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Abstract: Salt (NaCl) penetration was studied on dry-cured hams of different weight processed by two different salting processes. Chemical composition and water activity (aw) were analysed on two of the most representative ham muscles during the process. The normalized Weibull cumulative distribution was used to fit salt uptake in Biceps femoris m. (BF) and to calculate the salt diffusion coefficient. The aw values strictly depend on the Salt Index (S.I., gNaCl 100 gw-1). The S.I. of BF samples from hams taken at different processing steps, were modelled as a function of aw by both a linear and a first order polynomial model achieving good fitting (R2 = 0.92). The calibration root mean square error (RMSE) resulted being of 1% for both models. Cross validation was performed and the RMSEs were of 0.62% and 0.61% for the linear and polynomial models, respectively. These models can be useful to manage the salting process in dry-cured hams at industrial level.

Cover Letter

UNIVERSITÁ DEGLI STUDI DI TERAMO

Dear Editor,

I submit the revised manuscript and detailed answers to the Referees, for publishing of the research paper in your journal " Journal of Food Engineering".

Ms. Ref. No.: **JFOODENG-D-16-01041R1**

Title: "Prediction of the salt content from water activity analysis in dry-cured ham"

With best regards

Teramo, 21-12-2016

Dr.ssa Maria Martuscelli

For comuncation:

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Highlights

> The chemical composition and water activity were observed in ham muscles during resting and ripening process > The normalized Weibull cumulative distribution was used to fit salt uptake and to calculate the salt diffusion coefficient > The a_w values strictly depend on the Salt Index > The Salt Index data of *Biceps faemoris* muscle were modelled as a function of water activity > A linear and a first order polynomial model achieved a good fitting

Reply to Reviewer#1

In "*DETAILED ANSWERS TO Reviewer#1*", in point 11, we wrote Fig 2 (c, d) instead of Figure 3 (c, d) and there was a typing error since $p > 0.05$.

Reply to Reviewer#4

In all R2 manuscript the grammar has been improved, according your suggestion (see lines 17, 60-63, 73-77, 80, 87, 94-96, 104, 159, 175, 234, 304, 314-315, 334, 341-343, 391, 395, 397, 400, 410-413, 421-422, 426, 445-447, 450, 452, 477; Lines 503-506-R1 have been deleted in R2).

Line 41: (-) has been added
Line 142-147: The text has been split in more sentences.

Line 238: $S_{\sqcap}$ refers to S_{∞}, unfortunately some symbols were changed upon .pdf file building; this is an editorial problem and we wrote to the editor.

Line 245-246 - One directional refers to the flux direction (from the outer surface to the centre of BF m.), one dimensional refers to the Fick's law assumption that diffusion occurs along one dimension.

Line 251 - what the reviewer read as □l is β = Weibull shape parameter; unfortunately some symbols were changed upon .pdf file building; this is an editorial problem and we wrote to the editor.

Line 258 - what the reviewer read as $\square_e$ is β_e; unfortunately some symbols were changed upon .pdf file building; this is an editorial problem and we wrote to the editor.

Line 265 - the reference (Chen et al, 1990) was given at the end of the sentence.

Line 270 - the first □ is Π; the second □ is ≈. Unfortunately some symbols were changed upon .pdf file building; this is an editorial problem and we wrote to the editor.

Line 274 - The M_{ash} estimated by van der Sman and Boer (2005) was used for calculation purposes and this has been reported in the R2 text (line 277-278)

Line 277- The reference (van der Sman and Boer (2005) was given at the end of the sentence.

Line 369 in R1 -Line 370 in R2 begins with "*The β values were always* ...." Unfortunately some typing and symbols were changed upon .pdf file building; this is an editorial problem and we wrote to the editor.

Line 503-506 in R1 - Line 505 in R2. We completely agree with the reviewer and deleted the lines.

Figure 6 and 8. We do not deem this change necessary but we leave the decision to the editor.

Prediction of the salt content from water activity analysis in dry-cured ham

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ABSTRACT

Salt (NaCl) penetration was studied on dry-cured hams of different weight processed by two different salting processes. Chemical composition and water activity (a_w) were analysed on two of the most representative ham muscles during the process. The normalized Weibull cumulative distribution was used to fit salt uptake in *Biceps femoris* m. (BF) and to calculate the salt diffusion coefficient. The a_w values strictly depend on the Salt Index (S.I., g_{NaCl} 100 g_w^{-1}). The S.I. of BF samples from hams taken at different processing steps, were modelled as a function of a_w by both a linear and a first order polynomial model achieving good fitting (R^2 = 0.92). The calibration root mean square error (RMSE) resulted being of 1% for both models. Cross validation was performed and the RMSEs were of 0.62% and 0.61% for the linear and polynomial models, respectively. These models can be useful to manage the salting process in dry-cured hams at industrial level.

Keywords: dry-cured ham, salt diffusion, normalized Weibull distribution, water activity, prediction models

Nomenclature

a_w = water activity (-)

B = constant for water activity calculation (-)

β = shape factor of the Weibull equation (-)

β_e = constant for water activity calculation (-)

D_{calc} = calculated diffusivity ($m^2\ s^{-1}$)

D_{eff} = effective diffusivity ($m^2\ s^{-1}$)

l = length (m)

M= molar mass ($g\ mol^{-1}$)

n = dissociation number (-)

R_g = geometric factor (-)

S_0 = initial salt concentration ($g_{NaCl}\ 100g_{dw}^{-1}$)

S_t = salt concentration at time t ($g_{NaCl}\ 100g_{dw}^{-1}$)

S_∞ = salt concentration at equilibrium ($g_{NaCl}\ 100g_{dw}^{-1}$)

S.I. = salting index ($g_{NaCl}\ 100g_{w}^{-1}$)

$g_{NaCl}\ 100_{ffdw}^{-1}$ = salt concentration on fat-free dry weight ($g_{NaCl}\ 100_{ffdw}^{-1}$)

t = time (min)

x = mass fraction ($g\ g^{-1}$)

1. Introduction

In traditional dry-cured ham the penetration of salt along with other curing agents when used, is determinant for the achievement of physico-chemical properties related to the safety and stability of the final product as well as the development of the characteristic sensory quality. As known, salt influences the growth of microorganisms and bio-enzymatic activities (Toldrá, 2005) that affect

57 the safety and quality (texture, taste, flavour, colour) of the final product (Flores
58 et al., 2012; Serra et al., 2005; Countron-Gambotti et al., 1999).
59 There are two ways to proceed with salt treatment: undetermined salt or the
60 exact amount of salt supply (Toldrá, 2002); in Mediterranean countries (Spain,
61 Italy, France), during treatment with salt, hams are completely covered with dry
62 salt and placed in refrigerated rooms (0-4 °C, 70-95% R.H.) for a period of time
63 that differs based on product specifications defined by companies (Schivazappa
64 et al., 2010). In addition to the salting procedure (number of steps, length of
65 time between steps), other factors may affect salt uptake including raw material,
66 pH, skin trimming, extra- and intra-cellular fluid, fat layer, intra-muscle fat
67 content, the quality of the salt (type and size distribution) and the room
68 temperature (Arnau and Gou, 2001; Sánchez et al., 2008; Gou et al., 2008,
69 Garcia-Gil et al., 2012).
70 Diffusion is the most important mass transfer mechanism responsible for salt
71 uptake and water loss, due to the differences in concentration and osmotic
72 pressures among meat cells and salting agent (Raoult-Wack, 1994).
73 The normalized Weibull distribution is used to measure diffusive phenomena
74 since it's considered an adequate model in order to give an approximate
75 estimation of the diffusivity coefficient (Marabi et al., 2003); this model is also
76 considered as an alternative to Fick's equations for the non suitability of
77 assumptions (Petrova et al., 2015).
78 From the past decade to the present time many studies were carried out to
79 investigate salt diffusion and loss of moisture during dry-cured ham processing,
80 according to traditional analytical procedures (Grau et al., 2008) or by applying

non-destructive alternative methods (Fantazzini et al., 2009; Antequera et al., 2007, Picouet et al., 2013).

Recently, the improvement of salting control is a major goal for meat industry either to avoid oversalting or to meet the increasing demand for low-salt products. Health and nutritional concerns about sodium intake is currently leading food industries to optimise and/or to reduce the salt content in formulated and processed products, also for traditional ones.

Different models were developed to predict the salt content by alternative methods to its chemical analysis, that is time consuming and difficult to adapt to quality control routine checks. In particular, some studies tested the applicability of Magnetic Resonance Imaging (MRI) and computed tomography (CT) for the prediction of the salt content in hams (Caballero et al., 2016; Manzocco et al., 2013; Fantazzini et al., 2009; Fulladosa et al., 2010; Santos-Garcés et al., 2010; Santos-Garcés et al., 2012; Håseth et al., 2012); it has recently been evaluated the feasibility of using non-destructive technologies such as X-rays and ultrasound (US) for predicting the salt uptake in hams during the salting process (Fulladosa et al., 2015a, Fulladosa et al., 2015b).

However, these techniques, useful for research purposes, are not affordable for meat producers as routine analysis tools in order to monitor the process and the final product quality assessment.

