Research Article **Open Access**

Functional Foods in Health and Disease

Chemical composition, physicochemical evaluation and sensory analysis of yogurt added with extract of polyphenolic compounds from *Quercus crassifolia* oak bark

Eréndira Valencia-Avilés[1,2], Martha Estrella García-Pérez[3], Ma. Guadalupe Garnica-Romo[4], Juan de Dios Figueroa-Cárdenas[5], Maria Paciulli[6], Héctor Eduardo Martínez-Flores[3*]

[1]Programa Institucional de Doctorado en Ciencias Biológicas, Universidad Michoacana de San Nicolás Hidalgo, Ciudad Universitaria, Morelia, Michoacán, Mexico; [2]Universidad Politécnica de Uruapan. Carretera Uruapan – Carapan. No. 12000. Col. Capacuaro, Uruapan, Mich; [3]Facultad de Químico Farmacobiología. Universidad Michoacana de San Nicolás de Hidalgo. Tzintzuntzan 173, Col. Matamoros. Morelia, Mich., México. C.P. 58240; [4]Facultad de Ingeniería Civil, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, Morelia, Michoacán, Mexico; [5]Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Libramiento Norponiente 2000, Fraccionamiento Real de Juriquilla, Querétaro, Mexico; [6]Dipartimento di Scienze degli Alimenti e del Farmaco, Università di Parma, Parma, Italy.

***Corresponding author:** Professor Héctor Eduardo Martínez-Flores, Facultad de Químico Farmacobiología. Tzintzuntzan 173. Col. Matamoros. Morelia, Mich., México.

Submission date: June 6th, 2022; **Acceptance date:** August 23rd, 2022; **Publication date:** September 8, 2022

Please cite this article as: Valencia-Avilés E., García-Pérez M.E., Garnica-Romo M.G., Figueroa-Cárdenas J.D., Paciulli M., Martínez-Flores H.E. Chemical composition, physicochemical evaluation and sensory analysis of yogurt added with extract of polyphenolic compounds from *Quercus crassifolia* oak bark. *Functional Foods in Health and Disease.* 2022; 12(9): 502-517. DOI: 10.31989/ffhd.v12i9.951

ABSTRACT

Introduction: A diet high in calories and saturated fats has been associated with health problems that have been increasing worldwide. Therefore, it is required to increase the number of formulated foods that generate well-being to health. Yogurt is a widely consumed food by all sectors of the population and it can be used as a vehicle to incorporate bioactive compounds. The phenolic compounds present in forest residues, such as those from oak bark, can be used and incorporated into yogurt, to increase its benefits as a functional food.

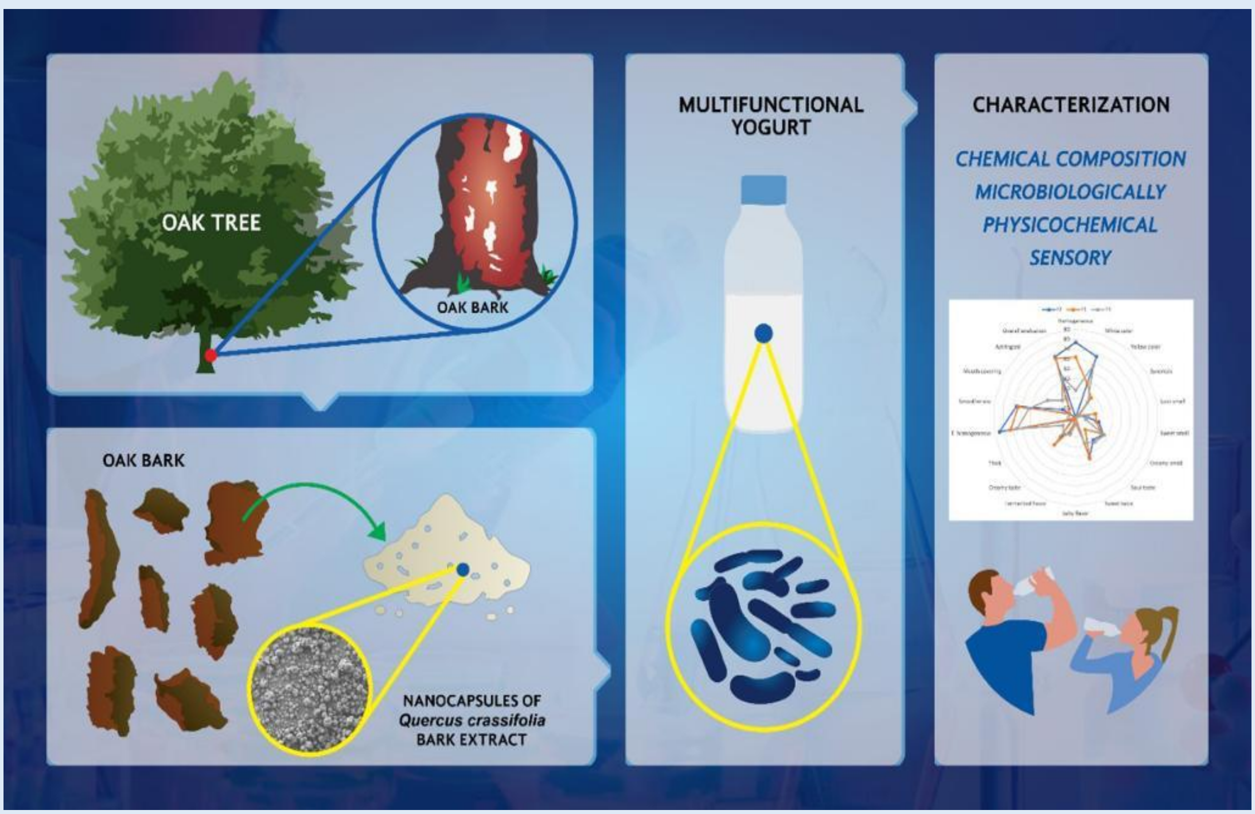
Objective: The objective of this study was to develop a multifunctional yogurt enriched with vegetable oil (2.3% w/w) as a source of omega 6 and 3 and adding nanocapsules (24.5% w/w) of an extract of oak bark from *Quercus crassifolia*, rich in in phenolic compounds and high antioxidant capacity.

Methods: Three yogurt formulations were prepared: F1: yogurt was made with non-fat milk, used as a control, F2: yogurt was prepared with non-fat milk and added with vegetable palm oil, and F3: non-fat yogurt was added with vegetable oil and nanoencapsulated oak bark phenolic extract. The yogurts were characterized in their chemical composition, microbiological analysis, and sensory analysis.

Results: The multifunctional product F3 and product F2 presented lactic acid bacteria in concentration of $3.01X10^6$ and $4.73x10^6$, respectively, preserving characteristics of probiotic food. Product F3 presented low levels of syneresis (7.34%) and it was significantly different from the control yogurt (9.01%). The viscosity increased from 150 cP in the control yogurt to 341 cP in F3, due to the increase in the concentrations of solids by nanoencapsulating the phenolic. The wall material used for nanoencapsulation was sodium caseinate and mantodextrin. However, this increase in viscosity did not affect the sensory evaluation of the product. There were no significant differences between the control yogurt and the F2 and F3 products.

Conclusion: A yogurt added with vegetable oil and nanoencapsulated oak bark phenolic extract was obtained. It was enhanced by the presence of probiotics, bioactive compounds, and essential fatty acids, and then evaluated and accepted by a sensory panel. Nanoencapsulation is a viable alternative to mask the characteristic astringent taste of phenolic compounds because it was not detected by the panelists.

