

ELECTRICAL BIOIMPEDANCE AND MECHANICAL PENETROMETRY TESTS FOR REMOTELY DETECTING THE TISSUE DAMAGE DUE TO MIS-MANAGEMENT OF THE COLD CHAIN IN OBLONG RED CHICORY

Andrea Manuello Bertetto¹, Andrea Fois², Filippo Tocco^{3,7}, Fernanda Velluzzi³, Andrea Deledda³, Fabrizio Farris⁴, Daniela Vacca⁴, Gianni Picci⁴, Antonio Hèctor Dell'Osa⁵, Ricardo Marcello⁶, Salvatore Melis^{6,7}, Alberto Concu^{6,7}

¹Department of Mechanics and Aerospace Engineering, Politecnico di Torino, Turin, Italy

²Nomadyca Ltd, Remote Biosignals Acquisition Unit, Mulago Hospital, Kampala, Uganda

³Department of Medical Sciences and Public Health, University of Cagliari, Italy

⁴Ortosestu Farming Mutual Company, Sestu-Cagliari, Italy

⁵Lab Electrónica Aplicada y Biomedicina, Universidad Nacional de Tierra del Fuego, Ushuaia, Argentina

⁶AC Technologies Ltd, Bioimpedance Devices Manufacturing, Cagliari, Italy

⁷Sporting Life And Medicine Lab, Sardegna Ricerche, Agency for Research & Technological Development, Cagliari, Italy

ABSTRACT

The oblong red chicory (*Cichorium intybus*) is a vegetable of considerable value since it is rich in water and low in calories (just 18 kCal per 100g) and the higher fibre content could provide of satiety during the meal. Moreover, such fibres are rich in inulin which is capable of positively selecting our intestinal microbiota. However, due to the limited diffusion of territories for its cultivation oblong red chicory may be subject to conservation along a specific cold chain to delay them for as long as possible biological processes of maturation, but low-temperature could cause internal breakdown that may limit the time in which this vegetable can be held and still ripened normally. In this experiment a suitable quantity of this vegetable was subjected for 12 hours temperatures stages respectively at -2°C, -8°C and -18°C, while 7°C was chosen as control temperature. After a thawing period, electrical bioimpedance and mechanical penetrometry measurements, i.e. the gold standard test but tricky to use, were applied to the chicory samples. Results showed that, as freezing temperature fell, at each unitary mechanical pressure change (1 kg cm⁻²) electrical impedance directly changed of about 18 Ω (linear regression test: P=0.018). So electrical bioimpedance measurements could be a new and easily method to control possible mis-management of the cold chain in logistic processing of the oblong red chicory.

Keywords: oblong red chicory, cold chain, mechanical penetrometry, electrical impedance

1 INTRODUCTION

The oblong red chicory (ORC), scientific name: *Cichorium intybus*, is a vegetable of considerable value due to its particular flavour but also for its high biological value on a nutritional level.

In fact, the red chicory is rich in water and low in calories, i.e. just 18 kCal per 100 g [1].

The higher fibre content stands out capable of providing satiety during the meal. A particular type of fibre contained in red chicory is the inulin which is capable of positively selecting the intestinal microbiota, i.e. the wealth of bacteria and other microorganisms that coexist in our bowel [2].

This vegetable is also characterized by a good content of vitamins and mineral salts, and a single portion of this kind of chicory is able to satisfy the daily requirement of vitamin K, a fundamental compound for the correct blood clotting process. Therefore ORC is also an important food for the diet of athletes engaged in long lasting sports performances.

Contact authors: A Concu¹, Andrea Manuello Bertetto²

¹AC Technologies Ltd, Via Pais 12-09128 Cagliari, Italy

²Politecnico di Torino, DIMEAS, Corso Duca degli Abruzzi 2424-10129 Torino, Italy

E-mail: aconcu44@gmail.com, andrea.manuello@polito.it

The characteristic bitter taste of the red chicory is due to its content in chicoric acid, a derivative of caffeine. This latter compound is also contained in chicory, from whose ground root a coffee substitute is obtained. Laboratory studies have shown how chicoric acid has antithrombotic [3] and anti-inflammatory effects and the consumption of red chicory root in capsules leads to a potential improvement in the treatment of the symptoms of patients suffering from arthritis, which translates into a lower sensation of pain and a less rigidity [4].

Interestingly, the colour of vegetables often is indicative of specific biochemical and functional properties that arouse the interest of researchers. In fact, the red colour of the ORC is due to anthocyanins [5] which are phenolic compounds capable of protecting the heart and which could even extend life thanks to the ability, which emerged from laboratory studies, to silence aging genes.

Moreover, it has been found [6] that a diet rich of anthocyanins, like as our oblong red chicory, is associated with reduced cancer growth incidence and metastasis as well as a longer period of cancer latency, and linked to a higher survival rate [7].

However, due to the limited diffusion of territories for ORC cultivation since the need for a very specific agronomic micro environment, having to be distributed in consumption areas often very distant from the place of production, it may be subject to conservation along a specific cold chain which, starting from temperatures around 0°C or slightly less, for rather long storage times these temperatures can be further reduced up to -7°C, -8°C, in order to delay them for as long as possible biological processes of maturation.

In this regard, it is not entirely unlikely that, due to accidents of various kinds, breakages may occur in the cold chain so programmed which could cause the temperature of the container to jump up to the minimum values, no longer controlled, corresponding to the characteristics refrigeration machine techniques, whereby the temperature to which the vegetables are subjected can drop to -18°C which is the maximum refrigeration temperature level that the machines most commonly used for this function generally reach.

In this experiment a suitable quantity of ORC heads was divided into four groups and three of these were subjected for a prolonged time to temperatures below 0°C, respectively -2°C, -8°C and -18°C, while the fourth group was kept at a temperature above 0°C. After a thawing period, electrical bioimpedance and mechanical penetrometry measurements were applied to the ORC samples, both to indirectly define possible variations in texture due to prolonged freezing periods.

2 METHODS

2.1 OBLONG RED CHICORY CHARACTERISTICS AND TEMPERATURE TESTING

In this experiment, 64 ORC heads were tested. These were divided into 4 groups of 16 samples each (see figure 1). Each group was subjected to a freezing process at different temperatures except one which was stored at room

temperature of 7°C. The freezing temperatures, obtained by using a previously built mechatronic device [8], were, respectively, of -2°C, -8°C and -18°C.

The freezing process lasted 12 hours and, subsequently, a thawing process was carried out inside a large cold room whose temperature was set at 7°C and where the ORC heads lasted 12 hours before moving on the experimental laboratory to effectuate both impedenziometric and penetrometric measurements.

The instrumental tests were conducted after the following steps:

- the room temperature was detected at the beginning of the measurements using a commercial digital thermometer;
- each sample of each group was measured for geometry noting length and width and, by assimilating the chicory head to a rotation ellipsoid, the major and minor axes in cm were detected (see figure 2);
- each plant was weighed, measuring its mass in kg.



Figure 1 The photos show, inside transparent plastic containers, the 16 samples of oblong red chicory heads included in one of the groups to be subjected to dystermia experiments. In the photo at the bottom right is shown the digital thermometer, with its metal rod equipped with a thermal sensor and which indicates, in this case, an ambient temperature of 15.5°C. The photo at the top right shows the thermometer rod stuck in a chicory head in order to sometime measure its internal temperature before freezing.

2.2 BIOELECTRICAL IMPEDANCE ASSESSMENT

The changes in the bioimpedance of the ORC samples due to the different normal or dystermic imposed conditions on them by the experimental protocol, were acquired. A device suitable for the purpose, called “e-Physio Tool” [9, 10, 11] and owned by the company AC Technologies Ltd, was used which allows an alternating electrical current at a constant value of 1 mA and having a frequency of 65 KHz to be injected into a biological sample.

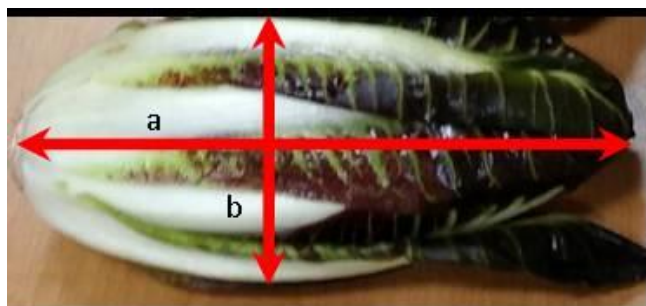


Figure 2 The photo shows a head of oblong red chicory with superimposed, in red, the major (a) and minor (b) axes of the rotation ellipsoid whose volume of geometric solid was calculated from these axes.

These bioimpedance variation values, recorded in Ω , were reported as a function of the temperatures to which each testing groups was subjected. This relatively high frequency of the current injected into the ORC samples was such as to completely short-circuit the electrical capacities of the cell membranes so nullifying the reactive component of the electrical impedance, in this way this latter is made up only of its resistive component. In fact, it is interesting to take into account that, in samples of ex-vivo biological tissues, it was found that the injection of an electrical current with a frequency of only 10 KHz gave rise to a bioimpedance module value which, practically, coincided with the modulus of its resistive component[12]. Therefore, it can be argued that, when we injected an electrical current with a frequency of 65KHz into red chicory samples, their capacitive reactance dependent on the cell membrane could be totally nullified and, at the same time, the impedance modulus practically reflected only its component of electrical resistivity which, as is known, allows the expression of the structural quality attributes of a vegetable in the form of numerical values [13]. As previously shown [13, 14], bioimpedance measurements of the red chicory samples were made using the tetra polar method in which, as shown in figure 3, two copper electrodes, inserted at the end of the sample, injected the electric current (I_{orc}) into the sample while the other two, placed inside the first ones, detected the difference in electric potential (ΔV_{orc}) due to the resistance opposed by the medium to the passage of the current or by the electrical impedance of the chicory's tissue (Z_{orc}) involved here which, as mentioned, is made up of the resistance alone (R_{orc}). The software implemented in e-Physio Tool proceeds to calculate the R_{orc} using the following equation based on Ohm's law:

$$R_{orc} = \Delta V_{orc} \div I_{orc} \quad (1)$$

In figure 3 is shown the blocks diagram representing the ICT platform, implemented into the e-Physio Tool, for the acquisition and the remote control of tissues status in frozen ORC trough their electrical bioimpedance measurements. In the diagram are identifiable three separate blocks: a sampling front-end, a command and control system and a data transmission system. The sampling front-end contains the ADAS-1000 electronic board (Analog Devices, USA)

