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Does freezing and thawing affect the volatile profile of strawberry fruit (*Fragaria* × *ananassa* Duch.)?

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ABSTRACT

This research was aimed at determining if there were any changes in the volatile profile of strawberries cv. Camarosa, when subjected to various freezing and thawing treatments. Strawberries were cut in half, one half of the berries were frozen directly at −20 °C or −80 °C or rapidly frozen in liquid nitrogen (N_2) (−196 °C). They were then stored overnight or for a week. Berries were later left to thaw at room temperature (natural thawing) for about 1 h and some were forced-thawed in a 30 °C water bath. Headspace volatile compounds were determined using an Atmospheric Pressure Chemical Ionisation-Mass Spectrometer (APCI-GPA) and validated with a Gas Chromatograph–Mass Spectrometer (GC–MS). In a comparison of thawed half berries and fresh berries, most esters such as hexyl acetate, ethyl methyl hexanoate, methyl acetate were increased significantly by week-long and not by overnight freeze/thaw treatments. Ethyl butyrate was not affected by any treatment. The abundance of aldehydes such as the acetaldehyde compounds was increased significantly when thawed naturally compared to when forced-thawed in all the cold storage treatments.

1. Introduction

Due to the highly fragile structure of strawberry fruit and their high rates of respiration, their postharvest life is relatively short (Tucker, 1993; Richardson and Kosikattrun, 1995). This is because the fruit generally contains a high percentage of water as fresh weight, which may be as high as 98%, and also exhibits a high metabolic rate, thus making them highly perishable (Tucker, 1993; Pomper and Breen, 1997; Hancock, 1999).

The characteristic flavour of a fruit is due to the production of specific volatile flavour compounds and a complex interaction of sugars, organic acids and phenolics (Kader, 1991; Moing et al., 2001). The perishability and inherent short life of the fruit can result in rapid changes in the volatile compound profile. There is little work on differences in the volatile profile of fresh strawberries compared to those stored frozen for different periods, before volatile compound analyses. The effect of different thawing regimes (slow versus rapid thawing) for volatile compound analysis is also not well documented. Previous experiments have tended to concentrate on comparing flavour constituents of different cultivars subjected to the same freeze/thaw regime, e.g. Schreier (1980), Honkanen and Hirvi (1990) and Larsen and Poll (1992). Freezing of strawberries with liquid nitrogen for individual quick freezing (IQF) is considered the most suitable method compared to conventional cold storage because the delicate texture and flavour is maintained (Wang, 1991).

This study was conducted to determine if there were any changes in the volatile profile of strawberries when subjected to various freezing and thawing treatments. Understanding whether freezing of strawberry fruit prior to volatile compound analysis, might change the volatile profile would enable researchers to decide whether to analyse the fruit immediately after harvesting or store them frozen for later analysis without fear of altering the volatile profile. The experiment was therefore set to test the hypothesis that the volatile profile of frozen and thawed strawberry fruit does not differ from that of fresh berries.

2. Materials and methods

2.1. Plant material

The research was carried out at the Food Science Laboratory, University of Nottingham, in England. Strawberry (*Fragaria* × *ananassa* Duchesne.), a hybrid of *Fragaria chiloensis* × *Fragaria virginiana* cv. Camarosa was used in the experiment. Fresh strawberry fruit were purchased from a local supermarket in Loughborough and fruit of uniform size, shape and ripening stage (basing on the standard colour chart of KG Fruits Ltd., UK) were selected before the calyces were removed with a sharp knife. Fruit were thereafter cut in half

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Table 1
Freeze/thaw treatments

Treatment	Freezing and storage	Thawing conditions
1	None (fresh berry)	None
2	−20 °C overnight	Ambient room temperature
3	−20 °C for 1 week	Ambient room temperature
4	−20 °C for 1 week	30 °C water bath
5	−80 °C overnight	Ambient room temperature
6	−80 °C overnight	30 °C water bath
7	−80 °C for 1 week	Ambient room temperature
8	−80 °C for 1 week	30 °C water bath
9	Liquid N_2/−80 °C overnight	Ambient room temperature
10	Liquid N_2/−80 °C overnight	30 °C water bath
11	Liquid N_2/−80 °C for 1 week	Ambient room temperature
12	Liquid N_2/−80 °C for 1 week	30 °C water bath

and each half was placed in a sample vial (Sterillin) and weighed before exposure to the different freeze/thaw regimes.

2.2. Treatments

The half berries were frozen at −20 °C in a conventional freezer, or at −80 °C in a low temperature deep freezer, or rapidly frozen in liquid nitrogen (N_2) (−196 °C), they were then stored overnight or for a week. Berries were later left to thaw at room temperature (natural thawing) for about 1 h and some were forced-thawed in a 30 °C water bath (Table 1).