Keywords: waste recovery; functional dairy foods; essential fatty acids; probiotics; antioxidants; sensory.



INTRODUCTION

Functional foods are consumed with the aim of delaying, reducing, or preventing chronic degenerative diseases. From a practical viewpoint, a functional food can be a natural, whole food that contains enough beneficial components or bioactive compounds. Other foods may be specially formulated with specific components to provide health benefits [1]. Some examples of bioactive compounds are dietary fiber, ascorbic acid, terpenes, ß-carotenoids, lutein, omega-6 and omega-3 fatty acids, probiotics, and phenolic compounds. Phenolic compounds are recognized for their antioxidant properties, and they can also act in multiple pathways involved in prevention of various pathologies [2-3]. In recent years, the study and commercialization of prebiotics and probiotics has become important due to their healthy properties. Easy-to-drink yogurts are among the products based off of probiotics on which research is focusing [4].

Yogurt is considered one of the most popular fermented milk products and it has gained widespread consumer acceptance as a healthy food [5,6]. The consumption of whole fat yogurts is declining due to the awareness of the probable harmful effect of saturated fat on consumer's health. Thus the dietary habits of consumers have changed, and market interest moved in favor of low or nonfat dairy products [5].

The supplementation of yogurt with healthy ingredients represents a strategy to convey functional foods to a large sector of consumers. Several studies report the technological and nutritional benefits of adding polyphenols extracts to dairy products [7-9]. Recent evidence shows that the probiotics' metabolism can transform polyphenols into physiologically active metabolites [10-11]. It has been suggested that most dietary polyphenols are intact when they reach the small intestine and colon[11-12]. It is estimated that around 90-95% polyphenols reach the colon, and the gut microbiota participates in their metabolism [13]. Some studies have shown that oral administration of both probiotics and polyphenols leads to a bioconversion of complex polyphenols to more bioavailable compounds (postbiotics) [11,14]. Scientific data has provided evidence that postbiotics possess different functional properties including antimicrobial, antioxidant, and immunomodulatory. These properties can positively affect the microbiota homeostasis and/or the host metabolic and signaling pathways. Thus affecting specific physiological, immunological, neuro-hormone biological, regulatory, and metabolic reactions [15-16].

Oak bark, which is considered waste by the forestry industry, contains a wide variety of bioactive compounds, including phenolic compounds [17]. Mexico is the largest center for the number of oaks (*Quercus* spp.), with about 161 species and 109 of them being endemic to the country. The main use of oaks is as timber. Oak generates a large amount of bark that can be valorized as a very interesting source of bioactive compounds, for the development of new food formulations. In previous work, we found that a purified extract from *Quercus crassifolia* bark is a good source of polyphenols (860.39 ± 5.68 mg GAE/g) with a higher in vitro free radical scavenging ability toward reactive species of biological importance (hydroxyl and peroxyl radical and the superoxide anion) [18]. Moreover, in another study, we tested the antibacterial activity and subacute-oral toxicity of the oak bark's phenolic extract, in order to understand the potential impact of its addition to fermented foods as a functional ingredient [19].

Phenolic compounds are photosensitive, heat labile, and susceptible to oxidation when exposed to various environmental factors. Furthermore, phenol compounds often have low bioavailability mainly due to low water solubility. Finally, many of these molecules are astringent and possess a bitter taste, which limits their use in food. For this reason, phenolic compounds must be protected.

For these reasons, it is convenient to apply methodologies that protect phenolic compounds from environmental and gastrointestinal conditions so that they do not affect their availability. Also, so that they do not interfere with the sensory properties of the foods in which they are incorporated.

Encapsulation and nanoencapsulation has been developed as a promising technique to protect phenolic compounds from external factors [20, 21]. Nanoencapsulation can improve solubility, increase the digestive transit time, improve the sensory characteristics of food, mask unpleasant flavors. This includes a bitter taste and an astringency of polyphenols and protects bioactive compounds from degradation avoiding undesirable physical and chemical reactions [22,23].

The objective of the present study was to elaborate a yogurt made with defatted milk added with vegetable oil as a source of essential fatty acids while added with a nanoencapsulated phenolic extract from oak bark from *Quercus crassifolia*. Then the objective was to evaluate the chemical, physicochemical, microbiological, and sensory properties of yogurt.

METHODS

Materials: The oak bark collection was carried out in a forest plantation located in Ciudad Hidalgo, Michoacán, México. The species commonly called "white" was chosen considering its importance in the forestry industry of Michoacán state. The botanical identification was done from branches and leaves. The oak bark was identified as *Q. crassifolia* at the Institute of Ecology in Pátzcuaro, Michoacán, and deposited in this herbarium. The collected barks were washed, and all the remaining silica, lichens, and moss were removed. Subsequently, the barks were divided into rectangles of 5 by 5 cm and dried in ovens at a temperature of 40 °C for 48 h. Grinding (Thomas Digital ED-5 Wiley® Cutting Mill) and sieving (# 40 mesh - 400 µm) were then carried out.

The milk used was Sello Rojo (Alimentos Sello Rojo S.A. de C.V., Guadalajara, Jalisco, Mexico) with a fat content of 0.20%, 3.01% protein, and 4.87% carbohydrates. Vegetable palm oil was used, which contained 21.35% of saturated fat, 48.88% of monounsaturated fat found all as oleic acid, and 26.08% of polyunsaturated fat, of which 21.34% was linoleic acid (omega 6) and 3.59% alpha-linoleic acid (omega 3). The gelatin used was from the company K'nox (Con Alimentos, S.A de C.V., Ecatepec, Edo. De México, México).

Polyphenol's extraction and nanoencapsulation: Phenolic extraction was carried out by mixing 50 g of powder bark with 500 ml of water under reflux for 1 h, and the solids were separated by filtration with a filter paper Whatman No. 42, and then it was subsequently washed with 500 ml of hot water [2]. Purification and concentration of the *Q. crassifolia* phenolic compounds was carried out [2,24]. In this step, 100 mL of crude extract of oak bark was filtered with a Gooch crucible (Pyrex 40-60 µm). The solution was defatted with hexane (Meyer) (5X100 ml), the aqueous fraction was recovered, and subsequently, phenolic compounds were extracted with ethyl acetate (Meyer) (5X100 ml). The solvent was evaporated, and the remaining solids were nanoencapsulated by spray drying (Büchi Nano Sparydryer B90). According to preliminary tests, the phenolic extract was nanoencapsulated at a concentration of 30% solids of the extract. In this step, was tested wall material formulations containing sodium caseinate and maltodextrin in a ratio of 25% - 75 and 50% - 50%, respectively, varying the pH 3.0, 3.5, 4.0, 4.5 and 5.0% values. The nanocapsules were observed by scanning electron microscopy to determine their morphology and integrity. The best results, showing that nanocapsules with greater integrity, were obtained at a ratio of 25% sodium caseinate and 75% maltodextrin at pH 5.0. The encapsulation efficiency of the phenolic compounds was 68.9%. The mean size of the nanocapsules was 328 nm. Also in that same study, the chemical composition of the extract was identified by GC-MS, finding nine phenolic compounds: 1-Hexanone, 5-methyl-1-phenyl; 2-methoxy-phenol, acetate; Phenyl-1,2-diamine, N,4,5-trimethyl-; Phenol, 2,6-dimethoxy- (syringol); Benzeneethanol, 4-hydroxy- (tyrosol); Pyrogallol 1,3-dimethyl ether; 8-Phenyl-5,5a,6,10,10a,11-hexahydro-5,11-(Obenzeno)-6,10-ethenocyclohepta(b)naphthalen-7-one; Scyllo-Inositol; Apigenin 7-glucoside.

