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Effect of fresh pork meat conditioning on quality characteristics of salami

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Abstract

The aim of this work was to evaluate the effect of pork meat conditioning under different relative humidity (RH) values on salami quality characteristics. During a 6 days conditioning period at 0°C under two levels of RH (95% vs. 80%), meat pH and weight loss were measured. Salami characteristics (moisture, weight loss, texture, appearance properties) were evaluated during 20 days of ripening. Results showed that conditioning at 80% RH yielded a significantly drier meat, being the weight loss rate 1.6 times higher than at 95% RH. The lower water content of meat allowed a shorter salami ripening phase, guaranteeing an appropriate weight loss and the development of the desired texture, while maintaining good appearance properties. The acceleration of this production phase represents a clear economic advantage for producers and consumers, leading to higher profit margins and lower retail prices. The possibility of using FT-NIR spectroscopy as a valid tool for the rapid evaluation of salami ripening was also demonstrated.

Keywords: Meat conditioning, salami, ripening, texture, color, FT-NIR spectroscopy.

Abbreviation list: a_w , water activity; B, Blue; B1, Batch 1; B2, Batch 2; FT-NIR, Fourier transform-near infrared; G, Green; LSD, Least Significant Difference; M-NEW, meat conditioned with the new process; M-STD, meat conditioned with the standard process; ANOVA, Analysis of Variance; MIA, Multivariate Image Analysis; NEW, new meat conditioning; PCA, principal component analysis; PC1, first principal component; R, Red; RH, relative humidity; S-NEW, salami obtained from meat conditioned with the new process; S-STD, salami obtained from meat conditioned with the standard process; SNV, standard normal variate; STD, standard meat conditioning.

1. Introduction

Salami are typical European dry fermented sausages manufactured mainly with pork meat and fat, with the addition of salt, curing agents (nitrate and/or nitrite), spices, sugars and eventually starter cultures. The manufacture of salami is highly complex because, apart from product parameters, several external factors affect the characteristics of the final product.

Temperature, relative humidity and air velocity in the fermentation and ripening rooms as well as ripening time determine the drop in pH and water activity (a_w) of salami, thus greatly affecting color, taste, flavor and texture (Feiner, 2006).

In particular, ripening is considered one of the most important stages in salami production, because it has a primary influence on physical, chemical, and microbiological characteristics of final dry fermented pork products. Some major quality and safety standards, such as the product weight loss, the seasoning uniformity, the presence of inner fissures, as well as the whole chemical and microbiological transformations, can be related to the way the ripening stage is carried out and controlled. Water loss is a crucial aspect in ripening because it is responsible for the lowering of a_w , which determines limitations to the growth of many spoilage and pathogenic microorganisms (Grassi & Montanari, 2005).

As the relative humidity in the fermentation and ripening rooms is constantly kept lower than the a_w of salami, there is a difference in vapor pressure that causes the removal of moisture through the outside layers of the products. The water loss has to occur at the right speed and to be as uniform as possible in order to avoid case hardening that is negative for both safety and texture of salami. In addition, if a product is not dried at a suitable speed, the desired firmness (or loss in weight) will be obtained in longer time and every day of extended drying or ripening is very costly. For this reason, different experimental strategies have been proposed in order to shorten the ripening stage. In particular, the addition of lipolytic and proteolytic enzymes have been successfully applied even if there can be the risk of

overmaturation and some legal limitations (Fernández, Ordoñez, Bruna, Herranz, & de la Hoz, 2000). Another suggested strategy involves the removal of as much water as possible from meat prior to fermentation by a cold pre-conditioning of fresh meat (Feiner, 2006). However, to the best of our knowledge, no studies have investigated the influence of meat pre-conditioning on salami features. On the contrary, as well reported in some reviews (Ordoñez, Hierro, Bruna, & de la Hoz, 1999; Fernández et al., 2000), several papers deal with the effects of ripening conditions on microbiological, physical and chemical properties of dry fermented sausages.

The aim of this work was to evaluate the effect of different relative humidity values during pork meat conditioning on the quality characteristics of salami. In particular, two levels of relative humidity (95% vs. 80%) were adopted during a 6 days conditioning period at 0°C.

2. Materials and Methods

2.1. Meat and salami samples

Fresh pork shoulders (about 6-7 kg each) conditioned under two different levels of relative humidity were used for industrial salami production. Standard conditioning (STD) lasted 6 days at 0°C with a relative humidity (RH) of 95%; the new conditioning process (NEW) was carried out at 0°C for 6 days, but at 80% RH. Two different batches of shoulders (B1 and B2) were used for each conditioning process, and the corresponding treatments were named M-STD1, M-STD2, M-NEW1 and M-NEW2, respectively. For each treatment, three shoulders were analyzed at four different times during conditioning (0, 1, 3, and 6 days). Meat batch, type of conditioning process, and conditioning time have been considered as independent factors in order to study their main and interaction effects.

