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Bio-Preservation of Refrigerated Peeled Shrimp (*Parapenaeus Longirostris*) Using Cactus Fruit Peels Polyphenolic Extract

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Abstract: *The objective of this study was to evaluate the bio-preservation efficiency of polyphenols extracted from cactus fruit peels in extending the shell life of peeled shrimp during refrigerated (2°C) storage. Changes in microbial, biochemical, and sensorial indicators of cactus polyphenols-treated peeled shrimp (PS) were studied comparatively to a control lot (CS). Results revealed that treatment limited biochemical degradation and reduced the final microbial load in shrimps during storage. They showed the dominance of Brochothrix and Psychrobacter within spoiling bacteria in the CS lot while only limited proliferation of Psychrobacter was observed in PS lot. Correspondence factorial analysis of sensory data showed that polyphenol-treatment extended product shelf life without altering sensorial properties. According principal component analysis, biochemical and bacterial changes were correlated positively with storage time but negatively with polyphenols treatment, which in its turn was positively correlated with sensory evaluation; confirming the beneficial effects of polyphenols treatment on shrimp bio-preservation.*

Keywords: *Shrimp; Bio-preservation, natural extract, shelf life, bacterial community*

I. Introduction

In worldwide fishing industry, shrimps are generally among the resources of high economic value. The deep water rose shrimp *Parapenaeus longirostris* is distributed throughout the eastern Atlantic Ocean and the Mediterranean, where according FAO fishery statistics it was the most important crustacean biomass landed by trawl fisheries in 2000-2008, constituting 23% of total crustacean regional landings (Knittweis et al., 2013). It is highly demanded in international trading markets, particularly in Europe where it is generally sold as refrigerated or frozen peeled products. Shrimp is a perishable product and postmortem changes occur rapidly compared with fish, including under cooling conditions (Zeng et al., 2005). A large variety of biochemical indicators may be used for characterization of shrimp quality and healthiness (Benner et al., 2003; Snellings et al., 2003). But as processing and in particular peeling may promote the development of a wide range of undesirable microorganisms including spoiling bacteria which lead to both health and economic concerns, microbiological indicators (Zeng et al., 2005, Dupard et al., 2006; Jiang et al., 2011; Guo et al., 2013) are also considered essential. Generally, the total number of microorganisms, as total viable counts (TVC) has been used in mandatory seafood standards and in microbiological specifications of trading agreements. However, it is known that only a small fraction of the microorganisms present on newly processed seafood is actually responsible of product spoilage and TVC may correlate poorly with the degree of freshness or remaining shelf life (Cadun et al., 2005). Therefore, characterization of microbial communities is important for providing objective quality indices to determine shelf life in seafood. For instance, the microflora of processed (chilled, cooked, peeled, packed, salted and fermented) shrimp has been largely studied using selective culture media (Mejlholm et al., 2005; Laursen et al., 2006; Han et al., 2014). Culture-independent methods are also being increasingly used to develop a complete overview of the bacterial community characteristics. Fingerprinting molecular methods, such as denaturing gradient gel electrophoresis (DGGE) and temporal temperature gradient gel electrophoresis (TTGE) are powerful tools for comparing changes in microbial communities and monitoring bacterial population dynamics (Jaffrès et al., 2009; 2011).

In shrimp, as in most seafood products, shelf life depends on the proliferation level reached during storage by the bacteria involved in spoilage. Minimizing this level and limit the environmental bacterial contamination may help processing industry to extend product shelf life. Several methods have been developed including modified atmosphere packaging (Caballero et al., 2002; Gonçalves et al., 2003; Mejlholm et al., 2005; Qian et al., 2013), the use of chemical additives (Montero et al. 2004; Gomez-Guillén et al., 2005) or a combination of both of these techniques (Thepnuan et al., 2008), as well as the use of bio-active packaging (Souza et al., 2015) in order to maintain qualitative proprieties and to avoid health hazards for consumers (Yerlikaya et al., 2010). In recent years bio-preservation, consisting in preventing growth of unwanted microorganisms in foodstuffs by using natural compounds, gained growing attention. Successful results are reported for proteins addition or soaking (Eakpetch et al., 2008; Kaewmanee et al., 2009), sepia soaking (Sadok

et al., 2004), chitosan glazing or coating (Huang et al., 2012) or applications of plant extract and essential oils for coating or glazing (Nirmal and Benjakul 2010; Sundararajan et al., 2011; Maqsood et al., 2012; Zhou et al., 2013) or lactic bacteria (Calo-Mata et al., 2008; Boulares et al., 2016) on either fresh or processed fish and shrimp.

Originating from the tropical and subtropical Americas, the cactus *Opuntia ficus indica* was spread further by people to Africa, Asia, Europe and Australia, where more than 900 000 ha of cultivated and wild plants growing in diverse agro-climatic condition provide food, forage, materials and bioactive compounds (FAO 2002). The nutritional, industrial and pharmacological valorization of the different parts (fruits, flowers and cladodes) of the plant is largely recognized and documented (FAO 2013). The succulent texture and long lasting permanence on the plant let the fruit available throughout most of the year (Jana 2012). Large amounts of biomasses are under-exploited (FAO 2002). In addition, in most of the industrial uses for the production of juices or processed beverages as well as jellies, sweeteners or jams, the thick peels are discarded (Sawaya et al., 1983) and thus constitute large amounts of by-products from which bioactive compounds could be extracted and valorized like as it is suggested for wastes from other fruits juice industries such as pears and apples (Ma et al., 2012; He et al., 2014). Objective of present study was to evaluate the efficacy of application of polyphenols extracted from cactus (*Opuntia ficus indica*) fruit peels in extending the shelf life of peeled shrimp (*Parapenaeus longirostris*) during refrigerated storage. Treatment consisted in soaking of peeled shrimp in a solution containing the polyphenols extract. Changes in biochemical, microbial and sensorial analysis of treated shrimps were studied comparatively to a control lot during refrigerated storage. The developing microbial communities were described using numeration of total aerobic viable counts on medium used for seafood analysis; as well as the PCR-TTGE method to identify and monitor the dominant bacterial populations.

