

**Food Science and Nutrition**

**REGULAR ARTICLE**

**Development of a mayonnaise with chitosan as natural antioxidant**

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**Abstract**

The aim of this work was to evaluate the influence of addition of chitosan as a natural antioxidant in the stability of mayonnaise. Three formulations were developed with EDTA and chitosans with different molecular weight. Mayonnaises were stored at 37; 47 and 57°C for 63 days. During this period, it was analyzed weekly for the peroxide and acidity index. The mayonnaises with chitosan obtained better results for the attributes of typical odor and taste respect to the mayonnaise control. It has been observed that addition of chitosan slowed down the lipid oxidation process of mayonnaises during 63 days of accelerated storage. The mayonnaise with chitosan with the bigger molecular weight showed better stability during accelerated storage at all temperatures. Further research on the effect of the deacetylation degree of chitosan in the preservation of mayonnaise is recommended to assess the efficacy of chitosan as a natural preservative for food.

*Key words:* Mayonnaise, Chitosan, Antioxidant activity, Lipid oxidation

**Introduction**

Mayonnaise is formulated to develop characteristics, such as, mouthfeel and spreadability which are considered desirable by the consumer. Due to instable characteristics of mayonnaise there might be some changes in product during shelf storage, and any kind of change in product's properties might have negative effect on consumers' attitude towards the product. Due to the commercial importance of mayonnaise, many investigations have been conducted on the stability and rheological properties of the product (Kiosseoglou and Sherman, 1983).

Oxidative rancidity is one of the major problems in relation to the use of vegetable oils. Even a hint of a rancid flavor can ruin an entire batch of salad dressing or mayonnaise. Time, temperature, light, air, surface exposure, moisture, nitrogenous organic material, and traces of metals are responsible for oxidative rancidity of salad dressings and mayonnaise (Yang and Lal, 2003). In association with alterations of food, there has been a demand from customers to produce mayonnaise

with less fat content and without chemical preservatives. In accordance with these trends, instead of applying traditional chemical preservatives, producers are focused on the application of naturally occurring preservatives.

Nowadays there are a number of natural antioxidants deriving from vegetable sources that have demonstrated very good effect to retard oxidation in many food rich in fats and oils. Spices and herbs added into food emulsions improve their consumer acceptance, flavor and oxidative stability. Some authors have evaluate the antioxidant activity of spices and herbs added to pure oils, but there are not many references relating to the influence of spices on the oxidative stability of oil-in-water emulsions (Mihov et al., 2012).

Different authors have observed the antioxidant activity of chitosan derivatives. For instance, Yin, Lin, Zhang and Yang (2002) reported scavenging activity of low molecular weight chitosan (LMWC) on superoxide radicals was more marked than that of chitosan with high molecular weight. In addition, it was informed that LMWC (0.5 mg/mL) has a scavenging activity on superoxide radicals of 80.3% (Esumi et al., 2003). Yin et al. (2002) reported that the activity of hydroxyl radicals can be suppressed by gold-chitosan nanocomposites. Xing et al. (2004) informed that low molecular weight sulfated chitosan had stronger scavenging activity on superoxide/hydroxyl radicals than that of high molecular weight sulfated chitosan.

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Nevertheless, very few attempts have been made to assess the antioxidant activity of chitosan in foodstuffs.

In this sense, the aim of this work was to evaluate the influence of the addition of chitosan as natural antioxidant in the physicochemical and sensory stability of mayonnaise. It could be an innovative and interesting commercial product and an alternative to the use of synthetic or chemical products.

## Materials and Methods

### Raw materials

In the elaboration of the mayonnaises it was used refined soybean oil, fresh eggs, pasteurized vinegar (5,1%, v/v of acetic acid), white sugar, sodium chloride, starch as thickener, potassium sorbate as preservative, guar and xanthan gums as stabilizers, citric and lactic acids as acidity regulators, etilendiaminetetracetic acid (EDTA) and chitosan as antioxidants. All ingredients were selected according to the statements of NC 277 (2008). Chitosans I (MW=310 kDa and DD=77.7%) and II (MW=123 kDa and DD=83.2%) were obtained at Drugs Research and Development Center (Havana, Cuba).

