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Artículo Original

Chocolate couvertures development from combination of CCN51 and Super Tree cocoa varieties in Pastaza-Ecuador

Desarrollo de una cobertura de chocolate a partir de la combinación de las variedades de cacao CCN51 y Super Tree en Pastaza-Ecuador

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Abstract

Dark chocolate coatings contain a high percentage of cocoa, which is pleasing to the consumer's taste. The aim of the research was to develop a bitter-type chocolate coating from the combination of CCN51 and super tree cocoa. In this study, an experimental method, with 18 treatments was applied. In the sensory analysis a hedonic scale test with 30 untrained judges and 15 trained ones was carried out, applying a completely random unifactorial design. The judges determined that T1 treatment with a content of 70% cocoa liquor, had a mean value of 3.8, while the value for the untrained judges was not significance. A bromatological analysis of the best treatment was made based on the NTE INEN 621:2010, resulting in moisture content of 37.86%, fat 9.26%, protein 2.04% and ash 99.38%, defatted cocoa dry extract 98.41%, dry extract of total cocoa and microbiological results less than 100 ufc.ml-1 in total mesophiles, yeasts and total coliforms, parameters that are within the permissible limit.

Keywords: Saquifrancia farm; cocoa; Super Tree; cocoa liquor; cocoa butter.

Resumen

Las coberturas de chocolate amargo contienen un alto porcentaje de cacao, lo que resulta agradable al paladar de los consumidores. El objetivo de la investigación fue elaborar una cobertura de chocolate de tipo amargo a partir de combinar el cacao CCN51 y super tree. El método que se aplicó en el estudio fue de tipo experimental con un total de 18 tratamientos. El resultado se determinó por una prueba sensorial con una escala hedónica, la catación se realizó con 30 jueces no capacitados y 15 capacitados, aplicando un diseño unifactorial completamente al azar. Los Jueces determinaron que el T1 con un contenido de 70 % de licor de cacao, tuvo un valor medio de 3.8, mientras que el valor para los jueces no entrenados no tuvo significancia. Se generó un análisis bromatológico del mejor tratamiento en base a la NTE INEN 621:2010, dando como resultado humedad, 37,86% grasa, 9,26% proteína, 2,04% ceniza, 99,38% extracto seco de cacao desgrasado, 98,41% extracto seco de cacao total y resultados microbiológicos menores a 100 ufc. ml-1 en mesófilos totales, levaduras y coliformes totales, parámetros que están dentro del límite permisible.

Palabras clave: Granja Saquifrancia; cacao; Súper Árbol; licor de cacao; manteca de cacao.

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1. INTRODUCTION

Cocoa (*Theobroma cacao L.*) is of Amazonian origin, introduced in Europe, since then it is a product of great global demand. Cocoa trade is based on quality, classified into fine aroma and ordinary (Campaña, Hidalgo, & Sigcha, 2016). Currently, there are cocoa plantations in Mexico, Brazil, Ecuador among other countries. It is known as "Food of the Gods" by the Olmecs, Mayas and Aztecs. The seeds were used as currency for gold by the Spaniards, who sent it to Spain. They added sweeteners to the drink and developed new recipes with the addition of sugar, cinnamon, almonds, eggs, and vanilla, which was gradually spread to France and the rest of world (Costaguta, 2007).

Over the years, chocolate has evolved into a smooth and creamy food with an unsettling pleasure on our senses. Chocolate is a mixture of cocoa liquor and cocoa butter with sugar, sometimes milk or fruit. Depending on the percentage of each ingredient added, different types of chocolates are made (Costaguta, 2007).

