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Effect of preservatives, tween 20, oil content and emulsion structure on the survival of *Lactobacillus fructivorans* in model salad dressings

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ABSTRACT

The effect of potassium sorbate (PS), nisin, Tween® 20 and oil level on the survival of *Lactobacillus fructivorans* in model salad dressings was studied. In general, *L. fructivorans* growth was prevented in formulated emulsions, but the addition of nisin was necessary to inactivate the bacterium throughout the storage time evaluated. The bacteriocin activity showed to be strongly dependent on system composition. Addition of PS alone did not influence *L. fructivorans* survival. But, when this preservative was added together with nisin to an emulsion containing 110 g/kg of oil, it exerted an antagonistic action on nisin effectiveness while for other levels of oil, a synergistic action was verified. The increase of oil level did not affect *L. fructivorans* survival in those emulsions without nisin. However, when the bacteriocin was present, it produced different effects which depended on system composition. Addition of tween did not affect *L. fructivorans* survival for emulsions free of additives or containing as preservative only PS. However, when nisin was present, the emulsifier effect was entirely dependant on oil content. Furthermore, the structure of the food matrix appeared as an additional factor which could influence either the growth of the microorganisms or the functionality of the preservatives. Tween addition turned the systems more fluid or solid, depending on the oil content of the systems considered. Results obtained highlight the importance of considering ingredient interactions when evaluating microbial stability of food systems.

1. Introduction

Salad dressings have grown in popularity in the recent years. Many consumers have turned to salads as a healthy option, which means that also the dressings have to be healthy (Gledhill, 1998). This implies that the food industry is facing a challenge to produce a wide variety of dressings, including dressings with a low fat content in order to meet consumer demands (Wendin & Gunnar, 2001). Significant microbiological consequences occur with this reduction. Mainly, as the oil content decreases, the water phase is increased, which in turn decreases the salt and the organic acid concentration in the water phase. This water phase is the critical microbiological concern (Smittle & Cirigliano, 1992). The microflora causing salad dressings spoilage consists of a few species of *Lactobacillus, Saccharomyces* and *Zygosaccharomyces. Lactobacillus fructivorans* is the predominant bacterium found in spoiled products (Smittle, 2000; Smittle & Flowers, 1982).

Salad dressings are oil-in-water emulsions stabilized with thickeners. The water phase comprises weak organic acids, such as acetic and citric acids, salts, sugar, chelators, thickening agents, surfactants and preservatives. Frequently, potassium sorbate or sodium benzoate are used as antimicrobial agents and emulsifiers, such as the non-ionic surfactant Tween® family, are added to the water phase of emulsions in order to stabilize it, preventing the coalescence of the droplets from the dispersed phase (Mc Clements, 1999). In most emulsified food, surfactants are above its critical micelle concentration (CMC) forming aggregates known as micelles. The non-polar inner regions of micelles present an environment closely similar to that given by an organic solvent, where solutes such as sorbic acid will tend to partitionate (Wedzicha, Ahmed, & Zeb, 1990). For this reason, the preservative effectiveness will be associated to its distribution in the different phases of the food emulsion, considering the interior of the micelles as a third

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phase. Castro, Garro, Gerschenson, and Campos (2003) found that potassium sorbate diminished its antimicrobial effectiveness when the surfactant Tween®20 was added to the emulsion. This fact was attributed to the surfactant binding the preservative.

Nowadays, consumer demands for more "natural" and "minimally processed" food. As a result of this trend, there has been a great interest in naturally produced antimicrobial agents (Cleveland, Montville, Nes, & Chikindas, 2001). Nisin, a small antimicrobial peptide produced by lactic acid bacteria, is generally recognized as safe and is approved for use in some pasteurized cheese spreads in the United States (Delves-Broughton, 1990). Nisin has been tested on meat and meat products, dairy foods (Abee, Krockel, & Hill, 1995) and vegetarian foods (Fang, Chen, & Chen, 1997); however, there are no published data on weather nisin has reliable antimicrobial effects in salad dressings.