The water activity (a_w) in meat products, hightly correlated with salt and moisture contents, is a critical parameter for microbial growth, according to Commission Regulations (EU) No 2073/2005 and No 852/2004 (EU, 2004, 2005); furthermore, a_w value is an important parameter to assure the safety of a long time ripening product as dry-cured ham (Pittia and Paparella, 2016).

In order to evaluate the stability of products in the meat industry, the determination of the a_w is widely used as a tool for quality control, through the use of low cost instruments and with a limited time of analysis (eg. dew point hygrometer or electrical hygrometer), though some authors also evaluated non-destructive method (CT) for predicting a_w in dry-cured hams (Vestergaard et al., 2005; Santos-Garces et al., 2010).

The aim of this research, carried out on a traditional Sauris PGI dry-cured ham, was: i) to study the salt uptake in different ham muscles with different weights and differently processed in terms of salting procedures; ii) to calculate salt diffusivity in *Biceps faemoris* (BF); and iii) to develop mathematical models to predict salt uptake (as defined by salting index, S.I. %) in BF muscles, sampled in different process steps, using water activity (a_w) values measured by dew point hygrometer.

2. Materials and methods

2.1. Materials and dry-cured ham process

A batch of one hundred and ten fresh hams (pH 5.6 ± 0.1) of pigs (crossbreed of Landrace, Large White and Duroc) from the same breeding were selected and used for this study. Upon arrival hams were sorted according to their weight in two classes: fifty five were classified as "small" (S) with an average weight in the range of 13.0 - 14.0 kg and the other ones were classified as “large” (L) with an average weight in the range of 14.5 ± 15.5 kg. The process was carried out with a partial trimming of hams, such as Prosciutto di Sauris (PGI) specification

131 (EU, 2010).

132 Five raw hams of each weight batch were used for the analyses of the initial raw
133 material characteristics.

134 The two weight-batches of raw hams (50 hams each) were then further divided
135 into two process lines different only for the salting procedure (Figure 1).
136 Keeping constant the length of the salting process: half of each weight-batch
137 (25 hams each) underwent the traditional (3s) salting, that includes 3 steps of
138 dry solid salt coverage procedure, according to the PGI regulation (Martuscelli
139 et al., 2009), and indicated as S-3s and L-3s (according to the respective
140 weight); the other half underwent a modified salting process, that was
141 developed only with 2 salting steps (2s) and indicated as S-2s and L-2s.

142 The salting process was carried out only with marine salt and no nitrite or
143 nitrate, as described by Martuscelli et al. (2015). An initial complete coverage of
144 the hams with dry salt was initially carried out by a salting machine Saimec
145 RSIX 2582 (Saimec Srl, Parma, Italy); this operation was followed by the
146 manual sprinkling of hams with salt onto specific critical areas (e.g. femur
147 bone). The salt-covered hams were then stored in a salting room at 3 ± 1 °C
148 and 95 % RH for 19 days. During this time hams were manually sprinkled twice,
149 at regular time intervals, with tiny amounts of salt to keep them always covered
150 by salt in three total coverage salt steps, (3s- samples). A modified salting
151 procedure was also carried out by performing only one additional salt coating
152 step at the middle of the salting time (e.g. 10 days) (2s- samples).

153 At the end of salting, each ham of the four batches was cleaned of residual
154 superficial salt by washing and underwent the following process conditions
155 (Martuscelli et al., 2015): (*resting*) 60 days, at increasing temperature from 1 ºC

to 16 - 18 ºC at a decreasing R.H. up to 85-80%; (*drying* and *smoking)* 15 days, at 20-22°C, 80-85% RH; (*smearing* and *ripening*) after application on the parts without rind of a mixture of pork fat, cereals flour and pepper *(sugna*), hams have been stored in the ripening rooms under environmental conditions (T: 12-15°C; RH: 80-85%) for fourteen months since the first salting.

To validate the NaCl predictive models (see section 2.6) a set of samples of dry cured ham, all collected in local supermarkets, was analysed. In particular this set includes: Sauris PGI hams (n = 16) produced in the same factory as above but coming from different raw materials batches, as well as Parma PDO hams (n = 3), PDO San Daniele hams (n = 3) and *Nostrano Abruzzese* hams (n = 3).

2.2. Sampling

Samples were taken at arrival (green hams, 0 days), at the end of salting (19 days), pre-resting (35 days), resting (97 days), middle ripening (180 days) and end of ripening (420 days). At each sampling time, five hams per weight and salting condition were collected, deboned, and cut on the cross section (10 cm from the bone of the thigh). Two slices (thickness, 3 cm) were taken for sampling at the widest section, according to the procedure described by Grau et al. (2008). Slices were individually packed under vacuum, frozen and stored at -30 °C and analyses were carried out within one week. Before the final sampling and further analysis, slices were left for two hours at room temperature, sufficient to equilibrate their temperature at 4°C.

Analyses were carried out on three portions of the cross-section slice, taken as representatives of the external and inner regions of the ham, which were individually sampled and homogenized. In particular, two muscle aliquots

corresponding to the *Semimembranosus m.* (inner-*SMi* and outer-*SMe*) and one to the *Biceps femoris m*. (*BF*) were sampled (see Figure 2). Coverage fat was discharged from all the samples but its height (cm) was measured in the fresh raw hams prior to sampling.

2.3 Chemical and physico-chemical analyses

All reagents for chemical analyses, were provided by Sigma (Steinheim, DE). Distilled water was used throughout the study when required for analytical purposes.

Moisture, NaCl, fat and protein content were determined according to AOAC official procedures (AOAC, 2002). In particular, moisture content was determined by drying about 3 g of sample in a forced-air drying oven at 105°C up to the constant weight. NaCl content was determined as chloride concentration by Volhard titration. Salt Index (S.I.%) was then computed as salt concentration (%) on water content (%), and expressed as g_{NaCl} 100 g_w^{-1}.

Fat content was determined by the Soxhlet method, using 40–60 petroleum ether. Ashes content was determined by mineralization of samples at 550 °C. Total nitrogen (TN) content (g 100 g_{dw}^{-1}) was determined by the Kjeldahl method and proteins by multiplying TN x 6.25.

Water holding capacity (W.H.C.) was performed on meat taken from the lean portion of the fresh hams. A sample of parallelepipeds shape (20 mm x 30 mm x 10 mm) was exactly weighed and placed on a net inside an inflated plastic bag and suspended for 48 h at 3°C. After the 48 h the sample was re-weighed, and W.H.C. computed as the weight change (%) due to drip loss.

Water activity (25 °C) was measured by a dew point hygrometer AquaLab CX 2 (Aqualab Scientific Pty Ltd., Castle Hill, NSW). The pH was determined using a pH electrode for solids on a Jenway pH-meter mod. 3510 (Bibby Scientific Ltd, Staffordshire, UK).

2.4 Image analysis

The lean and ham surface area were measured on pork ham cross-sectional slices using image processing technology according to Håseth et al. (2012). To calculate the percentage of dark-lean area in sample cross sectional area, images were captured using an image acquisition system in which the samples were illuminated using two pairs of parallel compact fluorescent globes (mod. PL E-D Pro, 23W/865, Philips, New York) with a colour temperature of 6500 K (D_{65}), a luminous efficacy of 60 Lm/W and a colour rendering index of 76%. The four bulbs (121 mm max diameter) were situated 45 cm above the sample and at an angle of 45º with it. A Color Digital Camera (CCD) QICAM Fast 1394 (QI Imaging, Burnaby, Canada), having a resolution of 1.4 million of pixel in a 12 bit digital output, was located vertically over the sample at a distance of 85 cm. The angle between the camera lens and the lighting source axis was around 45º. Lamps and CDC were held in a black box in order to exclude the surrounding light. White balance was carried out using a white standard tile (L* = 98.82; a* = -0.18; b* = -0.31) and the acquired images were submitted to spatial calibration. The cross sectional areas were isolated from the black background and processed using the software Image-Pro Plus® v. 6.2 (Media Cybernetics, Rockville, MD). After elimination of the bone area and conversion in grey scale (16 bit), images were submitted to the count of the dark-lean surface areas

using a grey scale threshold value of 45% while a threshold value of 100% was used for the total cross sectional surface area. The threshold value used for the area count permitted to isolate lean from the coverage and inter-muscular fat but not from the marble fat. The fat area was calculated by subtracting the lean area from the whole surface area

2.5 Calculations and mathematical modelling

The salt diffusivity ratio, SDR, was calculated as

$$SDR = \frac{S_t - S_0}{S_{¥} - S_0} \quad (1)$$

where: S_0, S_t and S_∞ are salt content at time zero, time t, and at equilibrium, respectively.

Thus, the salt diffusivity kinetics is expressed using as a base the median concentration of the salt (g_{NaCl} $100g_{dw}^{-1}$) in BF m. at the beginning of the process.

The normalized Weibull cumulative distribution was used to fit the NaCl diffusivity data according to Marabi et al. (2003) by assuming both one dimensional and one directional transport.

$$\frac{S_t - S_0}{S_\infty - S_0} = 1 - e^{-\left(\frac{t \cdot D_{calc}}{l^2}\right)^b} \quad (2)$$

where:

l = distance from the outer trimmed part to the core of BF (m);

D_{calc} = calculated salt diffusivity (m^2 s^{-1});

β = Weibull shape parameter (dimensionless).

