UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Caracterización nutricional de dos especies de insectos (*Platycoelia lutescens* y *Rhynchophorus palmarum*) consumidas en la Sierra y Amazonía ecuatoriana

.

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RESUMEN

Hoy en día se buscan alternativas sustentables de algunos alimentos, por lo que los insectos se han vuelto atractivos. El conocer más sobre sus posibles aportes beneficiosos a nuestra alimentación, como presencia de fenoles y su capacidad antioxidante, es un paso inicial hacia su uso. En este estudio se hizo una caracterización nutricional para proteínas, grasas y cenizas de *Platycoelia lutescens* y *Rhynchophorus palmarum* basándose en los métodos propuestos por la AOAC (Asociación de Químicos Agrícolas Oficiales) para carnes crudas para facilitar su comparación con fuentes de proteínas tradicionales. También se usó el método Folin-Ciocalteu para fenoles totales y el ensayo FRAP (Ferric Reducing Antioxidant Power) para la capacidad antioxidante, componentes bioactivos no registrados aún en insectos. Se encontraron valores previamente registrados en ambas especies para sus niveles de proteínas (*P. lutescens* ~50%, *R. palmarum* 27% - 44%), grasas (*P. lutescens* ~25%, *R. palmarum* ~50%) y cenizas (*P. lutescens* ~4.5%, *R. palmarum* ~1%), aunque entre muestras no presentan homogeneidad posiblemente debido a factores externos (sustrato o localidad). Los valores obtenidos de fenoles totales y capacidad antioxidante son nuevos, por lo que no se puede comparar con otros insectos, pero indican su gran potencial como alternativas alimenticias a seguir estudiando.

Palabras clave: capacidad antioxidante, Ecuador, entomofagia, fenoles totales, *Platycoelia lutescens*, *Rhynchophorus palmarum*.

ABSTRACT

Nowadays, sustainable alternatives for some foods are being sought, which is why insects have become attractive. Knowing more about their possible beneficial contributions to our diet, such as the presence of phenols and their antioxidant capacity, is an initial step towards their use. In this study, a nutritional characterization for protein, fat and ash of *Platycoelia lutescens* and *Rhynchophorus palmarum* was performed based on the methods proposed by the AOAC (Association of Official Agricultural Chemists) for raw meats, in order to facilitate the comparison of the results with traditional meat sources. The Folin-Ciocalteu method was used for total phenols and FRAP assay (Ferric Reducing Antioxidant Power) for antioxidant capacity, bioactive components not yet recorded in insects. Previously recorded values were found in both species for their protein (*P. lutescens* ~50%, *R. palmarum* 27% - 44%), fat (*P. lutescens* ~25%, *R. palmarum* ~50%) and ash levels (*P. lutescens* ~4.5%, *R. palmarum* ~1%), although between samples they do not present homogeneity, possibly due to external factors (substrate or locality). The values obtained for total phenols and antioxidant capacity are new, so they cannot be compared with other insects, but they indicate their great potential as food alternatives to be studied further.

Key words: antioxidant capacity, Ecuador, entomophagy, *Platycoelia lutescens*, *Rhynchophorus palmarum*, total phenols.

TABLE OF CONTENT

INTRODUCTION 10
- Entomophagy in Ecuador 10
- Information gap 12

METHODOLOGY 14
- Study area 14
- Species identification 14
- Nutritional characterization 15
  - Sample handling 15
  - Sample preparation 16
  - Nutritional characterization tests 16
  - Data analysis 17

RESULTS 18

DISCUSSION 21

CONCLUSIONS 26

REFERENCES 27

Annex A: Detailed methodology 32
- Protein analysis procedure (Kjeldahl Method) 32
- Ash analysis procedure 32
- Fat analysis procedure 32
- Folin-Ciocalteu Method for total phenols analysis procedure 32
- Ferric Reducing Ability of Plasma (FRAP) for antioxidant capacity analysis procedure... 33
- RStudio code for the calibration curves 33

Annex B: Images of the process of identification and nutritional characterization 34

Annex C: Tables and figures of the detailed results 40

TABLES

Table 1. Data of collection for *Platycoelia lutescens* and *Rhynchophorus palmarum*............14
Table 2. Nutritional characterization for *P. lutescens* and *R. palmarum*................................19
Table 3. Results of ash test for each sample of *P. lutescens* and *R. palmarum*.......................40
Table 4. Results of protein test for each sample of *P. lutescens* and *R. palmarum*................41
Table 5. Results of fat test for each sample of *P. lutescens* and *R. palmarum*.......................42
Table 6. Results of total phenols test for each sample of *P. lutescens* and *R. palmarum*. 43
Table 7. Results of FRAP assay for each sample of *P. lutescens* and *R. palmarum*..............44

FIGURES

Figure 1. Specimen of *Platycoelia lutescens* ("catzo blanco") collected for this project found on the ZUSFQ. 34
Figure 2. Mouthparts of *Rhynchophorus palmarum* ("chontacuro") after the dissection. 35
Figure 3. Prepared sample of *P. lutescens* before the lyophilization. 35
Figure 4. *R. palmarum* samples before lyophilization. A: frozen larvae due to the difference between collect date and analysis date (see Table 1), B: living larvae from *R. palmarum*. 36
Figure 5. Grinded and lyophilized sample of *P. lutescens*. 37
Figure 6. Fat test for two samples (8 and Cot, see Table 1) with three repetitions each. Start of immersion step. 37
Figure 7. Ash test for samples of *R. palmarum*, three repetitions each. A: preheated samples before entering the muffle furnace, B: chilled samples after muffle furnace. 38
Figure 8. Protein test for 8 (CH P) repetitions and all *P. lutescens* samples. A: steps for the protein test after protein digestion with Tashiro's indicator; B: all titrations for these samples, not labeled samples are for "hormiga culona" samples not presented in this study. 39
Figure 9. Calibration curve for total phenols test. 45
Figure 10. Calibration curve for FRAP assay. 46
Figure 11. Preliminar data for "hormiga culona", *Atta* cf. *cephalotes*. 46

INTRODUCTION

Due to constant and current climatic changes and the pressures to change the way we produce food; humans must find new dietary sources. The unequal distribution of resources, the excessive consumption of certain products, climate change, and other factors have led humanity under difficult situations to start looking for alternatives to include in their diets. The United Nations (UN) has set 17 Sustainable Development Goals (SDG) that will transform the world in order to make more sustainable food sources (UN, 2015), and one of them is to achieve zero hunger (SDG 2), which complements the SDG 12 of responsible consumption and production. For the issue of finding sustainable food sources, different alternatives have been sought, for example the consumption of insects, also known as entomophagy. Works such as that of Ramos-Elorduy & Viejo Montesinos (2007) and Huis (2013) show us how insects can be a potential food source and the use that has been made of them in situations of precisely combating famine or insufficient resources. Likewise, seeing these animals as a potential alternative would help in the advancement of achieving responsible production and consumption, in this case of food (Premalatha et al., 2011; Rumpold & Schlüter, 2013).

Entomophagy in Ecuador

Entomophagy is a millenary practice around the world. Thanks to colonial literary records, it is known that the great majority of tribes distributed in Ecuador consumed insects during designated times of the year (Onore, 1997). The most common forms of consumption have been larvae and some adults after being prepared in a specific way. Over time, both in Ecuador and around the world, entomophagy became an unusual practice and usually viewed with some contempt because they are "unpleasant" animals (Borgmeier, 1959; Macedo et al., 2017).

