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QUALITY ATTRIBUTES OF FRESH-CUT LETTUCE TREATED WITH COLD PLASMA

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Abstract

Cold plasma is a novel method that has proved to be capable as a sanitizing process due to its antimicrobial effects. This study aims to highlight the optimization of this technique for maintaining freshness and safety of fresh-cut lettuce without using any other chemical preservatives. The used atmospheric pressure plasma jet is driven by a radio frequency generator (27.12 MHz) with argon as working gas. The jet has been used at different operating powers in order to evaluate the optimal process parameters that do not affect the product quality. The quality of the lettuce leaves was assessed by optical methods such as chlorophyll fluorescence imaging analysis, fluorescence spectroscopy and colour measurement before and after plasma treatment, and also during the storage period. Depending on the applied process parameters, the effects of the cold plasma treatment on the quality of lettuce leaves can be controlled. However, the treatment conditions have to be adapted to each type of commodity.

Key words: *chlorophyll fluorescence imaging analysis, cold plasma, fluorescence spectroscopy, lettuce*

INTRODUCTION

Nowadays, consumers have been demanding more than ever both convenience and high quality ready-to-eat products. Production and consumption of minimally processed vegetables has increased in many countries in recent years [21].

As a consequence, the exploration of new technologies to preserve the quality of fresh-cut commodities and to extend their shelf life, without using any additives is highly desirable [1, 4]. Fresh produce is more susceptible to disease organisms because of the increased respiration rate after harvesting [24].

The main spoilage mechanisms affecting the shelf-life of the fresh-cut products are oxidation phenomena, such as browning, degradation and oxidation of pigments, due to the enzymatic activity of the cut leaves [6, 23].

Water loss, softening, translucency or surface dehydration may also occur as a result of their processing, conditioning and storage [23, 27].

In the context of latest foodborne outbreaks associated with these type of commodities, due to the presence of the pathogenic microorganism (*e.g. Escherichia coli* O157:H7) [10, 16], food scientists and food engineers turned their attention to novel decontamination and sterilization methods, suitable for application to fresh-cut products and different from the traditional ones [5, 8], which are based on a thermal treatment or are using different chemical sanitizers in order to inactivate foodborne microorganisms.

These innovative preservation methods have to maintain the requested characteristics of the food products as long as possible and must be effective in terms of food safety and should be economically profitable.

Cold plasma treatment is an emerging process technology for the sterilization and decontamination of different food products. Its application has a potential for the treatment of fresh produce and fresh-cut fruits and vegetables [9, 19].
Plasma is composed of gas molecules which have been dissociated by an energy input. It is constituted of particles in permanent interaction, such as photons, electrons, ions, atoms, free radicals and excited or non-excited molecules [5, 20].
As a dry, non-thermal process, cold plasma is representing an interesting and flexible sanitizing method that uses electricity and a carrier gas, such as air, nitrogen, argon or oxygen [7, 14, 22].
Recently, interactions of reactive species immanent in cold plasma and secondary plant metabolites of lamb's lettuce were investigated [11].
A correlation between applied treatment time and leaf surface morphology has been found. Secondly, a considerable reduction of phenolic acids in case of the exposed lamb's lettuce leaves was observed.
Consequently quality parameters must be considered when evaluating new treatment techniques.
While the antimicrobial effectiveness of cold plasma has successfully been demonstrated in previous reports of various research groups [12, 13, 15, 17, 26, 28], according to our knowledge, there is limited information available related to the influence of cold plasma treatment on the freshness and viability of fresh-cut vegetables.
Therefore, the objective of this study was to evaluate the impact of plasma treatment on the quality and viability as well as the effect of storage on the treated samples of fresh-cut lamb's lettuce leaves using chlorophyll fluorescence, fluorescence spectroscopy and colour measurement.