II. Preparation of polyphenols extract

Based on previous study (unpublished data) on the optimization of polyphenols recovery from the cactus (*Opuntia ficus indica*) fruit peels, portions of 10g of peel from fresh fruits were thoroughly minced with a mixer in 40 ml of ice-cold ethanol/water (80:20v/v) and kept for 24 h of maceration under shaking conditions at 4°C. The mixtures were then sonicated for 20 min and centrifuged at 10 000g for 15 min at 4°C. The supernatants were collected, pooled, and concentrated at 40°C under reduced pressure during 3 hours in a rotary evaporator (i.e. until total elimination of ethanol). To prevent oxidation of the polyphenols, the extract was conserved refrigerated in the dark and 1% aqueous solution from this extract was prepared upon needs and immediately used.

III. Total phenolic content determination

Phenolic compounds were assayed using the Folin-Ciocalteu reagent, following Singleton's method which was slightly modified (Singleton et al., 1999). Briefly, 0.125 ml of a 10-fold diluted extract was mixed with an equal volume of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 ml of 7% sodium carbonate (Na_2CO_3) solution. The mixture was then incubated at room temperature for 45 min and the absorbance measured at 725nm. Total phenolic content (three replicates) was expressed as mg catechin equivalents (CATE)/100 g of fruit peel.

IV. Radical Scavenging Activity

The antioxidant activity of the polyphenolic extract or standard pure phenolic compounds was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. The reaction for scavenging DPPH radicals was performed in polypropylene tubes by adding 2 ml of DPPH (4×10^{-5} M) in methanolic solution to 50 µL of sample. The mixture was shaken vigorously and left for 60 min at room temperature. The absorbance of the resulting solution was measured at 517 nm. Methanol was used as a blank solution, and DPPH solution without any sample extract served as control. The Trolox equivalent antioxidant capacity (TEAC) values were calculated from the equation determined from linear regression after plotting known solutions of Trolox with different concentrations (0.02-0.8 mM). The antiradical activity was also expressed as the inhibition percentage and was calculated using the following formula: % radical scavenging activity = (control OD - sample OD/control OD) ×100.

V. Shrimp treatment and sampling

Fresh whole shrimp (*Parapenaeus longirostris*) were purchased directly from the port of Bizerte (Northern Tunisia), immediately packed in polyethylene sealed bags and transferred in ice to the laboratory where they were manually headed and peeled in sterile conditions. Processing was performed within 24 h after capture. The peeled shrimp were divided into two lots: shrimps of first lot were left untreated and used as control (C) while those of second lot were soaked 5 min in distilled/sterilized water containing dilute (1⁄10) cactus phenol solution (P). The ratio of fish to soaking solution was 1⁄10 (w⁄v). Each lot (C and P) was

subdivided into 10 portions of 100 g (consisting in about 20 specimens) which were directly vacuum packaged in pouches of polyethylene and immediately stored at 2 °C. Sampling was undertaken after 0, 3, 5, 7 and 9 days of storage. Two pouches from each lot were randomly selected and analyzed in triplicates each sampled pouch (thus n = 6 for each sampling day and treatment).

VI. Chemical analysis

1. Lipid extraction and fatty acid analysis

Total lipids were extracted according to the method of Folch et al., (1957) and were expressed in g/100 g wet weight. They were made soluble in Vorbeck solution (Toluene- ethanol (4:1 v/v) (Metcalfe et al.,. 1966) and stored at -40 °C until analysis of fatty acids as fatty acids methyl esters (FAMEs) described by Metcalfe et al., (1966). A fraction of the lipid extract was saponified with 0.5 mol/L NaOH in methanol, followed by a methylation in 12% boron trifluoride in methanol (BF_3/MeOH). The methylated sample was then extracted with n-hexane. Analysis were performed on the non spoiled shrimps and limited to the three first sampling days (i.e. at 0, 3 and 5 days of storage). The FAMEs were resolved using an Agilent-Technologies chromatograph 6890N (Palo Alto, CA, USA having a flame ionization detector (FID), a splitless injector and a polar INNOWAX 30 M silica capillary column (0.25 mm i.d. & 0.25 µm film thickness). The temperature of the injector was 220°C and that of the detector w was 275°C. Helium at a flow rate of 1.5 ml/min was used as the carrier. Peaks of chromatograms were identified by comparison to a standard mixture (SUPELCO-47085-U Sigma-Aldrich) containing 19 FAMEs. The values are given as percentages of the total FAMEs.

2. Biochemical indicators of quality

The nitrogenous compounds in samples of flesh as well as the peroxidation indicators in lipids were studied throughout storage.

The analyzed nitrogenous compounds consisted in total volatile basic nitrogen (TVB), trimethylamine (TMA) and total free amino acid measured as ninhydrin positive substances (NPS). They were determined by flow injection analysis (FIA) according to the methods of Ruiz-Capillas & Horner (1999), Sadok et al., (1996) and Sadok et al., (1995) respectively.

