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Study of the encapsulation of hydrophilic nutraceuticals.

Estudio de la encapsulación de nutracéuticos hidrofílicos.

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Most people say that it is the intellect which makes a great scientist. They are wrong: it is character.

Albert Einstein

A mi tutora de proyecto, la Dra. Esther Santamaría, por guiarme, aconsejarme y corregirme. A mis compañeros de laboratorio, por la ayuda recibida y por hacer el trabajo más ameno. A todos los compañeros del grado con lo que he convivido durante este periodo, por los cafés y las charlas soñadoras. A mi familia por aguantar mi mal humor ante el trabajo acumulado y por apoyarme en conseguir mis metas.

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SUMMARY

Polyphenols are examples of hydrophilic nutraceuticals, which are contained in a relatively large amount in fruits or vegetables. The concentration or extraction of the fruits allows working at higher concentrations of polyphenols. Polyphenols are compounds rich in antioxidant properties. As antioxidants, they help protect the body's cells from free radicals, thus controlling the rapidity of aging and the development of heart disease, cancer and Alzheimer, among others.

Raspberries, belonging to the berry fruit group, are foods rich in polyphenols and therefore provide great health benefits.

The introduction into the food of active ingredients that may be beneficial to health requires in many cases an encapsulation that prevents its degradation or facilitates absorption by the body.

The objective of this work is to study the encapsulation of concentrates or extracts of fruits to avoid their degradation and to facilitate their incorporation in foods.

The number of polyphenols and the percentage of antioxidant activity containing lyophilized raspberry, as well as the degradation of these properties, will be analyzed.

Previous experiments will be carried out to analyze the properties correctly, as well as the extraction method, the amount of reagent to be used in the analysis and the most suitable hydrocolloid among the most common, alginate, gellan, etc., which will be gelled by the addition of Cations.

Finally, the physical properties of the beads in the texturometer will be analyzed.

Keywords: Raspberry, Antioxidant activity, Polyphenols, Encapsulation, Hydrocolloid, Hydrophilic Nutraceuticals

RESUMEN

Los polifenoles son ejemplos de nutracéuticos hidrofílicos, que están contenidos en una cantidad relativamente grande en frutas o verduras. La concentración o extracción de los frutos permite trabajar en concentraciones mayores de polifenoles. Los polifenoles son compuestos ricos en propiedades antioxidantes. Como antioxidantes ayudan a proteger las células del cuerpo de los radicales libres, controlando así la rapidez del envejecimiento y el desarrollo de enfermedades cardiacas, cáncer y Alzheimer, entre otras.

Las frambuesas, pertenecientes al grupo de frutas de bayas, son alimentos ricos en polifenoles y por tanto proporcionan grandes beneficios para la salud.

La introducción en los alimentos de ingredientes activos que pueden ser beneficiosos para la salud requiere en muchos casos de una encapsulación que impide su degradación o facilita la absorción por el cuerpo.

El objetivo de este trabajo es estudiar la encapsulación de concentrados o extractos de frambuesas para evitar su degradación y facilitar su incorporación en los alimentos.

Se han analizado la cantidad de polifenoles y el porcentaje de actividad antioxidante que contiene la frambuesa liofilizada, así como la degradación de estas propiedades.

Previamente se realizaron experimentos para analizar las propiedades correctamente, así como el método de extracción, la cantidad de reactivo a usar en los análisis y el hidrocoloide más adecuado entre los más comunes, alginato, gellan, etc., que se gelificarán mediante la adición de cationes.

Finalmente, se analizaron las propiedades físicas de las capsulas en el texturometro.

Palabras clave: Frambuesa, Actividad antioxidante, Polifenoles, Encapsulación, Hidrocoloide, Nutracéuticos Hidrofilicos.

1. INTRODUCTION

Many common foods contain beneficial properties for our health. For this reason, food studies are conducted to try to preserve these beneficial properties. Berry fruits such as raspberry, blackberry or strawberry are an example of these beneficial aliments.

1.1. NUTRACEUTICALS AND PROBIOTICS

Nutraceuticals are biological substances obtained from the natural sources that are characterized by anti-denaturing biotechnological processes to preserve their original properties without any chemical manipulation. [1]

Because of the virtues of nutraceuticals, some of these bio-derivatives act as potential medicines and can be prescribed for preventive or healing purposes.

The classification of nutraceuticals belongs to the aliment group that it takes part, being structurally and functionally similar. The classification according to the chemical composition is antioxidants, C and E vitamins, phenolic compounds, saponins, inulin and oligofructose, oils, fatty acids and phospholipids. [1]

Nutraceuticals may be used to improve health, delay the aging process, prevent chronic diseases, increase life expectancy, or support the structure or function of the body. Nowadays, nutraceuticals have received considerable interest due to potential nutritional, safety and therapeutic effects [2], but especially for the advantage over the medicine, because they avoid side effect, have naturally dietary supplement, etc.

Probiotics are living microorganisms that once ingested perform positive actions to the intestinal physiology promoting or favouring the health. The original observation of the beneficial properties conferred by some bacteria is attributed to the Nobel Prize winner Eli Metchnikoff, who is regarded as the grandfather of modern probiotics. In the early 20th century, Metchnikoff discovered that “healthy bacteria,” especially lactic acid bacteria (LAB), can have a positive influence on digestion and the immune system [3]. Most microorganisms recognized to date as probiotics are Gram-positive, with Lactobacillus and Bifido- bacterium being the main species

used as treatments of intestinal dysfunctions [4]. However, some Gram-negatives are also used as probiotics.

Probiotics are usually sold as beads, powders and combinations of different species which may have multiple advantages [5]. It has been probed in various animal studies that the use of one or more species of probiotics can have beneficial effect and it is logical to assume that a mosaic of probiotics could help in exerting multiple benefits they possess [6, 7].

The main goal of any food industry is to increase the versatility in consumption of different forms of food without losing its basic properties of providing nutrition and ensuring health.

Use of berries with probiotics has been tested for uropathogenic and urogenital disorders [8]. The proanthocyanins present in berries, such as cranberries, can modulate the immune system in conjunction with probiotics [8]. Anthocyanin rich raspberries also have a high amount of dietary fibers (6.5 g/100 g) with good absorption characteristics which could potentially serve as a carrier and microencapsulating agent as well as a prebiotic [9].

1.2. RED RASPBERRY

Berry fruits have long been regarded as having considerable health benefits due to their nutritional attributes, particularly their total antioxidant activity against cellular oxidation reactions [10]. These benefits have stimulated research to regularly investigate the phenolic status and antioxidant activity of distinct berry fruit species and new varieties in different countries.

A great variety of species exist from diverse botanical families (blueberries [*Vaccinium corymbosum* L., Ericaceae], strawberries [*Fragaria ananassa* Duch., Rosaceae], red raspberries [*Rubus idaeus* L., Rosaceae], and blackberries [*Rubus* sp., Rosaceae]), whose small purple or red fruits are denoted as berries [11]. Due to the various botanical families and botanical fruits to which polyphenol-rich fruit species belong, a wide range of phenolic contents and corresponding antioxidant activities may be expected according to the specific compounds that are present in these species.

There is great interest in studying berries as food ingredients for their bioactive constituents. Collected evidences from in vitro and in vivo studies have supported the cardio-metabolic benefits of red raspberries [12].

Considering genetic differences among wild relatives from natural population and cultivars of berries, a potential variability can be observed in their contents and composition of bioactive compounds [13]. Berries are rich sources of phytochemicals such as sugars, organic acids, and

phenolic compounds. Sugars and organic acids are their main soluble constituents and have major effect on taste and fruit ripeness, or even represent a suitable index of consumer's acceptability [14]. Type and quantity of individual compounds also affect fruit taste; therefore, the composition and concentration of these compounds may reflect changes in fruit quality [14].

Berries are a rich source of ascorbic acid [15]. Although the ascorbic acid content is on average 1.5 to 3-fold lower in strawberries, raspberries, blackberries and red currants, compared to black currants, their average values over 40 mg per 100 g of fresh weight are still high, and make them a rich source of vitamin C.

The anthocyanins are responsible for the color of polyphenol-rich fruit species [16-21]. In addition to their colorant properties, anthocyanins have been associated with a wide range of biological, pharmacological, anti-inflammatory, antioxidative, and chemoprotective properties [22].

An important question that arises from the preliminary screening of berries relates to the polyphenolic profiles of the species, specifically, which components within these profiles contribute most to differences in bioactive potential. These compounds are mainly represented by flavonoids, phenolic acids, and tannins, which are known as natural antioxidants [23]. Ellagic acid is the predominant phenolic acid in strawberries, raspberries and blackberries [24], and chlorogenic acid in blueberries [25]. Apart from phenolic acids, these berries contain high levels of flavonols, such as quercetin, kaempferol, and myricetin, as well as their derivatives (primarily glycosides), which may provide health benefits as dietary antioxidants [26]. Combining this information with an increasing knowledge of the structure of these compounds has enabled the selection of parents for breeding new, highly health-protective cultivars for the future.

1.3 POLYPHENOLS

Polyphenols are largely found in fruits, vegetables, cereals, and beverages derived from plants such as coffee, tea and wine [27].

Polyphenols show significant diversity in their structure, which influences their mechanism of action and the bioavailability [28]. Epidemiological studies on animals support the fact that polyphenols have inverse relation with cardiovascular or cancer diseases. The health effects of polyphenols are highly variable which depend on their intake and bioavailability [27, 29].

Recent studies provide evidence that consumption of polyphenols-rich diets, as found in fruits, may lower the risk of developing neurodegenerative diseases due to their anti-inflammatory properties [30]. Indeed, diet may affect human health considering that it can have detrimental effects, or in contrast, being able to attenuate inflammation [31]. Polyphenols, such as flavonoids and phenolic acids, have shown to play important roles in the regulation of inflammatory processes associated with several diseases, including neurodegenerative disorders [30].

Raspberries are fruits rich in these bioactive compounds [32] and some studies with raspberry extracts have shown anti-inflammatory capacity [33], and neuroprotective effects [34]. However, it remains unclear as to how these compounds exert beneficial effects, what concentrations are necessary, and which are the biologically active forms. In addition, when studying the potential effects of polyphenol-rich foods in human health, it is essential to bear in mind that the bioavailability of polyphenols is dependent on the modifications that ingested food suffers along the gastrointestinal (GI) tract [35].

1.4 PRESERVATION TECHNIQUES

Since the discoveries of Metchnikoff [36], probiotic microorganisms have been attributed to the beneficial properties of humans and animals, which has led to their application in various foods, which allows the consumer to get these benefits. In addition, the appropriate combination of prebiotics and probiotics produces greater consumer benefit and may produce a synergistic effect [37]. Encapsulation techniques are an alternative when attempting to protect these microorganisms from the effect of environmental agents that may affect viability during processing, storage, consumption and its passage through the gastrointestinal tract by allowing them to maintain their viability and functionality in the time [38] reducing cellular damage to retain the cells within the encapsulating materials that generate their isolation [39].

There is a wide variety of microorganism encapsulation techniques. Many techniques consider liquid mixtures to be dried in the encapsulation treatment, in this case the likely application of high temperatures is associated with a significant reduction in the viability of microorganisms due to rapid dehydration and thermal stress [40]. Lyophilisation is a suitable technique for the encapsulation of thermosensitive microorganisms, which by freezing the product and after the application of vacuum, the water is removed by sublimation, obtaining a dry paste that flavours the preservation in time of the product [38]. Lyophilisation technology

allows the encapsulation of biological materials by reducing the rate of chemical reactions and heat degradation [41]. Many factors have been reported as responsible for microbial survival during and after lyophilisation, including bacterial species, physiological status, cell density, protective effect [42], freezing rates and other parameters, as well as rehydration [42]. The effect of these parameters can cause cell damage, reduced viability and microbial activity to varying degrees, inducing changes in the physical state of the lipid membrane and protein structure [41].

Research results show that in addition to the encapsulation technique, the protective effect depends largely on the functional properties of the wall material [43, 44]. Non-digestible food ingredients such as prebiotics that are credited with protective qualities on the beneficial gastrointestinal flora of humans and animals could be applied as coating material [45]. These, because they are non-digestible aliments, resistant to digestive enzymes, benefit the host by selectively stimulating of the growth and / or activity of bacteria in the colon [46]. Due to the synergistic potential between probiotics and prebiotics, the combination establishes a symbiotic relationship, which benefits the host due to increased survival and implantation of live microorganisms in the gastrointestinal system [47].

