

Evaluation of seed and vegetable agglutination in yogurt and its effect on physicochemical parameters

By

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Abstract

To take advantage of the nutritional value of chia and canary seed, the technology for making yogurt was researched and developed, which meets the requirements of fermented milk established by INEN standards by adding seed extracts (chia and canary seed), vegetables (watercress and alfalfa), and two sweeteners (saccharin and stevia). A completely randomized block factorial design A*B*C was applied with three replicates corresponding to 24 treatments. To determine the effects between levels, Tukey significance tests ($p < 0.05$) were performed using the Stat Graphics statistical program. The physical-chemical, sensory, and microbiological analyzes carried out among the most important are Protein content with a value of 6.68% in birdseed and 5.24% in chia. In contrast, sensory characteristics such as smell, texture and flavor do not present a significant difference, only present in color regarding vegetables; a score of 2.5 out of 5 was obtained in the sensory analyzes in birdseed. The results establish that the best yogurt was made from birdseed enriched with watercress and stevia because it improves the chemical and sensory characteristics.

Keywords. Alfalfa, watercress, sweeteners, sensory analysis.

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1. Introduction

Yogurt is currently consumed for its health benefits and pleasant organoleptic properties; in this way, it has become one of the most desired dairy products in the world thanks to the variety of flavors and presentations of different consistencies: liquid, smoothie and semi-solid that exist in the market (Pinilla, 2008). Furthermore, agricultural products such as chia, canary seed, watercress, and alfalfa should be of great importance in the daily diet of children and adults due to their high content of “carbohydrates, proteins, fiber and omega 3” (Zavalía et al., 2010).

Yogurt is a dairy product obtained from the fermentation of milk through the action of suitable microorganisms and resulting in a reduction in pH with or without coagulation (isoelectric precipitation) INEN, Ecuadorian Technical Standard (INEN, 2011). It also provides a balance between nutrients such as proteins, fats, carbohydrates, vitamins, and minerals necessary for the functioning and maintenance of the body (Codex Alimentarius, 2011).

Hispanic sage, the scientific name for chia, is grown in Santa Elena and Imbabura. Currently, around 1,000 producers of Hispanic sage (Líderes Revista, 2018). Chia is an annual herbaceous plant that belongs to the *Lamiaceae* family; the importance is given to this species because it produces an oil with the highest known content in nature of fatty acid ω -3- α -linolenic; the chia seed has acquired a notorious importance for health and nutrition because its content of omega-3 fatty acids reduces cardiovascular diseases (Lara *et al.*, 2021). It contains around 33% oil with 82.3% essential fatty acids (α -linolenic and linoleic acids) (Busilacchi et al., 2013), between 19-23% protein with a good profile of essential amino acids and a higher proportion of lysine, methionine and cystine than in other oilseeds (Victor Aredo, Lía Velásquez, Obando Narro & E, 2014).

Canary seed can be used to prepare drinks such as canary seed drink or canary seed milk, which is very beneficial for the body as it contains many nutrients, not like regular milk, but it contains minerals, proteins, and vegetable fats, which is favorable for it since it helps reduce cholesterol (Cogliatti *et al.*, 2014).

Canary grass contains 18% protein and 8.7% unsaturated lipids; Canary grass extract contains lipase activity and a high nutrient content, so it can be an alternative for people who are lactose intolerant or who are on a hypocaloric diet; lipases are carboxylesterases that catalyze the hydrolysis of fatty acids esterified from triacylglycerols containing acid of 10 carbon atoms or more, the amino acids that this grain possesses highlight the unique structure of its proteins, mainly due to its high content of tryptophan (Cogliatti et al., 2014).

Alfalfa is rich in Vitamins A, B1, B2, B6, C, D, E and K, 10% of its weight are minerals, among which calcium, phosphorus, iron, sulfur, silica, potassium, and magnesium stand out. Moreover, sodium, 22% of its weight, are proteins whose content increases if the seed has germinated. It also contains isoflavones (genistein), coumarins (coumestrol), saponins and digestive enzymes (Inkanat Peru, 2021).

