Occurrence of Flavonols in Tomatoes and Tomato-Based Products

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The flavonol contents of 20 varieties of tomato fruit were investigated in relation to variety, size, season, and country of origin. Ten commonly consumed tomato-based food products were also assessed. Free and conjugated flavonols were identified and quantified using reversed-phase HPLC. Ninety-eight percent of flavonols detected in tomatoes were found to occur in the skin. Tomatoes contained, primarily as conjugates, quercetin and kaempferol. The main quercetin conjugate was identified as rutin (quercetin 3-rhamnosylglucoside) by LC-MS. The total flavonol content of the different varieties of tomato that were analyzed varied from 1.3 to 22.2 μg/g of fresh weight (fw). Smaller cherry tomato fruits originating from warm sunny climates, such as Spain and Israel, were found to contain the highest concentration of flavonols. Among the tomato-based products investigated, tomato juice and tomato purée were rich in flavonols, containing 14−16 μg/mL and 70 μg/g fw, respectively. In contrast to fresh tomatoes, most tomato-based products contained significant amounts of free flavonols.

Keywords: *Flavonols; rutin; quercetin; kaempferol; fresh tomatoes; canned tomatoes; tomato juice; tomato purée; tomato soup; HPLC*

INTRODUCTION

The flavonoids are a large family of low molecular weight polyphenolic compounds that are found in plant tissues and include the flavones, flavonols, flavonones, isoflavones, flavan-3-ols, and anthocyanins (Haslam, 1998). More than 4000 flavonoids have been described, most are conjugated to sugar molecules and are commonly located in the upper epidermal layer of leaves. The role of flavonoids in plants is unclear, although many functions have been proposed, including protection against UV-B radiation and defense against pathogen attack (Li et al., 1993; Dixon and Paiva, 1995).

High dietary intake of fruits and vegetables is known to reduce the incidence of some cancers (Block et al., 1992; Giovannucci., 1999). Although it is thought that non-nutritive compounds, such as flavonoids, may play a role in the protective effect of fruit and vegetables, epidemiological studies have drawn conflicting conclusions on the association between flavonoids and the incidence of cancer. The Zutphen study found no correlation between flavonoid intake, in the form of flavonols and smaller amounts of flavones, and lung, colorectal or all-cause cancer (Hertog et al., 1993), and a Spanish study similarly found no significant link between flavonol intake and protection against lung cancer (Garcia-Closas et al., 1998). In contrast, a clear inverse association between flavonol intake and the incidence of lung cancer was observed in a Finnish study (Knekt et al., 1996), and a protective effect of flavonols against gastric cancer was also found in a Spanish study involving patients with gastric adenocarcinoma (Garcia-Closas et al., 1999). Thus, information on the potency of flavonols as anticarcinogens is at present equivocal, and further studies are required before any firm conclusions can be drawn.

Indications of a protective effect of dietary flavonols against the development of free radical-derived damage to endothelial cells of the coronary arteries and the development of atherosclerosis (DeWhalley et al., 1990; Vinson et al., 1995) have led to epidemiological studies on the relation between dietary flavonol intake and the incidence of coronary heart disease (CHD). The Dutch Zutphen Elderly Study highlighted a positive correlation between the high flavonol/flavone intake and a reduction in CHD (Hertog et al., 1993). A similar relationship was observed in a Finnish cohort study (Knekt et al., 1996). Contrasting results on the prevention of CHD by dietary flavonols were found in a study of male health professionals in the United States. Although this investigation did not support a protective effect of high flavonol intake on total incidence of CHD, it could not rule out a possible protective effect on men with established CHD (Rimm et al., 1996).

Daily intake of flavonols and flavones in The Netherlands was estimated at 26 mg, with major sources being identified as tea (18−50 mg/L), onions (284−486 μg/g), and apple (21−72 μg/g) (Hertog et al., 1993). Recent work (Crozier et al., 1997b) has shown that certain types of tomatoes contain much higher levels of flavonols than were measured in the Dutch study (Hertog et al., 1992a). Also, as sizable amounts of the flavonols in tomatoes are recovered after cooking (Crozier et al., 1997b), the consumption of tomatoes and

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tomato-derived products in foods, such as pizza and lasagne, may provide a more significant contribution to the intake of flavonols in the Western diet than is currently realized.

This paper describes the flavonols found in tomato and the distribution of these compounds within the fruit. The flavonol content of fruits was investigated in relation to variety, season, and country of origin. In addition, flavonols in commonly consumed processed tomato products were also analyzed.

MATERIALS AND METHODS

Tomato Suppliers. Scottish tomatoes were provided by Scotland's Tomatoes plc (Clyde Valley, Lanarkshire, U.K.), and English tomatoes were obtained from English Village Salads plc (Banks, Southport, Lancs, U.K.). Seeds of tomato fruits with various skin colors were obtained from the C. M. Rick Stock Center (University of California, Los Angeles, CA) with the exception of Noire Charbonneuse, which was purchased from Simpson's Seeds (Surrey, U.K.). Spanish tomato fruits, cv. Bodar, Bond, Royesta, and Havanera, were kindly donated by Dr. Jesús Chammaro, Institute of Celluology, CSIC, Valencia, Spain. Other tomatoes from Spain, as well as Israel and South Africa, were supplied by Mr. B. Sparkes (English Village Salads plc). Tomatoes from the south of France were obtained from a local market in Toulouse by Sophie Bozonnet. All other tomato fruits were purchased from Safeway Stores plc (373 Byres Road, Glasgow G12, U.K.).

