

***In silico* analysis of the chemical profile of extracts from coffee bean residual press cake aiming at the application in the treatment of skin wounds**

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1 Introduction

1.1 Context and Motivation

Coffee is one of the most widely consumed beverages worldwide. Brazil is the world's largest producer of coffee, exporting almost 3 M tons only in 2020 [1]. Global coffee production generates annually 23 million tons of waste [2]. Therefore, coffee industries must make efforts to valorise their by-products to increase the sustainability of the process.

For this reason, research studies on by-products of coffee, such as coffee bean residual press cake (CPC), are taking place continuously to identify possible applications in the biotechnological fields, such as pharmaceutical, food and cosmetics areas. This could lead to a valorisation of CPC, increasing the sustained economic return on coffee crop production [3].

The extracts of the coffee bean press cake (ECPC) have been analysed using classical analytical techniques, such as NMR spectroscopy, UV-Vis spectrophotometry, and liquid chromatography (RP-HPLC). Also, there have been performed biochemical assays to evaluate the antioxidant potential of ECPC, as well as *in vitro* assays to determine cytotoxicity and cell proliferation. Additionally, *in vivo* assays have been carried out to evaluate the potential of ECPC on skin repair in murine study models [4].

The current challenge is understanding how the ECPC exerts its activity in biological systems as a wound healing agent. In this context, this project aims to explore existing metabolomics datasets of ECPC and of L929 fibroblast cell cultures focusing on the healing potential in acute wounds.

1.2 Goals

Keeping the above context in mind, this project aims to explore the functionalities of the R package *specmine* and complementary tools to process and mine metabolomics data, namely addressing datasets of spectroscopic techniques of ECPC, focusing on the skin wound healing process. The following scientific and technological objectives will be pursued:

Analysis of the selected datasets, creating a repository of datasets with available analysis pipelines:

- Implementing data pre-processing (e.g. removing baseline variations, dimensionality, normalisation, scaling, mean centering and some peak spectra processing) pipelines for the selected data.
- Applying unsupervised learning methods over the resulting data.

2 State of the art

2.1 Coffee: General Perspective

Coffee is currently one of the most appreciated and consumed beverages worldwide, being attributed to its highest standard organoleptic characteristics and beneficial health effect [5].

Brazil is the largest producer and exporter globally, followed by Vietnam and Colombia. The international market is dominated by *Coffea arabica* L. (arabica), and *Coffea canephora* Pierre (robusta) species [5]. Specifically, Brazilian arabica coffee accounts for 34,38% of the total production worldwide [1].

Coffee by-products: CPC

During the coffee processing, the bean is separated from the remaining parts, and more than 50% of coffee fruit is discarded, resulting in numerous by-product amounts [5]. Global coffee production generates 23 million tons of waste per year [2]. Therefore, coffee industries must make efforts to valorise the by-products to increase the sustainability of the process. For this reason, research studies on by-products of coffee are taking place continuously to identify possible applications in the biotechnological fields [5].

For example, the coffee beans that are not selected according to the criteria for beverage (0.5 million tons per year) have been used to extract coffee oil by cold mechanical pressing [4]. The oil extracted has been implemented in cosmetic and food formulations. This procedure has proven to be feasible, but it generates a solid processing residue (CPC), which is the object of this study.

The interest in this residue is mainly due to the presence of bioactive compounds in their composition (e.g. phenolic compounds and alkaloids) (Table 1) [6, 7]. A few recent studies have shown that CPC can be employed in biodiesel [8] and adsorbent production, precisely to remove cationic dyes and metal ions (e.g. copper(II) and zinc(II)) [9].

2.2 Skin: General Perspective

12 Skin is considered the biggest organ in the human body, covering, in an adult on average, 1.75 m^2 and weighing 5 kg [10]. Human skin acts as a barrier between internal and external environments and plays an essential role in regulating body homeostasis, particularly the water content and body temperature.