Encapsulation technology has received considerable interest in polymer science in wide range of disciplines and in numerous fields of applications. Over the past few decades, advances in biopolymer encapsulation based technologies have spurred development in many important fields such as pharmaceutical, foods, cosmetics, agricultural, electronic, and molecular diagnostic applications [48-51]

The process of encapsulation involves the entrapment of a substance (active agent) within a carrier material (wall material). By using encapsulation technology, the active agents can be protected from several drastic conditions such as light, shear, oxygen, moisture, heat or other extreme conditions, thus enhancing their stability and maintaining viability.

Encapsulation is also utilized for achieving various goals such as for masking of any unpleasant odours or tastes [51], transforming liquid droplets into solid particles, safe handling of the toxic materials, reducing the evaporative loss and flammability of liquids, reducing the reactivity of the core material, and extending the duration of the activity of the active agent. Encapsulation is a useful tool to improve the delivery of the encapsulated material to the required site with the required rate or optimal kinetics. Conditions for the encapsulation of active

molecules depend on the sensitivity (thermal and redox stability) and nature (solubility in oil and water) of the active components but release can be controlled by mechanical process, pH variations (acidic conditions in the stomach, near neutral in the intestine), enzymatic actions [52] or can be triggered by other external stimuli.

2. OBJECTIVES

The main aim of this project is the encapsulation of raspberry juice extract to avoid their degradation and to facilitate their incorporation in foods. To achieve this, there are different specific objectives:

- Study the behavior of different hydrocolloids in a range of concentrations to find the one that best gels the raspberry juice extract.
- Compare the degradation of the raspberry extract with the degradation of the beads.
- Study the physical properties of the raspberry beads.

3. EXPERIMENTAL

3.1 MATERIAL AND EQUIPMENT

Materials used are listed in table 1 and table 2.

Table 1: Solid reagents

REAGENT	PURITY	SUPPLIER
Calcium Citrate tetrahydrate	99,0%	Aldrich Chemistry
Calcium Chloride anhydrous	93,0%	Sigma Aldrich
Gallic Acid (GA)		Sigma
Gellan		Solegraells
Goma Kappa		Sosa
Lyophilized whole raspberry		Sosa
Sodium Alginate PB	99,0%	Panreac
Sodium Carbonate		Sigma Aldrich
2,2-Diphenyl-1-picrylhydrazyl (DPPH)		Aldrich

Table 2: Liquid reagents

REAGENT	PURITY	SUPPLIER
Hydrochloric Acid (HCl)	37,0%	Panreac
Folin & Ciocalteu's phenol reagent		Sigma Aldrich
Methanol (Reag. Ph. Eur) (MeOH)	99,9%	Panreac AppliChem
Milli-Q water		

The equipment used is detailed below.

- COBOS Balance. Model AX 2000, capacity 200 g and accuracy of 0.1 mg.
- ULTRA-TURRAX. Model T 25 basic IKA-WERKE.
- Magnetic stirrer. Model Stirrer RCT basic of IKA 0-2500 rpm.
- Spectrophotometer. Model Perkin Elmer model UV/VIS Lambda 20.
- PH / Ion meter. Model meter-ISE, Prolab 3000, Schott Intruments.
- Texturometer. Model TA. XT Plus.

3.2 METHODS

3.2.1 Experimental plan

The study was carried out in three steps. Firstly, it was analysed the degradation of the extract of the lyophilized raspberry by studying the effect of temperature and light. Secondly, it was analysed the degradation of the hydrated lyophilized raspberry and the degradation of the juice. Finally, it was analysed the degradation of the raspberries beads and it was contrasted to the first analyses. Furthermore, it was analysed the beads texture to study the beads physical properties.

3.2.2 Extraction procedure

The extraction of phenolic compounds was performed according to the method developed by Jin et al. (2015) with slight modifications [53].

To get the raspberry extract, 1 g of lyophilized raspberries was weighed and crushed. Then, that amount of crushed raspberry was placed in a 25-mL beaker. The extraction was performed with 1% acidified methanol following the ratio 1 g of fruit-10 mL of MeOH/HCl. This extraction was repeated 3 times. The obtained solution was filtered under vacuum with the Buchner funnel and the solution was level to 50 mL. Then the extract was separated into three closed containers. One was stored in the refrigerator, another was stored outside and the last one was stored outside but covered by light. Both were stored until the analysis were carried out.

To obtain the extract of the beads, the same procedure was followed but instead of crushing the lyophilized raspberries, the beads were crushed.

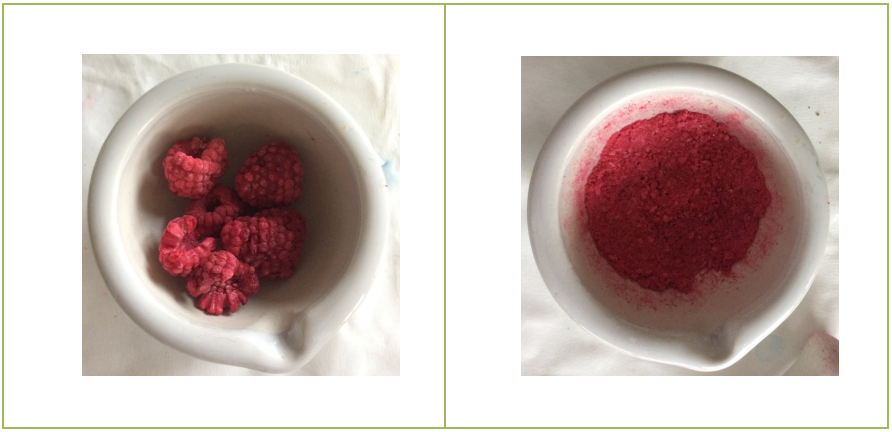


Figure 1: Left whole raspberry and right crushed lyophilized raspberry

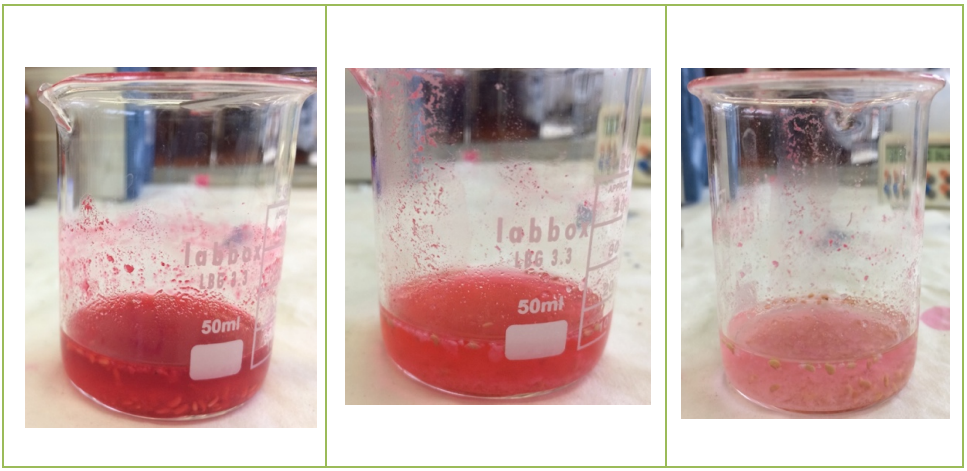


Figure 2: Left to right: 1 g of raspberry with 10 mL of MeOH/HCl, 1 g of filtered raspberry with 10 mL of MeOH/HCl and 1g of raspberry filtered for second time with 10 Ml of MeOH/HCl.

3.2.3 Preparation of raspberry juice

The natural fruits of raspberry have only 10% of dry mass and the rest was water. As the lyophilized raspberries did not contain any water, they were re-hydrated at 90% [54]. A certain number of raspberries was weighed and milli-Q water was added with the ratio 10 g of fruit - 90 mL of water. The mixture was stored for 24 hours in the refrigerator to ensure that the

raspberries were completely re-hydrated. After 24 hours, the mixture was placed in a liquidizer and the juice was obtained. Then, the juice was transferred to a closed container and stored in the refrigerator until analyses.

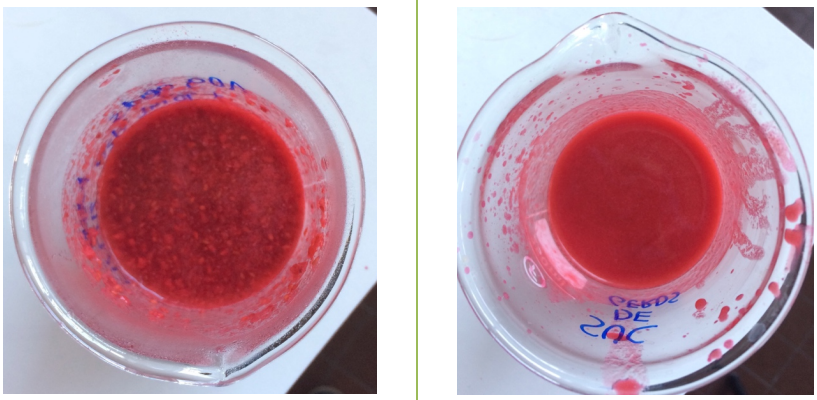


Figure 3: Left re-hydrated raspberry and right raspberry juice

3.2.4 Encapsulation

A certain amount of juice was added in two beakers. With sodium citrate the pH was adjusted above 4 because the pH of the juice was 3.22. Then, in one of the beakers, 0.5% alginate was added and in the other, 1% alginate. To homogenize the mixture, the juice was placed with alginate in the Ultra-Turrax. Thereafter, the mixture was stored for 24 hours to ensure gelling occurred.

After 24 hours, a solution of calcium chloride was prepared. The solution was of 8% to ensure the gelation [55]. The encapsulation was performed on the calcium chloride solution, because the gelification process needs an external calcium source from where the calcium ion diffuses to reach the polymer chain, and because of this union, there is a structural rearrangement in the space resulting a solid material with the characteristics of a gel [56]. To remove the air bubbles from the refrigerated mixture, the juice was heated to 25 ºC with gentle stirring on the magnetic stirrer. Then, with a manual pipette, juice-alginate mixture was pipetted and placed in the calcium chloride solution, thus obtaining the raspberry beads.

3.2.5 Degradation essays

3.2.5.1 Determination of antioxidant activity

A method according to Shimada et al. was used to detect the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [57].

Two solutions were prepared. A solution of 80% methanol with which the blank was made in the spectrophotometer and a solution of 3.94 mg of DPPH in 50 mL of 100% methanol. The absorbance of 2 mL of DPPH solution in 4 mL of 80% methanol solution was about 0.64-0.66 at 515 nm and 20 ºC, and this solution was the control test.

The assay was performed in test tubes. An amount of sample was added between 40 μL and diluted with the 80% methanol solution obtaining a total volume of 4 mL. Then 2 mL of DPPH solution was added to each tube at a 1 minute interval per tube. The mixture was stirred vigorously and allowed to react for half an hour. After half an hour, the absorbance was measured in the spectrophotometer at 515 nm.

3.2.5.1 Determination of total phenolic content

Total phenolic content (TPC) was determined according to the Folin–Ciocalteu colorimetric method using Gallic acid as a standard [58]. First a calibration curve was performed based on Gallic acid concentrations, relating absorbance to concentration (Appendix 1).

Two solutions were prepared. Solution A, composed of 0.5 g of Gallic acid, 10 mL of 100% methanol and 10 mL of milli-Q water, and a solution of Na_2CO_3 (20% w / v), (solution B). In different tubes, certain ml of solution A in milli-Q water were diluted, obtaining a final volume of 100 mL.

For the calibration, from each tube, 40 μL were caught and diluted in 3,16 ml of milli-Q water. The blank was carried out using 40 μL of 10% methanol solution instead of solution A. 200 μL of the Folin-Ciocalteu reagent was introduced into each tube. The mixture was stirred vigorously and allowed to react in the dark for 5 minutes. 600 μL of solution B was then added. The mixture was again stirred vigorously, and allowed to react for 60 minutes. After an hour, the absorbance was measured in the spectrophotometer at 765 nm.

The assay for the determination of concentrations was performed using the same method as the calibration, but using 40 μL of raspberry extract instead of solution A.

3.2.6 TPA test

Texture profile analysis (TPA) is an instrumental test originally developed at the General Foods Corporation Technical Centre (1963) to provide objective measurements of texture parameters, a major factor of food acceptability. It was designed as a two-cycle compression performed to simulate successive "chews" [59].