Growth of Soil-Grown *Lycopersicon esculentum*. Seeds obtained from the C. M. Rick Center (Los Angeles, CA) and Simpson's Seeds (Surrey, U.K.) were planted beneath 1 cm of sterile soil and moistened with distilled water. Plant pots were covered with cling film and placed in a growth cabinet with controlled conditions of white light (80−100 μeinstein/m^2/s) at 20 °C. The photoperiod was 16 h of light and 8 h of darkness. Following production of the first fruiting truss, plants were fed at alternate waterings with Tomorite liquid tomato fertilizer (Levington, Ipswich, U.K.). Plants were grown for 4−5 months prior to collection of fruits for flavonol analysis.

Processed Tomato Produce. Canned tomato soup (Safeway), tomato juice (Del Monte and Libby's), canned cherry tomatoes, and canned peeled plum tomatoes (Napolina), pasta sauce (Dolmio), tomato ketchup (Heinz), and tomato purée (Casino, Masque D'or, and Safeway) were purchased from Safeway Stores plc.

Sample Preparation. Tomato fruits (4−5 normal sized, 9−10 cherry tomatoes) and processed products, excluding tomato juice and tomato soup, were lyophilized and ground to a fine powder prior to acid hydrolysis. Tomato soup and tomato juice were hydrolyzed fresh.

Extraction and Hydrolysis Conditions. Optimization of acidic conditions for the hydrolysis of flavonol conjugates has been described by Hertog et al. (1992b) following an earlier study by Harborne (1965) on the release of free flavonols by acid and enzymatic hydrolyses. Preliminary screening was carried out to ascertain the most effective acid hydrolysis conditions for the tissues involved in this study. Samples of tomato fruit and processed products (20 mg of lyophilized material) and tomato juice and tomato soup (450 μL) were hydrolyzed at 90 °C for 2 h in a 3 mL glass V-vial containing 2 mL of 1.2 M HCl in 50% aqueous methanol, 5 μg of morin as an internal standard, and 20 mM sodium diethyldithiocarbamate as an antioxidant. A Teflon-coated magnetic stirrer was placed in the vial, which was sealed tightly with a PTFE-faced septum prior to heating in a Reacti-Therm heating/stirring module (Pierce, Rockford, IL). Extract aliquots of 100 μL, taken both before and after acid hydrolysis, were made up to 250 μL with distilled water adjusted to pH 2.5 with trifluoroacetic acid and filtered through a 0.2 μm Anopore filter (Whatman, Maidstone, Kent, U.K.), prior to the analysis of 100 μL volumes ($^1/_{50}$ aliquot of total sample) by gradient elution reverse-phase high-performance liquid chromatography (HPLC). All samples were analyzed in triplicate.

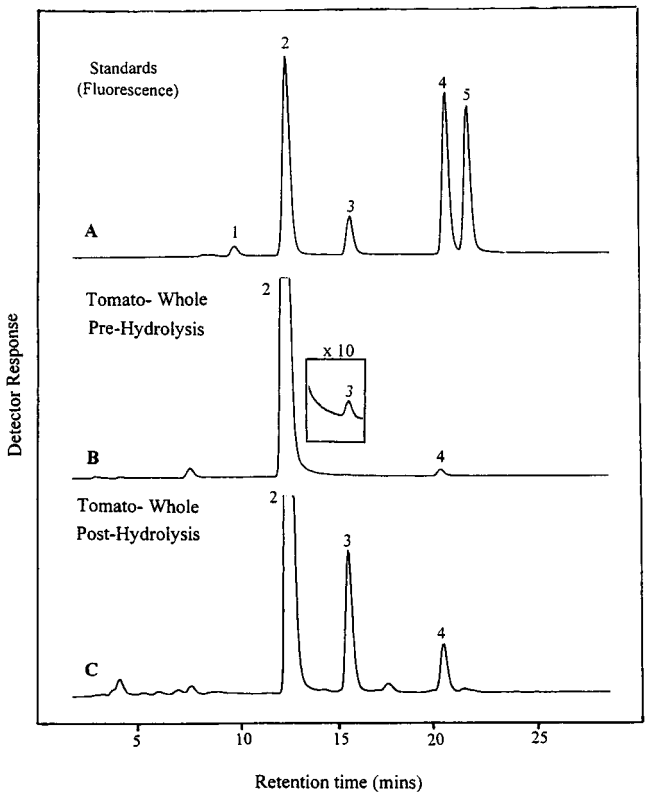

Figure 1. Gradient reverse-phase HPLC analysis of flavonols with detection by fluorescence after postcolumn derivatization. Samples: (A) 100 ng of (1) myricetin, (2) morin, (3) quercetin, (4) kaempferol, and (5) isorhamnetin; (B) 1 mg sample of lyophilized tomato tissue, cv. Paloma; (C) same as (B) but after acid hydrolysis. Numbers indicate peaks that cochromatograph with standards listed for sample A. Morin was added to samples B and C as an internal standard.

