

Use of blends of legume flours and manioc starch to elaborate gluten-free sweet biscuits.

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Abstract

The base for this research work was set in the study of the interaction between legume flours and tuber starch blends to be used for sweet biscuits formulations and the successive evaluation of quality parameters. As main inputs the following were used: legume flours, manioc starch, margarine, sugar, eggs, and baking soda. Basic utensils: plastic and aluminum trays, biscuits shapers, cutters, other biscuit items. Analytical balance, mixer, and oven. Methods: Establishment of experimental units, analysis of manioc starch and legume flours, moisture analysis, ash content determination, rheological analysis of blends, gluten analysis in the blend of starch and legumes flours, dough formulation and biscuit making, textural and color analysis, sensory analysis of biscuits, statistical analysis. The results of moisture and ash analyzes in the inputs for blends were within the limits established of 14,5%. Ash content found was 2,3% in starch, 6,3% in soybean meal, and 2,9% in chickpea flour. Gluten content was around 10 mg/g in average in the 3 samples. Concerning rheological characterization, water absorption for the 3 blends was relatively low. Values resulted for the torque were within 1.1 Nm; time required to reach this value did not exceed two minutes of kneading stability. The 3 blends denoted low amylasic activity, as well as low starch damage. Gel stability results were due to the high amylasic activity occurred in manioc starch. The retrogradation intensity predicted that the 3 blends would have a long shelf life since retrogradation processes were low for the 3 cases. Hardness presented in samples was due to protein supply provided by soybean meal. According to the sensory analysis average results for texture, flavor, and color attribute, differences were found. The ANOVA for sensory evaluation in biscuits showed differences regarding texture, flavor, and color attributes in biscuits. The moisture values obtained for each biscuit sample were similar to each other and did not exceed 3%. Tuber starch and legume flour incorporation in formulation to produce gluten-free biscuits contributes to the development of a flavoursome product and provides an alternative for people with gluten intolerance. The results obtained may be used as the basis for the production of gluten-free biscuits.

Keyword: manioc, starch, chickpea, soybean, flour, gluten-free, biscuits.

Uso de mezclas de harina de leguminosas y almidón de yuca en la elaboración de galletas dulces libres de gluten.

Resumen

El presente trabajo de investigación se basó en el estudio de la interacción entre mezclas de harinas de leguminosas y almidón de tubérculo para ser utilizadas en las formulaciones de galletas dulces y la evaluación sucesiva de parámetros de calidad. Se usaron como insumos principales: harinas de leguminosas, almidón de mandioca, margarina, azúcar, huevos y bicarbonato de sodio. Utensilios básicos: bandejas de plástico y aluminio, moldeadores de galletas, cortadores, otros artículos de galletería. Balanza analítica, batidora y horno. Métodos: establecimiento de unidades experimentales, análisis de almidón de mandioca y harinas de leguminosas, análisis de humedad, determinación del contenido de cenizas, análisis reológico de mezclas, análisis de gluten en la mezcla de harinas de almidón y legumbres, formulación de masas y fabricación de galletas, análisis de color textural, sensorial análisis de galletas, análisis estadístico. Los resultados de los análisis de humedad y cenizas en los insumos para las mezclas estuvieron dentro de los límites establecidos del 14,5%. El contenido de cenizas encontrado fue de 2,3% en almidón, 6,3% en harina de soja y 2,9% en harina de garbanzo. El contenido de gluten fue de alrededor de 10 mg/g en promedio en las 3 muestras. Con respecto a la caracterización reológica, la absorción de agua para las 3 mezclas fue relativamente baja. Los valores resultantes para el par estuvieron dentro de 1,1 Nm; el tiempo requerido para alcanzar este valor no excedió los 2 minutos de estabilidad de amasado. Las 3 mezclas denotaron una baja actividad amilásica, así como un bajo deterioro del almidón. Los resultados de la estabilidad del gel se debieron a una alta actividad amilásica en el almidón de mandioca. La intensidad de retrogradación predijo que las 3 mezclas tendrían una vida útil prolongada ya que los procesos de retrogradación fueron bajos para los 3 casos. La dureza presentada en las muestras se debió al suministro de proteínas proporcionado por la harina de soja. De acuerdo con los resultados promedio del análisis sensorial para el atributo de textura, sabor y color, se encontraron diferencias entre los tratamientos. El ANOVA para la evaluación sensorial en galletas mostró diferencias con respecto a la textura, el sabor y los atributos de color en las galletas. Los valores de humedad obtenidos para cada muestra de galletas fueron similares entre sí y no superaron el 3%. La incorporación de almidón de tubérculo y harina de leguminosas en la formulación para producir galletas sin gluten contribuye al desarrollo de un producto de buen sabor y proporciona una alternativa para las personas con intolerancia al gluten. Los resultados obtenidos pueden usarse como base para la producción de galletas libres de gluten.

Palabras clave: mandioca, almidón, garbanzo, soja, harina, libre de gluten, galletas.

