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Properties of Chalconaringenin and Rutin Isolated from Cherry Tomatoes

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ABSTRACT: Fresh cherry tomatoes cv. 'Susanne' contain more of the two flavonoids chalconaringenin (CN) and rutin than lycopene. Therefore some properties including antioxidant behavior of the flavonoids were studied. The two flavonoids were extracted from peel and isolated by use of different chromatographic methods. Molecular absorbtivities were found to be 26907 for CN and 20328 abs M^{-1} cm^{-1} for rutin. Both compounds exhibited properties as antioxidants through several assays, and rutin was found to be the strongest antioxidant except in one assay. None of the assays revealed pro-oxidative effects. As naringenin rather than CN is frequently reported as a tomato constituent, the stability of CN was investigated in order to detect potential ways of isomerization during sample preparation. CN isomerized slowly both under UVB radiation and in alkaline solutions. Thus, such factors do not explain the occurrence of naringenin in tomato samples. The deficiency in reports on CN may be explained by the similarity in chromatographic behaviors of CN and naringenin, and due to the fact that they have same molecular weights.

KEYWORDS: *Solanum lycopersicum* L., chalconaringenin, rutin, molar absorptivities, isomerization, antioxidants, FRAP, ABTS, DPPH, Price and Butler, Folin−Ciocalteu, nitric oxide

INTRODUCTION

Tomato is well recognized for its content of phytonutrients, especially the carotenoid lycopene. The fruit may also contain similar amounts of flavonoids, especially chalconaringenin (CN) and rutin (Figure 1). CN belongs to the primary class of flavonoids known as the chalcones. Most of the known flavonoid structures are metabolites from this class of flavonoids which is recognized as consisting of a C6−C3−C6 carbon system without the typical C-ring midstructure. Although its presence in tomato fruits was reported for the first time in 1980,[1] many publications report on naringenin though spectroscopic features points out CN to be the actual structure, e.g.[2] CN is a pale yellow compound, whereas its isomer, the flavanone naringenin, is colorless.

Rutin (quercetin 3-rutinoside) is a flavonol. It has been known as a tomato constituent since 1931.[3] Due to its ability to quench free radicals, its content in tomato fruits receives attention within horticultural and biotech research.[4−9] Both CN and rutin are basically located in the tomato peel. It thus follows that small tomatoes give a higher content of these compounds per unit serving compared to greater fruits. Their amounts in cherry tomatoes have been reported to be as high as 200 mg 100 g^{-1} fresh weight (FW) which exceeds the content of lycopene in average fruits (3 mg 100 g^{-1}).[10]

Instability of CN might be one of the reasons that naringenin rather than CN is reported. To investigate the stability of CN during sample preparation, a study was performed to look for any factors that could possibly give naringenin as an artifact. Moreover, little information is available with respect to spectrophotometric data as molar absorptivities of CN and rutin. Such features were therefore investigated. As the content of flavonoids in tomato in general is accepted to strongly contribute to the antioxidant status of the fruit, the radical quenching and redox ability of these major flavonoid structures were surveyed through several assays.

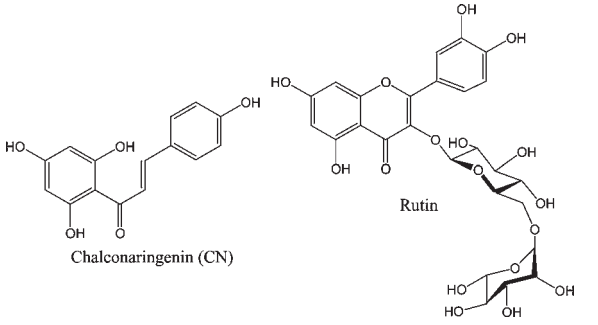

Figure 1. Chemical structures of the two most common flavonoids found in the cherry tomato cv. 'Suzanne': chalconaringenin and rutin.