Salami production consisted of the following steps: grinding and mixing of all ingredients (pork shoulders, salt, sucrose, dextrose, spices, sodium ascorbate, natural flavors, potassium

nitrate and sodium nitrite), stuffing in artificial casings, fermentation/drying for five days (at 18-20°C and 85-88% relative humidity) and ripening for fifteen days (at 10-12°C for 75% relative humidity).

For each salami type (named S-STD1, S-STD2, S-NEW1, and S-NEW2), a total of thirty samples (about 230 g each) were analyzed at five different times during drying and ripening: 0, 5, 10, 15 and 20 days (ripening time). In this case, meat batch, meat conditioning type, and salami ripening time have been considered as independent factors in order to study their main and interaction effects.

2.2. Meat and salami weight loss

Change in water content of pork shoulders during conditioning and of salami during ripening was evaluated by monitoring the weight loss. Three shoulders per conditioning treatments and six samples per salami type were weighted at each sampling time. Results are expressed as a percentage of the initial weight.

2.3. Meat pH

During conditioning of pork shoulders, pH was measured by means of a pH-meter (EC500, Extech Instruments, Nashua, NH) equipped with the electrode for solid samples.

Measurements were carried out in triplicate for each conditioning treatment and time.

2.4. Salami moisture

For each salami type and ripening time, nine slices 0.5 cm thick were cut out from three samples and separately minced by a heavy duty blender (Waring Laboratory, Torrington, CT) for 30 s at the highest speed. Moisture content of each minced slice was then determined by a

gravimetric method drying samples in an oven at 105°C for 16 h. Results are the average of the nine determinations and are expressed as g/100g.

2.5. Salami texture

Mechanical behavior of salami was evaluated by means of two different empirical rheological tests performed by a TA.HDPlus Texture Analyser (Stable Micro System, Godalming, UK) equipped with a 5 kN load cell and controlled by the Texture Exponent TEE32 V 3.0.4.0 software (Stable Micro System, Godalming, UK).

2.5.1. Compression of the whole salami

One sample for each salami type and ripening time was subjected as a whole to a compression test in five different points along the major axis. A spherical probe (2.5 cm diameter) was used at a crosshead speed of 2 mm/s. Results are expressed as whole firmness, calculated as the average load at 30% deformation (N).

2.5.2. Compression of salami test pieces

Nine test pieces (2.5 cm width, 2.5 cm length, 1.5 cm height) were cut out from the center of three different samples for each salami type and ripening time. Each test piece was compressed with a flat plate (10 cm diameter) using a crosshead speed of 1 mm/s. Results are expressed as hardness, corresponding to the load at 50% deformation (N).

2.6. Salami image analysis

At each ripening time, the images of eight slices (0.5 cm thick) cut out from the center of two different samples for each salami type were acquired. Image acquisition and elaboration were carried out as reported in Fongaro, Alamprese, and Casiraghi (2015). In particular, Red (R), Green (G), Blue (B), and intensity mean values measured in the RGB space color, as well as the heterogeneity parameter (ranging from 0 for homogeneous surfaces to 1 for heterogeneous

surfaces) (Fongaro & Kvaal, 2013) were calculated by using Image-Pro Plus (v. 7.0, MediaCybernetics, Inc., Rockville, MD, USA). The multivariate image analysis (MIA) method developed in our previous work (Fongaro et al., 2015) is able to distinguish different areas in salami slices, corresponding to different saturation levels of red, which can be associated to different level of meat oxidation. In fact, the color of the salami lean part becomes darker during ripening, due to dehydration and oxidation of myoglobin. By means of the MIA method, different areas of salami slice surface were quantified: high level of meat oxidation (Area 1), intermediate level of meat oxidation (Area 2), meat not yet oxidized (Area 3) and fat (Area 4). MIA was applied using the MACCMIA software (v. 1.81, McMaster Advanced Control Consortium, McMaster University, Ontario, Canada) for MATLAB (v. 7.8.0, The MathWorks Inc., Natick, MA, USA) and the Image Pro-Plus software.

2.9. FT-NIR spectroscopy

For each salami type and ripening time, nine slices 1.5 cm thick were cut out from the center of three salami and analyzed by near infrared (NIR) spectroscopy. A Fourier transform (FT)-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany) fitted with an integrating sphere was used. Spectra were recorded in quadruplicate for each slice, at a resolution of 16 cm^{-1} , with 120 scans for both background and samples, in a $12500\text{-}3600\text{ cm}^{-1}$ spectral range. Spectral data were smoothed (moving average with segment size of eleven), standardized by standard normal variate (SNV) and first derivative (Savitzky-Golay method, polynomial order = 2, gap size = 11 data points), and mean-centered before performing Principal Component Analysis (PCA) by means of The Unscrambler v. 9.8 software (Camo Software AS, Oslo, Norway). The PC1 scores were then translated to the origin, averaged along samples and ripening time, and modelled as a function of salami ripening time using Table Curve software (v. 4.0, Jandel Scientific, San Rafael, CA, USA).