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I. INTRODUCCIÓN

The base for cereal-derived staple foods for most of the inhabitants of the world is usually wheat (Samuel, 1996). Among others, this cereal contains two proteins, glutenin and gliadin conforming gluten. Gluten is a complex compound providing functional properties in doughs for bakery purposes, e.g., viscoelastic behavior. Gluten is the main agent for the protein-starch interaction, which is of high relevance in baking process (gas cell formation, stabilization, gas retention) (Gan, Ellis, Vaughan, & Galliard, 1989). However, gluten consumption may be an issue for some individuals. To avoid the effects of an enteropathy, i.e. a life-long intolerance to the gliadin fraction of wheat and the prolamins of rye, barley, and oats, a gluten-restrictive diet is suggested (Murray, 1999). Celiac disease is an autoimmune enteropathy affecting the small intestine villi (Jiménez-Ortega, Martínez-García, Quiles-Blanco, Abu-Naji, & González-Iglesias, 2016). This condition is characterized by mucous membrane damage in the small intestine, resulting in poor absorption of nutrients and, consequently, weight loss, diarrhea, anemia, fatigue, flatulence, deficiency of folate and osteopenia (Blades, 1997; Thompson, 1997). The manifestation of celiac disease has been reported to be the outcome of 3 processes: genetic predisposition, environmental factors and immunologic-based inflammation (Murray, 1999). An effective treatment for this condition is a strict adherence to a gluten-free diet throughout the lifetime of the patient. The total exclusion of gluten consumption results in clinical and mucosal recovery (Gallagher, Gormley, & Arendt, 2004). Current trends in food consumption are related to healthy and equilibrated diets (Olmedilla-Alonso, Farré-Rovir, Asensio-Vegas, & Martín-Pedrosa, 2010). Trends over a gluten-free diet affects the consumption of grains as food source; as an outcome, the effects of diet change over functional nutrients intake, as well as carbohydrate consumption, have been opposed (Bardella et al., 2000). While specially formulated gluten-free products are readily available elsewhere, little is known about to what extent these are incorporated into the diets of persons with special conditions (Anton & Artfield, 2008). Legumes feature a significant nutrient profile influencing positively in metabolic and physiological effects in the human organism, such as preventing heart and digestive

conditions (Campos-Vega, Loarca-Piña, & Oomah, 2010). The nutritional quality of legume grains is affected by certain processes and treatments during industrial processing. These processes hinder the provision of health benefits previously mentioned (Oghbaei & Prakash, 2016). Chickpea (*Cicer arietinum L.*) contains dietary bioactive compounds relevant to healthy diets. Chickpea production in Ecuador is carried out at small scale and mainly intended for self-consumption (Basantes-Morales, 2015). Soy (*Glycine max*) is characterized by a high content of both, protein and fat, and its content of trypsin inhibitors, saponins, isoflavones, and phytic acid which all have positive effects against chronic diseases; soy food products may be considered as functional food for their beneficial effects and the prevention of chronic and degenerative diseases, as well as used to improve the quality in products, however, the use of soybeans and chickpeas in food products is limited (Ahmad et al., 2014; Olatidoye & Sobowale, 2011). On the other hand, starch is an important source in global food systems due mainly to the provision of physical-chemical and functional properties; manioc (*Manihot esculenta*), a basic crop with higher production potential in Ecuador, is a relevant starch source, representing an opportunity for industrial development to counter the dependence from food products derived from wheat (Hernández-Medina, Torruco-Uco, Chel-Guerrero, & Betancur-Ancona, 2008). Given the variety of gluten-free legumes and tubers occurred in the Ecuadorian agricultural scenario, as well as the health benefits these vegetable species provide, an opportunity to establish innovative sources to the industrial processing of nutritive foods arises. As gluten is the main structure-forming protein contributing to appearance and crumb structure in baked products, it is a major challenge for scientists and technologists alike to find products which can be seen as an alternative for those unable to consume products containing gluten. The base for this research work was set in the study of the interaction between legume flours and tuber starch blends to be used for sweet biscuits formulations and the successive evaluation of the following parameters: dough rheological behavior and biscuits properties, e.g. substitution effect over texture, colorimetry, and sensory analysis in biscuits obtained from proposed

formulations. As means to an end, an option for nutritional and flavor requirements of celiac people can be envisaged without limiting the product consumption for other consumers aside, whereas further use of national production of chickpea, soy, and manioc can be enhanced, thus contributing to reduce partially import costs incurred in wheat purchase. The aim of the present work was to design and develop formulations to elaborate gluten-free sweet biscuits from chickpea flour, soybean meal, and manioc starch and to prove the feasibility of the use of these blends as a suitable substitute for wheat and other cereals containing gluten.

II. MATERIAL AND METHODS

The research work described in this article was carried out in the R&D food laboratories facilities in the Faculty of Chemical and Health Sciences at the Universidad Técnica de Machala (Ecuador). Legume flours (chickpea flour: 20.8% protein, 11.6% carbohydrates, 5.5% fat, 15.5% fiber; soybean meal: 35.9% protein, 15.8% carbohydrates, 18.6% fat, 17.3% fiber) and manioc starch (0% protein, 90% carbohydrates, 0% fat, 0% fiber) were purchased in local retail shops. To complete the formulation, margarine, sugar, eggs, and baking soda were also used. Plastic and aluminum trays, biscuits shapers, cutters, and other biscuit items were used to produce the biscuits samples. Equipment used included an analytical balance (Boeckel Co., Hamburg, Germany), an 8-speed mixer (Whirlpool, Benton Harbor, USA), and a semi-industrial oven (Andino, Quito, Ecuador). Table 1 shows the percentage of the inputs used in the formulations used as treatments.

