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ADDITION OF HIGH ESTERIFICATION PECTIN AS A STABILIZER IN THE PRODUCTION OF LOW FAT YOGURT

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ABSTRACT

A shake-type low-fat yogurt prepared with highly esterified pectin extracted from valence orange by acid hydrolysis with conventional heating was evaluated. A completely randomized design was used with experimental and commercial pectin (0.15, 0.3, 0.45%), and a control. Cow's milk was adjusted to 1.5% fat, with skimmed milk powder and pasteurization at 85ºC, 30 min and sucralose and lyophilized lactic culture were added with stirring, until acidity 0.58% and pH 4.74. Pectin was added as a stabilizer and subsequent refrigeration and storage at 5ºC ± 1ºC. Acidity, pH, syneresis and viscosity were evaluated for 28 days and sensory analysis by seven trained judges (NTC 4129), ten days after the yogurt was elaborated, using discriminatory test of multiple comparisons. The highest acidity and syneresis was found in the control and the two pectins (commercial and experimental) behave the same at equal percentages. Treatment with 0.15% and 0.3% of experimental pectin show a color close to the control and show slight statistical differences between them. Yogurt with less than 0.3% pectin presents an identical flavor to the commercial one and higher ones present differences with the control and affect the final flavor of the product. Yogurt with 0.3% experimental pectin presents a body without lumps, without syneresis, firm and smooth consistency, maintains the characteristic smell, aroma and flavor and meets the requirements of the NTC 805 standard, with a maximum of 0.5% fat, minimum of 2.6 % protein and 0.6% acidity with a shelf life of 22 days.

I. INTRODUCTION

Over the shelf life of a yogurt or fermented milk, pH decreases over time and acidity increases, due to the action of the initiating culture Streptococcus thermophillus and Lactobacillus bulgaricus, which cause lactic acid fermentation in milk by the enzymatic hydrolysis of lactose in glucose and gactose, with glucose subsequently decomposed in lactic acid [1]. This decrease in pH occurs not only during incubation, but also during yogurt storage, because microorganisms become viable, although the decline is less marked due to the effect of low temperature. The lactic acid produced gives a refreshing acidic flavor, while the compounds carbonyls, acetaldehyde, acetone and diacetyl (produced during fermentation) provide the product with the characteristic aroma and flavor [2].

Normally, exudate increases with storage time, for this reason the addition of Pectin, especially low esterification has the tendency to bind the water embedded in the structure of the gel improving the hydration of proteins and preventing the secretion of water from the structure, finding less serum loss, better firmness, good rheology, quality and taste to yogurt low in fat, increasing the content of soluble solids, a lower pH and lower serum loss [3].

The structure of pectin, in particular the degree of esterification, defines many of its physical and/or chemical properties. In a commercial pectin extracted from citrus fruits, with a esterification grade of 70-74%, the length of blocks of free carboxylic groups can vary from molecule to molecule, and each pectin molecule normally includes several blocks of different lengths. Useful pectin as a stabilizer of an acidified milk drink, at least some of its free carboxyl groups must be arranged in blocks (i.e. contiguously), and not randomly distributed discreetly along the polymer chain [4].

The basic structure of pectin consists of four types of polymers: a linear chain of methylsterified galacturonic acid called homogalacturone I (HGI), a branched region with ramnose attached to arabines or galactose called (RGI); a linear region of galacturonic acid bound to xylose (XGA) and a last branched region whose galacturonic acids are attached to different types of monosaccharides such as apiosa, fucose, glucuronic acid, ramnosa [5].

An acidified milk drink requires adequate viscosity; when there is an excess of pectin, there is the undesirable effect of causing significant increases in viscosity, reaching to gelling extremes. Maintaining a narrow working range is commercially difficult, because insufficient pectin produces sedimentation or excess causes undesirable high viscosity [6].

Concentrations of 0.1 - 0.2% genu pectin improved the rheological, chemical and sensory yogurt properties of Semi-skimmed yogurt [7-8]. A concentration of 0.3% ospino ñame starch and 0.6% ITAL 1000Y pectin as stabilizer in the production of beaten yogurt, increased viscosity during storage without affecting the rheological behavior characteristic of yogurt or its physical-chemical and sensory characteristics [6].