MATERIAL AND METHOD

Plant material

Being a main ingredient for many ready-to-eat salads, fresh-cut lamb's lettuce (*Valerianella olitoria* Poll.), was chosen as a model leafy vegetable for these experimental activities, in order to characterise quality attributes of the plasma treated samples.
Lamb's lettuces grown in a patch at Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Germany, without any preservatives added and free from pesticides, were harvested right before the beginning of the experiments. Leaves about 7 cm length and 2 cm width were cut right from the ground using a gardening scissors.
Leaves were washed with cold tap water and dried at room temperature. The leaves were chosen randomly and all tests were conducted in triplicate.

Cold plasma treatment and storage conditions

The used equipment was an atmospheric pressure plasma jet (APPJ) driven by a radio frequency (RF) generator (27.12 MHz) with argon as working gas (Photo 1).
The plasma device consisted of a ceramic nozzle equipped with two electrodes: the inner needle electrode, placed in the centre of the nozzle and a grounded ring electrode, placed near the outlet surrounding edge of the nozzle.
The configuration of the device also includes a power supply, represented by the RF generator and the matching unit, and a gas supply.

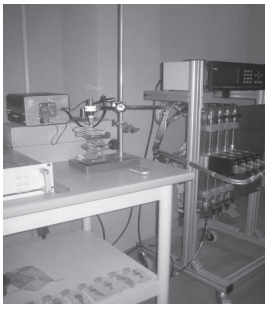

Photo 1. Set-up of the atmospheric pressure plasma jet.

Fresh-cut lamb's lettuce leaves were fitted into transparent acrylic glass sample holders right before the treatment.
The used distance between the tip of the plasma jet and the samples was set to 2 cm, and the plasma treatment was applied for 1, 2, 3 and 4 min. Untreated plasma samples were noted as 0

min samples. Experiments were conducted at room temperature.
Plasma treated samples and also control samples were air packed by hand in transparent plastic bags (Roth, Germany).
The wall thickness of the bags was 70 μm and the bags had a length of 19 cm and a width of 13.5 cm with a volume of 390 ml. Air packaging consisted of sealing the bags with their wire without eliminating air. Packed samples were stored in the dark at 5°C for 4 days.

Chlorophyll fluorescence imaging analysis

Chlorophyll fluorescence imaging analysis has been adopted as a fast and non-invasive method [25] to determine stress effects in lamb's lettuce leaves after different plasma treatments This method was used to describe the physiological status of treated leaves immediately after the plasma treatment and during the storage period in relation to untreated control samples.
Leaf chlorophyll fluorescence measurements were conducted on the top of each sample, the exact plasma treated area being analysed. The method was used to correlate the dynamics of the physiological effects of the plant tissue with the applied external treatment.
The used device was a FluorCAM fluorescence imaging system (640MF, PSI, Brno, Czech Republic). Right after the samples were taken out from the storage conditions, before measuring chlorophyll fluorescence parameters, the leaves were pre-darkened for about 5 min, according to the used protocol.
The maximum chlorophyll fluorescence signal (F_m) and the minimum chlorophyll fluorescence signal (F_0) were measured. Using these two parameters, the maximum PSII photochemical efficiency was calculated, according to the ratio: $F_v/F_m = (F_m - F_0)/F_m$. The raw data were analysed using the manufacturer's software package (FluorCAM 6, PSI, Brno, Czech Republic).

Fluorescence spectroscopy assessment

Fluorescence measurements were conducted using a PerkinElmer LS55 fluorescence spectrometer (Rodgau-Jügesheim, Germany) equipped with a pulsed xenon lamp and a red-sensitive photomultiplier (R928). The excitation wavelengths and the corresponding parameters that were used are given in table 1. The illuminated area was 10 mm high and 3 mm wide.

Table 1. Parameter settings of fluorescence emission spectra measurements

Excitation (nm)	Emission (nm)	Emission Slit (nm)	Low pass filter (nm)
280	300-500	10	290
470	500-800	10	515
490	550-800	10	515

In order to assess the samples by using the fluorescence spectroscopy, each lettuce leaf had to be cut out using a cork borer of 14 mm diameter. Metal cuvettes were filled with transparent gel plates so that the sample leaves could be placed on top of them, right under a quartz glass disks. Subsequently, the sample holders were closed and the fluorescence spectra of the leaves were recorded.