### Sample preparation

The 10 kg of mayonnaise for each formulation were developed according to NC 277 (2008). It was added 75 mg of EDTA (Formulation control) and 100 mg of chitosans I (Formulation Q1) and II (Formulation Q2) *per* kg of product, respectively.

EDTA was dissolved in a small aliquot of the oil used in the formulation, and then was mixed with the rest of the oil. Since chitosan is only soluble in an acidic medium, vinegar was used to dissolve chitosan.

The eggs were selected and washed with chlorinated water (5-10 ppm) (NEIAL 1645, 2007) for 3 min before cracking, to avoid possible contamination of the emulsion with microorganisms on the shell.

All product samples were elaborated under the same conditions to avoid possible differences, such as, process variables and loss of ingredients. First, solid ingredients were mixed, and then, the water was added to obtain a paste due to hydration of the starches. After that, the eggs were added and finally, the oil at 12°C and vinegar was added slowly, homogenizing the mixture until obtaining the emulsion.

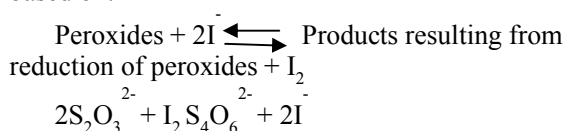
The product was packed in presence of air in the headspace of glass containers of 314 mL with metallic twiss off top ( $\varnothing = 63$  mm) and then stored in electric ovens at 37; 47 and 57°C during 63 days.

## Evaluation of the product during the accelerated storage

### Physicochemical analyses

Free acidity (% w/w acetic acid) and peroxide index (meq O<sub>2</sub> / kg of oil) were measured by standard techniques (EEC/2568, 1991) in the samples after the packaging (day 0) and every 7 days until 63 days of accelerated storage.

Free acidity was determined by titration of a sample of mayonnaise dissolved in ethanol/ether mixture with ethanolic solution of potassium hydroxide. Peroxide value was determined as follows: a mixture of mayonnaise and chloroform/acetic acid 3:2 (v/v) was left in darkness to react with saturated potassium iodine solution. During this reaction, the free iodine was titrated with a sodium thiosulfate solution (0.1 N), based on:



### Sensory evaluation

Quantitative Descriptive Analysis (Stone and Sidel, 1998) was used to evaluate sensory qualities of the mayonnaises at day zero and at the end of accelerated storage at 37°C. Ten trained panelists evaluated products and responses were analyzed with some modifications according to ISO 4121 (1987) through a structured scale of 10 cm delimited at both ends. Samples were served to each panelist separately under white illumination. Water was provided for cleansing the palate between samples. The sensory attributes used in the current study were color, flavor and overall acceptance. The evaluations were carried out according to a balanced blocks design (Costel and Durán, 1981).

### Microbiological evaluation

Microbiological evaluation was carried out for all treatments at the beginning of accelerated storage. A 10 g sample and 90 mL of sterilized distilled water were homogenized. The homogenate was serially diluted with sterilized distilled water, and the dilutions were spread on the specific medium in aerobic conditions. Total aerobic bacteria (35 ± 1°C, 48 hours), (NC-ISO 4833, 2003) were performed with plate count agar (PCA, Biocen, Havana, Cuba). The malt extract agar (MEA, Biocen) was used to grow fungi and yeasts (30°C, 48-72 hours), (NC-ISO 7954, 2002). Colony forming units (CFU) per gram in plates were counted, at a dilution giving 30-300 CFU per plate, with Micro Counter.

### Statistical analysis

Analyses of Variance were performed using STATISTICS software (STATISTICS, 1998) and the Duncan's multiple range tests were used to compare differences among mean values. Mean values were reported, and the significance was defined at  $p \leq 0.05$ .

### Results and Discussion

#### Characterization of mayonnaises

Table 1 shows the results of the physicochemical and microbiological evaluations of the mayonnaises at day zero. The values of peroxide index oscillated between 0.27 and 0.33 meq O<sub>2</sub>/kg, values that are very below the maximum established limit; the values of acidity index stayed in the three formulations inside the range allowed in the regulations. The results of the microbiological determinations, in all the cases stayed in the range of specifications settled down by NC 50 (1999).