However, just as it is heard in Asia and some specific localities around the world, in Ecuador there is still the consumption of insects as extraordinary and ancestral dishes in certain places.

Around the Sierra and Amazon regions of Ecuador, there are several insects that have been consumed for generations (Barragán et al., 2009). The famous “chontacuros” and “catzos” are known mainly for being consumed in very specific areas or cities in Ecuador. However, in this territory there are between 50 up to 100 species of insects that are being consumed by humans (Jongema, 2017).

Based on the literature, the “catzo blanco” is a single species, *Platycoelia lutescens*, which was renamed after a taxonomic revision where it previously could be found as *Leucopelea albencens* (Agila Lisintuña, 2020; Carvajal et al., 2011; Darquea Bustillos, 2018; Onore, 1997). Specifically, in the Andean cities, such as Quito and Otavalo, there is an ancestral food that continues to be consumed to this day. We are talking about the famous “catzo con tostado” (toasted corn with toasted beetle), and specifically the consumption of “catzo blanco” which is the common name given to this species of beetle. Depending on the locality there are other species of consumption, such as *Golofa unicolor* (Agila Lisintuña, 2020; Carvajal et al., 2011; Izurieta Wong & Játiva Delgado, 2014; Onore, 1997), but undoubtedly in most of the northern Sierra, more specifically in Quito and Otavalo, *P. lutescens* is consumed in October/November for All Souls’ Day (Agila Lisintuña, 2020; Darquea Bustillos, 2018). Although much is known about this historical consumption, the nutritional information frameworks of this insect are not well explored, as most studies remain on the general nutritional value and characterization of fatty acids that cannot be compared due to the unstandardized methodologies (Velastegui Chávez, 2018; Velsateguí C. et al., 2020).

The second species of high consumption in Ecuador is the South American palm weevil or “chontacuro”, *Rhynchophorus palmarum*. This is consumed as larvae along the Amazon by many communities in countries such as Brazil, Peru and Ecuador (Sancho et al., 2015). Unlike the species presented above, the “chontacuro” has something like a cultivation system, since it

is one of the most commercialized products between entomophagous regions. Together with the great demand of local consumers, it has led to the intervention of collectors by knocking down the palms of chonta (*Bactris gasipaes*) and morete (*Mauritia flexuosa*) so these beetles lay their eggs there and thus obtain larvae directly (Sancho, 2012). While for *P. lutescens* there are no standardized records of their nutritional information, for this species there are some investigations such as the one from Espinosa Matabay (2019) where they present these data in the same way as the packaged foods that can be obtained in supermarkets. In spite of that, most studies remain in a more general field where only the results of the analyzes are mentioned basing their possible benefits as a sustainable food (Vargas et al., 2013).

Information gap

Information about alternative food sources for some animal groups, such as insects, is quite limited. Considering that entomophagy is currently considered taboo in certain areas, the generally available information on the subject is very select and scarce. For *P. lutescens* there are several types of literature, mostly anecdotal and historical records about its consumption in the Ecuadorian Sierra (Barragán et al., 2009; Onore, 1997). Additionally, some literature mentions its taxonomic adjustment and other data such as being an emblematic species for Quito (Paredes Ponce, 2018). However, when it comes to the nutritional part, the few available studies work on the same topics: lipid profile, fatty acid analysis, gas chromatography and the culinary perception of customers, but none of them follow a standardized methodology (Lamilla Polanco, 2020; Velastegui Chávez, 2018; Velsateguí C. et al., 2020). This means we need more information on its nutritional composition with standardized methods for its comparison with traditional meats and other studies.

Meanwhile, for *R. palmarum* there are more studies on its possible nutritional and medicinal benefits (Ganchala Tigse, 2021). The most common analyses are composition of fatty acids,

the degree of oil saturation, amino acid composition and bromatological analysis (Espinosa Matabay, 2019; Vargas et al., 2013). Although these data are also available for the other species, this species presents a more standardized work on how to portray the nutritional values, as they appear similar to the labels of already processed products (Espinosa Matabay, 2019).

Nonetheless, although there are records of nutritional values for all these insects, there is not enough work maintaining a standardized format to present these data. Another factor that was not found in any work is the analysis of certain beneficial functional components in the human diet, such as carotenoids, vitamins A, B6, B12, D and E. Also, the existence of little literature with scientific data on these species in Ecuador may be a limiting factor when trying to see their potential as alternative foods.

Considering these information gaps, this thesis provides in general data for these fields. The main objective is to characterize the nutritional properties and the presence of bioactive substances with functional and chemical potential in samples of "catzo blanco" (*P. lutescens*) and "chontacuro" (*R. palmarum*) from markets located in the Ecuadorian Amazon and Andes where these species are actively consumed. This has been done by correctly identifying the species that are being consumed in each of the respective locations and comparing them with previous records of the species consumed there. Tests were also performed to identify the basic nutrients (e.g., proteins, lipids, and carbohydrates) and bioactive compounds with functional potential (e.g., antioxidant capacity and total phenols) that each of the samples of "catzo blanco" (*P. lutescens*) and "chontacuro" (*R. palmarum*).

METHODOLOGY

Study area

Samples of *P. lutescens* were collected from markets in two cities in the northern Sierra of Ecuador. One sample was obtained from a market from the city of Otavalo; while in Quito, samples were taken from Guamaní market (0°19'53"S 78°33'10"W) and San Roque market (0°13'07"S 78°31'18"W). On the other hand, samples for *R. palmarum* were collected in the markets of Puyo (Chapintza and Koyampari,1°29'25''S 78°0'08''W), El Coca (0°28'27''S 76°59'04''W), and Archidona (0°54'29''S 77°48'28''W) (see **Table 1**). Based on the information given by the sellers, it was recorded the palm of origin for some samples of *R. palmarum* showed in **Table 1**.

Table 1. Data of collection for *Platycoelia lutescens* and *Rhynchophorus palmarum*

Species	Date of collection	Locality	Province	Bioregion	Sample code
P. lutescens	2021.11	Otavalo	Imbabura	Andes	Cot
P. lutescens	2021.11	San Roque, Quito	Pichincha	Andes	Csr
P. lutescens	2021.11	Guamaní, Quito	Pichincha	Andes	Cg
R. palmarum	2022.03	Puyo, Chapintza (Chonta)	Pastaza	Amazon	1
R. palmarum	2022.03	Archidona (Chonta)	Napo	Amazon	2
R. palmarum	2022.03	Puyo, Koyampari (Chonta)	Pastaza	Amazon	3
R. palmarum	2022.02	El Coca	Orellana	Amazon	4
R. palmarum	2022.03	El Coca (Morete1)	Orellana	Amazon	5
R. palmarum	2022.03	El Coca (Morete2)	Orellana	Amazon	6
R. palmarum	2022.03	Puyo, Chapintza (Morete)	Pastaza	Amazon	7
R. palmarum	2021.11	Puyo	Pastaza	Amazon	8

Species identification

Taxonomic keys, identification guides, photographs, and specimens in collections were used to corroborate that the species mentioned in the literature are the ones still consumed in these

localities. For *P. lutescens* it was used taxonomic keys presented on pages 30-31 and 38-42 of Smith (2003) and compared with adults at the entomological collection of the Pontificia Universidad Católica de Ecuador (QCAZ). Finally, the larvae of "chontacuro" (*Rhynchophorus palmarum*) were identified based on their mouthparts using keys presented by Chamorro (2019) and Wattanapongsiri (1966), with an additional validation by the specialist Aymer Vásquez (professor at Universidad del Valle del Cauca).