Colour measurement

For the colour measurements the Hunter Lab-system was used. The values of L (light), a (redness) and b (yellowness) were taken at three spots of each leaf using a CR-300 Minolta Chroma Meter (Minolta Camera Co., Ltd., Japan) with illuminant D65 and 8 mm diameter measuring area and 0° viewing angle.
Three random areas of the treated leaf surface were measured for each sample at day 0 before the plasma treatment (L_0, a_0, b_0) and immediately after the treatment and also each day during the storage period (L, a and b). L (lightness, from 0 for black to 100 for white), a (redness) and b (yellowness) colour readings were recorded. From the measured values of L, a and b two colour terms were calculated: total colour differences, expressed as:
$\Delta E = [(L - L_0)^2 + (a - a_0)^2]^{1/2}$
and the difference in chroma between the samples, expressed as
$\Delta C = (a^2 + b^2)^{1/2} - (a_0^2 - b_0^2)^{1/2}$.
This colour index was used because it detects colour changes similar to the differences perceptible by human eye [2]. Each sample was measured three times, the given results being the average of the assessed measurements.

Data analysis

All plasma treatments were performed in triplicate. Chlorophyll fluorescence measurements and spectral data were firstly exported using programs of the manufacturers. All the given results represent the average of the assessed measurements along with the standard deviations.

RESULTS AND DISCUSSIONS

Effect of plasma treatment on photochemical efficiency

Photosynthesis is a complex physiological process that occurs in green plants based on different biophysical and biochemical reactions [25]. Chlorophyll fluorescence was measured in order to assess the response of the fresh-cut lamb's lettuce leaves to different process parameters of plasma treatment, in order to quantify the produce quality.

According to figure 1, which indicates the response of the plant tissue to the applied external stress, the level of maximum photochemical efficiency (F_v/F_m) was significantly affected by direct plasma treatment at 2 cm distance between plasma jet tip to the leaf surface.

Extended treatment durations for 2 min, 3 min, and 4 min led to similar inhibition of the metabolic activity of the tested samples. In contrast, 1 min plasma treatment showed a moderate alteration of F_v/F_m.

For all assessed samples, the initial inhibition of photochemical efficiency was maintained during the entire storage period, without significant variations during the determined period of time (Fig. 2).

These results indicate that chosen treatment conditions led to irreversible damage on the metabolic active plant system after 1 min duration of plasma treatment.

Hence, this quantification is valuable for further investigations, were shorter treatments and higher distances between plasma tip and sample surface can be applied to avoid inhibition of the metabolic activity of the plant.

Effect of plasma treatment on fluorescence emission spectra

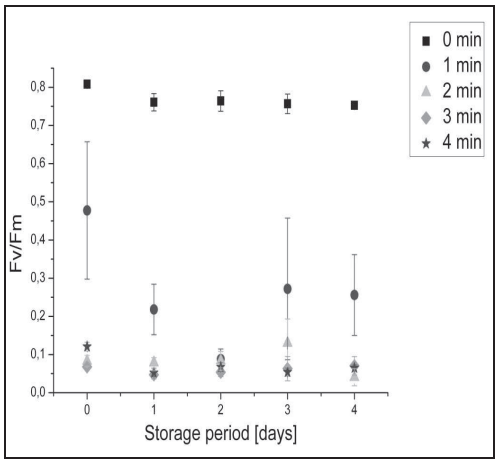

Fig. 1: Maximum photochemical efficiency of lamb's lettuce leaves after different plasma treatment times and subsequent storage.

In this study, fluorescence emission measurements were used as a potential indicator of the internal quality of plasma treated lettuce.

The fluorescence of photosynthetic pigments was assessed in order to evaluate quality changes of lamb's lettuce leaves according to the different plasma treatments that were applied. Fluorescence emission spectra during the storage period for each plasma treatment are presented in Fig. 3.