The effect of the use of potato, sweet potato and corn starch on the rheological and textural properties of denatted yogurt was evaluated, finding that 1% starch could significantly reduce syneresis and improve the firmness of yogurt [9]; and with 15% aloe vera and 5% granadilla, having less exudate due to aloe vera [10], and adding whey protein was obtained a difference in viscosity with a yogurt control [11].

The effect of yellow peach flour (INIAP-Quillu) was evaluated at 0.3%; 0.6%, and 0.9% on the physicochemical and rheological properties of low-fat yogurt, finding that the most accepted yogurt was yogurt with 0.9% flour, observing an increase in viscosity with 15% aloe vera and 5% granadilla, having less exudate due to aloe vera [10], and adding whey protein was obtained a difference in viscosity with a yogurt control [12].

Polymerized whey protein (PWP) obtained from cheese whey was evaluated on the physicochemical, texture, microstructure and sensory properties of low-fat curdled yogurt, finding that texture, apparent viscosity and sensory properties are similar to witness [13].

The objective of this work was to extract valencia orange peel pectin (Citrus sinensis L. Osbeck) by chemical method, identify neutral sugars and pectin structure and establish the optimal percentage of this high esterification pectin as stabilizer in the production of low-fat yogurt.

II. MATERIALS AND METHODS

Pectin was obtained in batch reactor with hot acid solution using the method described by [14]. 10 g of dried orange skin collected in "Nueva Esperanza" farm (flat coordinates: N 1008903 – W1524140) of the municipality of Chimichagua (Cesar), was treated with 100 mL of water (1:10) and heated to 80°C by 10 min for enzyme inactivation. The pH of the liquid was adjusted to 2.0 with HCl (0.5 M), and the suspension was heated to 90°C with magnetic agitation at 200 rpm for 1h. The suspension was then repeatedly filtered and washed with acidified water. Pectin was precipitated with aqueous ethanol solution 96% at 1:1 ratio and stored for 2 h, then filtered and washed with ethanol at 70% and 96%, sedated at 40°C, weighed and milled [15].

Pectin was characterized by determining the degree of esterification [16] y Galacturonic acid (m-hydroxydiphenyl colorimetric method). For the composition of neutral and acidic sugars, the sample was drying a test tube, 20 g of inositol was added as a standard reagent; methylglycosides were prepared by methenalysis in HCl (1 M) in methanol at 80°C (18 h), followed by N-acetylation with pyridine and acetic anhydride in methanol. The samples were then per-O-trimethylsililated by treatment with Tri-Sil (Pierce) at 80°C (0.5 h). The analysis was performed by GC/MS in Agilent 6890N GC with interface at 5975B MSD, using an Agilent DB-1 capillary column of 30 m x 0.25 mm ID silica [17]. For molecular weight determination, a solution of 2 mg.mL-1 of the sample, was passed through a spin filter at 0.45 m, followed by injection of 100 L into the HPLC. Exclusion size chromatography was performed on Agilent 1100 HPLC with Superose 12 column with 50 mM ammonium acetate eluent, flow rate of 0.9 mL.min-1 and ELSD detector (Detector Scatering Light Evaporative). Molecular weight fractions were determined from retention times, elution volume, and peak width and height. These tests were performed at the Complex Carbohydrate Research Center lab at the University of Georgia in the USA.

