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Evolution of physico-chemical, morphological and aromatic characteristics of Italian PDO dry-cured hams during processing

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Review

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3 1 **Evolution of physico-chemical, morphological and aromatic characteristics of Italian PDO**
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5 2 **dry-cured hams during processing**
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23 9 0250319182 Fax: +39 0250319190. *E-mail address:* gabriella.giovanelli@unimi.it
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29 11 **ABSTRACT**
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31 12 The aim of this work was to follow the evolution of physico-chemical (dry matter, NaCl
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33 13 concentration, pH, water activity), morphological (image analysis) and aromatic (e-nose)
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35 14 characteristics of the three main Italian PDOs during processing, from slaughtering to end of
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37 15 ripening. Main phenomena distinguishing the PDOs are NaCl concentration increase, which is
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39 16 higher in Toscano than in Parma and San Daniele hams, starting from the salting phase. Water
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41 17 activity values decrease during processing and the lowest values are detected in Toscano ham.
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43 18 Changes in morphological parameters (area, shape) and in color progressively occur during
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45 19 processing, and are more pronounced in T ham. A clear evolution of aroma of the three PDOs has
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47 20 been observed by e-nose and the complexity of the aromatic profile of the ripened hams is clearly
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49 21 highlighted.
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55 23 **Keywords:** dry-cured ham; PDO; ripening; physico-chemical parameters; morphological
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57 24 parameters; e-nose.
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1. Introduction

Dry-cured ham is a traditional and largely consumed product in Southern Europe and represents a major item of the meat industry in the Mediterranean area [1]. Italy is a primary dry-cured ham producer, with almost 50% of slaughtered pigs devoted to the production of Protected Designation of Origin (PDO) hams. Parma, San Daniele and Toscano are the three most important Italian PDOs, with over 9 million thighs processed for Parma ham, followed by San Daniele (over 2.5 millions) and Toscano (almost 300000) [2]. Protocols, specifications and control systems included in the PDOs ensure high quality standards, reproducible and typical characteristics, which are appreciated by local consumers and promote diffusion of these Italian food products in the world.

Phenomena that determine the transformation of pork meat into ham are mainly due to the absorption and diffusion of salt and the progressive dehydration of the meat. The ripening process, from salting to end-ripening, lasts at least 12 months (Parma and Toscano) and 13 months (San Daniele); in this period, modifications of physico-chemical characteristics such as NaCl concentration, pH, moisture content and water activity, together with biochemical reactions, mainly proteolysis and lipolysis, produce changes in color, taste, flavor and texture, which give the final products their typical characteristics [3, 4].

The specifications established by PDOs for Parma, San Daniele and Toscano hams define place of origin and processing, raw material and process characteristics, and some physico-chemical and sensory parameters of the final hams [5-7]. The three PDOs share a similar process technology, but differ in some aspects such as: a) the salting phase, which is longer (*i.e.* 3-4 weeks) for Toscano ham; in the case of Toscano, the addition of pepper, natural flavors and nitrates is also allowed; b) the pressing phase, which is only applied in the San Daniele process, and contributes to its typical shape; c) the seasoning phase, which is carried out under controlled temperature and relative humidity conditions, which are typical of the place of production.

Salting is one of the key processing steps of ham production for several reasons: NaCl has a bacteriostatic function and inhibits growth of pathogenic germs; it drives the dehydration process of

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3 51 the meat while reducing the water activity, thus preserving meat from degradation; it contributes to
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5 52 the overall sensory quality giving to ham its characteristic salty taste and acting as aroma enhancer
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7 53 [8]; it affects the rate and extension of enzymatic and chemical reactions such as proteolysis and
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10 54 lipolysis, which are in turn related to flavor formation and textural characteristics [9, 10]; it is
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12 55 involved in the typical dark red color formation [11]. According to PDO requirements, the final
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14 56 products are mainly distinguished by NaCl content, which must be comprised in specific ranges for
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16 57 the three PDOs (4.5-6.4% in Parma, 4.9%-6.9% in San Daniele, and maximum 8.3% in Toscano).
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18 58 The effect of different processing technologies on the physico-chemical and sensory properties of
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20 59 dry-cured hams has been investigated in several studies [12-18]. A number of studies have been
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22 60 carried out to investigate the development of volatile components, physico-chemical and/or sensory
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24 61 properties during ripening of Spanish, American and Italian dry-cured hams [19-24]. From
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26 62 literature, it is known that the volatile compounds of dry cured hams belong to 8 chemical families:
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28 63 aldehydes, alcohols, hydrocarbons, ketones, esters, sulphur compounds, carboxylic acids and
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30 64 terpenes [25, 26]. During processing, the aromatic profile evolution is due to biological and
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32 65 chemical changes. An intense proteolysis has been reported, especially during the initial seasoning
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34 66 period, whereas lipolysis of adipose tissue is mainly observed in the processing steps of salting and
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36 67 resting, when a substantial increase in free fatty acids occurs [27]. One of the most important
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38 68 reactions involved in the aroma development is the autoxidation of unsaturated fatty acids yielding
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40 69 to the formation of secondary products such as short-chain hydrocarbons, aldehydes, ketones, acids,
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42 70 alcohols and furans; moreover, the oxidative deamination-decarboxylation of amino acids via
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44 71 Strecker degradation involves the formation of aldehydes and ketones. Aldehydes may also result
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46 72 from the reaction between proteins and carbohydrates [25].
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48 73 Some studies evidenced that a longer maturation phase yields better aroma and taste properties, as
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50 74 well as better texture characteristics of dry-cured hams [21, 22, 28].
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52 75 Little information is available about the comparison of the Italian PDOs during processing, though
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54 76 these products are well identified and recognized by consumers. Therefore, the purpose of the
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3 77 present study was to monitor the evolution during ripening of the main physico-chemical (moisture,
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5 78 water activity, NaCl concentration, pH), morphological (image analysis) and aromatic (electronic
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7 79 nose) characteristics of Parma (P), San Daniele (SD) and Toscano (T) hams, considering the entire
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9 80 ham slices as well as the main muscular areas, corresponding to *Biceps femoris* and
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11 81 *Semimembranosus* muscles.
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16 83 **2. Materials and methods**

18 84 *2.1 Dry-cured hams*

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21 85 This study was carried out in the frame of a larger research program, concerning the
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23 86 characterization of dry cured hams belonging to the three main Italian PDOs (Parma, San Daniele
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25 87 and Toscano). In order to standardize the raw material (pig thighs) and eliminate sources of
26
27 88 variability other than the typical PDO processing conditions, all thighs were obtained from pigs
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29 89 belonging to Italian Landrace x Italian Large White cross genotype, reared in the same farm and fed
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31 90 with a standard cereal-soybean based meal. Pigs (at least 9 months age and 160 kg weight,
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33 91 according to PDOs requirements) were slaughtered in the same period, under similar and controlled
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35 92 conditions and all thighs were evaluated at the plant entrance for their compliance to the PDO rules
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37 93 for raw thigh acceptance (these rules are similar for all PDOs). Weight and circumference average
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39 94 values of the thighs after trimming were 13.0 ± 1.0 kg and 88.0 ± 3.0 cm, respectively. Length
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41 95 average value for P and T thighs was 48.9 ± 2.3 cm, whereas average length of SD thighs was 69.6
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43 96 ± 3.8 cm, due to the presence of the trotter. From slaughtering onward, processing of dry-cured
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45 97 hams was performed following the three PDO protocols.
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50 98 *2.2 Sampling procedure*

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53 99 For this study, 64 thighs, obtained as reported above, were processed and evaluated. Four thighs
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55 100 were sampled at t₀, and corresponded to the initial point for all PDOs (Table 1); the remaining 60
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57 101 thighs were processed according to the three PDO protocols (20 thighs for each PDO). At each
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59 102 sampling time from t₁ to t₅ (Table 1), four hams for each PDO were taken from the processing
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3 103 plant and used for analysis. The four hams for each phase and PDO were analyzed separately,
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5 104 therefore each result was obtained as the average value of the 4 replicates.
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8 105 To obtain the samples, hams were cut transversally from the thigh at about 8 cm from the femoral
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10 106 head. A slice about 5 cm thick was obtained from each thigh; slices were coded, vacuum packed,
11
12 107 frozen and stored at -18°C . Prior to analysis, samples were thawed for 24 hours at 4°C . The image
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14 108 was first acquired on the entire slice for morphological evaluation; the slice was then deboned, a
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16 109 first 3 mm slice was cut by a slicer and discarded. The image was acquired again for color
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18 110 evaluation, and then slices (5 or 10 mm thick) were cut and used for e-nose and analytical
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20 111 determinations. The e-nose evaluation was carried out on whole slices (comprising the
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22 112 subcutaneous fat); physico-chemical analyses were carried out on the whole defatted slice (lean
23
24 113 part) and on two specific regions, corresponding to *Biceps femoris* muscle (BF) and
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26 114 *Semimembranosus* muscle (SM) (Fig. 1). To obtain the lean part, the subcutaneous and
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28 115 intramuscular fat was manually removed from a 5 mm thick ham slice by a knife and the lean part
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30 116 was homogenized by Waring blender. To obtain BF and SM samples, the corresponding areas (Fig.
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32 117 1) were isolated from a 10 mm thick slice with a knife, and each portion was homogenized by
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34 118 Waring blender.
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42 120 2.3 Physico-chemical analyses

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44 121 Moisture content was determined by drying about 3 g of sample to constant weight, following
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46 122 AOAC procedure [29].
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49 123 Water activity was determined by a dew-point hygrometer (AquaLab, Decagon Devices Inc.,
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51 124 Pullman, WA, USA), calibrated with standard solutions ($a_w=0.984$ and $a_w=0.760$), at 25°C .
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54 125 pH was determined directly on the homogenized sample by a pH meter (PHM62, Radiometer,
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56 126 Copenhagen, Denmark), using an electrode for solid material.
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3 127 NaCl content was determined as chloride concentration by Volhard titration [30] . Samples were
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5 128 extracted as described by VESTERGAARD et al. [31] with minor modifications, as previously
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7 129 reported [32]. Results were expressed as NaCl g/100g.

