1	Characterizing the tissue of apple air-dried and osmo-air-dried rings by X-ray CT and OCT and
2	relationship with ring crispness and fruit maturity at harvest measured by TRS.
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22 Abstract

23 Air-dried apple rings were prepared from 'Golden Delicious' apples selected at harvest as less mature and 24 more mature according to the absorption coefficient measured at 670 nm by TRS, stored in air for 5 25 months, and subjected to air-drying with (OSMO) and without (noOSMO) osmodehydration pre-treatment 26 (60% sucrose syrup). Selected rings were submitted to microstructural analysis by X-ray computed 27 tomography (X-ray CT), to subsurface structure analysis by Optical coherence tomography (OCT) and to 28 texture and sound emission analysis by bending-snapping test. Higher crispness index, higher number of 29 sound events and higher average SPL characterized the OSMO rings. Total porosity was related to 30 $SPL_{av>60}$, tissue and pore anisotropy to $SPL_{av>60}$, pore fragmentation index to fracturability and specific 31 surface area to the work required to snap the ring. A differentiation of the drying treatments, as well as of 32 the products according to the TRS maturity class at harvest were obtained analysing by PCA 33 microstructure parameters and texture and acoustic parameters. The differences in mechanical and acoustic 34 characteristics between OSMO and noOSMO rings were due to the different subsurface structure as found 35 with OCT analysis. 36 37 Key words: Microstructure, X-CT, OCT, raw material selection, TRS, acoustic-mechanical properties, 38 crispness, osmodehydration pre-treatment, air-dried apple rings 39 40 **Industrial relevance**

41 There is an increasing demand of dried crispy fruit as they are considered by consumers healthy, natural 42 and tasty foods. The textural characteristics, exerting a strong effect on crispy and crunchy sensory 43 characteristics, have a great impact at consumption of dried crispy fruit. As their textural characteristics 44 depend on both fruit maturity at processing and processing conditions, food industry is demanding 45 nondestructive techniques which could be used for on-line/off-line sorting of fruit into classes each one 46 more suited for obtaining a specific product. Furthermore, the textural properties of dried foods depend on 47 microstructure, defined as the spatial arrangement of structural components and their interactions. Due to 48 the microscopic complexity, unambiguous methodologies that relate quality to food microstructure do not

49	exist today, in contrast to what already existing for several engineering materials. Hence there is the need
50	of developing methods that measure directly the microstructural properties of dried foods.
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52	Highlights:
53	• For the first time, X-rayCT, OCT and acoustic emission coupled to texture analysis were combined
54	to investigate the structure-property relationships of air-dried apple rings in relation to fruit
55	maturity at harvest measured by TRS and to pre-drying osmodehydration.
56	• X-rayCT and OCT indicated changes in the microstructure related to crispness parameters
57	measured by acoustic-texture analysis, which could be related to both fruit maturity at harvest and
58	osmotic pre-treatment.
59	• The results show that a differentiation of the products according to the TRS maturity class at
60	harvest was obtained.
61	

62 1. Introduction

63

64 processed fruits and vegetables. There is an increasing demand for innovative products that respond to 65 changed lifestyles and working rhythms. In addition, consumers are also more and more interested in 66 consuming healthy, natural and tasty foods. Dried crispy fruits could satisfy these requirements, as they are 67 perceived as healthy because of their nutritional value, combined with high fiber content, but also tasty. 68 Among these products, dried apples are part of several prepared foods including snack preparations and 69 integral breakfast foods, as well they are used alone as snacks (Lewicki & Jakubczyk, 2004). 70 Drying is a process involving heat and mass transfer that can cause physical and chemical alteration of the 71 material. The stress developed when water is removed from the fresh material causes shrinkage and change 72 in shape, both of which influence the porosity of the dried material and its rehydration properties (Lewicki 73 & Jakubczyk, 2004; Mayor, Silva & Sereno, 2005). Other consequences of the drying process involve 74 changes in the rheological properties of the product, which are bound to changes in composition, phase 75 transition of the material and microstructural changes due to loss of cell turgor pressure because of the loss 76 of water from the inner parts towards the surface, possibly causing stiffness, spoilage and disruption of cell 77 walls, or even a collapse of the cell tissue and a cell breakage (Lewicki & Lukaszuk, 2000; Maltini, 78 Torreggiani, Venir & Bertolo, 2003). The extent of these changes depends on the species and on the 79 maturation degree at processing, factors affecting the textural properties of the raw material. 80 The internal quality of fruit can be assessed non-destructively by using time-resolved reflectance 81 spectroscopy (TRS), which provides a complete characterization of diffusive media with the simultaneous 82 non-invasive measurement of the bulk optical properties. TRS is based on the measurement of the temporal 83 delay and the broadening experienced by a short laser pulse (pulse duration in the order of 100 ps) while 84 travelling through a turbid medium (Torricelli et al., 2008). By using an appropriate theoretical model of 85 light penetration for the analysis of photon time distribution, it is possible to simultaneously estimate the 86 absorption coefficient (μ_a) and the reduced scattering coefficient (μ'_s). Light penetration achieved by TRS 87 in most fruit and vegetables can be as great as 1-2 cm, depending on the optical properties (Cubeddu et al., 88 2001). Hence, TRS provides information on the internal properties of the medium and is not significantly

In the last few years, a new interest has arisen in the field of functional products, such as minimally

89 affected by surface features (Saeys, Velazco-Roa, Thennadil, Ramon & Nicolaï, 2008). TRS has been used 90 to assess maturity, texture and cell wall structure as well as internal defects in intact fruit (Vanoli, Zerbini, 91 Rizzolo, Spinelli & Torricelli, 2010), and to sort fruit at harvest according to maturity class, usually by 92 measuring fruit at 670 nm (near the chlorophyll-a peak) and classifying fruit with high $\mu_a 670$ values as less 93 mature and those having low $\mu_a 670$ values as more mature (Torricelli et al., 2008). As for air-dried apple 94 rings, it was shown that the classification of apples at harvest based on μ_a 670 was able to segregate fruit 95 generating fresh and air-dried rings of different quality (Rizzolo, Vanoli, Cortellino, Spinelli & Torricelli, 96 2011; Rizzolo, Vanoli, Cortellino, Spinelli & Torricelli, 2012): the differences found in the raw material 97 affected the changes occurring in apple rings with air-drying, mainly influencing weight loss, area 98 shrinkage and how much ring color changed due to browning phenomena. For 'Golden Delicious' and 'Pink Lady[®]' cultivars, by processing the more mature fruits, i.e. either after long cold storage or by using 99 100 apples having lower $\mu_a 670$ at harvest, air-dried rings with low shrinkage and low color changes (i.e. 101 showing less browning) with lower ring hardness and crispness index were obtained. 102 However, maximal shrinkage during drying decreases as its solids increase and structure collapse was 103 shown to decrease when fruit was impregnated with sugar prior to air drying (Torreggiani & Bertolo, 2004; 104 Wolf, Behsnilian & Speiss, 2001) by means of an osmotic process, which is carried out by immerging the 105 fruit into aqueous solutions of high sugar concentration, so achieving a partial dehydration coupled to a solute intake (Wolf, Behsnilian & Speiss, 2001). Moreover, great changes in the tissues structure could be 106 107 produced by combining the osmotic dehydration with air drying (Lewicki, 1998), with texture changing 108 from elastic-visco-plastic to rigid, becoming fragile and brittle, which are textural features linked to the 109 crispy and crunchy sensory attributes proper of snack food (Saeleaw & Schleining, 2011). In addition, the 110 rheological characteristics of osmo-air-dried apple rings were shown to change according to the cultivar: 111 'Golden Delicious' rings acquired rigidity but remained brittle and fragile, developing small fractures, whereas 'Pink Lady[®]' ones become rigid, but harder and stiffer, with abrupt failures of major intensity 112 113 (Farris, Gobbi, Torreggiani & Piergiovanni, 2008; Gobbi, Farris, Limbo & Torreggiani, 2012). These 114 mechanical characteristics are strictly bound to the porosity of dried apple rings, which has been 115 characterized using different imaging 2-D techniques, such as light microscopy (Mayor, Silva & Sereno,