The peroxide value (PV) was determined according to the ferric thiocyanate method with slight modification (Chapman and Mckay, 1949). Fractions of 50 to 100 mg from lipid extract were homogenized for 2 min at 2000 rpm in a final volume of 10 ml of a chloroform/methanol mixture (7:3). Then 50 ml of ammonium thiocyanate (10 mM) solution and 2 ml of ferrous chloride solution (10 mg/ml iron III) were added and mixed for 1 min at 2000 rpm. After an incubation of 5 min at room temperature the absorbance was measured at 500 nm against a blank and a standard curve. The results were expressed in terms of meq of oxygen per kg of oil.

The acid reactive substances in lipids were determined as thiobarbyturic acid reactive substances (TBARs) according to the AOAC (1998) method, which allows their determination without preliminary isolation of secondary oxidation products (Attouchi and Sadok 2010). Fractions of 50 to 200 mg from lipid extract were diluted in 10 ml of 1-butanol and mixed with 10 ml of 0.2% thiobarbyturic acid (TBA) in 1-butanol before an incubation of 2 h at 95^{o}C. After cooling for 10 min under running tap water, the absorbencies were measured at 532 nm against a blank and a standard curve determined by the TBARs reaction of aliquots (0.1 to 1 ml) of 0.2 mM 1,1,3,3-tetramethoxypropane (TMP) in 1-butanol. Results were expressed as mg malonaldehyde (MA)/kg of fresh weight.

VII. Microbial analysis

1. Enumeration of total aerobic viable bacteria

For bacterial analysis, 15-g portions from each sampled pouch was aseptically weighed and pooled per treatment into 120 ml of sterile physiological saline solution (0.85% NaCl) with 0.1% peptone in a sterile plastic bag. This pooled samples were blended with a stomacher 400 (Seward-Medical, London, UK) for 2 min. After 30 min at room temperature for bacterial resuscitation, 30 ml of each blend were pooled into a sterile vial and thoroughly mixed to constitute the homogenized analysis solution. Several appropriate 10-fold dilutions of this solution were carried out in sterile physiological saline solution and triplicates of 0.1 ml of each were spread on plates containing on Long and Hammer agar (LH) with 1% NaCl (Macé et al., 2012). The total aerobic viable counts (TAVC) were determined after each 2 days of incubation at at 20°C (Van Spreekens, 1974).

2. Biochemical characterization of bacterial isolates

When shrimp samples were considered as spoiled, approximately 25 to 30 isolates were randomly selected by picking colonies from LH plates of the highest dilution showing growth. These isolates were collected and purified twice on brain heart infusion agar (BHI Biokar-Diagnostic, Beauvais, France) and then

each isolate was examined for motility, Gram reaction with KOH (Gregersen 1978), catalase activity by the 3% H_2O_2 method and cytochrome oxidase production by Bactident-Oxidase reagent (Merck, Darmstadt, Germany).

3. Molecular identification of bacterial isolates

In order to characterize the isolates at species or genus level, molecular tests based on polymerase chain reaction (PCR) were used. The chromosomal DNA of the isolated strains was extracted using the Qiagen DNeasy Tissue Kit (Courtaboeuf, France) and oligo-nucleotide PCR obtained from Invitrogen (Cergy Pontoise, France).

For each strain or group of strains identified as belonging to a bacterial species or genus, the 16S rDNA gene was partially sequenced (about 700 bp) for one or several representative, depending on the group size. The 16S rDNA was amplified using the sequences of oligonucleotide primers pairs fD1 and rD1 (Table 1), as described in Macé et al., (2012). The nucleotide sequence of the amplified 16S rDNA was partially determined with an automated sequencer (Beckman Coulter Genomics, Takeley, UK). The sequences were then submitted to the National Center for Biotechnology Information (NCBI, Bethesda, USA, http://www.ncbi.nlm.nih.gov/). The computer program CLUSTAL W (Thompson et al., 1994) was used for sequence alignment and the Basic Local Alignment Search Tool 2 program (BLAST) for representation of sequence and similarity searches in the GenBank database.

Primer	Position	Oligonucleotide sequence (5'-3')	Annealing (°C)	Reference
fD1	16S rDNA gene, forward (positions 8-27)	AGAGTTTGATCCTGGCTCAG	56	Weisburg et al. [1991]
rD1	16S rDNA gene, reverse (positions 1525-1542)	TAAGGAGGTGATCCAGCC	56	Weisburg et al.[1991]
V3P2	16S rDNA gene, forward (positions 517-533).	ATTACCGCGGCTGCTGG	62	Parayre et al.[2007]
V3P3	16S rDNA gene, forward (positions 340-356)	CGCCCGCCGCGCGCGGCGGGCGG GGCGG	62	Parayre et al.[2007]
	with GC clamp.	GGGCACGGGGGCCTACGGGAGGC AGCAG		

Table 1: Sequences of oligonucleotide primers used for PCR amplification of bacteria strain isolated from shrimp.

4. Molecular identification from shrimp matrix direct bacterial DNA extraction

Temporal temperature gradient gel electrophoresis (TTGE) analysis was performed using the homogenized solution, prepared for the enumeration of inoculated strains was used to extract bacterial DNA, directly from the shrimp matrix, detailed by Jaffrès et al., (2009). Bacterial DNA extract was analysed by PCR-TTGE. Primers V3P2 and V3P3 GC-Clamp (Parayre et al., 2007) (Table 1) were used to amplify 16 S rRNA V3 region (194bp) by PCR as described previously (Jaffrès et al., 2009). The size of the PCR products was determined in a 1.5% (w/v) agarose gel (Invitrogen) using a PCR DNA ladder (Fisher Scientific, Illkirch, France).The PCR products obtained from the V3 16S rDNA fragment amplification were subjected to TTGE analysis performed as described by Jaffres et al. (2009). Standardization, analysis and comparison of TTGE fingerprints were monitored using BioNumerics Software, version 6.0 (Applied Maths NV, Sint-Martens-Latem, Belgium).

