# Risk management tool to define a corrective storage to enhance *Salmonella* inactivation in dry fermented sausages

Cristina Serra-Castelló[a], Sara Bover-Cid[a], Margarita Garriga[a], Tina Beck Hansen[b], Annemarie Gunvig[c1], Anna Jofré[a*]

[a]IRTA, Food Safety Program, Finca Camps i Armet s/n, 17121 Monells, Spain

[b]National Food Institute, Technical University of Denmark, Kemitorvet, Building 202, DK-2800 Kgs. Lyngby, Denmark

[c]Danish Technological Institute, DMRI, Gregersensvej 9, DK-2630 Taastrup, Denmark

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*Corresponding author: Anna Jofré. IRTA, Food Safety Program, Finca Camps i Armet s/n, 17121 Monells, Spain

Email: anna.jofre@irta.cat

[1]Present address: Annemarie Gunvig, Dagrofa, Kærup Industrivej 12, 4100 Ringsted, Denmark

**Abstract**

The resistance of *Salmonella* to the harsh conditions ocurring in shelf-stable dry fermented sausages (DFS) pose a food safety challenge for producers. The present study aimed to model the behaviour of *Salmonella* in acid (with starter culture) and low-acid (without starter culture) DFS as a function of $a_w$ and storage temperature in order to build a decision supporting tool supporting the design of a corrective storage strategy to enhance the safety of DFS. *Salmonella* spp. were inoculated in the raw meat batter at ca. 6 Log cfu/g with a cocktail of 3 strains (CTC1003, CTC1022 and CTC1754) just before mixing with the other ingredients and additives. After stuffing, sausages were fermented and ripened following industrial processing conditions. Different drying-times were applied to obtain three batches with different $a_w$ (0.88, 0.90 and 0.93). Afterwards, DFS were stored at 4, 8, 15 and 25 ºC for a maximum of three months and *Salmonella* spp. were periodically enumerated. The Weibull model was fitted to Log counts data to estimate inactivation kinetic parameters. The impact of temperature and $a_w$ on the primary inactivation parameters was evaluated using a polynomial equation. The results of the challenge tests showed that *Salmonella* spp. levels decreased during storage at all the assayed conditions, from 0.8 Log (in low-acid DFS at 4 ºC) up to 6.5 Log (in acid DFS at 25ºC). The effect of both $a_w$ and temperature was statistically significant. Delta ($\delta$) parameter decreased by decreasing $a_w$ and increasing temperature, while the shape ($p$) parameter ranged from above 1 (concave) at 10 ºC to below 1 at 25 ºC (convex). A common secondary model for the $p$ parameter was obtained for each type of DFS, acid and low-acid, indicating that acidification during the production of DFS affected the time for the first Log reduction ($\delta$) during the subsequent storage, but not the overall shape ($p$ parameter) of the inactivation. The developed models covered representative of real conditions, such as *Salmonella* contamination in the raw materials and its adaptation to the harsh processing conditions. The good predictive performance shown when appling the models to independent data (i.e. up to 80% of the predicions within the 'Acceptable Simulation Zone' for acid sausages) makes them a suitable and reliable risk management tool to support manufacturers to assess and design a lethality

treatment (i.e. corrective storage) to enhance the *Salmonella* inactivation in the product before DFS are released to the market.

**Keywords**

Pathogens; meat products; non-thermal inactivation; modelling; decision support tool; control measure

## *1. Introduction*

The production of dry fermented sausages (DFS) is one of the oldest forms of preserving meat (Ojha et al. 2015). As shelf-stable food, DFS do not support the growth of pathogenic microorganisms and refrigeration is not required to retain organoleptic acceptability. However, shelf-stability is not a guarantee of safety, which should be addressed in the production steps through a process not only inhibiting the growth but ensuring a sufficient reduction of the pathogens of concern that may be present in the raw materials.

The microbiological safety of DFS is mainly associated with the quality of raw materials and manufacturing practices, which determine the type and the initial levels of pathogenic microorganism potentially present (Barbuti & Parolari, 2002; Mutz et al., 2020) and the product formulation and the fermentation and drying conditions, which determine the time course of physicochemical characteristics changes during the DFS production process. Within this framework, pH (acidification due to the production of organic acids, mainly lactic acid) and water activity ($a_w$, reduction due to salting and drying) are the two intrinsic factors of high importance governing pathogen behaviour as part of the hurdle technology (Bonilauri et al., 2019; Leistner, 2000). Within the wide variety of DFS types, in Europe a differentiation between acid (usually northern) and low-acid (usually from Mediterranean area) DFS (Demeyer et al., 2000; Lebert et al., 2007) has been described. In acid non-thermally treated DFS and especially in mildly fermented low-acid (usually pH≥5.3) traditional DFS, typical from the Mediterranean region such as fuet, a low diameter DFS typical from Catalonia (Aymerich et al., 2003; Martin et al., 2011), the pathogen-controlling efficacy could be diminished and the safety of the product compromised (Jofré et al., 2009).

*Salmonella* is one of the most relevant pathogens in DFS due to its ability to survive acid and low $a_w$ conditions (Mutz et al., 2020). Although a decrease of *Salmonella* loads during the DFS production process is usually reported, it was also shown to survive certain processes (Gunvig et al., 2016; Jofré et al., 2009; Martin et al., 2011; Skandamis & Nychas, 2007). DFS contaminated with *Salmonella* has been epidemiologically linked to several salmonellosis outbreaks. From 2016 to 2020, up to eight notifications were recorded in the EU Rapid Alert System For Food

and Feed portal (RASFF, https://webgate.ec.europa.eu/rasff-window/portal/) about outbreaks in France, Sweeden, Denmark related with the presence of *Salmonella* in DFS from France, Spain, Italy and Poland (notification references 2020.5038; 2020.3378; 2018.1111; 2018.0246; 2017.1846; 2017.1511; 2016.1340; 2016.0492). These notifications highlight the ability of *Salmonella* to survive during the DFS production process and storage due to its resistance to acidity and low $a_w$ conditions (Mutz et al., 2020; Tiganitas et al. 2009), posing an important challenge for food business operators to accomplish with the zero-tolerance policy for *Salmonella* (no detection in 25 g of n=5 analysed units per verified lot) required by current European food safety microbiological criteria regulation (European Commission, 2005). Therefore, the development of strategies based on post-processing treatments can be useful. For instance, non-thermal technologies such as high pressure processing of DFS has been studied (Bonillauri et al, 2019; Jofré et al., 2009; Porto-Fett et al., 2010) though the low $a_w$ usually found in DFS exerts piezoprotection effect against *Salmonella* inactivation and reduces its efficacy (Bonilauri et al., 2019; Bover-Cid et al., 2012; Bover-Cid et al., 2017). Moreover, the investment cost of this technology is not always affordable by food producers. In this regard, strategies based on the enhancement of the hurdle technology, making the most of the physicochemical characteristics of DFS can be developed. For instance, few studies have proposed the implementation of a corrective storage period after the manufacturing process to enhance the reduction of verotoxigenic *Escherichia coli* in DFS (Hansen et al., 2011) and *Listeria monocytogenes* in dry-cured ham (Serra-Castelló et al., 2020), in both cases developing decision support tools for a proper implementeation of such control measure. Hwang et al. (2009) modelled the survival of *Salmonella* during the storage of soudjouk-style fermented sausages, an acid type (pH <5.2) sausage made of beef, which does not cover the conditions of the small-diameter acid (pH<5.3) and low-acid (pH≥5.3) traditional European pork DFS.

In this framework, the present study aimed to evaluate the behaviour of *Salmonella*, inoculated in the raw materials before stuffing, during the storage of low-acid and acid DFS. The behaviour of *Salmonella* was tackled through a modelling approach, which quantified the pathogen inactivation as a function of the product $a_w$ and storage temperature. The final objective was to

provide a risk management tool assisting the design of a feasible and cost-effective control measure contributing to ensuring the accomplishment of zero-tolerance policies and commercial requirements.

## *2. Material and methods*

### *2.1* Salmonella *strains*

A cocktail of three strains of *Salmonella enterica* from IRTA-Food Safety Program`s collection isolated from pork meat products and belonging to different serotypes, i.e. CTC1003 (London), CTC1022 (Derby) and CTC1754 (Rissen), was used in the present study. Inoculum cultures were prepared by growing each strain independently in Brain Heart Infusion (BHI) broth (Beckton Dickinson, Sparks, Md., USA) at 37 °C for 7 h and subsequently sub-cultured again at the same temperature for 18 h (i.e. till the stationary phase of growth was reached). Final cultures were preserved frozen at −80 °C in the growth medium supplemented with 20% glycerol until being used (Hereu et al., 2012).

### *2.2 Preparation, inoculation, processing and storage of dry fermented sausages*

Meat batter was prepared by mixing minced lean pork meat and fat (4:1). Following the mixing, the meat batter was inoculated with a cocktail of the three *Salmonella* strains (0.3% v/w) prepared by mixing equal number of cells for each strain using frozen cultures prepared as described in section 2.1 and diluted in water to achieve a final concentration of *ca*. 6 Log cfu/g. The meat batter was mixed for 1 min in the mixing machine (Mix-35P, Tecnotrip, Spain) in order to homogenize the inoculum in the batter before adding the following ingredients and additives (in g/kg): water, 30; NaCl, 18; dextrose, 5; black pepper, 2; sodium ascorbate, 0.5; $NaNO_2$, 0.1; $KNO_3$, 0.1. Finally, in low-acid DFS batches no starter culture was added, while a mixture with the starter cultures *Lactobacillus sakei*, *Pediococcus pentosaceus* and *Staphylococcus xylosus* (Aymerich et al., 2003; Marcos et al., 2007) was added to produce acid DFS batches. After addition of the cultures, the meat batter was mixed for an additional 1 min.

