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Improvement of the lactic acid fermentation of capers (*Capparis spinosa* L) through an experimental factorial design

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RESUMEN

Mejora de la fermentación ácido láctica de alcaparras (*Capparis spinosa* L) mediante un diseño factorial.

El estudio del proceso de fermentación mediante un diseño factorial nos permitió determinar una función f (Y= $f(X_1, X_2, \ldots, X_n)$) que existe entre la magnitud Y que es la disminución del pH (llamada respuesta), y las variables X, que son la salmuera, ácido láctico, ácido cítrico y los fermentos lácticos (llamados factores). Un completo plan factorial 2^4 fue hecho con objeto de determinar los factores y las interacciones entre los factores que tienen una influencia estadísticamente significativa en la respuesta estudiada. La salmuera, ácido láctico y ácido cítrico tienen un efecto significativo en la caída del pH; por el contrario, los fermentos lácticos no tienen efecto significativo. Por otra parte, las interacciones entre salmuera y ácido láctico, salmuera y fermentos lácticos, ácido láctico y ácido cítrico, y ácido láctico y fermentos lácticos tuvieron un efecto significativo en la caída del pH ($p < 0.0001$). La fermentación fue hecha en el laboratorio de investigación de la Sociedad Marocapres-Fez líder Internacional en la transformación de alcaparras, a una temperatura de aproximadamente 30°C entre los meses de junio y julio.

PALABRAS CLAVE: Ácido cítrico – Ácido láctico – Alcaparras – Capparis spinosa *– Fermentos lácticos – Salmuera.*

SUMMARY

Improvement of the lactic acid fermentation of capers through an experimental factorial design (*Capparis spinosa* L).

The study of the caper fermentation process through an experiment factorial plan allows us to determine a function f such that (Y= $f(X_1, X_2, \ldots, X_n)$) existing between magnitude Y which is the decrease of pH (called response), and variables X_i , which are brine, lactic acid, citric acid and lactic ferment (called factors). A complete factorial plan 2^4 was made in order to determine the factors and the interactions among the factors which have a statistically significant influence on the studied response. Brine, lactic acid and citric acid have a significant effect on the fall of pH; by contrast, lactic ferment does not have a significant effect. On the other hand, the interactions between brine and lactic acid, between brine and lactic ferment , between lactic acid with citric acid and between lactic acid with lactic ferment have significant effects on the fall of pH ($p < 0.0001$). The fermentation was done in the research laboratory of the society Marocapres-Fez, international leader in the processing of capers, at a temperature of about 30°C, between June and July.

KEY-WORDS: Brine – Capers – Capparis spinosa *– Citric acid – Lactic acid – Lactic ferment*

1. INTRODUCTION

The caper is a shrub which is well known in the countries of the Mediterranean Basin. It belongs to the family of Capparidaces and the genus Capparis which contains more than 350 species used for different purposes (food, traditional medicine, ornamentation, cosmetics ...). The flower bud is called “capers” while the fruit of this shrub is called “Caperberries”. The picking of capers and caperberries is manual and done between May and August. Morocco, Spain, Italy and Turkey are the main worldwide producers (Giuffrid *et al.*, 2002).

Concerning their use in the kitchen, the caper and caperberries are mainly used (Akgül, 1996).

The habitual treatment of capers consists of submerging the capers freshly harvested in a solution of salt (24% by weight) for about 40 days (Giuffrid *et al.*, 2002) until fermentation takes place and the bitter taste resulting from glycoside, called glucocapparin, disappears (Arslan *et al.*, 2007).

The direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product (Oberman and Libudzisz, 1998; Leroy and De Vuyst, 2004). Lactobacillus plantarum has been successfully used as a starter culture in several food fermentations (McDonald *et al.*, 1993; Aukrust *et al.,* 1994; Giraud *et al*., 1994; Gardner *et al*., 2001; Leal-Sánchez *et al.*, 2003; Mugula *et al*., 2003; Tolonen *et al*., 2004). L. plantarum has been noted for its acid production and tolerance to high salt concentrations (Fleming and Mc Feters, 1981) and for its superior ability to utilize the substrates (Oyewole and Odunfa, 1990), including dextrins after the depletion of fermentable sugars (Akinrele, 1970) and raw starch (Giraud *et al*., 1994; Mugula *et al*., 2003). Also, Lactobacillus acidophilus and L. plantarum have the ability to produce a greater amount of lactic acid than Lactobacillus casei and Lactobacillus delbrueckii (Yoon *et al*., 2005). For this reason, L. plantarum is used in this assay.

