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The Chemistry of Fresh Tomato Flavor

Emin YILMAZ

Çanakkale Onsekiz Mart University, Faculty of Engineering, Department of Food Engineering, 17100, Çanakkale - TURKEY

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Abstract: Tomato flavor results from taste components, aromatic volatiles and a complex interaction between them. Lack of characteristic flavor in supermarket tomatoes is a common consumer complaint. Sugars, organic acids, free amino acids, and salts are the main taste components. With over 400 volatile compounds identified, only thirty are present in concentrations over one ppb and of these, only 16 contribute significantly to the perceptible flavor. Radiolabeled substrate and enzyme denaturation studies have indicated involvement of some pathway enzymes in the biosynthesis of tomato aroma compounds. Studies of fresh tomato flavor are important for the efforts at improving fruit quality through genetic modifications by molecular techniques.

Key Words: tomato, flavor, volatile, taste, chemistry

Taze Domates Lezzet Kimyası

Özet: Domates lezzeti tad bileşenleri, aromatik uçucu bileşikler ve bunların karşılıklı etkileşiminden oluşur. Süpermarketlerdeki domateslerin lezzetinin yeterli bulunmaması yaygın bir tüketici şikayetidir. Ana tad bileşenleri şekerler, organik asitler, serbest amino asitler ve tuzlardır. Dörtüzdenden fazla tanısı yapılmış olan uçucu aroma bileşenlerinden sadece otuz kadarının konsantrasyonu bir ppb üzerindedir ve bunlardan da onaltısı lezzeti önemli düzeyde etkilemektedir. Radyoaktif -işaretli substrate ve enzim denatürasyon çalışmaları, domatesdeki uçucu aroma maddelerinin biyosentezinde bazı reaksiyon enzimlerinin rolünü göstermiştir. Taze domates lezzeti üzerinde yapılan araştırmalar meyve kalitesini yükseltmek için moleküler tekniklerle yapılacak genetik çalışmalar için önemlidir.

Anahtar Sözcükler: domates, lezzet, uçucu bileşikler, tad, kimya

Introduction

The tomato (*Lycopersicon esculentum*, Mill) originated in Tropical America, probably in Mexico or in Peru. The name is of South American origin and is derived from the Aztec word 'xitomate' or 'zitotomate'. It was taken to Europe in the early sixteenth century, and later in the eighteenth century to North America, and from these areas spread out to the rest of the world (Gould, 1983).

The tomato is a member of the *Solanaceae* family. Botanically, it is a berry fruit, but it is cultivated and used as a vegetable (Petro-Turza, 1978). It is a tender, warm-season perennial. Nutritionally, the tomato is a good source of vitamin A and C (Gould, 1983). Composition data varies due to the wide range of species, stage of ripeness, year of growth, climatic conditions, light, temperature, soil, fertilization, irrigation, and other conditions of cultivation, and handling and storage.

Average dry matter content of the ripe fresh fruit is between 5.0 and 7.5% (Petro-Turza, 1987). Percent composition of dry matter is shown in Table 1. The free sugars of commercial varieties of tomatoes are predominantly reducing sugars, and the quantity of sucrose is negligible. The pectins, arabinogalactans, xylans, arabinoxylans, and cellulose are the major polysaccharides. Glutamic acid comprises up to 45% of the total weight of free amino acids in fresh tomato juice with the next highest in concentration being aspartic acid. Citric acid is the most abundant organic acid with some malic acid also present (Gould, 1983).

Characteristic tomato flavor is formed by the action of volatile aroma compounds and non-volatile constituents listed in Table 1. Dissatisfaction of consumers with an absence of characteristic fresh tomato flavor is the main marketing complaint. Tomato consumers have indicated that they are willing to pay a premium for a full-flavored fruit (Bruhn et al., 1991).

Table 1. Composition of Dry Matter Content of Tomato (Petro-Turza, 1987).

Constituent	%
Fructose	25
Glucose	22
Saccharose	1
Citric acid	9
Malic acid	4
Protein	8
Dicarboxylic amino acid	2
Pectic substances	7
Cellulose	6
Hemicellulose	4
Minerals	8
Lipids	2
Ascorbic acid	0.5
Pigments	0.4
Other amino acids, vitamins, and polyphenols	1
Volatiles	0.1

Chemistry and Evaluation of Tomato Flavor

Flavor is a combination of taste and aroma sensations. The four tastes, sweet, sour, salty, and bitter are perceived by certain regions of the tongue, while volatiles are perceived by the olfactory nerve endings of the nose (Acree, 1993). The pleasant sweet-sour taste of tomatoes is mainly due to their sugar and organic acid contents. Of the over 400 volatiles determined, 30 have proved to be the most important compounds contributing to the aroma of tomatoes. The characteristic tomato flavor, thus, is produced by the complex interaction of the volatile and non-volatile components (Petro-Turza, 1987; Buttery and Ling, 1993a).

