CHEMICAL-PHYSICAL CHARACTERISATION OF ISOLATED PLANT CUTICLES SUBJECTED TO LOW-DOSE γ-IRRADIATION

José A. Heredia-Guerrero[a*], Rocío de Lara[b], Eva Domínguez[c], Antonio Heredia[c], Juana Benavente[b] and José J. Benítez[a].

[a]Instituto de Ciencia de Materiales de Sevilla, Centro Mixto CSIC-Universidad de Sevilla. Avda. Americo Vespuccio, 49, 41092 Sevilla, Spain.

[b]Grupo de Caracterización Electrocinética en Membranas e Interfases. Departamento de Física Aplicada I. Facultad de Ciencias. Universidad de Málaga. E-29071 Málaga. Spain.

[c]Instituto de Hortofruticultura Subtropical y Mediterránea (IHSM) "La Mayora", Centro Mixto CSIC-Universidad de Málaga, E-29760 Algarrobo-Costa, Málaga, Spain.

*Corresponding author: José A. Heredia-Guerrero, address: Instituto de Ciencia de Materiales de Sevilla, Centro Mixto CSIC-Universidad de Sevilla. Avda. Americo Vespuccio, 49, 41092 Sevilla, Spain, telephone: +34 95 448 9551, fax: +34 95 446 0665, e-mail: ja.heredia@csic.es

ABSTRACT

Isolated tomato fruit cuticles were subjected to low dose (80 Gy) γ-irradiation, as a potential methodology to prevent harvested fruit and vegetables spoilage. Both irradiated and non-irradiated samples have been morphologically and chemically characterized by Scanning Electron (SEM), Atomic Force (AFM), Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) and X-ray Photoelectron (XPS) spectroscopies. Additionally, electrochemical measurements comprising membrane potential and diffusive permeability were carried out to detect modifications in transport properties of the cuticle as the fruit primary protective membrane. It has been found that low dose γ-irradiation causes some textural changes on the surface but no significant chemical modification. Texture modification is found to be due to a partial removal of outermost (epicuticular) waxes which is accompanied by mild changes of electrochemical parameters such as the membrane fixed charge, cation transport number and salt permeability. The modification of such parameters indicates a slight reduction of the barrier properties of the cuticle upon low dose γ-irradiation.

Keywords: plant cuticle; de-waxing effect; gamma-irradiation.

1. INTRODUCTION

The plant cuticle is the continuous extra-cellular membrane covering the epidermal cell walls of leaves, fruits and non-wooden stems of higher plants (Domínguez et al., 2011). Cuticle structure and composition varies largely among plants, organs and growth stage (Jeffree, 2006; Pollard et al., 2008) but it is composed of a polyhydroxyester matrix (cutin) with embedded (intracuticular) and surface (epicuticular) waxes, a polysaccharide fraction from cell walls and a small proportion of phenolic compounds.

In the particular case of tomato fruits, alkanes, *iso*-alkanes and amyrins are the main constituents of the amorphous epicuticular wax layer (Kosma et al., 2010). The cutin matrix is a polyester mainly from C_{16} polyhydroxy-fatty acids (9(10),16-dihydroxy and 16-hydroxypalmitic acids) (Domínguez et al., 2011) while the polysaccharide fraction is essentially composed by cell wall cellulose, hemicellulose and pectin (López-Casado et al., 2007). The tomato cuticle is completed by small amounts of phenolic compounds like chalconaringenin, naringenin, naringenin-7-glucoside, and *m*- and *p*-coumaric acids (Hunt and Baker, 1980).

Gamma-irradiation has been employed as an alternative to the use of chemicals to prevent fruit and vegetables spoilage produced by insects during storage (Crawford and Ruff, 1996; Arvanitoyannis et al., 2009) and, in particular, low dose γ-irradiation of fresh food is proposed as the safest and most promising processing technology (Niemira and Fan, 2006).