Yogurt Formulation: Three yogurt formulations were made: nonfat yogurt (F1), yogurt with vegetable oil (F2), and multifunctional yogurt with vegetable oil that was added with nanocapsules containing phenolic extract from *Q. crassifolia* and denominated as multifunctional yogurt (F3).

The elaboration of the multifunctional yogurt was carried out according to the formulation of Tranquilino-Rodríguez et al. [25] with some modifications. The yogurt was prepared as follows: the defatted milk (91.95%) was mixed with gelatin (0.25%) and sucrose (5%), and then was placed at 63 °C under constant stirring (stage 1). A lactic culture (*L. delbrueckii* subspecies bulgaricus and *S. thermophilus* subspecies salvarius (Danisco®) were used) was added at a concentration of 0.03 g/L, and was kept in incubation for 9 h at 43 °C. After that, the product was cooled to 4 ºC (stage 2). This product was denominated F1. To prepare product 2, labeled as F2, the following was done: after having obtained the mixture in stage 1, vegetable oil (2.3%) and a mixture of mono and diglycerides (0.5%) were added. Next, the same yogurt making process conditions were continued as described in stage 2. To prepare product 3, labeled as F3, the following procedure was carried out: after having obtained the mixture in stage 1 and continued with the same yogurt making process conditions described in stage 2, the oak extract nanocapsules were added, in a concentration of 2.45 g nanocapsules in 10 g of yogurt.

Chemical composition of yogurt: Moisture, ashes, crude protein, and fat contents were determined according to the Association of Official Agricultural Chemists methods 926.08, 942.05, 991.20, and 2000-18, respectively [26]. The dietary fiber quantification was made by the enzymatic method of Prosky *et al*. [27]. The carbohydrate content was determined by difference, as follows:

$$\textbf{Eq. 1.}\ \boldsymbol{Carbohydrates}\ (\%) = \mathbf{100} - (\boldsymbol{protein}\% + \boldsymbol{fat}\% + \boldsymbol{moisture}\% + \boldsymbol{ash}\%)$$

Physicochemical análisis: Titratable acidity was determined in yogurt samples at 22 °C using the method 947.05 described in AOAC [26]. Ten grams of yogurt samples were diluted with 10 mL distilled water and titrated with 0.1 M NaOH in the presence of phenolphthalein. Titratable acidity was expressed as the percent of lactic acid based on the sample weight. The pH of the yogurt samples was determined using a Hanna pH meter 210 (Hanna Instruments) at 22 °C.

To measure syneresis, the technique described by Guinee et al. [28] was followed. Ten grams of yogurt were centrifuged at 5000 rpm for 20 min and the supernatant was taken. Calculations were performed by using Equation 2.

$$\textbf{Eq. 2. } Degree\ of\ syneresis = \frac{Supernatant\ Weight}{Sample\ weight} x100$$

The viscosity of the yogurt samples (18 ml) was measured at 4°C using a Brookfield concentric Rheometer (model DV3T, Brookfield Engineering Incorporation, Middleboro, USA), equipped with a SC4-18 spindle, and measured at 4-100 rpm and torque at 40-50%. The viscosity of the yogurt was reported in centipoise units (cP).

Microbiological analysis: To determine the number of viable microorganisms in yogurt, Lactobacillus delbrueckii subsp. Bulgaricus and Streptococcus thermophilus were detected [29]. Molds and yeasts were quantified according to the Official Mexican Standard 111 [30].Sensory analysis of multifunctional yogurt

Selection of sensory judges: A group of sensory judges that regularly consumed yogurt were selected. The selection was conducted starting from a group of 50 people. A sociodemographic survey was applied to find out age, sex, type of diet, and health status (if they had any disease or consumption of controlled drugs and tobacco) [31]. The selected panelists were subjected to a bitterness sensitivity test according to the methodology proposed by several authors [32-34]. Tasters who registered a "high" to "very high" perception of bitterness using the 6n-propylthiouracil (PROP) test were selected. Subsequently, the panelists underwent a basic flavor identification test, with the aim of determining the ability of the candidates to discriminate various stimuli presented at intensities above threshold levels [35]. A basic flavor identification test was carried out with forty-two people, 22 women and 20 men, the participants who obtained more than 60% correct answers were chosen.

Training for the use of scales Sensory profile of yogurt: The sensory profile of the different formulations of the multifunctional yogurt was carried out using the “Check all that apply (CATA)” methodology. Each judge filled out a form for each formulation: nonfat yogurt (F1), yogurt with vegetable fat (F2), multifunctional yogurt (F3). This form contained the sensory descriptors of the yogurt, such as aroma, texture, color, flavor, and appearance [36]. Sensory judges marked on the form the descriptor that they perceived and considered appropriate for each yogurt formulation.

Statistical análisis: Each of the determinations were carried out in triplicate. Experimental results were expressed as mean ± standard deviation. The variables were analyzed using the Shapiro-Wilk and Levene tests. A one-way analysis of variance (ANOVA) followed by a Tukey post hoc test was performed for the three formulation yogurt data. Cochran's Q non-parametric statistical test was used for the analysis of the sensory study data. The statistical analysis software XLSTAT 2018.5 from Addinsoft and Statistica 13.3 from Tibco was used.

RESULTS AND DISCUSSION

Three yogurt formulations were made: F1: yogurt made with non-fat milk, used as control, F2: yogurt prepared with non-fat milk added with vegetable palm oil, and F3: non-fat yogurt added with vegetable oil and nanoencapsulated oak bark phenolic extract, denominated as multifunctional yogurt. Chemical composition, physicochemical, microbiological, and sensory tests were carried out.

Chemical composition and physicochemical analysis of yogurt: The results of chemical composition of F1, F2 and F3 products are presented in Table 1. The fat content was significant ($p < 0.05$) lower (0.32 %) in F1, because the vegetable oil was not added. While for F3 the percentage of fat (1.13 %) resulted lower but not more significant than F2 (1.43 %). This is because in F3 the wall material that was used increased the amount of protein in the sample and had a dilution effect on the other components, including fat. Ash content also decreased significant in F3 (2.88 %) in comparison with the formulations without nanocapsules (F2 = 5.48 % and F1 = 5.63 %, respectively). The protein content shows a trend to increase in F3 as compared to F1 and F2, nevertheless the difference is not significant. It is worth mentioning that calcium caseinate was used as a wall material in the formulation of nanocapsules, thus it increases the protein content in F3. The carbohydrates varied from 66.58 % to 67.14 %, showing no significant differences between the three yogurt samples.