In the case of the untreated plasma samples (Fig. 3, A) there can be seen that during the storage period, the fluorescence intensity is decreasing, as a result of the degradation of chlorophyll. Plasma treated leaves however, showed different intensities of fluorescence emission as a response to the duration of the treatment.

After 1 min plasma treatment, the fluorescence intensity can be compared to that of untreated leaves, but with increasing treatment time, the fluorescence intensity was inversely proportional to the duration of the related plasma treatment.

This result can indicate that a decline of the fluorescence rate, irrespective of excitation wave and emission wavelength hence may suggest a decrease of the chlorophyll content. Grzegorzewski et al. (2011), however, reported that due to the plasma's highly reactive species, a disintegration of cell membranes may take

place, followed by a release of cellular components.
These component may than lead to different results, were the fluorescence intensities of plasma treated samples will have higher values than those of the control samples. Hence, because experimental results on the impact of plasma treatment on physiological activity of fresh-cut vegetables are sparse, further studies are needed, to complete a correlation between the antimicrobial effect of this method and its effects on food products.

Effect of plasma treatment on colour

The colour of fresh-cut lamb's lettuce leaves was affected by the direct (distance of 2 cm) plasma treatment (Fig. 4). Considerable total colour differences were found on day 0 for all plasma treated samples. Immediately after the plasma treatment, the 4 min treated samples showed the most notable colour difference.
During the storage period, untreated lettuce leaves recorded a more pronounced colour variation with respect to day 0 than the treated samples.
During the storage period, the variation in colour was mostly due to changes in green-red (a) values, being more sensitive over storage time.

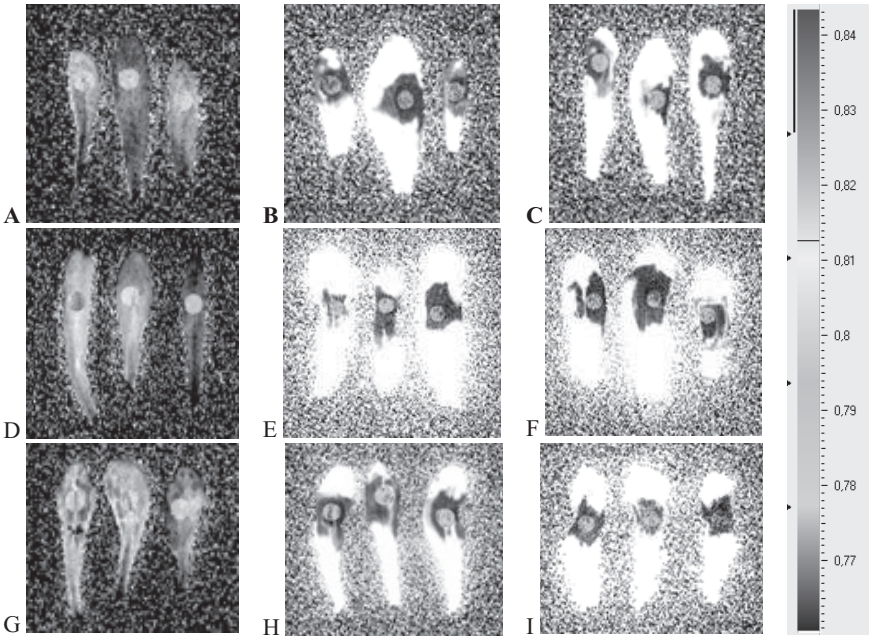

Fig. 2: Alteration of the F_v/F_m ratio on day 0 for the 0, 2 and 4 min plasma treatment (A, B, C), on day 2 for the 0, 1 and 3 min plasma treatment (E, F, G) and on day 4 for the 0, 1 and 2 min plasma treatment.