Table 1. Inputs percentage used in biscuits formulation established.

Inputs	Treatments		
	T1	T2	T3
Manioc starch	70.00	65.00	60.00
Chickpea flour	20.00	20.00	20.00
Soybean meal	10.00	15.00	20.00
Sugar	25.68	25.68	25.68
Margarine	21.40	21.40	21.40
Egg	08.56	08.56	08.56
Baking soda	02.05	02.05	02.05
Essence	01.20	01.20	01.20
Total	100	100	100

Establishment of experimental units

A mixture design was proposed to find the more suitable replacement degree. Experimental units were determined with a D-optimal design. To define percentages of the variables (inputs), minimum and maximum restriction levels as independent variables were set accordingly. Values of levels are shown in Table 2.

Table 2. Variables and restriction levels proposed for the mixture design.

Variable	Minimum restriction level (%)	Maximum restriction level (%)
Manioc starch	50	70
Chickpea flour	20	30
Soybean meal	10	20

Analysis of manioc starch and legume flours

Moisture analysis

For each input, a 5 g sample was taken over a capsule. Subsequently, capsules were placed into a stove (Mettler GmbH + Co., Schwabach, Germany) at 105° C for 150 minutes. Dried samples were then cooled at room temperature of 25° C within a desiccator for 20 minutes. Samples were weighted afterwards up to when constant weights were reached. To determine the moisture content, the following equation was used:

$$MC = \frac{w - d}{w} * 100 \quad (1)$$

where:

MC = moisture content,

w = weight of the original sample, and

d = weight of dried sample.

Ash content determination

A 3-5 g sample of each input was taken over a crucible. Crucibles were put into a muffle furnace at 550-600°C for 120 minutes. Dried samples were then cooled at room temperature of 25° C within a desiccator for 20 minutes. Calcinated samples were weighted afterwards up to when constant weights were reached. To determine the ash content, the following equation was used:

$$A = \frac{W_2}{W_1} * 100 \quad (2)$$

where:

A = Ash content;

W_1 = Weight of original sample.

W_2 = Weight of ash.

Rheological analysis of blends

The rheological profiles of blends selected as experimental units were determined by the Mixolab 2 equipment (Chopin Technologies, Cedex, France). The following dough characteristics (behaviors) were established: maximum torque during mixing (C1), protein weakening, based on mechanical work and temperature (C2), starch gelatinization (C3), stability of formed starch gel (C4), starch retrogradation during cooling stages (C5). These dough behaviors were selected to simulate the processing conditions.

Gluten analysis in the blend of starch and legumes flours

The protein fractions corresponding to gluten in manioc starch/legume flours blends were separated with washes of deionized water. The remnant gluten was weighted in an analytical balance (Kaushik, Kumar, Sihag, & Ray, 2015). The wet gluten yield was determined by the following equation:

$$WGY = \frac{W_g}{W_b} * 100 \quad (3)$$

where:

WGY = wet gluten yield;

W_b = Weight of blend sample, and;

W_w = Weight of washed blend.

Dough formulation and biscuit making

Biscuit making techniques were based on the Ecuadorian Technical Standard (INEN, 2005). The blend of manioc starch and legumes flours, according to the treatment selected by the mixture design, was used instead of wheat flour. As the first step, margarine and sugar were mixed in a semi-industrial mixer at 180 rpm for 10 minutes until a creamy consistency (“creaming-method”) was reached. The rest of ingredients and the blend to be tested were incorporated until a homogeneous dough was obtained. This dough was let to rest for 10 minutes so all ingredients could interact. Afterwards, the dough was flattened with a rolling pin. When a thin dough

layer was obtained, and small pieces were cut out with a biscuit cutter. These pieces were then baked in a semi-industrial oven at 180 ° C for 15 minutes.

Textural analysis

To simulate the action of the human teeth bite over the biscuits, a texture analyze (texture profile analysis - TPA) was carried out with an EZ-LX texturometer (Shimadzu Co., Kyoto, Japan) at 10 mm/s and 10 mm displacement; Trapezium X software (Shimadzu Co., Kyoto, Japan) was used to take the measurements. Breaking effects were applied in 3 different zones over the biscuit surfaces.