Sensory evaluation shows a satisfactory quality of the mayonnaises. It was not observed remarkable differences in the mayonnaises with chitosan in respect to the control. Adding chitosan in the formulation did not influence in the sensory characteristics and there were no stranger odor and taste observed (Table 2).

#### Evaluation of the product during accelerated storage

##### Acidity index

Figure 1 shows the behavior of the acidity index of the mayonnaises during the storage at 37; 47 and 57°C, respectively. It was observed that the acidity index of the mayonnaises spreads stayed almost constant for all treatments during accelerated storage. It can be observed that the acidity index of formulations Q1 and Q2 presented a

similar behavior and remained almost invariable during the storage; however, in the mayonnaise with EDTA (formulation control), although the acidity index varied slightly, this formulation presented an uncertainly behavior during storage compared with formulations Q1 and Q2 at all temperatures; the biggest variability was observed at 57°C.

After 63 days of accelerated storage at three temperatures, the acidity index did not surpass, in none of the cases, the maximum limit admitted by NC 50 (1999), although a slight increase is evidenced in this value with regard to the values obtained to the beginning in the three formulations; one can observe that the used chitosan didn't influence in a negative way in this parameter. Although it is important to highlight that at the moment of elaboration of these formulations, the mayonnaises with chitosans Q1 and Q2, presented a superior value of acidity index compare to that of the mayonnaise with EDTA, which possible related with the molecular weight and deacetylation degree of chitosans, being observed that the formulation Q1, with the smaller deacetylation degree (77.7%) presented the biggest value for this index.

The statistical analysis of the results showed significant difference ( $p \leq 0.05$ ) among the mayonnaises (Table 3). It is observed that at 37 and 47°C, the mayonnaises with chitosan presented significant difference ( $p \leq 0.05$ ) compared with the mayonnaise control, which presented superior values of acidity index during the storage; at 57°C significant difference was not observed ( $p \leq 0.05$ ) among the three formulations for acidity index, although the mayonnaise control presented, as tendency, bigger values than the formulations Q1 and Q2 at the three temperatures.

Table 1. Results of the physicochemical and microbiological evaluations.

Treatments	Physico-chemical characteristics		Microbiological evaluation	
	Peroxide index (meq O <sub>2</sub> /kg)	Acidity index (% w/w of acetic acid)	Fungi and yeast (log CFU/g)	Total aerobic bacteria (log CFU/g)
Control	0.33	0.62	1	1.3
Q1	0.27	0.84	1	1.3
Q2	0.27	0.76	1.3	1.3

Control: Formulation with EDTA; Q1: Formulation with chitosan I (MW=310 kDa and DD=77.7%); Q2: Formulation with chitosan II (MW=123 kDa and DD=83.2%).

Table 2. Sensory scores of mayonnaises prepared with chitosan (n = 10).

Attributes	Treatments		
	Control	Q1	Q2
Typical color	8.5 (0.2)	9.5 (0.2)	9.6 (0.2)
Typical odor	8.8 (0.5)	9.6 (0.3)	9.6 (0.2)
Rancid odor	0.6 (0.3)	0.4 (0.2)	0.4 (0.2)
Typical taste	8.9 (0.6)	9.3 (0.3)	9.4 (0.4)
Rancid taste	0.8 (0.6)	0.4 (0.2)	0.4 (0.2)
Stranger taste	0.8 (0.6)	0.3 (0.2)	0.4 (0.1)
Overall acceptance	9.6 (0.1)	9.8 (0.1)	9.8 (0.1)

Mean (standard deviation).

Control: Formulation with EDTA; Q1: Formulation with chitosan I (MW=310 kDa and DD=77.7%); Q2: Formulation with chitosan II (MW=123 kDa and DD=83.2%).