Bacteriocins are usually applied in food systems as one of the stress factors of a system with multiple hurdles (Cleveland et al., 2001). Since the action of bacteriocins against sensitive microorganisms is influenced to a large degree by factors such as pH, cell concentration, lipid content, proteolytic enzymes, and liquid vs. solid system (Ray & Daeschel, 1992), hurdle technology must consider the effects of possible antagonistic interactions between different preservatives or food components (Bouttefroy, Mansour, Linder, & Milliere, 2000). As a result of the interaction of nisin with fats a decrease in its activity was verified in foods with high fat content (Bhatti, Veeramachaneni, & Shelef, 2004; Jung, Bodyfelt, & Daeschel, 1992; Zapico, de Paz, Medina, & Núñez, 1999). Furthermore, the interaction of nisin with surfactants in a food matrix containing nisin is relatively ambiguous and it still needs to be elucidated. Jung et al. (1992) and Bhatti et al. (2004) found that the non-ionic emulsifier, Tween® 80, partially counteracted decreases of nisin activity in milk caused by the high fat content. Nevertheless, Henning, Metz, and Hammes (1986a) confirmed an antagonistic effect of emulsifiers on the antimicrobial efficacy of nisin.

Sorbic acid in combination with nisin proved to retard spoilage of "British fresh sausage" stored at 5 °C (Jarvis & Burke, 1976). Potassium sorbate combined with nisin decreased bacterial counts on a vegetarian food (Fang et al., 1997) and had a listericidal effect in buffered BHI broth (pH 5.5) during incubation at 4 °C (Buncic, Fitzgerald, Bell, & Hudson, 1995). These combinations proved to have the best preservative effect when compared to results for each preservative agent when used alone.

The effects of food structure include constraints on water distribution (Hills, Manning, Ridge, & Brocklehurst, 1996, 1997), organic acids distribution, which comprises food preservatives (Brocklehurst, Parker, Gunning, & Robins, 1993; Brocklehurst & Wilson, 2000), and the motility of the microorganisms (Dodd, 1990; Dodd & Waites, 1991; Mattila & Frost, 1988; Robins, Brocklehurst, & Wilson, 1994; Robins & Wilson, 1994; Wimpenny et al., 1995). Wilson, Wilson, and Waspe (2000) reported that the survival and growth of microorganisms in the food are affected not only by the chemical composition or the storage conditions of food, but also by its structure. In this sense, rheological measurements can provide useful information for a better understanding of the structures or the distribution of molecular components (especially those of macromolecular constitution) in foods (Durán, Costell, Izquierdo, & Durán, 1994; Rood, Davis, Dunstan, Forrest, & Boger, 2000). Foods are complex materials whose characteristics and properties, especially the mechanical ones, depend on their structures. The spatial arrangement of the structural elements at a microscopic scale, determined by the chemical composition and the physical forces of interaction between them, creates a direct relationship between structure and texture of the food (Stanley & Tung, 1975).

From what it is mentioned above, it can be assumed that the antimicrobial effectiveness of preservatives in salad dressings could be affected by other preservative presence, oil content and the addition of surfactants, not only because of their chemical interactions with the rest of the food components but also because of the structure they give to the food matrix which would condition microbial growth.

The present study was undertaken to examine the effects of nisin, PS, tween and oil level on *L. fructivorans* survival in emulsions resembling salad dressings.

2. Materials and methods

2.1. Experimental design

To assess the effects of nisin, potassium sorbate, Tween® 20 and oil content on the development of *L. fructivorans* population, the experiment was arranged in a design that included the use of two levels of nisin or potassium sorbate (0 and 0.500 g/kg), two levels of tween (0 and 20.00 g/kg) and three levels of corn oil (110 g/kg, 230 g/kg and 460 g/kg).

2.2. Model system preparation

Model salad dressings composition was selected taking into account ingredients commonly used for salad dressing formulation (Meyer, Grant, Luedecke, & Leung, 1989). The composition comprised: 25 g/kg acetic acid solution (50 g/l), 0.075 g/kg disodium calcic salt of ethylendiamine tetraacetic acid (EDTA), 5 g/kg xanthan gum, the necessary amount of sodium chloride to depress water activity to 0.985 and different concentrations of Tween® 20 (Tw), potassium sorbate (PS), nisin and corn oil, as it is mentioned in Table 1. All these ingredients, with the exception of oil, were suspended in MRS nutrient broth, poured into glass flasks and sterilized for 15 min at 121 °C. The pH was adjusted to 3.50 by adding some drops of citric acid solution (500 g/l) prior to sterilization. Nisin (Novasin®, Rhodia, Brasil), with an activity of 1.10^6 IU/g, was dissolved in a solution containing 0.02 mol/L HCl and 7.5 g/kg NaCl. This suspension was sterilized separately and then an appropriate dilution was added to the aqueous phase to give a final

Table 1
Concentrations of oil, potassium sorbate (PS), nisin and Tween®20 (Tw) present in salad dressings model systems.