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9 130 All determinations were carried out in triplicate.
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13 132 *2.4 Electronic nose analysis*

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16 133 Measurements were performed with Portable Electronic Nose (PEN2) from Win Muster Airsense
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18 134 (WMA) Analytics Inc. (Schwerin, Germany), as previously reported [32]. E-nose evaluation was
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20 135 carried out in duplicate on two slices for each ham, and the average of the sensor responses was
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22 136 used for subsequent statistical analysis.
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25 138 *2.5 Image analysis*

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29 139 Images were acquired using a digital color camera (Scion 1394 Fire wire Camera; Scion
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31 140 Corporation, USA), with maximum resolution (1600x1200 pixels) in jpeg format, operating as
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33 141 previously described [32].

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36 142 Morphological data were collected on the whole slice and on two specific regions, corresponding to
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38 143 BF and SM muscles (Fig. 1). Total area, lean area, subcutaneous fatty area and the ratio between
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40 144 length and width were measured on the whole slice. The area and the ratio length/width were also
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42 145 measured on BF and SM muscles.

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45 146 For color evaluation, a second image was taken from a freshly cut slice to get the values of Red
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47 147 (R), Green (G) and Blue (B) components of lean and subcutaneous fatty areas. Data were expressed
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49 148 as RGB Intensity-mean value (average of RGB values), corresponding to color intensity.

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51 149 Images were processed using Image-Pro Plus 6.2 (Media Cybernetics, Inc. Bethesda, MD, USA).
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54 151 *2.6 Statistical analysis*

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3 152 Physico-chemical and image analysis data were submitted to Generalized Linear Model (GLM)
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5 153 considering *Replicates* (hams), *PDOs* (Parma, San Daniele, Toscano), *Time* (t0-t5) and the
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7 154 interaction *PDO*Time* as factors and parameters as dependent variables. Replicates were
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9 155 considered as random factor in the model and nested within PDO. When a factor was found to be
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11 156 significant ($P<0.05$), t-tests were used as multiple comparison test (pdiff SAS LS-means option).
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13 157 The SAS/STAT statistical software package version 9.3 (SAS Institute Inc., Cary, USA) was used.
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15 158 Data obtained by e-nose were elaborated by Principal Component Analysis (PCA). The MINI TAB
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17 159 14, v.12.0 statistical software was used.
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23 161 **3. Results and discussion**

24 162 *3.1 Physico-chemical analyses*

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27 163 The evolution of physico-chemical characteristics, i.e. moisture content, NaCl content, water
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29 164 activity and pH during processing of the three dry-cured ham PDOs was evaluated by analyzing
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31 165 thighs immediately after slaughter and at five subsequent phases (Table 1). F-values with relevant
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33 166 significance for each physico-chemical parameter as obtained by two-way ANOVA are reported in
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35 167 Table 2. The factors *Time*, *PDO* and the interaction *Time x PDO* were significant for all parameters
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37 168 with the exception of pH, which varied only according to *Time*. The factor *Replicates* (hams) was
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39 169 always not significant. Mean values for each physico-chemical parameter evaluated on the whole
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41 170 slice by PDO and processing phase are shown in Table 3.
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45 171 Concerning NaCl concentration, the initial content in the fresh muscle (t0) is lower than the
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47 172 detection limit. NaCl content increases after the salting phase (t1) and continuously until the end of
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49 173 ripening in all PDOs. It is also evident that NaCl concentration is significantly higher in T than in
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51 174 both P and SD hams (which in turn are comparable), starting from t1 and all along the processing
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53 175 period. This is due to the fact that three subsequent salting steps are carried out during 25 days for T
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55 176 ham manufacture, whereas P and SD thighs are covered with salt in a two-step intervention and the
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57 177 salting phase is shorter (generally 21 and 14 days for Parma and San Daniele hams, respectively).
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3 178 | At the end of the process, T ham shows a NaCl concentration, which is almost double with respect
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5 179 | to the other PDOs, which show a similar salt content.

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7 180 | From Table 3 it can be observed that moisture content decreases during processing in all samples,
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9 181 | starting from 71.12 g/100g in the fresh thigh (t0) and reaching final values of about 54-55 g/100g
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11 182 | (t5). T hams show the fastest decrease in moisture content, and this can be due to the previously
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13 183 | mentioned differences in the salting phase; nevertheless, final moisture values are similar in the
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15 184 | three PDOs. Due to the simultaneous decrease in moisture content and increase in NaCl
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17 185 | concentration, water activity values decrease during the processing period, from the initial value of
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19 186 | 0.991 (t0) to final values ranging from 0.873 in T hams to 0.928 and 0.935 in SD and P hams,
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21 187 | respectively. Our data put in evidence that final aw values are mostly influenced by final NaCl
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23 188 | concentrations: all PDOs show similar final moisture content but have different NaCl
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25 189 | concentrations, in particular T hams show the highest NaCl content which corresponds to the lowest
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27 190 | water activity. pH values show minor changes during processing and no significant differences were
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29 191 | observed between the three PDOs. It is well known that salting is a key step in dry cured ham
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31 192 | processing, contributing to microbial stability and to the sensory characteristics of the final product.
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33 193 | The lowering of water activity produced by the simultaneous increase in NaCl and decrease in
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35 194 | water concentrations assures bacteriostatic conditions and prevents spoilage of the meat. From the
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37 195 | sensory point of view, NaCl contributes to the salty taste of hams and plays a major role in the
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39 196 | textural properties of the end products. It is known that main changes in the textural properties of
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41 197 | ripening hams are due to water loss, which causes hardening of the product, and proteolysis, which
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43 198 | in turn determines softening of the product [17, 19, 33]. Both dehydration and proteolysis are
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45 199 | affected by the rate of diffusion and final NaCl concentration in the hams. Various studies
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47 200 | demonstrated that hams with high NaCl content are characterized by harder texture and lower
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49 201 | moisture content [19, 33]. This is mainly due to the inhibition of proteolytic enzymes caused by
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51 202 | water loss and lowering of a_w values, which are directly related to the intensity of salting). The
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53 203 | effects of NaCl concentration on the quality characteristics have been especially studied in Iberian
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3 204 hams [34], which have NaCl content (8% to 15% on dry weight of the lean part) similar to T hams.
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5 205 Various studies have demonstrated that higher NaCl concentrations produce dry cured hams with
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7 206 higher hardness [9, 19, 34], whereas insufficient NaCl concentration yields to excessive softness
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10 207 and pastiness and to taste defects such as bitterness and metallic flavor, which are ascribed to
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12 208 extended proteolysis [15, 35].

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14 209 The evolution of physico-chemical parameters in BF and SM muscular areas is shown in Fig. 2,
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16 210 together with the values referring to the whole slice.