The inoculated meat batters were stuffed in 36-38 mm diameter natural pork casings using a stuffing machine (H15, Tecnotrip, Spain) and sausages of ca. 25 cm in length were elaborated. After, sausages were dipped into a solution of *Penicillium candidum* and *P. nalgiovensis* spores (Danisco, France). Sausages were let to dry at room temperature (18 - 20 ºC) for the dripping/drying of the casings and subsequently hung in a Versatile Environmental Test Chamber MLR-350 H (Sanyo Electric Co., Ltd. Japan) adapted with an Hygrotest 600 PHT-20/120 transmitter (Testo) for the fermentation and drying processes. Sausages were fermented for 1 day at 22 ºC with 85-86 % of relative humidity (RH). Afterwards, during the drying process, the RH conditions were set up to gradually decrease RH from 85 to 65 % and increase temperature from 13 to 18 ºC. With the aim to obtain sausages with different $a_w$ (0.88, 0.90 and 0.93), the duration of the drying processes was 20, 19 and 10-11 days, respectively.

A total of six batches of DFS were obtained, combining different $a_w$ (0.88, 0.90 and 0.93) and pH (≥5.6 and ≤5.1 for low-acid and acid DFS, respectively). For details on the physicochemical and microbiological analysis, including *Salmonella* counts, of the products during the processing see Supplementary Table 1. The obtained DFS were stored in perforated plastic bags and ramdomly distributed in four groups to be stored at foreseeable storage temperatures (i.e. 4 ºC, 10 ºC, 15 ºC and 25 ºC) for a maximum of three months.

*2.3 Microbiological analysis during the ripening processes and the subsequent storage*

The levels of *Salmonella* were monitored by sampling along the production process (15 samples type of DFS and final $a_w$ value) and storage period (with a total of 10-30 samples depending on the storage temperature).

After aseptically removing the casing, 25 g of sausage were homogenized ten-fold in saline solution (0.85 % NaCl and 0.1 % Bacto Peptone (Beckton Dickinson)) in a bag Blender Smasher® (bioMérieux, Marcy-l'Etoile, France) for 1 min and 10-fold serially diluted in saline solution. *Salmonella* was enumerated on the selective and differential chromogenic *Salmonella* agar (CHROMagar™ *Salmonella* Plus; Scharlab, S.L., Sentmenat, Spain) after incubation at 37 ºC for 24 - 48 h. Samples with expected *Salmonella* concentration below the quantification limit

(4 cfu/g) were enriched in Rappaport-Vassiliadis (RV) broth (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 41 ºC for 24 h. After enrichment, the presence of *Salmonella* was checked by plating on the chromogenic *Salmonella* agar. The absence of *Salmonella* in non-inoculated meat batter was confirmed in all the batches.

Levels of Lactic Acid Bacteria (LAB) were determined during the production process of the sausages in MRS (de Man, Rogosa and Sharpe) agar plates (Merck, Darmstadt, Germany), which were incubated at 30 ºC for 72 h under anaerobiosis using sealed jars with an AnaeroGen sachet (Oxoid Ltd.).

### *2.4 Primary modelling of the* Salmonella *behaviour during storage*

For each combination of the conditions (acidity/$a_w$/storage temperature), the primary Weibull model (Eq. 1) was fitted to the *Salmonella* survival data (Log N) as a function of the storage time using the nls2 and nls packages of R (R Core Team, 2019).

$$Log(N) = Log(N_0) - \left(\frac{t}{\delta}\right)^p \quad \text{Eq. 1}$$

Where $Log(N)$ is the *Salmonella* concentration at given time, $Log(N_0)$ is the average value of the initial *Salmonella* concentration of three replicates at time zero of the storage period (i.e. end of drying), $\delta$ is the time (days) required for the first Log reduction of *Salmonella*, $p$ is a dimensionless parameter describing the shape of the inactivation curve (i.e. $p < 1$ concave; $p = 1$ linear and $p > 1$ convex) and $t$ is the storage time (days).

The goodness-of-fit of the developed models was assessed by standard error of the parameter estimates, residual sum of squares (RSS), root mean squared errors (RMSE).

### *2.5 Secondary model fitting*

Polynomial models were developed to quantitatively characterize the effect of $a_w$ and storage temperature on the kinetic inactivation parameters ($\delta$ and $p$) resulting from the primary modelling (Table 2).

193 Following the parsimony principle, the fit of the polynomial models to the kinetic inactivation
194 parameters, transformations (including square root, inverse, Ln and Log) were assessed
195 throughout the application of stepwise regression to obtain equations with only the significant
196 parameters. Estimation of model parameters and the associated standard errors was conducted
197 with the *nls* and *lm* function of the *nls2* and *stats* packages of the R software (R Core Team,
198 2019).
199 Besides the classical two-step modelling approach described above, the one-step or global
200 modelling approach (Jewell, 2012; Martino & Marks, 2007) was applied, i.e. secondary
201 polynomial models for the inactivation parameters ($\delta$ and $p$) were integrated into the Weibull
202 primary model equation.The goodness-of-fit of the developed models was assessed by the
203 standard error of the parameter estimates, RSS and the RMSE. The F-test (Eq. 2) was applied to
204 assess the need of two different models for low-acid and acid DFS (Zwietering et al., 1990).

205 $$F = \frac{{}^{(RSS_{NH} - RSS_{AH})}/_{(df_{NH} - df_{AH})}}{RSS_{AH} - df_{AH}} \quad \text{Eq. 2}$$

206 Where $RSS_{NH}$ and $df_{NH}$ were the Residual Sum of Squares and the degrees of freedom (number
207 of points-number of parameters of the model) respectively, of the global model common for
208 both types of DFS (null hypothesis) and $RSS_{AH}$ and $df_{AH}$ were the Residual Sum of Squares and
209 the degrees of freedom respectively, of the global model with specific parameter coefficients for
210 each type of DFS (alternative hyphotesis).
211 The effect of the environmental conditions on the shape inactivation curve of *Salmonella* was
212 assessed with the comparison of two global models: i) a global model with a polynomial model
213 for describing the effect of temperature and $a_w$ on the $p$ parameter and ii) a global model with a
214 fixed $p$ value independent of the environmental conditions. The comparison was assessed using
215 the F-test (Eq. 2), where $RSS_{NH}$ and $df_{NH}$ were the Residual Sum of Squares and the degrees of
216 freedom (number of points-number of parameters of the model) respectively, of the constrained
217 model (global model with fixed p value; null hypothesis) and $RSS_{AH}$ and $df_{AH}$ are the Residual
218 Sum of Squares and the degrees of freedom respectively, of the global model with a polynomial
219 model describing the effect of temperature and $a_w$ on the $p$ parameter (alternative hypothesis).

220

221 *2.6 Evaluation of the model performance*

222 Predictions obtained by the models developed were compared with totally independent data
223 obtained by the Technical University of Denmark (DTU) about *Salmonella* behaviour in acid
224 and low-acid fermented sausages during storage after being fermented and dried under the
225 conditions detailed in Gunvig et al. (2016). The data are included in Supplementary Tables 2
226 and 3. The Acceptable Simulation Zone (ASZ) approach was used to compare the predicted and
227 observed *Salmonella* reduction during the storage of the DFS. Due to the scattering of the
228 observed data, simulations were considered acceptable when at least 70% of the observed Log
229 N values were inside the acceptable zone of ± 1 Log (Møller et al., 2016).

230

231 *3. Results and Discussion*

232 *3.1.*Salmonella*, lactic acid bacteria and pH during the fermentation and drying processes*

233 In low-acid DFS without starter culture, a slight increase of *Salmonella* was observed during the
234 first days of the process, followed by a slight decrease, with a total reduction of less than 1 Log
235 unit. In this type of sausages, LAB took at least 7 days to reach the stationary phase (i.e. 8 Log
236 cfu/g) and pH did not decrease below 5.3 (Supplementary Table 1). In acid DFS, LAB reached
237 the stationary phase levels in just 1 day and the pH decreased down to 4.6-4.8. The highest
238 reduction of *Salmonella* levels, 2.5-2.7 Log units, was recorded for those processes leading to
239 acid DFS with the lowest $a_w$ (0.88 and 0.90) highlighting the role of acidification on the loss of
240 viability of *Salmonella*. On the other hand, in DFS with a higher $a_w$ (0.93) *Salmonella* counts
241 only decreased by ca. 1 Log, being statistically similar (p-value > 0.05) to the pathogen
242 inactivation observed in low-acid DFS with the same $a_w$. As a result of the different behaviour
243 of *Salmonella* occurring during the production of the different types of sausages, the levels and
244 the physiological status of the pathogen in the end-product (at the beginning of the storage)
245 were not equal in all the conditions studied and this might have influenced the subsequent
246 behaviour during the storage at different temperatures (section 3.2). It has been described that to
247 survive stresses intrinsically associated with fermentation and drying, *Salmonella* develops

complex mechanisms of stress adaptation increasing its tolerance and survival against harsh environmental conditions, thus affecting the behaviour during the subsequent storage of DFS (Mutz et al., 2020). The behaviour of *Salmonella* during the storage of DFS has been frequently investigated inoculating the pathogens on slices of the end product, e.g. (Calicioglu et al. (2002), Dalzini et al. (2014) and Porto-Fett et al. (2008). However, this approach does not represent the actual contamination event, as *Salmonella* comes from contaminated raw materials (Barbuti & Parolari, 2002) with a relevant prevalence in fresh pig meat used for DFS manufacture (up to 23.7%, Martin et al., 2011).