The percentage of lactic acid present in each sample complied with the provisions of the NMX-F-511-1988 Standard, since the minimum permitted percentage of lactic acid is 0.5% [37]. Regarding the pH, it varied from 4.38 in F1 to 4.78 in F3. These values were similar than those reported by Ruiz-Rivera and Ramírez-Matheus [38], who made a yogurt with probiotics in which the highest pH value was 4.64. It related higher acidity and a higher acidic pH value as reported by Mazloomi [39], such as we found in our study. The degree of syneresis for F1 and F2 were 9.09% and 8.42% respectively, with no significant differences. The least degree of syneresis (7.34%) was significantly ($p < 0.05$) observed in the yogurt with nanocapsules (F3). Syneresis was similar to the value described by Crispín-Isidro [40], who reported a value of 9.9% in a yogurt developed with whole milk containing 2.6% fat. The syneresis in F3 improved compared to the control sample F1 and the F2 sample, because the nanocapsules were coated with sodium caseinate and maltodextrins. This meaning it is possible that these components have established hydrogen bonds with water, thus contributing to better water retention in the product, and controlling syneresis. However, no macroscopic changes in the three formulations were observed. The viscosity of each formulation behaved like a pseudoplastic fluid, as the viscosity decrease with the increase of rpm. The viscosity was significantly ($p < 0.05$) higher in F3 as compared to F2 and F1, this behavior may be attributed to the greater number of solids in the sample, causing an increase in its viscosity.

Table 1. Chemical composition and physicochemical analysis of yogurt F1, F2 and F3.

Component (%)	Sample of yogurt		
	F1	F2	F3
Fat	0.32 ± 0.22^{b}	1.43 ± 0.06^{a}	1.13 ± 0.09^{a}
Ash	5.23 ± 0.08^{a}	5.09 ± 0.03^{a}	2.66 ± 0.06^{b}
Protein	20.48 ± 0.60^{a}	19.72 ± 0.16^{a}	21.43 ± 0.26^{a}
Fiber	0.02 ± 0.01^{b}	0.04 ± 0.002^{b}	0.09 ± 0.03^{a}
Carbohydrates	67.14 ± 6.47^{a}	66.58 ± 0.18^{a}	67.00 ± 0.80^{a}
Lactic acid	7.20 ± 0.20^{a}	6.92 ± 0.20^{a}	5.87 ± 0.50^{b}
pH	4.38 ± 0.08^{c}	4.69 ± 0.01^{b}	4.78 ± 0.01^{a}
Syneresis	9.01 ± 0.42^{a}	8.42 ± 0.13^{a}	7.34 ± 0.12^{b}
Viscosity (cP)	150.13 ± 4.42^{b}	154.93 ± 8.25^{b}	341.42 ± 7.84^{a}

F1 = Yogurt control, not vegetable oil and not nanocapsules added, F2 = Yogurt with vegetable oil and without nanocapsules and F3 = Yogurt added with vegetable oil and nanocapsules. Values are expressed as mean ± SD. Means with different letters in the same raw are significantly different at $p < 0.05$ (ANOVA, followed by Tukey's test).

Microbiological analysis: Table 2 shows the results of the microbiological analysis in which it was observed that for F3 only 1 CFU/g of fungi and yeast was detected, for F1 and F2 there were no colonies were detected. Total coliforms were not detected in F1, F2, and F3. The content of lactic acid bacteria for F1, F2, and F3 was $3.75x10^6$, $4.73x10^6$ and $3.01x10^6$ CFU/g of yogurt, respectively. The three products kept the amount of lactic acid bacteria above $1x10^6$ CFU/ml, which is a criterion that has been proposed to exert therapeutic effects [39]. On the other hand, all the microbiological analyses were within the control limits established by the Mexican Standard NMX-F-444-1983 for this product category [41].

Table 2. Microorganisms detected in the different yogurt formulations.

Microorganism	F1	F2	F3	NMX-F-444-1983
Fungi and yeasts	Nd	Nd	1 CFU/g	Maximum 10 CFU/g
Total coliforms	Nd	Nd	Nd	Maximum 10 CFU /g
Lactic bacteria (*S. thermophilus and* *L. bulgaricus*)	$3.75x10^6$	$4.73X10^6$	$3.01X10^6$	Minimum 2,000.000 CFU/g

F1 = Yogurt control, not vegetable oil and not nanocapsules added, F2 = Yogurt with vegetable oil and without nanocapsules and F3 = Yogurt added with vegetable oil and nanocapsules. Nd = Not detectable.

Sensory evaluation: A total of 50 participants were registered in the sociodemographic survey, of which eight were eliminated for being smokers or taking controlled medications. According to the results of the PROP test, the basic taste test, and the training for scales, 6 sensory judges were chosen to participate in the sensory profile of yogurt, 2 men and 4 women, from 24 to 31 years old, that are frequent yogurt eaters. Table 3 shows the 17 descriptors used by the evaluators, of which 4 are related to appearance, 3 to aroma, and 6 to flavor and texture in the mouth. As well as the frequency with each of the attributes were mentioned for each formulation. The descriptors that the panelists mentioned most frequently to describe the attributes of the yogurt were homogeneous appearance, white color, sour smell, sweet smell, creamy smell, sour taste, sweet taste, creamy taste, homogeneous texture, smoothness, and mouth covering.

Table 3. Frequency in which the sensory judges mentioned the descriptors.

Attribute	Mentioned frequency total	F1	F2	F3
Homogeneous appearance	12	5	5	2
White color	15	5	5	5
Yellow color	5	3	0	2
Syneresis	4	1	1	2
Sour smell	9	3	3	3
Sweet smell	9	4	3	2
Creamy smell	13	3	5	5
Sour taste	10	3	4	3
Sweet taste	13	5	6	2
Salty flavor	2	0	0	2

Attribute	Mentioned frequency total	F1	F2	F3
Fermented flavor	8	2	3	3
Creamy taste	12	5	4	3
Thick	5	1	1	3
Homogeneous texture	15	5	5	5
Smoothness	16	6	5	5
Mouth covering	12	3	3	6
Astringent	3	1	0	2

F1 = Yogurt control, not vegetable oil and not nanocapsules added, F2 = Yogurt with vegetable oil and without nanocapsules, and F3 = Yogurt added with vegetable oil and nanocapsules.

Table 4 shows the *p* values associated with Cochran's Q tests. It is observed that the attributes that had a high proportion value due to statistical evaluation, indicate that it was marked more frequently by the judges who evaluated the product. It is also appreciated that yogurt without nanocapsules (F2) was more homogeneous and sweeter than the yogurt with nanocapsules (F3). Yogurt with nanocapsules was perceived as salty by some judges, however, no significant differences ($p < 0.05$) were detected. F2 and F3 has identical proportions in the following attributes: white color, sour smell, creamy smell, sour taste, and homogeneous texture. F2 has better values in sweet smell, sweet taste, creamy taste, and smoothness, however the differences were not significant ($p<0.05$). In F3, a slight sensation of salty, astringent, fermented flavor, and a greater mouth covering had been detected. The mouth covering may be due to a greater number of solids in F3 that caused a greater thickness value, and it is possible that a greater salivation is required and can be related to the buccal coating. It is important to highlight that in the evaluated attributes there were no significant statistical differences between the values obtained for F2 and F3.

Table 4. *P*-values and proportions of the attributes associated with Cochran's Q tests**.**

Attributes	***p values***	***Proportions***	
		F2	F3
Homogeneous appearance	0.083	0.833[a]	0.333[a]
White color	1.000	0.833[a]	0.833[a]
Yellow color	0.564	0.500[a]	0.333[a]
Syneresis	0.317	0.167[a]	0.333 [(a)]
Sour smell	1.000	0.500[a]	0.500[a]
Sweet smell	0.083	0.833[a]	0.333[a]
Creamy smell	1.000	0.833[a]	0.833[a]
Sour taste	1.000	0.500[a]	0.500[a]
Sweet taste	0.317	1[a]	0.833[a]
Salty flavor	0.157	0[a]	0.333[a]
Fermented flavor	0.317	0.333[a]	0.500[a]
Creamy taste	0.317	0.833[a]	0.500[a]
Thick	0.157	0.167[a]	0.500[a]

Attributes	***p values***	***Proportions***	
		F2	F3
Homogeneous texture	1.000	0.833[a]	0.833[a]
Smoothness	0.317	1[a]	0.833[a]
Mouth covering	0.180	0.333[a]	0.833[a]
Astringent	0.564	0.167[a]	0.333[a]

F1 was taken as the ideal product. F2 = Yogurt with vegetable oil and without nanocapsules, and F3 = Yogurt added with vegetable oil and nanocapsules.

