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Conception and Development of a New Product: Canned Sardines with Lemon or with Ascorbic Acid

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Abstract: The aim of this research is to produce two new products of canned sardine: canned with lemon or with ascorbic acid. The microbiological quality analysis show the absence of spores of bacillus and *Clostridium thermophilics* forms in the tested boxes. The percentage of medium recovery and of water in oil is in accordance with the standards. Besides, both products meet the various criteria of food safety and the standards required. The Friedman test sensory analysis revealed that the two products are not significantly different. Canned sardine with ascorbic acid is appreciated for criterion taste while canned sardine with lemon is appreciated for preference criterion.

Key words: Canned sardines, lemon, ascorbic acid, Friedman, criteria, safety

INTRODUCTION

As a source of food, fisheries and aquaculture play an important socioeconomic role. Particularly, bluefish including sardines, anchovies, tuna and mackerel is the most popular fish in Tunisia. The processing of this species knew a significant growth during the last few years due to the better availability of raw material and the development of the exportation. Appreciated for its taste but- also thanks to the know-how of the professionals, the Tunisian sardine is continuously increasing worldwide. The transformation of this species has evolved during the last years, appreciated for its taste and thanks to a better availability of the raw material as well as to the conquest of new markets in Europe in Asia in particular in Japan by way of several countries of Africa. For several years already started actions of valorization of the sardine by the diversification of the proposed range. New sardine products are available on the market, combining the taste of this fish with the aromatic qualities of natural plants or specific preparations. Motivated by the excellent quality of the Tunisian raw material and attentive to new trends and market requirements.

In recent years, there has been an increasing interest in using food additives from natural sources to improve taste and also extend the shelf-life of semi-preserved foodstuffs (Maktabi *et al*., 2016). Marination, a food-preservation technique is based on treatment of muscle with solutions containing salt, spices, lemon juice, etc. and provides high sensory acceptability to a variety of meat products (Ozogul *et al*., 2004). Marination is also used to tenderize or to change taste, textural and structural properties of meat (Aksu *et al*., 1997). Several studies regarding seafood marinating by tomato sauce (Kilinc and Cakli, 2005), rosemary extract (Cadun *et al*., 2008) acetic acid with NaCl with harissa sauce and red pepper (Boubaker *et al*., 2014) have been documented. Sardines are very suitable for marination because of their fat contents (Kilinc, 2003). Sardines are fatty fish that contain high levels of ω-3 polyunsaturated fatty acids and their consumption has been considered very beneficial to human health (Lopez-de-Dicastillo *et al*., 2012). *Sardinella aurita* is one of the most widespread fish in Tunisia and is an important source of low-cost dietary protein.

The purpose of this study is to investigate the effects of the addition of two ingredients (ascorbic acid and lemon) on the organoleptic quality of canned *Sardinella aurita*. Thus, the main objective of the present research was to improve the safety and the sensory quality of canned sardine obtained by the addition of lemon slices or ascorbic acid in order to propose new products.

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MATERIALS AND METHODS

***Sardinelle aurita*:** Canned sardine products are prepared from fresh *Sardinelle aurita*. The fish has been obtained from the regional fishing centre. at reception, the raw materials must be checked and accompanied by a safety certificate form (NT54.02 in 1986). The control of the fishery products was carried out by the company's veterinarian in accordance with the OJTR of 19/09/1998 (Boubaker *et al*., 2014).

Lemon fruit: Lemon has several varieties of which the best known are: Verno, EureKa, Femminelle, Intedonoto (Clement, 1981). Eureka is the most common lemon variety in the world and be found all the year round. Eureka variety was used because has juicy acidic and fragrant flesh. The thickness of the bark is medium to thin and the fruit usually being seedless. Thus, the high quality of lemon fruit are selected and manually washed with the potable water which is in agreements with the provisions of the fish inspection regulations for water used in registered establishments and manually cut to a size of 2.5×0.3 cm. Two pieces were added in the first six boxes and three pieces were added in the last six boxes.

