Université Catholique de Louvain - Walloon Agricultural Research Center, Gembloux - Université de Liège

ANALYSIS OF ACAI BY MID INFRA-RED SPECTRSCOPY

OMAR DIARBAKERLI

TRAVAIL DE FIN D'ETUDES PRESENTE EN VUE DE L'OBTENTION DU DIPLOME DE MASTER COMPLEMENTAIRE EN SCIENCES ET TECHNOLOGIE DES ALIMENTS

ANNÉE ACADÉMIQUE 2013-2014

(CO)-PROMOTEUR(S):
Dr. Ir. Vincent BAETEN
Dr. Ouissam ABBAS

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Education, Audiovisual and Culture Executive Agency
Erasmus Mundus Programme

Acknowledgments and appreciations:

It would not have been possible to write this master thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here.
This thesis would not have been possible without the help, support and patience of my principal supervisor, Dr. Ouissam Abbas, not to mention her encouragement, kindness, advices and unsurpassed knowledge of simplify things to students like me.
The good advice and support of my promoter, Dr. Ir. Vincent Baeten, has been invaluable on both an academic and a personal level, for which I am extremely grateful.
I would like to thank Quentin Arnould for his kindness, friendship, support and generosity.
I would like to acknowledge the financial, academic and technical support of the Université Catholique de Louvain (UCL), Centre Wallon de Recherches Agronomiques (CRA-W) and its staff, lab mates and co-workers.
Last, but by no means least, I would like to thank my parents. They were always there cheering me up, encouraging me with their best wishes and stood by me through the good times and bad.

Contents

List of figures: 2
List of tables: 4
Resumé EN: 5
Resumé FR: 6
1. Introduction 7
2. Theoritical part 7
2.1 About açai 7
2.2 Nutritional composition & medical benefits: 8
2.3 Anti-oxidant effect 9
2.4 Polyphenols and Anthocyanins in açai 9
2.5 Direct methods for analysing polyphenolic compounds: 11
2.6 Indirect methods for analysing polyphenolic compounds: 11
2.7 Infrared Spectrometry 12
3. Materials & methods: 16
3.1 Samples and extraction 16
3.2 Infra-red analysis 17
3.3 Total phenolic content (TPC) 18
3.4 Total anthocyanin content (TAC) 19
4. Results 21
4.1 Total polyphenols and total anthocyanins content 21
4.2 Mid Infra-Red spectroscopy 24
4.2.1 PCA on fruit powder spectra 25
4.2.2 PCA on phenolic extracts spectra 32
4.3.3 Partial least squares regression using fruits and extracts spectra 34
5. Conclusion & perspectives 37
References: 38

List of figures:

Figure 1: The açai fruit 7
Figure 2 : Infrared spectrum among the other spectrum of waves (migunvictoria.com.au) 13
Figure 3: Different ways of molecular vibration by IR (Wikipedia) 14
Figure 4: Illustration of IR light pathway by ATR technique (americanlaboratory.com) 15
Figure 5: location of the harvested samples 16
Figure 6: Extraction of polyphenols 17
Figure 7: The Bruker (Vertex 70) used to perform the MIR analysis 17
Figure 8: The dried methanolic extract being analyzed by the MIR 18
Figure 9: Anthocyanins in different media with different pH (source: foodcolor.eu) 20
Figure 10: Histogram graph of polyphenols contents differentiated by areas 21
Figure 11: Histogram graph of polyphenols contents differentiated by days 22
Figure 12: Histogram graph of anthocyanins contents differentiated by areas 22
Figure 13: Histogram graph of anthocyanins contents differentiated by days 23
Figure 14: Correlation of TAC and TPC 24
Figure 15: Correlation of TAC and TPC after eliminating the outliers 24
Figure 16: Different regions used of the MIR spectrum illustrated on a random acai sample 26
Figure 17: PCA graph where samples are differentiated by values of polyphenols content for the powder (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra) 27
Figure 18: PCA graph where samples are differentiated by values of total anthocyanin content for the powder (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra) 27
Figure 19: PCA graph of powder showing the middle group and the PC-1 used (pre-treatment: SNV, region used: Fingerprint) 28
Figure 20: Loading graph using the PC-1 29
Figure 21: PCA graph of the middle group and he high-mid group (pre-treatment: Savitzky–Golay, region used: Useful spectra) 30
Figure 22: Loading graph of the middle group and the high-mid group with the peaks of the cellulose highlighted 30
Figure 23: PCA graph of powder differentiated by cultivation days (pre-treatment: none, region used: Fingerprint) 31
Figure 24: PCA graph of powder differentiated by cultivation area (pre-treatment: none, region used: Fingerprint) 31
Figure 25: Spectrum of powder and spectrum of phenolic extract for the same sample 32
Figure 26: PCA graph were samples are differentiated by values of polyphenols content for the extracts (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra) 32
Figure 27: PCA graph where samples are differentiated by values of total anthocyanin content for the extracts (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra) 33
Figure 28: PCA graph of extracts differentiated by cultivation days (pre-treatment: none, region used: Fingerprint) 33
Figure 29: PCA graph of extracts differentiated by cultivation days (pre-treatment: Savitzky–Golay, region used: Fingerprint) 34

Figure 30: PCA graph of extracts differentiated by cultivation area (pre-treatment: none, region used: Fingerprint).....34
Figure 31: The pathway of the infrared beam in FTIR.....42
Figure 32: PCA graph where samples are differentiated by values of total anthocyanin content for the extracts (pre-treatment: none, region used: Super Fingerprint).....47
Figure 33: PCA graph where samples are differentiated by values of total anthocyanin content for the extracts (pre-treatment: Savitzky–Golay, region used: Super Fingerprint) 47

List of tables:

Table 1: Anthocyanins content in açai (Schauss et al., 2006) *DW: dried weight.....10
Table 2: Concentration of standard compounds that is needed to obtain an inhibition rate of 50% against peroxyl radicals by TOSC. (Inácio et al., 2012) 10
Table 3: Samples number according to the area of cultivation.....16
Table 4: Steps for preparing the standard series for the standard curve 19
Table 5: Steps for preparing the samples.....19
Table 6: Ranges of polyphenols content.....23
Table 7: Ranges of total anthocyanins content 23
Table 8: R^2, RPD and RMSEcv values.....35
Table 9: Best (obtained after eliminating outliers) R^2 and RPD value 36
Table 10: Total phenolic content (TPC) results:.....45
Table 11: Total anthocyanins content (TAC) results:.....46
Table 12: PLS-R results, R^2 and RMSEcv, of the Supe Fingerprint region analysis ..48

Resumé EN:

The evaluation of the composition of plant matrices including bioactive molecules such as phenolic compounds, would bring an added value to the agro-industrial products.
Given the significant changes in the content of phenolic, it is essential to develop a method for fast careening and accurate prediction in order to identify and possibly quantify the composition of interest.
Infrared spectroscopy has many advantages such as speed, ease of use and environmental friendliness by the limited use of solvents.
In the context of this work, a methodology based on the Attenuated Total Reflectance Fourier Transform infrared spectroscopy (ATR-FT-MIR), was developed trying to quantify those phenolic and anthocyanins compounds present in a vegetable matrix such as fruits known as rich in anthocyanins.
A total of 105 fruit powders and dried extracts, ie the residue of the phenolic extract, after the solvent has been evaporated were analyzed by MIR spectroscopy analyses.
To obtain reference values of total anthocyanin and phenolic contents, an individual extraction was made for both anthocyanins and phenolic compounds and analyzed by Folin–Ciocalteu and pH-differential method.
Then, infrared spectroscopy measurements were conducted on the raw material (fruit) and dried phenolic extract. Spectra were treated then by unsupervised chemometric methods like PCA in order to visualise the natural grouping of samples.
These analyses have helped to develop an initial method of estimation of anthocyanins and polyphenol content of açai, depending on the reference values obtained.
Analysis of fruit and phenolic extracts of the pulp and peel by FT-MIR spectroscopy provides a first estimate of their phenolic composition.
Our study showed that the MIR spectroscopy can be used for screening and approximate quantitative predictions (R^2=0.72-0.75, RPD=2.01-2.2) but not as accurate as found by NIR compared to other studies.

Resumé FR:

L’évaluation de la composition exacte des matrices végétales notamment en molécules bioactives comme les composés phénoliques, permettrait d’apporter une plus-value aux produits agro-industriels.
Vu les variations importantes du contenu en composés phénoliques, il est essentiel de développer une méthode d’analyse précise et rapide pour pouvoir les identifier et éventuellement les quantifier.
La spectroscopie infrarouge présente de nombreux avantages tels que la rapidité, la facilité d’utilisation et le respect de l’environnement par l’utilisation limitée de solvants.
Dans le cadre de ce travail, une méthodologie basée sur la spectroscopie infrarouge à transformée de Fourier en mode de réflexion totale atténuée (ATR-FT-MIR), a été développée pour identifier les composés phénoliques présents dans une matrice végétale telle que l'açai riches en anthocyanines.
Un total de 105 fruits et leurs extraits secs, c’est à dire le résidu phénolique après évaporation du solvant, ont été analysés par spectroscopie MIR.
Pour obtenir des valeurs de référence du contenu des anthocyanines et des phénols totaux, une extraction a été réalisée pour les anthocyanines et les fractions phénoliques qui ont été ensuite analysée par Folin–Ciocalteu et pH-differential method.
Ensuite, des mesures de spectroscopie infrarouge ont été effectuées sur la matière première (fruits) et de l'extrait phénolique séchée.
Ces analyses ont permis de mettre au point une méthode initiale d’estimatione contenu des polyphénols et des anthocyanins dans l'açai à partir d’informations sur les valeurs de références.
L’analyse des fruits et des extraits phénoliques de chairs et pelures d'açai par un spectromètre FT-MIR, a permis de donner une première estimation de leur composition en composés phénoliques et anthocyanines.
Notre étude a montré que la spectroscopie MIR peut être utilisé pour le dépistage et des prédictions quantitatives approximatives (R^2=0.72-0.75, RPD=2.01-2.2) mais pas aussi précis que l'on trouve par NIR par rapport à d'autres études.

1. Introduction

The anti-oxidants in the açai are considered as a high important source of antioxidants in certain country. To deliver the best to consumers, the agricultural industry keeps searching to develop technological tools or methods to guide the agricultural industry in the post-harvest monitoring and improving quality.
The proposal and development of any new analytical procedure leads to an investigation and subsequent validation of the procedure's efficacy.
To conduct this study, a total of 106 samples of açai fruits from 13 different areas were collected and analysed, tests including total anthocyanin content and total polyphenolic content were carried out to compare the results obtained from the analysis of the fruit by the Mid Infra-Red spectroscopy MIR.
Since MIR spectroscopy is a rapid and non-destructive analytical method and now becoming more efficient, applicable and available in the analysing field, our research aims to find the correlation data with Mid Infra-Red and if MIR can be used to identify and quantify the polyphenolic compounds or the anthocyanins in açai, or if not, classify the richness and determine which is more favourable to the producers and consumers.

2. Theoritical part

2.1 About açai

The fruit of the açaí palm (Euterpe oleracea Mart.), which is native of South America, has recently become popular as a functional food due to its antioxidant potential (Schauss et al., 2006). The edible fruit is round, black-purple in color, about 1-inch (25 mm) in diameter and contains a single large seed (Figure 1).

Figure 1: The açai fruit

Açai berries grow in clusters of 700 to 900 fruits. Each is about 1 inch in diameter and has a single large seed. While açai berries resemble grapes, they produce far less

pulp; in fact, about 80% of each berry is seed. However, the way that they are prepared and served is similar.
The method used to prepare the açai juice or extract is so important to ensure the preserving of its full health benefit. Some methods are cheaper and easier, and are therefore more interesting to large industries who sell the drinks in huge quantities. Considering that the açai industry has increased in recent years, it's no wonder that some companies try to move as much product as possible with little concern for the integrity of the product.
In a study in the Brazilian Amazon, açaí palm was described as the most important plant species because the fruit makes up a major component of their diet, up to 42% of the total food intake by weight (Schauss et al., 2006). A powdered preparation of freeze-dried açaí fruit pulp and skin was reported to contain (per 100 g of dry powder) 533.9 calories, 52.2 g carbohydrates, 8.1 g protein, and 32.5 g total fat. The carbohydrate portion included 44.2 g of dietary fibres and low sugar value (pulp is not sweet) (Schauss et al., 2006). The powder was also shown to contain (per 100 g): negligible vitamin C, 260 mg calcium, 4.4 mg iron, and 1002 U vitamin A, the amino acid content was 7.59% of total dry weight (versus 8.1% protein). The fat content of açaí consists of oleic acid (56.2% of total fats), palmitic acid (24.1%), and linoleic acid (12.5%) (Schauss et al., 2006).

2.2 Nutritional composition & medical benefits:

Açaí consumption is increasing worldwide because of the growing recognition of its nutritional and therapeutic properties (Koizimi et al., 2013)
Macerating the pulp of the fruit produces a viscous liquid, which is approximately 2.4% protein and 5.9% lipid (by weight) (Del Pozo-Insfran et al., 2004), a juice with anti-inflammatory, antioxidant and potential chemo-preventive activities.
Açai is considered to be rich in polyphenolic compounds which have a great anti-oxidant activity. The antioxidant capacity of purple açaí samples was found to be excellent against peroxyl radicals, good against peroxynitrite and poor against hydroxyl radicals compared with common European fruit and vegetable juices recently analysed (Lichtenthäler et al., 2005), the freeze-dried powder showed high antioxidant capacity against superoxide, the initial producer of potent reactive oxygen species, and peroxyl scavenging in a superoxide dismutase assay (Schauss et al., 2006).
In a study, healthy volunteers were dosed at 7 mL/kg of body weight of açai juice and pulp, 12h later individual increases in plasma antioxidant capacity of up to 2.3- and 3-fold for açai juice and pulp, respectively were observed (Mertens-Talcott et al., 2008).
As anti-oxidant effect is known for eliminating the free radicals, which play a role in developing cancer, a study show that Açai fractions containing polyphenolic compounds reduced the proliferation of leukaemia cells (Del Pozo-Insfran et al., 2006).
Following a 30-day treatment with Açai, and compared to baseline, there were reductions in fasting glucose and insulin levels (both $p < 0.02$), there was also a reduction in total cholesterol ($p = 0.03$), as well as borderline significant reductions in LDL-cholesterol ($p = 0.051$) (Udani et al., 2011). Antioxidant capacity of the polyphenol-rich beverages was classified in the following order: black cherry juice, açaí juice, cranberry juice>orange juice, iced tea beverages, apple juice (Seeram et al., 2008).
Açai was found to have an anti-inflammatory effect (Schauss et al., 2006).

A study results suggest that people with very low intakes of flavonoids have higher risks of coronary disease (Knekt et al., 1996).

2.3 Anti-oxidant effect

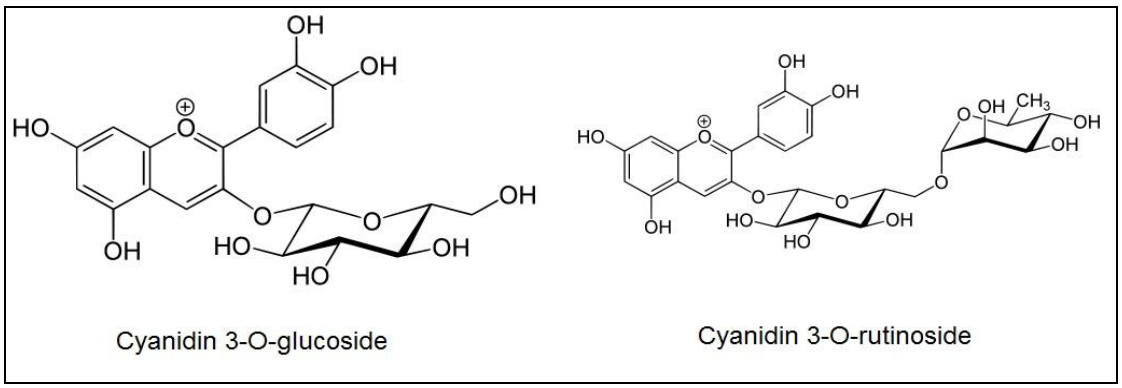
The healing and power of antioxidants is well known and documented. However, many are unsure of how this works.
Oxidation is usually a naturally occurring process in the human body and actually refers to the loss of an electron. Each cell in the body consists of a nucleus, neutrons, protons and electrons. Having an exact correct amount of electrons is important, as it will determine the cell's chemical behaviour. The cell will try to keep this exact number of electrons by either picking up missing electrons or giving away extras to keep the balance between the positive particles and the negative particles.
If the molecule for different reasons is missing an electron, a free radical is formed. It will attack the nearest stable molecule in an effort to steal back the needed electron to obtain the balance. This starts a chain reaction, as the cell that lost an electron to replace the missing electron in the first one has now produced a free radical of its own. This process eventually leads to the disruption of the cell as the free radicals chip away at the cell wall, causing damage that accumulates with age. Eventually, the free radicals can affect the cellular DNA. This cell damage shows itself in the human body is a number of ways like:
Cancer, Emphysema, Cataracts, Arteriosclerosis, Diabetes Mellitus, Crohn's disease, Rheumatoid arthritis, Heart Disease, Osteoporosis, Cerebrovascular disease, Stroke.
Antioxidants prevent this damage by stabilizing the free radicals before they cause cellular damage. They act as donors, supplying one of their own electrons to stabilize the molecule and effectively preventing them from stealing one from another healthy molecule.
Antioxidants are able to do this because they do remain stable after losing an electron of their own. They are able to function normally after donating an electron.
Among the popular anti-oxidants are the polyphenolic compounds (Flavonoïdes, Phenolic acids, Stilbene and Lignin) and the anthocyanins which belong to the Flavonoïdes.