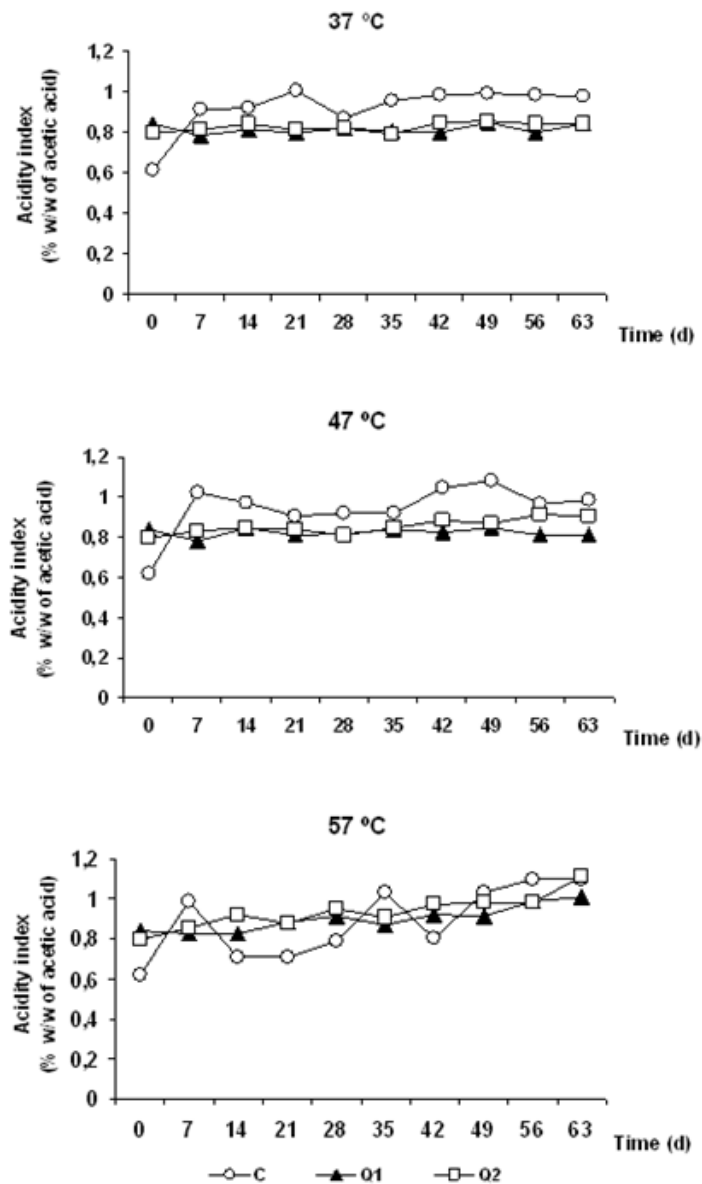


Figure 1. Behavior of the acidity index during the accelerated storage. C: Formulation with EDTA; Q1: Formulation with chitosan I (MW=310 kDa and DD=77.7%); Q2: Formulation with chitosan II (MW=123 kDa and DD=83.2%).

### Peroxide index

Figure 2 shows the behavior of the mayonnaises elaborated to the three temperatures used in this investigation. At 37°C a similar behavior was observed among the three formulations during storage, presenting at the beginning and the end, the superior values of peroxide index, the mayonnaise control and the inferior values, the formulation Q1. It was observed that between the 21-day and 42-day of storage, all formulations presented similar and almost constant values to this temperature. The formulation Q2 maintained intermediate values between the mayonnaises control and formulation Q1, respectively, as much to the beginning as to the end of the storage.

During storage at 47°C, certain tendency was observed to the gradual increase of the values of peroxide index in the three formulations, maintaining superior values, from the beginning, the mayonnaise control. The formulation Q1 maintained inferior values of peroxide index compared to the formulations control and Q2; and the formulation Q2 maintained, throughout the storage, intermediate values between the formulations control and Q1. At 57°C a similar behavior was observed among the three formulations to those observed at 47°C.

The difference in the behavior of the values of peroxide index among the mayonnaises with chitosan (although both mayonnaises presented inferior values to the mayonnaise control for this

parameter), it could be due to the influence of the molecular weight in the antioxidant capacity, taking like reference that described by Youn et al. (2001) that reported that the antioxidant activity of chitosan increased with the increasing of molecular weight.

Considering that the chitosan Q1 has bigger molecular weight with regard to the chitosan Q2, and being observed that the formulation Q1 maintained, to all the temperatures used in this study, inferior values of peroxide index to the values presented by the formulation Q2, it can be concluded that the one that bigger retard in the increase of the value of peroxide index presented in this investigation, was the formulation Q1 that is the chitosan with the bigger molecular weight.