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18 211 Graphs in Fig. 2a allow the comparison of NaCl diffusion in the different muscular areas of the
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20 212 thigh in the three PDO hams. In all cases, SM muscle, which is not protected by the skin and the fat
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22 213 and is directly exposed to the salting mixture, shows the deepest increase in NaCl concentration,
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24 214 which reaches maximum values at t2 (resting) in P (3 g/100g) and SD (2.3 g/100g) and at t1
25
26 215 (salting) in T (3.9 g/100g). The subsequent decrease in salt concentration in SM muscle is due to its
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28 216 diffusion into the inner parts of the thighs, as evidenced by NaCl evolution in BF muscle. In this
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30 217 area, NaCl increase is slower and almost linear up to t4 (pre-seasoning) in all PDOs. At this time,
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32 218 the two muscular areas considered in the study show similar NaCl concentrations, which are
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34 219 representative of the NaCl content of the whole slice. Further increase in salt content in all areas up
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36 220 to the end of ripening is due to water loss. Comparison between NaCl profiles in P and SD hams
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38 221 shows that, despite similar salting procedures and final NaCl concentrations, salt diffusion in SD
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40 222 thighs is more homogeneous during the early processing phases: at t2, NaCl concentration gradient
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42 223 between BF and SM is higher in P than in SD thighs, also considering that t2 is 60 days for P and
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44 224 only 40 days for SD. This can be ascribed to the pressing operation (typical for SD hams) and to
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46 225 manual and mechanical handling of the thighs during the salting phase. T hams show NaCl profiles
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48 226 in the two muscular areas which are more similar to those observed in P hams, with higher salt
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50 227 concentrations due to the three-step salting procedure and the longer salting time. RUIZ-RAMIREZ
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52 228 et al. [9, 17] studied the relationship between water content, NaCl content and textural parameters in
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54 229 dry-cured hams as well as in SM and BF muscles, finding positive correlation between the amount
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3 230 of added NaCl and final hardness and negative correlations between NaCl content and springiness
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5 231 and cohesiveness. They also observed different proteolysis indexes between SM and BF muscles,
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7 232 due to differences in NaCl absorption. Our data give additional information about the kinetics of
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9 233 NaCl absorption and water loss in the two main muscular areas of dry-cured PDO Italian hams:
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11 234 these phenomena are related to the typical sensory characteristics of end products, which have been
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13 235 specifically studied, as reported in a previous paper [32]. Concerning the textural characteristics,
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15 236 the sensory evaluation put in evidence that T ham was perceived as more fibrous, drier and harder
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17 237 than P and SD hams. Equally, T ham was characterized by the highest and lowest values of salty
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19 238 and sweet taste, respectively. The sensory evaluation also demonstrated that P and SD hams were
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21 239 similar for most of the attributes except for saltiness, sweetness and dryness, P ham being perceived
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23 240 as significantly sweeter and less salty and dry than SD ham [32].

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25 241 Moisture content evolution is shown in Fig. 2b. For SM and BF areas, data were collected starting
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27 242 from t1, but this doesn't preclude the analysis of water loss rates during processing. It can be
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29 243 observed that moisture content in SM and BF are different since the salting phase (t1) up to the end
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31 244 of ripening (t5), in all PDOs. The direct exposition to NaCl produces a fast dehydration of SM
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33 245 muscular area and water loss is continuous up to t4, when moisture values close to the final ones are
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35 246 reached. The final moisture content in SM is similar in all PDOs, ranging from 52.4 to 52.9 g/100g.
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37 247 On the other side, BF region is much more hydrated than SM region; in this area water loss occurs
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39 248 progressively in the course of processing and final values are much higher, ranging from 57.9 g/100
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41 249 g in P hams to 62.5 g/100g in T hams. Values of water activity referring to SM, BF and the whole
42
43 250 slice are shown in Fig. 2c. For this parameter too, differences can be observed between SM and BF
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45 251 regions; these differences are progressively reduced and final aw values can be considered in
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47 252 equilibrium in the whole ham. The evolution of aw values seems to be mostly related to the
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49 253 evolution in NaCl concentration: lower aw gradients between SM and BF are observed in SD hams,
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51 254 corresponding to lower NaCl gradients, and final aw values in T hams are lower than in SD and P
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255 hams because final NaCl concentration in T hams is higher, whereas moisture content are similar in
256 all the PDOs.

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258 3.2 Image analysis

259 The evolution of morphological parameters of the three PDOs was evaluated by image analysis on
260 samples collected immediately after slaughter and during processing. F-values with relevant
261 significance for each morphological parameter as obtained by GLM are reported in Table 4. The
262 factor *Time* was significant ($P < 0.0001$) for all parameters, whereas *PDO* significantly influenced
263 the total area and the ratio Length/Width. The interaction *Time x PDO* was significant ($P < 0.001$)
264 only for the ratio Length/Width, indicating that the evolution over time of this parameter differed
265 according to PDO. Mean values for each morphological parameter evaluated on the whole slice by
266 PDO and processing phase are shown in Table 5. Concerning the evolution of the total area, a
267 decrease during processing can be observed in all PDOs, starting from about 750 cm² in the fresh
268 thigh (t0) and reaching final values (t5) of about 460, 485 and 395 cm² for P, SD and T ham,
269 respectively. A similar trend can be observed considering the evolution of the lean area; starting
270 from the initial value of about 510 cm², a fast decrease is evident after salting (t1) and resting (t2) in
271 all PDOs, then a further decrease can be observed during seasoning (t4 and t5) for T ham in
272 particular, which reaches a final lean area of about 260 cm². In all PDOs, fatty area reduction is due
273 to trimming (t1), which endows the ham with its typical shape by removing part of the fat and the
274 skin. Considering the evolution of the ratio between length (major axis) and width (minor axis) of
275 the slices, a significant and progressive increase can be observed for the three PDOs during
276 processing, reaching the maximum value at the end of ripening. In particular, the highest
277 length/width ratio reached by T ham (2.71) may depend on the fact that high NaCl concentration
278 has an inhibitory effect on proteolytic activity and favors aggregation of myofibrillar proteins [15,
279 35]; for SD ham, the high ratio (2.36) can also be related to the pressing phase typical of this PDO.
280 From the comparison of the three PDOs, it is evident that at the end of the ripening (t5), T ham is

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3 281 characterized by the lowest total and lean area and the highest length/width ratio, while the
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5 282 morphological characteristics of P and SD hams are more similar.

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7 283 Fig. 3 shows the morphological data referred to BF and SM muscles. Graphs in Fig. 3a allow the
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9 284 comparison of the area evolution of the two muscles in the three PDOs. For each PDO the two
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11 285 muscular areas follow a similar trend during processing, showing a fast decrease during salting (t1)
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13 286 and resting (t2). At the end of ripening (t5), percentage area reduction is similar for the two
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15 287 muscular areas, and ranges from about 50% for T ham to about 40% and 37% for SD and P ham,
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17 288 respectively.

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20 289 Graphs in Fig. 3b show the length/width evolution of SM and BF during processing. Considering
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22 290 the three PDOs a similar trend can be observed for the two muscles; in particular, the fast increase
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24 291 of length/width observed after salting (t1) can be related to the trimming process and to salt
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26 292 diffusion with consequent muscle dehydration. A further increase of length/width ratio in BF, up to
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28 293 the end of ripening, is probably due to the slow increase of NaCl concentration in this muscle, with
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30 294 consequent dehydration.

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33 295 Color data collected during processing are shown in Fig 4. Considering the lean area (Fig. 4a), a
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35 296 progressive and similar decrease of RGB Intensity-mean can be observed for the three PDOs up to
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37 297 the drying phase (t3), then a further decrease can be evidenced during seasoning (t4 and t5), for T
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39 298 ham in particular. At the end of ripening, T ham is characterized by a lower RGB Intensity-mean
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41 299 value, which implies a darker color of this PDO compared to P and SD hams. This result is in
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43 300 accordance with those of the sensory study conducted on the three PDOs and reported in a previous
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45 301 paper [32]. Literature data report that color changes occurring during processing are related to the
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47 302 increased concentration of the pigments due to dehydration and muscle shrinkage; in addition, color
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49 303 intensification is due to the gradual transformation of muscle myoglobin throughout the ripening
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51 304 period, resulting in darker myoglobin derivatives [36, 37].

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54 305 Fig. 4b reports the RGB intensity-mean of the subcutaneous fatty area during processing. A similar
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56 306 trend can be observed for the three PDOs, showing a progressive decrease, in particular for T ham.
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3 307 This evolution corresponds to a progressive variation of the color of subcutaneous fat that becomes
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5 308 yellowish at the end of ripening, as shown by the sensory evaluation [32].
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9 310 *3.3 Electronic nose analysis*

11 311 To evaluate the aromatic profile evolution of P, SD and T hams, e-nose data collected during
12 312 processing, were elaborated by PCA. Fig. 5 shows the score plot (a) and loading plot (b) in the area
13 313 defined by the first two Principal Components (PC1 and PC2, 84.7% explained variance). The score
14 314 plot (Fig. 5a) shows the ability of e-nose to follow the evolution of the aromatic profile of the three
15 315 PDOs; samples are distributed on PC1 from left to right according to the processing phases and
16 316 three clusters can be identified. The fresh thigh (t0) and the samples collected after salting (t1) and
17 317 resting (t2) are located in the negative part of PC1 and are characterized by a similar aromatic
18 318 profile; considering this cluster, T samples (t1 and t2) are the only ones partially discriminated,
19 319 showing a more rapid evolution of volatile compounds in the early phases of ripening as evidenced
20 320 by PUGLIESE et al. [2]. From loading plot (Fig. 5b), it can be noticed that the samples belonging
21 321 to the first cluster are mainly characterized by WC sensors (W1C, W3C, W5C), sensitive to
22 322 aliphatic, aromatic and slightly polar compounds. The second cluster, located in the upper right
23 323 quadrant of the score plot (Fig. 5a), is composed by samples collected after drying (t3) and pre-
24 324 seasoning (t4); the aromatic profile of the three PDOs in these two phases is similar and probably it
25 325 is mainly related to aldehydes, produced up to 6 months of ripening, and esters, formed during
26 326 seasoning [2, 24]. Considering the loading plot (Fig. 5b), the volatile compounds of the second
27 327 cluster are perceived by W1S sensor, characterized by a broad range sensitivity, and by W2W
28 328 sensor, sensitive to sulfur-organic compounds. The third cluster, located in the lower left quadrant
29 329 of the score plot (Fig. 5a), is composed by samples collected at the end of ripening (t5) and
30 330 characterized by a typical aroma that cannot be ascribed to few compounds, but depends on a large
31 331 number of volatiles present in proper amount and proportion. Considering the three PDOs, it can be
32 332 noticed that SD is discriminated by P and T hams, which are closely located and characterized by a

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3 333 similar aromatic profile.- The sensory evaluation carried out on the same end-products in a previous
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5 334 work [32] did not evidence significant differences in odor and flavor descriptors, whilst the three
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7 335 fully ripened PDO hams could be discriminated by e-nose evaluation; in particular, the PCA
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9 336 elaboration of e-nose data of the three products clearly separated SD from T and P hams [32].