116 2005; Gobbi, Farris, Limbo & Torreggiani, 2012) and scanning electron microscopy (Bai, Rahaman,

117 Perera, Smith & Melton, 2002; Acevedo, Briones, Buera & Aguilera, 2008; Askari, Eman-Djomeh &

118 Mousavi, 2008; Witrowa-Rajchert & Rząca, 2009; Huang, Zhang, Wang, Mujumdar & Sun, 2012).

119 Even if scanning electron microscopy is a useful tool to analyze the sample microstructure, it does not give

120 reliable information about the total pore volume and pore size distribution in the sample. These information

121 on the microstructure can be obtained using the X-ray microtomography, which has been applied to study

122 the effect of far-infrared radiation assisted drying on microstructure of banana slices (Léonard, Blacher,

123 Nimmol & Devahastin, 2007), and to quantify the pore space of apple tissue (Mendoza et al., 2007;

124 Mendoza et al., 2010; Herremans et al., 2013).

125 Complementary information on dried ring microstructure could also be obtained using optical coherence

tomography (OCT), a novel approach to assess the subsurface microstructure (Huang et al., 1991). OCT is

127 a purely optical, non-destructive, non-invasive, and contactless high resolution imaging method, which is

based on the physical phenomenon of white light interferometry. The technique employs special light

sources with very short temporal coherence, which enables an excellent depth resolution in the range of

130 only a few microns (Drexler et al., 1999). In the field of food and plant photonics so far OCT has been used

to study the morphological and functional state of higher plant tissues (Sapozhnikova, Kamenskii &

132 Kuranov, 2003; Kutis, Sapozhnikova, Kuranov & Kamenskii, 2005; Verboven et al., 2013) and to detect

disease in melon seeds (Lee, Lee, Kim, Jung & Kim, 2011) and disease, defects and rots in onion

134 (Meglinski, Buranachai & Terry, 2010; Landahl, Terry & Ford, 2012).

The objectives of this work were: a) to evaluate the subsurface structure and the microstructure of air-dried and osmo-air-dried apple rings by OCT and X-ray micro-tomography; and, b) to study the relationships among the microstructure features and the crispness of dried apple rings in relation to fruit maturity

assessed at harvest by TRS.

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140 2. Materials and Methods

141 2.1. Fruit and experimental plan

142 'Golden Delicious' apples (*Malus×domestica*, Borkh.) coming from Laimburg (Trentino Alto-Adige

143 region, Italy) were harvested on 8 September 2011, which corresponds to the commercial picking window 144 for this cultivar in Trentino-Alto Adige region. Sixty fruits were selected and measured at harvest by TRS 145 at 670 nm (close to the absorption peak of chlorophyll-*a*), ranked according to decreasing $\mu_a 670$ (increasing 146 maturity) and randomized into three batches of 20 fruit each. The batches were stored for 5 months at +1°C 147 in air. Each batch corresponded to a different pre-treatment: without osmodehydration pre-drying 148 (noOSMO) and with 1 h (OSMO1) or 3 h (OSMO2) osmodehydration pre-drying. Three 5 mm thick 149 rings/fruit were prepared. The rings from each fruit were packed together into a tulle bag and immersed for 150 1 h (OSMO1) or 3 h (OSMO2) at 20°C in a sucrose solution ($a_w=0.90$, 60% w/w), which was continuously 151 recirculated at 1.5 L/min through a peristaltic pump. The ratio fruit/solution was 1/3. Before air drying, the 152 OSMO rings were drained, rinsed gently with tap water, and placed a few minutes over adsorbent paper to 153 remove excess water. OSMO and noOSMO rings were air-dried at 80°C up to a constant weight using a 154 pilot alternate upward-downward air circulated drier (Thermolab, Codogno, Italy) operating at an air speed 155 of 1.5 m/s. 156 Due to the duration of a single analysis, for the microstructural analysis by means of X-ray micro-CT a 157 selection of dried apple samples was made: rings obtained from the most differing pre-treatments before 158 drying (noOSMO and OSMO2) and from the most differing apples in terms of maturity measured by 159 means of TRS (ranks 1 and 2, less mature (LeM) fruit and ranks 19 and 20, more mature (MoM) fruit) were 160 chosen. For the subsurface microstructure analysis by OCT, instead, within each batch, the rings obtained 161 from the most differing apples in terms of TRS maturity (rank 1, the least mature; rank 20, the most 162 mature) were selected. On the dried rings prepared from the selected fruit, the mechanical and acoustic 163 properties were measured using a texture analyzer in conjunction with acoustical emission analysis 164 (bending-snapping test). 165

166 2.2. Assessment of maturity at harvest by time-resolved reflectance spectroscopy and samples 167 formation

For TRS measurements, a compact system was used, working at 670 nm, based on a pulsed laser diode
(mod. PDL800, PicoQuant GmbH, Germany), with 80 MHz repetition frequency, 100 ps duration, and 1

170 mW average power, a compact photomultiplier (mod. R5900U-L16, Hamamatsu Photonics, Japan) and an

171 integrated PC board (mod. SPC130, Becker&Hickl GmbH, Germany). Typical acquisition time for time-

172 correlated single photon counting is 1 s per point. A couple of 1 mm plastic fibers (Mod. ESKA GK4001,

173 Mitsubishi, Japan) delivers light into the sample and collects the emitted photons at a distance of 1.5 cm. A

band pass filter tuned at 670 nm was used to cut off the fluorescence signal due to chlorophyll. Overall, the

instrumental response function duration was <160 ps. The reduced scattering coefficient (μ'_s) and the

absorption coefficient (μ_a) were obtained by fitting the experimental TRS data with a standard solution of

177 the diffusion approximation to the transport equation for a semi-infinite homogenous medium. The

178 extrapolated boundary condition was used (Contini, Martelli, & Zaccanti, 1997) to take into account the

179 refractive index mismatch at the surface.