No specific research focused on the effect of low dose γ-irradiation on integral isolated cuticles has been carried out. Available data are restricted to individual components. Thus, the degradation of naringenin by γ-radiation in air produces eriodictyol (a hydroxylated derivative) though the process is meaningless under an inert

atmosphere (Nagy et al., 2008). In polysaccharides, high dose γ-radiation produces the formation of primary macroradicals, the degradation of polymers, and/or the amorphization of crystalline phases (Korotchenko and Sharpatyi, 2004). Meanwhile, low dose γ-irradiation of regenerated cellulose membranes causes surface cleaning by removal of an outermost carbonaceous contamination layer and a partial loss of crystallinity (Vázquez et al., 2011). Aliphatic polyesters can be degraded through chain-shortening reactions at low doses of γ-radiation, while cross-linking takes place at higher doses (Plikk et al., 2006). Finally, as a reference for aliphatic waxes behavior, polyethylene in air undergoes an oxidative degradation at low doses of γ-radiation (Singh, 1999). However, according to the literature, no research focused on the effect of low dose γ-irradiation on isolated cuticles has been carried out.

Hence, in this article, we have studied the effect of low-dose γ-irradiation on isolated mature tomato fruit cuticles. Induced morphological and chemical modifications were characterized by comparison with non-irradiated specimens. Special attention was paid to electrochemical properties modifications (i.e. cation transport number and solute permeability) as key indicators of alterations in its protective properties.

2. MATERIALS AND METHODS

2.1. Cuticle isolation

Cuticles were enzymatically isolated from red ripe *Solanum lycopersicum* L. tomato fruits following the protocol described by Petracek and Bukovac (1995). It uses an aqueous sodium citrate buffer (50 mM, pH 3.7) with a mixture of fungal cellulase (0.2%, w/v, Sigma, St Louis, Missouri, USA) and pectinase (2.0%, w/v, Sigma) and 1

mM NaN_3 to prevent microbial growth. To facilitate enzyme penetration, fruits were incubated in vacuum with continuous agitation at 37ºC for 10-14 days. Cuticles were then manually separated from the epidermis, rinsed in distilled water, and stored in a drying chamber with silica gel. These untreated isolated cuticles will be hereafter named as C.

Isolated cuticle pieces were separated in three batches, one of them for direct use and the others to be subjected to wax elimination and γ-irradiation, respectively. Five specimens were selected from every batch and cut into pieces to be analyzed electrochemically and by SEM, ATR-FTIR, AFM and XPS. This procedure favours the consistency of results obtained from the several analytical techniques used.

2.2. Cuticular wax extraction

Cuticular waxes were removed from isolated cuticles (200 mg) by a standard method with a chloroform:methanol mixture (2:1, v/v, 60 mL) for 4 h at 65ºC followed by washing several times with chloroform and methanol and drying at room conditions overnight. Cuticular wax free specimens are used as a reference and are labelled as CNW.

Soluble waxes were recovered from the chloroform:methanol phase by evaporation at 35°C in vacuum. The semisolid brownish residue (5-6 mg) was characterized by ATR-FTIR for reference purposes (see supporting info).

2.3. γ-irradiation of cuticles

Isolated cuticles (C) were irradiated at the Hospital Universitario "Carlos Haya" (Málaga, Spain) with a clinic ^{60}Co unit (photons energy between 1.17 MeV and 1.33

MeV) following the procedure already described by Casado *et al.* (2006) and using a dose of 80 J/kg at room temperature. Irradiated samples are named as C-Ir.

2.4. Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Infrared spectra of samples were obtained with an ATR accessory (MIRacle ATR, PIKE Technologies, USA) coupled to FTIR spectrometer (FT/IR-4100, JASCO, Spain). All spectra were recorded in the 4000 to 600 cm^{-1} range with 4 cm^{-1} resolution and accumulating 50 scans. Five samples of each type (C, C-Ir, and CNW) were analysed observing no significant variance within each batch.

The analysis depth, d_p, of ATR-FTIR depends on several factors such as the wave number, λ, the angle of incidence θ, the refractive index of the ATR crystal, n_1, and the sample, n_2, as indicated in the following equation:

$$d_p = \frac{\lambda}{2\pi n_1 \sqrt{\sin^2\theta - (n_2/n_1)^2}} \quad (1)$$

In our ATR-FTIR spectrometer $\theta = 45°$ and $n_1 = 2.4$ and, considering the refractive index of the cuticle as 1.5 (Holloway, 1982), the penetration depth of ATR into the sample varies between 0.5 µm at 4000 cm^{-1} and 2.9 µm at 700 cm^{-1}.