According to Figure 1, considering F1 as the ideal yogurt, the favorite properties were sour taste, white color, homogeneous appearance, creamy smell, homogeneous texture, sour smell, creamy taste, sweet taste, smoothness, and fermented flavor. Sensory attributes such as mouth covering, sweet smell, and sour smell were considered with less intensity. In addition, F2 shares some attributes with F1, such as, sweet taste, homogeneous appearance, creamy flavor, and smoothness. While F3 was similar to F1 in white color, homogeneous appearance, and fermented flavor. It is evident that three attributes are not related to the desirable parameters of F1, which are astringency, salty taste, and yellow color.

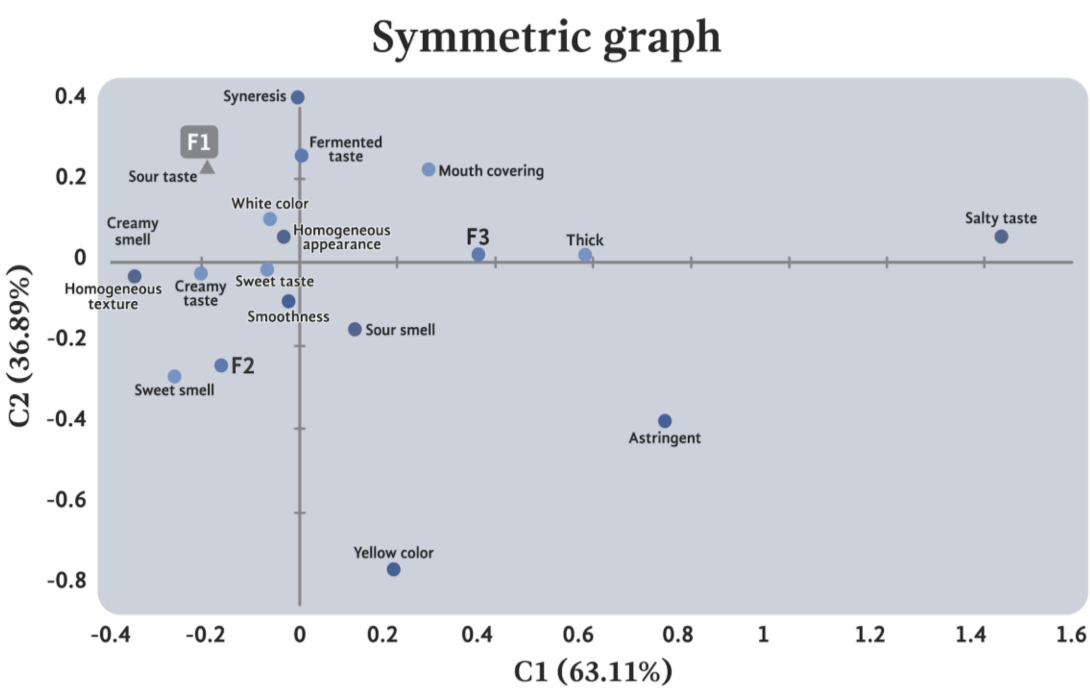


Figure 1. Sensory attributes of each formulation. F1 = Yogurt control, not vegetable oil and not nanocapsules added, F2 = Yogurt with vegetable oil and without nanocapsules, and F3 = Yogurt added with vegetable oil and nanocapsules.

Table 5 shows the correlations between the sensory attributes of products and the global hedonic evaluation of yogurt formulations. The homogeneous appearance, sweet smell, creamy smell, sweet taste, creamy taste, and smoothness resulted positively correlated with the global hedonic evaluation. While attributes such as syneresis, acid taste, salty taste, and mouth coating showed a negative correlation with the hedonic evaluation. Hedonic scores appear to be positively correlated with attributes that are linked to the ideal product (sour, homogeneous, creamy, white, fermented flavor), except for the sour taste.

Table 5. Sensory map of yogurt formulations F1, F2 and F3.

	Homogeneous appearance	White color	Yellow color	Syneresis	Sour smell	Sweet smell	Creamy smell	Sour taste	Sweet taste	Salty flavor	Fermented flavor	Creamy taste	Thick	Homogeneous texture	Smoot hness	Mouth covering	Astrin gent	Overall evaluation
Homogeneous appearance	1	0.14	0.05	-0.99	0.27	0.48	0.14	-0.72	0.96	-0.99	0.05	0.70	-0.19	0.14	0.96	-0.05	0.17	0.58
White color	0.14	1	-0.99	-0.45	-0.97	0.14	-0.88	0.00	-0.84	0.88	-0.14	0.29	0.94	0.65	-0.84	0.14	-0.45	0.23
Yellow color	0.05	-0.99	1	-0.17	-0.27	0.57	0.96	-0.27	0.92	-0.96	0.86	0.39	0.19	-0.14	0.92	0.05	-0.17	0.16
Syneresis	-0.99	-0.45	-0.17	1	0.33	-0.48	-0.45	0.99	-0.98	0.45	-0.17	-0.63	-0.96	0.91	0.86	0.17	0.20	-0.48
Sour smell	0.27	-0.97	-0.27	0.33	1	-0.72	-0.97	0.50	-0.94	0.00	-0.72	-0.55	-0.99	0.00	0.94	-0.27	0.33	-0.17
Sweet smell	0.48	0.14	0.57	-0.48	-0.72	1	0.99	-0.27	0.96	-0.99	0.57	0.88	0.39	0.14	0.96	-0.57	0.17	0.46
Creamy smell	0.14	-0.88	0.96	-0.45	-0.97	0.99	1	-0.97	0.99	-0.65	0.96	0.99	0.94	-0.88	-0.84	0.14	-0.45	0.43
Sour taste	-0.72	0.00	-0.27	0.99	0.50	-0.27	-0.97	1	-0.93	0.00	-0.27	-0.55	-0.55	0.97	0.94	-0.72	-0.33	-0.36
Sweet taste	0.96	-0.84	0.92	-0.98	-0.94	0.96	0.99	-0.94	1	-0.99	0.92	0.97	0.89	-0.84	-0.79	-0.92	-0.98	0.51
Salty flavor	-0.99	0.88	-0.96	0.45	0.00	-0.99	-0.65	0.00	-0.99	1	-0.96	-0.99	0.29	-0.65	-0.99	0.96	0.45	-0.63
Fermented flavor	0.05	-0.14	0.86	-0.17	-0.72	0.57	0.96	-0.27	0.92	-0.96	1	0.39	0.70	0.96	0.92	0.57	-0.17	0.07
Creamy taste	0.70	0.29	0.39	-0.63	-0.55	0.88	0.99	-0.55	0.97	-0.99	0.39	1	0.21	0.29	0.97	-0.39	0.00	0.77
Thick	-0.19	0.94	0.19	-0.96	-0.99	0.39	0.94	-0.55	0.89	0.29	0.70	0.21	1	-0.29	-0.97	0.39	0.00	0.10
Homogeneous textura	0.14	0.65	-0.14	0.91	0.00	0.14	-0.88	0.97	-0.84	-0.65	0.96	0.29	-0.29	1	0.99	0.14	-0.45	0.21
Smoothness	0.96	-0.84	0.92	0.86	0.94	0.96	-0.84	0.94	-0.79	-0.99	0.92	0.97	-0.97	0.99	1	-0.92	0.86	0.34
Mouth covering	-0.05	0.14	0.05	0.17	-0.27	-0.57	0.14	-0.72	-0.92	0.96	0.57	-0.39	0.39	0.14	-0.92	1	0.17	-0.17
Astringent	0.17	-0.45	-0.17	0.20	0.33	0.17	-0.45	-0.33	-0.98	0.45	-0.17	0.00	0.00	-0.45	0.86	0.17	1	-0.34
Overall evaluation	0.58	0.23	0.16	-0.48	-0.17	0.46	0.43	-0.36	0.51	-0.63	0.07	0.77	0.10	0.21	0.34	-0.17	-0.34	1

