Furanones in Strawberries: Evolution during Ripening and Postharvest Shelf Life

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Furaneol, mesifurane, and furaneol glucoside contents during ripening of four strawberry varieties (Oso Grande, Chandler, Tudla, and I-101) have been analyzed. Patterns of furanone contents were similar for the four varieties, amounts increasing during ripening to reach the highest values at the overripe stage. However, furaneol and derivatives differed quantitatively among varieties. The amount of furaneol shown by Oso Grande at the overripe stage is the highest so far reported (37.05 μg/g fw). The highest content in mesifurane and furaneol glucoside was found in the I-101 variety, at the overripe stage, 23.5 and 13.2 μg/g fw, respectively. Results obtained in eight different varieties, at commercial maturity stage, also showed quantitative differences. Strawberries were stored at 1 °C for 2 days to simulate refrigerated transport and then kept for 7 days at 17 °C to simulate the shelf life period. At 17 °C, the amount of mesifurane and furaneol glucoside increased more than 50% with concomitant loss of furaneol.

Keywords: *Strawberry; flavor; furanones*

INTRODUCTION

Aroma development is one of the most prominent changes that occurs during ripening of fruit. The aroma is also an important quality factor that influences consumer acceptability of fruits. Strawberry aroma is mainly determined by a complex mixture of esters, alcohols, aldehydes, and sulfur compounds (Schreier, 1980; Dirinck et al., 1981). Although no character impact compound has been found for strawberry aroma, some researchers consider 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (furaneol) and 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (mesifurane) as two of the most important aroma contributors (Pyysalo et al., 1979; Larsen and Poll, 1990, 1992). Both furaneol and mesifurane have strong, sweet, and pleasant odors. Furaneol imparts caramel burnt sugar notes at high concentrations and becomes fruity at lower concentrations (Re et al., 1973). Mesifurane is described as having a more sherry-like aroma (Hunter et al., 1974). Recently Larsen and Poll (1992) found that a mixture of furaneol and ethyl butanoate presented a strawberry-like odor.

Several studies have identified the presence of furaneol, mesifurane (Hirvi and Honkanen, 1982; Douillard and Guichard, 1990), and furaneol glucoside (Mayerl et al., 1989) in strawberries, but these compounds have not been found in all cultivars. Factors such as furaneol water-soluble nature (Pyysalo et al., 1979; Douillard and Guichard, 1989) and thermal instability (Flath and Forry, 1970; Shu et al., 1985) could well account for the failure of some authors to detect these compounds.

The new analysis procedure described by Sanz et al. (1994) involves HPLC separation and quantitation of furaneol and its derivatives which avoids some of the risks of thermal oxidative decomposition inherent in GC analysis. In this work, changes in furanone contents during strawberry ripening and postharvest shelf life have been studied using this method.

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EXPERIMENTAL PROCEDURES

Materials. Plants from Oso Grande, Chandler, Tudla, and I-101 strawberry varieties were greenhouse grown in optimum conditions of light, soil, and diseases control. Strawberry flowers were marked the day of blooming, and fruits were harvested at four visual ripening stages: white, pink, red, and dark-red (overripe). Strawberry fruits from Camarosa, Cartuno, Cartcua, Carlsbad, Laguna, Sunset, Seascape, and Cuesta varieties were grown in field at Cartayfres (Cartaya, Huelva) and sampled at commercial maturity stage (more than 75% red color). Oso Grande fruits used for the shelf life study were also harvested at commercial maturity stage in Torreagro (San Bartolome de las Torres, Huelva). Fruits were placed in 0.5 kg poly(ethylene) terphthalate baskets mechanically filmed with poly(propylene) (Poligal, thickness 25 μm), by means of a flow-pack device. The poly(propylene) film had an O_2 transmission rate of 750 $cm^3/m^2/24$ h at 23 °C and a moisture vapor trasmission rate of 1.1 $g/m^2/24$ h at 23 °C and 85% RH. Fruits were immediately stored at 1 °C for 2 days to simulate refrigerated transport and then stored for 7 days at 17 °C to simulate the shelf life period. Control fruits were stored at 1 °C.