2.10. Statistical analysis

Significant effects of meat batch, conditioning process and conditioning (for meat) or ripening (for salami) time, and of their 2-order interactions were assessed by multi-factor analysis of variance (ANOVA). For each significant factor ($P < 0.05$), the Fisher's Least Significant Difference (LSD) multiple range test was applied at a confidence interval of 99.9% to compare average values of the responses. Statistical analysis was carried out using Statgraphics Plus (v. 5.1, Statistical Graphics Corp., Herndon, VA, USA).

3. Results and Discussion

3.1. Meat weight loss and pH

Weight loss and pH of pork shoulders were measured during the two different conditioning processes at 0, 1, 3 and 6 days. ANOVA and LSD results are reported in Tables 1 and 2. Meat pH was affected by the three considered factors (Table 1), but highly significant differences ($P < 0.001$) were observed only as a function of conditioning time, with the major drop observed the first day (Table 2). At the end of the treatment, pH was lower than 5.8, indicated by Feiner (2006) as a correct value for meat to be processed. As expected, meat weight loss was significantly influenced ($P < 0.001$) by conditioning process, conditioning time and their interaction (Table 1). As better shown in Fig. 1, the two different levels of RH accounted for a significantly ($P < 0.01$) different rate in meat weight loss. Under standard conditions (95% RH) pork meat lost on average 0.21% weight per day reaching a total of 1.66% weight loss, whereas with 80% RH weight loss rate was 1.6 times higher (total weight loss of 2.62%). Thus, at the end of the conditioning period, the meat treated at 80% RH resulted to have, as desired, a significant lower moisture content.

3.2. Salami moisture and weight loss

Moisture and weight loss of salami during drying and ripening were measured to check the correct development of these production steps and to evaluate the effect of the two different meat conditioning processes. Results obtained are reported in Tables 3 and 4.

Meat batch exerted only a slight effect on salami moisture and weight loss (Table 3); in fact, no significant differences were observed as a function of this factor at a 99.9% confidence interval (Table 4). The most important factors affecting these salami parameters were the meat conditioning process and the ripening time. In particular, as expected on the basis of the meat weight loss results, salami produced with the meat conditioned under 80% RH (NEW conditioning process) reached a lower moisture content and weight loss, since the meat lost more weight during conditioning.

As reported in Fig. 2a, a linear decrease of the salami moisture content was observed during ripening, without difference in the decreasing rate between the two types of salami (S-STD and S-NEW). On the contrary, Fig. 2b highlights a significantly ($P < 0.05$) different rate of weight loss as a function of the meat conditioning process. In fact, after 5 days of drying the two types of salami lost the same weight percentage (about 13.0%), but at the end of ripening S-STD lost 34.0% weight vs. 32.1% of S-NEW. Experimental data were successfully fitted ($r = 1.000$) by the following equation:

$$y = a + b(1 - \exp^{-ct}) \quad [\text{Eq. 1}]$$

where y is the weight loss, t is the ripening time, a and b are constant values ($a+b$ corresponds to salami weight loss for t tending to infinity), and c is the rate of weight loss. Rate values of 0.096 ± 0.005 and 0.070 ± 0.006 were calculated for S-STD and S-NEW, respectively, thus confirming a significant ($P < 0.05$) faster weight loss for salami obtained by the STD meat conditioning process.

3.3. Salami quality parameters

Salami texture, color, and appearance were measured as important aspects of consumer acceptance. Results about mechanical properties are shown in Table 3 and 4. No significant differences were observed as a function of the meat batch used for salami production. Firmness of the whole salami was significantly affected ($P < 0.001$) only by the ripening time, whereas the hardness of the test pieces was significantly affected ($P < 0.001$) both by the ripening time and meat conditioning process. In particular, a significantly ($P < 0.001$) higher hardness was obtained with the NEW meat conditioning process as a clear consequence of the higher meat weight loss and the lower salami moisture. Moreover, a significant interaction of conditioning process and time has been observed for both firmness ($P < 0.0001$) and hardness ($P < 0.05$). Results of salami mechanical properties as a function of ripening time are graphically reported in Fig. 3, where the higher hardness of S-NEW at the end of ripening is clearly visible. From the experimental data fitting, carried out also in this case with [Eq. 1], it is possible to calculate that, in order to obtain a certain hardness in the center of salami, a shorter ripening period is needed. For instance, referring to the hardness of S-STD after 20 days of ripening (76.1 N), it is possible to calculate through the fitted equations that S-NEW samples reach the same value after only 8.3 days, with an evident economic advantage for the producer. Moreover, considering that dehydration and mechanical properties of fermented sausages change gradually along a radial direction going from the center to the surface of a product (Houben & van't Hoof, 2005), the similar trend of whole firmness measured for the two types of salami means that S-NEW has a more homogeneous structure from the center towards the outside of the product, certainly reflected in more uniform sensory properties. As regards appearance properties, RGB color, intensity mean and heterogeneity were measured by means of image analysis. Results are reported in Tables 3 and 5 together with the percentage of different salami slice areas identified by a multivariate image analysis