F1 = Yogurt control, not vegetable oil and not nanocapsules added, F2 = Yogurt without nanocapsules and with vegetable oil and F3 = Yogurt added with vegetable oil and nanocapsules.

The Principal Component Analyses is shown in Figure 2. Overall evaluation hedonic of yogurt is related to sweet taste, fermented flavor, creamy taste, sweet smell, yellow color, sour taste, and homogeneous texture. The two main components explained 100% of the variance of the data, which is satisfactory.

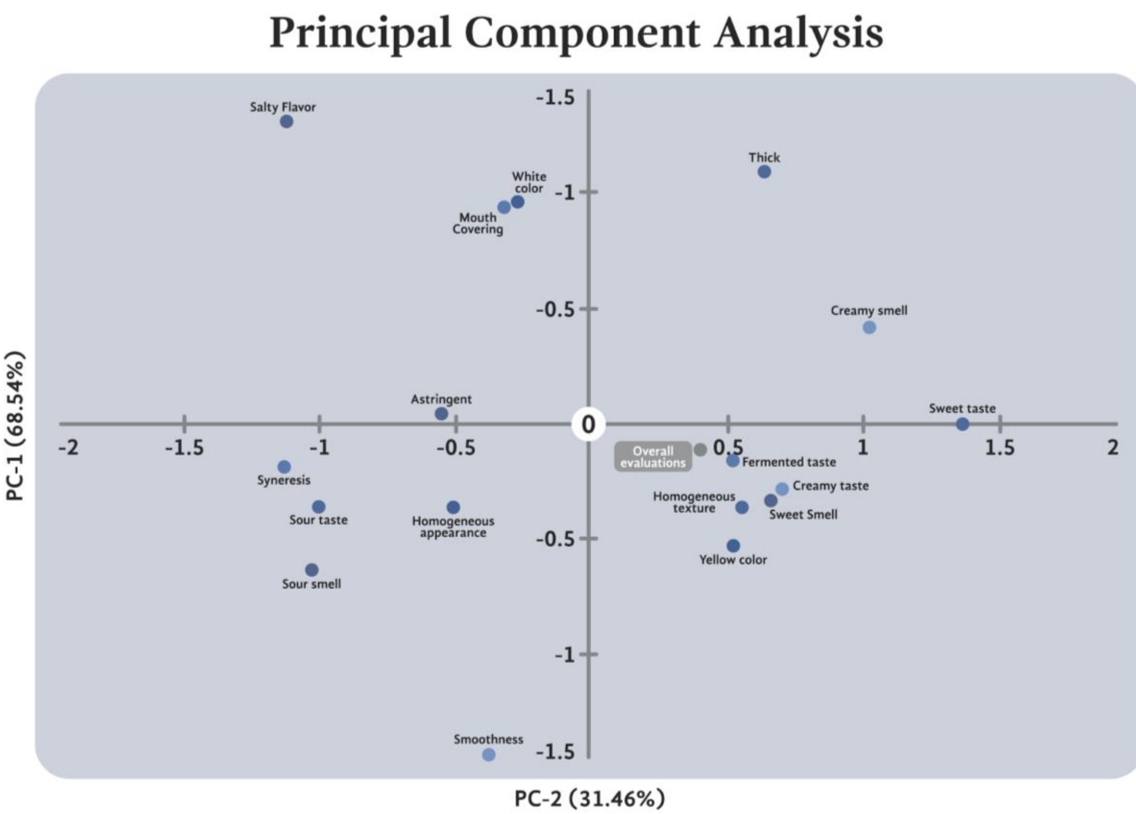


Figure 2. Principal analysis components with descriptors of the hedonic scale.

Table 6 shows the Analysis of Variance of the hedonic global evaluation. Although, there is a trend of greater acceptance of the products F1 and F2 compared to F3, there were no significant differences ($p < 0.05$) between the three yogurt formulations.

In Figure 3, the sensory profile of the yogurt formulations are observed. The panelists used the descriptors to characterize each yogurt formulation, and they described F2 with a homogeneous appearance, white color, homogeneous texture, and smoothness. It had a greater acceptance and it was similar to F1. The F3 sample was white, had homogeneous texture, and smooth texture. An important characteristic to highlight in F3 is that it presents a low perception in the attributes of saltiness and astringency, which is favorable in the acceptance of a yogurt by the consumer.

Table 6. Analysis of Variance of the hedonic scale of the different yogurt samples.

Sample	Overall assessment score
F1	63.94 ± 16.57^a
F2	65.91 ± 25.65^a
F3	44.09 ± 28.46^a

(Means with different letters in the same column are significantly different at $p < 0.05$ (ANOVA, followed by Tukey's test).