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11 337 Literature data report that alcohols are the most abundant volatiles of SD ham, representing about
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13 338 40% of the total volatile fraction; their percentage is significantly higher than in other Italian and
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15 339 European hams and their presence is probably due to the high degree of lipid oxidation [38].
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17 340 Aldehydes are the most representative volatile compounds in P and T hams [2, 23, 24] and their
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19 341 presence is probably related to proteolysis and amino acids degradation [26]. Esters are present to a
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21 342 much higher extent in P and T ham compared to SD [38]. Sulphur compounds have been detected
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23 343 among P ham volatiles [24], while a large amount of organic acids, arising from lipid oxidation
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25 344 and from the hydrolysis of triglycerides, has been identified in T ham similarly to Iberian dry-cured
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27 345 ham [2]. The complexity of the aromatic profile of the ripened hams is clearly highlighted by the
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29 346 electronic nose since the majority of the sensors (W1S, W2S, W3S, W5S, W1W) characterize
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31 347 the three DPOs final products (Fig. 5b).
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38 349 *3.4 Relationship between physico-chemical, morphological and aromatic data*

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40 350 In order to obtain a more exhaustive characterization of the three PDOs, physico-chemical,
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42 351 morphological and aromatic data collected during ripening were jointly elaborated by PCA.
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44 352 The score and loading plots in the plane defined by PC1 and PC2 (75.5% explained variance) are
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46 353 shown in Fig. 6. Considering the score plot (Fig. 6a), a similar evolution on PC1 and PC2 can be
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48 354 observed for the three DPOs during processing. Moving on PC1 from right to left, the fresh thigh
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50 355 (t0) and the samples collected after salting (t1) and resting (t2) are discriminated by samples
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52 356 gathered after drying (t3), during seasoning (t4) and at the end of ripening (t5). Comparing the three
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54 357 PDOs, it can be observed that T ham shows a more rapid evolution during the early phases of
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56 358 production (t1 and t2), while P and SD hams are characterized by a more similar trend. At the end
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3 359 of ripening (t5), the three PDOs are discriminated on the negative part of PC2 and appear scattered
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5 360 in the third quadrant. From the loading plot (Fig. 6b) showing the relationship between variables, it
6
7 361 can be observed that in the positive part of PC1, the morphological parameters (total, lean and fatty
8
9 362 area) are correlated to moisture content and a_w and, together with the WC sensors, characterize
10
11 363 samples in the early ripening phases. On the opposite side of PC1, NaCl is correlated to
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13 364 length/width ratio and inversely related to moisture, a_w and to the morphological (total, lean and
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15 365 fatty area) and color parameters. These variables, together with the WW and WS sensors
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17 366 characterize samples after drying (t3), during seasoning (t4) and at the end of ripening (t5). The
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19 367 final products are well discriminated from the unripened products and are well separated one from
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21 368 another. All final products are characterized by WS and WIW e-nose sensors. The ripened T ham,
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23 369 located at the very left of the plot, is typified by the highest NaCl content and lowest a_w , total and
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25 370 lean area and RGB intensity mean.

30 371 4. Conclusions

32 372 Characterization of Italian dry-cured hams belonging to the three main PDOs during processing
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34 373 indicates that chemical, morphological and aromatic parameters show a similar evolution. T ham is
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36 374 distinguished by higher NaCl concentration, starting from the salting phase and all along the
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38 375 processing period. Consequently, a_w values are the lowest in this PDO. Changes in morphological
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40 376 parameters (area, shape) and color progressively occur during processing and are more pronounced
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42 377 in Toscano ham. The two main muscular areas (SM and BF) ~~show~~ are differently affected by NaCl
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44 378 diffusion and moisture loss and these differences are progressively reduced during ripening. A
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46 379 clear evolution of aroma of the three PDOs has been observed by e-nose and the complexity of the
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48 380 aromatic profile of the ripened hams is clearly highlighted. ~~Considering~~ Taking into account all the
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50 381 evaluated parameters, Toscano ham is more discriminated from Parma and San Daniele hams;
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52 382 significant differences are evidenced in the early processing stages and in the final product, due to
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54 383 the specific manufacturing process which implies a longer salting phase.

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3 384 Considering that the sensory properties of PDO hams play a pivotal role in consumers' preference
4 and choice, the availability of ready-to-use analytical methods for the characterization of sensory
5 385 profiles is a growing need. Sensory evaluation and physico-chemical analysis provide useful
6 information but are labour- and time-requiring; the use of artificial senses, such as electronic nose
7 386 and image analysis, allows a rapid assessment of aromatic and visual characteristics of hams during
8 processing. In particular, due to its non-destructive nature, electronic nose could be applied for the
9 387 on-line monitoring and control of ham ripening.
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20
21 392 **Acknowledgments:** This study was conducted within the research project “Study of sensory and
22 393 instrumental parameters during and after ripening of different PDO dry-cured hams and their
23 394 influence on consumers liking”, funded by the Italian Ministero dell'Istruzione, dell'Università e
24 395 della Ricerca (MIUR), protocol n° 2007HCW9HN.
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31 397 **Conflict of interest:** None.

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33 398 **Compliance with Ethics requirements:** This article does not contain any studies with human or
34 399 living animal subjects.
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3 499 **Legends to figures**

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6 501 **Figure 1**

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8 502 Muscular areas: 1, *M. Semimebranosus*; 2, *M. Semitendinosus*; 3, *M. Biceps femoris*; 4, *M. Rectus*
9 *femoris* and *M. Vastus medialis* (*M. Quadriceps femoris*). A, bone area; B, internal fatty area; C,
10 503 subcutaneous fatty area.
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14 506 **Figure 2**

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16 507 Evolution of moisture content (a), NaCl concentration (b), and aw (c) in *Biceps femoris* (----) and
17 508 *Semimembranosus* (·····) muscles and in the whole slice (—) during processing of Parma (◆),
18 509 San Daniele (■) and Toscano (▲) hams. Error bars represent std. error.
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22 511 **Figure 3**

23 512 Area (a) and length/width (b) evolution of *Biceps femoris* (----) and *Semimembranosus* (·····)
24 513 muscles during processing of Parma (◆), San Daniele (■) and Toscano (▲) hams. Error bars
25 514 represent std. error.
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29 516 **Figure 4**

30 517 RGB-Intensity mean of lean area (a) and fatty area (b) during processing of Parma (◆), San Daniele
31 518 (■) and Toscano (▲) hams.
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35 520 **Figure 5**

36 521 PCA of electronic nose data: score plot (a) and loading plot (b) of Parma (◆), San Daniele (■) and
37 522 Toscano (▲) hams during processing.
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41 524 **Figure 6**

42 525 PCA-Score plot (a) and loading plot (b) of physico-chemical morphological and aromatic data
43 526 collected during processing of Parma (◆), San Daniele (■) and Toscano (▲) hams.
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Table 1. Sample codification, processing phases and time from slaughtering of ham samples.

Sample code	Processing phase	Time from slaughtering (days)		
		Parma (P)	San Daniele (SD)	Toscana (T)
t0	Slaughter	0	0	0
t1	Trimming and salting	21	14	25
t2	Resting	60	40	55
t3	Drying	118	131	122
t4	Pre-seasoning	237	240	237
t5	Seasoning	393	391	384

Table 2. F and p values for each physico-chemical parameter as obtained by two-way ANOVA.

Source of variation	NaCl		Moisture		a_w		pH	
	F value	p value	F value	p value	F value	p value	F value	p value
<i>Time</i>	113.13	<0.0001	7.83	<0.001	101.03	<0.0001	2.49	n.s.
<i>PDO</i>	164.68	<0.0001	158.33	<0.0001	169.44	<0.0001	5.04	<0.001
<i>Time*PDO</i>	8.66	<0.0001	2.76	<0.01	9.15	<0.0001	1.40	n.s.

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Table 3. Mean values for each physico-chemical parameter by PDO (P=Parma, SD=San Daniele, T=Toscano) and processing phase (t0-t5). Values are referred to the whole defatted slice.