2.5. X-ray Photoelectron Spectroscopy (XPS)

Membrane surface chemical characterization was carried out by XPS. Spectra were recorded with a Physical Electronics PHI 5700 spectrometer using a concentric hemispherical analyzer operating in the constant pass energy mode at 29.35 eV with an analysis area of 720 µm in diameter. MgK_α X-ray (hv = 1253.6 eV) was used as excitation source and binding energies are referenced to adventitious C_{1s} peak at 284.8

eV. The residual pressure in the analysis chamber was maintained below $5 \cdot 10^{-7}$ Pa during data collection. PHI ACCESS ESCA-V6.0F software package was used for acquisition and data analysis. Atomic concentration percentages of the characteristic membrane elements (C and O) were determined after subtraction of a Shirley-type background and taking into account the corresponding sensitivity factor for each spectral region. Two study modifications induced by low-dose γ-irradiation, two samples of C and C-Ir batches were analyzed and good reproducibility was observed.

2.6. Atomic Force Microscopy (AFM)

The AFM used is a Cervantes model from Nanotec Electrónica (Spain) operated in non-contact dynamic mode at the free lever resonance frequency. Images were obtained at room temperature and humidity (20-25 ºC and 40-50% RH) and processed using the WSxM software (Horcas et al., 2007). Nanosensors Si_3N_4 rectangular cantilevers with nominal force constant of 2.8 N m^{-1} and resonance frequency around 80 kHz were used.

Parameters such as the cantilever driving oscillation frequency and amplitude and set point have been carefully selected to operate in the long-range attractive van der Waals forces regime, thus ensuring a minimum perturbation of the sample by the probe. Due to the inherent local analysis of AFM, areas covering the maximum range of the scanner (10 μm x 10 μm) were inspected in, at least, four well separated points of three different samples. When reproducible patterns were observed, image size was reduced to improve resolution. Images presented reflect the most representative topographic patterns obtained.

2.7. Scanning electron microscopy (SEM)

The isolated cuticles were placed on a metallic holder using a double side adhesive tape and sputter coated with gold. A JEOL JSM-840 (Japan) scanning electron microscope operated at 10-20 kV was used for examination of samples. Three samples of C and C-Ir were examined in such conditions. Analysis was repeated in at least four points each.

2.8. Membrane potential and salt diffusion measurements

Experiments were performed with NaCl aqueous solutions in a dead-end test cell with the cuticular membranes tightly clamped between two glass half-cells by using silicone rubber rings. A magnetic stirrer was placed at the bottom of each half-cell to minimise concentration-polarisation at the cuticular membrane surfaces. Data are collected at room temperature (25.0 ± 0.5) ºC, standard pH (5.8 ± 0.2) and solution stirring at 525 rpm from five samples of each type (C, C-Ir, and CNW).

Salt diffusion measurements were carried out with the cuticular membranes separating a feeding NaCl concentrated solution (c_f = 0.01 M) from a diluted receiving solution (initially distilled water, c_r=0). Changes in the conductivity of the receiving solution (σ_r) as a result of solute flow were recorded versus time with a conductivity cell connected to a digital conductimeter (Radiometer CDM 83). The constancy of C_f value was checked in the feeding solution (variations lower than 5 % were determined). Measurements were carried out at two NaCl feeding concentrations (0.01 M and 0.05 M). Salt permeability (P_s) across a membrane can be obtained from diffusion measurements by means of the Fick's first law for a quasi-steady state and using the

slope of the experimental conductivity (σ_r) vs time relationships. A very detailed description of the method is reported elsewhere (Vázquez et al., 2011).

Membrane potential ($\Delta\Phi_{mbr}$), or the equilibrium electrical potential difference at both cuticular membranes sides due to a salt concentration gradient, was obtained with two reversible Ag/AgCl electrodes connected to a digital voltmeter (Yokohama 7552, 1GΩ input resistance). The concentration of the solution at one side of the membranes is fixed (c_c = 0.01 M) and on the other side is gradually varied ($0.002 \leq c_v$ (M) ≤ 0.1). $\Delta\Phi_{mbr}$ values were corrected by subtracting the electrode potential ($\Delta\Phi_{eled} = (RT/F) \ln(c_v/c_c)$) to the measured values.

