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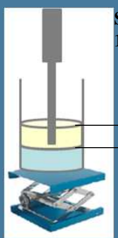
Introduction & Objective

In foodstuffs, interactions between spores, inhibitors and food components may occur (i.e. physicochemical interactions and/or physical constraints). Food emulsions are typically one of these complex systems produced through homogenization, and represent a major group of food colloidal systems formed from two immiscible liquids, with one dispersed and droplets in the second one. In emulsions, the site of microbial growth in food is commonly recognized as the aqueous phase[1, 2]. Food structure may influence both rate of growth and conditions under with growth is initiated[1]. Organic acids are widely used as hurdles to inactivate foodborne pathogens or spoilage microorganisms in food industry. Their inhibitory effect on bacterial spores is based on their lipophilic character by affecting membrane integrity[3]. Nevertheless, interactions between emulsion structure/composition and bioactive compounds (and/or microorganisms) may impact on microorganism viability. The objective of this work was to study such phenomena using a model oil in water (O/W) emulsion as a microbial medium to improve, in the long term, bacterial spore control.

Methods

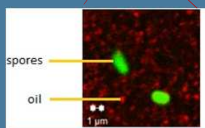
► Emulsification



Properties of organic acids[4]

	Acetic acid	Hexanoic acid	Lactic acid
pKa	*4.75*	*4.88*	*3.90*
log P	*-0.17*	*1.92*	*-0.72*

O/W emulsion



Confocal microscopy observation of inoculated 50/50 emulsion
(Nile red/oil phase; Thioflavin T/spores)

► Organic acid concentration (mM)

pH	Acetic acid	Hexanoic acid	Lactic acid
6	14	4	255
5.5	7.3	1.8	82

► Bacterial cells enumeration by a most probable assay in microplate:

Germination and growth of *Bacillus* were detected by the loss of fluorescence of wells due to the metabolism of esculin.

Ex: 78/96 positive wells with germination/growth occured.

% of germinate/developed spores in aqueous phase or emulsions was calculated from an enumeration by a most probable number (MPN) assay.

► Characterisation of cells growth and germination by flow cytometry[5] and bioscreen growth experience[6].

Which combinations of factors influence germination/growth of B. weihenstephanensis spores in O/W emulsion? (Table 1)

✓ **Effect of pH value:** the percentage of germinated/developed spores decreased with pH value whatever oil fraction volume (ϕ).
✓ **Effect of organic acids:** the addition of organic acids improved the antimicrobial response due to pH.
✓ **Effect of oil volume fraction (ϕ):**
at pH = 5.5 with HCl, no germination/growth happened with ϕ = 0 and ϕ = 0.35, while 5 % of spores germinated/grew in the 50/50 O/W emulsion;
at pH = 6.0 with heaxanoic acid, less than 25 % of spores germinated/grew with ϕ = 0 and ϕ = 0.35, while 50-75 % of spores germinated/grew in the 50/50 O/W emulsion.

Table 1. Germination/growth of *B. weihenstephanensis* KBAB4 in aqueous phases (3% w/w Tween 80), 50/50 O/W emulsion, 35/65 O/W emulsion at three pH (7; 6; 5.5) with or without organic acids. (ϕ, oil volume fraction).

pH	Acid	Total acid concentration (mM)	Non-dissociated form concentration (mM)	ϕ 0	35	50
7.0	HCl	-----	-----	++++	++++	++++
6.0	HCl	-----	-----	++++	++++	++++
	Hexanoic	4	0.28	+	+	+++
	Acetic	14	0.75	+	+	+
	Lactic	255	2.00	+	+	+
5.5	HCl	-----	-----	-	-	+
	Hexanoic	1.8	0.35	-	-	-
	Acetic	7.3	1.10	-	-	-
	Lactic	82	2.00	-	-	-

-: <0.03% of germination/growth
+: 0.03-25 % of germination/growth spores
++: 25-50% of germination/growth spores
+++: 50-75% of germination/growth spores
++++: 75-100% of germination/growth spores

Effect of ϕ more marked with hexanoic acid

Comparison between NMR experiments carried out on acetic and lactic acid showed differences in their diffusion coefficient evolution[7]. The distribution of organic acid in emulsions should have a key role in antimicrobial effect, especially for lipophilic hexanoic acid (log P = 1.92).

Effect of ϕ due to Tween 80 activity

At acidic pH values, Tween 80 seemed to have an antimicrobial activity on KBAB4 vegetative cells growth (effect on μ_{max} (Table 2) and on individual lag times (data not shown)) and germination (Figure 1).

Table 2. Maximal specific growth rate (μ_{max}) at pH=6.0 and 5.5 in nutrient broth in the presence of Tween at different concentration and minimum inhibitory concentrations.

[Tween] (% w/w)	pH 6.0	pH 5.5
0	1.63 ±0.05	1.48 ±0.04
1	1.64 ±0.02	1.41 ±0.05
2	1.57 ±0.17	1.29 ±0.07**
5	1.47 ±0.04 *	1.09 ±0.01**
10	1.40 ±0.01 *	0.75 ±0.00**

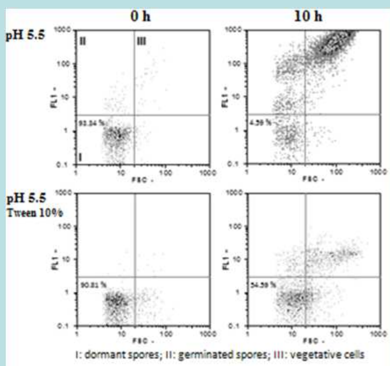


Figure 1. FCM-derived scatterplots of green fluorescence (FL1) versus forward-scatter intensities (FSC) of Syto®9 stained *B.weihenstephanensis* KBAB4 spores during germination and growth.

Conclusions & perspectives

► The inhibition of spores germination and growth in the model emulsion depended on several factors: pH value and oil volume fraction.

► The effect of the oil volume fraction was due to a combination of:
(i) the lipophilicity of the hexanoic acid having an impact on the distribution of organic acid between oil and water phases;
(ii) the antimicrobial activity of the emulsifier Tween 80 detected at acidic pH value.

► Tween 80 effect has been hardly described in the literature and this study highlighted the precaution with which this emulsifier must be used.

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