System	Oil [g/kg]	PS [g/kg]	Nisin [g/kg]	Tween®20 [g/kg]
1	110	0	0	0
2	110	0	0	20
3	110	0	0.5	0
4	110	0	0.5	20
5	110	0.5	0	0
6	110	0.5	0	20
7	110	0.5	0.5	0
8	110	0.5	0.5	20
9	230	0	0	0
10	230	0	0	20
11	230	0	0.5	0
12	230	0	0.5	20
13	230	0.5	0	0
14	230	0.5	0	20
15	230	0.5	0.5	0
16	230	0.5	0.5	20
17	460	0	0	0
18	460	0	0	20
19	460	0	0.5	0
20	460	0	0.5	20
21	460	0.5	0	0
22	460	0.5	0	20
23	460	0.5	0.5	0
24	460	0.5	0.5	20

bacteriocin concentration of 500 μg/g. All chemicals used were reagent grade with the exception of acetic acid and corn oil which were food grade. The emulsions were obtained by aseptically adding the corresponding amount of oil to the aqueous phase and mixing with a domestic blender for 3 min at 550 rpm. This procedure was undertaken onto ice to dissipate the heat generated by the emulsification.

2.3. Inoculum preparation

L. fructivorans CRL 941 G was obtained from CERELA (Centro de Referencia de Lactobacilos, Tucumán, Argentina). Stock cultures of this bacteria were grown on MRS broth (Biokar Diagnostics, Beauvais, France) at 25 °C for 2 days, were subcultured once more on the same media for 36 h and then, they were cultured in MRS broth at pH 3.5, adjusted with vinegar and citric acid (500 g/l). The latter procedure was done in order to mimic the acidic environment of the modeled food. Approximately after 36 h, the microbial suspension reached the early stationary phase of the microorganism (Castro et al., 2003). *Inoculation, storage and sampling.*

The bacterial suspension was inoculated into the different systems in order to have an initial population of approximately 10^6 CFU/g. For each emulsion, 15 g were placed in sterile caramel glass flasks. For every emulsion formulation, two flasks were prepared. All the systems were incubated at 30 ± 1 °C. At selected times, viable bacterial counts were determined by using pour plates with MRS agar (Biokar Diagnostics, Beauvais, France) supplemented with 0.5 g of fructose/100 g of system. All plates were incubated in CO_2 enriched atmosphere at 30 ± 1 °C and growth was inspected after 5 days, as described by Smittle & Flowers (1982).

2.4. Rheology measurements

The study was undertaken for oil-in-water emulsions containing PS and nisin so as to asses the effect of oil level and tween addition on rheological behavior. Emulsions were formulated with the three oil levels used (110 g/kg, 230 g/kg and 460 g/kg) and with 0 or 20.00 g/kg of Tween® 20 (systems 7, 8, 15, 16, 23 and 24). The pH of the systems was adjusted to 3.5 with drops of citric acid solution (500 g/l).

A Paar Physica shear-rheometer (MC200, Stuttgart, Germany) was utilized to run the experiments. Stainless steel parallel plates geometry (30 mm diameter) and a gap of 2 mm were employed. The linear viscoelastic region (LVR) for each sample was first determined by performing oscillatory stress sweeps from 0.01 to 4 Pa at a constant frequency of 6 s^{-1}. A constant strain of 4.0% was then chosen for those emulsions containing 110.00 and 230 g/kg oil and a value of 0.5% was chosen for the emulsions with 460 g/kg oil. The cited strains and the frequency sweeps (0.03–40 s^{-1}) were performed within the LVR of all samples. Storage (G') and loss (G'') moduli as well as the tangent of the phase shift angle δ ($\tan\delta = G''/G'$) were determined from the frequency sweeps for all samples tested.

2.5. Data treatment

The data were first screened to determine if there was a practical difference between the bacterial populations of the different model systems at each time point during storage. It was deemed that a practical difference in population levels was present if the difference between the means for the control and treatment was equal to or greater than one log CFU/g. A value of one log was chosen for practical significance as differences of one order of magnitude are generally regarded as being of microbial significance (Gill & Holley, 2000; Jarvis, 1989). If the difference between the two means were of practical significance, analysis of variance (ANOVA) was used to study the log reductions in viable counts and to find which factors had a significant effect in these reductions, in all the different treatments. Based on the ANOVA results, the Least Significant Difference (L.S.D.) method was applied to study the differences between treatments simultaneously. For this last study, experimental units were the series of log reductions. For each model system formulated, the data of two replicate experiments were used.