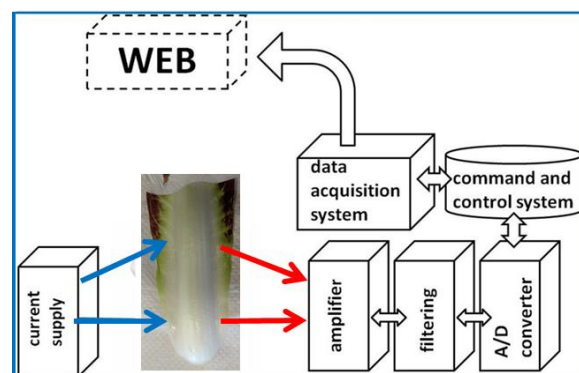


Figure 3 The blocks diagram represents the different modules and their interconnections which make up the e-Physio Tool for the acquisition of the electrical bioimpedance from oblong red chicory leaves as well as the remote acquisition system for this information. Blue arrows represent the current injection electrodes into the central venation of a leaf fragment, red arrows represent the acquisition electrodes for the electrical potential difference due to the impedance opposed from leaf tissues to the injecting current.

to inject the electrical current into the leaf fragment (the current supply box in figure) and the voltage difference induced by bioimpedance which is assessed by a module containing the electrical signals amplifier (amplifier box) together with a three stages filtering section to ensure the elimination of frequencies outside of the range of interest from the acquired signals (filtering box) followed by a 10bit analogical-digital converter appropriately calibrated (A/D converter box). The box for command and control system is for supervise the sampling sessions by initializing and driving the ADAS-1000's operations sending all the information to a controlling software operating on a PC trough a data acquisition system which, in turn, contains a Bluetooth microchip fully supporting the radio frequency communication protocol which enabled a totally wireless operation mode of e-Physio Tool (see the data acquisition system box in figure). The WEB box (in figure with dashed lines) consents the device to be remotely controlled by a Personal Computer running a proprietary software. The proprietary control software is developed in C language, operating under the Linux Operating System. The communications are carried via a radio frequency communication interface (RFCOMM). The PC sent commands directly to the e-Physio Tool using a binary protocol designed for this experiment. The device's microcontroller received the commands, performed a parsing of the binary protocol and issued the specific native commands to the ADAS-1000 using a serial peripheral interface (SPI). The ADAS-1000, after receiving the setup and session commands, started streaming the bioimpedance sampled data to the e-Physio Tool microcontroller using the SPI interface. The microcontroller processed in real time the stream of sampled data converting it in a different packet format that were sent to the controlling PC using the RFCOMM interface.

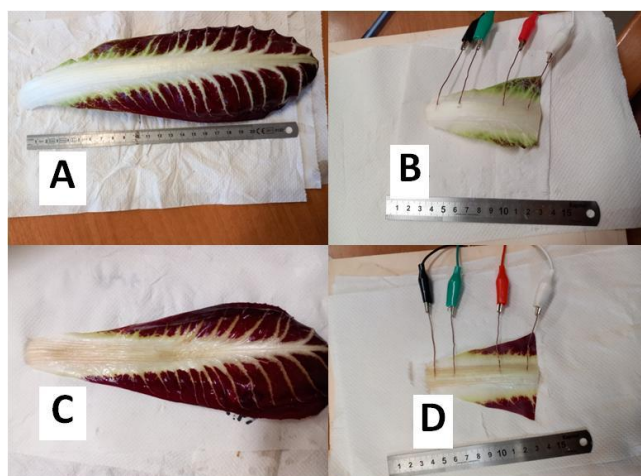


Figure 4 In A the photo shows a superficial leaf of red chicory excised from its head while it was in environmental thermal conditions while the photo in B shows its basal portion, 10 cm long, in which the bioimpedance measuring electrodes were inserted, respectively the electrical current injection electrodes (black and white) are inserted 1cm from the respective leaf portion ends, and the impedance derivation electrodes (green and red) were inserted 2 cm inside the homologous injection electrodes. In this way, the distance between the impedance derivation electrodes was always 4 cm. The photos in C and D concern the same condition but in a red chicory leaf kept at -18°C for 12 hours in which the dehydration condition of the plant fibres constituting the central vein of the leaf can still be seen.

The four electrodes here utilized are of annealed and cold drawn copper wire and have a diameter of 0.5 mm. Each electrode was freed from the insulating sheath for 5 mm, this non-insulated portion was manually inserted into a leaf fragment, along the central venation, as shown in figure 4. The 4 photos in this figure concern two superficial leaves of a ORC (A and C on the left) and two fragments excised from them (B and D on the right), these latter being used for the measurement of electrical impedance. Leaf's fragment, cut starting from the base of leaf A (10 cm length), in which the current injecting electrodes (black and white) are inserted at 1cm from the respective fragment ends, and the impedance derivation electrodes (green and red) are placed 2 cm inside the homologous injection ones. In this way, the distance between the impedance derivation electrodes was always 4 cm. The Photo D concerns the measurement of electrical bioimpedance carried out on a fragment excised from a red chicory leaf kept for 12 hours at -18°C (C) in which the dehydration condition of the plant fibres constituting the central venation can still be seen. Figure 5 shows a photographic sequence of the manual procedure applied by an expert operator to a leaf of an ORC to be tested for impedance assessment.

2.3 MECHANICAL ASSESSMENT

Penetrometric tests on fruit and vegetable products make it possible to quantify the resistance of the product structure



Figure 5 A photographic sequence is shown of the manual procedure applied to obtain a red chicory leaf fragment to measure its electrical impedance. Photos from 1 to 4 concern the cutting a leaf from the chicory head; photos from 5 to 11 concern the excision of a leaf fragment and its preparation for the bioimpedance electrodes insertion; photos from 12 to 18 concern the bioimpedance electrodes insertion being aided by a graduated metal ruler to identify the respective positions of the electrodes to be inserted into the leaf fragment.

to penetration carried out with a reference indenter, i.e. its hardness. This resistance is linked to the organoleptic, quality and freshness characteristics of the preservation process of these products [15, 16]. Particularly, the organoleptic characteristics of a food reflect the qualities perceivable through one or more sense organs, such as appearance, colour, shape, aroma, flavour, consistency and characteristics connected to it, including fluidity, viscosity, friability and others immediately connected to the perceivable quality of the product. The penetrometric tests performed on fruits and vegetables are basically a hardness tests. Hardness is a characteristic of materials that is quantified by a numerical value that indicates the plastic deformability characteristics of the material and is defined as the resistance to permanent deformation of the material itself. Hardness tests determine the resistance of a material to penetration by a tool called an indenter [17]. For materials used in engineering there are different scales to measure their hardness. Of these the most used are [18]:

- The Brinell scale;
- The Vickers scale;
- The Rockwell scale;
- The Mohs scale.

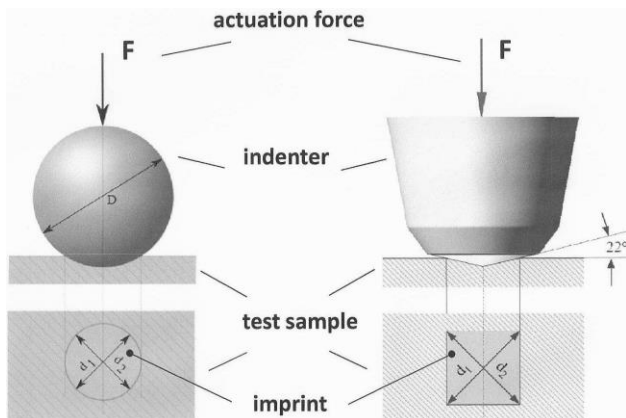


Figure 6 Schematic representations of two devices for measuring the hardness of a material specimen by indenter. The drawing on the left concerns the Brinell measurement method generating a circle imprint while the drawing on the right concerns the Vickers measurement method generating a square imprint. F: applied actuation force.

The hardness characteristics of the ORC heads have been correlated to the geometry of the plastic imprint left by the indenter or punch, based on a given actuation force F , as illustrated in figure 6 for the frequent cases of Brinell and Vickers hardness. However it is of keep in mind that in the International System of Units there is no fundamental unit of measurement for hardness. The instrument here used for the penetrometric tests (JUANJUAN, model GY-2/GY-3, China) is represented in figure 7. It is a precision penetrometer with a dynamometric operating principle which measures the maximum penetration pressure value reached when the sample fails. The penetration pressure value at the failure in the structure was recorded in kg cm^{-2} . These values were reported as a function of the temperatures to which a given ORC group was subjected. The penetration zones are those at the base of the chicory head, corresponding to the section where the plant was cut from the root. For the tests on red chicory it was necessary to prepare dedicated probes, which were calibrated specifically (using apples as the gold standard sample). This need was linked to the high resistance to penetration offered by chicory, compared to fruits which are commonly subjected to penetrometric tests. In figure 8 it can be seen the two modified penetration probes, respectively with a like blade and a like hexagonal section, with also the apples used as gold standard for their calibration. The penetrometric tests were then conducted with the modified probes. The probe used after the calibration activity which was carried out using as reference the probes supplied with the instrument and represented by flat-tipped punches with diameters $\Phi = 8 \text{ mm}$ and $\Phi = 11 \text{ mm}$ respectively. The 8 mm one was carried out with penetration of the modified probe shown from a flat tip punch, with an area 4.69 times smaller than the reference probe with diameter $\Phi = 8 \text{ mm}$.



Figure 7 Images on top concern the joint professional rack and the main pointer of the device here utilized for penetrometer tests. Images below concern the two different reading scales of the pressure applied by the penetrometer on the sample to be tested depending on the diameter of each of the two usable pressure heads.

The value of the pressure ratio of the two probes does not result in the ratio of the two areas, as the phenomenon is highly non-linear. Figure 9 shows one red chicory test area, at the base of the plant cut, and it can be seen the holes left by the action of the probe following penetration.