3. RESULTS AND DISCUSSION

3.1. Surface morphological analysis

As the purpose of this research is the study of changes induced by low-dose γ-irradiation of tomato cuticles, the surface morphological analysis has been concentrated on the comparison between irradiated (C-Ir) and non-irradiated (C) samples. Fig. 1 shows the corresponding SEM images and both display the characteristic rounded-shaped undulations surrounding a depression that are originated by the plastic deformation caused by the epidermal cell removal. However, the main difference between non-irradiated and γ-irradiated cuticles is the presence, in the first case, of bright elongated and interconnected features, Fig. 1A. On the other side, low-dose γ-irradiation produces a clear removal of such elongated motifs, Fig. 1B.

To study the textural modifications induced by γ-irradiation in detail, cuticle surface has been analysed by AFM. The operation mode used provides a high resolution topographic characterization with negligible sample perturbation. For instance, this method has been previously employed to image soft lipid nanoparticles supported on

flat substrates (Heredia-Guerrero et al., 2011). Fig. 2A shows the surface topography of the non-irradiated (C) sample. Irregular, wrinkled and interconnected deposits about 10 nm high are observed and associated to those observed in a much larger scale by SEM. γ-irradiated samples, Fig. 2B, displays a significantly smoother surface with some residual rounded features 10 nm high. Due to cuticle structure, and by analogy with the adventitious carbon decontamination from cellulosic membranes in analogous experimental conditions (Vázquez et al., 2011), these deposits are associated to epicuticular waxes.

These results suggest then that low-dose γ-irradiation causes a noticeable wax removal from the surface of cuticles. Though the interaction mechanism between γ-radiation and waxes is out of the scope of this paper, it could be related to the formation of oxygenated species as in the reported oxidative degradation of aliphatic chain in the presence of air (Singh, 1999).

3.2. Chemical analysis

ATR-FTIR spectroscopy is very powerful tool for the precise chemical characterization of plant cuticles (Ribero da Luz, 2007; Fernández et al., 2011) and it has been used here to investigate the chemical modifications associated to low dose γ-irradiation. Overall ATR-FTIR spectra of C, C-Ir and CNW are quite similar (see "supplementary material", Figure S1) and indicates no substantial chemical modification, however, small differences can be appreciated. Fig. 3A and 3B shows the spectra of C, C-Ir and CNW in the C-H (3025-2750 cm^{-1}) and C=O (1800-1650 cm^{-1}) stretching regions, respectively. While the polyester cutin matrix remains unaltered, Fig. 3B, γ-irradiation causes a noticeable reduction of the intensity of methylene peaks, Fig. 3A, affecting to components at 2917 cm^{-1} and 2848 cm^{-1}. The C-H spectrum of the de-

waxed cuticle (CNW) displays broader and shifted bands (2923 cm^{-1} and 2853 cm^{-1}) corresponding to the basal polyester cutin matrix and, in a lesser extent, to the intracuticular waxes. To better characterize the epicuticular layer, the CNW spectrum has been subtracted from the irradiated and non-irradiated ones, Fig. 3C. Sharp peaks at 2917 cm^{-1} and 2848 cm^{-1} ($-CH_2-$) as well as the characteristic stretching of $-CH_3$ at 2957 cm^{-1} are clear indications of well packed aliphatic compounds. It is also observed that γ-irradiation causes the removal of about half of the epicuticular wax layer. Actually, the ratio between the asymmetrical $-CH_2-$ and C=O stretching bands was calculated to be 1.09 for C, 0.95 for C-Ir and 0.75 for CNW. The elimination of such epicuticular wax layer was further confirmed by the ATR-FTIR spectra of the extraction residue (see "supplementary information", Figure S2).