VIII. Sensory analysis

Sensory analysis was conducted by a taste panel consisting of 80 Tunisian consumers gathered during a National Seafood Exhibition. Treated and control shrimps were individually coded and randomly presented to panelists who shared samples. A four-point hedonic scale was used for rating: 1 = low, 2 = lightly, 3 = moderate and 4 = high quality (Peryam & Pilgrim, 1957) according to its spoilage level (SS: strongly spoiled, LS: lightly spoiled, MS: moderately spoiled, NS: non-spoiled). Panelists were instructed to consume the whole sample and to rinse their mouth with water between samples to minimize any residual effect. Sensory assessment included the following parameters: taste, astringency, color, odor and firmness. Sour odor and firmness loss were chosen as being directly related to autolysis development and astringency as indicating a marked polyphenolic character.

IX. Statistical analysis

For each lot and at each sampling time, the results were presented as mean ± standard deviation (SD) of n = 6 and were analyzed using two-way analysis of variance (ANOVA) using time and treatments as variables along with their interaction effect. Tukey procedure was applied for post hoc comparisons of the data with a significance level fixed at 5%. Results of sensory analysis were first submitted to a correspondence factorial analysis (CFA). Finally, all the data were also analyzed by principal component analysis (PCA). Leverage correction was applied to all the data. Variables were weighted with the inverse of the standard deviation of all data in order to compensate for their different scales. Martens Uncertainty test was used to examine the influence of considered variables on the treatment with polyphenols. Statistics were performed using XL-stat software

X. Results And Discussion

Many food preservation strategies can be used for the control of microbial spoilage and oxidation but even if synthetic antimicrobial and antioxidant agents are approved in many countries, the use of natural safe and effective preservatives is a demand of food consumers and producers (Ortega-Ramirez et al., 2014). Cactus pear (*Opuntia* spp.) cladodes and fruits are rich in bio-active compounds among which polyphenols are the most important (Feugang et al., 2006). Polyphenols, include a large variety of molecules such as flavonoids, betalains and tannins, which are natural secondary metabolites subject of intense investigations owing to their wide spectrum of biological activities which include in particular strong antioxidant and antimicrobial effects (Azeredo, 2009; FAO, 2013). The -OH groups in the phenolic compounds are thought to be largely responsible for these actions (Gelssman, 1963). In present study, polyphenols extracted from cactus fruits peels were used for treatment of peeled shrimp and the effects on biochemical, microbial and sensorial changes during refrigerated storage were studied comparatively to a control lot.

1. Total polyphenolic content and antioxidant activity of cactus fruit peel extract

The total polyphenolic content of the cactus pear peel extract was 1472 ± 7 mg per 100 g of fruit peel. The cactus fruit peel extracts showed a much higher polyphenol proportions compared to the extracts of *Opuntia* cactus fruit pulp (909.47 ± 29.34 mg/l and 15.34 ± 0.73 mg/kg respectively) found in others studies (Khatabi et al., 2011). As expected and in agreement with the strong correlation reported between antioxidant activity and phenolic content in cactus fruit extracts (Butera et al., 2002), the peel extract also showed a strong antioxidant activity with an average value of 100 µmol ± 0.015 of TEAC/g fw which represent 4 to 20 fold stronger source of antioxidants when compared to the pulp, for which values ranged from 4.20 to 26.3 µmol TEAC/g of fresh weight as reported by Butera et al., (2002).

2. Changes in lipid content and in fatty acids composition

The lipid content of the control and treated shrimp during refrigerated storage are presented Fig.1. Initial lipid content of the shrimp *P. longirostris* showed an average value of 0.54±0.04 g/100g. It is within the range of expected values for this species. Indeed, lipid content is reported to range between 0.3 and 3.2% according to Oksuz et al. (2009) with a value of 0.3% for autumn. Cadun et al. (2005) and Huidobro et al. (2002) also found values of 0.35 and 0.31% for frozen shrimps and shrimps covered with liquid ice respectively.

During refrigerated storage, a marked decrease in lipid content was observed in the flesh of both experimental lots; with however distinct profile as decrease was faster and reached a lower level in untreated shrimp lot. Such a decrease is commonly observed during storage of seafood products. This is due to their enzymatic and bacterial degradation according to Chaijan et al. (2006). The different changes observed between the two shrimp lots clearly indicate that polyphenols treatment showed preserving effect on lipids during storage. Such effect could be due to the inhibition of enzymatic and bacterial degradation of shrimp lipid by polyphenols. It is well known that polyphenols may react with protein to give complex (Richard et al., 2006). Such complex formed at the surface of flesh may act as a barrier inhibiting the loss of liquids, including water and lipids as reported in sea bream fillets treated with aromatic plants rich in polyphenolic compounds (Attouchi and Sadok, 2010). It may be supposed that a similar phenomenon occurred in shrimps of polyphenols treated lot of present study.

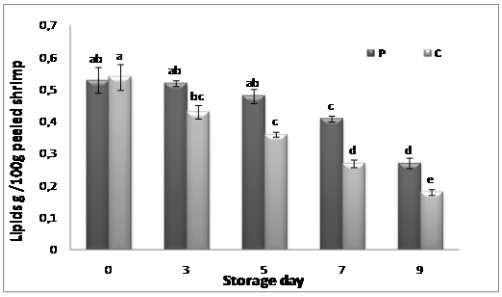

Figure 1: Flesh total lipid content in Control (C) and Polyphenol (P) Treated shrimp during various periods of refrigerated storage (2°C). Data are mean ± Standard Deviation, (N = 6; In Each Case). Values with different superscript letters are significantly different (P < 0.05).