Color analysis

Color aspects in biscuits are quality factors influencing preferences of consumers and must be controlled accurately (Jan, Panesar, & Singh, 2018). Color in biscuits was measured with a CR-400 colorimeter (Konica Minolta, Japan). Biscuit samples were placed on a flat surface and lectures were taken subsequently. The results were expressed quantitatively with the following parameters (*tristimuli*): clarity (L^*), red/green coordinates (+a indicates red, -a indicates green, a^*), yellow/blue coordinates (+b indicates yellow, -b indicates blue, b^*) (Hunter Lab color space). To calculate chromaticity ($a^* \times b^*$), the following equation was used:

According to the tonality angle of biscuits, the following equation were used:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

$$h = \tan^{-1} (b^*/a^*) \quad (5)$$

$$h = \arctan (b^*/a^*) \quad (6)$$

To calculate the total numerical difference in color between the samples in contrast with the standard sample, To, the following equation was used:

$$\Delta E = (C_1 + C_2) = \sqrt{(L * 2 - L * 1)^2 + (a * 2 - a * 1)^2 + (b * 2 - b * 1)^2} \quad (7)$$

Where:

C1 = value obtained from the 100% gluten-free standard sample To and

C2 = in treatments under study.

Sensory analysis of biscuits.

The sensory analysis was carried out by a panel of 20 untrained panelists. The most important attributes in biscuits were evaluated, namely texture and taste. A nominal 5-scale hedonic chart was used where value 01 meant “I totally dislike it” and value 05 meant “I like it a lot.”

Statistical analysis

To analyze the data obtained, the Statgraphics Centurion XVI.II (Statgraphics Technologies, Inc., The Plains, USA) statistical package was used. Data obtained were expressed as the average of 3 repetitions (n = 3) ± standard deviation. The univariate ANOVA test with a significance level of p = 0.05 was applied. When differences were found between treatments, the Tukey Post Hoc multiple comparison test was used.

III. RESULTS AND DISCUSSION

Moisture and ash analysis in inputs

The results of moisture and ash analyzes in the inputs used to elaborate the blends are shown in Table 3. Moisture content in manioc starch was 13%, this was closer to values reported previously around 13.2% (Castaño-Peláez, Mejía-Gómez, & Ríos-Márquez, 2012). Taken as reference the Ecuadorian Technical Standard INEN-0616 (INEN, 2006) for flour regarding moisture contents, values found for chickpea and soybean meals were within the limits established of 14,5%. Values previously reported for soybean meal were around 11.1 and 13.7% (Sana, Xhabiri, Seferi, & Sinani, 2012). Ash content is a quality factor in flours; for starch, ash content found was 2.3%. Ash content shall be 3% as the upper limit (Codex Alimentarius, 1989). Soybean meal presented the highest ash concentration, 6.3%, value consistent with those previously reported of circa 6.29% (Raya-Pérez, Aguirre-Mancilla, Tapia-Aparicio, Ramírez-

Pimentel, & Covarrubias- Prieto, 2012). For chickpea flour, ash content was 2.9%, results similar to those previously reported of 2.79% - 3.1% (Gadallah, 2017; Wani & Kumar, 2014).

Table 3. Moisture and ash content in inputs.^a

Inputs	Moisture (%)	Moisture (%)
Manioc starch	13.00±1.04	2.30±0.23
Chickpea flor	10.50±1.05	2.90±0.44
Soybean meal	11.34±1.06	6.30±0.45

^aAverage results (n=3, ±standard deviation).

Establishment of experimental units and blends conformation

The experimental units determined by D-optimal design program and the mixture design proposed is described are shown in Table 4 and Table 5, respectively.

Table 4. Experimental units found by D-optimal.

Std.	Run	C1 ^a (%)	C2 ^b (%)	C3 ^c (%)
15	1	62.50	25.00	12.50
17	2	60.00	25.00	15.00
9	3	65.00	22.50	12.50
14	4	57.50	27.50	15.00
12	5	60.00	22.50	17.50
8	6	55.00	25.00	20.00
5	7	65.00	20.00	15.00
1	8	70.00	20.00	10.00
-4	9	60.00	20.00	20.00
7	10	65.00	25.00	10.00
13	11	62.50	22.50	15.00
16	12	57.50	25.00	17.50
10	13	55.00	27.50	17.50
6	14	55.00	30.00	15.00
11	15	60.00	27.50	12.50
2	16	50.00	30.00	20.00
3	17	60.00	30.00	10.00

^aC1 A: Manioc starch (%), ^bC2 B: Chickpea flour (%), ^cC3 C: Soybean meal (%)

Table 5. Treatments selected by the mixture design

Treatment	Manioc starch (%)	Chickpea flour (%)	Soybean meal (%)
T1	70	20	10
T2	65	20	15
T3	65	20	20

Gluten determination

Results of gluten determination in the blends are shown on Table 6. According to standards, results are within the established limits of ≤ 20 ppm. T3 treatment presented a lower content of the protein complex.

Table 6. Gluten amount in treatments

Treatments	Gluten (%)	Gluten (mg/g)
T1	0.0010	10
T2	0.0011	11
T3	0.0009	09

These results patterned that both the 3 treatments could be suitable for the elaboration of products destined for celiac consumers. The results obtained for this parameter showed that products elaborated using any of the blends subject of research are safe to be consumed for celiac patients (Codex Alimentarius, 2015).