procedure (Fongaro et al., 2015). As already reported in §2.6, Area 1 corresponds to the salami slice surface with high level of meat oxidation, Area 2 to intermediate level of meat oxidation, Area 3 to meat not yet oxidized, and Area 4 to fat (Area 4). ANOVA results (Table 3) highlighted that color, intensity, and heterogeneity were mainly affected by meat batch and salami ripening time, as well as by the interaction between meat conditioning process and ripening time. Actually, two different batches of meat are likely to be different in color, because this is one of the most variable meat attributes and each batch is composed of more than one shoulder, belonging to different animals. In any case, the observed differences between the two meat batches, even if statistically significant, are little and probably not perceivable by the human eye.

During salami ripening, changes in pH, water activity, and oxygen availability deeply affect the myoglobin forms responsible for the color of the final product (Feiner, 2006). In fact, the different areas of salami slice surface were significantly affected by ripening time and its interaction with the meat conditioning process. As expected, an increase of the areas corresponding to high and partially oxidized meat (Area 1 and Area 2), and a decrease of the surface covered by not yet oxidized meat (Area 3) were observed. As a result, color indexes R, G, and B, and intensity (related to the brightness of the product) significantly ($P < 0.001$) decreased during salami ripening (Table 5), with a higher extent for S-NEW than S-STD (data not shown). It is also interesting to notice that the heterogeneity significantly ($P < 0.001$) increased at the end of the ripening time, meaning that the surface texture of salami slices became sharper: with drying and shrinkage, the homogeneous aspect of meat immediately after grinding changed in a clear distribution of different elements, such as oxidized meat, not oxidized meat and fat. Because of salami shrinkage, the area corresponding to fat (Area 4) significantly ($P < 0.001$) decreased during salami ripening (Table 5), mainly for S-NEW (data not shown).

The meat conditioning process did not show a deep effect on appearance properties of salami slices. Actually, in nitrite-cured products the relatively low pH and the added ascorbate are the main factors leading to the formation of the cured pigment $\text{MbFe}^{\text{II}}\text{NO}$ (Götterup, Olsen, Knøchel, Tjener, Stahnke, & Møller, 2008).

In general, the appearance properties changed mainly within the first 10-15 days of ripening, thus corroborating the possibilities to shorten the ripening phase applying the pre-conditioning of meat.

3.4. FT-NIR spectroscopy

FT-NIR spectroscopy is an emerging analytical technique with great potential in food processing control (Porep, Kammerer, & Carle, 2015) and in particular in fermentation monitoring (Grassi, Alamprese, Bono, Picozzi, Foschino, & Casiraghi, 2013; Grassi, Amigo, Lyndgaard, Foschino, & Casiraghi, 2014). Thus, in order to evaluate the potential of this technique to model phenomena occurring during salami ripening, FT-NIR spectra of all samples were acquired. PC1 scores obtained from spectral data elaboration by PCA were fitted as a function of ripening time using [Eq. 1]. Fig. 4 shows the averaged results obtained for S-STD and S-NEW. The two curves showed different trends and rate, meaning that ripening phenomena proceeded in S-NEW with a definitely higher speed with respect to S-STD (0.216 vs. 0.092). Actually, after about 10-15 days, the model curve for S-NEW approached the asymptote, whereas for S-STD an increasing trend is evident until the end of the ripening period.

FT-NIR spectroscopy results are therefore in agreement with the conclusions drawn by the other analytical techniques, with the advantage to be definitely less time-consuming and to observe ripening phenomena as a whole instead of considering only one parameter at a time.

4. Conclusion

In conclusion, the work demonstrated that the ripening phase during salami production could be shortened by applying fresh meat conditioning under low relative humidity (80%) for 6 days. The lower water content of the meat used in salami production guarantees the product safety and accounts for a more rapid development of the desired textural properties, which are also more homogeneous along the radial direction of the final product. Salami appearance properties were not affected by meat conditioning, thus confirming the feasibility of the ripening phase shortening. Considering that time and energy requirements of the ripening phase contribute very much to the total costs of salami manufacturing process (Fernandez et al., 2000), the acceleration of this phase represents a clear economic advantage for producers and consumers, leading to higher profit margins and lower retail prices.