The effective salt diffusivity, D_{eff}, was derived from Marabi et al. (2003).

$$D_{eff} = R_g^{-1} \times D_{calc} \quad (3)$$

where:

R_g =13.1 for planar samples

D_{eff} = effective salt diffusivity ($m^2\ s^{-1}$)

The theoretical $a_{w\ NaCl}$ values were calculated according to Chen (1989).

$$\frac{1}{a_{w,Nacl}} = 1 + M_w \left(b_e + B m^n \right) m \quad (4)$$

where:

M_w= molar mass of water (18 g mol^{-1})

β_e= constant (1.868)

B = constant (0.0582)

m = molality (mol kg_w^{-1})

n = dissociation number of NaCl in non-ideal solutions (1.618)

Based on the assumption that there is little interactions between solutes, and therefore their contributions to the chemical potential are additive, the theoretical a_w values were computed by multiplying the individual contributions of NaCl, and the remaining salts (ash) to obtain the water activity of the aqueous phase of the food according to Chen (1990).

$$a_w = \prod_s a_{w,s} \approx a_{w,Nacl} a_{w,ash} \quad (5)$$

The $a_{w,ash}$ values were calculated according to Gulati and Datta (1989).

$$a_{w,ash} = \frac{x_w}{x_w + \frac{n_{ash} M_w x_{ash}}{M_{ash}}} \quad (6)$$

where:

M_{ash}= molar mass of ash

n = dissociation number of ash

x_w = mass fraction of water (g g^{-1})

For calculation purposes, the M_{ash} value of 72 g mol^{-1} estimated by van der Sman and Boer (2005) was used. Moreover, as the majority of natural occurring salts in meat is monovalent, the dissociation number of ash was assumed equal to 2 according to van der Sman and Boer (2005).

The modelling of the experimental values of S.I.% as a function of a_w, was carried out by using linear and second degree polynomial models.

2.6 Statistical analysis

All determinations were done in triplicate, except where differently indicated. Means and standard deviations were calculated and shown in figures or tables.

Analysis of variance was performed to test the significance of the effects of the factor variables (weight, salting steps, muscle, time) and multiple mean comparisons with Tukey's honestly significant difference (HSD) test was used to test the significance of differences among the mean values of samples.

Non-linear regression was performed on the experimental data using the least square method and the “Levenberg-Marquadt” algorithm. The goodness of fit of the models was evaluated by the determination coefficient (R^2), the root mean square error (RMSE), and the residual distribution.

The NaCl predictive models were validated by cross validation using external samples (see section 2.1) and the error of prediction was calculated by the root mean square error of validation (RMSEV).

Data were processed by using Microsoft Excel 2011 for Mac and Statistica 8.0 for Windows (StatSoft^{TM}, Tulsa, UK).

3. Results and discussion

3.1. Raw material characteristics

The weight of the raw hams did not affect compositional and physico-chemical characteristics of BF and SM muscles (Table 1), this is consistent with the results reported by Martuscelli et al. (2015).

On the other hand, the weight of the raw ham significantly affected ($p < 0.05$) the ratio between fat and lean muscle, as evaluated by image analysis, which was lower in the L batch (0.40 ± 0.03) than in the S one (0.47 ± 0.03). Whereas the thickness of cover fat was very variable and ranged from 0.8 to 2.5 cm and from 1.0 to 3.0 cm in L and S batches, respectively.

3.2 Salt penetration during salting and resting as affected by factor variables

Figure 3 shows the evolution of salt concentration in the three muscle portions of the hams salted differently until the end of the resting step. The NaCl concentration was determined on fat-free dry weight ($g_{NaCl}\ 100_{ffdw}^{-1}$) to avoid the variability of the raw material composition as determined by the fat content (Grau et al., 2008). As expected, independently of the weight and salting procedure, the salt content varied more significantly and with a higher rate in the SMe muscle than in SMi and BF ones. This due to the external position of SMe in the ham, directly in contact with the dry salt and the saturated salt solution that appears as soon as water comes out from the meat and solubilizes part of the salt. In this portion salt reached its maximum content (11 - 13 $g_{NaCl}\ 100_{ffdw}^{-1}$) at the end of the salting stage. The salt content of all samples reached

a plateau condition except for that of 3s-L hams, in which it continued to increase until the end of resting step.

In all samples, the salt concentration of the SMi portion progressively increased upon time by reaching, after the resting time, values similar to those detected in the corresponding SMe (Figure 3). The same tendency was observed in the inner BF muscle but with a slower rate, and this led to a salt concentration of about 8-10% at the end of the resting step.

Analysis of variance (ANOVA) highlighted a significant effect of muscle ($p < 0.001$), salting step ($p < 0.05$) and weight ($p < 0.05$) on the salt content at the end of the resting step; the combined effect salting step x weight was also significant ($p < 0.01$). Salt migration occurs faster in the outer muscle portions (*Semimembranosus*, *Semitendinosus*), generally less abundant in intra-muscular fat (Fantazzini et al., 2009) and these muscles act as "reserve" of salt. During the post-salting steps NaCl will diffuse and penetrate in the inner muscle portions with a flow which occurs in the opposite direction to that of water (Schivazappa et al., 2010). According to the process conditions applied during dry-ham processing, the change in NaCl content after the salting step (over 21 days of processing) depends on the diffusive phenomena among the various muscle portions within the same ham as the exceeding salt, not dissolved on the surface of the meat and penetrated into the product, was completely removed by washing at the end of salting.

3.3 Salt penetration in Biceps femoris muscle during the whole process

Since the salt uptake in the inner muscle portion (corresponding to BF m.) is important in order to achieve the physico-chemical conditions (i.e. a_w)

350 determining product safety, in this study the overall salt diffusivity during the
351 whole ripening process was calculated. Salt concentration in the BF m. was
352 determined over 420 days of processing time and values in the range of 12 and
353 15 g 100 g_{dw}^{-1},depending on salting conditions, were found at the end of
354 ripening.
355 The salt diffusion in BF during the salting and early post-salting time is rather
356 slow, thus, in order to compute the apparent diffusion coefficient, the normalized
357 Weibull cumulative distribution was used. The latter gives analytical solutions
358 quite similar to Fick's equation but contains a β parameter, whose value
359 indicates the existence of a lag phase and which is also considered for the non-
360 ideality of the assumptions (Petrova et al., 2015). For calculation purposes the
361 maximum distance from the outer perimeter and the core of BF was measured
362 and used for D_{calc} computation by assuming one dimensional and one
363 directional flux; this because the diffusivity through the skin and outer layer
364 cover fat was considered negligible being up to two orders lower than that of
365 muscles (Wood, 1966; Fox 1980).
366 Figure 4 shows the kinetics of NaCl penetration during processing time and the
367 fitting of the normalized Weibull distribution model in the four batches under
368 investigation.
369 The salt diffusivity values along with the model parameters and goodness of fit
370 indices are reported in Table 2. T□□□β values were always higher than one,
371 indicating the existence of a lag phase in the early stage of processing prior to
372 the salt penetration in BF muscle by diffusion. The inverse of the β value, which
373 corresponds to the initial rate of salt penetration, is higher in S than in L hams

374 as well as higher in 3s- than in 2s- hams and the same results were observed
375 for the salt content at equilibrium values.
376 Independently of the salting procedure, the calculated salt diffusivity (D_{calc}) was
377 the highest in the L hams (Table 2), and, within the latter samples, it was the
378 highest in those subjected to the 3s-salting process. The highest salt diffusivity
379 in L samples could be related to the lower surface : area ratio between fat and
380 lean muscles in L samples (0.40) when compared to S samples (0.47), or to the
381 higher thickness of the external fat layer of S samples, as previously reported,
382 or, maybe, to the higher specific surface area of salt penetration related to the
383 thickness of the hams.
384 By assuming that salt penetration is exclusively driven by diffusion, an attempt
385 to calculate the effective diffusion coefficient was carried out by adopting a R_g
386 factor of 13.1 according to Marabi et al. (2003); the effective salt diffusivity (D_{eff})
387 resulted in the order of 10^{-10} (Table 2) which is similar to those reported by
388 Woods (1966) and Fox (1980). These authors calculated the salt diffusion on
389 outer muscles (e.g. SMe) during the salting process, which is generally carried
390 out at about 1-5 °C, whilst in the present study the overall salt diffusion
391 coefficient was evaluated in the most inner muscle during the whole process,
392 which was carried out in a wider temperature range i.e from 2 °C (salting) up to
393 15 °C (resting and ripening) (Martuscelli et al., 2015).
394 The S_∞ value, which represent the salt concentration at time $t \rightarrow \infty$, was
395 considered as the salt concentration at equilibrium as it represents the
396 asymptote of the cumulative Weibull distribution function.
397 As a consequence of salt diffusion in the muscle, moisture loss occurs (Toldrá,
398 2002), resulting in an increase of dry matter. The water loss in BF muscles was

399 described as a function of salt uptake by using linear regression analysis
400 (Figure 5). The average regression coefficient is about 9 and this result
401 indicates that, under the considered experimental conditions, the inflow of one
402 mole of sodium chloride implies a counter flow of 27 moles of water. Under the
403 experimental conditions of this study, the molal volume of sodium chloride is
404 higher than the molar volume of water and the latter is expected to diffuse faster
405 than the former as predicted by the Stokes-Einstein equation. However, water
406 loss is not only dependent on diffusion but is driven by changes in osmotic
407 pressure and by water evaporation rate at the vaporization surface.
408 The regression coefficient of the water loss vs salt uptake linear regression
409 resulted the same (8.8 ± 0.7) for S and L hams, whilst it was nominally higher in
410 2s- samples (9.2 ± 0.5) than in 3s- ones (8.3 ± 0.3). Being water loss equal, the
411 2s- samples showed a lower salt content, than 3s- samples, which indicates
412 that the reduction in the number of salting steps was effective in reducing the
413 salt uptake of about 10% at an equal moisture level.
414
415 *3.4. Relationship between salt concentration, theoretical and experimental a_w*
416 The evolution of the diffusive phenomena of salt (inwards) and moisture
417 (outwards) during the ripening step (420 days) caused the decrease of the a_w in
418 the final products, so as to ensure their quality and safety (Martuscelli et al.,
419 2015). Water activity describes the 'freedom' of water in a food matrix in terms
420 of relative water vapour pressure (Reid, 2007). In processed meat products, the
421 determination of the water activity (a_w) is important since its sufficiently low
422 values are required to limit both the growth of pathogenic micro-organisms

(Pittia and Paparella, 2016) and the activity of some enzymes implied in the maturation process (Blesa et al., 2008).