Chocolate is a complete nutritional food that has approximately 30% fat (cocoa butter with 35% oleic ac., 35% stearic ac. and 25% palmitic ac.), 6% protein, 61% carbohydrates and 3% humidity and ashes composed of minerals such as phosphorus, iron and calcium. It also contains vitamin A and B complex vitamins. Inexpensive chocolates are made with synthetic cocoa butter, which have characteristics with pleasant and fatty flavors on the palate (Valenzuela, 2007). The Saquifrance estate cultivates the CCN51 variety and super tree on its properties. The first is known for its productivity and the second for its bitterness (Morales *et al.,* 2016). These varieties differ in their characteristics, so the coverage formulation is optimal (Alcides & Ramírez, 2016). Saquifrance also has a chocolate processing plant where cocoa paste or liquor is obtained. Currently, there is a need to expand production lines due to international requirements and trends in the use of raw materials with other characteristics.. There are products on the market that contain innovative inputs or ingredients which have achieved a high purchasing value, and there is a trend towards the consumption of bitter chocolate, especially in chocolate couvertures where the cocoa aroma characteristic is appreciated. Chocolate couverture is the most demanded; however, it is not the variety grown on the farm. In this context, it is urgent to respond to the current market dynamics by innovating. The proposal is to combine the advantages of the two Saquifrancia cocoa varieties: CCN51 cocoa of high productivity (volume) and Super Tree (aroma and flavor), trying to find a mixture that be sensorially accepted by the consumer and find the desired characteristics in a chocolate coating (Enríquez y Ojeda, 2020). Ecuadorian cocoa is known as one of the best in the world for its organoleptic characteristics. It has been used since ancient times in gastronomy, taking into account the factors that intervene to have a quality product such as: post-harvest, fermentation, drying, roasting and conching (Díaz & Pinoargote, 2011).According to Quintana, Gómez, García and Martínez (2015), CCN51 cocoa has been classified for its high perceived acidity, astringency, bitternessand fruity flavors. Therefore, aspects such as origin and other factors that are performed until having a chocolate couverture have been improved, with the help of the Super Tree variety, which is characterized by its fruity and floral flavors, as well as similar characteristics to fine aroma cocoa, it is possible to achieve an exquisite chocolate couverture to the palate (Alcides & Ramírez, 2016).

CCN51 cocoa is a clone that stands out for its productivity, resistance to pests and diseases, an unusual bitter taste and with a good fermentation it would become an appetizing product (Perea, Ramirez, & Villamizar, 2011).

The Super Tree or Trinitario cocoa has the same characteristics as the fine aroma cocoa, when processed it develops a pronounced chocolate flavor, allowing a quality product to be obtained in terms of flavor (Elwers, Zambrano, Rohsius, & Lieberei, 2009). Cocoa clone CCN51 was discovered by the agronomist Homero Castro Zurita in 1962, after 70% of the cocoa crop destruction, caused by the pests known as witches' broom (*Crenipellis perniciosa*) and monilla (*moniliophthora roreri*). The CCN51 clone whose acronym stands for Castro Naranjal collectionand the number 51 as a result of the trial conducted in Naranjal at Hacienda Sofí (Quintana & Goméz, 2011).

The two varieties of cocoa mentioned above have potential as raw materials and in combination can offer innovative products for the chocolate industry. The development of new products for the company, such as a bitter chocolate couverture, covers the market by solving the lack of products . This coating type is popular in food products worldwide due to its nutritional value (protein and fat), sensory characteristics are pleasant for the consumer (Wells & Van der Gaag, 2006). It is a versatile product used in different areas of gastronomy, medicine, confectionery, ice cream and pastry shops.

The objective of this research was to elaborate chocolate couvertures with CCN51 and Super Tree cocoa varieties by using an experimental design (what is type? Here should be mentioned) with three treatments and two formulations.

2. MATERIALS AND METHODS

Location

The research was carried out in Pastaza province, city of Puyo, located in the northwest of Ecuador. The product was manufactured at the Saquifrancia agroindustrial and tourist farm. The analyses were carried out in the chemistry, bromatology and microbiology laboratories of the Universidad Estatal Amazónica.

Experimental research

An experimental design was carried out to determine the treatments and formulationsdeveloped for the product.

Qualitative research

Statistical methods were used to validate the results, which allowed obtaining accurate and objective information (to identify the best product).

Completely randomized One-factor design (ACD)

Eighteen experimental units with three treatments, two formulations and three replicates were used for the experimental design.

Study variables

Independent variables:

- Percentage of cocoa liquor
- Percentage of vegetable fat (cocoa butter)
- Percentage of sugar
- Percentage of lecithin

Dependent variables:

- Physical-chemical composition
- Organoleptic characteristics: brightness, color, aroma, flavor (cocoa and sweet), bitterness, astringency, texture, and fluidity.