Rheological profile in starch/flour blends

Results of the rheological characterization of

the 3 treatments chosen are detailed in Tables 7 (rheological behavior values) and Table 8 (rheological indices). In behavior C1, water absorption for the 3 blends was relatively low. For biscuits, blends with low water absorption capacity are preferred to obtain a product with a low moisture; on the other hand, formulations for bakery products, higher values are required to allow greater water addition into the dough (Moreira-de-Oliveira, Ribeiro-Pirozi, & Da-Silva-Borges, 2007). Values resulted for the torque in behavior C1 were within 1.1 Nm, however, the time required to reach this value did not exceed two minutes of kneading stability; weak flours would be the end-result in such processes periods. This might be attributed to the absence of gluten in doughs. Doughs analyzed, resulting from the 3 treatments established fulfilled the specifications required for biscuits production (1 - 3 min) (Dubat & Boinot, 2012). Regarding behavior C2, i.e., protein fraction weakening, T3 presented the lowest value. Torque values for this behavior were between 0.35 - 0.45 Nm. Graphical description of the rheological behavior of the 3 treatments (blends) are depicted in Figure 1. Should the torque values be lower than 0.5 Nm, characteristics in biscuits dough are improved concerning adequate volume and toughness (Dubat & Boinot, 2012). T1 and T2 treatments were found to be within this range as well.

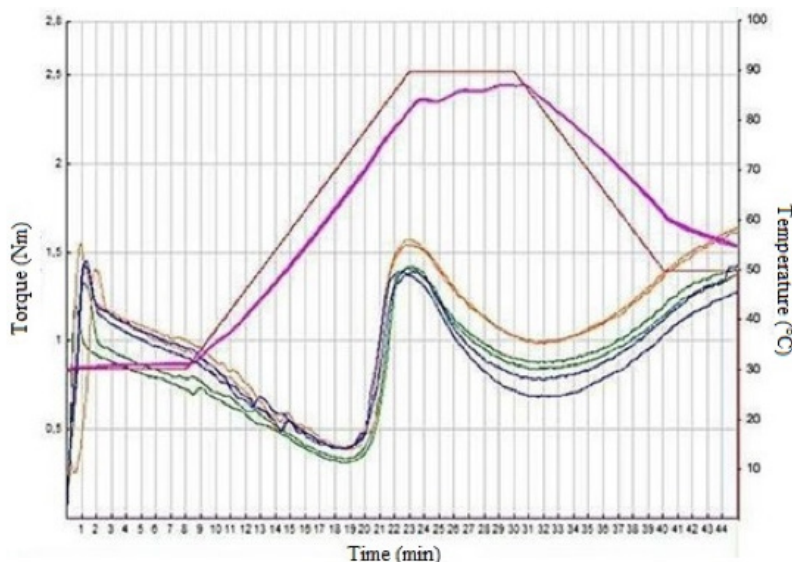


Fig 1. Graphical description of the rheological behavior of the 3 treatments (blends) described by Mixolab 2

Table 7. Values of rheological behavior obtained in Mixolab 2

T	C1 (Nm)	Time (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
T1	1.47±0.10 ^a	1.47±0.74 ^a	0.39±0.01 ^a	1.56±0.02 ^a	0.99±0.0 ^a	1.63±0.02 ^a
T2	1.44±0.02 ^a	1.23±0.07 ^a	0.39±0.00 ^a	1.39±0.00 ^b	0.73±0.07 ^b	1.35±0.11 ^a
T3	1.26±0.09 ^a	0.89±0.41 ^a	0.33±0.01 ^b	1.42±0.01 ^{bc}	0.86±0.03 ^{ba}	1.39±0.02 ^a

Average results (n=3, ± standard deviation).
Different superscripts in the same row show statistical differences (p ≤ 0.05).

Table 8. Rheological indices in flour/starch blends with different substitution values.

Treatment	Gluten matrix stability (kneading) (C1 – Cs)	Gluten matrix stability (kneading and temperature increase) (Cs – C2)	Gelatinization intensity (C3 – C2)	Amylasic activity indication (C3 – C4)	Retrogradation activity (C5 – C4)
T1	0.504	0.580	1.166	0.571	0.640
T2	0.507	0.542	0.998	0.659	0.621
T3	0.507	0.432	1.091	0.557	0.529

Results in behavior C3 evidenced that T2 showed the lowest starch quality; a value of 1.49 Nm denoted a low amylasic activity, as well as low starch damage (Sacón-Vera, Bernal-Bailón, Dueñas-Rivadeneira, Cobefia-Ruiz, & López-Bello, 2016). A limit lower than 2.5 Nm in the torque in C3 for biscuits (Dubat & Boinot, 2012). The 3 blends were within these limits concerning this behavior. Values obtained from behavior C4, gel stability, were due to the high amylasic activity occurred in manioc starch. Behavior C5, retrogradation intensity, predicted that the 3 blends would have a long shelf life since retrogradation processes were low for the 3 cases. According to the ANOVA carried out, C2, C3, and C4, parameters means showed differences, whereas C1 and C5 showed any differences. Table 5 shows results of rheological indices. Stability parameters in the gluten network during kneading and temperature increase values are used as quality attributes in products made from wheat flour. The rheological values obtained in the 3 blends were within lower indices; this might result from the absence of gluten in the blends. In the production of biscuits, the protein network conformation is not required since the demand for a crumb volume increase is not needed. Indices found, regarding gelatinization intensity, were between 0.99 to 1.16. The gelatinization temperature requirements of manioc starch may cause issues when food products

demand high temperature values during warming and cooking (Hernández-Medina et al., 2008). The values for the amylasic activity index found were 0.55 - 0.65, meaning that the lower the index, the lower the viscosity in the mass. The retrogradation intensity is due to the amylopectin recrystallization, which predicts a low retrogradation behavior in all 3 treatments.