180 The absorption coefficient at 670 nm was measured on two opposite sides of each fruit and the average per 181 fruit was used for fruit ranking from less mature to more mature fruit. The 60 ranked apples were grouped 182 by 3, with a total of 20 groups, corresponding to 20 levels of μ_a . Each fruit from each group was randomly 183 assigned to a different pre-drying treatment. In this way, fruit from the whole range of μ_a were available for 184 each pre-drying treatment.

185

186 2.3. Microstructural analysis

187 2.3.1 X-ray micro-CT

188 For the X-ray micro-CT analysis, by using a cork borer, small cylindrical samples (3 mm diameter) were 189 excised from the dried apple, approximately 5 mm from the peel, excluding regions in which vascular 190 tissue could be discerned visually. The thickness of the apple slices was not altered in preparing the 191 samples. The samples were mounted on the rotating holder and stabilized using parafilm. Because of the 192 dry state of the samples, hardly any sample degradation was expected within the time frame of the scan (29 193 minutes). X-ray micro-CT measurements were performed on a SkyScan 1172 system (Bruker microCT, 194 Kontich, Belgium), operated at 55 keV source voltage and 181 µA current and with an isotropic image 195 pixel resolution of 2.44 μ m. The samples were rotated over 0.35° steps over a total of 180°, each time 196 averaging 3 frames to acquire a radiographic image of 1048 by 2000 pixels. The projection images were

197 loaded into dedicated software (NRecon1.6.3.2, Bruker microCT, Kontich, Belgium) to reconstruct virtual 198 cross-sections of the sample. This resulted in a 3D greyscale datastack, digitized to 880 slices of 2000 by 199 2000 pixels. The images were smoothed by a Gaussian smoothing kernel, and corrected for rings and beam 200 hardening, which are common artifacts in X-ray CT images. For image analysis a cylindrical volume of 201 interest (diameter 2.5 mm) was cropped centrally in the imaged volume to exclude interference with the 202 excised borders of the sample. The remaining volume for analysis measured 5.4 mm³. The images were 203 filtered in 3D space using a median filter with filter radius of 2 pixels. Otsu's algorithm (Otsu, 1979) was 204 applied for binarizing the image by separating two peaks in the grey scale frequency distribution: pixels 205 with lower intensities than the Otsu threshold were assigned to the background (air) and pixels with a 206 higher intensity than that threshold were assigned to the apple tissue material. Individual 3D objects smaller 207 than 27 voxels were considered to be noise and were filtered out of the datastack. Morphometric 208 parameters describing the microstructure were calculated on the 3D data using CTAn v.1.12.0.0 (Bruker 209 microCT, Kontich, Belgium). A description of the parameters and the main concept of the calculation can 210 be found in Herremans et al. (2013) and Skyscan (2010).

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212 2.3.2 Optical coherence tomography

213 All measurements have been performed with a modular spectral domain OCT system (henceforth referred 214 to as "SD-OCT"), which is composed of a light source, a probe head and a spectrometer. The 215 supercontinuum light source (Koheras SuperK Versa, NKT Photonics, Denmark) emits light at a central 216 wavelength of 860 nm. Its spectral bandwidth of 170 nm allows for an axial resolution of 2 µm (in air). In 217 the probe head, the beam is split by a non-polarizing bulk beam splitter (BS) into a reference and a sample 218 arm. In the reference arm light is reflected from a gold coated mirror (M), whereas in the sample arm it is 219 reflected from the different layers within the sample. The light returning from both arms is recombined and 220 sent to the spectrometer, where it is spectrally dispersed by a transmission grating and recorded by a CCD 221 camera. The recorded spectrum is modulated by interference fringes, with the frequency of the modulation 222 depending on the path length differences between reference and sample arm. For recording a cross-section 223 image the beam of light is scanned over the sample surface over a range of 5 mm by means of a

galvanometer mirror (GM). During this process 1000 interferograms are recorded, which are used for
reconstructing the images. A schematic diagram of a spectral-domain OCT system is depicted in Fig. 1.
This particular system was equipped with a multi focal-length probe head. With such a probe head it was
possible to switch between different imaging optics, and thus, to change the lateral resolution and depth of
focus of the probing beam. Single depth scans were acquired at a rate of 20 kHz.

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2.4. Texture and sound emission analysis

231 A TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) was used for bending-snapping 232 test fitted with a 50N load cell and equipped with an acoustic emission detector (AED, Stable 233 Microsystems), using the HDP/3PB Three Point Bending Rig. The lower supporting blades were separated 234 by a distance of 45 mm, and the compressing blade was driven down between the two supports at a speed 235 of 0.17 mm/s, bending each apple ring until it snapped. A microphone unit Type 4188-A-021 (Brüel & 236 Kjær) was connected to an AED for sound pressure measurements and was placed at the sample level 237 located 100 mm away from the central axis of the probe. The sound measurement system was calibrated 238 using the Brüel & Kjær Type 4231 sound calibrator at sound pressure levels of 94 and 114 dB at 1000 Hz. 239 The gain of AED was set at 0 dB and the sampling rate was set at 500 Hz for sound and force measurements. Acoustic signals were captured in "RAW" format used in the TA.XT plus Texture 240 241 Analyzer. Data were than converted to dB. The mechanical and acoustic characteristics were extracted 242 from the data using Texture Exponent 32 software (Stable Microsystems). All tests were performed in a 243 laboratory with no special soundproof facilities at room temperature. Force/displacement and 244 sound/displacement curves were simultaneously plotted. From the force curve the following parameters 245 were extracted: number of peaks, ring hardness corresponding to the maximum force (hardness, N), 246 distance at the first major point (*Travel1*, mm), distance at the break (*fracturability*, mm), work required to 247 the first major fracture point (Area1, N×mm), work required to snap the ring (Total area, N×mm), slope of 248 the first part of the force curve (*slope*, N/mm) and gradient to the maximum force (*gradient_max*, N/mm). 249 From the sound curves the following data were extracted: total number of sound peaks (*N_sounds*), number 250 of sound peaks having SPL higher than 60 dB (*N_sounds>60dB*), average SPL of sound peaks lower than