Chocolate formulation for couverture

Three treatments were carried out with different concentrations of cocoa liquor (combination of cocoa CCN51 and super tree), as detailed in Table 1.

After establishing the treatments, the formulations were developed as shown in Table 2. each formulation.

Development of technological process

Cocoa beans must be fermented and dry (7 %humidity) (Ohene, Patersona, & Fowlerb, 2007). The beans should be selected free of impurities for subsequent drying and roasting (between 130°C and 140°C), which aids flavor development from precursors formed during fermentation (Ohene et al., 2007).

Then it goes through shelling and grinding, resulting in cocoa liquor. The cocoa paste goes to the mixing and refining stage in which sugar is added. These operations are carried out between 60°C and 70°C to obtain a homogeneous mass with a suitable particle size for subsequent conching. The conching operation is where the missing ingredients (cocoa butter, lecithin) are incorporated at temperatures between 60°C and 70°C, which improves flavor, color, stabilizes viscosity and eliminates undesirable flavors. Finally, the process goes through tempering to improve consistency and finally molding (Ohene et al., 2007).

Sensory analysis of the product

Sensory tests were carried out using the structured hedonic scale; this allows to quantify the magnitude of the difference between the intensity (rating 0 to 5) and acceptability (rating 1 to 5) of the treatments. The evaluation is detailed in Table 3.

Table 1. Concentration of varieties to obtain cocoa liquor.

VARIETIES	T1	T2	T3
Cocoa CCN51	80%	70%	60%
Super Tree Cocoa	20%	30%	40%

Table 2. Chocolate formulations for couverture.

Formulation	Ingredients	Treatments (Combination in % of cocoa liquor CCN51 and Súper tree)		
		T1 (80-20)	T2 (70-30)	T3 (60-40)
F1	Cocoa liquor	70%	70%	70%
	Sugar	24,5%	24,5%	24,5%
	Vegetable shortening (cocoa)	5,0%	5,0%	5,0%
	Lecithin	0,5%	0,5%	0,5%
	TOTAL	100 %	100 %	100 %
F2	Cocoa liquor	65%	65%	65%
	Sugar	27,5%	27,5%	27,5%
	Vegetable shortening (cocoa)	7%	7%	7%
	Lecithin	0,5%	0,5%	0,5%
	TOTAL	100 %	100 %	100 %

Table 3. Hedonic scale.

Intensity		Aceptability	
0	Absent	1	Poor
1	Barely detectable	2	Bad
2	Present	3	Regular
3	Characteristics of the sample	4	Good
4	Dominant	5	Excellent
5	Extrema		

According to the intensity, the parameters evaluated were color, aroma, flavor (cocoa and sweet), bitterness and astringency. Regarding acceptability, they were color, aroma, flavor (cocoa and sweet), texture and fluidity.

Sensory test design.

The sensory analysis was carried out with trained people, who know the product, and with untrained panelists (three replicates were carried out).

The samples were coded with three digits taken from the random number table.

Tasting procedure.

The untrained judges were each assigned the samples (10g) previously coded, accompanied by a glass of water to drink between each sample, plus a piece of apple to cleanse the taste buds and evaluation sheet using a structured hedonic scale. The tasting was carried out between 9:00 am- 10:00 am and 11:00 am -12:00 pm, so the results would not be affected by external factors (lunch time). The parameters evaluated were brightness, color, aroma, flavor (cocoa and sweet), bitterness, astringency, and texture.

The trained judges were each assigned the pre-coded samples (100g) and the evaluation sheet using a structured hedonic scale (see Annex 4?). They worked according to their sales product. The evaluated parameters were color, aroma, flavor (cocoa and sweet), bitterness, astringency, texture, fluidity and gloss.

Statistical analysis of sample tasting.

Data analysis was performed through an analysis of variance (ANOVA) using the Excel spreadsheet ..

If a significant difference was observed, Tukey's test was applied at a significance level of 0.05 if significant differences existed using the InfoStat 2011 computerized software package.Physical, chemical and microbiological analysis of chocolate couverture

The following analyses were performed on the selected treatment:

Moisture analysis (gravimetric method): Moisture analysis (gravimetric method): it was performed based on the AOAC 931.04 method, calibrating the temperature at 103°C±2°C. content was calculated (CODEX, 2016).

