INTERACTION BETWEEN POTASSIUM SORBATE, OIL AND TWEEN 20: ITS EFFECT ON THE GROWTH AND INHIBITION OF *Z BAILII* IN MODEL SALAD DRESSINGS

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Accepted for publication February 10, 2003

ABSTRACT

The effect of the interaction between potassium sorbate (KS), oil and Tween®20 on the growth and inhibition of Z bailii *in model salad dressings was studied.* Z. bailii *grew in emulsions containing 110 and 230 g/kg of oil and the addition of 0.5 g/kg of KS inhibited growth. When the oil content was raised to 460 g/kg, a steep death rate curve was observed and the effect of KS addition was hidden by the inhibitory action of the high oil level. Tween®20 action depended on oil level: for emulsions containing 110 or 230 g/kg, a depression in the activity of KS was observed. This behavior was attributed to the decrease in the free form of the preservative due to the partition between surfactant micelles and the water. In contrast, when the oil content was 460 g/kg, KS activity was enhanced in spite of the decrease in the amount of its free form. These results highlight the importance of considering ingredient interactions when evaluating shelf-life.*

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INTRODUCTION

Salad dressings are complex food systems since they are made from a dispersed oil phase, a surfactant (generally nonionic) and a high concentration of organic acids in an aqueous phase, which currently comprises salts, sugar and an hydrocolloid. Water activity and pH depression together with sodium benzoate or potassium sorbate addition are the stress factors usually employed to get a shelf stable salad dressing. However, microbial spoilage of these products occasionally occurs due to the growth of a select group of microorganisms (Smittle 2000). The microflora causing salad dressings spoilage consists of a few species of *Lactobacillus*, *Saccharomyces* and *Zygosaccharomyces*. According to Kurztman *et al.* (1971), *Z. bailii* is the yeast primarily responsible for dressing spoilage.

The high reactivity of sorbic acid and its derivatives is important in complex food systems, where it can participate in reactions with food components. These reactions can be influential on its antimicrobial activity and on the quality and safety of the food (Binstok *et al.* 1998; Ferrand *et al.* 2000; Sofos 1989).

Surfactants form micelles in aqueous solutions when their concentration exceeds some critical level, known as the *critical micelle concentration* or CMC (Myers 1988). This concentration is almost always found in food formulations. According to the literature, preservatives tend to become associated with surfactant micelles and this fact might affect preservative activity (Kurup *et al.* 1991 a, b; Wedzicha *et al.* 1991; Wedzicha and Ahmed 1994; Wedzicha and Couet 1996; Yoon and Burgess 1996).

This paper evaluated the microbiological activity of sorbates in an oil-in-water emulsion resembling salad dressings, to evaluate the effect of antimicrobial interactions with other ingredients on shelf-life. This evaluation was performed through the study of the inhibitory effect of the preservative potassium sorbate on the growth of *Z. bailii* in different model systems.

MATERIALS AND METHODS

Inoculum Preparation

Zygosaccharomyces bailii NRRL 7256 inoculum was prepared by growing cells in Sabouraud broth (Biokar Diagnostics, Beauvais, France) at 30C until they reached stationary phase (24 h).

Model System Preparation

Food model system composition was as follows: 25 g/kg acetic acid solution (50 g/L), 0.075 g/kg disodium calcic salt of ethylendiamine tetraacetic acid (EDTA), 5 g/kg % w/w xanthan gum, the neccessary amount of sodium chloride

to depress water activity to 0.985 and different concentrations of Tween® 20, potassium sorbate (KS) and oil, as mentioned in Table 1. All these ingredients, with the exception of oil, were suspended in Sabouraud nutrient broth, poured into glass flasks and sterilized for 15 min at 121C. The pH was adjusted to 3.5 by adding some drops of citric acid solution (500 g/L) prior to autoclaving. The emulsions were obtained by aseptically adding the corresponding amount of oil to an aqueous phase and mixing with a domestic blender for 3 min at 550 RPM.