Regression analysis of the data was performed for those systems were *L. fructivorans* population decreased with time and it was verified the existence of linear relationships between Log (N/N_0) and t (N_0 being the initial number of microorganisms and N the number of microorganisms at time t), indicating that the lethal process followed the pattern of a first order kinetics (Xiong, Xie, Edmonson, Linton, & Sheard, 1999). For each model system, the death rate constant (k) was calculated from the equations for the best linear fit and significant differences between them were analyzed using the Co-Variance Analysis (ANCOVA).

For both statistical analyses, the confidence level used was 95%. The tests were computed by using STATGRAPHICS® *Plus* version 4.0 software (1994).

3. Results and discussion

In Figs. 1, 2, and 3, it can be seen that microbial population of the emulsions without nisin (systems 1, 2, 5, 6, 9, 10, 13, 14, 17, 18, 21 and 22) did not vary during the storage time compared to the initial values.

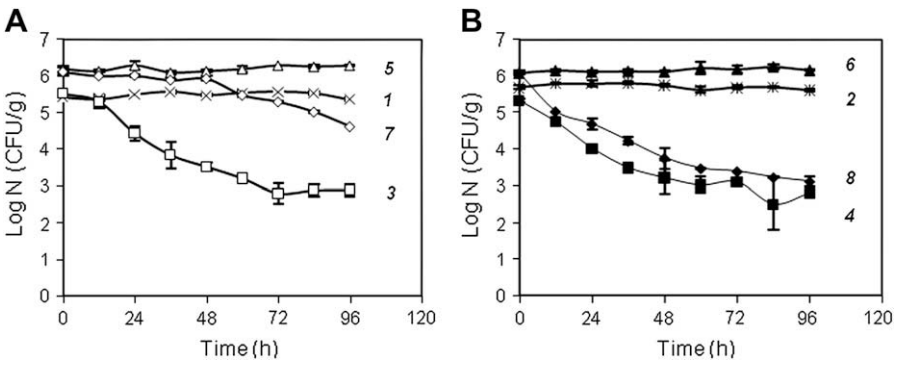

Fig. 1. Effect of additives on the viable counts of *L. fructivorans* CRL 941 G during storage at 30 °C in model systems containing 110 g/kg oil. Panel **A:** systems free of tween; **B**: systems containing tween. Systems without additives (×); systems containing Tw (∗); systems containing nisin (□); systems containing PS (△); systems containing nisin and PS (◇); systems containing nisin and Tw (■); systems containing PS and Tw (▲); systems containing nisin, PS and Tw (◆). Numbers in italics at the right of the time series indicate the corresponding system (see Table 1).

This fact emphasizes that the stress factors imposed by the formulation prevent the bacterial growth but they were not enough to inactive it throughout the storage time evaluated. This tendency was also observed for system 12, which had nisin (230 g/kg oil + nisin + Tw).

Several reports showed that *L. fructivorans* is well adapted to the salad dressing environment being able to grow and cause bulging of plastic bottles as a result of gas formation for extended storage times (Meyer et al., 1989; Smittle, 2000). Probably, in our study, *L. fructivorans* adaptation phase was longer than the storage time considered and this behavior could be linked with the fact that in emulsified systems microbial growth is developed in colonies instead of as free entities (Brocklehurst, Parker, Gunning, Coleman, & Robins, 1995; Brocklehurst et al., 1993; Wilson et al., 2002).

3.1. Effect of nisin

In general, all the systems containing nisin showed inhibition of bacterial growth (Figs. 1, 2 and 3 systems: 3, 4, 7, 8, 11, 15, 16, 19, 20, 23 and 24). However, the degree of log reduction and, consequently, the death rate constants depended on the composition of each formulation (Table 2).

Considering the samples with 110 g/kg oil (Fig. 1, panels A and B), it was observed that those which contained nisin (system 3), nisin + Tw (system 4) and nisin + Tw + PS (system 8) showed death rate constants with no significant differences between them (Table 2) underlying that the addition of Tw or Tw + PS did not affect the bacteriocin activity. Nevertheless, the addition of PS in the presence of nisin (system 7) promoted a significant reduction in the death rate constant.

