SUBSTANTIAL EQUIVALENCE APPLICATION FOR THE APPROVAL OF GUAYUSA LEAF TEA (*ILEX GUAYUSA* LOES.) FOR USE AS A DRY LEAF INFUSION

6 March 2017

Contact:
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Board Chairman
IKIAM EP

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1. Summary

Universidad Regional Amazónica IKIAM (IKIAM University) from Ecuador leads a consortium of guayusa growers, processors, and exporters located in Napo, Ecuador as well as food product importers and distributors in the UK and Europe. IKIAM EP is the commercial entity of IKIAM University.

IKIAM EP and the applicants outlined under "2. Administrative Information" hereby seek to obtain a scientific evaluation of the substantial equivalence of guayusa leaf tea (*Ilex guayusa* Loes.) with yerba mate (*Ilex paraguariensis* A. St. Hil.). Guayusa is a novel food, as interpreted by EU legislation, yerba mate has been an established product within EU consumer markets since before 1997. Scientific evaluation is sought for the approval of guayusa leaf tea for sale in European Union countries.

The production process for guayusa tea has been standardized for all the applicants and one of the consortium members (Jumandipro S.A.) has already been granted organic certifications by Quality Certification Services (QCS), compliant with organic standards for USA, Canada, and the EU. This organic certification refers to the group of growers, processing, and exports, so that the final product can be considered 100% organic. It is expected that any other member of the group may obtain this organic certification as well, if required by the buyer.

Guayusa tea products have been available commercially and consumed in the United States and elsewhere worldwide over the last 8 years, as a major industry has developed centered around its production. Yerba mate (*Ilex paraguariensis* A. St. Hil.) has been widely used in the UK and further in the European market for tea preparations, in the following presentations:

- Loose leaf tea
- Tea boxes with small teabags

Main composition, nutritional values, metabolism, intended use as well as level of undesirable substances are equivalent in both *Ilex* plants. Since guayusa has not been consumed to a significant degree in the EU before 1997, but being a "sister leaf" to yerba mate, an evaluation of substantial equivalence is sought between guayusa leaf tea and yerba mate teas available widely on the global market.

This dossier follows the "*ACNFP guidelines for the presentation of data to demonstrate substantial equivalence between a novel food or food ingredient and an existing counterpart*" and provides scientific evidence to support all claims that guayusa leaf tea is substantially equivalent to yerba mate teas.

2. Administrative Information

2.1. Applicants:

IKIAM EP and the group of applicants outlined below have the purpose of selling guayusa in Europe.

- **#1 IKIAM EP**

Km. 3 Vía Muyuna
Tena, 150150
Napo, Ecuador
Phone: +593 (0)6 370 0040

IKIAM EP is a public enterprise incorporated in the Republic of Ecuador. It is the commercial arm of IKIAM University and is responsible for managing commercial activity associated with IKIAM and organizations that work in partnership with IKIAM. IKIAM University is leading a consortium of guayusa growers and producers with a view to sponsoring guayusa industry participants to build ethical international export value chains for Guayusa.

- **#2 Asociación Kallari**

Barrio San Jorge Huachiyacu y s/n, vía al pano
Tena, 150150
Napo, Ecuador

- **#3 Asociación Agro Artesanal Wiñak**

Barrio San Agustín, vía a Balneario Sinchi Sacha
Archidona, 150350
Napo, Ecuador

- **#4 Zazaguayusa S.A.**

Sn Y Rayuquidy
Tena, 150150
Napo, Ecuador

- **#5 Jumandipro S.A. (trading as "Waykana" brand)**

Troncal Amazónica vía Archidona, Sector Chimbadero
Tena, 150150
Napo, Ecuador

- **#6 Kallari Futuro Gmbh**

Melanchthonstr. 30
Tübingen D-72074
Germany

- **#7 Quito Berlin Gmbh**

Prinzenstr. 85c,
Berlin, D-10969
Germany

- **#8 JoyFlor SRL**

Via Varese, n. 8
San Giuliano Milanese, 20098 (MI)
Italy

- **#9 Sebastian Jörg, Peter Friendank**

Gustav-Müller-Str. 3310829
Berlin, Germany

- **#10 Asoprojuk**

Napo, Ecuador

2.2. Contact:

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Address: Km 8 Vía Muyuna, Tena, Ecuador

2.3. Name of novel food ingredient:

Guayusa tea leaves (*Ilex guayusa* Loes.)

2.4. Date of application:

6 March 2017

3. Composition

3.1. Information on source and comparator organisms

Guayusa (*Ilex guayusa* Loes.) is a tropical tree native to the Northwestern Amazon region across Ecuador, Colombia, and Peru. Ilex guayusa is a species of holly, as are all species of the *Ilex* genus within the *Aquifoliaceae* family. Guayusa tea leaves are dried leaves harvested from guayusa shrubs or small trees, and they are valued traditionally by native peoples as a naturally caffeinated beverage for daily use, akin to the drinking of green, black, and white teas (*Camellia sinensis*) or coffee (*Coffea arabica*). Guayusa tea and its close relative yerba mate tea (*Ilex paraguariensis* A. St.-Hil.) are both consumed as beverages for their caffeine and antioxidant content. Both species have a long historical record of traditional usage as beverages drunk daily by native peoples in South America. Consumption is widespread across many South American countries, in modern times and also historically for hundreds or thousands of years. Beyond the primary nutritional equivalency of yerba mate tea, yerba mate is justified as a comparator product for guayusa tea for the following reasons:

- taxonomically, as both occur within the same *Ilex* genus;
- ethnographically, as both teas are consumed as beverages to harness their equivalent stimulant and antioxidant properties; and
- chemically, as recent chemical evaluations have shown that the functional equivalency between guayusa and yerba mate teas is founded on their chemical similarity as sources of bioactive compounds including flavonoids, phenolics, carotenoids, chlorogenic acids, and related caffeoylquinic acid derivatives (Burris et al., 2012; García-Ruiz et al., 2017, Heck & Mejia 2007; Viera et al., 2010), which substantiate their equivalence beyond the primary nutritional components, vitamins, and minerals.

Yerba mate has been consumed broadly in the European Union before 1997. It was historically introduced to Europe during the Spanish exploration of South America along with Coffee. Its consumption in Europe was revitalised at the turn of the 20th century as a low cost alternative to coffee among European troops. From 1936 – 1938, Argentinian export records show that approximately 20 metrics tons of Yerba mate were being exported to France, Germany, Spain, and the United Kingdom. These European imports decreased during the years of the Second World War, but, yerba mate in modern times continues to be imported to Europe, with Spain and Germany being key European markets from 1996 – 2006 (Folch, 2010). For this reason it is not classified as a novel food according to the EU Novel Food legislation. Specifically, yerba mate is not listed as a novel food or ingredient in the EU Novel Food Catalogue found in the following official website:
http://ec.europa.eu/food/safety/novel_food/catalogue/search/public/index.cfm

3.2. History of use

In Ecuador, guayusa leaf tea has been consumed for a very long time by native peoples of the Amazon. According to Victor Manuel Patiño's study of guayusa (Patiño, 1968), early Jesuit missionaries in the region used and commercialized the production of guayusa in Ecuador. The value of guayusa was well-documented and known by Westerners as to have been cultivated throughout the region and traded over large distances. The Jesuits had cultivated and brought dried guayusa from the lowland Amazon to the highlands of Quito where they started to commercialize it, but unfortunately once the Jesuit missions were expelled from the Amazon during 1766-1768, this halted the commercial production of guayusa, delaying the reintroduction and utilization of guayusa leaf tea in the Andean Highlands of Ecuador.