Recently, capers have started to gain increasing economic importance in the world market and demands are continuously increasing (Arslan *et al*., 2007). This pushed the caper processing industry to improve their fermentation conditions. Thus, this research has been designed to integrate this perspective of improvement and it aims at determining, for the first time, the best values of factors and the best combinations between the factors susceptible to reach a decrease in pH in order to inhibit the growth of undesirable microorganisms and consequently to obtain a good fermentation.

2. MATERIALS AND METHODS

2.1. Materials

The capers used in this study were collected in the early morning from wild growing plants in the fez region in June 2008 and transported in cool bags to the laboratory. The buds were separated from foreign materials such as stalk, leaves and dust before being processed. A commercial lyophilized starter culture (L. plantarum) was used in the assay,(Chr-Hansen Vege-Start 10, Horsholm, Denmark). Clear water and small-grain salt were used in the preparation of brine. Lactic and citric acids (S D Fine – Chem limited– Numbai-India) were commercial products.

2.2. Experimental Design

Many factors can significantly influence the fermentation of capers. The bibliographical data have urged us to study four factors: brine, lactic acid, citric acid and lactic ferment. This study was realized according to a plan of factorial experience wherein the calculation of the coefficients of the polynomial model has been accomplished through the method of "least squares" with the use of coded variables. In fact, the act of replacing the natural variables by coded variables allows for the same domain of variation for each factor (between –1 and +1) and hence being able to compare the effect of factors among themselves. The lowest level is coded –1 while the highest level is coded +1 (Table 1).

A complete factorial plan with 4 factors and N (number of tries) has been designed which will be equal to 16 = (24), in this study we have conducted 3 replicates for each trial.

A summary of the the total tests can be seen in Table 2, which we have called "Table of Experiments".

Table 1
Levels of variables (factors)

FACTORS	LEVELS -1	LEVELS +1
Brine	10%	20%
Lactic acid	0.1%	1%
Citric acid	0.1%	1%
Lactic ferment	0.5%	2,5%

2.3. Brining procedure of capers

Caper buds of medium size were put in 1-L sterilized glass jars and brined. Sixteen brine (called cover juice) types were used for the fermentation of caper buds (see table 2). After adding the cover juice (brine added to lactic acid, citric acid and lactic ferment), the jars were hermitically closed and left to ferment at the room temperature of the laboratory (about 30 ° C) for 6 weeks. 3 repetitions were carried out for each trial.

A sample was taken every 2 weeks to measure the chemical and microbiological parameters of the samples.

2.4. Chemical analysis of the cover juice

A measurement of the pH of the juice was taken every 2 weeks of fermentation time (T0, T1, T2, and T3), using a pH meter (Hanna HI 831) regularly calibrated with buffer solutions as pH 4.0 and 7.0. The average pH values measured are represented in table 3 (column response Y).

2.5. Statistical Analysis

The statistical calculations (calculation of coefficients, t test, analysis of variance, curve ...) were done using the JMP software.

3. RESULTS AND DISCUSSION

On the basis of table 3, the mathematical model is written as follows and has the form:

(1) $Y = a_0 + a_1 X_1 + a_2 X_2 + a_3 X_3 + a_4 X_4 + a_{12} X_1 X_2 + a_{13} X_1 X_3 + a_{14} X_1 X_4 + a_{23} X_2 X_3 + a_{24} X_2 X_4 + a_{34} + X_3 X_4$

To be able to conduct the statistical calculations and prevent that n = p, (n = number of tests and p the number of estimated parameters starting from the model, in other words, the number of the model's coefficients), it is necessary to neglect the high interactions of order (3 or more). This is the case in this study.

Or:

Y: is the measured response during the experiment.

a_0, a_1 .... a_4, a_{12} ... ... a_{34}: are the mathematical coefficients of the model (the factors' average effects and the factors' interactions).