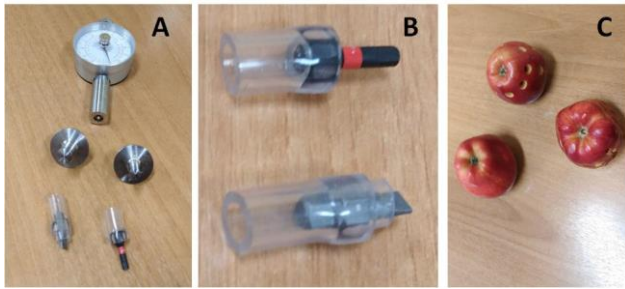


Figure 8 A: photo of the penetrator main body (upper), the two pressure heads (middle) and the two modified penetration probes, during the penetration handling the modified probe with hexagonal section (down) was used by applying it to the pressure head (middle) with a diameter of 8 mm (via a transparent plastic joint) and this in turn was screwed to the stem of the main penetrator body (upper); B: photo of enlarged details of the two modified penetration probes; C: some apples utilized here as gold standard samples to calibrate modified probes; here are seen several penetration holes.



Figure 9 The photo shows the test area with traces of 4 holes produced by the modified penetration probe along the major axis of the rotation ellipsoid in the cutting area at the base of the red chicory plant.

2.4 INFORMATION AND COMMUNICATION TECHNOLOGIES PLATFORM

2.4.1 Communication Layer

To remotely carry out the bioimpedance measurement sessions of the ORC samples was used a distributed ICT infrastructure which was developed by our own lab. It was designed to optimize the data acquisition processes (single sampling, repeated sampling and time series) coming from devices and remote devices interconnected to a central analysis system via the Internet. The architectural features of this platform will be briefly described below. From a structural point of view, the platform used is configured as a distributed Web Application which consists of four software levels with different functional roles, as visible in figure 10. The Communication Layer of the platform used is delegated to manage the interactions existing between the applications that compose it and the external clients that interface with them. As visible in figure 10, the platform supports communication and interaction with two different

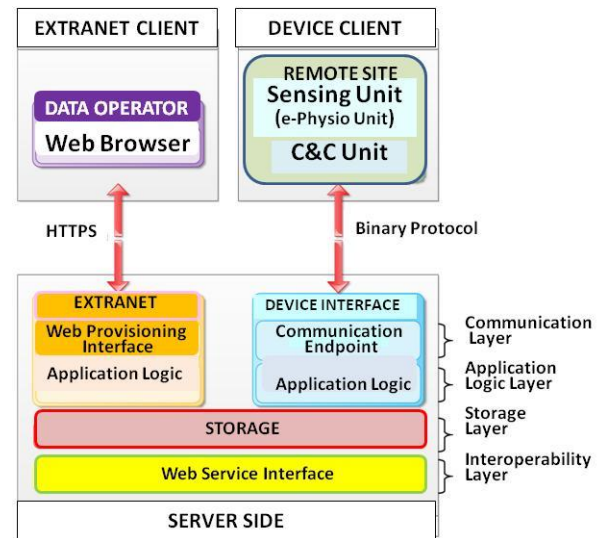


Figure 10 ICT architecture utilized.

classes of clients, which interface with two distinct application sections of the telesampling system. The first class of clients consists of the tools used to access the services provided by the extranet module of the platform, typically used by operators expert in data management who actively participate in the testing/sampling process. The platform's extranet exposes the functions of system configuration, data visualization and processing and administration of the devices assigned to the individual test sites. These tasks are managed by the platform's administrative staff (or by the individual data operator). The entire graphical interaction interface (user interface - UI) which is generated as a support tool for User eXperience (UX) is accessible to users through the use of a thin client consisting of a recent web browser, which has a sufficient level of compliance with the main industry standards (such as Google Chrome or Mozilla Firefox or Microsoft Internet Explorer in versions later than 10).

All the interaction processes that occur between the clients of the extranet and the application logic of the platform are conveyed using a secure transport protocol, the HTTPS protocol using a configuration that involves the exchange of HTTP messages (see Fielding, Irvine, Gettys, & Mogul, 1999) [19] over a TLS encryption channel (Dierks & Rescorla, 2008) [20]. The second class of clients is instead made up of systems for the acquisition and transmission of biosignals acquired through the use of devices in remote testing/sampling sites. As shown in Figure 1, the clients assigned to the different operators to detect and transmit the signals of interest are made up of two main units, a sensing device (i.e., in the case considered, the e-Physio Tool device) which takes care, through a specific detection logic, to acquire impedance data using specific sensors, characterized by the name of Sensing Unit (SU) and by a second command and control unit, called Command and Control Unit (CCU), typically consisting of a smartphone or from a tablet PC or other device with an internet connection and sufficient computational resources. In the specific case, this device is represented by a Linux micro

server based on Raspberry PI 4 SBC, connected to the internet via 4G connectivity via WiFi and connected directly to the e-Physio Tool device via SPI type wired connection. The data collected by the SU are then transmitted and stored by the CCU which subsequently sends them to the server side of the platform, interfacing with a specific data exchange module belonging to the Communication Layer.

Since the type of connectivity available for the CCU is normally represented by access to the internet operating over a digital wireless telephone network, the information exchange system between this class of client and the server side of the platform was modeled taking account of the peculiarities that distinguish this type of communication channel. In fact, despite the maturation of the sector and the technological advances that have characterized the digital

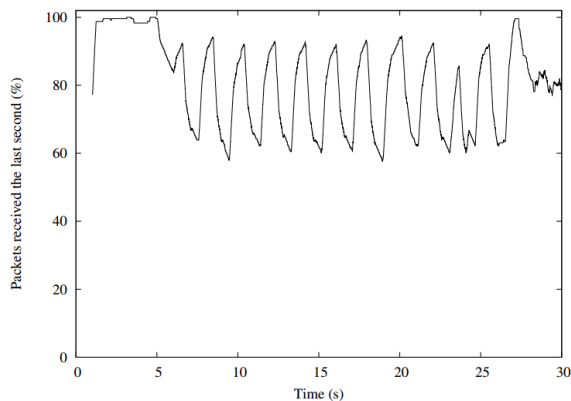


Figure 11 Example packet loss for an HSDPA connection with a single stationary user connected and a target rate of 2.8 Mbps

data networks of mobile telephony operators in recent years, some critical issues still remain present which must be taken into consideration right from the design phase.

In particular, the first problem that was addressed consists in the possible lack of availability of the data connection itself, for transient reasons (weakness of the signal, placement of the CCU in gray areas, temporary overloads or malfunction of a cell or portions of the network etc.) or permanent (long-term network failures, defective cells, complete lack of coverage in the CCU operations area). The second problem taken into consideration is that relating to the limitation of the bandwidth actually available to carry out communication between the CCU and the server side of the platform. In fact, although the evolution of wireless communication standards for digital data which in the space of a few years went from GSM (Global System for Mobile Communications) to Edge, to the Universal Mobile Telecommunications System (UMTS) to the High Speed Downlink Packet Access (HSDPA) versions up to the most recent Long Term Evolution (LTE), has brought about notable improvements in terms reduction of lost packets, latency and bandwidth available for each mobile user connected to a specific cell, as demonstrated in the literature, conditions of connection instability still exist today which can cause significant packet loss experiences

even in stationary conditions in which there is a ideal signal coverage, as seen for example in figure 11.

Furthermore, the progress made in terms of available bandwidth has been mainly focused on the downstream data flow, to make the model of use of Web contents as rapid as possible, which is strongly unbalanced in communication

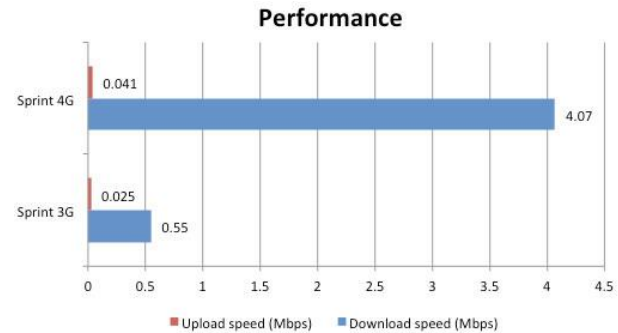


Figure 12 Comparative measurements between 3G and 4G connections.

direction that goes from Web Applications towards the users' terminals, as demonstrated for example by a comparative test reported in the literature and shown in the figure 12. The third issue addressed in the design phase consists of the costs of data communication through digital mobile telephone networks. Also in this case, recent market developments have led, in recent years, to the appearance of types of offers for data traffic that are more convenient for users, thanks to the introduction of flat pricing models based on flat-rate fees for predefined quantities of traffic carried out. However, the cost of traffic on mobile devices is higher than that of data traffic on fixed lines and, therefore, it is necessary to implement all mechanisms to reduce the amount of bandwidth occupied in order to contain the overall costs linked to data transfers digital information. To meet the requirements posed by the management of the critical issues just listed, which derive from the peculiar characteristics of internet connections that use digital networks for wireless telephony, a specific highly optimized binary protocol was therefore here developed, capable of combining strong encryption features of data and control of the integrity of messages to a system of compression and incremental sending of the information to be transmitted. This protocol allows to obtain superior performances compared to standard protocols characterized by less conciseness in the transmission of information and allows to simultaneously satisfy all the requirements listed previously.

2.4.2 Application Logic Layer

The second level of the platform consists of the application logic of the system. Also in this level, similarly to what is reported for the Communication Layer, a division into two distinct functional areas is visible. The first area relates to the business logic that underlies the operation of the systems' extranet. This part of the system contains all the application modules which, by exposing an interface

through the communication layer of the extranet, allow you to interact with the deployment configuration of the platform used, to manage the system users, the acquisition sites, the acquired data and devices used for the sampling sessions.

The second application area is the one that supervises the mechanism for receiving data from remote devices, the transmission of remote device configurations, the processing of received data and the archiving of unprocessed and processed data. The combination of these two areas of the platform is responsible for providing the entire set of functions that can be used by the two types of client classes authorized to access the remote sensing system.

2.4.3 Storage Layer

The storage layer of the system is responsible for archiving and permanently storing all the information contained within the remote sensing system, ranging from the configuration data of a specific deployment up to the trace data acquired by remote sensors. The two modules of the application layer rely entirely on the storage module to store the information they manage and to recall it from the storage state following targeted queries, for example queries in a relational database management system (RDBMS). The storage level is divided into three different archiving systems, a module for storing data on a file system and a second module for storing structured data in a specific RDBMS and, finally, a graph-based storage.

The first module is used by the platform to mainly store processed and unprocessed files such as sampling of signals acquired from remote sensors, while the second module is functional for saving and querying data from acquisition sites, access data, authorization, summary data extracted from processes and so on. Mechanisms to support data integrity requirements have been implemented in both storage modules. The third and final storage module uses the graph model to define data and their relationships. This storage module is used exclusively by the platform's authorization system, presenting adequate performance levels to satisfy the requirements of this portion of the system.