Yerba mate has an equivalent long historical record of hundreds of years for utilization as a caffeinated tea by natives of South America in Paraguay, Uruguay, Argentina, and southern Brazil. Similarly, yerba mate was commercialized by Jesuit missionaries in the same manner that they used to drink and commercialize the production of yerba mate (*Ilex paraguariensis* A. St. -Hil.) tea in Paraguay. Over the last few decades, yerba mate has seen a revival of use leading to large-scale worldwide commercialization, concomitant scientific research interest on its health properties.

Though guayusa has a long history of traditional usage as part of the cultural heritage of native peoples in Ecuador and was known and esteemed by early explorers in the region, its commercialization has been delayed due to several historical influences and has been awaiting revival until the present day. Nevertheless, the natives of the northwest Amazon have maintained their traditional knowledge and utilized guayusa as part of their daily routine. A figure obtained from Patiño's published document shows the reports made by explorers on the native distribution of guayusa in the northwest Amazon over time in comparison to yerba mate native and cultivated areas:

Figure 1 – Native distribution of guayusa in the northwest Amazon over time in comparison to yerba mate native and cultivated areas

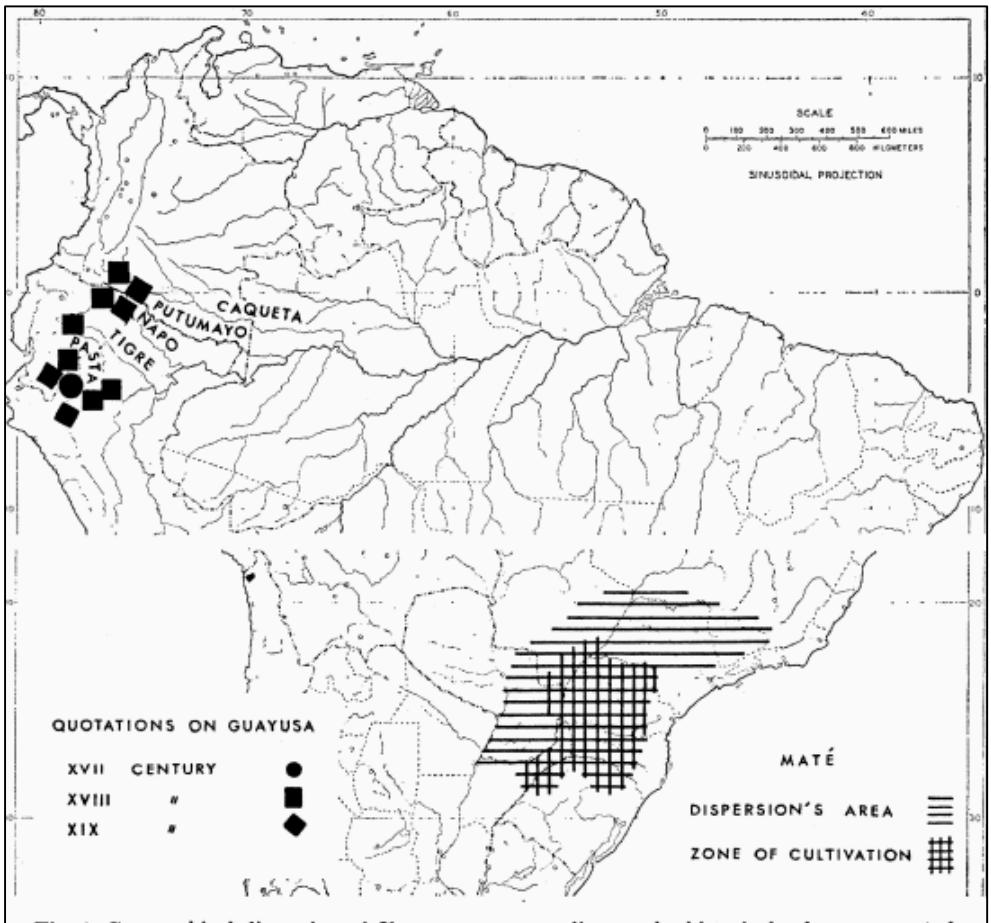

Fig. 1. Geographical dispersion of *Ilex guayusa*, according to the historical references quoted in the text. For comparison, see the distribution of mate (*I. paraguariensis*), both as spontaneous and cultivated plant.

Source and elaboration: Patiño (1968)

Patiño made a reference to the Indian Jívaros of the region, saying that they were people with good physical condition who consume guayusa tea regularly to stay awake and alert.

The description of the preparation of guayusa is performed in the same manner as a standard hot water tea infusion and was deemed to have a pleasant taste.

Currently, guayusa is exported internationally from the Napo region of Ecuador by its major commercial producer called Runa. According to Bloomberg Businessweek, Runa began to commercialize guayusa in the United States market more than eight years ago. Runa sales in 2015 were reported at USD 6 million and in 2016 expected sales were of around USD 10 million. Though Runa began their production in Ecuador, the commercialization of guayusa actually began more than forty years ago in the country, and nowadays can be found in all supermarkets of the region. In the United States, guayusa tea products can be found in more than 8,000 stores from "Wholefoods" to "Safeway", and even on major Internet sites including Amazon.com. Currently, supermarkets in Europe, especially in the United Kingdom and Germany, have shown interest in commercializing the product once import authorization has been obtained.

3.3. Production process

Yerba mate and guayusa teas are both made from dried and crushed leaves, originating from trees/bushes having similar characteristics. This results in equivalent production process chains for guayusa and yerba mate tea.

The production process chain is made up of several phases of production consisting of sowing, harvesting, and post-harvesting. In sowing and harvesting, the individual farmer works through the "chacra" or "chakra" system consisting of localized agroforestry family units. The farmers replicate and maintain the natural environment by cultivating a mix of trees and annual crops alongside the guayusa production. The same techniques that are used to reproduce yerba mate (*Ilex paraguariensis*) are also used in the planting of new guayusa plants. Farmers reproduce guayusa by vegetative propagation beginning with the transplantation of small plant cuttings into holes in the earth, controlling for ambient light, and providing mechanical weed control when necessary. Before harvesting, it is necessary to verify that the plant complies with several standards regarding the plant's full growth and maturity, and once harvested it is immediately transported to a processing plant for dehydration. When the leaves arrive at the processing plant, an inspection is carried out in order to verify compliance with the quality characteristics required of the suppliers. At this point, the product is verified and materials undergo a pre-selection stage, whereby only the highest quality leaves harvested are kept for use in the production process.

Several quality control measures are then followed to ensure a high quality of the product. After initial inspection verifying compliance of harvested product, samples of guayusa leaf material are inspected for their organoleptic properties, including color, smell, and visual state of the fresh leaf. At this stage the preparations mentioned for guayusa are similar with those for yerba mate, though some processing steps may be modified based on the manufacturer and desired final product. Leaf material is then dried using commercially available ovens/dehydrators and a production manager monitors and records the oven temperature to ensure adequate dehydration. Once dried, the leaf material is minced and ground to a uniform size in a clean commercial plant tissue grinder, and tested for further quality control. Samples are obtained and kept throughout the process, and the final products are sent to external accredited laboratories for analytical testing. A relevant sample of all batches is kept for traceability. To carry out better physicochemical and microbiological control of the final product the following analyses are then carried out in an external laboratory: microbiological tests for determination of total aerobic bacteria, *Escherichia coli*, enterobacteria, *Clostridium*, *Salmonella*, *Shigella*, and moulds, physical-chemical tests for determination of moisture and ash content, contaminant testing for detection of arsenic, lead, and pesticide testing of organochlorines and phosphorochlorates. Such testing follows the

guidelines set according to the Ecuadorian Technical Norm INEN 2 392: 2007 First Edition. "Aromatic herbs. Requirements", and to national and international organic regulations.

