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Highlights

- First application of response surface method to modelling bisphenols migration
- Valuable prediction of bisphenols migration in simulants
- Model predictions are conservative for canned vegetables
- Desirability study of usage conditions to comply with regulation

Empirical models to predict the effect of sterilization and storage on bisphenols migration from metallic can coating into food simulants

Based on response surface methodology, empirical models were built to predict the influence of can processing (heat treatment) and storage conditions (time and temperature) on the migration of bisphenol compounds from the inner lacquer of tinplate cans (4 brands) into several food simulants. Analysis using liquid chromatography revealed the presence of BADGE.2H_2O and BPA in all samples. Models were significant in fitting the levels of these two bisphenols in food simulants depending on the input variables, with high adjusted coefficients of determination. Their prediction performance was validated through running new data sets. Further comparison of predicted values with bisphenols levels measured in canned vegetables revealed that the proposed models are conservative. By the desirability of the response output, the models are capable of proposing the range of can processing and storage conditions that limit migration for further compliance with the regulation. So, the proposed approach could be a convenient tool for the industries to control processing conditions in order to ensure conformity of canned foods.

Keywords: experimental design; food packaging; modelling; response surface methodology; tinplate cans.

Introduction

Migrants from food packaging are, for a long time, of great concern by food safety authorities all over the world and among them, bisphenol A (BPA) and its substitutes (BPF, BPS) paid a special attention. BPA (or BPF) has been widely used as a monomer in polycarbonate plastic or epoxy-phenolic resins made of bisphenol A diglycidyl ether (BADGE) (or bisphenol F diglycidyl ether (BFDGE)). These resins are coating commonly applied on the inside metallic walls of food and beverage cans in

order to prevent metal corrosion occurring with aggressive food ingredients (such as low-pH food).

Many studies have already proved that heat treatments generally applied during canned food processes lead to migration of important quantity of free BADGE (or BFDGE) and BPA (or BPF), resulting in about hundreds of µg kg^{-1} (Munguia-Lopez and Soto-Valdez 2001; Cabado et al. 2008). Additionally, the instability of BADGE (or BFDGE) epoxide groups can easily produce hydrolysed derivatives (such as BADGE·$2H_2O$ or BADGE·H_2O) in contact with aqueous and acidic foodstuffs, while use of hydrochloric acid may form chlorinated derivatives such as BADGE.HCl. Finally, all these compounds can be released, along with oligomers and derivatives, into the canned foods (Gallart-Ayala et al. 2011; Noureddine El Moussawi et al., 2019). Due to their health concern, bisphenol compounds have been regulated with specific migration limits (SMLs) established for materials and articles intended to come in contact with foods, especially plastics and inner resins used by the can industries (e.g. for BPA, the European Regulation 10/2011 fixed a SML value of 600 µg/kg, this value being recently reduced to 50 µg/kg by European Regulation 2018/213) (European Union 2011; European Union 2018).

In such a context, early prediction of compounds migration from packaging into food is a key issue for canning and food industries. Testing the material before use with food simulants is required, to ensure migration levels remain below these SML values. Numerous models have also been developed, to predict migration and limit experimental tests (Poças et al. 2008). These models are mostly deterministic, based on theoretical diffusion equations and compounds partitioning between packaging and food simulants (Ernstoff et al. 2017; Fang and Vitrac 2017; Brandsch et al. 2002; Helmroth et al. 2002). They were reported to be valuable for assessing material compliance with

regulation, especially for plastic packaging. However they require the assessment of some fundamental constants that are dependent on the packaging (at least the diffusion coefficient of the migrant in the polymer, and the partition coefficient of the migrant in the packaging/food simulant system). In practice, such constants remain unknown for several canning industries that buy to other companies their raw materials (as an illustration, we have experienced a lack of information relative to raw material composition in such industries in Lebanon).

So, there is an interest for developing simple models capable of predicting migration of bisphenols through monitoring tractable parameters such as sterilization and storage conditions (e.g. contact time and temperature). Such models could be valuable tools for canning industries, enabling them to efficiently control parameters affecting the migration of bisphenol compounds (such as sterilization conditions, brand of coating, type of packed food, and storage conditions).

This work aims at showing the capability of empirical models based on surface response methodology (RSM) for predicting migration levels of bisphenols from cans. Only one previous study showed their efficiency in modelling migration of oligomers from high-density polyethylene containers, with temperature and contact time as input variables (Fauconnier et al. 2001). Here, we considered can brand, food simulant type, sterilization time, storage temperature and storage time as input variables. Models were built from data set based on experimental designs to limit the number of experiments, and further validated on a new data set. They applicability to predict migration in canned foods is also presented.

Materials and method

Tinplate cans

Four types of tinplate cans were investigated, in order to reflect different commercially available coatings. All cans were three-pieces with easy open lids, collected from three main canning factories in Lebanon. According to the manufacturers, the can lacquers were imported from France, Germany or Turkey. Most of the cans (50 cans coded as C1, C2 and C4: 10.1 cm height, 7.3 cm diameter, 3.15 dm^2, 423 mL) were coated with epoxy-phenolic resin pigmented with titanium oxide (giving a white appearance) and were intended for packing vegetables and fruits. The C3 cans (12 cans: 5.3 cm height, 7.3 cm diameter, 2.05 dm^2, 222 mL), intended to pack processed meats, were coated with lacquer of same source as C1 cans, but the coating was modified with microcrystalline wax (E 905) slipping agent in addition to aluminium pigmentation (grey appearance). This category allows studying the possible effect of slipping agent on food contamination.

Food simulants

Type of simulants and can filling

Four simulants were considered that mimic aqueous (water and 10% v/v ethanol in water), acidic (3% w/v acetic acid) and semi-fatty foods (50% v/v ethanol in water), being the food categories concerned by our tinplate cans. Ethanol (10 or 50% in water) and acetic acid (3%) are simulants recommended by the European regulation (European Union 2011). Water was additionally considered since a previous study reported overestimation of BPA migration with 10% v/v ethanol in water (Goodson et al. 2004). Cans were filled either with 350 ml (C1, C2, C4) or 175 ml (C3) of food simulants. They were then sealed, without sterilization or once sterilized.

Sterilization

Sterilization, using an ACB autoclave pilot, was performed at 121°C since it is the most common practice in food industries, being also recommended by European regulation 10/2011 to simulate the worst case for sterilization between 100 and 121°C. Sterilization duration of 30 min was selected as the most common practice at this temperature, and 90 min was also considered as it is helpful for ensuring the complete cook of partially cooked food.

Storage

All filled sealed cans were stored under the appropriate controlled conditions according to the experimental designs built. The storage temperature was selected to simulate storage in a fridge (5°C), at room temperature (the most common: 22.5°C) and at room temperature in hot countries such as Lebanon during summer (40°C). Storage duration considered were 1, 15 and 60 days to mimic short and long storage.

Food products

Typical Lebanese vegetables concerned with C1 and C2 cans, namely fava beans, red beans, chickpeas and okra, were purchased from two local industries in Lebanon. Detailed information relative to the pH, moisture and fat content of these food products is given in our previous report (Noureddine El Moussawi et al., 2019). C1 or C2 cans filled with each food product were sterilised for 30 min at 121°C.