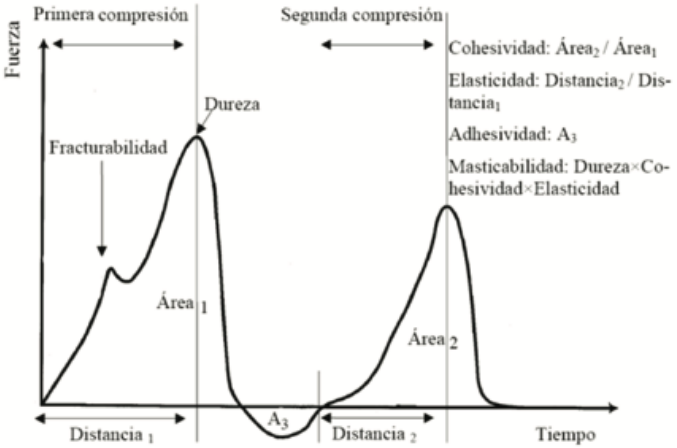


Figure 4: General graph of texture analysis [60]

Force-time curves group the textural properties as initial (on first bite: hardness, viscosity, brittleness), masticatory (during chewing: gumminess, chewiness, adhesiveness) and residual (rate of breakdown, type of breakdown). These properties are measured in the texturometer. The original TPA parameters defined by the General Foods Corporation group are:

- Hardness [N]: Force required for a pre-determined deformation.

  How measured: Force at P1
- Fracturability [N]: Force at first significant break in the curve

  How measured: Force at F1
- Cohesiveness [no unit]: Strength of internal bonds in sample

  How measured: A_2/A_1

- Adhesiveness [J]: Work required to overcome the sticky forces between the sample and the probe.

  How measured: A_3

- Gumminess [N]: Energy needed to disintegrate a semisolid food until it is ready to swallow

  How measured: Hardness·Cohesiveness

- Chewiness [J]: Energy needed to chew a solid food until it is ready for swallowing

  How measured: Gumminess·Springiness

- Springiness [m]: Originally called “Elasticity”. Rate at which a deformed sample returns to its original size and shape.

  How measured: d_2

4. RESULTS AND DISCUSSION

4.1 PREVIOUS EXPERIMENTS

4.1.1 Extraction with water

Comparison experiments were conducted to determine which extraction method was the most effective to analyse the degradation, extraction with acidified methanol or extraction with water.

The results obtained by determining phenolic content were as follows:

Table 3: Obtaining polyphenols by extraction with 1% acidified methanol and water

EXTRACTIVE REAGENT	POLYPHENOL CONCENTRATION [mg GA/mg raspberry]
MeOH/HCl (1%)	0.355 ± 0.023
Milli-Q water	0.200 ± 0.003

The concentration results show that the capacity of extraction with water is about 56.5% less in comparison of the capacity of extraction with MeOH/HCl (1%), thus, water have practically half the capacity for extracting.

Table 4: Obtaining antioxidant activity by extraction with 1% acidified methanol and water

EXTRACTIVE REAGENT	PERCENTAGE OF DICOLORATION OF DPPH [% / mg lyophilized raspberry]
MeOH/HCl (1%)	48.94 ± 1.13
Milli-Q water	20.83 ± 0.87

The antiradical activity (AAR) results show that the capacity of extraction with water is about 42.5% less in comparison of the capacity of extraction with 1% acidified methanol, thus, water have practically half the capacity for extracting.

Therefore, the extraction of beads was done with 1% acidified methanol, because with water half of the extraction was obtained.

4.1.2 Gelling agent

To encapsulate the raspberry juice some experiments were done in order to find the best gelling agent and the adequate working pH.

Firstly, the juice pH was regulated by sodium bicarbonate and it was seen that the juice turned very stiff. Without regulating the pH, the beads were dissolved and the geometry was not like beads.

Secondly, the juice pH was regulated between 4 and 10 by sodium citrate, because the bicarbonate produced carbon dioxide, which can deform the resulting beads. Then, different tests were done using different hydrocolloids. The analysed hydrocolloids were Gellan, Iota, Kappa and Alginate in different weight percentages, the maximum and minimum [61]. The following tables show the differences between them.

Table 5: Encapsulation with Gellan

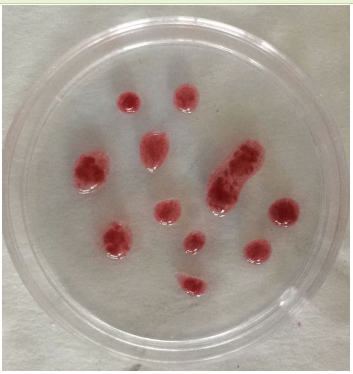
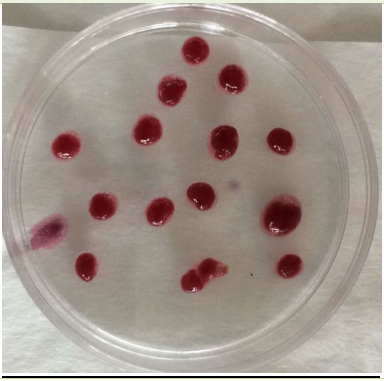
Gelling Agent	Gellan	Gellan
Percentage	0.25	1.30
Image		

Table 6: Encapsulation with Iota

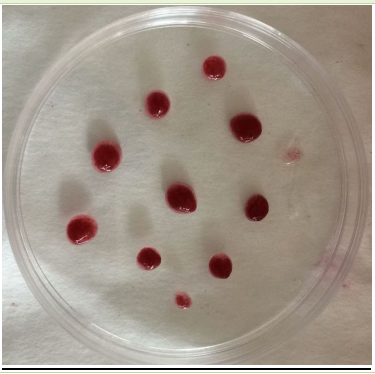
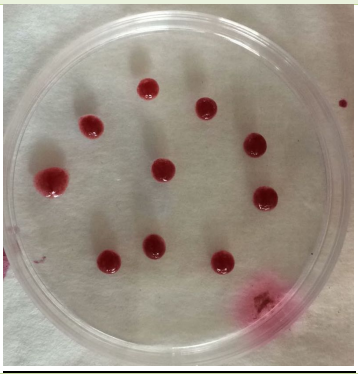
Gelling Agent	Iota	Iota
Percentage	0.50	0.76
Image		

Table 7: Encapsulation with Kappa

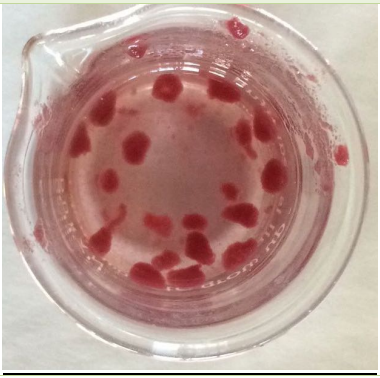
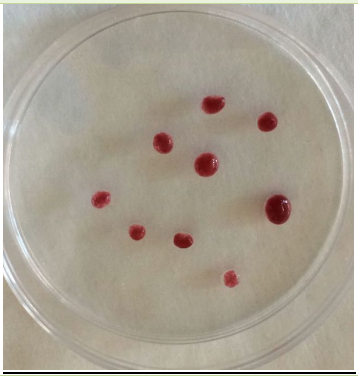
Gelling Agent	Kappa	Kappa
Percentage	0.51	0.76
Image		

Table 8: Encapsulation with Alginate

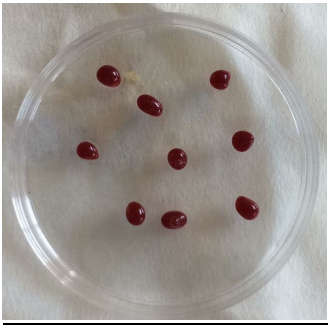
Gelling Agent	Alginate	Alginate
Percentage	0.45	0.55
Image		

Table 4 shows the encapsulation with Gellan. As can be seen, the beads do not gelify correctly at any of the two concentrations of Gellan, and therefore, the content is transferred to the $CaCl_2$ solution.

Table 5 shows the encapsulation with Iota. As can be seen, the beads gelify but the texture is not completely compact, so a part of the beads content is also transferred to the $CaCl_2$ solution. In addition, the size of beads obtained is very diverse.

Table 6 shows the encapsulation with Kappa. Gelling with this hydrocolloid provides a sphericity quality like Gellan, improving the properties at higher concentration of hydrocolloid.

Table 7 shows the encapsulation with Alginate. As can be seen, the beads gelify correctly at both concentrations of Alginate. Furthermore, the texture of the beads is totally compact and the content is not transferred to the $CaCl_2$ solution.

Observing the results in the different gelling agents, for the encapsulation of the raspberry juice, the alginate was used.

4.1.3 Extraction time for beads

To ensure that the necessary time to obtain the extract of the beads was the same as the lyophilized raspberry, an experiment was realized. 10 g of beads were introduced in a beaker with 100 mL of 1% acidified methanol. The beads were left into the beaker and collected at different times, taking samples of 3 mL. The samples were analysed in function of the collected time in order to know how much time was necessary for extraction.

The results obtained were as follows:

Table 9: Percentage extraction of raspberry extract as a function of time

TIME (min)	% EXTRACTION
2	49.70 ± 0.57
4	52.27 ± 0.96
6	61.21 ± 0.96
8	66.22 ± 0.96
10	71.70 ± 1.44
30	84.83 ± 2.78
60	95,60 ± 0,38
90	97,97 ±0,48
120	100,00 ± 2,20

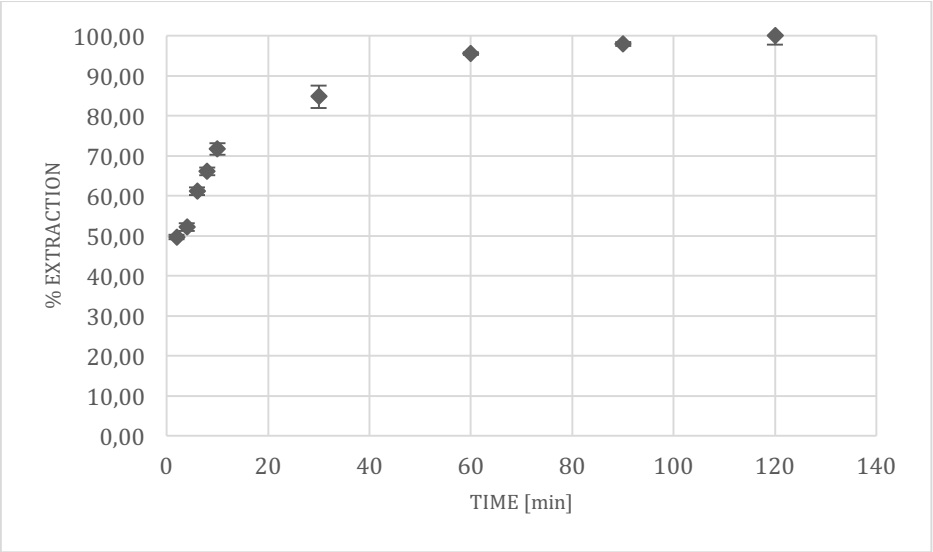


Figure 5: Graphical representation of the extraction percentage of the raspberry extract as a function of time

As can be seen in figure 5, the highest percentage of extraction occurs between the first 30 or 40 minutes, obtaining an extraction greater than 80%. For this reason, it will be considered that 30 minutes are enough to perform the extraction of the beads.

4.1.4 Sample amount for DPPH analysis

For the DPPH analysis, some experiments were realized in order to use the adequate quantity of raspberry extract. It was tested 15, 30, 50 and 90 μL of extract in comparison of control test.