Nutritional characterization

In a collaboration with a project of Professor José Miguel Álvarez Suárez of the Colegio de Ciencias e Ingenierías of the Universidad San Francisco de Quito (USFQ), the procedures to be followed for the analysis of the nutritional composition of the samples were established as follows.

Sample handling

All samples were collected alive to preserve their nutritional, microbiological and taxonomic characteristics intact. The organisms were taken to the Instituto de Microbiología of the USFQ to take samples for potential pathogens (*Salmonella* sp., *Streptococcus* sp., *Bacillus subtilis*, *Escherichia coli*, *Enterobacter liquefaciens*, *E. cloacae* and *Klebsiella pneumoniae*) as part of another investigation of this institute. Then, between three to 10 individuals were separated for taxonomic identification of each species and kept at the Museo de Zoología y Laboratorio de Zoolgía Terrestre of the USFQ (ZUSFQ). Immediately, the remaining individuals of each sample were placed in labeled containers (with date and place of collection) to be frozen at about -40°C until the nutritional analysis. All samples used in this study were collected under the research permit MAAE-ARSFC-2021-1808.

Sample preparation

To perform the nutritional characterization correctly, only the parts of the insects that are consumed are used. The following preparation procedures were used on each sample.

- "Catzo blanco" (*Platycoelia lutescens*): small portions of individuals were taken from the frozen samples to maintain the proper cold chain. Legs, elytra, and wings were removed from each individual. Once each portion was finished, they were returned to the freezer in a new labeled container ready for the analysis.
- "Chontacuro" (*Rhynchophorus palmarum*): complete individuals were kept frozen until further analysis if they were not fresh collected (samples from November 2021 in this study), or alive individuals were transported directly to the laboratory for processing the sample (samples from March 2022).

All samples went through lyophilization (freeze drying) for two days. Afterwards, they were bathed in liquid nitrogen and grinded to help the process and began a second process of freeze-drying for another two days. In some cases, such as for some *R. palmarum* samples, extra lyophilization time was needed up until having the desired consistency. Lastly, all samples went through a finer grinding process and were kept frozen until further analysis.

Nutritional characterization tests

With the grinded sample tests were performed for moisture content, crude protein, crude fat, ash, according to the Association of Official Analytical Chemists (de Castro et al., 2018). In addition to these traditional tests, the aim was to generate a broader nutritional profile with important nutrients such as polyphenols and antioxidant capacity. For the total phenols analysis it was used the methodology proposed by Singleton et al. (1999), most known as the Folin-Ciocalteu Method. In addition, for the antioxidant capacity was used the Ferric Reducing

Antioxidant Power (FRAP) assay (Benzie & Strain, 1996). For further details on the methodology consult annex A.

Data analysis

The statistical analysis was carried on Excel for calculating the values of each test. Ashes percentage was calculated with the weight in grams of the empty crucible (A), the sample (B), and the crucible with sample after muffle furnace (C) in the formula $\left(\frac{C-A}{B}\right) * 100$. In the case of proteins, it was considered the sample weight (S), volume of titration (V1), normality of HCl (N), volume of blank titration (V0) and the conversion factor (F=6.25 for proteins) following the formula $\left(1.4 * (V1 - V0) * \frac{N}{S}\right) * F$. For fats it was used the sample weight (S) and the fat obtained (f) in each beaker, $\left(\frac{f}{S}\right) * 100$. All samples had three repetitions for what the results of each were averaged. For the graphics of the calibration curves, it was used RStudio with the library ggplot2 (see annex A).

RESULTS

For the taxonomic identification, the individuals from the entomological collection that entered the Museo de Zoología Terrestre de la USFQ as part of this project were used. All the individuals of *P. lutescens* came out of the sample from Guamaní, Quito. After using the previously mentioned key and comparing them with the individuals at the QCAZ collection, it was confirmed that the organisms collected belong to the species *Platycoelia lutescens*. Meanwhile, *R. palmarum* larvae that entered the collection were part of the November 2021 sample from El Puyo (see **Table 1**). A dissection of the mouthparts of the larvae was performed with the guidance of Aymer Vasquez and the work of Chamorro (2019). After taking photos, checking with the pre-existing work and corroboration by the expert, it was determined that the species was *Rhynchophorus palmarum*.

Regarding the nutritional characterization, Table 2 shows the general values obtained for each sample. The percentage of ash in *P. lutescens* samples remains above 4% for each of the localities, while in *R. palmarum* it remains at about 3%. *P. lutescens* samples have a protein value above 50% while the ones for *R. palmarum* from El Coca have less than 30% in its composition, and the ones from Puyo around 40%. On the other hand, *R. palmarum* has about 50% fat, which unlike *P. lutescens* its values are around 25%. In *P. lutescens* samples, no significant differences were found between markets for any of the values for ashes, proteins, and fats. On *R. palmarum* samples, there was also no significant difference between localities for ashes and fats, but protein levels highly differ from each other with around 10%. Images for the whole process are presented on Annex B.

Table 2. Nutritional characterization for *P. lutescens* and *R. palmarum*.

Species	Sample	Ash (%)	Protein (%)	Fat (%)	Total phenols (GAE/100g*)	FRAP (µM Trolox/100g*)
P. lutescens	Cg	4,65 ± 0,01	53,64 ± 6,41	23,54 ± 3,25	29,18	125,90
P. lutescens	Csr	4,22 ± 0,37	51,46 ± 2,33	23,38 ± 5,67	29,09	85,65
P. lutescens	Cot	4,52 ± 0,34	54,90 ± 5,32	25,32 ± 2,92	29,41	61,15
R. palmarum	1	1,98 ± 0,28	27,75 ± 1,26	54,91± 0,99	38,44	52,03
R. palmarum	2	2,47 ± 0,03	28,48 ± 1,01	53,79 ± 0,93	26,25	ND
R. palmarum	3	2,18 ± 0,10	29,03 ± 0,19	50,88 ± 1,21	37,23	ND
R. palmarum	4	3,23 ± 0,32	40,20 ± 1,23	52,91± 1,32	19,55	61,15
R. palmarum	5	2,90± 0,04	44,00 ± 0,23	49,20 ± 2,30	26,46	54,40
R. palmarum	6	2,38 ± 0,31	28,95 ± 0,23	49,40 ± 0,60	17,92	55,15
R. palmarum	7	2,52 ± 0,21	31,10 ± 0,67	46,07 ± 0,13	21,92	53,15
R. palmarum	8	2,80 ± 0,22	29,21 ± 0,44	50,94 ± 0,99	41,79	89,40

All values are presented as mean ± SD. See sample codes on **Table 1**. GAE: miligrams of gallic acid equivalent per liter; g*: grams of dried sample.

On Table 6 it shown the results for the Folin-Ciocalteu Method, and its calibration curve is found on **Figure 9**. On average *R. palmarum* samples had 29,07mg of gallic acid per 100g of dried sample, but there were very different values on the different samples with the highest being 41,79mg (sample 8) and the lowest 17,92mg (sample 6). *P. lutescens* samples had a similar value from 29,23g, but, unlike the other species, all three samples had similar values (see Table 6). Meanwhile, antioxidants on average were seen on all samples of both species. *R. palmarum* presented in general lower values than *P. lutescens* (see Table 7 for data and calibration curve on **Figure 10**). Contrary to total phenols, on samples of *P. lutescens* there were very different values of Trolox (antioxidant for measuring antioxidant capacity) per 100g of dried sample between the localities, from 61,15µM up to 125,9 µM. For *R. palmarum* though, most samples were between 52,03 µM and 61,15 µM, except for number 8 with 89,4 µM. Samples 2 and 3 have not been considered for this test since there were not sufficient

reagents for these two samples and its repetitions. For further details on each test and sample repetitions, see Annex C.