Watercress is rich in vitamin A (from beta-carotene) and vitamin C and is a source of folate, calcium, iron, and vitamin E. It also contains valuable amounts of vitamin K, thiamin, B6, potassium, and naturally occurring iodine. It is low in sodium. Due to its high-water content

(93%); it is low in calories (El Telégrafo, n.d.); it also provides minerals such as calcium, iron, and, to a lesser extent, potassium, and phosphorus (Miguel *et al.*, 2020).

Saccharin is considered the oldest sweetener; it was synthesized at the beginning of 1879 and had a sweetening power 300 times greater than that of sucrose (Stephens Camacho *et al.*, 2018). The Recommended Daily Intake (RDI) for saccharin is 0-15 mg/kg/day. Stevia is a non-caloric sweetener with a large amount of fiber, protein, and minerals; it is a natural sweetener without calories, 100 to 300 times sweeter than sugar. International organizations support its consumption as a safe supplement (Durán *et al.*, 2012). With the widespread appearance of natural sweeteners such as stevia, which is available in different commercial formulations, the suitability of yogurt needs to be validated by several studies (Narayanan *et al.*, 2014).

The research deals with isoelectric precipitation in which a food drink will be obtained, enriched with watercress and alfalfa, and sweetened with products such as saccharin and stevia, thus improving the physicochemical and microbiological characteristics of the product, considering the different formulations.

2. Materials and methods

2.1. Vegetal material

Seeds: Chia is rich in mucilage, starch, and oil; About 2 mm long by 1.5 wide, and oval and glossy, grayish brown to reddish brown, the canary seed the inflorescences are green at first, then turn slightly purple; the seeds are shiny brown and sheathed in a small shell. The yogurt-like drink was made with chia seeds imported from Peru and canary seeds from Canada.

Vegetables: Alfalfa, due to its extraordinary richness in minerals, such as iron, phosphorus, potassium, and calcium, is credited with the ability to strengthen bone mass and, at a preventive level, to help prevent fractures in the elderly and watercress is a plant rich in antioxidants, vitamins and minerals that provide various health benefits, **Sweeteners:** Stevia and saccharin as natural sweeteners to reduce the content of free sugars in yogurt.

2.2. Experimental design.

Control yogurt production using skimmed milk placed in the storage tank, pasteurized, and disinfected in a very high temperature (UHT) pasteurization system, adding cultures of *Streptococcus thermophilus* *Lactobacillus delbrueckii* subsp. *Bulgaricus*, transfer to the fermentation tanks at a temperature of 43 °C for 4 hours, mix with the different additives and flavorings and finally store at 4 °C.

Yogurts with 50% milk and 50% natural extract (chia and canary seed) were prepared separately, heated for 30 minutes at 80°C, lowered to 43°C, and then inoculated with 1% (vol. / vol.) of lactic ferment containing *Streptococcus thermophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus* aseptically.

The mixes were divided into two equal portions; one portion was further inoculated with 8% (vol/vol) watercress and the other with 8% (vol/vol) alfalfa of each probiotic culture. Then, 500 mL of the mixtures were poured into polystyrene glasses and incubated at 43 °C until a pH of 4.5 ± 0.05 was reached. Subsequently, the yogurts were cooled to 4°C, the sweeteners with stevia at 1.7% (vol. / Vol.). Moreover, 1.7 (vol. / vol.) of the saccharin is easily added to the other container, and it should be noted that whereas a control was carried out without adding any ingredient, the treatments were carried out in triplicate.

2.3. Determination of physicochemical parameters

2.3.1. pH measurement. - The measurement was carried out at 25 °C (room temperature) using a pH meter, calibrated before use with pH 4.0 and pH 7.0 buffers. The AOAC 981.12 method (AOAC Official Method, 2012) was followed.