Total fatty acids (FA) compositions of lipids are presented in Table 2. In both lots, saturated fatty acids (SFA) represented the major type of fatty acids followed by polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA). Within SFA, the major FAs were palmitic acid C16:0, stearic acid C18:0 while in MUFA the major FAs were palmitoleic acid C16:1 n-7 and oleic acid C18:1 n-9. The three major PUFA consisted in DHA - docosahexaenoic acid C22:6(n-3), EPA - eicosapentaenoic acid C20:5(n-3) and ARA - arachidonic acid C20:4 n-6. As expected, FA profiles of both lots were similar at the beginning of the experiment. During storage, marked changes appeared in control lot, consisting mainly in a significant ($p<0.05$) decrease in PUFA levels with a concomitant increase in SFA levels while only minor changes appeared in treated lot, with no incidence on relative composition of FA types. These results are clearly indicating that the polyphenolic treatment had preservative effects on PUFAs of peeled shrimps. Similar preservative effects of natural polyphenols, extracted from quince fruit (*Cydonia oblonga* Miller), were reported recently in a study concerning their use freshwater mullet (*Mugil cephalus*) smoked fillets refrigerated during 75 days (Bouzgarrou et al., 2015).

Table 2: Fatty Acids composition of Control (C) and polyphenol treated peeled (P) shrimp following various periods of refrigerated storage (+2°C) at Day 0, 3 and 5. Sfa: Saturated Fatty Acid; Mufa: Monounsaturated Fatty Acid; Pufa: Polyunsaturated Fatty Acid; Others: C15:0, C17:0, C19:0. Data Are Mean±Standard Deviation, (N=3).

Fatty Acids	**Storage time (0 days)**		**Storage time after 3 days**		**Storage time after 5 days**	
	C 0	**P 0**	**C3**	**P 3**	**C 5**	**P 5**
(C14:0)	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
(C16:0)	35.36^{a}	34.07^{a}	40.36^{b}	$37.95^{a,b}$	40.79^{b}	38.17^{b}
(C18:0)	$18.15^{a,b}$	14.44^{a}	18.77^{b}	14.62^{a}	18.77^{b}	14.80^{a}
(C16:1 n-7)	3.99^{a}	4.06^{a}	$4.85^{a,b}$	4.71^{a}	4.24^{a}	5.96^{b}
(C16:3 n-4)	$1.33^{a,b}$	1.6^{a}	1.3^{b}	1.57^{a}	1.24^{b}	1.42^{a}
(C18:1 n-9)	16.42^{a}	15.54^{a}	$14.33^{a,b}$	15.53^{a}	12.27^{b}	$14.18^{a,b}$
(C18:1 n-7)	2.60^{a}	2.34^{a}	3.07^{a}	3.01^{a}	2.47^{a}	2.38^{a}
(C18:2 n-6)	2.29^{a}	1.62^{a}	1.56^{a}	1.60^{a}	1.32^{b}	1.59^{a}
(C18:3 n-3)	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
(C18:4 n-3)	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
(C20:4 n-6)	3.32^{a}	3.44^{a}	3.25^{a}	3.31^{a}	3.05^{a}	3.22^{a}
(C20:5 n-3)EPA	9.38^{a}	9.32^{a}	8.41^{a}	8.99^{a}	7.75^{b}	8.65^{a}
(C22:5 n-3)	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
(C22:6 n-3)DHA	8.33^{a}	8.29^{a}	6.41^{b}	7.82^{a}	6.41^{b}	7.69^{a}
SFA	53.51^{a}	48.51^{a}	59.14^{b}	52.57^{a}	54.55^{a}	52.96^{a}
MUFA	19.95^{a}	16.76^{a}	21.22^{a}	21.24^{a}	18.16^{a}	19.80^{a}
PUFA	23.00^{a}	23.74^{a}	19.64^{b}	21.71^{a}	18.94^{b}	22.10^{a}
n-3	17.71^{a}	17.61^{a}	14.83^{b}	16.81^{a}	14.15^{b}	16.43^{a}
n-6	4.84^{a}	5.07^{a}	4.81^{a}	4.91^{a}	4.37^{a}	4.82^{a}

$^{a–e}$ Means within the same row with different superscripts indicate significant differences ($p<0.05$).

3. Changes in biochemical indicators of quality

Changes in biochemical indicators of quality throughout storage are compiled in Table 3. Initial PV levels were similar for both experimental lots with a value of 0.25 meq active O_2/kg oil. It increased significantly during storage to reach 1.07 meq O_2/kg oil in the shrimp of control lot at the end of the experiment. The shrimp treated with polyphenols showed significantly ($p<0.05$) lower final PV level (0.7 meq O_2/kg oil) than did the control lot, reflecting a reduced lipid oxidation related to treatment effect. The initial values of

TBARs in control and treated shrimp lipid were 0.53 and 0.51 mg MA/kg oil respectively. These values are low and emphasize the excellent quality and freshness of the starting material. Similarly to PV trends, during refrigerated storage TBARs levels increased significantly ($p<0.05$) in peeled shrimp of the two lots but in a lower extent in polyphenols-treated shrimp. Thus pattern of changes of TBARs clearly showed that cactus fruit peel polyphenols exhibited significant antioxidant properties and were able to retard lipid oxidation in flesh of peeled shrimps during the 9 days of refrigerated-storage. Indeed, TBARs levels quantify the accumulation of secondary products of oxidation which are formed along time. The lipid oxidation occurring in peeled shrimps during refrigerated storage is generally due to the high content of unsaturated fatty acids and pro-oxidants in the muscle, (Oksuz et al., 2009). Indeed, it is admitted that relatively high levels of PUFA with important numbers of double bounds in the lipid fraction determine susceptibility of flesh oxidation (Chaijan et al., 2006). It may be supposed that bioactive compounds migrate from the aqueous solution of polyphenols extract to shrimp flesh surface they are adsorbed and act as free radical scavengers. Globally, results of both PV and TBARs showed that cactus-polyphenols exhibited significant antioxidant properties and that the treatment limited and retarded lipid oxidation in peeled shrimps. These results are additional proofs of preservative action of the polyphenols treatment.