251	60 dB ($SPL_{av<60}$), average SPL of sound peaks higher than 60 dB ($SPL_{av>60}$), average SPL of total sound
252	peaks (<i>avSPL</i>). The crispness index (E_{mod} , MPa) was calculated from gradient_max (E_{mod} max) and slope
253	$(E_{\text{mod}}$ slope) according to Farris, Gobbi, Torreggiani & Piergiovanni (2008).
254	
255	2.5. Statistical analysis
256	Data were submitted to Analysis of Variance and means were compared by Tukey's (mechanical and
257	acoustic parameters) and Duncan's (morphometric parameters) tests at P≤0.05% (Statgraphics v.7,
258	Manugistic Inc., Rockville, MD, USA).
259	Principal Component analysis (PCA) was carried out in order to study the relationships between X-ray CT
260	morphometric parameters and mechanical and acoustic properties of dried rings considering 10
261	morphometric parameters, 6 mechanical and 5 acoustic parameters and was performed by The Unscrambler
262	X version 10.0.1 (CAMO, Oslo, Norway) software package using the nonlinear iterative partial least-
263	squares (NIPALS) algorithm. The principal component (PC) scores were then submitted to ANOVA, and
264	means were compared by Duncan's test at $P \le 0.05\%$.
265	
266	3. Results
267	3.1 Absorption coefficient at harvest
268	The absorption coefficient at 670 nm ranged from 0.35 cm ⁻¹ for the least mature fruit to 0.092 cm ⁻¹ for the
269	most mature apple; the optical properties at harvest of LeM and MoM apples selected for this study were
270	(average \pm standard error): LeM maturity class: 0.032 ± 0.0083 cm ⁻¹ ; MoM maturity class: 0.11 ± 0.0064
271	cm^{-1} .
272	3.2. Microstructure
273	Fig. 2 (left) presents an X-CT slice of an OSMO2 LeM dried apple ring. The skeleton of dried apple tissue

could be accurately detected because of the high contrast with the pore space. So, a threshold was applied

to segment skeleton from the pore space resulting in a binary image (**Fig. 2, right**). **Fig. 3** shows

276 representative cross sections of noOSMO and OSMO2 MoM dried apple rings. By comparing the images,

277 it is clear that the microstructure of dried apple ring somewhat changes with the osmodehydration pre-

drying. In fact, in the OSMO2 sample an higher presence of large pores than in the noOSMO ones isevident.

280 Considering the morphometric parameters computed (Table 1), on average (mean±standard error) the 281 osmotic pre-treatment increased porosity (noOSMO, 77.8±1.1 %; OSMO2, 82.0±1.6 %) and tissue specific 282 surface area (noOSMO, 144.82±8.61 mm⁻¹; OSMO2, 159.81±2.79 mm⁻¹) and decreased pore anisotropy 283 (noOSMO, 0.532±0.016; OSMO2, 0.499±0.014), tissue thickness (noOSMO, 0.0233±0.0011 mm; 284 OSMO2, 0.0205±0.00034 mm), tissue anisotropy (noOSMO, 0.564±0.012; OSMO2, 0.501±0.012) and 285 tissue intersection surface (noOSMO, 2.67±0.23 mm²; OSMO2, 1.73±0.13 mm²). On the other hand, tissue 286 specific surface area and tissue thickness, along with tissue fractal dimension, depended also by the TRS 287 maturity class: in fact LeM rings on average had higher tissue specific surface area (LeM, 159.92±6.58 288 mm⁻¹; MoM, 144.71±6.12 mm⁻¹) and lower tissue thickness (LeM, 0.0209±0.00083 mm; MoM, 289 0.0228±0.00113 mm) and tissue fractal dimension (LeM, 2.490±0.012; MoM, 2.527±0.011). In addition, 290 the comparison of the TRS maturity classes within the same pre-treatment highlighted that in noOSMO 291 samples only porosity was influenced by TRS maturity class, with LeM rings showing higher porosity than 292 MoM rings, while in OSMO2 samples LeM rings were characterized by lower pore fragmentation index 293 and tissue fractal dimension, and higher tissue specific surface area, tissue fragmentation index and tissue 294 structure model index than MoM rings. Furthermore, the osmotic pre-treatment had a diverse impact on 295 morphometric parameters in the two TRS maturity classes. In fact, the osmotic pre-treatment induced in 296 LeM rings a decrease in pore anisotropy, tissue anisotropy and tissue intersection surface, whereas in MoM 297 rings an increase in tissue specific surface area and a decrease in tissue thickness and tissue fractal 298 dimension (Table 1).

A more profound insight in the microstructure is shown by the pore space thickness and tissue structure thickness distributions. These are approximated by a 3D sphere-fitting algorithm on the skeletonized structure, hereby calculating local structure diameters for every position on the skeleton.

302 The tissue thickness distributions (Fig. 4) show that more than 75% of cell spaces in OSMO2 rings were

smaller than 25 µm, independently from the TRS maturity class. In contrast, for noOSMO rings only

304 60.2% (MoM) and 72.8% (LeM) of cell spaces were smaller than 25 μm. As for pore space thickness

distributions (Fig. 5), in noOSMO rings, whatever the TRS maturity class, more than 50% of pores had
thickness smaller than 0.10 mm, and only about 3% of pores were larger than 0.20 mm, with a maximum
value of 0.27 mm. With the osmotic pre-treatment, for both LeM and MoM rings the distribution was
shifted towards larger values. In LeM rings 39.6% of pores had thickness lower than 0.10 mm, and 8.7% of
pores were larger than 0.20 mm, reaching a maximum value of 0.32 mm, whereas in MoM rings only 37%
of pores were smaller than 0.10 mm, and more than 17% of pores were larger than 0.20 mm, with about
5.7% of pore thickness ranging from 0.40 to 0.458 mm.

312

313 3.3 Subsurface structure

Fig. 6 shows representative OCT images of the subsurface of the dried rings from the least (R1) and most (R20) mature apple fruit in each batch. The shown images consist of 1000 adjacent depth scans and feature (optical) dimensions of 5×0.88 mm². OCT clearly distinguished noOSMO and OSMO air-dried rings: noOSMO samples feature a dense structure and thus a limited penetration depth, while the OSMO ones feature a loose surface structure with large inclusions of air. The differences between the noOSMO and OSMO samples seemed to be a surface effect, since they could not be clearly reproduced at freshly prepared sites of fractures. From OCT images it was not possible to deduce the time of the

321 osmodehydration pre-treatment or the effect of the TRS maturities.

322

323 3.4 Crispness parameters of air-dried and osmo-air-dried apple rings

324 If the mechanical and acoustic properties of dried rings from the selected fruit are considered (Table 2), no

325 significant influence of TRS maturity at harvest within each pre-treatment was found for the parameters

taken into consideration, with a few exceptions concerning the acoustic parameters. In noOSMO treatment

327 LeM rings were characterized by higher value of $SPL_{av<60}$ than MoM ones, whereas in OSMO2 samples,

328 LeM rings showed lower values of $SPL_{av>60}$ and avSPL than MoM rings.

329 In contrast, the osmotic pre-treatment strongly influenced some mechanical properties and almost all the

acoustic parameters: in OSMO rings *fracturability*, *Area1* and *Travel1* were lower, and *gradient_max*,

slope, E_{mod} .max and E_{mod} .slope were higher than in noOSMO rings, being in OSMO rings (mean \pm standard