For guayusa suppliers listed in this dossier, an annual visit is carried out by accredited laboratories to undertake sampling and microbiological analyses of surfaces in direct contact with processed guayusa, analysing materials both live and inert. Regular tests are performed on the following: hands of operators, working tables, and other machinery and equipment according to its usage and operation. Product traceability management is carried out according to the "Product Identification, Status and Traceability Procedure" in order to verify and document the traceability of all sourced guayusa products. With regards to allergens, no other species, raw materials, adjuvants, or allergenic substances are involved in the guayusa tea processing process and thus it is a product free of other materials since only the dehydration of the guayusa leaves is carried out during processing. The processing of yerba mate follows the same principles and key steps as described here for guayusa. Yerba mate is harvested, dried, ground, and packaged in an equivalent set of processing steps. The similarity between the processes is driven by the physical similarities of the source material, the similarities of the final products, and the fact that these processing steps are very simple.

3.4. Dietary composition

Detailed laboratory analysis has been carried out using five batches of guayusa leaf tea by an accredited European lab complying with the ISO/IEC 17025:2005 standard. All five guayusa samples were sourced from the Napo region of Ecuador. To permit a within-test comparison, yerba mate tea was obtained and included in the laboratory testing with the following manufacturing product details and batch number:

- Taragui Yerba Mate, Pure Leaf Tea, Net Weight 1.1 lb
- Product of Argentina
- Lot Number: 13116 17:01 7 3312

Accurate and relevant compositional analysis of a food ingredient for consumption as a typical cup of tea (scientifically described as an aqueous extraction in warm water) is very challenging:

a) It is possible to use a standard method to brew a cup of tea and then analyse the resulting aqueous extraction. However, it is not possible to guarantee that consumers will brew a cup of tea in the same way, resulting in inaccuracy and possible under-reporting of undesirable contaminants, nutritional value, and under-estimation of differences between guayusa and yerba mate samples.
b) It is also possible to test raw dried leaves as sold in consumer outlets. However, this dossier is not applying for approval of the consumption of raw tea leaves, nor is it even remotely likely that a consumer would choose to eat raw tea leaves. This would result in a gross overstatement of any nutritive value and undesirable components, and gross overstatement of any absolute differences between guayusa and yerba mate.

In this dossier, for complete transparency and greatest relevance, two sets of values are presented:

In tables labelled ‘X-a’, dry leaf values for guayusa and yerba mate are presented in units/100g of dry leaf. This table represents reproducible values irrespective of how a consumer might brew a cup of tea. However, values are grossly overstated, not representing the values that a consumer would consume when drinking a cup of tea.

In tables labelled ‘X-b’, secondary values are calculated for a 1.5g tea bag dissolved in 200ml of water. This table assumes complete dissolution of the dry tea leaves in the water. While it is not possible in reality to totally dissolve tea leaves in water, this analysis represents an absolute most extreme scenario for consumption of undesirable contaminants. Values in Tables ‘X-b’ are all presented as ‘less than’ values to accurately reflect the reality that tea leaves are only ever partially dissolved. Values in ‘X-b’ tables are presented in weight/100ml of aqueous solution.

Table 1-a - Comparative Dietary Composition of Guayusa & Yerba Mate Teas (dry leaf only)

Nutritional Content (units / 100g)	**Guayusa (range)**	**Guayusa (Average)**	**Yerba Mate**	**Units**
Energy	299 < 320	313.60	294	kcal/ 100g
Energy	1239 < 1332	1303.40	1229	kJ/ 100g
Total Fat	7.8 < 8.3	7.96	2.7	g/ 100g
Saturated Fatty Acids	3.15 < 3.72	3.36	1.83	g/ 100g
Monounsaturated F. Acids	0.75 < 0.97	0.81	0.46	g/ 100g
Polyunsaturated F. Acids	3.22 < 3.57	3.44	0.25	g/ 100g
Sodium	3 < 4.19	3.90	<3	mg/ 100g
Total Carbohydrate	61.1 < 65.1	64.10	71.7	g/ 100g
Total Dietary Fibre	34.3 < 38.8	36.98	36.5	g/ 100g
Available Carbohydrate	22.3 < 30.5	27.12	35.2	g/ 100g
Total Sugar	3.4 < 5.3	4.84	4	g/ 100g
Protein	14.7 < 15.1	14.92	14.1	g/ 100g
Moisture	4.7 < 6.8	5.40	5.1	g/ 100g
Ash	7.2 < 9.5	7.72	6.4	g/ 100g

*Certificates of analysis provided in Appendix 1

Table 1b - Comparative Dietary Composition of Guayusa & Yerba Mate Teas (estimated for a standard 1.5g tea bag in a 200ml cup of water)

Nutritional Content	**Guayusa**	**Yerba Mate**	**Difference**	**Units**
Energy	<2.36	<2.21	<0.15%	kcal/ 100ml

Energy	<9.77	<9.22	<0.56%	kJ/ 100 ml
Total Fat	<0.06	<0.02	<0.04%	g/ 100 ml
Saturated Fatty Acids	<0.03	<0.01	<0.02%	g/ 100 ml
Monounsaturated F. Acids	<0.006	<0.003	<0.003%	g/ 100 ml
Polyunsaturated F. Acids	<0.03	<0.01	<0.02%	g/ 100 ml
Sodium	<0.02	<0.02	-	mg/ 100 ml
Total Carbohydrate	<0.48	<0.53	<0.05%	g/ 100 ml
Total Dietary Fibre	<0.28	<0.27	<0.01%	g/ 100 ml
Available Carbohydrate	<0.20	<0.26	<0.6%	g/ 100 ml
Total Sugar	<0.04	<0.03	<0.01%	g/ 100 ml
Protein	<0.11	<0.11	-	g/ 100 ml
Moisture	<0.04	<0.04	-	g/ 100 ml
Ash	<0.06	<0.05	<0.01%	g/ 100 ml

3.5. Compositional Analysis Conclusions

Energy: While the calorific value of different foods varies by orders of magnitude, the tested values of dry yerba mate and guayusa samples, 313.6 kcal/100g and 294 kcal/100g respectively, result in a less than 0.15% difference in a 200ml tea cup. These results show that both yerba mate and guayusa leaves, when consumed as an aqueous extraction in warm water (i.e. a cup of tea), both have an exceptionally low energy value and have strong equivalency.

Fat: The tested values for fat (total, saturated, monounsaturated, and polyunsaturated) of yerba mate and guayusa samples (as dry leaves) are all lower than 7.96 g/100g, classifying both guayusa and yerba mate as low fat products. When consumed as a cup of tea, these values result in a less than 0.003% – 0.04% difference. These results show that guayusa and yerba mate have equivalency, with negligible difference in all fats.

Sodium: At 3mg sodium /100g of dry leaves, both guayusa and yerba mate samples have negligible sodium content, raw or in aqueous form.

Carbohydrates: Total carbohydrates comprise 64.1% and 71.1% of total dry weight for both guayusa and yerba mate, characterizing these products equivalently as being primarily composed of carbohydrates. Differences in total carbohydrate, total dietary fibre, and available carbohydrates are negligible between guayusa and yerba mate, all estimated below 0.6% when consumed as a cup of tea.

Total sugar, protein, moisture, and ash: With differences below 0.01% for guayusa and yerba mate when consumed as a cup of tea, and with total concentrations below 0.11g/100ml, these tests show that both guayusa and yerba mate samples have equivalent and negligible sugar, protein, moisture, and ash values when prepared as a 200ml cup of tea.

