mixture was kept cool by external ice cooling or refrigeration throughout the isolation after blending. The ether extract was dried over anhydrous sodium sulfate and transferred to a clean 5 L round bottom flask. The ether was then carefully evaporated and the solute spread out on the walls of the flask by rotating the flask in a 60 °C water bath. A suitable head was then immediately attached to the flask which allowed the entrance of the sweep gas (air at 3 L/min) which passed over the inside walls of the flask and exited through a large Tenax trap (Tenax GC, 60–80 mesh, 10 g, 14 cm × 2.2 cm i.d.). The isolation was carried out for 2 h at room temperature (25 °C). The trap was then removed and eluted with 50–100 mL of diethyl ether. The ether extract was concentrated on a warm water bath to ca. 50 μL.

The isolation from paste was carried out in a similar way except that the tomato paste was diluted with three times its volume of water.

Capillary Gas Chromatography (GC) Analysis. GC analysis was carried out using a HP 5890 gas chromatograph with a fused silica capillary column 60 m long by 0.32 mm i.d. coated with DB-1 and also with a column of the same dimensions coated with DB-Wax. The temperature program with the DB-1 capillary was 30 °C for the first 25 min, then increased at 4 °C/min to 200 °C, and held at this temperature for a further 20 min. The temperature program with the DB-Wax capillary was 30 °C for the first 4 min, then increased at 2 °C/min to 170 °C, and then held for a further 30 min. The injector temperature was 170 °C for both columns.

Quantitative analyses were made using 2-octanone as an internal standard added to the filtered blended tomato before the ether extraction. Recovery factors were determined for Furaneol and norfuraneol using stock solutions of both compounds in distilled water which were stored at refrigerator temperatures. Measured amounts were added to 150 mL of a 1% solution of NaH_2PO_4 in water (pH 4.5), and the mixture was taken through the isolation process used for tomato as described above.

HPLC Method. The sample preparation procedure was similar to that described by Lee and Nagy (1987). The column used was a Keystone ODS/B (250 × 4.6 mm, 5 mm) coupled to a RP-18 guard column (20 × 2 cm i.d., Upchurch Scientific, Oak Harbor, WA) with a ternary solvent mixture consisting of 77:17:6 (v/v/v) 0.05 M acetate buffer (pH 4.0)/methanol/acetonitrile. Detection was performed at 280 nm.

Odor Threshold Determination. Odor thresholds were determined in water and in buffered solutions following the general methods previously described (Guadagni and Buttery, 1978). The water used was distilled water, which was boiled to remove ca. 10% of it to carry away any volatiles. Distilled water containing 1% NaH_2PO_4 was used to determine the threshold at pH 4.5. This solution plus in addition 0.5% phosphoric acid was used to measure the threshold at pH 3. These solutions were boiled as for the water to carry away any volatiles, cooled, and stored in clean Pyrex glass contain-

Table 1. Odor Thresholds Found for Furaneol and Norfuraneol

study	concn, μg/L of water (ppb) Furaneol	norfuraneol
present, USDA (1994)		
pH 7	60 (45–110)[a]	23000 (12000–140000)[a]
pH 4.5	31 (20–52)[a]	2100 (830–3800)[a]
pH 3	21 (13–63)[a]	2500 (1150–4300)[a]
Pittet et al. (1970)	100–200	
	30 (taste)	
Honkanen et al. (1980)	0.03	
Wilson et al. (1990)	1700	

[a] 95% confidence interval.

ers. The number of (experienced) judges used varied from 18 to 23. Probit analysis (a statistics program for drug dose tests) was used to determine the threshold points (taken where 75% of the panelists give the correct judgment) and also to determine the 95% confidence intervals.

Authentic Samples. Furaneol was obtained from Aldrich Chemical Co. and stored at −20 °C under an argon atmosphere. Norfuraneol was synthesized following the procedure described by Peer et al. (1968). Both compounds were purified by recrystallization from ether−pentane and were better than 98% pure by GC analysis.