Table 3: Changes in Total Volatile Base Nitrogen (TVB), Trimethylamine (TMA), Total Free Amino Acids as ninhydrin positive substances (NPS), Peroxide Value (PV) and Thiobarbyturic Acid (TBARS) in Control (C) and Polyphenols-Treated Peeled Shrimps (P) during various periods of refrigerated storage (2°C). Data are Mean ± Standard Deviation, (N = 6).

Days		0	3	5	7	9
PV	**C**	0.32±0.04 a	0.68±0.03 d	0.67±0.30 cd	0.96±0.06 e	1.07±0.06 e
(meq active O_2/kg oil)	**P**	0.25±0.04 a	0.46±0.02 abc	0.45±0.02 ab	0.60±0.02 bcd	0.73±0.05 d
TBARS	**C**	0.53±0.02 a	0.93±0.02 cd	1.54±0.06 g	1.68±0.04 gh	1.79±0.02 h
(mg MA/kg oil)	**P**	0.51±0.01 a	0.73±0.05 b	0.85±0.01 bc	0.99±0.01 de	1.34±0.02 f
TVB-N	**C**	11.49±0.24 ab	15.54±0.22 c	21.68±0.24 e	28.83±0.14 f	32.07±0.22 g
(mg/100g)	**P**	10.85±0.31 a	12.19±0.30 b	15.96±0.24 c	18.35±0.29 d	22.12±0.29 e
TMA-N	**C**	0.27±0.08 ab	1.75±0.10 d	3.83±0.17 f	4.43±0.10 g	5.92±0.06 h
(mg/100g)	**P**	0.21±0.03 a	0.43±0.04 b	1.07±0.05 c	2.06±0.04 d	3.04±0.11 e
NPS	**C**	0.58±0.01 a	1.34±0.05 c	1.47±0.06 cd	1.68±0.04 de	1.87±0.02 e
(mM/100g)	**P**	0.58±0.01 a	0.67±0.02 ab	0.68±0.03 ab	0.81±0.03 b	1.38±0.07 c

Levels of TVB of both control and polyphenols treated lots remained below the European Commission (CEC, 1995) limit (35 mg-N TVB 100 g^{-1}). Nevertheless, polyphenols treated lot showed significantly lower levels comparatively to control, indicating that the treatment produced an obvious preservative effect on peeled shrimps. There is no regulation concerning the TMA level in crustacean but, beyond 12 mg-N TMA 100 g^{-1} the product quality is generally considered damaged. Similarly to TVB, throughout storage TMA levels of both control and polyphenols treated lots did not exceed this limit and polyphenols treated lot showed significantly lower levels comparatively to control. After 5 and 9 days of storage, when the control and treated batch were rejected by the panelists, none of the lots exceeded this limit. Thus, results indicate that levels of TVB or TMA are not closely linked to the sensory quality of refrigerated peeled shrimp. During the entire refrigerated storage, NPS level of control lot was significantly ($p<0.05$) higher than that of polyphenols treated lot. Free amino acids are produced in fish and shellfish as a result of muscle proteolysis and spoilage progress while tissues serve as a substrate for microbial growth (Ruiz-Capillas & Moral, 2003). These results further confirm preservative action of polyphenols treatment and reveal that it is also related to antibacterial properties of its bio-active compounds which seem effective against shrimp spoiling bacteria.

4. Sensory analysis

Using the four-point hedonic scale, initially nearly similar patterns of percentages were observed in each sensorial attribute for treated and non- treated samples, which exhibited high scores quality. In order to obtain a better understanding of changes of the different quality parameters during storage; the systematic structure in the sensory data was studied by the correspondence factorial analysis (CFA). Together, the first two factorial axes allowed restoration of around 90.37% of the total information (Fig.2). The first and second axis, which restored 77.75% and 12.62% of the total information respectively, allowed the separation of strongly-spoiled SS samples (right part of Fig.2) from non-spoiled NS samples (left part of Fig.2). For instance, control samples (C0) and polyphenols-treated samples (P0 and P3) were located in the NS area; whereas control samples (C5) and treated-shrimp samples (P9) were located in the SS area. Lightly-spoiled LS and moderately-spoiled MS were grouped in intermediate areas. Along storage, changes in sensorial attributes of samples

showed a progressive shift throughout these grouping categories with faster changes for the control comparatively to treated lot. Thus the sensorial results clearly demonstrate that the use of polyphenols extract extended the shelf life of shrimp without altering their initial sensorial properties.