2.3.2. measurement of soluble solids. An electronic refractometer was used, and measurements were made at a temperature of approximately 25 °C. This device has a scale from 0 to 100 °Brix. AOAC: Official Methods of Analysis (Rybak-Chmielewska, 2003).

2.3.3. Determination of titratable acidity. - Determination of titratable acidity the titratable acidity (TA), as a percentage of lactic acid, of the yogurt samples was determined according to the Official Methods of Analysis of the Association of Official Agricultural Chemists (Bodily, 1956).

$$\%A = (V \times N \times \text{mleq (lactic acid)}) / MW \times 100 \quad (1)$$

2.3.4. Humidity determination: It was carried out by the gravimetric method Official Methods of Analysis of the Association of Official Agricultural Chemists (Bodily, 1956).

2.3.5. Determination of viscosity. - The viscosity of the yogurt was determined with a rotational viscometer, model 802 Laboratories; measurements were made at 25 °C, and all measurements were recorded at 50 rpm. Yogurt was gently stirred for 20 s (20 s continuous sweeps) before analysis. All determinations were repeated at least three times.

2.3.6. Determination of ashes. - It was carried out following the AOAC method: Official Methods of Analysis (The Official Methods of Analysis of AOAC International, 2016), previously drying the samples at 110°C and subsequently calcining at a temperature of 550°C until constant weight. The results were determined through the following:

$$\% \text{ Ashes} = (W2-W1) / W0 \times 100 \quad (2)$$

2.3.7. Fat determination. - The fat content was determined using the Gerber method described by Egan et al. (1987). First, 9.5 ml of concentrated sulfuric acid, 11 ml of yogurt and 2 ml of fat-free isoamyl alcohol were placed in the butyrometer, the butyrometer was closed and the mixture was stirred for 5 minutes at 1000-1200 rpm. Subsequently, it is placed in a water bath (T = 75-80 °C) for two h, after which the fat content is read on the butyrometer scale.

2.3.8. Determination of crude protein: The method is based on the destruction of organic matter with concentrated sulfuric acid, forming ammonium sulfate, which, more than sodium hydroxide, releases ammonia, which is distilled into boric acid, forming ammonium borate, which is titrated with diluted sulfuric acid at a concentration of 0.1 N. It was determined using the Kjeldahl analytical method, which comprises three steps: digestion, distillation, and titration. This protein determination is called crude because it is not a direct measure of protein but rather an estimate of total protein based on the nitrogen content of the feed (nitrogen x 6.25 = crude protein) (Bodily, 1956).

$$\text{Nitrogen in the sample (\%)} = ((A \times B \times 0.14) / C) \text{ Crude protein (\%)} = \text{Nitrogen in the sample} \times 6.25 \quad (3)$$

2.4. Determination of microbiological parameters

2.4.1. E. coli / coliform count

To confirm coliform colonies, a representative number of coliform colonies incubated at 37°C ± 1°C were selected, transferred to a tube containing BGLB broth, and then incubated at 37°C ± 1°C. The number of coliforms per Gram or milliliter was determined by multiplying the number of confirmed colonies in BGLB by the dilution factor. For confirmation of E. coli,

a representative number of colonies were selected from plates incubated at 37 °C. Petri film plates can be counted in a standard colony counter or lighted magnifying glass. Incubation time and temperature vary by method. The best-known approved methods are:

- AOAC official method 991.14 For coliforms: Incubate 24 h \pm 2 h at 35 °C \pm 1 °C. For E. coli: Incubate 48 h \pm 2 h at 35 °C \pm 1 °C.
- AOAC Official Method 998.08 For E. coli (meat, poultry, marine): Incubate 24 h \pm 2 h at 35 °C \pm 1 °C.
- NMKL Method (147.1993) For coliforms: Incubate 24 h \pm 2 h at 37 °C \pm 1 °C. For E. coli: Incubate 48 h \pm 2 h at 37 °C \pm 1 °C.