The skin also acts as the first line of defence against infection, setting up an integral part of the innate immune system, protecting the body from noxious substances and invasion by microorganisms. Skin is also crucial to mechanical

Table 1. Concentrations of phenolic acids and alkaloids (mg g⁻¹ dry matter) in aqueous extracts of green and roasted coffee press cake [6].

Compounds	EGPC	ETPC
Chlorogenic acid	11.11±0.28	1.95±0.31
Caffeine	4.50±0.006	5.60±0.08
Trigonelline	1.55±0.51	1.02±0.14
Ferulic acid	1.40±0.29	0.92±0.16
Protocatechuic acid	1.20±0.003	0.21±0.02
Syringic acid	0.96±0.04	0.84±0.08

protection, absorption and secretion of liquids, protection against solar radiation and vitamin D metabolism, besides aesthetic and sensorial functions [6].

Cicatrization process

The skin is continuously subject to various lesions and can be wounded physically or chemically. The wounds consist of the disruption of skin integrity, followed by a cascade of events to repair and reestablish integrity and functionality [11]. Wound healing is a complex biological process after an injury. This process consists of continuous, overlapping, and precisely programmed phases: inflammation, proliferation and remodelling.

After the homeostasis, where vascular constriction and fibrin clot formation occurs, the inflammatory response begins. In the inflammation phase occurs the accumulation of inflammatory mediators, such as interleukin (IL)-1, prostaglandins, tumour necrosis factor α and β , promoting the vasodilation and recruitment of immune cells (keratinocytes and fibroblasts) [12].

The final stage of the wound healing process is tissue remodelling, which can last up to a year, depending on the severity of the wound and the presence of local and systematic factors [13]. Clinically, this is the most crucial phase due to the variation of the rate, quality, and total amount of matrix deposition and, consequently, the scar's strength [11, 14].

2.3 Metabolomics

Besides quantifying and identifying the chemical compounds in biological samples, the current challenge is understanding how they exert their activities in biological systems as wound healing agents. To face this challenge, metabolomics has become one of the major areas for characterizing chemically complex biomasses, such as coffee by-products, due to its robustness, precision, and high data acquisition [15].

Metabolomics is one of the omics technologies, which includes studies of chemical processes involving small molecules in biological systems and their subsequent identification and quantification. Metabolomics analyses can be divided into targeted and untargeted ones, according to the type of sample extraction and preparation, and the analytical techniques adopted [16].

Targeted analyses are applied to identify and quantify a specific group of compounds or metabolites involved in a particular metabolic pathway or biochemical phenotype. Targeted metabolomics typically requires a higher level of purification of compounds. Contrarily, untargeted analyses are useful for quantifying a large number of metabolites. This way, untargeted analyses have been applied to detect unknown compounds and metabolic fingerprints [17].

The workflow of these studies can include several steps: sample handling and preparation, metabolite extraction, metabolite separation, detection technologies, data pre-processing and treatment (Figure 1). However, depending on the type of study and samples, some steps may not be necessary. Only detection and data analysis have been essential steps in all reported metabolomics studies.

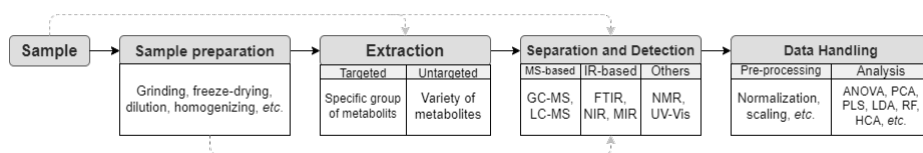


Fig. 1. Schematic presentation of ECPC metabolomic analysis.

2.4 Separation and detection technologies

The separation and detection methodologies selection represents a crucial step in the metabolomic analysis. A different range of separation techniques has been applied in ECPC profiling, including chromatographic techniques (HPLC and GC) coupled with UV-Vis and mass spectrometry (MS) detectors, as well as spectroscopic techniques (e.g. nuclear magnetic resonance - NMR, Fourier transform infrared - FTIR, and UV-Vis) [18].