- error): fracturability, 0.66±0.04 mm; Area1, 1.70±0.18 N×mm; Travel1, 0.51±0.04 mm; gradient_max,
- 333 11.92±0.69 N/mm; *slope*, 10.72±0.38 N/mm, E_{mod} .max, 279.1±26.5 MPa; and E_{mod} .slope, 252.6±24.6 MPa.
- 334 No differences in mechanical properties between the times of osmotic pre-treatment were found.
- 335 The *N_sounds* did not differ among the pre-treatments, even if there was a tendency to increase with the
- osmodehydration time, with OSMO2 rings showing a mean value almost twice the value of noOSMO
- samples. However, OSMO2 rings were characterized by a significantly higher $N_{sounds} > 60 dB$ (14.3 ±
- 338 3.7), higher $SPL_{av<60}$ (50.37 ± 0.45 dB), higher avSPL (57.86 ± 0.74 dB), and lower $SPL_{av>60}$ (71.39 ± 1.17
- dB), than noOSMO rings, which showed lower $N_{sounds} > 60dB$ (4.5±1.4), corresponding to only about
- 340 15% of *N_sounds*, lower *avSPL* (51.07 \pm 1.69 dB) and *SPL*_{*av*<60} (45.86 \pm 0.74 dB), but higher *SPL*_{*av*>60}
- 341 (80.00±1.74 dB). The osmosis time significantly influenced $SPL_{av<60}$ and $SPL_{av>60}$, being the former lower
- in OSMO1 rings (48.40 ± 0.57 dB), value higher than the noOSMO ones, and the latter higher in OSMO1
- rings $(77.97 \pm 1.56 \text{ dB})$, value not different from the noOSMO..
- 344

345 3.5 PCA on mechanical and acoustic properties and morphometric parameters

346 PCA based on X-CT morphometric parameters reported in Table1 and the mechanical and acoustic 347 properties of each ring analysed by X-CT allowed the selction of four principal components (PC), which 348 explained 89.8% of total variation (Fig.7). In PC1 (45.18% of total variance) slope, gradient_max and E_{mod} 349 slope mechanical parameters were positively related to avSPL, N_sounds and N_sounds>60dB acoustic 350 parameters and negatively related to $SPL_{av>60}$ and Areal parameters. In addition, PC1 highlighted 351 relationships between some morphometric parameters and acoustic characteristics: total porosity was 352 related to $SPL_{av<60}$, and was opposite to tissue anisotropy and pore anisotropy, which were related to 353 $SPL_{av>60}$, while Areal was related to tissue intersection surface. PC1 had positive scores for OSMO2 rings, 354 with LeM OSMO2 ones having the highest value, and negative for noOSMO rings, without any difference 355 between the TRS maturity classes (Fig.8). PC2 (26.55% of total variance), instead, underlined positive 356 relationships between morphometric parameters and mechanical characteristics: pore fragmentation index 357 was related to *fracturability* and the work required to snap the ring (*Total area*) to specific surface area. 358 Moreover, PC2 opposed pore fragmentation index, fracturability, and tissue fractal dimension to tissue

359 fragmentation index, tissue structure model index, Total area and specific surface area, and distinguished 360 dried apple rings according to the TRS maturity class. In fact, PC2 had negative scores for LeM rings and 361 positive scores for the MoM ones, but this difference was statistically significant only for the OSMO2 rings 362 (Fig.8). PC3 (10.67% of total variance) was mainly linked to pore anisotropy, $SPL_{av>60}$ total porosity and 363 tissue anisotropy, which were opposite to tissue structure model index and tissue intersection surface, 364 whereas in PC4 (7.48% of total variance) specific surface area was inversely related to Nsounds>60dB and 365 *N_sounds* acoustic parameters. PC3 and PC4 distinguished the TRS maturity classes, but only for the 366 noOSMO rings, which were characterized by positive scores for LeM rings and negative scores for the 367 MoM ones (Fig.8).

368

369 4. Discussion

370 The usefulness of the osmotic step as a pre-treatment prior to air-drying is related to the physico-chemical 371 modifications occurring in the plant tissue. In fact the simultaneous counter-current mass transfer process, 372 in which water outflows to the surrounding solution and the solute infuses into the product, causes in a 373 short time a fully plasmolysis of the cells on the surface of the material due to osmotic dehydration, with 374 little or no influence on the interior cells, so developing a gradient of turgor pressure, which can deform the 375 structure. Shrinkage and stretching forces are not strong enough to break cell walls or to split middle 376 lamella, but, when the osmotic process lasts at least 3 h, some detachments of cells occur, resulting in the 377 deformation and creation of new and small intercellular spaces (Lewicki & Porzecka-Pawlak, 2005). It was 378 shown that osmosis is a surface process as sugars penetrate to a depth of 2-3 mm, where a decreasing of 379 water binding by the apple can be observed (Salvatori, Andrés, Chiralt & Fito, 1999). During subsequent 380 air drying, sugars added during the osmotic dehydration pre-treatment helped to decrease structural 381 collapse (del Valle, Cuadros & Aguilera, 1998; Lewicki, 1998; Lewicki & Lukaszuk, 2000), which resulted 382 in a more porous structure. The OCT analysis, applied in this work for the first time as an alternative 383 imaging method to study the sub-surface structure of air-dried and osmo-air-dried apple rings, confirmed 384 the fact that the osmosis is a surface process, as the differences found by OCT imaging between noOSMO 385 and OSMO rings could not be clearly reproduced at freshly prepared sites of fractures. The observed

difference in the subsurface structure, i.e. a dense structure for noOSMO rings and a loose surface structure
with large inclusions of air for the OSMO ones, could be due to the fact that upon immersion in the osmotic
medium the first layers of cells die, and resulting in the creation of a volume near the surface (Mavroudis,

389 Dejmek & Sjöholm, 2004).

390 Scanning electron microscopy (SEM) studies carried out by Moreno, Simpson, Estrada, Lorenzen, Moraga 391 & Almonacid (2011) on 'Granny Smith' apples showed the effects of osmodehydration in sucrose solution 392 at microstructural levels. In fresh apples tissues are composed of numerous cells and intercellular spaces, 393 with cells closely bound to each other by middle lamella. In these cells, a large vacuole occupies most of 394 the protoplast, and the plasmalemma and tonoplast are close to the cellular wall. Cellular collapse as well 395 as protoplast contraction and cell wall edge distortion were observed as a consequence of 396 osmodehydration. On the other hand, Witrowa-Rajchert and Rząca (2009) found that air-drying at 70°C 397 caused in apple slices (cv 'Idared') changes in the structure properties of the material, bound to physical 398 alteration, such as shrinkage, increased porosity, decreased ability to imbibe water, and damage to 399 microscopic structure. The same authors reported that in fresh tissue half of the population of the cells has a 400 larger cross-section area than 0.034 mm², while most of the dried cells have a cross-section area of 0.0025 mm² with 50% having cross-section areas up to 0.0020 mm². SEM images underlined that in air-dried 401 402 apple rings the shrinkage stress causes numerous breaks of cell walls, with microstructure being 403 characterized by small cavities and very high density, with larger cells only in the boundary area of the 404 slices, suggesting that shrinkage of air-dried apple rings was anisotropic (Witrowa-Rajchert and Rzaca, 405 2009; Bai, Rahman, Perera, Smith & Melton, 2002; Lewicki & Jakubczyk, 2004). In addition, there has 406 been reported a strong negative correlation between porosity, computed from apparent and true density 407 values, and volume shrinkage, ranging the porosity from 69 to 74% with 73-76% volume shrinkage values 408 (Witrowa-Rajchert and Rząca, 2009). 409 Our results showed that the pre-drying osmodehydration treatment caused an increase in the porosity and

specific surface area of dried rings, which corresponded to lower volume and area shrinkage (data reported
in Rizzolo, Vanoli, Cortellino, Spinelli & Torricelli, 2013), confirming the negative correlation between
porosity and shrinkage found by Witrowa-Rajchert and Rząca (2009). The osmotic pre-treatment also