The results obtained were as follows:

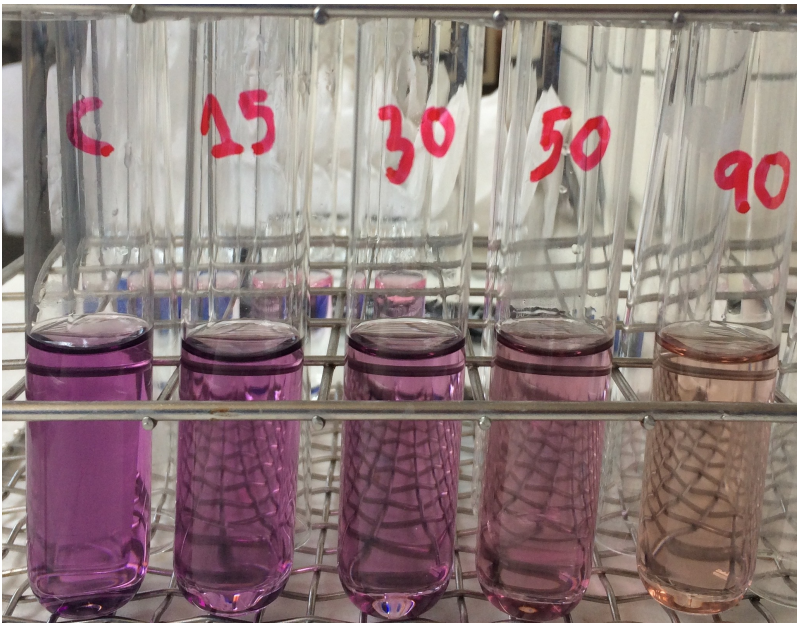


Figure 6: Left to right 0-15-30-50 and 90 μL of raspberry extract diluted with 80% methanol to 4mL and 2mL of DPPH solution.

DPPH• reduction is based on the decrease in absorbance at a characteristic wavelength. In its free radical form, DPPH• absorbs at 515 nm and when it is reduced by an antioxidant, this absorption disappears. Consequently, the disappearance of the DPPH• provides an index to estimate the ability of the test compound to entrap radicals. The model that explains the activity of a compound as antiradical is exemplified by the following equation:

$$DPPH\bullet + (AH)_n \rightarrow DPPH\text{-}H + (A\bullet)_n \qquad \text{(Equation 1)}$$

Where AH is the antioxidant which act as an antiradical, giving hydrogen atoms [62].

As can be seen in figure 6, with 15 and 30 μL, solutions obtained was similar to control tube. Very high diluted solutions are obtained with 50 and 90 μL. The reason for the colour change is due to the ability to trap radicals. In the tubes with 15 and 30 μL, the amount of extract is so diluted that a very small amount is being measured, producing a large range of errors. In the tubes with 50 and 90 μL, the amount of extract is so large that it inhibits the DPPH solution. To avoid errors at both ends, an amount of extract of 40 μL was used.

4.2 DEGRADATION OF LYOPHILIZED RASPBERRY EXTRACT

To determining the degradation, three tests were done, in order to found what was the most affecting property for degradation, the temperature or the light.

To calculate the antiradical activity, the percentage of discoloration of the 2,2-diphenyl-1-picrylhydrazole radical (DPPH •) was calculated using the following equation:

$$\%AAR = \frac{(A_{control} - A_{muestra})}{A_{control}} \cdot 100 \qquad (Equation\ 2)$$

Total phenolic content was determined using Gallic acid as a standard.

4.2.1 Determination of antioxidant activity

The following table shows the results obtained on DPPH discoloration depending on the conditions.

Table 10: Results of antiradical activity of lyophilized rasperry in function of time

	PERCENTAGE OF DISCOLORATION OF DPPH [% / mg lyophilized raspberry]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
0	48.94 ± 1.13	48.94 ± 1.13	48.94 ± 1.13
3	49.14 ± 1.56	49.91 ± 2.51	43.66 ± 0.70
6	47.85 ± 0.68	49.27 ± 2.79	41.22 ± 0.78
14	44.50 ± 1.48	42.83 ± 1.97	29.43 ± 1.95
22	44.82 ± 1.21	41.34 ± 1.65	16.36 ± 3.64
43	41.60 ± 1.48	40.96 ± 0.97	13.46 ± 1.40

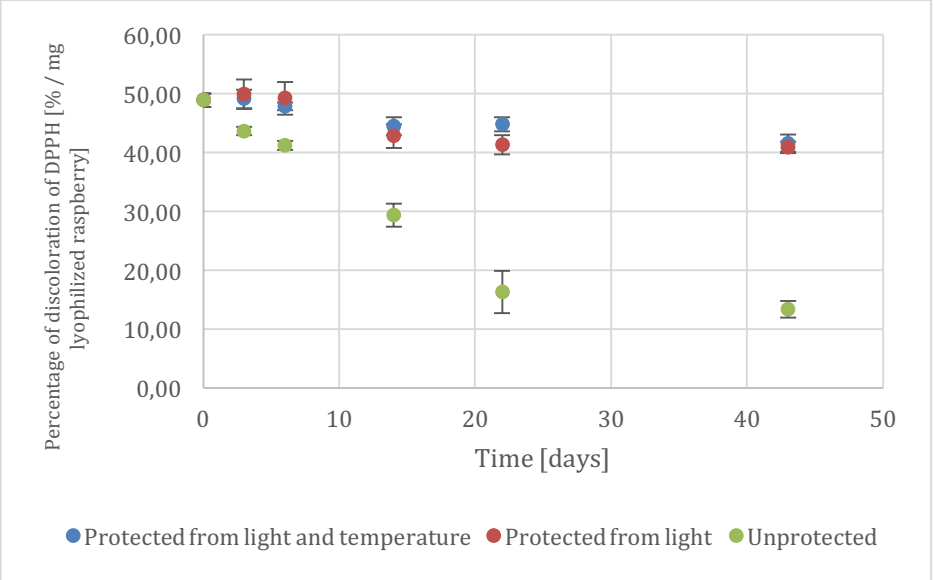


Figure 7: Graphical representation of results of antiradical activity of lyophilized rasperry in function of time

As can be seen in figure 7, the antiradical activity of the sample at first time is approximately 50%. Being protected from light and temperature, the antiradical activity decrease a little, but not exaggeratedly. The same happens to the sample protected only from the light. The great difference in antiradical activity occurs when the sample is exposed to light and at room temperature rather than at refrigeration temperature. In this case, the antiradical activity goes from 50% to 15%.

Table 11: Results of loss of antiradical activity of lyophilized raspberry in function of time. (1/2)

	LOSS OF AAR [%]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
3	2.15 ± 1.87	4.17 ± 2.49	10.79 ± 1.42
6	2.24 ± 1.39	4.43 ± 1.90	15.79 ± 1.60

Table 11: Results of loss of antiradical activity of lyophilized raspberry in function of time. (2/2)

	LOSS OF AAR [%]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
14	9.06 ± 3.01	12.50 ± 4.03	39.87 ± 3.99
22	8.42 ± 2.47	15.53 ± 3.37	66.58 ± 7.43
43	15.00 ± 3.01	16.32 ± 1.97	72.50 ± 2.86

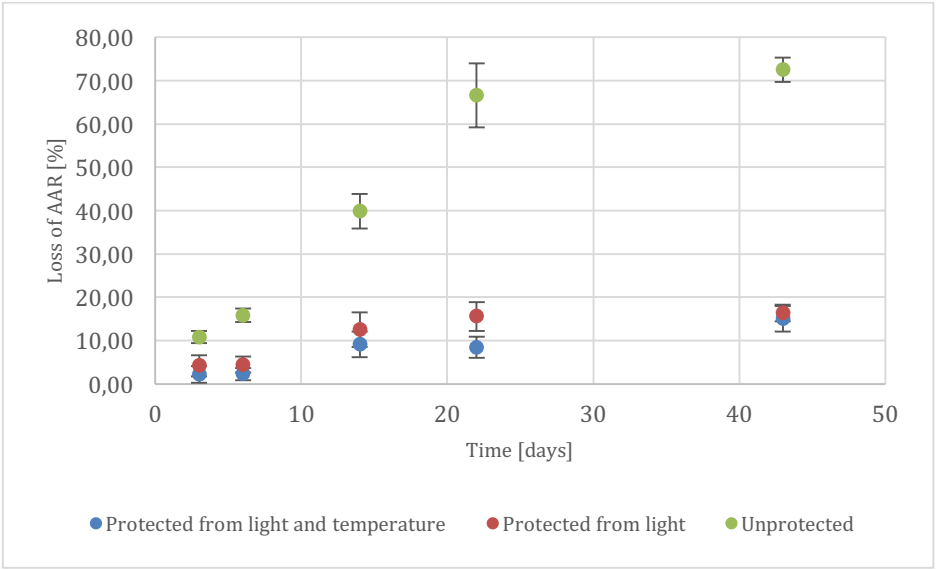


Figure 8: Graphical representation of results of loss of antiradical activity of lyophilized raspberry in function of time

Figure 8 shows the trend of loss of antiradical activity. The two protected samples lose up to 15% of radical activity over 50 days, while the unprotected sample loses up to 75%.

4.2.2 Determination of total phenolic content

The following table shows the results obtained on polyphenol concentration depending on the conditions.

Table 12: Results of phenolic content of the lyophilized raspberry in function of time

	POLYPHENOL CONCENTRATION [mg GA / mg lyophilized raspberry]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
0	0.355 ± 0.023	0.355 ± 0.023	0.355 ± 0.023
3	0.399 ± 0.009	0.337 ± 0.022	0.297 ± 0.007
8	0.336 ± 0.018	0.318 ± 0.011	0.220 ± 0.015
16	0.313 ± 0.018	0.317 ± 0.005	0.202 ± 0.008
22	0.342 ± 0.014	0.333 ± 0.020	0.188 ± 0.013
36	0.399 ± 0.010	0.308 ± 0.014	0.161 ± 0.018
50	0.307 ± 0.005	0.237 ± 0.020	0.071 ± 0.006

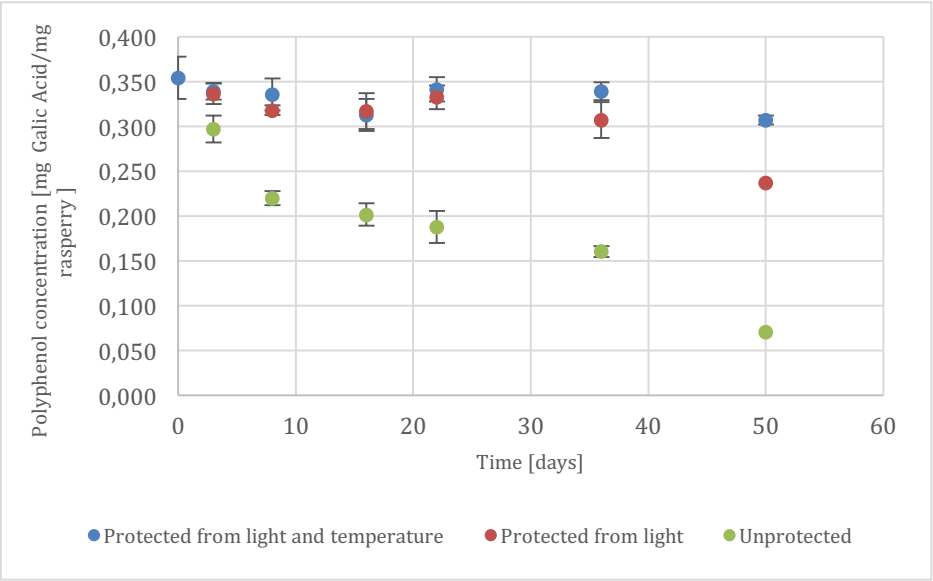


Figure 9: Graphical representation of results of phenolic content of the lyophilized raspberry in function of time

As can be seen in figure 9, the concentration of GA of the sample at first time is 0.335 mg GA/mg of lyophilized raspberry. Being protected from light and temperature, the concentration decrease to 0.307 mg GA/mg of lyophilized raspberry. The decrease of concentration is larger in the sample protected only from temperature where the concentration after 50 days is 0,237 mg GA/mg of lyophilized raspberry. The great difference is also when the sample is exposed to light and at room temperature, when de concentration is 0.071.