Another interesting result is the possibility to use FT-NIR spectroscopy as a valid tool for the rapid evaluation of the salami ripening phase. This technique is able to observe the different occurring phenomena as a whole and to give clear indications about the end of ripening.

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Legends to Figures

Figure 1. Trend of weight loss in fresh pork shoulder conditioned at 0°C for 6 days under 95% (M-STD) or 80% (M-NEW) relative humidity.

Figure 2. Trend of moisture (a) and weight loss (b) in salami produced from pork shoulder conditioned at 0°C for 6 days under 95% (S-STD) or 80% (S-NEW) relative humidity.

Figure 3. Trend of whole firmness (a) and hardness (b) in salami produced from pork shoulder conditioned at 0°C for 6 days under 95% (S-STD) or 80% (S-NEW) relative humidity.

Figure 4. Results of FT-NIR spectroscopy applied to salami produced from pork shoulder conditioned at 0°C for 6 days under 95% (S-STD) or 80% (S-NEW) relative humidity: PC1 score modelling as a function of salami ripening time.

Table 1. Results of multi-factor ANOVA (probability value) for pork shoulder analytical parameters

	pH	Weight loss
Main Effects		
Batch (A)	0.0017	0.7704
Conditioning process (B)	0.0111	<0.0001
Conditioning time (C)	0.0034	<0.0001
Interactions		
AB	0.0006	0.9224
AC	0.0001	0.9408
BC	0.9091	<0.0001

Table 2. Pork shoulder weight loss and pH (mean and standard error values) as a function of batch and conditioning type and time

Experimental factor	pH	Weight loss (%)
Batch		
B1	5.76±0.01 ^a	1.44±0.01 ^a
B2	5.82±0.01 ^a	1.44±0.01 ^a
Conditioning process		
STD	5.81±0.01 ^a	1.13±0.01 ^b
NEW	5.77±0.01 ^a	1.74±0.01 ^a
Conditioning time (days)		
0	5.85±0.02 ^b	
1	5.77±0.02 ^{ab}	0.73±0.01 ^a
3	5.78±0.02 ^{ab}	1.45±0.01 ^b
6	5.76±0.02 ^a	2.14±0.01 ^c

^{a-c}: for each experimental factor and each variable, different superscript letters indicate significant differences amongst mean values as calculated by LSD test ($P < 0.001$).

Table 3. Results of multi-factor ANOVA (probability value) for salami analytical parameters

	Moisture	Weight loss	Whole firmness	Hardness	R	G	B	Intensity	Heterogeneity	Area 1	Area 2	Area 3	Area 4
Main Effects													
Batch (A)	0.0356	0.0086	0.0537	0.9132	<0.0001	<0.0001	<0.0001	<0.0001	0.0503	0.2904	0.0001	0.4107	<0.0001
Conditioning process (B)	<0.0001	<0.0001	0.5538	<0.0001	0.0001	0.6961	0.4187	0.1747	0.2964	0.1124	0.0002	0.0029	0.7778
Ripening time (C)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interactions													
AB	<0.0001	0.4247	0.0091	0.0057	0.2734	0.3722	0.0965	0.2089	0.1566	0.0012	0.1246	0.0260	0.0135
AC	<0.0001	0.0314	0.0477	0.0749	0.3985	0.1139	0.1085	0.3272	0.7646	0.2524	0.1309	0.3645	0.5054
BC	0.0971	0.0025	<0.0001	0.0118	<0.0001	0.0002	0.0002	<0.0001	0.1405	0.0054	0.0004	0.0931	0.0002

Table 4. Salami weight loss, moisture and mechanical properties (mean and standard error values) as a function of batch, conditioning process and ripening time

Experimental factor	Moisture (g/100g)	Weight loss (%)	Whole firmness (N)	Hardness (N)
Batch				
B1	44.6±0.2 ^a	24.6±0.1 ^a	58.1±0.7 ^a	62.0±1.6 ^a
B2	44.1±0.2 ^a	24.1±0.1 ^a	56.4±0.6 ^a	61.7±1.7 ^a
Conditioning process				
STD	45.3±0.2 ^b	25.0±0.1 ^b	57.5±0.6 ^a	54.2±1.5 ^a
NEW	43.5±0.2 ^a	23.6±0.1 ^a	57.0±0.7 ^a	69.5±1.8 ^b
Ripening time (days)				
0	56.5±0.3 ^e		34.6±1.3 ^a	8.6±3.3 ^a
5	50.2±0.3 ^d	12.9±0.2 ^a	40.7±0.9 ^b	57.9±2.7 ^b
10	43.6±0.3 ^c	22.6±0.2 ^b	49.0±0.8 ^c	71.7±2.4 ^c
15	37.8±0.3 ^b	28.7±0.2 ^c	71.6±0.9 ^d	80.9±2.2 ^{cd}
20	33.8±0.3 ^a	33.0±0.2 ^d	90.3±1.0 ^e	90.1±2.2 ^d

^{a-e}: for each experimental factor, different superscript letters indicate significant differences amongst mean values as calculated by LSD test ($P < 0.001$).

