Damage to Strawberries Caused by Simulated Transport

G. La Scalia, M. Enea, R. Micale, O. Corona, L. Settanni

Abstract—The quality and condition of perishable products delivered to the market and their subsequent selling prices are directly affected by the care taken during harvesting and handling. Mechanical injury, in fact, occurs at all stages, from pre-harvest operations through post-harvest handling, packing and transport to the market. The main implications of this damage are the reduction of the product's quality and economical losses related to the shelf life diminution. For most perishable products, the shelf life is relatively short and it is typically dictated by microbial growth related to the application of dynamic and static loads during transportation. This paper presents the correlation between vibration levels and microbiological growth on strawberries and woodland strawberries and detects the presence of volatile organic compounds (VOC) in order to develop an intelligent logistic unit capable of monitoring VOCs using a specific sensor system. Fresh fruits were exposed to vibrations by means of a vibrating table in a temperature-controlled environment. Microbiological analyses were conducted on samples, taken at different positions along the column of the crates. The values obtained were compared with control samples not exposed to vibrations and the results show that different positions along the column influence the development of bacteria, yeasts and filamentous fungi.

Keywords—Microbiological analysis, shelf life, transport damage, volatile organic compounds.

I. INTRODUCTION

THE recognition of an increasing and worldwide demand for high quality fruits and vegetables has grown in recent years. While in the past, flavor and appearance were the most important attributes of fruits and other fresh vegetables, nowadays consumers are more concerned about food safety and nutritional value. In fact the preference for organic products and fruits, rich in nutrients and other beneficial compounds [1], is increasing. Information on the flavor chemistry of natural and processed fruits plays an important role for the food industry in determining optimal harvesting dates and storage conditions and in ensuring that the quality required for consumer acceptability is maintained. These considerations result in a growing interest in determining the qualitative composition of volatile organic compounds (VOCs) emitted from fruits and vegetables; in particular extensive studies have been conducted on strawberries [2]-[6].

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The volatile components of strawberries are constituted by a set of aromatic compounds belonging to different chemical groups such as aldehydes, alcohols, esters, ketones, aromatic compounds, terpenes, furans, lactones, and sulphur compounds [7].

Perishable product's quality depends also upon extrinsic factors. The mechanical damage of these products, as a consequence of inappropriate harvest, manipulation and transport, is one of the most common and severe extrinsic factors that induces negative changes in sensory attributes (skin and flesh browning and off-flavours), internal breakdown reactions and consequently a reduction of their quality [8], [9]. The loss of fresh fruits and vegetables during transport and distribution has been estimated to be above 30% [10] and it is well known that one of the major causes of the mechanical damage are the vibrations that fruits suffer during transport from farms to market [11]. Many researches have been carried out on assessing the effect of transport vibrations on perishable products. Kawano et al. [12], for example, reported a relationship among the degrees of mechanical injury of strawberries, the vibrating force of simulated transport and the duration of vibrations. Moreover the frequencies of transport vibrations have been monitored for trucks carrying fresh fruit [13], [14] and the mechanical damage due to transport vibrations was investigated on different species of fruit and vegetables such as potatoes [15], peaches [16], [17], apples [18], [19], loquats [20], and pears [21], [22]. In the last fifty years several methodologies were set up to evaluate the damage on fruits due to transport and different systems to simulate transport were used: mechanical [23], [24], electro-hydraulic [25]-[28], and electro-dynamic [29].

Quality variations are often difficult to assess by merely visual or tactile inspections, in fact the human senses have only a limited capability to assess the intrinsic product properties, modern sensor technologies can help to provide the required information. The measurement of intrinsic factors can indirectly give information on effects, in fruits and vegetables, of extrinsic factors; this can help to determine the quality of the products and to predict their residual shelf life. As the costs of sensor and communication technologies have decreased dramatically over the past decade, electronic quality monitoring techniques have become attractive to a wider range of supply chain applications [30].