MATERIALS AND METHODS

Plant Material. Cherry tomato plants cv. 'Susanne' with visible flowers were planted at a plant density of 3.0 plants m^{-2} on rock wool slabs (15 × 7.5 × 90 cm) on October 20, 2008, in greenhouse compartments at Bioforsk Vest Saerheim. Natural light was supplemented with light from high pressure sodium lamps (Philips SON/T 400 W), providing a photon flux density of 220 μmol m^{-2}-s^{-1} photosynthetic active radiation at plant height during 18 h day^{-1}. The temperature set points in the greenhouse were 25 °C at day and 18 °C at night, with venting at 25 °C. In the greenhouse, a water vapor pressure deficit of

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Table 1. Antioxidant Values and Radical Scavenging Capacities of Chalconaringenin (CN) and Rutin in Different Assays ($4 < n < 8$, ± SD)

assay	CN (μM)	CN result	rutin (μM)	rutin result	ref compd (μM)	ref compd result
Price and Butler (μM GA)	44	103 ± 12	42	65 ± 2		
	89	134 ± 16	84	102 ± 15	76	111 ± 7[a]
Folin−Ciocalteu (μM GA)	0.92	0.84 ± 0.02			0.3	1.11 ± 0.17[a]
	1.83	1.71 ± 0.02			0.75	1.72 ± 0.02[a]
	3.66	3.60 ± 0.17			1.49	3.17 ± 0.10[a]
FRAP (μM $FeSO_4$)	183	434 ± 24	104	388 ± 35	102	196 ± 10[b]
	366	833 ± 48	208	835 ± 56	205	457 ± 23[b]
	732	1322 ± 48	416	1769 ± 96	409	930 ± 30[b]
	1831	2318 ± 45	1039	4333 ± 86	1023	2367 ± 119[b]
	3662	3507 ± 52	2079	5776 ± 11	2045	4361 ± 202[b]
ABTS (% inhibition)	4	24.1 ± 0.6	5	33.1 ± 2.1		
	10	40.1 ± 0.5	9	54.5 ± 0.4		
	19	66.0 ± 0.3	17	84.9 ± 0.5		
DPPH (% inhibition)	5	5.4 ± 0.4	4	7.8 ± 0.3		
	9	7.8 ± 0.6	9	13.0 ± 0.9		
	19	13.0 ± 0.4	17	21.0 ± 4.2		
	38	22.1 ± 2.0	34	48.2 ± 1.1		
	190	43.8 ± 1.7	68	90.0 ± 6.3		
	379	57.0 ± 2.8	170	93.7 ± 0.1		
NO (% inhibition)	183	2.1 ± 0.8	104	5.5 ± 1.4	102	3.1 ± 0.6[b]
	366	3.8 ± 0.9	208	11.2 ± 0.8	205	5.7 ± 0.8[b]
	732	5.4 ± 0.6	416	19.2 ± 1.6	409	11.8 ± 1.7[b]
	1831	18.4 ± 1.0	1039	29.8 ± 0.4	1023	34.0 ± 3.5[b]
	3662	23.8 ± 0.7	2079	36.7 ± 0.5	2045	37.5 ± 2.1[b]

[a] Rutin from SigmaAldrich. [b] Ascorbic acid from SigmaAldrich.

3.0 g m^{-3} and a CO_2 level of 800 μmol mol^{-1} were maintained. Plants were watered sufficiently using a complete nutrient solution with an EC level of 3.5 mS cm^{-1} containing the following elements (in mg L^{-1}): N, 335; P, 61; K, 404; Ca 284; Mg,70; S, 91; Fe 4.0; Mn,1.2; Zn, 0.5; B, 0.4; Cu 0.05; Mo 0.06.

Chemicals. Chalconaringenin (CN) and rutin were isolated from cherry tomatoes as described below. In addition, rutin and all other chemicals were obtained from Sigma-Aldrich.

Extraction and Isolation. About 5 kg FW greenhouse grown 'Susanne' (Saerheim, December 2008) harvested at maturity stage V (red) was frozen at −20 °C and then partly thawed until the fruit easily could be peeled. The peel was extracted twice with 1.5 L of methanol (0.1% TFA) for 24 h at ambient temperature. The combined extracts were concentrated and then partitioned against *n*-hexane followed by ethyl acetate. After removal of solvent, the ethyl acetate extract was cleaned on a bed of Amberlite XAD-7 according to standard procedure.[11] Further purification of the extract was achieved by flash chromatography (VersaPak, Silica Cartridge, 40 × 150 mm, VersaFlash, Sigma Aldrich). Aliquots of 50 mg dissolved in 5 mL of ethyl acetate were loaded on the column, and ascending elution through two linearly connected cartridges was performed by use of ethyl acetate saturated with water. Fractions of 50 mL of colored eluent were collected. All fractions were concentrated on a rotary evaporator, and traces of water were removed by freeze-drying. In total, a yield of >555 mg CN was achieved from the plant material.