In samples with the intermediate level oil content (230 g/kg) nisin activity was significant in systems 11 (just with nisin), 15 (nisin + PS) and 16 (nisin + PS + Tw) compared to the control system (system 9) and to system 10, that only contains Tw as a variable factor (Fig. 2, panels A and B). System containing nisin and tween (system 12) was the only one containing nisin in which the bacteria survived along the storage. This behavior suggested that tween exerted an antagonistic action on the antimicrobial effectiveness of nisin; this trend will be analyzed later when the effect of tween is discussed in Section 3.4.

All the systems which contained the highest oil content (460 g/kg) + nisin presented significant differences when compared to the control system (system 17) and to the same system which had Tw added (system 18). This is evident in Fig. 3, panels A and B. Higher death rate constants were observed in the system containing nisin + PS (system 23) and the system with nisin + PS + Tw (system 24). The addition of Tw to the system that only contained nisin or to the system with nisin + PS did not affect the death rate constants (Table 2: system 19 vs. system 20; system 23 vs. system 24). From these results it could be proposed that, in emulsions of this oil content, the addition of sorbate enhances nisin activity while the addition of the emulsifier does not affect it.

Regarding the biopreservative effectiveness, the results exposed here indicate that, in general, the addition of 0.500 g/kg nisin to emulsions which mimic salad dressings inhibited *L. fructivorans* growth throughout storage of four days at 30 °C. This bacteriocin proved to have a bactericidal action against the strain tested in almost all the system formulations examined (Figs. 1, 2 and 3). The inhibition patterns differed from one treatment to another suggesting that the efficacy of the natural preservative depends on several factors. Factors that may influence the recovery and efficiency of bacteriocins in emulsified systems are binding to other additives or ingredients and partitioning into polar or non-polar food components (Aasen et al., 2003; Delves-Broughton, 1990).

Table 2
Death rate constants for *L. fructivorans* in model salad dressings.

System number and composition (g/kg)	Death rate constants, k [h^{-1}] × 10^2
3 (oil: 110; nisin 0.5)	0.33^{a}
4 (oil: 110; nisin: 0.5; Tw: 20)	0.31^{a}
7 (oil: 110; PS: 0.5; nisin: 0.5)	0.11^{c}
8 (oil: 110; PS: 0.5; nisin: 0.5; Tw: 20)	0.29^{ab}
11 (oil: 230; nisin: 0.5)	0.21^{bc}
15 (oil: 230; PS: 0.5; nisin: 0.5)	0.48^{d}
16 (oil: 230; PS: 0.5; nisin: 0.5; Tw: 20)	0.47^{d}
19 (oil: 460; nisin: 0.5)	0.16^{c}
20 (oil: 460; nisin: 0.5; Tw: 20)	0.14^{c}
23 (oil: 460; PS: 0.5; nisin: 0.5)	0.29^{ab}
24 (oil: 460; PS: 0.5; nisin: 0.5; Tw: 20)	0.27^{ab}

Same letters indicate that there were no significant differences between death rate constants ($p > 0.05$). In systems 1, 2, 5, 6, 9, 10, 12, 13, 14, 17, 18, 21 and 22 *L. fructivorans* population remained constant throughout storage.

3.2. Effect of sorbate

In Figs. 1, 2 and 3, it can be seen that the use of potassium sorbate alone resulted in no significant differences on viable cells counts between treated samples (systems 5, 6, 13, 14, 21 and 22) and samples with Tw (systems 2, 10 and 18) or without the emulsifier (control systems: 1, 9 and 17). This trend can be expected since it is known that many lactic acid bacteria are not inhibited by PS (Sofos, 2000).

The joint addition of nisin and PS to an emulsion made with 110 g/kg oil (system 7), gave a smaller death rate constant than the system formulated just with nisin (system 3). This trend suggested that, at this level of oil, PS would exert an antagonic effect diminishing nisin antibacterial effectiveness. More work must be done to elucidate this trend.

It must be stressed that, addition of PS to a nisin + Tw treated sample, in the presence of 110 g/kg oil, did not affect k (Table 2, system 4 vs. system 8).