This article assesses the impact of vibrations on strawberries in terms of microbiological growth and determines the volatile organic compounds (VOCs) of these fruits. Transport vibrations were simulated by means a vibrating table to investigate their effects on strawberry and woodland strawberry. VOCs were identified by means of a gas chromatography analysis. This study is the first step in

development of an intelligent logistic unit capable of monitoring the phenomena that affect the product's quality by means of a sensor system.

II. MATERIALS AND METHODS

A. Vibration Measurement

The two commercial cultivar considered in this study, "Florida Fortuna" and "Regina delle Valli" for strawberries and woodland strawberries respectively, were picked manually by the Bufalata Company at the end of spring 2014 in Marsala (TP, Italy) and placed in PVC containers to reduce the damage that may occur from their transporting to the laboratory. To evaluate the damage that fruits undergo during a real transport, a column of 10 crates made of cardboard (500mm by 300 mm by 92 mm) containing 10 containers of strawberries was placed on the top of a vibrating table (Euro Percussion, CT 38/31) driven by a frequency converter (Euro Percussion, 0-100 Hz). The column was held in place by means of PVC band (Figs. 1 and 2).
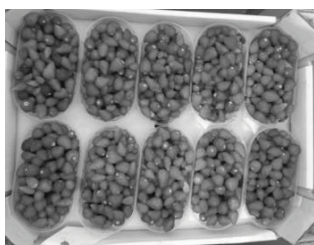

Fig. 1 Containers for the packaging of strawberries

Fig. 2 Column of creates placed on the vibrating table and contained in cooling cell
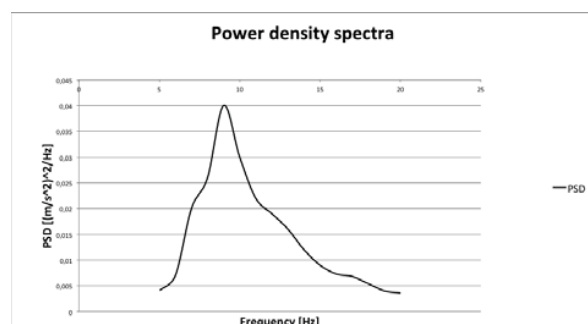

Fig. 3 Power Spectra Density profile

To maintain a constant temperature (4°C), the vibrating table was placed in the cooling cell of the laboratory of the Department of Agricultural and Forest Science, Palermo University. Tests were carried out by stressing the column for 48 h in order to simulate real transport conditions. To evaluate the damage of fruits due to the vibrations, the column of creates was stressed with a frequency of 9 Hz that represents the power density spectra (PSD) peak (Fig. 3) obtained with an experimental test on the track used for the transport by the company. This value is coherently with the study reported by Fischer et al. [31] in which they have demonstrated that the 5 to 10 Hz range caused the most relevant damages to the strawberries.

To determine the existence of a correlation between the fruits damage and their position along the column, samples of strawberries and woodland strawberries were taken at different levels of the column: bottom (1st create), medium (6th) and top (10th). To assess the acceleration behaviour along the column two micro elctro-mechanical systems (MEMS) accelerometers, MMA745, were fixed on the first and the last creates. The samples were submitted to microbiological tests to determine the microbiological contamination levels. Each test was carried out in triplicate. To determine the correlation between the microbiological grown and vibrations the research focused on the analysis of samples throughout the whole supply chain. In the initial transport phase the crates are loaded on refrigerated truck in order to be carried to the distribution warehouse until shipment to the final retailers. Each one of these phases is characterized by deterministic temperature and duration. In particular, the considered phases are three: phase I, in which the initial transportation is considered with an average duration of 48 h at approximately 4°C; phase II, where the fruits are stored in a refrigerated warehouse for approximately 4 day at 4 °C; and the phase III, in which the products are sold to final market in about 1 day at 25°C. A representative mapping of the supply chain activities in terms of their duration, temperature and experimental microbiological testing is given in Table I.