Ascorbic acid: Ascorbic acid with high purity from fluka (70152; Steinheim, Switzerland) as tested at two concentrations; 1 and 0.75%. The additive is added directly to the virgin olive oil (300 mL) using a 5 mL graduated pipette). Olive oil is in agreement with the Ref. CAC/RS 33-1970. Six canned sardines were prepared for each concentration used. Ascorbic acid is a strong antioxidant and has a direct synergistic relationship with other antioxidants (Aubourg *et al*., 2004). The use of ascorbic acid as an antioxidant was reported by Aubourg *et al*. (2004) and Rostamzad *et al*. (2011). Ascorbic acid can be used as an antioxidant agent to increase shelf life of processed food and canned. Thus, the use of ascorbic acid is justified.

Physico-chemical analysis

pH: pH was measured according to the method of laboratory manual of analytical methods and procedures for fish products (Miwa and Ji, 1992). The pH was monitored by homogenizing 5 g of samples in 20 mL distilled water for 1 min at room temperature.

To study the stability test, 9 boxes were randomly selected, including 3 boxes incubated at 37°C for 7 days, 3 boxes at 55°C for 7 days and 3 boxes stored at room temperature and used as controls.

The physico-chemical analysis focused on measuring the pH of the cans, in order to compare their values between the incubated and non-incubated samples (controls). According to current standards, the pH difference must not exceed 0.5 units.

The average of the percentage recovery: The Total Net Weight (TNW) in grams is the difference between the total weight before opening the box (TW) and the and the net weight of the empty box (NW).

Before opening, the boxes were cleaned, dried and their labels removed. The 2.5 mm mesh diameter sieve has been placed on a flat surface with a 20% inclination to the horizontal. After reversing the contents of the box on the screen, wait 2 min and measure the Drained Net Weight (NDW). The percentage (%) of weight recovery is the ratio of drained net weight in % to Total Net Weight (TNW).

The percentage of the water: After the extraction of the liquid phase, we wait 5 min for the separation between juice and water to be carried out. The percentage (%) of water is the ratio between the volume of water and the total volume of juice obtained after separation. The results are satisfied when the percentage (%) of water is ≤8%.

Microbiological analysis: Thermophilic Bacillus and *Clostridium* are determined according to French standards (NF-V08-404 in 1986) and (NF-V08-405 in 1986), respectively.

The sensory analysis: For this test sardinelle is selected according to guidelines concerning fresh and refrigerated fish (EU., 1991). The sensory panel composed by 12 students which were selected on the basis of their sensory performances. We used the methodology described by (Anonymous, 1995) to select the most important descriptors.

The test used is a ranking test. It consists in ranking the samples presented simultaneously in order of increasing intensity. The intensity of different sensory criteria are identified using a structured intensity scale with a score ranging from 0-5 (not collected: 0; low: 1; rather low: 2; medium: 3; rather high: 4 and high: 5). The agreement on ratings is obtained by training of the sensory panel in other to evaluate the reference products before testing the final products. We recommend the Friedman test.

For statistical interpretation, we used the Friedman test. The test indicates whether a significant difference exists between all samples. If this is the case, it will be necessary to determine which couples are different using a multiple comparison test.

Statistical analysis: All the experiments were done in triplicate samples were analyzed at each sampling time.

The statistical analysis of the results were carried out using the SPSS (Statistical Package for the Social Sciences) Software Version 20.0 (IBM, USA). The statistical significance is evaluanted with Tukey's test at $p<0.05$. The results of sensory analysis were processed using Friedman's non-parametric tes. This is a Chi-square test (χ^2) of difference between the sum of the ranks obtained for each product and an average sum of the ranks.