RESULTS AND DISCUSSION

It is known that Furaneol has acidic properties. The authors found that a 2% solution of Furaneol in water had a pH of 2.8. This is similar to acetic acid, which showed a pH of 2.7 for a 2% water solution. It therefore seemed likely that the volatility of Furaneol (and thus the concentration reaching the olfactory senses) in aqueous solution would vary with the pH.

Odor Threshold Determination. The odor thresholds of Furaneol and norfuraneol were determined at three different pH values, 7.0, 4.5, and 3. These data are shown in Table 1. It can be seen that there is a factor of ca. 3 times difference between the thresholds at pH 7.0 and pH 3 for Furaneol and a larger factor for norfuraneol. Our value for Furaneol is within experimental error of that found by Pittet et al. (1970) for taste. Of course, the process involved in taking the solution into the mouth (for "taste" evaluation) in this case is really olfactory, the vapors reaching the olfactory senses through the back of the throat (a process often termed as "retronasal"). Glassware which has been washed in the commonly used trisodium phosphate can sometimes retain an alkaline pH which (with the very dilute solutions involved) may explain the very high figure for the odor threshold found by Wilson et al. (1990).

Quantitative Studies. The authors had previously reported some quantitative data for the concentration of Furaneol in fresh and processed tomato (Buttery et al., 1994). However, this had been from preliminary studies intended only to give some idea of the order of the concentration. The authors have continued their study of the quantitative analysis of Furaneol and norfuraneol in tomato products and have obtained more accurate data. Various methods have been developed for the quantitative analysis of Furaneol in foods. Probably the most reliable and accurate method is that described by Sen et al. (1991), which uses the stable isotope dilution assay method [cf. Schieberle and Grosch (1987)] involving a synthetic form of Furaneol in which both methyl groups contain ^{13}C. Not having the facilities for this method, the present authors used a modification of the method of Pickenhagen et al. (1981) which used continuous liquid−liquid extraction followed by gas chromatography using the internal standard isopropyl decanoate.

Table 2. Concentration Found for Furaneol and Norfuraneol in Fresh Red Vine Ripe Tomato, in Mature Green Tomato, and in Tomato Paste

	concn, in μg/kg of tomato product (ppb) ripe tomato	green tomato	tomato paste
Furaneol	700	<50	1000
norfuraneol	1200	<2	3300

The authors' modification of the Pickenhagen et al. (1981) method gave an average recovery of 77% for standard mixtures of Furaneol using a 1% solution of NaH_2PO_4 (which gives a pH of 4.5, which is close to that of tomato) instead of tomato. If the pH was close to 7, the recovery was much less because the liquid−liquid extraction step was not efficient. Using this recovery factor the concentrations found in fresh, green, and processed tomato are shown in Table 2. The figures shown are the means of separate analyses of at least three different batches of tomatoes or paste. It can be seen that the concentrations of both Furaneol and norfuraneol are very much smaller in green tomatoes, indicating that the formation of these compounds probably occurs during the ripening process. The slightly higher concentration in tomato paste is understandable. The water soluble Furaneol and norfuraneol would not be lost as readily with the water vapor during concentration. Under certain conditions, however, Furaneol is unstable at higher temperatures and there may be some loss from decomposition in the tomato paste process. The metal walls of the processing equipment might also be involved.

HPLC Quantitative Method. Some preliminary studies were also carried out using analysis by HPLC. The sample procedure was similar to that described by Lee and Nagy (1987) for pineapple. The HPLC separation was carried out using a Keystone ODS/B column with a ternary solvent mixture consisting of acetate buffer/methanol/acetonitrile with detection at 280 nm. The quantitative data obtained for both fresh tomato and paste were of the same order as that found with the GC method (Table 2).