2.4.4 Interoperability Layer

The interoperability layer constitutes the platform's interface to external systems. This layer mainly relies on standard communication mechanisms in the context of the exchange of information between IT systems from a Machine To Machine (M2M) perspective. The form of communication used therefore consists of a RESTful Web Services interface that uses the JSON (Java Script Object Notation) representation format to make it possible to exchange certain types of data between the platform and third-party information systems. This layer requires a further structural description, considering its peculiarity in the overall scheme of the platform.

In fact, in some types of use cases envisaged for the platform, an interoperability gateway can occupy a hybrid infrastructural position. In fact, since this component allows

interactions from the outside with the remote sensing platform it must be, in numerous concrete topologies, in a position that makes it physically accessible to calls coming from third-party systems. Large IT infrastructures, such as those present in some large ICT structures and data centres, can divide internal networks into areas with different security, with the use of demilitarized zones (DMZs) and network or application level gateways which constitute filters between network partitions having different security requirements.

In complex scenarios such as those described, it is extremely unlikely that the interoperability gateway will be placed on infrastructures that are located, from the point of view of the topology of the host network, in proximity to those used for the deployment of the remaining parts of the platform. This consideration implies that, especially if mixed-use IT infrastructures are used in the deployment, it is more complex to provide stringent requirements on the host which are perfectly reasonable for the infrastructures used for the rest of the platform. These peculiarities of the Interoperability Layer were therefore taken into due consideration in the analysis of the infrastructure supporting this platform.

2.5 APPLIED STATISTICS

Statistical methods here applied concerned tests for comparison between groups of data and prediction of inference between variables. The statistical tests that allow such evaluations fall mostly within the scope of parametric statistics which the application needs that data groups considered have a so-called normal or Gaussian distribution. For this, the MedCalc software package (Belgium) was chosen; a $P < 0.05$ was given as significant.

3 RESULTS

3.1 IMPLEMENTATION OF THE FUNCTIONAL ELEMENTS OF THE "REMOTE DEVICE SERVER" PLATFORM

Ere it has been made the Remote Device Server (RDS) which was the communication endpoint between the platform and the remote command and control devices, and was intended to acquire data from the sensors operating in the remote acquisition sites. This endpoint was implemented by a daemon developed specifically for managing bidirectional communication with remote devices. The tasks delegated to the RDS were the following:

- reception of connection requests from remote command and control units;
- authentication of remote command and control units connected to the platform;
- point 1 reception of individual sampling files transmitted by command and control units authenticated;
- insertion of the received sampling files into a specific pre-processing area of the file-based permanent storage layer;

- insertion of data from each sampling received in the relational storage area of the platform;
- sending modified configuration data to the remote client;
- sending sampling scheduling data to the remote client.

Thus, the RDS consisted of a network daemon based on a multiprocess edge-triggered architecture, centred on a network event processing state machine, which took advantage of the abstractions provided by the libevent library [21]. The daemon has been extremely optimized for carrying out the tasks listed above through the use of special state machines for representing the various phases of communication between client and server. Each instance of a process dedicated to managing a single connection determined a server memory occupation that could be estimated at less than 2MB. Since each connection coming from the remote clients was totally idempotent and the state is maintained throughout the communication session by the protocol used, the communication layer between the platform and the remote devices enjoyed a linear horizontal scalability property, which could be obtained simply by adding additional RDS instances listening for remote client requests, configured in load balancing with any technique, i.e. from a simple DNS (Domain Name System) round robin to the use of dedicated load balancers. Based on these considerations it was therefore easy to understand how the use of a decoupled two-level architecture such as the one described for managing the provision of extranet functions would not have been particularly sensible in the case of RDS. The negligible memory impact and the linear horizontal scalability, in fact, suggested the use of a multi-process management technique such as the one here adopted, which avoids the architectural complexity of a complete decoupling between the communication layer and the information processing layer, which in this case it would not bring any benefit. The RDS architecture, therefore, was composed of two main elements, a listening module for incoming connections which, when it received an incoming request from a remote command and control unit, started a dedicated lightweight process to its management and delegated the protocol exchange with the client, as visible in figure 13. Each connection management process was terminated when the operations required by the connection itself was completed (i.e. when it is terminated). The communication protocol operated over a TLS (Transport Layer Security) channel for secure data exchange between the client and the server. The TLS layer of the communication channel also provided an important MAC (Media Access Control) layer for the data which was subjected to a much more effective checksum than that provided exclusively by the underlying TCP transport protocol, which may not even be sufficient to identify with the precision necessary to the level of data integrity required by the platform errors in transmissions and, consequently, corrosion in the data. All messages transmitted in the protocol were also accompanied by an additional HMAC based on SHA512 cryptographic level

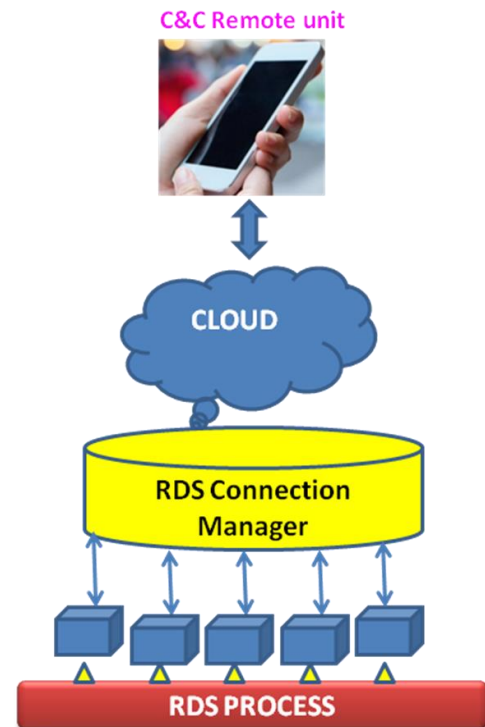


Figure 13 Remote Device Server communication architecture

hashing algorithms. The files transmitted by the remote client, containing the data acquired during a sampling session, had an internal file format, accessible after carrying out the decryption operation on the external envelope.

3.2 GEOMETRICAL AND MASS ACQUISITIONS

Graphs concerning the geometrical data of elongate red chicory samples belonging to the different groups, assessed just after they were thawed from the distermia exposition, are drawn. In particular, data acquired concerned: chicory head's major and minor axis, its volume, its mass. Furthermore, for each group the mean and standard deviation for the different measures are reported. Concerning the geometrical data of elongate red chicory samples belonging to the different tested groups, descriptive data shown in the figures 13, 14, 15 and 16, highlight that as the measurements carried out manually on each sample of elongate red chicory indicate, the length of the major axis of this vegetable oscillated among all the four groups of samples around 25 and 30 cm while the range of the minor axis oscillations was even narrowed, i.e. around 10 cm or little more. About the estimated geometrical volume of the elongate red chicory's heads, indirectly calculated as a rotation ellipsoid volume and estimated in litres, it showed wide fluctuations in all four vegetable groups with differences between maximum and minimum values reaching up to 100% (see volume graphs in figures 14, 15 and 16). The elongate red chicory's head masses, which values were estimated in g by a commercial digital scale, showed large oscillations between vegetable samples of the same group as they were thawed at room temperature.

3.2.1 7°C Group of Red Chicory Samples

In Figure 13 graphs concern geometric axes (top), volume (middle) and mass (bottom) of the obtained red chicory's rotation ellipsoid in 7°C group. Graphs points concern each issue values.

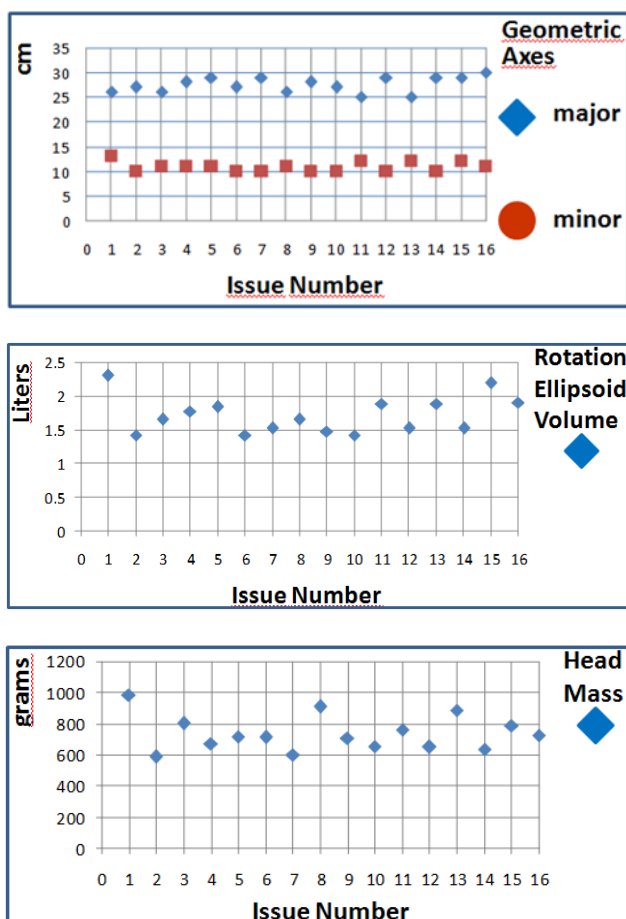


Figure 13 Graphs about red chicory's rotation ellipsoid in 7°C group.

Table I - Red chicory dimensions statistics, group 7°C

	major axis (cm)	minor axis (cm)	Head volume (L)	Head mass (g)
Mean	27.5	10.8	1.7	735.6
±SD*	1.5	0.9	0.2	113.5

*Mean values ± standard deviation (SD) concern a sample of 16 elongate red chicory heads.

3.2.2 -2°C Group of Red Chicory Samples

In Figure 14 graphs concern geometric axes (top), volume (middle) and mass (bottom) of the obtained red chicory's rotation ellipsoid in -2°C group. Graphs points concern each issue values.

However, the rate of weight fluctuation remained in all four groups of vegetables between 600 and 800 g. In tables from I to IV, are reported the mean values ± SD of the geometric and weight variables of each disthermically tested group of red chicory.