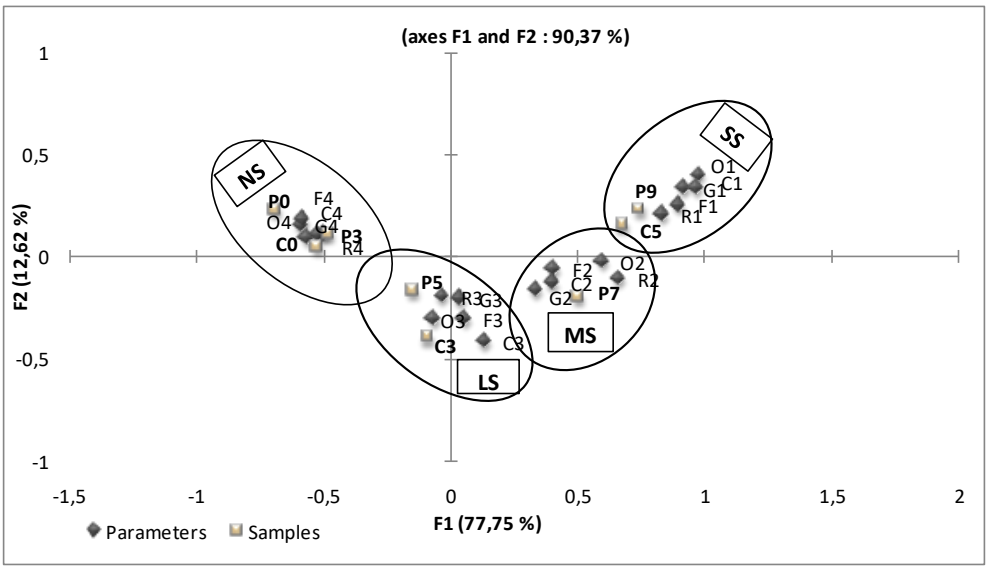

Figure 2: Simultaneous representation of samples and spoilage levels of shrimp samples during various periods of refrigerated Storage (2°C). On Plane 1-2 Of Correspondence Factorial Analysis (CFA). Ns: Non-Spoiled; Ls: Lightly Spoiled; Ms: Moderately Spoiled; Ss: Strongly Spoiled. Sample Nomenclature: C, Control Samples (Non-Treated); P, Treated Sample With Polyphenol. Number Express Duration of Storage (Days). Sensorial Parameters: F: Firmness, G: Taste, O: Odor, R: Astringency, C: Color.

5. Microbial analysis

Initially, control and treated lots displayed total aerobic viable counts (TVC) of 3.7 Log-CFU g^{-1} and 3.8 Log-CFU g^{-1} respectively (Fig.3). During refrigerated storage, the control lot showed a faster microbial development with a TVC value reaching 6.5 Log-CFU g^{-1} after 5 days of storage and thus already overcoming the upper acceptable limit (5 Log-CFU g^{-1}) which was overcome after 9 days in the polyphenols-treated lot (5,4 Log-CFU g^{-1}). The spoilage depends not only on the level of contamination but also on which microorganisms are present on the product. Indeed, microflora of spoiled products consist in a mix of microorganisms that have actively contributed to the spoilage, called Specific Spoilage Organisms (SSO), and microorganisms that have grown without affecting the sensory quality (Gram et al., 2002). Modifying this assemblage of microorganisms and their development may impact product shelf life.

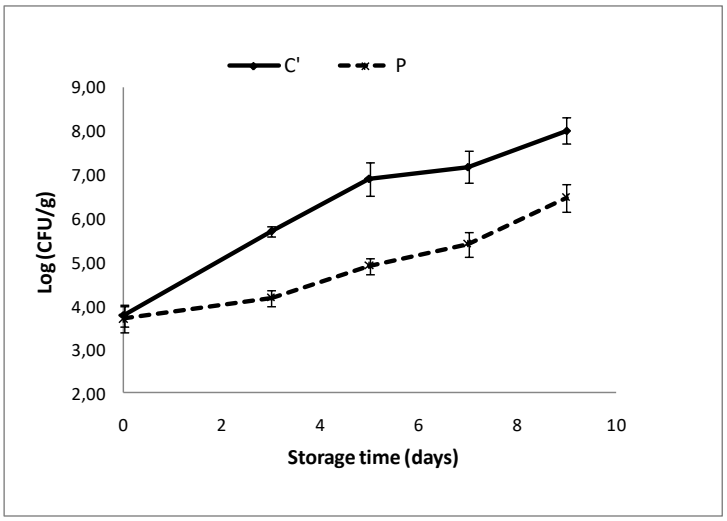

Figure 3: Bacterial growth evolution in Total Aerobic Viable Count (TAVC), of Control (C) and Treated shrimps (P) during various periods of refrigerated Storage (2°C). Values with different superscript letters are significantly different ($P < 0.05$).

The bacterial isolates obtained from control and treated samples were divided into two main groups based on phenotypic tests (Gram reaction, oxidase, catalase). The first consisted in Gram negative and oxidase positive bacterias while the second groups consisted in Gram positive and catalase negative bacterias. Using total 16S rRNA gene sequencing, the strains isolates were identified with 98-100% sequence identity as *Carnobacterium*, *Psychrobacter* and *Brochothrix*. The TTGE analysis enabled the visualization microbiota dynamics by examining fingerprints of dominating bacterial groups evolving during storage (Fig.4). In order to analyze TTGE patterns and to detect the bacterial species, fingerprints of the different samples were compared with those of pure strains of *Carnobacterium*, *Psychrobacter*, *Brochothrix*, isolated from both C and CP lots. The TTGE profiles of shrimp samples during storage revealed 5 different major bands (A to E) among which three bands could be confirmed as corresponding to *Brochothrix* (band D), *Psychrobacter* (band C) and *Carnobacterium* (band B) by comparing the band migration position to that of the reference strain profiles. The two other bands (A and E) were unassigned and were considered as bands related to unknown strains. At beginning of analysis, TTGE profiles of control (C0) and treated (P0) shrimp revealed similar bacterial diversities (bands B, C, D and E) with few differences in bands intensities. This type of profile was observed along storage with variations in band intensities in control lot whereas in treated lot, the profile changed with mainly the disappearance of bands B and D and the apparition of a new band (band A).