Phase	NaCl (g/100g)			Moisture (g/100g)			a_w			pH		
	P	SD	T	P	SD	T	P	SD	T	P	SD	T
t0	n.d.	n.d.	n.d.	71.12 ^a	71.12 ^a	71.12 ^a	0.991 ^a	0.991 ^a	0.991 ^a	5.64	5.64	5.64
t1	1.23 ^a	1.06 ^a	2.24 ^b	71.65 ^b	68.76 ^a	67.15 ^a	0.984 ^b	0.982 ^{ab}	0.963 ^b	5.56	5.57	5.53
t2	1.78 ^a	1.45 ^a	2.55 ^{ab}	65.65 ^a	68.65 ^b	64.76 ^{bc}	0.967 ^b	0.974 ^b	0.948 ^c	5.65	5.65	5.70
t3	1.66 ^{ab}	2.16 ^b	2.87 ^c	66.17 ^b	64.37 ^{ab}	62.93 ^c	0.964 ^b	0.955 ^c	0.936 ^d	5.79	5.68	5.69
t4	2.03 ^{bc}	2.35 ^{bc}	4.12 ^c	60.92 ^b	59.83 ^{ab}	57.34 ^d	0.943 ^c	0.941 ^d	0.899 ^e	5.70	5.71	5.61
t5	2.44 ^c	2.63 ^c	4.48 ^c	54.11 ^d	54.68 ^e	55.29 ^d	0.935 ^c	0.928 ^e	0.873 ^f	5.66	5.70	5.51

n.d., not detectable

For each parameter, subscript letters indicate significant differences at each phase (comparison by column); superscript letters indicate significant differences by PDO (comparison by row).

Table 4 F-values and p-values for each morphological parameter as obtained by two-way

ANOVA.

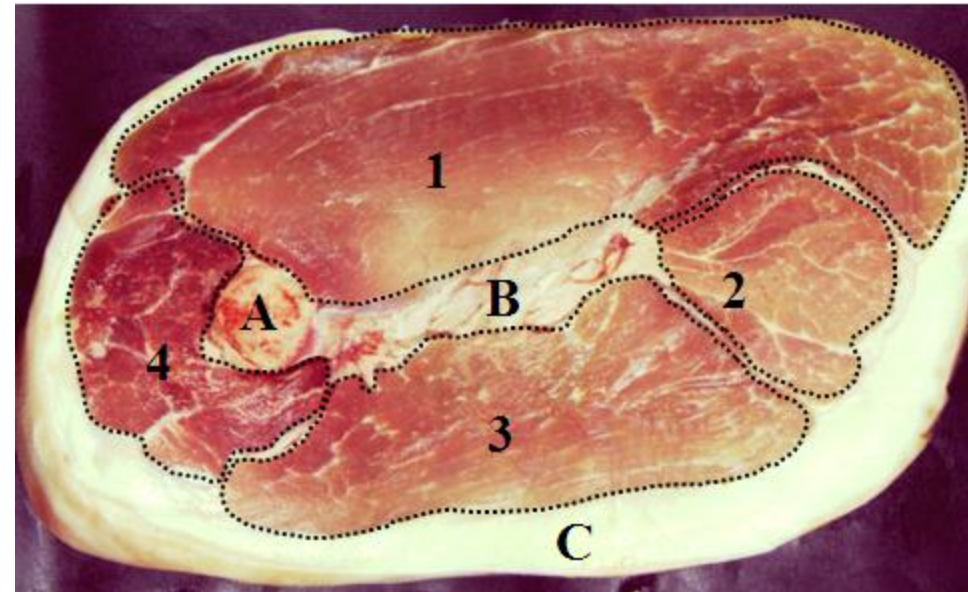
Source of variation	Total area		Lean area		Fatty area		Lenght/Width	
	F value	<i>p</i> value	F value	<i>p</i> value	F value	<i>p</i> value	F value	<i>p</i> value
<i>Time</i>	6.85	<0.01	2.85	n.s.	3.04	n.s.	45.75	<0.001
<i>PDO</i>	78.77	<0.0001	58.07	<0.0001	17.13	<0.0001	42.75	<0.001
<i>Time*PDO</i>	1.32	n.s.	1.36	n.s.	0.80	n.s.	4.71	<0.001

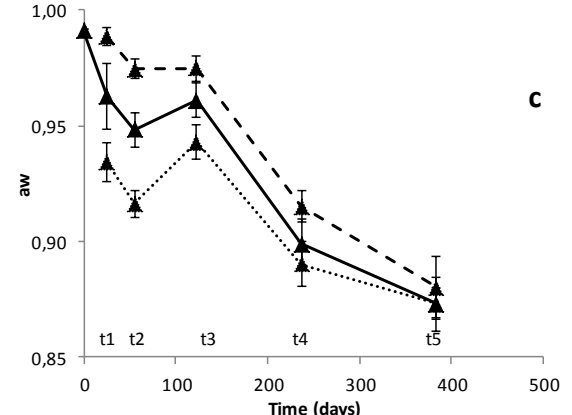
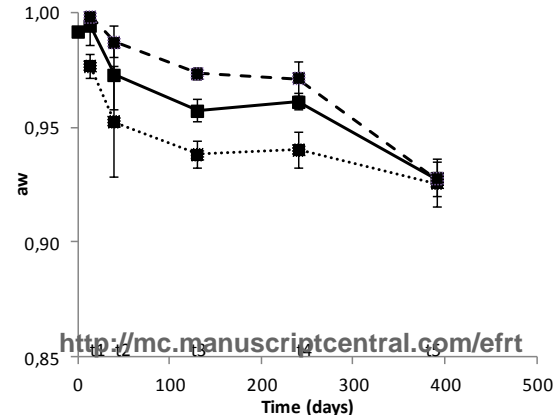
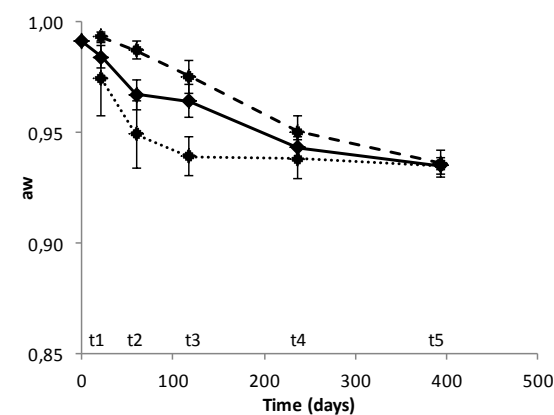
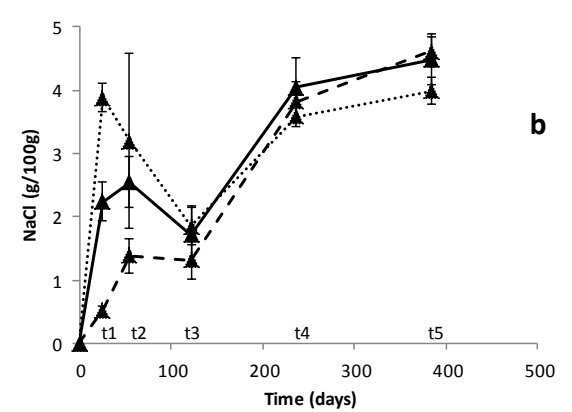
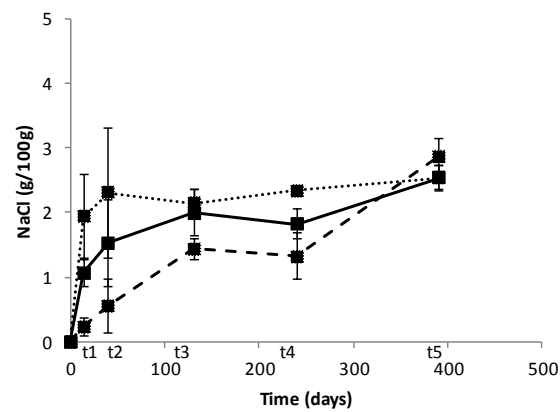
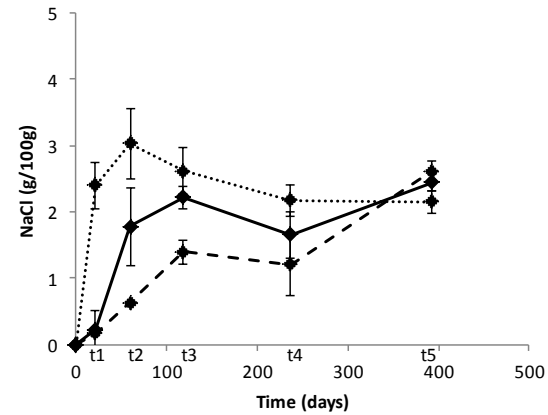
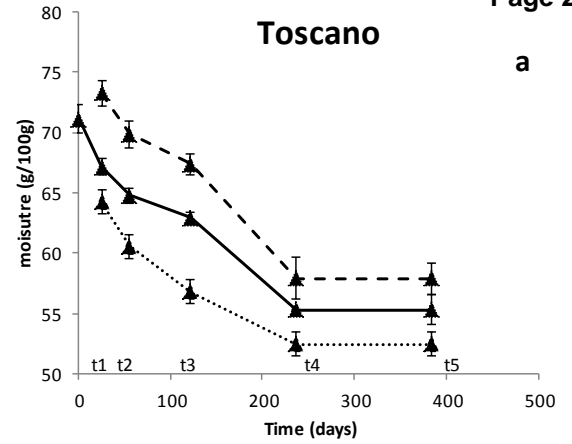
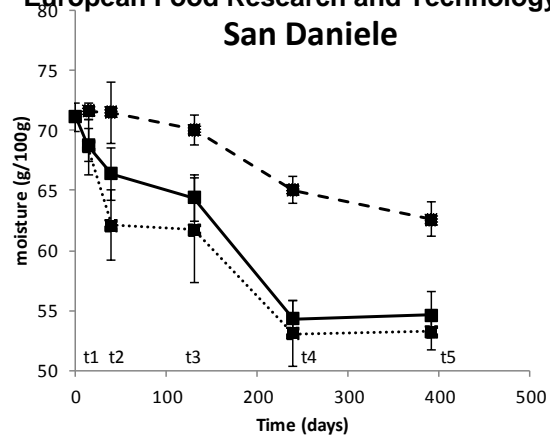
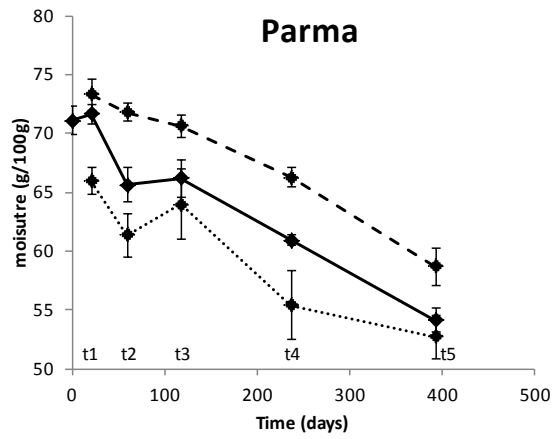
Table 5. Mean values for each morphological parameter by PDO (P=Parma, SD=San Daniele, T=Toscano) and ripening phase (t0-t5). Values are referred to the whole slice.