Equation (1)

$$\%H = \frac{wet\ sample\ g - dry\ sample\ g}{wet\ sample\ g} * 100$$

Protein analysis (volumetric method): according to the AOAC 960.52 method (Kjeldahl digestion), three stages were carried out: digestion, distillation, and titration (Helrich, 1990). To obtain the crude protein content, it was calculated by means of the following equation (2):

Where:

$$\%P = \frac{(V_{HCL} - V_b) * meqN * N_{HCL} * F}{M}$$

- meqN: atomic weight of nitrogen
- HHCL: normality of 0.1N hydrochloric acid.
- F: conversion factor (6,25)
- VHCL: volume of hydrochloric acid consumed in the titration.(ml)
- Vb: target volume (0,1) – (ml)

Fat analysis (gravimetric method): governed to the AOAC 963.15 method (Soxhlet extraction) or NTE INEN 535, with help of an organic solvent the fat was extracted semi- continuously. By means of equation (3) the fat content was calculated (CODEX, 2016).

Equation (3)

$$\%G = \frac{m_2 - m_1}{M} * 100$$

Where:

- $\mathbf{m_1}$: weight in g of the empty round bottom flask (with porcelain piece and support).
- $\mathbf{m_2}$: weight in g of the round bottom flask with fat after drying (with porcelain piece and support).
- **M**: weight of the sample in g.

Ash analysis (gravimetric method): the sample is incinerated in a muffle at 600°C according to and equation 5 is used to determine its result.

Equation (4)

$$\%Ceniza = \frac{weight\ g\ of\ ash - weight\ g\ of\ the\ crucible}{sample\ weight\ g} * 100$$

Analysis of defatted dry extract and total cocoa dry extract (gravimetric method): the fat was extracted by solvent and the sample was taken to the oven to determine the extract content by weight difference, using the following equations to determine each analysis (1 and 5):

Equation (1)

$$\%H = \frac{wet\ weight - dry\ weight}{wet\ weight} \times 100$$

Equation (5)

$$\%ESD = 100 - \%H$$

Analysis of molds and yeasts: according to NTE INEN 1529-10, the sterilized sample is placed in a culture between 22°C and 25°C, the agar must contain yeast extract, glucose, and mineral salts. The plate count technique was used by deep sowing.

Coliforms: according to NTE INEN 1529-7, it consisted of a plate count by deep sowing of the sample on agar, incubating it at 30°C±1°C for 24±2h.

Mesophilic aerobes: according to NTE INEN 1529-5, the sample was inoculated in a solid nutrient medium, incubated at 30°C±1°C for 78 hours. The plate count was performed by deep sowing in the agar, this serves to calculate the amount of m/o per g or cm^3 of food.

In summary, these analyzes are commonly performed and only the method should be mentioned and not the complete method reported.

Treatment design

The percentages in which the cocoa varieties CCN51 and super tree were combined to obtain three treatments (cocoa liquor). Perea *et al.,* (2011) point out that CCN51 cocoa is characterized by its bitter taste, for this reason it does not resemble the organoleptic characteristics of national cocoa (Morales et al., 2016). Then Alcides and Ramirez (2016), mentions that organoleptic characteristics of the Super Tree in relation to the fine aroma present similarity (unique flavor and aromas). For this reason, the addition of a certain percentage of Super Árbol cocoa is proposed to mask the bitter taste and contribute to the final product with sensory characteristics like the chocolate of a fine aroma.

Description of the chocolate couverture process

The production of chocolate couverture was developed using the following process technology as shown in Figure 1.