Texture analysis

Biscuits quality lies on textural parameters, e.g., hardness, that is chewing breakage easiness, good taste and appearance (Dapčević-Hadnadev, Torbica, & Hadnadev, 2013). Table 9 shows the average data obtained from the textural analysis for each treatment. T3 presented the highest value and was statistically different to T0, T1, and T2; this might be due to the protein supply provided by the soybean meal in this treatment resulting in a higher hardness degree. As previously reported, to incorporate soybean meal to blends to prepare gluten-free biscuits, texture properties in the final product were improved (Paucar-Menacho, Salvador-Reyes, Guillén-Sánchez, & Mori-Arismendi, 2016; Schober, O'Brien, McCarthy, Darnedde, & Arendt, 2003). Adhesive strength of the biscuits produced were statistically equal in all treatments; relatively low values were found. When comparing the treatments with T0, statistical differences were found. Elasticity

is the property describing the degree in which food recovers its height among the first and second bites (González, Alvis, & Arrázola, 2015); T2 (with 15% soybean meal) showed a greater value of elasticity, whereas statistical differences in each treatment were found. Chewing properties with lower values were related to higher times for deglutition early stages before swallowing (Mariela & Daniel, 2012); those were greater in T1 and T2. T3 showed a value of 4.36 ± 0.38 . Gluten-free biscuits texture might have been influenced by the protein content from soybean

meal. The salient protein content in biscuits made from legume flour (soy and okara) lead to improved hardness and density, due to water absorption properties of soybeans, conducting also to an increase in mass, in contrast to the effect starch had in such processes (Park, Choi, & Kim, 2015), thus, products with a sound texture profile from gluten-free flours are obtained when improvers are used. In such ways, hardness, cohesiveness, chewiness, elasticity, among other properties, would be optimized. The ANOVA showed that the blends studied had effects ($p = 0.05$) over the texture profile in biscuits.

Table 9. Texture properties of biscuits produced from treatments studied

T	Hardness (N)	Adhesivity (J)	Cohesivity (N)	Adhesive strength (N)	Gumminess (N)	Elasticity	Chewiness (N)
T0	07.16±2.26 ^a	0.00±0.00 ^a	0.57±0.04 ^a	0.02±0.01 ^a	44.22±1.35 ^a	0.99±0.01 ^b	4.90±0.08 ^{ab}
T1	11.86±0.93 ^{bc}	0.01±0.00 ^b	0.74±0.04 ^c	0.02±0.02 ^a	61.07±1.31 ^c	0.99±0.09 ^c	5.31±0.19 ^c
T2	14.05±0.60 ^b	0.01±0.00 ^c	0.85±0.03 ^b	0.02±0.01 ^a	64.76±1.52 ^c	1.17±0.05 ^b	6.34±0.42 ^b
T3	15.21±0.34 ^c	0.01±0.00 ^d	0.69±0.02 ^b	0.02±0.01 ^a	56.49±1.66 ^b	0.77±0.05 ^a	4.36±0.38 ^a

Average results (n=3, ± standard deviation).
Different superscripts in the same row show statistical differences ($p \leq 0.05$).

Color analysis

Table 10 shows the results obtained regarding colorimetry for each treatment. Color analysis is determined by the five most important parameters in colorimetry. T0 showed a less luminous coloration ($L^* 70.14$) when compared to the other treatments, however, T1 presented a higher clarity ($L^* 77.41$) due to physical properties intrinsic to starch. Parameter L^* decreased the substitution percentage of soybean meal increased; T2 and T3 were darker than T0 and T1; the protein content had a negative correlation concerning to the luminosity parameter in the final product (Chevallier, Colonna, Buléon, & Della Valle, 2000). Brightness parameters reported previously for L^* of 76.31 for gluten-free biscuits made with 100% oat bran, L^* , 77.77 for biscuits made with 70% oat bran/30% oat meal, 77.85 for 50% oat bran/50% oat meal, which might be due to the protein content

in bran providing less brightness as the percentage of bran increases (Duta & Culetu, 2015). Lower results were found for parameter L^* in gluten-free biscuits made of blends made of pigeon pea flour and cornstarch, with the former in higher proportion (Liendo-Bastardo & Silva-Chávez, 2015); values generated were lower than 60. L^* values reported on biscuits made of 88% wheat flour and 12% arracacha (*Arracacia xanthorrhiza*) meal as high as 67.86 (García-Méndez & Pacheco-de-Delahaye, 2007); in standard biscuits made of 100% wheat flour, the value reported was 66.94, concluding, therefore, that legume flours and tuber starches addition in biscuits formulation affected the surface luminosity for legume proteins produce reactions, e.g., Maillard reaction, during baking process, resulting in a less bright finished product.