HPLC and Postcolumn Derivatization. Samples were analyzed using a Shimadzu (Kyoto, Japan) LC-10A series automated liquid chromatograph comprising a SCL-10A system controller, two LC-10A pumps, a SIL-10A autoinjector with sample cooler, a CTO-10A column oven, and a SPD-10A UV−vis detector linked to a Reeve Analytical (Glasgow, U.K.) 2700 data handling system. Reversed-phase separations were carried out at 40 °C using a 150 × 3.0 mm i.d., 4 μm Genesis C_{18} cartridge column fitted with a 10 × 4.0 mm i.d., 4 μm C_{18} Genesis guard column in an integrated holder (Jones Chromatography, Mid-Glamorgan, U.K.). The mobile phase was a 20-min, 20−40% gradient of acetonitrile in distilled water adjusted to pH 2.5 with trifluoroacetic acid, eluted at a flow rate of 0.5 mL/min (Crozier et al., 1997a). Column eluent was first directed to the SPD-10A absorbance monitor operating at 365 nm, after which postcolumn derivatization was achieved by the addition of methanolic aluminum nitrate containing 7.5% glacial acetic acid, as described by Hollman and Trijp (1996), pumped at a flow rate of 0.5 mL/min by a pulse-free model 9802 precision mixer/splitter (Reeve Analytical). The mixture was passed through a 1.9 m × $^{30}/_{1000}$ in. i.d. loop of peek tubing in the column oven before being directed to an RF-10A fluorometer, and fluorescent flavonol complexes were detected at excitation 425 nm and emission 480 nm. The limit of detection at A_{365nm} was <5 ng, and linear 5−250 ng calibration curves were obtained for morin, rutin, quercetin, kaempferol, and isorhamnetin. The fluorescence intensity of the individual flavonoid derivatives varied; however, 0.1−100 ng linear calibration curves were obtained for morin, myricetin, quercetin, kaempferol, and isorhamnetin.

Estimates of Free and Conjugated Flavonol Levels. Free flavonols were detected in the unhydrolyzed sample, whereas the hydrolyzed samples contained both free and conjugated. Thus, conjugated flavonol levels were estimated

