User's Guide of the Inter-Batch Physico-Chemical Variability Calculator

1. Scope

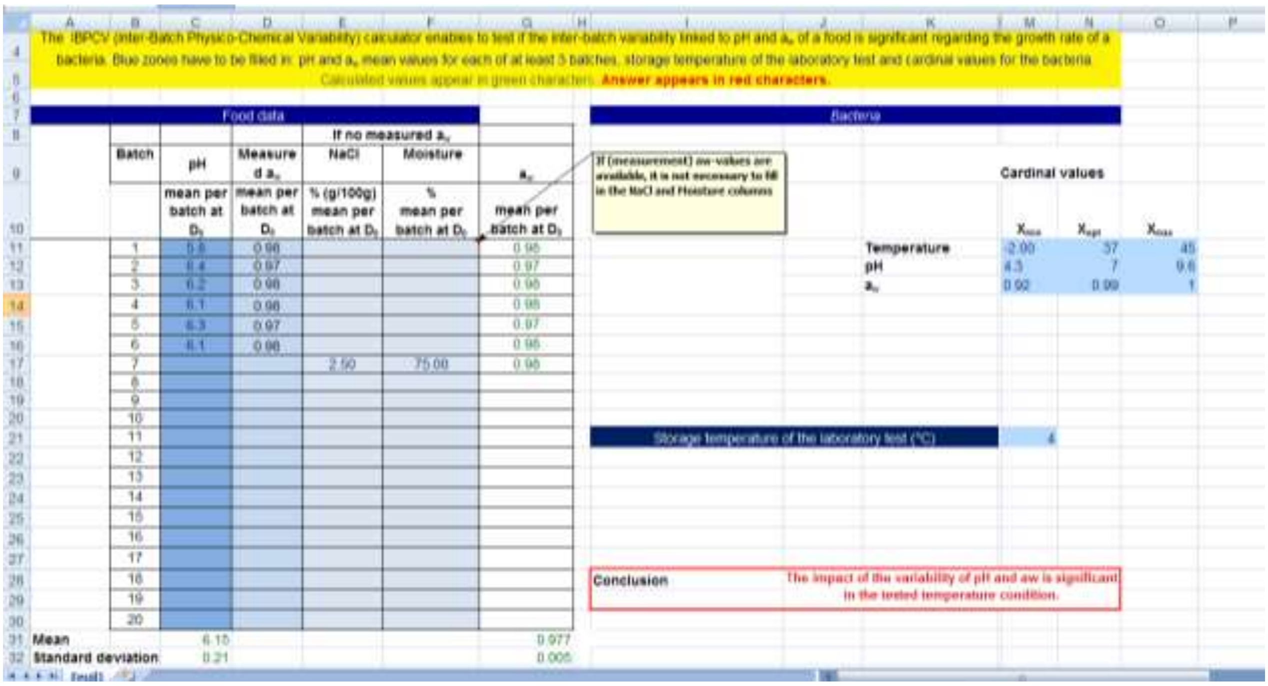
The Inter-Batch Physico-Chemical Variability (IBPCV) Calculator enables assessment of the impact of batch to batch variability in the physico-chemical characteristics (pH and a_w) of a food on the maximum growth rate of a bacteria.

This calculator is intended to be used in the growth range of the studied bacteria. This growth range is delimited by the cardinal values (pH_{min}, pH_{max} and $a_{w\,min}$, $a_{w\,max}$) of the bacteria.

This calculator requires pH and a_w values to characterize the batches.

2. Use of the IBPCV calculator

An overview of the IBPCV calculator is presented below.



2.1 Input data (blue zones) related to the food, the bacteria and the temperature of the test

The food must be characterized by two physico-chemical parameters: pH and a_w. The values of these parameters for a batch are based on measurements taken on more than one sample (ideally more than 10 samples).

The IBPCV calculator cannot be used for pH or a_w values lower than the cardinal values of the studied bacteria, and two warnings ("pH mean ≤ pH min" and "a_w mean ≤ a_w min") are included in the calculator.

2.2 Food data

Enter the food data as follows:

- Column B "Batch". List the number of batches (maximum of 20) that have been tested. Each batch is therefore characterized by mean values for pH and a_w. The a_w values for the food can either be measured or derived from its salt (NaCl) and moisture content. Note that a warning in red ("it is recommended to compare at least 3 batches") will appear if fewer than 3 batches have values for both pH and a_w

- Column C "pH". Enter the mean values for pH (to one decimal point) measured on the day of manufacture of the food. A warning ("pH mean ≤ pH min") will appear if the entered value is lower than the minimum pH for growth of the bacteria of interest.

- Column D "Measured a_w". Enter the mean values a_w (to two decimal points) measured the day of the manufacture of the food. A warning ("a_w mean ≤ aw min") will appear if the entered value is lower than the minimum a_w for growth of the bacteria of interest.

  If no measured a_w data is available but the initial NaCl and initial moisture contents have been measured, the mean values of these two parameters can be filled in columns E and F - "NaCl" and "Moisture".