The water phase, from the partition of the crude extract against ethyl acetate, was concentrated and purified over a bed of XAD-7. The purified extract was further fractionated over an open top column (5 × 100 cm, Pyrex) of Sephadex LH-20 material. A step gradient of methanol in water starting with 30% methanol (v/v) (2 L) followed by 3 L of 50% and ending with 2 L of 70% methanol was used in order to fractionate the extract. Fractions of 200 mL were collected and analyzed by HPLC. Similar fractions were combined, and about 358 mg of rutin was achieved mainly from fractions eluted with 50% methanol. The characterization of the isolated compounds has previously been published.[12]

Molar Absorptivities. From each fraction of CN purified by flash chromatography, small samples (1−3 mg) were accurately determined by weight and dissolved in 100 mL of methanol. Molar absorptivities were established by use of a UV spectrophotometer (Agilent 8453, Agilent Technologies) at λ_{max} (365 nm) given a molar weight of 273.0708 g mol^{-1} of CN ($C_{15}H_{12}O_5$).[12] Samples with similar coefficients were pooled together giving four fractions of CN. Correspondingly, the molar extinction coefficient of rutin obtained from tomato peel was determined given a molar weight of rutin ($C_{27}H_{30}O_{16}$) of 611.1602 g mol^{-1}.[12]

Price and Butler Assay. The total phenol method of Price and Butler was evaluated for determination of CN and rutin.[13] A 20 mM solution of $FeCl_3$ was prepared in 0.10 M HCl, whereas a 16 mM $K_3Fe(CN)_6$ was prepared in water. Stabilizer was prepared according to Graham:[14] 30 mL of distilled water, 10 mL of 85% H_3PO_4, 10 mL of 1% gum arabic. Sample aliquots of 0.1 mL were mixed with 3 mL of distilled water and 1 mL of each of the $FeCl_3$ and $K_3Fe(CN)_6$ solutions. After 15 min at ambient temperature 5 mL of stabilizer was added to each sample, and absorbance was read at 700 nm. The assay was standardized against gallic acid (Table 1) with various concentrations of the substrates.

Folin−Ciocalteu Assay. The total phenol assay was prepared and used according to Waterman and Mole based on the original descriptions by Folin and Denis, and Folin and Ciocalteu.[15−18] To a 0.5 L round-bottom flask, 700 mL of deionized water was added together with sodium tungstate (25 g), phosphomolybdic acid (6.25 g), 25 mL of concentrated HCl and 85% *o*-H_3PO_4 (12.5 mL). The solution was refluxed for 10 h and cooled, and lithium sulfate (37.5 g) was added. A

few drops of Br_2 (liq) were added so that the final reagent was yellow in color (not green). The solution was diluted to 250 mL with deionized water. Anhydrous sodium carbonate and deionized water were mixed in an Erlenmeyer flask in the proportion 20 g/100 mL.

Sample (250 μL) was added to a 50 mL reagent tube containing 15 mL of water. After vortexing 1.25 mL of the Folin−Ciocalteu reagent was added, and the solution was mixed again. After 8 min, 3.75 mL of the sodium carbonate solution was added, and the volume was adjusted to 25 mL with deionized water. After 2 h the absorbance of the solution was recorded at 760 nm. The assay was standardized against gallic acid (Table 1) with various concentrations of the substrates, which gave absorbance ranges below 0.2 AU.

FRAP Assay. An assay based on ferric reduction ability developed for use on plasma samples (FRAP) was used according to Benzie and Strain.[19] A working solution consisting of 25 mL of NaOAc/HOAc buffer (pH 3.6), 2.5 mL 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3$ was prepared. 1.5 mL of the working solution was mixed with 50 μL of sample solution and 150 μL of distilled water and kept for exactly 10 min at 37 °C, and absorbance was read at 596.5 nm (against blank sample). The readings were calibrated against solutions of $FeSO_4$, which generate a blue complex with TPTZ (Table 1).