DISCUSSION

Within the similar values of *P. lutescens* samples for nutritional characterization, a slight difference is noted between the sample from Otavalo and those from the Quito markets. The proteins and fats of the first one are slightly higher. This may be due to the locality of collection, since this city is located to the north of Quito, while it is known that in the other markets the collection sites are in the outskirts of the capital towards the south (Darquea Bustillos, 2018; Onore, 1997). On the other hand, although the ash values are much closer to each other, this pattern does not hold true for these samples, raising a question about the factors that may be affecting these values. To learn more about how the type of soil in which these beetles inhabit and other variables may directly impact their nutritional composition, more research should be done, as it is not something that has been considered in this degree work or in other literature found to this date.

Similarly, in *R. palmarum* samples, slight differences in protein, fat and ash values can be seen among them. The highest percentage values in fats are in three different localities (samples 1, 2 and 4, see Table 2), but they share the substrate from which the individuals were collected: chonta palm, *Bactris gasipaes*. This is contrasted with the samples belonging to "morete", *Mauritia flexuosa* (samples 5 to 7, see Table 2), where the three lowest fat values are seen. Likewise, as mentioned for *P. lutescens*, there is ND on how the substrate or other variables affect the values found in the nutritional characterization of *R. palmarum*, therefore more research should be done in this field.

Comparing these results with other nutritional characterization data, the percentage values of protein, ash and fat of both species are higher than those recorded for raw beef and pork in Ecuador. Based on the study of Orozco (2013) study, fat levels for fresh beef are around 8.94% and for pork 12.52%. If we compare the samples of *P. lutescens*, these have between 23.54%

and 25.32%, being considerably higher; as well as in all the samples of *R. palmarum* we see values ranging between 46.07% and 54.91%. This indicates that the percentage fat levels in both insects are higher than those of a common protein.

In the same study, values for protein percentage are given, for beef 16.72% and for pork 18.36%. As in fats, the protein values for *P. lutescens* are higher than these meats (51.46%-54.90%) and in *R. palmarum* as well (27.75%-44%) (see Table 2). Although there are samples of *R. palmarum* where there are higher values of protein out of all samples (samples 4 and 5), we can suppose that this may be due to the substrate on which they were on or the time of larval harvest. None of these reasons have been investigated, so the exact origin of these differences in protein values cannot be known. On the other hand, the ash tests in *P. lutescens* are significantly high (around 4.5%, see Table 2) compared to raw beef and pork (0.65% and 0.83%, respectively). In *R. palmarum* there are also higher ash levels (1.98%-3.23%), but they do not present as great a difference with these conventional meats as *P. lutescens*. This means that *P. lutescens* has a greater amount of minerals than *R. palmarum* and traditional meats. These values indicate that, percentagewise, both *P. lutescens* and *R. palmarum* represent a better food source due to their high levels of protein, fat and ash compared to these meats, which are currently consumed as part of our daily diet.

Meanwhile, when considering other studies conducted on insect species, *P. lutescens* has a higher protein value than some species of butterflies (*Aegiale hesperiaris*, *Comadia redtembacheri*) and ants (*Liometopum apiculatum*) reported in other locations, such as Hidalgo, Mexico (Rostro et al., 2012). Its fat values however remain low with respect to others, and ash values are similar to those of these insect species. On the other hand, as expected from *R. palmarum*, compared to other insects that are consumed as larvae, it has similar values of protein (~35%), ash (~1%) and fat (~40%). Although these values do not vary greatly between

larvae and adults on those species of ants and butterflies, for *R. palmarum* the field should be opened to investigate its potential consumption in the adult stage, since no record of the consumption of the adults have been found in Ecuador (Ganchala Tigse, 2021). This may indicate that, depending on the insect, locality, and stage of consumption, it may contain more or less fat and protein content, providing a greater contribution than conventional meats, but it remains unknown for this species.

Meanwhile, phenolic compounds were found on all samples of both species, but, as shown on Table 6, *R. palmarum* has a broader spectrum of data. The highest value was found in sample 8 belonging to Puyo (from November 2021), while the lowest is from El Coca (collected in March 2022, sample 6). Although we do not know the substrate on which sample 8 was, we know that sample 6 is belongs to “morete” (*Mauritia flexuosa*). Considering all other samples of *R. palmarum* that came from “morete” (6, 5 and 7), we can see that all three have lower values of phenols than those of sample 8. This might not be an indicator of the substrate of being a deciding factor since there are samples from “chonta” that are also low (see Table 6). Therefore, these data, unlike the results for protein, fat, and ash, do not clearly indicate whether there can be a pattern based on locality or substrate to define the presence of total phenols in *R. palmarum*. In contrast, the three *P. lutescens* samples had a greater consistency of data, as the values did not vary much regardless of locality. This could be a fairly accurate indicator of the true value of total phenols in *P. lutescens*, but more research is needed to truly corroborate this find.

Although there are no defined values of total phenols for *R. palmarum*, the fact that relatively similar values were found on average in both species, shows that, as in some foods, phenolic compounds are present. The presence of these compounds in these species makes them more attractive for their potential use as an alternative food since phenols are desired in certain

quantities. It has been shown that they may have benefits in human health, such as reducing the possibility of developing cardiovascular diseases, cancer, and Alzheimer's disease (Creus, 2004). However, further research is still needed to generate the phenol profile for these species and to determine the full contribution they would make to the human diet and health.

In contrast, for antioxidant capacity the data from *R. palmarum* samples were more consistent, although due to lack of reagents, samples 2, 3 and a repeat of sample 1 could not be performed before the end of this thesis. In any case, the results were sufficient to demonstrate that this characteristic has no real variation by locality or substrate (see Table 7), although as in the total phenol test, sample 8 indicated the highest value of all. The case of *P. lutescens* was different, as here all locations differ with values of 61.15 µM, 85.65 µM, and 125.90 µM of Trolox per 100g of dry sample. This variation is quite high, so it should be something to be further investigated with more samples from each market to find statistically significant values. Likewise, it should be considered that in none of these species nor for sister species has this type of analysis been performed, leaving without real references of the values that would be expected to be found in these animals. As mentioned for total phenols, despite not having the exact values for these insects in their antioxidant capacity, the contribution of these data shows their presence. Antioxidant capacity based on Trolox is used to determine the quality of foods according to their antioxidant levels (Benítez-Estrada et al., 2020), so for these species future research work must be done so it could be determined their quality as potential alternative foods.

As in all research, there were some obstacles encountered at the beginning of the work. Due to time constraints at the beginning of this project, it was not possible to obtain sufficient samples from the different markets for *P. lutescens*. Because of this, there are not enough records to generate a larger spectrum of exact comparisons between localities, but this may be something

that can be reviewed in future research. Similarly, regarding the arrival times of *R. palmarum* samples to the lab, they did not coincide with the times for taxonomic identification nor were enough individuals from each locality assigned to the collection destined to the ZUSFQ, to carry out a more detailed taxonomic identification. These aspects should be considered for future research related to these species, because as they are market samples there are certain limits to the number of individuals to be obtained; and in the case of *P. lutescens* there is also the seasonality of its sale that must be considered. More work along the same lines is encouraged in order to obtain more information on the "harvest" and sale periods of both species, as well as to include other factors such as the lack of habit of collecting them on other seasons, as it may be for *P. lutescens*, or the substrate on which they are found, as in the case of *R. palmarum*.