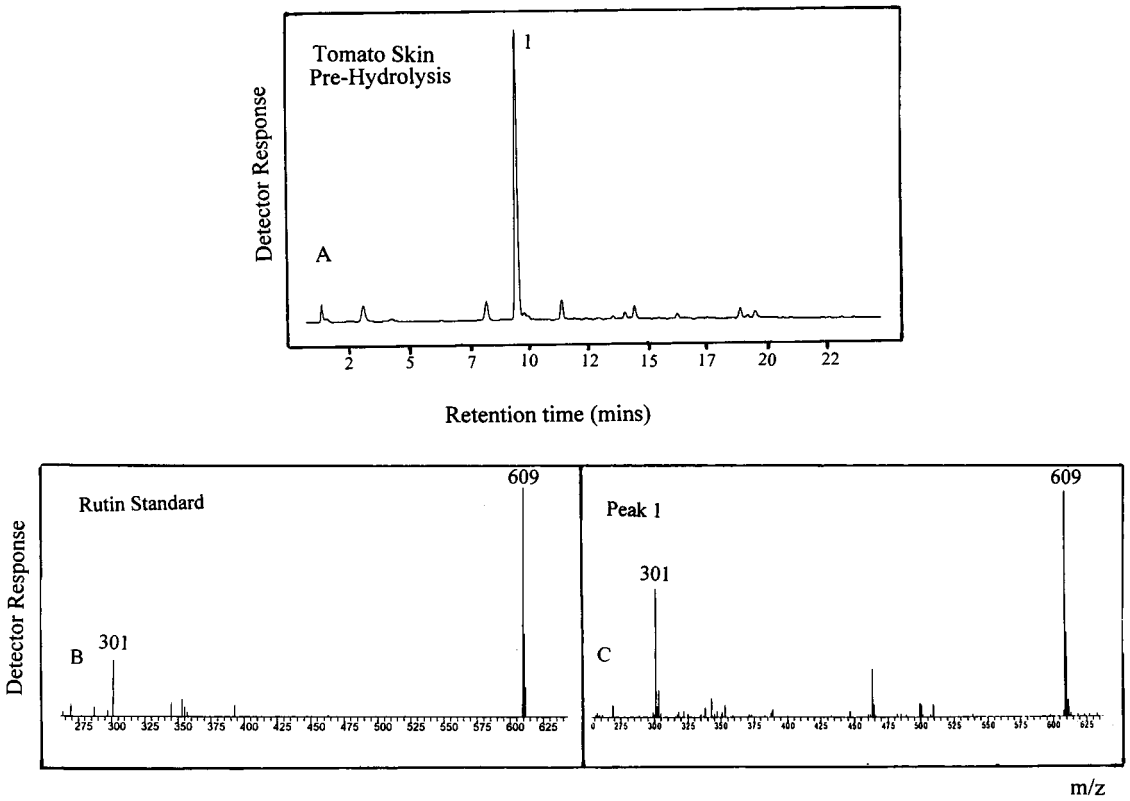

Figure 2. Gradient reverse-phase HPLC analysis of flavonols with detection by absorbance (371 nm) and mass spectrometry (APCI, negative ion mode). Samples: (A) an extract from 1 mg of lyophilized tomato fruit skin analyzed prior to acid hydrolysis with detection using an absorbance monitor operating at 371 nm; (B) *m/z* 250−650 mass spectrum of a rutin standard; (C) *m/z* 250−650 mass spectrum of prehydrolyzed tomato skin sample, peak 1 in trace A.

by subtracting the amount found in the unhydrolyzed sample from that detected after acid hydrolysis.

Liquid Chromatography−Mass Spectrometry (LC-MS). Samples were analyzed using a Shimadzu LC-10A *vp* series automated liquid chromatograph comprising an SCL-10A *vp* system controller, two LC-10A *vp* pumps, an SIL-10AD *vp* autoinjector with sample cooler, a CTO-10AC *vp* column oven, and an SPD-10A *vp* UV−vis detector. Reverse-phase separations were carried out at 40 °C using a 150 × 3.0 mm i.d., 5 μm Nemesis C_{18} column. The mobile phase was a 20-min gradient of 12−35% acetonitrile containing 1% formic acid, maintained for a further 5 min at 35%. The flow rate was 0.8 mL/min, and column eluent was first passed through the SPD-10A *vp* absorbance monitor operating at 371 nm before being directed to a Shimadzu LCQ 8000 quadrupole mass spectrometer with atmospheric pressure chemical ionization (APCI) and a nebulizing gas flow of 2.5 L/min. Full scan *m/z* 250−650 negative ion spectra were obtained every 4 s. Data obtained were analyzed using Shimadzu LCMS QP 8000 software.

Reference Compounds. Morin, myricetin, quercetin, rutin, and kaempferol were purchased from Sigma-Aldrich (Poole, Dorset, U.K.).

RESULTS AND DISCUSSION

Early work on the flavonol content of tomatoes, summarized by Woeldecke and Herrmann (1974), indicated that the fruit contained quercetin 3-rhamnosylglucoside (rutin) together with smaller amounts of quercetin 3-rhamnosyldiglucoside, kaempferol 3-rhamnosylglucoside, and kaempferol 3-rhamnosyldiglucoside. In the present study, highly sensitive reverse-phase HPLC with postcolumn derivatization detected trace amounts of quercetin and kaempferol in Spanish cherry tomato samples prior to acid hydrolysis. However, much larger quantities were detected after acid hydrolysis (Figure 1), indicating that the endogenous flavonols are present in tomato tissues primarily as conjugates.

Analysis of Spanish cherry tomato skin extracts by LC-MS prior to acid hydrolysis confirmed that the predominant quercetin conjugate was rutin (Figure 2). As they were present in only trace amounts, it was not possible to identify additional quercetin or kaempferol conjugates. However, LC-MS analysis of acid-hydrolyzed samples confirmed the presence of quercetin and kaempferol (Figure 3) released by cleavage of their conjugated derivatives.

Screening of the flavonol content of fruits and vegetables by Hertog et al. (1992a) included the quantification of flavonols in Dutch tomatoes at four time points over a 12 month period. The amounts detected were between 4.6 and 8.2 μg of quercetin/g (fresh weight) and <2 μg of kaempferol/g. The samples were analyzed only after acid hydrolysis and therefore provided no information on the relative proportion of free and conjugated flavonols. More recently, work by Crozier et al. (1997b) on the flavonol content of Spanish, Scottish, and Dutch tomatoes showed that quercetin is present almost exclusively as conjugates, an observation confirmed by the present study. Flavonols were previously found to be present primarily as conjugates in a wide range of fruits and vegetables as reported by Herrmann (1976, 1988).