The IBPCV calculator will calculate an a_w value according to the formula of Resnik and Chirife (1988), given in the EURL Lm technical guidance document:

$$a_w = 1 - 0.0052471 \times WPS - 0.00012206 \times WPS^2$$

$$a_w = 1 - 0.0052471 \; x \; WPS - 0.00012206 \; x \; WPS^2$$

where WPS (Water Phase Salt) content (in g/100ml)

$$= \frac{NaCl \text{ content } (\text{in g per } 100\, g)}{\text{moisture content } (\text{in ml per } 100\, g)} \times 100$$

Note that this formula can only be used for food products where NaCl is the main component responsible for the a_w of the food.

- Column G"a_w" gives the initial mean a_w value of the product (measured or calculated data) that is used for the inter-batch variability calculation.

For the calculations, the system prioritizes the measured a_w values over the calculated a_w.

2.3 Data about cardinal values of the bacteria

Input the cardinal values (X_{min}, X_{opt} and X_{max}) for temperature, pH and a_w of the considered bacteria. Data in table 1 (ANSES reference) below may be used.

It is recommended that the values for temperature are entered as whole numbers, pH with one decimal and a_w value with two decimals places.

Table1: Cardinal values for temperature, pH and a_w of the food pathogenic bacteria.

	T_{min} (° C)	T_{opt} (° C)	T_{max} (° C)	pH_{min}	pH_{opt}	pH_{max}	aw_{min}	aw_{opt}	aw_{max}
L. monocytogenes	-2	30 - 37	45	4.0-4.3	7.0	9.6	0.92	0.99	1
Salmonella spp	5	35 - 37	50	3.8	7-7.5	9.5	0.94	0.99	0.99
S. aureus	6	35 - 41	48	4	6-7	10	0.83	0.99	0.99
E. coli 0157/H7	6	40	45.5	4.4	6-7	9	0.95	0.99	/
Bacillus cereus / vegetative cells	4 (10 for emetic strains)	30 - 37	55	4.3	6-7	9.3	0.92	0.99-1	/
Cl. perfringens	10	40 - 45	52	5.0	6-7	8.3	0.95/0.97	0.99 -1	/
Cl. botulinum/ (proteolytic A and B)	10	35 - 40	48	4.6	6.8	9	0.94	0.99-1	/
Cl. botulinum/ (non proteolytic B)	3	18 - 25	45	5.0	7	9	0.97	0.99 -1	/
Cl. botulinum (E)	3	18 - 25	45	5.0	7	9	0.97	0.99 -1	/

2.4 Storage temperature data

Enter the temperature at which the challenge test is planned to be performed. The input value is a whole number and always lower than the T_{opt} of the studied microorganism.

A warning appears if the storage temperature of the test is below T_{min} or above T_{opt} of the studied bacteria.

3. Result in the calculator (in red)

The IBPCV calculator will conclude, on the impact of the variability of pH and a_w on the growth rate of a bacteria in the tested conditions of the challenge test, based on the physico-chemical input data characterizing the product, the cardinal values of the bacteria and the storage temperature of the challenge test.

The conclusion is either that "The impact of the variability of pH and a_w is significant in the tested temperature condition" or "The impact of the variability of pH and a_w is not significant in the tested temperature condition."

It is important to note that when the pH or a_w values of the tested product are close to the growth/no growth boundaries of the bacteria, slight changes in pH or a_w values can have a significant impact on the growth rate of the bacteria. This will lead to the conclusion that "The impact of the variability of pH and a_w is significant in the tested temperature condition".

4. References

- ANSES, Data sheets on foodborne microbiological hazards https://www.anses.fr/en/content/microbiological-hazards-files
- EURL Lm Technical guidance document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat-foods, EURL for *Listeria monocytogenes*, (2014).

https://ec.europa.eu/food/sites/food/files/safety/docs/biosafety_fh_mc_technical_guidance_document_listeria_in_rte_foods.pdf

- NF V01-009 (mai 2014), *Lignes directrices pour la réalisation de tests de croissance microbiologiques*.
- Regulation (EC) 2073/2005 on microbiological criteria for foodstuffs (2005). OJ L 338 22.12.2005, p. 1.
- Resnik SL, Chirife J. 1988. *Proposed theoretical water activity values at various temperatures for selected solutions to be used as reference sources in the range of microbial growth*. J. Food Prot. 51:419–423.

Annex

Calculation

- Calculate: $\varphi_{\mathrm{T}} = \left(\frac{T_{\mathrm{opt}} - T}{T_{\mathrm{opt}} - T_{\min}} \right)^3$

T (°C) being the temperature used for the challenge test ($T \leq T_{\mathrm{opt}}$), $T_{\min}$ (°C) and T_{opt} (°C) being respectively minimum growth temperature and optimum growth temperature of the studied bacteria.