RESULTS AND DISCUSSION

Physico-chemical analysis

pH of the raw material during storage at t°≤4°C: Fish is more perishable than chicken or red meat as it contains relatively large quantities of free amino acids and volatile nitrogen bases compared with other meats (Ashie *et al*., 1996). Gram and Huss, stated that fish are perishable food commodities which generally spoil faster than other muscle foods. pH value has been employed often as a complementary analysis to fish spoilage detection (Boubaker *et al*., 2014). pH could be useful to evaluate the qualitative changes in fish during storage where the quality of fish degrades due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated (Gonzalez-Fandos *et al*., 2005). The potential of Hydrogen ions (H^+) is also a physical parameter for evaluating the development of metabolic reactions, especially, those that occur after the death of fish where ATP and glycogen degradation are of particular importance (Abbas *et al*., 2008) established a correlation between fish freshness and pH during cold storage suggested that this physical characteristic could be used as suitable tool for the analysis and fish freshness evaluation rather than sensory evaluation method which inherent many uncertainties. Oucif *et al.* found a pH value of 6.1 in sardine. According to Baygar *et al.* (2012) the pH of raw fish flesh (*Sardina pilchardus*) is 6.3. According to Kilinc (2003) pH value of raw material *Sardina pilchardus* during marination was 6.72. To Assess the effectiveness of a cold chain for fresh fish salmon (*Salmo salar*) and sardine (*Sardina pilchardus*) in a food processing plant, the final value of pH in the finished product of sardine depends more on the quality of the raw material than the effect of processing in the company as long as they have a good manufacturing practice and adequate cold chain (Calanche *et al*., 2013). Initial quality of raw materials, considering their freshness, microbiological load and physical damage is an important factor which influences the quality of the end product (Fuselli *et al*., 1994). For a better understanding of the effects of storage frozen sardine (*Sardina pilchardus*), Fatiha *et al.* conclude that the Frozen sardine stored at lower temperatures (-30°C) was maintained better sensory and nutritional properties (omega 3 EPA-DHA). Similarly, frozen sardines retains better organoleptic and nutritional quality when stored whole. However, according to Ortiz and Bello storage time had effect on the stability of sardine fatty acids and the variance analysis indicated that percentage of polyunsaturated fatty acids of n-6 series in the total lipids of cachama and n-3 series in sardine were affected by the storage temperature from -10 to -20°C. As mentioned Table 1, we noted that the pH values averages of 24 h of storage at t°C≤4°C were ranged between 6.18 and 6.25. Similarly, results were found by other studies. Farag studied the estimation of formatting biogenetic amines concentration in fresh and processed sardine fish products during different storage conditions. These results revealed that the pH in cooled sardine fish during storage at 4±1°C was found 6.58 and 6.72 at 0 time and after 4 days, respectively. The pH value in sardine found by Marrakchi *et al.* and Gokoglu *et al.* were 5.83 and 6.2, respectively. It has been reported by Hansen *et al.* that the increase in pH indicated the loss of quality where the decrease of pH in stored fish flesh could be observed due to the acid which is a common metabolite from growth of a number of bacteria include lactic acid bacteria. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh (Shenderyuk and Byokowsky, 1990). The correlation of pH towards the freshness of fish had been clearly shown (Abbas *et al*., 2008). As conclusion, in general, the pH in fresh fish flesh is almost neutral. Also, the pH depend on fish species and others factors (Simeonidou *et al*., 1997). According to our results, we did not note pH increase after 24 h of storage at t°≤4°. Thus, we can say that the raw material (*Sardine pilchardus*) used to produce canned sardine testifies to the high quality. Moreover, as mentioned Table 1, the temperatures recorded are ≤5°C which is in agreement with internal specifications and proved the respect of the cold chain (Table 1).

Table 1: pH and temperature of raw material during storage at t°C≤4°C

No. of cases	Temperature (°C)	pH
1	4.8±0.10	6.23±0.10
2	3.8±0.12	6.21±0.12
3	4.0±0.10	6.25±0.09
4	2.8±0.11	6.20±0.07
5	4.5±0.10	6.22±0.08
6	3.4±0.10	6.20±0.10
7	4.0±0.11	6.18±0.04
8	3.8±0.12	6.25±0.10
9	4.4±0.11	6.22±0.08
10	4.6±0.10	6.18±0.10

Results are expressed as mean±standard deviation of three samples of three replicates during 24 h

The canned sardines with lemon or with ascorbic acid are prepared according to the Table 2. During the production process some parameters are controlled. As

Table 2: Composition of new products developed

	Product				
Ingredients	1	2	3	4	Witness
No. of sardines/box	6-11	6-11	5-9	5-9	8-9
Slices of lemon	2 slices	3 slices	-	-	0
Ascorbic acid (%)	-	-	0.75	1	0
Olive oil (mL)	300	300	300	300	300
Nested weight (g)	82±2	84.5±3	79.5±7	82±7	84±1