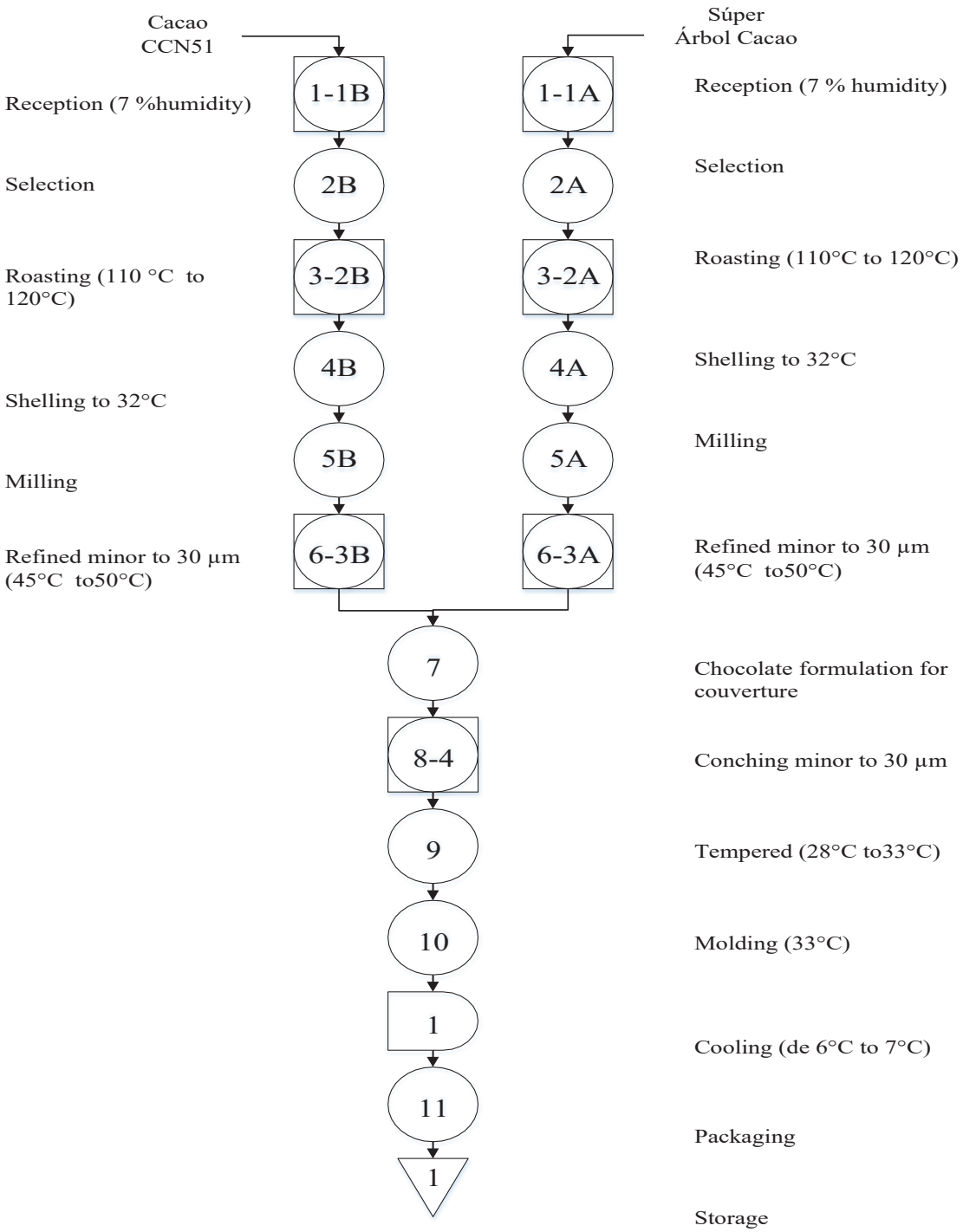

Figure 1 OTIDA (Operation, Transportation, Inspection, Delay and Archive) diagram for chocolate couverture elaboration.

The elaboration of chocolate couverture consisted of 17 processes, 7 inspections, 1 delay and 1 storage as shown in Table 6.

By means of Figure 1 it is possible to verify the specific parameters of the process such as humidity and impurities. From the cocoa liquor, each treatment begins to be formulated and therefore is incorporated into the machine (conching, loss of 2.5% of cocoa liquor) where the missing ingredients (sugar, cocoa butter and lecithin) are incorporated, which improves flavor, color, stabilizes viscosity and eliminates undesirable flavors. To finish the process, it was tempered (loss of 1.5% of cocoa liquor) to improve consistency. Finally, it was molded (100g), cooled (6°C- 7°C), packaged and stored.