TABLE 1.
CONCENTRATIONS OF POTASSIUM SORBATE (KS), TWEEN® 20 AND OIL, PRESENT IN SALAD DRESSING MODEL SYSTEMS

System	KS [g/kg]	Oil [g/kg]	Tween® 20 [g/kg]
1	0	110	0
2	0.5	110	0
3	0	460	0
4	0.5	460	0
5	0	110	40
6	0.5	110	40
7	0	460	40
8	0.5	460	40
9	0	230	0
10	0.25	230	0
11	0.5	230	0
12	0	230	20
13	0.25	230	20
14	0.5	230	20
15	0	230	40
16	0.25	230	40
17	0.5	230	40

Inoculation and Storage

For each emulsion, 15 g were placed in sterile caramel glass flasks. For every emulsion formulation, two flasks were prepared. A high number of *Z. bailii* viable cells (approximately equal to 10^7 CFU/g) were inoculated in order to asses their survival and growth capability in each system (López-Malo and Palou 2000). All the systems were incubated at 33 (± 1)C and, at selected storage times, viable yeast

counts were determined by surface plating on Sabouraud agar at several sample dilutions (0.1% peptone water). Three plates were used for each dilution and were incubated at 33C for 2 days before enumerating.

Determination of Partition Coefficient and the Distribution of Undissociated Sorbic Acid

The apparent partition coefficients of the preservative in the emulsions were determined according to the method of Huang *et al.* (1996). Samples (40 g) of emulsions were centrifuged (10,000 x g for 10 min at 4C) to separate the phases. The preservative content was measured by the AOAC oxidation method (1990) in the emulsions and in the water phase, in order to calculate its amount in the oil phase. Then, the apparent partition coefficient was obtained as $P_{ap}=C_{oil}/C_{aq}$, being C_{oil} the concentration of sorbic acid in the oil phase and C_{aq} the concentration of sorbic acid in the aqueous phase. Corrections were made to convert the apparent partition coefficient into the true partition coefficient by using the dissociation constant and the concentration of H^+ (Wedzicha *et al.* 1991).

In the emulsions containing Tween®20, the total concentration of sorbic acid in the oil phase was used to calculate the concentration of the monomer in the oil phase from the equation: $K_d= [(HA)_2]/[HA]^2$, being K_d the dissociation constant and [HA] the concentration of undisssociated sorbic. This data was used to obtain the corresponding concentration of free acid in the aqueous phase by using the true partition coefficient (P), which refers to the equilibrium between the same sorbic acid species in the two phases. Then, the concentration of undissociated sorbic acid bound to micelles was evaluated performing the difference between the total concentration of undissociated sorbic acid and the concentration of free undissociated sorbic acid in the aqueous phase (Wedzicha and Couet 1996).

Determination of Death Rate Constants (k). Data Analysis

The fate of the microorganisms in the systems containing varying concentrations of the ingredients was studied by determining the number of viable microorganisms at specific time intervals. Regression analysis showed the existence of linear relationships between log (N_0 /N) and t, where N_0 is the initial number of microorganisms and N is the number of microorganisms at time t, indicating that the lethal process followed the pattern of a first order kinetics (Xiong *et al.* 1999). In first order kinetics, *k* is the death rate constant. The death rate constants for different systems were calculated from the equations for the best fitting lines and statistically significant differences between them were studied by Analysis of Co-Variance (ANCOVA) using StatGraphics Plus (version 4.0, STSC, 1994, Rockville, MD).

RESULTS AND DISCUSSION

Activity of Potassium Sorbate

The presence of potassium sorbate in the model systems was effective against *Z. bailii* growth. Figure 1 panel A shows how these microorganisms grew in the dressing environment. Stationary phase was reached approximately after 60 h in an emulsion containing 110 g/kg of oil (system 1). This was inhibited when KS was present in a concentration of 0.5 g/kg (system 2). The same trend was observed when Tween®20 was present (Table 2, systems 5 and 6). Results obtained show the importance of potassium sorbate for stability of low oil content salad dressings.

Figure 1, panel B shows growth and survival of *Z. bailii* in emulsions containing 230 g/kg of oil and 0.25 or 0.50 g/kg of sorbate. At an intermediate concentration of KS (0.25 g/kg), the preservative did performed its inhibitory effect but at a slower rate (Table 2, systems 10 and 11). The same behavior was observed in systems of similar composition but containing Tween®20 (Table 2, systems 13, 14, 17).