- If $\overline{pH} < pH_{\mathrm{opt}}$, calculate:

$$\varphi_{\mathrm{pH,s}} = \left(\frac{pH_{\mathrm{opt}} - \left(\overline{pH} - 2s_{\mathrm{pH}}\right)}{pH_{\mathrm{opt}} - pH_{\min}} \right)^3 \text{ and } \varphi_{\mathrm{pH,i}} = \begin{cases} 0 & \text{, if } \overline{pH} + 2s_{\mathrm{pH}} \geq pH_{\mathrm{opt}} \\ \left(\frac{pH_{\mathrm{opt}} - \left(\overline{pH} + 2s_{\mathrm{pH}}\right)}{pH_{\mathrm{opt}} - pH_{\min}} \right)^3 & \text{, if } \overline{pH} + 2s_{\mathrm{pH}} < pH_{\mathrm{opt}} \end{cases}$$

$\overline{pH}$ being the between-batch mean pH of the food, s_{pH} being the between-batch sample standard deviation for the pH of the food, $pH_{\min}$ and pH_{opt} being respectively minimum and optimum pH for growth of the studied bacteria.

- If $\overline{pH} \geq pH_{\mathrm{opt}}$, calculate:

$$\varphi_{\mathrm{pH,s}} = \left(\frac{\left(\overline{pH} + 2s_{\mathrm{pH}}\right) - pH_{\mathrm{opt}}}{pH_{\max} - pH_{\mathrm{opt}}} \right)^3 \text{ and } \varphi_{\mathrm{pH,i}} = \begin{cases} 0 & \text{, if } \overline{pH} - 2s_{\mathrm{pH}} \leq pH_{\mathrm{opt}} \\ \left(\frac{\left(\overline{pH} - 2s_{\mathrm{pH}}\right) - pH_{\mathrm{opt}}}{pH_{\max} - pH_{\mathrm{opt}}} \right)^3 & \text{, if } \overline{pH} - 2s_{\mathrm{pH}} > pH_{\mathrm{opt}} \end{cases}$$

$pH_{\max}$ being the maximal pH for growth of the studied bacteria.

- If $\overline{a_{\mathrm{w}}} < a_{\mathrm{w\,opt}}$, calculate:

$$\varphi_{a_{\mathrm{w}},\mathrm{s}} = \left(\frac{a_{\mathrm{w\,opt}} - \left(\overline{a_{\mathrm{w}}} - 2s_{a_{\mathrm{w}}}\right)}{a_{\mathrm{w\,opt}} - a_{\mathrm{w\,min}}} \right)^3 \text{ and } \varphi_{a_{\mathrm{w}},\mathrm{i}} = \begin{cases} 0 & \text{, if } \overline{a_{\mathrm{w}}} + 2s_{a_{\mathrm{w}}} \geq a_{\mathrm{w\,opt}} \\ \left(\frac{a_{\mathrm{w\,opt}} - \left(\overline{a_{\mathrm{w}}} + 2s_{a_{\mathrm{w}}}\right)}{a_{\mathrm{w\,opt}} - a_{\mathrm{w\,min}}} \right)^3 & \text{, if } \overline{a_{\mathrm{w}}} + 2s_{a_{\mathrm{w}}} < a_{\mathrm{w\,opt}} \end{cases}$$

$\overline{a_w}$ being the mean between-batch mean a_w of the food, s_{a_w} being the between-batch sample standard deviation for the a_w of the food, $a_{w\,min}$ and $a_{w\,opt}$ being respectively minimum and optimum a_w for growth of the studied bacteria.

- If $\overline{a_w} \geq a_{w\,opt}$, calculate:

$$\varphi_{a_w,s} = \left(\frac{\left(\overline{a_w} + 2s_{a_w}\right) - a_{w\,opt}}{a_{w\,max} - a_{w\,opt}} \right)^3 \text{ and } \varphi_{a_w,i} = \begin{cases} 0 & \text{, if } \overline{a_w} - 2s_{a_w} \leq a_{w\,opt} \\ \left(\frac{\left(\overline{a_w} - 2s_{a_w}\right) - a_{w\,opt}}{a_{w\,max} - a_{w\,opt}} \right)^3 & \text{, if } \overline{a_w} - 2s_{a_w} > a_{w\,opt} \end{cases}$$

$a_{w\,max}$ being the maximal a_w for growth of the studied bacteria.

- Calculate the two parameters:

$$y_s = \frac{j_T}{2(1-j_{pH,s})(1-j_{a_w,s})} + \frac{j_{pH,s}}{2(1-j_T)(1-j_{a_w,s})} + \frac{j_{a_w,s}}{2(1-j_T)(1-j_{pH,s})}$$

and,

$$y_i = \frac{j_T}{2(1-j_{pH,i})(1-j_{a_w,i})} + \frac{j_{pH,i}}{2(1-j_T)(1-j_{a_w,i})} + \frac{j_{a_w,i}}{2(1-j_T)(1-j_{pH,i})}$$

- Then calculate: $Dj_{pHaw} = j_{pH,s} + j_{aw,s} - j_{pH,i} - j_{aw,i}$ and $Dy = y_s - y_i$

The physico-chemical inter-batch variability (pH and a_w) of the food has a relevant impact on the growth of the studied strain if at least one of the parameters Dj_{pHaw} or Dy is over 0.2.