As Beckett (2008) mentions, if left longer in the conching machine, the homogeneity of the fat in the chocolate particles improves the stability of the fat crystallization in the tempering process, and with the prescribed cooling temperatures of 28°C and heating of 33°C, they favor the shine, fluidity, and stability of fat crystals, acquiring that crunchiness of the chocolate when it is broken (Schuhmacher et al., 2007).

3. RESULTS AND DISCUSSION

Analysis of sensory attributes according to their intensity

Table 7 shows the ANOVA of the intensity parameter.

Based on the values obtained in Table 7 for the organoleptic attributes evaluated according to intensity, they were statistically equal in the opinion of the trained tasters, while for the untrained tasters there was a significant difference in aroma and color with a reliability of 95%, as evidenced in Table 8, which shows the Tukey test, where the best treatment in each organoleptic attribute was verified.

Respect to color, treatment 6 (65% cocoa liquor; 60% CCN51 and 40% Super Árbol) presented a high evaluation among the measures, which means that untrained tasters appreciated a color characteristic of bitter chocolate. Caballero and Maldonado (2012) mentioned that the color of this product should be dark brown, therefore it complies with this parameter.

Regarding the aroma, treatment 1 (70% cocoa liquor; 80% CCN51 and 20% Super Tree) shows a higher value among the measures, indicating that the untrained tasters considered a cocoa aroma that stands out among the others. Zambrano et al. (2010) indicated that the aroma is produced during fermentation and roasting, where at 120°C the precursor amino acids of the chocolate aroma are released, and it also depends on the type of cocoa variety with which it is going to be elaborated; then with a mixture of 20% Super Tree cocoa liquor added it is enough to have that typical fruity aroma of almonds. According to Jinap, Bakar, and Saari (2004),

Table 6. Summary table of the OTIDA diagram for chocolate couverture elaboration.

Meaning	Symbology	Quantity
Process	○	17
Inspection	□	7
Delay	D	1
Storage	▽	1

Table 7. Summary ANOVA table of the organoleptic attributes evaluated (Intensity).

Analysis of Variance Table (SC Type III)						
Organoleptic attributes	Untrained tasters			Trained tasters		
	Variance factor: Treatments			Variance factor: Treatments		
	F	P-value	Critical value for F	F	P-value	Critical value for F
Brightness	1,3519	0,24483*	2,2661	0,6238	0,6820*	2,3231
Color	9,5039	0,00000004 97**	2,2661	0,7133	0,6151*	2,3231
Aroma	2,9440	0,01409**	2,2661	1,7909	0,1235*	2,3231
Cocoa flavor	1,2575	0,2845*	2,2661	1,3498	0,2516*	2,3231
Sweet taste	1,2326	0,2958*	2,2661	1,7452	0,1332*	2,3231
Bitterness	0,2600	0,9343*	2,2661	1,7385	0,1347*	2,3231
Astringency	0,4288	0,8281*	2,2661	0,6829	0,6376*	2,3231

*: no significant difference
**: there is significant difference
------: parameter not evaluated.

the pyrazine contained in cocoa is responsible for giving the characteristic aroma and flavor of chocolate in the roasting process.

Beckett (2008) points out that conching is one of the important operations for minimizing undesirable parameters, refining the smell, and homogenizing the ingredients to give a good texture and flavor, losing the bitter and astringent taste (Zambrano et al., 2010).

Analysis of sensory attributes according to their acceptability

The following table shows the scores of the untrained and trained judges:

According to the values obtained in Table 9 on the organoleptic attributes evaluated according to acceptability, there is significant difference in certain sensory attributes; the untrained tasters considered significant difference in color, aroma and texture, while the trained tasters found significant differences in brightness, color, aroma and texture with a reliability of 95%, as evidenced in Table 10, which shows the Tukey' test, where the best treatment was verified in each organoleptic attribute.

