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Preliminary Study on the Reduced Pressure Cold Plasma Processing of Fresh Cut Salads: Rheological Assessment of Modifications in the Plant Tissue Structure

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Cold plasma is a novel nonthermal food processing technology that uses energetic, reactive gases to inactivate contaminating microbes and to achieve an enzymatic depletion on foods. The primary modes of action are due to UV light and reactive chemical products of the cold plasma ionization process. A wide array of cold plasma systems that operate at atmospheric pressures or in low-pressure treatment chambers are under development. Optimization and scale up to commercial treatment levels require a more complete understanding of plasma interaction modes with food. In particular, little attention to date has been paid to structural and textural modification induced by plasma in food matrices.

Results that are presented in this paper refer to the study of low pressure cold plasma treatment of fresh vegetable, precisely fresh cut salad leaves (*Valerianella locusta* Laterr).

Throughout dynamic rheology tests, it has been evaluated the impact of the non-thermal plasma treatment on the structural modifications occurring in the salad tissue at a mesoscopic scale-length where cell wall materials (CWM) account for the perceived texture of the salad.

The rheological behaviour of CWM water dispersions in steady-state regime proved that plasma treatment is able to induce an immediate modification in flow behaviour, which persists during the shelf-life period. CWM dispersions behave as weak gels. Specifically, cold plasma treatment of salad leaves generates CWM gels with higher coordination of the gel polymers network, as quantified by means of the Bohlin's theory on week gels.

Moreover, it has been proved that the relaxation time distribution spectrum may provide useful information on understanding the underlying stress relaxation mechanisms in gels.

An in-depth investigation of physical events could be of help in view of the optimization of the cold plasma technology application in plant foods preservation.

1. Introduction

Plasma is comprised of several excited atomic, molecular, ionic and radical species, coexisting with numerous reactive species (electrons, ions, free radicals, molecules in excited state and quanta of electromagnetic radiation). Food industry is particularly interested in the possibilities of exploitation of a particular type of plasma, known as non-equilibrium plasma or cold plasma (Misra et al, 2016). Literature results are related to the cold plasma antimicrobial function and enzymatic inactivation (Schlüter et al. 2013). Because of the higher efficiency of treatments under vacuum, most of the research for technological applications has focused on equipment at reduced gas pressure. Indeed, a higher gas pressure is closely related to a greater collision frequency among particles and, consequently, it increases the probability that with time the particles reach a steady-state energy equilibrium. Such mechanism is reflected into a lower ion energy in atmospheric plasmas (Bardo & Bakarova, 2010). That notwithstanding, recent economic and technological advantages of atmospheric plasma treatments warrant the growing interest in these systems (Schütze et al. 1998).

For minimally-processed food commodities, the non-thermal cold plasma treatment may be provided as environmental-friendly unit operation, aimed to deliver high texture quality and safe commodities, other than

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offering an operation of improved sustainable strategies for reducing the amount of unsold product at the retail. Fresh minimally processed salad leaves (*Valerianella locusta* Laterr) are very perishable products. The macroscopic salad wilting, from “crispy “ to “soggy”, has been related to the aging of the cellular tissue of the leaves, mainly to the modifications occurring on the cell walls materials, the main structural components of the plant tissue, due to their non-equilibrium nature (Roversi and Piazza, 2016, Roversi et al, 2016).

The aim of this paper is to explore, in a preliminary way, the structural effect due to reduced pressure plasma treatment of fresh-cut salad leaves. The structural events at the mesoscopic level, which underlay the perceived texture modification, are investigated following a rheological approach.

2. Experimentals

2.1 Materials

Fresh salad (*Valerianella locusta* Laterr) was supplied by a local farm (Agronomia Srl, Bergamo, Italy). After harvesting leaves were washed with chlorine solutions (150 ppm), and then packaged in sealed polypropylene trays (100 g).