Measuring bisphenols migration

Standards and reagents

Acetonitrile (ACN) (HPLC plus Gradient grade and LC-MS grade), methanol (MeOH) (HPLC plus Gradient grade and LC-MS grade), ethanol (EtOH) (anhydrous absolute

and HPLC plus Gradient grade), water (LC-MS grade), formic acid (FA) (LC-MS grade) and acetic acid (RPE glacial) were obtained from Carlo Erba (France). BPA (purity ≥99.9%), BPF (purity ≥98%), BPS (purity ≥98%), BADGE (purity ≥95%), BADGE.2H_2O (purity ≥97%), BADGE.2HCl (purity ≥95%), BFDGE (purity ≥95%, mixture of diastereoisomers), BFDGE.2H_2O (purity ≥95.0%), and BFDGE.2HCl (purity ≥90.0%, total assay of the three isomers) were obtained from Sigma Aldrich (France). Ultra-pure Milli-Q water (18.2 MΩ, 25.0°C) was produced by an Integral 3 from Merck-Millipore®.

Instrumentation

All bisphenols considered here (BPA, BPF, BPS, BADGE, BFDGE and their derivatives) were systematically investigated in the samples collected from the cans. For that purpose, the same UHPLC/Fluorescence and UHPLC/TOF-MS systems as described previously (Noureddine El Moussawi et al. 2017) were used, but with different chromatographic gradients.

For UHPLC/Fluorescence, solvents A (water) and B (ACN) were pumped using the following gradient: 0 min - 43% B, 1 min ramp to 50% B (maintained for 4 min), 2 min ramp to 60% B (maintained for 5 min), 1 min ramp to 100% B (maintained for 2 min) and back to 43% B in 1 min (total duration 16 min). Quantification was performed with external calibration of integrated peak areas of ten points in the range 0.1 to 80 µg L^{-1}.

For UHPLC/TOF-MS, both ESI^- and ESI^+ ionization modes were operated. The ESI^- operation parameters and mobile gradient were as previously described (Noureddine El Moussawi et al. 2017), while ESI^+ conditions were reported in another study (Noureddine El Moussawi et al. 2019).

Sample treatment for simulants

All samples were either directly treated the same day after opening the cans, or stored in glass tubes at 5°C until their treatment (sample stability was checked under these conditions). Sterilized samples contained high amounts of organic contaminants, requiring ten times dilution with mobile phase (ACN/water 43/57 v/v) before their analysis. On the other hand, unsterilized cans contained only traces, thus requiring SPE pre-concentration step. For that purpose, 10 mL samples were loaded on Supelco-HLB (60 mg/3 mL) cartridges (SPE manual Visiprep^{TM} system used) previously conditioned with 5 mL of MeOH and equilibrated with 5 mL of blank food simulant. After a washing step with 5 mL MeOH/water (5/95 v/v), organic contaminants were eluted with 2 mL MeOH. Then the cartridges were dried under vacuum for 15 min, followed by another 2 mL of MeOH to elute any remaining residues. The two elution fractions were combined and gently evaporated to dryness under nitrogen stream at 35°C. The residues were then recovered with 1 ml mobile phase (ACN/water 43/57 v/v). The same protocol was used for treatment of samples from EtOH-based food simulants, except that the samples were water diluted to reach only 5% EtOH before SPE (to avoid bisphenols losses during sample percolation), and that the residues after evaporation were reconstituted in 0.5 mL mobile phase.

Sample treatment for food products

The whole food content of three cans of same lot number was homogenized in a stainless steel blender. About 100 g of the food mixture was freeze dried using a Labanconco freeze dryer. Three replicates of 0.2 g subsample were taken from each sample and vortexed with 4 mL of methanol (Munguia-Lopez et al. 2002) then shaken for 20 min and finally soaked for 12 h. After soaking, the samples were shaken again for 20 min. After ensuring the good separation between liquid and solid portions, 3 mL

of liquid part were taken and another 3 mL of methanol were added. The mixture was shaken for final 20 min, then a second 3 mL were collected from the liquid phase. The total collected portions (i.e. 6 mL) of methanolic extracts were then evaporated to dryness under gentle nitrogen flow at 35°C. The dried residues were reconstituted with 10 mL of ACN/water (5/95 v/v). Further clean-up on Supelco-HLB (60 mg/3 mL) cartridges was performed as described previously (Noureddine El Moussawi et al. 2019). The final extract was evaporated to dryness under gentle nitrogen stream at 35°C. Then, the residues were recovered with 1 ml of chromatographic mobile phase. The final sample solution was diluted three folds (or more if necessary) and further filtrated using 0.2 µm PTFE syringe-less hand compressor filters (Whatman® Mini-UniPrep) before further analysis.

Migration modelling

General strategy

The response surface methodology requires two steps (Bezerra et al. 2008). First, some preliminary study should be carried to determine the range of variables to be used in the final migration protocol. It is then essential to elaborate an experimental design that minimizes the number of tests and allows modelling of migration.

We used the JMP 13 software (SAS Institute. Cary, NC 1989-2007) to build our experimental designs on custom design based on RSM. This type of designs is general, flexible, and matches the experimenter requests (regarding the types of variables and the time available for experiments): in addition, it authorizes modelling in presence of categorical variables. First, second and interaction order of variables were all selected on software. Experimental designs were developed through running the minimum possible number of randomized experiments to create balanced models.

Input variables

Five factors have been introduced as input variables in the models: type of tinplate cans (x_1), nature of food simulant (x_2), sterilization duration (x_3), storage temperature (x_4) and storage time (x_5). They can be divided into categorical and discrete variables as detailed in Table 1, also showing the tested levels. As RSM models need centered variables while experiments cannot always provide them, it is common to use rescaled variables coded as (-1,+1) for describing numerical independent variables in the experimental interval. So, variables x_3, x_4, and x_5 were replaced by the following terms:

$x'_3 = (x_3\text{-}45)/45$; $x'_4 = (x_4\text{-}22.5)/17.5$; $x'_5 = (x_5\text{-}30.5)/29.5$

Response modelling

The general polynomial equation giving response surfaces is:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j$$

where x_i and x_j are the variables, while β_0, β_i, β_{ii}, and β_{ij} are coefficients to determined. Output responses are bisphenol levels in food simulants (in µg kg^{-1}), so that different models were considered, each adapted to a bisphenol compound.

Building the models

Empirical models were built considering, as a first approach, a second order model to include both variable interactions and quadratic terms. Since x_1 and x_2 are categorical variables, these parameters represent only a specific set of constants (for example $\beta_1 x_1$ represents only 4 constants depending on whether C1, C2, C3 or C4 cans were used). On the opposite, x'_3, x'_4 and x'_5 are numerical variables with infinite continuous input values: their corresponding linear, quadratic and interaction regression coefficients are constants multiplied with the variables themselves.