When the oil content was raised to 460 g/kg of oil, no effect of preservative addition was observed (Table 2, systems 3 vs 4). Probably, the inhibitory effect exerted by this oil content (as it will be analyzed in the next section), masked the preservative effect. However, in the presence of 40 g/kg of Tween®20, the inhibitory effect of KS addition at the 0.50 g/kg level, was observed (Table 2, systems 7 and 8).

Effect of Oil Concentration

The tested microorganism grew in those systems containing 110 and 230 g/kg of oil without KS. The higher oil content in the latter affected the normal growth of the yeast showing a reduction in the population able to reach stationary phase. When the oil content was raised to 460 g/kg, a dramatically steep death rate curve could be seen (Fig. 2, panel A). This behavior might be attributed to the increase in the volume of the oil phase that seemed to have a generalized preservation effect on the emulsions modeled: the consequent reduction in the aqueous phase determined an increase of additive concentration in that phase. Furthermore, oil could be considered here as an inert ingredient because of the conditions in which the assay was performed: the main sources of contamination in salad dressings and mayonnaise are poor sanitation and contaminated ingredients (Smittle 2000), both being controlled by the sterilization of the aqueous phase and the aseptic manufacture of the model systems.

Adding 40 g/kg Tween® 20 to the systems (Fig. 2, panel B) did not change the above pattern. At 460 g/kg oil level, an inhibition of death was observed when compard with systems without Tween®20.