413 affected the degree of pore and tissue anisotropy, which are a measure of preferential alignment of the 414 structure, and they are scaled from 0 for total isotropy to 1 for total anisotropy. Here noOSMO rings 415 showed higher pore and tissue anisotropy values than OSMO2 rings; this difference could be ascribed to 416 how apple rings shrank with air-drying. In fact the light microscopy images of the section of two apple 417 rings air dried at 80°C, one after 90 min of osmosis and the other without the osmotic pre-treatment 418 reported by Gobbi, Farris, Limbo & Torreggiani (2012) showed that an important shrinkage took place 419 along the thickness axis in the not pretreated sample, which was characterized also by far fewer voids, with 420 shape not as around as the pre-treated ring. The positive effect of the osmotic pre-treatment on the dried 421 ring structure was confirmed also by the values of tissue fragmentation index, the tissue thickness 422 distribution and pore space thickness distributions, indicating that in OSMO rings there was a more 423 connected solid structure, with a lower local thickness of the cell spaces and an higher proportion of larger 424 pores than in noOSMO rings. These different morphometric characteristics found for air-dried and osmo-425 air-dried apple rings greatly influenced the mechanical and acoustic parameters considered as indices of 426 ring crispness. Hardness, gradient to the maximum force, E_{mod} , fracturability, work required to the first 427 force breakdown and work to snap the ring values indicated that air-dried ring were tough (strong and 428 highly deformable), while the osmo-air-dried one were brittle (hard and weak), as previously found by 429 Farris, Gobbi, Torreggiani & Piergiovanni (2008) and Gobbi, Farris, Limbo & Torreggiani (2012). In 430 addition our results showed that *slope*, *gradient_max* and E_{mod} slope mechanical parameters, which had 431 higher values in OSMO rings, were positively related to N_sounds, N_sounds>60dB and avSPL, all 432 acoustic parameters which have been associated to a high sensory crispness (Salvador, Varela, Sanz & 433 Fiszman, 2009; Saeleaw & Schleining, 2011). 434 Also raw material characteristics (cultivar and ripeness degree) have an influence on apple air-dried ring 435 quality. Konopacka & Plocharski (2001) found that prolonging the storage time of apple fruit (i.e.,

436 increasing ripeness) the derived ring showed increasing density and decreasing thickness retention, and that

437 apple rings produced by fruit after picking (less ripe) and also those produced from soft, overripe fruit after

438 storage were harder than those produced from ripe fruit. Higher ring hardness and crispness index were

439 also found by Rizzolo, Vanoli, Cortellino, Spinelli & Torricelli (2011, 2012) in air-dried apple rings

440 prepared either from fruit classified at harvest as less mature based on μ_a 670 or from fruit processed at 441 harvest. Our results indicate that using less mature apples based on μ_a 670 measured at harvest by TRS, air-442 dried ring with higher porosity and higher $SPL_{av<60}$ could be produced, as well as osmo-air-dried ring 443 having a more connected solid structure, with lower tissue and pore degree of anisotropy, and defined less 444 crispy by acoustic parameters (lower $SPL_{av>60}$ and lower avSPL) than osmo-air-dried rings produced by 445 more mature fruit.

446

447 5. Conclusions

X-ray CT images were used to compute microstructural descriptors, OCT images were used to visualize the 448 449 subsurface structure, and force and sound pressure level profiles were used to evaluate crispness of air-450 dried apple rings obtained with or without an osmodehydration pre-treatment. Higher crispness index, 451 higher number of sound events and higher average SPL characterized the OSMO rings. Porosity was 452 related to $SPL_{av<60}$, tissue and pore anisotropy to $SPL_{av>60}$, pore fragmentation index to *fracturability* and 453 specific surface area to the work required to snap the ring. By using principal component analysis a 454 differentiation of the drying treatments, as well as of the products according to the TRS maturity class at 455 harvest were obtained. The differences in mechanical and acoustic characteristics between OSMO and 456 noOSMO rings could be also due to the different subsurface structure as found with OCT analysis. 457 It can be concluded that there is a clear relation between the maturity at harvest nondestructively assessed 458 on intact fruit by TRS, the processing conditions and the microstructure features determined by X-ray CT 459 and OCT, and texture quality (crispness) of dried apple rings. TRS therefore holds a large promise for 460 application as a straightforward sorting tool for obtaining high quality dried apple rings.

461

462 Acknowledgements

This publication has been produced with the financial support of the European Union (project FP7-226783
- InsideFood). The opinions expressed in this document do by no means reflect the official opinion of the
European Union or its representatives. Alexandra Nemeth and Michael Leitner furthermore acknowledge
support from the European Regional Development Fund (EFRE) in the framework of the EU-program

467 REGIO 13, and the federal state of Upper Austria.

468

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Table 1. 3-D morphometric parameters (means ± standard error) for noOSMO and OSMO2 dried apple rings in function of TRS maturity class (LeM=less mature; MoM=more mature) (n=2) for tissue structure

and pore space.

584

	Means ±standard error						¥
	noOSMO		OSMO2			ain ects I	iteraction
	LeM	MoM	LeM	MoM	0	М	O×M
Porosity (%)	79.63±0.82	76.04±0.81	81.88±0.76	82.03±3.74	*	ns	ns
Pore anisotropy	0.556±0.013	0.508±0.016	0.470 ± 0.016	0.490 ± 0.028	*	ns	ns
Pore specific surface area (mm ⁻¹)	42.56 ± 1.65	45.15±1.39	39.71±1.85	37.87±9.03	ns	ns	ns
Pore fragmentation index (mm ⁻¹)	-13.83±17.18	-1.71±9.50	-6.14±1.75	0.17±1.25	ns	ns	ns
Tissue specific surface area (mm ⁻¹)) 155.42±14.80	134.21±0.79	164.42±0.17	155.20±2.03	*	*	ns
Tissue thickness (µm)	21.8±1.6	24.7±0.7	20.0±0.1	21.0±0.5	**	*	ns
Tissue anisotropy	0.582±0.009	0.547±0.016	0.493±0.014	0.509 ± 0.024	**	ns	ns
Tissue Intersection surface (mm ²)	2.36±0.06	2.98±0.34	1.70 ± 0.18	1.76±0.27	**	ns	ns
Tissue fragmentation index (mm ⁻¹)) -2.58±10.76	$-12.10{\pm}11.43$	-13.36±0.53	-20.24 ± 2.10	ns	ns	ns
Tissue structure model index	0.29±0.38	0.06±0.37	0.012±0.027	-0.227 ± 0.035	ns	ns	ns
Tissue fractal dimension	2.496±0.027	2.545±0.007	2.484±0.001	2.510±0.008	ns	*	ns