In addition to the species presented in this project, we tested a sample of "hormiga culona" (*Atta* cf. *cephalotes*) collected in November 2021 in the city of Puyo (see preliminary data in **Figure 11**). As this sample also belonged to a market, only queens were obtained as part of the individuals used. This implied that, at the time of taxonomic identification, a major problem was encountered: it is not possible to identify *Atta* species only with queens. This, together with the fact that they are seasonal and therefore only one sample was found in markets, meant that this species had to be left aside for this study, but work will continue be done when more samples are obtained for further analysis. Also, to facilitate identification, it should be ensured that workers are obtained as part of the sample, either by getting them in the market itself trapped in the legs of the queens or by gaining access to the nest where these ants were collected and thus, being able to take a specific sample for identification.

CONCLUSIONS

It was determined that both *P. lutescens* and *R. palmarum* have higher ash, fat, and protein values than conventional beef and pork, as well as other insects that have been studied in other countries. Similarly, it was possible to confirm the presence of antioxidant capacity and total phenols in both species, which are desired characteristics in foods because they demonstrate their quality and their potential benefits to human health. Even with this information, more studies for these characteristics are encouraged in order to better understand their possible use as an alternative food in our diet. Consecutively, this may trigger a greater interest in continuing the search for new alternatives to traditional food sources and follow the SDGs, and thus lead to a transformation of the current human lifestyle.

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ANNEX A: DETAILED METHODOLOGY

Protein analysis procedure (Kjeldahl Method)

1. For each sample it was used the weight indicated on Table 4 of grinded and lyophilized insect. Each sample was made by triplicate. It was transferred to a VELP glass tube.
2. 15 ml of sulfuric acid was added to each sample, and a Kjeldahl tablet and a defoamer tablet too.
3. Place each tube on the DKL Automatic Digestion Unit, start the program and let the digested sample cool afterwards.
4. With a Semi-Automatic Kjeldahl Distillation Unit each sample was extracted into a 250ml Erlenmeyer with 30ml of boric acid and three drops of Tashiro's indicator.
5. After distillation, all samples must look green.
6. It was titrated with 0,1N HCl solution until each sample returned to a fuchsia color. Based on AOAC (2019).

Ash analysis procedure

1. For each sample it was used the weight indicated on Table 3 of grinded and lyophilized insect. Each sample was made by triplicate. It was transferred to an empty crucible that was previously weighted.
2. They were burned on a muffle furnace until calcinated (over 12 hours).
3. Each crucible was weighted after cooling.
   Based on AOAC (2019).

Fat analysis procedure

1. For each sample it was used the weight of grinded and lyophilized insect indicated on Table 5. Each sample was made by triplicate.
2. The empty Soxhlet Laboratory Extractor beakers were weighted before starting the new samples cycle.
3. For each sample and its triplicate was used 50ml of ethanol on a Soxhlet Laboratory Extractor.
4. After the fat extraction of each sample, the beakers with fat were left to cool down before weighing them.
   Based on AOAC (2019).

Folin-Ciocalteu Method for total phenols analysis procedure

1. Each sample was prepared with 0,5g of grinded and lyophilized insect and 10ml of ethanol. Kept on refrigeration until needed.
2. The samples went through centrifugation all night, and before using them another 5 minutes centrifuge at 5000 rpm.

3. For each sample triplicate it was separated 100 µl of the supernatant liquid on a 1.5ml Eppendorf.
4. Based on Singleton et al. (1999), the procedure was adapted to the 100 µl of sample for each triplicate.

Ferric Reducing Ability of Plasma (FRAP) for antioxidant capacity analysis procedure

1. Each sample was prepared with 0,5g of grinded and lyophilized insect and 10ml of ethanol. Kept on refrigeration until needed.
2. The samples went through centrifugation all night, and before using them another 5 minutes centrifuge at 5000 rpm.
3. For each sample triplicate it was separated 100 µl of the supernatant liquid on a 1.5ml Eppendorf.
4. Based on Benzie & Strain (1996), the procedure was adapted to the 100 µl of sample for each triplicate.

RStudio code for the calibration curves

```
# TOTAL PHENOLS CALLIBRATION CURVE
    # English
library(ggplot2)
ggplot(data=X2CALIBRAR_FENOLES_ACIDO_GALICO, aes(uM,ABS)) +
  geom_point() +
  geom_smooth(method = "lm") +
  ggtitle("Calibration curve of gallic acid") +
  xlab("Concentration in µm") +
  ylab("Absorbance (760 nm)")


# ANTIOXIDANT CAPACITY
    # English
library(ggplot2)
ggplot(data=X1CALIBRAR_ANTIOXIDANTES_TROLOX, aes(uM,ABS)) +
  geom_point() +
  geom_smooth(method = "lm") +
  ggtitle("Calibration curve of Trolox") +
  xlab("Concentration in µm") +
  ylab("Absorbance (593 nm)")
```

ANNEX B: IMAGES OF THE PROCESS OF IDENTIFICATION AND NUTRITIONAL CHARACTERIZA

TION

Figure 1. Specimen of *Platycoelia lutescens* ("catzo blanco") collected for this project found on the ZUSFQ.

Figure 2. Mouthparts of *Rhynchophorus palmarum* (“chontacuro”) after the dissection.

Figure 3. Prepared sample of *P. lutescens* before the lyophilization.

Figure 4. *R. palmarum* samples before lyophilization. A: frozen larvae due to the difference between collect date and analysis date (see Table 1), B: living larvae from *R. palmarum*.

Figure 5. Grinded and lyophilized sample of *P. lutescens*.

Figure 6. Fat test for two samples (8 and Cot, see Table 1) with three repetitions each. Start of immersion step.

Figure 7. Ash test for samples of *R. palmarum*, three repetitions each. A: preheated samples before entering the muffle furnace, B: chilled samples after muffle furnace.