### *3.2.* Salmonella *behaviour during storage of low-acid and acid sausages*

Figure 1 shows the survival of *Salmonella* during storage in the 24 combination of conditions assayed. Results indicated that under the evaluated conditions, both low-acid and acid DFS were not only bacteriostatic but also bactericidal against *Salmonella*. However, different extent of *Salmonella* inactivation was observed depending on the acidity and the $a_w$ of the DFS as well as the storage temperature. Specifically, at the storage time of ca. 60 d, a reduction of 3.0 Logs in the *Salmonella* level was observed in low-acid DFS with a $a_w$ of 0.88 and stored at 25 ºC. At the same storage time and temperature, higher reductions of *Salmonella* were observed for the same type of DFS with higher $a_w$, 0.90 (3.7 Log) and 0.93 (4.0 Log), indicating that *Salmonella* could have adquired higher resistance during the manufacture of DFS with lower $a_w$ that those showing higher $a_w$ at the end of the drying. These findings are in agreement with those found by Farkos et al. (2013) dealing with low moisture foods inoculated with dried cells of *Salmonella*, showing an increased survival capacity with decreasing $a_w$ of the matrix. In the work performed with *L. monocytogenes* inoculated in slices of dry-cured ham, the lower $a_w$ the higher the inactivation (Serra-Castelló et al. 2020). In that case, however, *L. monocytogenes* was exposed to the product characteristics and storage conditions after the manufacturing, thus without previous adaptation, which can be the reason for the different impact of the $a_w$. In acid DFS, these reductions were enhanced by ca. 2 Logs, showing the relevance of the acidity of the product in promoting the pathogen inactivation. The effect of storage temperature was also very

275 remarkable since no relevant *Salmonella* reduction (< 1 Log) was found after 60 d of storage at
276 4 ºC in any of the products assessed. In summary, higher reduction of *Salmonella* was recorded
277 in DFS with higher $a_w$ and stored at higher temperatures and this inactivation was enhanced in
278 acid DFS. These results highlight the importance of the product intrinsic factors ($a_w$ and pH) and
279 its combination with the storage temperature. In this regard, the observed bactericidal effect
280 could be related with the metabolic exhaustion phenomenon associated with the combination of
281 antimicrobial hurdles in agreement with the principles of the hurdle technology developed by
282 Leistner (2000). Accordingly, in shelf-stable products with physicochemical characteristics not
283 supporting the growth of microorganisms the viability of bacterial cells is compromised because
284 they completely use up their energy trying to repair homeostasis mechanisms, causing a die-off
285 of the microorganisms along the storage. The inactivation rate is known to be higher when the
286 temperature increases towards the optimal growth for the microorganisms as well as when some
287 of the other physicochemical characteristics (pH or $a_w$) approach limits of the microbial growth
288 (Leistner, 2000; Serra-Castelló et al., 2020). In the present study, room temperature storage,
289 acidity and high $a_w$ (0.93, i.e. the minimum $a_w$ for *Salmonella* growth when other factors are
290 optimal (ICMSF, 1996) of the DFS, would be conditions favouring metabolic exhaustion.
291 Overall, results indicated that the storage of low-acid and acid DFS at selected temperature
292 conditions (e.g. 25 ºC) would favour the inactivation of *Salmonella* cells, even adapted to the
293 stress of fermentation and drying conditions. Therefore, sausage manufacturers can design a
294 control measure into their manufacturing operations based on this phenomenon to minimize the
295 risk of non-compliance with the *Salmonella* zero-tolerance policy.
296
297 *3.3. Primary modelling of Salmonella behaviour during storage*
298 The Weibull model (Eq. 1) was found to be appropriate to describe *Salmonella* reduction
299 (inactivation) during the storage (Table 1), as also reported in other low-moisture foods
300 (Santillana-Farakos, 2013), although the fit of the model was poor for low-acid DFS stored at
301 low temperature (4ºC) conditions due to the lack of inactivation within the time frame of the

present experiment (3 months). This was the reason for the associated high standard errors of the Weibull parameters estimated for this particular case.

At the three evaluated levels of $a_w$, higher values of the $\delta$ parameter of the Weibull model, i.e. the time for the first Log reduction of *Salmonella*, were obtained in low-acid DFS compared with acid DFS with the same $a_w$ and stored at the same temperature (Table 1), quantifying the enhanced *Salmonella* inactivation in acid DFS with reductions in $\delta$ of up to 2.4-fold in the driest DFS. Moreover, in both products, $\delta$ was increased with increasing $a_w$ and decreasing storage temperature, indicating the enhancement of the *Salmonella* lethality due to the low $a_w$ and high storage temperature (up to 25 ºC). For example, in low-acid DFS with the lowest $a_w$ (0.88), a ca. one week of storage at 25 ºC would be enough to decrease *Salmonella* counts by 1 Log, but little or no microbiologically relevant *Salmonella* inactivation would be expected after 90 d at 4 ºC.

These results were also supported by the *p* parameter of the Weibull model, that described different inactivation shape curves depending on the $a_w$ of the product and storage temperature. At 25 ºC, *p* values tended to be below 1 in most of the conditions, indicating a higher inactivation of the pathogen at the beginning of the storage followed by a slow down of the rate of inactivation of *Salmonella*. On the other hand, at lower storage temperatures, *p* values tended to be higher than 1 in most of the cases, corresponding to a convex curve (shoulder shape), indicating lower inactivation at the beginning of the storage, probably due to a slow down in the metabolism of *Salmonella* at temperatures close or below its minimum growth temperature, described to be below 7 ºC for most serotypes (ICMSF 1996).

*3.4. Secondary and global modelling*

The Log transformation for $\delta$ parameter and the inverse transformation for *p* parameter were chosen for both products, i.e. low-acid and acid DFS, as they gave the best fit (Table 2). The polynomial models developed indicated that $\delta$ and *p* parameters were linearly dependent on $a_w$ and storage temperature. In addition, the quadratic term found for $a_w$ in the polynomial model for *p*, described the great effect of $a_w$ on the shape of the *Salmonella* inactivation curve. Refined

model parameters (Table 2) were obtained through the one-step (global) approach, integrating of the secondary polynomials developed for the inactivation parameters in the Weibull primary model equation and the re-fitting of this combined model to the entire set of 350 data points of *Salmonella* in both types of DFS. Interestingly, the F-test indicated that equations obtained for $\delta$ parameters of both products were statistically different but not the ones describing the *p* parameter, thus, a unique model for the *p* parameter was considered for both low-acid and acid DFS. Despite not being significantly influenced by the type of product, *p* parameter showed to be affected by the environmental conditions, i.e. storage temperature and $a_{w.}$ The F-test (Eq. 2) statistical comparison between the global model with a polynomial model for the *p* parameter and the global model with a fixed *p* parameter, resulted in a high F value (121.09) showing that the constrained model (model with fixed *p*) could not explain the same variance as the complex model. Thus, results suggested that the effect of temperature and $a_w$ has to be considered when characterizing the shape of the inactivation curve of *Salmonella* in fermented sausages.

The inoculation of *Samonella* in the raw materials of different types of sausages lead to different levels in the final product, i.e. at the beginning of storage. Despite this could be a drawback as different initial levels could affect the characterization of the behaviour of the pathogen, this is especially relevant when dealing with low inoculum levels, where the variability in the counts together with being in a region close to the plate count detection limit highly affect the shape of the inactivation/growth curves (Mataragas et al., 2015). However, in the present study the levels of *Salmonella* recorded at the end of the drying process (i.e. at the beginning of the storage period assessed), were high enough to allow a proper characterization of the shape of the inactivation curve of the pathogen during the storage time. At the same time, data covered the impact that the sequential exposure of *Salmonella* to stresses during the DFS manufacturing processes (acidification and drying) could have on the subsequent inactivation during the storage, which should not be covered if *Salmonella* had been inoculated in the end product without being exposed to the fermenentation and drying. Tiganitas (2009) highlighted the impact of the order in the application of hurdles, showing that the lethality due to acid and osmotic stresses was higher when the stresses were applied sequentially compared to their

simultaneous application. Our results would indicate that fermentation (acidification due to organic acids produced by LAB) would increase *Salmonella* sensitivity during storage. The impact of drying (low $a_w$) was different at the beginning of the storage in comparison with the later stages of the study. In this respect, for the $\delta$ parameter, the lower the $a_w$ the shorter the time for the first log reduction, thus a lower $a_w$ favored the early inactivation. However, when taking into account the long term data, considering the whole inactivation curve (*p* parameter), the results indicate that lower $a_w$ resulted in lower total inactivation, indicating that the low $a_w$ favored the occurrence of a tail of resistant cells.

Therefore, the model was built considering representative of foreseeable industrial conditions leading to different *Salmonella* levels and physiological states as a result of the different resistance and adaptation of the pathogen to the process conditions. The model will provide useful information to manufacturers producing different types of DFS to assess the feasibility of applying a short storage period prior to their release to the market, thaking the advantage of the non-thermal inactivation effects of the product on *Salmonella*.