Table 5. Salami appearance properties (mean and standard error values) as a function of batch, conditioning process and ripening time

Experimental factor	R	G	B	Intensity	Heterogeneity	Area 1 (%)	Area 2 (%)	Area 3 (%)	Area 4 (%)
Batch									
B1	189.5±0.7 ^b	128.8±0.7 ^b	125.1±0.6 ^b	147.8±0.6 ^b	0.50±0.01 ^a	8.1±0.3 ^a	19.8±0.5 ^a	50.9±0.5 ^a	22.1±0.5 ^b
B2	181.7±0.7 ^a	123.8±0.7 ^a	119.8±0.6 ^a	141.8±0.6 ^a	0.48±0.01 ^a	8.5±0.2 ^a	22.8±0.5 ^b	51.4±0.5 ^a	18.0±0.6 ^a
Conditioning process									
STD	188.1±0.7 ^b	126.1±0.7 ^a	122.1±0.7 ^a	145.4±0.7 ^a	0.49±0.01 ^a	8.0±0.3 ^a	19.9±0.5 ^a	52.2±0.5 ^a	19.9±0.5 ^a
NEW	183.0±0.8 ^a	126.5±0.6 ^a	122.8±0.6 ^a	144.1±0.6 ^a	0.50±0.01 ^a	8.6±0.3 ^a	22.7±0.5 ^b	50.1±0.5 ^a	20.2±0.6 ^a
Ripening time (days)									
5	193.3±1.2 ^c	132.0±0.9 ^c	127.9±0.9 ^c	151.0±1.0 ^c	0.46±0.01 ^a	4.2±0.4 ^a	15.8±0.7 ^a	57.2±0.6 ^c	23.1±0.8 ^b
10	189.5±1.2 ^b	129.2±1.0 ^{bc}	125.3±0.9 ^{bc}	148.0±1.0 ^{bc}	0.48±0.01 ^a	8.0±0.4 ^b	18.9±0.7 ^a	53.1±0.7 ^b	20.5±0.8 ^a
15	184.3±1.2 ^{ab}	124.9±1.0 ^b	121.0±0.9 ^b	143.4±1.0 ^b	0.50±0.01 ^{ab}	9.4±0.4 ^b	23.4±0.7 ^b	47.7±0.6 ^a	21.2±0.9 ^a
20	175.2±1.2 ^a	119.1±1.0 ^a	115.6±0.9 ^a	136.6±1.0 ^a	0.53±0.01 ^b	11.6±0.4 ^c	27.1±0.7 ^c	46.6±0.7 ^a	15.4±0.8 ^a

^{a-c}: for each experimental factor and each variable, different superscript letters indicate significant differences amongst mean values as calculated by LSD test ($P < 0.001$).

Figure 1

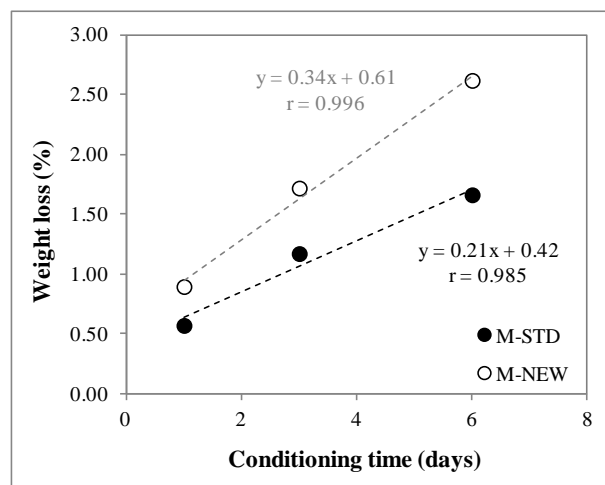
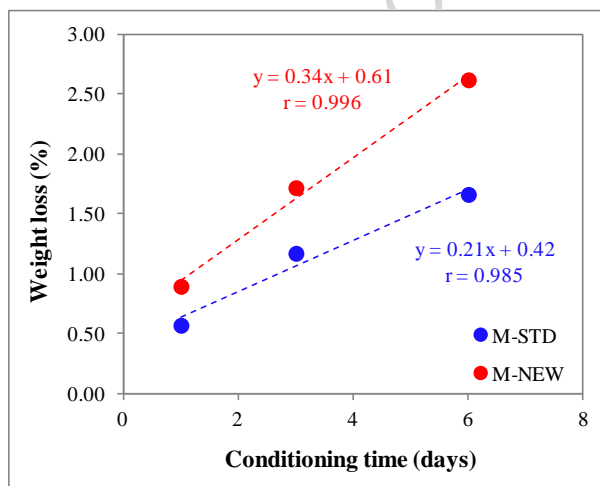