On the other hand, for the emulsions formulated with 230 g/kg oil, the presence of PS to systems containing nisin (system 15) and nisin + Tw (systems 16) produced higher log reductions than those observed for samples which only contain nisin (system 11) and nisin + Tw (system 12) (Fig. 2). In Table 2, values for systems 11, 15 and 16 can be observed. The same trend was observed for emulsions containing 460 g/kg oil (Table 2: systems 19 and 20 vs. systems 23 and 24). Mentioned results indicated that PS exerted a synergistic action on nisin antibacterial effectiveness. This trend was described earlier by Buncic et al. (1995) in buffered BHI broth against *Listeria monocytogenes* and by Fang et al. (1997) who reported that the combined treatment extended shelf-life of vegetarian foods inhibiting growth of *Staphylococcus aureus* C10 and *Bacillus cereus* B7. It is well known that nisin binds to the cytoplasmic membrane of vegetative cells and penetrates into the lipid phase of the membrane, forming pores which allow the efflux of intracellular materials, resulting in dissipation of the proton motive force and eventually cell death (Bruno & Montville, 1993). Nisin A can form transient multistate pores with diameters ranging from 0.2 to 1.2 nm. Such pores would allow the passage of solutes with molecular masses up to 0.5 kDa (Abee et al., 1995). It can be assumed that PS could be able to penetrate into the cell environment through these pores inducing inhibition of bacterial growth. Therefore, sorbate yielded higher inhibition responses in combination with nisin than nisin itself.

3.3. Effect of oil content

The increase of oil level from 110 g/kg to 230 g/kg or 460 g/kg in those emulsions without nisin did not affect *L. fructivorans* survival

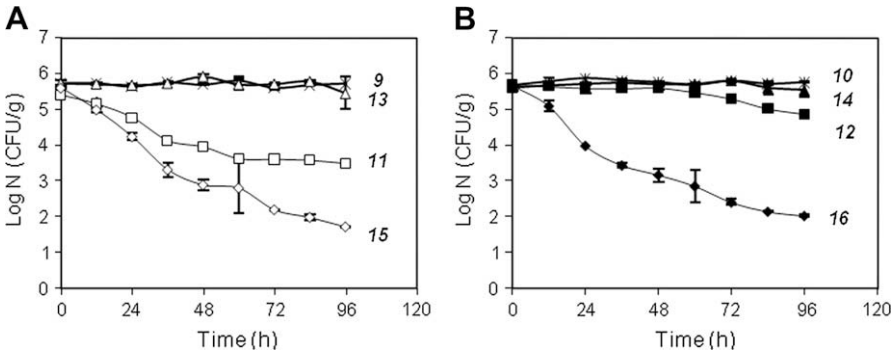

Fig. 2. Effect of additives on the viable counts of *L. fructivorans* CRL 941 G during storage at 30 °C in model systems containing 230 g/kg oil. Panel **A:** systems free of tween; **B**: systems containing tween. Systems without additives (×); systems containing Tw (✳); systems containing nisin (□); systems containing PS (△); systems containing nisin and PS (◇); systems containing nisin and Tw (■); systems containing PS and Tw (▲); systems containing nisin, PS and Tw (◆). Numbers in italics at the right of the time series indicate the corresponding system (see Table 1).

(systems 1 vs. 9 vs. 17; systems 2 vs. 10 vs. 18; systems 5 vs. 13 vs. 21; systems 6 vs. 14 vs. 22). On the contrary, the increase of oil level in the presence of nisin produced different effects which depended on system composition.

In the presence of nisin alone, the rise in oil concentration produced, in general, a decrease of the death rate constant of the microorganism (Table 2, systems 3 vs. 11 vs. 19). This trend is consistent with the one reported in literature, where it is said that nisin is less effective when the fat content of food is increased (Bhatti et al., 2004; Jung et al., 1992; Zapico et al., 1999).

In those emulsions containing nisin + PS and nisin + PS + Tw, it can be noticed that an increase in oil level from 110 g/kg to 230 g/kg determined a significant increase in death rate constants (Table 2, systems 7 vs. 15 and 8 vs. 16). Conversely, an increase of the oil content from 230 g/kg to 460 g/kg determined a decrease in the values of the death rate constants (Table 2, systems 15 vs. 23 and 16 vs. 24). These results are in agreement with those reported by Glass and Johnson (2004) who observed that an addition of 200 g/kg milkfat or soybean oil significantly decreased the antibotulinal activity of nisin, whereas the effect of 100 g/kg fat was variable, confirming that the antagonistic effect of fat on nisin activity is concentration dependent.

It is important to point out that this controversial and diverse effect could be due to:

I) Nisin being less effective as oil level is increased, as it was mentioned above.