Hence, general equation for the models in our case is the following:

$$\text{Migrant level} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3' + \beta_4 x_4' + \beta_5 x_5' + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3' + \beta_{14} x_1 x_4' + \beta_{15} x_1 x_5' + \beta_{23} x_2 x_3' + \beta_{24} x_2 x_4' + \beta_{25} x_2 x_5' + \beta_{34} x_3' x_4' + \beta_{35} x_3' x_5' + \beta_{45} x_4' x_5' + \beta_{33} x_3'^2 + \beta_{44} x_4'^2 + \beta_{55} x_5'^2$$

where β_0, β_i, β_{ij}, β_{ii} are regression coefficients corresponding to the model intercept, effect parameter, interaction effect parameter and quadratic effect parameter, respectively.

The development data sets were used to build the models in order to properly fit our data. At first, in order to determine whether some variables could be discarded from the model, we tested hypotheses for the individual regression coefficients. The simple analysis starts with determining the main effect for each level of a factor (i.e. the difference between the average of output responses at the target level of a factor and the overall average of the output responses of all experiments). Thereafter, F-test is performed in order to identify the significance of the main factors, and the p-value approach was used (i.e. p-value < 0.05).

Because evaluating all possible regressions can be burdensome computationally, various methods have been developed for evaluating only a small number of subset regression models by either adding or deleting variables one at a time (Carley et al. 2004). In our case, backward elimination method was followed where we begin with a model that includes all candidate variables. Then according to the p-value of each factor (i.e. significant or not), the factor is included or excluded (elimination is done in the order from most insignificant to least significant) until finding the suitable model. Some variables did not have a significant effect, but could not be deleted due to their significant interaction with other variables.

Models validation

Validation of models was conducted by an external validation thanks to new experiments performed in a different batch from development data set. Selection of these experiments was done on the basis of: 1) repetition of experiments with unexpected output responses, 2) available conditions (remaining cans and available time within 15 days).

The validation process was achieved on three successive steps. Firstly we performed visual confirmation of attendance of validation experiment points within the confident interval of the linear model. Then, we calculated relative error (η) between the measured concentrations and the predicted concentrations with a fixed threshold about 0.2 (Kat and Els 2012). Last but not least, the $R^2_{predicted}$ was calculated to assess the quality of model prediction for the new experiments (validation data set), according to the following equation:

$$R^2_{predicted} = 1 - \left(\frac{\sum_{i=1}^{n}(O_i - P_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2}\right)$$

where n is the number of validation experiments, O_i is the observed concentration for experiment i, P_i is the predicted value for its experiment, and $\bar{O}$ the mean concentration from the development data set.

If $R^2_{predicted}$ is higher or close to $R^2_{adjusted}$, the model correctly fits the new experiments, while if there is a significant decrease the model should not be used as predictive model.

Results and discussion

Migration of bisphenols in the simulants after can contact

The UHPLC/Fluorescence analyses revealed several peaks in the simulant extracts, but only a few of them appearing at the expected retention times of target compounds

(Figure 1). However, due to the low levels of migrants in the extracts, only peaks corresponding to BADGE.2H_2O and BPA could be confirmed through the UHPLC/TOF-MS analyses.

The average migration levels of bisphenols measured in our sterilized cans are gathered in Table 2 (since all conditions tested are considered here, large standard deviations are observed). Concentrations measured are within the range of previous levels reported in canned food simulants (Munguia-Lopez and Soto-Valdez 2001; Noureddine El Moussawi et al. 2017) and canned food (Noonan et al. 2011; Geens et al. 2010). A logic correlation between the levels of BADGE.2H_2O and BPA can be noticed, where higher values of BPA were correlated to the higher values of BADGE.2H_2O and vice versa. Observed BADGE.2H_2O release is higher than recently reported for other cans, while BPA migration is within the same range (7-8.4 and 14-16 µg dm^{-2} for BPA and BADGE.2H_2O, respectively) (Paseiro-Cerrato et al. 2017).

All cans do comply with the European regulation regarding migration of BADGE and its derivatives. In the case of BPA, only C2 cans do comply with the recent European regulation (i.e. SML below 50 µg kg^{-1}). Indeed, C2 cans offer the minimum migration regarding both contaminants, closely followed by C4 cans. Significant higher migration levels of BADGE.2H_2O were observed with C3 cans, with also high BPA levels in C1 and C3 cans. Both types of cans were from the same brand, being coated with lacquer of same source. However, the coating of C3 cans was modified with microcrystalline wax (E 905) slipping agent in addition to aluminium pigmentation (grey appearance).

Migration modelling

Preliminary tests

Preliminary experiments were conducted to establish the experimental domain. We faced problems with ethanol-based food simulants (S3 and S4), since most of cans filled with these simulants opened in the sterilization pot. Consequently, we decided to build two different experimental designs: Design I to study the effect of food simulant type on migration during storage of non-sterilized cans, and Design II to investigate the effect of sterilization with food simulants that comply with this process. As a matter of fact, Design I also aimed at investigating the possibility to further study the sterilization process on simulants S1 and S2 only, as representative of simulants S3 and S4. A total of 72 experimental scenarios (36 per design) were conducted to build the can processing and storage dependent migration models (see Table 3).

Modelling migration in non-sterilized cans - Effect of food simulant and storage (Design I)

After removal of non-significant parameters, empirical model equations obtained for BADGE.2H$_2$0 and BPA are (values of regression coefficients are detailed in Supplementary material - Table S1):

$$[\mathrm{BADGE.2H_2O}] = \beta_0 + \beta_2 x_2 + \beta_4 x_4' + \beta_{24} x_2 x_4'$$

$$[\mathrm{BPA}] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_4 x_4' + \beta_5 x_5' + \beta_{12} x_1 x_2 + \beta_{14} x_1 x_4' + + \beta_{15} x_1 x_5' + \beta_{24} x_2 x_4' + \beta_{25} x_2 x_5' + \beta_{45} x_4' x_5' + \beta_{44} x_4'^2 + \beta_{55} x_5'^2$$

These equations suggest that the food simulant (x_2) plays a role in the migration of both BADGE.2H$_2$O and BPA, as confirmed by the p-values obtained after the significance test (0.027 and 0.0002, respectively – see Table 4). Looking into the detailed results reveal that the effect of food simulant on BADGE.2H$_2$O migration is driven only by 50% ethanol (S4). For BPA the results are almost quite the same since a clear effect of

S4 is shown. A slight effect of S3 is also suggested based on significance of a few estimated regression coefficients. However, considering the standard errors, the values of regression coefficients for S3 are not significantly different from regression coefficient for S1 and S2. Only simulant S4 is significantly different from the three others. These results suggest that whether water (S1), 3% acetic acid (S2) or 10% ethanol (S3) are used, similar migration should be observed both for BPA and BADGE.2H_2O upon storage. This is in agreement with previous results from Biles et al. (1997) showing, for polycarbonate plastic stored 10 days at 65°C with unstirred food simulant, that BPA migration was quite moderate with water (0.23 µg cm^{-2}) or 10% ethanol (0.91 µg cm^{-2}) while it was greatly enhanced with 50% ethanol (5.9 µg cm^{-2}). Also results reported later by Paseiro-Cerrato et al. (2017) for non-sterilized epoxy-coated cans filled with 50% ethanol, clearly showed that in this simulant mass transfer is still occurring for prolonged storage; their results indicate that after 90 days of storage BPA and BADGE.2H_2O migration in 50% ethanol was even higher than in sterilized epoxy-coated cans filled with water.

