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Improvement organoleptic qualities of fermented caper through an experimental factorial design *Capparis spinosa* L

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Abstract: The objective of this study is to describe the physico-chemical, microbiological and organoleptic qualities of Moroccan capers and explore possibilities for improving organoleptic properties of capers that have undergone fermentation in brine of various salt chemical composition, lactic acid, citric acid and lactic ferment (lactic acid bacteria). The fermentation tests were performed using a factorial experimental design 24 and the sensory evaluations were performed using a 9-point hedonic scale where responses range from 1 for "dislike extremely" to 9 for "like extremely", the median being expressed by the statement 5 "Neither Like nor Dislike" (Peryam and Girardot, 1952).

The results concerning taste, texture and color have shown that there's one significant difference in taste and color between the different samples of capers ($P < 0.05$) and the much appreciated best samples, acording to tasters are: 16 (20% salt, 1% lactic acid, 1% citric acid and 2.5% of lactic acid bacteria) and 15 (10% salt, 1% lactic acid, 1% citric acid and 2.5% lactic acid bacteria). By contrast, there is no significant difference ($P < 0.05$) between the texture of different samples that is to say the texture of capers was not influenced by the chemical composition of the brine used. Meaning the score in the different samples is around 8.

Data on the overall acceptability of samples were processed by factorial design of experiments and confirmed in turn that preference scores are the highest achieved when salt, lactic acid, citric acid and lactic acid bacteria are at their top.

Key words: Capers – *Capparis spinosa* – Factorial design – Hedonic scale – Sensory evaluation

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Introduction

The Caper « *Capparis spinosa* » is a shrub which is well known in the countries of the Mediterranean Basin. It belongs to the family of *Capparidacées* and the genus *Capparis* which contains more than 350 species.

The flower buds, the fruits and occasionally the shoots of *Capparis spinosa* L, have been used as a condiment in the Mediterranean region (countries surrounding the Mediterranean Sea) and neighboring countries since ancient times; those products have been greatly appreciated for their pungent and bitter flavor. Generally the pickled flower buds are the most appreciated product; those buds, when still tightly closed are harvested and then brined and packed in vinegar (capers). These buds are selected by size, the smaller ones being the most greatly appreciated on the market. Morocco, Turkey, Spain and Italy are the leading world producers.

To improve the conditions of fermentation and improve the sensory profile of capers, the addition of organic acid would be of great interest (Arsalan et al., 2007, Douieb et al., 2010). The competitive environment and consumer satisfaction more and more demanding requiring the food industry to innovate in developing new products and improving existing products and in innovation it is important to consider many aspects of quality (psychology, technology, legal, etc.) (Moskowitz et al., 2005). There are also other factors like flavor, texture, and appearance which play a prominent role in the acceptability of foods and must therefore be assessed (Coleman, 1990; and Michelle Lawless, 1993; Pecore and Kellen, 2002, González et al., 2007). In this regard, it is indisputable that the consumer's opinion is critical (Moldão-Martins et al., 2004, Munoz et al., 1992; Yanti, 1992). Consequently, food companies pay great attention to the evaluation of the sensory attributes of foodstuffs by trained experts to predict consumer reactions, which is essential in the development, optimization and improvement of product quality (Coello et al., 1999; Abu

Salem and Abu-Arab, 2008). Thus, the goal of this study is to determine the effect of the composition of the brine of salt, organic acids (lactic and citric acids) and lactic acid bacteria on the organoleptic properties of fermented capers.

Materials and Methods

Materials. Capers used in this study were collected in the early morning from wild growing plants in the fez region, Morocco in June 2008 and transported in cool bags into the laboratory. The buds were divided into two lots A and B.

The vinegar used in section A of this work is from Spain (14%) and diluted in laboratory to get a (3.5%) vinegar.

In section B of this work, A commercial lyophilized starter culture (*L. plantarum*) was used in the assay, (Chr-Hansen Vege-Start 10, Horsholm, Denmark). Clear water and small-grained salt were used in the preparation of brine. Lactic and citric acids (S D Fine – Chem limited- Numbai-India) were commercial products.

Local. The sensory evaluations were conducted in a room (equipped with individual cabins) offering a pleasant atmosphere, neutral color, well lit, soundproof and protected against external odors (American Society for Testing and Materials, 1986), including, the odors from the sample preparation room which is located next door, so that the waiting time between preparation and sensory evaluation should be as short as possible (French Association for Standardization AFNOR, 1988).