A progressive and significant decrease of water activity values was observed until the end of the resting process with a_w values ranging from 0.990 (raw BF m.) to 0.91 (3s-S SMe at the end of ripening). However, at the end of resting step, the water activity value depended only on the type of muscle ($p < 0.01$), without any effect of the number of the salting treatments or weight of raw hams (data not shown).

Water activity describes the macroscopic translational mobility of water from the foods to outside the food due to differences in chemical potential (Schmidt, 2007). For intermediate moisture food (IMF) products like dry-cured hams, the chemical potential is mostly affected by their salt (NaCl) content, even if the water freedom also depends on the concentration of other solutes (e.g. ashes), which is influenced by the process of ripening (van der Sman and Boer, 2005).

The theoretical water activity values of BF muscles (from 2s-, 3s-, S and L weight samples at different times of processing) were calculated using Eq. 4 according to Chen (1989) and the equation permitted to predict a_w values with good accuracy (Figure 6). A positive bias of 0.012 was observed at high a_w values, since the contribution of ashes was not taken into account (Figure 6a,b). When the contribution of ashes to the theoretical water activity was calculated according to Eqs. 5 and 6 (Chen, 1990; Gulati and Datta, 1989), the bias at high a_w values was reduced to 0.006 (Figure 6c,d). No bias was observed at low a_w values (Figure 6c,d) and this indicates that other small organic ligands (e.g. amines, aminoacids and di- or tri-peptides) which are formed during ripening, don't contribute to the a_w.

448

449 *3.5 Prediction of salt content by water activity*

450 In this study it was predicted the salt index (S.I. %, g_{NaCl} $100g_w^{-1}$) of BF muscle
451 on the basis of water activity values, since the a_w of the dry-cured hams
452 resulted mainly correlated to the salt content expressed in terms of molality. The
453 salt index is in fact a largely used index in the quality control of dry-cured meat
454 process that relates the salt content to the actual moisture of the product.

455 This predictive modelling was attempted with the aim of helping the direct
456 estimate of NaCl concentration in dry-cured ham by reducing the number of
457 experimental determination as well as time-consuming analyses and reagent
458 costs.

459 The model was calibrated on 90 data from slices of independent samples. Table
460 4 shows the range (min-max) for water activity, pH and other chemical
461 parameters measured in the *Biceps faemoris* m. of the ham samples used for
462 the calibration of the model. The physico-chemical parameters and chemical
463 composition of the products were investigated in order to define the range of
464 validity of the obtained model and to make the application of this model possible
465 also for similar products (e.g. cured, seasoned and ripened meats, bacon, etc.).

466 The S.I. % values of the samples of BF m. of hams at different process steps
467 were modelled as a function of the corresponding a_w values by a first and a
468 second degree polynomial models which fit well as shown in Figure 7 *a* and *b*
469 (determination coefficients of 0.92, in both cases).

470 Fantazzini et al. (2009) used Magnetic Resonance Imaging (MRI) for the
471 prediction of salt content in hams and observed major deviations in the range of
472 2.5-4.5% NaCl content and an overestimation over 5% NaCl, which

corresponds to the salt concentration of commercial products. On the contrary, the residual distribution of the model proposed in this study is not dependent on the sodium chloride content as shown in Figure 7 *c* and *d*, where the residuals plot and the root mean square error of calibration are reported. Moreover, in this study, the prediction made regarding the salt content, was even more accurate at the end rather than at the beginning of the dry-curing process because, as previously discussed, the ashes contribution to a_w is higher in raw than in dry-cured hams.

In Figure 8 (a and b) the observed vs predicted plots of the calibration data set, with linear and polynomial model, respectively, are shown. The root mean square error of calibration (RMSEC) was calculated and resulted of 1% for both the linear and polynomial model (Table 3). This error in the S.I.% determination corresponds to an error in the determination of the NaCl content of 0.73 and 0.71% for the linear and polynomial models, respectively, and these values are within the RMSEC range observed by Santos-Garcés et al. (2010) who used a tomographic technique to detect salt content in dry-cured hams.

In order to confirm the model adequacy, a validation of the results was carried out by cross validation using 25 hams (validation group), which were not used for the calibration of the models. The validation group included IGP Sauris dry-cured hams, other PDO dry-cured hams (Parma and San Daniele) and also not branded hams (Nostrano Abruzzese).

The two calibration models permitted to predict the salt content (S.I. %) of the validation group with good accuracy as all values were within the 0.95 prediction interval of the observed vs predicted regression (Figure 8 a, b). The root mean square error of cross validation (RMSECV) was of 0.62% and 0.61%

for the linear and polynomial model respectively. In general, the polynomial model permitted to achieve the lowest RMSECV (0.49%) on Sauris IGP hams, which are the types of ham used for the calibration set, whilst the linear model permitted to achieve the lowest RMSECV (0.21%) on other types of commercial hams.

4. Conclusions

The use of mathematical models to predict the salt content of the dry-cured hams on the basis of a_w values can be proposed as a possible solution for the process and quality control of meat processing companies since it is rapid, easy to perform and at low cost.

The salt prediction method proposed in this study could be an alternative to both conventional analytical (e.g. Volhard method) and innovative instrumental techniques (MRI, computed tomography) which are useful for research purposes but not as routine tools readily.

Furthermore, the predictive error in the salt index determination is of 1%, which corresponds to an error in the determination of the NaCl content of about 0.7%. Even though the magnitude of the error in NaCl content prediction is higher than that of the conventional analytical techniques, the model could have practical utility for product quality controls.

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Figures captions

Figure 1. Block scheme indicating the numerousness of samples for each experimental batch.

Figure 2. Cross section of ham: sampling points for external and internal *Semimembranosus* (SMe and SMi) and *Biceps faemoris* (BF) muscles and distance (l) measured for the calculation of salt uptake in BF.

Figure 3. Change of NaCl content (g $100g_{ffdw}^{-1}$) after salting (19 d), during (35 d) and at the end of the resting step (97 d), in different muscles of hams of different size (S and L), subjected to two or three steps of salting process (2s- and 3s-, respectively). 2s-S (a), 3s-S (b), 2s-L (c), 3s-L (d); in the same graphic, data marked with different italic letters are significantly different (Tukey test, $p<0.05$).

Figure 4. NaCl (g $100g_{dw}^{-1}$), in the Biceps femoris muscle of small (S) and large (L) size hams subjected to two (2s) and three (3s) steps of salting process. 2s-S (a), 3s-S (b), 2s-L (c), 3s-L (d). The arrow indicates the end of salting process.

Figure 5. Relationship between salt uptake and water loss (g $100g_{dw}^{-1}$) in Biceps femoris muscle of hams of different size (S and L), subjected to two or three steps of salting process (2s- and 3s-, respectively). 2s-S (a), 3s-S (b), 2s-L (c), 3s-L (d). Dashed line represents 95% confidence interval.

Figure 6. Theoretically predicted vs observed water activity (a_w) values in Biceps femoris muscle of hams: $a_{w\ NaCl}$ predicted by S.I.% (a), predicted $a_{w\ NaCl}$ residual plot (b), a_w predicted by S.I.% and corrected by ash content (c), predicted a_w residual plot (d). Solid line represents the ideal model.

Figure 7. Predictive models of the salting index ($g_{NaCl}\ 100g_{H_2O}^{-1}$) of Biceps femoris by water activity (a_w). Linear model (a), linear model residual plot (b), second degree polynomial model (c), and its residual plot solid line represents the regression line and dashed line the root mean square error of calibration.

Figure 8. Calibration and validation data sets with linear (a) and polynomial model (b) respectively Regression and 0.90 prediction interval.

