ESCOLA
SUPERIOR
DE SAÚDE
POLITÉCNICO
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MESTRADO
BIOQUÍMICA EM SAÚDE-RAMO BIOTECNOLOGIA (325/20-6)

Biotech in Psy: tools to understand the Brain

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07/**2022**

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Internship Report submitted to fulfill the requirements necessary to obtain a Master's degree in **Biochemistry in Health– Biotechnology Branch** by Escola Superior De Saúde– Instituto Politécnico do Porto.

Acknowledgments

To Investigator-Professor Patricia Baptista, my supervisor, I express my gratitude for always being considerate and thoughtful of me, pushing me to learn more and create higher settings in the investigation field.

To Professor. Patricia Oliveira-Silva, I am thankful for being a part of your agenda, whichI know is completely full most of the year, and for being able to learn with you and absorb all the knowledge that you have to offer.

To Dra. Sofia Salsinha, for making her time available to guide me in her study and helping me to improve my laboratorial skills.

To my professors, mainly Prof. Mónica Vieira and Prof. Ricardo Ferraz, and colleagues in ESS, because I am extremely thankful for being on this journey with you, but most important for helping me to pursue a carrier in the Biotechnology field. Thank you for giving me the opportunity to be in this course, for your kindness and availability to help me whenever I asked, even when I was not as much present as I should be, due to the job and the internship. You have been extraordinary

To the most important people in my life- my mom, my brother, my boyfriend, and my best friend- for always supporting me, even on my crankiest and exhaustive days, tired from conjugating the internship and job. Thank you for listening and making me open my eyes every time that it was needed. Thank you for not ever leaving my side.

To M.T. I owe everything. You are my true inspiration in this life to be a better person, to fight for what I believe, and to never stop having confidence in myself and my skills.

Abstract

Collaboration between different scientific areas might profoundly influence the scientific study of human well-being and the treatment of many diseases. Based on that, this internship was designed with the goal to explore one of those possible collaborations between Psychology and Biotechnology. These are two scientific areas in which I share a special interest because it allows having a deeper understanding of the brain and its functions. In fact, this experience allowed me not only to acquire new and essential skills but also to recognize the importance of interdisciplinarity research, mainly applied to human well-being and preventativestrategies.

Within this scope, I was part of a research project implemented by the HNL and CBQF, to understand the effect of a probiotic product, called Kombucha, on the human brain, acting through the "gut-brain axis". This research resulted in an internationally refereed scientific publication. This project allowed me to be part of both laboratories (the HNL and the CBQF), where I was able to acquire scientific skills at various levels and personal skills.

Keywords: interdisciplinarity; biotechnology; psychology; Kombucha, gut-brain axis,probiotics.

Resumo

A colaboração entre diferentes áreas científicas pode influenciar profundamente o estudo científico do bem-estar humano e o tratamento de muitas doenças. Com base nisso, este estágio foi desenhado com o objetivo de explorar uma dessas possíveis colaborações entre a Psicologia e a Biotecnologia. Estas são duas áreas científicas em que partilho um interesse especial porque permite ter uma compreensão mais profunda sobre o cérebro e as suas funções. De facto, esta experiência permitiu-me não só adquirir novas e essenciais competências, mas também reconhecer a importância da investigação interdisciplinar, nomeadamente aplicada ao bem-estar humano e estratégias preventivas.

Neste âmbito, fiz parte de um projeto de pesquisa implementado pelo HNL e CBQF, com o objetivo de compreender o efeito de um produto probiótico, denominado Kombucha, no cérebro humano, atuando através do "eixo intestino-cérebro". Esta pesquisa resultou em uma publicação científica referenciada internacionalmente. Este projeto permitiu-me fazer parte de ambos os laboratórios (o HNL e o CBQF), onde pude adquirir competências científicas a vários níveis e também competências pessoais.

Palavras-chave: interdisciplinaridade; biotecnologia; psicologia; Kombucha, eixo intestino-cérebro, probióticos.