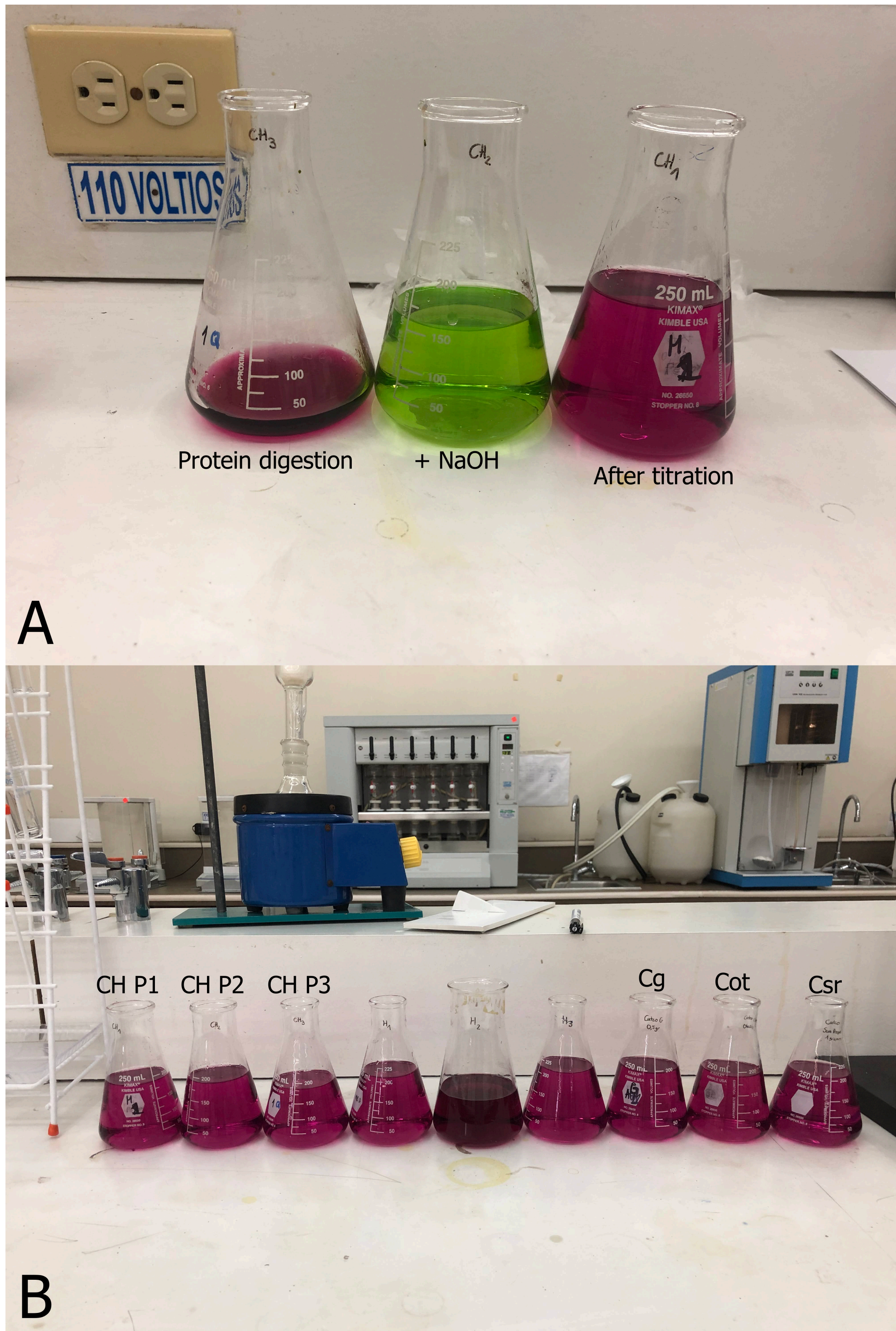

Figure 8. Protein test for 8 (CH P) repetitions and all *P. lutescens* samples. A: steps for the protein test after protein digestion with Tashiro's indicator; B: all titrations for these samples, not labeled samples are for "hormiga culona" samples not presented in this study.

ANNEX C: TABLES AND FIGURES OF THE DETAILED RESULTS

Table 3. Results of ash test for each sample of *P. lutescens* and *R. palmarum*.

Sample	Empty crucible (g)	Crucible + sample (g)	Sample (g)	Crucible + sample after muffle furnace (g)	Ash (g)	Ash (%)	Average (%)	SD
Cg 1	19,76	20,75	0,99	19,81	0,04	4,55	4,65	0,01
Cg 2	16,95	17,95	1	17,00	0,05	4,50		
Cg 3	17,74	18,35	0,61	17,77	0,03	4,92		
Csr 1	17,34	18,35	1,01	17,38	0,04	4,32	4,22	0,37
Csr 2	19,34	20,36	1,02	19,38	0,04	3,81		
Csr 3	18,83	19,85	1,02	18,88	0,05	4,54		
Cot 1	17,17	18,19	1,02	17,22	0,05	4,90	4,52	0,34
Cot 2	15,91	16,92	1,01	15,95	0,04	4,41		
Cot 3	27,17	28,21	1,04	27,21	0,04	4,24		
1.1	27,20	28,28	1,08	27,22	0,02	2,10	1,98	0,28
1.2	27,43	28,41	0,97	27,45	0,02	1,66		
1.3	27,44	28,47	1,02	27,47	0,02	2,18		
2.1	16,95	17,83	0,88	16,97	0,02	2,51	2,47	0,03
2.2	20,33	21,35	1,02	20,35	0,02	2,44		
2.3	21,47	22,50	1,03	21,49	0,03	2,46		
3.1	15,91	16,91	1,00	15,94	0,02	2,16	2,18	0,10
3.2	27,17	28,17	0,99	27,19	0,02	2,10		
3.3	17,54	18,54	0,99	17,57	0,02	2,29		
4.1	19,18	20,27	1,09	19,21	0,04	3,40	3,23	0,32
4.2	17,03	18,20	1,17	17,07	0,04	3,43		
4.3	19,69	20,61	0,93	19,71	0,03	2,85		
5.1	19,76	20,99	1,23	19,80	0,04	2,93	2,90	0,04
5.2	18,98	19,95	0,97	19,00	0,03	2,91		
5.3	18,71	19,77	1,06	18,74	0,03	2,85		
6.1	19,34	20,40	1,06	19,36	0,03	2,37	2,38	0,31
6.2	17,35	18,34	0,99	17,37	0,02	2,08		
6.3	17,78	18,77	1,00	17,80	0,03	2,70		
7.1	17,18	18,30	1,12	17,21	0,03	2,73	2,52	0,21
7.2	20,41	21,71	1,30	20,44	0,03	2,30		
7.3	17,75	18,82	1,07	17,78	0,03	2,53		
8.1	17,03	18,02	0,99	17,06	0,03	2,59	2,80	0,22
8.2	19,17	20,15	0,98	19,20	0,03	3,03		
8.3	16,82	17,81	0,99	16,85	0,03	2,80		

For the sample codes see **Table 1**. For each sample three repetitions were made.

Table 4. Results of protein test for each sample of *P. lutescens* and *R. palmarum*.

Sample	Sample weight (g)	V1(ml) - titration	N (normality (HCl))	V0 (ml) - blank titration	F (conversion factor)	Total protein (%) MS	Average (%)	SD
Cg 1	1	57,00	0,1	0,12	6,25	49,770	53,64	6,41
Cg 2	1	57,40	0,1	0,12	6,25	50,120		
Cg 3	0,5	35,00	0,1	0,12	6,25	61,040		
Csr 1	1	57,30	0,1	0,12	6,25	50,033	51,46	2,33
Csr 2	1	57,50	0,1	0,12	6,25	50,208		
Csr 3	1	62,00	0,1	0,12	6,25	54,145		
Cot 1	1	59,10	0,1	0,12	6,25	51,608	54,90	5,32
Cot 2	1	59,60	0,1	0,12	6,25	52,045		
Cot 3	0,5	35,00	0,1	0,12	6,25	61,040		
1.1	1	33,50	0,1	0,12	6,25	29,208	27,75	1,26
1.2	1	31,00	0,1	0,12	6,25	27,020		
1.3	1	31,00	0,1	0,12	6,25	27,020		
2.1	1	34,00	0,1	0,12	6,25	29,645	28,48	1,01
2.2	1	32,00	0,1	0,12	6,25	27,895		
2.3	1	32,00	0,1	0,12	6,25	27,895		
3.1	1	33,24	0,1	0,12	6,25	28,980	29,03	0,19
3.2	1	33,54	0,1	0,12	6,25	29,243		
3.3	1	33,12	0,1	0,12	6,25	28,875		
4.1	1	47,20	0,1	0,12	6,25	41,195	40,20	1,23
4.2	1	46,50	0,1	0,12	6,25	40,583		
4.3	1	44,50	0,1	0,12	6,25	38,833		
5.1	1	50,50	0,1	0,12	6,25	44,083	44,00	0,23
5.2	1	50,10	0,1	0,12	6,25	43,733		
5.3	1	50,60	0,1	0,12	6,25	44,170		
6.1	1	33,50	0,1	0,12	6,25	29,208	28,95	0,23
6.2	1	33,10	0,1	0,12	6,25	28,858		
6.3	1	33,00	0,1	0,12	6,25	28,770		
7.1	1	35,50	0,1	0,12	6,25	30,958	31,10	0,67
7.2	1	35,00	0,1	0,12	6,25	30,520		
7.3	1	36,50	0,1	0,12	6,25	31,833		
8.1	1	33,00	0,1	0,12	6,25	28,770	29,21	0,44
8.2	1	34,00	0,1	0,12	6,25	29,645		
8.3	1	33,50	0,1	0,12	6,25	29,208		

For the sample codes see **Table 1**. For each sample three repetitions were made.