Distribution of Flavonols within Tomato Fruits. When different parts of Spanish cherry tomatoes were examined, 98% of the main flavonol, conjugated quercetin, was found in the skin compared to ~1% in the seeds and flesh (Table 1). Quercetin contributes ~96% (138.5 ± 5.6 μg/g) of the skin-derived flavonols with the remainder consisting of kaempferol (4.8 ± 0.3 μg/g). In many plant species the accumulation of flavonols is

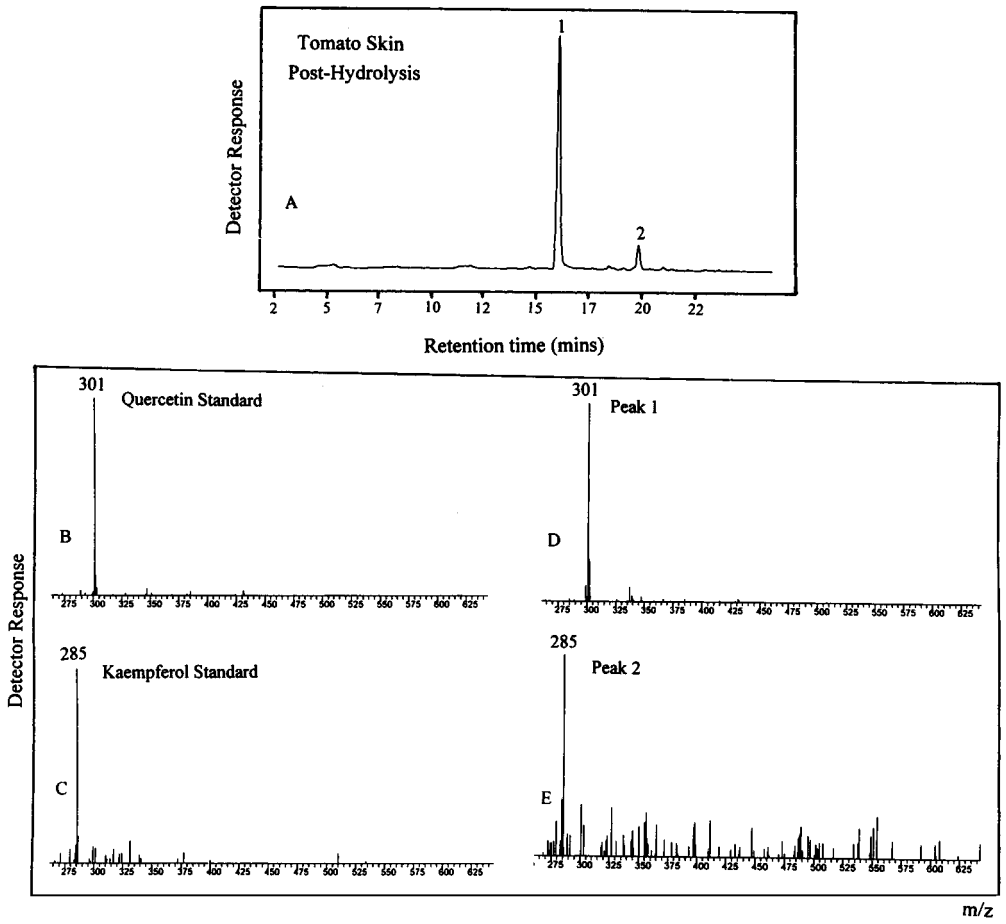

Figure 3. Gradient reverse-phase HPLC analysis of flavonols with detection by absorbance (371 nm) and mass spectrometry (APCI, negative ion mode). Samples: (A) extract from 1 mg of lyophilized tomato fruit skin after acid hydrolysis analyzed using UV detection at 371 nm; (B) *m*/*z* 250−650 mass spectrum of a quercetin standard; (C) *m*/*z* 250−650 mass spectrum of a kaempferol standard; (D) *m*/*z* 250−650 mass spectrum of peak 1 in sample A; (E) *m*/*z* 250−650 mass spectrum of peak 2 in sample A.

Table 1. Distribution of Flavonols in Spanish Cherry Tomatoes (*L. esculentum* Mill.) Cv. Paloma[a]

tomato	free quercetin	free kaempferol	conjugated quercetin	conjugated kaempferol	total flavonol
whole	0.2 ± 0.0	0.5 ± 0.1	23.4 ± 1.2	1.2 ± 0.1	25.3 ± 1.3
skin	0.7 ± 0.0	0.4 ± 0.0	137.8 ± 5.6	4.4 ± 0.3	143.3 ± 5.8
flesh	nd	0.1 ± 0.0	0.9 ± 0.0	0.2 ± 0.1	1.2 ± 0.1
seed	0.1 ± 0.0	0.2 ± 0.0	1.0 ± 0.1	0.2 ± 0.0	1.5 ± 0.1

[a] Tomatoes were purchased from Safeway Stores plc. Data are expressed as μg/g (fw) ± SE (n = 3).

enhanced in response to elevated light levels, in particular to increased UV-B radiation (Lois, 1994; Brandt et al., 1995). There is evidence that quercetin is present in much higher concentrations in the skin of Pinot Noir grapes grown in the open rather than shaded clusters (Price et al., 1995). Exposure to high levels of sunlight may therefore result in the accumulation of increased amounts of protective compounds, including flavonols in the skin of tomato fruit.

Fruit Size. The total flavonol content of Spanish Favorita, a red cherry tomato, was 21.5 μg/g, compared to the normal-sized fruit variety, Bond, which contained 10.9 μg/g (Table 2). Compared with an earlier study that included commercial English and Spanish cherry tomatoes (Crozier et al., 1997b), the flavonol contents of the English-grown cherry varieties, Favorita and Cherry Belle, were very low indeed, with the levels no different from those detected in normal-sized fruits from the same source (Table 2).

