Evaluation of the effectiveness of Proallium S Líquido DMC in sauces

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INTRODUCTION

During the past years an increase of the epidemiologic outbreaks related to the intake of ready to eat food such as mayonnaises and other table sauces has been recorded. In view of such situation, it is recommended to take the necessary measures for preventing the risks that affect the consumers' health.

A solution would be the use of food grade preservatives to prevent the deterioration and increase the safety of this kind of products. It is true that consumers prefer free of additive food and ask more and more for the use of preservatives of natural origin, harmless and effective against microorganisms.

Proallium S Líquido DMC is a commercial product of DOMCA S.A.U. (Spain) consisting of a nature identical flavour based on a selection of organosulfur compounds with antimicrobial properties that are proved to be present in different species of the plant genus *Alliaceae* (garlic, onion).

The goal of the present work has been the evaluation of the antimicrobial activity of Proallium S Líquido DMC in table sauces such as alioli and ketchup in the control of altering microorganisms and pathogens transmitted by food.

MATERIAL AND METHODS

Trial of controlled inoculation

By means of *Challenge Test* technique the effectiveness of the product against the different microorganisms inoculated in ketchup and alioli sauce has been proved. The manufacture and elaboration processes of food have been the usual ones determined by reference manufacturers in the sector.

After inoculation with the microorganisms, Proallium S Líquido DMC has been applied at 500 and 1.000 ppm. Besides the corresponding controls, some batches with potassium sorbate (500 and 1.000 ppm) have been included. All food has been packed and kept at 4ºC until the end of the trial. For monitoring the evolution of the different microorganisms a sampling has been programmed at different times established (0, 1, 5, 10, 15 and 20 days). All determinations have been carried out in duplicate.

Microorganisms

The collection strains *Lactobacillus plantarum* CECT 220, *Salmonella enteritidis* CECT 7159, *Yersinia enterocolitica* IP383 *Escherichia coli* DMC12 and *Zygosaccharomyces bailii* DSM 70834 have been used, in addition to a *pool* of lactic acid bacteria and yeasts isolated from ketchup and involved in phenomena of alteration of such food.

Adaptation of strains and preparation of inoculum.

After reception, acid lactic acid bacteria (LAB) were inoculated in Man Rogosa & Sharpe - MRSB culture medium and incubated at 30 ± 1 ºC for 2-3 days. The enterobacteria were maintained in Brain Heart Infusion - BHI and grown at 37 ± 1ºC for 24 hours. In the case of yeasts YGB (Merck, Germany) a glucose culture medium was used at 25 ± 1℃ for 2-4 days.

To guarantee the survival of the strains a previous adaptation to the conditions of ketchup and alioli sauce was done. For such purpose the different strains were inoculated directly in the food previously enriched with quantities of YGB, MRSB and BHI depending on the microbial group, respectively. The inoculated mixtures were incubated in the very best conditions for each microorganism until the production of gas was obvious. Consequently, the inoculum preparation was adjusted at 10^8 cfu/ml for bacteria and 10^7 cfu/ml for yeasts.

Means and culture conditions

For the determination of LAB the normalised method was followed as per ISO 15214:1998 by using Man Rogosa Sharpe agar (Merck, Germany). In the count of enterobacteria, Chromosalm Agar (Microkit, Spain) MacConkey Agar (Merck, Germany) medium and Cefsulodina-Irgasan-Novobiocina (CIN) of Sharlau (Spain) selective medium were used. The culture conditions in this case were 37 ± 1ºC during 24- 48 hours.

The determination of yeasts was done with oxytetracycline glucose yeast extract - OGY (Merck, Germany) agar medium. The incubation times varied depending on the strain (between 2 and 6 days). All determinations were carried out in duplicate.

RESULTS AND DISCUSSION

Effectiveness of Proallium S Líquido DMC in alioli sauce.