Phase	Total area (mm ²)			Lean area (mm ²)			Fatty area (mm ²)			Lenght/Width		
	P	SD	T	P	SD	T	P	SD	T	P	SD	T
t0	75272 ^a	75272 ^a	75272 ^a	51054 ^a	51054 ^a	51054 ^a	23303 ^a	23303 ^a	23303 ^a	1.61 ^a	1.61 ^a	1.61 ^a
t1	61687 ^{ab}	65750 ^b	56583 ^a	45190 ^a	46417 ^a	42883 ^b	15529 ^{ab}	18315 ^b	12750 ^a	1.97 ^b	1.74 ^a	2.09 ^b
t2	52694 ^b	48427 ^{ab}	42760 ^{cd}	37797 ^b	36217 ^{ab}	31434 ^{cd}	14084 ^a	11382 ^a	10576 ^a	1.90 ^a	2.03 ^a	2.41 ^b
t3	53970 ^a	50531 ^a	48735 ^a	38276 ^a	35587 ^a	35583 ^a	14881 ^a	14059 ^{bcd}	12405 ^a	1.83 ^a	2.05 ^b	2.29 ^c
t4	41983 ^d	45001 ^c	43190 ^{cd}	27222 ^d	30840 ^a	31358 ^{cd}	14025 ^a	13235 ^{cd}	11038 ^a	1.92 ^a	2.30 ^b	2.46 ^b
t5	45927 ^d	48541 ^b	39478 ^d	33368 ^b	31198 ^{ab}	26142 ^d	11722 ^a	16359 ^{bc}	12421 ^a	2.06 ^a	2.36 ^b	2.71 ^d

For each parameter, subscript letters indicate significant differences at each phase (comparison by column); superscript letters indicate significant differences by PDO (comparison by row).

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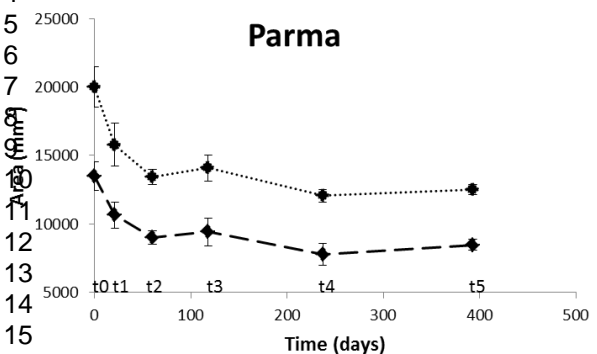
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c

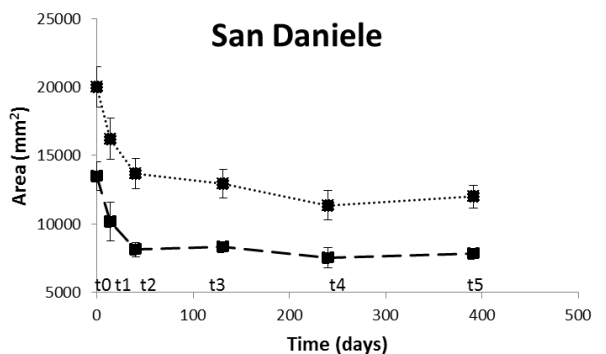
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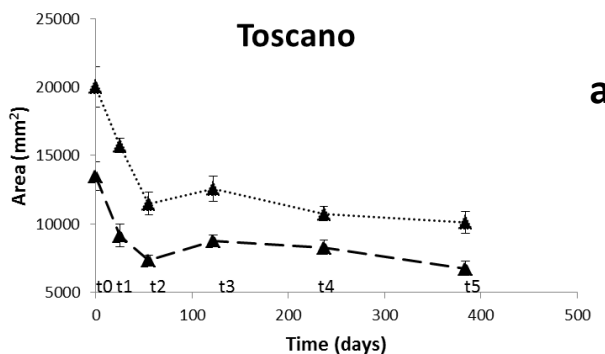
Parma



San Daniele

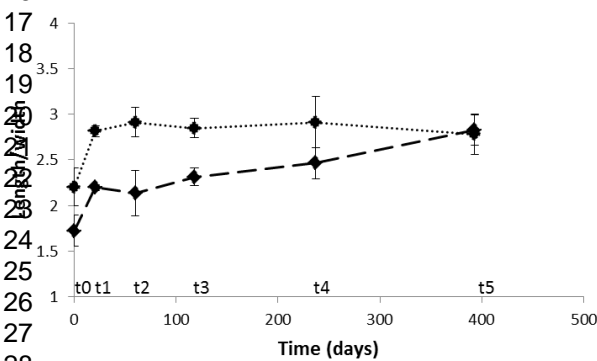


Toscana

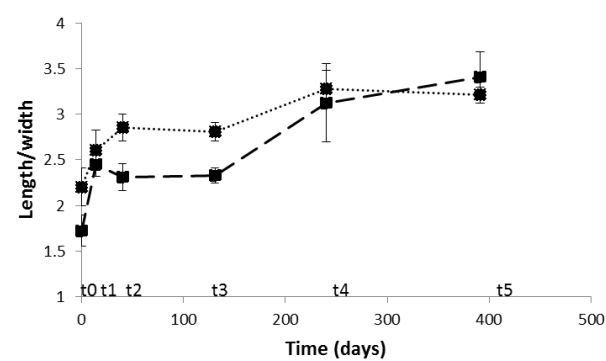


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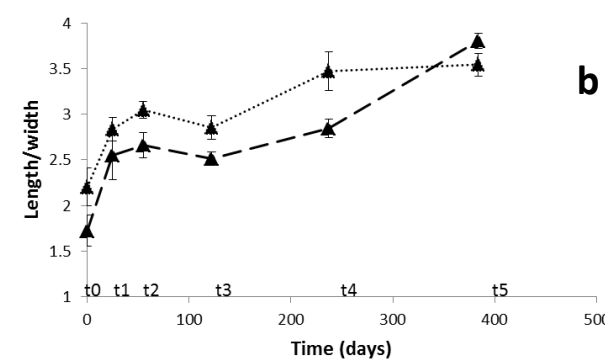
Parma