II) The emulsions studied, behave as viscoelastic systems. In general, rheological measurements tended to present a crossing between G' and G'' at frequencies between 25–30 s^{-1}. Nevertheless, this crossing disappeared for those systems with the highest oil level. The latter emulsions presented a peak at frequencies of 5–6 s^{-1}, behavior that can be compared to those of several gels of predominant elastic behavior (François, Rojas, Daraio, & Bernik, 2003). The dissimilar rheological properties observed for studied emulsions could affect the effectiveness of the different preservatives in two divergent ways: i) an increase in oil level could lead to a higher death rate constant due to the reduction of the nutrient availability given by the stronger solid character of the emulsion matrix; ii) an increase in oil level could lead to a lower death rate constant because it conditionates the meeting of the preservatives with the microorganisms as a result of the stronger solid character of the system.

III) An increase of the oil phase determines an increase in the external droplet surface (as long as droplets keep their size) leading to an increase of the interfacial surface which enables more nisin to be at the interface. This situation would change bacteriocin effectiveness (Bhatti et al., 2004; Glass & Johnson, 2004; Jung et al., 1992), but it would allow that the preservative and the microorganism meet at the oil droplet surface (or at the interface), enhancing nisin effectiveness against microbial growth. This scenario was proposed by Kurup, Wan, and Chan (1991a, 1991b) and it was reported by Brocklehurst et al. (1995). Hence, two possibilities can be expected: i)nisin effectiveness decreases as a consequence for not being at the water phase which would lead to a reduction of the death rate constant; ii) nisin effectiveness increases because of the fact that the interface gives like a "meeting

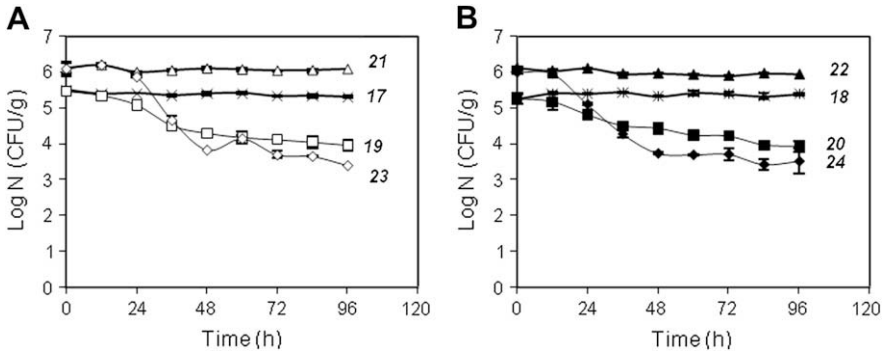

Fig. 3. Effect of additives on the viable counts of *L. fructivorans* CRL 941 G during storage at 30 °C in model systems containing 460 g/kg oil. Panel **A:** systems free of tween; **B**: systems containing tween. Systems without additives (×); systems containing Tw (✳); systems containing nisin (□); systems containing PS (△); systems containing nisin and PS (◇); systems containing nisin and Tw (■); systems containing PS and Tw (▲); systems containing nisin, PS and Tw (◆). Numbers in italics at the right of the time series indicate the corresponding system (see Table 1).

Table 3
Dynamic rheological parameters[a] determined for model salad dressings at 4.0% constant strain for emulsions containing 110 g/kg and 230 g/kg oil and at 0.5% constant strain for emulsions containing 460 g/kg oil.

System number and composition	G' (Pa)	G'' (Pa)	tan δ
7 (oil: 110; PS: 0.5; nisin: 0.5)	12.3 ± 0.5	6.29 ± 0.05	0.506 ± 0.002
8 (oil: 110; PS: 0.5; nisin: 0.5; Tw: 20)	10.1 ± 0.9	6.0 ± 0.2	0.59 ± 0.04
15 (oil: 230; PS: 0.5; nisin: 0.5)	18.2 ± 0.2	8.5 ± 0.3	0.47 ± 0.02
16 (oil: 230; PS: 0.5; nisin: 0.5; Tw: 20)	12.4 ± 0.3	6.9 ± 0.5	0.56 ± 0.03
23 (oil: 460; PS: 0.5; nisin: 0.5)	38.6 ± 0.6	14.7 ± 0.2	0.380 ± 0.001
24 ((oil: 460; PS: 0.5; nisin: 0.5; Tw: 20)	57.0 ± 3.0	17.9 ± 0.1	0.32 ± 0.01

[a] Average and standard deviations for $n = 4$ are shown.

point" where nisin encounters microorganisms, which would augment the death rate constant.

It has to be pointed out that an increase of the oil content in an emulsion produced a decrease of sorbate concentration in the water phase (Castro, 2006; Castro et al., 2003; Wedzicha et al., 1990) which would affect the antimicrobial effectiveness of nisin depending on the oil level.