ABTS Assay. Scavenging capacities of the stable $ABTS^{\bullet}$ radical were measured by a method slightly modified from that of Re and co-workers.[20] Phosphate buffered saline solution (PBS) was prepared by mixing 100 mL of a 100 mM KH_2PO_4 buffer (pH 7.4) with 1.5621 g NaCl (150 mM final concentration). ABTS was dissolved in PBS to give a 7 mM ABTS solution. $ABTS^{\bullet+}$ was produced by addition of about 1 mg of potassium peroxosulfate (2.5 mM), and the solution was kept at room temperature for 16 h before use. The solution was diluted with PBS to give an absorbance of 0.70 ± 0.02 at 734 nm. PBS was used to zero the instrument. 3.0 mL of the $ABTS^{\bullet+}$ solution was mixed with 10 μL of sample and kept at room temperature for exactly 6 min before measurement at 734 nm. Inhibition of the radical was calculated at different concentrations (Table 1). The IC_{50} values (i.e., the concentrations which give 50% inhibition) were calculated based on the equation of the best fitted curve to the experimental data.

DPPH Assay. Measurement of DPPH radical scavenging activity was performed as described by Blois.[21] The concentration of DPPH in methanol was adjusted to correspond to an absorbance value of approximately 1.0 absorbance unit (AU) at 515 nm. This gave a DPPH concentration close to 100 μM. Exactly 100 μL of sample solution was mixed with 2.9 mL of DPPH solution. The sample was kept at ambient temperature (about 20 °C) for 30 min before being measured at 515 nm. Control measurement consisted of methanol rather than sample. Scavenging of the DPPH radical was measured as % reduction in color at various concentrations of the substrates (Table 1): % inhibition = $100(A_0 - A_c)/A_0$ where A_0 = absorbance at $t = 0$ and A_c = absorbance for added sample concentration.[22]

Nitric Oxide Scavenging Activity. Nitric oxide (NO) scavenging activity was assayed in accordance with Marcocci et al. but scaled to a micro level.[23] Sodium nitroprusside (5 mM) was prepared in phosphate buffered saline (PBS) at pH 7.4 (see ABTS Assay above). Samples of rutin, CN and standard ascorbic acid were dissolved in methanol in concentrations ranging from 0.5 to 60 μM. 100 μL of sample was mixed with 100 μL of sodium nitroprusside and left for 1 h at ambient temperature. A ready-to-use Griess reagent (800 μL) was then added, and color development occurred in 15 min before the absorbance was read at 532 nm (background subtraction at 620 nm) by use of 10 mm microvolume cuvettes (Hellma 400 μL, Sigma-Aldrich). Sodium nitroprusside was used as blank, whereas samples in controls were replaced by methanol. NO scavenging was calculated as % inhibition = $100(A_{control} - A_{sample})/A_{control}$ where $A_{control}$ = absorbance of control, and A_{sample} = absorbance of sample (Table 1).

HPLC. A liquid chromatograph (Agilent 1100 system, Agilent Technologies) supplied with an autosampler and a photodiode array detector was used for the analysis of individual phenolics. Separation was achieved by an Eclipse XDB-C8 (4.6 × 150 mm, 5 μm) column (Agilent Technologies), and the use of a binary solvent system consisting of (A) 0.05% TFA in water and (B) 0.05% TFA in acetonitrile. The gradient (percent B in A) was linear from 5% to 10% in 5 min, from 10% to 25% for the next 5 min, from 25% to 85% in 6 min, from 85% to 5% in 2 min, and finally recondition of the column by 5% in 2 min. The flow rate was 0.8 mL/min, 10 μL samples were injected on the column, and separation took place at 30 °C. CN and rutin were detected at 370 nm, whereas naringenin was detected at 280 nm. All HPLC samples were filtered through a 13 mm syringe filter (Nylon 0.45 μm, VWR International) prior to injection. Individual standard curves were made for each of the single compounds that were analyzed by HPLC.

UVB Isomerization. From a 3883 μM methanol solution of chalconaringenin 2.5 mL was diluted with water to a total volume of 100 mL (97 μM CN). The solution was kept in an open top beaker (8 cm in diameter, 2 cm high) placed in an ultraviolet viewing chamber and continuously irradiated at 365 nm (Spectroline CX-20, Spectronics Corporation). Reaction occurred at ambient temperature, and the solution was analyzed each half hour for 20 h by use of a UV spectrophotometer at 370 and 280 nm.

NaOH Isomerization. Isomerization of CN was performed in accordance with previous reports.[24,25] However, concern was made with respect to concentration of the NaOH solution and on reaction rate. In brief, solutions of approximately 5 mL of 100 μM CN in 0.2 and 0.8 M NaOH, respectively, were prepared in test tubes and stored at ambient temperature during reaction time. Isomerization was followed by spectrophotometry at 426 nm, which was the absorbance maximum for CN at both NaOH concentrations, and at 340 and 310 nm which were the absorbance maxima for naringenin in 0.2 and 0.8 M NaOH, respectively. Analyses were performed with four (0.2 M NaOH) and two (0.8 M NaOH) replicates.