Abbreviation List

CBQF– Centro de Biotecnologia e Química Fina

CNS– central nervous System

DNA– deoxyribonucleic acid

ECG– Electrocardiogram

EDA– Electrodermal activity

EEG– Electroencephalogram

ENS–Enteric Nervous System

GABA– γ–Aminobutyric acid

GBA– gut–brain axis

GC–FID– Gas Chromatography With Flame Ionization Detection

GI– gastrointestinal

HNL– Human Neurobehavioral Laboratory

HPA– hypothalamic–pituitary–adrenal

HPLC– High–performance liquid chromatography

ml– millilitres

qPCR– quantitative polymerase chain reaction

RA– respiratory activity

Rpm– rotations per minute

SCFAs– Short–chain fatty acid

TSSTs– trier social stress tests

Figures Authorship

All figures displayed in this document are from HNL authorship.

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1. Introduction

Nowadays, it is extremely important to design interdisciplinary projects, in order to complement areas of knowledge and obtain better results. The interconnection of the areas of Psychology and Biotechnology makes perfect sense since technological development has an impact on human physical and mental health.

The fascination for these two fields has awakened a significant interest in deepening my knowledge, mainly related to human health, hence the selection of this internship. I was granted the opportunity of participating in a research project that is still being developed in the HNL and in the CBQF, with the intention to know and understand what is the effect that Kombucha, a known non-dairy probiotic, has on the brain and thereafter on human behavior and mental health.

In this way, the aim of this internship was to understand and improve laboratory skills, mainly, in the biotechnology domain, but also in the neuropsychology field, andlearn to combine them appropriately in different studies. Therefore, it included the participation in two laboratories, CBQF, and HNL.

Initially, this internship would be conducted through direct participation in the biological data collection for the study, and later biochemical and microbiological analysis of these samples. However, due to the lack of funding for the project, the data collection could not be carried out, yet. Nonetheless, the tasks proposed in the internship project were not conditioned because it was possible to follow other research work and as such, it was possible to acquire the skills outlined in the internship project.

In the CBQF, I acquired knowledge in biochemical and microbiological areas. I have beencontacted with several procedures and assays, such as qPCR, HPLC, microbial culture preparations, and human feces samples preparations, GC-FID, and DNA extraction. The objective of the CBQF internship was to familiarize me with different laboratory techniques of investigation, and to widen my range of knowledge and proficiency in the application of such techniques.

In the HNL, I acquired knowledge about the techniques involving the collection of physiological measures, like, as an example, the application of RA, EDA, EEG, and ECG. The aim of this part of the internship was enlighten about neurophysiological measures and how they're important in the study of the human brain.

2. Literature Review

2.1. Probiotics, Prebiotics, Symbiotics and Psychobiotics

Nowadays, people are getting more concerned with their alimentation and their dietary habits, because of the impact that it has on both physical and mental health (Leta etal, 2018). New forms and types of food have emerged whose focus is the improve of generalhealth, called function foods. This new category of foods includes probiotic, prebiotics, symbiotics and recently, psychobiotics foods and are significantly correlated with the reduction of pathologies and risk factors (Koirala & Anal, 2021).

Probiotics are defined as live strains of selected beneficial microorganisms, like bacterias and yeasts, that reside in the human organism (Cosme et al., 2022). Such microorganisms are various, but the most common are *Bifidobacterium spp*, *Lactobacillus spp* and *Lactococcus spp*, as bacterias, and *Saccharomyces spp*, as yeasts (Hill et al, 2014). Probiotics are the main category of functional foods being studied due to their human beneficial proved properties, including boosting the immune system (Galdeano et al. 2019) balancing the intestinal microbiota (Khaneghah et al., 2020), preventing of diseases like cancer, diabetes, and development of allergic reactions (Varsha et al., 2021). Other than physical health, continuous probiotic consumption, mainly with *Lactobacillus* and *Bifidobacterium spp*, has been associated with the promotion of cognitive functions, improving mood, reduction of anxiety and depression-like symptoms (Akkashesh et al., 2016; Eastwood et al., 2021; Lv et al., 2021; McKean et al., 2017; Pinto-Sanchez et al, 2017; Sarkar et al., 2016; Tong et al., 2020). For example, a probiotic treatment used in autistic children, showed GI symptoms reduction, and a decrease in the occurrence ofanti-social, and aberrant behaviors (Grimaldi et al, 2018). In another study related to neurodegenerative diseases, it was found that treatment with probiotics strains (mainly *Bifidobacterium spp*), and selenium demonstrated improvement in cognitive functions, suchas memory and neuroplasticity, in Alzheimer's patients (Tamtaji et al., 2019).