3.4. Effect of tween

The addition of the surfactant Tween®20 to the systems without preservatives or to the systems that contained PS as the only preservative did not affect the survival of *L. fructivorans* (systems: 1 vs. 2, 9 vs. 10, 17 vs. 18 or 5 vs. 6, 13 vs. 14, 21 vs. 22, respectively). When nisin was added to the samples mentioned above, the surfactant effect was entirely dependent upon oil content. For the intermediate level of oil (230 g/kg) the addition of Tw exerted an antagonistic effect on the antimicrobial action of nisin since the bacteriocin stopped being bactericidal (Fig. 2, system 11 vs. system 12). This effect is in agreement with that observed by Henning et al. (1986a) who also described that nisin effectiveness was disturbed by the presence of phospholipids or fatty acids (Henning, Metz, & Hammes, 1986b). On the other hand, for the lowest level of oil (110 g/kg) the addition of Tw to a system that contained nisin and PS enhanced the death rate constant (Table 2, system 7 vs. system 8). For the rest of the samples tested, including samples containing 460 g/kg of oil, Tw addition did not modify the tendencies observed for each of the additives or ingredients analyzed.

It has to be pointed out that there are several ways in which this surfactant can affect the antimicrobial activity of nisin and sorbate:

a) According to Courthadon, Girardet, Chapal, Lorient, and Linden (1995) and Coupland and McClements (1996), the adsorption of a polypeptide, like nisin, to the oil–water interface formed in an emulsion is counteracted by the use of surfactants, especially Tween®20. These emulsifiers displaced bacteriocins from the interface by changing its thermodynamic environment. This tendency could lead to: a1) an increase of nisin activity since it will be located at the water phase. It was mentioned earlier in this work that surfactants counteract the antagonistic effect exerted by fats on nisin activity; a2) a decrease of nisin activity since the interface can be considered as the "meeting point" between nisin and microorganisms.

b) Moreover, the presence of a surfactant determined a reduction on the size of the oil droplets for a given concentration of oil as it was previously demonstrated (Castro, 2006). This situation may influence diversely on the behavior of the systems:

b1) When oil content was 110 g/kg or 230 g/kg, the addition of Tw turned the systems more fluid (Table 3). As a consequence, it could be expected a lower rate of inactivation since more nutrients would be available for microorganisms due to the augmented fluid character of the systems or, on the contrary, this would lead to a higher rate of inactivation since the meeting of the microorganisms with the preservatives would be more favored.

b2) When the oil content was 460 g/kg, the addition of Tw turned the system more solid (Table 3), giving again two different possibilities. Thus, this situation could lead to a higher rate of inactivation considering that less nutrients would be available as a consequence of the strong solid character of the system. But, on the other hand, it could lead to a lower rate of inactivation since the solid character of the system would hinder the contact between the preservatives and the microorganisms.

In summary, the phenomenon reported here comprised the results of the balance of the opposing tendencies mentioned.

4. Conclusions

In general, *L. fructivorans* growth was prevented in formulated emulsion, but the addition of nisin was necessary to inactivate the bacteria throughout the storage time evaluated. The bacteriocin activity showed to be strongly dependent on system composition.

Addition of PS alone did not influence *L. fructivorans* survival. However, when this preservative together with nisin was added to an emulsion containing 110 g/kg of oil, it exerted an antagonistic action on bacteriocin effectiveness while for the rest of oil levels, a synergistic action was verified.

The increase of oil level from 110 g/kg to 230 g/kg or 460 g/kg in those emulsions without nisin did not affect *L. fructivorans* survival. On the contrary, in the presence of nisin, it produced different effects which depended on system composition.

Addition of tween did not affect *L. fructivorans* survival for emulsions free of additives or containing only PS. But, when nisin was present, the emulsifier effect depended on oil content. For the lowest level of oil, its addition counteracted the losses in nisin activity induced by fat content. For the intermediate level of oil (230 g/kg), the addition of Tw exerted an antagonistic effect on the antimicrobial action of nisin since the bacteriocin stopped being bactericidal. For the highest level of oil, Tw addition did not influence nisin activity.

Furthermore, the structure of the food matrix appeared as an additional factor which could influence either the growth of the microorganisms or the functionality of the preservatives. Tween addition turned the systems more fluid or solid, depending on the oil content of the systems considered. Thus, the effect of Tween® 20 must be considered not only from a chemical point of view but also from a structural one.

Results obtained highlight the importance of considering ingredient interactions when evaluating microbial stability of food systems.

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