### *3.5. Effect of acidity on* Salmonella *inactivation during storage*

Results from the secondary and global modelling (section 3.4) indicated that the level of acidification in DFS affected the time for the first Log reduction of *Salmonella* but not the inactivation curve shape. Therefore, contrary to $a_w$, the acidity of the product enhanced *Salmonella* inactivation without changing the overall shape inactivation behaviour of the pathogen towards $a_w$ and storage temperature, indicating these were the main factors influencing the shape of the curve of *Salmonella* during storage. Interestingly, a linear relationship was observed when plotting the ratio of $\delta$ predicted by the global model from acid and low-acid DFS with the same $a_w$ versus storage temperature (Figure 2) and it was quantified through a linear relationship described by Eq. 3 with a goodness-of-fit of $R^2_{adj}$ of 0.964.

$$\frac{\delta_{acid}}{\delta_{low-acid}} = 0.4494 + 0.0138 \cdot T \qquad \text{Eq. 3}$$

where $\delta_{acid}$ and $\delta_{low\text{-}acid}$ are the δ values predicted by the global model for acid DFS (Table 2) and T is the storage temperature.

In acid DFS, the time for the first Log reduction of *Salmonella* decreased by 50, 41, 34 and 21% at storage temperatures of 4, 10, 15 and 25ºC, respectively, in comparison with the values found in low-acid DFS, indicating that the effect of the acidification during the DFS production (leading to different levels and physiological status of the pathogen) on the subsequent *Salmonella* inactivation was stronger at lower storage temperatures.

Regarding $a_w$ and although the ratio $\delta_{acid}$ / $\delta_{low\text{-}acid}$ was systematically higher in sausages with higher $a_w$, it was not statistically different from ratio of $\delta$ found in sausages with lower $a_w$ at the same storage temperature (p-value>0.05), indicating that the ratio of $\delta$ was not significantly affected by the $a_w$ when sausages were stored at the same temperature. Thus, the effect of acidity on the first Log reduction of *Salmonella* in DFS was suggested to be mainly dependent on the storage temperature but not on the $a_w$.

### *3.6. Assessment of the predictive performance of the developed models*

Only a few scientific studies are available regarding the behaviour of *Salmonella* during the storage of DFS. In these studies considerably different fermentation and drying conditions, diameter and sausage formulation were used, which are reported to affect the inactivation of *Salmonella* in DFS (Mataragas et al., 2015), hindering the comparison of the pathogen reduction loads reported by literature with the ones obtained in the present study. Moreover, in most of them, *Salmonella* was inoculated into ripened DFS, thus, without taking into account the effect of the progressive adaptation to the harsh product caracteristics on the *Salmonella* behaviour during the storage period.

Low-acid and acid DFS with characteristics and physicochemical parameters similar to the ones assessed in the present study were studied by Gunvig et al. (2016) and the *Salmonella* counts obtained during the storage of these products were used as totally independent data to evaluate the predictive performance of the developed models (Supplementary Tables 2 and 3). Results showed that for low-acid DFS, 65/115 (62%) of the predictions obtained with the developed

412 model were within the ASZ (± 1 Log) (Supplementary Table 2). It is worth to highlight that
413 most of the residuals obtained with the comparison of the observed and predicted *Salmonella*
414 counts were negative, especially for temperatures above 16 ºC, indicating that the model
415 provided slightly fail safe predictions. On the other hand, this trend was not observed at 5ºC,
416 where slightly/or no inactivation of *Salmonella* was expected. These results could be explained
417 by the conservative pH values (i.e. worst case scenario, pH 5.6-5.7) of the DFS used in the
418 present study for developing the model, which were slighly higher than those of the DFS (pH
419 5.1-5.6) used for the evaluation of the predictive performance of the model for low-acid DFS.
420 Regarding the prediction of *Salmonella* counts in acid DFS, 94/117 (80 %) of the predictions
421 were within the ASZ (± 1 Log) (Supplementary Table 3), indicating a good predictive
422 performance of the model developed for acid DFS.
423 Overall, results showed the good predictive performance of the models and reported evidences
424 that models could be an objective and reliable tool to calculate the *Salmonella* reduction by the
425 application of a corrective storage period.
426 The developed model quantified the inactivation of *Salmonella* during the storage of DFS with
427 different physicochemical properties (i.e. different $a_w$ and pH at the beginning of storage). The
428 greatest strength of the model lies in the experimental design of the study, which through the
429 simulation of a *Salmonella* contamination in the raw materials, takes into account the harsh
430 conditions of the processing process.
431
432 *3.7. Application of the developed models*
433 The bactericidal effect against *Salmonella* observed during the storage of DFS could be used for
434 sausage manufacturers as a lethality treatment to enhance the *Salmonella* inactivation in the
435 product before being released into the market, particularly if suspected to be contaminated with
436 the pathogen. The predictive models developed in this study would assist manufacturers to set
437 the necessary time and temperature to achieve the desired reduction of *Salmonella* in different
438 types of sausages (low-acid and acid) as a function of the $a_w$ of the finished product. In this
439 framework, the developed model predicts that a short corrective storage time of 5 to 8 d

(depending on the $a_w$ of the DFS) would let to a 1 Log reduction of the *Salmonella* concentration in acid DFS. Overall, and considering the estimated shelf-life of the fermented sausages, the application of a such corrective storage time immediately after the drying process and before the commercialization of the product could be used by sausage manufacturers as a control measure to enhance the reduction of *Salmonella* levels.

## *4. Conclusions*

Dry fermented sausage manufacturers can take advantage of the time-temperature conditions of the storage and the physicochemical characteristics of the product, mainly $a_w$, to further enhance *Salmonella* inactivation. For this purpose, the developed models quantifying the bactericidal effect of the temperature and low $a_w$ during the storage of DFS can be used by food manufacturers as a risk management tool to design a corrective storage and hence, to establish a risk minimization strategy to enhance *Salmonella* reduction when the fermentation and drying processes are not enough to reduce the levels of *Salmonella* in the product.

## *5. Acknowledgements*

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606 *Figure Captions*

607 **Figure 1.** Behavior of *Salmonella* in low-acid and acid dry fermented sausages (DFS) with
608 different $a_w$ and stored at 4, 10, 15 and 25 °C. Symbols represent the observed pathogen counts
609 in Log cfu/g (n=3) and lines show the fit of the global model shown in Table 2.

610 **Figure 2.** Ratios of predicted $\delta$ of low-acid and acid dry fermented sausages with different $a_w$
611 and stored at different storage temperatures (4, 10, 15 and 25 ºC). The line shows the fit of the
612 linear model according to Eq. 3.

613

614

615 *Table Captions*

616 **Table 1**. Estimated inactivation kinetic parameters resulting from fitting the primary Weibull
617 model to the *Salmonella* counts obtained for low-acid and acid dry fermented sausages (DFS)
618 with different physicochemical characteristics and stored at different temperatures.

619 **Table 2.** Estimated coefficients of the global model resulting from the fitting to values of the
620 primary and secondary inactivation kinetics of *Salmonella* in dry fermented sausages.

621 **Supplementary Table 1**. Description of the physicochemical (pH and $a_w$) and microbiological
622 (*Salmonella* and LAB levels) characteristics of low-acid and acid dry fermented sausages along
623 the production process.

624 **Supplementary Table 2.** Comparison of observed and predicted *Salmonella* concentration
625 during the storage of low-acid dry fermented sausages.

626 **Supplementary Table 3.** Comparison of observed and predicted *Salmonella* concentration
627 during the storage of acid dry fermented sausages.

**Figure 1**

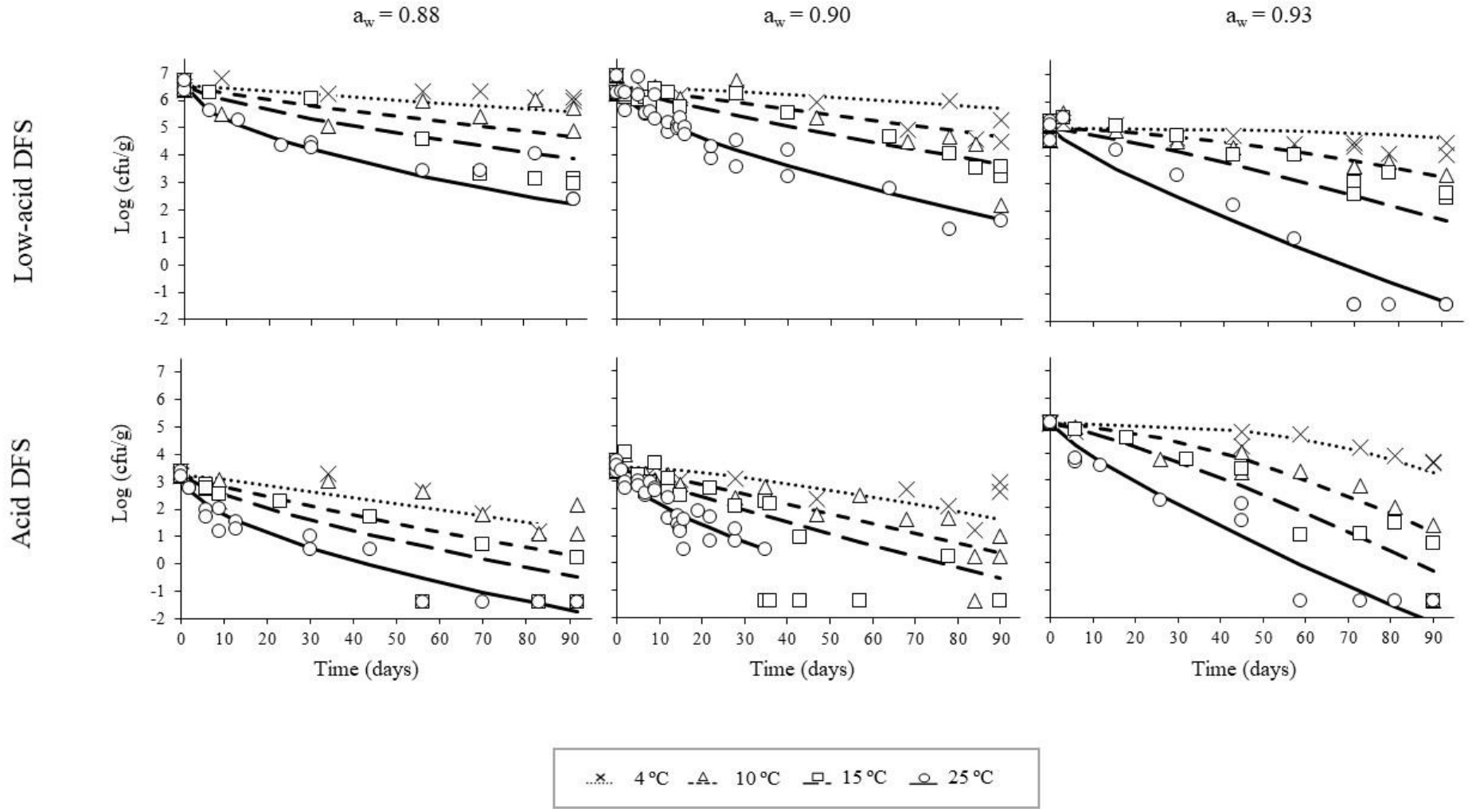
$a_w$ = 0.88
$a_w$ = 0.90
$a_w$ = 0.93
Low-acid DFS
Acid DFS
Log (cfu/g)
Time (days)
4 °C
10 °C
15 °C
25 °C