Experimental design.

Protocol A: Characterization of the qualities of Moroccan capers

Capers were treated in the usual processing (sizing, sorting, brining, fermentation and washing with water, conditioning in vinegar). After calibration, the capers were divided into four parts according to their diameter: non pareilles (6/7mm), Surfines (7 / 8 mm), capucines (8 / 9 mm) and fines (11/12 mm) had undergone fermentation in a brine of 20% (NaCl). After fermentation, the capers were drained from the brine they contained and soaked in water for 4 hours and then marinated with a solution of acetic vinegar to 3.5 %. After three days of osmosis, we performed a first-time measurements of pH, acidity and salt content in the juice and one count of microorganisms. In a second step, we proceeded to other determinations physicochemical and organoleptic characterization.

For each caliber, three repetitions were performed and the values given below are average values.

Microbiological determinations :

The counting of the total plate count was performed according to French standard NF V08-051, yeast and molds were determined according to standard NF V08-059, and sulfite-reducers were counted as NF V08-061, coliforms have been determined according to standard NF V08-060. The salmonella was determined using the method described in standard NF V08-052.

Physico-chemical determinations:

- PH, acidity and salt content were measured and determined by official method (AOAC, 1984)
- Nutritional general values: moisture, ash, proteins were determined by official method (AOAC, 1984) while the carbohydrate content and energy values were determined by calculation.
- Determination of lipids was performed by continuous extraction using a Soxhlet apparatus (AOAC, 1984).
- Determination of total fiber was performed by gravimetric method (AOAC 985-29)
- Determination of sodium was carried out by atomic absorption spectrometry
- Fructose, glucose, sucrose, maltose, lactose, total sugar mono-and disaccharides have been determined by the AOAC method enzymatic 980-13 and 982-14
- Saturated fatty acids, monounsaturated and polyunsaturated fats have been determined on ISO 5509 and ISO 5508

Protocol B : Study to improve the organoleptic properties of capers

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

The fermentation tests were realized according to a factorial design of experiment where each factor varies between a low level (-1) and a high level (1) (Table 1). . The calculation of coefficients of the polynomial model has been done by the method of "least squares" with the use of coded variables (-1 and +1).

It's about a complete factorial plan with 4 factors and N (number of tries) which will be equal $16 = (2^4)$, in this study we have conducted 3 replications for each try.

To recapitulate the total tests, we have used the following (table 2) named table of experiments.

Preparation and presentation of samples. Once the phase of fermentation was complete, the fermented caper buds were subjected to sensory evaluation and presented in full to experts in a random order and in identical clear glass plates at an ambient temperature of 20 ° C. The plates were coded with 3 digits random numbers, these codes were printed on labels by computer.

Sensory evaluation. The sensory evaluations of different samples of capers were performed by a panel of 20 experts of the corporation (12F, 8M), highly trained, good

connoisseurs of capers and well trained in sensory analysis for 15 sessions of 1 hour 30 each according to the recommendations outlined by (Meilgaard et al., 1991; Grosso and Resurreccion, 2002; Nepote et al., 2006a). The panelists were selected according to the following criteria: a) people with natural dentition, b) people without food allergies, c) non-smokers, d) people between the ages of 24-55, e) people who consume regular capers, f) people available for all sessions, g) people interested in participating, and h) people able to verbally communicate their observations regarding the product (Plemmons and Resurreccion, 1998).

Table 1. Levels of variables (factors)

FACTORS	LEVELS	
	-1	+1
Brine	10%	20%
Lactic acid	0.1%	1%
Citric acid	0.1%	1%
Lactic acid bacteria	0.5%	2.5%

Table 2.Table of experiments

N° test	Brine	Lactic acid	Citric acid	Lactic acid bacteria
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%

The sensory evaluations were conducted towards the end of the morning between 10 am and 11.30 am (Amerine et al., 1965) in 2 sessions to avoid fatigue and mental sensory of experts (Meilgaard et al., 1987a). Before the conduct of sessions of sensory evaluation, the experts were informed about the rules and have been requested to respect

these rules: a) abstain from smoking 1 hour before and during sessions, b) do not consume drinks of persistent taste, c) do not eat spicy food, d) do not suck sweets or chew gum, and e) refrain from using cosmetics or perfumes.