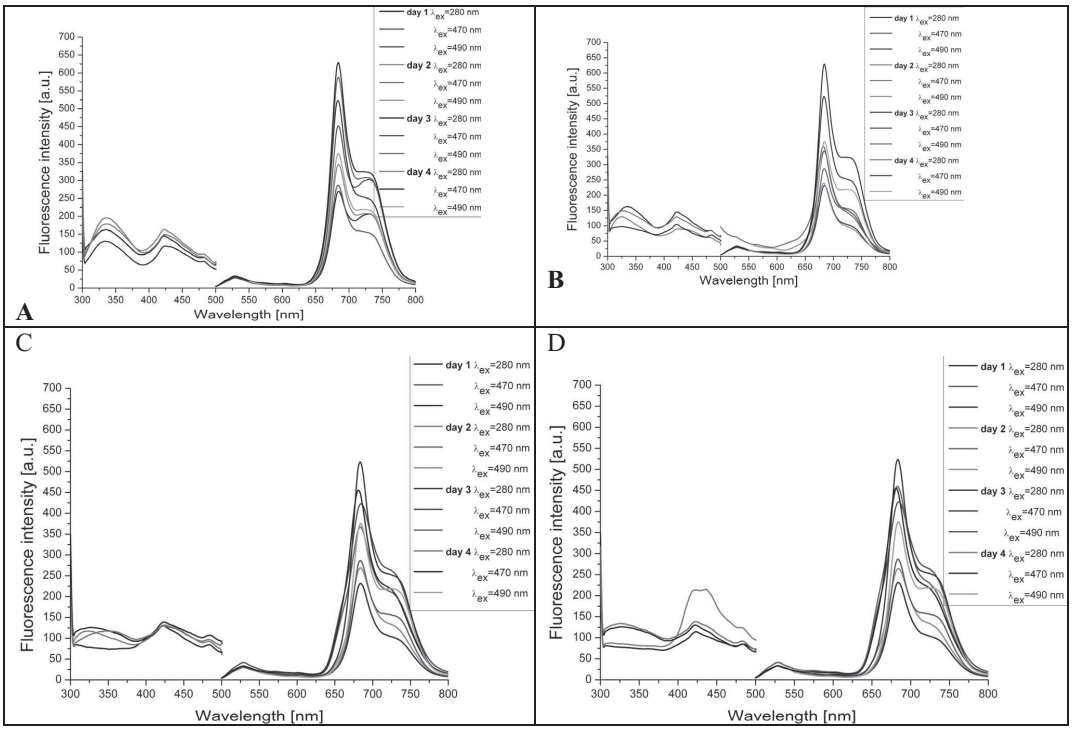

Fig. 3: Fluorescence of lamb's lettuce after 0 min (A), 1 min (B), 3 min (C) and 4 min (D) plasma treatment.

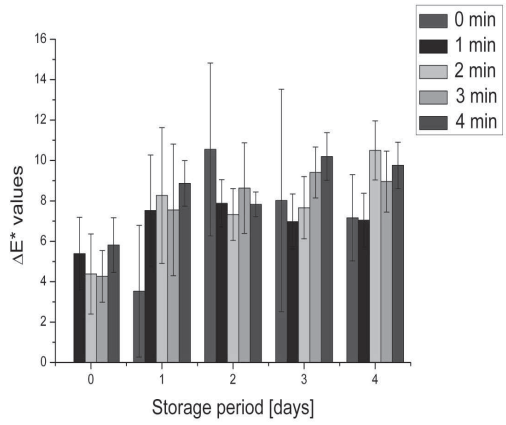

Fig. 4: Total colour differences during storage period for control and 2 cm plasma treated samples.

On the contrary, *L* and Δ*C* values (results not shown) proved to be more uniform during the tested period. As browning can be expressed by the a-value, our results are in agreement with the findings of Lonchamp et al. (2009), regarding the browning development of the leaves during the storage period, whereas in our case, the loss of green colour being more evident for the untreated samples.