II.1 PREPARATION OF LOW-FAT YOGURT

A completely random design was used with seven treatments, three with pectin extracted from orange peels (T1, T2 and T3) and three with commercial pectin at concentrations of 0.15, 0.3, 0.45 % (T4, T5 and T6) and a treatment without addition of pectin (T7) as a reference (witness), with variance analysis (Anova) and mean comparison test by the Tukey method, at 95% confidence. Fresh cow's milk with less than three hours of milking, filtered and skim was used in a centrifuge with a capacity of 100 L up to a fat percentage of 2%. Fat was adjusted to 1.5%, using 2.96% skim milk powder (Colanta, Medellin, with 0.31% fat) and pasteurized at 85°C and 30 min. Allow to cool to 42°C and add 0.0183% sucralose stirring to dissolve it. The freeze-dried lactic culture of Streptococcus Termophillus and Lactobacillus Bulgaricus was added and stirred constantly during inoculation to an acidity of 0.58% lactic acid and pH 4.74. After 3 hours and 15 minutes, the temperature decreased to 10°C and stirred vigorously. Pectin is added as a stabilizer at different concentrations. It was packed in pre-sterilized plastic containers, refrigerated and stored at 5°C to 10°C.

II.2 TREATMENT PROPERTIES ON EVALUATION DAYS

Yogurt was evaluated by, acidity (942.05/90 A.O.A.C), pH (10,041/84 A.O.A.C), syneresis (centrifugation, 15 min, 1500 r.p.m.) and viscosity (Ostwald) with three repetitions each week for 28 days and sensory analysis (smell, taste, body, aroma and smell) [18-19] evaluated by seven judges trained according to NTC 4129, was performed ten days after the yogurt had been prepared using a discriminatory test of multiple comparisons with commercial yogurt. The group of tasters were students of the Agroindustrial Engineering program, who were trained to form the sensory panel.

The yogurt corresponding to the desired or optimal was characterized as follows: Fat Content (gerber), Proteins (12.1.07

- A.O.A.C 1984), Total Solids (A.O.A.C 16032, 1984 Modified), Ash (AOAC,2000), Humidity (Method 16.023 - A.O.A.C 1984), Density (Thermo Lactodensimeter), Acidity (Potentiometric Titration), Determination of Theoretical Caloric Content (Relationship), Total Coliform Count and e. coli , Mold and yeast count.

III. RESULTS AND DISCUSSIONS

III.1 OBTAINING AND CHARACTERIZING PECTIN

Table 1: Pectin quality obtained from shells of conventional heating orange.

	Conventional heating	[19]
GaIA (% mol)	44,9	54,4
Ramnose (% mol)	5,8	1,6
Arabinose (% mole)	14,4	13,5
Galactose (% mol)	13,6	3,5
Xylose (% mol)	5,7	0,1
Glucose (% mol)	13,1	2,7
Fucose (% mol)	0,5	n,d
Mannose (% mol)	2,0	n,d
Glucuronic acid (% mol)	n,d,	-
Molecular weight (kDa)	5-10	
Degree of esterification (%)	60,6	65,1
Performance (%)	9,6	27,3

Source: Authors, (2020).

Table 1 shows that pectin contains 44.9% galacturonic acid, lower than obtained by [18] and similar to proposed by [20-21]. The RGI region is approximately 33.8 (ramnosa, arabinosa and gactose) and is within the range of 20-35% raised by [22] et al, 2006. In RGII, glucose predominates, followed by groping and fucosa with about 15.6%. The xylose content (XGA region) has a close value of 5.7. This means that in the pectin found, the HG region predominates, followed by RGI and then by RGII similar to what other authors reported by [23-24]. This pectin is high esterification (60.6%) indicating a low effect of the hydrolysis reaction with short chains (low molecular weight) indicating that perhaps the effect on glycoside joints or β- elimination predominates [25].

III.2 YOGURT PROPERTIES IN DIFFERENT TREATMENTS

In Figure 1, pH and acidity behavior is observed during the 28 days of evaluation, a decrease in pH to 4.3 (a) is observed, with the exception of pectin-free treatment (witness), which is below 4.3. In the same way the acidity increases over the course of days (b), being significantly different its value to the witness (above 1.2%) where other treatments are between 0.8 and 1% lactic acid.