Figure 1

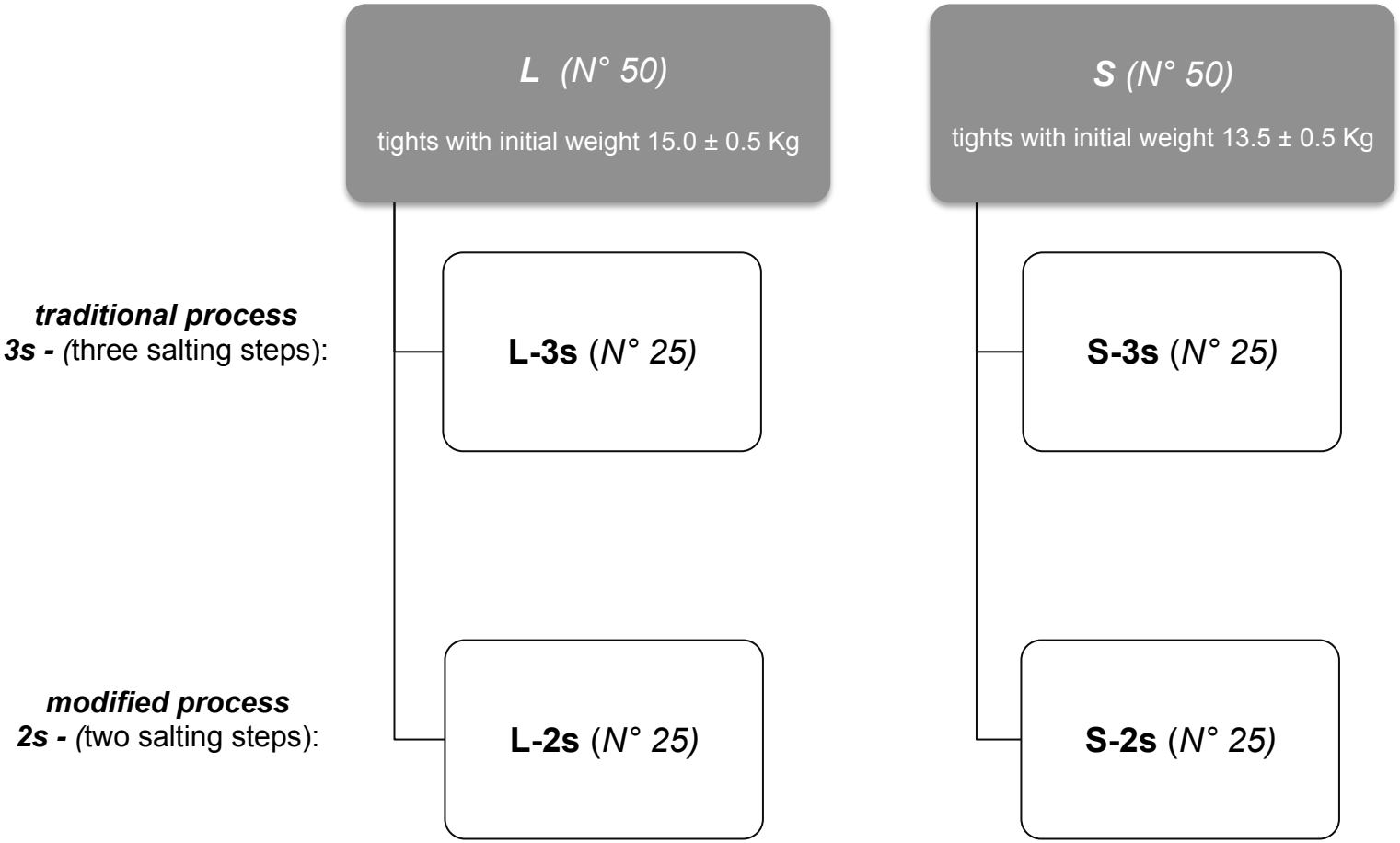
L (N° 50)
tights with initial weight 15.0 ± 0.5 Kg
S (N° 50)
tights with initial weight 13.5 ± 0.5 Kg
traditional process
3s - (three salting steps):
L-3s (N° 25)
S-3s (N° 25)
modified process
2s - (two salting steps):
L-2s (N° 25)
S-2s (N° 25)