Compared with traditional analytical techniques, spectroscopic methods stand out because demand less sample pre-treatment, no purification of analytes, also allowing a faster collection of spectral data [4].

NMR spectroscopy is based on the absorption and re-emission of electromagnetic radiation, providing information on the number of magnetically distinct atoms of the studied isotope in a molecule [19].

2.5 Machine Learning

ML is a sub-field of Artificial Intelligence (AI), a more prominent and well-known field concerned with the development of computational learning models. ML refers to a machine's ability to enhance its performance based on previous results from a computational standpoint. ML has recently become the most prominent sub-field of AI because of newer and better hardware, and larger amounts of available data [20].

Researchers in the field of ML have proposed a wide range of learning models and algorithms. Many distinct types of ML models/algorithms exist, including decision trees, inductive logic programming, Bayesian learning, artificial neural networks, and evolutionary learning, among others [21].

There are four main forms of ML based on the feedback available: supervised, unsupervised, semi-supervised learning, and reinforcement learning. The main goal of supervised learning is to detect feature patterns associated with each label. On the other hand, unsupervised learning works with unlabeled data to achieve tasks like grouping, dimensionality reduction, and data visualisation [20].

Unsupervised Learning

Unsupervised machine learning techniques may be used when the training data is unclassified and unlabeled. This investigates how the system can derive a function to explain the unlabeled data's hidden patterns [22]. The system does not determine the appropriate output, but it does propose data organisation or uncovers hidden patterns in unlabeled data [23].

Processes that retrieve relevant data aspects are included in this category, such as clustering (e.g. K-means and EM), dimensionality reduction (e.g. PCA and t-SNE), and outlier identification. These methods are commonly employed in data visualisation, data dimensionality reduction, noise removal, and data correlation interpretation. Such correlations may make the entire data set easier to comprehend. Unsupervised ML can be utilised as a precursor to Supervised ML models in light of the various existing applications [20].

2.6 Bioinformatics tools

The rapid growth of metabolomics-based approaches in biosamples characterisation implies the permanent and essential improvement of bioinformatics tools to treat and extract relevant information. It is then necessary to implement new data analysis tools and validate, reproduce, and standardise them [24].

The *specmine* package allows the integrated analyses of metabolomics and spectral data, guiding the development of flexible data analyses pipelines for specific case studies. This package covers different data formats afforded by analytical techniques, e.g., GC/LC-MS, IR, UV-Vis, NMR, and Raman spectroscopies. A set of functionalities can be performed, including data loading in various formats, data pre-processing, metabolite identification, univariate and multivariate data analyses, machine learning, and feature selection [25, 26].

To simplify the application of this package, a web platform interface, the webspecmine, was also developed. This site provides simple, interactive front-end, and easy-to-use tools to cover the main steps of the metabolomics data analysis data [27].

2.7 Related work

More recently, distinct approaches have been made to research the influence of ECPC in the skin wound healing process. Several studies focus on the chemical characterisation of ECPC associated with a *in vitro* and pre-clinical assays. However, the data resulting from these studies can be enhanced with an *in silico* approach to facilitate the analysis and possible association between chemical compounds and their activities in biological systems as wound healing agents.

Affonso *et al.* (2016) investigated the effect of aqueous ECPC on skin wound healing. In a preliminary step, the authors evaluated the extracts' phytochemical profile and antioxidant activity, followed by the *in vitro* determination of the cytotoxicity and cell viability, migration and proliferation. The analytical techniques implemented were UV-Vis spectrophotometry and RP-HPLC and revealed higher amounts of phenolic compounds, mainly chlorogenic acids, as well as caffeine.

In a second approach, the *in vivo* effect of topical treatment of hydrogels containing the aqueous extracts in a model of an excisional wound in Swiss mice was investigated. It was found that coffee extracts significantly reduced wound area size during the inflammatory phase and that green ECPC had a superior efficiency compared to roasted ECPC.