Table 3: Parameters controlled during the production

Steps	Parameters	Results
Cooking	T≤100±10°C	~105°C
Drying	T≤100 ±10°C	~102°C
		To comply with the standard
Crimping	1.08<Thickness of crimp>1.3	1.24
	1.70<Body hook>2.10	2.02
	1.7<Bottom hook>2.10	1.86
	Deviations in brackets>0.2	1.15
	Crossing>1	>1.17
		To comply with the standard
Waiting time before sterilization	Time (t)<3 h	To comply with the the standard
Sterilization	T>116°C	~116°C
	t = 60 min	t = 60 min
	1.2<Pressure (P)<2.4	P = 1.4 atm
		To comply with the standard

Table 4: Stability test of canned sardine with three lemon slices

Temperatures	Room temperature	37°C	55°C
Visual evaluation (convex box)	Normal box	Normal box	Normal box
pH	6.33±0.1	6.43±0.13	6.35±0.1
notice	Stable	Stable	Stable

pH are expressed as mean±standard deviation of three replicates

mentioned Table 3, the results show that the parameters determined for the various steps of production of canned sardines are controlled according to the references documents (Table 3).

Control of final product: In practice, the incubation test is a good indicator of process control. In the literature, the pH value has often been used as a complementary analysis to the detection of fish deterioration. The results of the stability tests and pH measurements are presented in Table 4 and 5. The results showed that the pH for canned sardines with 3 slices for all tests at various temperatures used (room temperature, 37 and 55°C) were found 6.33, 6.43 and 6.35, respectively. With 1% of ascorbic acids the pH values were found 6.43, 6.41 and 6.45 at various temperatures used. As seen Table 4 and 5 insignificant changes were noted in pH values between the two products (canned sardines with lemon and canned sardine with ascorbic acid). However, in both cases we noted that the pH is stable which means the products are stable. Other researchers reported insignificant changes in

Table 5: Stability test of sardine preserved with 1% of ascorbic acid

Temperatures	Room temperature	37°C	55°C
Visual evaluation (convex box)	Normal boxes	Normal boxes	Normal boxes
pH	6.43±0.1	6.45±0.12	6.41±0.11
Notice	Stable	Stable	Stable

pH are expressed as mean±standard deviation of three replicates

Table 6: Metrological analysis of canned sardine added of three slices of lemon

Metrological analysis	Room temperature	37 °C	55 °C
Total net weight (g)	125±1	125±3	124±2
Dry net weight (g)	90.5±1.2	85±1	82±1.5
DNW/TNW (%)	72.4±1.2	68±1.1	66.1±1
Exudate (%)	0.5	0.5	0.5

Results are expressed as mean±standard deviation of three samples of three replicates

Table 7: Metrological analysis of canned sardine added with 1% of ascorbic acid

	Room temperature	37 °C	55 °C
Total net weight (g)	128.5±5	122±2	122.5±3
Dry net weight (g)	98±1	88±1.2	84±1.2
DNW/TNW (%)	76.3±1.2	72±1.1	68.5±1
Exudate (%)	0	1±0.1	2.4±0.2

Results are expressed as mean±standard deviation of three samples of three replicates

pH levels of marinated fish. Poligne and Collignan found that the pH levels of anchovies pickled with acetic acid increased from 3.90-4.2. Aksu *et al.* (1997) determined that pH values of anchovy marinated with 2% and 4% and stored at 4°C increased from 4.25 and 4.18 from 4.25 and 4.18-4.53 and 4.31, respectively after 20 days of storage and then remained constant until the end of the storage. El Shehawy *et al.* studied the characteristics of some traditional salted fish samples from Egyptian market. These results revealed that the pH of salted sardine 1 and salted sardine 2 were 6.03±0.01 and 6.24±0.03, respectively. In our study, according to the obtained results of pH values the canned sardines with lemon and canned sardines with ascorbic acid are stable at the tested temperatures and are in agreement with the standard (NF-V08-401 in 1997). According to the obtained results, we noted that no box is convex and no leaking or floss boxes.