2.4.2. yeasts and molds

Petri film plates are faced in stacks of up to 20 plates at a temperature of 25 °C \pm 1 °C for 3-5 days. Read Petri film plates in a standard Quebec-type colony counter or under a magnifying light source. Yeast and mold colonies were numbered separately after 3 and 5 days. Plates were scored according to their number of colonies; cells from the presumably random yeast colonies were examined microscopically to confirm the absence of bacterial colonies.

2.5. Sensory evaluation

Consumer acceptance of yogurt was studied after 24 hours of storage at 4°C. In a 24-member panel, yogurt was evaluated with samples presented in coded containers in individual cabinets for sensory analysis, for which they had to rate color, aroma, texture, flavor, and general acceptability on a 5-point hedonic scale: 1 = lowest and 5 = highest.

2.6. Statistical treatment

The results were obtained from a completely randomized design (ANOVA) with a 2*2*2 factorial arrangement for the types of seeds (chia and Canarian seed), vegetables (watercress and alfalfa), and different low-calorie sweeteners (saccharin and stevia). Three repetitions were performed for a total of 24 experimental units for the results of the physicochemical characterization of the beverage and Tukey's test at the 5% significance level if there were differences between the combinations. The data were analyzed by the Stat Graphics Centurion XVI program.

3. Results and discussion

The characteristics of the yogurt that were developed based on seeds, vegetables and sweeteners are found in the combinations in Table 1; the physicochemical analyzes of the yogurt presented representative values in some parameters. However, it is important to consider that the properties evaluated are not all the parameters that define the quality of yogurt; these parameters are grouped into physical-chemical, microbiological and sensory criteria, which depend on numerous factors and their interactions, and are linked to the main physical components: chemical products of milk (fat, protein and lactose), as well as micro compounds commonly found, such as minerals, vitamins, cholesterol and terpenes (García *et al.*, 2014).

The *pH* presented a value of 4.76 in Chía, while birdseed obtained 4.60, which is higher than the control and the data of (Vásquez-Villalobos *et al.*, 2015), in their research on the physicochemical properties and sensory acceptability. of the fruity yogurt of skimmed goat's milk with mango and banana In accelerated tests, it is mentioned that the pH ranges between (4.35 - 4.36), the existing variation because the pH present in the fruits is lower than that of the seeds, the pH with a value of 4.73 in saccharin), while stevia obtained a value of 4.63, the

values are similar to (4.6 - 4.9) of (Parra Huertas *et al.*, 2011), this is due to the enzymatic hydrolysis of lactose into glucose and galactose, glucose is subsequently broken down into lactic acid (Rojas Castro *et al.*, 2007). This formation of lactic acid causes a decrease in pH.

Soluble solids with a value of 11.70% that corresponds to canary seed, in chia 10.50% were obtained; these data are found below (16 - 24) mentioned (Salamanca *et al.*, 2010), in their study on Elaboration of a functional drink of high biological value based on borojó (Borojó a *patinoi Cuatrec*), because borojó contains more soluble solids than seeds. On the other hand, the soluble solids of 11.33% corresponding to stevia and saccharin presented a value of 10.88%; these values are below the (12.13 - 18.20) reported Physicochemical and flow properties of a settled yogurt enriched with microcapsules containing omega three fatty acids (Macedo *et al.*, 2015) in their study of the physicochemical and flow properties of a settled yogurt enriched with microcapsules containing omega three fatty acids, the data differ because the sucrose added in the research contains more soluble solids than saccharin and stevia and the amounts applied are minimal.

Titrate acidity was observed, a value of 1.17% corresponding to chia, while in a Canary seed, the value was 0.76%, whose data are related to the control and between the parameters of the Andean Standards (Técnica, 2007). ranges from (0.5 - 1.5). In titrate acidity, a value of 0.97% was found in alfalfa, while in watercress, it indicated a value of 0.95%, the same ones between the parameters of (0.6 - 1.2) established by the Salvadoran Standard (Técnica, 2007). The research is within the Codex Alimentarius Standards (Codex Alimentarius, 2011), which indicate that yogurt must have at least 0.6% lactic acid.