Non-Volatile Compounds

Sugars, organic acids, free amino acids, and salts are the main components contributing to tomato taste. The characteristic sweet-sour taste of tomato is due to a combination of the sugars and organic acids present. About 50% of the dry matter is composed of sugars, primarily the reducing sugars, glucose and fructose. Also, minute quantities of saccharose, raffinose, arabinose, xylose, galactose, and sugar alcohol myoinositol have been reported. In the initial stages of development, glucose is dominant with a glucose:fructose ratio of 1.8, then total sugar content increases significantly, and the

ratio approaches 1.0. The total sugar content of ripe tomato is between 1.7 and 4.7% (Petro-Turza, 1987). Positive correlations between sweetness, reducing sugar content and soluble solids have been shown (Bisogni and Ambreester, 1976; Stevens et al., 1979). Similarly, the greater importance of fructose due to its greater potency in sweetness perception, which is more than approximately three times that of glucose, may be a factor (Petro-Turza, 1987). In contrast, Jones and Scott (1983) could not demonstrate any correlation between reducing sugar or dry matter and sweetness of the F1 hybrids which were high sugar and acid varieties. This was attributed to a 'terminal threshold' concept, which states that above a certain concentration, additional increases yield no increase in perceived intensity of stimulus. Malundo et al. (1995) demonstrated that addition of sugars increased flavor acceptability when the sample has a pH near 3.74 (or 0.80% titratable acidity) but did not affect the tomato-like character of fresh tomatoes.

Organic acids comprise about 15% of the dry content of fresh tomatoes. Citric and malic acids are the major organic acids, in addition to several other carboxylic acids, sugar acids, and alicyclic acids. In the ripe red tomato, the malic to citric acid ratio is 0.5 or lower. At higher levels of citric acid, the sweetness effect of glucose was found to be more than that of fructose (Petro-Turza, 1987). Stevens et al. (1979) observed high correlations between sourness, titratable acidity (TA) and pH. Bisogni et al. (1976) suggested that free acid content provided a greater impact on tomato sourness than hydrogen ion concentration. Malic acid has been reported to be 14% more sour than citric acid, but it has less influence on tomato taste because of its lower concentration. Malundo et al. (1995) found a negative correlation between acid and pH, and positive correlation between pH and TA. Baldwin et al. (1998) found significant correlations between TA and overall acceptability, pH with sweetness and sourness, and SS / TA with overall taste.

Free amino acids form about 2-2.5% of the total dry matter of tomatoes (Petro-Turza, 1987). Glutamic acid, γ -aminobutyric acid, glutamine, and aspartic acid comprise about 80% of the total free amino acids in tomatoes. The higher glutamic acid concentrations in fruit picked at the breaker or earlier stages were parallel to higher scores for 'off-flavor' as described by a sensory panel (Kader et al., 1978). Tomato fruitiness intensity

was significantly correlated to reducing sugars / glutamic acid ratio and content. The negative correlation of glutamic acid to tomato fruitiness was unexpected, in contrast to the well-known flavor enhancing ability of glutamic acid (Bucheli et al., 1999). Other effects of free amino acids on tomato taste were ascribed to their own taste, their taste-enhancing capacity, and their buffering capacity (Petro-Turza, 1987).

Minerals constitute about 8% of the dry matter content of tomatoes. Potassium and phosphate are the two major ones. Minerals have an effect on pH and titratable acidity and have buffering capacity as well; thus, they influence the taste of tomatoes (Petro-Turza, 1987).

Recently, a group of researchers published statistical models describing the taste of tomatoes based on simple instrumental measurements (Verkerke et al., 1998). They found that sweetness can be estimated by percent juice, SS, and percent sugar; mealiness can be described by percent juice, slope of the deformation curve, breaking force of the pericarp, and energy under the curve. Pleasantness can similarly be described by percent juice, SS, TA, breaking force of the pericarp, diameter of the fruit, and energy under the curve. The results suggest a simple and low cost means of tomato taste evaluation.