**Figure 2**

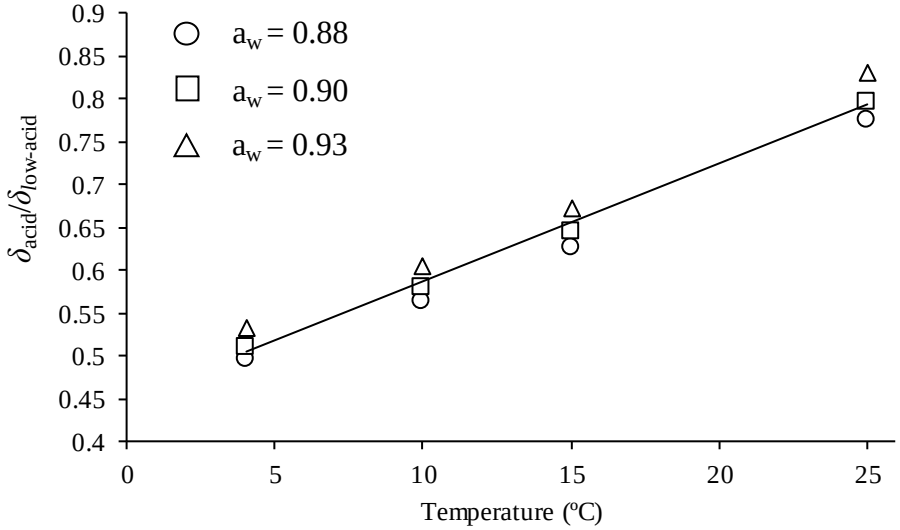
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
$\delta_{acid}/\delta_{low\text{-}acid}$
$a_w$ = 0.88
$a_w$ = 0.90
$a_w$ = 0.93
0
5
10
15
20
25
Temperature (ºC)

Table 1

| Product | Experimental conditions | | Kinetic parameters | | | | Goodness of fit[d] | |
|---|---|---|---|---|---|---|---|---|
| **DFS** | **$a_w$ (pH)[a]** | **T[b] °C** | **Log$N_0$[c]** Log cfu/g | ***δ*[d]** days | ***p*[d]** | **n[e]** | **RSS** | **RMSE** |
| Low-acid | 0.878 ± 0.002 | 4 | 6.53 | 156.11 ± 73.75 | 1.47 ± 1.05 | 10 | 0.220 | 0.166 |
| | (5.68 ± 0.11) | 10 | 6.53 | 130.00 ± 187.00 | 2.05 ± 6.63 | 10 | 0.227 | 0.168 |
| | | 15 | 6.53 | 32.25 ± 5.14 | 1.25 ± 0.21 | 10 | 0.721 | 0.300 |
| | | 25 | 6.53 | 6.81 ± 2.77 | 0.51 ± 0.09 | 13 | 2.178 | 0.445 |
| | 0.889 ± 0.001 | 4 | 6.47 | 64.85 ± 8.98 | 1.41 ± 0.67 | 12 | 1.690 | 0.411 |
| | (5.60 ± 0.07) | 10 | 6.47 | 49.16 ± 7.25 | 1.45 ± 0.47 | 12 | 1.306 | 0.362 |
| | | 15 | 6.47 | 39.09 ± 4.34 | 1.34 ± 0.20 | 17 | 1.201 | 0.283 |
| | | 25 | 6.47 | 8.93 ± 1.12 | 0.71 ± 0.05 | 30 | 4.879 | 0.417 |
| | 0.932 ± 0.000 | 4 | 5.02 | 105.67 ± 19.47 | 1.22 ± 0.56 | 13 | 0.585 | 0.231 |
| | (5.64 ± 0.03) | 10 | 5.02 | 59.47 ± 5.34 | 1.27 ± 0.36 | 12 | 0.722 | 0.269 |
| | | 15 | 5.02 | 48.63 ± 5.88 | 1.48 ± 0.34 | 13 | 1.394 | 0.356 |
| | | 25 | 5.02 | 13.82 ± 3.78 | 1.03 ± 0.17 | 13 | 4.942 | 0.670 |
| Acid | 0.883 ± 0.002 | 4 | 3.27 | 64.44 ± 6.10 | 3.01 ± 1.30 | 10 | 0.993 | 0.407 |
| | (4.83 ± 0.15) | 10 | 3.27 | 59.73 ± 9.05 | 1.43 ± 0.58 | 11 | 1.317 | 0.383 |
| | | 15 | 3.27 | 15.29 ± 7.30 | 0.80 ± 0.24 | 13 | 7.030 | 0.799 |
| | | 25 | 3.27 | 4.20 ± 0.97 | 0.51 ± 0.05 | 19 | 2.539 | 0.386 |
| | 0.903 ± 0.002 | 4 | 3.54 | 53.40 ± 17.81 | 0.50 ± 0.31 | 12 | 2.119 | 0.460 |
| | (5.06 ± 0.03) | 10 | 3.54 | 35.23 ± 7.82 | 1.28 ± 0.34 | 18 | 6.629 | 0.644 |
| | | 15 | 3.54 | 9.91 ± 3.73 | 0.76 ± 0.16 | 24 | 27.077 | 1.109 |
| | | 25 | 3.54 | 6.21 ± 1.17 | 0.67 ± 0.10 | 27 | 5.228 | 0.457 |
| | 0.930 ± 0.002 | 4 | 5.09 | 64.54 ± 16.66 | 0.48 ± 0.28 | 12 | 1.213 | 0.348 |
| | (4.70 ± 0.05) | 10 | 5.09 | 42.30 ± 8.06 | 2.04 ± 0.57 | 12 | 6.635 | 0.815 |
| | | 15 | 5.09 | 26.45 ± 5.38 | 1.35 ± 0.25 | 13 | 4.622 | 0.648 |
| | | 25 | 5.09 | 5.38 ± 2.08 | 0.67 ± 0.10 | 14 | 5.472 | 0.676 |

[a] $a_w$ and pH of the DFS at the beginning of the storage ± standard deviation .
[b] storage temperature.
[c] Log$N_0$ is the average value of the initial *Salmonella* counts of three replicates at the beginning of the storage.
[d] Parameter estimates ± standard error.
[e] n: number of count data, i.e. Log (N), included for fitting. RSS: residual sum of squares; RMSE: root mean of squared errors.

**Table 2**

| | | Sausage type | Coefficients of the polynomial models[a] | | | | | | Goodness of fit[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | a | b | c | d | e | f | n | P | RMSE | $R^2_{adj}$ |
| Sencondary modelling | $Log\ (\delta) = a + b \cdot T + c \cdot a_w$ | Low-acid | 2.03 ± 2.29 | -0.05 ± 0.01 | 0.29 ± 2.53 | - | - | - | 12 | 3 | 0.18 | 0.831 |
| | $1/p = a + b \cdot T + c \cdot {a_w}^2$ | | 3.71 ± 3.09 | 0.05 ± 0.01 | -4.00 ± 3.42 | - | - | - | 12 | 3 | 0.24 | 0.742 |
| | $Log\ (\delta) = a + b \cdot T + c \cdot a_w$ | Acid | 0.92 ± 1.89 | -0.05 ± 0.01 | 1.26 ± 2.09 | - | - | - | 12 | 3 | 0.15 | 0.887 |
| | $1/p = a + b \cdot T + c \cdot {a_w}^2$ | | 1.47 ± 3.15 | 0.05 ± 0.01 | -1.40 ± 3.48 | - | - | - | 12 | 3 | 0.16 | 0.915 |
| Global modelling | $\mathrm{Log}(N) = \mathrm{Log}(N_0) - \left(\frac{t}{10^{(a+b\cdot T+c\cdot a_w)}}\right)^{\frac{1}{d+e\cdot T+f\cdot({a_w}^2)}}$ | Low-acid | -0.40 ± 1.05 | -0.06 ± 0.00 | 2.95 ± 1.15 | 5.54 ± 0.92 | 0.04 ± 0.00 | -6.15± 1.09 | 350 | 9 | 1.05 | - |
| | | Acid | 1.30 ± 1.03 | -0.05 ± 0.00 | 3.59 ± 1.12 | | | | | | | |

[a] Parameter estimates ± standard error

[b] n: number of *Salmonella* counts (LogN). $\delta$ (days) or $p$ values included for fitting; P: number of estimated parameters of the model; RMSE: root mean of squared errors; $R^2_{adj}$: adjusted coefficient of determination.