2.2 Cold plasma treatment

A reduced pressure cold plasma treatment was performed in a Zepto plasma system (Diener electronic Italia Srl, Lomazzo, Italy) equipped with the 40 kHz HF-generator. Argon has been selected as reactive gas, in agreement with literature (Fröhling et al., 2012), with a flow rate of 0.2 l/min. The chamber contained a single leaf for each batch of treatment and the inner pressure was maintained at 0.5 mbar. After processing, leaves were stored in plastic trays, sealed with a polyethylene film. The shelf-life study was performed at a storage temperature of 10 °C for 15 days.

2.3 Extraction of the alcohol insoluble polysaccharide fraction (AIS)

The main self-assembled components of the salad tissue, cell wall materials (CWM), were extracted according to the procedure proposed by Campbell and his group (1990). Briefly, 20 g of salad leaves taken from the package at different storage times were boiled in ethanol to inactivate potential wall-modifying enzymes and filtrated under pressure by using Glass Microfibre Filters (Whatman), pore size 125 µm. A methanol–chloroform solution (1:1) was used to wash the resulting pellet until complete discoloration. The pellet was washed in acetone and finally dried at room temperature. In order to perform the rheological measurements, samples were rehydrated with distilled water at 23 °C for at least 24 h (final concentration: 2% w/w).

2.4 Rheological analysis

Flow curves of the CWM dispersions and dynamic mechanical spectra were obtained using a CMT (combined motor and transducer) rheometer (DHR-2, TA Instruments). All measurements were performed using stainless steel parallel plate geometry (40 mm diameter) at 23 °C. A solvent trap was used to prevent loss of solvent.
Flow curves have been generated by measuring shear rate under a ramped shear stress from 20 to 100 Pa.
The oscillatory strain sweep tests were performed to determine the linear viscoelastic region for each sample.
Frequency sweep tests were then performed in linear viscoelastic region, in the frequency range of 0.01 - 200 rad/s and mechanical spectra (G’(ω) and G’’(ω)), were obtained.

Relaxation time spectra, H(τ), were extracted from the dynamical mechanical data G’(ω) and G’’(ω). The relationship between the functions is expressed by the following equations:

$$G'(\omega) = G_0 + \int_0^{\infty} H(\tau) \frac{\omega^2\tau^2}{1+\omega^2\tau^2} \frac{d\tau}{\tau} \tag{1}$$

$$G''(\omega) = \int_0^{\infty} H(\tau) \frac{\omega\tau}{1+\omega^2\tau^2} \frac{d\tau}{\tau} \tag{2}$$

where G_0 is the high equilibrium modulus, ω represents the frequency and τ is the relaxation time. Relaxation spectra H(τ) were then quantified according to the Bayes Relax model (Hansen et al., 2008), which convert Eqs. (1) and (2) to yield H(τ) and depicts the relative contributions of Maxwell elements, with different relaxation times (τ) values, to the overall relaxation capability of the tissue. Accordingly, data regularization based on combination of the smoothness constraint and the maximum entropy metric is included in a Bayesian framework, for the estimation of the continuous relaxation spectra from dynamic experiments.

3. Results

Cell wall materials dispersed in water form physical networks of jammed particles with non-covalent, reversible interactions mainly made up by hydrogen bounds. In this work the effects of the cold plasma treatment on these self-assembled structures are monitored throughout a rheological approach.

3.1 Steady-state: viscosity

The flow curves for the control (untreated) CWM dispersion and the plasma treated CWM dispersion at 0 days shelf-life, are shown in figure 1. The resulting non-Newtonian, shear-thinning behavior suggests that CWM dispersions behave as structured fluids. The apparent viscosity does not show a steady value. A steady state plateau in viscosity could be reached if an equilibrium is established between structure breakdown and rebuilding and is dependent on the stabilization of internal network structures. It can be argued that the CWM network structure is not entirely destroyed by shearing the material under the tested deformation range for both the treated and the untreated samples. The complex nature of the self-assembled polysaccharides structures is therefore evidenced.