In this sense, the hypothesis of this study is based on the assumption that a biologically compatible extract (ECPC) is a rich source of bioactive compounds and that, administered topically on the lesion, assists in the process of tissue repair.

3 Methodology

The dataset utilized for analysis in the current project originated from a series of experiments conducted by Regina Afonso, aiming to further investigate the results highlighted in the related work section [24].

In summary, L929 fibroblast cell cultures were subjected to incisions to simulate a wound. Subsequently, separate experiments were conducted, adding the following solutions: extracts of residual coffee bean press cake from green coffee (EGCPC) and roasted coffee (ETCPC), caffeic acid, chlorogenic acid, and caffeine. These three compounds were selected due to their presence in the chemical composition of the analyzed extracts.

After the sample collection, proton nuclear magnetic resonance (NMR) spectroscopy was performed on both the extracellular content of the cells (supernatants of the culture media) to identify the exometabolome and the cellular extracts (endometabolome). This allows for a better understanding of intracellular and extracellular metabolism during cellular healing.

The dataset obtained is in the VARIAN format, which consists of a set of folders (fid, log, procpa.txt and text.txt), where each folder represents one sample. To facilitate the analysis of the obtained NMR data, it was divided into sub-datasets described as "exometabolome," "endometabolome," and "extracts," where the latter specifically refers to the EGCPC and ETCPC extracts.

A standard pipeline of NMR data analysis was followed (Figure 2), which started with the data loading, following the data visualization that can be used to display the spectral profiles and highlight differences or similarities among the samples and data pre-processing.

To load the NMR data it was used the method "read_varian_spec_raw" present in the specmine package [25, 26]. During the following pipeline, the same package was used, however since this package expects to have a metadata file, which is usually available for this type of analysis, some of the methods were adapted.

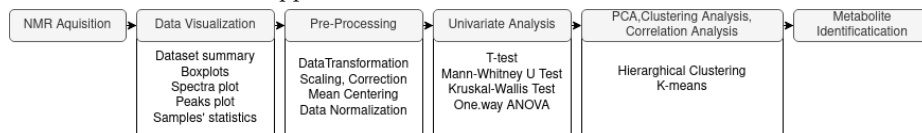


Fig. 2. A standard pipeline of NMR data analysis.

Due to the absence of a metadata file, throughout the data visualization, the functions used to plot the spectra and the peaks were adapted.

These adaptations and further implemented functions are publicly available at <https://github.com/Mendes2022/Projecto>.

After analyzing the spectrum and the distribution of the intensity values, the following pre-processing steps were performed: removal of variables with missing values, logarithmic transformation, scaling, background correction using the modified polynomial fitting, smoothing-interpolation using the binning method, mean-centering, and normalization using the median method. These pre-processing steps were applied to enhance the quality and comparability of the spectral data for further analysis.

Also, during the data pre-processing it was performed principal components analysis (PCA) and clustering analysis that was important to improve the interpretation of spectra by reducing dimensionality, removing irrelevant information and outliers, and enhancing the robustness and accuracy of classification analyses.

It was also performed univariate analyses and correlation analysis in order to compare the spectral intensities at specific peaks or regions of interest between different samples and identify clusters or groups of samples that exhibit similar NMR profiles.

The final step was to perform metabolite identification, to identify the compounds on the extracts, and to verify the presence of intracellular and extracellular metabolites produced by fibroblasts during the repair wound process. The execution of this task was performed by using the method "nmr_identification" present in the specmine package [25, 26] and implementing a set of functions, that search on the NMR peaks for each sample the peaks of metabolites (chemical shifts and intensities) that can be directly or indirectly related to the metabolism of the fibroblasts during the regeneration process.

4 Results and Discussion

The following section presents only a portion of the results obtained during the analysis of the dataset.

The methodology described above can be reviewed in the notebook provided at the following link: <https://mendes2022.github.io/Projecto/>. Then, the most relevant results will be presented regarding the identification of metabolites present in fibroblast metabolism that may be related to cell regeneration.

