Technology and development
for the food industry

REPORT:

Challenge Test to verify the microbiological stability of tomato sauce.
Evaluation of the antimicrobial activity of Proallium® S Líquido in pasteurized tomato sauces against selected strains of lactobacillus and yeast.

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1. JUSTIFICATION AND OBJECT OF THE STUDY

The application of preservatives which prevent the deterioration of food products has seen a great evolution during the last years. This development is based on a significant increase in the demand of healthy and natural ready-to-eat products. Going along with this progress the need of natural products with high antimicrobial properties has grown, as well as the need of optimizing their application and reducing their doses of application to a minimum.

The object of this study is the evaluation of the antimicrobial potential of the product Proallium® S líquido (DOMCA S.A.U) in the control of microorganisms in tomato sauce. The methodology of Challenge Test was used to evaluate the effect of Proallium® S líquido on a group of selected lactic acid bacteria and yeasts, inoculated in several batches, with different concentrations of Proallium® in their formulations. ®

2. METHOLOGY

Challenge Test

The methodology of *Challenge Test* was used in order to verify the potential of the product Proallium® as clean-label preservative in tomato sauce. The sauce was inoculated with two different pools of microorganisms (yeast and lactic acid bacteria). This type of test permits to study the capacity of microorganisms to survive or grow in the food product under certain circumstances. To do so, an analytical monitorizing system was established during a defined period of time (30 days)

The application of this type of test is recommended when a change in the formulation of an already commercialized product or a change in the condition of production is planned. This is even more important in food products with a long period of shelf life in order to assure the product's microbiological stability.

Samples

Batches of the pasteurized tomato sauce with different concentrations of Proallium® were prepared, as well as a negative control without any additives and a control with potassium sorbate.

- Tomato sauce (control)
- Tomato sauce + 200 ppm Proallium® S líquido
- Tomato sauce + 400 ppm Proallium® S líquido
- Tomato sauce + 600 ppm Proallium® S líquido
- Tomato sauce + 800 ppm Proallium® S líquido
- Tomato sauce + 500 ppm potassium sorbate.

Microorganisms

The mircoorganisms tested were isolated from different sauces and are implicated in their deterioration. Five strains of yeast and five strains of lactic acid bacteria were pooled. After their reception the lactic acid bacteria were inoculated in Man Rogosa Sharpe broth (MRSB) at 30ºC during 2-3 days. The strains of yeast were inoculated in YGB and incubated at 25ºC during 2-4 days.

Adaptation and Preparation of the Strains.

The microorganisms were adapted previously to the conditions of the tomato sauce. To do so, the different strains were directly inoculated in the sauce previously enriched with MRSB and YGB, respectively. The inoculated pool of yeasts was incubated at 25ºC, while the strains of lactic acid bacteria were incubated at 30ºC during the time necessary to observe visibly the formation of gas. Afterwards, the concentration of 10^8 cfu/ml of lactic acid bacteria and 10^7 cfu/ml of yeasts were established.

Inoculation in the tomato sauce

Once adjusted to final concentration of the preparations, different cocktails were prepared and adjusted to the final concentration desired in the food product of approximately 10^2 and 10^4 cfu/ml of lactic acid bacteria and 10^1 and 10^2 cfu/ml of yeasts.

Table 1. Innitial concentrations	UFC / g
Batch 1. Yeast Cocktail	10^1
Batch 2. Yeast Cocktail	10^2
Batch 3. Lactic acid bacteria Cocktail	10^2
Batch 4. Lactic acid bacteria Cocktail	10^4

Monitorization

The concentration of microorganisms was observed at 0, 1, 5, 10, 15 y 30 days after inoculation. For the determination of lactic acid bacteria the method according to ISO 15214:1998 using agar Man Rogosa Sharpe (Merck, Alemania) was applied. For the count of yeast the oxitetraciclin glucose yeast agar - OGY (Merck Alemania) was used. The time of incubation varied according to the strains (between 2 and 6 days). All data were collected by double determination.

3. RESULTS

Antibacterial activity of Proallium® against Lactic acid bacteria

The group of lactic acid bacteria is constituted of a great number of microorganisms that are of interest for the food industry due to their fermentative and acidifying capacity. Amongst them are *Lactobacillus*, *Leuconostoc*, *Lactococcus* or *Enterococcus sp.* On the other hand, others are implicated in the undesired alterations of food products and sauces in specific, such as strains like *Lb. fructivorans, Lb. fermentum* o *Leuconostoc pleofructi,* amongst others.

In the present study the capacity of tomato sauce to inhibit the growth of microorganisms which were inoculated intentionally was tested. To verify this potential, the use of strains from culture collections is not recommended because of their poor capacity of adaptation to the conditions present in food products. For this reason they tend not to be capable of stabilizing a population, although they were previously adapted to the media. For this reason, this study was realized with strains directly isolated from sauces which are directly responsible for undesired alterations. Using these strains permits a better adaptation of the microorganisms to the medium of the sauce and allows a real evaluation of the potential of Proallium® S líquido as natural preservative in tomato sauce.