To better characterize the chemical modifications suggested by ATR-FTIR, a more surface sensitive technique like XPS was used. XPS is a very accurate qualitative tool which also allows a good evaluation of the quantitative elemental composition from regions few tens of nanometers deep. Fig. 4 illustrates the O_{1s} and C_{1s} spectra of C and C-Ir samples (see "supplementary information" for XPS survey spectra of C and C-Ir samples, Figure S3). A strong reduction of C and simultaneous increase of O signals are simultaneously observed upon irradiation. Surface compositions are calculated to be 96.4% C and 3.6% O for non-irradiated and 89.2% C and 10.8% O after low dose γ-irradiation. Thus, in our experimental conditions, the C/O ratio changes from 26.8 to 8.3, respectively. The carbonaceous reduction detected by XPS is higher than the one deduced from ATR-FTIR, however, it must be taken into account that the analysis depth of ATR-FTIR in this type of samples is in the order of 1 μm (see 2.4 in "Materials and methods" section) while XPS profiles about 10 to 20 nm. This makes XPS to be much more surface sensitive and overestimate the epicuticular wax removal. Furthermore, the

apparition of oxygenated species arising from the oxidative degradation of aliphatic compounds (Singh, 1999), may be contributing to the O_{1s} signal and thus decreasing the C/O ratio in γ-irradiated cuticles. Unfortunately not much additional conclusions can be derived from O_{1s} and C_{1s} peaks positions (binding energies) due to the multiple contributions they contain (Hietala et al., 1997; Genet et al., 2002).

In conclusion, both ATR-FTIR and XPS data have revealed that low-dose γ-irradiation causes no significant chemical modifications of isolated tomato cuticles but the partial epicuticular wax layer removal. Such elimination can be associated to the surfaces textural modifications observed by AFM and SEM.

3.3. Transport characterization

The cuticle is the primary barrier protecting fruits and leaves from the atmosphere. Though low dose γ-irradiation has shown to have little effect on the texture and chemical composition of isolated tomato cuticles, modifications related to transport parameters (permeability, ion exchange capacity…) may be of relevance in terms of fruit degradation. To address this issue, salt diffusion and membrane potential measurements have been carried out. The study has been extended to fully de-waxed plant cuticles (CNW) to specifically evaluate the role of epicuticular waxes.

Salt diffusion permeability (Ps) values obtained for NaCl are compiled in Table 1. The complete removal of epicuticular waxes (CNW) produces a significant increment of salt permeability (≈ 40 %) as may be expected from the elimination of such hydrophobic phase. In low-dose γ-irradiated samples, the increment is only 15% which indicates a partial de-waxing. In this sense, it can be interpreted that low dose γ-irradiation slightly reduces the barrier capacity of isolated tomato cuticles.

The membrane potential ($\Delta\Phi_{mbr}$) provides additional information on changes induced by γ-irradiation on characteristic electrochemical parameters such as fixed charge concentration in the membrane (X_f) and ion transport numbers (t_i), that is, the fraction of the total current carried out for one particular ion ($t_i = I_i/I_T$) . Fig. 5 shows the variation of membrane potentials with salt concentration ratio (c_v/c_c). As it can be observed, two different $\Delta\Phi_{mbr}$ -ln(c_v/c_c) relationships were obtained depending on the c_v value. At $c_v < c_c$ (interval 10^{-3} M to 10^{-2} M), membrane potentials increase with the increase of c_c, a tendency comparable to that exhibited by a cation exchanger (solid line). The opposite tendency was obtained for $c_v > c_c$ (interval 10^{-2} M to 10^{-1} M), with a membrane potential dependence similar to that shown by the solution diffusion potential (dotted line). This behaviour is similar to that reported in the literature for weakly negatively charged membranes (Kimura et al., 1984).

Taking into account the Teorell-Meyer-Siever (TMS) theory (Meyer and Sievers, 1936; Teorell, 1956), the membrane potential can be assumed to be due to two Donnan potentials at each membrane-solution interface associated to the partial exclusion of co-ions (or ions with a similar electrical character to that of the membrane) plus an additional diffusion potential due to the different diffusivity of anions and cations in the membrane. Donnan exclusion of anions is relevant when solution concentration is lower that the membrane fixed charge concentration, but it can be neglected at high solution concentrations, when the membrane potential is mainly due to the ions diffusion in the membrane. Then, on the base of the TMS theory, the average values for the cation transport number across the studied membrane were determined from the slope of the straight line obtained at high concentrations (right hand side values in Fig. 5), and the results are indicated in Table 1. As observed, there is a reduction of <t+> as a consequence of both γ-irradiation and complete epicuticular wax elimination. The comparison of Na^+ transport

number in the studied cuticles with that reported for aqueous solutions ($t_{Na^+}^{o}$ = 0.38) confirms the electronegative character of tomato cuticle ($t_{Na^+}(C) > t_{Na^+}^{o}$), which favours the transport of cations. Such effect is a direct electrical contribution of waxes and the reduction of the cation transport number is therefore an indication of cuticle de-waxing.