2.4 Polyphenols and Anthocyanins in açai

The main source of polyphenols is dietary, since they are found in a wide array of foods. For example, honey; most legumes; fruits such as apples, blackberries, blueberries, pomegranate, cherries, cranberries, grapes, pears, plums, raspberries, and strawberries; and vegetables such as broccoli, cabbage, celery and onion are rich in polyphenols. Red wine, chocolate, black tea, white tea, green tea, olive oil, argan oil, bee pollen and many grains are sources. Ingestion of polyphenols occurs by consuming a wide array of plant foods.
Polyphenols contains a large number of compounds. Polyphenols are grouped into 4 groups: Flavonoïdes, Phenolic acids, Stilbene and Lignin (Sonia Collin, UCL).
The Flavonoides consists of 12 sub-groups: flavones, isoflavones, flavans, flavonones, flavanols, flavanolols, anthocyanins, catechins, leukoanthocyanins, chalcons, dihydrochalcons and aurons (Lim T. K, 2012).
Acai contains differents types of polyphenols including the anthocyanins whih are a sub-family of the polyphenols family.

Polyphenolic compounds vary a lot in the açai, and with the variety of these compounds comes the high healthy effects of it, a study shows the polyphenol compounds in açai are: Gallic acid, Protocatechuic acid, p-Hydroxybenzoic acid, Vanillic acid, Chlorogenic acid, Caffeic acid, Syrengic acid, Orientin, Homoorientin, Luteolin 7-glucoside, Vitein, Isovitexin, Taxifolin, Luteolin and Chrysoeriol (Inácio et al., 2012).

Researchers confirm the presence of cyanidin 3-glucoside and cyanidin 3-rutinoside as major anthocyanic compounds (Gallori et al., 2004).



Other polyphenolic compounds are presented but in lower concentration: cyanidin 3-sambubioside, peonidin 3-glucoside and peonidin 3- rutinoside (Table 1).

Table 1: Anthocyanins content in açai (Schauss et al., 2006) *DW: dried weight

Anthocyanin	Content mg/g DW*	Ratio per total anthocyanins %
Cyanidin 3-rutinoside	1.93	60.31
Cyanidin 3-glucoside	1.17	36.56
Cyanidin 3-sambubioside	0.04	1.25
Peonidin 3-glucoside	0.02	0.63
Peonidin 3- rutinoside	0.04	1.25
Total	3.19	100

Cyanidin 3-glucoside and cyanidin 3-rutinoside are considered among the best anthocyanins concerning their efficacy (Table 2) (Inácio et al., 2012) and the benefit of açai is clear here considering that the açai has 3.2 mg/g anthocyanins in the dried matter (Schauss et al., 2006).

Table 2: Concentration of standard compounds that is needed to obtain an inhibition rate of 50% against peroxyl radicals by TOSC. (Inácio et al., 2012)

Compound	mg/L
Luteolin	4.8
Cyanidin 3-glucoside	5.65
Orientin	6.52
Cyanidin 3-rutinoside	6.72
Homoorientin	7.11
Chrysoeriol	7.49
Isovitexin	7.87
Vitexin	9.12
Vanillic acid	10.8

2.5 Direct methods for analysing polyphenolic compounds:

The polyphenolic compounds content has been the interest of scientific researchers for several years, different methods were developed to obtain the most accurate results, many new methods have become the dominant methods considering their accuracy and specificity, but some old methods are still used by some researchers.
The most used method is the High Performance Liquid Chromatography (HPLC) attached to a VIS-UV detector or a Mass Spectrometer (MS), which comes with more precise results and can differentiate between different types of polyphenol compounds, making it a qualitative and quantitative method, but still require a professional preparation of the sample, a professional worker to perform the test and each test costs a non-negligible amount of money, so repeating the test will cost and the more tests to perform the more the cost will increase.

2.6 Indirect methods for analysing polyphenolic compounds:

Most research on the quantitation, purification, separation, and identification of anthocyanins has relied on expensive equipment, and/or lengthy sample preparation. These methods include paper chromatography, thin-layer chromatography, column chromatography, solid phase extraction, counter current chromatography, UV–visible absorption spectroscopy, high performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (Skrede et al., 2002) (Takeoka et al., 2002).

The Folin-Ciocalteu (FC) method of performing a total phenolics assay, originally developed for protein determination, has recently evolved as a total antioxidant capacity assay but was found to be incapable of measuring lipophilic antioxidants. But was then modified and standardized to enable simultaneous measurement of lipophilic and hydrophilic antioxidants (Berker et al., 2013).
Other researchers have tried to measure the potency of the polyphenolic compounds, several methods have been used to compare the potency, Total Oxidant Scavenging Capacity (TOSC), (Lichtenthäler et al., 2005) Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing Antioxidant Power (FRAP) (Seeram et al., 2008).

The pH differential method has been validated and demonstrated to be simple, quick, and accurate for measuring the total anthocyanin content of a sample (Lee et al., 2008). It has been found a high correlation ($R > 0.925$, $p < 0.05$) between the pH differential method and HPLC when determining the amount of anthocyanins found in samples (Lee et al., 2008). In 2005, the pH differential method received first action approval from the Association of Analytical Communities (AOAC) International official methods board (AOAC method 2005.02).
Values for quantifying anthocyanins acquired from different methods (pH differential and the two different HPLC solvents system) were highly correlated with one another. HPLC is an invaluable tool for identifying and quantifying different individual anthocyanin in a sample. But, the pH differential method is a simple, rapid, and economical means for determining the amount of anthocyanins in a sample, and this method has been verified by AOAC's strict validation guidelines (Jungmin et al., 2007).

Researchers are still looking for better methods, especially more rapid and non-destructive ones as the demands and variety of rapid analysing are increasing.
A rapid and non-destructive method to determine total anthocyanin content in açaí fruits using Near Infra-Red (NIR) spectroscopy and multivariate calibration was achieved in a study done in 2012 (Inácio et al., 2012). The values for accuracy and precision exhibited promising results (R^2=0.97, RPD=3.08), indicating that the model developed by NIR spectroscopy for Total Anthocyanins Content (TAC) can be used as an alternative to UV–Vis measurements (Inácio et al., 2012).
Another study showed that Near Infra-Red (NIR) spectroscopy is a reliable method to determine soluble solids in açaí pulp and thereby to classify açaí pulp (Koizimi et al., 2013).
Since Mid Infra-Red gives more structural and compositional information while NIR permit to estimate the physic-chemical properties, we think that it might be capable of giving a more precise and accurate results than NIR, and its capable of keeping the benefits of the NIR as being rapid and non-destructive analysis method.
Mid Infra-Red (MIR) has been also showed to be a rapid method to quantify phenolic and anthocyanin compounds in grape which may make it a suitable method for acai too (Fragoso et al., 2011).
A study conducted on blueberries (family Ericaceae; genus Vaccinium corymbosum L.) to examine the potential of MIR and NIR spectroscopy to evaluate the content of total phenols and total anthocyanins revealed that a model developed in the NIR region was able to evaluate the content of total phenols and total anthocyanins. The predicted results by MIR were not as good as those obtained in NIR (Sinelli et al., 2008).

2.7 Infrared Spectrometry

Infrared refers to that part of the electromagnetic spectrum between the visible and microwave regions. These waves differ from each other in the length and frequency.
Frequency, ν (nu), is the number of wave cycles that pass through a point in one second. It is measured in Hz, where 1 Hz = 1 cycle/sec. Wavelength, λ (lambda), is the length of one complete wave cycle.
Energy is related to wavelength and frequency by the following formulas:

$$\mathbf{E = h\nu}$$

Where h = Planck's constant, 6.6×10^{-34} joules-sec

Note that energy is directly proportional to frequency and inversely proportional to wavelength. A wavenumber is the inverse of the wavelength in cm.
The infrared range is 780 nm – 1mm, divided to three regions: Near IR, Medium IR and Far IR, the interest is more in the NIR and MIR which range between (780 nm – 2500 nm) and (2500 nm – 25000 nm) respectively (Figure 2).

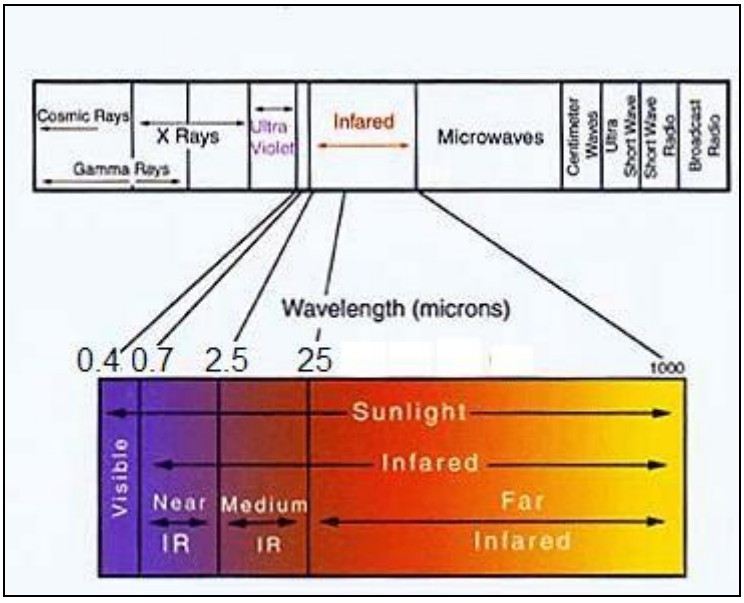


Figure 2 : Infrared spectrum among the other spectrum of waves (migunvictoria.com.au)

In wavenumbers, the MIR range is 4000–400 cm^{-1}. An increase in wavenumber corresponds to an increase in energy. Infrared radiation is absorbed by organic molecules and converted into energy of molecular vibration. In IR spectroscopy, an organic molecule is exposed to infrared radiation. When the radiant energy matches the energy of a specific molecular vibration, absorption occurs.

The wavenumber, plotted on the X-axis, is proportional to energy; therefore, the highest energy vibrations are on the left. The percent transmittance (%T) or reflectance (%R) is plotted on the Y-axis. Absorption of radiant energy is therefore represented by a curve, zero transmittance (reflectance) corresponds to 100% absorption of light at that wavelength.
Band intensities can also be expressed as absorbance (A). Absorbance is the logarithm, to the base 10, of the reciprocal of the transmittance:

$$A = \log_{10} (1/T)$$

When an infrared light beam passes through a molecule, the molecule absorb the energy from this beam and use it to vibrate in different manners, each wavelength has it specific energy and each molecule absorbs different amounts of energy and therefore vibrates in different ways and wavelength, but this vibration is always the same under the same wavelength, which makes the response of this molecule as an identity to it and makes it possible to identify it.

There are two types of molecular vibrations, stretching and bending.
In order for a vibrational mode in a molecule to be "IR active," it must be associated with changes in the dipole. A permanent dipole is not needed, as the process requires only a change in dipole for the moment.
The atoms in a CH_2- group, commonly found in organic compounds, can vibrate in six different ways: symmetric and asymmetric stretching, scissoring, rocking, wagging and twisting, (Figure 3).

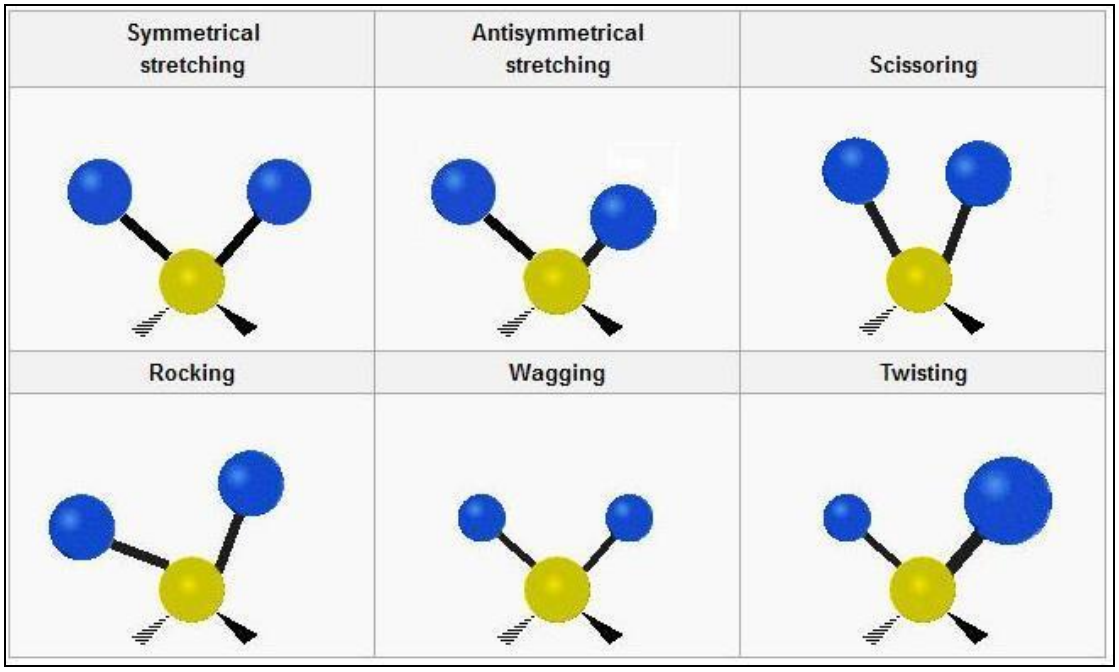


Figure 3: Different ways of molecular vibration by IR (Wikipedia)

Both the stretching and bending happened at specific wavelengths, which according to that the quantitative and qualitative analysing are determined.

Mid Infra-Red spectrometer (described in the Annexe 1) may be equipped by different accessories permitting measurements in reflexion or transmission. Attenuated total reflectance (**ATR**) is a useful technique to obtain the IR spectrum of the surface of a material, which is too thick (the sample absorbs too much and don't let any beam out of the sample) or too dark (strongly absorbing colour are the dark colours which absorb too much of the beam that nothing is left to be detected) to be analyzed by standard transmission methods. ATR accomplish the analysis regardless of the thickness, the effective penetration depth is a fraction of a wavelength. Sample materials can be in different form like powders, pastes, fibres, paper, or oils. The sample is placed in contact with an internal reflection element, a material with a high refractive index, e.g. ZnSe and diamond. The area of the measurement is small usually around 2 mm^2. The light is totally reflected, several times, and the sample surface interacts with the evanescent wave resulting in the absorption of radiation at each point of reflection. Some ATR use only one reflection point others use multiple reflectance points.
The intensity of the evanescent wave fades with the distance from the surface of the internal reflection element. Beam with longer wavelength penetrates deeper into the sample, which results in better bands (Sylvia-Monique Thomas, University of Nevada Las Vegas). The angle of entering is usually chosen to be between 45° and 60º (for obtaining a deeper penetration a smaller angle is used and vice versa). Sensitivity of ATR instrument depends on the physical contact between the crystal and the sample, to achieve an intact contact an sort of press arm is used to ensure a good pressing force, not too much to break the samples or smash it nor too loose (Figure 4).

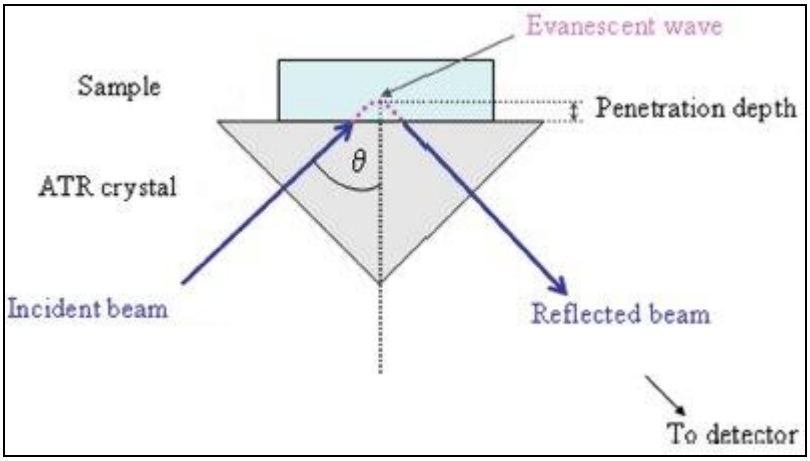


Figure 4: Illustration of IR light pathway by ATR technique (americanlaboratory.com)

3. Materials & methods:

3.1 Samples and extraction

The açai fruits were harvested from 13 different areas in Brazil mostly from Ponta de Pedras, Abaetetuba and Muaná (Figure 5), a total of 105 samples were collected over 2 months (from 29/10/2012 to 17/01/2013) stored at -18 °C. For each sample, the weight, harvest date and place were registered and presented on (Table 3), the pulp of fruits were prepared in Brazil, frozen and then sent to CRA-W (Belgium) as a frozen fruit without the seeds and were stored at -18 °C.
The samples were then ground to obtain fine homogeneous particles by using a simple grinder (IKA A11 Basic)
A part of the samples was used as it is to undergo an analysis by the Mid Infra-Red using the ATR accessory, another part was used for the extraction of the polyphenolic compound and the anthocyanins.