Viscosity with a value of 15270.83 Cp in Chía, while the canary seed obtained a value of 2541.75 Cp, these values are lower than (5633 - 11717) of (Ruiz Rivera & Ramírez Matheus, 2009) the variation in the viscosity of Chía is due to the ability to absorb water in the hydration process that then releases gels that are components of its structure, thus increasing its viscosity, unlike canary grass, the starches they have cannot retain water, and this is reflected in their viscosity.

Humidity in chia obtained a value of 83.11% and the canary seed presented a value of 79.86%, the same ones that are between the control ranges and those of (Perez-Landeras, 2016) in their publication of Fermented Milks in the Community of Madrid indicate a range of (78.7 - 89.1). The moisture data obtained in saccharin 81.63% and Stevia 81.35, whose values are like (81.06 -87.42) according to (Macedo *et al.*, 2015) in their study of the physicochemical and flow properties of a settled yogurt enriched with microcapsules containing Omega-3 fatty acids.

Ash with values of 0.93% in chia, while in Canary seed it obtained a value of 0.81, because the percentages of ash presented by the seeds evaluated as Canary grass that contains 13.48% in mass production of *Trichoderma harzianum Rifai* in different organic substrates (Científica *et al.*, 2008)], concerning chia, the content is 4.5%, cited by (Quitral, 2013), in his study of the chemical composition of the seeds of chia, flaxseed and rosehip and their contribution in omega-3 fatty acids, these contents are different from the fruits and milk with which the norm is governed.

Protein of 6.68% that corresponds to Canary seed, in chia it presented 5.24%, whose data are higher than (1.86 - 2.84) mentioned (Quicazán *et al.*, 2017), in their study on evaluation of the fermentation of soy beverage with lactic culture, due to the high protein content of the

seeds and the concentrations used in the formulas, the same ones found in the parameters of the Official Mexican Standard (SEGOB, 2010). The protein for alfalfa with a value of 6.55%, while watercress obtained a value of 5.36%; these values are like those (Gabriela Segovia, 2007) in her study of rheological changes during the process of production of Chocho yogurt (*Lupinus mutabilis* Sweet) mentions that the protein obtained from 5.79%, both investigations are within the parameters indicated by the Colombian Technical Standard (NTC 805, 2006) establishes a minimum of 2.6% of protein. In proteins, the value of 6.40 in saccharin, while stevia obtained a value of 5.51%, differs from (3.86 - 3.93) (Benjamín Castañeda et al., 2009); the variation is because sucrose and stevia do not contain proteins, unlike saccharin.

Fat was found that chia indicated a value of 1.93% and canary seed a value of 1.67%; these percentages are within the range established by (Codex Alimentarius, 2011), which indicates that yogurt should have less than 15% fat. On the other hand, values were found in alfalfa of 1.88 and watercress with a value of 1.71%, the same ones that are lower than the Nicaraguan Standards (NTN, 2007) stable with a minimum of 3.0% fat because those who in this investigation different low-fat vegetables were added, so the percentage varies.

Microbiological analysis

In *E. coli*, a value of 366.67 CFU was found, which corresponds to chia; in canary seed, 0.00 CFU was found; according to the NTE INEN 2395 Standards, the ranges must be found (<1), the values that conform to the standard are Canary seed while that chia exceeds the parameters due to water retention. In total *coliforms*, a value of 175.00 CFU was obtained, which corresponds to Chía, while in a canary seed, the value was 75.00 CFU; according to the Standards (INEN, 2019), it is established that the ranges must be between (10 - 100), the value that it conforms to the canary seed standards while the chia exceeds the parameters. Finally, in molds and yeasts, it was found that chia presented a value of 350.00 CFU and canary grass obtained a value of 150.00 CFU, which is consistent with the parameters established by (INEN, 2019) that indicates a range of (200 - 500).