To conclude, results from experimental Design I are in favour of further studying the sterilization process on simulants S1 and S2 only, as representative of simulant S3 (i.e. Design II). That way, migration modelling mimics application to aqueous canned food.

Modelling migration in sterilized cans - Effect of process and storage conditions (Design II)

After removal of non-significant parameters, the final models developed for this experimental design are the following (values of regression coefficients are detailed in Supplementary material - Table S2):

$$\mathrm{BADGE.2H_2O} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3' + \beta_4 x_4' + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3' + \beta_{23} x_2 x_3' + \beta_{24} x_2 x_4' + \beta_{33} x_3'^2$$

$$\mathrm{BPA} = \beta_0 + \beta_1 x_1 + \beta_3 x_3' + \beta_4 x_4' + \beta_5 x_5' + \beta_{13} x_1 x_3' + \beta_{15} x_1 x_5' + \beta_{33} x_3'^2 + \beta_{44} x_4'^2$$

It is noticeable that storage time (x_5) has no influence on BADGE.2H$_2$O migration. Similarly, the food simulant (x_2, S1 or S2) has no impact on BPA levels. On the contrary, for both compounds, the type of can, sterilization time and storage temperature or their combinations are significant in the fit model.

Many studies reported that heat processing of cans has an important influence on inducing the migration of residual compounds from can lining into foods. Hence, Cabado et al. (2008) found higher migration of BADGE in tuna fish when heat processed cans were considered, with similar migration levels for the two sterilization conditions tested in their study (115°C during 45 min, or 121°C during 30 min). Similarly, Simoneau et al. (2002) reported significant higher BADGE migration from cans into sunflower oil as simulant after processing at 115°C, as compared to non-processed cans, with similar migration levels whatever the two sterilization times they considered (30 min or 60 min). In our model, sterilization time plays a major role on BADGE.2H$_2$O and BPA migration (p-values < 0.0001 for both compounds as shown in Table 4), possibly since prolonged duration up to 90 min was considered here.

Due to severe conditions during sterilization (i.e. 121°C for 30 or 90 min), mass transfer from the can coating into simulants mainly occurred during this step. Then, levels of BADGE.2H$_2$O remained stable in the processed cans, whatever storage duration and temperature (p-value of 0.940 and 0.393, respectively – see Table 4). In the case of BPA, while storage temperature did not affect its levels in processed cans (p-value = 0.616), storage time was found significant (p-value = 0.002). Looking deeper into the data revealed that this significant effect of storage time was related to a specific case (C1 cans), where BPA level decreased over storage; considering the other can categories, BPA level remained stable over storage time which matches previous studies

(Goodson et al. 2004; Munguia-Lopez and Soto-Valdez, 2001; Noureddine El Moussawi et al. 2017).

Testing models adequacy and validation

Validation conditions for both Designs I and II are given in Table 5, as well as bisphenols levels expected and measured. As illustrated in Figure 2, most of the output responses of validation experiments lay within the confidence intervals (the red margins) of actual by predicted plots for both BADGE.2H_2O and BPA, particularly for models based on Design II. This is confirmed by calculation of relative error (η) between the measured and the predicted concentrations (see Table 5). Values are in the range 0.01-0.06 (mean = 0.04) for BADGE.2H_2O, and 0.004-0.34 (mean = 0.12) for BPA. Thus, except for extreme values observed for BPA, these relative errors are below the threshold and validate the models.

To test further the models adequacy, the analysis of variance, coefficient of determination, significance and lack of fit of the actual to predicted plots were carried out (see Supplementary material – Table S3). Adjusted coefficients of determination ($R^2_{adjusted}$) show good prediction of the models in the case of BPA for both experimental designs, and BADGE,2H_2O for Design II. Interestingly, whatever the models, statistical F-ratios correspond to very small p-values, indicating that these migration models are significant to predict the migration of BPA and BADGE,2H_2O based on canned food processing and storage parameters. Also, the values of $R^2_{predicted}$ calculated for Design II-based models are close to $R^2_{adjusted}$. In conclusion, the proposed model built on Design II enables good prediction concentration of BPA and BADGE.2H_2O migration into simulants for all can brands and process parameters considered.

Application to prediction of bisphenol migration in canned foods

Vegetable foods typical of Lebanese cuisine and frequently available in cans (i.e. fava beans, red beans, chickpeas and okra) were also considered in this work. Measuring bisphenols levels in the raw products as well in the canned foods enabled to investigate bisphenol migration caused by the sterilization process. Again, among the bisphenols considered, only BPA and BADGE were confirmed. Interestingly, levels observed in canned foods were lower than the levels measured in food simulants. As a consequence, the migration models built with simulants were conservative, as predicted bisphenols concentrations in canned foods were overestimated as illustrated in Figure 3.

Valuable models application for the industry

We believe that RSM methodology could be convenient for the food and canning industries. Experimental design is required for building the models initially and exploring the range of conditions to test. After that, modelling is a suitable tool to evaluate the impact of the different process factors on migrant concentrations, and also to determine the optimized combination of factors to comply with the regulation. Hence, it is possible with the JMP software to change the desirability of model output (e.g. BPA concentration<50 µg kg^{-1}) to obtain the best input combination to reach this maximum desirability (Figure 4): the software evaluated all possible combinations between our studied parameters (i.e. type of can, sterilization time, storage temperature and storage time) and proposed the best combination to achieve BPA levels in compliance with the regulation.

In this illustrative application, the storage temperature has a very weak effect on desirability which is in accordance with the non-significance of that parameter in the model. Other parameters play a key role on desirability. In particular, considering the types of can studied, only cans C2 are suitable to reach BPA migration in compliance

with SML as previously discussed. For sterilization at 121°C, 90 min is more suitable than 30 min, which was unexpected as previously discussed. This could be explained either by BPA back retention in the coating over time, or by BPA disappearance due to reaction of phenolic groups at high temperature. Interestingly, in the case of storage time, despite a weak influence on BPA concentrations its effect on desirability is important. Thus, according to this desirability study, in our case the optimum combination to ensure BPA levels complying with regulation should be cans C2, 90 min sterilization, and storage at room temperature for 60 days.

Conclusion

At the time where most migration models developed take into account the physicochemical properties of packaging, which is complex to control at the level of food canning industries, this paper provides interesting simple predictive models based on response surface methodology. Such models could be very helpful for industries to take precautions or provide advices with a view to minimizing bisphenols migration in order to comply with regulations. Even though experimental tests will be required at the beginning to transpose the approach to custom applications, then routine controls will require only few tests to check that model applicability is still valid (especially to take into account high variability in cans and protective layers).

Aknowledgements

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Appendix A. Supplementary material

Disclosure statement

No potential conflict of interest was reported by the authors.

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Tables

Table 1: Symbols and levels of studied input variables.