In conclusion: based on the equivalency of results shown in Table 1a, and due to the diluting factor of 200ml of aqueous solution for a standard cup of tea, shown in Table 1b, the differences in the composition of guayusa tea and yerba mate tea are all below 0.56% of weight per 200ml cup of tea, and for many components, the difference is much less, or there is no difference. For this reason, we conclude that the dietary composition of yerba mate and guayusa is equivalent.

4. Nutritional value and metabolism

4.1. Bioactive and nutritional compound analysis

Caffeine Content:

Table 2a - Caffeine Content
(dry leaf only)

	Guayusa (range)	Guayusa (Average)	Yerba Mate
Caffeine (mg / 100 g)	1860 < 1940	1902	1540

*Certificates of analysis provided in Appendix 1

Table 2b - Caffeine Content
(estimated for a standard 1.5g tea bag in a 200ml cup of water)

	Guayusa	Yerba mate	Difference
Caffeine (mg / 100 ml)	14.27	11.55	2.72%

Caffeine content conclusions:

A principal driver for commercial interest in both guayusa and yerba mate teas, is the stimulant effect of caffeine and other methylxanthines (purine alkaloids) present in their leaf and stem tissues. Samples of guayusa and yerba mate were submitted for analysis and quantification of caffeine content. The range for caffeine content in guayusa reflected values previously reported in research literature (Kapp et al., 2016). Caffeine content for yerba mate was also within previously accepted ranges (Bastos, 2005). The results presented in this

dossier and from previous studies, demonstrate that caffeine content in guayusa and yerba mate vary by a small 2.72%.

Most importantly, the caffeine content of guayusa and yerba mate (as dry leaves) are both almost an order of magnitude below the 200mg single dose the European Food Safety Authority's Panel on Dietetic Products, Nutrition, and Allergies considers to be an upper limit for caffeine concentrations that "do not raise concern" (EFSA, 2015). Furthermore, a 200ml cup of yerba mate or guayusa tea would have a caffeine range of 28mg per cup. This caffeine content sits well below an average 95mg expected from a 237ml cup of coffee. The USDA National Nutrient Database reports that an 8-ounce (237ml) cup of "coffee brewed from grounds" contains 95mg caffeine.

In conclusion: These results demonstrate that there is equivalency for caffeine content between guayusa and yerba mate. Equivalency holds with respect to the average value, and exists almost an order of magnitude below the acceptable EFSA safe limit and well below the caffeine content of a cup of coffee.

Amino Acid Content

Table 3a - Total Amino Acid Content (dry leaf only)

Total Amino Acids (g /100 g)	Guayusa (range)	Guayusa (average)	Yerba Mate
Alanine	0.56 - 0.61	0.60	0.55
Arginine	0.53 - 0.58	0.56	0.50
Aspartic Acid	1.08 - 1.10	1.09	0.98
Cystine	0.09 - 0.11	0.10	0.09
Glutamic Acid	1.15 - 1.24	1.21	1.08
Glycine	0.50 - 0.54	0.53	0.50
Histidine	0.20 - 0.22	0.22	0.19
Isoleucine	0.46 - 0.50	0.48	0.41
Leucine	0.81 - 0.89	0.86	0.79
Lysine	0.63 - 0.64	0.64	0.50
Methionine	0.19 - 0.22	0.21	0.19
Phenylalanine	0.52 - 0.57	0.55	0.49
Proline	0.51 - 0.54	0.53	0.49
Serine	0.51 - 0.55	0.54	0.50
Threonine	0.49 - 0.52	0.51	0.47
Tyrosine	0.36 - 0.39	0.38	0.34
Valine	0.59 - 0.64	0.62	0.54

*Certificates of analysis provided in Appendix 1

Table 3b - Total Amino Acid Content
(estimated for a standard 1.5g tea bag in a 200ml cup of water)

Total Amino Acids (mg /100 g)	Guayusa	Yerba Mate	Difference
Alanine	<4.485	<4.125	0.35%
Arginine	<4.215	<3.75	0.47%
Aspartic Acid	<8.16	<7.35	0.81%
Cystine	<0.75	<0.675	0.08%
Glutamic Acid	<9.09	<8.1	0.99%
Glycine	<3.975	<3.75	0.23%
Histidine	<1.62	<1.425	0.20%
Isoleucine	<3.615	<3.075	0.54%
Leucine	<6.48	<5.925	0.56%
Lysine	<4.77	<3.75	1.02%
Methionine	<1.575	<1.425	0.15%
Phenylalanine	<4.155	<3.675	0.48%
Proline	<4.005	<3.675	0.33%
Serine	<4.035	<3.75	0.29%
Threonine	<3.84	<3.525	0.32%
Tyrosine	<2.835	<2.55	0.29%
Valine	<4.62	<4.05	0.57%

Amino acid content conclusions:

In order to assess the content of other bioactive compounds of interest to health, samples of guayusa and yerba mate were submitted for laboratory testing and assayed for their amino acid content. Laboratory analysis was performed to identify 17 free amino acids in guayusa and yerba mate commercial leaf teas. Analysis revealed that guayusa and yerba mate teas are equivalent in regards to their total and free amino acid content.

In conclusion: Guayusa and yerba mate teas are equivalent in regards to their total and free amino acid content.

Mineral Content

Table 4a - Mineral Content
(dry leaf only)

Mineral Content (ug/g)	Guayusa (range)	Guayusa (average)	Yerba Mate
Aluminium	286 < 311	298	453
Antimony	1.0	1.0	<1.0
Barium	77.7 < 87.4	82.1	78.3
Beryllium	0.05	0.05	0.065
Bismuth	2.0	2.0	<2.0
Boron	21 < 22.6	21.88	77.9
Cobalt	0.5	<0.5	<0.50
Copper	8.74 < 9.18	8.68	9.44
Iron	29.5 < 32.1	31.2	184
Lithium	0.50	0.50	<0.50
Magnesium	5800 < 6140	6030	6580
Manganese	813 < 879	847.75	2070
Molybdenum	1.97 < 3.63	2.33	<0.50
Nickel	1.89 < 2.07	2.0	5.40
Phosphorous	3110 < 3340	3250	1370
Potassium	15800 < 16800	16200	15300
Selenium	5.0	5.0	<5.0
Silver	0.5	0.50	<0.50
Sodium	24.1 < 34.5	27.1	19.7
Strontium	105 < 111	108.25	33.8
Tin	2.0	2.0	<2.0
Titanium	0.29 < 0.31	0.30	4.4
Zinc	94.8 < 95.2	94.95	54.3

*Certificates of analysis provided in Appendix 1

Table 4b - Mineral Content
(estimated for a standard 1.5g tea bag in a 200ml cup of water)

Mineral Content (ug/g)	Guayusa (range)	Guayusa (average)	Yerba Mate
Aluminium	286 < 311	298	453
Antimony	1.0	1.0	<1.0
Barium	77.7 < 87.4	82.1	78.3
Beryllium	0.05	0.05	0.065
Bismuth	2.0	2.0	<2.0

Boron	21 < 22.6	21.88	77.9
Cobalt	0.5	<0.5	<0.50
Copper	8.74 < 9.18	8.68	9.44
Iron	29.5 < 32.1	31.2	184
Lithium	0.50	0.50	<0.50
Magnesium	5800 < 6140	6030	6580
Manganese	813 < 879	847.75	2070
Molybdenum	1.97 < 3.63	2.33	<0.50
Nickel	1.89 < 2.07	2.0	5.40
Phosphorous	3110 < 3340	3250	1370
Potassium	15800 < 16800	16200	15300
Selenium	5.0	5.0	<5.0
Silver	0.5	0.50	<0.50
Sodium	24.1 < 34.5	27.1	19.7
Strontium	105 < 111	108.25	33.8
Tin	2.0	2.0	<2.0
Titanium	0.29 < 0.31	0.30	4.4
Zinc	94.8 < 95.2	94.95	54.3

Mineral content conclusions:

Samples of guayusa and yerba mate leaf teas were submitted for analysis of mineral content, quantifying the presence of metals and inorganics in tea samples (excluding undesirable heavy metals). Magnesium and Potassium both tested within the mg/g range, with all other metals and minerals within the ug/g range. This range equivalency across every metal and mineral tested is consistent with the overall equivalency of guayusa and yerba mate.