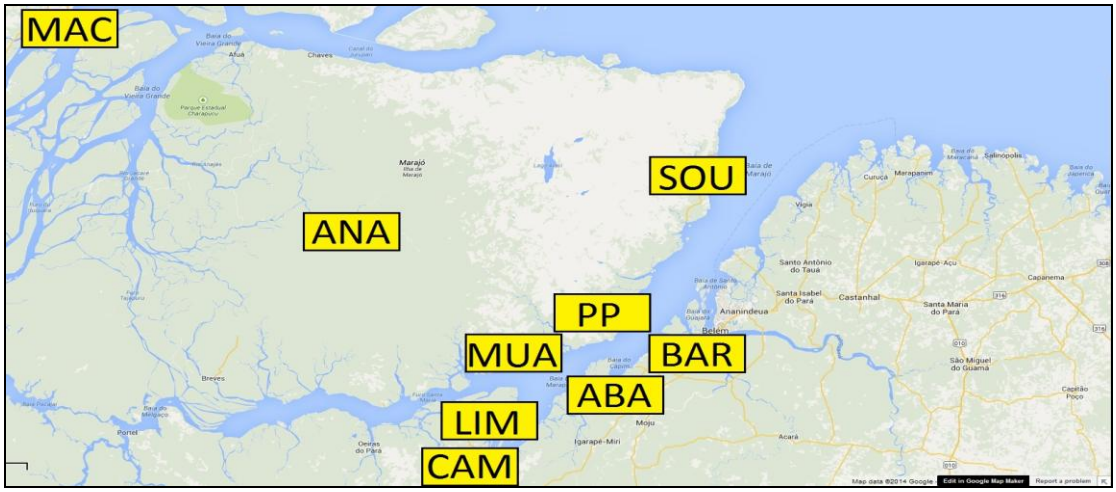


Figure 5: location of the harvested samples

Table 3: Samples number according to the area of cultivation

Locality	Locality code	Number of sample
Barcarena	BAR	5
Acará	ACA	2
Ponta de Pedras	PP	34
Abaetetuba	ABA	25
São Sebastião da Boa Vista	SSBV	2
São Domingos do Capim	SDC	1
Muaná	MUA	13
Cametá	CAM	7
Limoeiro do Ajurú	LIM	4
Soure	SOU	5
Macapá	MAC	3
Anajás	ANA	4
TOTAL		**105**

The extraction of the polyphenolic compounds was performed by 2 ml /g of acai in methanol, in 22 °C, and centrifuged two times as the (Figure 6) shows, the extracted solution was stored in 18 °C, until the measurement process.

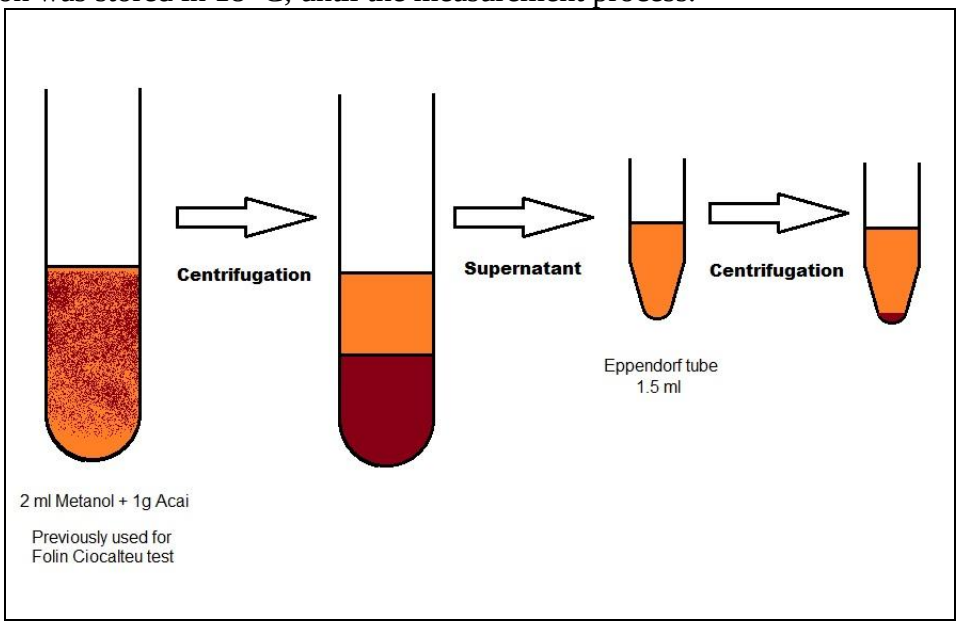


Figure 6: Extraction of polyphenols

The extraction of the anthocyanins was performed according to the Association of Analytical Communities (AOAC) International official methods board (AOAC method 2005.02) (described in Annexe 2) by two buffer solutions at pH 1 and 4.5 for 2 hours in room temperature.

3.2 Infra-red analysis

The infra-red spectral measurements were performed by an FT-IR spectrometer Bruker Vertex 70 (Figure 7), equipped with an ATR accessory (Golden Gate).

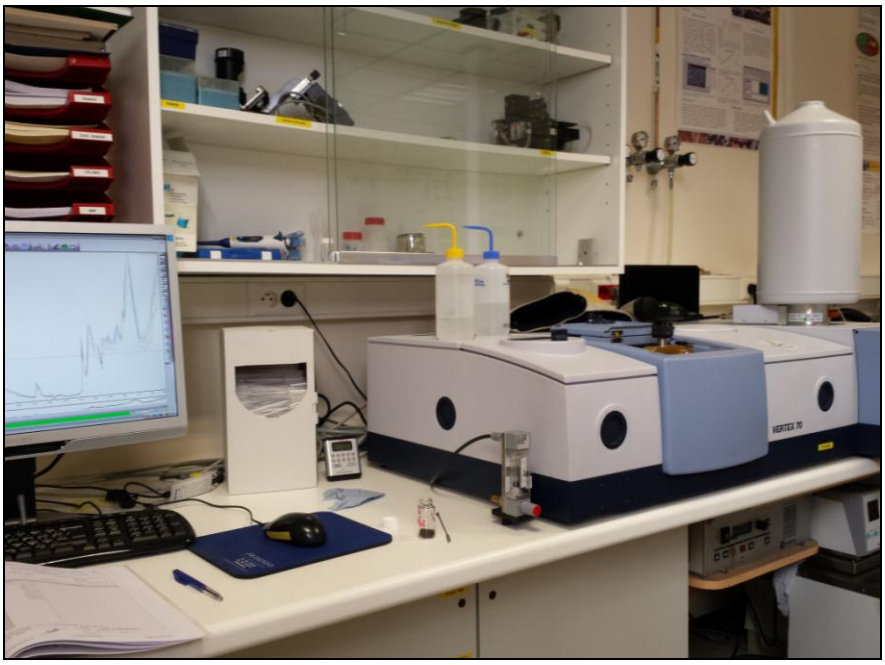


Figure 7: The Bruker (Vertex 70) used to perform the MIR analysis

The infra-red spectral measurements were performed randomly using an ATR accessory to obtain the spectra of the pulp of the fruit as it is with a 4 cm^{-1} resolution, 64 scans in the range of 4000-600 cm^{-1} and each sample was analyzed 3 times with the ambient air as a background and were pressed in order to ensure a close contact with the crystal.
Concerning the methanolic extracts of the polyphenolic fraction, 5 µl of extracts was added slowly with a courant of compressed air to dry the sample before it surpasses the area of measurement (Figure 8).

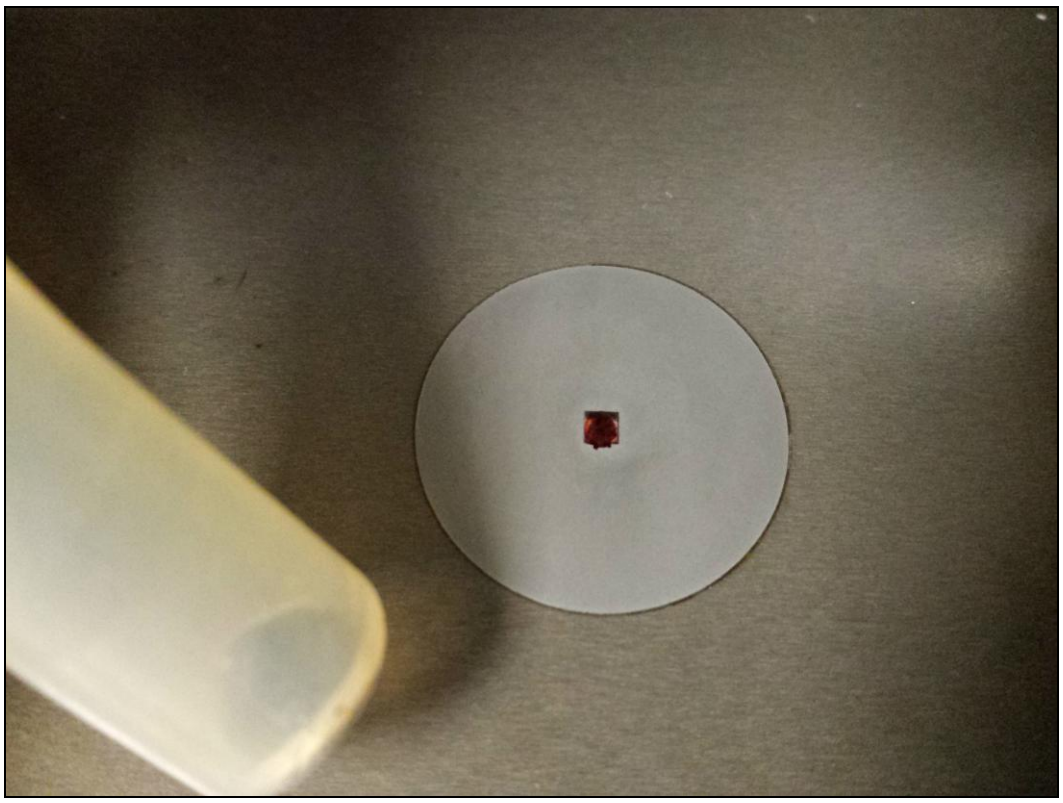


Figure 8: The dried methanolic extract being analyzed by the MIR

The measures were made with 4 cm^{-1} resolution, 64 scans in the range of 4000-600 cm^{-1} and each sample was analyzed 2 times.

The extracts of the anthocyanins were not analyzed by MIR since the separation of the supernatant was difficult and most of the extracts dried and precipitated as salts.

3.3 Total phenolic content (TPC)

Determination of polyphenolic compounds was carried out by Folin–Ciocalteu reagent, in presence of a saturated solution of sodium carbonate.
A standard curve is recommended (Table 4), so stock reference solution of Gallic acid of 100 mg/L was used, then a second solution of 35% of sodium carbonate was prepared by using a stirring plate to ensure the homogenisation and avoid crystallisation. Then the samples were prepared for being analysed (Table 5). 11 samples of Gallic acid were prepared for the determining standard curve as follow:

Table 4: Steps for preparing the standard series for the standard curve

	Volume of SS (ml)	Volume of water (ml)	Volume of Folin react (ml)	Volume of sodium carbonate after 3 minutes (ml)	Volume of water to complete to 10 ml (ml)
1	0.0	5.2	0.5	1.0	3.3
2	0.2	5.0	0.5	1.0	3.3
3	0.4	4.8	0.5	1.0	3.3
4	0.6	4.6	0.5	1.0	3.3
5	0.8	4.4	0.5	1.0	3.3
6	1.0	4.2	0.5	1.0	3.3
7	1.2	4.0	0.5	1.0	3.3
8	1.4	3.8	0.5	1.0	3.3
9	1.6	3.6	0.5	1.0	3.3
10	1.8	3.4	0.5	1.0	3.3
11	2.0	3.2	0.5	1.0	3.3

Table 5: Steps for preparing the samples

Volume of extract (µl)	Volume of water (ml)	Volume of Folin react (ml)	Volume of sodium carbonate after 3 minutes (ml)	Volume of water to complete to 10 ml (ml)
40	5	0.5	1.0	3.46

After 1 hour, the optical density has been measured at 725 nm.
The results are expressed in gallic acid equivalent.
To obtain the standard curve an equation of a line Y= aX+b was used to determine the concentration in mg of Gallic acid, which after calculation turned out to be Y=86.2X+0.42
The total concentration (mg/kg) was calculated by multiplying the concentration in mg of Gallic acid by 50 since the amount used to do the test was 2 g and the extraction solution was 2 ml.

3.4 Total anthocyanin content (TAC)

The total anthocyanin content determination by pH-differential method was performed using the Askar & Treptow methods described in the book Quality Assurance in Tropical Fruit Processing [190] which uses the official reference method by the AOAC, method 37.1.68 (AOAC,Official method 2005.02).The method is suitable to determine total anthocyanin content based on structural changes in the anthocyanin chromophore between pH 1.0 and 4.5.
Anthocyanins undergo a reversible structural transformation as a function of pH which is presented as a change in colour (Figure 9)

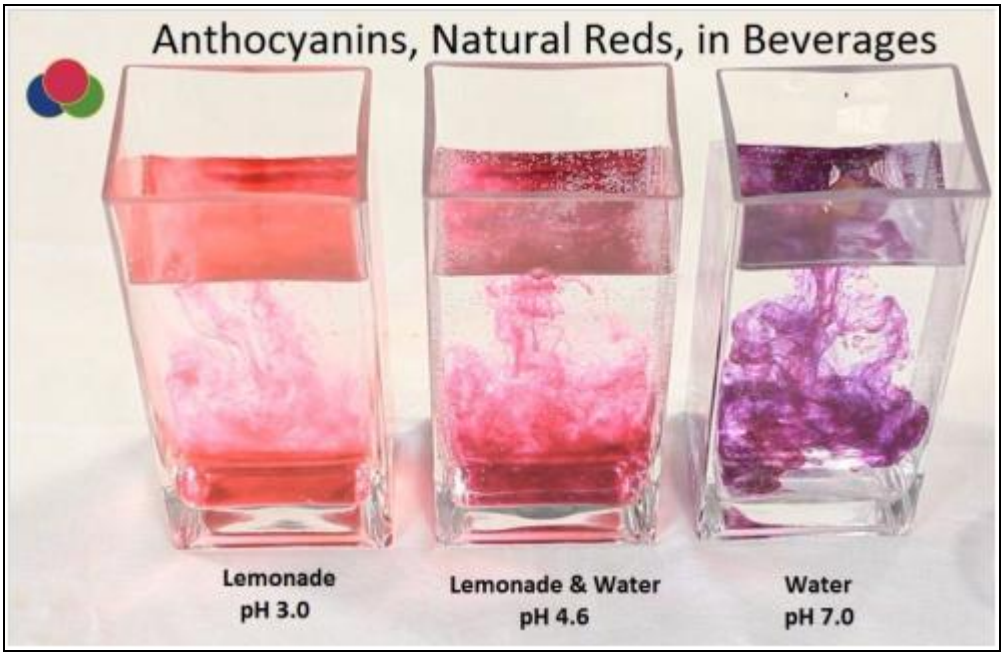


Figure 9: Anthocyanins in different media with different pH (source: foodcolor.eu)

Two buffer solutions were prepared with a pH of 1 and 4.5, the one with a pH of 1 was prepared by solubilizing 1.86 g of KCl with 950 mL of distilled water, then with HCl 1N was added until a pH of 1 was obtained, the volume was completed to 1 litre with distilled water. The other buffer was prepared by adding 54.43g of sodium acetate trihydrate $CH_3COONa.3H_2O$ to 930 mL of distilled water, HCL 1N was used to obtain a pH of 4.5 and the final volume was completed to 1 litre by distilled water. In the method procedure, a dilution (10-300) should take place since the extracts or juices are too dark to be measured by a spectrometer, a series of several dilutions was performed and the appropriate dilution factor was 150 times.

For each sample, 1g was dispersed in 3 mL of each buffer solution and mixed with a vortex to ensure the homogenization and to allow a more efficient extraction which took place at the room temperature for two hours, the mixed solution was centrifuged for 4 minutes at 3000 rpm, a filtration was performed with Whatman 1 paper but no residue was found left on the paper so we decide to skip this step.

To achieve a dilution of 150, 100 µL was taken and mixed with 5 mL of the appropriate buffer solution.

The absorbance was then measured using a spectrometer at wavelengths of 514 and 700 nm for each solution.