CONCLUSIONS

As Perni et al. (2008) noted, the adjustment of process parameters of a plasma device can modify its effects on the treated sample. More importantly, when food products are treated and especially in the case of fresh-cut commodities, which rapidly lose their quality mainly because of degradation processes (respiration, senescence, natural microbial flora), an assessment of the final product quality is needed.

A direct plasma treatment, with a 2 cm distance from the treated sample, which was applied for 1 min, presented good results in terms of final quality attributes that were assessed immediately after the treatment but also during the storage period. Depending on the applied process parameters, the quality of the lettuce leaves was less affected by the cold plasma. However, the treatment conditions have to be adapted to each type of commodity.

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REFERENCES

[1] Artés, F., Gómez, P., Aguayo, E., Escalona, V., Artés-Hernández, F., 2009, *Sustainable sanitation techniques for keeping qualityand safety of fresh-cut plant commodities*. Postharvest Biology and Technology, 51: 287-296

[2] Chisari, M., Todaro, A., Barbagallo. R. N., Spagna, G., 2010, Salinity *effects on enzymatic browning and antioxidant capacity of fresh-cut baby Romaine lettuce (Latuca sativa L. cv. Duende).* Food Chem., 119: 1502-1506

[3] Conte, A., Scrocco, C., Brescia, I., Mastromatteo, M., Del Nobile, M. A., 2011, *Shelf life of fresh-cut Cime di rapa (Brassica rapa L.) as affected by packaging. LWT: Food Science and Technology,* 44: 1218-1225

[4] Conte, A., Scrocco, C., Lecce, L., Mastromatteo, M., Del Nobile, M. A., 2009, *Ready-to-eat sweet cherries: Study on different packaging systems, Innovative Food Science and Emerging Technologies*, 10: 564-571

[5] Critzer, F. J., Kelly-Wintenberg, K., South, S. L., Golden, D. A., 2007, *Atmosfeic Plasma Inactivation of Foodborne Pathogens on Fresh Produce Surface*, Journal of Food Protection, 70(10): 2290-2296

[6] Del Nobile, M. A., Conte, A., Canarsi, M., Sinigaglia, M., 2008, *Use of biodegradable films for prolonging the shelf life of minimally processed lettuce*, Journal of Food Engineering, 85: 317-325

[7] Deng, S., Cheng, G., Ni, G., Meng, Y., Cheng, H., 2008, *Bacterial inactivation by atmospheric pressure dielectric barrier discharge plasma jet,* Japanese Journal of Applied Physics, 47(8): 7009-7012

[8] Fernández, A., Shearer, N., Wilson, D. R., Thompson, A., 2012, *Effect of microbial loading on the efficiency of cold atmospheric gas plasma inactivation of Salmonella enterica serovar Typhimurium*, International Journal of Food Microbiology, 152: 175-180

[9] Fernández-Gutierrez, S. A, Pedrow, P. D., Pitts, M. J., Powers, J., 2010, *Cold atmospheric-pressure plasma applied to active packaging of apples*, IEEE Transactions on Plasma Science, 38(4): 957:965

[10] Gelting, R. J., Baloch, M. A., Zarate-Bermudez, M. A., Selman, C., 2011*, Irrigation water issues potentially related to the 2006 multistate E. coli O157:H7 outbreak associated with spinach*, Agricultural Water Management, 98: 1395-1402

[11] Grzegorzewski, F., Ehlbeck, J., Schlüter, O., Kroh, L., W., Rohn, S., 2011, *Treating lamb's lettuce with a cold plasma – Influence of atmospheric pressure Ar plasma immanent species on the phenolic profile of Valerianella locusta*, LWT: Food Science and Tehnology, 44(10): 2285-2289

[12] Kelly-Wintenberg, K., Hodge, A., Montie, T. C., 1999, *Use of a one atmosphere uniform glow discharge plasma to kill a broad spectrum of microorgganisms*, J. Vac. Sci. Technol. A., 17(4): 1539-1544