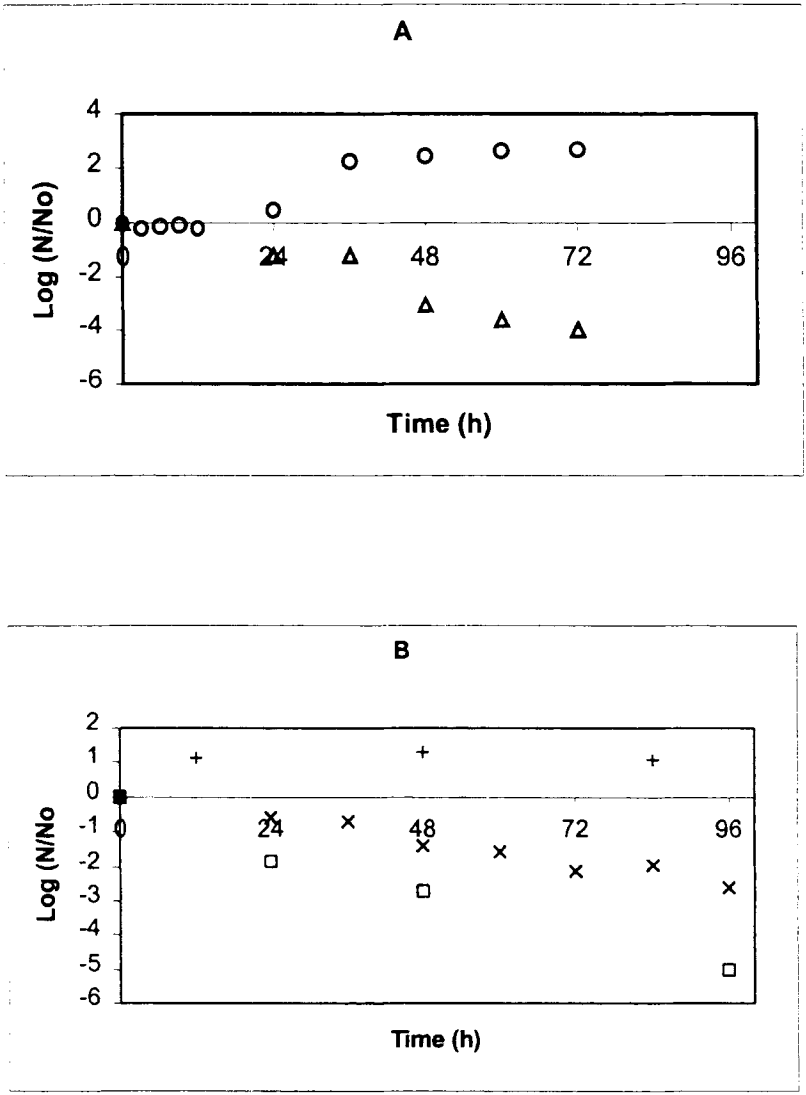

FIG. 1. EFFECT OF SORBATES IN THE GROWTH AND DEATH OF *Z. BAILII*
(A) Emulsions containing 110 g/kg of oil. Systems: 1(○) and 2 (△). (B) Emulsions containing 230 g/kg of oil. Systems: 9 (+), 10 (x) and 11 (□).

TABLE 2.
DEATH RATE CONSTANTS

System	Death Rate constants, k (h^{-1}).10^2
2	7.93[a,b]
3	6.10[b]
4	6.00[b]
6	2.91[c]
7	4.90
8	7.31[a]
10	2.80[c]
11	5.08[b]
13	2.90 [c]
14	4.37 [a]
17	2.27[c]

Systems 1, 5, 9, 12, 15 and 16 showed Z. *bailii* growth during storage.
Same letter denote nonsignificant differences between rate constants ($P< 0.05$).

When 0.5 g/kg of KS were present, no significant change in death rate was observed with the increase in oil level (Fig. 2, panel C). Probably, the inhibitory effect of preservative masked oil effect. The addition of 40 g/kg of Tween®20 did not change the trend when the oil level was 110 or 230 g/kg but diminished the death rate. Increasing the oil phase to 460 g/kg gave a death rate greater than the ones observed without Tween®20 or at lower oil concentrations (Fig. 2, panel D).

Effect of Tween® 20 Concentration

Surfactants are normally used to stabilize emulsion droplets against aggregation by providing a protective membrane around the droplet. In general, surfactants are present in foods above their critical micelle concentration (CMC). This causes surfactant micelle formation in the aqueous phase. These surfactant micelles can interact with the preservative affecting its activity (Florence and Atwood 1988; Wedzicha *et al.* 1991 and Wedzicha and Ahmed 1994; Kurup *et al.* 1991 a, b).

In Fig. 3, panel A, and B, a decrease in the death rate of *Z.bailii* can be observed when the concentration of the surfactant is raised from none to 40 g/kg in emulsions containing 0.5 g/kg of KS. In panel A, the oil content of the systems was 230 g/kg while in panel B it was 110 g/kg. Although preservative exerted its inhibitory effect on yeast growth, as it is clearly seen on the tendency of the curves, this action was not as effective as it was in the absence of Tween® 20. The same behavior was observed in the absence of KS: Tween®20 addition promoted *Z. bailli* growth for emulsions containing 230 g/kg of oil (data not shown) and delayed the death for the emulsions with 460 g/kg of oil (Table 2, system 3 and 7). Probably, the surfactant could be metabolized by the microorganism or acted like a "nutrient carrier" between the aqueous phase and the cellular membrane in the oily environment, promoting, in this way, its growth or extending microbial survival.