Prebiotics are defined as substrates that are selectively digested by the host microorganisms (Cosme et al., 2022), stimulating the growth and metabolism of probiotics in the gut (Pimentel et al., 2022) and inhibit the growth of certain pathologic microorganisms (Tran and Li, 2022). They are commonly found in any food, like soybeans, oats, bacon, wheat, and others (Manzoor et al., 2022). To be prebiotic it must be nondigestible, nonabsorbable and non-adsorption, fermented by probiotics in the gut and stimulate the activity of one or more bacteria in the GI tract (Tran and Li,2022). Some prebiotics have antioxidant activities, associated with cancer prevention,inflammation reduction and lower risk of GI problems, and with the improvement of the mineral bioavailability, preventing cardiovascular diseases and obesity(Manzoor et al., 2022). The knowledge about the prebiotic mechanism of action in the human organism is still very raw but they are associated with the activation and stimulationof immune cells through direct interaction with their receptors in the host's cell surface andthe production of SCFAs used as energy sources to improve human health gut microbiome (Tran and Li, 2022).

Symbiotics are functional products defined by the combination of prebiotic and probiotic products, that may exert positive health effects by altering the gut microbiota, like metabolic activation of gut health-promoting bacteria and improvement of the gut barrier (Laice et al., 2021; Amirpour et al., 2020). They can have better antioxidant activity, improvement of the immune system, and improve cardiovascular health, reducing the development of complications (Zhou et al., 2021; Devi et al., 2019).

Psychobiotics are a group of probiotics and prebiotics with effects on emotional, cognitive, and neural levels, to improve well-being and illness (Sarkar et al., 2016). These supplements have been the current object of study in gut-brain to understand the relation, and how altered gut microbiota can have an impact on mental illness (Eastwood et al, 2021;Sarkar et al., 2016). Different studies revealed that the intake of psychobiotics can regulateneurotransmitters and proteins, like Gamma-Aminobutyric Acid (GABA), serotonin (Cheng et al., 2019), and brain-derived neurotrophic factors, which are crucial for learning, memory, mood processes and other cognitive functions (Sarkar et al., 2016). Therefore, condition the development of neuropsychiatric and neurodegenerative pathologies (Amirani et al, 2020; Leblhuber et al. 2021).

So, what these different studies showed is that the consumption of probiotics, prebiotics, symbiotics and. psychobiotics when applied for mental health purposes can improve mood, stress-related symptoms, memory, and learning functions as well as regulateemotional behavior (Tong et al, 2020).

2.2. Kombucha

Kombucha is a non-dairy probiotic that has gained a lot of popularity throughout the years. It originated initially in China and then spread to western societies, like Europe and the United States of America, during World War II (Laavanya et al., 2021; Kapp & Sumner, 2019).

Kombucha is a non- or low-alcoholic, acidic, and carbonated beverage that is developed by the fermentation of sweetened tea with a symbiotic culture of bacteria and yeasts (SCOBY). Most SCOBY microorganisms like *Lactobacillus*, *Bifidobacterium*, *Bacillus cereus*, and yeasts of the genus Saccharomyces have *probiotic activity* (Laavanya et al., 2021; Vargas et al., 2021). The literature reported health benefits associated with Kombucha intake, such as anti-inflammatory, antioxidant and anti-bacterial, anti-diabetic, anti-carcinogenic activity, reducing the concentration of cholesterol, and improving the liver metabolism, the immune system, and GI functions (Chakravorty et al., 2016; Morales, 2020; Jakubczyk et al, 2020; Jayabalan et al, 2014; Villarreal-Soto et al, 2020; Villarreal-Soto et al, 2019;). According to the literature it is believable that Kombucha may have some effects on mental health. However, there is no clinical evidence about such claims, being still a very unknown field, that is only now starting to be explored. Some authors refer to Kombucha as a neuroprotective agent, due to its antioxidant activities against neurodegenerative diseases and neural cell death (Morales, 2020; Permatasari et al., 2021). For example, one of the most abundant metabolites present in Kombucha is glutamate, a neurotransmitter responsible for learning, memory, and neural development function, that is associated with the reduction of anxiety and depression symptomatology (Villarreal-Soto et al., 2020; Banerjee et al., 2021). Though Kombucha is a probiotic and there are significant studies that establish the relationship between probiotics and the treatment of certain mental pathologies, direct link is being made between Kombucha and mental health. However, studies that prove this link are scarce. So, research in this area is urgent, to improve the knowledge about the impact of Kombucha on mental health. Anti