Table 10. Colorimetric analysis in biscuits samples.

Treatment	Luminosity L*	a*	b*	Cromaticity C*	Tonality h*
T0	70.14±0.15 ^a	6.90±0.10 ^b	42.36±0.51 ^b	42.92±0.51 ^b	80.75±0.07 ^a
T1	77.41±0.33 ^c	2.29±1.04 ^a	32.45±1.09 ^a	32.54±1.16 ^a	85.99±1.69 ^{ab}
T2	76.51±0.27 ^c	3.14±0.55 ^a	33.26±0.58 ^a	33.42±0.62 ^a	84.61±0.88 ^b
T3	74.80±1.08 ^b	4.29±1.35 ^a	32.68±0.48 ^a	32.98±0.57 ^a	82.53±2.28 ^{ab}

Average results (n=3, ± standard deviation).
Different superscripts in the same row show statistical differences (p ≤ 0.05).

Products with higher starch substitution have the brightness parameter increased (Silva & Conti-Silva, 2016). Inclusion of chickpea flour in bread formulations influenced the final product features, provoking a less bright surface color as the replacement level increased. higher brightness tones (Mohammed, Ahmed, & Senge, 2014); this might be because of the carbohydrate content present in manioc starch. T1 presented a lower result regarding a* coordinate; this increased as the manioc starch percentage decreased. T2, T3, and T0 presented higher results of the b* coordinate, tending to a yellowish color (Fig. 11). This might be due to the natural pigments occurring in chickpea flour and soybean meal. Results of a* and b* coordinates in the dough blends studied were greater than the previously reported for gluten-free biscuits from starch of tuber and leguminous (1.40 - 2.45 in parameter a* and 20.37 - 21.39 in b*) (Liendo-Bastardo & Silva-Chávez, 2015). For chromaticity C* (pigmentation degree), T1 presented less color saturation in relation to the central axis; T0 is the most saturated, unlike the other sample. Results in h* tonality for T1 and T2 showed a greater tone angle, from 85.99 to 84.61, respectively. These values were higher than those in T0, corresponding to a yellow color. The ANOVA (p = 0.05) showed that manioc starch and legume flour blends had effects over color parameters L*, a*, and b* in biscuits. T1 was slightly clearer than the others. The 3 samples presented a yellowish tone, albeit T1 showed a tendency towards greenish tonalities, meanwhile T0 leant towards reddish colors. Results of color difference with respect to the pattern are shown in table 00. As shown in Table 11, T1 showed a greater total numerical color difference compared to the other treatments. T3, on the other hand, presented a smaller quantitative difference compared to T1 and to T2.

Table 11. Total numeric color difference in treatments

Treatments	ΔE*
T1	13.13
T2	11.72
T3	11.06

*Color difference

Sensory analysis

Figure 2. depicts the texture results for the treatments studied. According to the average results for texture, greater acceptability was found for T0 (4.65), followed by T1 (4.45). Similarly, T1 achieved the highest acceptability in terms of flavor; an average of 4.55 was determined for this attribute.

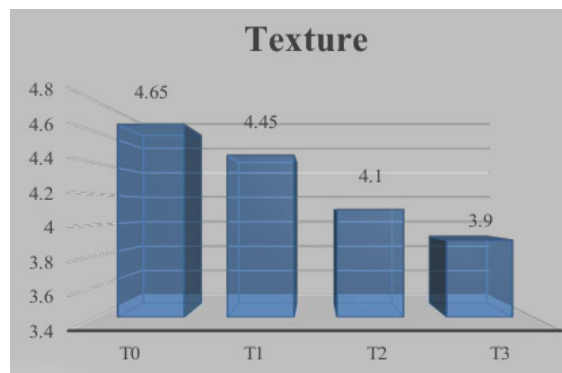


Fig 2. Acceptability averages for the texture attribute in biscuits samples

Regarding color attribute, T1 presented a lower acceptability degree; T0 and T3 had a greater acceptability degree with an average of 4.55 and 4.15, respectively. T3 obtained lower acceptability in terms of texture and flavor. Respecting the color attribute, T0 showed better results, followed by T3. Whitish color was reported for T1, this might be due to the higher percentage of manioc starch used in the blend. Figure 3. depicts the color results for the treatments studied.

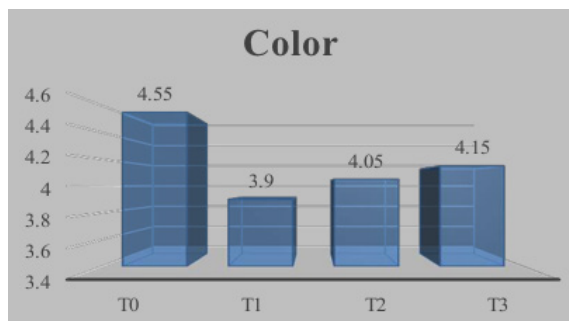


Fig 3. Acceptability averages for the color attribute in biscuits samples

Manioc starch use in the production of biscuits allowed favorable results in terms of acceptability and was proved it can be a substitute for wheat flour (Bénitez et al., 2008). Wheat flour products partially substituted with legume flour were reported to be sensory accepted by consumers (sensory evaluation values higher than 5 points on a scale of 7) in bread, brownies, and biscuits (Granito, Valero, & Zambrano, 2010). Similar results were collected in the sensory evaluation of biscuits made with pigeon pea flour and cornstarch; minor substitutions with pigeon pea flour were sensory pleasing, this could be for the attribute of characteristic flavor of legumes (Liendo-Bastardo & Silva-Chávez, 2015). Figure 4. depicts the flavor results for the treatments studied.