Variables	**Symbols**	**Levels**			
Categorical		L1	L2	L3	L4
Tinplate can	x_1	C1	C2	C3	C4
Food simulant	x_2	S1: water	S2: 3% w/v acetic acid	S3: 10% v/v ethanol	S4: 50% v/v ethanol
Discrete		L1	L2	L3	
Sterilization time (min)	x_3	0	30	90	
Storage temperature (°C)	x_4	5	22.5	40	
Storage time (day)	x_5	1	15	60	

Table 2: Average migration levels measured for BPA and BADGE.2H_2O (considering all tested conditions).

	BADGE.2H_2O*				**BPA****			
Type of can	Average concentration (µg kg^{-1})		Average migration (µg dm^{-2})		Average concentration (µg kg^{-1})		Average migration (µg dm^{-2})	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C1	520	116	57.8	18.4	197	85	21.8	9.4
C2	349	45	38.8	5.0	47	5.4	5.2	0.6
C3	1106	193	94.4	16.4	132	58	11.3	5.0
C4	355	87	39.4	9.7	65	12.7	7.2	1.4

** European SML = 9,000 µg kg^{-1} (sum of BADGE and its hydrolysed derivatives)*
*** European SML = 50 µg kg^{-1}*

Table 3: Details of experimental conditions (Designs I and II) for building the models.

	Design I				Design II				
Experiment	Type of can	Food Simulant	Storage		Type of can	Food Simulant	Sterilization time (min)	Storage	
			T°C	Time (day)				T°C	Time (day)
1	C1	S1	5	60	C1	S1	90	5	60
2	C1	S1	40	1	C1	S1	90	40	1
3	C1	S2	40	1	C1	S2	0	5	60
4	C1	S2	5	60	C1	S1	0	22.5	15
5	C1	S3	22.5	1	C1	S1	0	40	60
6	C1	S3	40	60	C1	S2	0	22.5	1
7	C1	S4	40	15	C1	S2	90	40	60
8	C1	S4	5	1	C1	S2	90	5	15
9	C1	S4	22.5	60	C1	S2	30	40	15
10	C2	S1	5	1	C1	S1	30	5	1
11	C2	S1	40	60	C2	S1	90	40	60
12	C2	S2	40	60	C2	S2	90	5	60
13	C2	S2	5	1	C2	S2	30	22.5	15
14	C2	S3	40	1	C2	S1	0	40	1
15	C2	S3	5	15	C2	S2	0	40	60
16	C2	S3	22.5	60	C2	S2	0	5	1
17	C2	S4	22.5	1	C2	S1	90	5	1
18	C2	S4	5	60	C2	S2	90	40	1
19	C3	S1	40	1	C2	S1	0	5	60
20	C3	S1	5	60	C3	S2	90	5	60
21	C3	S2	5	1	C3	S1	0	5	60
22	C3	S2	40	60	C3	S1	90	40	60
23	C3	S3	40	1	C3	S1	0	40	1
24	C3	S3	5	60	C3	S2	0	40	60
25	C3	S4	40	60	C3	S1	90	5	1
26	C3	S4	5	1	C3	S2	0	5	1
27	C4	S1	40	60	C3	S2	90	40	1
28	C4	S1	5	1	C4	S2	90	5	1
29	C4	S2	5	60	C4	S2	90	40	60
30	C4	S2	40	1	C4	S1	0	40	60
31	C4	S3	5	1	C4	S1	90	5	60
32	C4	S3	40	15	C4	S1	90	40	15
33	C4	S3	5	60	C4	S1	30	22.5	1
34	C4	S4	40	60	C4	S2	0	40	1
35	C4	S4	40	1	C4	S2	0	5	60
36	C4	S4	5	15	C4	S1	0	5	15

Table 4: Effect test significance on the basis of p-value for the two models.

Effect test	Variable symbol	Design I				Design II			
		BADGE.2H_2O		BPA		BADGE.2H_2O		BPA	
Source		**F ratio**	**P-value**	**F ratio**	**P-value**	**F ratio**	**P-value**	**F ratio**	**P-value**
Type of can	x_1	2.481	0.238	81.827	**0.002***	63.530	**<0.0001***	29.316	**0.0002***
Type of simulant	x_2	14.639	**0.027***	406.812	**0.0002***	13.969	**0.007***	0.223	0.651
Sterilization time	x'_3	NA	NA	NA	NA	700.202	**<0.0001***	121.263	**<0.0001***
Sterilization time*Sterilization time	x'^2_3	NA	NA	NA	NA	23.247	**0.002***	50.498	**0.0002***
Storage temperature	x'_4	14.280	**0.032***	268.594	**0.0005***	0.830	0.393	0.275	0.616
Storage temperature*Storage temperature	x'^2_4	1.136	0.365	292.602	**0.0004***	1.435	0.270	19.061	**0.003***
Storage time	x'_5	3.007	0.181	318.533	**0.0004***	0.0061	0.940	21.496	**0.002***
Storage time*Storage time	x'^2_5	4.497	0.124	71.812	**0.003***	0.367	0.564	5.198	0.0566
Type of can*type of simulant	$x_1 * x_2$	1.382	0.437	163.663	**0.001***	2.270	0.168	2.941	0.108
Type of can*Sterilization time	$x_1 * x'_3$	NA	NA	NA	NA	62.534	**<0.0001***	8.297	**0.011***
Type of can*Storage temperature	$x_1 * x'_4$	1.493	0.375	57.254	**0.004***	0.187	0.902	2.166	0.180
Type of can*Storage time	$x_1 * x'_5$	1.432	0.388	110.165	**0.001***	0.939	0.471	11.717	**0.004***
Type of simulant*Sterilization time	$x_2 * x'_3$	NA	NA	NA	NA	7.812	**0.027***	2.003	0.200
Type of simulant*Storage temperature	$x_2 * x'_4$	10.841	**0.041***	15.756	**0.024***	2.835	0.136	0.000	0.996
Type of simulant*Storage time	$x_2 * x'_5$	3.175	0.184	132.644	**0.001***	0.307	0.596	0.0364	0.854
Sterilization time*Storage temperature	$x'_3 * x'_4$	NA	NA	NA	NA	0.016	0.903	2.336	0.170
Sterilization time*Storage time	$x'_3 * x'_5$	NA	NA	NA	NA	0.090	0.772	5.043	0.056
Storage temperature*Storage time	$x'_4 * x'_5$	1.513	0.306	38.973	**0.008***	0.691	0.433	0.0235	0.883

** Significant effect if p<0.05; NA: not appropriate since Design I is dedicated to non-sterilized cans only*

Table 5: Details of experimental conditions (Designs I and II) for models validation, and comparison between observed and predicted values.