Figure 2

Click here to download high resolution image

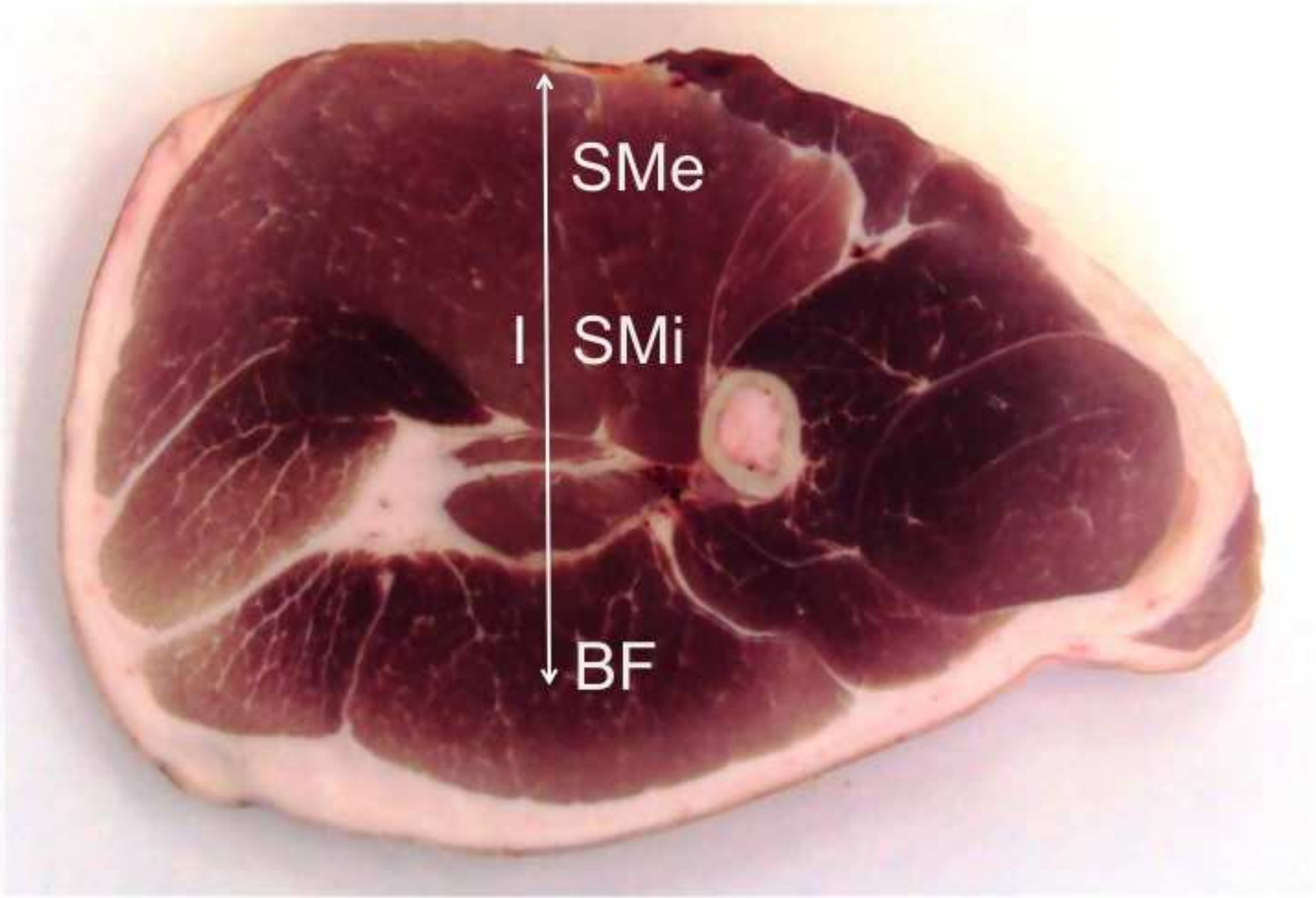

Figure 2

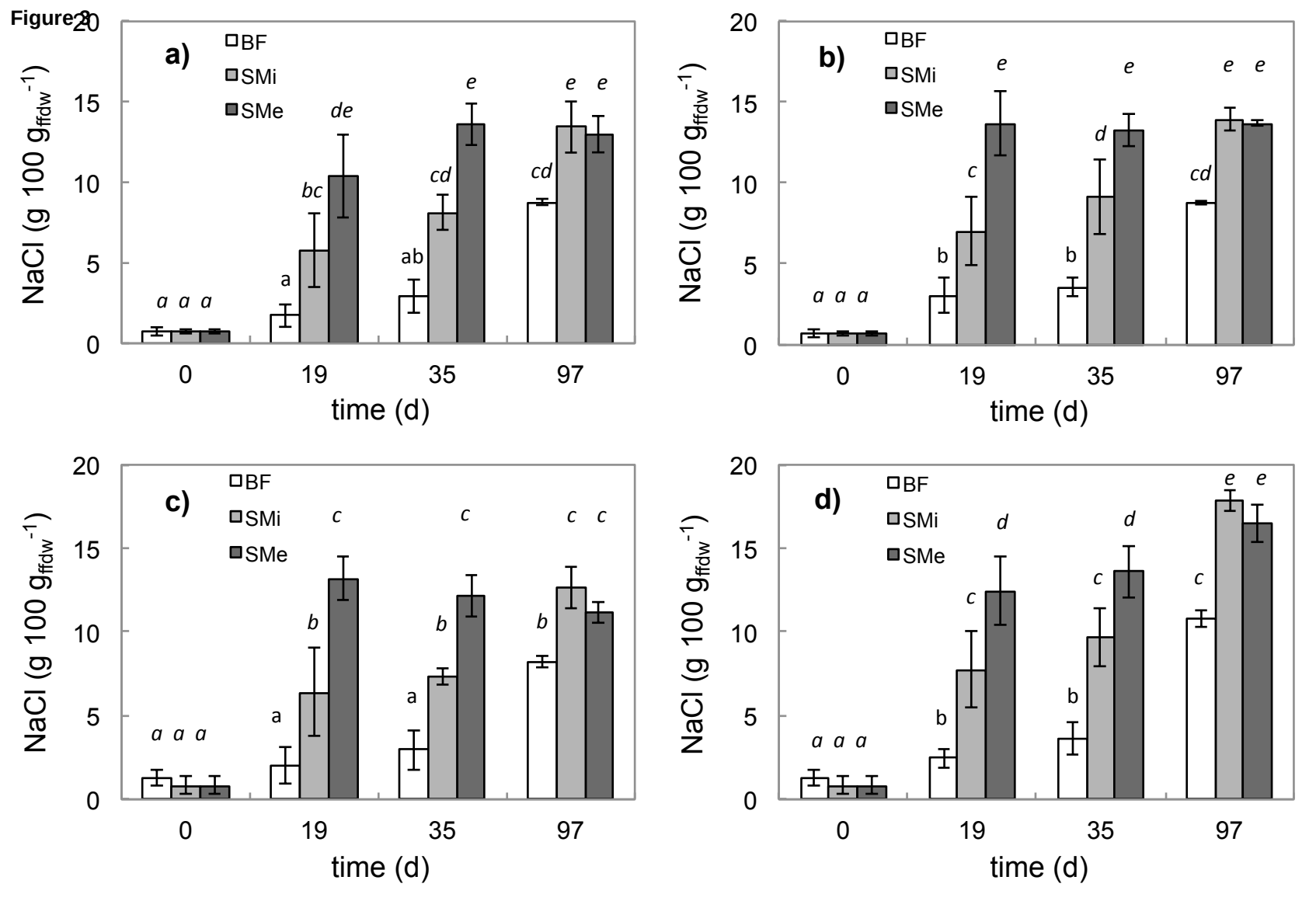

Figure 4

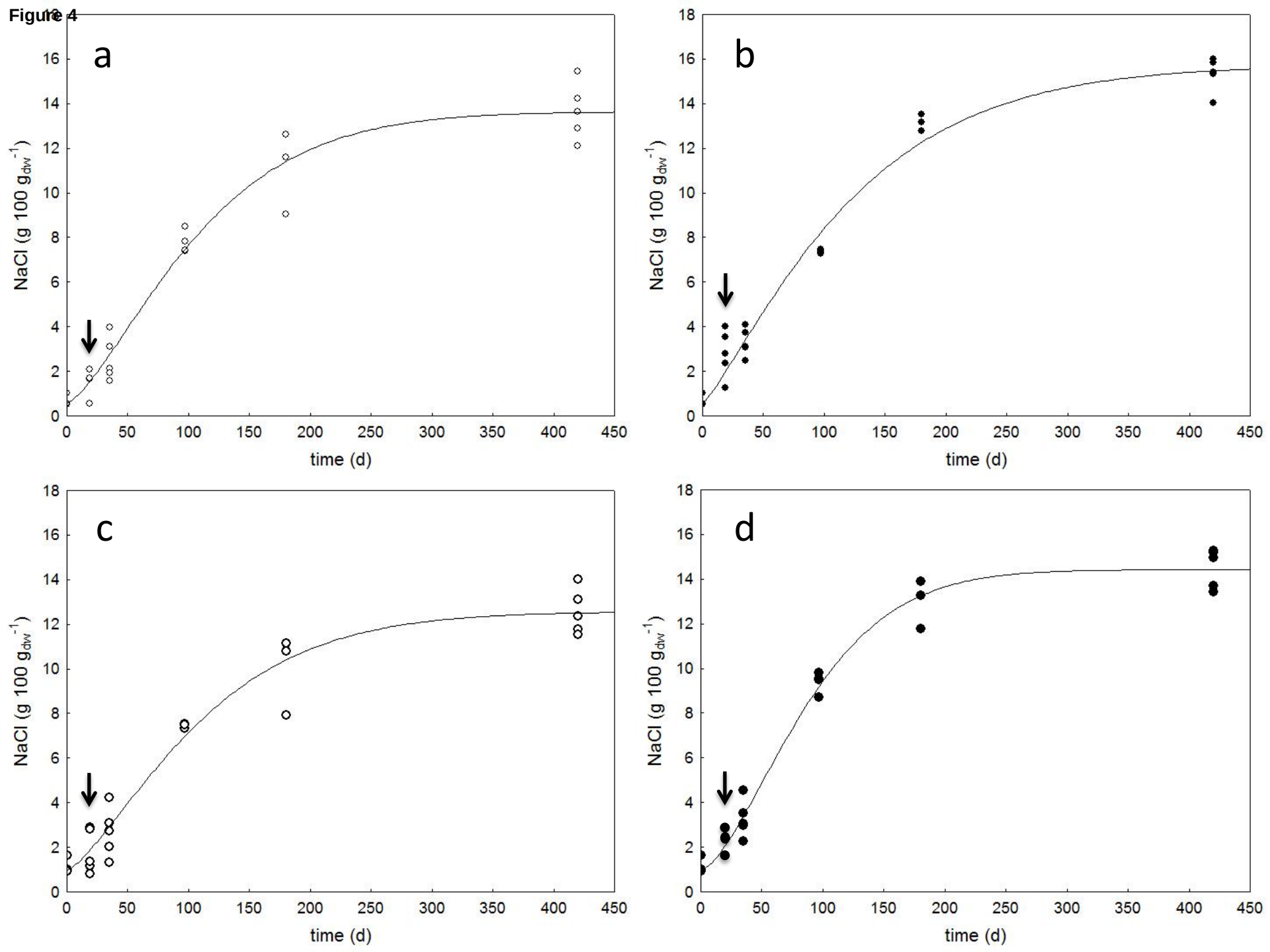
a
b
c
d
NaCl (g 100 g_{dw}^{-1})
time (d)
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300
350
400
450

Figure 5

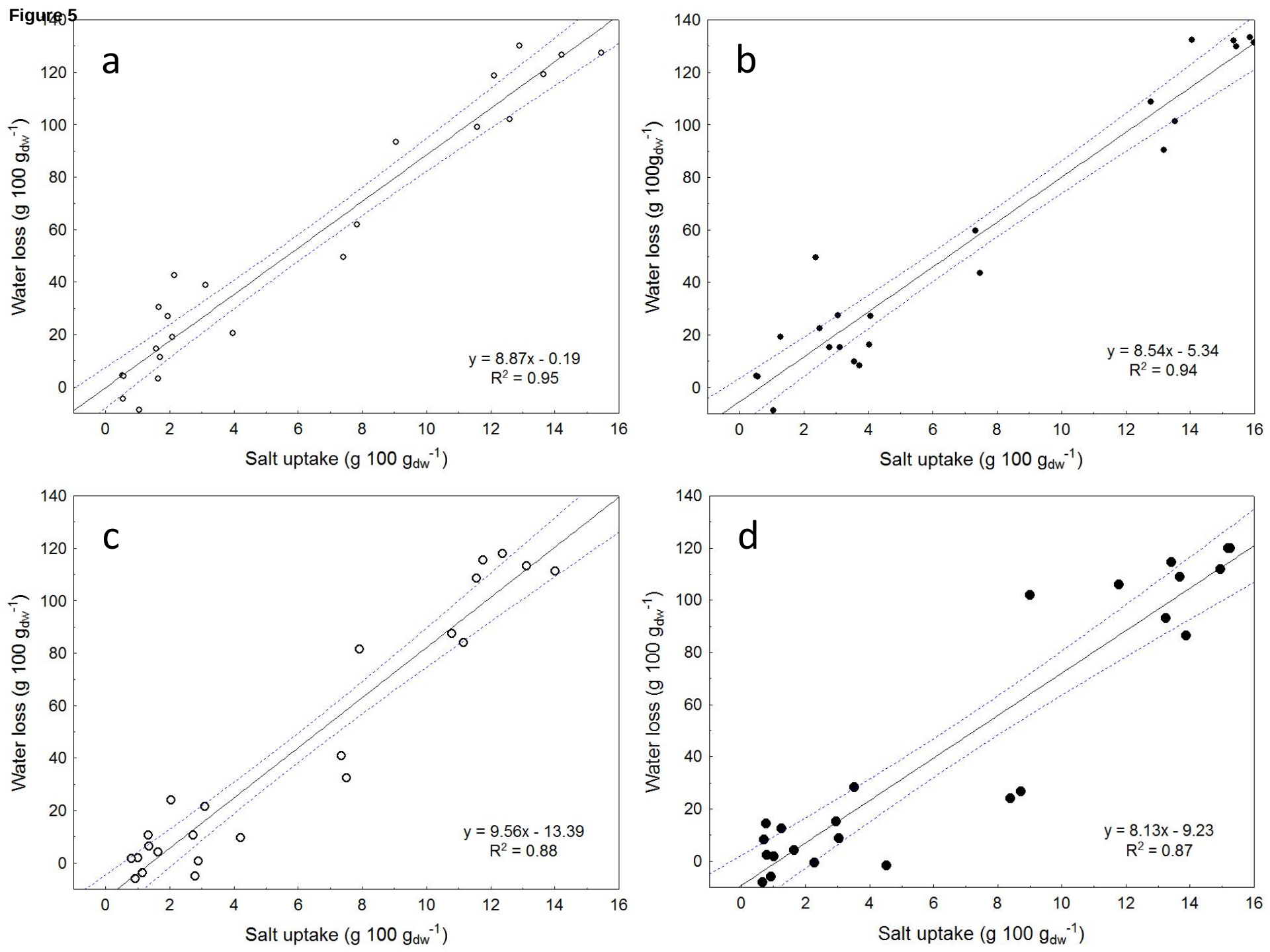
a
Water loss (g 100 g_{dw}^{-1})
Salt uptake (g 100 g_{dw}^{-1})
y = 8.87x - 0.19
R^2 = 0.95
b
Water loss (g 100g_{dw}^{-1})
Salt uptake (g 100 g_{dw}^{-1})
y = 8.54x - 5.34
R^2 = 0.94
c
Water loss (g 100 g_{dw}^{-1})
Salt uptake (g 100 g_{dw}^{-1})
y = 9.56x - 13.39
R^2 = 0.88
d
Water loss (g 100 g_{dw}^{-1})
Salt uptake (g 100 g_{dw}^{-1})
y = 8.13x - 9.23
R^2 = 0.87