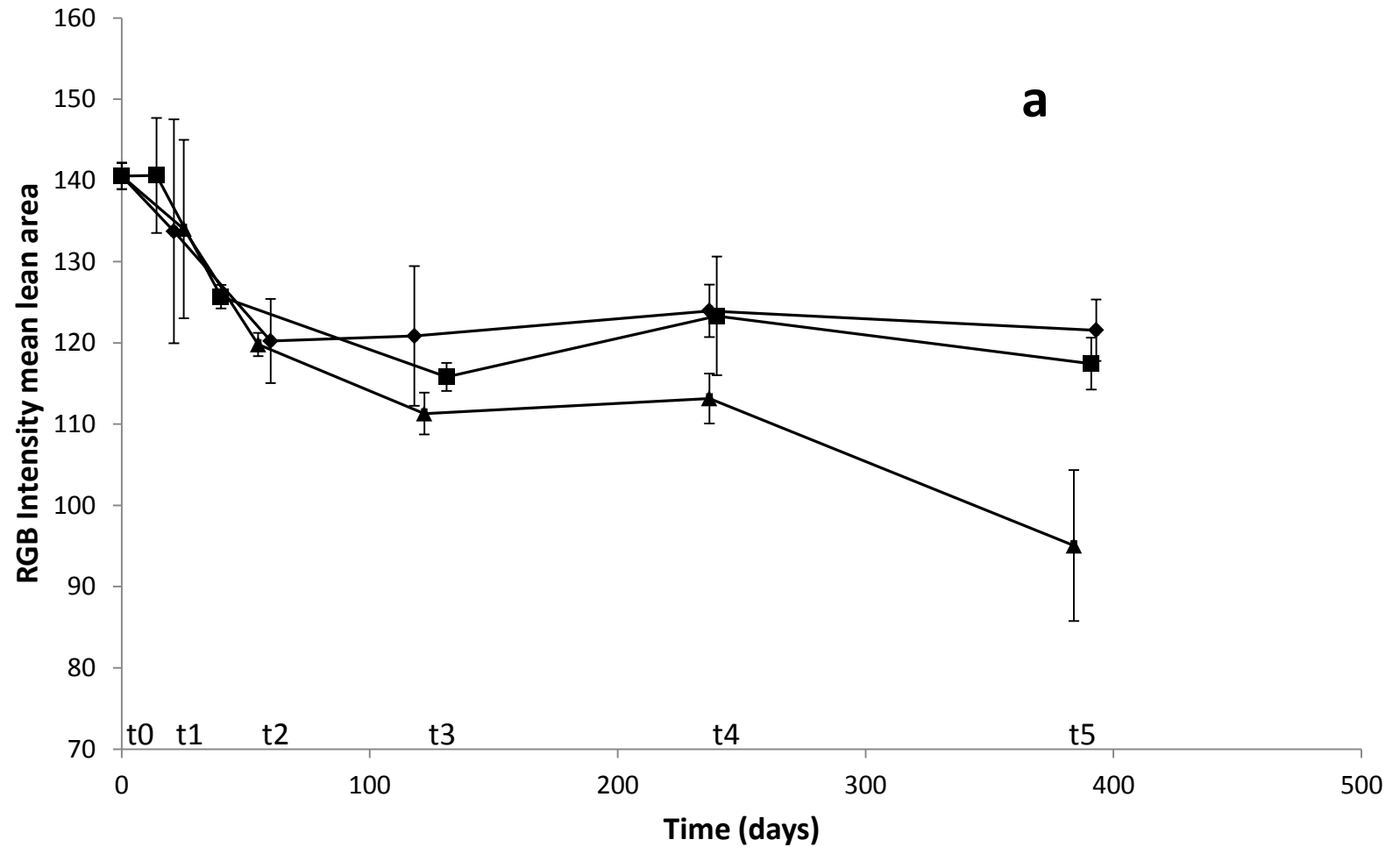
San Daniele



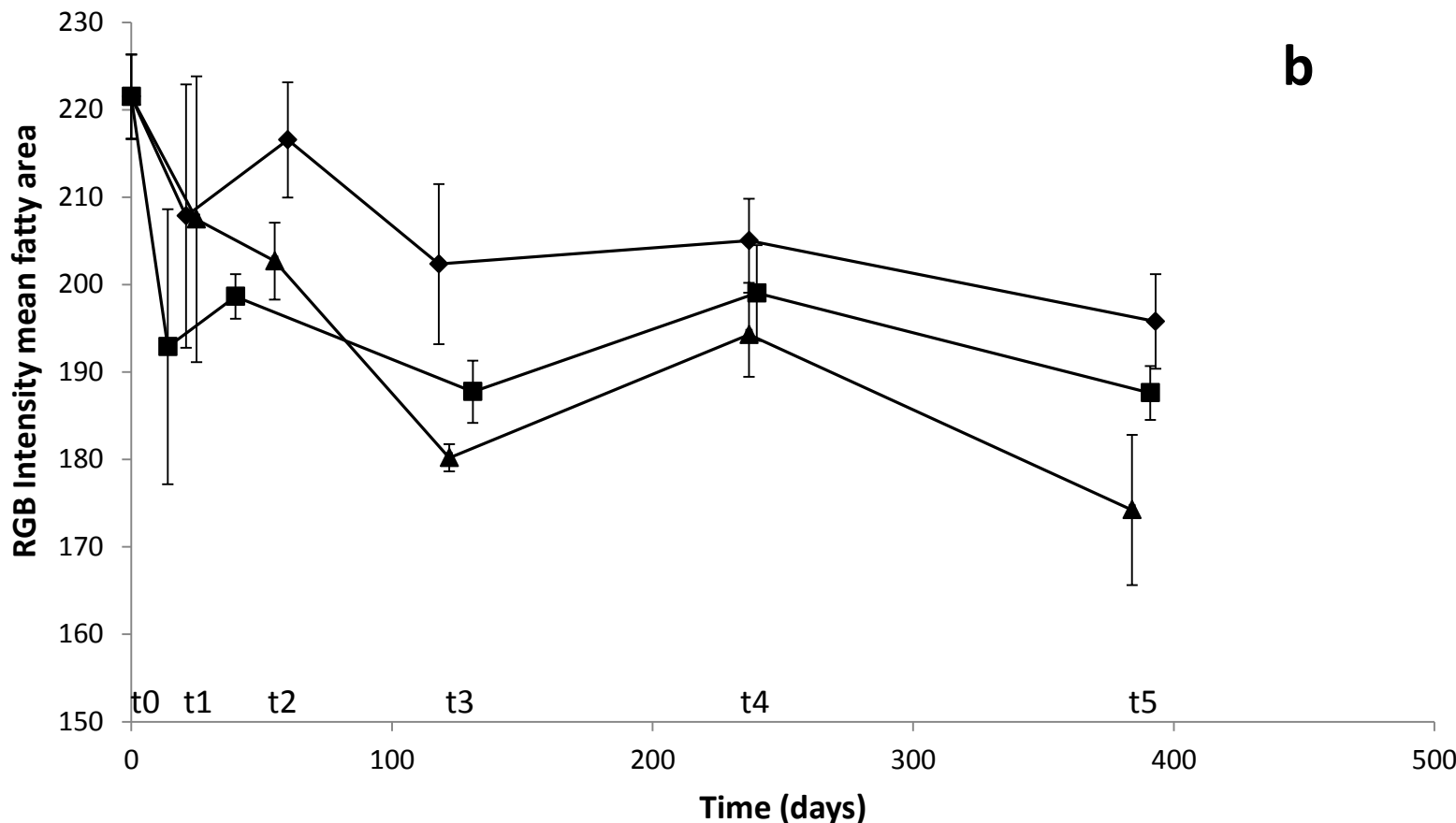
Toscana

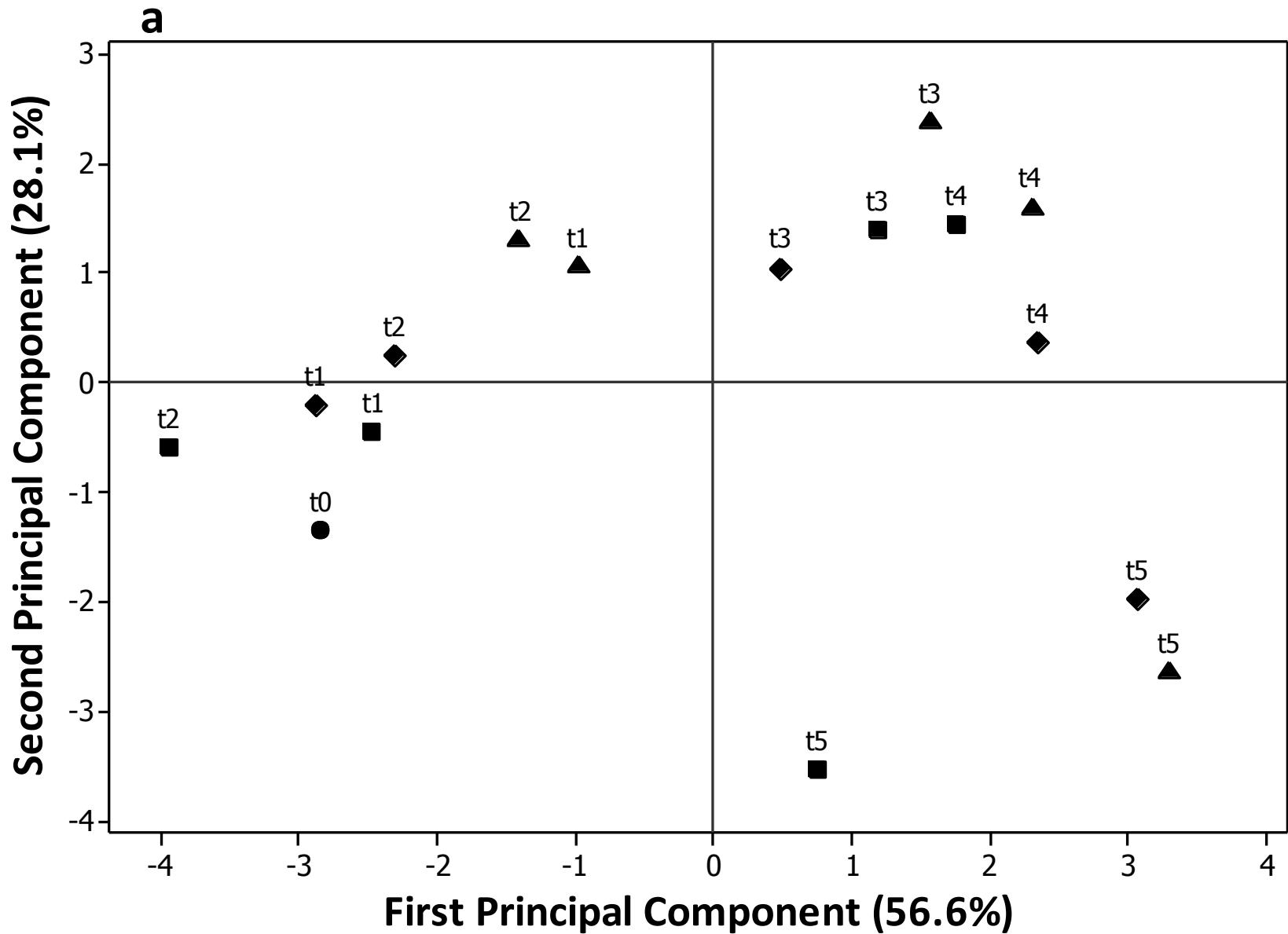


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