585 ¥ O=osmotic pre-treatment; M=TRS maturity class; significance of P-value: **, P $\leq 0.05\%$; *, P $\leq 0.1\%$, ns, not

586 significant

587

590	Table 2 . Mechanical and acoustic parameters (mean±standard error) of air-dried apple rings prepared from
591	apple fruit selected for X-CT and/or OCT analysis in relation to pre-drying treatment (noOSMO, no pre-
592	treatment; OSMO1; 1h osmodehydration; OSMO2, 3 h osmodehydration). Means in the same row
593	followed by different letters are statistically different (Tukey's test, $P \le 0.05\%$) (n=6).

	Means ±star	idard error					ANOVA [¥]			
	noOSMO		OSMO1		OSMO2		main effects		interaction	
	LeM	MoM	LeM	MoM	LeM	MoM	0	M	O×M	
Mechanical parameter	·s									
number of peaks	2.2 ± 0.6^{a}	3.2 ± 0.84^{a}	$4.0{\pm}1.1^{a}$	5.0±1.1 ^a	3.3±0.7 ^a	3.8±0.5 ^a	ns	ns	ns	
hardness (N)	5.98±0.55ª	6.83±0.61ª	6.65±0.65 ^a	7.13±0.37 ^a	7.41±0.33 ^a	6.82±0.54 ^a	ns	ns	ns	
fracturability (mm)	$0.95{\pm}0.06^{ab}$	1.06±0.07 ^a	0.59±0.06°	0.61 ± 0.07^{bc}	0.71 ± 0.11^{bc}	0.73±0.08 ^{abc}	***	ns	ns	
gradient_max (N/mm)	6.98±0.81b ^a	6.40 ± 0.76^{b}	11.49±1.06 ^{ab}	12.85±2.08ª	12.39±0.89 ^a	$10.92{\pm}1.41^{ab}$	***	ns	ns	
Area1 (N×mm)	2.68 ±0.19 ^{ab}	3.26±0.77 ^a	$1.25 \pm 0.35^{\text{b}}$	2.08±0.45 ^{ab}	1.68±0.39 ^{ab}	1.77±0.24 ^{ab}	**	ns	ns	
Total area (N×mm)	3.39±0.52ª	3.86±0.52ª	3.72±0.42 ^a	3.29±0.55ª	3.61±0.47 ^a	3.70±0.57 ^a	ns	ns	ns	
Travel1 (mm)	0.94±0.06ª	0.96±0.15 ^a	0.39±0.07 ^b	0.57±0.10 ^{ab}	0.49 ± 0.08^{b}	0.57±0.05 ^{ab}	***	ns	ns	
slope (N/mm)	5.29±0.63 ^b	6.30±0.38 ^b	11.37±1.34 ^a	11.38±1.77 ^a	10.76±0.58 ^a	9.35±0.65 ^{ab}	***	ns	ns	
E _{mod} max (MPa)	132.3±20.8 ^b	°125.5±12.2°	236.7±35.0 ^{ab}	^c 343.5±80.1 ^a	313.8±49.1 ^{ab}	222.6±28.4 ^{abc}	**	ns	ns	
$E_{\rm mod}$ slope (MPa)	101.6±19.7 ^b	124.7±8.5 ^{ab}	235.9±39.8ab	299.6±65.9ª	284.9±59.5ª	189.8±11.8 ^{ab}	**	ns	ns	
Acoustic parameters										
N_sounds	23.2±7.6 ^a	25.2±8.7 ^a	33.5±11.4 ^a	30.0±8.6 ^a	48.2±15.0 ^a	40.0±9.7 ^a	ns	ns	ns	
N_sounds>60dB	$3.5{\pm}1.8$ ^a	5.5±2.2 ^a	11.5±3.7 ^a	11.7±2.4 ^a	14.5±5.8 ^a	14.2±5.2 ^a	*	ns	ns	
$SPL_{av<60}$ (dB)	47.84±0.46 ^b	43.88±0.79°	48.07±0.88 ^{ab}	48.73±0.78ª	^b 50.92±0.47 ^a	49.81±0.72 ^{ab}	***	*	**	
$SPL_{av>60}(dB)$	81.82±3.09 ^a	78.19±1.57ª	^b 76.91±2.11 ^{ab}	79.03±2.41ª	69.00±1.51 ^b	73.78±1.20 ^{ab}	***	ns	ns	
avSPL (dB)	52.62±1.85 ^b	°49.51±2.85°	59.35±2.02 ^{ab}	63.59±3.59ª	56.17±0.92 ^{ab}	°59.54±0.68 ^{ab}	***	ns	ns	

599 List of Figures

- 600 Figure 1 Schematic diagram of a spectral-domain OCT system. The dashed boxes represent portable and
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- 617 symbols) and more (empty symbols) mature apples in the batch. Variables abbreviations: FI,
- fragmentation index; OSVR, tissue specific surface area.
- 619 Figure 8. Results of PCA: average PCs scores in function of pre-treatment and TRS maturity class (LeM,
- 620 less mature; MoM, more mature). Bars refer to standard error (n=2).

622 Figure 1

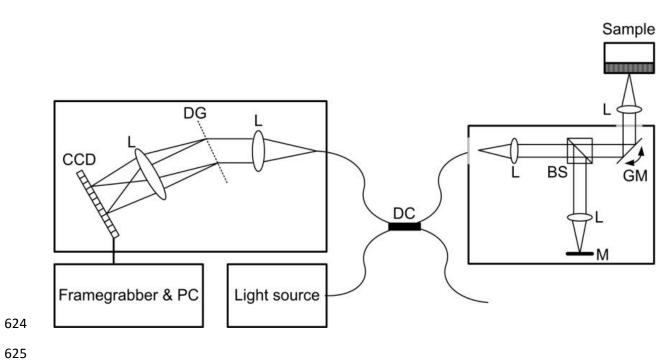
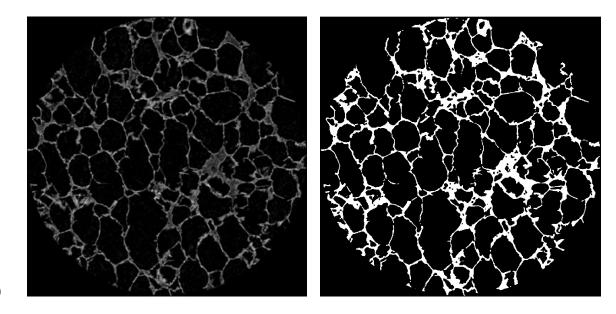
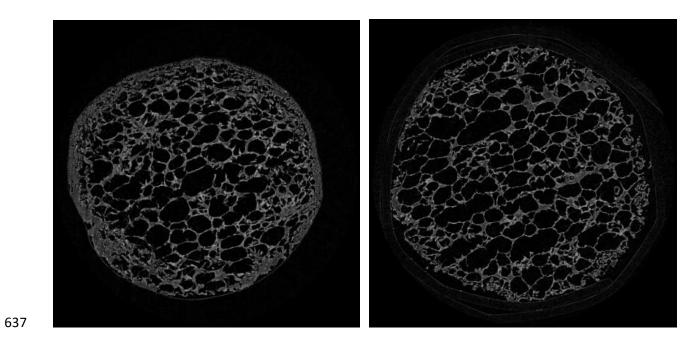