The deterioration of ready to eat food such as table sauces is a serious problem for the food industry due to the huge economic losses involved. Such products are usually preserved at pH values between 3,5 and 4,5. These parameters are not much favourable for the microbial growth. Nevertheless they are adequate for the development of certain lactic acid bacteria such as *Lactobacillus plantarum* or *Lactobacillus fermentum* and acid tolerant yeasts such as Z. *bailli, Y. lipolytica* and *aromyces exiguus,* responsible for alteration processes in this food.

In the present work, the antimicrobial effectiveness of Proallium S Líquido DMC has been evaluated against *Lactobacillus plantarum* CECT 220 and *Zygosaccharomyces bailli* DSM 70834 collection strains in an alimentary model like alioli sauce. It could be shown that its application at a dose of 500 ppm produced a reduction of the total microbial charge and the complete elimination of the inoculated microorganisms was reached from the 10^{th} day of preservation onwards. The batches treated with potassium sorbate at the same dose did not show significant decreases in the microbial counts compared with the controls, as shown in figure 1.

The antimicrobial capacity against *Salmonella enteritidis* CECT 7159 and *Yersinia enterocolítica* IP383 has been evaluated as well. In this case the results show that the application of Proallium S Líquido DMC at a dose of de 1.000 ppm performed an efficient control in the development of these enteropathogenic microorganisms with significant differences compared to the control batches and the ones treated with potassium sorbate. The bactericidal effect of the product increased from the first week of preservation on, with diminutions up to 4 logarithmic unities in the case of *Salmonella* and about 3 logarithmic unities for *Yersinia* at the end of the trial.

The batches treated with potassium sorbate showed a reduction in their counts as well. Nevertheless, the values remained always over the ones obtained for the batches treated with Proallium S Líquido DMC.

The organosulfur compounds that compose Proallium S Líquido DMC have been described in bibliography as wide spectrum antimicrobials and their effectiveness has been reported against pathogenic agents such as *E. coli, S. typhi, S. aureus* or *P. aeruginosa* (Gomaa *et al.,* 2003, Iwalokun *et al.,* 2004). For all that and as regards to the results obtained, it can be stated that the addition of this product, besides prolonging the shelf life of such food, means a natural and effective method for controlling food enteropathogenic agents.