4.1 Analysis of the sub-dataset "extracts"

In the initial phase, the data from the "extracts" sub-dataset were analyzed, which consists of two samples, one from each extract, with 32,000 data points.

The metabolomic profile of each extract (EGCPC and ETCPC) obtained by 1H NMR is shown in Figure 3. Visual analysis of the metabolomic profiles revealed that the samples exhibit similar chemical compositions.

However, ETCPC shows lower intensities in the range of phenolic compounds, which typically appear in the chemical shift range of 6.0 to 9.0 ppm [28].

The difference in the phenolic region is justified considering that those metabolites are thermolabile, and it is common for roasted coffee to have lower contents compared to green coffee. The ETCPC samples exhibited higher quantities of caffeine compared to EGCPC [24]. Additionally, the studied samples showed discrepancies in their alkaloid and chlorogenic acid contents, suggesting different potentials in terms of their biological effects and in cell regeneration and proliferation.

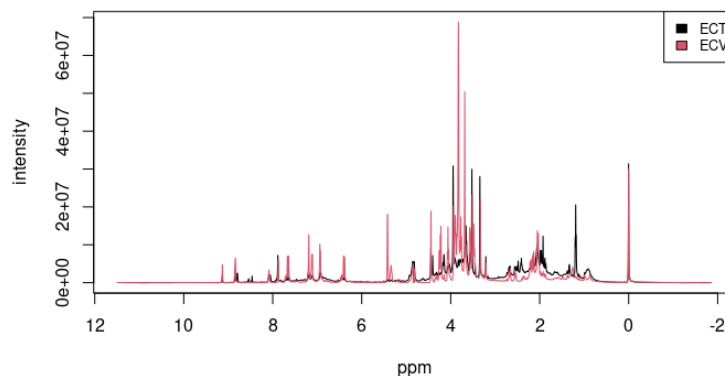


Fig. 3. Spectral plot of the extracts samples (ET - ETCPC, CV - EGCPC).

In order to verify the variation of caffeine and chlorogenic acid in the samples, it was implemented to functions named "find_caffeine" and "find_chlorogenic" and the results corroborate with the presence of these metabolites on the samples.

However, it's important to note that this comparison can only provide qualitative insights and not quantitative measurements. The intensity of a peak in an NMR spectrum is influenced by various factors such as the number of protons, their chemical environment, relaxation times, and instrumental parameters. Therefore, while comparing peak intensities it can only provide a general idea of relative concentrations [15].

A Welch's two-sample t-test was conducted to analyze these samples. The resulting p-value was 3.664e-06, indicating a highly significant difference between the means of the two groups. The results indicate that the average value of intensities peaks in EGCPC was higher than that of ETCPC.

In summary, the information that could be useful in subsequent analyses is related to the differentiation of the metabolomic profile between the extracts, specifically their phenolic and alkaloid composition.

4.2 Analysis of the sub-dataset “exometabolome”

The next step involved analyzing the “exometabolome” sub-dataset, which contains the NMR data of the supernatants of the culture media in the presence of the analyzed extracts and the compounds caffeic acid, chlorogenic acid, and caffeine. Analyzing these three compounds is relevant because, in addition to being present in the analyzed extracts, as previously verified, they have properties that can contribute to cellular regeneration.

Caffeic acid is an antioxidant with anti-inflammatory and photoprotective properties. Studies have shown that caffeic acid can promote cellular regeneration by stimulating the synthesis of collagen, an important protein for wound healing and skin regeneration [29]. Chlorogenic acid also has antioxidant and anti-inflammatory properties, stimulating the activity of antioxidant enzymes and increasing the proliferation of skin cells [30]. Caffeine, on the other hand, can have positive or negative effects depending on the dose and context. At low concentrations, caffeine can promote cellular regeneration by stimulating cell proliferation and increasing metabolic activity. However, at higher doses, caffeine can interfere with cellular regeneration by inhibiting collagen synthesis and negatively affecting wound healing [31].