Table 2
Table of experiments

N° test	Brine	Lactic acid	Citric acid	Lactic ferment
1	10%	0.1%	0.1%	0.5%
2	20%	0.1%	0.1%	0.5%
3	10%	1%	0.1%	0.5%
4	20%	1%	0.1%	0.5%
5	10%	0.1%	1%	0.5%
6	20%	0.1%	1%	0.5%
7	10%	1%	1%	0.5%
8	20%	1%	1%	0.5%
9	10%	0.1%	0.1%	2.5%
10	20%	0.1%	0.1%	2.5%
11	10%	1%	0.1%	2.5%
12	20%	1%	0.1%	2.5%
13	10%	0.1%	1%	2.5%
14	20%	0.1%	1%	2.5%
15	10%	1%	1%	2.5%
16	20%	1%	1%	2.5%

$a_{ij}.X_i.X_j$ correspond to the interactions.
n: the number of realized experiments
p: the number of estimated parameters from the model

After the point estimate of effects (Fig.1), the model is written as:

(2) $Y = 1.894 + 0.260\,X_1 + 0.127\,X_2 + 0.047\,X_3 + 0.025\,X_4 - 0.107\,X_1\,X_2 - 0.006\,X_1\,X_3 - 0.063\,X_1\,X_4 + 0.140\,X_2\,X_3 + 0.111\,X_2\,X_4 - 0.007\,X_3\,X_4$

3.1. The significance of effects

According to the test of significance of effects (test "t") of the study (Fig.1), we noticed that at $p<0.0001$, brine, lactic acid and citric acid have a significant effect on the decrease in pH, on the contrary to the lactic ferment, which does not have any significant effect.

We also noted that the interactions between brine and lactic acid, between brine and ferment lactic, between lactic acid and citric acid and between lactic acid and lactic ferment have significant effects on the decrease in pH, unlike the interactions between brine with citric acid and citric acid with lactic ferment which do not have a significant effect ($p <0.0001$).

The mathematical model is written as

(3) $Y = 1.894 + 0.260\,X_1 + 0.127\,X_2 + 0.047\,X_3 - 0.107\,X_1\,X_2 - 0.063\,X_1\,X_4 + 0.140\,X_2\,X_3 + 0.111\,X_2\,X_4$

Since Y represents the decrease in pH, and since the aim is to increase its value, it is the brine which has the most important effect, followed by lactic acid, while citric acid has relatively little effect.

As to the effect of the interactions, it is the interaction between lactic acid and citric acid that was the most important effect, followed by the effect of the interaction between lactic acid and lactic ferment, and then, the effect of the interaction between brine and lactic acid; the interaction between brine and lactic ferment has the least effect.

Interpretation of principal effects:

Total salt effect: when the content of salt in the juice varies from 10% to 20%, the value of Y increases by 2 x 0.260 = 0.520. To increase Y (in other words, to improve the decrease in pH), it is therefore necessary to take a brine of 20%.

Lactic acid total effect: when the content of lactic acid passes from 0.1% to 1%, the value of Y increases by 2 x 0.127 = 0.254. So to increase Y, it is therefore necessary to use 1% of lactic acid.

Interpretation of the interaction:

Brine with lactic acid: when the content of the two factors varies respectively from 10% to 20% and from 0.1% to 1%, the value of Y decrease by 2 x 0.047 = 0.094. To increase Y, both factors in the interaction have to be at their lowest levels.

We indeed noticed that the interaction between lactic acid and citric acid, and between lactic acid lactic ferment do not result in a stronger decrease in pH unless the factors in the interaction are at their

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Table 3
Matrix of experiments

No test	Average X0	Brine X1	Lactic acid X2	Citric acid X3	Lactic ferment X4	Response Y decrease in the pH
1	+1	–1	–1	–1	–1	1.89
2	+1	+1	–1	–1	–1	2.25
3	+1	–1	+1	–1	–1	0.94
4	+1	+1	+1	–1	–1	2.17
5	+1	–1	–1	+1	–1	1.14
6	+1	+1	–1	+1	–1	2.13
7	+1	–1	+1	+1	–1	2.21
8	+1	+1	+1	+1	–1	2.22
9	+1	–1	–1	–1	+1	1.35
10	+1	+1	–1	–1	+1	1.95
11	+1	–1	+1	–1	+1	2.14
12	+1	+1	+1	–1	+1	2.08
13	+1	–1	–1	+1	+1	1.22
14	+1	+1	–1	+1	+1	2.21
15	+1	–1	+1	+1	+1	2.18
16	+1	+1	+1	+1	+1	2.23
Effect	a0	a1	a2	a3	a4	
Low level		10%	0.1%	0.1%	0.5%	
High level		20%	1%	1%	2.5%	

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	10	6,4471667	0,644717	6,9898
Error	37	3,4127583	0,092237	Prob > F
C. Total	47	9,8599250		<.0001

Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1,89375	0,043836	43,20	<.0001
Lactic ferment	0,0245833	0,043836	0,56	0,5783
Citric acid	0,0475	0,043836	1,08	0,2856
Lactic acid	0,1266667	0,043836	2,89	0,0064
Brine	0,2604167	0,043836	5,94	<.0001
Lactic ferment*Citric acid	–0,006667	0,043836	–0,15	0,8799
Lactic ferment*Lactic acid	0,1108333	0,043836	2,53	0,0159
Citric acid*Lactic acid	0,1404167	0,043836	3,20	0,0028
Lactic ferment*Brine	–0,062917	0,043836	–1,44	0,1596
Citric acid*Brine	–0,005833	0,043836	–0,13	0,8949
Lactic acid*Brine	–0,1075	0,043836	–2,45	0,0190

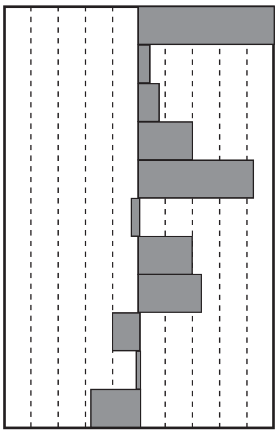

Figure 1.
Statistical calculations of the mathematical model of pH decrease for caper fermentation.

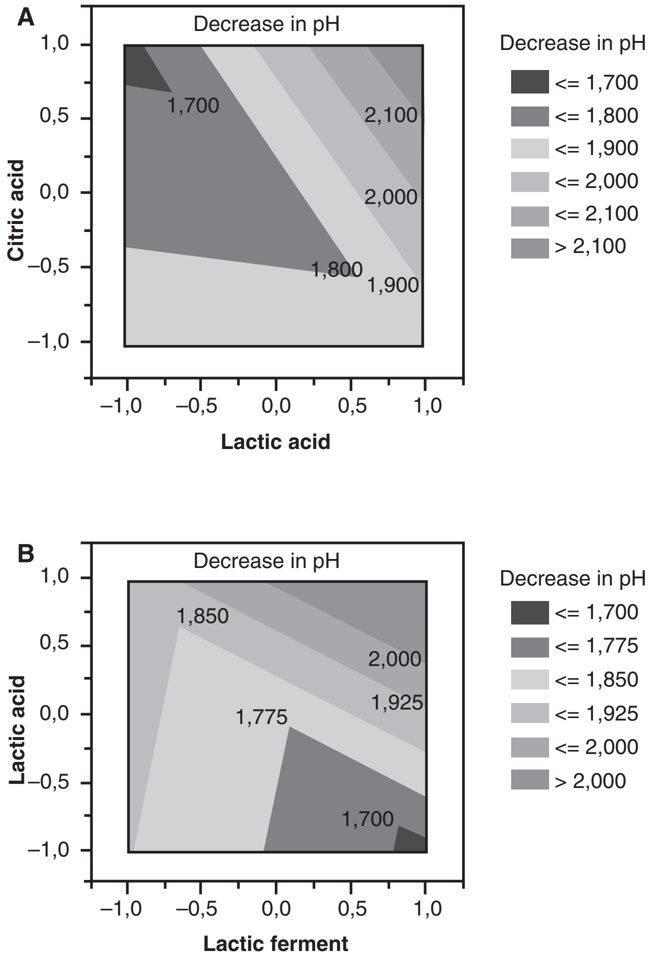

Figure 2.
Response surface. (A) Interaction of lactic acid with citric acid; (B) lactic acid with lactic ferment.

highest height levels (+1), that is to say, lactic acid with the value of 1%, citric at 1%, as well, and lactic ferment with the value of 2.5%.(Fig.2-A et Fig.2-B).

3.2. Analysis of variance

The aim is to compare the sum of differences squares due solely to regression (therefore to the model) with the sum of squares of the residues with the help of the F test. We noticed that F (observed) > F (0.0001) (Fig.1) and therefore we accept the hypothesis of the validity of the model (equation 3).

(3) $Y= 1.894 + 0.260 X_1 + 0.127 X_2 + 0.047 X_3 - 0.107 X_1 X_2 - 0.063 X_1 X_4 + 0.140 X_2 X_3 + 0.111 X_2 X_4$

4. CONCLUSIONS

By way of conclusion, we can say that the largest decrease in pH is produced when the factors are at their highest levels (+1) of the domain of their variations.

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