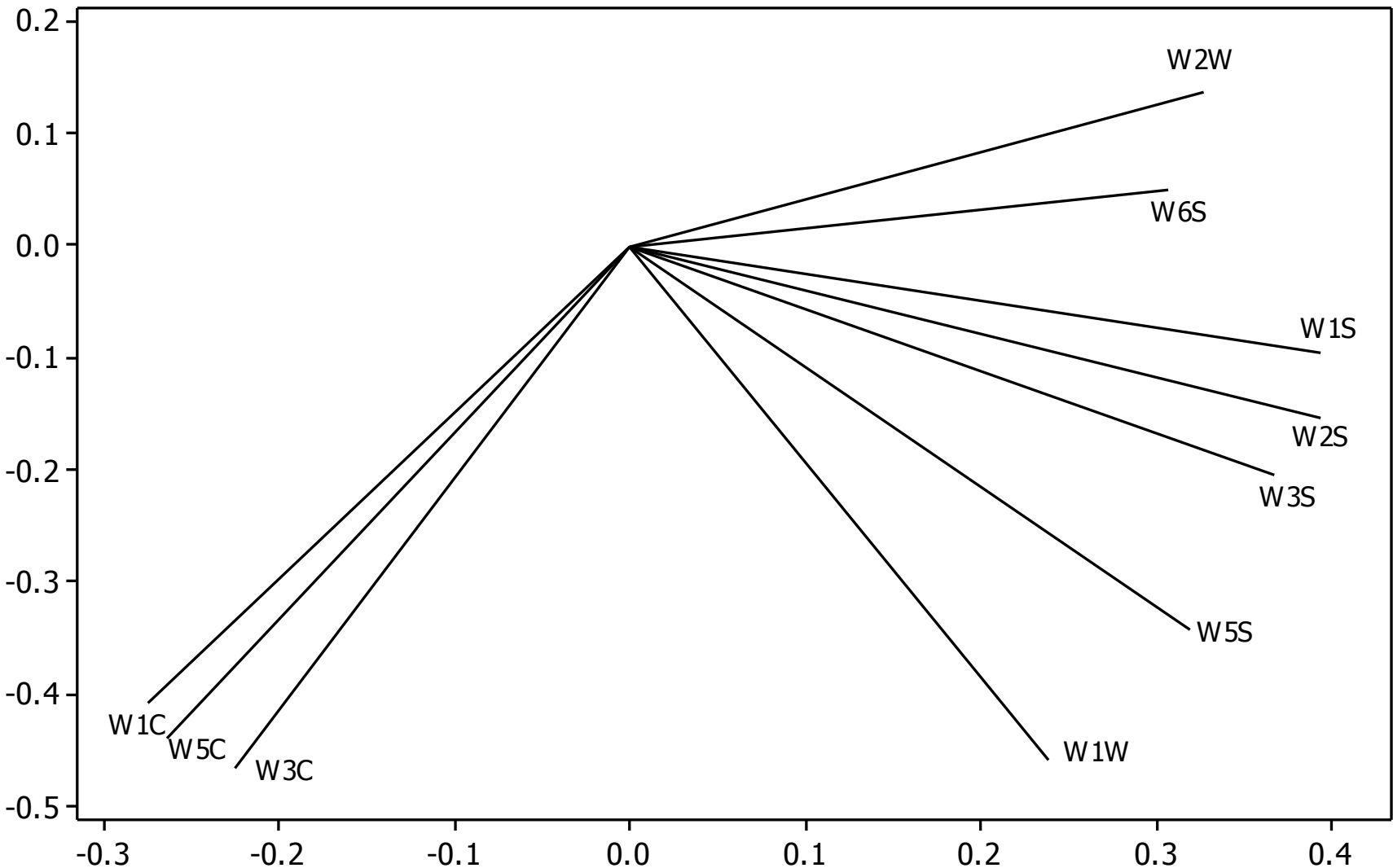
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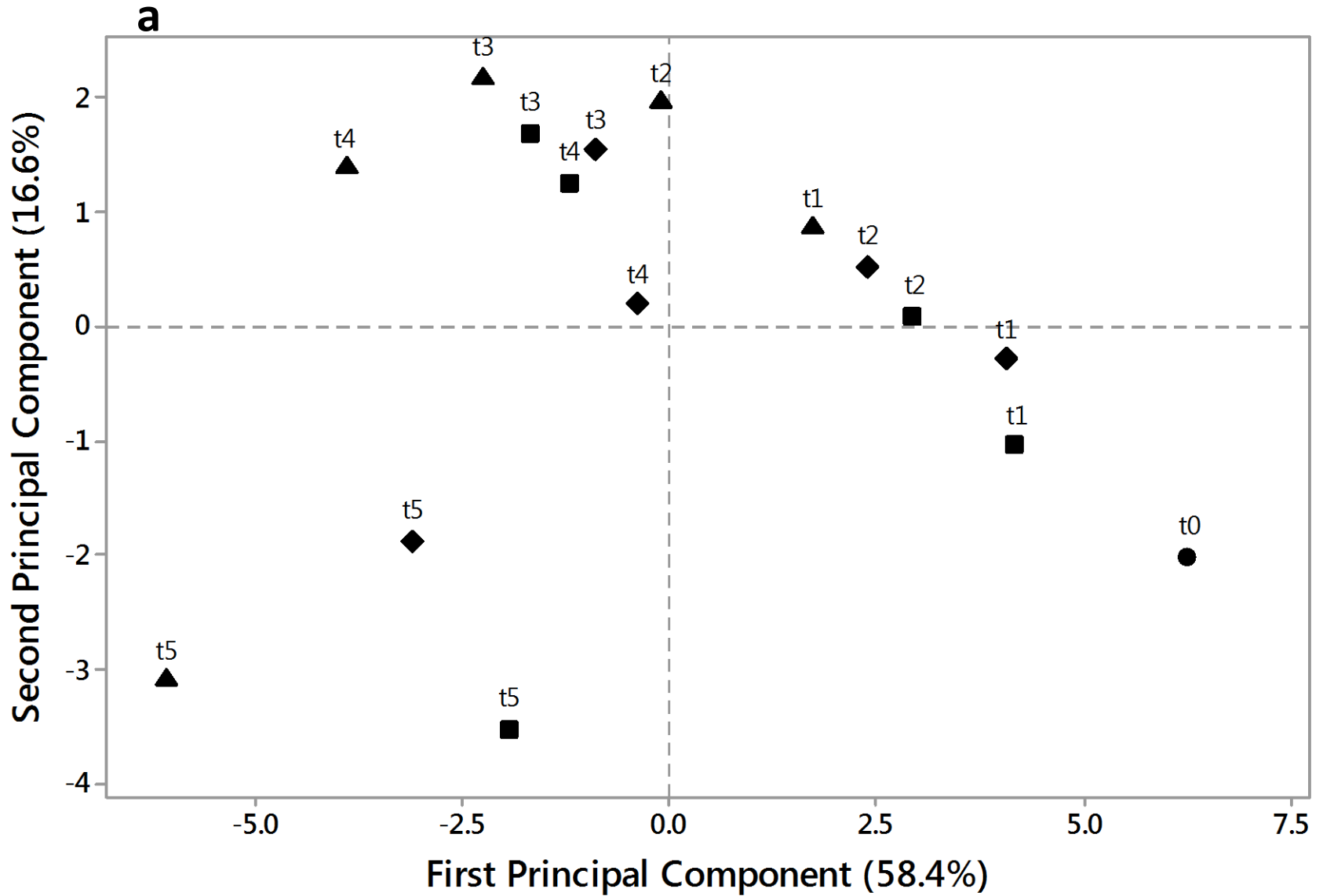




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