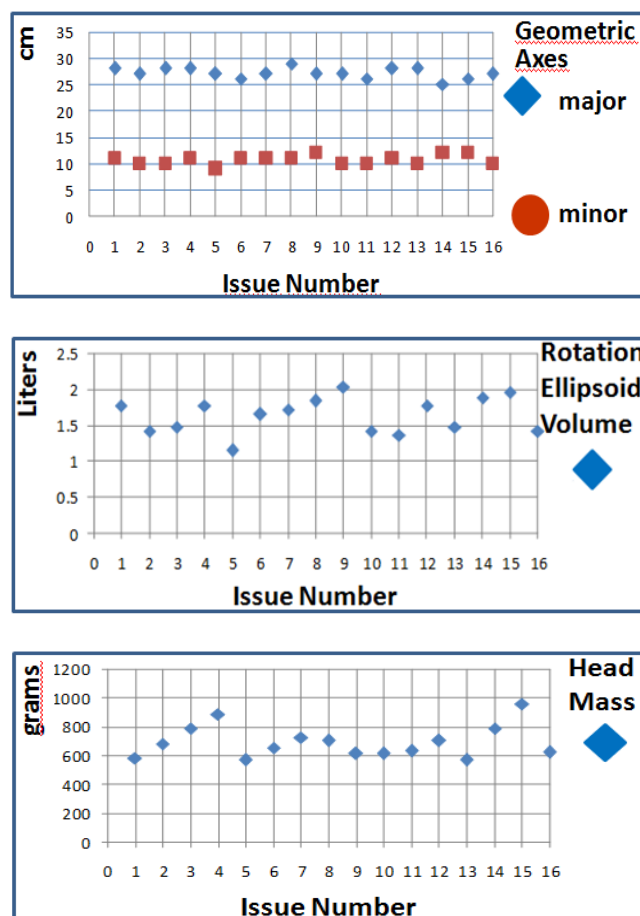


Figure 14 Graphs about red chicory's rotation ellipsoid in -2°C group.

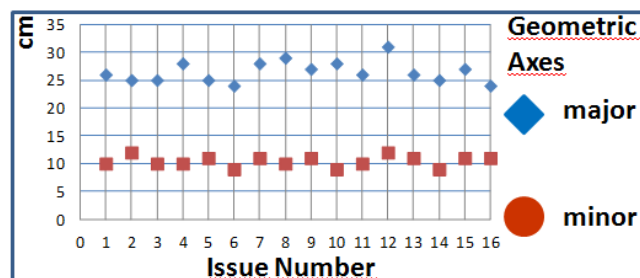
Table II - Red chicory dimensions statistics, group -2°C

	major axis (cm)	minor axis (cm)	Head volume (L)	Head mass (g)
mean	27.1	10.6	1.6	692.1
±SD*	1.0	0.8	0.2	113.7

*Mean values ± standard deviation (SD) concern a sample of 16 elongate red chicory heads.

3.2.3 -8°C Group of Red Chicory Samples

In Figure 15 graphs concern geometric axes (top), volume (middle) and mass (bottom) of the obtained red chicory's rotation ellipsoid in -8°C group. Graphs points concern each issue values.



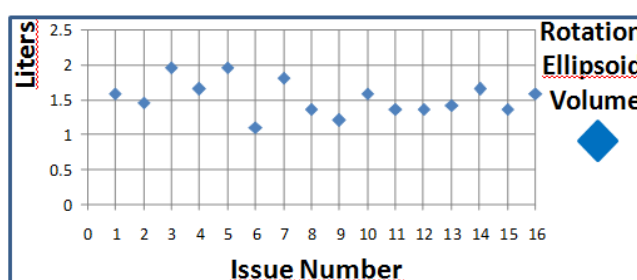
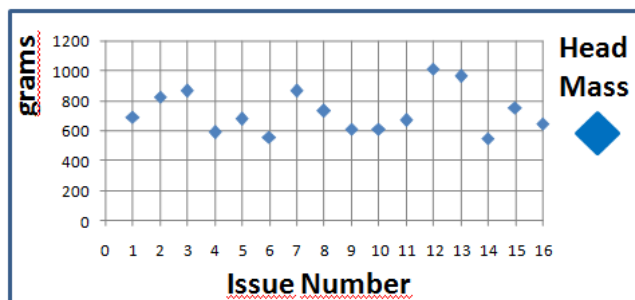
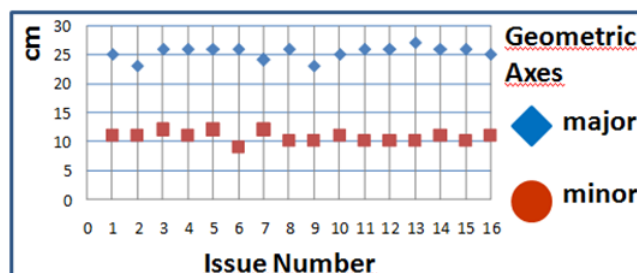
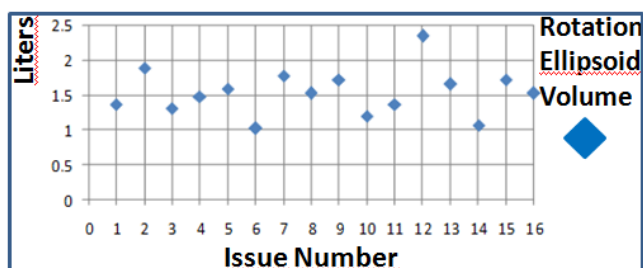


Figure 15 about red chicory's rotation ellipsoid in -8°C group.

Table III - Red chicory dimensions statistics, group -8°C

	major axis (cm)	minor axis (cm)	Head volume (L)	Head mass (g)
mean	26.5	10.4	1.5	723.6
± SD	1.9	0.9	0.3	144.1

*Mean values ± standard deviation (SD) concern a sample of 16 elongate red chicory heads.

Table V – Red Chicory Head's Major Axes

t°C	7	-2	-8
-2	0.382		
-8	0.109	0.272	
18	<0.001*	<0.001*	0.037*

Data concern P values among means differences for each tested temperature (t°C). *Mean difference statistically significant. From presented data it can be argued that the intergroup variability, done as the SD versus mean ratio in percentage, was the lowest for the major and minor axes (5.6% and 5.2% respectively) or quantities directly measured manually, increased considerably in the indirectly estimated

3.2.4 -18°C Group of Red Chicory Samples

In Figure 16 graphs concern geometric axes (top), volume (middle) and mass (bottom) of the obtained red chicory's rotation ellipsoid in -18°C group. Graphs points concern each issue values. Data concern P values among mean differences for each tested temperature (t°C). No differences statistically significant were found. Chicory volume (11.8%) and increased a lot in the size obtained through a commercial device, i.e. the red chicory heads mass (17.1%). Chicory volume (11.8%) and increased a lot in the size obtained through a commercial device, i.e. the red chicory heads mass (17.1%).

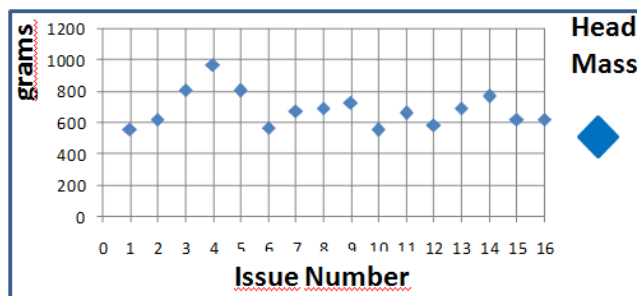


Figure 16 Graphs about red chicory's rotation ellipsoid in -18°C group.

Table IV - Red chicory dimensions statistics, group -18°C

	major axis (cm)	minor axis (cm)	Head volume (L)	Head mass (g)
mean	25.3	10.6	1.5	678.9
±SD*	1.1	0.8	0.2	112.1

*Mean values ± standard deviation (SD) concern a sample of 16 elongate red chicory heads.

Table VI – Red Chicory Head's Minor Axes

t°C	7	-2	-8
-2	0.511		
-8	0.218	0.512	
-18	0.511	1.000	0.510

However, in order to establish whether or not the exposure of red chicory heads to increasing negative temperature degrees for a prolonged period gave rise to statistically significant variations in the geometric and weight sizes of these vegetables, using the Student's *t* test for paired groups of data, in tables V, VI, VII and VIII the P values resulting from the comparisons between means ± SD obtained, for each variable, from the chicory samples subjected to the 4 programmed temperatures, are reported. Concerning the Red Chicory Head's Major Axes (table V) data show that

only compared to -18°C both room temperature (7°C) and the other two sub-zero temperatures

Table VII – Red Chicory Head's Volumes

t°C	7	-2	-8
-2	0.168		
-8	0.034*	0.276	
-18	0.008*	0.167	1.000

Data concern P values among mean differences for each tested temperature (t°C). *Mean difference statistically significant.

Table VIII – Red Chicory Head's Masses

t°C	7	-2	-8
-2	0.287		
-8	0.795	0.498	
-18	0.165	0.743	0.335

Data concern P values among mean differences for each tested temperature (t°C). No differences statistically significant were found.

(-2°C and -8°C) showed statistically significant differences in this red chicory axis. On the contrary, in considering the minor axis of the ellipsoid, geometrically representing the shape of this type of chicory (table VI), no significant differences were found in comparing mean values among each set temperature with all the others three ones. In considering mean values of the calculated volume of red chicory after each group of samples completed thawing (table VII), statistically significant differences were observed only for what concerned the ambient temperature condition (7°C) respectively towards temperatures of -8°C and -18°C. Finally, the data shown in Table VIII indicate that none of the hypothermic treatments gave rise to statistically significant weight changes among the four groups of ORC.

3.3 RED CHICORY ELECTRICAL IMPEDANCE AND MECHANICAL HARDNESS ACQUISITIONS

In order to verify whether the data acquired from the electrical bioimpedance and mechanical penetrometry measurements, carried out on the tufts of ORC, responded or not to be into a normal/Gaussian statistical distribution, in the graphs of the figures numbered from 17 to 24 the distribution plots respectively of the detected bioelectrical impedance and of the mechanical pressure applied to the oblong red chicory heads of each of the 4 groups tested are represented and the verification that the acquired data whether or not they followed a Gaussian distribution was carried out by applying the D'Agostino-Pearson normality test to verify the normality of a distribution [20, 22]. The null hypothesis relating to this test is that our data follows a normal distribution so must be $P > 0.05$; in this case the null hypothesis is accepted, i.e. that we are faced with a normal distribution. Since all the D'Agostino-Pearson tests applied showed a $P > 0.05$ so all tested distributions were parametric. These data consented to utilize parametric tests

to compare freezing induced changes in both P and Z changes.