In conclusion: Equivalency across every mineral tested is consistent with the overall equivalency of guayusa and yerba mate

4.2. Metabolism studies

In addition to the quantitative analytical laboratory data presented in this report which demonstrate the nutritional value and bioactive compounds in manufactured guayusa, we report and review on several metabolism studies which have been performed to assess the safety of consuming guayusa leaf tea and products. These scientific reports provide several independent sources of relevant information including previous quantitative analytical results on the chemistry of guayusa tea, and biomedical results performed in both *in vitro* and *in vivo* studies, and important anecdotal evidence for the general safety of daily consumption by native peoples in the Ecuadorian Amazon.

One of the earliest pharmacological studies documented the ethnobotanical use of guayusa in preparing a concentrated water extract made from a boiling water decoction of guayusa leaves which was used as a medicinal preparation (Lewis et al., 1991). The product thus prepared was intended for ritualistic stimulant effects at daybreak by Amazonian Achuar Jívaros. The presence of caffeine and other methylxanthines (theobromine & theophylline) were detected and levels quantified by HPLC-UV analysis in leaf and blood samples. A concentration of caffeine between 1.5-3.5% was reported for guayusa leaves collected and used by Achuar Jívaros in their study.

No other alkaloids were detected in their phytochemical analysis, that analysis confirmingthe absence of the psychoactive alkaloid, cocaine. It is important to clarify that *Ilex Guayusa*, within the *Ilex* genus, within the *Aquifoliacieae* family, is completely unrelated to any Coca plants within the far removed *Erythroxylaceae* family. Guayusa has never been consumed traditionally for stimulant properties other than the stimulant properties of caffeine.

The researchers obtained blood samples from the participating men during ritualistic consumption of these concentrated tea extracts, and following analytical testing concluded that caffeine is the compound responsible for immediate biological activity in guayusa (Lewis et al., 1991). As the Achuar Jívaros were largely consuming these concentrated guayusa tea extracts on a daily basis their common use of this plant provides preliminary anecdotal and analytical evidence for the safety of guayusa as a traditionally consumed plant in the northwest Amazon[1].

Recent investigations on the toxicology of a standardized preparation of guayusa tea extract liquid concentrates found no toxicity when subjected to *in vitro* genotoxicity assays by Bacterial Reverse Mutation Test (Ames Test) and a Chromosome Aberration Assay. Performing a 90-day *in vivo* study with male and female rats provided with various levels of guayusa concentrates failed to find incremental toxicity, and thus concluded that there are no harmful effects based on consumption of the guayusa leaf extract (Kapp et al., 2016)[2].

A recent study explored the effects of guayusa leaf extract on human health as compared with a green coffee extract and a synthetic caffeine control. The experiment made use of a double-blind clinical trial to assess the effect on overall safety, bodily absorption, and influence on endogenous neurotransmitters. All treatments contained the same content of caffeine (200mg) which was intended to test and approximate the effect of caffeine of an average daily dose in the United States. No differences were seen between the effects on heart rate or blood pressure due to the guayusa and coffee extracts as compared with the synthetic caffeine control. No significant difference was noted for absorption of caffeine between the natural plant caffeine sources (guayusa and green coffee) and the synthetic control. Monitoring neurotransmitter levels (serotonin, dopamine, norepinephrine, or GABA) after consumption showed no difference or change from baseline levels after treatments. While epinephrine and glutamate levels increased after consumption of green coffee extract and control, such a change was not observed for the guayusa leaf extract, potentially implying a different mode of activity in regard to epinephrine release for the guayusa than with green coffee extract and synthetic caffeine control. Based on their evidence, the authors

[1] See further details in: Lewis, W. H., Kennelly, E. J., Bass, G. N., Wedner, H. J., & Elvin-Lewis, M. P. (1991). Ritualistic use of the holly *Ilex guayusa* by Amazonian Jivaro Indians. Journal of Ethnopharmacology, 33(1-2), 25-30.

[2] See further details in: Kapp Jr, R. W., Mendes, O., Roy, S., McQuate, R. S., & Kraska, R. (2016). General and genetic toxicology of guayusa concentrate (*Ilex guayusa*). International Journal of Toxicology, 35(2), 222-242.

concluded that there were no safety concerns at dosages of guayusa leaf extract containing up to 200mg caffeine[3].

5. Intended use

This application for substantial equivalence is presented with the intention of marketing guayusa for use by consumers, in exactly the same way that the very common green, white, or black teas (*Camellia sinensis*), or herbal teas such as yerba mate (*Ilex paraguariensis*) are marketed. Stated simply, consumers will use guayusa to make a cup of tea as consumed commonly worldwide. More specifically, consumers will make a hot aqueous extraction of a standard 1.5g of guayusa tea leaves in a standard 200ml volume of water. As for tea and herbal teas, the intended commercial guayusa products for sale in Europe will take the form of:

1) Loose-leaf tea.
2) Leaf tea within 1.5 – 2.0g tea bags.

6. Undesirable substances

6.1. Chemical Contaminants & Heavy Metals

The European Union and its member states do not list undesirable levels of metals and heavy metals (mercury, lead, cadmium, tin, and arsenic) in tea or herbal tea products, undesirable levels for these compounds are listed for other classes of food such as cereals and fish products. Specifically, limits for metals and heavy metals in teas are not provided in the relevant legislation: Commission Regulation (EC) No 1881/2006, nor the COMMISSION REGULATION (EC) No 629/2008.

For this reason, it is not possible to test for levels of heavy metals against 'acceptable' levels. One reason for this regulatory freeness is that dried leaf products (such as tea and herbal teas) are not at risk of common routes of contamination, such as bioaccumulation of mercury in fish, bioaccumulation of heavy metals and toxins in plant seeds (i.e. cereal products), or leaching of tin into canned products from cans with tin or other metal linings. Another reason for regulatory freeness is that teas are sold for use in the preparation of cups of tea. Specifically, a cup of tea is an aqueous extraction in hot water. The extraction efficiency of metals and heavy metals through this method is very low resulting in a minimal possibility of consumption of contaminants, even if dried leaf tea was to be contaminated.

In this dossier we show levels of metals and heavy metals in guayusa, in comparison with the test results of Infoods Ltd Chia, reported to the UK FSA in their successful application authorisation as a novel food (InFoods Ltd, 2012), and in comparison with our test results for yerba mate.

For clarification: Tin was not tested. According to the FSA of Ireland, "*The principal concern in relation to tin in food is the possibility of high levels potentially present in canned food in incorrectly manufactured tins, where tin present in the can has leached into the food.*" (FSA of Ireland, 2009). There is risk also associated with seafood due to bioaccumulation of environmental organotin pollutants. Neither of these sources of tin are relevant for guayusa tea leaves.