[13] Kelly-Wintenberg, K., Montie, T. C., Brickman, C., Roth, J. R., Carr, A. K., Sorge, K., Wadsworth, L.C., Tsai, P. P. Y., 1998*, Room temperature sterilization of surfaces and fabrics with a One Atmosphere Uniform Glow Discharge Plasma*. Journal of Industrial Microbiology & Biotechnology, 20: 69-74

[14] Laroussi, M., 1996. *Sterilization of Contaminated matter with an Atmospheric Pressure Plasma*, IEEE Trans. Plasma Sci, 24(3): 1188-1191

[15] Laroussi, M., 2005, *Low Temperature Plasma-Based Sterilization: Overview and State-of-the-Art. Plasma Process.* Polym., 2: 391-400

[16] Lee, S. Y., Baek, S. Y., 2008, *Effect of chemical sanitizer combined with modified atmosphere packaging on inhibiting Escherichia coli O157:H7 in commercial spinach,* Food Microbiology, 25: 582-587

[17] Leipold, F., Kusano, Y., Hansen, F., Jacobsen, T., 2010, *Decontamination of rotating cutting tool during operation by means of atmospheric pressure plasmas*, Food Control., 21: 1194-1198.

[18] Lonchamp, J., Barry-Ryan, C., Devereux, M., 2009, *Identification of volatile quality markers of ready-to-use lettuce and cabbage*, Food Research International, 42: 1077-1086

[19] Niemira, B. A., 2012, Cold plasma reduction of Salmonella and Escherichia coli O157:H7 on almonds using ambient pressure gases, Journal of Food Science, 2012, 77(3): 171-175

[20] Niemira, B. A, Sites, J., 2008, *Cold plasma inactivates Salmonella Stanley and Escherichia coli O157:H7 inoculated on Golden Delicious Apples*, Journal of Food Protection, 71(7): 1357-1365

[21] Oliveira, M., Solsona, C., Alegre, I., Viñas, I., Abadias, M., 2010, *Effects of packaging type and storage temperature on the growth of foodborne pathogens on shredded 'Romaine' lettuce*, Food Microbiology, 27:375-380

[22] Perni, S., Liu, D. W., Shana, G., Kong, M. G., 2008, *Cold atmosferic plasma decontamination of the pericarps of fruit, Journal of Food Protection*, 71(2): 302-308

[23] Rojas-Graü, M. A., Oms-Oliu, G., Soliva-Fortuny, R., Martín-Belloso, O., 2009, *The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: a review,* International Journal of Food Science and technology, 44:875-889

[24] Sandhya, 2010, *Modified atmosphere packaging of fresh produce: Current status and future needs*, LWT: Food Science and Technology, 43: 381-392

[25] Schlüter, O., Foerster, J., Geyer, M. Knorr, D., Herppich, W. B., 2009, *Characterization of high-hydrostatic-pressure effects on fresh produce using chlorophyll fluorescence image analysis,* Food Bioprocess Technol, 2:291-299

[26] Schwabedissen, A., Łaciński, P., Chen, X., Engemann, J., 2007, *PlasmaLabel – a new method to disinfect goods inside a closed package using dielectric barrier discharges*, Contrib. Plasma Phys, 47(7): 551-558

27] Smeu, I., Popa, M. E., *Efect of minimally processing operations on the shelf-life and quality characteristics of Romanian lettuce*, Romanian Biotechnological Letters Supplement, 16(6): 139-143

[28] Yun, H., Kim, B., Jung, S., Kruk, Z. A., Kim, D. B., Choe, W., Jo, C., 2010, *Inactivation of Listeria monocytogenes inoculated on disposable plastic tray, aluminum foil, and paper cup by atmospheric pressure plasma*, Food Control, 21: 1182-1186

[29] Zhang, H., Zhu, L., Hu, H., Zheng, K., Jin, Q., 2011, *Monitoring Leaf Chlorophyll Fluorescence with Spectral Reflectance in Rice (Oryza sativa L.),* Procedia Engineering, 15:4403-4408