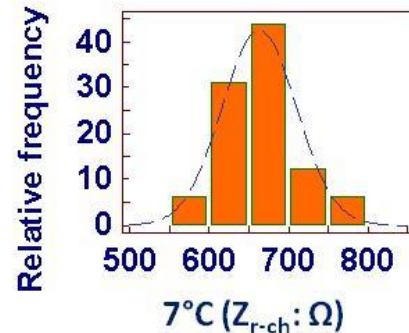


Figure 17 Distribution plot of electrical impedance (Z_{r-ch}) of red chicory issues after staying at 7°C. Since the D'Agostino-Pearson test showed a $P = 0.9537$ so the normal size of distribution is admitted

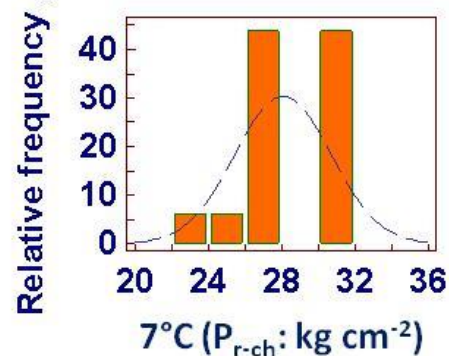


Figure 18 Distribution plot of mechanical pressure applied to red chicory issues (P_{r-ch}) after staying at 7°C. Since the D'Agostino-Pearson test showed a $P = 0.6495$ so the normal size of distribution is admitted

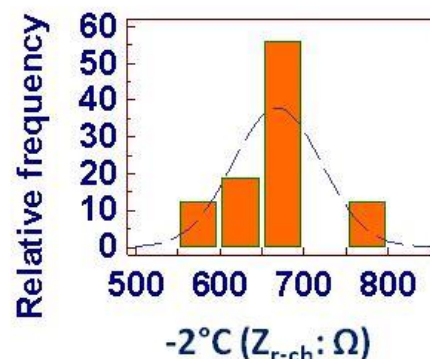


Figure 19 Distribution plot of electrical impedance (Z_{r-ch}) of red chicory issues after freezing at -2°C. Since the D'Agostino-Pearson test showed a $P = 0.2293$ so the normal size of distribution is admitted

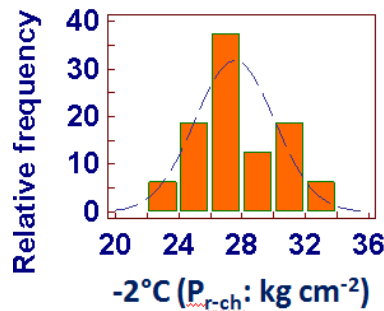


Figure 20 Distribution plot of mechanical pressure applied to red chicory issues (P_{r-ch}) after freezing at -2°C . Since the D'Agostino–Pearson test showed a $P = 0.6379$ so the normal size of distribution is admitted

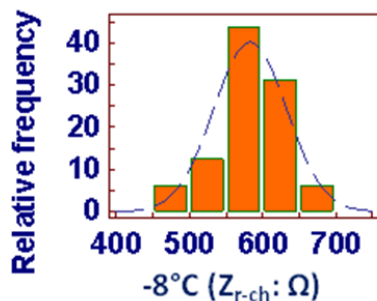


Figure 21 Distribution plot of electrical impedance (Z_{r-ch}) of red chicory issues after freezing at -8°C . Since the D'Agostino–Pearson test showed a $P = 0.6760$ so the normal size of distribution is admitted

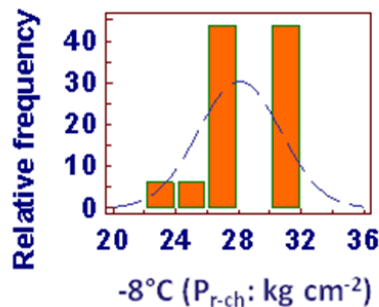


Figure 22 Distribution plot of mechanical pressure applied to red chicory issues (P_{r-ch}) after freezing at -8°C . Since the D'Agostino–Pearson test showed a $P = 0.9695$ so the normal size of distribution is admitted

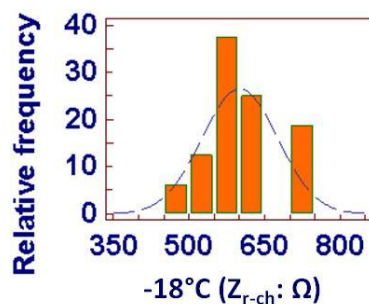


Figure 23 Distribution plot of electrical impedance (Z_{r-ch}) of red chicory issues after freezing at -18°C . Since the

D'Agostino–Pearson test showed a $P = 0.6174$ so the normal size of distribution is admitted

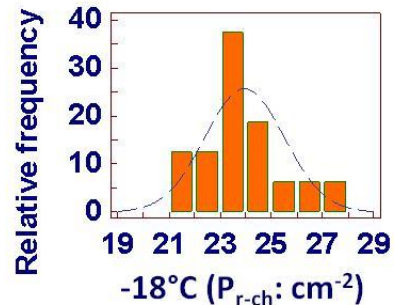


Figure 24 Distribution plot of mechanical pressure applied to red chicory issues (P_{r-ch}) after freezing at -18°C . Since the D'Agostino–Pearson test showed a $P = 0.2208$ so the normal size of distribution is admitted

3.3.1 Parametric comparison among oblong red chicory groups

Columns in the graphs of figures 25 and 26 respectively concern the mean values of assessed electrical impedance and of mechanical pressure in the ORC groups each of which was submitted at a specific prolonged temperature as shown in the abscissa's graphs.

These mean values were compared, twice, using the parametric Student's t test method for paired data and the significance level value among they are shown top each pair of compared columns. A statically significant reduction (Fig. 25) respect to 7°C impedance mean value occurs when comparing the latter with those concerning the condition at -8°C (-12%) and at -18°C (-9%), i.e. blue lines, while this did not occur when comparing 7°C with -2°C conditions which latter differed only of $5\ \Omega$ (blue lines in figure). As show graphs in figure 26, a statically significant reduction in mechanical pressure mean value occur when comparing the 7°C induced kgm^{-2} with those concerning the -8°C (-16%) and -18°C (-15%), while this condition is not observed when comparing 7°C condition with -2°C one in inducing kg m^{-2} changes, which latter differed only of $0.5\ \text{kg cm}^{-2}$ from the former one. Moreover, freezing oblong red chicories at -2°C gave also rise to statistically significant differences from both impedance and mechanical pressure mean values in comparison to that of the -8°C (-14%) and to the -18 (-13%) freezing conditions. Interestingly, slowing chicory temperature from -8°C towards -18°C did not reach relevant differences in both Z and P mean values in testing vegetables. The results of these parametric tests applied for the comparison of the respective variations of Z_{r-ch} and P_{r-ch} in relation to decreasing temperatures, show unequivocally that the storage of the oblong red chicory at temperatures below -2°C gave rise to significant decreases in these two parameters which, indirectly, indicate an increasing deterioration of the tissue and therefore organoleptic qualities of this vegetable.

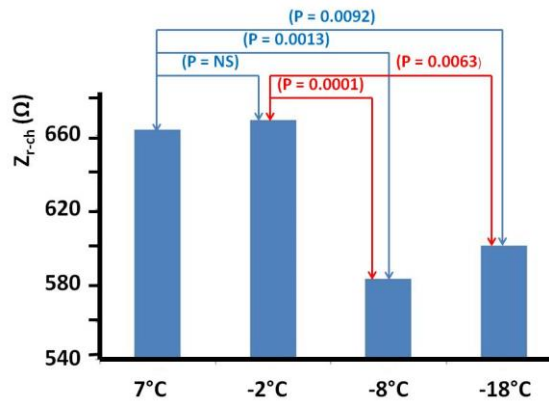


Figure 25 Columns concern mean values of electrical impedance (Z_{r-ch}) assessed in each group (16 issues) of red chicory submitted for 12 hours at the temperature shown at the foot of each column. In brackets the P values between two red chicory groups connected by the respective arrows obtained from the Student's t test for paired data groups

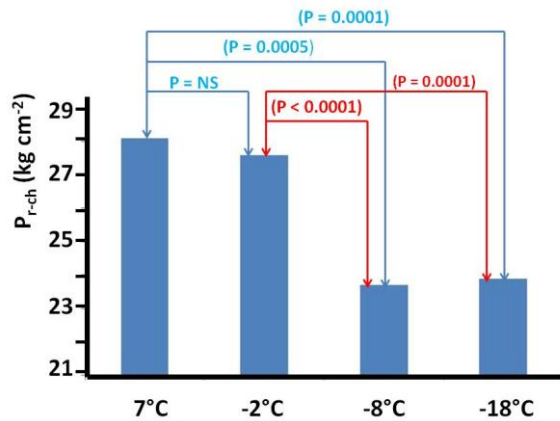


Figure 26 Columns concern mean values of mechanical pressure (P_{r-ch}) applied to each group (16 issues) of red chicory submitted for 12 hours at the temperature shown at the foot of each column. In brackets the P values between two red chicory groups connected by the respective arrows obtained from the Student's t test for paired data groups.

3.4 PREDICTIVE STATISTIC TESTS

Another interesting result that emerges from these two comparisons tests is a good stability of the Z_{r-ch} and P_{r-ch} values for temperature drops that do not exceed -2°C .

In this regard, it is notable that in considering that the areas where oblong red chicory is cultured there could occur night frosts which generally seriously damage other species of leafy vegetables, red chicory seems to possess a capacity for resistance to such meteorological conditions, and this is demonstrated for the first time here by the concordance of both the electrical and mechanical data used in this experiment. The normal distributions of the variables here utilized in the aim of acquire instrumental indicators to indirectly obtain critical information concerning the freezing effect on the chicory structural integrity, i.e. the electrical bioimpedance and the mechanical penetration pressure, also consented to describe the relationship between these two variables in a way of obtain the possible

law that could predict the one unknown from the other known by utilizing the statistic method of the regression. Table IX shows the linear regression equation data concerning the distribution of medians in the electrical impedance changes (in Ω) versus the mechanical pressure ones (in kg cm^{-2}) acquired at each of the four $t^{\circ}\text{C}$ to which the chicory samples were submitted. Equation (2) shows that at each unitary mechanical pressure change (1 kg cm^{-2}) the corresponding electrical impedance directly changed of about 18Ω . This correspondence is clearly demonstrated from the graph shown figure 27.