When flavonol levels in Spanish Favorita and English Spectra fruit are calculated on a square centimeter of skin basis, the cherry tomato contains 12.5 μg/cm^2 compared to 2.4 μg/cm^2 in the skin of the Spectra fruit. The greater skin/volume ratio of cherry tomatoes will further enhance their flavonol content compared to that of cultivars with larger fruit.

Country of Origin. Analysis of Favorita obtained from England, Spain, South Africa, and Scotland showed that fruits from Spain and South Africa contained 4−5-fold more flavonol than British fruits (Table 2). Commercial tomato growing in Scotland and England usually involves the use of greenhouses, with plants tightly packed together and fruits shaded by the foliage of surrounding plants. Fruit grown in greenhouses is effectively grown in conditions of relatively low light (~35 μeinstein/m^2/s), with UV-B from sunlight filtered first through glass and then through surrounding leaf tissue. This may limit the induction and reduce the accumulation of flavonols in the skin of the tomatoes. In contrast, fruit from warmer sunnier countries such as Spain are usually field-grown, and if necessary they are shielded from the elements using plastic rather than

Table 2. Flavonol Content of Tomato Fruits Produced in Various Countries[a]

country of origin	tomato variety	date of harvest	free quercetin	free kaempferol	conjugated quercetin	conjugated kaempferol	total flavonol
Spain	Bodar	July 1997	0.1 ± 0.0	0.2 ± 0.0	7.7 ± 0.9	0.4 ± 0.1	8.4 ± 1.0
	Royesta	July 1997	0.1 ± 0.0	0.3 ± 0.0	12.7 ± 0.4	0.7 ± 0.1	13.8 ± 0.4
	Bond	July 1997	0.1 ± 0.0	0.3 ± 0.0	10.2 ± 0.5	0.4 ± 0.0	10.9 ± 0.5
	Favorita[C]	Jan 1997	nd	0.2 ± 0.0	20.7 ± 0.6	0.6 ± 0.1	21.5 ± 1.8
	Havanera	July 1997	nd	0.2 ± 0.0	5.6 ± 0.9	0.8 ± 0.1	6.6 ± 1.0
Israel	unknown[C]	March 1997	nd	0.2 ± 0.1	21.5 ± 0.8	0.5 ± 0.0	22.2 ± 0.8
South Africa	Favorita[C]	Feb 1997	nd	0.2 ± 0.1	15.0 ± 0.7	0.8 ± 0.0	16.0 ± 0.7
southern France	unknown (market)	Sept 1997	nd	0.2 ± 0.0	10.3 ± 0.4	1.3 ± 0.0	11.8 ± 0.5
	unknown (Toulouse)	Sept 1997	0.2 ± 0.0	0.2 ± 0.0	13.1 ± 1.1	0.4 ± 0.0	13.9 ± 1.1
England	Favorita[C]	July 1996	nd	0.2 ± 0.0	3.0 ± 0.1	0.2 ± 0.1	3.4 ± 0.1
	Cherry Belle[C]	July 1996	nd	0.2 ± 0.0	3.9 ± 0.6	0.2 ± 0.0	4.3 ± 0.6
	102-Yellow[Y]	July 1996	nd	0.2 ± 0.0	2.1 ± 0.1	0.2 ± 0.0	2.5 ± 0.1
	Flavore	July 1996	nd	0.1 ± 0.0	1.6 ± 0.2	0.1 ± 0.0	1.8 ± 0.2
	Spectra	July 1996	nd	0.1 ± 0.0	3.0 ± 0.4	0.1 ± 0.0	3.2 ± 0.4
	Aromata	July 1996	nd	0.1 ± 0.0	1.2 ± 0.0	nd	1.3 ± 0.1
Scotland	Vanessa 2000[B]	June 1997	nd	0.1 ± 0.0	1.6 ± 0.2	0.1 ± 0.0	1.8 ± 0.2
	Vanessa Beefsteak[B]	June 1997	0.2 ± 0.1	0.1 ± 0.0	1.8 ± 0.1	0.2 ± 0.0	2.3 ± 0.1
	72/47	June 1997	0.2 ± 0.1	0.2 ± 0.0	3.0 ± 0.0	0.3 ± 0.0	3.7 ± 0.1
	E27 681[C]	June 1997	0.1 ± 0.0	0.2 ± 0.0	10.9 ± 0.0	0.7 ± 0.0	11.9 ± 0.1
	Favorita[C]	June 1997	nd	0.2 ± 0.0	5.4 ± 0.1	1.0 ± 0.1	6.6 ± 0.1

[a] Data are expressed as μg/g (fw) ± SE ($n = 3$). [C] denotes cherry tomatoes, [Y] yellow tomatoes, and [B] beefsteak tomatoes. nd, not detected. Tomato suppliers: Scottish fruits, Scotland's Tomatoes plc; English fruits, English Village Salads plc; Spanish and South African variety Favorita and unknown Israeli fruits, Mr. B. Sparkes, English Village Salads plc; Spanish varieties, Dr. Jesus Chammaro, Valencia; southern France, S. Bozonnet, Toulouse.