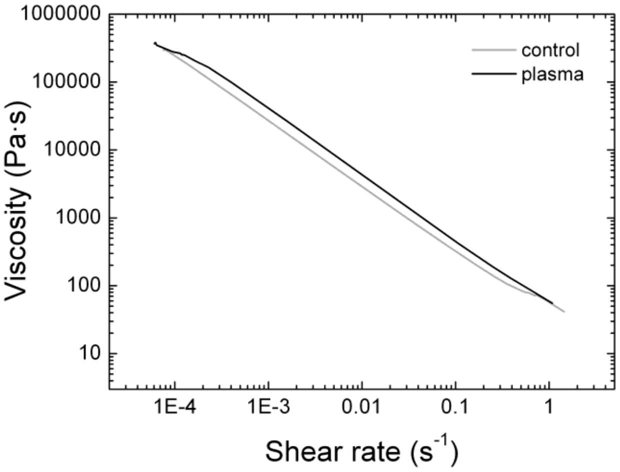

Figure 1: Steady-state viscosity versus shear rate for water suspensions (2% w/w) of cell wall materials extracted from salad leaves in presence or absence of cold plasma pre-treatment.

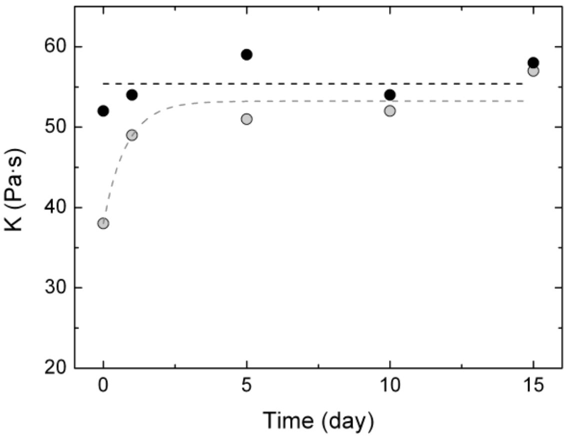

Figure 2: Consistency coefficient changes over the shelf-life time for plasma-treated (black circles) and control (grey circles) CWM water dispersions.

The relationship between shear stress (σ) and shear rate ($\dot{\gamma}$) plotted on log-log co-ordinates for a shear-thinning fluid can be approximated by a straight line, in terms of the apparent viscosity, and can be reliably described by means of Ostwald–de Waele model:

$$\eta = K\dot{\gamma}^{n-1} \tag{3}$$

where *K* is the consistency coefficient; *n*, called flow index, corresponds to the degree of non-Newtonian behaviour of the sample. The two flow parameters were obtained for samples tested at various shelf-life times. In figure 2 it is shown how the plasma treatment can cause an immediate increase of consistency index (*K*) (corresponding to 52 and 38 Pa·s for treated and control matrices, respectively). During storage time, the consistency coefficient increases until a plateau value around 55 Pa·s for control samples is reached. On the other hand, *K* looks quite constant in the case of plasma treated samples. It can be therefore inferred that less microscale structural rearrangements occur within the plasma-treated complex fluid. It seems to reflect

3.2 Oscillatory regime: frequency sweep

The viscoelastic properties of the CWM dispersions were investigated in the oscillatory regime by performing frequency sweep tests over the 0.01-100 rad·s^{-1} frequency range within the linear viscoelasticity region. A typical mechanical spectra is shown in figure 3 for a control untreated sample at 7 days shelf-life. The dependence of the viscoelastic moduli on the frequency is typical of a gel-like material.