Table 5. Results of fat test for each sample of *P. lutescens* and *R. palmarum*.

Sample	Empty beaker (g)	Beaker + fat (g)	Fat only (g)	Sample (g)	Fat (%)	Average (%)	SD
Cg 1	73,02	73,80	0,78	3	25,90	23,54	3,25
Cg 2	72,45	73,20	0,75	3	24,90		
Cg 3	72,21	72,80	0,59	3	19,83		
Csr 1	72,56	73,45	0,89	3	29,67	23,38	5,67
Csr 2	72,66	73,22	0,56	3	18,67		
Csr 3	72,35	73,00	0,65	3	21,81		
Cot 1	73,25	74,08	0,83	3	27,75	25,32	2,92
Cot 2	72,9	73,56	0,66	3	22,07		
Cot 3	72,21	72,99	0,78	3	26,14		
1.1	73,29	74,95	1,66	3	55,23	54,91	0,99
1.2	72,21	73,88	1,67	3	55,69		
1.3	73,03	74,64	1,61	3	53,80		
2.1	74,63	76,22	1,59	3	52,90	53,79	0,93
2.2	76,74	78,35	1,61	3	53,71		
2.3	72,34	73,98	1,64	3	54,77		
3.1	76,75	78,28	1,53	3	50,88	50,95	1,21
3.2	73,29	74,78	1,49	3	49,78		
3.3	74,64	76,21	1,57	3	52,19		
4.1	72,69	74,26	1,57	3	52,29	52,91	1,32
4.2	73,36	74,99	1,63	3	54,43		
4.3	73,28	74,84	1,56	3	52,02		
5.1	74,29	75,74	1,45	3	48,30	49,20	2,30
5.2	75,15	76,57	1,42	3	47,48		
5.3	74,69	76,24	1,55	3	51,81		
6.1	73,29	74,75	1,46	3	48,73	49,40	0,60
6.2	75,14	76,64	1,50	3	49,92		
6.3	74,68	76,17	1,49	3	49,54		
7.1	74,32	75,70	1,38	3	46,02	46,07	0,13
7.2	73,36	74,74	1,38	3	45,97		
7.3	72,68	74,07	1,39	3	46,22		
8.1	73,05	74,54	1,49	3	49,80	50,94	0,99
8.2	74,33	75,88	1,55	3	51,57		
8.3	73,28	74,82	1,54	3	51,45		

For the sample codes see **Table 1**. For each sample three repetitions were made.

Table 6. Results of total phenols test for each sample of *P. lutescens* and *R. palmarum*.

Sample	Rep1	Rep2	Rep3	Average	mM Ga	g Ga/L	GAE/g*	GAE/100g*	Average	SD
1	0,760	0,969	0,760	0,830	0,565	0,096	0,19	38,44	29,07	9,18
2	0,534	0,601	0,568	0,568	0,386	0,066	0,13	26,25		
3	0,808	0,804	0,801	0,804	0,547	0,093	0,19	37,23		
4	0,457	0,437	0,379	0,424	0,287	0,049	0,10	19,55		
5	0,666	0,621	0,623	0,637	0,433	0,074	0,15	29,46		
6	0,379	0,384	0,404	0,389	0,263	0,045	0,09	17,92		
7	0,480	0,456	0,489	0,475	0,322	0,055	0,11	21,92		
8	0,989	0,973	0,745	0,902	0,614	0,104	0,21	41,79		
CG	0,608	0,611	0,674	0,631	0,429	0,073	0,15	29,18	29,23	0,17
Cot	0,622	0,607	0,678	0,636	0,432	0,074	0,15	29,41		
Csr	0,663	0,609	0,614	0,629	0,427	0,073	0,15	29,09		

For the sample codes see **Table 1**. Ga: gallic acid; GAE: miligrams of gallic acid equivalent per liter; g*: grams of dried sample. For each sample three repetitions were made.

Table 7. Results of FRAP assay for each sample of *P. lutescens* and *R. palmarum*

Sample	Rep1	Rep2	Rep3	Mean	µM trolox/ L	µM trolox/ mL	µM Trolox/ g*	µM Trolox/ 100g*	Mean	SD
1	0,203	0,202	ND	0,203	130,06	0,130	0,26	52,03	60,88	14,33
2	ND	ND	ND	ND	ND	ND	ND	ND		
3	ND	ND	ND	ND	ND	ND	ND	ND		
4	0,212	0,257	0,247	0,239	152,88	0,153	0,31	61,15		
5	0,214	0,203	0,22	0,212	136,00	0,136	0,27	54,40		
6	0,224	0,215	0,207	0,215	137,88	0,138	0,28	55,15		
7	0,210	0,202	0,209	0,207	132,88	0,133	0,27	53,15		
8	0,323	0,356	0,378	0,352	223,50	0,224	0,45	89,40		
CG	0,477	0,537	0,480	0,498	314,75	0,315	0,63	125,90	90,90	32,69
Cot	0,247	0,240	0,229	0,239	152,88	0,153	0,31	61,15		
Csr	0,236	0,250	0,526	0,337	214,13	0,214	0,43	85,65		

For the sample codes see **Table 1**. g*: grams of dried sample. For each sample three repetitions were made. In samples 1 – repetition3, 2 and 3 are not enough data for this analysis due to lack of reagent before the end of this investigation (represented with “ND” for “No Data” and colored with orange).