The percentages of the recovery medium and the exudate: Table 6 and 7 report the results of the percentage of recovery medium in sardines with lemon or with ascorbic acid. The obtained results showed that the percentage of recovery medium were 66.1, 68 and 72.4 in canned sardines with 3 slices of lemon and 68.5, 72 and 76.3 in canned sardines with 1% of ascorbic acid at 55, 37°C and at room temperature, respectively. As noted the % of recovery medium decrease when the temperature rises. Probably, this is due to the effect of temperature which causing losses in sardine components. The percentage of dry net weight of canned sardine are in

accordance with the Regulation (EEC) n°2136/89 of the council of June, 21, 1989. However, according to Article 7 of Directives 79/112/EEC and 76/211/EEC, the trade description of canned sardines is determined by the ratio between the weight of the sardines contained in the container after sterilization and the net weight, expressed in grams. Article 4 requires that this ratio must exceed 70 %. The obtained results showed that the percentage of the water (exudates) were ranged between 0 and 2.4% at all used temperatures. In canned sardines with harissa or with red pepper, the percentage of exudates water were ranged between 1 and 1.5% (Boubaker *et al*., 2014). Water loss can be explained in terms of heat treatment and protein degradation in canned sardine muscle, this leading to a decreasing water holding capacity of proteins (Castrillo *et al*., 1996). According to the results, the percentages of water in oil obtained do not exceed 8% and are in accordance with Council Regulation (EEC) No. 2136/89 of 21 June, 1989.

Microbiological analysis: The Egyptian Organization of Standardizations for microbiological aspects of fish products (canned Tuna, canned Sardines, El-Feseekh, salted sardine, smoked fish and frozen fish), stated that these fish products shouldn't have Clostridium. The same researcher also reported the following microbiological standards: Anaerobic spore formers producing H_2S should not exceed 10^2 CFU g^{-1} in smoked and salted fish but canned fish shouldn't have it. Salted, smoked and frozen fish shouldn't have *E. coli*. Coliform group should be $<10^3$, 10^1 CFU g^{-1} in frozen and smoked fish, respectively. Total viable bacterial count should not exceed 10^5 and 10^6 CFU g^{-1} in smoked and frozen and smoked fish, respectively. Total viable bacterial count should not exceed 10^5 and 10^6 CFU g^{-1} in smoked and frozen fish products, respectively. Staphylococcus should be $<10^3$, 10^2 and zero CFU g^{-1} in frozen, salted and smoked fish, respectively. The general recommended value for the total viable bacteria in fish is 5 10^5 CFU g^{-1}. In marinated products, the bacterial growth is inhibited by acidic pH and the use of antimicrobial agent (Bjorkoth, 2005). It was reported that to restrict LAB (lactic acid bacteria) growth, a pH no higher than 4.0, a salt concentration of marinade not much under 6% and a low storage temperature has been recommended. In this study, the microbiological quality of canned sardines was assessed by analyzing the forms of Bacillus and *Clostridium thermophilis* spores that are known for their thermal resistance. *Clostridium botulinum* produce spores that are the most heat resistant of all pathogenic micro-organisms. Consequently, the fish canning industry must rely on thermal processes sufficient to ensure the lowest probability of survival of C. *botulinum* spores, so as to present no significant health risk to consumers. Therefore, a reliable safety and quality assurance program should systematically integrate the quality of raw material; the hygiene and sanitation, the proper thermal processing (including design, application and monitoring); the proper can seaming (welding). The microbiological analyzes showed the absence of spores. This proves the application of Good hygiene Practices (GMP) and product safety are provided. Therefore, the products are free of biological hazards and are fit for consumption.

Sensory analysis: Marinated fish is preserved by the simultaneous action of organic acids such as acetic acid and salt. The combined preservative action prevents the growth of pathogenic bacteria and most spoilage bacteria. The products obtained have a pleasant taste without being too tough and have a reasonable shelf-life (Fuselli *et al*., 1994). The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in colour, odor and texture (Ozogul *et al*., 2004). Sensory evaluation is the most important method for freshness and quality assessment in the fish sector (Kemp *et al*., 2018). Sensory inspection of processed fish used in the fish industry to find defects that have occurred during handling and processing (Edirisinghe *et al*., 2018). Marinades are the solutions including sugar, spices, oil and vinegar or fruit juice that already has been used to improve the tenderness, juiciness, flavor and aroma (Cadun *et al*., 2008) whereas, the off-odor and off-taste of the products affect the consumer acceptability (Rostamzad *et al*,. 2010). Marination is also used to tenderize or to change taste, textural and structural properties of raw material. It has reported that the marinades stored at cooler temperatures (4-6°C) keep a long time such as 4 months. In this study, the sensory assessment of final products, sardines with lemon and sardine with ascorbic acid was conducted using the sensory attribute, especially, the fresh (freshness) taste using the methodology described by Dominique with a panel of trained panellist (Boubaker *et al*., 2014). The freshness is the mainly required taste by consumers in this kind of product. Previously, the judges evaluated the reference products and after this we discussed with them to obtain an agreement on ratings. The products are prepared as shown Table 2. Thus, four boxes from each product are randomly collected where the average weigh is between 79 and 82 g. Each box contains a variable number of sardines (Table 2). Fish samples from the different treatments were individually presented in covered small porcelain dishes to each panellist. Tasters are asked to rank coded samples according to acceptance from the least acceptable to the most acceptable. In