As shown in Table 10, treatment 1 (70% cocoa liquor; 80% CCN51 and 20% Super Tree) met the expectations of the trained judges in terms of brightness, color, texture, and they perceived a pleasant aroma in treatment 3. While the untrained judges liked treatment 6 (65% cocoa liquor; 60% CCN51 and 40% Super Tree) in relation to color and aroma, agreeing with the trained judges that the best texture is in treatment 1. Caballero and Maldonado (2012) mentioned that a product is appetizing for its texture when it does not feel lumpy on the palate and when breaking it, a crunch is felt. To obtain this consistency, brightness and color of the chocolate depends on the size of the particles (less than 30 microns) and a good tempering process, which helps to give a good crystallization of the fat.

Analysis of the best treatment

With the averages obtained from the evaluation, the analysis of variance of the treatments was carried out.

Table 8. Tukey summary table of the organoleptic attributes that had a significant difference. (Intensity)

Test: Tukey				
Variance Factor	**Treatments**	**Stockings**	**Ranges**	
Color	6	**3,33**	A	
	5	3,13	A	
	2	3	A	
	1	2,6	A	
	3	2,47	A	
	4	1,4		B
Aroma	1	**3,63**	A	
	2	3,37	A	B
	5	3,13	A	B
	3	3,07	A	B
	6	2,93	A	B
	4	2,6		B

Table 9. Summary ANOVA table of the organoleptic attributes evaluated (Acceptability).

Analysis of Variance Table (SC Type III)						
Organoleptic attributes	**Untrained tasters**			**Trained tasters**		
	Variance factor: Treatments			**Variance factor: Treatments**		
	F	**P-value**	**Critical value for F**	**F**	**P-value**	**Critical value for F**
Brightness	1,4635	0,2041*	2,2661	2,9647	0,0163**	2,3231
Color	9,5326	0,000000 0471**	2,2661	2,4291	0,0416**	2,3231
Aroma	2,4013	0,0390**	2,2661	4,7738	0,00069**	2,3231
Cocoa flavor	1,7776	0,1198*	2,2661	2,0980	0,0736*	2,3231
Sweet taste	1,1572	0,3323*	2,2661	0,6079	0,6941*	2,3231
Texture	7,7213	0,000001 3967**	2,2661	4,5832	0,00096**	2,3231
Fluency	--------	---------	---------	1,7092	0,1414*	2,3231

*: no significant difference (according to P-value is greater than α= 0.05).
**: there is a significant difference (according to P-value is less than α= 0.05).
------: parameter not evaluated.

Table 10. Tukey summary table of the organoleptic attributes that had a significant difference. (Acceptability).

Organoleptic Attributes	Untrainedtasters					Trainedtasters				
	Test:Tukey					Test:Tukey				
	Treatments	Stockings	Ranges			Treatments	Stockings	Ranges		
Brightness						1	**3,8**	A		
						5	3,67	A		
						3	3,27	A	B	
						2	3,27	A	B	
						4	3,2	A	B	
						6	2,8		B	
Color	6	**3,93**	A			1	**4,27**	A		
	5	3,87	A			3	4,2	A	B	
	2	3,6	A			2	4,13	A	B	
	1	3,6	A			6	4,07	A	B	
	3	3,23	A			5	4	A	B	
	4	2,47		B		4	3,53		B	
Aroma	6	**3,8**	A			3	4	A		
	1	3,77	A	B		1	3,8	A	B	
	5	3,73	A	B		6	3,47	A	B	C
	3	3,5	A	B		2	3,47	A	B	C
	2	3,43	A	B		5	3,07		B	C
	4	3,07		B		4	2,67			C
Texture	1	**4,1**	A			1	**4,27**	A		
	2	3,87	A	B		5	2,93	A	B	
	5	3,8	A	B		2	2,93	A	B	
	6	3,73	A	B		6	2,8		B	
	3	3,3		B	C	3	2,67		B	
	4	2,8			C	4	2		B	

Source: Authors

According to the statistical results of ANOVA (Table 11), with respect to untrained judges there was a significant difference between treatments because according to p-value obtained is less than $\alpha = 0.05$. Meanwhile, for trained judges, the statistical results indicate that there is no significant difference between treatments (all are equal), because according to p-value obtained is greater than $\alpha = 0.05$.

The best treatment was determined using Tukey's method.