Table 3. Monthly Variations in the Flavonol Content of Spanish Cherry Tomato Cv. Paloma between February 1997 and February 1998[a]

date of harvest	free quercetin	free kaempferol	conjugated quercetin	conjugated kaempferol	total flavonol
February 1997	nd	0.3 ± 0.0	10.9 ± 1.1	0.2 ± 0.1	11.4 ± 1.2
March	nd	0.3 ± 0.0	19.8 ± 2.2	0.4 ± 0.1	20.5 ± 2.2
April	nd	0.3 ± 0.1	24.6 ± 2.2	0.5 ± 0.0	25.4 ± 2.2
May	0.5 ± 0.1	0.3 ± 0.1	18.6 ± 1.2	0.4 ± 0.1	19.8 ± 1.4
June	0.2 ± 0.0	0.5 ± 0.1	23.4 ± 1.2	1.2 ± 0.1	25.3 ± 1.3
July	0.1 ± 0.0	0.2 ± 0.0	14.8 ± 0.7	0.7 ± 0.1	15.9 ± 0.8
August	0.1 ± 0.0	0.2 ± 0.0	16.7 ± 1.9	0.6 ± 0.1	17.6 ± 2.0
September	0.1 ± 0.0	0.2 ± 0.0	13.8 ± 1.4	1.2 ± 0.2	15.2 ± 1.4
October	0.2 ± 0.0	0.3 ± 0.0	25.7 ± 1.2	1.7 ± 0.2	27.8 ± 1.4
November	0.7 ± 0.3	0.2 ± 0.0	15.0 ± 0.2	1.0 ± 0.0	16.3 ± 0.2
December	0.1 ± 0.0	0.3 ± 0.0	22.5 ± 0.5	1.0 ± 0.0	24.0 ± 0.5
January 1998	0.2 ± 0.0	0.4 ± 0.0	33.4 ± 1.3	2.3 ± 0.3	36.4 ± 1.2
February	0.1 ± 0.0	0.2 ± 0.0	9.7 ± 0.4	0.3 ± 0.0	10.2 ± 0.4

[a] Data are expressed as μg/g (fw) ± SE ($n = 3$).

glass. The developing fruits would receive more sunlight and would be exposed to UV-B radiation. Cherry tomato E27 681, obtained from Scotland's Tomatoes, was found to contain higher levels of flavonols (11.9 ± 0.1 μg of quercetin/g) than the other Scottish-grown cherry tomatoes. However, E27 681 was grown in a small experimental greenhouse with plants well spaced out with little shading of fruit.

Effect of Season. The flavonol content of Spanish-grown cherry tomato, Paloma, was assessed over a period of 13 months. The levels fluctuated but not markedly, with total flavonols ranging from 10.2 to 36.4 μg/g (Table 3). Flavonol contents in these field-grown fruits would be affected by a wide range of environmental influences including light levels, temperature, pathogen attack, and nutritional status (Landry et al., 1995; Dixon and Paiva, 1995; Christie et al., 1994; Bongue-Bartelsman and Phillips, 1995). Despite these variables, growing conditions in Spain induce the accumulation of a relatively high flavonol content in tomato fruits throughout most of the year.

Effect of Variety. Fruit variety was also identified as a factor affecting the flavonol content of tomatoes. Bond and Havanera are both normal-sized, field-grown Spanish tomatoes obtained from plants cultivated alongside each other in the same plot near Valencia (January 1997); nonetheless, the total flavonol content of Bond fruit was 10.9 ± 0.5 μg/g compared to 6.6 ± 1.0 μg/g in Havanera (Table 2).

The concentration of flavonols in tomato fruits with deep red and purple skins was investigated. It was hypothesized that the skins of such varieties may contain substantial amounts of anthocyanins, and as flavonols originate from the same branch of the phenylpropanoid pathway as anthocyanins (Holton and Cornish, 1995; Duthie and Crozier, 2000), the skins might also contain elevated levels of flavonols. Red-leaved, anthocyanin-rich varieties of lettuce have been shown to contain very high levels of conjugated quercetin compared to many green-leaved varieties (Crozier et al., 1997b).