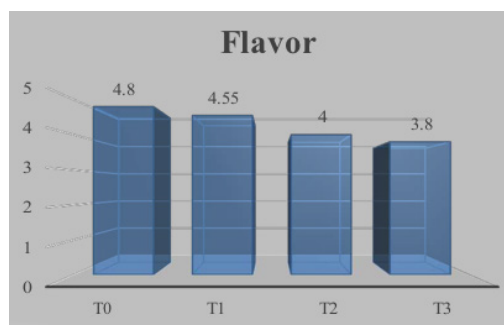


Fig 4. Acceptability averages for the flavor attribute in biscuits samples

A 20% replacement with chickpea flour in bread making provided acceptable results, similar to samples made with 100% wheat flour (Farzana & Mohajan, 2015; Man, Păucean, Muste, & Pop, 2015). As the level of soybean meal substitution increases in biscuits formulation, it is less acceptable in terms of organoleptic attributes, especially flavor perception (Silva & Conti-Silva, 2016). This might be because of the lipoxygenase present in soybeans, triggering

volatile compounds release and thus enhancing unpleasant flavors. These differences in biscuit samples can result from the influence of various compounds produced during baking, probably due to the development of the Maillard reaction when compound flours are used (Pérez, Matta, Osella, de la Torre, & Sánchez, 2013). Results of the ANOVA for sensory evaluation in biscuits are presented in Table 12. Tukey test results showed, regarding texture and flavor attributes in biscuits, that T0 had any difference with T1, but differs from T2 and T3. Regarding color attributes, T1, T2, and T3 showed differences compared to T0.

Table 12. Sensory analysis for biscuits studied

Treatments	Texture	Flavor	Color
T0	4.65 ± 0.49 ^a	4.80 ± 0.41 ^a	4.55 ± 0.51 ^a
T1	4.45 ± 0.51 ^{ab}	4.55 ± 0.60 ^a	3.90 ± 0.45 ^b
T2	4.10 ± 0.45 ^{bc}	4.00 ± 0.32 ^b	4.05 ± 0.22 ^b
T3	3.90 ± 0.55 ^c	3.80 ± 0.52 ^b	4.15 ± 0.49 ^b

Average results (n=3, ± standard deviation). Different superscripts in the same row show statistical differences (p ≤ 0.05).

Moisture analysis in biscuits samples

Data obtained is detailed in Table 13. According to Ecuadorian Technical Standard NTE INEN 2085 (2005) (INEN, 2005), a maximum of 10% is acquainted concerning moisture content in biscuits. Replacing with a higher percentage of soybean meal in blends influences the moisture content. The solid matter content in soybean meal was related to the low humidity percentage, this might be due to the emulsifying power present in this legume (Ndife, Kida, & Fagbemi, 2014; Taghdir et al., 2017). The moisture values obtained for each biscuit sample were similar to each other and did not exceed 3%.

Table 13. Moisture percentage in biscuits samples.

Treatments	Moisture (%)
T1	2.45
T2	2.23
T3	2.10

IV. CONCLUSIONS

Tuber starch and legume flour incorporation in formulation to produce gluten-free biscuits, not only contributes to the development of a flavorsome product but also provides a new alternative for people

with gluten intolerance.

Blends of manioc starch, chickpea flour, and soybean meal resulted below 20 ppm gluten; according to Codex Stan 118-1979 on Gluten-free foods, blends of the mentioned are safe for the production of biscuits for celiac patients.

The rheological analysis allowed to predict the behavior of doughs obtained from the blends; higher substitution percentage with legume flours derived in a lower water absorption capacity due to the low quality of gluten. T1 and T2 had better rheological behavior fitting to be destined for biscuits. In addition, rheological indices determined showed that all blends presented low retrogradation, therefore predicting an enhanced shelf life.

Biscuits hardness varied accordingly to the legume flour percentage included in the blend; the greater the amount of soybean meal, the greater the hardness the blend showed. However, the biscuits samples became fragile when the starch content was high.

As the percentage of starch increased, the luminosity parameter (L^*) increased and the tonality parameters $h(a^*, b^*)$ decreased accordingly. Darker, golden, and with greater hue biscuits were obtained with a higher content of legume flours.

In terms of color, as the incorporation of chickpea flour and soybean meal increased, the acceptability improved whereas pleasant results were not consequent for the flavor attribute. Overall, biscuits made in the 3 treatments studied presented good results, however, the best rated treatment was T1.

The results obtained in this research may be used as the basis for the production of gluten-free biscuits; to incorporate tuber starch and legume flours is viable to counter the needs of a consumer sector with specific problems, such as celiac patients.

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