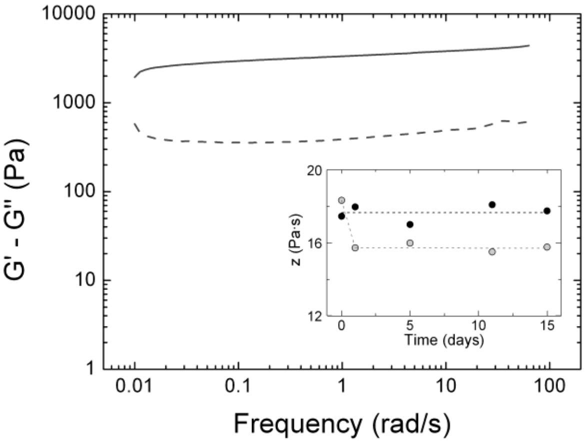

Figure 3: Storage modulus G' (continuous lines) and loss modulus G" (dashed lines) as a function of angular frequency (left, 0.5% amplitude). Inset: Bohlin's coordination index for plasma treated (black circles) and control (grey circles) CMW gels versus shelf-life time.

In the case of weak gels, this viscoelastic behaviour can therefore be described through the cooperative flow theory presented by Bohlin to explain the flow character and microstructure of a flowing substance by means of rheological data (Bohlin, 1980). In dynamic experiments, the theory of cooperative flow states that the complex modulus and the frequency are related by the following expression:

$$G^*(\omega) = A_f \omega^{1/z} \tag{4}$$

where G*(ω) is the complex modulus, ω the frequency, A_f represents the interaction strength between the flow units inside the gel, and z is the coordination number of cooperative flow units in the structure, a reticulation factor correlated to the number of interactions the substance microstructure. The two parameters of the theory can be determined from experimental data.

As it is evident from the inset of figure 3, plasma-treated samples are characterized by a quite constant *z* value (around 18 Pa·s) all along the shelf-life period investigated. Samples used as control, despite an initial values similar to the plasma-treated samples, display a lower coordination index (around 16 Pa·s). Data suggest an higher stability of the structural components of the plasma-treated salad leaves during the investigated shelf-life time.

The theory is valid, according to Bohlin, in the range of frequencies corresponding to times between the relaxation time and the stationary state (Bohlin, 1980). The macroscopic viscoelastic behaviour of CWM gel was therefore studied through relaxation time distribution spectrum obtained from frequency sweep test data.

The theoretical interest in calculating a relaxation spectrum is based on a supposition that it reflects molecular movements of macromolecules and thus can be connected with the molecular structure, eventuality modified by external disturbance of the system (plasma irradiation, in this case).

According to Maxwell model, the total stress of the whole system can be defined as the sum of the single stresses in each element. Every Maxwell element concurs to the structure relaxation, and as consequence, the relaxation modulus can be described by a sum of infinite Maxwell models, each with a characteristic relaxation time (τ):

$$G(t) = \sum_{k==1}^{N} G_k e^{-t/\tau} \tag{5}$$

In our study, relaxation spectra were obtained by fitting G'(τ) and G"(τ) experimental data with Fredholm integral equations. Figure 4a shows the relaxation spectra of plasma irradiated and not-treated samples at fifteen days shelf-life.