Figure 2

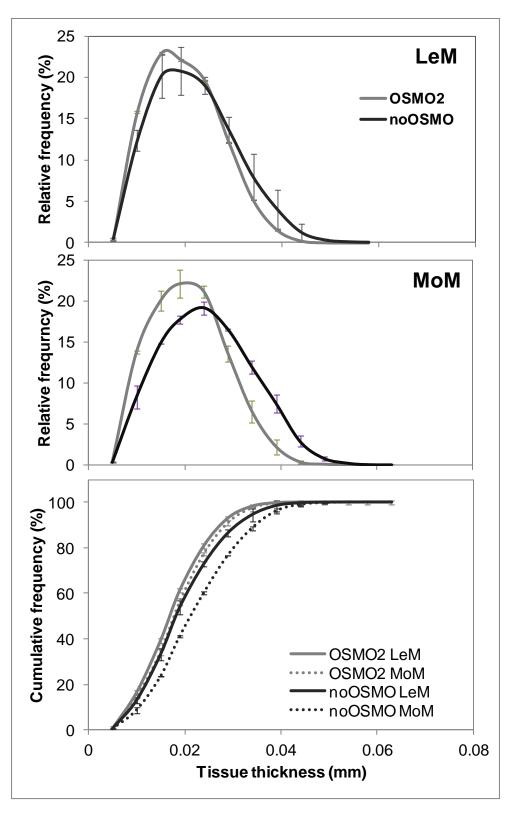


- Figure 3.

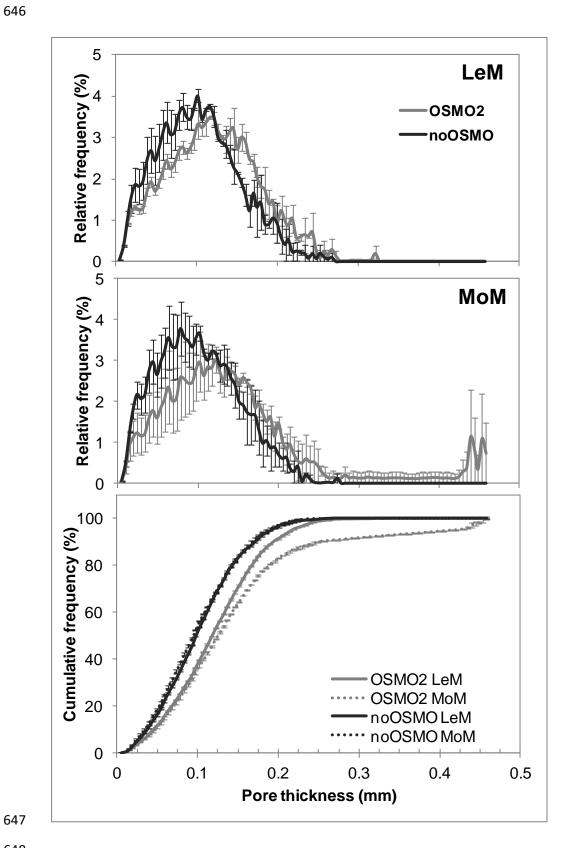




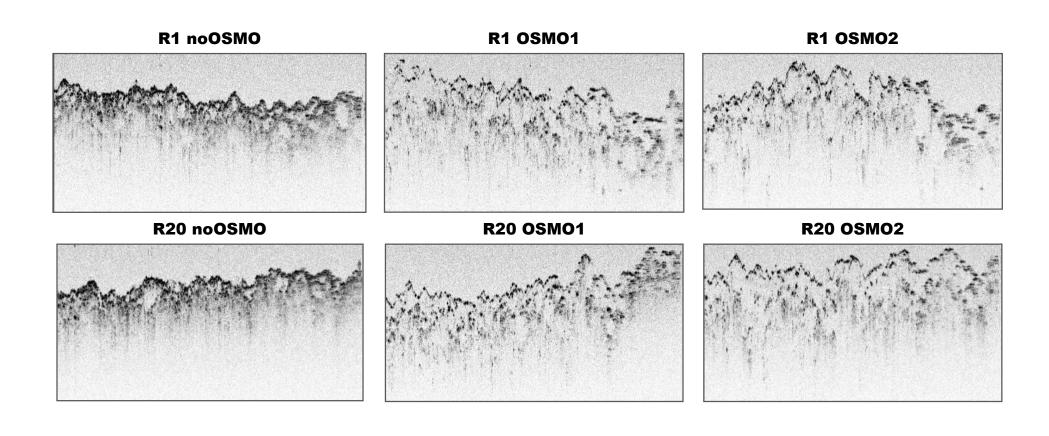
641 Figure 4



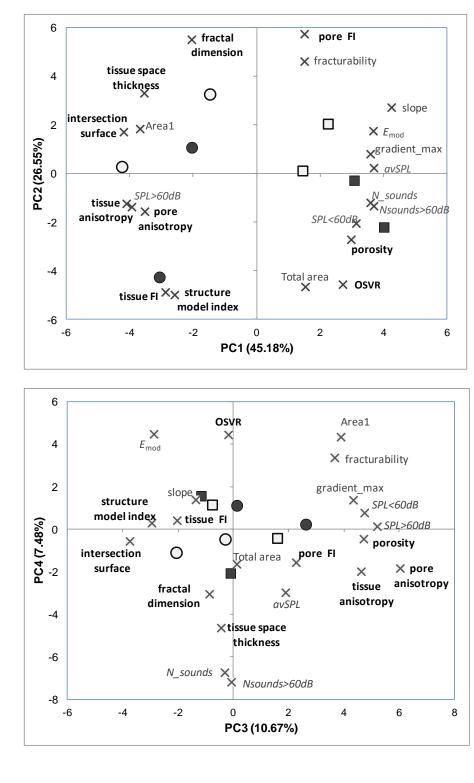








1 Figure 7





6 Figure 8