Experiment	Can type	Food simulant	Sterilization time (min)	Storage temperature (°C)	Storage time (min)	**BADGE.2H$_2$O (μg kg^{-1})**			**BPA (μg kg^{-1})**		
						observed	predicted	error η	observed	predicted	error η
Design I											
1	C1	S4		40	15	283	229	0.19	138	151	0.10
2	C1	S4		40	15	224	229	0.02	112	151	0.35
3	C1	S4		40	15	251	229	0.09	190	151	0.20
4	C1	S4		22.5	15	142	109	0.23	177	152	0.14
5	C1	S4		22.5	15	139	109	0.22	190	152	0.20
6	C1	S4		22.5	15	175	109	0.38	183	152	0.17
Design II											
1	C1	S1	30	5	1	354	343	0.03	225	257	0.14
2	C1	S1	30	5	1	363	343	0.05	338	257	0.24
3	C1	S1	90	40	1	467	483	0.03	325	250	0.23
4	C1	S1	90	5	60	471	489	0.03	45	47	0.05
5	C1	S2	90	5	15	574	611	0.06	55	47	0.14
6	C1	S2	90	5	15	623	611	0.01	47	47	0.01
7	C2	S1	90	5	1	317	323	0.02	232	215	0.07
8	C2	S1	90	5	1	334	323	0.03	211	215	0.02
9	C2	S1	90	5	1	326	323	0.01	107	120	0.12
10	C2	S1	90	40	60	312	294	0.06	37	49	0.34
11	C2	S2	30	22.5	15	274	289	0.06	42	40	0.05
12	C2	S2	30	22.5	15	292	289	0.01	40	40	0.004

Figures

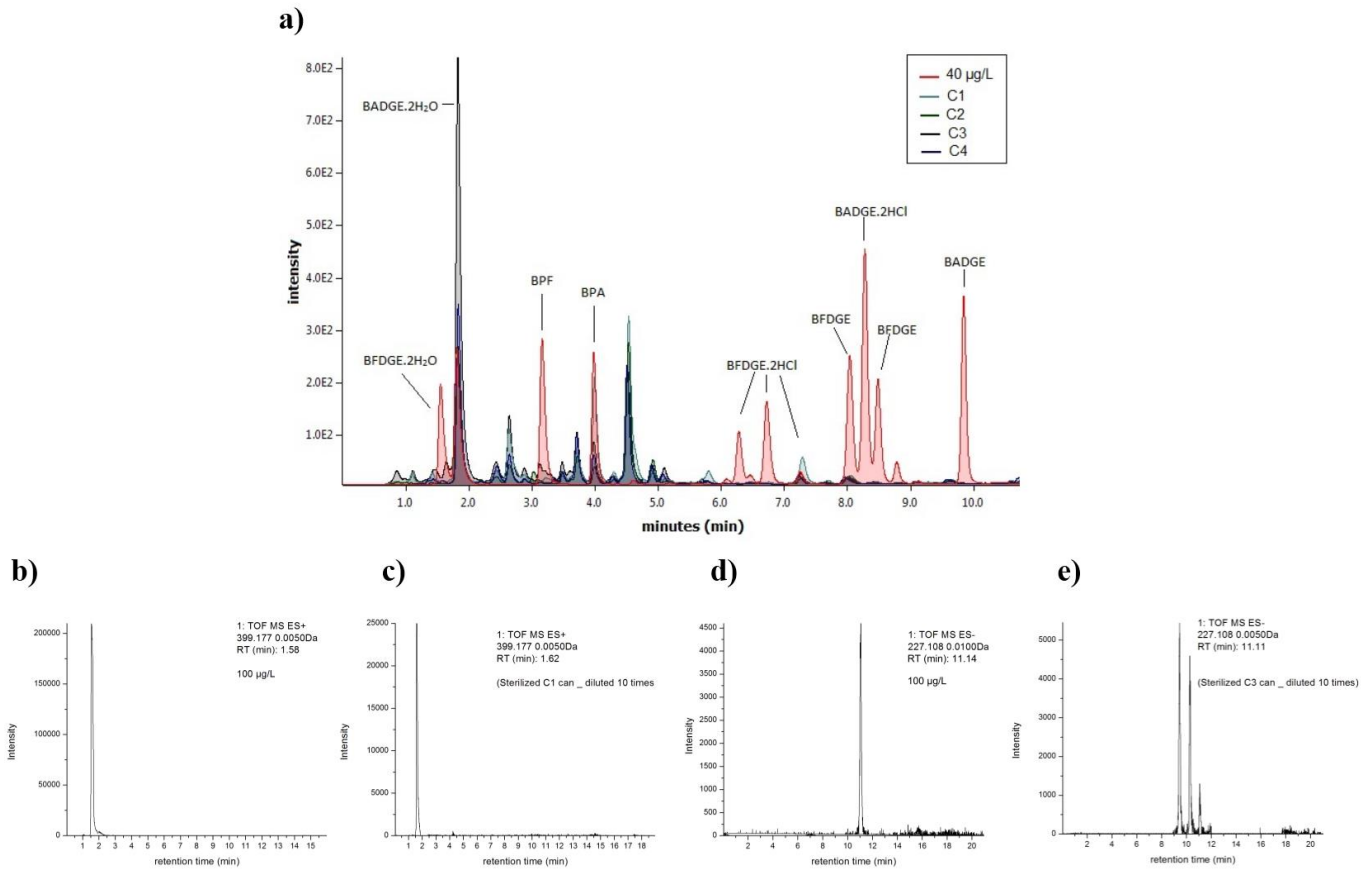

Figure 1: UHPLC-fluorescence chromatograms after analysis of four can categories and 40 µg L^{-1} standard (a), and UHPLC-TOF-MS chromatograms of detected compounds: (b) BADGE.$2H_2O$ peak of 100 µg L^{-1} standard; (c) BADGE.$2H_2O$ peak corresponding to a sterilized C1 can; (d) BPA peak of 100 µg L^{-1} standard; (e) BPA peak corresponding to a sterilized C3 can, with BADGE.$2H_2O$ main *m/z* of 399.177 and BPA main *m/z* of 227.108