The concentration of anthocyanin in the extraction is obtained by the following equation:

$$C_1 = ((A_{514} - A_{700})_{pH\ 1,0} - (A_{514} - A_{700})_{pH\ 4,5}) \times D \times F$$

Were C: concentration of anthocyanins mg/kg of acai, A: absorbance, D: dilution factor, F: conversion factor for anthocyanin in açai fruit, C: concentration of anthocyanin in mg/kg

4. Results

4.1 Total polyphenols and total anthocyanins content

In order to visualise the general distribution of the phenolic fraction of acai samples studied, total polyphenols contents (TPC) results analysed by Folin–Ciocalteu are shown as a histogram graph and differentiated by the area and date of harvest, the results are detailed in the Annexe 3
Only regions containing high number of samples are presented here. The harvest area did not show any preference, each region has samples of high and low concentration of phenolic content (Figure 10).

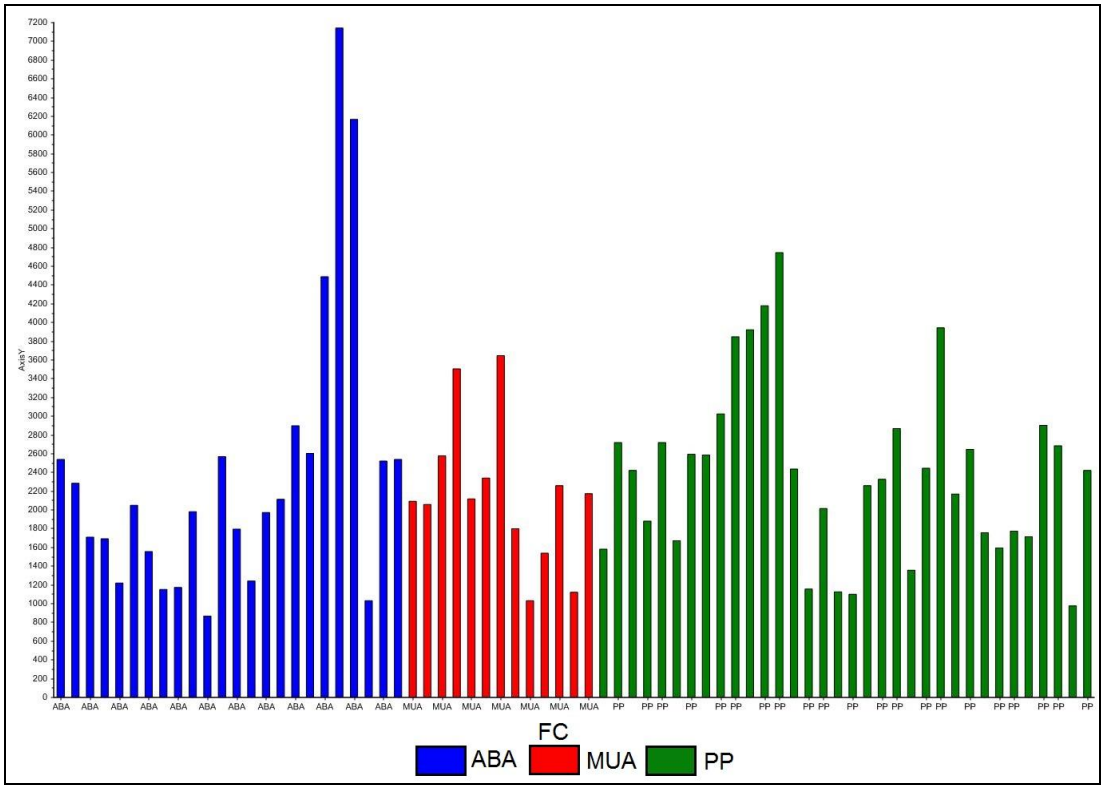


Figure 10: Histogram graph of polyphenols contents differentiated by areas

The harvest date did not show any preference, each day shows samples of high and low concentration of phenolic content (Figure 11).

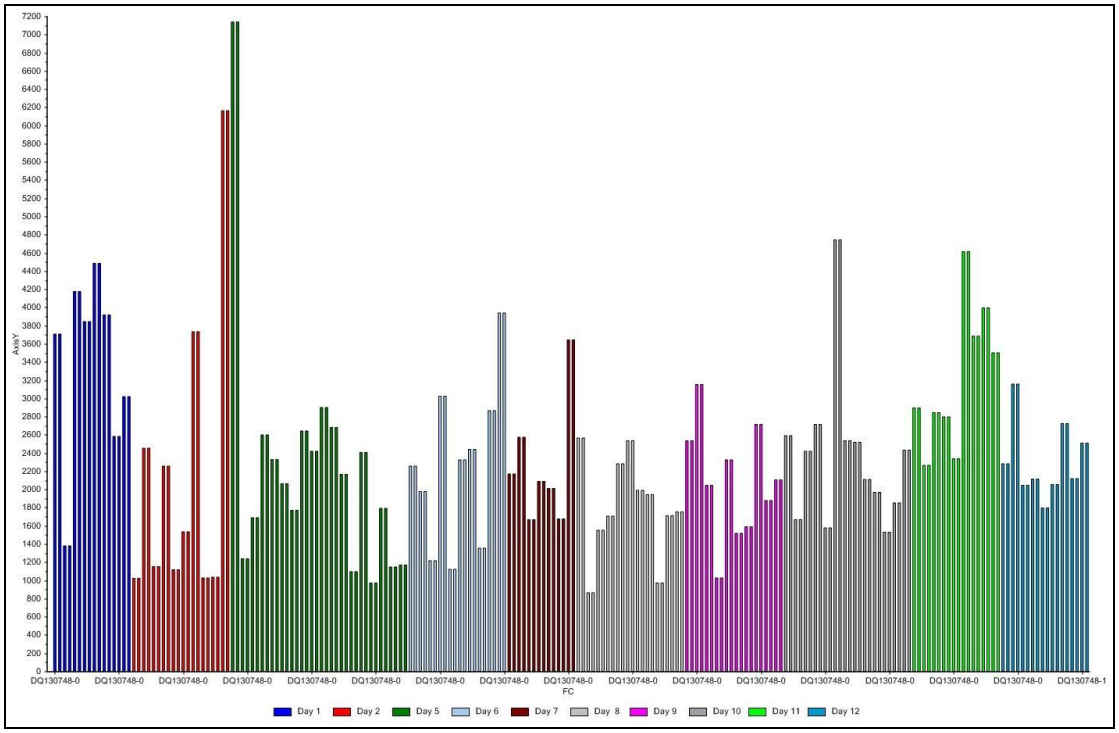


Figure 11: Histogram graph of polyphenols contents differentiated by days

In the same way, the total anthocyanin content (TAC) results are shown as a histogram graph and differentiated by the area and date of harvest, the results are detailed in the Annexe 4.

Only regions containing high number of samples are presented here. The harvest area did not show any preference, each region has samples of high and low concentration of anthocyanins content (Figure 12).

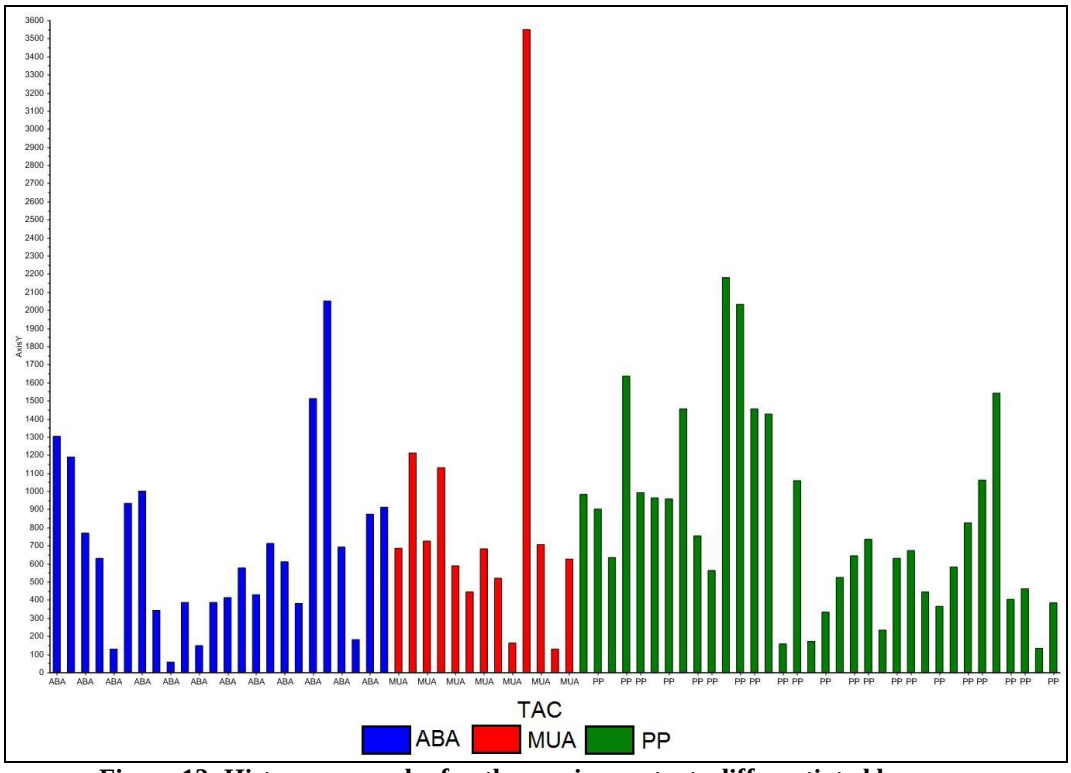


Figure 12: Histogram graph of anthocyanins contents differentiated by areas

The harvest days did not show any preference, each day has samples of high and low concentration of anthocyanins content (Figure 13).

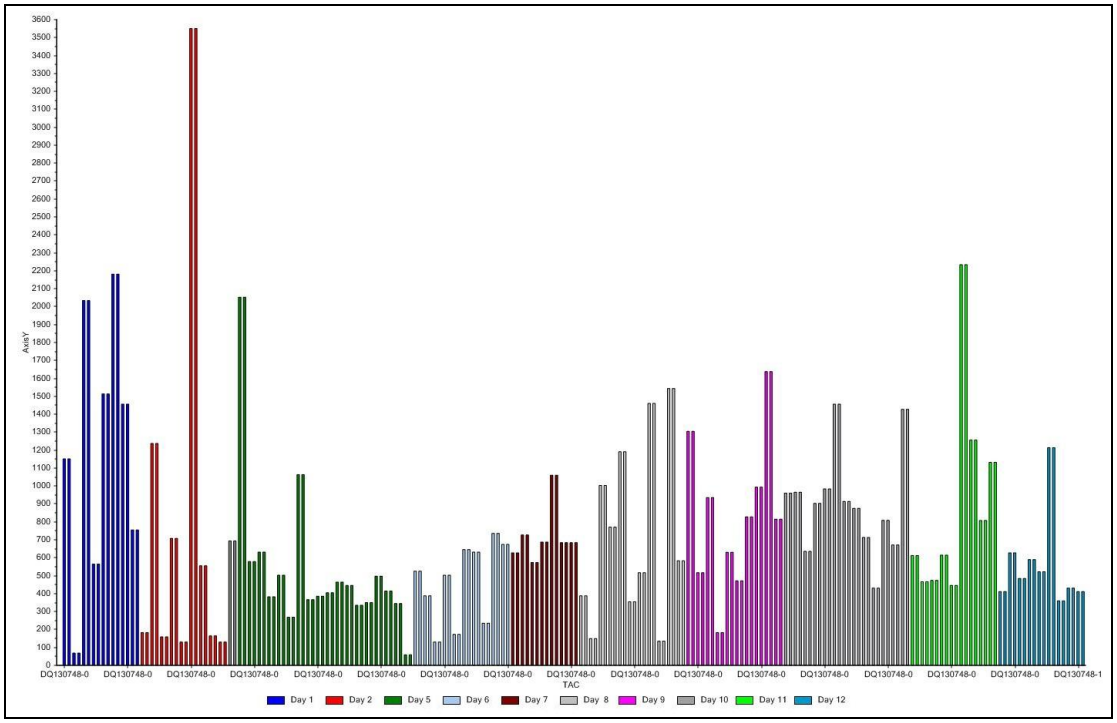


Figure 13: Histogram graph of anthocyanins contents differentiated by days

After taking into account the results of the polyphenols and anthocyanin compounds, and considering the minimum and maximum values and numbers of high and low values for both results, we defined 3 ranges for both tests to differentiate between low, medium and high value of both polyphenols and anthocyanin compounds.

Concerning the polyphenols the range was defined in tales 6, 7:

Table 6: Ranges of polyphenols content

Concentration	Low	Medium	High
mg/kg of acai	< 1400	1400 - 3000	>3000

Concerning the total anthocyanin contents the range was defined as below:

Table 7: Ranges of total anthocyanins content

Concentration	Low	Medium	High
mg/kg of acai	< 350	350 - 1250	>1250

A correlation graph was performed for the total anthocyanin and polyphenols contents (Figure 14) but the result shows no good correlation between TPC and TAC.

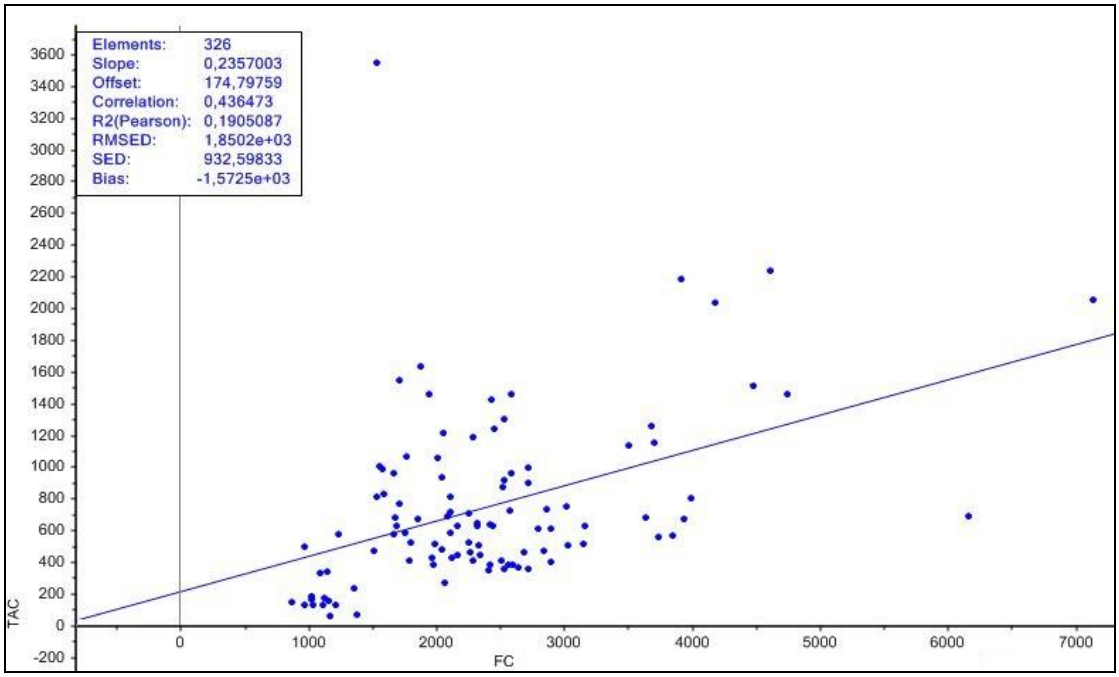


Figure 14: Correlation of TAC and TPC

The correlation was poor, so a second graph was done after eliminating the outliers which are 6 points that are the most far from the line (Figure 15) but the correlation was still poor which indicates even if two samples contain same amount of phenolic content they may differ in total anthocyanins content, which mean that anthocyanins are not presented as a fixed portion of polyphenolic compounds.

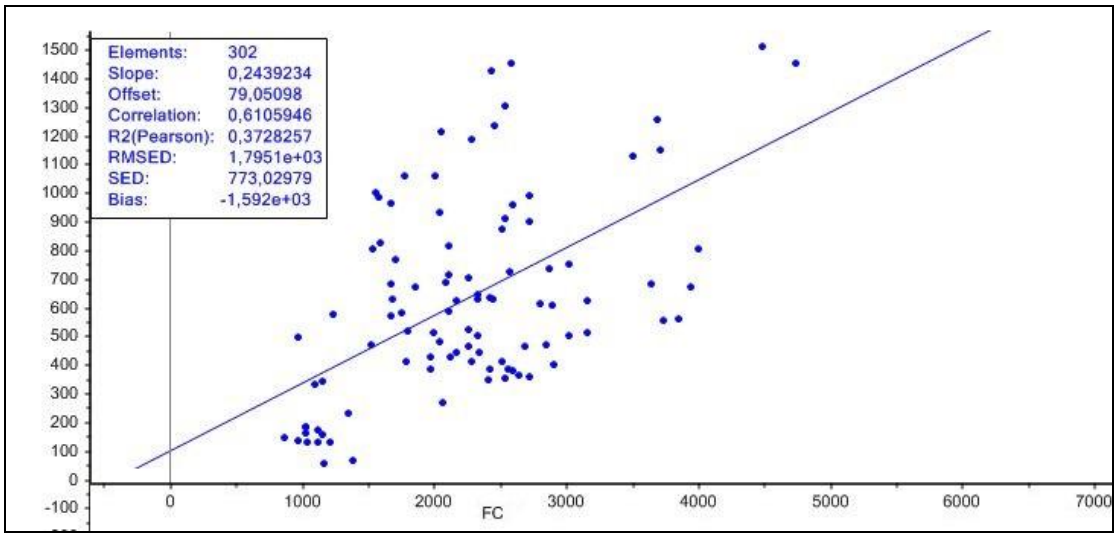


Figure 15: Correlation of TAC and TPC after eliminating the outliers

4.2 Mid Infra-Red spectroscopy

Sampling, experiment, environment and modelling all contribute to the MIR method accuracy. The effect of these parameters should be carefully studied during method development and validation. The impact of some of these factors, are easier to correct than others. For now, a wider selection of instruments with improved resolution/sensitivity is now available, environmental conditions during manufacturing are better controlled, and data acquisition settings can be optimized.