The results of the evolution of the pool of lactic acid bacteria are visualized in figure 2, showing that Proallium® S líquido, even at the lowest concentration applied (200 ppm) provides excellent control of the microorganisms inoculated, significantly reducing the concentration from the first day on and showing a much higher efficacy than potassium sorbate and the control without additional treatment. The pool of lactic acid bacteria was inoculated in the tomato sauce at two different concentrations: $2{,}2 \times 10^2$ and $4{,}1 \times 10^4$ cfu/ml. At day one, the concentration of lactic acid bacteria could be reduced to values lower than $0{,}3 \times 10^1$ and less than 1×10^1 cfu/ml, respectively. In the following determinations no growth at all could be detected in any of the counts realized. Numeric results of the counts expressed in LOG cfu/ml are shown in table 1.

Observing the evolution of bacterial growth in the control batches (Control and 500 ppm of potassium sorbate 500) it can be concluded that, although the strains are wild type strains isolated from sauces, they show certain difficulties to adapt to the conditions of the sauce. They show a poor capacity of survival and after initial growth (after 5 days), they disappear after 15 days. The acid conditions seem to be the limitating factor for the proliferation of these microorganisms. Still, the time of survival of the strains in the control batches (Control and 500 ppm of potassium sorbate) compared with the steep decrease of the batches treated with Proallium® clearly shows the bactericidal capacity of Proallium® against these strains.

Antibacterial activity of Proallium against Yeasts

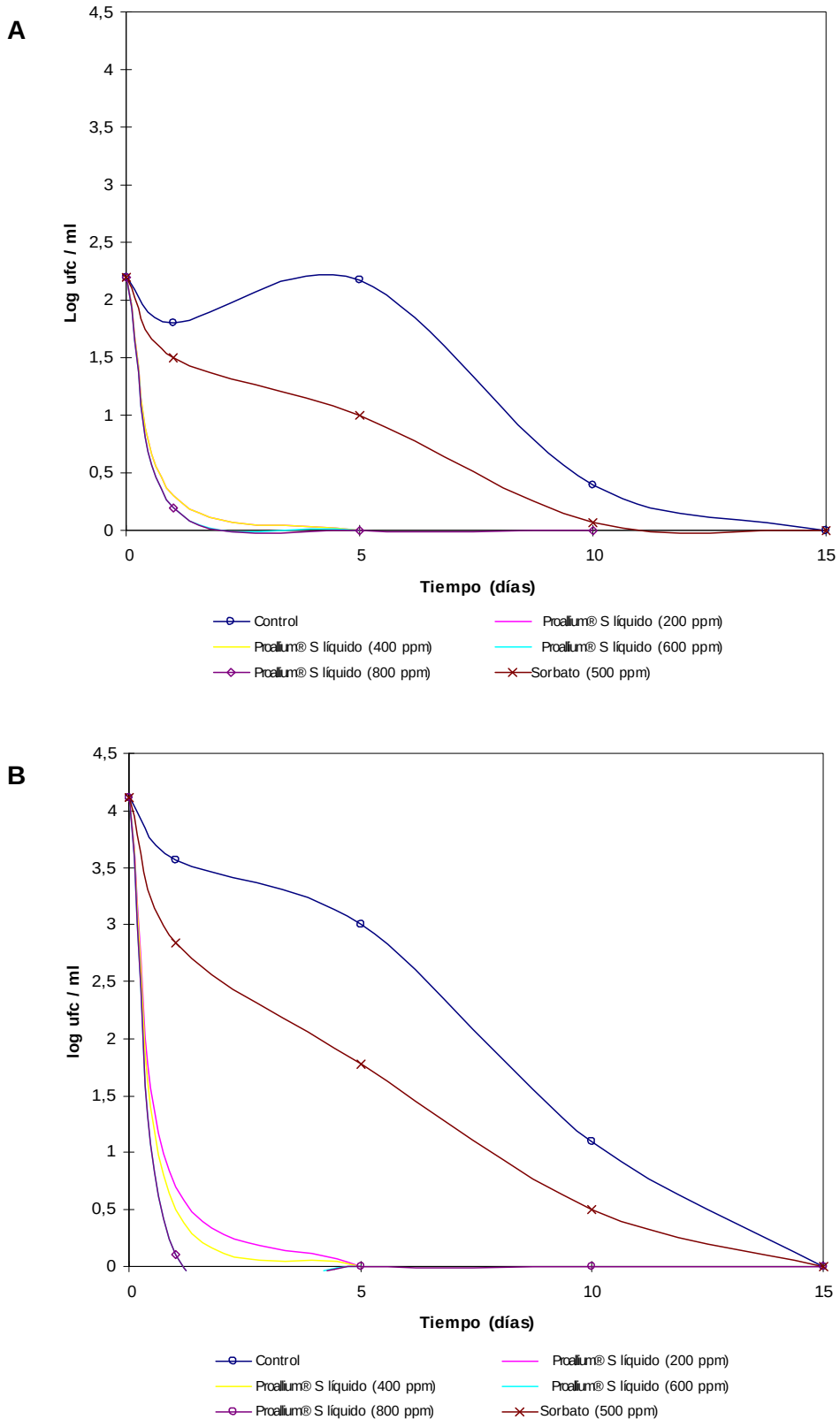
Yeasts are frequently involved in the undesired alteration of food products, mainly due to their fermentation processes, influencing taste and texture of food products such as sauces and others.