Supplementary Table 1

| Industrial process target $a_w$ | Sampling time (days) | Physicochemical characteristics | | | | Microbiological counts | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Low-acid DFS | | Acid DFS | | Low-acid DFS | | Acid DFS | |
| | | pH | $a_w$ | pH | $a_w$ | *Salmonella* (Log cfu/g) | LAB (Log cfu/g) | *Salmonella* (Log cfu/g) | LAB (Log cfu/g) |
| **0.88** | Stuffing day | 5.73 ± 0.02 | 0.978 ± 0.001 | 5.72 ± 0.03 | 0.976 ± 0.001 | 5.81 ± 0.11 | 2.75 ± 0.60 | 5.71 ± 0.04 | 7.22 ± 0.06 |
| | 1 | 5.76 ± 0.01 | 0.973 ± 0.002 | 4.93 ± 0.10 | 0.972 ± 0.002 | 7.55 ± 0.06 | 5.81 ± 0.10 | 5.71 ± 0.09 | 8.86 ± 0.05 |
| | 7 | 5.37 ± 0.06 | 0.954 ± 0.013 | 4.62 ± 0.06 | 0.947 ± 0.013 | 7.47 ± 0.25 | 7.39 ± 0.15 | 4.96 ± 0.13 | 8.85 ± 0.06 |
| | 14 | 5.37 ± 0.03 | 0.898 ± 0.011 | 4.87 ± 0.01 | 0.896 ± 0.057 | 7.33 ± 0.21 | 8.63 ± 0.06 | 3.69 ± 0.07 | 8.84 ± 0.01 |
| | 21 days [a] | 5.68 ± 0.11 | 0.878 ± 0.002 | 4.83 ± 0.15 | 0.883 ± 0.002 | 6.53 ± 0.17 | 8.64 ± 0.05 | 3.27 ± 0.08 | 8.68 ± 0.14 |
| **0.90** | Stuffing day | 5.88 ± 0.10 | 0.978 ± 0.001 | 5.95 ± 0.08 | 0.975 ± 0.001 | 5.93 ± 0.03 | 2.69 ± 0.38 | 5.85 ± 0.06 | 7.03 ± 0.04 |
| | 1 | 5.89 ± 0.01 | 0.978 ± 0.001 | 5.18 ± 0.02 | 0.976 ± 0.002 | 6.90 ± 0.20 | 5.50 ± 0.08 | 6.25 ± 0.26 | 8.88 ± 0.04 |
| | 4 | 5.92 ± 0.02 | 0.973 ± 0.002 | 4.77 ± 0.04 | 0.968 ± 0.000 | 7.47 ± 0.06 | 7.58 ± 0.09 | 5.70 ± 0.05 | 8.93 ± 0.07 |
| | 7 | 5.79 ± 0.09 | 0.964 ± 0.003 | 4.83 ± 0.03 | 0.956 ± 0.004 | 7.73 ± 0.39 | 8.31 ± 0.07 | 4.63 ± 0.10 | 8.91 ± 0.04 |
| | 14 | 5.52 ± 0.04 | 0.934 ± 0.008 | 4.83 ± 0.02 | 0.922 ± 0.008 | 6.83 ± 0.25 | 8.45 ± 0.09 | 4.11 ± 0.08 | 9.00 ± 0.09 |
| | 20[a] | 5.60 ± 0.07 | 0.889 ± 0.001 | 5.06 ± 0.03 | 0.903 ± 0.002 | 6.47 ± 0.37 | 8.30 ± 0.12 | 3.54 ± 0.19 | 8.89 ± 0.01 |
| **0.93** | Stuffing day | 5.78 ± 0.08 | 0.974 ± 0.001 | 5.76 ± 0.04 | 0.976 ± 0.001 | 5.64 ± 0.10 | 3.10 ± 0.14 | 5.84 ± 0.04 | 7.22 ± 0.03 |
| | 1 | 5.72 ± 0.01 | 0.969 ± 0.002 | 5.01 ± 0.03 | 0.966 ± 0.002 | 5.68 ± 0.13 | 5.37 ± 0.18 | 5.58 ± 0.01 | 8.67 ± 0.04 |
| | 3 | 5.77 ± 0.01 | 0.962 ± 0.003 | 4.75 ± 0.01 | 0.965 ± 0.003 | 5.70 ± 0.05 | 7.15 ± 0.07 | 5.54 ± 0.02 | 8.68 ± 0.05 |
| | 7 | 5.61 ± 0.01 | 0.954 ± 0.002 | 4.69 ± 0.04 | 0.955 ± 0.002 | 5.60 ± 0.27 | 8.20 ± 0.05 | 5.22 ± 0.03 | 8.63 ± 0.03 |
| | 11[a] | 5.64 ± 0.03 | 0.932 ± 0.002 | 4.70 ± 0.05 | 0.930 ± 0.002 | 5.02 ± 0.37 | 8.22 ± 0.06 | 5.09 ± 0.02 | 8.64 ± 0.01 |