To control these factors the samples were well homogenised, the temperature in the lab was controlled to around 24 °C, and to decrease the effect of humidity, a background spectrum was collected before analysing each sample. Other factors, like instrumental error, on the other hand, are more difficult to control and/or correct. To achieve accurate method accuracy, the interference from these factors must be eliminated or minimized using appropriate sample presentation and/or math pre-treatments. The goal of data pre-treatment is to eliminate or minimize variability unrelated to the property of interest, so that pertinent changes can be more effectively modelled.
The most commonly used pre-processing methods in spectroscopy are derivatives, Standard Normal Variety (SNV) and normalized values.
Derivatives like Savitzky–Golay are among the most common signal pre-treatments applied to spectral data. Derivatives are mainly used to resolve peak overlap (or enhance resolution) and eliminate constant and linear baseline drift between samples.
SNV is another frequently used pre-treatment method due to its simple algorithm and effectiveness in scattering correction. SNV is often used on spectra where baseline and pathlength changes cause differences between otherwise identical spectra.
The spectra obtained by the infrared measurement were analysed by The Unscrambler X, the raw data was analysed with and without pre-treatment, the pre-treatment includes treating raw data with SNV analysis, first derivative analysis (SGolay), and normalization.

PCA analysis

4.2.1 PCA on fruit powder spectra

The carbon dioxide and humidity in the air are also measured during the measurement of the açai. CO_2 absorb in the wavelengths between 2400 cm^{-1} and 1800 cm^{-1}, so we eliminate that part from spectra treated.
Two different ranges of the spectra were distinguished after elimination (Figure 16), the first part we called it the "Useful spectra" which consists of two regions that lies between 3750 - 2400 cm^{-1} and 1800 – 600 cm^{-1}, the second part is called the "Fingerprint" region and lies between 1800 – 600 cm^{-1}. The graph below shows the two regions on a spectrum.

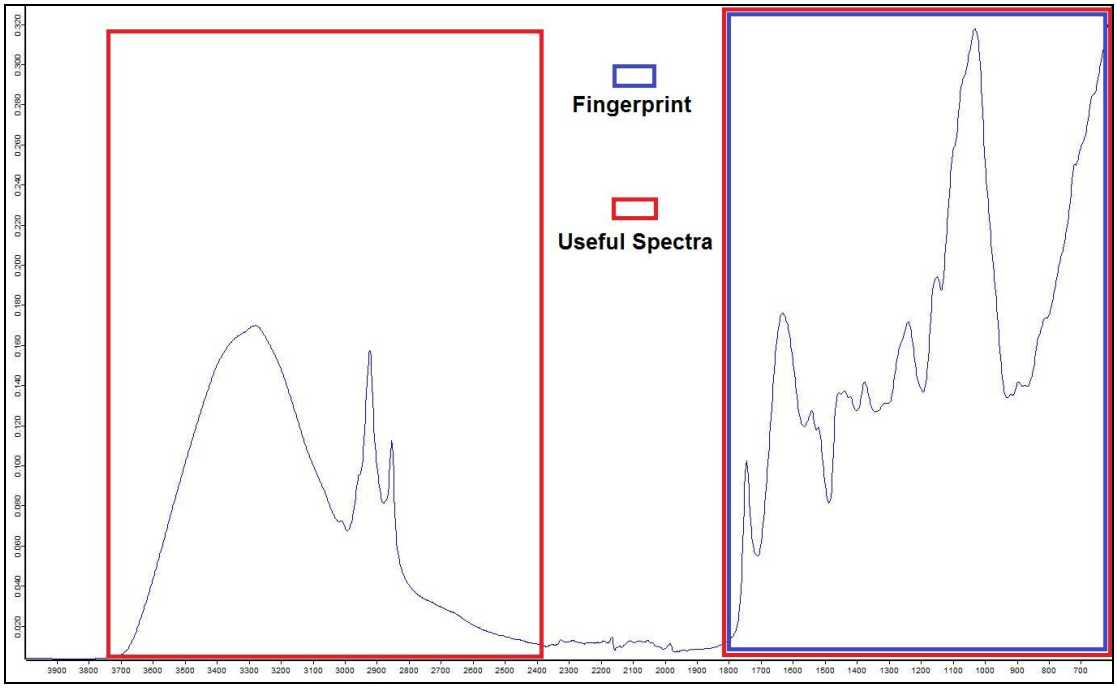


Figure 16: Different regions used of the MIR spectrum illustrated on a random acai sample

A study on grape measuring the phenolic content showed that the best PLS regression model was the one obtained when working in the region of 1168-1457 cm^{-1} because it gave the most accurate and robust prediction for total phenolic compounds (Fragoso et al., 2011), so we did the analysis on this region too and we called it super fingerprint, but we didn't find any considerable differences or improvement using it (Annexe 5).

PCA (Principal Component Analysis) was performed on data of all matrixes obtained (with and without treatment).
The PCA results for the four matrixes (with different pre-treatments) were very similar.
After differentiation of the PCA results by the polyphenol content (Figure 17) and the anthocyanin content (Figure 18) using the Folin–Ciocalteu and the pH-differential method respectively as reference methods, the results below were obtained.

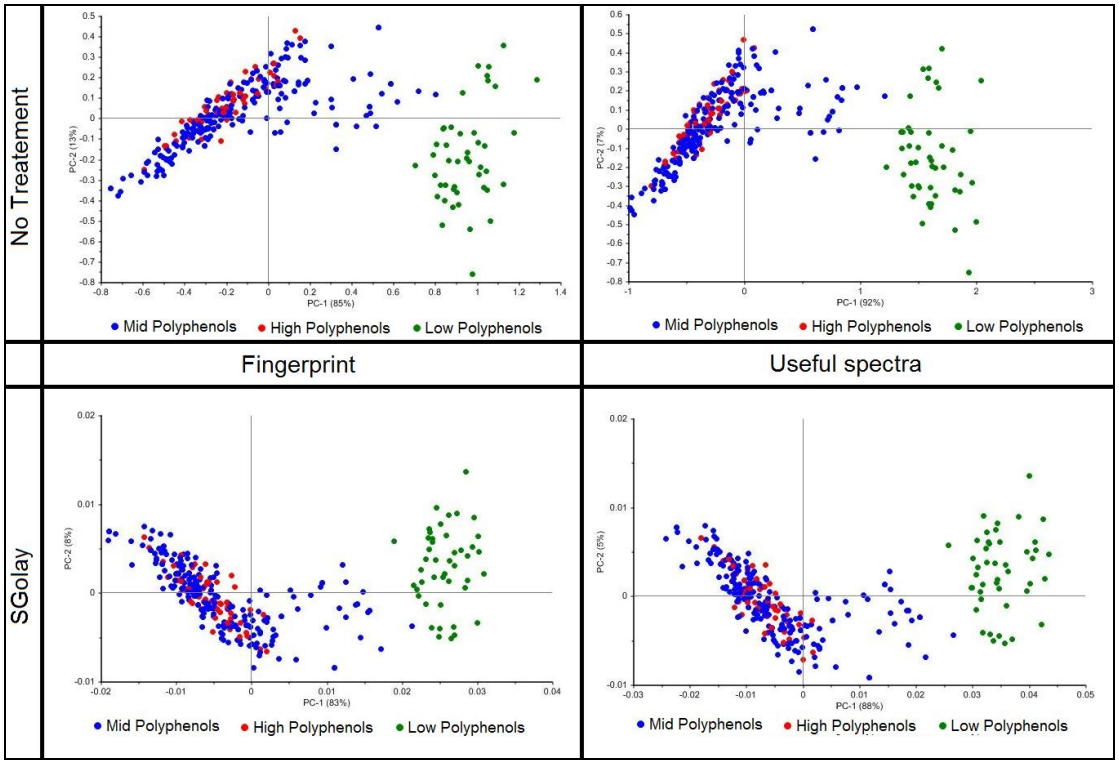


Figure 17: PCA graph where samples are differentiated by values of polyphenols content for the powder (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra)

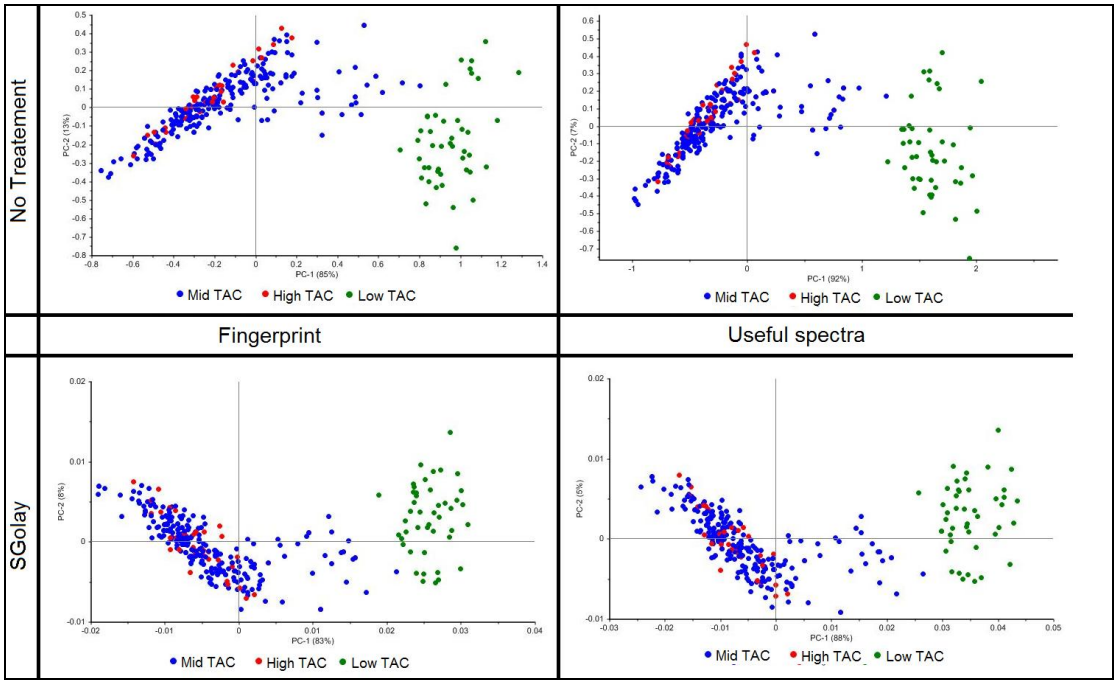


Figure 18: PCA graph where samples are differentiated by values of total anthocyanin content for the powder (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra)

As illustrated above the green dots which represent the low values of both TAC/TPC are gathered together to form like a distinct group, the blue and red dots which represent the mid and high values respectively for both TAC/TPC are kind of merged together.

This grouping may indicate that the method is useful for differentiation between the low and mid/high value, which we confirmed later by the RPD, RMSEcv and R^2 values (definitions will be given later).

The first cloud of dots (blue and red) represent a distinct group aside from a few samples (7 samples in the midle group), which we believe they contained amounts of fibres from the seeds during sampling (separating the pulp form the seed) and we obtained visual confirmation on some of them that they had fibres.
To confirm the presence of fibres, a loading graph was used to determine the distinct peaks of the samples in the middle (Figure 19), using the PC1 as it shows in the PCA (Figure 20) that it separates the mid-high group from the samples expected to obtain fibres.

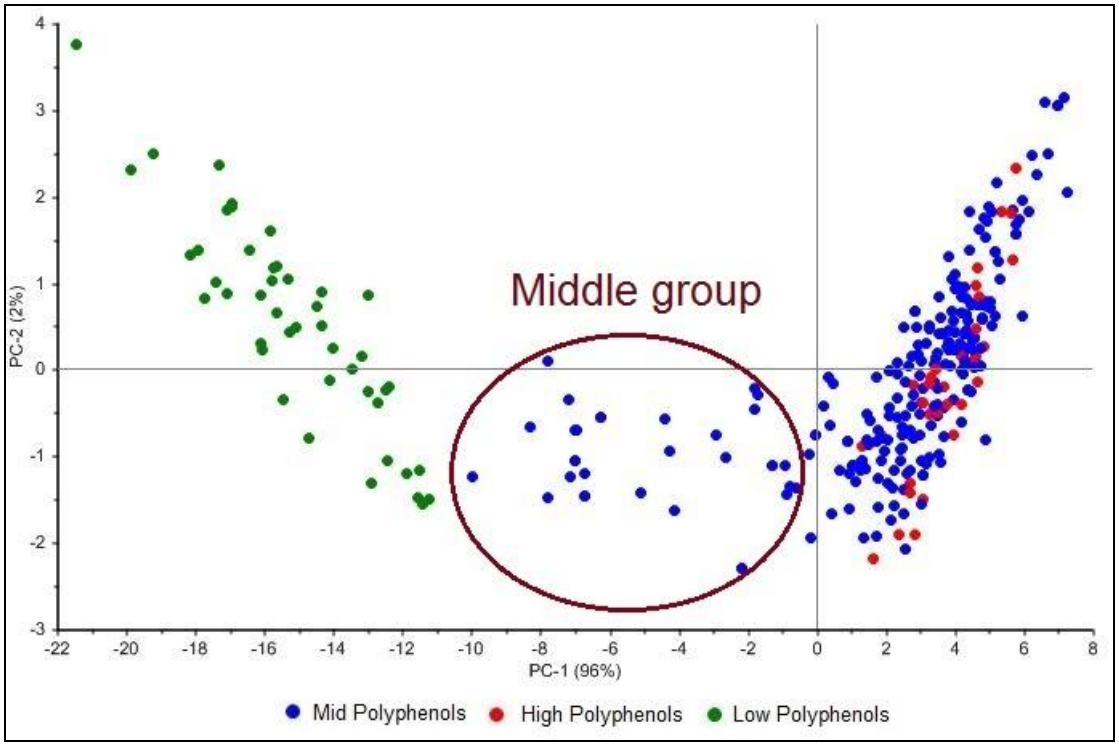


Figure 19: PCA graph of powder showing the middle group and the PC-1 used (pre-treatment: SNV, region used: Fingerprint)

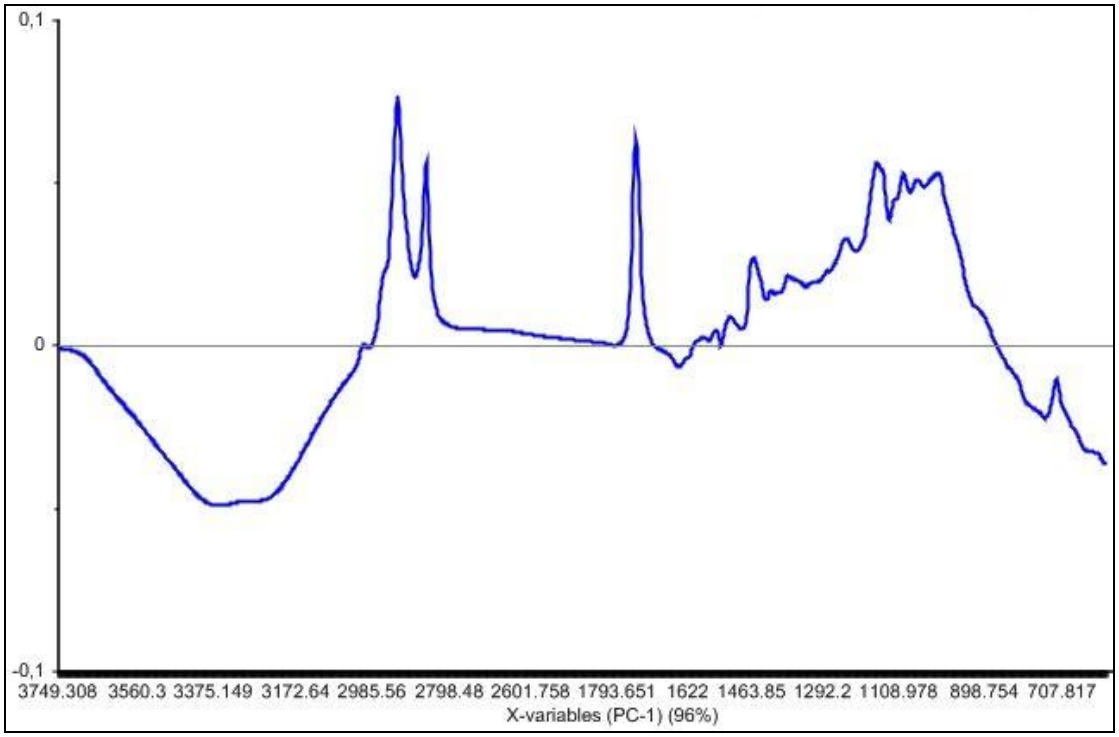


Figure 20: Loading graph using the PC-1

The distinctive peak that characterize the middle group is the peak in the region of 3400 – 3200 cm^{-1} which is the region for the functional group –OH, and since the fibres, which consist mainly of cellulose and hemi-cellulose, contains a lot more hydroxyl group than the polyphenols, this indicate that those samples contain substance with more hydroxyl group which match our suspicion of containing fibres. We did a PCA without the low concentration group (Figure 21), since the middle group was in one quarter of the PCA (right lower in our case) separated by the PC-1 and PC-2, we took in consideration only the positive peaks of PC-1 and the negative peaks of PC-2 on the loading graph.