Table 12 shows statistically that, for the untrained judges, the best couverture chocolate is the one with 70% cocoa liquor (treatment 1) in which it is mixed with two types of varieties (80% CCN51 cocoa and 20% Super Árbol). According to the analysis of the attributes, this is the one that has stood out in brightness, color, aroma, and texture: then with 20% of Super Árbol cocoa liquor combined with CCN51 it shows better sensory characteristics.

Physical-Chemical Analysis

The results of the physical-chemical analysis of bitter couverture chocolate were compared with the Mexican standard NMX-F-061-1964 and NTE INEN 621:2010. Table 13 shows the experimental results. The moisture and ash contents are within the established ranges compared to the Mexican standard NMX-F-061-1964. When fermentation is carried out under appropriate conditions, the cocoa bean does not incorporate substances that can increase the percentage of minerals (Beckett, 2008). With respect to moisture content, dry cocoa beans have been acquired with the required percentage of 7% to 8% moisture. The roasting process allowed the elimination of excess water (Schuhmacher et al., 2007).

The protein (9.26%) and fat (37.86%) contents have a high percentage according to the minimum established in the Mexican standard NMX-F-061-1964 and NTE INEN 621:2010. The high percentage ratio may be due to the mixture of the two raw materials, CCN51 and Super Tree, which contain different concentrations of protein and fat (Graziani, Ortiz, & Parra, 2003).

The contents of defatted dry extract and total cocoa dry extract analyzed compared to the NTE INEN 621:2010 standard is higher than the minimum level of 2.5% and 35% established according to the requirements that a couverture chocolate must contain. The product itself partially has a high content of non-fat components with

Table 11. ANOVA of the treatments evaluated.

Analysis of Variance Table (SC Type III)				
Variance factor	Untrained tasters		Trained tasters	
	F	p-value	F	p-value
Treatments	12,208	0,000230	1,99	0,1527

Table 12. Comparisons of means by Tukey's test.

Untrainedtasters				
Treatments(T)	Stockings	n	Ranges	
1	**3,8**	A		
5	3,73	A	B	
6	3,7	A	B	
2	3,57	A	B	
3	3,33		B	C
4	2,9			C

Table 13. Comparison of the physicochemical results of the best treatment with the standards.

Parameters	Result of the analysis	Mexican Standard NMX-F-061-1964		Standard NTE INEN 621:2010	
		Min.	Max.	Min.	Max.
% Humidity	1,59	---	2,0	---	---
% Protein	9,26	7,25		---	---
% Grease	37,86		---	31	
% Ash	2,04	1,9	2,1	---	---
% Dry defatted cocoa extract	99,38	---	---	2,5	
Total % of cocoa dry extract	98,41	---	---	35	

minimal moisture content, favoring the preservation of the product (INEN, 2010).

Microbiological analysis

Microbiological analyses were performed to verify that the product does not represent a health hazard caused by microorganisms.

Table 14. Comparison of the microbiological results of the chocolate sample for couverture with INEN 621.

CHOCOLATE AN ALYSIS FOR COU OUVERTURE		
Parameters	Sample analyzed	According to standard
Total mesophiles	<100 CFU	$<2.0*10^4$UFC
Yeast	<25 UFC	$<1.0*10^2$UFC
Total coliforms	<100 CFU	$<1.0*10^2$UFC

Source: Authors.

As can be seen in Table 14, the results of the microbiological analysis of the chocolate couverture report the presence of total mesophiles, total coliforms, and yeasts, which are less than 100 CFU.

4. CONCLUSIONS

Bitter chocolate couverture was made from cocoa liquor containing a combination of two varieties (CCN51 and Súper Árbol), plus the addition of sugar, cocoa butter and lecithin, according to the proposed treatments; the conching process was controlled

where the paste is refined and undesirable flavors are eliminated, giving it the product's own characteristics.

The untrained judges, through statistical analysis, reported that the best organoleptic characteristics in brightness, color, aroma, flavor and texture were found in treatment 1 containing 70% cocoa liquor; that is, the Super Tree variety influences the characteristics of the CCN51 variety; unlike the trained judges, no significant difference was found between treatments.

According to the formulation, the best treatment for bitter type chocolate couvertureis within the parameters, both physical-chemical and microbiological according to NTE INEN 621:2010, concluding its feasibility in the production line at the Saquifrancia farm.

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Conflict of interest
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