Preparation of Sample for HPLC. Strawberries were cut symmetrically in four pieces. Four portions from four different fruits (ca. 20 g) were randomly sampled and ground with 5 mL of distilled water in a Waring blender at 0−4 °C. Celite 545 (10 g) was added and after mixing allowed to settle for 5 min. The mixture was filtered, washed three times with 10 mL of distilled water, and again filtered first through a 0.45 μm and then through a 0.2 μm nylon membrane before HPLC analysis.

HPLC Analysis. Quantitative HPLC analysis was carried out according to the method described by Sanz et al. (1994) with slight modifications. A liquid chromatograph, Beckman Golden System, equipped with an ODS (4.6 mm × 250 mm) 5 μm column was used. UV detector was selected at 280 nm, and the injection volume was 20 μL. The mobile phase utilized for the separation of furanones consisted of two eluents: 0.2 M sodium acetate/acetic acid, pH 4 (solvent A), and methanol (solvent B). Chromatographic conditions were 0−11 min, isocratic 13% B; 11−26 min, gradient 13−23% B; 26−30 min, isocratic 23% B; and 30−33 min, gradient 23−80% B (cleaning process).

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Table 1. Furaneol, Mesifurane, Furaneol Glucoside, and Total Furanone Content (μg/g fw) during Strawberry Ripening

compound	maturity stage: white	pink	red	dark-red
Oso Grande				
furaneol	2.42 ± 0.24[a]	5.98 ± 2.09	16.14 ± 4.57	37.05 ± 4.68
mesifurane			3.21 ± 2.61	15.61 ± 0.83
glucoside			2.31 ± 1.27	12.44 ± 1.38
total	2.42 ± 0.24a[b]	5.98 ± 2.09a	21.67 ± 8.40a	65.08 ± 6.64a
aroma value[c]	243	598	1615	3720
Chandler				
furaneol	3.47 ± 0.38	5.45 ± 1.07	13.69 ± 4.07	21.98 ± 0.86
mesifurane			0.30 ± 0.20	2.11 ± 1.00
glucoside			2.37 ± 0.30	2.73 ± 1.50
total	3.47 ± 0.38b	5.45 ± 1.07a	16.33 ± 4.19a	27.36 ± 0.93c
aroma value	347	549	1371	2253
I-101				
furaneol	0.48 ± 0.58	5.74 ± 1.7	12.71 ± 0.68	28.29 ± 2.06
mesifurane		0.41 ± 0.30	6.44 ± 2.39	23.50 ± 7.18
glucoside		0.52 ± 0.38	3.62 ± 1.05	13.21 ± 3.54
total	0.48 ± 0.58c	6.56 ± 2.75a	22.78 ± 2.81a	65.01 ± 7.98a
aroma value	47	570	1277	2852
Tudla				
furaneol	3.45 ± 0.19	9.73 ± 4.98	14.89 ± 0.70	22.98 ± 3.01
mesifurane			1.50 ± 0.90	3.60 ± 1.03
glucoside			3.40 ± 1.92	8.90 ± 1.51
total	3.45 ± 0.19b	9.73 ± 4.98a	19.29 ± 5.66a	35.49 ± 5.83b
aroma value	345	973	1490	2302

[a] Values represent the mean and standard deviation of four analyses. [b] Means of total furanone contents for the same maturity stage with the same letter are not statistically different at significance level, $p = 0.05$. [c] Aroma value was calculated by dividing the concentrations of furaneol and mesifurane by their threshold values according to Larsen and Poll (1992)

Calibration curves ($r = 0.999$) were obtained for furaneol and mesifurane in the concentration range 0.5−60 μg/mL in distilled water. Furaneol glucoside content in the extracts was determined based on a molar extinction coefficient for furaneol glucoside 2.66 times greater than that of furaneol at 280 nm (Sanz et al., 1994)

Atmosphere Composition. Gas composition inside each basket was analyzed during storage. CO_2 and O_2 contents were analyzed by a gas chromatograph, Hewlett-Packard 5890, equipped with a thermal conductivity detector, on a stainless steel Carbosieve S-II (3 m × 3 mm i.d.) column and helium as carrier gas. Calibration curves were obtained for CO_2 and O_2 in the range 0−700 and 0−250 μL/mL.