In the present trial the capacity of Proallium® S líquido to inhibit the growth of various strains of yeasts involved in the alteration of sauces was verified. Different concentrations of the pool of yeasts were inoculated in tomato sauce (10^1 y 10^2 ufc/ml). In total, seven batches were prepared: five of them with different concentrations of Proallium® S liquid (200, 400, 600 and 800 ppm), one control without any addition of additives and one control with 500 ppm of potassium sorbate. All batches treated with Proallium® S líquido showed excellent results, completely inhibiting the growth of the inoculated strains.

The results of the evolution of the pool of yeasts are visualized in figure 3, showing that Proallium® S líquido, even at the lowest concentration applied (200 ppm) provides an excellent control of the microorganisms inoculated, totally eliminating the strains from the first day on and showing a much higher efficacy than potassium sorbate and the control without additional treatment. The pool of yeast was inoculated in tomato sauce at two different concentrations: 1,2 x 10^1 and 2,23 x 10^2 cfu/ml. In both cases, from the first day on, no presence or only very low levels of yeasts could be detected in the counts realized.

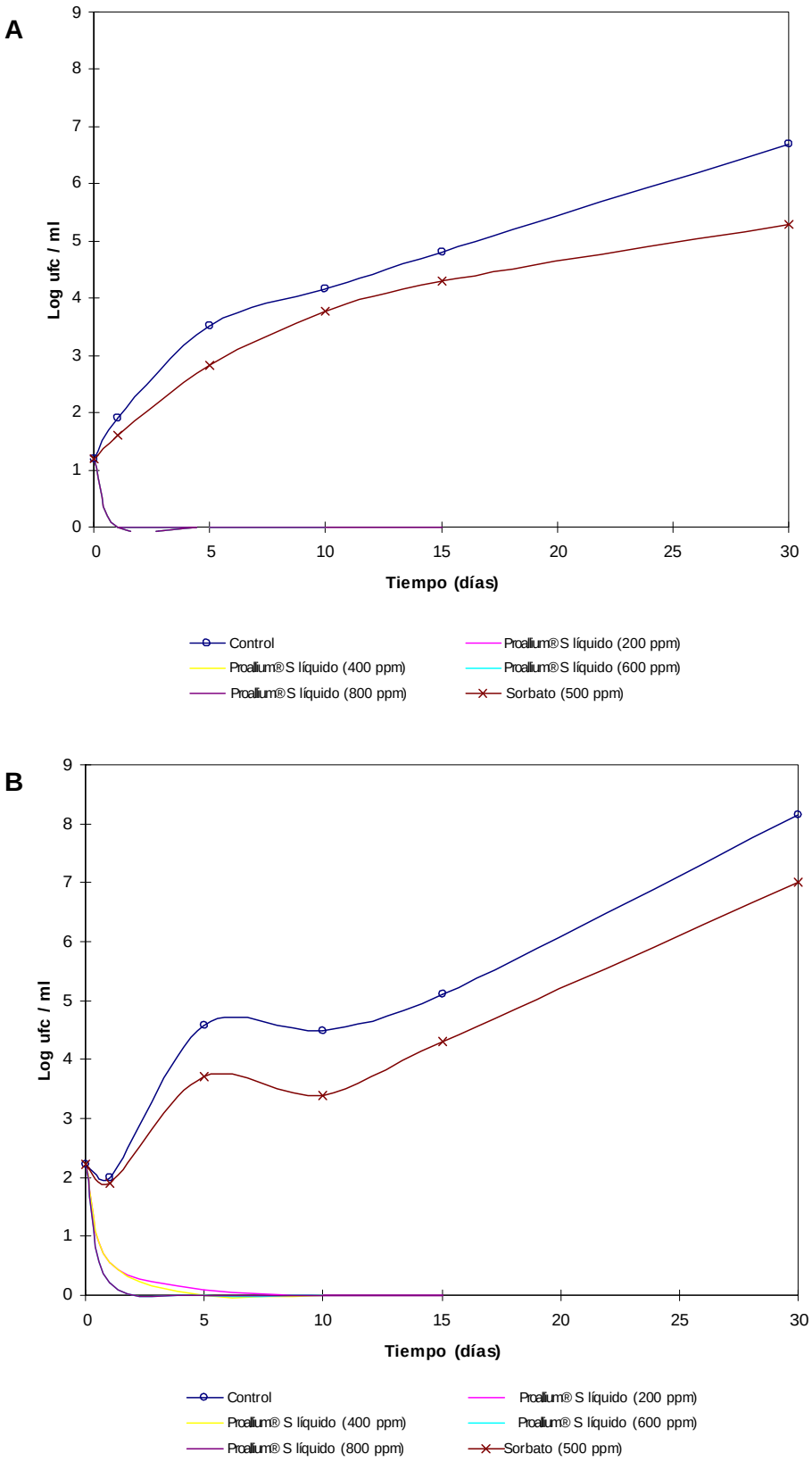
In the control batches (control and 500 ppm of potassium sorbate) the yeast strains perfectly adapted to the conditions of the sauce, already showing elevated concentration after 5 days, and reaching values of approximately 10^5 to 10^8 cfu/ml after 30 days. In contrast, in the batches treated with Proallium® the yeast strains were eliminated or drastically reduced from the first day on, maintaining total absence until the end of the study (30 days). This shows the excellent capacity of Proallium® S líquido in the control of yeasts compared with traditional preservatives such potassium sorbate. Numeric results of the counts expressed in LOG cfu/ml are shown in table 1.