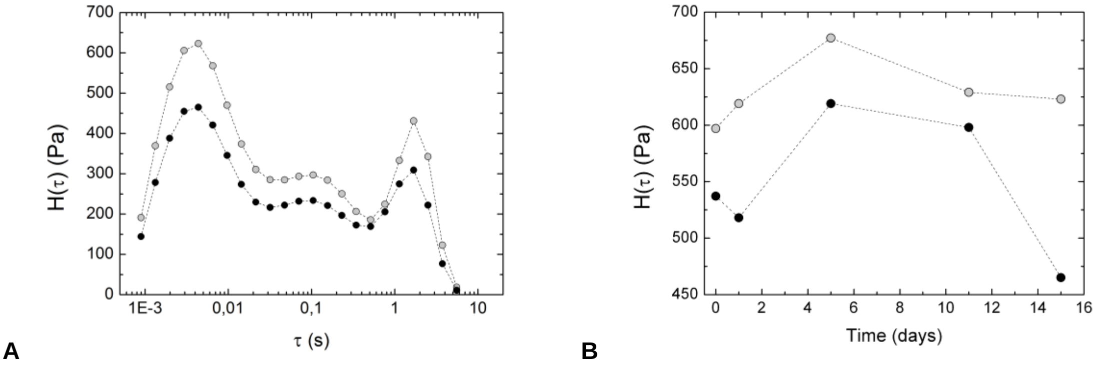

Figure 4: (A) Relaxation spectrum H(τ), for CWM gels obtained from cold plasma traded (black circles) or not treated (grey circles) salad leaves; (B) intensity of the short relaxation time peak over 15 days shelf life time.

The intensity of the peaks reflects the amount of dissipated energy during relaxation. The number of peaks and time constants are often correlated with specific molecular architectures; as a result, it can be used as an approach to understand the structural behaviour of biomaterials.

The CWM weak gels exhibited, at each investigated storage time, three dominant relaxation peaks, characterized by different intensity and specific relaxation time values. The peaks in the continuous distribution curve are located at short relaxation time (τ ~ 0.004 s), medium relaxation time (around τ ~ 0.1 s), and long relaxation time (τ ~ 1.69 s). Even though relaxation phenomena of treated and non-treated samples are similar (τ are not shifted), their intensities are noticeably different and are higher for the control sample all over the aging time considered in this study. We hypothesize that the peaks in the relaxation spectra reflect the relaxation behaviour of CWM constitutive biopolymers, pectins, cellulose and hemicellulose. The higher peaks intensity of the relaxation spectra of hydrogels from untreated salad leaves may correlate to the lower elastic properties of CWM structure. Conversely, a higher coordination of the structural components in plasma-irradiated materials can be inferred, which is in agreement with the higher Bohlin's coordination number of cooperative flow units in the structure which was previously presented. In addition, the differences in peak intensities of cold plasma-treated material are stronger with increasing the shelf life time.

As an example, the evolution of the short relaxation time peaks intensity during storage time are shown in figure 4b.

The identification of the nature of the fast, intermediate and slow relaxation modes is far from being fully described. From data obtained so far, it may be hypothesized that the configuration of the network, changes the density of physical entanglements. It has been clearly shown that polymer chain dynamic is definitively responsible for the fast and slow relaxation modes (Li et al., 2010). A full description should consider plasma-induced polymers' buckling network, rearrangement and formation of new physical crosslinks.

It can be concluded that the relaxation spectra analysis captures the essential features of the linear viscoelastic properties and is promising to provide fundamental understandings of the viscoelastic structure-function of plasma-treated food matrices.

4. Conclusions

The hierarchical structure of cell wall materials and the dynamics of the flowing components at the mesoscale structural level are believed to be responsible for texture of salad leaves (Roversi & Piazza, 2016). It has been demonstrated that the reduced pressure cold plasma technology applied to salads leaves modifies, all over the investigated storage time, the viscoelastic behaviour of the CWM self-assembled structural component of the plant tissue. This suggests positive sensory-appreciated changes in salad macroscopically perceived quality. Relaxation time distribution spectrum provides useful information in understanding the underlying stress relaxation mechanisms. In conclusion, the control of structure dynamics can be seen as a critical factor to be considered during the future set up of cold plasma technology for fresh plant-derived food products.

Key limitations for cold plasma are represented by the relatively early state of technology development, the variety and complexity of the necessary equipment and the largely unexplored impacts of cold plasma treatment on the sensory qualities of treated foods. Optimization and scale up to commercial treatment levels require a more complete understanding of a huge number of chemical and physico-chemical events taking place during the plasma processing. Basic knowledge could help in engineering this new non-thermal technology and to further clarify the advantages and the disadvantages of reduced pressure versus atmospheric pressure cold plasma processes on the overall food quality. Obviously, the economic side of the two technologies hindering the scale-up of the operation cannot be neglected. Nevertheless, this area of technology shows promise and is the subject of active research to enhance efficacy.

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