Figure 1 (Color version for Web only)



ACCEPTED MANUSCRIPT

Figure 2

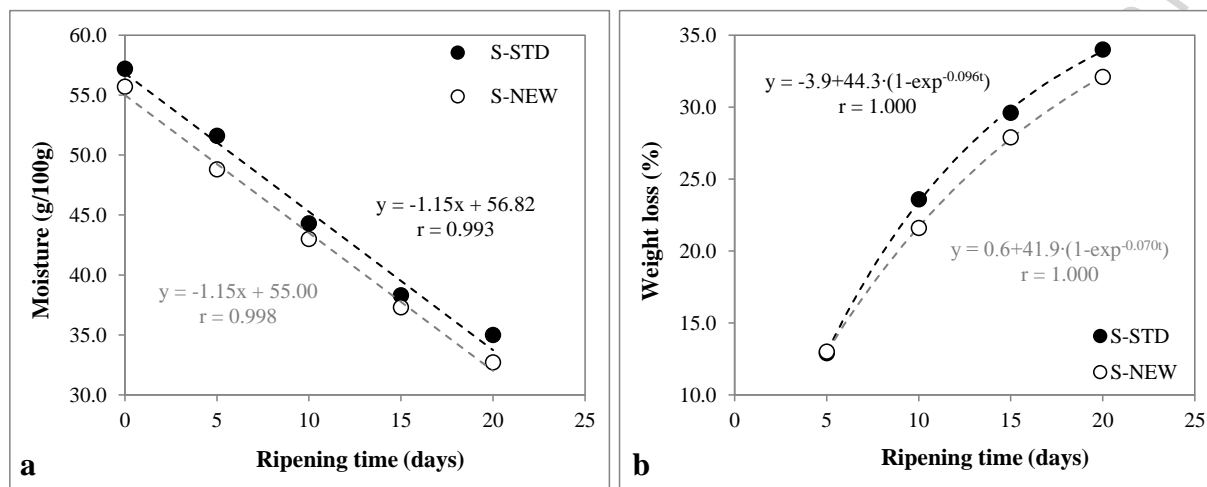


Figure 2 (Color version for Web only)

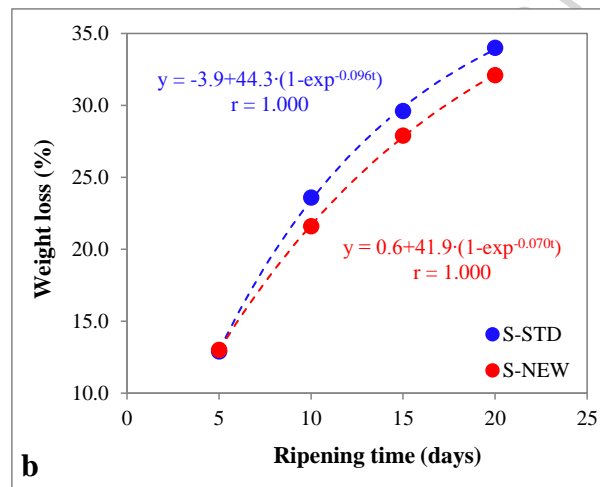
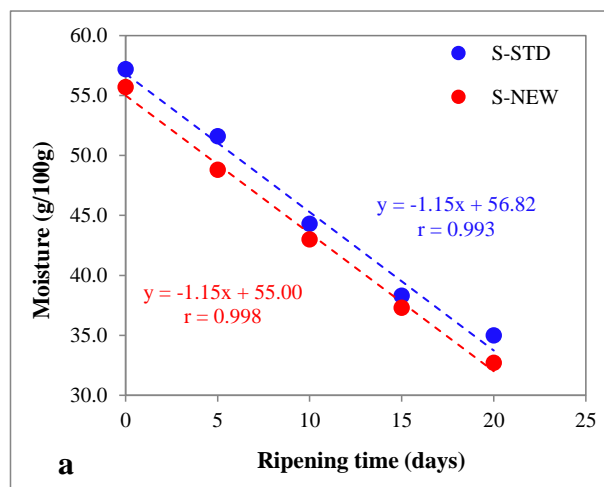


