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1,2-Propanediol in strawberries and its role as a flavour precursor

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1,2-Propanediol has been identified in strawberries after solvent extraction, derivatisation to 1,2-bis(trimethylsily)oxypropane and analysis of its TMS derivative using GLC-MS and GLC-FID. The level of 1,2-propanediol in strawberries was fond to be 0.5 $\mu g\,g^{-1}$ of fresh weight of fruit. Thirty-nine per cent of added 1,2-propanediol was recovered from strawberries. When exogenous 1,2-propanediol was fed to strawberry callus cultures, 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one-glucoside, the glucosylated form of one of the character-impact compounds of strawberry flavour, was formed. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

A current topic of flavour research is to investigate the biosynthetic pathways of character-impact compounds of fruit flavours. When such pathways are established, it should be feasible either to produce these flavour compounds by cultivating plant cells in a bioreactor or to genetically modify the plant so that the gene responsible for the particular flavour compound formation will be over-expressed (Berger, 1995).

Strawberry flavour consists of approximately 350 aromatic compounds, 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one (DMHF) and its methyl ether 2,5-dimethyl-4-methoxy-2*H*-furan-3-one (mesifuran) are the two major character-impact compounds of strawberry flavour, each with a very low odour threshold (Latrasse, 1991). 2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one alone has the typical flavour of fresh strawberries and is considered a key aroma component of strawberries (Honkanen & Hirvi, 1990); it has also been reported to be one of the most important strawberry flavour compounds (Larsen *et al.*, 1992). Despite the great significance and high commercial value of these two furanones, their biosynthesis is still relatively unknown. Methylpentoses have been proposed as precursors of furanones (Pisarnitskii *et al.*, 1992) and it has been reported that 6-deoxy-D-fructose can enhance the production of DMHF-glucoside in strawberry tissue cultures (Zabetakis & Holden, 1995). There is also evidence that the biosynthesis of 6-deoxy-hexoses arises from the condensation of dihydroxyacetone phosphate (DHAP) with lactaldehyde (2-hydroxy-propanal) (Wong *et al.*, 1983, Ghalambor & Heath, 1962, Twerdochlib *et al.*, 1994).

There are reports that 1,2-propanediol functions as a key intermediate in the metabolism of deoxyhexoses (Huff & Rudney, 1959; Gupta & Robinson, 1960; Weimer, 1984). When ingested, 1,2-propanediol is oxidised and decarboxylated to pyruvic and acetic acids (Matusik *et al.*, 1993). The reported enzymatic reduction of lactaldehyde to propanediol (Gupta & Robinson, 1960) prompted us to investigate whether 1,2-propanediol is present in strawberries, where 6-deoxy-hexoses are considered to be important flavour precursors. To the best of our knowledge, 1,2-propanediol has not been reported as a natural component of foodstuffs. However, it may be present in soft drinks as a result of migration from plastic containers to the drink (Van Rillaer & Beernaert, 1983) and in beer, as a fermentation by-product (Williamson & Iverson, 1993).

The aim of the present work is to establish whether 1,2-propanediol, a potential precursor of lactaldehyde, is present in strawberries, and to examine the effect of exogenous 1,2-propanediol on flavour bioformation in strawberry tissue cultures.

MATERIALS AND METHODS

Analysis of 1,2-propanediol in strawberry fruit

Strawberry fruits (250 g) were obtained from a local market and macerated in a Sorball mixer with distilled water (200 ml). Celite (12 g) (Sigma, UK) and the optimum amount of sodium chloride (160 g) (Sigma, UK) was added and mixed with the homogenate. The mixture

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was vacuum-filtered through a (Whatman (BDH, UK) No 1) filter paper and the filtrate (400 ml) extracted with 4×300 ml dichloromethane (AR grade, Fisons, UK). The combined organic phase was concentrated to 10 ml using a rotary evaporator, dried using anhydrous sodium sulphate (Sigma, UK) and filtered. The filtrate was further concentrated to 500 μl using a Kuderna–Danish evaporation concentrator. The concentrate (300 μl) was transferred to a screw-top vial and 1,3-propanediol (Sigma, UK; internal standard; 200 ppm in dichloromethane: 200 μl) was added, together with 750 μl of acetonitrile (AR grade, Fisons, UK) and 250 μl bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Sigma, UK). The mixture was heated at 70°C for 50 min and isopropanol (250 μl) was then added to destroy excess BSTFA. The solution containing the trimethylsilyl-ethers (TMS-ethers) of 1,2-propanediol and 1,3-propanediol was analysed by coupled Gas Liquid Chromatography–Mass Spectrometry (GLC–MS) and coupled Gas Liquid Chromatography–Flame Ionization Detector (GLC–FID) Similarly, standard 1,2-propanediol (Sigma, UK) was derivatised with BSTFA and analysed by GLC–MS; the mass spectrum of standard 1,2-propanediol-TMS ether was obtained. All solvents used were shown by GLC–FID to be free of 1,2-propanediol.