Table 8: Ranking test of the canned sardine products

	Product 1		Product 2		Product 3		Product 4	
Tasters	Note	Rank	Note	Rank	Note	Rank	Note	Rank
1	6.5	4	9	1	8	2	7.5	3
2	6	2	8	1	5	3	5	4
3	3	3.5	3	3.5	6	2	7	1
4	7	2	9	1	6	3	5	4
5	6	2.5	6	2.5	8	1	4	4
6	5	4	8	1	6	3	7	2
7	6	2.5	6	2.5	8	1	4	4
8	8	3.5	8	3.5	9	1	8.5	2
9	5	4	6	2.5	9	1	6	2.5
10	4	3.5	4	3.5	6	2	8	1
Σ	56.5	31.5	67.0	22.0	71.0	19.0	62.0	27.5
Ranking		4		2		1		3

Table 9: Sensorial analysis of canned sardines

Products	1	Note	Criterion	2	Note	Criterion	3	Note	Criterion	4	Note	Criterion
Color	5	0.50	0	6.00	0.60	1	18.50	1.85	2	12.00	1.20	1.00
Aspect	2.5	0.25	0	5.50	0.55	1	4.00	0.40	0	3.50	0.35	0.00
Taste	17	1.70	2	29.00	2.90	3	35.00	3.50	3	32.00	3.20	3.00
Salt degree	7	0.70	1	8.00	0.80	1	7.00	0.70	1	6.00	0.60	1.00
Preference	19	1.90	2	30.50	3.05	3	29.00	2.90	3	28.50	2.85	3.00
Global note	56.50	5.65		67.00	6.70		74.00	7.40		59.0	5.9	0
Ranking	4			2			1			3		

general, equalities are not allowed. The ranking ratings given to each sample by the 10 tasters were grouped in the Table 8. The obtained results show to us that the tasters enjoyed first sardine canned with 1% of ascorbic acid and second they appreciated the canned sardine with 3 slices of lemon. This finding is proved by the award of the highest note of 3.50 and of 3.20, respectively. Also, we noted that sardines with 1% of ascorbic acid is better appreciated than sardine with 0.75%. (3.50>2.90). This is due to the concentration effect of ascorbic acid. Statistically, we c ompare the products by Friedman test (Friedman *et al*., 1980). The Friedman test is a non-parametric test for analyzing randomized complete block designs. The Friedman test (F) is a test for comparing three or more related samples. The number of subjects; n is 10 and number of products p is 4). Thus, the Friedmen's coefficient: $F_{cal} = 5.61$.