Volatiles Compounds

Petro-Turza (Petro-Turza, 1987) lists over four 400 compounds identified in tomato fruit. Of those, only around 30 are present in concentrations over one part per billion (ppb) as summarized by Buttery (1993). The concentrations, odor thresholds, and log odor units of tomato volatiles present at levels of above one ppb are listed in Table 2. Of these, 16 have positive log odor units and are likely to contribute to tomato flavor. A combination of *cis*-3-hexenal, *cis*-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and β -ionone at appropriate concentrations produces the aroma of fresh ripe tomato (Buttery, 1993). Similarly, the most important volatile compounds in tomato aroma are listed as hexanal, *cis*-3-hexenal, *trans*-2-hexenal, hexanol, *cis*-3-hexenol, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, β -ionone, geranylacetone, 1-penten-3-one, 3-methylbutanal, 3-methylbutanol, phenylethanol, 2-pentenal, acetone, ethanol, and methanol (Buttery and Ling, 1993b).

Furaneol has since been described as an important volatile in tomato aroma. It ranks seventh in the fresh tomato and fifth in the paste based on its concentration / threshold ratio. Norfuraneol has a very low probability of contributing to tomato aroma (Buttery et al., 1995). 2-Isobutylthiazole and *cis*-3-hexenal have been described as 'flavor impact compounds' (Kazeniak and Hall, 1970). Volatile nitro compounds, which have not been found in other foods, are present in tomatoes. 1-Nitro-2-phenylethane, 1-nitro-2-methylpropane, and 1-nitro-3-methylbutane have also been identified as potential contributors to tomato aroma (Buttery, 1993).

Kazeniak and Hall (1970) showed the association of tomato 'fresh' flavor with *cis*-3-hexenal, *trans*-2-hexenal, hexanal and 2-isobutylthiazole. When the flavor effect of these compounds diminishes, then alcoholic 'processed' or 'enzymic' flavors become dominant. *Cis*-3-hexenal has been associated with the 'fresh green' aroma of tomato. Its sensory threshold is very low (0.25 ppb), and a 1ppm solution in water resembled the aroma of freshly cut green tomato (Petro-Turza, 1987).

Another important 'green' flavor compound in tomato is *trans*-2-hexenal. Although Kazeniak and Hall (1970) find it less 'fresh green' in character than *cis*-3-hexenal and less intense, it has desirable blending or 'mouthfeel' properties. Its threshold concentration in aqueous solution has been determined to be 17 ppb and its aroma was found not to be like tomato (Petro-Turza, 1987). The other 'green' flavor in tomatoes is hexanal, and found to be present in the range of 0.1-0.5ppm in tomato juice (Kazeniak and Hall, 1970).

2-Isobutylthiazole has been described as having a spoiled vine-like, slightly horseradish-type flavor in tomatoes. When added to tomato paste or juice in the 25-50 ppb concentration range, it increases 'fresh tomato-like' aroma, but at higher levels, the aroma becomes objectionable, rancid, medicinal, and metallic off-odors became dominant. 6-Methyl-5-hepten-2-one was present in tomatoes, and was characterized as having a fruit-like aroma (Kazeniak and Hall, 1970).

Ethanol and methanol are found in low quantities in tomatoes. Their effect is thought to influence the perception of other substrates, although methanol does not appear to contribute to the flavor of tomato juice or paste (Kazeniak and Hall, 1970). Ethanol and methanol at concentrations found in ripe tomatoes have been found to

Table 2. Tomato Volatiles Present in Fresh Tomato at Levels $>$, or $=1$ ppb, Their Odor Threshold (ppb in water) and the Descending Order of Log Odor Units (Buttery, 1993).