Sensory analysis

The smell presented a value of 4.33 in the bird seed, while the chia obtained a value of 2.67, concerning the value of the birdseed it is like (4.03 - 4.11) cited by (Benjamín Castañeda et al., 2009), while chia varies because it has a bean smell that the cupping panel did not like. A color value of 3.33 was established in the watercress, while alfalfa obtained a value of 2.67; these data are like (2.45 - 3.11). These values are low because, in one of the investigations, goat milks and the other extracts are added to vegetables; therefore, the color varied from that of traditional yogurt.

In texture, it was found that chia had a value of 3.67 and canary seed a value of 3.33; these values are like (2.20 - 3.38) (Rojas Castro *et al.*, 2007); this is to the seeds since the milk of goat influences the texture being different from that of conventional yogurt. With other investigations on texture, a value of 3.67 was established in saccharin, while stevia obtained a value of 1.67 on a scale of 1 to 5; the sensory responses are like those established (Rojas Castro *et al.*, 2007).

Conclusions.

The types of chia and canary seeds have a significant direct impact on the protein content of yogurt; in this study, it was determined that the canary seed is the seed that most

increases the protein content of the drink, with a value of 6.68 according to the test of significance (Tukey).

The types of vegetables did not influence the taste, smell, and texture, while there was a color difference between watercress and alfalfa. Therefore, a watercress with 3.50 is the most suitable for its application in the yogurt production process.

The types of sweeteners influence the energy of the final product; therefore, any of the sweeteners (saccharin and stevia) can be applied, but in small concentrations, to keep the product's calories low, unlike traditional sweeteners.

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Contributions of the authors: Yandry Dayan Zambrano contributed to the development of the experimental phase in which he acquired the different raw materials, in addition to the interpretation of the results of the isoelectric precipitation in which a food drink was obtained, enriched with watercress and alfalfa. , sweetened with saccharin and Stevia, José Villarroel Bastidas developed the structure and discussion of the results as well as the conclusions of the combination of the different components such as watercress at 8% (vol/vol) and the other with alfalfa at 8% (vol/ vol) of each probiotic culture, adding the sweeteners 1.7% Stevia (vol/vol) and 1.7 (vol/vol) saccharin.

Data Availability Statement

The yogurt-type drink was made with chia seeds imported from Peru and canary seeds from Canada, while alfalfa, due to its extraordinary richness in minerals, such as iron, phosphorus, potassium and calcium, was obtained in the province of Cotopaxi. Likewise, watercress is a plant rich in antioxidants, vitamins and minerals that provide various health benefits, sweeteners such as stevia and saccharin to reduce the content of free sugars in yogurt, obtained in the Quevedo canton, to later elaborate on the different combinations in which the incubation time and temperature were incubated in digital incubation equipment, once the isoelectric precipitation had occurred, the alfalfa was mixed with the watercress, adding the sweeteners stevia and saccharin. Once the mixtures were finished, the physicochemical, microbiological, and sensory analyzes were carried out.

Declaration of conflicts of interest

Following the provisions of the conflict-of-interest section of the Publication Rules and under the rules of the International Committee of Journal Editors, it is necessary to communicate in writing the existence of any relationship between the authors of the article and any public or private entity from which any possible conflict of interest could arise.

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The first author of the referenced manuscript, on his behalf and behalf of all the signatory authors, declares that there is no potential conflict of interest related to the article.