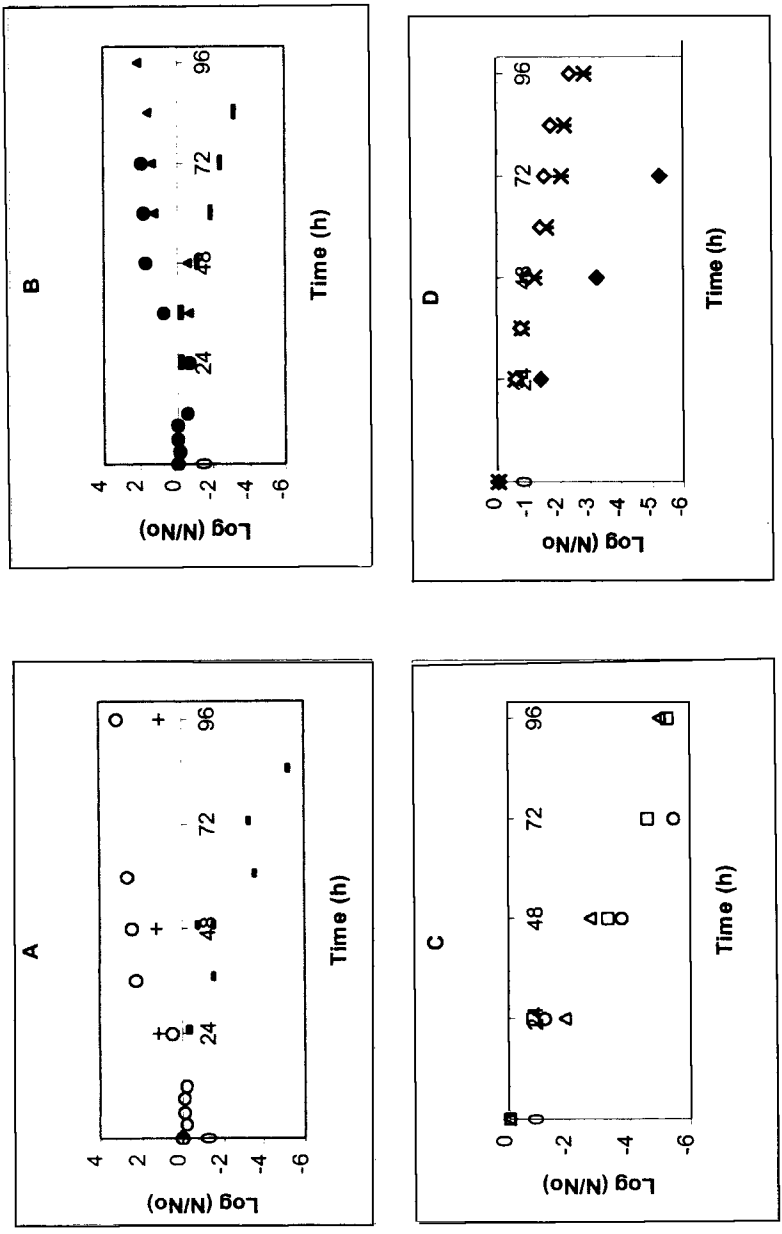

FIG. 2. EFFECT OF OIL CONTENT IN THE GROWTH AND DEATH OF *Z. BAILII* IN THE ABSENCE (A, C) OR PRESENCE (B, D) OF TWEEN 20 (A) systems: 1 (○), 9 (+) and 3 (-) ; (B) systems: 5 (•),15 (▲) and 7 (-).(C), systems: 2 (O), 11 (Δ), and 4 (□). (D) systems: 6 (*), 17 (◇), and 8 (◆)