TABLE I
MAPPING OF THE SUPPLY CHAIN ACTIVITIES OF THE CASE STUDY

		Steps of monitoring				
		I phase			II phase	III phase
Microbiological analysis	T_0	8h	24h	48h	144 h	168 h

B. Microbiological Analysis

Strawberries were microbiologically investigated by viable plate counts. Samples of approximately 25 g were subjected to decimal serial dilution performed in Ringer's solution (Sigma-Aldrich, Milan, Italy) and were homogenized in a stomacher (BagMixer® 400, Interscience, Saint Nom, France) for 2 min at the highest speed. Total mesophilic counts (TMC) were estimated on Plate Count Agar (PCA), while yeasts and moulds on Malt Agar (MA) supplemented with tetracycline hydrochloride (50 µg mL^{-1}) to inhibit bacterial growth. Incubation occurred aerobically at 30 and 25°C for PCA and MA, respectively.

C. Volatile Organic Compounds (VOCs) Analysis

VOCs, detected at 0, 2, 4, 6 and 8 days for untreated fruits

kept in room conditions (25°C, RH 80%), were sampled using a solid phase microextraction (SPME) which involved the adsorption of analytes onto a fused silica fiber coated with suitable stationary phase and their subsequent desorption immediately before chromatographic analysis. The use of head space SPME, that exposes the fiber to sample head space (HS), can drastically reduce interferences that affect the absorbance of flavor compounds on the fiber; consequently, the head space SPME gas chromatography and mass spectrometry (HS-SPME-GC/MS) method allows a useful comparison of aroma profiles trough a capillary GC/MS analyses carried out using a gas chromatograph [32], [33]-[43].

The SUPELCO SPME (Bellefonte, PA, Italy) fiber holder and fiber used were coated with divinylbenzene/polydimethylsiloxane (DV/PDMS), 65 µm. Before the first extraction, the fiber was conditioned in the GC injector port at 300°C for 1 h, according to the manufacturer's recommendation. Analysis of samples was carried out by homogenizing 3 g of fruit, 3 mL of water and transferring it into 22-mL vials with pierceable silicone rubber septa coated with polytetrafluoroethylene (PTFE) film. 0.1 mL of 1-Heptanol hydroalcoholic solution (35 µg/mL) was used as an internal standard. Extraction temperature of head-space and time were 40°C and 30 min, respectively. 0.2 g of NaCl was added to increase extraction rate of volatile compounds. The samples were gently vortexed during extraction using a magnetic stirrer. Fiber exposition was prolonged for 30 min at 40°C. The SPME fiber was directly inserted into a Finnegan Trace MS for GC/MS (Agilent 6890 Series GC system, Agilent 5973 Net Work Mass Selective Detector; Milan, Italy) equipped with a DB-WAX capillary column (Agilent Technologies; 30 m, 0.250 mm i.d., film thickness 0.25 mm, part no 122-7032). The GC temperature was 40°C for the first 2 min (during split less injection), then from 40°C to 60°C, increasing 4°C min-1, 60°C for 2 min, from 60°C to 190°C, increasing 2°C min-1, from 190°C to 230°C, increasing 5°C min^{-1}, and finally 230°C for 15 min. The GC injector was at 250°C, the Fid at 250°C, the transfer line at 230°C, with carrier helium being transported at a rate of 1 mL min-1, and EM at 70 eV. Mass spectra were recorded by electronic impact at 70 eV using the ion source temperature of 200°C. All compounds of m/z 33-495 atomic mass units (amu) were detected with this scan mode. Individual peaks were identified by comparing their retention indices to those of control samples and by comparing their mass spectra with those within the NIST/EPA/NIH Mass Spectral Library database (Version 2.0d, build 2005), and on the basis of the retention time, compared to the database values for standard reference materials. Confirmation was carried out using a laboratory built MS spectral database, collected from chromatographic runs of pure compounds performed with the same equipment and conditions. All solvents and reagents were purchased from WWR International (Milan, Italy). Chemical and physical tests were performed in triplicate, with the results expressed as mean±standard deviation.