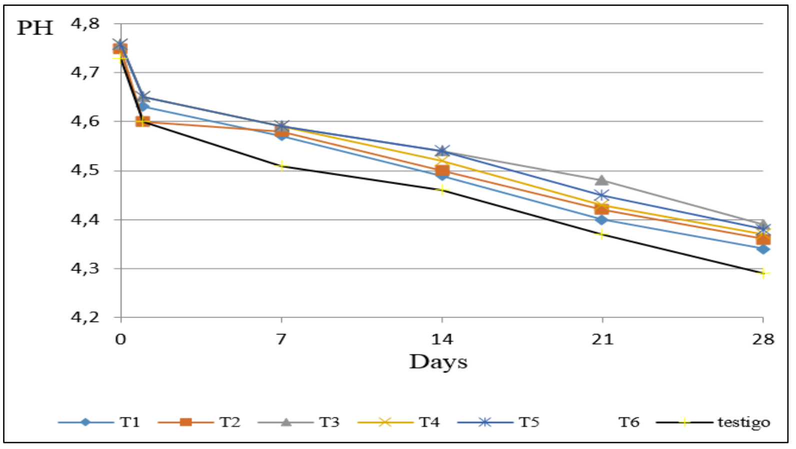

Figure 1: Behavior of pH and acidity during the evaluation days.
Source: Authors, (2020).

The witness was acidified before 14 days and is above the optimal acidity of 0.9% lactic acid [2] (experimental pectin) is acidified from day 21, the other formulations with pectin (commercial or experimental) are within the mentioned value (b).

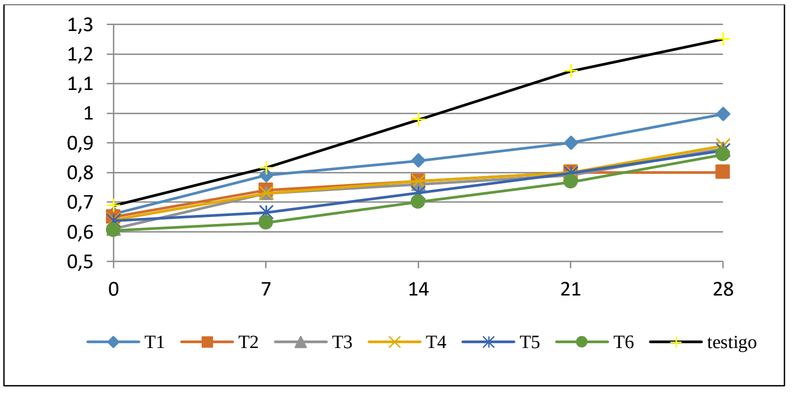

Figure 2: Behavior of exudation during the evaluation days.
Source: Authors, (2020).

Figure 2 shows that commercial pectin at high concentration is the least exudate (T6) and similar behavior presents experimental pectin (T3) up to 21 days. T2 (0.3% experimental) and T5 (0.3% commercial) exhibit the same behavior over time reaching 3.4%. T1 has a higher exudate than other treatments (up to 4.6%) which is lower than that found by [6], with ITAL 1000Y pectin for whole natural yogurt (6,472-1.05%). The witness exceeds the above values with a value of 7.8% water.

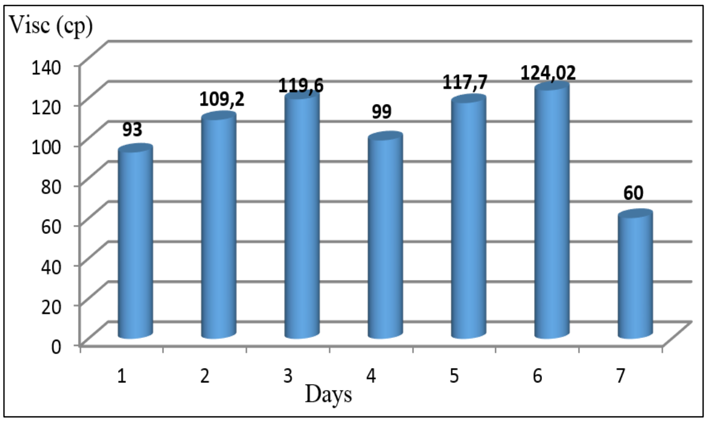

Figure 3: Viscosity behavior after 10 days of evaluation.
Source: Authors, (2020).