Table 13: Results of polyphenol degradation of lyophilized raspberry in function of time

	POLYPHENOL DEGRADATION [%]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
3	4.31 ± 2.65	5.08 ± 6.24	16.12 ± 2.00
8	5.30 ± 4.97	10.27 ± 3.16	37.87 ± 4.19
16	11.70 ± 4.97	10.49 ± 1.49	43.06 ± 2.17
22	3.64 ± 3.48	6.18 ± 5.63	46.93 ± 3.54
36	4.31 ± 2.83	13.25 ± 3.82	54.65 ± 5.10
50	13.36 ± 1.38	33.12 ± 5.63	80.05 ± 1.70

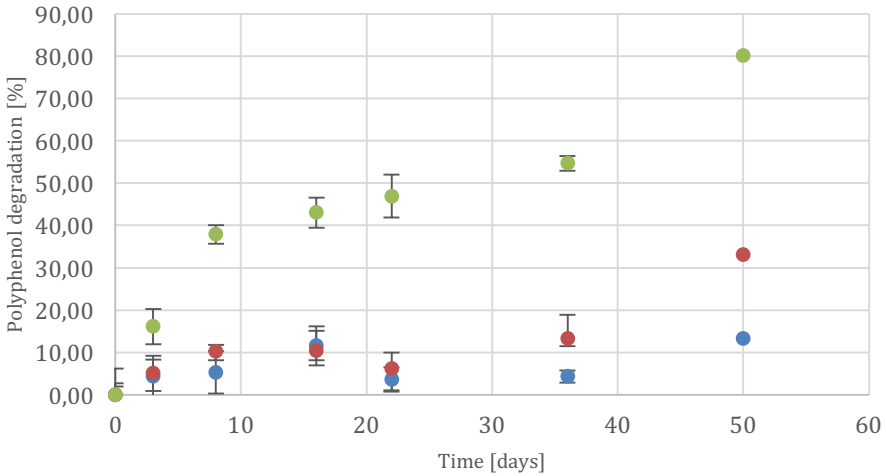


Figure 10: Graphical representation of results of polyphenol degradation of lyophilized raspberry in function of time

Figure 10 shows the degradation of lyophilized raspberry. The temperature and light protected samples lose up to 15% of polyphenols over 50 days, while the temperature protected sample

loses up to 30%. The largest degradation is that of the unprotected sample, which loses up to 80% of polyphenols.

In figure 11 the colour change of lyophilized raspberry extract can be seen after 50 days. To the left the extract unprotected and to the right the extract protected from light and temperature.

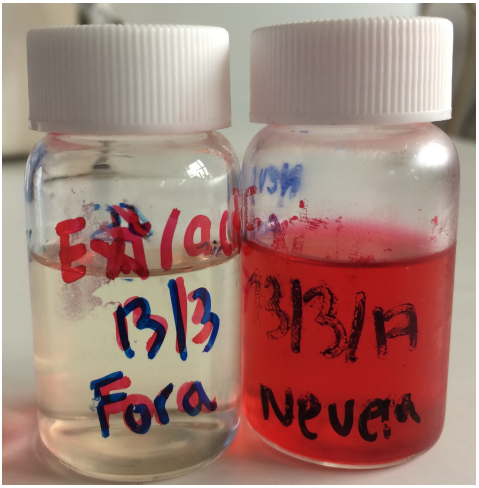


Figure 11: Left unprotected extract and right extract protected from light and temperature after 50 days

4.3 RE-HYDRATED RASPBERRIES ANALYSIS

To know the characteristics of the raspberry pulp, the re-hydrated raspberries were analysed. The blender with which the juice was made, separated the pulp from the juice.

By difference between the characteristics of the re-hydrated raspberries and the characteristics of the juice, the following results were obtained.

4.3.1 Determination of antioxidant activity

Knowing that the total absorbance corresponds to the raspberry extract and that when the juice was prepared, the pulp was separated from the juice, the absorbance of the pulp was calculated as follows:

$$AAR_p = AAR_{rh} - AAR_j; \qquad (Equation\ 3)$$
$$\delta AAR_p = \delta AAR_{rh} + \delta AAR_j \qquad (Equation\ 4)$$

Where AAR_p, AAR_{rh} and AAR_j are the absorbance of the pulp, the absorbance of the re-hydrated raspberry and the absorbance of the juice respectively.

Table 14: Results of antiradical activity of raspberry pulp

EXTRACT	PERCENTAGE OF DISCOLORATION OF DPPH [% / mg lyophilized raspberry]
Re-hydrated raspberry (AAR_{rh})	58.57 ± 1.29
Raspberry juice (AA_{Rj})	38.22 ± 1.10
Raspberry pulp (AA_{Rp})	20.35 ± 2.39

4.3.2 Determination of total phenolic content

Knowing that the total concentration of polyphenols corresponds to the raspberry extract and that when the juice was prepared, the pulp was separated from the juice, the concentration of the pulp was calculated as follows:

$$C_p = C_{rh} - C_j; \qquad (Equation\ 4)$$
$$\delta C_p = \delta C_{rh} + \delta C_j \qquad (Equation\ 5)$$

Where C_p, C_{rh} and C_j are the concentration of polyphenols of the pulp, the concentration of the re-hydrated raspberry and the concentration of the juice respectively.

Table 15: Results of phenolic content of raspberry pulp

EXTRACT	CONCENTRATION [mg GA/mg raspberry]
Re-hydrated raspberry (C_{rh})	0.388 ± 0.014
Raspberry juice (C_j)	0.281 ± 0.008
Raspberry pulp (C_p)	0.107 ± 0.022

The results, show that the raspberry pulp contains 30% of the properties of the extract, so it is expected that the initial characteristics of raspberry juice will be about 30% lower than the extract. In addition, the content of the beads, will not be the same as the extract, because what is encapsulated is the juice.

4.4 DEGRADATION OF RASBERRY JUICE

4.4.1 Determination of antioxidant activity

The following table shows the results obtained on DPPH discoloration depending on the conditions.

Table 16: Results of antiradical activity of rasperry juice in function of time

	PERCENTAGE OF DISCOLORATION OF DPPH [% / mg lyophilized raspberry]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
0	39.24 ± 1.10	39.24 ± 1.10	39.24 ± 1.10
5	38.57 ± 0.61	39.18 ± 1.70	27.75 ± 3.68
13	37.90 ± 1.86	39,02 ± 1.79	23.23 ± 1.91
34	35.93 ± 1.08	35,11 ± 1.25	18.53 ± 1.72

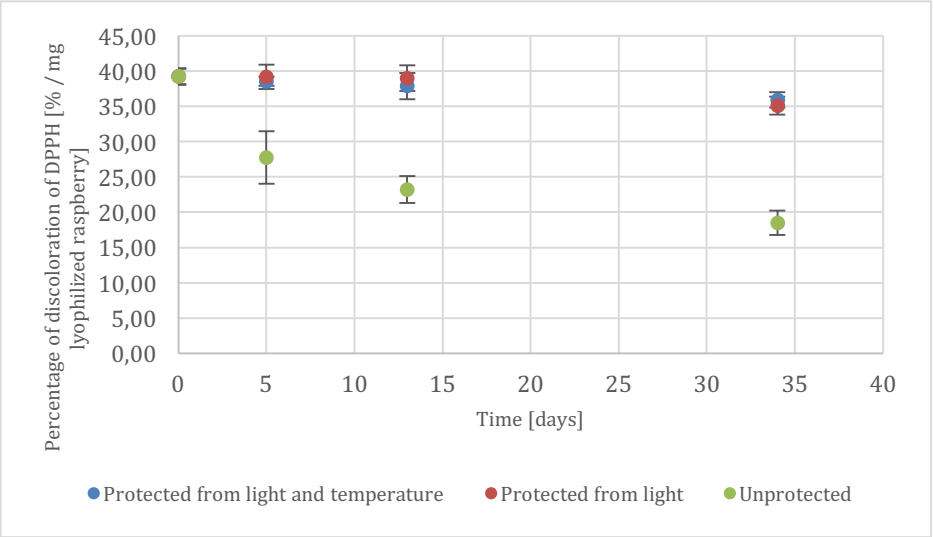


Figure 12: Graphical representation of results of antiradical activity of rasperry juice in function of time

Table 17: Results of loss of antiradical activity of raspberry juice in function of time

	LOSS OF AAR [%]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
5	1.70 ± 1.56	3.07 ± 2.17	29.26 ± 9.37
13	3.40 ± 2.74	3.28 ± 2.27	40.80 ± 4.86
34	8.41 ± 2.76	10.51 ± 3.18	52.76 ± 4.32

Raspberry juice has a similar tendency to raspberry extract, but in this case, the sample protected only from temperature behaves similar to the sample protected from light and temperature. As can be seen in the table 17, in the case of the unprotected condition, the error obtained between replicates is very large. In the previous tests this fact has been repeated, where the highest error is committed in the unprotected sample. This fact may be due to the low concentration of the sample, which can be altered by poor cleaning of the cuvette.

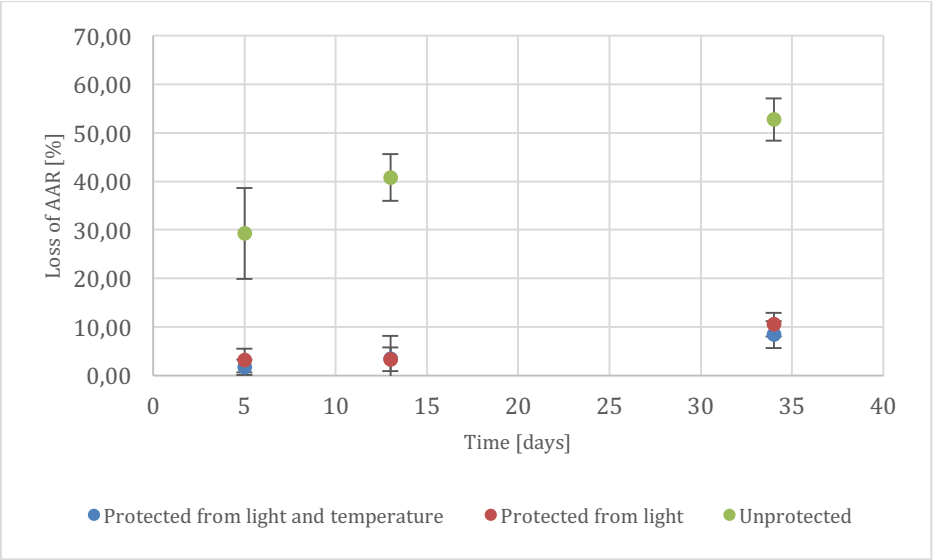


Figure 13: Graphical representation of results of loss of antiradical activity of raspberry juice in function of time

4.4.2 Determination of total phenolic content

The following table shows the results obtained on polyphenol concentration depending on the conditions.

Table 18: Results of phenolic content of raspberry juice in function of time

	POLYPHENOL CONCENTRATION [mg GA / mg lyophilized raspberry]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
0	0.281 ± 0.008	0.281 ± 0.008	0.281 ± 0.008
5	0.292 ± 0.014	0.256 ± 0.025	0.206 ± 0.003
12	0.278 ± 0.009	0.262 ± 0.017	0.156 ± 0.005
26	0.294 ± 0.003	0.270 ± 0.010	0.121 ± 0.030
36	0.292 ± 0.010	0.268 ± 0.010	0.089 ± 0.013

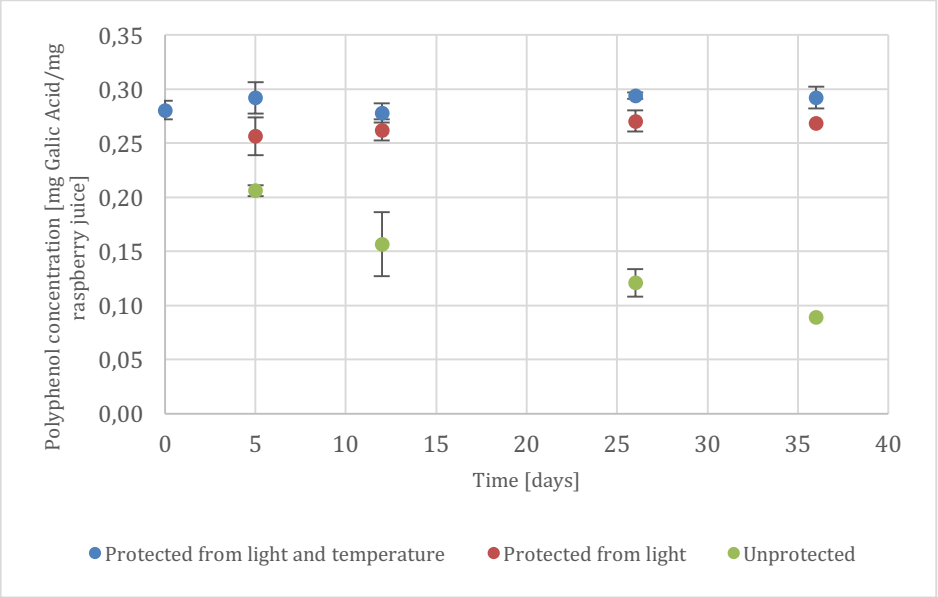


Figure 14: Graphical representation of results of phenolic content of raspberry juice in function of time

Table 14 shows, in the same way as in the loss of DPPH discoloration, that the condition that affect the most to the concentration variation is the unprotected condition. It can also be seen that the error in the unprotected case is greater than in the other cases.