RESULTS AND DISCUSSION

In total 555 and 358 mg of pure chalconaringenin (CN) and rutin (Figure 1) were obtained from 5 kg FW of the cherry tomato cv. Susanne. A content of 200 mg 100 g^{-1} of the compounds was found in a similar cultivar,[26] which clearly indicates a low yield of the isolation procedure. The isolated CN was found to have a molar extinction coefficient (ε) at 26907 abs M^{-1} cm^{-1}, whereas that of rutin was determined to be 20328 abs M^{-1} cm^{-1}. The values were calculated from a number of dried samples (15 and 8, respectively). These values are of importance in determining the concentration or purity of the compounds in solutions, and were used in this work to calculate accurate concentrations of the two flavonoids.

CN contributed to formation of Prussian blue complex in the assay of Price and Butler (P&B) in an amount that was nearly twice that of the common reference compound gallic acid (GA) (Table 1). Similarly, rutin revealed results higher than GA. These results are in agreement with what would be expected due to the polyphenolic nature of the compounds (Figure 1). The principle of the assay is based on the reduction of ferric and the generation of a measurable blue complex, ferrous cyanide, $Fe_7(CN)_{18}$-$(H_2O)_x$ ($14 \leq x \leq 16$).[13] Ferric reducing abilities are often found to correlate with general antioxidant ability. Thus, the compounds' ability to reduce ferric in this assay can be arranged as CN > rutin > GA.

The FRAP assay revealed a somewhat different behavior (Figure 2, Table 1). Also here the ability of the substrate to reduce

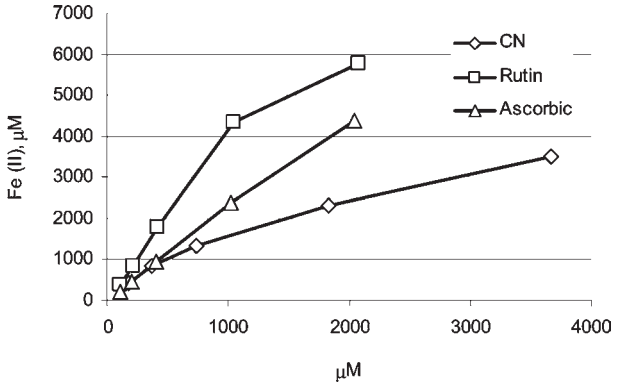

Figure 2. Reduction of ferric by chalconaringenin (CN), rutin and ascorbic acid in the FRAP assay.

Table 2. IC_{50} Values (μM) for Chalconaringenin (CN) and Rutin Measured in Three Different Antioxidant Assays, and Calculated from Curve Fitted Equations

	CN	rutin
ABTS	13	9
DPPH	216	59
NO	7409	2773

ferric is measured, and that ability was lower by CN compared to rutin and also compared to ascorbic acid. At low concentrations, the FRAP values of CN and ascorbic acid were similar. Rutin exhibited a much stronger antioxidant power in the FRAP assay, about twice that of ascorbic at concentrations of 1000 μM, and 2.5 times that of CN. Thus, the order of antioxidant capacity in the FRAP assay was found to be rutin > ascorbic > CN.

The Folin−Ciocalteu (F-C) assay is widely used to estimate total phenolic content in natural products.[15] The basic mechanism is however an oxidation of phenols by a molybdotungstate reagent, and the assay can as such be considered as an antioxidant method. Assaying revealed rutin as a stronger antioxidant than CN and gallic acid (Table 1). This result is in contrast to the P&B assaying. The F-C assay revealed higher linearity and lower standard deviation than the P&B assay.