The addition of pectin increases the viscosity of yogurt formulations. A higher concentration of pectin (extracted and commercial) give greater viscosity (Figure 3). Generally speaking, the two pectins behave equal to equal percentages. There is only substantial difference with the witness, who shows pectin's work as a stabilizer and the identical behavior of experimental pectin with commercial pectin.

III.3 SENSORY ANALYSIS

Table 2: Comparison of averages of sensory attributes between treatments.

Attribute	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**Tes**
Colour	$5,6^{a}\pm0,8$	$4,9^{ab}\pm0,9$	$4,0^{b}\pm0,6$	$4,4^{b}\pm0,7$	$4,4^{b}\pm0,13$	$4,3^{b}\pm0,5$	5
smell	$4,7^{a}\pm0,5$	$2,9^{c}\pm0,7$	$1,6^{d}\pm0,5$	$4,7^{a}\pm0,5$	$3,9^{b}\pm0,7$	$3,3^{bc}\pm0,9$	5
flavor	$4,7^{ab}\pm0,4$	$4,6^{ab}\pm0,5$	$3,7^{c}\pm0,8$	$5,0^{a}\pm0,0$	$4,4^{ab}\pm0,53$	$4,1^{bc}\pm0,7$	5
Texture	$5,6^{d}\pm0,5$	$6,6^{C}\pm0,5$	$7,7^{b}\pm0,9$	$6,7^{b}\pm0,4$	$7,9\pm0,38$	$8,7^{a}\pm0,6$	5
Odor	$5,4^{c}\pm0,5$ $1,0^{ab}\pm0,1$	$6,6^{b}\pm0,5$ $1,0^{ab}\pm0,1$	$7,3^{a}\pm0,5$ $1,0^{ab}\pm0,1$	$5,4^{c}\pm0,5$ $1,0^{ab}\pm0,1$	$6,1^{b}\pm0,4$ $1,0^{b}\pm0,1$	$6,6^{b}\pm0,5$ $1,0^{a}\pm0.1$	5

Source: Authors, (2020).

Equal letters do not present significant differences at 95% confidence.

Treatment T1 with 0.15% and T2 with 0.3% experimental pectin, show a color closer to the Characteristic White Color in the Witness Sample and present light statistical differences between them and very different with the other treatments (Table 2). By increasing the % of pectin, the aroma weakens compared to the commercial yogurt sample, because pectin transfers its orange aroma to yogurt. The T3 treatment has the lowest acceptance value, since apparently percentages of 0.45% change the characteristic aroma of yogurt and with the lowest values in both pectins (commercial and experimental) the aroma is perceived very little and is quite close to the value of the witness and are statistically the same.

Only the T4 treatment has identical flavor with commercial yogurt and other treatments with low pectin content have slight significant differences with commercial yogurt and between them. Pectin values greater than 0.3% in both pectins (commercial and experimental) have substantial differences with the witness and affect the final taste of the product, the T3 treatment being more evident.

In terms of texture, all treatments have significant differences between them and are superior to the witness with a Homogeneous Body, without lumps, without separation of the serum and firm and smooth consistency characteristic of yogurt. This is best evidenced at high percentages of pectins. Treatments closest to the witness with respect to odor are those that contain the least amount of pectin and there are no significant differences between them. Increasing the pectin content makes the orange odor more present, being greater than percentages of 0.45. This can be masked with aromas and fruit content or flavorings.

Treatment 2 was chosen with 0.3% experimental pectin as the ideal for yogurt preparation because it has good stability (viscosity, and syneresis) without significantly altering the organoleptic characteristics of it, contrary to the found by fathia, 2017, that used 0.6% commercial pectin. Generally speaking, T3 (0.45% pectin) did better in terms of pectin stabilizer function, but organoleptic characteristics of the product (smell, aroma and taste) are altered and T1 (0.15%) didn't showed different significant results with respect to the witness.

Table 3: Characterization Yogurt produced with experimental pectin.