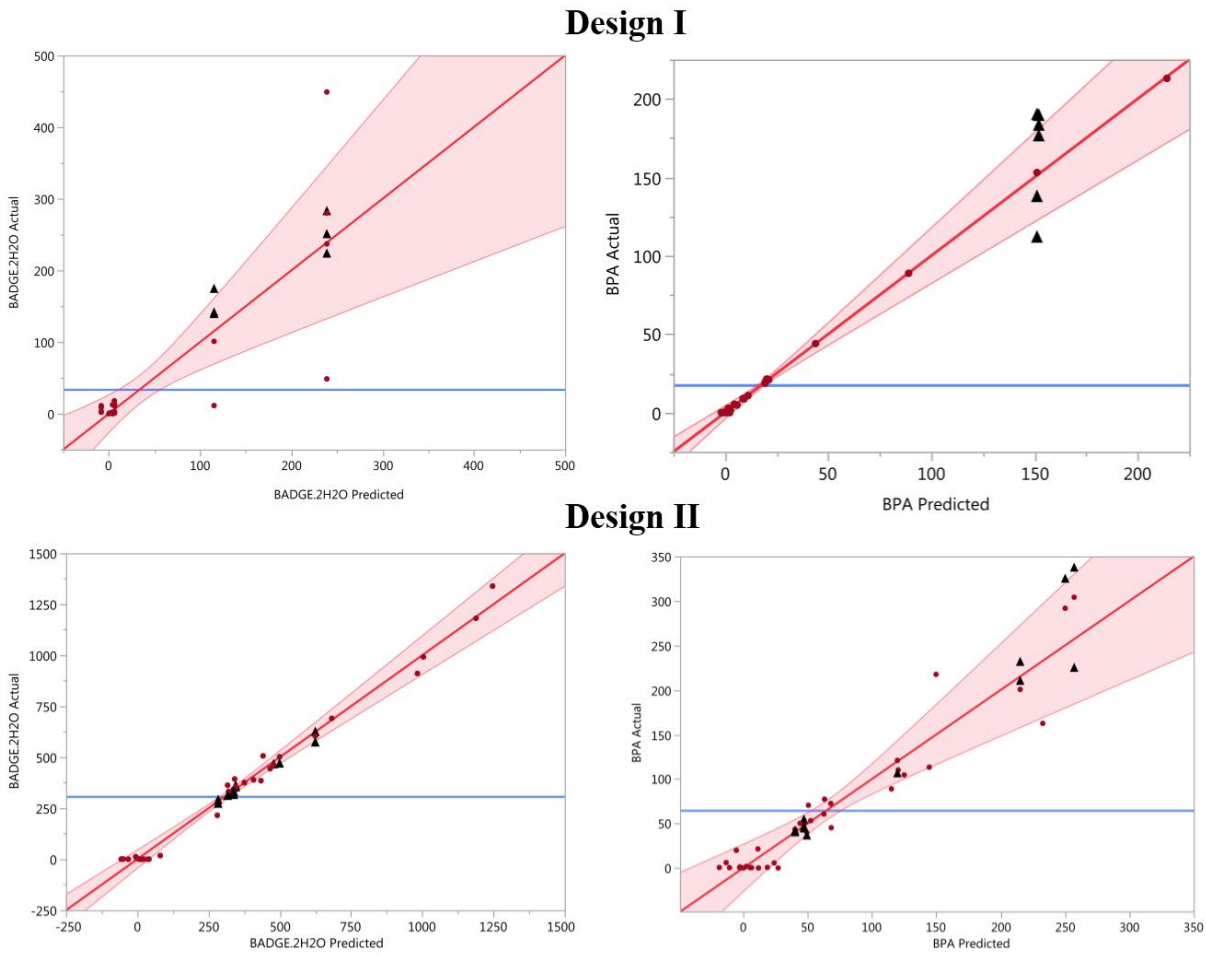

Figure 2: Predictive capability of models built for both design of experiments (with red margins representing the confidence intervals): (•) data from development set, (▲) data from validation set.

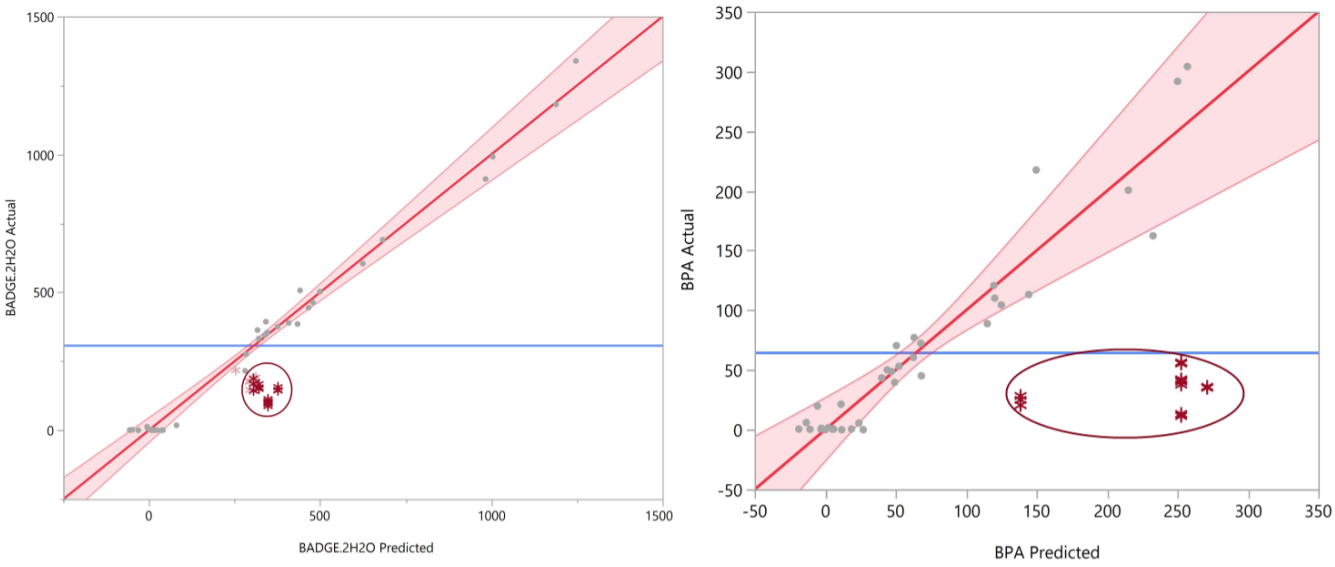

Figure 3: Prediction of BPA and BADGE.2H_2O levels in sterilized canned legumes and vegetables based on the migration models built using food simulants. * *corresponds to the values obtained for food samples.*

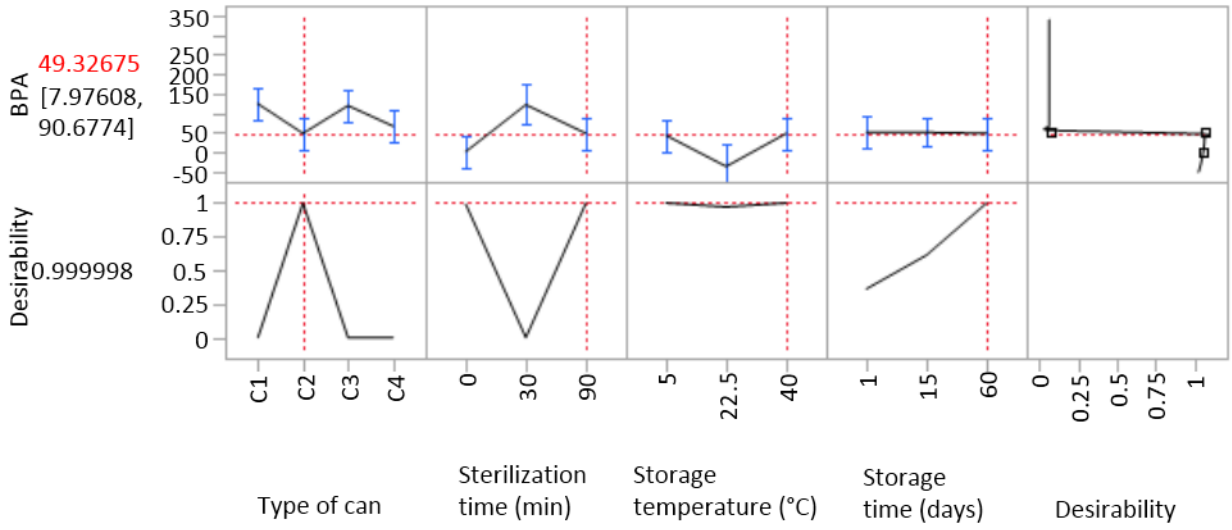

Figure 4: Desirability study for optimization of process parameters to comply with European Regulation 2018/213.