Table 19: Results of degradation of raspberry juice in function of time

	POLYPHENOL DEGRADATION [%]		
TIME [days]	**Protected from light and temperature**	**Protected from light**	**Unprotected**
5	4.08 ± 4.53	8.62 ± 6.74	26.55 ± 1.18
12	0.91 ± 2.55	6.58 ± 6.18	46.44 ± 1.80
26	4.77 ± 0.76	3.63 ± 1.93	56.56 ± 10.55
36	4.08 ± 2.85	4.31 ± 2.39	69.74 ± 4.63

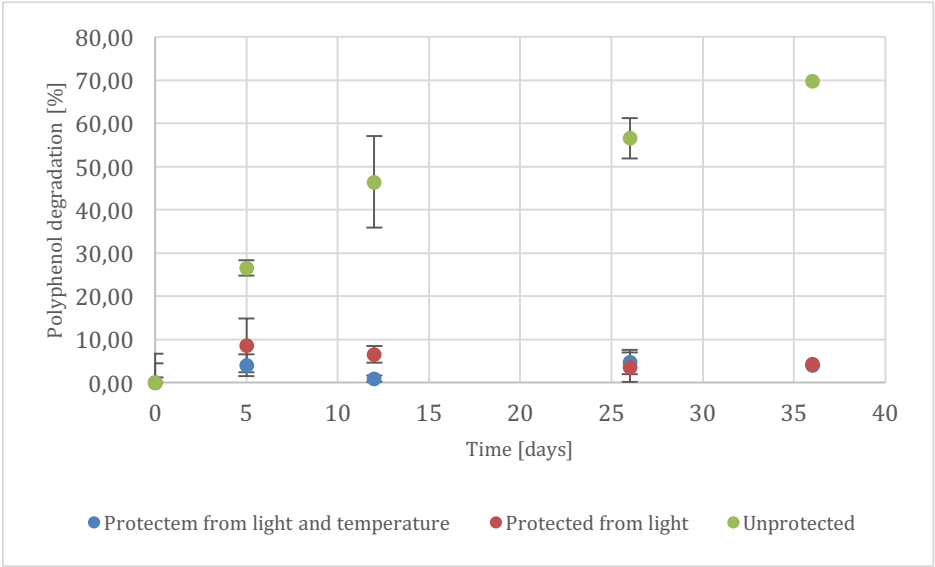


Figure 15: Graphical representation of results of degradation raspberry juice in function of time

As the radical activity, the loss of concentration of GA is like the extract case. It can be seen from figure 15 that raspberry juice does not degrade so much if it is not protected from temperature.

In Figure 16 the colour change of raspberry juice extract can be seen after 36 days. To the right the extract unprotected and to the left the extract protected from light and temperature.

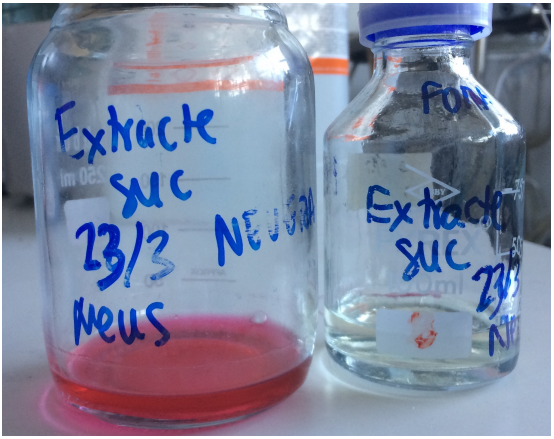


Figure 16: Right: unprotected extract and left: extract protected from light and temperature after 36 days

4.5 DEGRADATION OF BEADS

As it was observed that the most critical degradation was the one that was totally unprotected, the analysis of the beads was performed only in this condition.

4.5.1 Determination of antioxidant activity

The following table shows the results obtained on DPPH discoloration for unprotected beads.

Table 20: Results of antiradical activity of beads in function of time

TIME (days)	PERCENTAGE OF DICOLORATION OF DPPH [% / mg beads]
1	9.75 ± 0.26
2	7.98 ± 0.22
5	7.81 ± 0.05
7	7.63 ± 0.10

The results, show that the percentage of antiradical activity is lower than raspberry juice, approximately 75% lower.

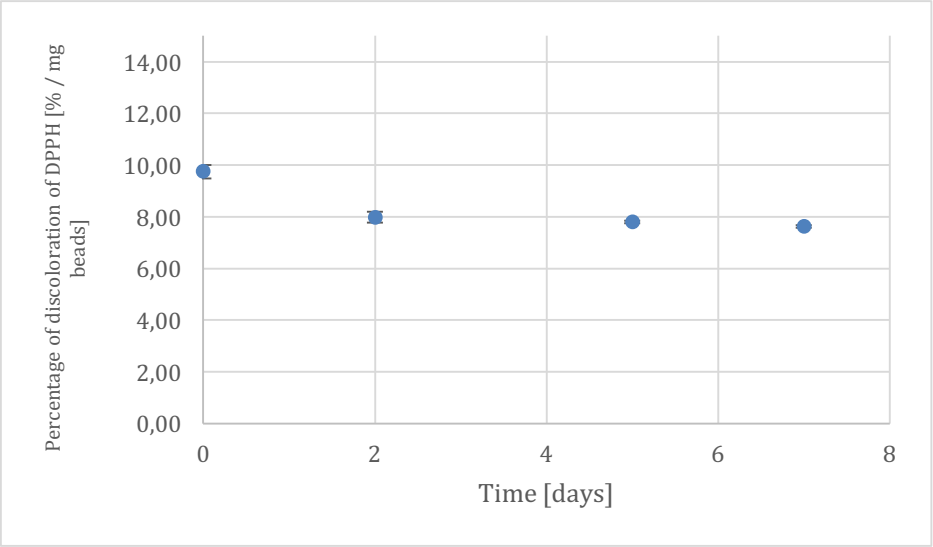


Figure 17: Graphical representation of antiradical activity of spheres in function of time

As can be seen in figure 17, the antiradical activity is reduced from 9.75 to 7.63, practically 20%. In the case without encapsulating, at the same time of degradation, the reduction is about 30-35%. This fact indicates that encapsulation is not very effective in preventing the loss of antiradical activity.

Table 21: Results of loss of antiradical activity of spheres in function of time

TIME (days)	LOSS OF AAR [%]
2	18,16 ± 2.21
5	19,95 ± 0.52
7	21,77 ± 0.55

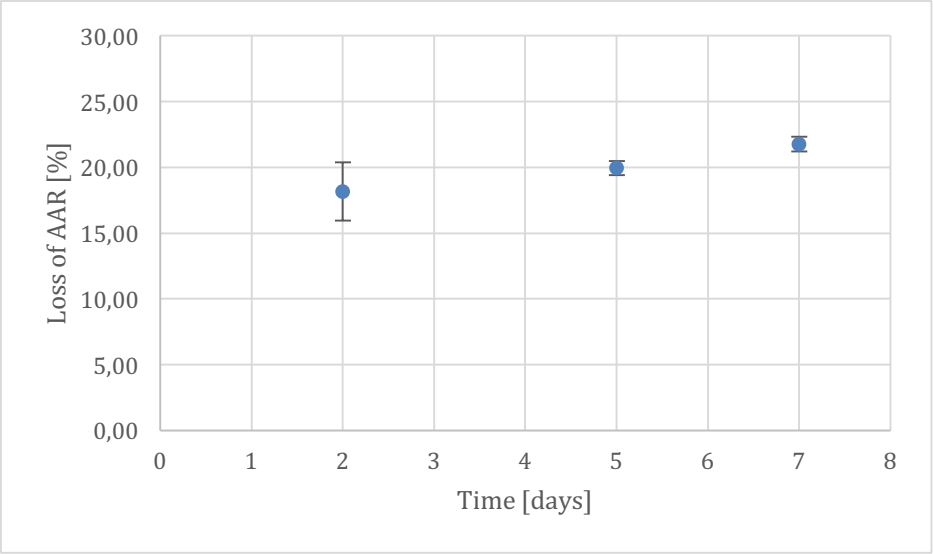


Figure 18: Graphical representation of results of loss of antiradical activity of spheres in function of time

Figure 18 shows the loss of radical activity, being around 20%. If this value is compared with that of raspberry juice in the same time interval, it can be said that encapsulation reduces the loss of antiradical activity by 10-15%.

4.5.2 Determination of total phenolic content

The following table shows the results obtained on polyphenol concentration depending on the conditions.

Table 22: Results of phenolic content of beads in function of time

TIME (days)	POLYPHENOL CONCENTRATION [mg GA/mg beads]
1	0.041 ± 0.002
2	0.041 ± 0.001
5	0.039 ± 0.001
7	0.038 ± 0.001

The results, show that the percentage of phenolic content is lower than raspberry juice, approximately 85% lower.

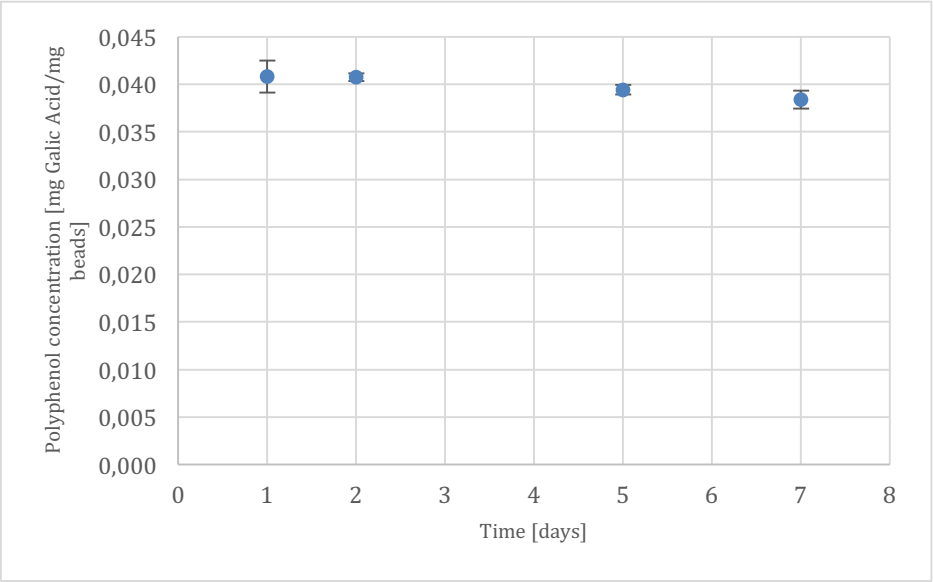


Figure 19: Graphical representation of results of phenolic content of beads in function of time

As can be seen in figure 19, the phenolic content is practically invariant. Despite being a small valour, it remains also unchanged.