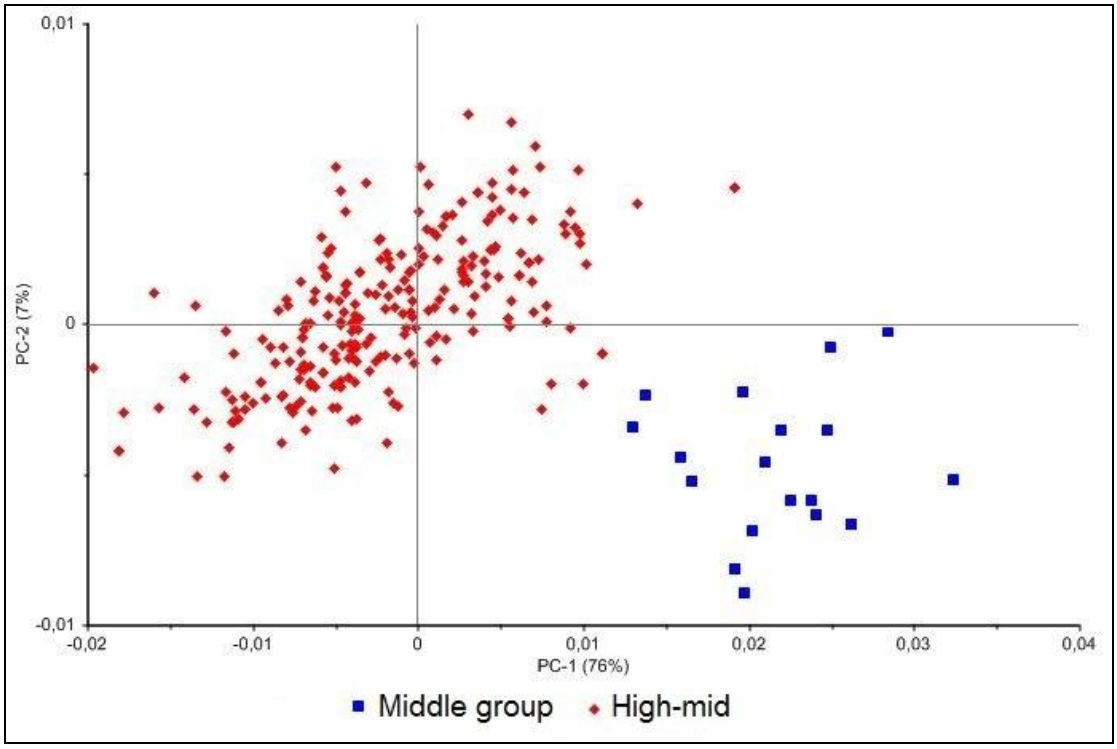


Figure 21: PCA graph of the middle group and he high-mid group (pre-treatment: Savitzky–Golay, region used: Useful spectra)

The he characterization of the spectral profile of cellulose was done essentially through "Infrared Characteristic Group Frequencies, (Socrates G., 1997)".We searched in the loading graph for the peaks of the cellulose since we suspect its presence in the samples of the middle group, the peaks of the cellulose were clearly present and identified (Figure 22), which indicate that the middle group contained amount of cellulose.

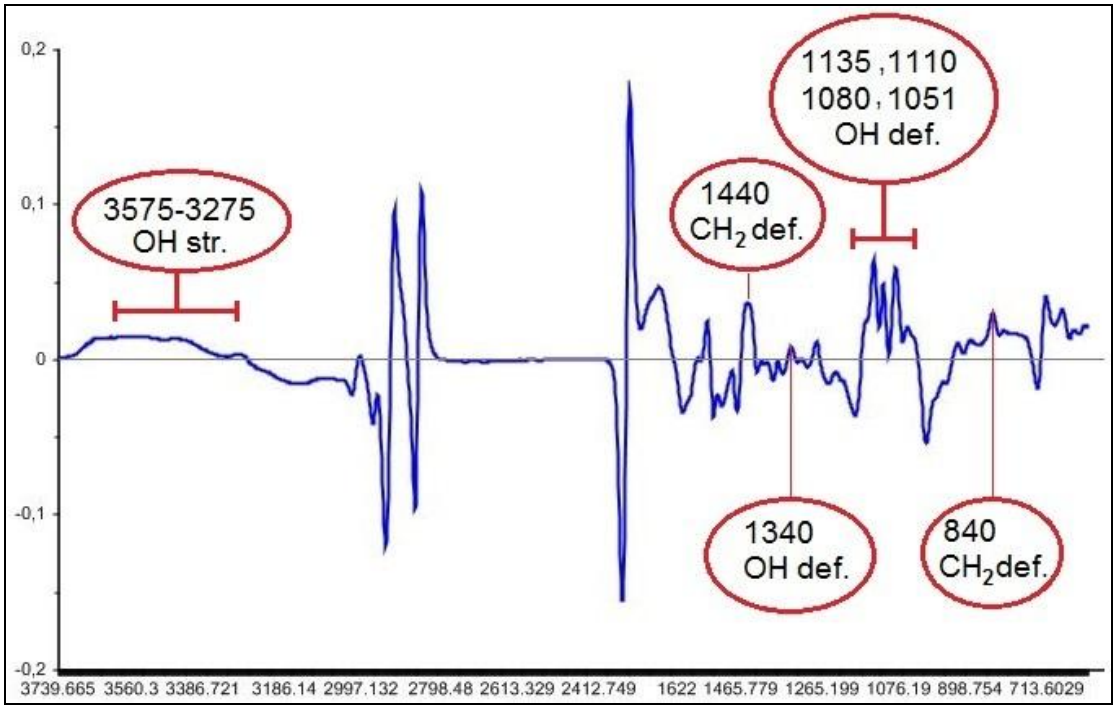


Figure 22: Loading graph of the middle group and the high-mid group with the peaks of the cellulose highlighted

We did a PCA differentiated by the days of cultivation and the area of cultivation but no useful information were obtained (Figure 23, 24)

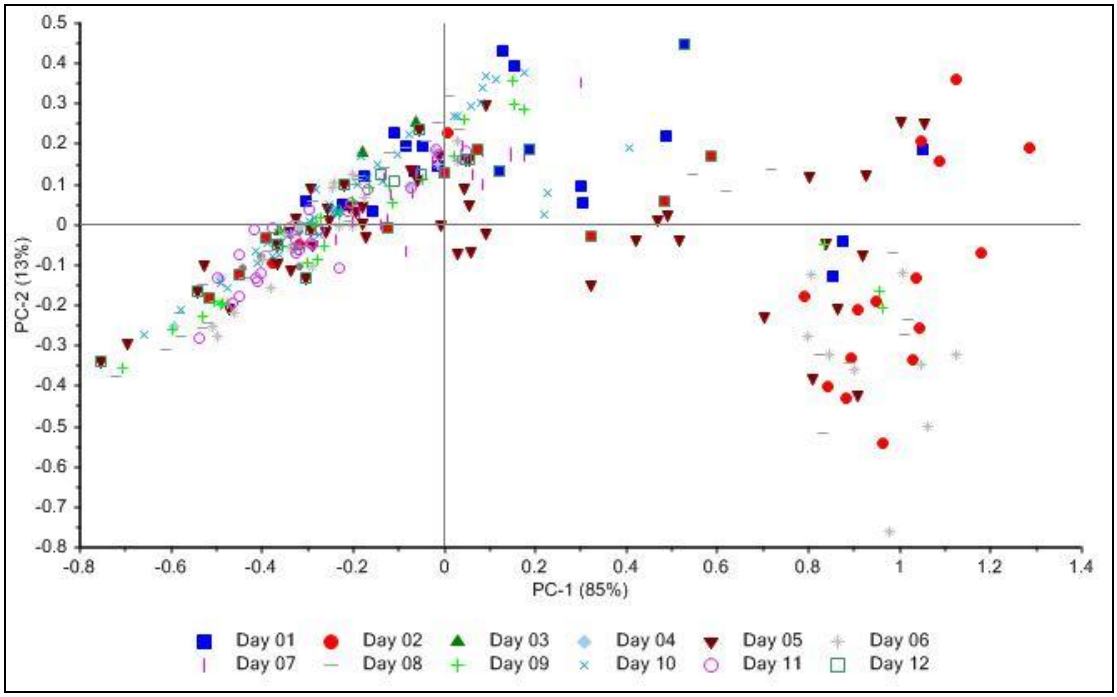


Figure 23: PCA graph of powder differentiated by cultivation days (pre-treatment: none, region used: Fingerprint)

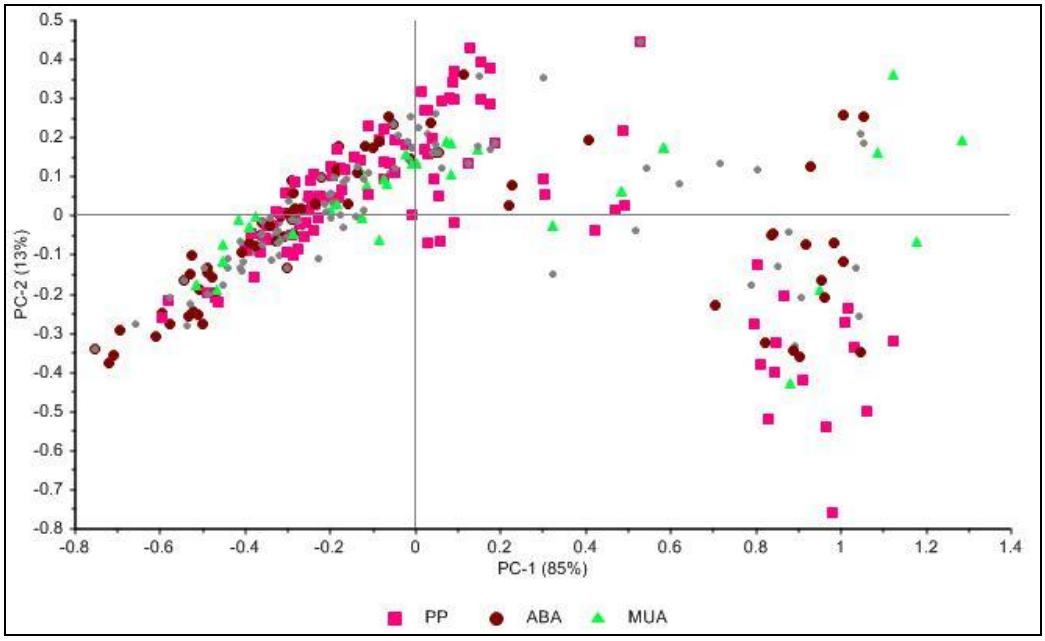


Figure 24: PCA graph of powder differentiated by cultivation area (pre-treatment: none, region used: Fingerprint)

As the reference analysis by Folin–Ciocalteu and pH-differential method revealed, there is no differentiation between the days or regions of harvest PCA's of the fruits, so the analysis on the extracts was performed.

4.2.2 PCA on phenolic extracts spectra

No big differences were observed between the spectrum of powder and spectrum of phenolic extract for the same sample (Figure 25). Spectra of extracts seem to be more resolved due to the reduction of the matrix effect encountered with raw material.

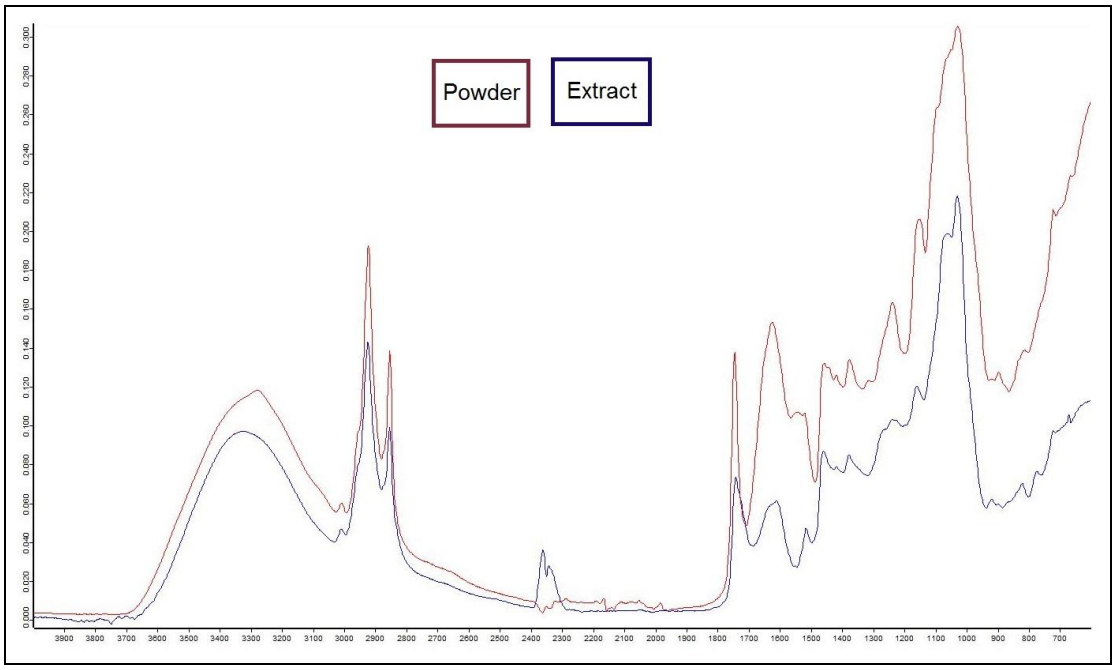


Figure 25: Spectrum of powder and spectrum of phenolic extract for the same sample

PCA (Principal Component Analysis) was performed on data of all matrixes obtained (with and without treatment) as the results below show (Figure 26, 27)

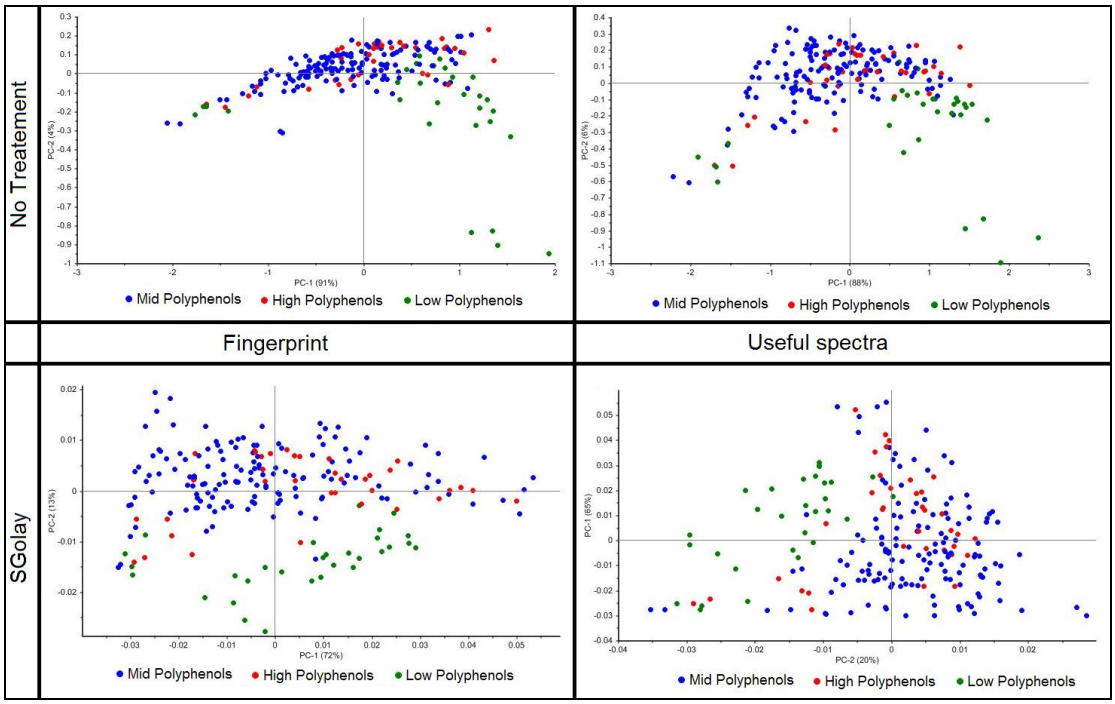


Figure 26: PCA graph were samples are differentiated by values of polyphenols content for the extracts (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra)