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Tables and figures

Physico-chemical parameters

Table 1. Physical chemical analysis

	BAST	BAS	BBS	BBS T	CWS T	CWS	CAST	CAS	CONT ROL
pH	4.48 ^{AB} _C	4.66 ^{AB} _C	4.64 ^A _{BC}	4.61 ^A _{BC}	4.70 ^B _C	4.83 ^C	4.79 ^C	4.71 ^B _C	4.44 ^A
Soluble solids	11.33 ^B _C	12 ^C	11.53 _{BC}	11.93 _C	10.53 _{AB}	9.7 ^A	10.27 ^A	11.5 ^B _C	16.22 ^D
Acidity	0.77 ^{DE}	0.79 ^E	0.75 ^D	0.71 ^C	0.53 ^A	0.56 ^B	0.57 ^B	0.53 ^A	0.84 ^F
Viscosity	2463.3 _{3^{AB}}	1421.3 _{3^{AB}}	3799. _{33^B}	2483 _{A^B}	1144 _{0^C}	17323. _{33^D}	14986. _{67^D}	17333. _{33^D}	1465 ^A
Humidity	79.68 ^B	80.12 ^B	80.02 _B	79.62 _B	82.94 _C	83.03 ^C	83.34 ^C	83.14 ^C	78.32 ^A
Ashes	0.83 ^B	0.8 ^B	0.81 ^B	0.8 ^B	0.96 ^C	0.97 ^C	0.89 ^B _C	0.88 ^B _C	0.45 ^A
Protein	6.2 ^D	8.59 ^E	6.3 ^D	5.61 ^B _C	4.22 ^A	5.31 ^B	5.41 ^B	6.01 ^{CD}	10 ^F
Fat	1.87 ^B	1.57 ^A	1.67 ^A	1.57 ^A	1.97 ^B	1.63 ^A	2.43 ^C	1.67 ^A	2 ^B

(**P < 0.05**) **BAST** = Birdseed, alfalfa, and Stevia **BAS** = Birdseed, alfalfa, and saccharin **BBS** = Birdseed, watercress and saccharin **BBST** = Birdseed, watercress and Stevia **CWST** = Chia, watercress and Stevia **CWS** = Chia, watercress and saccharin **CAST** = Chia, alfalfa and stevia **CAS** = Chia, alfalfa and saccharin, Control.

Table 2. Microbiological analysis

	BAS T	BA S	BB S	BBS T	CWS T	CWS	CAST	CA S	CONTR OL
<i>E. coli</i> (UFC/g)	0 ^A	0 ^A	0 ^A	0 ^A	466.6 _{7^B}	333.3 _{3^B}	666.6 _{7^C}	0 ^A	0 ^A
Total coliforms (CFU / g)	0 ^C	0 ^A	0 ^H	300 ^K	500 ^L	100 ^J	100 ^I	0 ^B	0 ^D
Molds / Yeasts (CFU / g)	0 ^H	600 _J	0 ^F	0 ^G	600 ^K	100 ^I	700 ^L	0 ^C	0 ^A

(**P <0.05**) **BAST** = Birdseed, alfalfa, and Stevia **BAS** = Birdseed, alfalfa and saccharin **BBS** = Birdseed, watercress and saccharin **BBST** = Birdseed, watercress and Stevia **CWST** = Chia, watercress and Stevia **CWS** = Chia, watercress and saccharin **CAST** = Chia, alfalfa and stevia **CAS** = Chia, alfalfa and saccharin, Control.

Table 3. Sensory analysis

	BAST	BAS	BBS	BBST	CWST	CWS	CAST	CAS
Colour	1.67 ^A	2.67 ^A	3.33 ^A	3.67 ^A	4 ^A	3 ^A	3 ^A	1.67 ^A
Smell	4.33 ^{AB}	4 ^{AB}	5 ^B	4 ^{AB}	4 ^{AB}	4 ^{AB}	3 ^A	4 ^{AB}
Taste	3 ^A	2.33 ^A	3.33 ^A	2.67 ^A	3.67 ^A	3 ^A	1.67 ^A	2.67 ^A
Texture	1.67 ^{AB}	3.33 ^{ABC}	4 ^{BC}	1.33 ^A	4.67 ^C	3.67 ^{ABC}	3.33 ^{ABC}	3.67 ^{ABC}

(**P <0.05**) **BAST** = Birdseed, alfalfa, and Stevia **BAS** = Birdseed, alfalfa, and saccharin **BBS** = Birdseed, watercress, and saccharin **BBST** = Birdseed, watercress, and Stevia **CWST** = Chia, watercress, and Stevia **CWS** = Chia, watercress, and saccharin **CAST** = Chia, alfalfa and stevia **CAS** = Chia, alfalfa and saccharin.