Figure 3

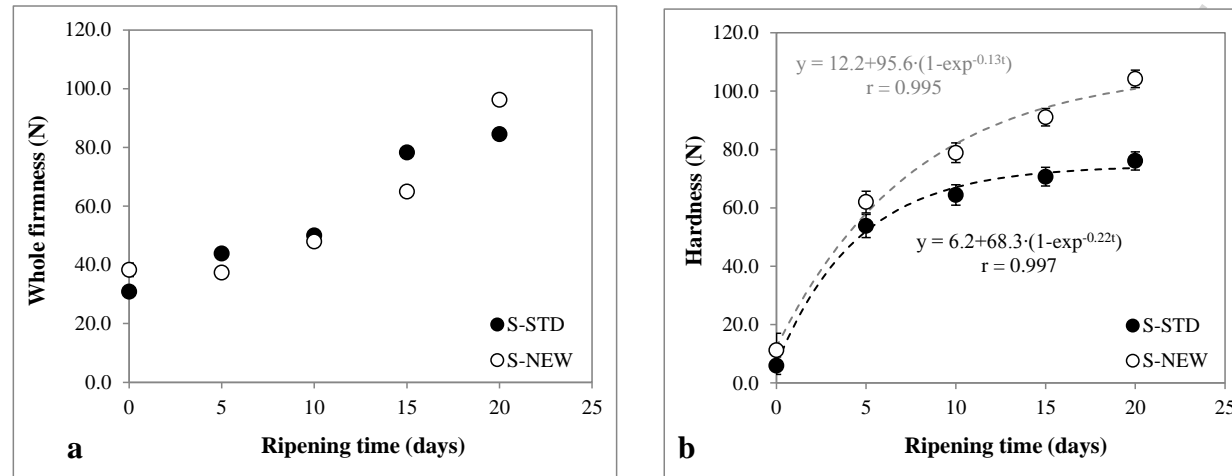


Figure 3 (Color version for Web only)

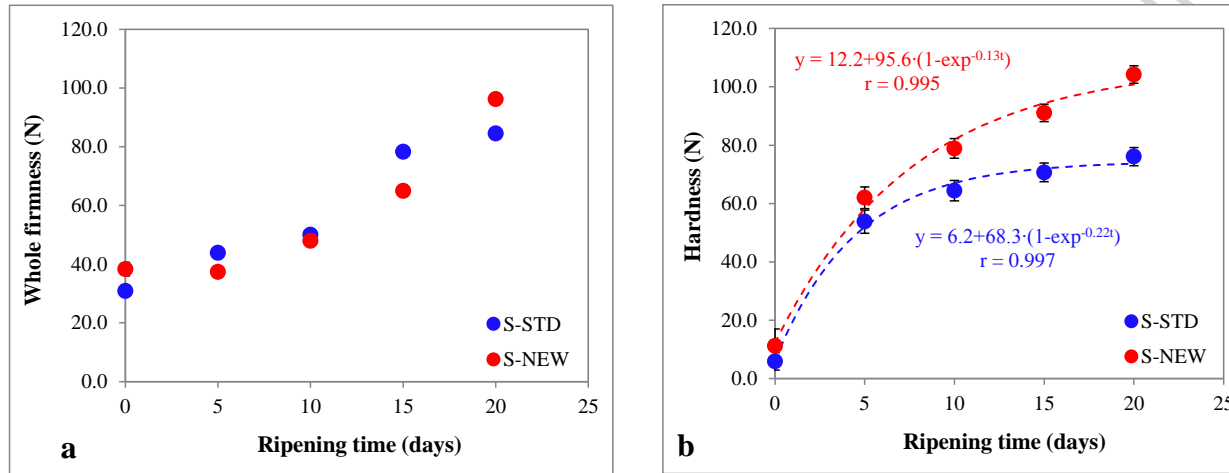


Figure 4

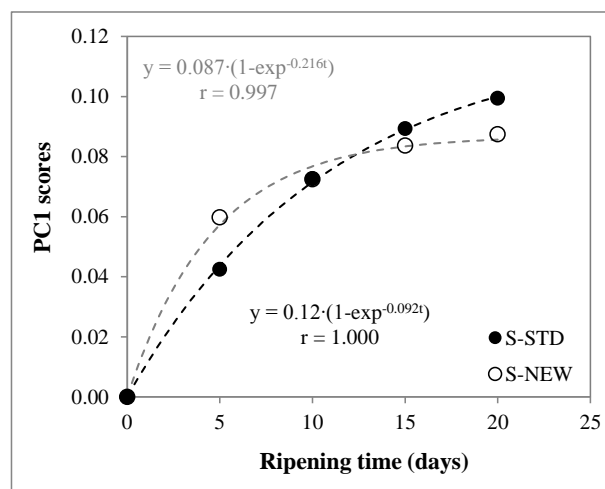


Figure 4 (Color version for Web only)