The fact that Kombucha is a beverage without legal regulation (except for Brazil) may be a barrier to the execution of clinical trials (Tran et al., 2020). Governments' authorities need to define legislative guidelines to guarantee the safety control of considered natural products, like Kombucha and other probiotics, to gain more concrete evidence about this drink's benefits and safety. The lack of no strict criteria to be followed that guarantees product control and processing can condition the product quality and safety.

Discovering and knowing what benefits Kombucha has on mental health willbe advantageous, because this natural product can be a treatment option and/or preventivecompound of several mental pathologies.

2.3 Gut-Brain Axis

Nowadays there is a growing interest in studying the connection gut-brain. It has been found that the human gut metabolites and possible alterations in the microbiota have been involved in the development of many diseases like inflammatory bowel disease, obesity, colon cancer, and currently nervous inflammatory diseases (Cheng et al., 2022). The gut microbiota can affect the development of the CNS, the activity of immune cells, the regulation of the formation and integrity of the blood-brain barrier and can also influence mental health (Agirman and Hsiao, 2021). The CNS, on the other hand, affects the composition of the microbiota by controlling the movement and secretion of the GI tract. the intestinal immune response through the functional activity of effector cells and it has been shown that the mental state can directly regulate the intestinal microbiota and trigger inflammation and unbalanced intestinal movement through the HPA axis (Dong et al., 2022). The literature has reported that the bidirectional system of the brainand the gut communicate through several pathways, such as the autonomic nervous system; the neuroendocrine system; the immune system; and chemical signaling (Cryan et al, 2019;Casertano et al., 2022; Snigdha et al., 2022; Yu & Zhao et al., 2021; Barrio et al., 2022).

2.3.1. Autonomic Nervous System Pathway

Neural pathways are the best-known route of the GBA and includethe vagus nerve and the ENS (Yu & Zhao, 2021). The vagus nerveis mainly formed by 80% of afferent fibers, that transfer the information from the gut to the CNS, and 20% of efferent fibers that project the information fromthe brain to the smooth muscle of the gut (Bonaz et al., 2018), sensing both mechanical stimuli, luminal volume, and chemical stimuli, like neurotransmitters and cytokines, that may continuously affect the microbiota (Snigdha et al., 2022). The vagus nerve is also involved in the communication between the gut and the caudal nucleus of the solitary tract,involved in the brain circuits dedicated to reward and pleasure (Tan et al., 2020).

The ENS is responsible for controlling GI tract functions, like its motility, epithelial secretions, and blood flow (Wang, 2018) and communicates with the CNS through the vagusand some sympathetic nerves (Yu & Zhao, 2021), and its functions can be controlled by the gut, over SCFAs and neuroactive compounds (Morais et al., 2021).

2.3.2. **Neuroendocrine System Pathway**

The other pathway involved in the GBA is the neuroendocrine system. Gut metabolites can enter systemic circulation activate the HPA axis and modify the secretion of gut hormones and neurotransmitters, and consecutively regulate body metabolism functions (Chakrabarti et al, 2022; Cheng et al., 2022; Cryan et al., 2019). The gut microbiota also can influence the HPA axis by vagal nervesignaling and realizing immune mediators, neuroactive metabolites, and others (Misiack et al, 2020). Activation of the HPA needs a cascade of endocrinological signaling that implies the production of corticotropin-releasing hormone (in the hypothalamus leading to the production of adrenocorticotropic hormone in the pituitary gland (Naveed et al., 2021). The HPA axis can also be activated by the microbiota due to increased permeability of the intestinal barrier and a proinflammatory state (Farzi et al, 2018). When confronted with a stressor, there is an increment of cortisol hormone that can augment blood sugar leading tothe suppression of immunologic functions through cytokine release (Cryan et al., 2019; Casertano et al., 2022; Snigdha et al., 2022).