III. RESULTS

A. Vibration Analysis

Experimental test involved the acquisition and processing of the acceleration data gathered by means of two accelerometers. The test consisted in 3 trials of 40 s with the frequency used in the simulation analysis in order to obtain the accelerations for the X and Y axis in the crate on the top of the column and in the crate on the floor. The vibration spectrum for each axis was extracted from the raw acceleration data by means of Fast Fourier Transformation (FFT). The FFT spectrum, for the vibrations along the X and Y axis measured in the experimental tests for the bottom crate show that vibration acceleration in the X-axis is more prominent than in the other direction, and vice versa for the crate on the top of the column. In particular for the bottom crate, the vibration acceleration was approximately 26 m/s^2 on the X-axis and 16m/s^2 on the Y-axis. Different considerations can be done for the top crate, where the vibration acceleration was approximately 18 m/s^2 on the X-axis and 24 m/s^2 on the Y-axis.

B. Microbiological Analyses

The results of the plate counts carried out during the application of vibrations for 48 h are reported in Fig. 4, where the symbols represent: ♦, control crate; □, bottom position; ▲, central position; ○, top position. Data reported are the mean values while bars represent the standard deviation of the mean. Vertical bars not visible are smaller than symbol size TMC showed that bacteria were at higher levels (Figs. 4 (a)-(d)) than yeasts (Figs. 4 (b)-(e)) and moulds (Figs. 4 (c)-(e)). A different microbiological growth was found on the crates located in different position along the column object of investigation (top, medium and bottom). In particular bacteria at the bottom of the column increased their numbers during the 48 h of observation, while bacteria located at the top and medium remained almost constant for strawberries (Fig. 4 (a)) and slightly increased for woodland strawberries (Fig. 4 (d)). On the contrary, moulds increased faster when located at the top of the column (Figs. 4 (c)-(f)). A negligible increase in cell number was registered regarding yeasts located at the centre and bottom of the column of strawberries (Fig. 4 (b)), while a trend similar to the moulds' one was registered for woodland strawberries (Fig. 4 (e)). Figs. 5 and 6 show the microbiological developments for the different vibrations considered in the II and III phase, respectively. It is graphically evident, especially for strawberries (Figs. 5 (a), (b) and 6 (a), (b)), that the trend observed in I phase was confirmed during II and III phase: bacteria and yeast increase their concentrations from the top to the bottom of the column and mould from the bottom to the top. Regarding woodland strawberries, this phenomenon was confirmed only for moulds (Figs. 5 (b) and 6 (b)). However, the longer the vibration time is, the higher the final cell densities are.
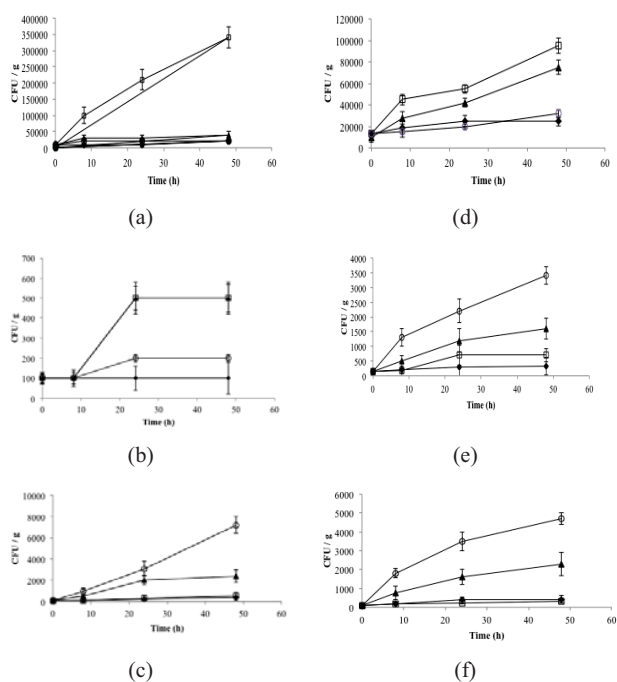
(a)   (d)

(b)   (e)

(c)   (f)