Fig. 2. Effect of Proallium® S líquido against Lactic acid bacteria in tomato sauce.



A. Innitial inoculation: 10^2 ufc/ml **B**. Innitial inoculation: 10^4 ufc/ml

Fig. 3. Effect of Proallium® S líquido against yeasts in tomato sauce.



A. Innitial inoculation: 10^1 ufc/ml **B**. Innitial inoculation: 10^2 ufc/ml

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	Mean results Log CFU / ml						
	LACTIC ACID BACTERIA						
1,00E+02	Days of preservation	0	1	5	10	15	30
	Control	2,2	1,8	2,17	0,4	0	0
	Proallium® S líquido (200 ppm)	2,2	0,3	0	0	0	0
	Proallium® S líquido (400 ppm)	2,2	0,3	0	0	0	0
	Proallium® S líquido (600	2,2	0,2	0	0	0	0
	Proallium® S líquido (800 ppm)	2,2	0,2	0	0	0	0
	Sorbato K (500 ppm)	2,2	1,5	1	0,07	0	0
1,00E+04	Days of preservation	0	1	5	10	15	30
	Control	4,11	3,5667	3	1,1	0	0
	Proallium® S líquido (200 ppm)	4,11	0,7	0	0	0	0
	Proallium® S líquido (400 ppm)	4,11	0,5	0	0	0	0
	Proallium® S líquido (600 ppm)	4,11	0,1	0	0	0	0
	Proallium® S líquido (800 ppm)	4,11	0,1	0	0	0	0
	Sorbato K (500 ppm)	4,11	2,84139	1,77815	0,5	0	0
	YEAST						
1,00E+01	Days of preservation	0	1	5	10	15	30
	Control	1,2	1,90206	3,52119	4,177	4,8	6,69897
	Proallium® S líquido (200 ppm)	1,2	0	0	0	0	0
	Proallium® S líquido (400 ppm)	1,2	0	0	0	0	0
	Proallium® S líquido (600 ppm)	1,2	0	0	0	0	0
	Proallium® S líquido (800 ppm)	1,2	0	0	0	0	0
	Sorbato K (500 ppm)	1,2	1,61278	2,82531	3,78533	4,3	5,3
1,00E+02	Days of preservation	0	1	5	10	15	30
	Control	2,23	2	4,57171	4,5	5,1	8,1451
	Proallium® S líquido (200 ppm)	2,23	0,55	0,1	0	0	0
	Proallium® S líquido (400 ppm)	2,23	0,52	0	0	0	0
	Proallium® S líquido (600 ppm)	2,23	0,2	0	0	0	0
	Proallium® S líquido (800 ppm)	2,23	0,2	0	0	0	0
	Sorbato K (500 ppm)	2,23	1,90309	3,69897	3,39794	4,3	7

Table 1: Mean results of counts expressed in LOG cfu/ml.

4. CONCLUSIONS

Based on the results of this study it can be concluded that Proallium® S líquido is an excellent product for the control of undesired growth of lactic acid bacteria and yeasts in tomato sauce and sauces in general. Proallium® S líquido presents a highly effective alternative to commercially present preservatives such as potassium sorbate and others, even exceeding its efficacy in tomato sauce as shown here.

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