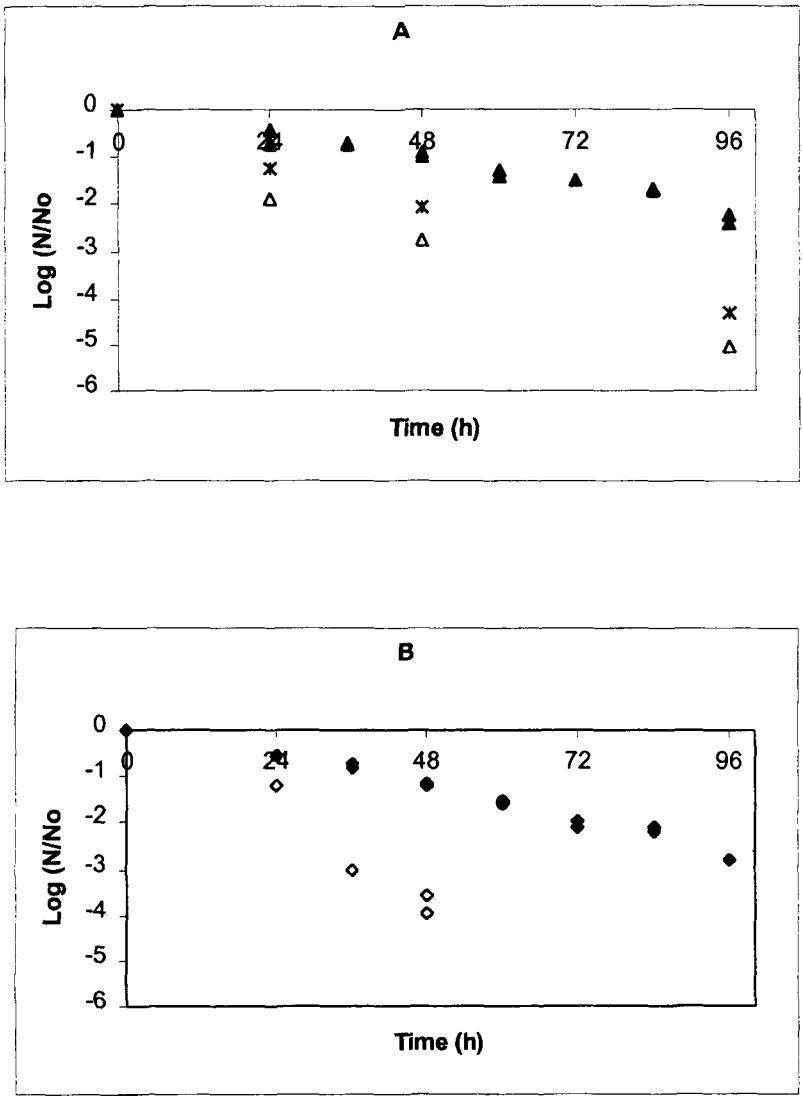

FIG. 3. EFFECT OF TWEEN ON THE SURVIVAL OF *Z BAILII* IN EMULSIONS CONTAINING 0.5 G/KG OF POTASSIUM SORBATE
(A) Systems: 11 (△), 14 (*) and 17 (▲). (B) Systems: 2 (◇) and 6 (◆).

In relation to the decrease of the activity of preservatives due to the presence of a surfactant, other researchers (Bean *et al.* 1969) found that, an addition of 0.4% v/v polysorbate 80 to oil-water mixtures containing 25% v/v liquid paraffin and 0.1% w/v total chlorocresol reduced preservative activity one hundred-fold. Moreover, the activity of methyl-para-hydroxybenzoate, phenoxyethanol and chlorocresol against *Candida albicans* and *Aspergillus niger* was decreased by the addition of Span 80 and Tween 80 in emulsions (Kurup *et al.* 1991, b)

The decrease in antimicrobial activity induced by the presence of a surfactant could be attributed to the partition of the preservative between the micelles and the water. As it is shown in Table 3 (systems 2 and 6 ; 11 and 14, 17; 4, and 8), the P_{ap} decreased when the emulsion contained Tween®20. This behavior can be attributed to the surfactant binding the preservative: the total amount of potassium sorbate in the aqueous phase increased but a part of it was bounded to the micelles, resulting in a decrease of the free form in the aqueous phase. An important part in the aqueous phase was entrapped in the micelles and therefore was not available to act on the microorganisms. In the studied systems, the amount of KS bound to the micelles was around 74% for the emulsion containing 110 g/kg of oil and 62-72% for the emulsions containing 230 g/kg of oil (Table 3). This trend was previously reported by Wedzicha *et al.* (1991) for the Tween 80 - sunflower oil - water system containing sorbic acid. In this case, the amount of sorbic acid associated to the micelles was 36% when the level of Tween 80 was 2% and it increased to 51% when the level of Tween 80 was 4%.

It should be stressed that addition of 40 g/kg instead of 20 g/kg of the surfactant to a system with 230 g/kg of oil, produced a higher degree of sorbate association to the micelles. In the presence of 40 g/kg of Tween®20, that association produced a significant decrease in the death rate constant which went from 5.08×10^{-2} h^{-1} in the absence of surfactant to a value of $4.37 \times 10^{-2} h^{-1}$ or 2.27×10^{-2} in the presence of 20 or 40 g/kg Tween 20, respectively (Table 2, systems 11, 14 and 17) when the level of preservative was 0.50 g/kg. When the content of KS was 0.25 g/kg, sorbate - micelle association determined yeast growth, when Tween 20 increased from 20 to 40 g/kg (Table 2, systems 13 and 16).