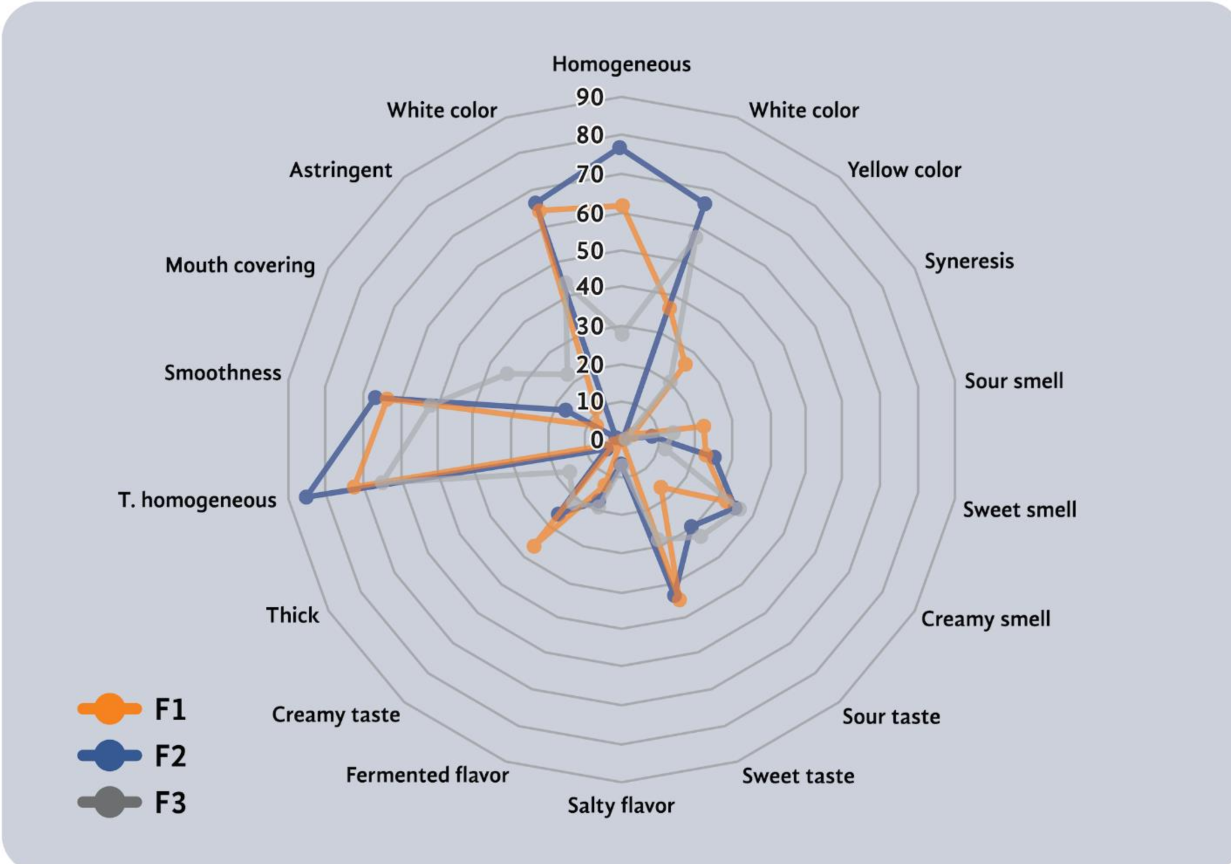


Figure 3. Sensory profile of yogurt formulations F1, F2 and F3.

CONCLUSION

A multifunctional yogurt supplemented was designed with palm vegetable oil replacing the fat milk and it was added with nanocapsules containing a phenolic extract of *Quercus crassifolia*. The yogurt formulated with vegetable palm oil (F2) and the yogurt added with vegetable palm oil and nanocapsules (F3) showed sensory attributes equal to the control yogurt. The nanoencapsulation of the phenolic extract of *Quercus crassifolia* was efficient since the panelists did not detect the characteristic astringent taste of phenolic compounds. In addition, the multifunctional yogurt retained its probiotic properties by containing a quantity of at least $1X10^6$ lactic acid bacteria

List of Abbreviations: AOAC, Association of Official Analytical Chemists; CATA, check all that apply; CFU, colony-forming unit; F1, nonfat yogurt; F2, yogurt with vegetable fat; F3, multifunctional yogurt with vegetable fat and added with nanocapsules containing; polyphenolic extract from *Q. crassifolia*; GAE, gallic acid equivalents; GC-MS, gas chromatography coupled to Mass Spectrometry; Nd = not detectable; No = number; PROP, 6n-propylthiouracil; ROS: reactive oxygen species; SD = standard deviation.

Author Contributions: H.E.M.F. and M.E.G.P. designed the experiments, E.V.A. designed and performed the experiments, M.G.G.R., J.D.F.C., M.P. and H.E.M.F. discuss and analyzed the data obtained during the experiments. All authors approved the paper.

Competing Interests: There are no conflicts of interest to declare.

Acknowledgments: The authors wish to thank CONACyT for the Ph.D. scholarship of Eréndira Valencia Avilés. This research was supported with equipment NanoSpraydryer Buchi B-90 acquired through Project No. 253736 "Equipamiento para el fortalecimiento del grupo de investigación en caracterización de propiedades de los alimentos con impacto en los Programas Institucionales de Maestría y Doctorado en Ciencias Biológicas de la UMSNH", approved for author Héctor Eduardo Martínez-Flores in "Apoyo al Fortalecimiento y Desarrollo de la Infraestructura Científica y Tecnológica 2015" of the Consejo Nacional de Ciencia y Tecnología de México - CONACYT.

REFERENCES:

1. Singh H: Nanotechnology Applications in Functional Foods; Opportunities and Challenges. Prev Nutr Food Sci 2016, 21(1):1-8. DOI: https://doi.org//10.3746/pnf.2016.21.1.1
2. García-Pérez ME, Royer M, Duque-Fernandez A, Diouf PN, Stevanovic T, Pouliot R: Antioxidant, toxicological and antiproliferative properties of Canadian polyphenolic extracts on normal and psoriatic keratinocytes. J. Ethnopharmacol 2010, 132:251–258. DOI: https://doi.org//10.1016./j.jep.2010.08.030
3. García-Pérez ME, Allaeys I, Rusu D, Pouliot R, Janezic, TS, Poubelle PE: *Picea mariana* polyphenolic extract inhibits phlogogenic mediators produced by TNF-α-activated psoriatic keratinocytes: Impact on NF-κB pathway. J. Ethnopharmacol 2014, 151:265–278. DOI: https://doi.org// 10.1016/j.jep.2013.10.034
4. Mishra C, Lambert J: Production of anti-microbial substances by probiotics. Asia Pac J Clin Nutr 1996, 5:20–24.
5. Saleh A, Mohamed AA, Alamri MS, Hussain S, Qasem AA, Ibraheem MA: Effect of different starches on the rheological, sensory and storage attributes of non-fat set yogurt. Foods 2020:9. DOI: https://doi.org/10.3390/foods9010061
6. Saddiqa M, Umbreen H, Zahoor T, Bhatty N, Aftab K: Immunomodulatory Effect of Yogurt Enriched with Garlic Extract on Ulcerative Colitis Induced Rats. J Food Nutr Res 2022, 10(5):377-385. DOI: https://doi.org/10.12691/jfnr-10-5-6
7. Almusallam IA, Mohamed AIA, Saleh A, Al-Juhaimi FY, Ghafoor K, Maiman SA, Elfadil E, Babiker EE: Potential of date palm spikelet extract as an anti-oxidative agent in set-type yogurt during cold storage. CyTA Journal of Food 2021, (1):190-197. DOI: https://doi.org/10.1080/19476337.2021.1877826
8. Mediza Romero ML, von Staszewski M. Martínez MJ: The effect of green tea polyphenols addition on the physicochemical, microbiological and bioactive characteristics of yogurt. Br Food J 2021, 123(7):2380-2397. DOI: https://doi.org/10.1108/BFJ-07-2020-0648
9. Georgakouli K, Mpesios A, Kouretas D, Petrotos K, Mitsagga C, Giavasis I, Jamurtas AZ: The Effects of an Olive Fruit Polyphenol-Enriched Yogurt on Body Composition, Blood Redox Status, Physiological and Metabolic Parameters and Yogurt Microflora. Nutrients 2016, 8, 344; DOI: https://doi.org/10.3390/nu8060344
10. Rossi M, Amaretti A, Leonardi A, Raimondi S, Simone M, Quartieri A: Potential Impact of probiotic consumption on the bioactivity of dietary phytochemicals. J Agric Food Chem 2013, 61:9551–9558. DOI: https://doi.org// 10.1021/jf402722m
11. Gil-Sánchez I, Cueva C, Tamargo A, Quintela JC, de la Fuente E, Walker AW, Moreno-Arribas MV, Bartolomé B: Application of the dynamic gastrointestinal simulator (simgi®) to assess the impact of probiotic supplementation in the metabolism of grape polyphenols. Food Res Int 2020, 129:108790. DOI: https://doi.org/10.1016/j.foodres.2019.108790
12. Marín L, Miguélez EM, Villar CJ, Lombó F: Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int 2015, 905215. DOI: https://doi.org/10.1155/2015/905215
13. Dueñas M, Muñoz-González I, Cueva C, Jiménez-Girón A, Sánchez-Patán F, Santos-Buelga C, Moreno-Arribas MV, Bartolomé B: A survey of modulation of gut microbiota by dietary polyphenols. Biomed Res Int 2015, 850902. DOI: https://doi.org/10.1155/2015/850902
14. Suthanthangjai W, Davies K, Phillips A, Ansell J, Kilmartin PA: Biotransformation of blueberry anthocyanins to bioavailable phenolic compounds by Lactobacillus. Planta Med 2013, 79-PJ46. DOI: https://doi.org/10.1055/s-0033-1352250
15. Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, Mata-Haro V, González-Córdova AF, Vallejo-Cordoba B, Hernández-Mendoza A: Postbiotics: An evolving term within the functional foods field. Trends Food Sci Technol 2018, 75:105–114. DOI: https://doi.org/10.1016/j.tifs.2018.03.009