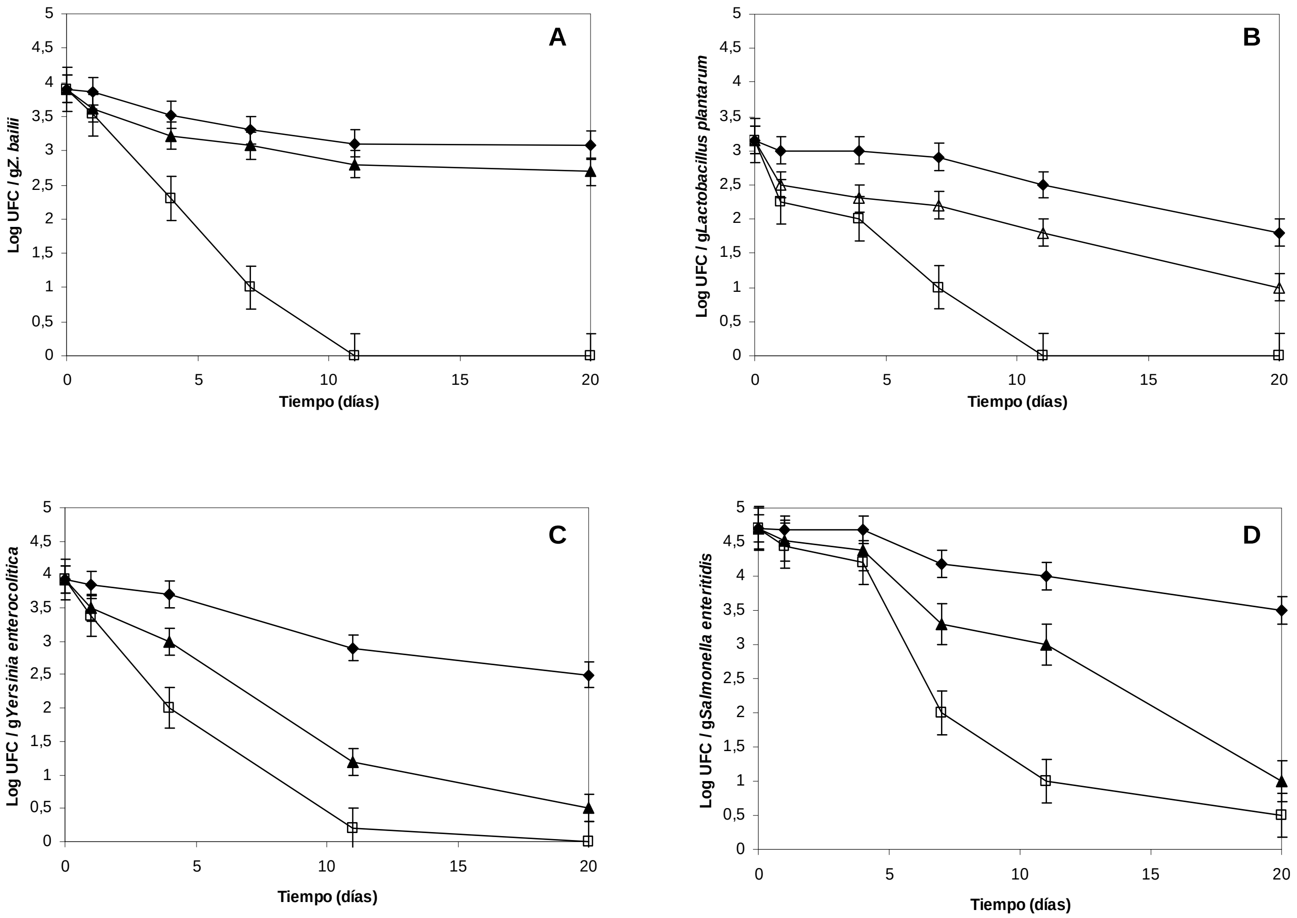

Figure 1. Antimicrobial activity of Proallium S Líquido DMC in table sauce like alioli.

(A) *Zygosaccharomyces bailli* and **(B)** *Lactobacillus plantarum;* ♦ Positive control (approximate initial inoculum of 10^3-10^4 UFC/g) □ Proallium S Líquido (500 ppm) ▲ Potassium sorbate (500 ppm) **(C)** *Yersinia enterocolitica* and **(D)** *Salmonella enteritidis;* ♦ Positive control (approximate initial inoculum of 10^4-10^5UFC/g) □ Proallium S Líquido (1000 ppm). ▲ Potassium sorbate (1000 ppm)

Effectiveness of Proallium S Líquido DMC in ketchup

The lactic acid bacteria constitute an interesting group of microorganisms for food industry due to their capacity to behave as food modifiers. Strains of *Lactobacillus fructivorans, Lactobacillus fermentum* or *Leuconostoc* between others have been described many times as being responsible for undesirable alteration processes of ketchup (Bjorkroth *et al.,* 1996). On the other side, yeasts constitute one of the microbial groups that are more usually involved in deterioration processes of such food, being substantially responsible for undesirable fermentations.

In relation to all this, we have decided to evaluate the effectiveness of Proallium S Líquido DMC against a *pool* of bacteria and yeasts of lactic acid isolated from ketchup and responsible for real alteration phenomena of this food. At the same time its antimicrobial effectiveness against *Escherichia coli* DMC-12 has been verified.

The results obtained showed that the application of Proallium S Líquido DMC at a dose of 500 ppm performed an excellent control against the inoculated lactic acid microorganisms, with significant decreases in the counts from the first day of application on and a total elimination from 5th preservation day on, as shown in figures 2 A and B.

In the case of *Escherichia coli* a progressive reduction has been observed throughout the time in spite of the high initial concentration of the microorganisms (>10^4 UFC / g). At the end of the trial differences up to 3 logarithmic unities have been reached as regards to the control batches and about 1,5 logarithmic unities as regards to those batches treated with potassium sorbate (Figure 2 C).