Table 23: Results of degradation of beads in function of time

TIME (days)	DEGRADATION [%]
2	0.201 ± 0.304
5	3.267 ± 1.198
7	5.955 ± 0.288

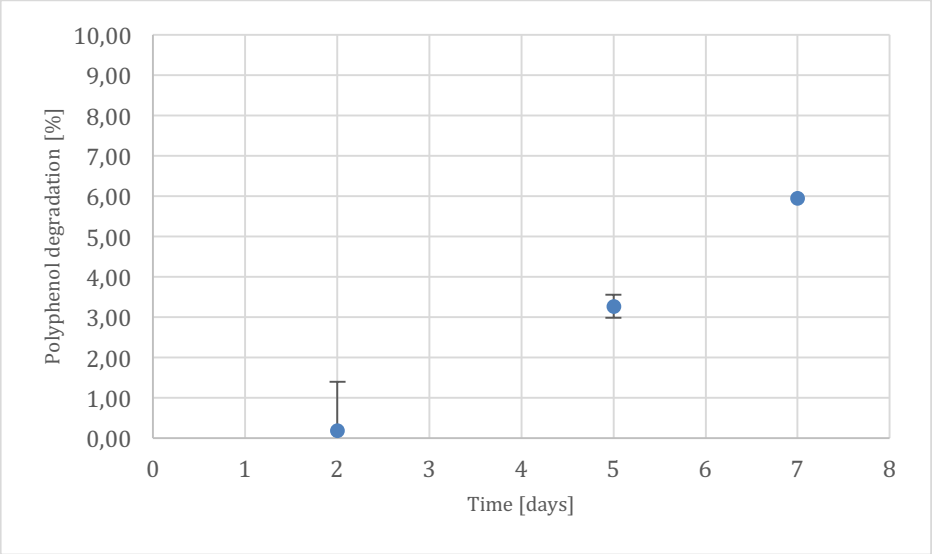
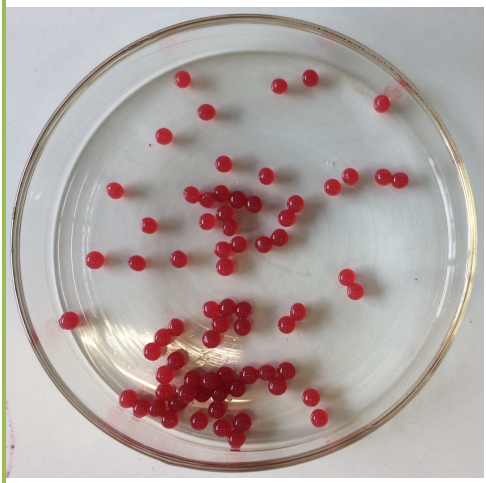


Figure 20: Graphical representation of results of polyphenol degradation of beads in function of time

Figure 20 shows that the degradation is very low, being around 6 %. If this value is compared with that of raspberry juice in the same time interval, it is obtained that in the case of juice, the loss was 30-35%. It can be said that encapsulation reduces the degradation of phenolic content by 20-25%

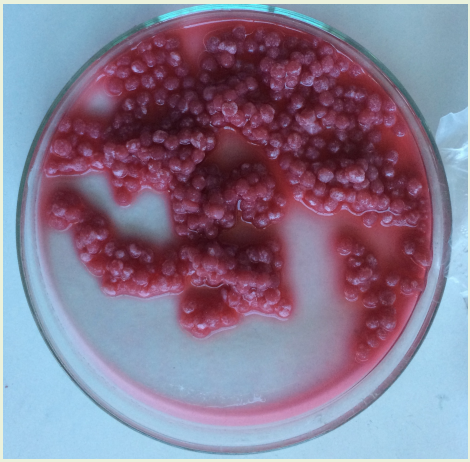
The values of the initial properties of the beads give an idea of the capacity of the beads, in other words, the amount of juice contained in each bead. If the reduction of properties in the beads resembles the reduction of amount of juice in the beads it could be stated that 1g of beads contains 75-85% less juice than 1 gram of juice, which would mean that for every gram of beads, there would be only 0.15-0.25 g of juice.

PICTURE 1



PICTURE 2

PICTURE 3



PICTURE 4

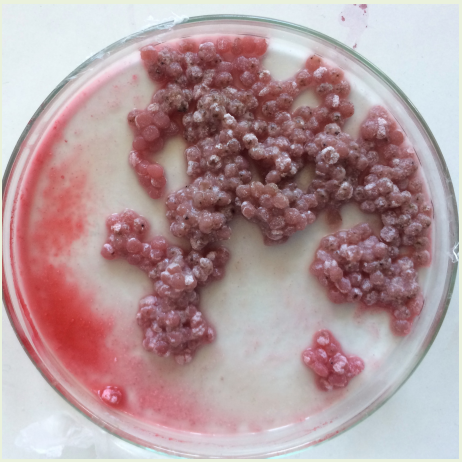


Figure 11: picture1: beads at first day, picture 2: beads at day 2, picture 3: beads at day 5 and picture 4: beads at day 7.

4.6 TPA TEST

Once the raspberry juice was encapsulated, its physical properties were analysed in the texturometer. Once the raspberry juice was encapsulated, its physical properties were analysed in the texturometer. In each analysis 15 beads were analysed. The beads were analysed in two different percentages of deformation (distance travelled by the probe in the first bite. In the first analysis, at 50% and in the second at 90%.

The results obtained are shown in the following table:

Table 24: Percentage and valour of force applied in each experiment.

Force [%]	Applied Force [N]
50	1.282 ± 0.215
90	0.611 ± 0.050

Below is an example of the variation of force versus time during the analysis.

- 50% of deformation:

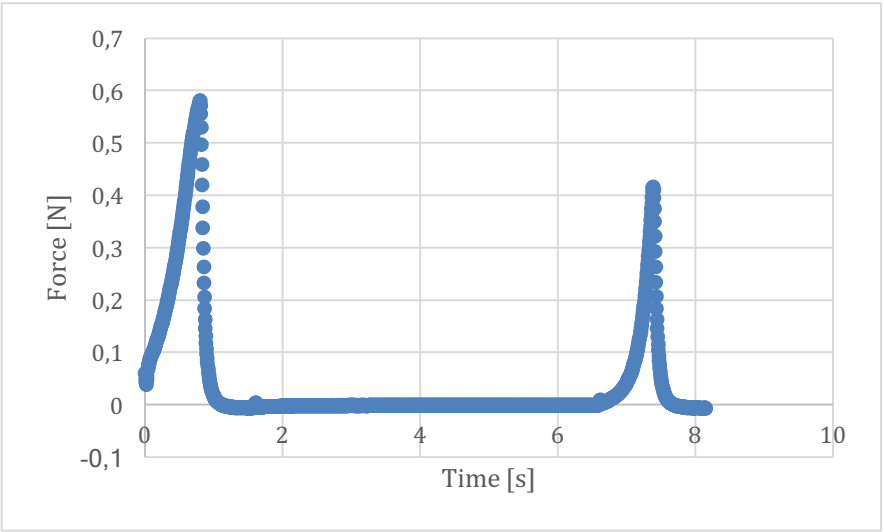


Figure 22: Graphical representation of force applied during the experiment of 50% of deformation.

The following table shows the results obtained in the texturometer to 50% of deformation.

Table 25: Results of parameters measured during 50% deformation experiment

TPA PARAMETER [SI unit]	VALOR
Hardness [N]	1.741 ± 0.288
Adhesiveness [g·s]	-1.803 ± 0.275
Springiness [N]	0.228 ± 0.025
Cohesiveness [N]	0.136 ± 0.010
Gumminess [N]	0.213 ± 0.020
Chewiness [J]	0.054 ± 0.010
Resilience [N]	0.098 ± 0.011

- 90% of deformation:

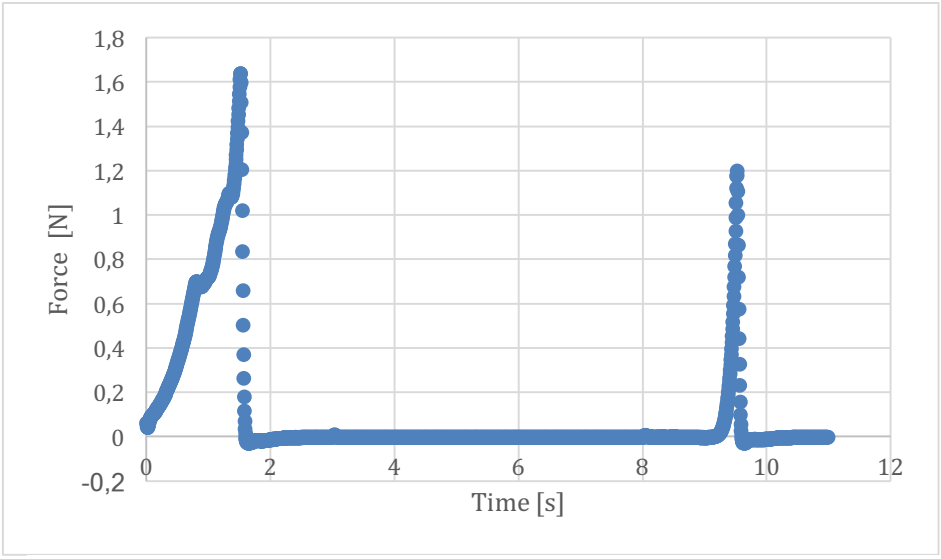


Figure 23: : Graphical representation of force applied during the experiment of 90% of deformation.

The following table shows the results obtained in the texturometer to 90% of deformation.

Table 26: Results of parameters measured during 90% deformation experiment

TPA PARAMETER [SI unit]	VALOR
Hardness [N]	0.502 ± 0.266
Adhesiveness [g·s]	-1.080 ± 0.231
Springiness [N]	0.967 ± 0.047
Cohesiveness	0.337 ± 0.014
Gumminess [N]	0.238 ± 0.254
Chewiness [J]	0.164 ± 0.088
Resilience [N]	0.181 ± 0.010

The fracturability, is the first significant break in the first bite. As can be seen in the figure 22, at 50% of deformation a significant break is not observed, which mean that at this deformation, the beads do not break. In contrast, the figure 23, shows a significant break at 0,699 N. Therefore, it can be stated that for breaking the bead, a force of approximately 1.6 N, corresponding to 90% deformation, is required.

The hardness, is the maximum force applied during the first bite. This value refers to the force required to compress a food between the tongue and the palate [63]. As expected, the force required to deform a given percentage is less the less we want to deform.

If the sample is sticky, the force represented on the graph becomes negative. The area of this negative force is taken as a measure of the adhesiveness of the sample [64]. As can be seen, the adhesiveness is less in the 50% of deformation than in the 90%. Therefore, it can be stated that work to unstick the whole sphere is greater than the break sphere.

Springiness (elasticity) is the height that recover the sample during the time between the first and second cycle. Is the measure of the quantity of sample that has been broken in the first bite. As expected, the quantity of broken sample is larger when it comes to 90% of deformation, when the sample is broken.

The cohesiveness represents the bond strength between the particles, which means the limit to which it can deform before breaking [65]. As expected, the force required to deform a given percentage is less the less we want to deform. The limit of deformability is greater when the sample is deformed to 90%, because in the first deformation no rupture is observed.

The gumminess is the energy required to disintegrate a semi-solid aliment in order to be ready to be swallow. The chewiness represents de work to disintegrate the aliment in order to be ready to be swallow. These parameters are a function of the cohesiveness, so it is expected that it will be greater in the deformation of 90%. These parameters are a function of the cohesiveness, so it is expected that it will be greater in the deformation of 90%. The reason is simple, because if we deform more, more energy and work is needed.

The resilience is the capacity of the sample to endure the deformation. The comparison is also simple. The sample endure more when the deformation is at 90% until the point of fracture.

5. CONCLUSIONS

The experimental study has allowed to reach a series of conclusions:

- The method of extraction with MeOH, is more effective than the extraction with water, because from the extraction with water an extract is obtained with a concentration 50% smaller with respect to the extraction with MeOH.

- For the extraction of the beads, a time of 30 minutes is sufficient, because during this time an extraction of more than 80% of the content of the beads is obtained.

- The hydrocolloid that best gels the extract of raspberry juice is alginate, which provides beads with perfectly spherical geometry and resistant texture. The concentration with which the best results are obtained is in all cases the highest.

- The factor that most affects the degradation of the extract is in all cases the light. The temperature influences slightly the degradation, but not as the light, with which practically all the polyphenols are degraded.

- The encapsulation process introduces a small amount of active ingredients. For each gram of sphere, it is estimated that 25% of polyphenols are obtained which is obtained in 1 gram of extract. These data indicate that the capsule contains a relatively low extract percentage.

- The degradation of the polyphenolic content in the beads is much smaller than in the extract, despite the little amount of extract that they contain. This statement indicates that encapsulation has achieved its goal, prevent degradation of the active ingredients. The prevention of polyphenolic content degradation has been reduced by 20-25%.

- The percentage of prevention of loss of antioxidant activity is lower than that of loss of polyphenols. In this case, the loss has been reduced by 10-15%.

REFERENCES AND NOTES

[1] Biruete Guzmán A, Juárez Hernández E, Sieiro Ortega E, Romro Viruegas R, Silencio Barrita, JL. Los nutracéuticos. Lo que es conveniente saber. Vol 76. Núm 3 pp 136-145. 2009.

[2] Hardy G. Nutraceuticals and functional foods: Introduction and meaning. Nutrition. 16:688–9. 2000;

[3] Anukam KC, Reid G. Probiotics: 100 years (1907– 2007) after Elie Metchnikoff's observations. In Communicating current research and educational topics and trends in applied microbiology pp. 466–474. 2008.

[4] Marco ML, Pavan S, Kleerebezem M. Towards understanding molecular modes of probiotic action. Curr Opin Biotechnol 17: 204–210. 2006.