Properties	**Result**	**Y/N**	**NTC standard**		**Resolution 2310 of 1986**	
Content	1.5%	Y	**Min**	**Max**	**Min**	**Max**
			>0.5%	<2.5%	1.5%	2.4%
Protein	2.7%	Y	Min=2.6 Max= --		not specified	
Total Solids	10.05%	--	not specified		not specified	
Ashes	0.94%	--	not specified		not specified	
Humidity	89.5%	--	not specified		not specified	
Density	1034 g/cm^3	--	not specified		not specified	
Acidity	0.772% Lactic acid	Y	Min=0.6% A. L		(0.70-1.50)% A. L	
Total Coliform Count	4 x 10^1 NMP/ml	Y	(10-100) NPM/ml		(20-93) NPM/ml	
E. coli count	<1 x 10^1 NMP/ml	Y	0		<3	
Mold and yeast count	10 x 10^1 NMP/ml		(200-500) NPM/ml		(200-500) NPM/ml	

Source: Authors, (2020).

S = Compliance with requirements.
N = Non-compliance with the requirements.

Yogurt prepared with selected experimental pectin (T2) contains 96% skim milk, 2.96% skim milk powder, 0.3% experimental pectin, 3% Cultivation, 0.0183% Sucralose and was evaluated and compared with NTC 805 and resolution 2310 of 1986 (Ministrio de Protección Social, 1987), which shows that it meets all properties (Table 3). The period of study of the product was 28 days, however the shelf life of the product was 22 days since after this period of time it has undesirable organoleptic characteristics, such as strong acid odor [26].

Based on the content of commercial yogurt properties and developed with experimental pectin, the caloric content was determined, demonstrating that has 60% decrease in calorie content, more than 50% decrease in fat content and more than 70% decrease in total carbohydrates compared to commercial whole yogurt with very close levels of proteins, being the 107.2 calorie, caloric content for commercial yogurt versus 43.94 for low-fat yogurt in this study.

IV. CONCLUSIONS

Pectin obtained from orange peels and extracted by conventional heating with HCL, is high methoxyl, with properties to be used as a stabilizer to produce low-fat yogurt type beaten (ligth) at a concentration of 0.3%, a shelf life of 21 days and a low percentage of exudate.

V. AUTHOR'S CONTRIBUTION

Conceptualization: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Methodology: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Investigation: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Discussion of results: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Writing – Original Draft: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Writing – Review and Editing: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Resources: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Supervision: Ricardo Durán, Carlos Guillen and Juana Bermúdez.
Approval of the final text: Ricardo Durán, Carlos Guillen and Juana Bermúdez.

VI. REFERENCES

[1] Codex. (2012). Codex Standard for Fermented Milks. CODEX STAN 243-2003.

[2] Tamine, A., Robinson, R. (2002). Science and technology yogurt. Second edition. Editorial Acribia, S. A. Zaragoza, Spain.

[3] Khubber, S., Chaturvedi, K., Thaku, Sharma,N , Kumar, S. (2021). Low-methoxyl pectin stabilizes low-fat set yoghurt and improves their physicochemical properties, rheology, microstructure and sensory liking. Food Hydrocolloids, 111.

[4] Durán, R., Villa, A.L. (2017). Experimental study for the design of an industrial plant for the production of pectins from mango and orange. doctorate in engineering, UdeA.

[5] Mohnen, D. (2002). Biosynthesis of pectins. In Pectins and their Manipulation. Edited by Seymour GB, Knox JP. Oxford: Blackwell Publishing and CRC Press, 52-98. 2002.

[6] Mendoza R., Trujillo y., Durán D. (2007). Evaluation of hawthorn yam starch (dioscorea rotundata) as a stabilizer in the production of smoothie-type whole yogurt. University of Pamplona. Research Group in Food Engineering and Technology.

[7] Bellavita R.y Gunanja G. (1998). Yogurt with GENU pectin. Latin American Lactea Technology Magazine. 11: 29-30.

[8] Castillo, M., Borregales, C., Sanchez, M (2004). Influence of pectin on the rheological properties of yogurt. Rev. Fac. Farma. 46(2): 33-37.