Fruits were harvested at ripeness, and the flavonol content of the skins was analyzed. The darkly pigmented skin of Noire Charbonneuse had a total flavonol content of 440 μg/g (Table 4). The flavonol content of Anthocyanin Gainer, a deep red fruit with yellow "freckles", was also found to be high. However, not all of the darkly pigmented fruits were high in flavonols; skin from Aubergine, a variety characterized by purple striations, contained only 108 μg of flavonol/g. In contrast, skin from Anthocyanin Free and Dark Green, which were not heavily pigmented, contained 224 and

Table 4. Flavonol Content of Fruit Skins from Tomato Varieties with Different Colored Skins[a]

tomato variety	skin color	free quercetin	free kaempferol	conjugated quercetin	conjugated kaempferol	total flavonol
Noire Charbonneuse	red/purple	3.9 ± 0.2	0.2 ± 0.0	402 ± 14	14.2 ± 2.0	440 ± 29
Anthocyanin Gainer	deep red	3.0 ± 0.4	0.4 ± 0.0	252 ± 30	20.9 ± 1.0	276 ± 32
Aubergine	red/dark patches	0.3 ± 0.0	nd	103 ± 7	4.5 ± 0.2	108 ± 7
Anthocyanin Free	red	0.6 ± 0.0	0.1 ± 0.0	206 ± 20	17.3 ± 0.9	224 ± 19
Dark Green	red/yellow	0.8 ± 0.1	nd	183 ± 11	4.9 ± 0.4	189 ± 12

[a] Data are expressed as μg/g (fw) ± SE ($n = 3$).

Table 5. Free and Conjugated Quercetin, Kaempferol, and Isorhamnetin Content of Tomato-Based Food Products[a]

tomato product	brand	free quercetin	free kaempferol	conjugated quercetin	conjugated kaempferol	total flavonol	free flavonol %
tomato soup	Safeway	0.3 ± 0.0	nd	1.2 ± 0.1	nd	1.5 ± 0.1	19.6
tomato juice	Del Monte	2.9 ± 0.1	0.4 ± 0.0	11.5 ± 1.8	0.4 ± 0.1	15.2 ± 1.9	21.6
	Libby's	3.5 ± 0.2	0.4 ± 0.0	12.7 ± 1.0	0.3 ± 0.0	16.9 ± 1.0	22.9
canned cherry tomatoes	Napolina	nd	nd	1.7 ± 0.1	0.1 ± 0.0	1.8 ± 0.1	0
canned plum tomatoes	Napolina	nd	nd	0.3 ± 0.0	nd	0.4 ± 0.0	0
pasta sauce	Dolmio	1.2 ± 0.2	nd	7.9 ± 0.6	0.1 ± 0.0	9.2 ± 0.5	12.6
ketchup	Heinz	0.4 ± 0.0	nd	8.2 ± 0.5	0.1 ± 0.0	8.8 ± 0.5	4.5
purée	Casino	3.8 ± 0.2	0.6 ± 0.3	32.5 ± 4.0	0.2 ± 0.0	37.1 ± 4.3	11.9
	Masque D'or	5.4 ± 0.5	nd	10.9 ± 2.1	0.3 ± 0.0	16.6 ± 1.7	32.5
	Safeway	9.5 ± 1.6	nd	61.4 ± 5.5	1.3 ± 0.2	72.2 ± 5.8	13.2

[a] Data for tomato juice and tomato soup are expressed as μg/mL ± SE ($n = 3$); all other data are expressed as μg/g (fw) ± SE ($n = 3$). nd, not detected.

189 μg/g, respectively. It is clear that the flavonol contents of tomato fruits do not necessarily correlate with their anthocyanin contents; nevertheless, this approach allowed the identification of Noire Charbonneuse as a tomato variety rich in flavonols.

Processed Tomato Produce. A range of processed tomato products were analyzed, and the data obtained are summarized in Table 5. Tomato juice was found to be a rich source of flavonols with total flavonol contents of 15.2−16.9 μg/mL, comparable with that of red wine, which can vary from 4.6 to 41.6 μg/mL (McDonald et al., 1998). In contrast to tomato fruit, which contains almost exclusively conjugated quercetin, up to 30% of the quercetin in processed produce was in the free form. Hydrolysis of flavonol conjugates during cooking of tomatoes was not observed in an earlier study (Crozier et al., 1997b), so the accumulation of quercetin in juices, purée, and paste may be a consequence of enzymatic hydrolysis of rutin and other quercetin conjugates during pasteurization and processing procedures. The concentration of flavonols in tomato juice is likely to depend on the extraction of flavonols from the skin into the juice during initial processing, which often involves heating, and also on the amount of skin remaining in the tomato juice following filtration. Safeway tomato purée was also identified as being particularly rich in flavonols, containing ∼70 μg/g. Analysis of canned tomatoes revealed that canned cherry tomatoes and canned peeled plum tomatoes (Napolina) both contained extremely low levels of flavonols compared to fresh fruit. This could be due to boiling of the fruit prior to canning as cooking in this manner results in up to an 80% loss of flavonols (Crozier et al., 1997b), presumably by leaching from the skins.

In summary, tomatoes and tomato products are a rich source of conjugated quercetin and kaempferol. Flavonol contents were found to vary according to fruit variety, size, and country of origin, with cherry tomatoes originating from warm, sunny climates such as Spain containing the highest concentrations. Tomato flavonols were able to withstand industrial processing methods, allowing their detection in a variety of tomato-based products. Tomato juice and tomato purée were found to be particularly rich in flavonols. Because of the addition of tomato sauce to many foods and the widespread use of tomato pastes in dishes such as pizza and lasagne, tomatoes may directly and indirectly make a more sizable contribution to daily flavonol intake than was previously realized.

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