[3] See further details in: Krieger, D. R., Kalman, D. S., Feldman, S., Arnillas, L., Goldberg, D., Gisbert, O., & Nader, S. (2016). The Safety, Pharmacokinetics, and Nervous System Effects of Two Natural Sources of Caffeine in Healthy Adult Males. Clinical and Translational Science, 9(5), 246-251.

For clarification: Mercury was not tested. According to the FSA of Ireland (FSA, 2009), "*A recent SCOOP report (EU Scientific Cooperation Task, 2004) on exposure of the European population to heavy metals in their diet showed that mercury is relatively widely distributed in food at very low levels, and primarily in the less toxic inorganic form, but that the most toxic form of mercury, methylmercury, is found at significant levels only in fish and seafood.*" Bioaccumulation of mercury from environmental contamination of seafood is not relevant for guayusa.

Table 5 – Metals and Heavy Metals
(estimated for a standard 1.5g tea bag in a 200ml cup of water)

Metals (mg/kg)	Guayusa	Infoods Ltd Chia	Yerba Mate
Arsenic	<0.0006	<0.1	<0.0003
Cadmium	<0.0003	<0.1	<0.0001
Chromium	<0.0001	Not reported	<0.0003
Lead	<0.0003	<0.1	<0.0003
Thallium	<0.0003	Not reported	<0.0003
Vanadium	<0.0001	Not reported	<0.0002

6.2. Microbiological Content

Results of microbiological testing detected no adverse levels of microbiological contamination, according to safe limits established in "Working Document on Microbial Contaminant Limits for Microbial Pest Control Products, European Commission: Health & Consumer Protection Directorate-General, Directorate E, Safety of the food chain, Unit E.3 - Chemicals, contaminants, pesticides, SANCO/12116/2012-rev.0, September 2012". Equivalent results were reported for guayusa and yerba mate. Positive controls confirmed the effectiveness of the assay.

Equivalency between guayusa and yerba mate for this class of contamination is due to the equivalent dry nature of the products, and the equivalent plant leaf material from which they are sourced.

Table 6 – Microbiological contaminants
(dry leaves only)

Microbiological (CFU/g)	Guayusa	Yerba Mate
Coliforms	<10	<10
Coagulase + *Staphylococcus*	<20	<20
Bacillus cereus	<20	<20
Aerobic colony count	300	50

ß-Glucuronidase + *Escherichia coli*	<10	<10
Salmonella	not detected/25 g	not detected/25 g
Yeasts	<100	<100
Moulds	<100	500

*Certificates of analysis provided in Appendix 1

6.3. Mycotoxins

Samples of guayusa and yerba mate leaf teas were submitted for detection of potential mycotoxins. The results did not detect the presence of any mycotoxins in any samples submitted for analytical testing. Positive controls confirmed the effectiveness of the assay.

Table 7 - Mycotoxins

Mycotoxins (µg/kg)	Guayusa	Yerba Mate
Deoxynivalenol (DON)	<10	<10
Diacetlyoxyscirpenol (DAS)	<10	<10
3-Acetyldeoxynivalenol (3AcDON)	<10	<10
15-Acetyldeoxynivalenol (15AcDON)	<10	<10
Fusarenone X (Fus X)	<10	<10
Nivalenol (NIV)	<10	<10
Nesolaniol (NEO)	<10	<10
T2 Toxin (T2)	<10	<10
HT2 Toxin (HT2)	<10	<10
T2-triol	<10	<10

*Certificates of analysis provided in Appendix 1

6.4. Pesticide Analysis

Screening of guayusa and yerba mate leaf tea samples for pesticides was conducted by targeted analysis using Liquid Chromatography and Gas Chromatography tandem to Mass Spectrometry (LC-MS/MS & GC-MS/MS). Methods for analysis employed Multiple Reaction Monitoring (MRM) of parent and daughter ion transitions of a broad range of common pesticides which are used in the cultivation of plant crops worldwide and specifically targeting pesticides used commonly in the production of green tea (*Camellia sinensis*). Using such targeted approaches, the analytical methods can detect and quantify more than 259 compounds and isomers using LC-MS/MS, and 105 compounds and isomers using GC-MS/MS detection (Tables 9 & 10) which are known for their sensitivity and low limits of quantification, thus enabling detection of compounds at trace levels. As certain pesticides exist as various isomers, several marker compounds were summed together for presentation of data by the laboratory performing the analysis. A detailed list of the compounds, their analytical results, and the limits as set in the United Kingdom can be found in the Certificates of Analysis for the Pesticides Screens located in the Appendix.

The results of targeted LC-MS/MS and GC-MS/MS analysis did not detect any pesticides in any samples of the guayusa leaf tea submitted for laboratory screening. Positive controls

confirmed the effectiveness of the assays. All guayusa tea samples thus met the requirements for being below the Maximum Recovery Level of pesticides in tea samples as standardized for *Camellia sinensis* dried leaves and stalks, fermented or otherwise, in the United Kingdom. The results provide a quantitative measure of the absence of pesticides in the product and support the organic status of guayusa leaf tea as grown and produced under the standards of the current manufacturer and suppliers.

In contrast to the results obtained for guayusa leaf tea, the targeted GC-MS/MS analyses of the yerba mate sample detected the presence of the pyrethroid pesticide, lambda-cyhalothrin, typically used as an insecticide. The quantitative data revealed a content of 0.022mg/kg of lambda-cyhalothrin in the yerba mate tea sample which still falls below the Maximum Recovery Level of this compound in the United Kingdom.