When the oil content was 460 g/kg and sorbate was present, the addition of 40 g/kg of the surfactant increased the activity of the preservative (Table 2, systems 4 and 8), although the amount of free preservative in the aqueous phase decreased due to sorbic acid bounding to the micelles (Table 3). Probably, at this oil level, the amount of preservative bound to the micelles did not play an important role and the effect of Tween®20 on preservative activity could be attributed to other physicochemical properties of the surfactant such as the reduction in surface and interfacial tension which would increase the adsorption and uptake of preservatives by microbial cells thereby killing the cells at a faster rate. An enhancement in the antibacterial activities of methyl-para-hydroxibenzoate, phenoxyethanol and

chlorocresol against *Pseudomonas aeruginosa* in the presence of 1% of Tween 80 was attributed by Kurup *et al.* (1991b) to the increase in the permeability of bacterial membranes to preservatives.

TABLE 3.
PARTITION COEFFICIENT AND THE DISTRIBUTION OF UNDISSOCIATED SORBIC ACID IN STUDIED SYSTEMS

System	P^{a}_{ap}	$[HA] . 10^3$ (mol/dm^3)	$[HA]_{free} . 10^3$ (mol/dm^3)	$[HA]_{bound} . 10^3$ (mol/dm^3)	% of $[HA]_{bound}$
2	2.5 ± 0.2	2.57	2.57	----	----
11	2.5 ± 0.2	1.95	1.95	----	----
4	2.7± 0.2	1.53	1.53	----	----
6	0.8 ± 0.1	2.91	0.76	2.15	74
14	1.2 ± 0.2	2.64	1.01	1.63	62
17	0.8 ± 0.2	2.90	0.80	2.10	72
8	1.2 ± 0.2	2.52	0.95	1.56	62.

[a] values represent mean ± standard deviation
All the concentrations correspond to the aqueous phase

In summary, the effect of Tween®20 addition depended on the oil content in the emulsion and preservative presence. This trend was also observed by Bean *et al.* (1969) and Kurup *et al.* (1991 a) in relation to other antimicrobials.

Results mentioned show that activity of the preservative in the emulsion is not only dependent on the concentration of its free undissociated form but also on oil and surfactant influence on sorbate activity and that oil and surfactant exert specific effects concerning yeast growth.

CONCLUSIONS

Sorbate exerted an inhibitory action on *Z. bailii* growth in oil in water emulsions.

The presence of a high oil concentration of 460 g/kg gave the product a relatively stable microbiological condition with respect to studied yeast, even when

the preservative was not added to the formulation. This oil content is likely to be found in many American dressings. A sorbate level of 50 g/kg in an emulsion containing this oil content showed no effect on emulsion stability unless 40 g/kg Tween®20 were present.

An important interaction for emulsion stability is the one established between the surfactant and the preservative. This interaction was caused by the reduction of the concentration of free preservative in the aqueous phase. Tween®20 also exerted specific actions that affected microorganism growth. For example, it reduced antimicrobial activity at the 110 and 230 g/kg oil level, extending the time necessary to inhibit yeast growth (i.e., from 48 to 96 h. See Fig. 3, panel B systems 2 and 6, respectively). In contrast, when the oil content was 460 g/kg, an increase in the preservative activity occurred, although the amount of free preservative decreased due to binding to surfactant micelles. The persistence of the microorganism in the food product might lead to spoilage concerns. As a consequence, it is necessary to conduct further research to elucidate which are the mechanisms involved in the protective effect on yeast survival exerted by the surfactant, when it is present in low oil content emulsions. These contrast its synergistic effect with preservatives, when it is added to systems which contain higher oil concentrations.

These results stress the importance of taking into account the interactions between ingredients as well as specific effects when formulating salad dressings in order to select the adequate concentration of the preservative needed to assure a reasonable shelf-life.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from Universidad de Buenos Aires and Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, Author M.Castro wants to thank the support of Univ. Nac. del Nordeste, who is also grateful to Ing. M.E.Cayré and Ing. M.C. Giménez for their continuing interest.

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