For GLC–MS analysis, a Carlo Erba 4200 GLC was coupled to a Kratos MS80 RFA mass spectrometer with a source temperature of 180°C. A BPX5 capillary column (25 m×0.32 mm×0.5 μm film thickness) (SGE, UK) was held at 50°C for 5 min and programmed from 50 to 170°C at 5°C min^{-1}, using helium at 2 ml min^{-1}. Injection (0.2 μl) was made on the column. Mass spectra were recorded with an ion source energy of 70 eV.

Solutions of 1,2-propanediol and 1,3-propanediol were derivatised with BSTFA and analysed by GLC-FID in order to construct a calibration curve. A Fisons GLC 9000 series, equipped with a BP20 column (25 m×0.35 mm×0.25 μm film thickness) (SGE, UK), was used. Injections (0.5 μm) were split with a split ratio of 30:1. The temperature was programmed at 50°C for 5 min and then from 50°C to 150°C at 4°C per min. The carrier gas was helium with a flow rate of 2 ml min^{-1}.

The recovery factor for added 1,2-propanediol was determined by spiking the initial strawberry juice with a known amount of 1,2-propanediol and analysing this spiked solution as described above.

Callus culture

Precursor feeding experiments were carried out using plants of *Fragaria×ananassa* (cv. Elsanta) (R.W. Walpole Strawberry Plants Ltd., UK). Petioles were cut from plants and sterilized with sodium hypochlorite (Bois, 1992); afterwards they were cut into discs approximately 2–3 mm thick and each disc was placed on agar-solidified modified Murashige and Skoog (1962) (MS) medium. The tissues were cultured at 25°C under a continuous irradiance of 150 μmol m^{-2} s^{-1}, supplied by fluorescent tubes (Warmwhite, Thorn EMI).

The medium used for the strawberry cultures consisted of MS basal salt mixture supplemented with agar (1% w/v), sucrose (3.5% w/v) and plant growth regulators such as benzylaminopurine (BA, 2.22 μM) and 2,4-dichlorophenoxyacetic acid (2,4-D, 2.26 μM). The pH of the medium was adjusted to 5.7–5.8 by adding HCl (0.2 M) and the medium was sterilised at 104 kPa for 15 min. All chemicals were from Sigma, UK. Calluses were transferred aseptically to fresh medium every 4 weeks (Bois, 1992).

The medium for the control cultures was as above and was sterilised as before. The precursor-fed culture medium contained 1,2-propanediol (0.5 or 1%) and the above chemicals at the same levels and was filter-sterilised.

Analysis of callus cultures

The simultaneous analysis of DMHF, DMHF-glucoside and mesifuran in the homogenised cultured calluses was performed by HPLC with UV detection at 280 nm (Zabetakis & Holden, 1996) DMHF-glucoside content in the extracts was determined based on a molar extinction coefficient at 280 nm or DMHF-glucoside 2.66 times greater than for DMHF (Sanz *et al.*, 1994).

RESULTS AND DISCUSSION

Identification and quantification of 1,2-propanediol

The mass spectrum of 1,2-propanediol-TMS ether extracted from strawberries was identical to the one published by Van Rillaer & Beernaert (1983). This spectrum, identical with that of standard 1,2-propanediol-TMS ether, is characterised by the silyl fragment ions at m/e 73 $[(CH_3)_3Si]^+$, m/e 117 $[CH_3CHO\text{-}Si(CH_3)_3]^+$ and m/e 147 $[(CH_3)_2SiOSi(CH_3)_3]^+$ (Van Rillaer & Beernaert, 1983). The retention times of the derivative from strawberries and the authentic compound were identical.

Linear responses ($r = 0.9826$) were obtained when 1,2-propanediol-TMS ether in the concentration range 7.5–130 μg ml^{-1} with 1,3-propanediol-TMS ether (internal standard) at a constant concentration (30 μg ml^{-1}) were chromatographed and a standard curve [peak area ratio (PAr) vs the concentration of 1,2-propanediol-TMS ether; peak area ratio is the ratio of the area of 1,2-propanediol-TMS ether over the area of 1,3-propanediol-TMS ether] was constructed. The regression line equation is $y = -0.00506 + 0.00863x$, with $r = 0.9826$; where y is the PAr and x is the concentration of 1,2-propanediol-TMS ether. The detection limit (3×baseline noise) for 1,2-propanediol-TMS ether was 5 μg ml^{-1}. Propanediol is very polar and very soluble in water. Therefore, strawberry juice was saturated with sodium

Table 1. Amounts of DMHF, DMHF-glucoside and mesifuran in control and 1,2-propanediol fed cultures (μg of analyte per gram FW of tissue)

	DMHF	DMHF-glucoside	Mesifuran
Control	n.d.	n.d.	n.d.
0.5% 1,2-propanediol	n.d.	n.d.	n.d.
1% 1,2-propanediol	n.d.	0.94 ± 0.03^a	n.d.

n.d., not detected
[a]Mean of three analyses (95% confidence level).