Table 1 - LC-MS/MS Pesticide Screen

LC-MS/MS Pesticides Screen					
Abamectin	Desmedipham	Flubendiamide	Mepronil	Pirimicarb	Terbufos
Acetamiprid	Desmetryn	Flucythrinate	Metaflumizone Metamitron	desmethyl Pirimicarb	Terbufos sulfone
Acrinathrin	Dialifos	Fludioxonil	Metazachlor	Pirimicarb & desmethyl pirim.	Terbufos sulfoxide
Aldicarb, ald. sulfoxide & sulfone	Diclofop-methyl	Flufenacet	Metconazole	Prochloraz	Terbuthylazine
Allethrin	Dicrotophos	Flufenoxuron	Methabenzthiazuron	Profenofos	Terbutryn
Amicarbazone	Diethofencarb	Flumorph	Methacrifos	Promecarb	Tetraconazole
Aminocarb	Diflubenzuron	Fluometuron	Methiocarb, sulfoxide & sulfone	Prometon	Tetramethrin
Atrazine	Diflufenican	Fluopicolide	Methomyl and thiodicarb	Prometryn	Thiacloprid
Azaconazole	Dimethachlor	Fluoxastrobin	Methoprotryne	Propamocarb Hydrochloride	Tetramethrin
Azamethiphos	Dimethenamid	Fluquinconazole	Methoxyfenozide	Propanil	Thiacloprid
Beflubetamid	Dimethoate and Omethoate	Flurochloridone	Metobromuron	Propaquizafop	Thiamethoxam
Bendiocarb	Dimethomorph	Flurtamone	Metolachlor	Propargite	Thiobencarb
Benzoximate	Dimethylvinphos	Flusilazole	Metolcarb	Propazine	Thiophanate-Methyl
Bifenazate	Dimoxystrobin	Flutolanil	Metoxuron	Propetamphos	Tolclofos-methyl
Bifenox	Diniconazole	Flutriafol	Metrafenone	Propham	Triazophos
Bixafen	Dinotefuran	Formetanate hydrochloride	Mevinphos	Propiconazole	Trichlorfon
Boscalid	Dioxacarb	Formothion	Mexacarbate	Propoxur	Tridemorph
Bromacil	Diphenamid	Fosthiazate	Molinate	Propyzamide	Trietazine
Buprofezin	Disulfoton, sulfoxide and sulfone	Furalaxyl	Monolinuron	Prosulfocarb	Trifloxystrobin
Butocarboxim	Diuron	Furathiocarb	Monuron	Prothioconazole-desthio	Triflumizole
Butralin	Emamectin	Guthion	Moxidectin	Pyracarbolid	Triflumuron
Carbaryl	Epoxiconazole	Halofenozide	Napropamide	Pyraclostrobin	Trimethacarb
Carbetamide	Ethidimuron (sulfodiazole)	Haloxyfop-methyl	Nitenpyram	Pyraflufen ethyl	Triticonazole
Carbofuran & 3-OH carbofuran	Ethion	Hexaflumuron	Norflurazon	Pyridaben	Uniconazole
Carbosulfan	Ethofumesate	Hexazinone	Novaluron	Pyrifenox	Vamidothion
Carboxin	Ethoprophos	Hexythiazox	Oxadiazon	Pyriproxifen	Vernolate
Carfentrazone-ethyl Chlorantraniliprole	Etofenprox	Imidacloprid	Oxamyl	Quassin (quassia)	Trifloxystrobin
Chloridazon	Famoxadone	Indoxacarb	Paclobutrazol	Quinalphos	XMC (3,5-xylyl methylcarbamate)
Chlortoluron	Fenamidone	Ipconazole	Paraoxon	Quinoxyfen	Zoxamide
Chlorthiophos	Fenamiphos, fen. sulfone & fen. sulfoxide	Iprobenfos	Paraoxon	Quizalofop ethyl	
Cinidon-ethyl	Fenarimol	Iprovalicarb	methyl	Resmethrin	
Clofentezine	Fenazaquin	Isocarbophos	Pencycuron	Rotenone	
Clomazone	Fenbuconazole	Isofenphos-methyl	Penthiopyrad	Silthiofam	
Clothianidin	Fenchlorphos oxon	Isoprothiolane	Pethoxamid	Simazine	
Coumaphos	Fenhexamid	Isoproturon	Phenmedipham	Spinetoram	
Crufomate	Fenobucarb	Isopyrazam	Phenothrin	Spinosad	
Cyanazine	Fenoxaprop-P-ethyl	Isoxaben	Phenthoate	Spirodiclofen	
Cyazofamid	Fenoxycarb	Isoxaflutole	Phorate, sulfoxide & sulfone	Spiromesifen	
Cycloate	Fenpropathrin	Ivermectin	Phosalone	Spirotetramat	
Cycluron	Fenpropidin	Lenacil	Phosfolan	Spiroxamine	
Cyflufenamid	Fenpyroximate	Linuron	Phosmet	tau-Fluvalinate	
Cymiazole	Fenthion, fenth. sulfone & fenth. sulfoxide	Lufenuron	Phoxim	Tebufenozide	
Cymoxanil	Fenuron	Malathion and malaoxon	Picolinafen	Tebufenpyrad	
DEET (N,N-dimethyl-m-toluamide)	Fipronil	Mandipropamid	Picoxystrobin	Tebupirimfos	
Demeton-S-methyl	Flamprop isopropyl	Mecarbam	Pinoxaden	Tebuthiuron	
Oxydemeton-me and dem-S-me sulfone	Flonicamid	Mephosfolan	Piperonyl Butoxide	Temephos	

*Complete descriptions of identified markers and summation of isomers provided in Certificates of Analysis in Appendix 1.

Table 2 - GC-MS/MS Pesticide Screen

GC/MS-MS Pesticide Screen					
Acephate	Chlorpropham	Difenoconazole	Hexachlorocyclohexane - gamma	Pendimethalin	Tefluthrin
Aldrin	Chlorpyrifos	Dimethoate & Omethoate	Hexachlorocyclohexane - gamma	Permethrin	Terbuthylazine
Atrazine	Chlorpyrifos-methyl	Diphenylamine	Hexaconazole	Phosalone	Terbutryn
Azinphos-ethyl	Chlorthal-dimethyl	Endosulfan	Imazalil	Phosmet	Tetradifon
Azinphos-methyl	Chlorthion	Endrin	Iodofenphos	Phosphamidon	Tolclofos-methyl
Azoxystrobin	Cyanazine	Ethion	Iprodione	Pirimicarb	Tolylfluanid
Benalaxyl	Cyfluthrin	Ethoprophos	Kresoxim-methyl	Pirimiphos-methyl	Triadimefon and Triadimenol
Bifenthrin	Cyhalothrin - lambda	Etridiazole	Malathion	Procymidone	Triazophos
Bromophos	Cypermethrin	Etrimfos	Metalaxyl & metalaxyl-M	Prometryn	Trifluralin
Bromophos-ethyl	Cyproconazole	Fenarimol	Methacrifos	Propachlor	Vinclozolin
Bupirimate	DDT	Fenchlorphos	Methamidophos	Propham	
Buprofezin	Deltamethrin	Fenitrothion	Methidathion	Propiconazole	
Cadusaphos	Desmetryn	Fenvalerate	Methoxychlor	Propyzamide	
Carbofuran	Diazinon	Fluazifop-p-butyl	Metribuzin	Quinalphos	
Carbophenothion	Dichlofluanid	Flucythrinate	Mevinphos	Quintozene & pentachloroaniline	
Carbosulfan	Dichlorvos	Fonofos	Oxadixyl	Simazine	
Chlordane	Dicofol	Heptachlor & heptachlor epoxide	Parathion	Sulfotep	
Chlorfenvinphos	Dieldrin	Heptenophos	Parathion-methyl	Tebuconazole	
Chlorothalonil	Aldrin & Dieldrin	HCB	Penconazole	Tecnazene	

*Complete descriptions of identified markers and summation of isomers provided in Certificates of Analysis in Appendix 1.

6.5. Supporting Evidence of Guayusa as a Traditional Food

Additional supporting evidence is provided which documents the daily and commonplace consumption of guayusa leaf tea which reveals a general regard of safety by native consumers. As guayusa has a long history of commonplace usage such reports document the great respect and esteem of this traditional plant consumed in the northwest Amazon and neighbouring highland regions.

A publication from Saltos et. al. (2016) documents the traditional usage of plants by natives in Pastaza Province of the Ecuadorian Amazon. The aim of the study was to collect relevant information on commonly used species focusing on medicinal plants, and then to categorize them based on their region, use by ethnic groups, and intended usage categories. Considering the 52 species which were documented in the study, *Ilex guayusa* was reported in the highest frequency by natives interviewed and was described as a stimulant plant providing "energizing" qualities. Such anecdotal evidence confirms earlier reports of this plant species as an esteemed plant documenting current viewpoints of guayusa leaf in the Ecuadorian Amazon[4].

Another publication from Schultes (1972) documents the discovery of guayusa samples at a burial site dated to 500 A.D. in the highlands of Bolivia. A phytochemical analysis of the plant material obtained from the field site confirmed the presence of caffeine, and the botanical/taxonomic identification was supported by morphological evidence. This discovery has important implications as it is believed that this plant may have been carried from long distances and brought to the site in the Bolivian highlands. As guayusa is not known to grow at such altitudes it would imply that the plant was highly esteemed as to have been transported with the intent to be traded. In addition, Schultes describes and delimits the biogeographical range of guayusa, estimating from field collections that guayusa was grown in the lowlands from southern Colombia to northern Peru, the main region of cultivation being found in the Ecuadorian Amazon. Based on his preliminary observations and review of the research literature he suggests that guayusa is largely cultivated and is associated with human habitations, not being found growing in a wild state. The document further explains the long history of exploration in the region which recognized the esteem and value of guayusa as a commonly consumed stimulant beverage, citing a variety sources of ethnobotanical reports on guayusa over the last centuries of Amazonian research[5].