The critical value at 5% is $F_{th} = 7.81$ determined from χ^2 to (p-1) degrees of freedom. $F_{cal}<F_{th}$ proves that there are no significant differences between the different products presented. It can be concluded that all products are perceived to be identical. Apparently, the treatment effects have no identical effects. Based on this fact that no significant differences were detected for the sensory attributes between the products; we found useful to continue our investigations. Thus, we asked the tasters to classify the different canned sardines prepared in order of preference according to the intensity of five criteria (color, taste, preference, degree of salt and aspect). The results are shown Fig. 1 (Table 9). For the color, aspect and salt criteria, lemon and ascorbic acid added in the coating medium do not influence the organoleptic characteristics of the samples tested. However, the taste of these products is strongly influenced by the addition of these two additives. The results in Fig.1 explain the ranking of the tasters. Probably, ascorbic acid with 1% (product ranked 1 by the tasters) ensures the product a taste of freshness (acid taste). It has been reported that ascorbic acid increases the acidity of the food and/or confers an acid taste and increases their flavor while improving their organoleptic quality. Many brands play on the beneficial effects of the vitamins or vitamin C perceived by the consumer, the industrialists enrich their foods in vitamin C in order to make a sales argument: 67% of the respondents prefer to consume foods with a guaranteed content or enriched with vitamins (including C) and 39% opt for food supplements. Normally, the first role attributed to ascorbic acid is an antioxidant. The use of ascorbic acid as an antioxidant was reported by Aubourg *et al.* (2004) Rostamzad *et al.* (2011). Ascorbic acid often is used as an antioxidant agent to increase shelf life of processed food and canned. This acid is a strong antioxidant and has a direct synergistic relationship with other antioxidants (Aubourg *et al*., 2004). Ascorbic acid is a known antioxidant commonly used in the food industry and it can be associated with prooxidant actions (Chen *et al*., 2008). The effect of aqueous solutions of Ascorbic Acid (AA) on the rancidity development in cobia (*Rachycentron canadum*) fillets during frozen storage was studied. Results of investigation revealed that