There were 12 treatments and each treatment had 10 half berries, giving a total of 120 samples.

2.3. Volatile compound analysis

Headspace profiles (Hakala et al., 2002) of the volatile compounds of the first half of fresh/thawed strawberries were analysed immediately using an Atmospheric Pressure Chemical Ionisation-Gas Phase Analysis (APCI-GPA, Platform LC2 Micromass, Manchester, UK) as described by Brauss et al. (1998) and utilised by Modise et al. (2004). The APCI-GPA was fitted with a custom-built sampling interface that allowed the introduction of gas phase samples. It is characterised by a soft ionisation technique (operating in the API positive ion mode) with real time monitoring, and high sensitivity, enabling it to measure very low headspace concentrations of volatile compounds among its other characteristics. Headspace profiles were obtained from macerated strawberry samples prepared using a mill and blender (BL 350 Blender, Kenwood Ltd., UK). A half berry corresponding to the other half that was used for the freeze/thaw treatments was placed inside the mill and blender. The lid of the mill had a modified entry port that enabled entrance of the APCI-GPA interface sample line (Watson et al., 2002). The blender was activated for two 1-s pulses to disrupt the fruit cells in order to release volatile compounds and compartmentalised enzymes which produce additional volatiles (Grab and Gfeller, 1999; Baldwin et al., 2000; Forney et al., 2000). Headspace inside the mill was sampled via a heated deactivated heated (160 °C) fused silica transfer line (electronic nose) at an air flow rate of 14 mL min^{-1}. Ion mass (m/z) peaks recovered for 10 replicate samples on the APCI, were identified and quantified. Ethyl butyrate was used as a reference standard where 10 μL was injected into the APCI using a syringe of 0.49 mm diameter set to inject 1.5 μL min^{-1}, at a flow rate of 14 mL min^{-1} (Humonics Precision Flow, Veriflow 500, USA). A preliminary full scan spectra of the headspace sampled from fresh strawberry fruit (cv. Camarosa) was thus obtained so as to validate the actual identity of the compounds using the Gas Chromatograph–Mass Spectrometer (GC–MS, GC 5890 Series II, Hewlett Packard and MS-Fisons Instruments, MD 800). Fruit were macerated with a pestle so as to release volatiles and a tenax trap was inserted in an airtight 100 mL bottle. A pressure pump was activated to blow the volatiles onto the tenax trap in the bottle for 10 min. The flow rate was set at 40–50 mL s^{-1}. The GC–MS uses tenax to adsorb volatile compounds and sample injection is through re-concentration in a cooling trap. The GC–MS was used to separate the ions via the Masslynx library (Masslynx V3.2, Micromass Ltd., Manchester, UK) and a quality fit indicated the correlation of the volatile compound with the quality fit, when the highest peak is selected.

2.4. Data analysis

A multi-factorial design was used for statistical data analysis where factors under consideration were storage environment, period of storage and the repetition of the intensity of volatile compounds (for freeze/thaw experiments), using Genstat 5 Release 4.1 (Rothamstead Institute), and means were separated using Standard Error Differences (S.E.D.) at ($P < 0.05$).

3. Results

Ions with prominent peaks were chosen (Fig. 1) from the scan for selected ion recording (SIR) of the headspace of 10 replicates of each sample. The identities of the selected ions are shown in Table 2. Generic terms rather than the International Union of Pure

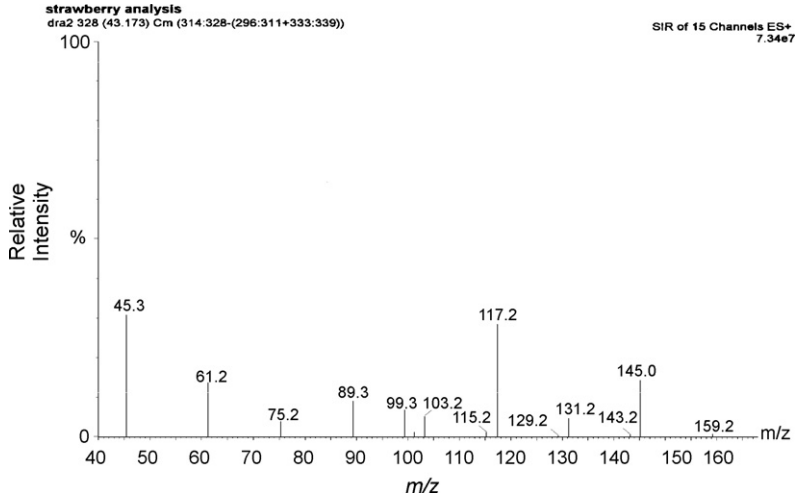

Fig. 1. A full spectral profile of the strawberry fruit (cv. Camarosa).