[4] See further details in: Saltos, R. V. A., Vásquez, T. E. R., Lazo, J. A., Banguera, D. V., Guayasamín, P. D. R., Vargas, J. K. A., & Peñas, I. V. (2016). The use of medicinal plants by rural populations of the Pastaza province in the Ecuadorian Amazon. Acta Amazonica, 46(4), 355-366.

[5] See further details in: Schultes, R. E. (1972). *Ilex guayusa* from 500 AD to the present. Etnologiska Studier 32, Göteborgs Etnografiska Museum, Göteborg, Sweden, pp. 115-138.

A paper from Patiño (1968) is one of the earliest ethnobotanical reviews of guayusa consumption and utilization in the northwest Amazon. Citing many examples in great detail, Patiño considers guayusa to be a neglected stimulant plant containing caffeine, which awaits rediscovery and commercialization. As mentioned in the document, the Jívaros would make use of daily consumption of guayusa to stay healthy as well as to stay awake and alert. Another anecdote cited mentions the general beauty of the plant and that its flavor was "agreeable, somewhat similar to tea, but finer and more pleasant. " A similar quote mentions that its leaves “are the most valued part of the plant” and "are eagerly sought in various parts of New Granada by those acquainted with its beneficial properties. " Such documentation provides important evidence of the general consumption of guayusa over the last centuries and confirms the broad appreciation for its qualities much like other caffeine-containing species (coffee, green/black tea, chocolate, cola, yerba mate) which are revered worldwide[6].

An interesting report from Sidali et. al. (2016) documents the modern utilization of guayusa by indigenous communities as well as by the local general populace as a tea used commonly at homes and in restaurants. It is important to recognize the daily and common use of guayusa by indigenous communities as they have planted trees in their home gardens so that they can have consistent yearly access to this caffeine-containing tea. This paper makes special emphasis to recognize that guayusa is of great historical and traditional value to native peoples, so much so that it should be considered a major part of their heritage, and recognized as a part of their culture while living in the Amazon and in relation to the Andes. Not only is guayusa described within the context of morning rituals, but it is considered a major symbol and indicator of their native value system and culture. As such, the evidence discussed in this paper supports the use of guayusa as a traditional food of long-term esteem which is recognized as safe by indigenous communities. Because of the utilization of guayusa leaf tea as part of their daily diets, indigenous cultures recognize guayusa as a significant part of their cultural practices and strongly recognize guayusa as a major part of their self-identity[7].

6.6. Stability of Manufactured Tea Product

As the guayusa tea products currently being offered are processed and completely dried upon being manufactured, the stability of the commercial bulk tea and tea bag products facilitates long-term storage without degradation. Based on our previous results, the stability of the dried guayusa leaf tea has been assessed and a sanitary certificate has been registered and provided claiming a minimum shelf-life of 1 year time or longer for guayusa leaf teas at typical room and temperature conditions.

7. Other Relevant Data

7.1. Labelling

Without prejudice to other requirements by EU law concerning the labelling of foodstuffs, the following additional specific labelling requirements shall apply:

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[6] See further details in: Patiño, V. M. (1968). Guayusa, a neglected stimulant from the eastern Andean foothills. Economic Botany, 22(4), 311-316.

[7] See further details in: Sidali, K. L., Morocho, P. Y., & Garrido-Pérez, E. I. (2016). Food Tourism in Indigenous Settings as a Strategy of Sustainable Development: The Case of *Ilex guayusa* Loes. in the Ecuadorian Amazon. Sustainability, 8(10), 967.

(a) The product shall be labelled "guayusa tea".
(b) Recommended preparation of guayusa per individual cup (eight ounces) is of 1,5-2g brewed in hot water for 3-7 minutes.
(c) There shall be an easily visible and legible statement that the product may not be nutritionally appropriate for certain sections of the population (pregnant and breastfeeding women and children under the age of five years), because it contains natural caffeine.
(d) Advice shall be given that the product should be used as part of a healthy diet.

7.2. Quality Certificates

Organic Certification

Several of the producers and farmers already have organic certification of their guayusa product. An example of organic certification is provided in the Appendix to this documents. As can be verified from the attached certificate, the product "guayusa" is already certified organic under European, American, and Canadian regulations.

Sanitary Certificate

As part of a Sanitary Certificate Notification for National Food Products (CERTIFICADO DE NOTIFICACIÓN SANITARIA NO 13447-ALN-0117 DE PRODUCTOS ALIMENTICIOS NACIONALES) provided by the Ministry of Public Health of the Ecuadorian Government, the following document provides support for the stability of guayusa leaf teas, listing a shelf life of one year (365 days) or longer.

7.3. Taxonomy of *Ilex guayusa* and *Ilex paraguarensis*

Common Name:	**Guayusa**
Kingdom:	Plantae
Subkingdom:	Tracheobionta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order:	Aquifoliales
Family:	Aquifoliaceae
Genus:	*Ilex* L.
Species:	*Ilex guayusa* Loes.

Common Name:	**Yerba Mate**
Kingdom:	Plantae
Subkingdom:	Tracheobionta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order:	Aquifoliales
Family:	Aquifoliaceae
Genus:	*Ilex* L.
Species:	*Ilex paraguariensis* A. St. -Hil.

8. Conclusions

The data presented in this dossier confirm the substantial equivalence of guayusa leaf tea (*Ilex guayusa* Loes.) with yerba mate (*Ilex paraguariensis* A. Sti. -Hil.), paying specific attention to the areas of:

- Taxonomic relationship (within genus)

- Gross physical composition (dried leaves only)
- Production process (harvested, dried, and crushed).
- Dietary composition (Energy, fats, carbohydrates, sugar, protein, moisture, and ash)
- Nutritional value (amino acid and mineral composition)
- Caffeine content (natural caffeine)
- Metabolism
- Intended use
- Undesirable substances (metals and heavy metals)
- Undesirable substances (microbiological content and mycotoxins)
- Undesirable substances (pesticides)

in accordance with REGULATION (EC) No 258/97. The proposed commercialization of these two *Ilex* species follows a long history of traditional utilization by indigenous peoples, which hold the plants in esteem for their caffeine content and have revered them as part of their cultural heritage. Guayusa cultivation and consumption has been maintained for millennia as part of the household agricultural system in the northwest Amazon, being planted nearby to homes to enable year-round availability of this domestic crop. The historical record of usage and utility provides long-term evidence demonstrating the general regard of safety by native peoples who have consumed guayusa as a tea as part of their daily diets.

The laboratory analyses performed and reported in this dossier are intended to provide qualitative and quantitative analytical evidence confirming the substantial equivalence of guayusa leaf tea with yerba mate. Samples of guayusa leaf tea were submitted for analytical testing in accredited laboratories in the European Union which demonstrated that guayusa meets the designated safety and health standards regarding undesirable substances, as required by published guidelines. The analyses of nutritional composition, amino acids, minerals, and caffeine content further exemplify the nutritional and functional aspects of consuming guayusa tea and its equivalency to yerba mate tea.

Based on these analytical results for the parameters tested, it has been demonstrated that guayusa and yerba mate are substantially equivalent, that guayusa tea can be regarded as safe for consumption as a tea, and that guayusa is a product fit for public consumption in the European Union.

9. References

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10. Appendices