[a]: End of the drying

**Supplementary Table 2**

| Recipe[a] | Temperature (ºC) | pH | $a_w$ | Time (days) | Observed concentration (Log N) | Predicted concentration (Log N) | Observed-predicted concentration |
|---|---|---|---|---|---|---|---|
| 4 | 5 | 5.3 | 0.898 | 0 | 3.30 | 3.30 | 0.00 |
| | 5 | 5.3 | 0.898 | 15 | 2.80 | 2.26 | 0.54 |
| | 5 | 5.3 | 0.898 | 15 | 2.96 | 2.26 | 0.70 |
| | 5 | 5.3 | 0.898 | 28 | 3.10 | 1.61 | 1.49 |
| | 5 | 5.3 | 0.898 | 28 | 3.38 | 1.61 | 1.77 |
| | 16 | 5.3 | 0.898 | 0 | 3.30 | 3.30 | 0.00 |
| | 16 | 5.3 | 0.898 | 10 | 2.46 | 3.15 | -0.69 |
| | 16 | 5.3 | 0.898 | 10 | 2.67 | 3.15 | -0.48 |
| | 16 | 5.3 | 0.898 | 15 | 2.23 | 3.05 | -0.82 |
| | 16 | 5.3 | 0.898 | 15 | 2.32 | 3.05 | -0.73 |
| | 22 | 5.3 | 0.898 | 0 | 3.30 | 3.30 | 0.00 |
| | 22 | 5.3 | 0.898 | 8 | 2.18 | 3.25 | -1.08 |
| | 22 | 5.3 | 0.898 | 8 | 2.43 | 3.25 | -0.82 |
| | 22 | 5.3 | 0.898 | 15 | 1.70 | 3.18 | -1.48 |
| | 22 | 5.3 | 0.898 | 15 | 1.60 | 3.18 | -1.58 |
| 5 | 5 | 5.2 | 0.898 | 0 | 3.30 | 3.30 | 0.00 |
| | 5 | 5.2 | 0.898 | 15 | 2.86 | 2.26 | 0.60 |
| | 5 | 5.2 | 0.898 | 15 | 3.03 | 2.26 | 0.76 |
| | 5 | 5.2 | 0.898 | 30 | 2.92 | 1.52 | 1.41 |
| | 5 | 5.2 | 0.898 | 30 | 3.03 | 1.52 | 1.51 |
| | 16 | 5.2 | 0.898 | 0 | 3.30 | 3.30 | 0.00 |
| | 16 | 5.2 | 0.898 | 10 | 2.72 | 3.15 | -0.42 |
| | 16 | 5.2 | 0.898 | 10 | 2.69 | 3.15 | -0.46 |
| | 16 | 5.2 | 0.898 | 15 | 2.53 | 3.05 | -0.52 |
| | 16 | 5.2 | 0.898 | 15 | 2.69 | 3.05 | -0.36 |
| | 22 | 5.2 | 0.898 | 0 | 3.30 | 3.30 | 0.00 |
| | 22 | 5.2 | 0.898 | 8 | 2.51 | 3.25 | -0.75 |
| | 22 | 5.2 | 0.898 | 8 | 2.74 | 3.25 | -0.51 |
| | 22 | 5.2 | 0.898 | 15 | 1.90 | 3.18 | -1.28 |
| | 22 | 5.2 | 0.898 | 15 | 1.95 | 3.18 | -1.23 |
| 23 | 5 | 5.2 | 0.912 | 0 | 4.34 | 4.34 | 0.00 |
| | 5 | 5.2 | 0.912 | 13 | 2.70 | 3.40 | -0.70 |
| | 5 | 5.2 | 0.912 | 13 | 3.38 | 3.40 | -0.02 |
| | 5 | 5.2 | 0.912 | 26 | 3.42 | 2.89 | 0.53 |
| | 5 | 5.2 | 0.912 | 26 | 3.41 | 2.89 | 0.52 |
| | 16 | 5.2 | 0.912 | 0 | 4.34 | 4.34 | 0.00 |
| | 16 | 5.2 | 0.912 | 5 | 2.60 | 4.25 | -1.65 |
| | 16 | 5.2 | 0.912 | 5 | 2.90 | 4.25 | -1.34 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 16 | 5.2 | 0.912 | 13 | 3.05 | 4.08 | -1.03 |
| | 16 | 5.2 | 0.912 | 13 | 2.93 | 4.08 | -1.15 |
| | 22 | 5.2 | 0.912 | 0 | 4.34 | 4.34 | 0.00 |
| | 22 | 5.2 | 0.912 | 5 | 2.90 | 4.30 | -1.40 |
| | 22 | 5.2 | 0.912 | 5 | 2.95 | 4.30 | -1.35 |
| | 22 | 5.2 | 0.912 | 12 | 2.08 | 4.23 | -2.15 |
| | 22 | 5.2 | 0.912 | 12 | 2.18 | 4.23 | -2.05 |
| 24 | 5 | 5.3 | 0.889 | 0 | 5.20 | 5.20 | 0.00 |
| | 5 | 5.3 | 0.889 | 13 | 5.60 | 4.28 | 1.32 |
| | 5 | 5.3 | 0.889 | 13 | 5.64 | 4.28 | 1.36 |
| | 5 | 5.3 | 0.889 | 26 | 5.74 | 3.51 | 2.23 |
| | 5 | 5.3 | 0.889 | 26 | 5.76 | 3.51 | 2.26 |
| | 16 | 5.3 | 0.889 | 0 | 5.20 | 5.20 | 0.00 |
| | 16 | 5.3 | 0.889 | 8 | 5.03 | 5.10 | -0.08 |
| | 16 | 5.3 | 0.889 | 8 | 5.48 | 5.10 | 0.37 |
| | 16 | 5.3 | 0.889 | 13 | 4.89 | 5.02 | -0.12 |
| | 16 | 5.3 | 0.889 | 13 | 4.90 | 5.02 | -0.11 |
| | 22 | 5.3 | 0.889 | 0 | 5.20 | 5.20 | 0.00 |
| | 22 | 5.3 | 0.889 | 6 | 4.69 | 5.18 | -0.48 |
| | 22 | 5.3 | 0.889 | 6 | 5.59 | 5.18 | 0.42 |
| | 22 | 5.3 | 0.889 | 13 | 4.77 | 5.12 | -0.35 |
| | 22 | 5.3 | 0.889 | 13 | 4.40 | 5.12 | -0.72 |
| 25 | 5 | 5.3 | 0.894 | 0 | 3.20 | 3.20 | 0.00 |
| | 5 | 5.3 | 0.894 | 14 | 2.43 | 2.22 | 0.21 |
| | 5 | 5.3 | 0.894 | 14 | 2.43 | 2.22 | 0.21 |
| | 5 | 5.3 | 0.894 | 27 | 2.26 | 1.51 | 0.75 |
| | 5 | 5.3 | 0.894 | 27 | 2.11 | 1.51 | 0.60 |
| | 16 | 5.3 | 0.894 | 0 | 3.20 | 3.20 | 0.00 |
| | 16 | 5.3 | 0.894 | 9 | 1.48 | 3.08 | -1.60 |
| | 16 | 5.3 | 0.894 | 9 | 2.15 | 3.08 | -0.93 |
| | 16 | 5.3 | 0.894 | 14 | 1.90 | 2.98 | -1.08 |
| | 16 | 5.3 | 0.894 | 14 | 1.70 | 2.98 | -1.28 |
| | 22 | 5.3 | 0.894 | 0 | 3.20 | 3.20 | 0.00 |
| | 22 | 5.3 | 0.894 | 7 | 1.60 | 3.16 | -1.56 |
| | 22 | 5.3 | 0.894 | 7 | 1.30 | 3.16 | -1.86 |
| | 22 | 5.3 | 0.894 | 14 | 1.00 | 3.10 | -2.10 |
| | 22 | 5.3 | 0.894 | 14 | 1.00 | 3.10 | -2.10 |
| 26 | 5 | 5.6 | 0.891 | 0 | 5.60 | 5.60 | 0.00 |
| | 5 | 5.6 | 0.891 | 14 | 4.72 | 4.62 | 0.11 |
| | 5 | 5.6 | 0.891 | 14 | 4.73 | 4.62 | 0.11 |
| | 5 | 5.6 | 0.891 | 29 | 4.71 | 3.77 | 0.94 |
| | 5 | 5.6 | 0.891 | 29 | 4.54 | 3.77 | 0.77 |
| | 16 | 5.6 | 0.891 | 0 | 5.60 | 5.60 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 16 | 5.6 | 0.891 | 9 | 4.00 | 5.48 | -1.48 |
| | 16 | 5.6 | 0.891 | 9 | 4.12 | 5.48 | -1.36 |
| | 16 | 5.6 | 0.891 | 14 | 3.95 | 5.39 | -1.44 |
| | 16 | 5.6 | 0.891 | 14 | 3.93 | 5.39 | -1.46 |
| | 22 | 5.6 | 0.891 | 0 | 5.60 | 5.60 | 0.00 |
| | 22 | 5.6 | 0.891 | 7 | 3.92 | 5.57 | -1.64 |
| | 22 | 5.6 | 0.891 | 7 | 3.77 | 5.57 | -1.80 |
| | 22 | 5.6 | 0.891 | 14 | 3.22 | 5.50 | -2.29 |
| | 22 | 5.6 | 0.891 | 14 | 3.34 | 5.50 | -2.16 |
| 27 | 5 | 5.3 | 0.888 | 0 | 2.00 | 2.00 | 0.00 |
| | 5 | 5.3 | 0.888 | 13 | 2.28 | 1.08 | 1.20 |
| | 5 | 5.3 | 0.888 | 13 | 2.11 | 1.08 | 1.03 |
| | 5 | 5.3 | 0.888 | 28 | 1.95 | 0.18 | 1.77 |
| | 5 | 5.3 | 0.888 | 28 | 1.48 | 0.18 | 1.30 |
| | 16 | 5.3 | 0.888 | 0 | 2.00 | 2.00 | 0.00 |
| | 16 | 5.3 | 0.888 | 8 | 1.60 | 1.90 | -0.30 |
| | 16 | 5.3 | 0.888 | 8 | 1.48 | 1.90 | -0.43 |
| | 16 | 5.3 | 0.888 | 13 | 1.30 | 1.82 | -0.52 |
| | 16 | 5.3 | 0.888 | 13 | 1.00 | 1.82 | -0.82 |
| | 22 | 5.3 | 0.888 | 0 | 2.00 | 2.00 | 0.00 |
| | 22 | 5.3 | 0.888 | 6 | 1.00 | 1.98 | -0.98 |
| | 22 | 5.3 | 0.888 | 6 | 1.00 | 1.98 | -0.98 |
| | 22 | 5.3 | 0.888 | 13 | 1.00 | 1.92 | -0.92 |
| | 22 | 5.3 | 0.888 | 13 | 1.00 | 1.92 | -0.92 |

[a] Recipes described in Gunvig et al., 2016.