[9] Saleh, A., Mohamed, A., Alamri S., Hussain, S., Akram A. and Mohamed A. (2020). Effect of Different Starches on the Rheological, Sensory and Storage Attributes of Non-fat Set Yogurt. Foods, 9, 61.

[10] Gutierrez-alzate, K, Beltran-cotta, L and Granados, C. Caracterización bromatológica de una bebida láctea fermentada tipo yogur a partir de lactosuero con cristales de aloe vera (Aloe barbadensis Miller) y granadilla (Passiflora ligularis Juss). Revista chilena de nutrición, 47,3, pp.390-395.

[11] Araya, T. (2013). Evaluation of the effect of incorporating sweet whey protein in a low-fat shake yogurt. Food degree program. Costa Rica University).

[12] Fang, M., Shen,X., Hou,J.,Guo, M. (2019). Effects of polymerized whey protein prepared directly from cheese whey as fat replacer on physiochemical, texture, microstructure and sensory properties of low-fat set yogurt. Food Science and Technology 115.

[13] Durán, R., Villa, A.L. (2014). Microwave assisted extraction of essential oil and pectin from orange peel in different stages of maturity. Faculty of Agronomy review 31: 145-158.

[14] Durán, R*, Villa, A.L., Ribeiro, R and Rabi, J. (2015). Pectin Extraction from Mango Peels in Batch Reactor: Dynamic One-Dimensional Modeling and Lattice Boltzmann Simulation. Chemical Production Process Model. 10(3): 203–210.

[15] Bochek, A. M.; Zabivalova, N. M. y Petropavlovskii, G. A. (2001) Determination of the Esterification Degree of Polygalacturonic Acid. Russian Journal of Applied Chemistry. 4(5): 796-799. Translated from Zhurnal Prikladnoi Khimii.

[16] Merkle, J., Larick, J. 1994. Conditions for Extraction and Concentration of Volatile Materials from Bovine Fat with Supercritical Carbon Dioxide. journal of food science. 59(3).

[17] Carpenter, P.; Lyon, H. y Hasdell, A. (2002) Sensory analysis in the development and control of food quality. Zaragossa (España). Ed. Acribia S.A. 191p.

[18] Morales A. (2005). Sensory Evaluation of Foods in Theory and Practice. Edition Editorial Acribia, S. A. Zaragoza (Spain).

[19] Yapo, B. M., Lerouge, P., Thibault, J.F., Ralet, M.C. (2007). Pectins from citrus peel cell walls contain homogalacturonans homogenous with respect to molar mass, rhamnogalacturonan-I and rhamnogalacturonan-II. Carbohydrate Polymers. 69(3): 426–435.

[20] Voragen, A. G. J., Coenen, G.J., Verhoef, R. P., Schols. A. (2009). Pectin, a versatile polysaccharide present in plant cell walls. Structural Chemistry. 20: 263–275.

[21] Wolf, S., Mouille, G., Pelloux, J. (2009) Homogalacturonan methyl-esterification and plant development. Molecular Plant. 2: 851–860.

[22] Hellın, P.; Ralet, M. C.; Bonnin, E., Thibault, J. F. (2005) Homogalacturonans from lime pectins exhibit homogeneous charge density and molar mass distributions. Carbohydrate Polymers, 60: 307–317.

[23] Thibault, J.F., Renard, C. M. G. C., Axelos, M. A. V., Roger, P., Crépeau, M.J. (1993). Studies of the length of homogalacturonic regions in pectins by acid hydrolysis. Carbohydrate Research. 238: 271–286.

[24] Deng, C.; O'Neill, M.A.,York, W. S. (2006) Selective chemical depolymerization of rhamnogalacturonans. Carbohydrate Research. 341: 474–484.

[25] York, W.S., Darvill, A.G., McNeil, M., Stevenson, T., Albersheim, P. (1985) Isolation and Characterization of plant cell walls and cell wall components. Methods Enzymol. 118: 3-40.

[26] Icontec. (2007). COLOMBIAN TECHNICAL STANDARD.