Table IX – Linear regression equation data

Dependent Y	Z (electrical impedance: Ω)
Independent X	P (penetration pressure: kg cm^{-2})

Least squares regression

Sample size	4
Coefficient of determination R^2	0.9638
Residual standard deviation	10.0507

Regression Equation

$y = 163.32 + 18.02x$					
Parameter	Coeff.	Std. Error	95% CI	t	P
Intercept	163.32	64.00	-112.07 to 438.71	2,55	0.1253
Slope	18.02	2.48	7.39 to 28.65	7,29	0.0183

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	5374.72	5374.72
Residual	2	202.03	101.02

F-ratio	53.2067
Significance level	$P=0.0183$

$$Z_{r-ch} = 163.32 + 18.02 \times P_{r-ch} \quad (2)$$

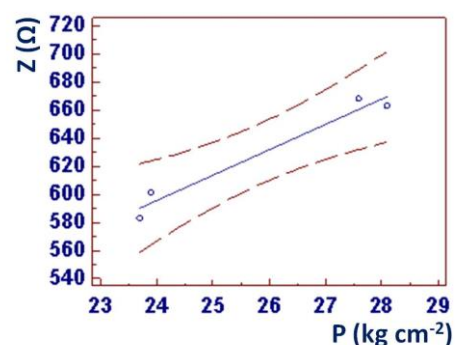


Figure 27 The regression straight line of the electrical impedance (Z) versus applied mechanical pressure (P) is represented concerning tested elongated red chicory samples at each of the four temperature steps which statistical medians values are respectively represented as four empty circles. Dashed lines concern the 95% confidence interval.

4 DISCUSSION

In a recent review Franzoni et al. [23] highlighted that temperature to which vegetables are submitted in their storage or transportation is very meaningful for preserving the quality of these agricultural products. Nevertheless, low temperature in sensitive leafy vegetables such as elongate red chicory can induce loss of quality. Despite the low temperature produces a slowdown in the metabolic processes relating to respiration and the biosynthesis of ethylene, both conditions necessary for continuous maturation with benefits on shelf life, the temperature range that can provide positive effects is a very critical and a complex condition to obtain. In fact, it could happen that exposing these vegetables to specific temperature ranges and for a longer period can induce chilling injury (CI) symptoms. The susceptibility to CI at low temperature varies among vegetable cultivars and genetic backgrounds. It has been found that often CI is visible only when post-storage ripening reaches 20 °C after storage from 2 to 6 °C for 2 weeks as well as when stored at 0 °C for 3 weeks or longer [24]. This chilling injury concerns a disorder that occurs internally to the vegetables and fruits and it becomes visible when products are cut. CI has been related to cell membrane breakdown and disruption [25]. In fact, low temperatures give rise to become rigid the lipid constituents of the membrane, reducing in this way their fluidity and affecting their structural and functional integrity [26]. This in turn produces the membranes breakdown and compromises the cell's functionality. Cold induces fatty peroxide synthesis in cell's membrane which is revealed by increasing thiobarbituric acid reactive substances (TBARS) leakage towards membrane cells. In fact, TBARS are reliable estimators of lipid peroxidation [23].

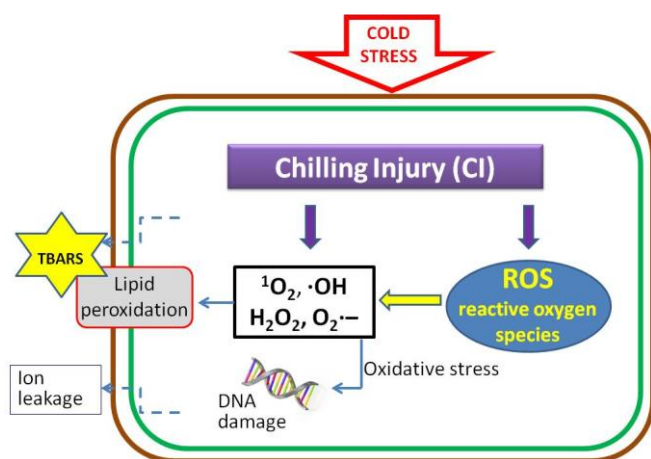


Figure 28 Schematic metabolic and functional damages mainly occurring in vegetable cells submitted to cold stress. External brown line is the cell wall while the green one is the cell plasmalemma. ROS: accumulation of reactive oxygen species; $^1\text{O}_2$: singlet oxygen radical; $\cdot\text{OH}$: hydroxyl radical; H_2O_2 : hydrogen peroxide; $\text{O}_2^{\cdot-}$: superoxide ion; TBARS: thiobarbituric acid reactive substances.

The loss of membrane integrity promotes ion leakage from the cells thus disrupting the balance of electrolytes within the vegetable tissues [27, 28] and this imbalance in ion concentrations gives rise to several adverse effects on physiological processes concerning the cellular functions up to reach a DNA damage.

Flesh browning is the main symptom associated with CI and it is caused by polyphenol oxidases being this enzymatic activity as responsible for phenolic conversion into quinines, gain in this way access to their substrates located in the vacuole when membranes are damaged. Cold stressed fruits also lose juiciness (increased fluffiness) and flavour, both phenomena as linked to a loss of correct ripening metabolism [29]. It has been found that fruit show flesh reddening and bleeding associated with CI [29, 30] and this was attributed to an accumulation of anthocyanins. Figure 28, deduced from the work of Franzoni et al. [23], schematically shows the main metabolic and structural damages toward which a plant cell subjected to cold stress encounters. Nothing excludes that similar phenomena to those described above could also have done in our oblong red chicory samples after they were subjected to low temperature treatments. The importance of a proper temperature management while postharvest handling, is proven by then. In any case, since the markets dedicated to consumers of these products are often located far away from the production sites, and this requires complex logistics implemented by multiple actors, the management of storage/transport conditions is critical to purposes of their good quality when they reach the consumer. The measurement of electrical impedance for the purpose of determining the physiological condition of vegetal tissues [31] has been used in relation to cold acclimation [32] and to frostbite injuries [13, 33, 34] and heat injuries [35]. When plant products are removed from the devices that have kept them at a low storage temperature, they undergo a series of changes including: recovery of physiological function, initiation of maturation, senescence and cellular dysfunction [36]. These changes can occur over a short period of time and therefore they might be expected to produce a unique biological system suitable for demonstrating the interaction between a given physiological condition and the corresponding electrical properties of plant tissues. Electrical models are able to partition the complex numbers that characterize the electrical impedance, respectively into the real resistive component and the imaginary or capacitive one respectively concerning the internal compartments of the cell (resistances) and the membranes (capacitive reactances) [37, 38, 39]. Harker and Maindonald [40] using this approach during the ripening of nectarine peaches, were able to simultaneously evaluate changes in the state of the cell wall, cell membranes and intracellular compartments. They found that during normal ripening of these vegetal products the electrical resistance of the cell wall and vacuoles decreases by 60% and 26%, respectively, and the electrical capacity of the membranes decreases by 9%.

Starting from these experimental assumptions, numerous studies have demonstrated that electrical impedance measurements are able, in different types of plant products, to distinguish between fruit ripening with and without chilling lesions [40, 41, 42]. These latter studies aimed to further investigate the ability of electrical impedance measurements to provide useful and physiologically relevant insights regarding plant ripening status.

5 CONCLUSIONS

Recently [13], it has been designed and built a homemade prototype of a portable device for measuring the resistive component of the electrical impedance in vegetable preparations: the e-Physio Tool, which is suitable for obtain numerical values of the real/resistive component of the electrical impedance which indirectly returns, in mathematical terms, the biological/organoleptic condition in which a specific plant to which distemic conditions have been imposed is found. In fact, the aforementioned experiment highlighted a quadratic mathematical correlation between the bioimpedance values thus detected in lettuce leaves subjected to increasing levels of hypothermia and the quantity of specific proteins expressed by clusters of genes responsible for defending against these abiotic stresses, these latter biomolecular data obtained with the reverse transcriptase-polymerase chain reaction method (RT-PCR) [43]. Starting from these latter results, by utilizing the above described bioimpedance tool, here it has been tested the electrical resistance in the ORC after each time of chosen temperature to which they were submitted, in the aim of discover possible relationships between that electrical variable together with the already standardized variation of the penetration pressure in these plants from which such information could be obtained which concern the biological/organoleptic status of tested samples [44]. In fact, this type of indirect mechanical measurement is not simple to handle. In spite of this, it is known that the reduction of the penetration pressure in a vegetable is an indicator of the loss of structural compactness of the same one [15, 16] and, since from the regression equation (2) obtained in these experiments it can be deduced that with the lowering of the temperature the Z_{orc} also reduced linearly together with the P_{orc} , then Z_{orc} values measured after the chicory thawing, can be used as an easy to obtain indicator of the structural deterioration induced from the progressive freezing of this vegetable and, thank to the ICT platform to which the e-Physio Tool is integrated, these electrical data could be made available to a remote operator in real time, thus allowing a constant verification of the degree of possible deterioration of this agricultural product due to mis-management of the cold chain in the logistic processing of the oblong red chicory.