Considering this, it is necessary to take into account that the present sub-dataset also includes intensity values for the culture medium and for the experiment where no compound was added apart from the culture medium (control) and that each experiment was conducted in quadruplicate.

After visualizing the obtained data, there is uncertainty in distinguishing between the sample that corresponds to only the culture medium and the one representing the control. Considering the complexity of the spectrum of sample “Branco_Meio.2,” it is possible to infer that the other sample corresponds to the sample with only the culture medium (Figure 4). However, the “nmr.identification” method was used to confirm whether the metabolites present in that sample are consistent with the composition of the DMEM culture medium. Since the identified metabolites with a higher score are part of the complex composition of the culture medium, the data analysis proceeded with the assumption that this sample corresponds to DMEM medium.

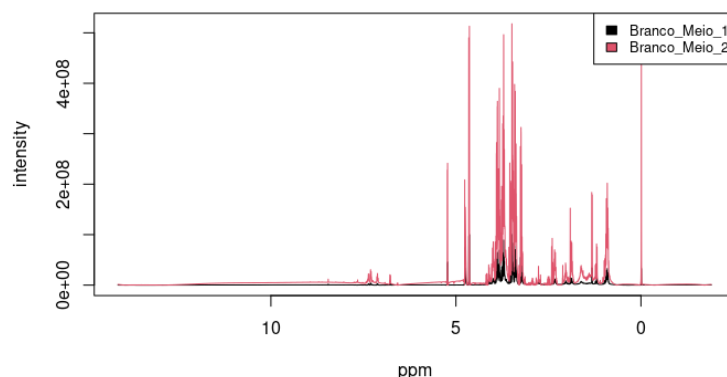


Fig. 4. Spectral plot of the samples, culture medium and control.

The metabolomic profiles of the remaining samples were visualized, with each graph representing their respective quadruplicates. Overall, variations among the quadruplicates and variations in the complexity of the spectra among the samples were observed.

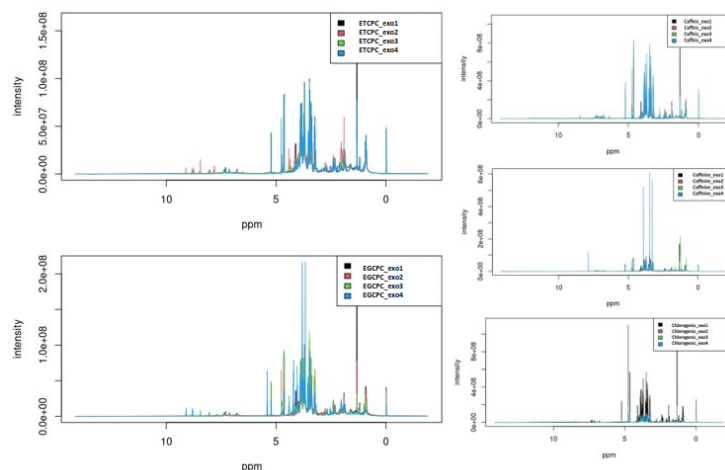


Fig. 5. Spectral plot of the experiments with the extracts and specific compounds (quadruplicates).

In order to reduce the complexity of the metabolomic profile analysis, the mean value was calculated for each quadruplicate, following their preprocessing.

To determine if the intensity values of the experiments with the addition of compounds and extracts were statistically different from the control group, non-parametric tests were performed for each case. The Mann-Whitney U test, also known as the Wilcoxon rank-sum test, was used, and in all cases, the p-values were very small, indicating significant differences between the compared groups.

The next step involved performing the PCA, which is a widely used technique in data analysis and dimensionality reduction. PCA allows exploration of the underlying structure of the "exometabolome" dataset by transforming the original variables into a new set of uncorrelated variables known as principal components.

Analyzing the metrics resulting from the pca performance, in the case of the proportion of variance, PC1 explains 50.58% of the variance and PC2 explains 23.72%.