FIGURES

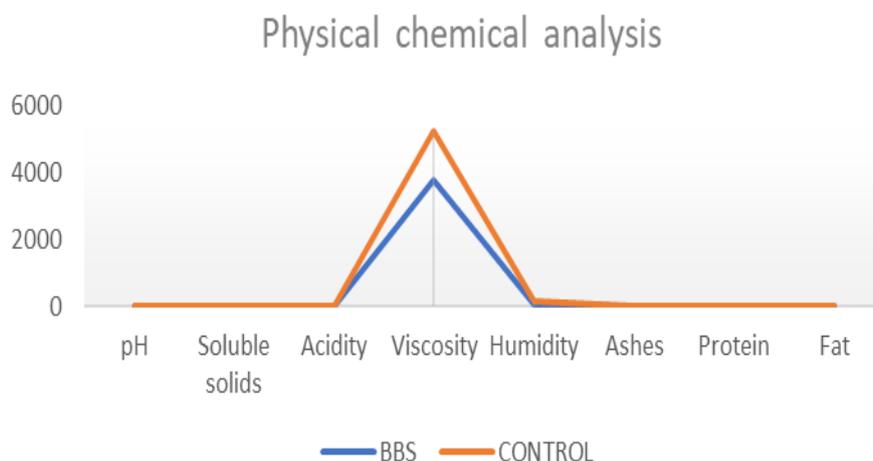


Figure 1. Physicochemical analysis **BBS** = Canary seed, watercress, and saccharin; Control.

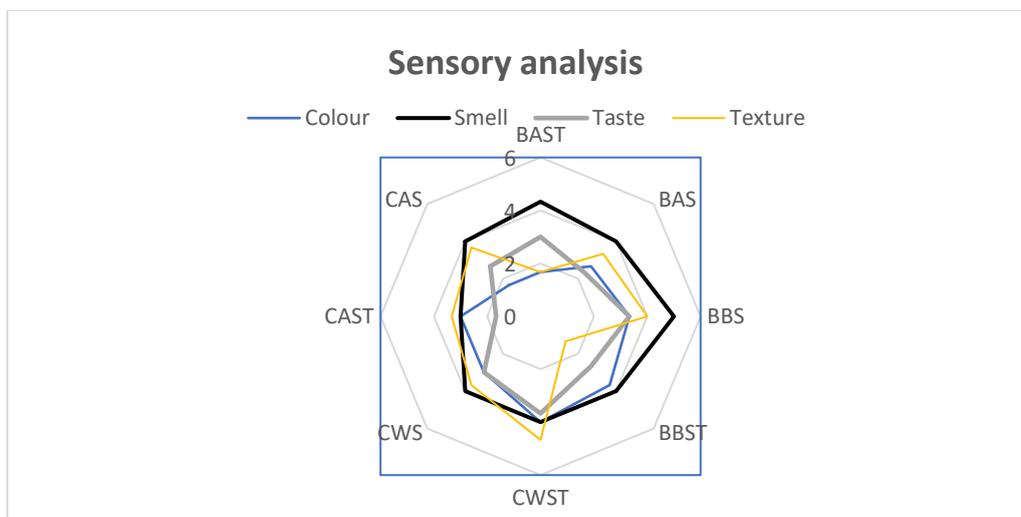


Figure 2. Sensory analysis **BAST** = Birdseed, alfalfa, and Stevia **BAS** = Birdseed, alfalfa, and saccharin **BBS** = Birdseed, watercress, and saccharin **BBST** = Birdseed, watercress and

Stevia **CWST** = Chia, watercress and Stevia **CWS** = Chia, watercress and saccharin **CAST** = chia, alfalfa and stevia **CAS** = Chia, alfalfa and saccharin

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