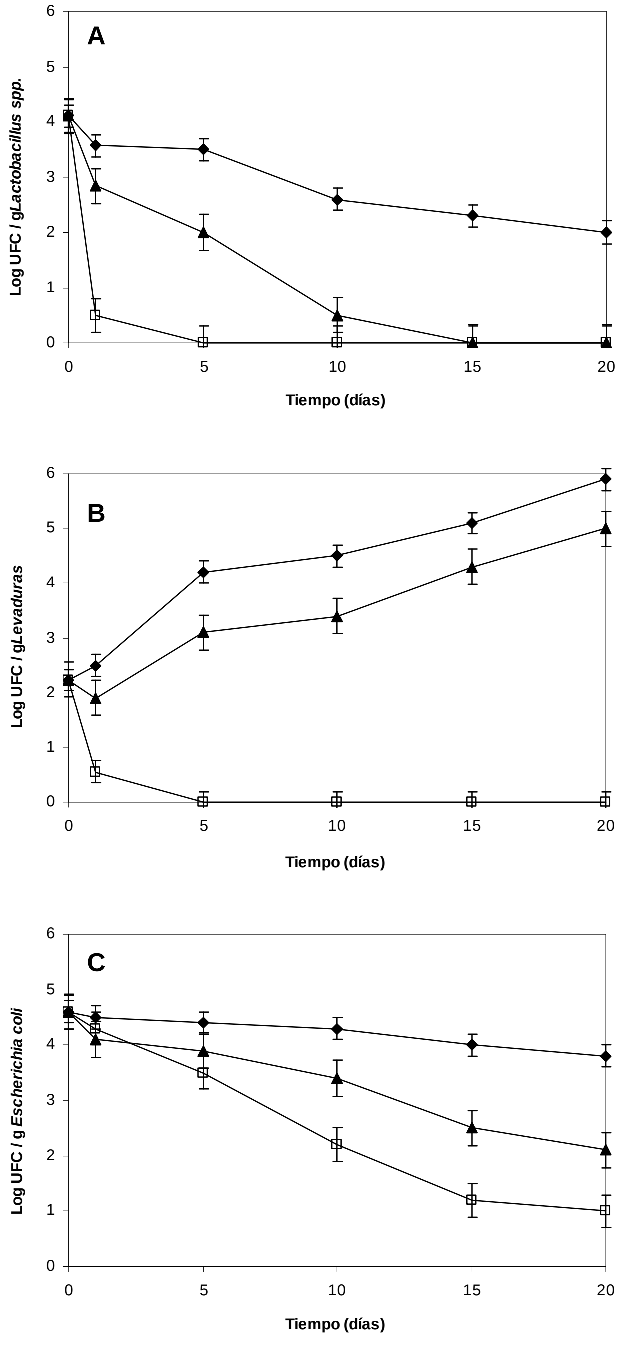

Figure 2. Antimicrobial activity of Proallium S Líquido DMC in tomato ketchup sauce (A) *Pool* of lactic acid bacteria isolated from alteration processes of ketchup **(B)** *Pool* of lactic acid yeasts isolated from alteration processes of ketchup **(C)** *Escherichia coli* DMC 12. ♦ Positive control, ▲ Potassium sorbate (500 ppm), □ Proallium S Líquido (500 ppm).

These results point out the sensibility of these microorganisms to the product, that is specially relevant for yeasts, where the differences at the end of the trial compared with the control batches and those treated with potassium sorbate were significant (between 6 and 4,5 logarithmic unities respectively).

CONCLUSIONS.

The data obtained in the present work prove that Proallium S Líquido DMC has an antimicrobial activity capable of effectively controlling the development of microorganisms and modifying yeasts in table sauces such as alioli and ketchup, contributing to the prolongation of the shelf life of such type of products.

Moreover, its capacity against enteropathogenic microorganisms such as *Salmonella enteritidis, Yersinia enterocolitica* and *Escherichia coli* has been pointed out, proving its effectiveness as an alternative to the use of traditional preservatives such as potassium sorbate.

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