Evaluation of Quality Parameters during Storage. Strawberry fruits, stored at two different temperatures (17 and 1 °C), were evaluated for skin color and firmness. Fruits (30) from three baskets were evaluated on days 0, 2, 4, 7, and 9.

Color. Strawberry skin color was evaluated with a Minolta CR-200 portable tristimulus colorimeter (Minolta, Ramsey, NY) using color space L^*, a^*, b^*. Hue, expressed as an angular measure (h), and chroma (C^*) were calculated as follows (CIE, 1978):

$$h = \arctan(b^*/a^*)$$

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

Firmness. Firmness was measured as penetration force with a Zwick 3303 penetrometer, using a 5 mm plunger tip, and is expresed as newtons (N).

RESULTS AND DISCUSSION

To understand the aroma of a fruit, it is necessary to know not only the nature of the constituents but also how the significant components change in kind and quantity during the development of the fruit. Previously, Pérez et al. (1992) studied volatile ester formation through strawberry development and ripening. Methyl and ethyl esters of butanoic and hexanoic acids were quantitatively the most important volatile compounds identified in mature Chandler strawberries, but neither furaneol nor related compounds were identified. Because of the high content of furaneol found in some strawberry cultivars (Larsen and Poll, 1992), we have quantified the content of these compounds in strawberry fruits from Oso Grande, Chandler, I-101, and Tudla varieties, during ripening.

Data on furaneol, mesifurane, furaneol glucoside, and total furanone content in each variety at four ripening stages are presented in Table 1. The development of furanone compounds in each variety during ripening appears similar. Total furanone content increased during ripening reaching the highest value at the overripe stage. These results agree with those reported on the evolution of these and other aroma compounds during ripening in different strawberry varieties (Pérez et al., 1992; Sanz et al., 1994). The reason for the almost absence of these compounds at the first ripening stages could be the lack of the forming enzyme activity in unripe fruits, as proposed by Yamashita et al. (1977) for the volatile esters.

Although conclusive biochemical evidences have not been presented yet, mesifurane and furaneol glucoside probably derive from furaneol. In this sense, the capacity of the studied strawberry cultivars to produce furaneol and derived compounds, expressed as total furanones, was analyzed at each ripening stage. In pink and red fruits, total amounts of furanones calculated for each variety were not statistically different, but in white and dark-red fruits, significative differences were found. At the overripe stage Oso Grande and I-101

Table 2. Furanone Contents (μg/g fw) in Strawberries Sampled at Commercial Ripening Stage[a]

variety	furaneol	furaneol glucoside	total furanones
Camarosa	18.98 ± 0.41	7.13 ± 0.16	26.11 ± 2.11
Cartuno	12.18 ± 0.86	3.20 ± 0.62	15.39 ± 1.70
Cartcua	10.62 ± 0.33	0.60 ± 0.12	11.22 ± 0.48
Carlsbad	10.48 ± 0.43		10.48 ± 0.43
Laguna	7.98 ± 0.06	0.41 ± 0.11	8.39 ± 0.23
Sunset	3.67 ± 0.10		3.67 ± 0.10
Seascape	3.18 ± 0.16		3.18 ± 0.16
Cuesta	3.22 ± 0.12		3.22 ± 0.12

[a] Values represent the mean and standard deviation of four analyses. No mesifurane was detected in any of the studied varieties.

showed the highest values of total furanones, 65.08 ± 6.64 and 65.01 ± 7.98, respectively, these values being statistically different from those found in Tudla and Chandler strawberries.

As in other studies on volatile compounds in different strawberry varieties (Schreier et al., 1980; Douillard and Guichard, 1990), large quantitative differences were noticed between furaneol and derivative content among varieties. The amount of furaneol shown by Oso Grande at the overripe stage is the highest so far reported (37.05 μg/g fw), comparable with the amounts found in Douglas strawberries in an earlier study (Sanz et al., 1995). Oso Grande variety is very well adapted to the climate of Southern Spain, and because of its good organoleptic properties (López-Aranda et al., 1995), it has been extensively cultivated in Huelva province for fresh market.