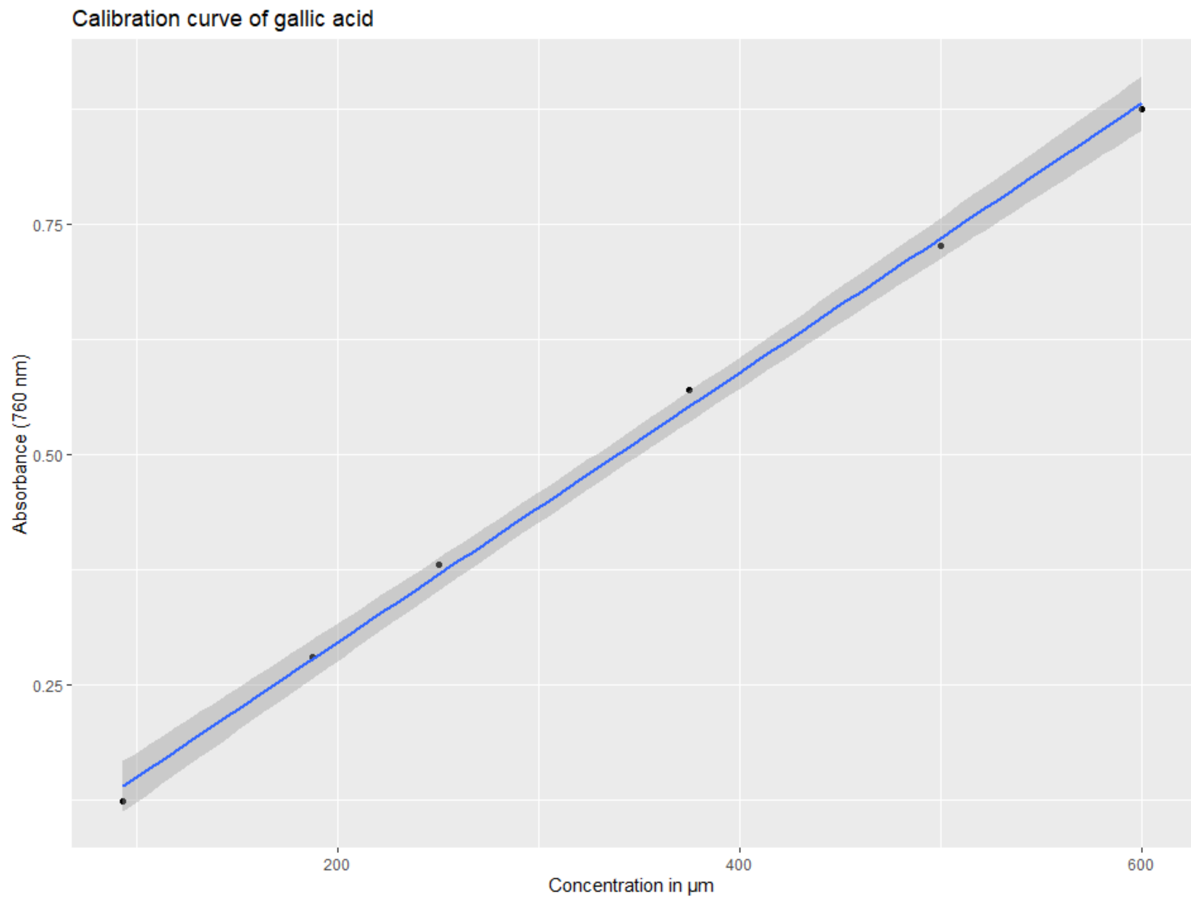

Figure 9. Calibration curve for total phenols test.

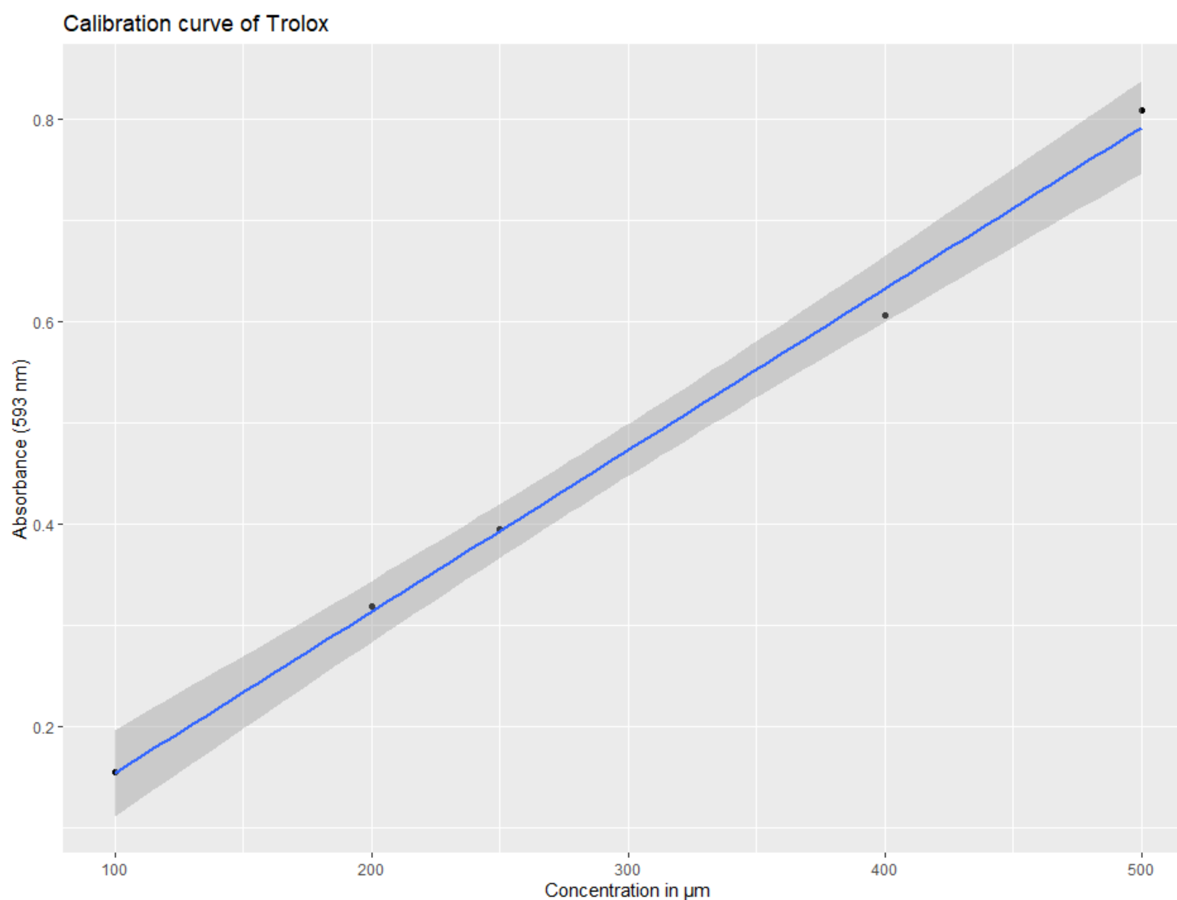

Figure 10. Calibration curve for FRAP assay.

	Sample	Empty crisol (in g)	Crisol + sample (in g)	Sample (in g)	Crisol + sample after muffle furnace (in g)	Ash (in g)	Ash (%)	Average (%)	SD
ASH	H1	18,7	19,7	1	18,76	0,06	6,43	6,24	0,18
	H2	19,68	20,69	1,01	19,74	0,06	6,08		
	H3	20,5	21,54	1,04	20,56	0,06	6,21		

	Sample	Sample weight (in g)	V1(ml) - titration	N (normality (HCl))	V0 (ml) - blank titration	F (conversion factor)	Total protein (%) MS	Average (%)	SD
PROTEIN	H1	1	45,50	0,1	0,12	6,25	39,708	41,17	2,91
	H2	1	45,00	0,1	0,12	6,25	39,270		
	H3	1	51,00	0,1	0,12	6,25	44,520		

	Sample	Empty beaker (in g)	Beaker + fat (in g)	Fat only (in g)	Sample (in g)	Fat (%)	Average (%)	SD
FATS	H1	74,59	75,56	0,97	3	32,24	30,21	1,77
	H2	73,30	74,18	0,88	3	29,26		
	H3	76,75	77,62	0,87	3	29,11		

	Sample	Average	mM Ga	g Ga/L	GAE/g*	GAE/100g*
PHENOLS	H	0,665	0,452	0,077	0,15	30,76

	Sample	Average	mM Ga	g Ga/L	GAE/g*	Trolox/100g*
ANTIOX	H	0,392	248,50	0,249	0,50	99,40

Figure 11. Preliminar data for "hormiga culona", *Atta* cf. *cephalotes*.