Fresh Ripe Tomatoes with Enzyme Deactivation. It is not known whether there is a specific enzyme in tomatoes that can bring about hydrolysis of Furaneol glucoside when the tomato is cut up such as by blending. It is well-known that lipid oxidation enzyme action occurs very rapidly with such tissue disruption. To study whether Furaneol or norfuraneol is free in the intact tomato, a batch of fresh ripe tomatoes was frozen in a −20 °C freezer until the tomatoes were completely solid. A weighed quantity of the frozen tomatoes was then added to a blending jar containing a small quantity of NaCl saturated water and an excess of NaCl. The frozen tomatoes were then broken up by blending. The salt aids in the liquefaction of the tomato but the cooling effect of ice−salt mixing keeps the temperature below 0 °C. While still being kept close to 0 °C, the mixture was taken through the isolation process as described under Experimental Procedures for the tomato blended at room temperature. It was felt that the cold conditions and the NaCl saturation should considerably retard enzyme action. Quantitative analysis under these conditions showed concentrations of Furaneol and norfuraneol not significantly different from that when room temperature blending was used. This study then

Table 3. Log Concentration/Threshold Ratios for Furaneol and Norfuraneol in Fresh Tomato and in Tomato Paste and Comparison with Values for Other Major Tomato Components

	log concn/threshold	
	fresh ripe tomato	tomato paste
Furaneol	1.4	1.5
norfuraneol	−0.24	0.2
Comparison to Values for Other Major Fresh Tomato Components[a]		
(*Z*)-3-hexenal	4.7	
β-ionone	2.8	
hexanal	2.8	
β-damascenone	2.7	
1-penten-3-one	2.7	
3-methylbutanal	2.1	
(*E*)-2-hexenal	1.2	
2-isobutylthiazole	1.0	
Comparison to Values for Other Major Tomato Paste Components[a]		
dimethyl sulfide		3.8
β-damascenone		3.8
β-ionone		2.5
3-methylbutanal		2.1
1-nitro-2-phenylethane		1.5
eugenol		1.2
methional		1.2
3-methylbutyric acid		0.9

[a] Buttery et al. (1990).

supports the view that Furaneol and norfuraneol are present in the intact tomato, probably formed by slow enzyme hydrolysis of the glucoside occurring during ripening.

Concentration/Threshold Ratios. From the data in Tables 1 and 2 the concentration/threshold ratios can be determined for Furaneol and norfuraneol in fresh tomato and tomato paste. This ratio has been referred to in previous publications by the authors as "odor units". These are shown in Table 3 (in their $\log_{10}$ form) using the thresholds determined at pH 4.5, which is close to the pH of the normal tomato products. Also shown for comparison are values for other components in fresh tomato and paste in the order of their concentration/threshold ratios. It can be seen that Furaneol ranks seventh in fresh tomato and fifth (together with 1-nitrophenylethane) in the paste. It is doubtful whether the exact rank of a compound is that meaningful except that the probability of a compound contributing to the odor is likely to be a function of the number of threshold concentrations present, which, of course, is equal to the concentration/threshold ratio. Norfuraneol occurs below threshold in the fresh ripe tomato and thus has a very low probability of contributing to the odor, but in tomato paste norfuraneol occurs just above threshold and has a small probability of contributing to the odor.

Synthetic Tomato Essences with Furaneol. Some of the authors had previously found that a mixture of seven synthetic components in water solution closely matched the aroma of tomato paste (Buttery et al., 1990). Preliminary qualitative sensory panel studies were carried out in the present work with 18−23 judges and 45 judgments on each comparison. It was found that addition of 1 mg/L of Furaneol to the synthetic tomato paste mixture, although detectable by the panel, did not confer any improved aroma.

In similar studies with a synthetic mixture for fresh tomato aroma (Buttery, 1993), addition of 0.7 mg/L of Furaneol (again detectable by the panel) showed a slight improvement to the aroma.

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