28 grams of capers were taken from each test (the 3 repetitions of each trial were subjected to sensory evaluation) (E-18 committee of the American Society for Testing and Materials, 1968) and placed by plastic spatulas on plates and served with water at room temperature on a clear plastic tray, the panelists were invited to look, feel and taste the capers without being able to consume and to rinse the mouth (after spat out) with the water after each tasting. Participants expressed judgments on acceptance samples using a 9-point hedonic scale with scores ranging from 1 = dislike extremely to 9 = like extremely (Peryam and Pilgrim, 1957), data were recorded on a bulletin paper. The sensory attributes evaluated were taste, texture, color and overall acceptability.

Statistical analysis. Data collected for the taste, texture and color were subjected to variance analysis ($P<0.05$) and multiple comparison test of Tukey ($P<0.05$) using (SPSS 16.0). While the data on the overall acceptability were subjected to a statistical analysis using a computerized analysis of variance and t-test ($P<0.0001$) (JMP 5.0.1). All values are the means of three replicate analyses.

Results and discussion

1) Physico-chemical, microbiological and organoleptic qualities of Moroccan capers:

Microbiological properties (see table 3)

Organoleptic Properties:
Color : green color
Flavor : characteristic (good taste)
Odor : characteristic (pleasant odor)
Texture : firm

physico-chimical and nutritional properties (see table 4)

2)Taste-Texture-Color :

Taste. Regarding the taste of capers (ANOVA test), we find that there is a significant difference in taste between the 16 samples treated differently (sig.0.000< 0.05) and post test Tukey (Table 5), tasters have especially appreciated the sample 16 (with 20% brine of salt, 1% of lactic acid, 1% of citric acid and 2.5% of lactic acid bacteria) with a score of 8.25 which corresponds to "Like Very Much", followed by sample 15 (with 10% brine of salt, 1% of lactic acid, 1% of citric acid and 2.5% of lactic acid bacteria) with the score 7.40 which corresponds to"Like Moderately". These findings are in agreement with (Douieb et al., 2010) who reported that the use of organic acids in large quantities (1% lactic and 1% citric acids and 2.5% for lactic acid bacteria) leads to a good capers fermentation.

3)

Table 3. Microbiological properties

	Non pareilles	Surfines	Capucines	Fines
Total plate count	0	0	0	0
Yeast	0	0	0	0
Mold	0	0	0	0
Sulfite-reducing	0	0	0	0
Coliform	0	0	0	0
Fecal Coliform	0	0	0	0
Salmonella	0	0	0	0

Counting CFU/g

From (ANOVA test), we conclude that there is no significant difference between the 20 tasters (sig. 0.373> 0.05). This mxeans that the tasters have used the same scale and have given a score coherent with the different samples.

Texture. Although the tasters used the same scale (ANOVA test), (Sig. 0.102 > 0.05), there is no significant difference in texture between the different samples (Sig. 0.749 > 0.05). The type of brine used has no effect on the texture of this caper which is confirmed with a score around 8 corresponding to "Like Very Much" in all samples.

Color. Regarding the color of capers, we notice that there is a significant difference between the 16 samples of capers (ANOVA test) (Sig.0.000 < 0.05) and Tukey's post test (Table 5), the same tendency was also observed by tasters vis-à-vis the two samples numbers: 16 (20% brine with salt, 1% of lactic acid, 1% of citric acid and 2.5% of lactic acid bacteria) and 15 (brine with 10% salt, 1% of lactic acid, 1% of citric acid and 2.5% of lactic acid bacteria) that were most appreciated with scores of 7.70 and 6.75 which correspond to "Like very much" (Note 8) and "like moderately"(note 7) on the hedonic scale, while samples 4 and 8 were the least acceptable and did not differ (score 3.80 for both). These findings are in agreement with (Ö zcan, 1999) and (Douieb et al., 2010) who reported that the use of organic acids (1% lactic and citric acids and 2.5% for the lactic acid bacteria) in large amount leads to a good capers fermentation.