Volatile	Concentration (ppb)	Odor Threshold (ppb)	Log Odor Units
<i>cis</i> -3-Hexenal	12,000	0.25	3.7
β -Ionone	4	0.007	2.8
Hexanal	3,100	4.5	2.8
β -Damascenone	1	0.002	2.7
1-Penten-3-one	520	1.0	2.7
3-Methylbutanal	27	0.2	2.1
<i>trans</i> -2-Hexenal	270	17.0	1.2
2-Isobutylthiazole	36	3.5	1.0
1-nitro-2-Phenylethane	17	2.0	0.9
<i>trans</i> -2-Heptenal	60	13.0	0.7
Phenylacetaldehyde	15	4.0	0.6
6-methyl-5-Hepten-2-one	130	50.0	0.4
<i>cis</i> -3-Hexenol	150	70.0	0.3
2-Phenylethanol	1,900	1000.0	0.3
3-Methylbutanol	380	250.0	0.2
Methyl salicylate	48	40.0	0.008
Geranylacetone	57	60.0	-0.02
β -Cyclocitral	3	5.0	-0.2
1-nitro-3-methyl-Butane	59	150.0	-0.4
Geranial	12	32.0	-0.4
Linalool	2	6.0	-0.5
1-Penten-3-ol	110	400.0	-0.6
<i>trans</i> -2-Pentenal	140	1,500.0	-1.0
Neral	2	30.0	-1.2
Pentanol	120	4,000.0	-1.5
Pseudoionone	10	800.0	-1.9
Isobutyl cyanide	13	1,000.0	-1.9
Hexanol	7	500.0	-1.9
Epoxy- β -ionone	1	100.0	-2.0

suppress certain aroma volatiles (hexanal, 3-methylbutanol, phenylethanol, 1-penten-3-one) present in tomatoes when evaluated in odor threshold studies, while enhancing other volatiles (*trans*-2-hexenal, hexanol, and 3-methylbutanal) (Tandon, 1997).

The first step in studying tomato flavor by instrumental analysis is the separation of volatile materials from non-volatile materials. Usually, diced or homogenized samples are vacuum-steam distilled with a continuous extraction apparatus (Nickerson-Likens apparatus) (Buttery et al., 1971), vacuum condensed (Buttery et al., 1998), distilled at low temperature by the Forss and Holbway method (Etievant et al., 1996), sampled with dynamic headspace techniques (Buttery, 1993), or analyzed by regular headspace technique

(Baldwin et al., 1991, 1996). Recently, a relatively new sampling technique, solid phase microextraction (SPME), was examined to investigate volatiles in tomatoes and strawberries (Beaudry et al., 1998), and found suitable for rapid and quantitative analysis.

Tissue disruption results in major changes in flavor profiles, as summarized in Table 3 by Buttery and Ling (1993a). Accordingly, tomato volatiles are produced during the ripening process in the intact tomato, and when the tomato is macerated in some way such as cutting, chewing or blending. These two main routes of volatile production point out the involvement of certain enzymes in the biogenesis of volatiles as well (Buttery and Ling, 1993a). Upon tissue disruption, enzymes, substrates and air are mixed together; hence, enzyme

A.) Volatiles that appear after tissue disruption	
	<i>trans</i> -2-pentenal
B.) Volatiles that increase after tissue disruption	
	<i>cis</i> -3-hexenal
	<i>trans</i> -2-hexenal
	hexanal
	<i>trans</i> -2-heptenal
	1-penten-3-one
	1-penten-3-ol
	geranylacetone
C.) Volatiles that show no significant change due to tissue disruption	
	3-methylbutanol
	pentanol
	<i>cis</i> -3-hexenol
	hexanol
	6-methyl-5-hepten-2-one
	phenylacetaldehyde
	2-phenylethanol
	2-isobutylthiazole
	1-nitro-2-phenylethane

Table 3. Changes in Volatiles in Tomato after Tissue Disruption (Buttery and Ling, 1993a).

deactivation is needed during the isolation process. Heating, organic solvents and strong acids have been shown to cause certain problems and are not used widely (Buttery, 1993; Baldwin et al., 1991, 1996). Saturated CuSO_4 is effective, but may react with sulfur compounds and catalyze chemical autoxidation (Buttery et al., 1987). Saturated CaCl_2 , NaCl , and trichloroacetic acid are very effective in denaturing enzymes with few problems (Buttery, 1993; Baldwin et al., 1991). Heating the whole tomato at 100°C in an 800 watt microwave oven for 1 min before blending is effective but may produce some new volatiles generated by the heat (Buttery and Ling, 1993a).