Since there is no experimental result indicating a modification of the cuticle components (phenolic compounds, polysaccharides, cutin...) but the partial removal of the epicuticular waxes layer upon low dose γ-irradiation, we can conclude that the slight reduction of the electrochemical parameters is exclusively due to a partial elimination of such epicuticular wax layer, which confirms the conclusion previously deduced from texture and chemical analysis.

4. CONCLUSIONS

Low dose γ-irradiation of isolated tomato cuticles has shown some textural changes but no significant chemical modifications. Precisely, only partial removal of the outermost epicuticular wax layer has been detected by SEM, AFM, ATR-FTIR and XPS analyses. Such alteration of this primary protective layer is accompanied by a slight reduction of its barrier capacity as deduced from salt diffusion permeability and membrane potential measurements. The extent of these modifications is small enough to propose the use of low-dose γ-irradiation as an effective treatment for fruit preservation and to expect low impact in the aspect and drying susceptibility of fruits under storage.

ACKNOWLEDGMENTS

We would like to thank Dr. P. Galán (Medical Physics Department of Hospital Regional Universitario Carlos Haya, Spain) for carrying out the γ -irradiation on the samples.

REFERENCES

Arvanitoyannis, I.S., Stratakos, A.C., Tsarouhas, P., 2009. Irradiation applications in vegetables and fruits: a review. Critical Reviews in Food Science and Nutrition 49, 427-462.

Casado, J., Martínez de Yuso, M.V., Galán, P., Benavente, J., 2006. Modificación estructural y química de membranas de celofán debido a radiaciones ionizantes. Revista de Física Médica 7(1), 11-16.

Chamel, A., Maréchal, Y., Triantaphylides, C., Dudon, V., 1994. Demonstration using FTIR spectroscopy of the effect of a gamma irradiation on the formation of the cherry tomato fruit cuticle. Comptes rendus de l'Académie des Sciences, Sciences de la vie/Life sciences 317, 346-350.

Crawford, L.M., Ruff, E.H., 1996. A review of the safety of cold pasteurization through irradiation. Food Control 7(2), 87-97.

Domínguez, E., Heredia-Guerrero, J.A., Heredia, A., 2011. The biophysical design of plant cuticles: an overview. New Phytologist 189(4), 938-949.

Fernández, V., Khayet, M., Montero-Prado, P., Heredia-Guerrero, J.A., Liakopoulos, et al., 2011. New insights into the properties of pubescent surfaces: peach fruit as a model. Plant Physiology 156 (4), 2098-2108.

Heredia-Guerrero, J.A., San-Miguel, M.A., Luna, M., Dominguez, E., Heredia, A., et al., 2011. Structure and support induced structure disruption of soft nanoparticles obtained from hydroxylated fatty acids. Soft Matter 7, 4357-4363.

Holloway, P.J., 1982 Structure and histochemistry of plant cuticular membranes: an overview, in: Cutler, D.F., Alvin, K.L., Price, C.E. (Eds.), The plant cuticle, Academic Press, London, pp 1-32.

Horcas, I., Fernández, R., Gómez-Rodríguez, J.M., Colchero, J., Gómez-Herrero, J., et al., 2007. WSXM: A software for scanning probe microscopy and a tool for nanotechnology. Review of Scientific Instruments 78(1), 013705.

Hunt, G.M., Baker, E.A., 1980. Phenolic constituents of tomato fruit cuticles. Phytochemistry 19(7), 1416-1419.

Jeffree, C.H., 2006. The fine structure of the plant cuticle, in: Riederer, M., Müller, C. (Eds.) Biology of the Plant Cuticle, Blackwell Publishing, Oxford. pp. 11-125.

Kimura, Y., Lim, H.J., Iijima, T., 1984. Membrane potentials of charged cellulosic membranes. Journal of Membrane Science 18, 285-296.