Figure 6

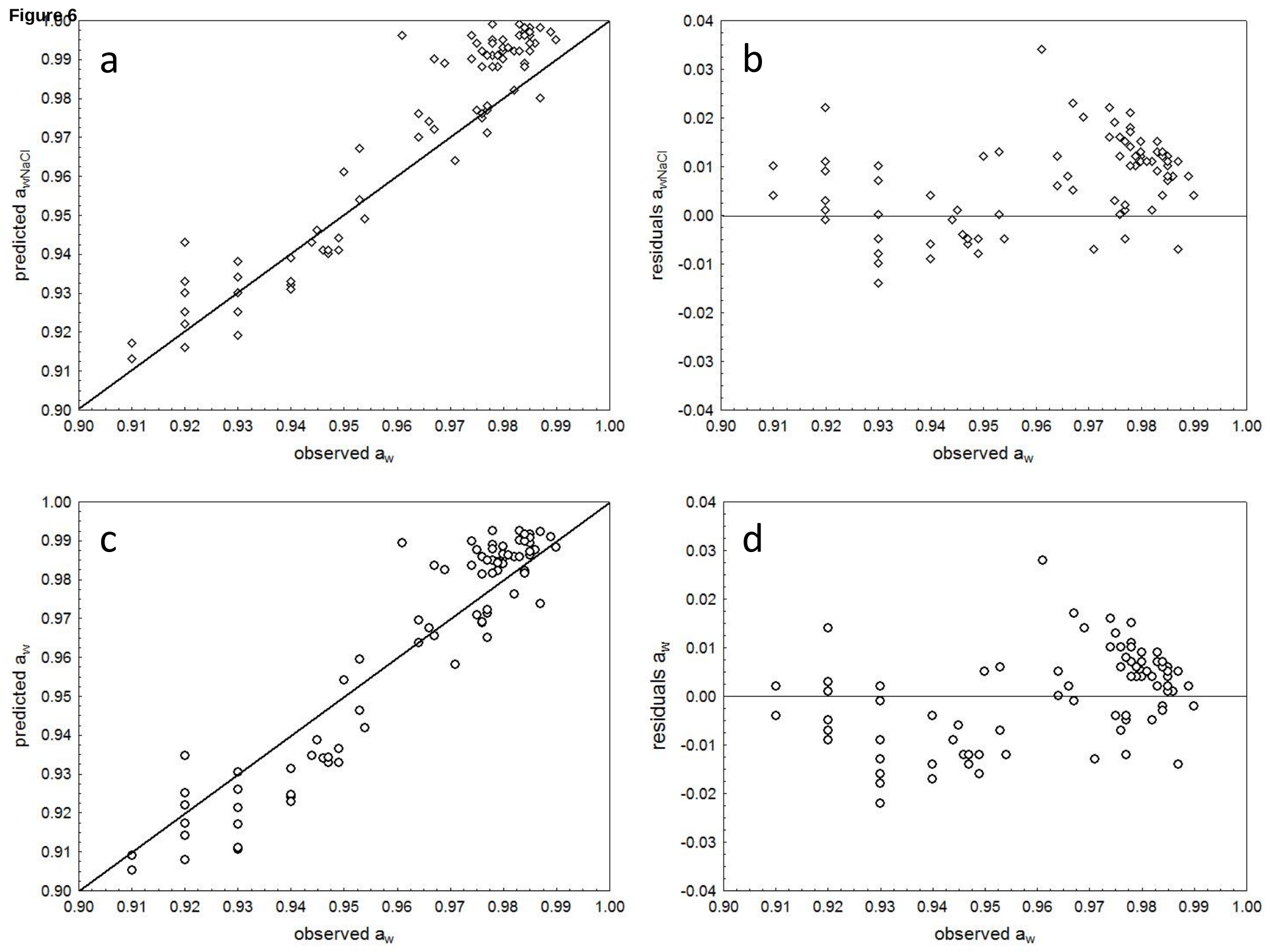
a
predicted a_{wNaCl}
observed a_w
b
residuals a_{wNaCl}
observed a_w
c
predicted a_w
observed a_w
d
residuals a_w
observed a_w

Figure 7

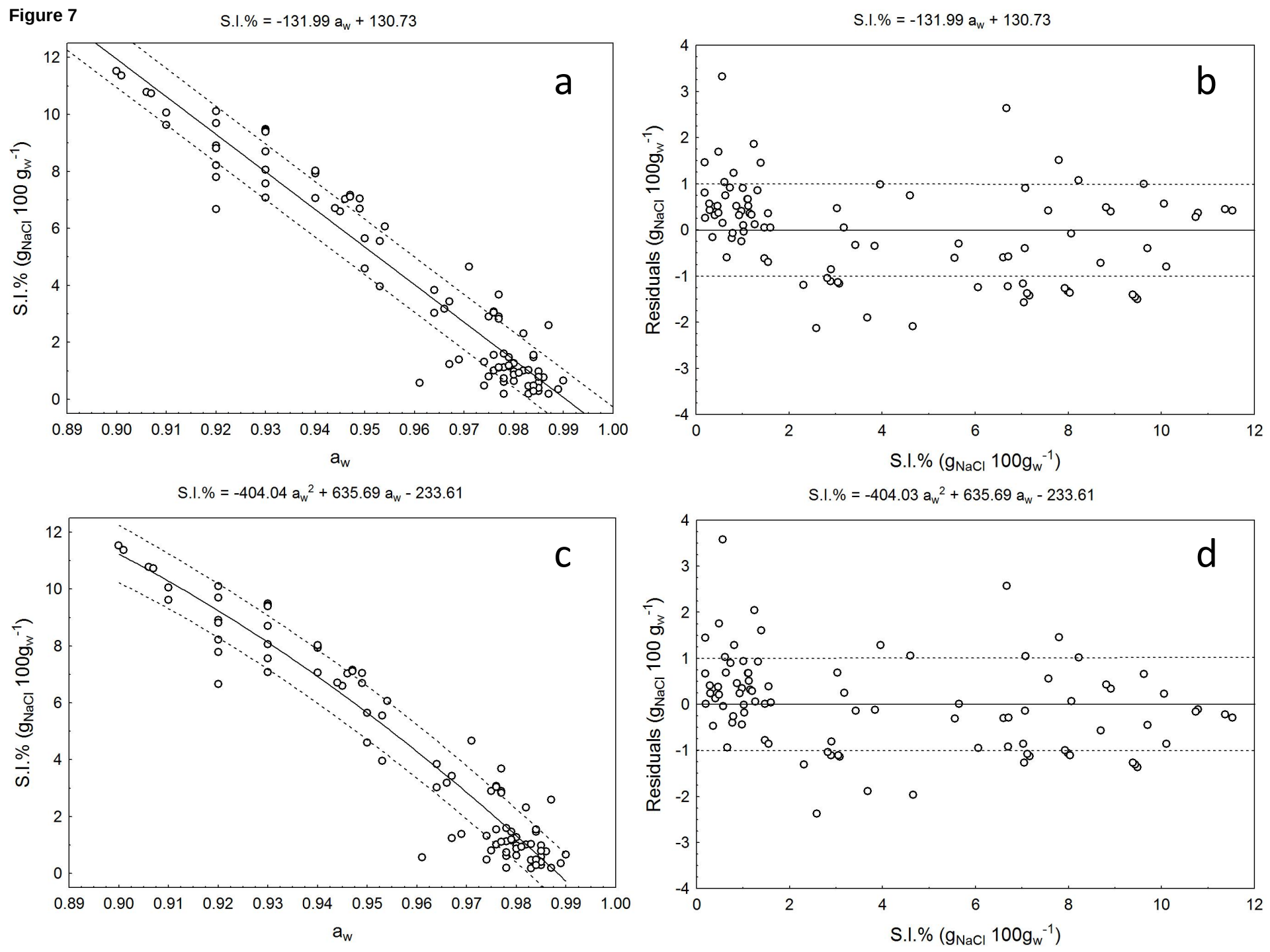
S.I.% = -131.99 a_w + 130.73
a
S.I.% (g_{NaCl} 100 g_w^{-1})
a_w
S.I.% = -131.99 a_w + 130.73
b
Residuals (g_{NaCl} 100g_w^{-1})
S.I.% (g_{NaCl} 100g_w^{-1})
S.I.% = -404.04 a_w^2 + 635.69 a_w - 233.61
c
S.I.% (g_{NaCl} 100g_w^{-1})
a_w
S.I.% = -404.03 a_w^2 + 635.69 a_w - 233.61
d
Residuals (g_{NaCl} 100 g_w^{-1})
S.I.% (g_{NaCl} 100g_w^{-1})