The highest content of mesifurane and furaneol glucoside was found in the I-101 variety at the last ripening stage, 23.5 and 13.2 μg/g fw, respectively (Table 1). Considering the pattern of formation of those three compounds, this variety could be compared with the Totem variety from British Columbia (Sanz et al., 1995) which is mainly used for processing industry, since its short shelf life makes it undesirable for fresh market.

From the data shown in Table 1, it can be observed that in all studied varieties the content of furaneol was much higher than those of mesifurane and furaneol glucoside. Although no volatile compounds may be regarded as totally unimportant in the aroma of strawberries, compounds having the highest aroma value seem to be the most important (Larsen and Poll, 1992). For each variety we evaluated the individual importance of furaneol and mesifurane by considering its aroma value. This value, included in Table 1, was calculated by dividing the concentration of the aroma compounds by the upper threshold values according to Larsen and Poll (1992), 0.01 μg/g for furaneol and 1.0 μg/g for mesifurane. In this study furaneol glucoside was not included because of its nonvolatile nature. The better correlation for furaneol content and strawberry aroma is explained by the furaneol lower threshold concentration (Sanz et al., 1995). Thus, despite similarities in total furanone content between Oso Grande and I-101 overripe fruits, clear differences were found in the aroma value, 3720 and 2852, respectively, due to the higher furaneol content of Oso Grande fruits. This variety showed the highest aroma value in the last ripening stages (red and dark-red), which could explain the superior organolpetic quality of this strawberry cultivar (López-Aranda et al., 1995).

In all varieties the amounts of mesifurane and furaneol glucoside tended to increase at the overripe stage when high levels of furaneol are available. It seems that during the senescence furaneol could be rapidly converted into these two compounds. The requirement for high levels of furaneol previous to mesifurane and furaneol glucoside biosynthesis was also observed when eight different strawberry cultivars sampled at commercial maturity stage (75% red) were analyzed. Furanone contents of six new Californian strawberry cultivars and two new Spanish ones (Cartuno and Cartcua) that are being tested in southern Spain are listed in Table 2. No mesifurane was detected in any of the studied varieties, and only very low levels of furaneol glucoside were found. The almost absence of furaneol derivatives found in most varieties could be related to the low amount of furaneol available at the studied maturity stage. Camarosa and Cartuno showed the higher furanone contents. At the commercial maturity stage Camarosa strawberries had higher furaneol and furaneol glucoside values than those obtained for red

Table 3. Atmosphere Composition, Color (Expressed as L^*, Hue = $\arctan(b^*/a^*)$, Chroma = $(a^{*2} + b^{*2})^{1/2}$), and Firmness of Strawberries during Storage in Poly(propylene)-Filmed Baskets Kept at 1 and 17 °C

		atmosphere			color		
day	temp (°C)	CO_2 (%)	O_2 (%)	firmness (N)	*L*	hue	chroma
0		0.03 ± 0.01[a]	21.01 ± 1.22	50.00 ± 8.72[b]	35.76 ± 3.81[b]	35.33 ± 2.95	37.93 ± 5.43
2		3.47 ± 1.71	17.71 ± 1.55	38.64 ± 8.48	34.67 ± 3.63	37.16 ± 4.14	39.44 ± 5.02
4	17	26.23 ± 6.42	1.44 ± 0.66	37.22 ± 7.94	35.42 ± 3.14	33.26 ± 2.97	37.56 ± 4.35
	1	5.92 ± 2.02	16.23 ± 1.48	37.75 ± 8.02	37.95 ± 5.71	38.37 ± 7.13	35.72 ± 2.60
7	17	47.79 ± 9.09	0.89 ± 0.23	36.54 ± 5.62	33.38 ± 3.97	32.33 ± 3.64	35.70 ± 2.58
	1	7.81 ± 2.83	13.41 ± 0.61	40.02 ± 7.66	36.21 ± 4.10	37.45 ± 4.30	36.74 ± 3.50
9	17	57.64 ± 4.21	0.83 ± 0.14	37.54 ± 8.82	32.67 ± 3.74	29.11 ± 3.07	33.01 ± 4.01
	1	8.94 ± 2.41	14.02 ± 0.81	37.44 ± 10.64	35.38 ± 3.79	35.37 ± 4.46	38.47 ± 2.10

[a] Mean and standard deviation of three replicates. [b] Mean and standard deviation of 30 determinations.