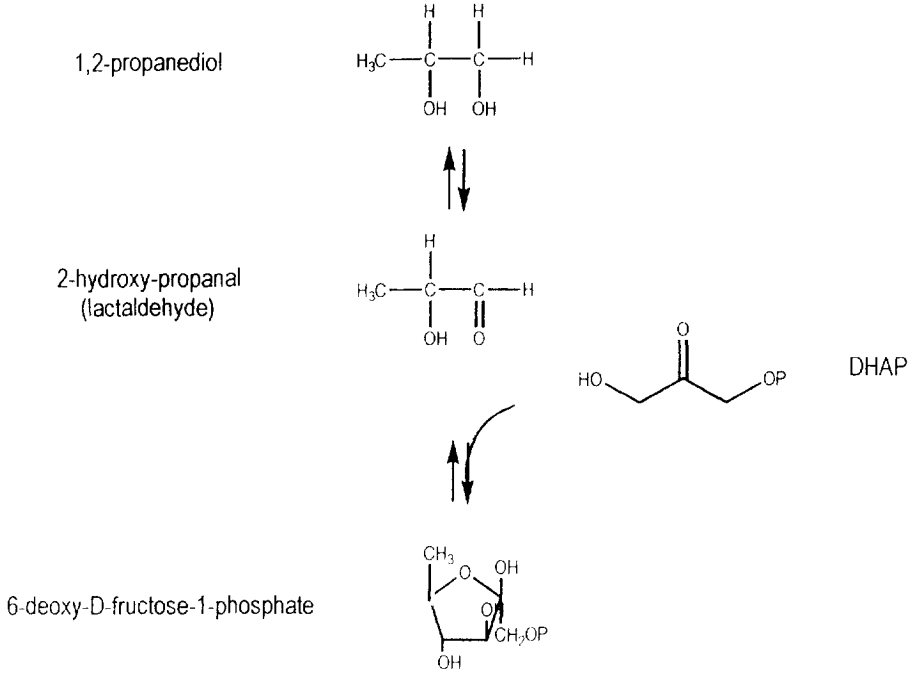

Fig. 1. Proposed biosynthetic pathway of 6-deoxy-D-fructose-1-phosphate, a suggested DMHF precursor.

chloride before the extraction in order to improve the extraction of 1,2-propanediol into dichloromethane. However, the presence of cell material, proteins and fat reduced the recovery factor to a relatively low level compared with the recovery of 1,2-propanediol from a clear aqueous solution. Recoveries of 72% are obtained from soft drinks (Van Rillaer & Beernaert, 1983). The average recovery factor of added 1,2-propanediol was 39%. On this basis, the level of 1,2-propanediol in strawberries was $0.49 + 0.04\ \mu g\ g^{-1}$ fresh weight (FW) of fruit (mean of three analyses, 95% confidence level).

The role of 1,2-propanediol as a flavour precursor

The identification of the DMHF-glucoside in 1,2-propanediol-fed cultures was carried out as described by Zabetakis and Holden (1996). The levels of DMHF-glucoside, DMHF and mesifuran, expressed as μg of analyte per gram FW of callus tissue, have been compared in control cultures (i.e. cultures with no added 1,2-propanediol) and 1,2-propanediol-fed cultures (Table 1).

Given that exogenous 1,2-propanediol enhances the formation of DMHF-glucoside in strawberry callus cultures (Table 1) and, further, that lactaldehyde is proposed as the precursor of 6-deoxy-1-phosphate and thereafter, DMHF in strawberries (Zabetakis & Holden, 1995), the diol may be considered to be an important precursor of a DMHF biosynthetic pathway. Taking into account also the presence of alcohol dehydrogenase enzymes in strawberries (Yamashita *et al.*, 1978; Mitchell & Jelenkovic, 1995), 1,2-propanediol may be oxidised to lactaldehyde by these enzymes. It is proposed that lactaldehyde may react with dihydroxyacetone phosphate (DHAP), an omnipresent key metabolic compound (Stryer, 1988) to produce 6-deoxy-1-phosphate (Fig. 1). This deoxy sugar may in turn be converted to DMHF-glucoside as suggested by Zabetakis and Holden (1996).

1,2-Propanediol is therefore proposed as one of the initial compounds of the biosynthetic pathway of DMHF. Given that DMHF is synthesized after several enzymatic steps and the yield of each step may be relatively low in tissue callus, it is not surprising that the effect of exogenous 1,2-propanediol on

DMHF-glucoside formation becomes detectable only at high levels of added 1,2-propanediol (i.e. 1%). Experiments on the bioconversion of 1,2-propanediol to lactaldehyde in strawberries should lead to a better understanding of the factors governing the bioavailability of these two precursors of the DMHF biosynthetic pathway.

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