Table 4. physico-chimical and nutritional properties

	Non pareilles (6/7mm)	Surfines (7/8 mm)	Capucines (8/9 mm)	Fines (11/12 mm)
Chlorides on juice (%)	6.37	6.30	6.30	6.41
Acidity on juice (%)	1.27	1.30	1.31	1.42
pH	3.30	3.25	3.26	3.23
Humidity (%)	87.20	88.50	85.50	89.10
Total ash (%)	5.60	5.80	8.70	6.30
Protein (%)	1.80	1.50	1.50	1.50
Fat (%)	0.20	0.20	0.20	0.20
Carbohydrates (%)	1.80	0.60	1.30	0.80
Total fiber (%)	3.40	3.40	2.80	3.10
Energy (KJ/100g)	69	43	54	47
Energy (Kcal/100g)	16	10	13	11
Sodium (mg/100g)	2220	2130	3400	2090
Fructose (%)	< 0.3	< 0.3	< 0.3	< 0.3
Glucose (%)	< 0.3	< 0.3	< 0.3	< 0.3
Saccharose (%)	< 0.3	< 0.3	< 0.3	< 0.3
Maltose (%)	< 0.3	< 0.3	< 0.3	< 0.3
Lactose (%)	< 0.3	< 0.3	< 0.3	< 0.3
Total sugar mono and disaccharides (%)	Trace	Trace	Trace	Trace
Fatty acids Saturates (%)	0.07	0.12	0.08	0.12
Fatty acids monounsaturates (%)	0.03	0.04	0.04	0.04
Fatty acids polyunsaturates (%)	0.09	0.05	0.08	0.05

Table 5. Sensory properties (Mean ± SD) des câpres ayant fermenté dans différentes saumures

Sensory properties	Treatments (test)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Taste	4.20^{cdg} ± 1.45	4.90^{cdfg} ± 2.34	3.90^{cd} ± 2.10	3.80^{cd} ± 2.14	5.35^{cdefg} ± 2.32	5.65^{cdefg} ± 2.08	4.75^{cdfg} ± 2.12	3.80^{cd} ± 2.91	4.00^{cdg} ± 1.80	4.50^{cdfg} ± 1.88	4.85^{cdfg} ± 1.81	4.75^{cdfg} ± 2.36	6.15^{bg} ± 1.93	6.50^{bf} ± 2.04	7.40^{be} ± 1.70	8.25^{ab} ± 1.12
Texture	8.55^{a} ± 0.87	8.40^{a} ± 0.75	8.20^{a} ± 0.70	8.55^{a} ± 0.51	8.40^{a} ± 0.68	8.15^{a} ± 0.99	8.35^{a} ± 0.67	8.35^{a} ± 0.67	8.15^{a} ± 0.75	8.20^{a} ± 0.83	8.30^{a} ± 0.66	8.35^{a} ± 0.75	8.50^{a} ± 0.61	8.55^{a} ± 0.61	8.40^{a} ± 0.75	8.45^{a} ± 0.76
Color	4.20^{cdfg} ± 1.44	4.90^{cdefg} ± 2.34	3.90^{cdg} ± 2.10	3.80^{cd} ± 2.14	5.35^{cdefg} ± 2.32	5.65^{bcfg} ± 2.08	4.75^{cdefg} ± 2.12	3.80^{cd} ± 2.91	4.00^{cdg} ± 1.81	4.70^{cdefg} ± 2.13	4.85^{cdefg} ± 1.81	4.75^{cdefg} ± 2.36	6.10^{bg} ± 1.71	6.30^{bf} ± 1.81	6.75^{be} ± 1.62	7.70^{ab} ± 1.53

Within each row, means superscript with different letters are significantly different ($P<0.05$).Values are means of three replicates.

Table 6. Matrix of experiments

No test	Average X0	Brine X1	Lactic acid X2	Citric acid X3	Lactic ferment X4	Response Y Overall acceptability
1	+1	-1	-1	-1	-1	5.65
2	+1	+1	-1	-1	-1	6.10
3	+1	-1	+1	-1	-1	5.35
4	+1	+1	+1	-1	-1	5.40
5	+1	-1	-1	+1	-1	6.40
6	+1	+1	-1	+1	-1	6.50
7	+1	-1	+1	+1	-1	6.45
8	+1	+1	+1	+1	-1	5.85
9	+1	-1	-1	-1	+1	5.40
10	+1	+1	-1	-1	+1	5.80
11	+1	-1	+1	-1	+1	6.00
12	+1	+1	+1	-1	+1	5.95
13	+1	-1	-1	+1	+1	6.95
14	+1	+1	-1	+1	+1	6.15
15	+1	-1	+1	+1	+1	8.30
16	+1	+1	+1	+1	+1	8.75
Effect	a0	a1	a2	a3	a4	
Low level		10%	0.1%	0.1%	0.5%	
High level		20%	1%	1%	2.5%	

3) Overall acceptability :

On the basis of table 6, the mathematical model is written as follows has the form:

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

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's of use 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 (factors average effects and factors interactions).