Hence, TTGE showed that initially *Brochothrix* was among the major detected microflora and *Carnobacterium*, was detected with lesser intensity. Both these microflora, maintained in control lot during storage while they were not further detected in treated lot. Thus the antimicrobial activity of the extracts against microbial growth appeared the most effective against *Brochothrix* and *Carnobacterium*. For the other microflora, a reduction was seen on not only the rate of increase, but also in the bacterial counts in the treated shrimp during storage.

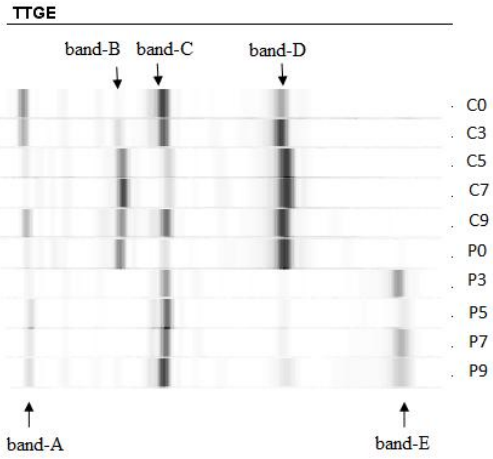

Figure 4: Fingerprints and bacterial population dynamics of peeled shrimps during storage. Digitized TTGE profiles of 16s Rrna Gene V3 Regions obtained by PCR amplification from bacterial DNA of six samples (Day 0, Day 3, Day 5, Day 7, Day 9) from 2 batches (C: Control Shrimps; P: Treated Shrimps) stored at 2°C. Assignment of PCR-TTGE Bands obtained from shrimp matrix to bacterial reference strain profiles: Band-A, Unassigned; Band-B, *Carnobacterium*; Band-C, *Psychrobacter*; Band-D, *Brochothrix*; and Band-E, Unassigned. The unassigned bands are considered as related to unknown strains.

When growing, the metabolic activity of *Brochothrix* spoils the product with the emission of off-odours (Jaffrès et al., 2011). Thus the inhibition of *Brochothrix* in treated shrimps may contribute to explain the extension of sensory shelf-life. Polyphenol treatment by reducing bacterial development as well as the related activity of endogenous enzymes slows down the rate of deterioration during storage. Antimicrobial coatings techniques are commonly applied to improve food safety, including seafood products (Dupard et al., 2006; Jiang et al., 2011; Guo et al., 2013). The polyphenolic extract used in present study seems to be potentially useful for this kind of applications.

6. Principal component analysis (PCA)

All the obtained data were submitted to principal component analysis (PCA) in order to assess the effect of the treatment on the biochemical, microbial and sensory quality of shrimp flesh (Fig.5a and 5b). Together, the first two principal components (PC1 and PC2) accounted for 83% of total system variability. For PC1, the biochemical and bacterial growth variables were positively correlated with the storage time while lipid

and sensory attributes were negatively correlated with it. For PC2, the polyphenols treatment was positively correlated with sensory analysis. Globally, samples of control and treated shrimps were grouped into two distinct clouds confirming the positive effect of polyphenols treatment on bio-preservation of peeled shrimp during refrigerated storage.

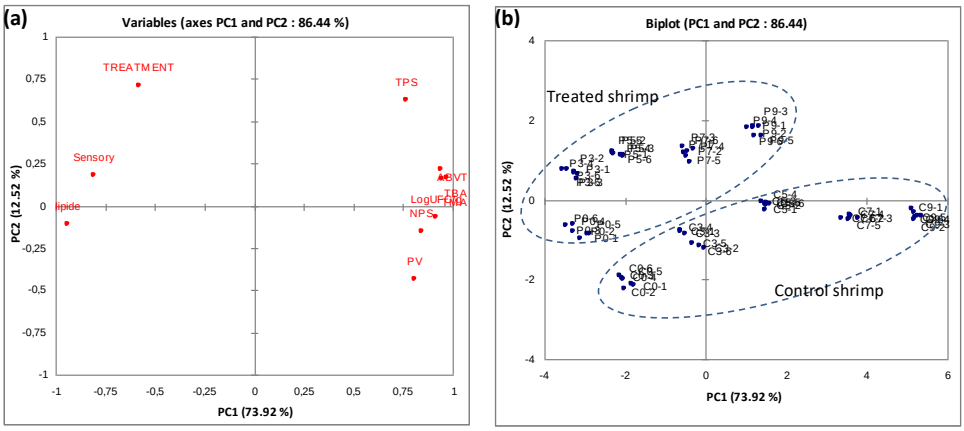

Figure 5: Score (A) and loading (B) Plots from the Principal Component Analysis (PCA) carried out on all variables (Tps: Storage time, TMA, ABVT, NPS, Sensory analysis, Bacterial growth and treatment). Treated (P) and Control groups (C) are encircled.

XI. Conclusion

Polyphenols extract from cactus peel fruits was evaluated for its antibacterial and antioxidant activities. It appeared to have promising applications in aquatic food processing as the natural antioxidants reduced the final microbial load during refrigerated storage as well as biochemical degradation in peeled shrimps. The use of such extract, either in the frame of sound application in local contexts or in the frame of industrially produced extracts, may also be regarded as a new opportunity for the valorization of an under-exploited agro-resource. In addition, the study of bacterial communities and the PCR-TTGE technique used in this work appeared as an excellent tool to distinguish species that are difficult to enumerate separately by culture-dependent methods and to monitor the evolution of the microbial ecosystem in seafood products during storage. This polyphasic study has improved our knowledge of the spoilage microbiota of peeled shrimp and allowed an objective evaluation of the efficiency of a natural additive

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