Table 4 informs the statistical analysis of the influence of temperature in the index of peroxide. It is observed that at 37°C, there was not significant difference ( $p \leq 0.05$ ) among the elaborated mayonnaises; at 47°C the mayonnaise control differs significantly ( $p \leq 0.05$ ) respect to the mayonnaises with chitosan, showing the biggest value of peroxide index; at 57°C, the mayonnaise control and the formulation Q2 did not show significant difference ( $p \leq 0.05$ ), while they both differed significantly ( $p \leq 0.05$ ) with the formulation Q1, showing this the smallest value of index of peroxide. This result can be influenced by the ones explained in the analysis of Figure 2, referred to the antioxidant capacity of the chitosan with regard to the molecular weight.

Table 3. Means for acidity index of mayonnaises from duncan's multiple range tests.

Treatments	Acidity index (% m-m of acetic acid)		
	37 °C	47 °C	57 °C
Control	0.91 (0.1) c	0.94 (0.1) c	0.90 (0.1) bc
Q1	0.82 (0.02) a	0.82 (0.02) a	0.90 (0.06) bc
Q2	0.83 (0.02) a	0.85 (0.03) ab	0.93 (0.08) c

Different letters indicate significant difference ( $p \leq 0.05$ ).

Control: Formulation with EDTA; Q1: Formulation with chitosan I (MW=310 kDa and DD=77.7%); Q2: Formulation with chitosan II (MW=123 kDa and DD=83.2%).

Table 4. Means for peroxide index of mayonnaises from duncan's multiple range tests.

Treatments	Peroxide index (meq O <sub>2</sub> /kg)		
	37°C	47°C	57°C
Control	0.60 (0.1) bcde	0.65 (0.1) de	0.68 (0.1) e
Q1	0.54 (0.1) ab	0.50 (0.1) a	0.51 (0.1) a
Q2	0.58 (0.1) abcd	0.55 (0.1) abc	0.62 (0.1) cde

Different letters indicate significant difference ( $p \leq 0.05$ ).

Control: Formulation with EDTA; Q1: Formulation with chitosan I (MW=310 kDa and DD=77.7%); Q2: Formulation with chitosan II (MW=123 kDa and DD=83.2%).