$a_{ij}.X_i.X_j$ correspond to interactions.

n: number of realized experiments

p: the number of estimated parameters from model

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

(2) $\mathbf{Y= 6.313 - 0.001 X_1 + 0.194 X_2 + 0.605 X_3 + 0.349 X_4 -0.019-X_1X_2 - 0.108 X_1 X_3 + 0.002 X_1X_4 + 0.225 X_2 X_3+ 0.393 X_2 X_4 +0.266 X_3 X_4}$

According to the test of significance of effects (t-test) of student (Fig. 1), we have noticed that at risk ($p<0.0001$), lactic acid, citric acid and lactic acid bacteria (lactic ferment) have a significant effect on the acceptability by the taster; by contrast, the effect of brine on the acceptability of the product is not significant.

We have also noticed that interactions between the brine with citric acid, lactic acid with citric acid, lactic acid with lactic ferment and citric acid with lactic ferment have a significant impact on the acceptability Product unlike interactions between the brine with lactic acid and brine with lactic ferment which have no significant effect ($P<0.0001$).

The model chosen will then form:

(3) $\mathbf{Y= 6.313 + 0.194 X_2 + 0. 605 X_3 + 0.349 X_4 - 0.108 X_1 X_3 + 0.225 X_2 X_3+ 0.393 X_2 X_4+ 0.266 X_3 X_4}$

So Y will vary from a maximum of 8.24 "like very much" when the factors are all at high level (+1: replace X with the value +1) and a minimum of 4.39 "Dislike slightly" when all factors are at low level (-1: replace X with the value -1).

Since Y is the overall acceptability of the product and since we seek to increase its value (in other words: increase the acceptability of the product by the tasters). Factors in variation should therefore be at their high levels (+1) that is to say (20% salt, 1% lactic acid, 1% citric acid and lactic ferment 2.5%). This is in perfect agreement with the results

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	10	39,101888	3,91019	19,2537
Error	37	7,514260	0,20309	Prob > F
C. Total	47	46,616148		<.0001

Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	6,3127083	0,065046	97,05	<.0001
Brine	-0,001042	0,065046	-0,02	0,9873
Lactic acid	0,1939583	0,065046	2,98	0,0050
Citric acid	0,6052083	0,065046	9,30	<.0001
Lactic ferment	0,3497917	0,065046	5,38	<.0001
Brine *Lactic acid	-0,018958	0,065046	-0,29	0,7723
Brine *Citric acid	-0,107708	0,065046	-1,66	0,1062
Brine *Lactic ferment	0,001875	0,065046	0,03	0,9772
Lactic acid *Citric acid	0,2247917	0,065046	3,46	0,0014
Lactic acid *Lactic ferment	0,3935417	0,065046	6,05	<.0001
Citric acid*Lactic ferment	0,2664583	0,065046	4,10	0,0002

Fig.1. Statistical calculations of the mathematical model of overall acceptability for capers

of sensory evaluation described in Section 2 (Taste-Texture-Color).

Analysis of variance. It's to compare with the help of F test, the sum of differences squares due solely to regression (therefore to the model) with the sum of square of the residues. We notice effectively that F (observed) > F (0.0001) (Fig.1), therefore we accept the hypothesis of the validity of the model (equation 3).

(3) $Y = 6.313 + 0.194\ X_2 + 0.605\ X_3 + 0.349\ X_4 - 0.108\ X_1 X_3 + 0.225\ X_2 X_3 + 0.393\ X_2 X_4 + 0.266\ X_3 X_4$

Conclusions

We can reveal that the sensory evaluations and the factorial design of experiment show that the highest scores for acceptance and preferences of capers are obtained when the factors are in their high levels (+1) at the domain of their variation (20 % salt, 1% for lactic acid, 1% as for citric acid and 2.5% for lactic ferment).

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