[5] Timmerman, H. M., Koning, C. J. M., Mulder, L., Rombouts, F. M., & Beynen, A. C. Monostrain, multistrain and multispecies probiotics e a comparison of functionality and efficacy. International Journal of Food Microbiology, 96, 219e233. 2004.

[6] Famularo, G., de Simone, C., Matteuzzi, D., & Pirovano, F. Traditional and high potency probiotic preparations for oral bacteriotherapy. Bio Drugs, 12, 455e470. 1999.

[7] Sanders, M. E. Probiotics. Food Technology, 53, 67e75. 1999.

[8] Reid, G. The role of Cranberry and probiotics in intestinal and urogenital tract health. Critical Reviews in Food Scienc e and Nutriti on, 42, 293e300. 2002.

[9] Chiou, D., & Langrish, T. A. G. Development and characterisation of novel

nutraceuticals with spray drying technology. Journal of Food Engineering, 82, 84e91. 2007.

[10] Seeram, N. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. Journal of Agricultural and Food Chemistry 56:627-629. 2008.

[11] Carolina F, Gloria M, Juan Pablo Z, Francisca S, and Paz R. Comparison of the total phenolic content, total anthocyanin content and antioxidant activity of polyphenol-rich fruits grown in Chile. vol.41 no.1. 2014.

[12] Burton-Freeman, B. M., Sandhu, A. K., & Edirisinghe, I. Red raspberries and their bioactive polyphenols: cardiometabolic and neuronal health links. Advances in Nutrition, 7(1), 44–65. 2016.

[13] Rember, S.F.; Mage, F.; Haffner, K; Blomhoff, R. Highbush blueberries *Vaccinium corymbosum* L., raspberries *Rubus idaeus* L. and black currants *Ribes nigrum* L. - influence of cultivar on antioxidant activity and other quality parameters. Acta Horticulturae, v.744, p.259-266. 2007.

[14] Kafkas, E.; Kosar, M.; Türemis, N.; Baser, K.H.C. of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. Food Chemistry, v.97, p.732-736. 2006.

[15] Milivojevic, J.; Slatnar, A.; Mikulic-Petkovsek, M,; Stampar, F.; Nikolic, M.; Veberic, R.; The influence of early yield on the accumulation of major taste and health related compounds in black and red currant cultivars (*Ribes* spp.). Journal of Agricultural. 2008.

[16] Capocasa, F., J. Scalzo, B. Mezzetti, and M. Battino. Combining quality and antioxidant attributes in the strawberry: The role of genotype. Food Chemistry 111:872–878. 2008.

[17] Koca, I. and B. Karadeniz. Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey. Scientia Horticulturae 121:447-450. 2009.

[18] Caliskan, O., and S. Bayazit. Phytochemical and antioxidant attributes of autochthonous Turkish pomegranates. Scientia Horticulturae 147:81–88. 2012

[19] Josuttis, M., C. Carlen, P. Crespo, R. Nestby, T.B. Toldam- Andersen, H. Dietrich, and E. Krüger. A comparison of bioactive compounds of strawberry fruit from Europe affected by genotype and latitude. Journal of Berry Research 2:73–95. 2012.

[20] Skrede, G., B.K. Martinsen, A-B. Wold, S-E. Birkeland, and K. Aaby. Variation in quality parameters between and within 14 Nordic tree fruit and berry species. Acta Agriculturae Scandinavica Section B-Soil and Plant Science 62:193-208. 2012.

[21] Wang, S.Y., H. Chen, M.J. Camp, and M.K. Ehlenfeldt. Genotype and growing season influence blueberry antioxidant capacity and other quality attributes. International Journal of Food Science and Technology 47:1540-1549. 2012.

[22] De Pascual-Teresa, S., and M.T. Sanchez-Ballesta. Anthocyanins: from plant to health. Phytochemistry. Reviews 7:281–299. 2008.

[23] Maatta-Riihinen, K.R.; Kamal-eldin, A.; Torronen, A.R. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family *Rosaceae*). Journal of Agricultural and Food Chemistry, v.52, p.6178-6187. 2004.

[24] Kopenen, J.M.; Mattila, P.H.; Torronen, A.R. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. Journal of Agricultural and Food Chemistry, v.55, p.1612-1619. 2007.

[25] Zheng, W.; Wang, S.Y. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. Journal of Agricultural and Food Chemistry, v.51, p.502-509. 2003.

[26] Siriwoharn, T.; Wrolstad, R.E.; Finn, C.E; Pereira, C.B. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. Journal of Agricultural and Food Chemistry, v.52, p.8021-8030. 2004.

[27] X. Han, T. Shen, H. Lou, Dietary polyphenols and their biological significance, Int. J. Mol. Sci. 8 950–988. 2007.

[28] C. Moctezuma, A. Hammerbacher, M. Heil, J. Gershenzon, R. Méndez-Alonzo,K. Oyama, Specific polyphenols and tannins are associated with defenseagainst insect herbivores in the tropical oak Quercus oleoides, J. Chem. Ecol. 40, 458–467. 2014.

[29] A. Scalbert, G. Williamson, Dietary intake and bioavailability of polyphenols, J.Nutr. 130, 2073S–2085S. 2000.

[30] Spencer, J. P. E., Vafeiadou, K., Williams, R. J., & Vauzour, D. Neuroinflammation: modulation by flavonoids and mechanisms of action. Molecular Aspects of Medicine, 33(1), 83–97. 2012.

[31] Wu, X., & Schauss, A. G. Mitigation of inflammation with foods. Journal of Agriculture and Food Chemistry, 60(27), 6703–6717. 2012.

[32] Bobinaite˙ , R., Viškelis, P., & Venskutonis, P. R. Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (Rubus spp.) cultivars. Food Chemistry, 132(3), 1495–1501. (2012).

[33] Jo, Y., Park, H., Choi, S., Kim, S., Bao, C., Kim, H. W., ... Auh, J. Metabolomic analysis reveals cyanidins in black raspberry as candidates for suppression of lipopolysaccharide-induced inflammation in murine macrophages. Journal of Agricultural and Food Chemistry, 63(22), 5449–5458. 2015.

[34] Chen, W., Su, H., Huang, Z., Feng, L., & Nie, H. Neuroprotective effect of raspberry extract by inhibiting peroxynitrite-induced DNA damage and hydroxyl radical formation. Food Research International, 49(1), 22–26. 2012.

[35] Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. American Journal of Clinical Nutrition, 81(1), 230S–242S. 2005.

[36] Tripathi, M. K. y S. K. Giri, Probiotic functional foods: Survival of probiotics during processing and storage, J Funct Foods, 9, 225-24. 2014.

[37] Tripathi, M. K. y S. K. Giri, Probiotic functional foods: Survival of probiotics during processing and storage, J Funct Foods, 9, 225-241, 2014.

[38] Semyonov D., K. Z. Ramon O., Levin-Brener L., Gurevich N. y S. E., Microencapsulation of Lactobacilllus paracasei by spray freeze drying, Food Res Int, 43, 193- 202, 2010.

[39] Ranadheera, R. D. C. S., S. K. Baines y M. C. Adams, Importance of food in probiotic efficacy, Food Res Int, 43(1), 1-7, 2010.

[40] Ray, S., U. Raychaudhuri y R. Chakraborty, An overview of encapsulation of active compounds used in food products by drying technology, Food Biosci, 13, 76-83. 2016

[41] Strasser, S., Neureiter M., M. Geppl , R. Braun y H. Danner, Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria, J App Microbiol, 107, 167-177. 2009.

[42] Shamekhi, F., M. Shuhaimi, A. B. Ariff y A. M. Yazid, Optimization of a cryoprotective medium for infant formula probiotic applications using response surface methodology, Ann Microbiol, 62, 911–921. 2012.

[43] Quigley, E. M., Prebiotics and probiotics; modifying and mining the microbiota, Alimentary Pharmabiotic. Centre, University College Cork: 6, 213– 218, (2010)

[44] Vasiljevic, T. y Shah, N. P., Probiotics— From Metchnikoff to bioactives, Int Dairy J, 18(7), 714-728, (2008).

[45] Vasiljevic, T. y Shah, N. P., Probiotics—From Metchnikoff to bioactives, Int Dairy J, 18(7), 714-728, 2008.

[46] Fritzen-Freire, C. B., E. S. Prudencio, R. D. M. C. Amboni y S. S. Pinto, Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics, Food Res Int, 45(1), 306-312. 2012.

[47] Casiraghi, M. C.; Canzi, E.; Zanchi, R.; Donati, E.; Villa, L. Effects of a symbiotic milk product on human intestinal Ecosystem, J Appl Microbiol, 103, p. 499-506. 2007.

[48] Nedovic V, Kalusevic A, Manojlovic V, Levic S, Bugarski B. Procedia Food Sci; 1:1806. 2011.

[49] Wandrey C, Bartkowiak A, Harding SE. Materials for encapsulation. In: Zuidam NJ,

Nedovic VA, editors. Encapsulation technologies for food active ingredients and

food processing. Dordrecht, The Netherlands: Springer; p. 31. 2009.

[50] Fang Z, Bhandari B. Trends Food Sci Technol; 21:510. 2010

[51] Gibbs BF, Kermasha S, Alli I, Mulligan CN. Int J Food Sci Nutr; 50:213. 1999.

[52] Ghosh AK, Bandyopadhyay P. Polysaccharide–protein interactions and their relevance in food colloids. The complex world of polysaccharides. Karnataka: Bangalore: Unilever R & D Bangalore; p. 395 Cap. 14. 2012.

[53] Jin, L., Zhang, Y. L., Liang, Z. X., Zhang, X. X., Si, G. C., & Niu, L. X. Phenolic compounds and antioxidant properties of bulb extracts of lilium leucanthum (Baker) Baker native to China. Quality Assurance & Safety of Crops and Food, 7, 141–151. 2015

[54] G. Peña-Varela; Y. Salinas-Moreno; R. Ríos-Sánchez; Contenido de antocianinas totales y actividad antioxidante en frutos de frambuesa con diferente grado de maduración. Chapingo Serie Horticultura 12(2): 159-163. 2006

[55] Brishila Lupo Pasin; Estudio de la gelificacion de alginatos para encapsulación; caracterización, preparación y aplicación en alimentos funcionales. Departament d'Enginyeria Quimica. 2014.

[56] Funami, Takahiro; Fang, Yapeng; Noda, Sakie; Ishihara, Sayaca; Nakauma, Makoto; Draget Kurt I. Nishinari, Katsuyoshi and Phillips, Glyn O. Rheological properties of sodium alginate in an aqueous system during gelation in relation to supermolecular structures and Ca2+ binding. Food Hydrocolloids. 23(7):1746- 1756. 2009

[57] Shimada, K.Fujikawa, K.Yahara, K.Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agriculture and Food Chemistry, 40, pp. 945-948. 1992.

[58] Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods in Enzymology, 299, 152-178. 1999

[59]: Brennan, J. G., Jowitt, R. & Williams, A. An Analysisof the Action og the General Foods Tecturometer Journal of Texture Studies 6, 83-100. 1975.

[60] RUIZ, Jorge Luís. Textura de músculos de cerco y de jamón curado con distintos niveles de NaCl, pH y contenido de agua. Tesis doctoral (Facultad de veterinaria). Autónoma de Barcelona. 212 p. 2005.

[61] Texture – A hydrocolloid recipe collection, edited by Martin Lersch. v. 3.0. February 2014

[62] Brand-Williams W., Cuvelier M.E., Berset C. Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.* 28, 25-30. 1995

[63] Civille, Gail y Szczesniak, Alina. Guidelines to training a textute profile panel. Journal texture Studies. Vol. 4: 204-223. 1976.

[64] Rosenthal, A. Food texture, measurements and perception. Editorial Aspen Publisher, INC. 1999.

[65] Szcesniak, Alina. Texture is a sensory Property. Food Quality and Preference. Vol 13: 215-225. 2002.

ACRONYMS

AAR	Antiradical Activity
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GA	Galic Acid
HCl	Hydrochloric Acid
LAB	Lactic Acid bacteria
MeOH	Methanol
TPA	Texture Profile Analysis
UTC	Ultra turrax

APPENDICES

APPENDIX 1: CALIBRATION CURVE

Concentration of GA [mg/mL]	Absorbance at 765 nm [nm]
0,075	0,070
0,125	0,126
0,175	0,178
0,250	0,270
0,375	0,392
0,425	0,453
0,500	0,521
0,575	0,604