Fig. 4 (a) Bacteria loads of strawberry during the application of vibrations (b) Yeasts loads of strawberry during the application of vibrations (c) Moulds loads of strawberry during the application of vibrations (d) Bacteria loads of woodland strawberry during the application of vibrations (e) Yeasts loads of woodland strawberry during the application of vibrations (f) Moulds loads of woodland strawberry during the application of vibrations
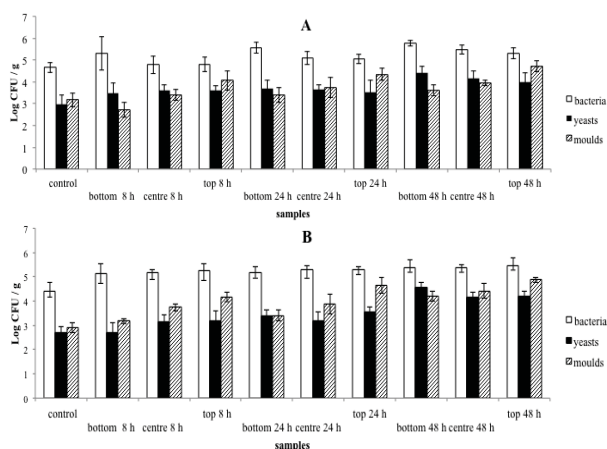

Fig. 5 (A) Microbiological loads of strawberries in the II phase; (B) Microbiological loads of woodland strawberries in the II phase

C. VOC Analyses

During storage, strawberries and woodland strawberries generated 110 and 130 compounds, respectively. VOCs were composed of acids, alcohols, aldehydes, esters, ketones, terpenes, aromatic hydrocarbons and hydrocarbons (Table II).
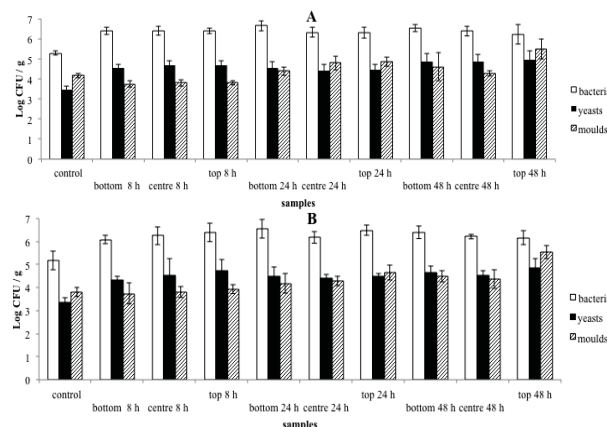

Fig. 6 (A) Microbiological loads of strawberries in the III phase; (B) Microbiological loads of woodland strawberries in the III phase

TABLE II
VOLATILE ORGANIC COMPOUND ON STRAWBERRY AND WOODLAND STRAWBERRY

Main categories	Major compounds identified
Acids	Hexanoic acid, butanoic acid, acetic acid
Alcohols	1-Hexanol, 1-Octanol, Decanol
Aldehydes	Benzaldehyde, Nonanal, Decanal
Esters	Ethyl hexanoate, octyl butanoate, isoamylacetate, ethyl dodecanoate, ethyl cinnamate, octyl acetate, ethyl octanoate, octyl 2-methylbutanoate, octyl isovalerate, ethyl 9-Hexadecenoate, hexyl acetate, octyl hexanoate, isoamyl butyrate, butyl hexanoate, hexyl hexoate, methylhexanoate, butyl butyrate, hexyl butanoate, decyl acetate
Ketones	Acetophenon, β-Ionone
Terpenes	Linalol, α-Tepineol, nerolidol, citronellol, myrtenol