Table 4. Furanone Contents (μg/g fw) of Strawberries during Storage in Poly(propylene)-Filmed Baskets Kept at 1 and 17 °C[a]

		day				
	temp (°C)	0	2	4	7	9
furaneol	1	8.67 ± 1.43	13.95 ± 3.06	9.59 ± 1.68	15.55 ± 1.09	12.75 ± 1.32
	17			10.06 ± 0.72	6.95 ± 2.59	2.88 ± 0.11
mesifurane	1	1.32 ± 0.37	2.21 ± 0.80	1.02 ± 0.61	1.99 ± 1.72	2.64 ± 1.56
	17			1.24 ± 0.35	5.57 ± 2.35	5.47 ± 0.92
furaneol glucoside	1	2.53 ± 0.04	2.77 ± 0.45	1.94 ± 1.13	2.09 ± 0.50	3.12 ± 0.51
	17			2.30 ± 0.47	4.74 ± 2.16	3.84 ± 0.28

[a] Values represent the mean and standard deviation of four analyses.

fruits from two older Californian varieties, Oso Grande and Chandler (Table 1).

Strawberries are highly perishable, and thus effective handling procedures are required to prevent excessive deterioration. For Spanish strawberries, a 2−3 day surface-refrigerated transport is required. Fruit harvest prior to full ripening is a common practice to extend the market life, since those fruits are less susceptible to decay during transportation and storage, though in this maturity stage a lack of organoleptic quality is not unusual. In this sense, Oso Grande has proven to be an excellent variety, with firm and flavored fruits at early stages of ripening.

Strawberry fruits of the Oso Grande variety were used to study the evolution of furanone compounds during transportation and shelf life. Fruits harvested at usual commercial picking date were submitted to the habitual postharvest process of fruits destined to export, fast cooling, and package. Baskets with 500 g of fruits, filmed with poly(propylene), were stored at different temperatures in a system designed to mimic its market life. Development of a modified atmosphere (MA), consisting of elevated CO_2 and/or reduced O_2, within polymeric film pouches extends strawberry shelf life (Ke at al., 1991), although detrimental effects on fruit flavor have also been reported (Shamaila et al., 1992). Atmosphere composition, firmness, and color of the fruits during 9 days of storage are listed in Table 3. After 2 days at 1 °C, few changes are noticed in furaneol, mesifurane, and furaneol glucoside contents (Table 4). When strawberries were conserved at 17 °C, the atmosphere in the basket change rapidly from 3.5% CO_2 on day 2 to 26.2% on day 4. The benefits of this high CO_2 level on strawberry firmness are shown in Table 3, with similar values for 17 °C strawberries and control (1 °C) fruits. Up to this level of CO_2, no changes in furaneol and derived compound levels, or off-flavor development, were observed. On day 7, the CO_2 content reached 50%, and the amount of mesifurane and furaneol glucoside increased more than 50% with concomitant loss of furaneol. This increase in furaneol derivatives could indicate that the enzymatic system responsible for these processes is still active. The last analysis carried out 2 days later (60% CO_2) showed similar values for mesifurane and furaneol glucoside contents and a very low amount of furaneol, 2.88 μg/g. Control fruits had 12.75 μg/g furaneol on day 9.

On days 7 and 9 a strong off-flavor was detected in strawberries stored at 17 °C. Off-flavor may be induced by anerobic respiration with accumulation of certain volatile compunds such as acetaldehyde and ethanol (Larsen and Watkins, 1995). However Ke et al. (1994) found that CA storage altered the aroma of strawberry not only by overproduction of acetaldehyde and ethanol but also due to reduced production of some volatile esters. Besides these changes in volatile ester biosynthesis due to high CO_2 and low O_2 (Pérez et al., 1996), loss of organoleptic quality during MA storage of strawberries could also be related to a severe reduction of furaneol content.

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