Korotchenko, K.A., Sharpatyi, V.A., 2004. Radiation chemistry of polysaccharides: 3. On the strange dose dependence of the buildup of some radiolysis products. High energy chemistry 38(4), 231-235.

Kosma, D.K., Parsons, E.P., Isaacson, T., Lü, S., Rose, J.K., et al., 2010. Fruit cuticle lipid composition during development in tomato ripening mutants. Physiologia Plantarum 139(1), 107-117.

López-Casado, G., Matas, A.J., Dominguez, E., Cuartero, J., Heredia, A., 2007. Biomechanics of isolated tomato (*Solanum lycopersicum* L.) fruit cuticles: the role of the cutin matrix and polysaccharides. Journal of Experimental Botany 58(14), 3875-3883.

Meyer, K.H., Siever, J.F., 1936. La permeabilité des membranes. I. Theorie de la permeabilité ionique. Helvetica Chimica. Acta 19, 649-664.

Nagy, T.O., Ledolter, K., Solar, S., 2008. Oxidation of naringenin by gamma-radiation. Radiation Physics and Chemistry 77, 728-733.

Niemira, B.A., Fan, X., 2006. Low-dose irradiation of fresh-cut produce: safety, sensory, and shelf life, in: Sommers, C.H., Fan, X. (Eds.) Food Irradiation Research and Technology, Blackwell Publishing, Iowa, pp. 169-184.

Petracek, P.D., Bukovac, M.J., 1995. Rheological properties of enzymatically isolated tomato fruit cuticle. Plant Physiology 109(2), 675-679.

Plikk, P., Odelius, K., Hakkarainen, M., Albertsson, A.C., 2006. Finalizing the properties of porous scaffolds of aliphatic polyesters through radiation sterilization. Biomaterials 27, 5335-5347.

Pollard, M., Beisson, F., Li, Y., Ohlrogge, J.B., 2008. Building lipid barriers: biosynthesis of cutin and suberin. Trends in Plant Science 13, 236-246.

Ribero da Luz, B., 2007. Attenuated total reflectance spectroscopy of plant leaves: a tool for ecological and botanical studies. New Phytologist 172(2), 305-318.

Singh, A., 1999. Irradiation of polyethylene: some aspects of crosslinking and oxidative degradation. Radiation Physics and Chemistry 56, 375-380.

Teorell, T., 1956. Transport phenomena in membranes. Discussions of the Faraday Society 21, 9-26.

Vázquez, M.I., Heredia-Guerrero, J.A., Galán, P., Benítez, J.J., Benavente, J., 2011. Structural, chemical surface and transport modifications of regenerated cellulose dense membranes due to low-dose gamma-radiation. Materials Chemistry and Physics 126, 734-740.

LEGENDS

Figure 1. SEM images of A) non-irradiated (C) and B) low dose γ-irradiated (C-Ir) isolated tomato cuticles. No major textural modification is observed but the removal of the bright small elongated deposits upon irradiation.

Figure 2. Dynamic AFM images of A) non-irradiated (C) and B) low dose γ-irradiated (C-Ir) isolated tomato cuticles. Wrinkled features are eliminated by irradiation leaving rounded isolated residues.

Figure 3. ATR-FTIR spectra of non-irradiated (C), low dose γ-irradiated (C-Ir) and fully de-waxed (CNW) isolated tomato cuticles in the A) C-H and B) C=O stretching regions. Spectra resulting from the matrix (CNW) subtraction C) are included to highlight the chemical modifications induced by low-dose γ-irradiation.

Figure 4. O_{1s} and C_{1s} XPS spectra of non-irradiated (C) and low dose γ-irradiated (C-Ir) tomato cuticles. The increase of the O/C signal ratio is consistent with a partial removal of the outermost epicuticular wax layer upon γ-irradiation.

Figure 5. Membrane potential *vs* $\ln(c_v/c_f)$ for unaltered (C) (●), low dose γ-irradiated (C-Ir) (●) and fully dewaxed (CNW) (□) tomato cuticles.

Table 1. NaCl diffusion permeability (P_s) and average cation transport number ($<t_+>$) in the studied membranes.