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

Scope

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

This calculator is design to be used in the growth area of the studied bacteria. This growth area is restricted by the cardinal values of the bacteria (pH_{min} - pH_{max} and $a_{w\ min}$ - $a_{w\ max}$).

The use of this calculator, previously requires to have available data (pH and a_w values) to characterize the batches.

Use of the IBPCV calculator

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

1.1 Food data

The first column named "Batch" refers to the number of batches that could be tested. To assess the between-batches variability, it is recommended to characterize at least 3 batches. As a reminder a warning appears in red ("it is recommended to compare at least 3 batches") if data from only 2 batches are filled in.
The maximum number of batches to be tested by the IBPCV is 20 batches.

The food needs to be characterized by two physico-chemical parameters: pH and a_w. To estimate the characteristics of a batch it is necessary to measure these parameters on more than one sample (ideally more than 10 samples). Each batch is therefore characterized by mean values for pH and a_w.

So, in column "pH" and "Measured a_w", enter the mean initial values for pH and a_w of the food. These initial values noted "values at D_0" correspond to values measured the day of the manufacture of the food.

It is recommended to use pH mean values with only one decimal and a_w mean values with two decimals. To avoid using the IBPCV calculator with pH or aw mean values lower than the cardinal values of the studied bacteria, two warning were included ("pH mean ≤ pH min" and "aw mean ≤ aw min").

Regarding the water activity (a_w), if no 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 column "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:

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

with WPS content (in g/100ml) $= \frac{NaCl\ content\ (in\ g\ per\ 100g)}{moisture\ content\ \ (in\ ml\ per\ 100g)}\ x\ 100.$

Be careful, this formula can be used for food product where NaCl is the main component responsible for the a_w of the food.

The last column labeled "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 rather than the calculated $a_{w.}$

1.2 Data about cardinal values of the bacteria

Input the cardinal values (X_{min} - X_{opt} - X_{max}) for temperature, pH and a_w of the considered bacteria. You may refer to data proposed in table 1 (ANSES reference) below.

It is recommended to use temperature value as whole number, pH with one decimal and a_w value with two decimals.

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}	$a_{w\,min}$	$a_{w\,opt}$	$a_{w\,max}$
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	/

1.3 Storage temperature data

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

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

2. Answer of the calculator (in red)

From the physico-chemical input data characterizing the product, from the cardinal values of the bacteria and the storage temperature of the challenge test, 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 condition of the challenge test.

The answer is either "The impact of the variability of pH and a_w is significant in the tested temperature conditions" or "The impact of the variability of pH and a_w is not significant in the tested temperature conditions."

It is important to underline that when 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 could have a significant effect on the growth rate of the bacteria, leading to the conclusion "The impact of the variability of pH and a_w is significant in the tested temperature conditions".

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

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

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

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

$\overline{pH}$ being the between-batch mean pH of the food, s_{pH} being the between-batch 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 $\overline{pH} > pH_{opt}$, calculate:

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

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

- If $\overline{a_w} < a_{wopt}$, calculate:

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

$\overline{a_w}$ being the mean between-batch mean a_w of the food, s_{a_w} being the between-batch 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 $\overline{a_w} > a_{w\,opt}$, calculate:

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

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

- Calculate the two parameters:

$$\psi_s = \frac{\varphi_T}{2(1-\varphi_{pH,s})(1-\varphi_{a_w,s})} + \frac{\varphi_{pH,s}}{2(1-\varphi_T)(1-\varphi_{a_w,s})} + \frac{\varphi_{a_w,s}}{2(1-\varphi_T)(1-\varphi_{pH,s})}$$

and,

$$\psi_i = \frac{\varphi_T}{2(1-\varphi_{pH,i})(1-\varphi_{a_w,i})} + \frac{\varphi_{pH,i}}{2(1-\varphi_T)(1-\varphi_{a_w,i})} + \frac{\varphi_{a_w,i}}{2(1-\varphi_T)(1-\varphi_{pH,i})}$$

- Then calculate: $\Delta\varphi_{pHaw} = \varphi_{pH,s} + \varphi_{aw,s} - \varphi_{pH,i} - \varphi_{aw,i}$ and $\Delta\psi = \psi_s - \psi_i$

The physico-chemical inter-batch variability of the food has a relevant impact on the growth of the studied strain if at least one of the parameters $\Delta\varphi_{pHaw}$ or $\Delta\psi$ is over 0.2.