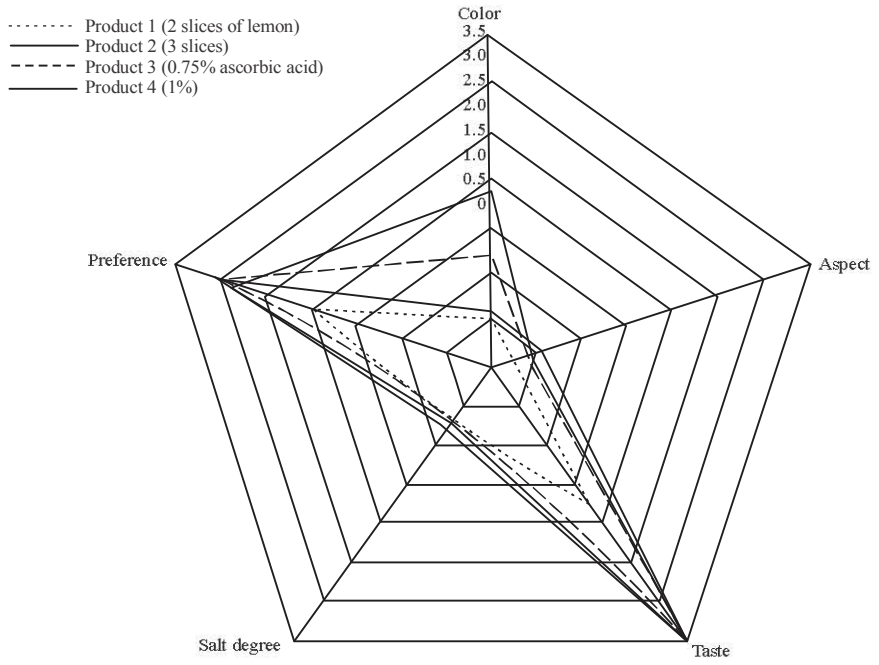

Fig. 1: Sensorial analysis of canned sardines

ascorbic acid retarded oxidative changes in frozen cobia fillets. Best oxidation inhibition results on fish fillets were obtained when employing a 0.5% AA solution (Taheri *et al*., 2012). On the other hand, the positive effects of Ascorbic Acid (AA) and Citric Acid (CA) on fish oil and emulsions is mentioned (Corbo *et al*., 2008) where the advantages of CA and AA) are discussed and further studies based on the positive role of a CA-AA mixture will be carried out to extend the shelf life of medium- and high-fat content fish species during frozen storage (Aubourg *et al*., 2004). It has been reported that ascorbic acid is a preservative that limits browning (color change) of fruits, rancidity (bad taste) of fat. Thus, foods retain the maximum of their natural color even when canned. According to the obtained results, we noted that the ascorbic acid gives shine to the color which is confirmed by the obtained results (Fig. 1) where the rating assigned to each product for the color criterion were 1.85, 1.20, 0.60 and 0.5. For product 4 (canned sardine with 1% of ascorbic acid), product 3 (canned sardine with 0.75% of ascorbic acid), product 2 (canned sardine with 3 slices of lemon), product 1 (canned sardine with 2 slices of lemon), respectively. Sardines are fatty fish that contain high levels of ω-3 polyunsaturated fatty acids which are very susceptible to loss of nutritional quality and shortening of shelf life because of lipid oxidation. (Gimenez *et al*., 2004). So, the off-odor and off-taste of the products from lipid oxidation affect the consumer acceptability (Rostamzad *et al*., 2010)). Moreover, more precisely, the use of the ascorbic acid in many food products allows to the manufacturers to provide food with safeguarded conservation lives and organoleptic qualities by its principal role of antioxidant. Thus, the use of ascorbic acid and the publication of this study were justified. Now, for the preference criterion, the canned sardine with 3 slices of Eureca lemon obtained the highest average score of 3.05 and ranked n°1. Probably, this score of 3.05 was assigned for the flavor taste persuaded by the tasters and justifies their choices for this product. Lemon juice and lemon fruit are extensively used as flavoring ingredients in a wide variety of foods. These ingredients are commonly added to fishes consumed raw and after cooking, especially in the Mediterranean basin (Goulas and Kontominas, 2007) and for salted fish (Alfonzo *et al*., 2017). Thus, lemon aroma is well accepted for fishery products. The food and pharmaceutical industries in Pakistan are using citrus oils as flavoring and masking agents in abundant quantity (Ahmad *et al*., 2006). Nowadays, the application of Eos (Essential oils) and the addition of lemon EOs could be positively applied also on salted fishes. In this way, oils composition of four species of citrus fruits such as malta (*C. sinensis*), mousami (*C. sinensis*), grape fruit (*C. paradisi*) and Eureka lemon (*C. limon*) were investigated. The researchers concluded that the Eureka lemon and mousami peel oils had the pleasant sharp and strongest flavor compared to others fruits and the components identified in Eureka lemon peel oils were

more than other oils (Ahmad *et al*., 2006).The effect of the lemon essential oils on the safety and sensory quality of salted sardines (*Sardina pilchardus*) was studied. As a result of these experiences, the highest scores of sensory evaluation were registered for flavor and overall acceptability of the experimental trials in presence of EOs (Alfonzo *et al*., 2017). They conclude that the use of EOs to produce salted fishes represents a valid strategy to improve safety and sensory characteristics of salted sardines.

CONCLUSION

Tunisian canned sardine enjoys a goo brand image and are well appreciated by the consumer worldwide. Taking advantage of this fact, we have made two new products canned sardine with Eureka lemon and canned sardine with ascorbic acid. The test of stability is in accordance with the standard. Also, we did not notice any major non conformity at least about the boxes tested and no spore of Bacillus and *Clostridium thermophilics* forms were detected. The percentage of medium recovery is in accordance with "CEE" n° 2136/92 Council (OJEC 22-07-89) and the percentage of water (exudate) does not exceed 8% at the temperatures used. The percentage of water in oil is in accordance with the EEC n° 2136/92 of the Council (OJEC of 22-07-89). The tasters have ranked canned sardine with 1% of ascorbic acid n°1, canned sardine with 3 slices of lemon n°2, canned sardine with 0.75% n°3 and finally, canned sardine with 2 slices n°4. Statistically at 95%, the sensory analysis of samples tested through the Friedman test showed that the two products are not significantly different. However, canned sardine with 1% of ascorbic acid is appreciated for the criterion taste while canned sardine with 3 slices of Eureca lemon is appreciated for the preference criterion. This research has also economic implications, since, the flavor improvement due to the addition of lemon might increase the consumption of sardines by regular and new consumers. Besides, Recent reports (Settanni *et al*., 2014) demonstrated that EOs extracted from Citrus lemon cultivated in Sicily (South Italy) are highly effective against food spoilage and/or pathogenic microorganisms. We advise this company to develop the canned sardine with 1% of ascorbic acid and canned sardine with 3 slices of Eureca lemon. Besides, based on the increasing interest toward novel food preservatives, we advice a company to conquer other markets to diversify the range of products in particular the production of canned fish with natural antimicrobial agents such as EOs or others agents extracted from lemon such as eriocitrin which is stable at high temperature (121°C, 15 min) in acidic solution (pH 3.5).

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