Qualitative and quantitative identification of isolated essential oils of tomato samples are usually accomplished by GC-MS, GC-IR, and NMR techniques (Petro-Turza, 1987; Buttery, 1993; Buttery et al., 1971, 1998; Etievant et al., 1996; Baldwin et al., 1991, 1996). Another commonly used method for aroma analysis is GC-Olfactometry. A typical design and protocol for aroma extraction dilution analysis (AEDA) includes the use of a splitter to divide a gas chromatographic effluent between a sniff port or olfactometer and a chemical detector. The sample is diluted serially and evaluated with each chromatographic sampling while both retention times and odor intensities are plotted (Acree, 1993; Ullrich and Grosch, 1987). The first AEDA and GC-O analyses of tomato fruit volatiles determined the most important

compounds having a flavor dilution greater than 100 as *cis*-3-hexenal, hexanal, 1-octen-3-one, methional, 1-penten-3-one, and methylbutanal (Krumbein and Auerswald, 1998). CharmAnalysis, developed by Acree (1993), provides a psychophysical estimation of the order of intensity of individual compounds. It yields peak heights and areas and is analyzed by Charmware software. Odor thresholds can also be determined. Another method to measure the contribution of individual components to the perceived aroma is odor threshold determination. A threshold is defined as the minimum concentration that can be perceived or the amount of energy necessary for task accomplishment at a given level of probability, usually at the 95% confidence level (Dember, 1979). From threshold values and determined concentration values, the odor units (U_o) can be calculated. U_o is defined as the ratio of the concentration of the compound divided by its threshold concentration (Guadagni et al., 1966).

If an odor unit is less than 1, it is considered to provide no significant contribution as it exists in quantities below the odor threshold. Similarly, when the $\log U_o$ value is positive, its contribution is significant (Stern et al., 1994). A number of odor threshold studies have been conducted to determine important contributors to tomato aroma (Buttery et al., 1971; Stern et al., 1994; Buttery et al., 1990). A recent study determined odor thresholds of 15 important tomato

volatiles in deionized water, ethanol / methanol / deionized water mix and deodorized tomato homogenate. It suggested odor-suppressing effects for ethanol and methanol and found *cis*-3-hexenal with the highest odor unit in all the mediums studied (Tandon, 1997).

A common problem encountered in all the methods mentioned is that they have little or no relationship to perception of aroma by the human nose during eating. The process which incorporates mastication, saliva, enzymes in saliva, and air flow dynamics can not be adequately simulated by mechanical maceration. Therefore, a term 'nosepace' is used to define the volatile profile existing in the nose during eating of a food product, and techniques to measure this profile have been developed (Taylor et al., 1994). The volatiles were trapped on Tenax through breath expiration via the nose and analyzed. Proportions of 2+3-methylbutanal, 2+3-methylbutanol, 2-isobutylthiazole, 3-methylnitrobutane and *cis*-3-hexenal increased in the nosepace during tomato eating, while hexanal and other C6 compounds of lipid oxidation did not (Linforth et al., 1994). Compounds formed during ripening (e.g., isobutylthiazole) were shown to be rapidly released in the mouth, whereas compounds formed by lipid oxidation are released rapidly (hexanal) or more slowly (hexenol) depending on the enzyme reactions that form them (Brauss et al., 1998).

It has recently been made clear (Baldwin et al., 2000) that plant breeders and molecular biologists do not have clear genetic markers for selection and manipulation of tomato flavor quality. Henceforth, it is essential to identify each important aroma volatile, and to analyze its

sensory properties. Biochemical pathways for the synthesis of these molecules must also be elucidated before clear targets for genetic improvements can be identified.

Conclusion

Tomatoes are important products providing vitamin A and C with a specially appealing flavor. Characteristic tomato flavor is a result of the interaction of taste components and aromatic volatiles. Sugars, organic acids, free amino acids, and salts are the non-volatile constituents of tomato flavor. Glucose and fructose make up about 50% of the dry matter. The main organic acid is citric acid. Glutamic acid is the major free amino acid found in tomato juice. Potassium and phosphate are the most abundant minerals in fresh tomatoes. There are over 400 aroma volatiles in fresh tomatoes, but only about 30 contribute significantly to the flavor. The most important volatile compounds are *cis*-3-hexenal, hexanal, *cis*-3-hexenol, β -ionone, β -damascenone, 1-penten-3-one, 3-methylbutanal, 2-isobutylthiazole and others. Tissue disruption mixes enzymes, substrates and air to initiate reactions; hence, enzyme inactivation is required during essential oil isolation. Qualitative and quantitative analyses of aroma compounds are usually achieved by GC-MS, GC-IR, NMR, GC-O, AEDA, and threshold studies. Determination of fresh tomato flavor is essential for genetic marker identification to improve the flavor quality of the product. Hence, further studies with tomato aroma volatiles, and their effects on the senses, in addition to details on biosynthesis are necessary.

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