K-means clustering, a common approach to partitioning datasets into distinct groups or clusters, was also performed. However, when focusing on the analyzed extracts, a clear separation between the clusters was not observed.

Correlation analysis was conducted, revealing a strong positive correlation between the chlorogenic acid data and the samples of the analyzed extracts. As expected, the samples of ETCPC and EGCPc exhibited a higher correlation of 0.87.

After performing univariate and unsupervised analyses of the "exometabolome" dataset, a strategy was devised to determine if the addition of the analyzed components to the fibroblast cell culture had any effect on cell regeneration and proliferation.

Firstly, a review of the literature was conducted to identify metabolites in the extracellular medium of fibroblasts that could indicate the process of wound healing. In fibroblasts, several extracellular metabolites can indicate cell regeneration and can be detected by 1H NMR spectroscopy.

Fibroblasts play a crucial role in cell regeneration and tissue repair, being responsible for synthesizing and organizing the extracellular matrix (ECM), such as collagen, elastin, and fibronectin. Various metabolic pathways, such as glycolysis, the tricarboxylic acid (TCA) cycle, amino acid metabolism, and the pentose phosphate pathway, are involved in supporting fibroblast functions during cell regeneration. An example of amino acid metabolism (arginine) and related pathways linked to wound repair in fibroblasts is shown in the (Figure 6) [32].

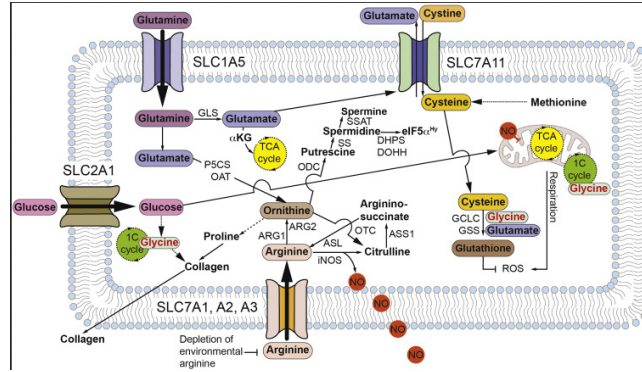


Fig. 6. Arginine metabolism and related pathways linked to wound repair. Adapted from Emming et al. 2021 [32]

Overall, fibroblasts' involvement in cell regeneration requires active metabolic pathways to support their energy needs, synthesize ECM components, and perform essential cellular functions. These processes are tightly regulated and coordinated to ensure efficient tissue repair and regeneration.

Taking this information into consideration, here are some examples of extracellular metabolites directly or indirectly related to fibroblasts metabolism during the regeneration process:

1. Lactate: Lactate is a key metabolite in cell metabolism and can indicate increased glycolysis, which is often associated with active cell growth and regeneration [33].
2. Alanine: Alanine is an amino acid involved in energy metabolism[32].
3. Glutamate and glutamine: Glutamate and glutamine are amino acids involved in various cellular processes, including energy metabolism, nitrogen transport, and collagen synthesis[32].

4. Succinate: Succinate is an intermediate of the tricarboxylic acid (TCA) cycle and can indicate active cellular metabolism and regeneration[32].

To identify the presence of these metabolites in the analyzed samples, separate functions were implemented for each metabolite based on their 1H NMR profiles. The Human Metabolome Database (HMDB) and SpectraBase databases were consulted to obtain the chemical shift values and intensities of each isolated metabolite's profile. Of the analyzed metabolites, only lactate and alanine were detected. The absence of glutamine in the extracellular medium can be justified by its transport into the intracellular medium for collagen synthesis [32].

4.3 Analysis of the sub-dataset "endometabolome"

The analysis of the "endometabolome" sub-dataset aims to identify intracellular metabolites to gain insights into the metabolic pathways involved in the cellular regeneration process. In this case, only the 1H NMR data from the experiments with the analyzed extracts were analyzed. Regarding the composition of the sub-dataset, it consisted of 2 samples of ETCPC and 3 samples of EGPCPC. Due to the agreement between the visual analysis of the spectra and the results of the statistical analyses, it was decided to use only one sample from each experiment (Figure 7).