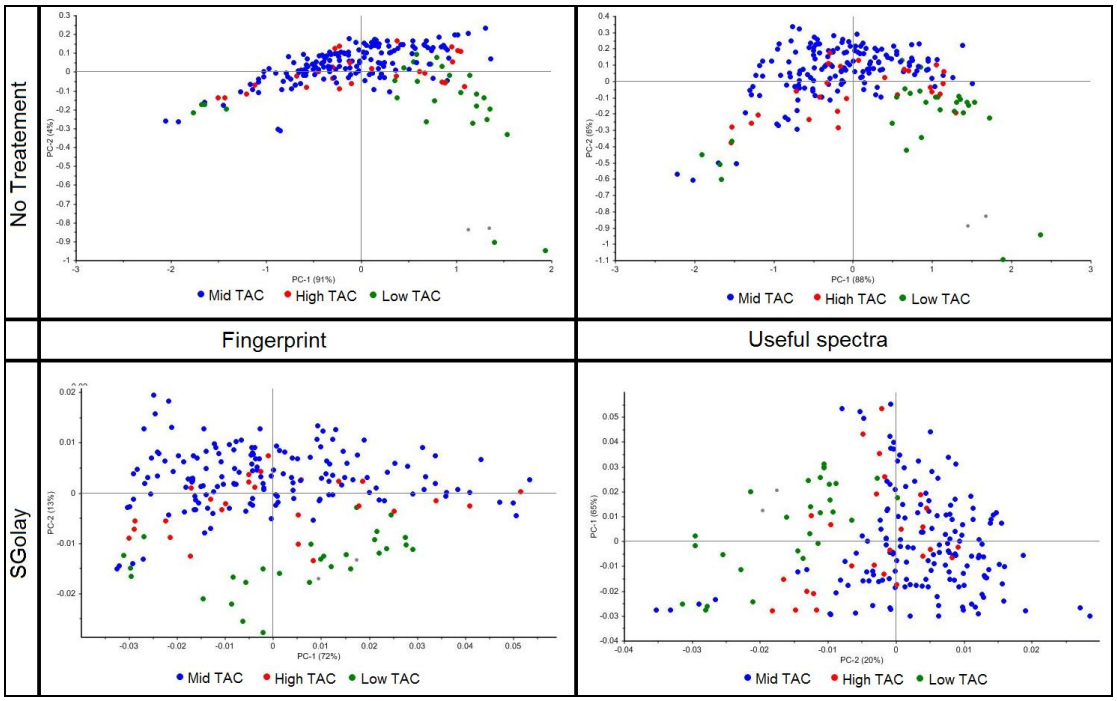


Figure 27: PCA graph where samples are differentiated by values of total anthocyanin content for the extracts (pre-treatment: none/Savitzky–Golay, region used: Fingerprint/Useful spectra)

Compared to the PCA obtained on the fruit spectra, the three groups formed are not visible here and it's possible to distinguish here only 2 groups, which indicates that the intermediate group presented in the fruit's PCA has no relation with the phenolic content but is a result of the presence of different compound that were present in the fruits but are no longer present in the extracts like fibres.

We did then a PCA differentiated by the days of cultivation and the area of cultivation but no useful information were obtained (Figure 28, 29, 30)

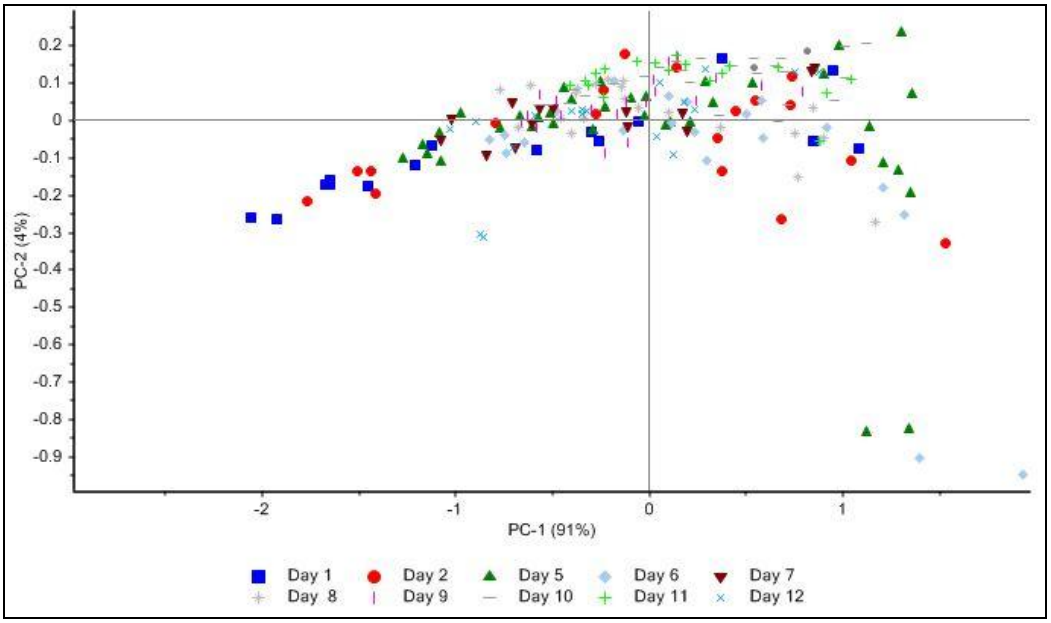


Figure 28: PCA graph of extracts differentiated by cultivation days (pre-treatment: none, region used: Fingerprint)

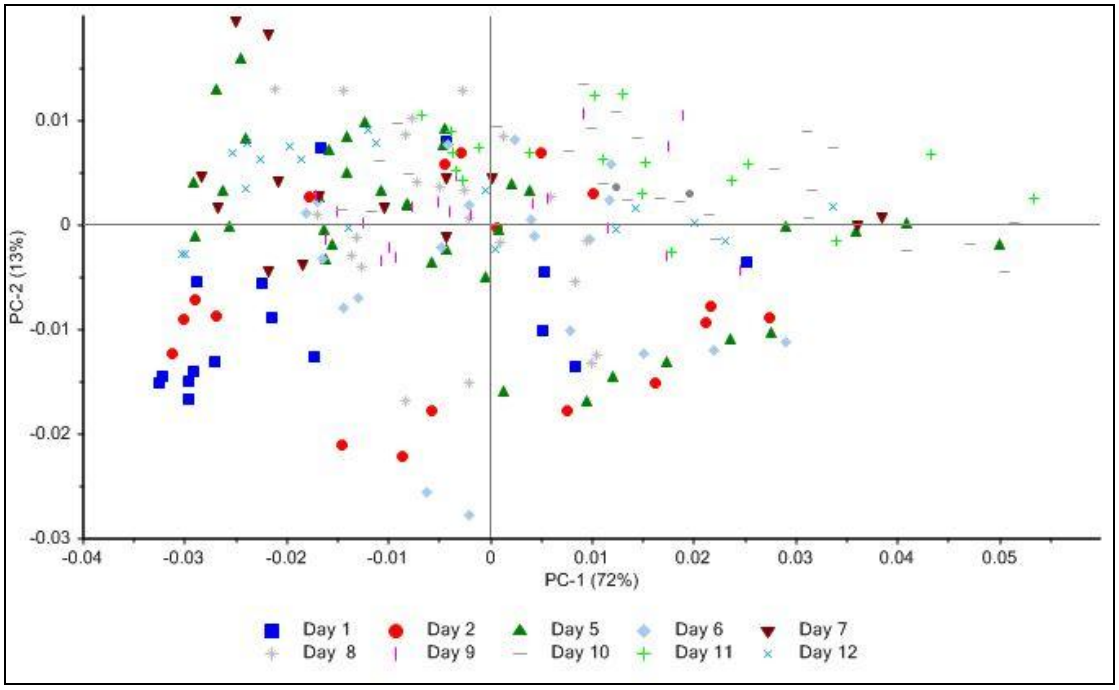


Figure 29: PCA graph of extracts differentiated by cultivation days (pre-treatment: Savitzky–Golay, region used: Fingerprint)

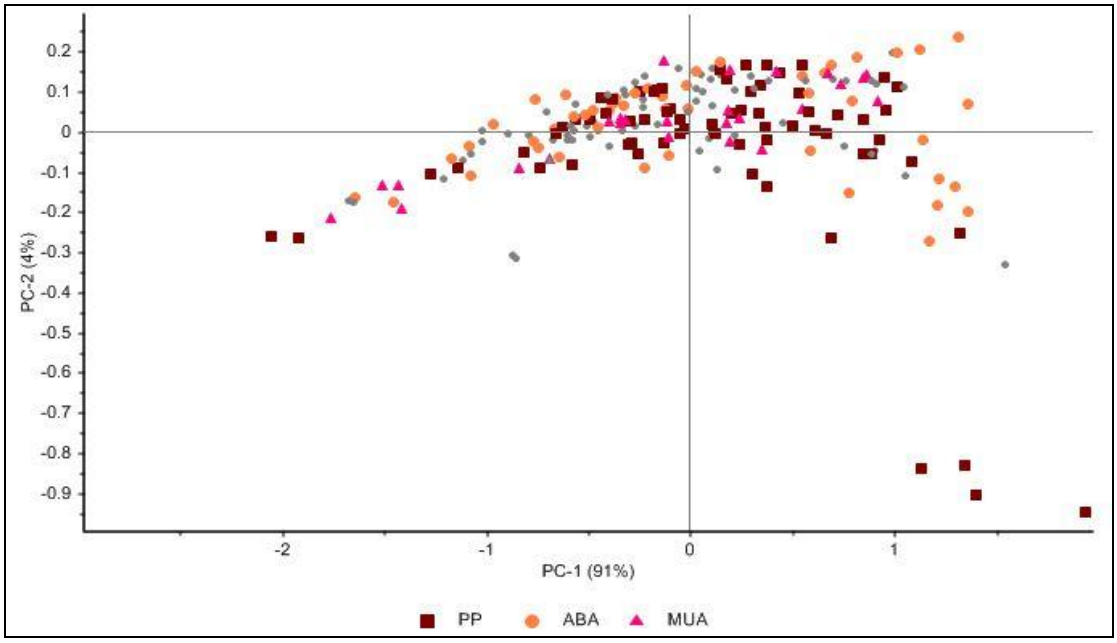


Figure 30: PCA graph of extracts differentiated by cultivation area (pre-treatment: none, region used: Fingerprint)

As the PCAs on the fruits and their phenolic extract revealed, there are some classes in function of phenolic or anthocyanin, so a PLS was performed next in order to investigate the ability of the method to estimate the richness of acai fruits in phenolic compounds and particularly anthocyanins.

4.3.3 Partial least squares regression using fruits and extracts spectra

A PLS-R was adopted for proper calibration performance since it's a recent technique that generalizes and combines features from principal components analysis and

multiple regressions. The performance of the PLS-R models can be evaluated by analysing different factors, in our study we choose the root square R^2,RMSEcv and the RPD-CV (residual predictive deviation) which is the ratio of standard deviation to root mean square error of cross validation (Table 8)

There are 7 levels of accuracy based on the R^2 (Williams, 2003), if the R^2 value is less than 0.25, the calibration is considered not usable, if it lies between 0.26 and 0.49 the correlation is very poor, between 0.5 and 0.64 the calibration is acceptable for rough screening, between 0.66 and 0.81 the calibration can be used for screening and approximate predictions, between 0.82 and 0.9 it's usable for most application, between 0.92 and 0.96 it's usable for most application including quality assurance, above 0.98 it's usable for any application.

Five levels of prediction accuracy based on RPD value were also considered. An RPD value below 1.5 indicates that the prediction accuracy has not been increased by the sensor compared to using the mean value for all samples, thus the calibration is not usable, a value between 1.5 and 2 reveals that the prediction error has decreased to half the original one, which can be interpreted as a possibility to distinguish between low and high values, a value between 2 and 2.5 approximate quantitative predictions for the manure composition can be made, between 2.5 and 3, above 3, the prediction accuracy is classified as good and excellent respectively.

The best R^2 and RPD were obtained from the first derivative SGolay after eliminating the outliers, for both the fruit and the extract, Table 9 illustrates the values obtained for R^2 and RPD, in both fruits and extracts.

Table 8: R^2, RPD and RMSEcv values

Powder	FC	All							
		Useful Spectra			Fingerprint			Super Fingerprint	
		R^2	RPD	RMSEcv	R^2	RPD	RMSEcv	R^2	RPD	RMSEcv
		0.68	1.65	640	0.72	1.78	592	0.68	1.71	619
	TAC	All							
		Useful spectra			Fingerprint			Super Fingerprint	
		R^2	RPD	RMSEcv	R^2	RPD	RMSEcv	R^2	RPD	RMSEcv
		0.37	1.21	445	0.4	1.20	448	0.32	1.18	455
Extract	FC	All							
		Useful Spectra			Fingerprint			Super Fingerprint	
		R^2	RPD	RMSEcv	R^2	RPD	RMSEcv	R^2	RPD	RMSEcv
		0.75	1.89	559	0.75	1.90	556	0.70	1.82	579
	TAC	All							
		Useful Spectra			Fingerprint			Super Fingerprint	
		R^2	RPD	RMSEcv	R^2	RPD	RMSEcv	R^2	RPD	RMSEcv
		0.42	1.16	464	0.29	1.13	478	0.25	1.12	481

Table 9: Best (obtained after eliminating outliers) R^2 and RPD value

Powder TPC		
Fingerprint		
R^2	RPD	RMSECV
0,72	2,15	491

Powder TAC		
Useful Spertra		
R^2	RPD	RMSECV
0,6	1,55	39

Extract TPC		
Fingerprint		
R^2	RPD	RMSECV
0,73	2,2	476

Extract TAC		
Fingerprint		
R^2	RPD	RMSECV
0,8	2,003	270

As the results obtained on spectra of fruits show, without eliminating the outliers both RPD and R^2 values were kind of low, but after the elimination of the outliers, both values get significantly better.
Concerning the powder for total polyphenol contents (TPC), the R^2 reached a value between 0.66 and 0.81 (0.72) which indicates that the calibration can be used for screening and approximate predictions, and the RPD reached a value between 2 and 2.5 (2.15) which indicate that approximate quantitative predictions for the manure composition can be made. But for the total anthocyanin contents (TAC), both values were low with RPD between 1.5 and 2 which can be interpreted as a possibility to distinguish between low and high values.
Concerning the extracts for both total anthocyanin contents (TAC) and total polyphenol contents (TPC), the R^2 reached a value between 0.66 and 0.81 (0.75 and 0.73 respectively) which indicates that the calibration can be used for screening and approximate predictions, and the RPD reached a value between 2 and 2.5 (2.2 and 2.003) which indicates that an approximate quantitative predictions for the manure composition can be made.

5. Conclusion & perspectives

The analysis of a total of 105 samples of acai by Mid Infra-Red spectroscopy using an ATR accessory revealed no differences in concentration of TPC and TAC between the regions or date of harvest.
Good sampling preparation showed a high importance, since contamination with foreign substances like fibres when preparing samples may affect the spectrum of each sample.
The problem may be overcome by studying the phenolic extracts to do the analysis which will decrease the interference of the fibres and other non-soluble substances.
Although Near Infra-Red (NIR) spectroscopy was found to be a reliable method to determine soluble solids and total anthocyanins content (R^2=0.97, RPD=3.08) in açaí pulp (Koizimi et al., 2013), (Inácio et al., 2012), and Mid Infra-Red (MIR) to be a rapid method to quantify phenolic and anthocyanin compounds in grape ($R^2 > 0.95$ and RPD > 4.0 for TPC, $R^2 > 0.90$ and RPD > 3.0 for TAC) (Fragoso et al., 2011), our study showed that the MIR spectroscopy was not as good as NIR for total anthocyanins and phenolic content, and still can be used for screening and approximate quantitative predictions but not as accurate as found by NIR, which was the same for a study conducted on blueberries to examine the potential of MIR and NIR spectroscopy and to evaluate the content of total phenols and total anthocyanins which revealed that a model was developed in the NIR region (RMSECV = 0.50% and RMSEP = 0.65%) and was able to evaluate the content of total phenols and total anthocyanins. The predicted results by MIR were not as good as those obtained in NIR (RMSECV = 0.30% and RMSEP = 0.36%) (Sinelli et al., 2008).
PCA graphs of the powder showed a grouping of samples with low TPC and TAC, contrary to the phenolic extracts where PCA graphs were dispersed randomly. PLS-R results were higher in phenolic extracts (R^2 0.73 - 0.75, RPD 2 - 2.21) than powder (R^2 0.55 – 0.72, RPD 1.55 – 2.15)
This decreasing in values for R^2 and RPD (0.72-0.75 and 2-2.2 respectively), compared to the previous studies, might be due to the non-homogeneity nature of the açai pulp, which even after grinding stayed non-homogenized for naked eyes.
Since previous studies showed that using NIR is a great method to predict the composition of phenolic content in acai (Koizimi et al., 2013) (Inácio et al., 2012) and that the NIR is more effective and accurate than MIR for prediction o phenolic content (Sinelli et al., 2008), our perspective will be to analyse the same samples by the NIR and compare the results of both studies, so we will be able to determine if the problem that we obtained not accurate results is due to the samples or it's because the MIR analyses is really not as effective as the NIR for phenolic content prediction.
Since our study showed that the MIR can be used for screening and approximate quantitative predictions, the next step will be to use the High Throughput Screening (HTS-XT) as an accessory for FT-IR spectrometers to allow automated measurements of samples in either the NIR or the MIR region depending on the result of the NIR analysis.
Another attempt to obtain better results will be to analyse the extracts of the anthocyanins that we used to obtain the references results of the TAC, but one of the obstacle that could face us, is that the solution used to do the extract is a buffer solution that contains potassium chloride and sodium acetate with hydrochloride, so it contains a lot of functional groups that could interfere with the spectra of the samples by absorbing light in the IR region, maybe covering and hiding some important peaks that can be used to predict the content of the analysed samples.