REFERENCES

- [1] Perović J., Šaponjac V.T., Kojić J., Krulj J., Moreno D.A., García-Viguera C., Bodroža-Solarov M. and Ilić N., Chicory (*Cichorium intybus* L.) as a food ingredient - Nutritional composition, bioactivity, safety, and health claims: A review. *Food Chem*, Vol. 336, pp. 127676, 2021.
- [2] Sheng W., Ji G. and Zhang L., Immunomodulatory effects of inulin and its intestinal metabolites. *Front Immunol*, Vol. 14, pp. 1224092, 2023.
- [3] Schumacher E., Vigh E., Molnár V., Kenyeres P., Fehér G., Késmárky G., Tóth K. and Garai J., Thrombosis preventive potential of chicory coffee consumption: a clinical study. *Phytother Res*, Vol. 25, No. 5, pp.744-748, 2011.
- [4] Olsen N.J., Branch V.K., Jonnala G., Seskar M. and Cooper M., Phase 1, placebo-controlled, dose escalation trial of chicory root extract in patients with osteoarthritis of the hip or knee. *BMC Musculoskeletal Disord*, Vol. 11, pp. 156, 2010
- [5] Migliorini A.A., Piroski C.S., Daniel T.G., Cruz T.M., Escher G.B., Vieira do Carmo M.A., Azevedo L., Marques M.B., Granato D. and Rosso N.D., Red Chicory (*Cichorium intybus*) Extract rich in anthocyanins: chemical stability, antioxidant activity, and antiproliferative activity in vitro. *J Food Sci*, Vol. 84, No: 5, pp. 990-1001, 2019.
- [6] Flore G., Deledda A., Lombardo M., Armani A. and Velluzzi F., Effects of functional and nutraceutical foods in the context of the mediterranean diet in patients diagnosed with breast cancer. *Antioxidants (Basel)*, Vol.12, No. 10 pp.1845, 2023.
- [7] Ávila-Gálvez M.Á., Giménez-Bastida J.A., Espín, J.C. and González-Sarrías A., Dietary Phenolics against Breast Cancer. A Critical Evidence-Based Review and Future Perspectives. *Int J Mol Sci*, Vol. 21, pp. 5718, 2020.
- [8] Manuello Bertetto A., Fois A., Tocco F., Velluzzi F., Farris F., Vacca D., Picci G., Marcello R., Pinna R. and Concu A., Peaches electrical bioimpedance assessment to control freeze induced texture changes: a easy and not expensive method to discover fruits undeclared cold treatments. *International Journal of Mechanics and Control*, Vol. 24. No. 02, pp. 77-83, 2023.
- [9] Campagna M., Lecca L.I., Velluzzi F., Serra C., Bianco P., Manuello Bertetto A., Dell'Osa A.H., Fanni B., De Pau A., Fois A., Melis L., Kalb A., Marcello R., Melis S., Concu A. and Tocco F., A mechatronic simulator of an aircraft cockpit to study, by a virtual flight test, cardiovascular fitness in airline pilots banned to fly since covid-19 lockdown. *International Journal of Mechanics and Control*, Vol. 23, No. 02, pp. 85-99, 2022.
- [10] Tocco F., Mattana D.V., Solinas R., Velluzzi F., Usai P., Fois A., Melis L., Bianco P., Serra C., Manuello Bertetto A., Dell'Osa A.H., Pereira A.F., Cerina A.,

- Loviselli L., Marcello R., Melis S. and Concu A., Cardiometabolic benefits from a coastal sailing in a radical cystectomy patient remotely controlled by an internet of thing mechatronic tool: a case study. *International Journal of Mechanics and Control*, Vol. 24, No. 02, pp. 39-61, 2023.
- [11] Tocco F., Solinas R., Velluzzi F., Massidda M., Mattana D.V., Fois A., Melis L., Manuello Bertetto A., Bonisoli E.[†], Venturini S., Bianco P., Dell'Osa A.H., Pereira A.F., Melis S., Cerina A., Loviselli L., Marcello R. and Concu A., A mechatronic tool for revealing inverse relationships among heart's stroke volume and head's linear acceleration induced by moored boats rolling in elderly sailors with unchanged body sizes: a non-drug anti-hypertensive advantage. *International Journal of Mechanics and Control*, Vol. 25, No. 01, pp. 133-142, 2024.
- [12] Banach J.K. and Zywicka R., The effect of electrical stimulation and freezing on electrical conductivity of beef trimmed at various times after slaughter. *J Food Eng*, Vol. 100, pp. 119-124, 2010.
- [13] Albani D., Perrotta L., Dell'Osa A.H., Fois A., Velluzzi F., Loviselli A. and Concu A., Relationships among bioimpedance variables and gene expression in lactuca sativa exposed to cold weather. IFMBE Proceedings, 17th International Conference on the Electrical Bioimpedance (ICEBI), June 9-13, 2019, Joinville, Brazil. Vol. 73, pp. 141-145. Ed. Magjarevic R., Springer Nature Singapore Pte Ltd. 2020.
- [14] Dell'Osa A.H., Battacone G., Pulina G., Fois A., Concu A., Kalb A., Melis S., Loviselli A. and Velluzzi F., Bio-electrical impedance device for remote control of an indirect index of mechanical tenderness in ripening beef meat. *International Journal of Mechanics and Control*, Vol. 21, pp. 139-146, 2020.
- [15] Marani C., Agnelli M. and Mascheroni R., Osmo-Frozen Fruits Mass Transfer and Quality Evaluation. *J Food Eng*, Vol. 79, pp. 1122-1130, 2007.
- [16] Phan P.A. and Mimault J., Effets de divers traitements de congélation-décongélation sur l'évaluation des paramètres de texture et l'exsudation des fruits. Relation avec la qualité. *Int J Refrigeration*, Vol. 3, pp. 255-260, 1980.
- [17] Van Vlak L.H., *Materials Technology*. Addison-Wesley Publishing Company Inc., 1973.
- [18] Moore P. and Booth G., *The Welding Engineer's Guide to Fracture and Fatigue*. Woodhead Publishing, 2015.
- [19] Younge E.S., A Testbed for Protocol Analysis for the Internet of Things. *Wireless and Mobile Technologies*, Vol. 5, No. 1, pp.1-6. 2023.
- [20] Dierks T. and Rescorla E., *The Transport Layer Security Protocol*, Version 1.2, RFC 5246, Network Working Group, Montreal, Canada, 2008.
- [21] Libevent – an event notification library. libevent.org. Retrieved 17 September 2021.
- [22] D'Agostino R. and Pearson E.S., Tests for departure from normality. Empirical results for the distributions b_2 and $\sqrt{b_1}$. *Biometrika*, Vol. 60, No. 3, pp. 613-622, 1973.
- [23] Franzoni G., Spadafora N.D., Sirangelo T.M., Ferrante A. and Rogers H.J., Biochemical and molecular changes in peach fruit exposed to cold stress conditions. *Molecular Horticulture*, Vol. 3, No. 24, pp. 1-17, 2023.
- [24] Lurie S., Proteomic and metabolomic studies on chilling injury in peach and nectarine. *Front Plant Sci*, Vol. 13, pp. 958312, 2022.
- [25] Jung J.H., Seo P.J., Oh E. and Kim J., Temperature perception by plants. *Trends Plant Sci*, Vol. 28, No. 8, pp. 924-940, 2023.
- [26] Luo Z., Dongdong L.D., Du R., Mou W. and Mao L., Hydrogen sulfide alleviates chilling injury of banana fruit by enhanced antioxidant capacity and proline content. *Sci Hortic*, Vol. 183, pp. 144-151, 2015.
- [27] Ma Y., Hu S, Chen G., Zheng Y. and Jin P., Cold shock treatment alleviates chilling injury in peach fruit by regulating antioxidant capacity and membrane lipid metabolism. *Food Quality Safety*, Vol. 6, pp. 1-11, 2022.
- [28] Wang Y., Luo Z., Khan Z.U., Mao L. and Ying T., Effect of nitric oxide on energy metabolism in postharvest banana fruit in response to chilling stress. *Postharvest Biol Technol*, Vol. 108, pp. 21-27, 2015.
- [29] Lurie S. and Crisosto C.H., Chilling injury in peach and nectarine. *Postharvest Biol Technol*, Vol. 37, pp. 195-208, 2005.
- [30] Manganaris G.A., Vicente A.R., Crisosto C.H. and Labavitch J.M., Effect of delayed storage and continuous ethylene exposure on flesh reddening of "Royal Diamond" plums. *J Sci Food Agric*, Vol. 88, No. 12, pp. 2180-2185, 2008.
- [31] Van Buggenhout S., Lille M., Messagie I., Van Loey A., Autio K. and Hendrickx M., Impact of pretreatment and freezing conditions on the microstructure of frozen carrots: Quantification and relation to texture loss. *Eur Food Res and Technol*, Vol. 222, No. 5-6, pp. 543-553, 2006.
- [32] Harker F.R. and Forbes S.K., Ripening and development of chilling injury in persimmon fruit: an electrical impedance study. *New Zealand J Crop Horticultural Sci*, Vol. 25, pp. 149-157, 1997.
- [33] Greenham C.G. and Daday H., Electrical determination of cold hardiness in *Trifolium Repens* L. and *Medicago Sativa* L., *Nature*, Vol. 180, pp. 541-543, 1957.
- [34] Privé J.P. and Zhang M.I.N., Estimating cold stress in Beautiful Arcade apple roots using electrical impedance analysis. *Hort Technology*, Vol. 6, pp. 54-58, 1996.
- [35] Greenham C.G., Randall P.J. and Ward N.M., Impedance parameters in relation to phosphorus and calcium deficiencies in subterranean clover (*Trifolium*

- subterraneum L.). *J exper botany*, Vol. 23, pp. 210-210, 1972.
- [36] Zhang M.I.N. and Willison J.H.M., Electrical impedance analysis in plant tissues: the effect of freeze-thaw injury on the electrical properties of potato and carrot root tissues. *Can J Plant Sci*, Vol. 72, pp. 545-553, 1992.
- [37] Brady C.J., Fruit ripening. *Annual Review of Plant Biology*, Vol. 38, pp.155-178, 1987.
- [38] Zhang M.I.N., Stout D.G. and Willison J.H.M., Electrical impedance analysis in plant tissues: Symplasmic resistance and membrane capacitance in the Hayden model. *J Exp Bot*, vol. 41, pp. 371-380, 1990.
- [39] Zhang M.I.N. and Willison J.H.M., A microcomputer-based precision impedance analyser for measuring electrical impedance in plant tissues. *Can J Plant Sci*, Vol. 71, pp. 1285-1288, 1991.
- [40] Harker F.R. and Maindonald J.H., Ripening of nectarine fruit (changes in the cell wall, vacuole, and membranes detected using electrical impedance measurements). *Plant physiology*, Vol, 106, pp. 165-171, 1994.
- [41] Furmanski R.J. and Buescher R.W., Influence of chilling on electrolyte leakage and internal conductivity of peach fruits. *HortScience*, Vol. 14, pp. 167-168, 1979.
- [42] Von Mollendorff L.J., De Villiers O.T. and Jacobs G., An anatomical and histological study of changes in mesocarp tissue during storage and ripening of 'Flavortop' nectarines. *J S Afr Soc Hort Sci*, Vol. 2, pp. 14-18, 1992.
- [43] Guo X., Zhu W., Wang F. and Wang H., Genome-wide investigation of the PLD gene family in tomato: identification, analysis, and expression. *Genes (Basel)*, Vol. 15, No. 3, pp. 326, 2024.
- [44] Zhang M., Duan Z.H., Zhang J.F. and Peng J., Effects of freezing conditions on quality of areca fruits. *J Food Eng*, Vol. 61, pp. 393-397, 2004.