Both the ABTS and DPPH assays revealed CN and rutin as antioxidants (Tables 1 and 2, Figure 3). The sensitivity of the ABTS assay was found to be about ten times that of the DPPH assay, which reveals the difference in stability of the two radicals used in the two assays, respectively. From a practical point of view the DPPH assay demanded high concentrations of both substrates, and the assay is as such not a suitable choice for measuring antioxidant capacity of these and similar expensive flavonoids. As compared to trolox, which is a water-soluble derivative of vitamin E, both assays gave the antioxidant capacity order rutin > CN > trolox. The half maximal inhibitory concentration (IC_{50}) of CN in the ABTS assay was found to be about 1.5 times that of rutin, whereas the numbers from the DPPH assay showed that the IC_{50} of CN is 3.7 times that of rutin (Table 2). Thus, rutin is a strong radical scavenger in these systems compared to CN. The ability of rutin to effectively scavenge the DPPH radical has recently been compared to the similar effects of ascorbic acid and butylated hydroxytoluene (BHT).[27] At a concentration of 0.05 mg mL^{-1} the three compounds showed 92.8%, 58.8% and 90.4% inhibition,

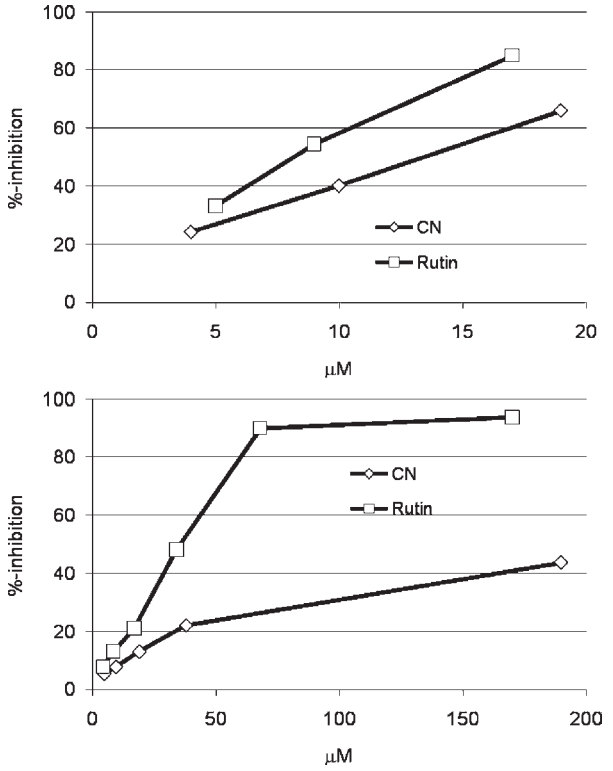

Figure 3. Scavenging of $ABTS^{\bullet+}$ (upper) and $DPPH^{\bullet+}$ (lower) by chalconaringenin (CN) and rutin.

respectively. Though not measured here, the range in DPPH radical scavenging ability thus seems to be rutin > ascorbic > CN, whereas the order in the ABTS assay is rutin > CN with ascorbic expected to be close to that of CN.

The nitric oxide (NO) assay is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which in turn interacts with oxygen to produce nitrite ions.[23] These can be estimated by use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of nitric oxide. The use of this assay revealed positive NO radical scavenging effects of both CN and rutin (Table 1 and 2, Figure 4). Compared to ascorbic acid the antioxidant order was found to be rutin > ascorbic acid > CN.

Quercetin is a recognized antioxidant compound, and its molecular features with an extended chromophore, a C2−C3 double bound, and a catechol arrangement of the B-ring have partly been used to explain the antioxidant properties.[28] In their structure−activity relationship study on antioxidant and pro-oxidant behavior of flavonoids, Cao and co-workers (2000) pointed out that the conjugation between rings A and B does not affect the antioxidant activity but is very important for the copper-initiated pro-oxidant action of a flavonoid.[29] As rutin contain the quercetin structure it is expected that rutin and quercetin should have similar properties in *in vitro* assays, and that the enhanced water solubility of rutin should make it more suitable as a test compound. CN has previously been reported to act as a pro-oxidant by increasing TBARS formation during incubation of LDL with Cu^{2+} at concentrations 5 and 25 μM.[25] All six assays used in this compilation show however an antioxidant effect of CN. The effect is in all cases except the P&B assay weaker than those found for rutin, which in part may be explained by the lack of the *ortho*-dihydroxy function of the aromatic ring structure.