16. Sharma M, Shukla, G: Metabiotics: One Step ahead ofProbiotics; an Insight intoMechanisms Involved inAnticancerous Effect inColorectal Cancer. Front Microbiol 2016, 7:1940.
DOI: https://doi.org/10.3389/fmicb.2016.01940
17. Stevanovic T, Diouf PN, Garcia-Perez ME: Bioactive Polyphenols from Healthy Diets and Forest Biomass. Curr Nutr Food Sci 2009, 5:264–295.
DOI: https://doi.org/10.2174/157340109790218067
18. Valencia-Avilés E, García-Pérez M.E, Garnica-Romo MG, Figueroa-Cárdenas JD, Meléndez-Herrera E, Salgado-Garciglia R, Martínez-Flores HE: Antioxidant Properties of Polyphenolic Extracts from *Quercus laurina*, *Quercus crassifolia*, and *Quercus scytophylla* Bark. Antioxidants 2018, 7:81.
DOI: https://doi.org/10.3390/antiox7070081
19. Valencia-Avilés E, Martínez-Flores HE, García-Pérez M, Melendez-Herrera E, García-Pérez ME: Investigation of the Antibacterial Activity and Subacute Toxicity of a Quercus crassifolia Polyphenolic Bark Extract for its Potential Use in Functional Foods. J Food Sci 2019, 84:1692-1702.
DOI: https://doi.org/10.1111/1750-3841.14652
20. Munin A, Edwards-Lévy F: Encapsulation of natural polyphenolic compounds; a review. Pharmaceutics 2011, 3:793-829.
DOI: https://doi.org/10.3390/pharmaceutics3040793
21. Roshanpour S, Tavakoli J, Beigmohammadi F, Alaei S: Improving antioxidant effect of phenolic extract of Mentha piperita using nanoencapsulation process. J Food Meas Charact 12021, 5:23–32.
DOI: https://doi.org/10.1007/s11694-020-00606-x
22. de Souza Simões L, Madalena DA, Pinheiro AC, Teixeira JA, Vicente AA, Ramos ÓL: Micro- and nano bio-based delivery systems for food applications: In vitro behavior. Adv Colloid Interface Sci 2017, 243:23-45.
DOI: https://doi.org/10.1016/j.cis.2017.02.010
23. Ballesteros LF, Ramirez MJ, Orrego CE, Teixeira JA, Mussatto SI: Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chem 2017, 237:623-631. DOI: https://doi.org/10.1016/j.foodchem.2017.05.142
24. Diouf PN, Stevanovic T, Cloutier A: Study on chemical composition, antioxidant and anti-inflammatory activities of hot water extract from *Picea mariana* bark and its proanthocyanidin-rich fractions. Food Chem 2009, 113:897–902.DOI: https://doi.org/10.1016/j.foodchem.2008.08.016
25. Tranquilino-Rodriguez E, Martinez-Flores HE, Rodiles-Lopez JO, Zamora-Vega R, Salgado-Garciglia R, Perez-Sanchez RE: Survival rate of *Saccharomyces boulardii* adapted to a functional freeze-dried yogurt: experimental study related to processing, storage and digestion by Wistar rats. Funct Foods Health Dis 2017, 7:99-114.
DOI: https://doi.org/ 10.31989/ffhd.v7i2.319
26. AOAC: Official Method of Analysis. 16th Edition. Edited by Association of Official Analytical Chemists. Washington, DC, USA; 2000.
27. Prosky L, Asp NG, Schweizer TF, DeVries JW, Furda I: Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. J Assoc Offic Anal Chem 1988, 71:1017–23.
DOI: https://doi.org/10.1093/jaoac/71.5.1017
28. Guinee TP, Mullins CG, Reville WJ, Cotter MP: Physical properties of stirred curd unsweetened yoghurts stabilised with different dairy ingredients. Milchwissenschaft 1995, 50:196–200.
29. ISO 7889:2003:Yogurt - Enumeration of Characteristic Microorganisms - Colony-Count Technique at 37 °C, 2009.
30. NOM-111-SSA1-1994: Bienes y Servicios: Método Para La Cuenta de Mohos y Levaduras En Alimentos) of the Ministry of Health; 1994.
31. Meiselman HL: Critical evaluation of sensory techniques. Food Qual Prefer 1993, 4:33-40.
DOI: https://doi.org/10.1016/0950-3293(93)90311-S.
32. Bartoshuk LM, Duffy VB, Lucchina LA, Prutkin J, Fast K: PROP (6-n-propylthiouracil) supertasters and the saltiness of NaCl. Ann N Y Acad Sci 1998, 30:855:793-6.
DOI: https://doi.org/10.1111/j.1749-6632.1998.tb10660.x
33. Predero FDL, Pangborn RM: Evaluación sensorial de los alimentos, métodos analíticos (Alhambra M). México; 1997.
34. Fortin J, Desplancke C: Guía de selección y entrenamiento de un panel de catadores. Acribia. España; 2001.
35. Lawless HT, Heymann H: Sensory Evaluation of Food Principles and Practices. 2nd edition. Kluwer Academics/Plenum Publishers; 2010.
36. Karagül-Yüceer Y, Drake M: Sensory Analysis of Yogurt. Manufacturing Yogurt and Fermented Milks, 2nd Edition; 2013: 353–367.
37. Mazloomi SM, Shekarforoush SS, Ebrahimnejad H, Sajedianfard J: Effect of adding inulin on microbial and physicochemical properties of low fat probiotic yogurt. Iran J Vet Res 2011, 12:93-98.

DOI: https://doi.org/10.22099/IJVR.2011.47

38. Mexican Standard NMX-F-444-1983 (NMX-F-444-1983). Foods. Yogurt or Bulgarian Milk, 1983.
39. Mexican Standard NMX-F-511-1988. Food Determination of Acidity in Reconstituted Milk, 1988.
40. Ruiz Rivera JA, Ramírez Matheus AO: Elaboración de yogurt con probióticos (*Bifidobacterium* spp. y *Lactobacillus acidophilus*) e inulina. Revista de La Facultad de Agronomia 2009, 26:223–242.
41. Crispín-Isidro G, Lobato-Calleros C, Espinosa-Andrews H, Alvarez-Ramirez J, Vernon-Carter EJ: Effect of inulin and agave fructans addition on the rheological, microstructural and sensory properties of reduced-fat stirred yogurt. LWT - Food Sci Technol 2015, 62(1), 438–444. DOI: https://doi.org/10.1016/j.lwt.2014.06.042