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Annexe

Annexe 1:

The typical setup of an IR experiment includes a light source, a sample, the spectral apparatus, a detector and a computer.

A light source emits polychromatic IR light, normally it's an instrument called interferometer, which is shot on a sample. The light beam is partially absorbed or reflected by the sample when it passes through it. Molecules in the sample interact with the light beam by taking up energy of the light beam and use this energy to vibrate. A detector detects the light that is either reflected or passed through the sample and registers how much light is transmitted through the sample. The data is processed and shown of as a characteristic spectrum showing the transmittance (absorbance) or the reflectance of the light beam, which is characteristic for every molecule.

The main part of a FT spectrometer is the interferometer, in which the light beam is split by a semi-permeable beamsplitter into two partial beams that are reflected back to the beamsplitter via a fixed and a mobile mirror. At the beamsplitter the two beams recombine. The position and motion of the mobile mirror changes the optical path of the light beam, so both partial beams have a phase difference and the interference amplitude is changing. (Figure 31). The detector in the spectrometer records an intensity signal as function of the change of the optical pathlength, which is shown as an interferogram, and then processed to other type graphs.

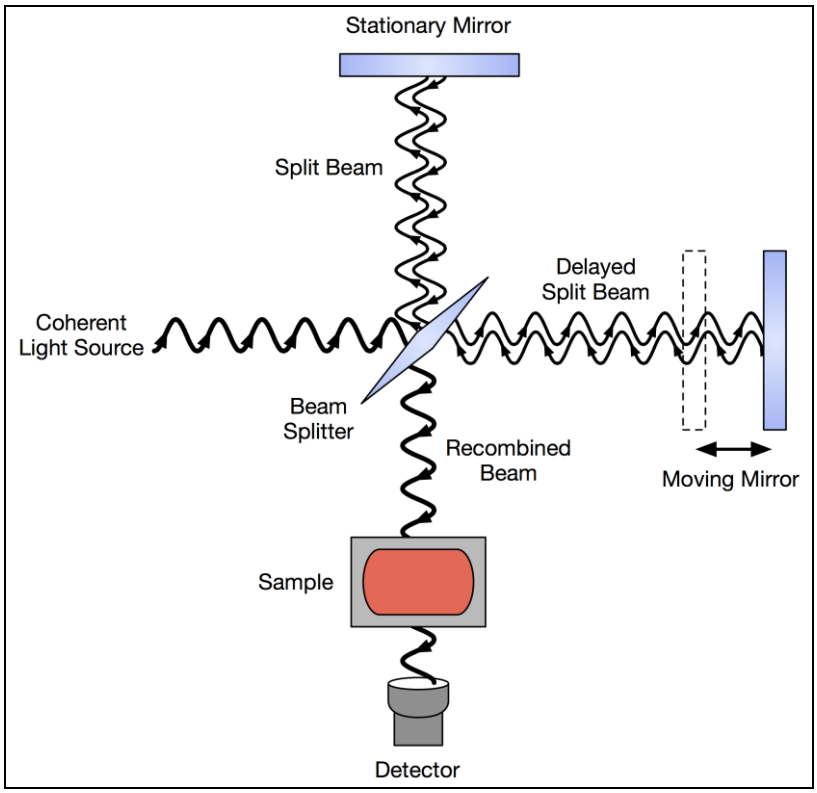

Figure 31: The pathway of the infrared beam in FTIR

Annexe 2:

Total Anthocyanin content (pH-differential method) :

La détermination des anthocyanines se base sur la méthode de Askar & Treptow (1993). Deux types d'échantillons sont analysés : des extraits de pigments obtenus comme décrits au point 2.3 et de l'açaï fraîchement préparé.

On pèse 0,95 à 1,05 g d'extrait que l'on dilue entre 10 et 300 fois dans une solution à pH = 1 (HCl 0,2 N et KCl 0,2 N) pour que l'absorbance finale lue soit comprise dans l'intervalle 0,300-0,800 à λ = 514 nm (pic d'absorbance maximale des anthocyanines de l'açaï). Chaque dilution est faite sans agitation brusque pour éviter l'incorporation d'oxygène. Chaque solution est filtrée sur filtre Whatman n°1 juste avant les lectures d'absorbance. La lecture de l'absorbance est faite à 514 nm et à 700 nm. Pour un même échantillon, cette opération est reproduite de manière équivalente après dilution identique de l'extrait dans un tampon pH 4,5 (HCl 1 N/CH_3COONa 1 N). On obtient la concentration en anthocyanines par le biais de l'équation suivante (Eq. 1) :

$$\textbf{(Eq. 1)} \quad C_1 = ((A_{514} - A_{700})_{pH\ 1,0} - (A_{514} - A_{700})_{pH\ 4,5}) \times D \times F$$

C_1 = concentration en anthocyanines en mg/kg d'açaï ou d'extrait
A = absorbance
D = facteur de dilution du jus
F = 18,67864 = facteur de conversion pour les anthocyanines de l'açaï (cyanidine-3-rutinoside et cyanidine-3-glycoside) (*cfr.* Chap. IV, A., point 1.3.2., p. 106).

Chaque échantillon de jus est analysé en double. Les deux valeurs de C_1 sont calculées directement. Une nouvelle détermination est faite à partir du même échantillon si l'écart-type entre les valeurs est supérieur à 12%. Cette opération est répétée jusqu'à ce qu'un nouvel écart-type de trois valeurs entre elles soit inférieur à 12% de la concentration moyenne.

Enfin, pour de nombreuses expériences, il s'avère intéressant de pouvoir exprimer la concentration en anthocyanines par kg de fruits frais. Si la détermination de C_1s'est faite à partir d'un extrait de pigments dans de l'éthanol acidifié, la conversion se fait simplement en divisant C_1 par la masse de fruits introduite par litre de solvant. Si la détermination de C_1 s'est faite sur base d'un açaï, on effectue la conversion suivante (Eq. 2) :

$$\textbf{(Eq. 2)} \quad C_2 = C_1 \times M_2$$

C_2 = concentration en anthocyanines en g/kg de fruits
M_2 = masse de jus recueillie par kilo de fruits dépulpés (*cfr*. Chap. III, B., point 3, p. 74).

ASKAR, A., TREPTOW, H., Quality assurance in tropical fruit processing, Ed. Springer-Verlag, 1993, 238 p.

Pour info, on utilise depuis 2004 un facteur F de 21,2 prenant en compte les 62% de Cy-3-rut pour 38% de Cy-3-glu (l´autre facteur faisait comme si c´était 50-50%).

Annexe 3 :
Table 10: Total phenolic content (TPC) results:

Sample	TPC (mg/kg)
1	3708,134
2	1380,786
3	4176,18
4	3845,542
5	4485,348
6	3918,54
7	2583,106
8	3021,094
9	1024,384
10	2454,286
11	1153,204
12	2256,762
13	1118,852
14	1535,37
15	3733,898
16	1028,678
17	1037,266
18	6164,302
19	7139,04
20	1239,084
21	1689,954
22	2600,282
23	2329,76
24	2063,532
25	1771,54
26	2643,222
27	2419,934
28	2900,862
29	2681,868
30	2166,588
31	1097,382
32	2407,052
33	972,856
34	1793,01
35	1148,91
36	1170,38
37	2256,762
38	1977,652
39	1217,614
40	3025,388
41	1123,146
42	2325,466
43	2441,404
44	1355,022
45	2866,51
46	3940,01
47	2170,882
48	2574,518
49	1668,484
50	2089,296
51	2012,004
52	1677,072
53	3643,724
54	2565,93
55	865,506
56	1552,546
57	1707,13
58	2282,526
59	2535,872
60	1990,534
61	1943,3
62	972,856
63	1711,424
64	1754,364
65	2535,872
66	3154,208
67	2046,356
68	1028,678
69	2325,466
70	1518,194
71	1591,192
72	2716,22
73	1878,89
74	2106,472
75	2591,694
76	1668,484
77	2419,934
78	2716,22
79	1578,31
80	4742,988
81	2535,872
82	2518,696
83	2110,766
84	1969,064
85	1531,076
86	1853,126
87	2432,816
88	2896,568
89	2265,35
90	2845,04
91	2797,806
92	2338,348
93	4614,168
94	3686,664
95	3995,832
96	3502,022
97	2282,526
98	3158,502
99	2046,356
100	2115,06
101	1797,304
102	2054,944
103	2724,808
104	2119,354
105	2510,108

Annexe 4 :
Table 11: Total anthocyanins content (TAC) results:

Sample	TAC (mg/kg)
1	1149,57
2	66,78
3	2032,02
4	562,86
5	1512,09
6	2179,89
7	1454,85
8	753,66
9	181,26
10	1235,43
11	157,41
12	705,96
13	128,79
14	3548,88
15	553,32
16	162,18
17	128,79
18	691,65
19	2051,1
20	577,17
21	629,64
22	381,6
23	500,85
24	267,12
25	1062,12
26	365,7
27	384,78
28	403,86
29	462,69
30	443,61
31	333,9
32	348,21
33	496,08
34	413,4
35	343,44
36	57,24
37	524,7
38	386,37
39	128,79
40	500,85
41	171,72
42	643,95
43	629,64
44	233,73
45	734,58
46	672,57
47	626,46
48	725,04
49	572,4
50	686,88
51	1058,94
52	489,72
53	682,11
54	386,37
55	147,87
56	1001,7
57	769,56
58	1189,32
59	352,98
60	515,16
61	1459,62
62	133,56
63	1542,3
64	581,94
65	1303,8
66	515,16
67	934,92
68	181,26
69	629,64
70	470,64
71	826,8
72	992,16
73	1636,11
74	814,08
75	958,77
76	963,54
77	634,41
78	901,53
79	982,62
80	1454,85
81	912,66
82	874,5
83	712,32
84	429,3
85	807,72
86	670,98
87	1426,23
88	610,56
89	464,28
90	472,23
91	613,74
92	443,61
93	2232,36
94	1254,51
95	806,13
96	1130,49
97	410,22
98	626,46
99	483,36
100	588,3
101	519,93
102	1211,58
103	357,75
104	429,3
105	410,22

Annexe 5 :

PCA of Super Fingerprint region :

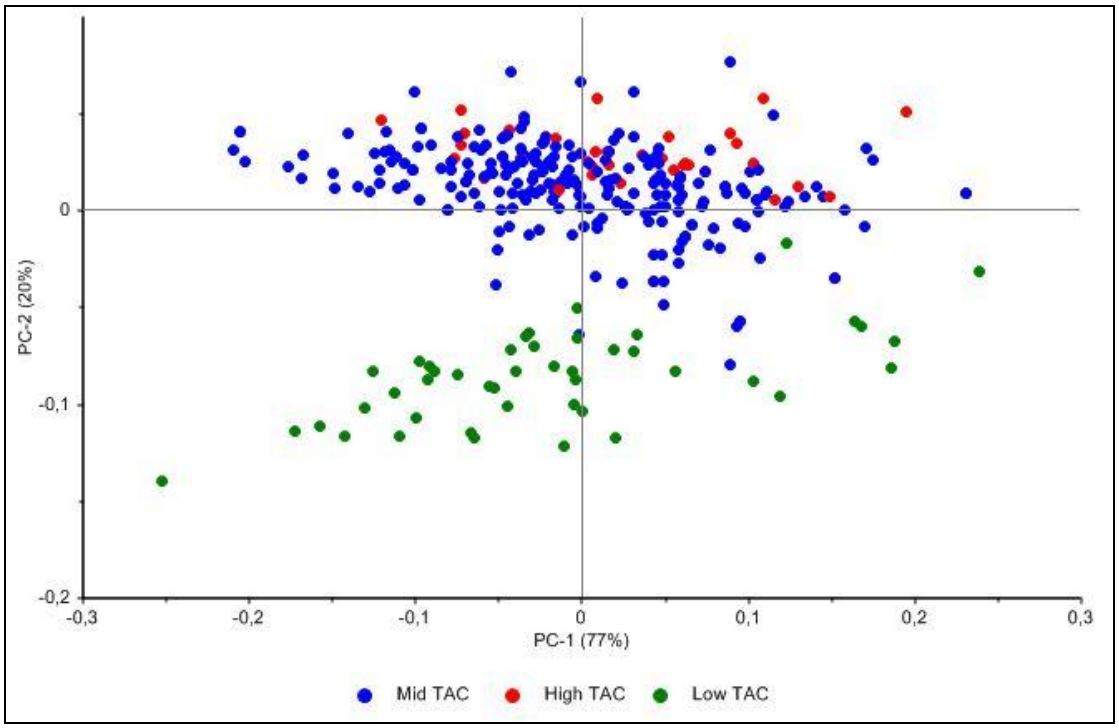

Figure 32: PCA graph where samples are differentiated by values of total anthocyanin content for the extracts (pre-treatment: none, region used: Super Fingerprint)

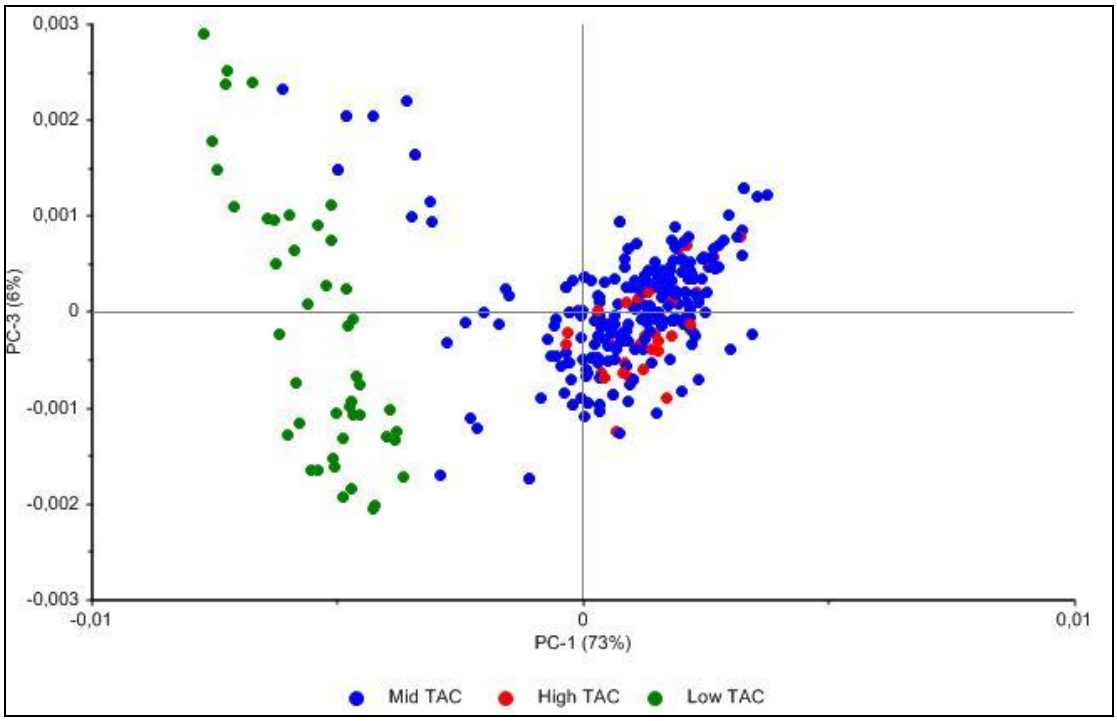

Figure 33: PCA graph where samples are differentiated by values of total anthocyanin content for the extracts (pre-treatment: Savitzky–Golay, region used: Super Fingerprint)

Table 12: PLS-R results, R^2 and RMSEcv, of the Supe Fingerprint region analysis

		R^2	RMSEcv
Pre-treatment: none	TPC	0,68	619
	TAC	0,32	455
Pre-treatment: Savitzky–Golay	TPC	0,68	613
	TAC	0,3	466