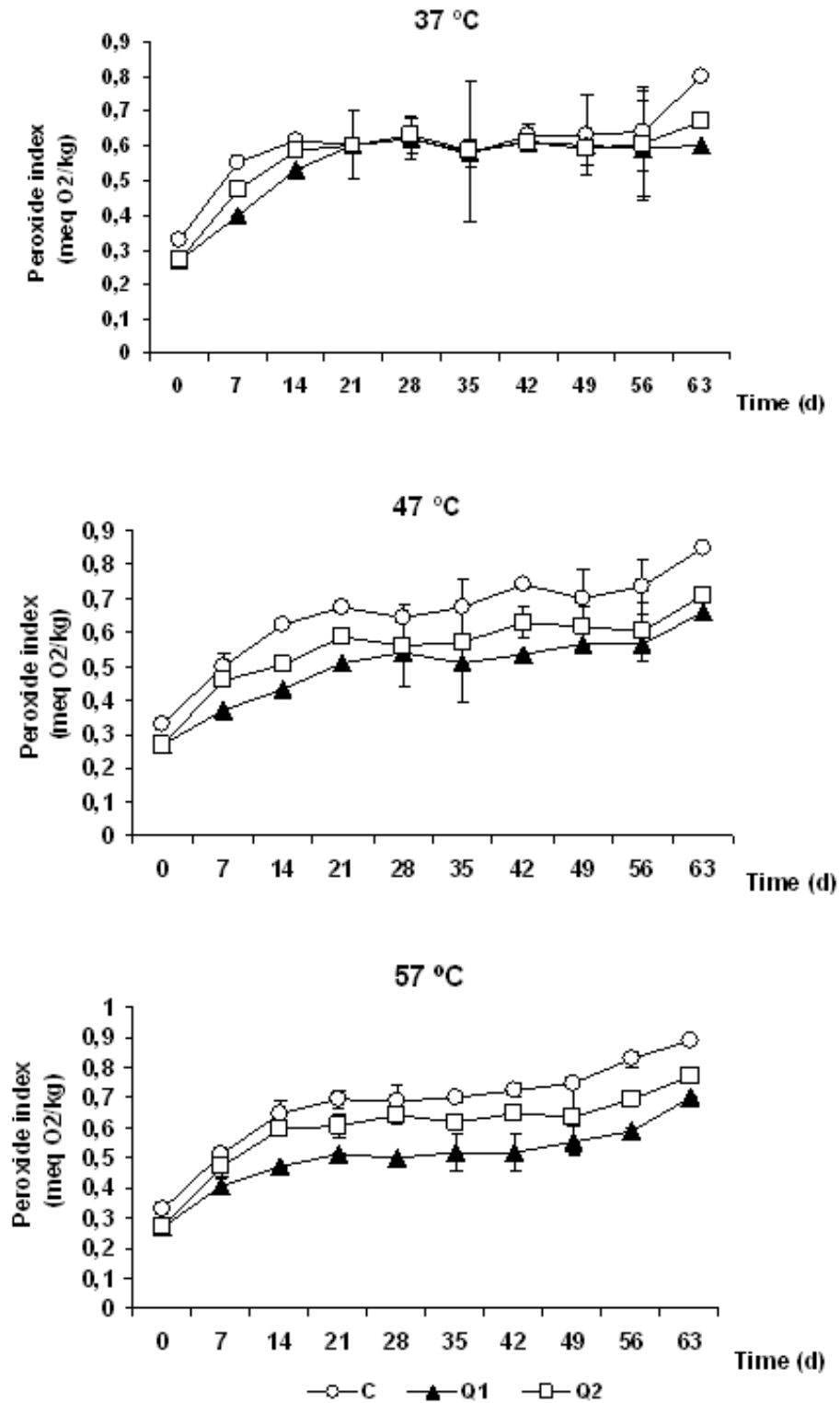


Figure 2. Behavior of the peroxide index during the accelerated storage. C: Formulation with EDTA; Q1: Formulation with chitosan I (MW=310 kDa and DD=77.7%); Q2: Formulation with chitosan II (MW=123 kDa and DD=83.2%).

Considering the results explained previously, it can be observed that the chitosan can be used in the mayonnaises as an antioxidant, because compared with the value of peroxide index obtained in the mayonnaise elaborated with EDTA, of 0.89 meq O<sub>2</sub>/kg for 57°C, the mayonnaise elaborated with Q1 presented a value of 0.70 meq O<sub>2</sub>/kg to the same temperature, and the mayonnaise elaborated with Q2, a value of 0.77 meq O<sub>2</sub>/kg. The mayonnaises with chitosans showed inferior values of peroxide index with regard to the one elaborated with EDTA. Chitosans slowed the oxidation from the fats to the temperatures used at the same time in this study that EDTA.

Velásquez (2007) reported that mayonnaises elaborated with BHT and alpha tocoferol at 5; 25

and 35°C for 60 days of storage, presented 0.6 and 1.3 meq O<sub>2</sub>/kg, respectively to 5°C; at 25°C, the values were of 1 and 2.5 meq O<sub>2</sub>/kg; and at 35°C the values were 4 and 12 meq O<sub>2</sub>/kg, respectively. García and Molina (2008) obtained values of peroxide index at 60 days of 6 and 14 meq O<sub>2</sub>/kg, at 21 and 35°C, respectively; at 45°C they carried out the experiment until the 42 days because the product had already reached levels of peroxides related with the sensory rejection of mayonnaise.

Chitosans were able to slow the lipid oxidation in the mayonnaise, still at the higher temperatures. It should be pointed out that with regard to EDTA, both chitosans (Q1 and Q2) maintained a similar behavior as for values of index of peroxide, staying below 1 meq O<sub>2</sub>/kg in all the cases.