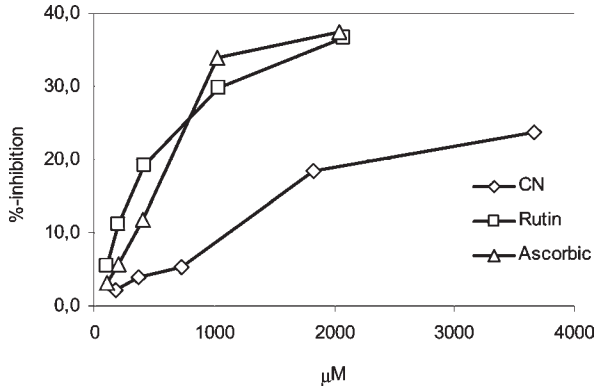

Figure 4. Scavenging of nitric oxide by chalconaringenin (CN), rutin and ascorbic acid.

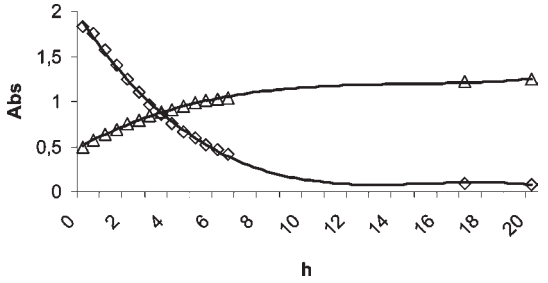

Figure 5. Isomerization of chalconaringenin under UV irradiation (365 nm) as determined by spectrophotometry by reduction of CN (squares) and appearance of naringenin (triangles) at 290 nm (n = 4).

Irradiation of CN close to its UV absorbance maximum (365 nm) revealed a conversion of the compound to its three ring isomer naringenin which belong to the noncolored class of flavonoids called the flavanones. The reaction was followed by spectrophotometry until the yellow sample solution faded, and the absorbance approached a constant value (Figure 5). The appearance of naringenin as followed at 290 nm was strongly negatively correlated (−0.995) to the concentration of CN. An HPLC analysis of the mixture revealed no phenolic compounds other than CN and naringenin. The reaction medium was water, but in the first place methanol was used as solvent but with no conversion of CN. This reveals a dependence of using a strong hydrophilic solvent. During the linear part of the reaction time (the first five hours) the reaction was completed by 71.3%.

The reaction rate seems to be too small to explain the possible isomerization of CN in tomato samples which are exposed to light irradiation during sample preparation or sample storage. This topic is a matter of concern as a minority of publications report on the occurrence of CN in tomatoes, rather most report on the content of naringenin. (Literature reviewed by Slimestad and Verheul,[26] and molecular structures identified by Slimestad, Fossen and Verheul.[12]) The present UV-B radiation experiment shows that is unlikely that sample preparation is affected by UV-B light and that it should occur under reduced light irradiation.

Isomerization of CN was also performed by use of NaOH at two different concentrations (0.2 and 0.8 M) (Figure 6). Disappearance of CN and appearance of naringenin were strongly negatively correlated at both concentration levels of the alkaline solution: −0.999 and −0.988, respectively. No peaks other than CN and naringenin were detected upon HPLC analysis of the reaction

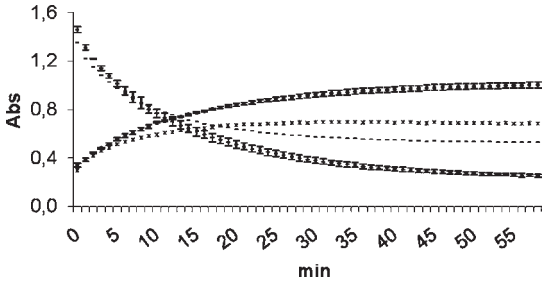

Figure 6. Isomerization of chalconaringenin (CN) by use of 0.2 M NaOH (—) and 0.8 M NaOH (— ± SD) at ambient temperature and determined spectrophotometrically (n = 4). Reduction in CN was followed by an increase in the concentration of naringenin (× and × ± SD for 0.2 and 0.8 M NaOH, respectively).

mixtures. The initial reaction rate (<20 min) with 0.2 M NaOH was found to be 3.29 nM s^{-1}, whereas the overall reaction rate was found to be 1.39 nM s^{-1}. This rate is too low to explain naringenin as an artifact upon tomato analysis.

Overall, as CN seems to be quite stable during sample preparation of tomato products, it is more likely that other factors contribute to the lack in reports of tomato CN. Such factors can be the existence of real differences in tomato composition though this has not been caught up by our analyses of European tomatoes. More realistic seems to be that there is a misinterpretation of CN as naringenin due to their close retention upon reversed phase HPLC, and the fact that the molecular ions are the same upon MS analysis.

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