**Supplementary Table 3**

| Recipe[a] | Temperature (ºC) | pH | $a_w$ | Time (days) | Observed concentration (Log N) | Predicted concentration (Log N) | Observed-predicted concentration |
|---|---|---|---|---|---|---|---|
| 1 | 5 | 5.0 | 0.872 | 0 | 2.60 | 2.60 | 0.00 |
| | 5 | 5.0 | 0.872 | 13 | 2.26 | 1.94 | 0.32 |
| | 5 | 5.0 | 0.872 | 13 | 2.66 | 1.94 | 0.72 |
| | 5 | 5.0 | 0.872 | 28 | 2.15 | 1.11 | 1.04 |
| | 5 | 5.0 | 0.872 | 28 | 2.74 | 1.11 | 1.63 |
| | 16 | 5.0 | 0.872 | 0 | 2.60 | 2.60 | 0.00 |
| | 16 | 5.0 | 0.872 | 8 | 1.95 | 2.55 | -0.60 |
| | 16 | 5.0 | 0.872 | 8 | 2.00 | 2.55 | -0.55 |
| | 16 | 5.0 | 0.872 | 13 | 2.11 | 2.50 | -0.38 |
| | 16 | 5.0 | 0.872 | 13 | 1.95 | 2.50 | -0.54 |
| | 22 | 5.0 | 0.872 | 0 | 2.60 | 2.60 | 0.00 |
| | 22 | 5.0 | 0.872 | 6 | 1.00 | 2.59 | -1.59 |
| | 22 | 5.0 | 0.872 | 6 | 1.60 | 2.59 | -0.99 |
| | 22 | 5.0 | 0.872 | 13 | 1.00 | 2.56 | -1.56 |
| | 22 | 5.0 | 0.872 | 13 | 1.60 | 2.56 | -0.96 |
| 3 | 5 | 4.8 | 0.869 | 0 | 2.40 | 2.40 | 0.00 |
| | 5 | 4.8 | 0.869 | 14 | 1.85 | 1.69 | 0.15 |
| | 5 | 4.8 | 0.869 | 14 | 1.90 | 1.69 | 0.21 |
| | 5 | 4.8 | 0.869 | 29 | 1.78 | 0.83 | 0.95 |
| | 5 | 4.8 | 0.869 | 29 | 2.11 | 0.83 | 1.28 |
| | 16 | 4.8 | 0.869 | 0 | 2.40 | 2.40 | 0.00 |
| | 16 | 4.8 | 0.869 | 9 | 1.48 | 2.34 | -0.87 |
| | 16 | 4.8 | 0.869 | 9 | 1.60 | 2.34 | -0.74 |
| | 16 | 4.8 | 0.869 | 14 | 1.30 | 2.29 | -0.99 |
| | 16 | 4.8 | 0.869 | 14 | 1.78 | 2.29 | -0.51 |
| | 22 | 4.8 | 0.869 | 0 | 2.40 | 2.40 | 0.00 |
| | 22 | 4.8 | 0.869 | 7 | 1.00 | 2.39 | -1.39 |
| | 22 | 4.8 | 0.869 | 7 | 1.00 | 2.39 | -1.39 |
| | 22 | 4.8 | 0.869 | 14 | 1.00 | 2.35 | -1.35 |
| | 22 | 4.8 | 0.869 | 14 | 1.00 | 2.35 | -1.35 |
| 6 | 5 | 4.9 | 0.890 | 0 | 2.60 | 2.60 | 0.00 |
| | 5 | 4.9 | 0.890 | 15 | 2.08 | 1.79 | 0.29 |
| | 5 | 4.9 | 0.890 | 15 | 2.11 | 1.79 | 0.32 |
| | 5 | 4.9 | 0.890 | 28 | 1.70 | 1.21 | 0.49 |
| | 5 | 4.9 | 0.890 | 28 | 2.20 | 1.21 | 0.99 |
| | 16 | 4.9 | 0.890 | 0 | 2.60 | 2.60 | 0.00 |
| | 16 | 4.9 | 0.890 | 10 | 1.95 | 2.50 | -0.55 |
| | 16 | 4.9 | 0.890 | 10 | 2.04 | 2.50 | -0.46 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 16 | 4.9 | 0.890 | 15 | 1.78 | 2.43 | -0.65 |
| | 16 | 4.9 | 0.890 | 15 | 1.70 | 2.43 | -0.73 |
| | 22 | 4.9 | 0.890 | 0 | 2.60 | 2.60 | 0.00 |
| | 22 | 4.9 | 0.890 | 8 | 1.48 | 2.57 | -1.09 |
| | 22 | 4.9 | 0.890 | 8 | 1.48 | 2.57 | -1.09 |
| | 22 | 4.9 | 0.890 | 15 | 1.00 | 2.53 | -1.53 |
| | 22 | 4.9 | 0.890 | 15 | 1.30 | 2.53 | -1.22 |
| 10 | 5 | 4.9 | 0.870 | 0 | 3.10 | 3.10 | 0.00 |
| | 5 | 4.9 | 0.870 | 14 | 2.46 | 2.39 | 0.07 |
| | 5 | 4.9 | 0.870 | 14 | 2.28 | 2.39 | -0.11 |
| | 5 | 4.9 | 0.870 | 29 | 3.20 | 1.54 | 1.66 |
| | 5 | 4.9 | 0.870 | 29 | 2.60 | 1.54 | 1.07 |
| | 16 | 4.9 | 0.870 | 0 | 3.10 | 3.10 | 0.00 |
| | 16 | 4.9 | 0.870 | 9 | 2.63 | 3.04 | -0.41 |
| | 16 | 4.9 | 0.870 | 9 | 2.43 | 3.04 | -0.61 |
| | 16 | 4.9 | 0.870 | 14 | 1.78 | 2.99 | -1.21 |
| | 16 | 4.9 | 0.870 | 14 | 1.90 | 2.99 | -1.08 |
| | 22 | 4.9 | 0.870 | 0 | 3.10 | 3.10 | 0.00 |
| | 22 | 4.9 | 0.870 | 7 | 2.20 | 3.09 | -0.88 |
| | 22 | 4.9 | 0.870 | 14 | 1.70 | 3.05 | -1.36 |
| | 22 | 4.9 | 0.870 | 14 | 1.78 | 3.05 | -1.28 |
| 12 | 5 | 4.7 | 0.855 | 0 | 2.00 | 2.00 | 0.00 |
| | 5 | 4.7 | 0.855 | 13 | 1.60 | 1.38 | 0.22 |
| | 5 | 4.7 | 0.855 | 13 | 1.00 | 1.38 | -0.38 |
| | 5 | 4.7 | 0.855 | 28 | 2.11 | 0.40 | 1.71 |
| | 5 | 4.7 | 0.855 | 28 | 1.30 | 0.40 | 0.90 |
| | 16 | 4.7 | 0.855 | 0 | 2.00 | 2.00 | 0.00 |
| | 16 | 4.7 | 0.855 | 8 | 1.00 | 1.97 | -0.97 |
| | 16 | 4.7 | 0.855 | 8 | 1.00 | 1.97 | -0.97 |
| | 16 | 4.7 | 0.855 | 13 | 1.00 | 1.92 | -0.92 |
| | 16 | 4.7 | 0.855 | 13 | 1.00 | 1.92 | -0.92 |
| | 22 | 4.7 | 0.855 | 0 | 2.00 | 2.00 | 0.00 |
| | 22 | 4.7 | 0.855 | 6 | 1.00 | 1.99 | -0.99 |
| | 22 | 4.7 | 0.855 | 6 | 2.30 | 1.99 | 0.31 |
| | 22 | 4.7 | 0.855 | 13 | 1.00 | 1.97 | -0.97 |
| | 22 | 4.7 | 0.855 | 13 | 0.70 | 1.97 | -1.27 |
| 13 | 5 | 4.8 | 0.902 | 0 | 2.40 | 2.40 | 0.00 |
| | 5 | 4.8 | 0.902 | 15 | 2.18 | 1.57 | 0.61 |
| | 5 | 4.8 | 0.902 | 15 | 2.00 | 1.57 | 0.43 |
| | 5 | 4.8 | 0.902 | 28 | 2.00 | 1.08 | 0.92 |
| | 5 | 4.8 | 0.902 | 28 | 1.95 | 1.08 | 0.87 |
| | 16 | 4.8 | 0.902 | 0 | 2.40 | 2.40 | 0.00 |
| | 16 | 4.8 | 0.902 | 10 | 1.48 | 2.28 | -0.80 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 16 | 4.8 | 0.902 | 10 | 2.08 | 2.28 | -0.20 |
| | 16 | 4.8 | 0.902 | 15 | 1.85 | 2.20 | -0.35 |
| | 16 | 4.8 | 0.902 | 15 | 1.00 | 2.20 | -1.20 |
| | 22 | 4.8 | 0.902 | 0 | 2.40 | 2.40 | 0.00 |
| | 22 | 4.8 | 0.902 | 8 | 1.78 | 2.36 | -0.58 |
| | 22 | 4.8 | 0.902 | 8 | 2.00 | 2.36 | -0.36 |
| | 22 | 4.8 | 0.902 | 15 | 1.90 | 2.31 | -0.40 |
| | 22 | 4.8 | 0.902 | 15 | 1.78 | 2.31 | -0.53 |
| 18 | 5 | 4.5 | 0.904 | 0 | 1.00 | 1.00 | 0.00 |
| | 5 | 4.5 | 0.904 | 13 | 1.00 | 0.24 | 0.76 |
| | 5 | 4.5 | 0.904 | 13 | 1.00 | 0.24 | 0.76 |
| | 5 | 4.5 | 0.904 | 26 | 1.00 | -0.24 | 1.24 |
| | 5 | 4.5 | 0.904 | 26 | 1.00 | -0.24 | 1.24 |
| | 16 | 4.5 | 0.904 | 0 | 1.00 | 1.00 | 0.00 |
| | 16 | 4.5 | 0.904 | 5 | 1.00 | 0.94 | 0.06 |
| | 16 | 4.5 | 0.904 | 5 | 1.00 | 0.94 | 0.06 |
| | 16 | 4.5 | 0.904 | 13 | 1.00 | 0.82 | 0.18 |
| | 16 | 4.5 | 0.904 | 13 | 1.00 | 0.82 | 0.18 |
| | 22 | 4.5 | 0.904 | 0 | 1.00 | 1.00 | 0.00 |
| | 22 | 4.5 | 0.904 | 5 | 1.00 | 0.98 | 0.02 |
| | 22 | 4.5 | 0.904 | 5 | 1.00 | 0.98 | 0.02 |
| | 22 | 4.5 | 0.904 | 12 | 1.00 | 0.93 | 0.07 |
| | 22 | 4.5 | 0.904 | 12 | 1.00 | 0.93 | 0.07 |
| 22 | 5 | 5.0 | 0.927 | 0 | 5.81 | 5.81 | 0.00 |
| | 5 | 5.0 | 0.927 | 13 | 5.28 | 4.97 | 0.31 |
| | 5 | 5.0 | 0.927 | 13 | 5.40 | 4.97 | 0.43 |
| | 5 | 5.0 | 0.927 | 26 | 5.25 | 4.66 | 0.58 |
| | 5 | 5.0 | 0.927 | 26 | 5.16 | 4.66 | 0.50 |
| | 16 | 5.0 | 0.927 | 0 | 5.81 | 5.81 | 0.00 |
| | 16 | 5.0 | 0.927 | 5 | 5.73 | 5.70 | 0.03 |
| | 16 | 5.0 | 0.927 | 5 | 5.45 | 5.70 | -0.25 |
| | 16 | 5.0 | 0.927 | 13 | 5.00 | 5.55 | -0.55 |
| | 16 | 5.0 | 0.927 | 13 | 4.98 | 5.55 | -0.57 |
| | 22 | 5.0 | 0.927 | 0 | 5.81 | 5.81 | 0.00 |
| | 22 | 5.0 | 0.927 | 5 | 5.30 | 5.77 | -0.47 |
| | 22 | 5.0 | 0.927 | 5 | 5.57 | 5.77 | -0.20 |
| | 22 | 5.0 | 0.927 | 12 | 4.42 | 5.69 | -1.27 |
| | 22 | 5.0 | 0.927 | 12 | 4.85 | 5.69 | -0.85 |

[a] Recipes described in Gunvig et al., 2016.