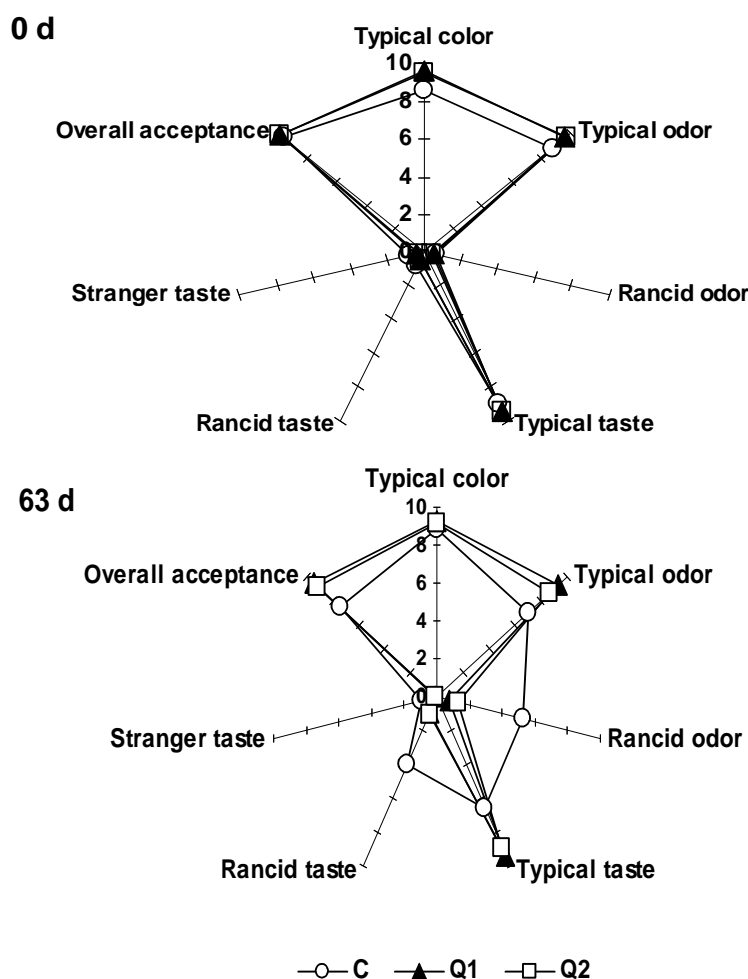


Figure 3. Descriptive quantitative profile of the recently elaborated mayonnaises and after 63 days of accelerated storage at 37°C. C: Formulation with EDTA; Q1: Formulation with chitosan I (MW=310 kDa and DD=77.7%); Q2: Formulation with chitosan II (MW=123 kDa and DD=83.2%).

### Sensory evaluation

Figure 3 shows similar values for the global quality of all treatments, although the panelists assigned a bigger punctuation in the color attributes and characteristic scent to the mayonnaises with chitosan, without these they differed between both. The chitosan addition did not affect the organoleptic characteristics of mayonnaises.

At 63 days, in the evaluation carried out to the mayonnaises stored at 37°C, it is observed that the mayonnaises with chitosan showed a similar behavior to the one described at time zero (Figure 3), while in the mayonnaise control, the judges detected a strange slight flavor, related with a bitter taste and odor and slight rancid taste, although the value of peroxide index was not superior to 1 meq O<sub>2</sub>/kg (Figure 2).

Likewise a decrease is appreciated in the punctuation assigned to the characteristic odor and taste, that which could be related with the appearance of rancid odor and taste.

The global quality of the mayonnaises with chitosans was excellent during storage, while for the mayonnaise control, it varied from excellent to acceptable at the beginning until the end of the storage, respectively.

The formulation Q1 presented the smallest punctuations in the attribute of rancid taste and the biggest punctuations for characteristic odor and taste; likewise the judges granted the biggest punctuation for global quality (Figure 3).

### Conclusions

This paper shows the possible use of chitosan as a natural antioxidant ingredient in the formulation of mayonnaises. The mayonnaises with chitosan obtained bigger punctuations for the attributes of typical odor and taste respect to the mayonnaise control. Chitosan addition slowed the lipid oxidation of mayonnaises during 63 days at 37; 47 and 57°C. The mayonnaise with chitosan with the bigger molecular weight showed better stability during accelerated storage at evaluated temperatures. Further research on the effect of the deacetylation degree of chitosan in the preservation of mayonnaise is recommended to assess the efficacy of chitosan as a natural preservative for food and the shelf life of the products should be estimated.

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