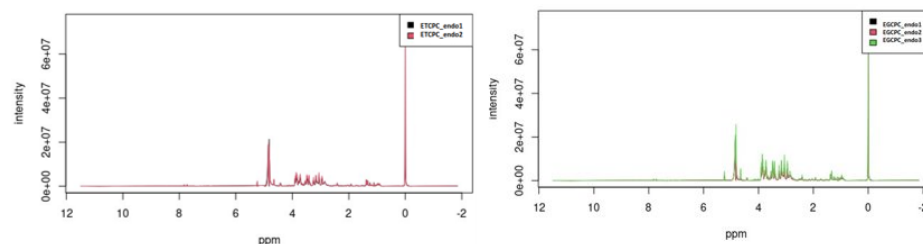


Fig. 7. Spectral plot of the experiments with the extracts.

Furthermore, the correlation between the samples was determined to be 0.510, indicating a moderate relationship between the two samples.

After conducting the unsupervised analysis of the "endometabolome" dataset, the same strategy as described above was implemented. The objective is to assess whether the addition of extracts to the fibroblast cell culture had any impact on cell regeneration and proliferation, specifically in terms of intracellular metabolism and extracellular matrix (ECM) composition.

In addition to the previously mentioned metabolic pathways, the metabolic pathways involved in phospholipid and collagen synthesis will be further elucidated. These pathways play a crucial role in the intracellular medium of fibroblasts and are important for the process of wound healing.

It is important to note that directly detecting specific ECM components such as collagen and fibronectin using 1H NMR spectroscopy may be challenging due to their larger size and complex structure.

However, certain metabolites can indirectly indicate ECM remodeling and cell regeneration processes. While these metabolites may not be exclusive to

collagen synthesis, they can provide insights into overall cellular activity and tissue regeneration. Examples of such metabolites include:

1. Choline-containing compounds: Choline is involved in phospholipid synthesis, which is essential for cell membrane formation. Increased choline levels can suggest active cell growth and regeneration [34].
2. Glycine: Glycine is an amino acid that serves as a fundamental building block for collagen synthesis. Its presence may indirectly indicate the potential for collagen production [32].

Among the analyzed metabolites, choline and glycine were detected in the intracellular medium, indicating ECM remodeling and cell regeneration processes.

5 Conclusion

The analysis of the metabolomic profiles of the extracts confirmed the presence of relevant bioactive compounds, such as caffeine and chlorogenic acid.

The results from the samples obtained in the experiments with fibroblast cell culture suggest that chlorogenic acid, caffeine, and caffeic acid can stimulate cellular regeneration when used at low concentrations. Similarly, the analysis of the metabolomic profiles in the presence of coffee paste extracts indicates a positive influence on the process of skin repair.

Thus, it can be inferred that the three compounds under analysis are examples of some of the bioactive compounds present in coffee paste extracts that may promote cellular regeneration and proliferation. However, it is necessary to consider the complexity of the involved metabolic pathways and the synergistic effect of multiple compounds on metabolic pathway regulation.

Some of the metabolites indicating ECM remodeling and cell regeneration processes were identified in the samples with coffee paste extracts. Therefore, it was possible to identify potential metabolic pathways in which the coffee paste metabolites may be directly or indirectly interfering. Some examples of the identified metabolic pathways include glycolysis, amino acid metabolism (arginine, glutamine, and glycine), and phospholipid synthesis.

In order to understand the direct effect of the bioactive compounds in coffee paste extracts on the metabolic pathways of fibroblasts, more detailed analyses could be performed with the assistance of databases such as Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and BioCyc.

Furthermore, it is important to emphasize that the effects of cell regeneration are complex and can depend on various factors, including concentration, exposure time, and interaction with other compounds. Therefore, future studies could be conducted to investigate the variation in concentration of coffee paste extracts and the exposure time of cells to the metabolites.

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