Appendix A - Supplementary material

Supplementary Material - **Table S1:** Selection of estimated regression coefficients with their standard error and significance* against zero for models based on Design I.

BADGE.2H$_2$O				
Term		Scaled Estimate	Std error	p-value scaled = 0
β_0		30.95	9.74	0.0036
$\beta_2 x_2$	If [S1]	-28.93	17.48	0.190
	If [S2]	-27.64	17.48	0.125
	If [S3]	-27.73	16.23	0.0985
	If [S4]	84.30	16.23	**<0.0001**
β_4		32.85	10.26	**0.0034**
$\beta_{24} x_2$	If [S1]	-30.83	17.78	0.093
	If [S2]	-30.15	17.78	0.101
	If [S3]	-29.79	17.78	0.104
	If [S4]	90.7798	17.78	**< 0.0001**

BPA				
Term		Scaled Estimate	Std Error	p-value scaled = 0
β_0		53.16	10.16	**0.0004**
$\beta_1 x_1$	If [C1]	10.16	5.13	0.075
	If [C2]	-16.82	4.90	**0.006**
	If [C3]	6.49	5.08	0.229
	If [C4]	0.16	4.81	0.974
$\beta_2 x_2$	If [S1]	-6.22	5.08	0.248
	If [S2]	-6.85	5.08	0.207
	If [S3]	-15.63	4.76	**0.0082**
	If [S4]	28.71	5.03	**0.0002**
β_4		8.35	2.84	**0.003**
β_5		10.5	3.18	**0.025**

$\beta_{12}x_1x_2$	If [C1]* [S1]	-13.14	8.70	0.161
	If [C1]*[S2]	-12.15	8.70	0.192
	If [C1]* [S3]	-24.28	8.68	**0.0189**
	If [C1]*[S4]	49.58	8.68	**0.0002**
	If [C2]*[S1]	13.39	8.56	0.148
	If[C2]*[S2]	14.57	8.56	0.119
	If [C2]*[S3]	5.90	7.68	0.459
	If[C2]*[S4]	-33.87	8.63	**0.002**
	If [C3]*[S1]	-7.24	8.66	0.422
	If [C3]*[S2]	0.59	8.66	0.946
	If [C3]*[S3]	2.93	8.48	0.735
	If [C3]*[S4]	3.69	8.63	0.677
	If [C4]*[S1]	6.99	8.51	0.430
	If [C4]*[S2]	-3.01	8.51	0.730
	If [C4]*[S3]	15.43	7.76	0.074
	If [C4]*[S4]	-19.40	8.00	**0.035**

$\beta_{15}x_1$	If [C1]	16.65	5.02	**0.007**
	If [C2]	-14.41	5.00	**0.016**
	If [C3]	0.894	4.95	0.860
	If [C4]	-3.14	4.88	0.533
$\beta_{25}x_2$	If [S1]	-8.72	4.95	0.108
	If [S2]	-8.05	4.95	0.134
	If [S3]	-8.73	4.96	0.109
	If [S4]	25.51	5.01	**0.0005**
β_{55}		-43.20	11.05	0.0029

** based on p-value from a t-test assessing if the estimated values are statistically different from zero*

Supplementary Material - **Table S2:** Estimated regression coefficients of the models built to fit data from Design II.

BADGE.2H$_2$O			**BPA**		
Term		Scaled estimate	Term		Scaled estimate
β_0		494.10	β_0		97.38
$\beta_1 x_1$	If [C1]	-8.33	$\beta_1 x_1$	If [C1]	53.93
	If [C2]	-123.24		If [C2]	-37.09
	If [C3]	249.25		If [C3]	7.73
	If [C4]	-117.68		If [C4]	-24.57
$\beta_2 x_2$	If [S1]	-38.84	β_3		45.98
	If [S2]	38.84	β_4		2.64
β_3		300.79	β_5		-19.75
β_4		9.25	$\beta_{13} x_1$	If [C1]	23.23
$\beta_{12} x_1 x_2$	If [C1]*[S1]	-10.86		If [C2]	-22.48
	If [C1]*[S2]	10.86		If [C3]	16.93
	If [C2]*[S1]	32.84		If [C4]	-17.67
	If [C2]*[S2]	-32.84	$\beta_{15} x_1$	If [C1]	-42.80
	If [C3]*[S1]	-40.53		If [C2]	18.24
	If [C3]*[S2]	40.53		If [C3]	5.04
	If [C4]*[S1]	18.56		If [C4]	19.51
	If [C4]*[S2]	-18.56	β_{33}		-117.62
$\beta_{13} x_1$	If [C1]	-27.25	β_{44}		82.02
	If [C2]	-117.37			
	If [C3]	249.60			
	If [C4]	-104.98			
$\beta_{23} x_2$	If [S1]	-32.73			
	If [S2]	32.73			
$\beta_{24} x_2$	If [S1]	-19.76			
	If [S2]	19.76			
β_{33}		-187.99			

Supplementary Material - **Table S3**: Adequacy and significance tests of regression models.

Model Fit				
	Design I		**Design II**	
	BADGE.2H$_2$O	BPA	BADGE.2H$_2$O	BPA
$R^{2\,a}$	0.693	0.999	0.989	0.912
$R^2_{adjusted}$[b]	0.617	0.994	0.981	0.854
$R^2_{predicted}$	0.948	0.956	0.977	0.937

Analysis of variance												
	Design I						**Design II**					
	BADGE.2H$_2$O			BPA			BADGE.2H$_2$O			BPA		
	Model	Error	Total	Model	Error	Total	Model	Error	Total	Model	Error	Total
Degree of freedom (d*f*)[c]	7	28	35	32	3	35	15	20	35	14	21	35
Sum of Squares (SS)[d]	213651	94384	308035	6924	34.9	69280	4492456	49790	4542246	212182	2044	232626
Mean Square (MS)[e]	30522	3371		2164	11.6		299497	2489		15156	973	
F-ratio[f]	9.05			186			120.3			15.57		
P-value[g]	<0.0001 *			0.0006 *			<0.0001 *			<0.0001 *		

** Significant effect if p<0.05*
a: coefficient of determination defined as $R^2 = 1 - SS_{(error)}/ SS_{(Total)}$ $(0 \leq R^2 \leq 1)$
b: adjusted coefficient of determination defined as $R^2_{adjusted} = 1 - (SS_{(error)} / n\text{-}p)/ (SS_{(Total)}/n\text{-}1)$, where (n) is the number of observations and (p) is the number of regression coefficients
c: number of free units of information
d: calculated by summing the squared factor effect for each run
e: MS = SS/df
f: F-ratio = $MS_{(model)}/ MS_{(error)}$
g: looked up in the F table