At T_0, the compounds present at higher concentrations were esters (11000 μg/kg) and aldehydes (1200 μg/kg) for strawberries and woodland strawberries, while other classes of compounds exhibited different concentrations depending on the fruits analysed. In particular, alcohols, ketones and terpenes resulted significantly higher for woodland strawberries. During sampling from T_0 to T_8, volatile compounds showed a different evolution as a function of the relative chemical class. Esters and alcohols increased significantly from T_0 to T_2, while the increase of terpene, ketone and acid concentration was slower. In fact, acids increased only in woodland strawberries. Subsequently, a rapid decrease of the esters and alcohols was recorded at T_4, and a slight continuous reduction was noted until T_6 and T_8. During storage, the other classes of compounds showed a reduction in concentration especially at T_6 and T_8, with the exception of aldehydes of woodland strawberries, which remained almost unchanged. Figs. 7 and 8 showed the different concentration of VOCs for strawberries and woodland strawberries, respectively:
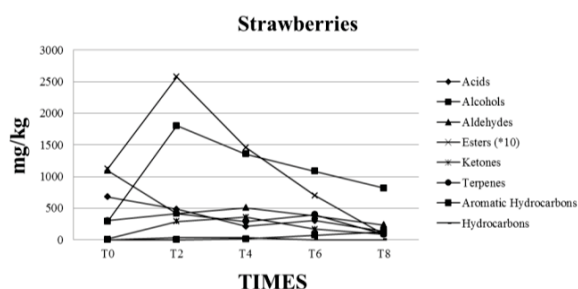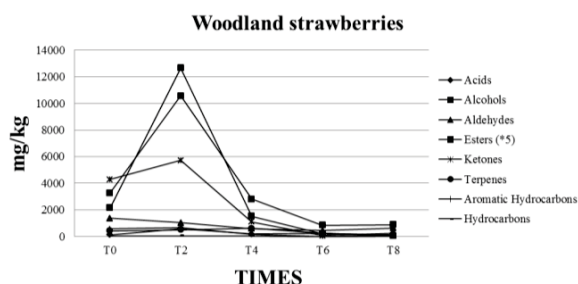
Fig. 7 VOC Concentration on strawberries

Fig. 8 VOC Concentration on woodland strawberries

IV. CONCLUSION

Strawberries are the most popular type of berry fruit in the world because of their high content of essential nutrients and beneficial phytochemicals, which seem to have relevant biological activity in human health but they are also fragile, perishable and delicate. The short shelf life of strawberries is hence a main reason of complexity in their management throughout the supply chain. Strawberries are highly sensible to mechanical injuries that occur during post-harvest handling and transportation. The present paper aims to determine the presence of principal VOCs on strawberries and woodland strawberries and to assess a correlation between vibration levels and quality of fruits by means of a microbiological investigation of TMC, bacteria, yeasts and moulds. The chemical analysis showed the presence of volatile compounds for strawberries and woodland strawberries categorized in six different main classes. Each one of these classes is constituted of several compounds with different level of concentration on the samples analysed. To simulate the vibration of a real transport, a column of crates containing strawberries and woodland strawberries were placed on a vibrating table in a cooling cell and the tests were conduct along 3 different levels of the column (top, medium and bottom). The results shows that, from a microbiological point of view, both cultivars have a significant reduction in quality increasing the time of vibration and there is a variation of bacteria, yeasts and moulds concentration caused by the position of the crates along the column. In fact, the trend observed during the application of vibrations shows the bacteria and the yeasts increasing their concentrations from the top to the bottom of the column while moulds from the bottom to the top. This result can be explained using the acquired data from the MEMS accelerometers that show higher values of acceleration along the horizontal axis for the crate on the top of the column and higher values along the axial direction for the crate on the bottom. The higher the oscillation is, the greater the dispersion of spores inside the crate due to structural characteristics of the mould is. The research confirms the importance of vibration effects that fruit suffers during the transportation phase, in predicting the shelf life of perishable products. Moreover, the significant variations recorded in the content of the volatile compounds analysed, especially those belonging to esters, alcohols and aldehydes can be exploited to monitor indirectly the quality of fruits during their storage. Further investigations on the correlation between VOC concentration and microbiological grown has to be conducted in order to develop a mathematical model that can help to predict the remaining shelf life of perishable products along the whole supply chain. In fact, literature shelf life models focus on the influences that one-parameter have on products, with evident lacks about the contemporary influence of intrinsic and extrinsic factors. The needed data for the shelf life model could be gathered by means an intelligent logistic unit equipped with specific sensors.

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