Figure 8

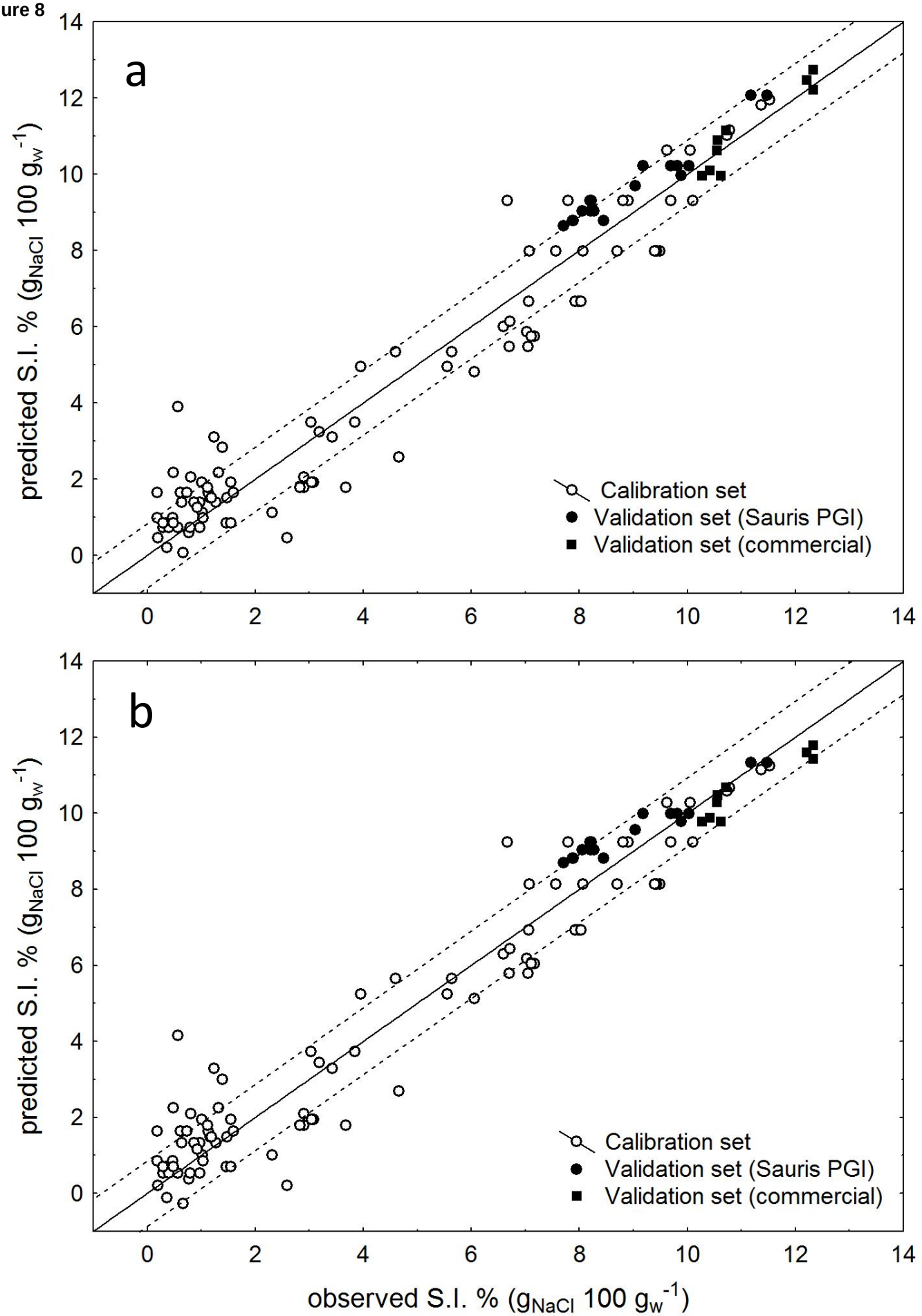
a
b
predicted S.I. % (g_{NaCl} 100 g_w^{-1})
observed S.I. % (g_{NaCl} 100 g_w^{-1})
Calibration set
Validation set (Sauris PGI)
Validation set (commercial)
0
2
4
6
8
10
12
14

Table 1

Table 1. Chemical and physico-chemical characteristics (mean ± standard deviation) of *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles of raw hams of small (S) and large (L) weight and significance of the muscle and weight effects as evaluated by ANOVA.

	muscle	weight		Effect	
		S	L	muscle	weight
Dry weight	BF	25.47 ± 0.52	26.04 ± 2.01	n.s.*	n.s.
(g 100 g^{-1})	SM	25.41 ± 0.50	25.62 ± 0.60	n.s.	n.s.
Protein	BF	91.11 ± 1.76	88.10 ± 4.08	n.s.	n.s.
(g 100g_{dw}^{-1})	SM	92.39 ± 3.22	90.87 ± 4.57	n.s.	n.s.
NaCl	BF	0.46 ± 0.17	1.09 ± 0.45	n.s.	n.s.
(g 100g_{dw}^{-1})	SM	0.66 ± 0.14	0.82 ± 0.51	n.s.	n.s.
Ashes	BF	4.35 ± 0.07	4.38 ± 0.13	n.s.	n.s.
(g 100g_{dw}^{-1})	SM	4.62 ± 0.05	4.21 ± 0.68	n.s.	n.s.
Total fat	BF	5.13 ± 3.54	8.32 ± 4.40	n.s.	n.s.
(g 100g_{dw}^{-1})	SM	3.28 ± 1.99	4.59 ± 3.45	n.s.	n.s.
W.H.C.	BF	7.95 ± 3.36	10.03 ± 6.02	n.s.	n.s.
(drip loss, %)	SM	9.98 ± 2.64	7.38 ± 2.53	n.s.	n.s.
pH	BF	5.50 ± 0.11	5.64 ± 0.01	n.s.	n.s.
(-)	SM	5.51 ± 0.03	5.54 ± 0.11	n.s.	n.s.
a_w	BF	0.985 ± 0.003	0.987 ± 0.001	n.s.	n.s.
(-)	SM	0.988 ± 0.001	0.986 ± 0.003	n.s.	n.s.

*n.s., no significance.

Table 2

Table 2. Estimation of the parameters and goodness of fit of the cumulative Weibull distribution applied to model salt uptake in *Biceps femoris* muscles of different ham samples over time.

Size	Salting	β	D_{calc}	S_E	R^2	RMSE
			$m\ s^{-1}\ 10^{-9}$	$g_{NaCl}\ 100\ g_{dw}^{-1}$		$g_{NaCl}\ 100\ g_{dw}^{-1}$
S	2s	1.35±0.15	1.07±0.04	13.6±0.5	0.968	1.76
	3s	1.20±0.17	1.17±0.04	15.7±0.6	0.976	1.24
L	2s	1.37±0.13	1.60±0.07	12.6±0.5	0.958	1.58
	3s	1.53±0.15	1.94±0.05	14.4±0.3	0.982	1.97

± standard error

Table 3

Table 3. Estimation of the parameters and goodness of fit of the models applied to predict the salting index (S.I.%) in BF muscles of different ham samples from a_w values.

Parameter	Unit	Predictive model	
		Linear	Polynomial
Intercept	(g_{NaCl} 100 g_w)	131	-230
a_w	(-)	-132	636
a_w^2	(-)	-	-404
R^2	(-)	0.918	0.921
RMSEC	(g_{NaCl} 100 g_w)	1.05	1.03
$RMSEV_{(all\ samples)}$	(g_{NaCl} 100 g_w)	0.62	0.61
$RMSEV_{(Sauris\ IGP\ samples)}$	(g_{NaCl} 100 g_w)	0.55	0.49
$RMSEV_{(commercial\ samples)}$	(g_{NaCl} 100 g_w)	0.21	0.33

Table 4. Range of chemical and chemico-physical parameters measured in BF muscle of dry-cured hams employed to data modelling.

	range (min-max)
Moisture (g 100 g^{-1})	60.3 - 74.5
NaCl (g 100 g^{-1})	0.1 - 6.7
Fat (g 100 g^{-1})	1.3 - 3.9
Protein (g 100 g^{-1})	22.9 - 30.8
Ashes (g 100 g^{-1})	1.1 - 7.7
a_w (-)	0.90 - 0.99
pH (-)	5.6 - 6.0