2.3.3. **Immune Pathway**

The GI tract has a dense concentration of immune cells and is in constant communication with the microbes present in the gut (Cryan et al, 2019). The literature reported that SCFAs, and bacteriocins, as well as neuromodulators, seem to activate the immune system conditioning and regulate cytokine secretion and microglial activation passing through the blood and lymphatic systems and be in constant communication with the brain and the gut (Chang et al., 2022; Cryan et al., 2019; Song et al., 2022). Themicrobiota communicates with the innate immunologic system through microbial-associated molecular patterns (Naveed et al., 2021). When in dysbiosis, bacterial metabolites such as endotoxins can induce paracellular permeability through the compromised intestinal epithelium leading to the chronic release of proinflammatory cytokines, which are related to anxiety and depression (Schoultz & Keita, 2020; Naveed et al., 2021).

2.3.4. **Chemical Signaling Pathway**

The gut microbiota regulates the CNS homeostasis through various chemical compounds like neurotransmitters, such as serotonin and GABA, microbial metabolites, likeSCFAs, and other compounds (Naveed et al., 2021; Yu & Zhao, 2021; Casertano et al., 2022).

GABA is the main inhibitory neurotransmitter and is speculated to be produced in the gut, by *Lacotbacillus spp.* and *Bifidobacterium spp.* (Yu & Zhao, 2021; Casertano et al., 2022; Snigdha et al., 2022). Similarly, another neurotransmitter, serotonin, is produced in the gut and its process is regulated by strains of *Candida*, *Streptococcus*, *Escherichia,* and *Enterococcus spp.* (Casertano et al, 2022; Snigdha et al., 2022; Naveed et al., 2021; Yu & Zhao,2021). This neurotransmitter is extremely correlated with mood, appetite, and sleep functions and its deregulation can lead to developing neuropathologies.

Other metabolites, that influence CNS homeostasis, like SCFAs are products of anaerobic fermentation generated by bacteria in the gut (Barrio et al., 2022; Louis & Flint, 2009), and act as neurohormonal signaling molecules and other neuroactive properties (Casertano et al., 2022; Mirzaei et al., 2021; Naveed et al., 2021). Likewise, SCFAs, such as acetate, butyrate, and propionate (Casertano et al., 2022), interact with their receptors on colonocyte cells promoting indirect signaling to the brain via systemic circulation or vagalpathways, by inducing the secretion of certain gut hormones.

This brief view about the ways of interaction between the GBA axis, allows to not only understand the biological and chemical intrinsic process but also how the food and alimentary habits can impact mental health. A lot of research is still needed, and interdisciplinary work is essential to have a broader and more coherent knowledge about how food does not only impact the GI tract but also neurologic processes.

2.4. **Interdisciplinary of Biotechnology and Psychology**

As has being argued, bringing together different scientific research areas allows for a more comprehensive understanding of complex processes, particularly biological ones. For instance, the interconnection of areas such as biotechnology and psychology in the research field helps to have broader knowledge about the human organism and interconnectivity among different systems and allows correlation of biomedicine and biotechnology methods with neuropsychological and neurophysiological tools.

In this study, this interdisciplinarity does not apply only to behavioral psychology aspects but to neuromolecular aspects, which by itself conditions human behavior. So, the integration between the two scientific fields helps to understand the neuromolecular aspects of human mental state, including memory, perception, learning, creative and emotional processing, among other, generating a more comprehensive knowledge and creation of new therapeutics, for treatment and prevention of mental diseases, like depression, anxiety, neurodegenerative diseases, autism, and so one.

Biotechnology and psychology relationship involves a more holistic approach to the whole human condition, giving more consistent and accurate information of how the human organism communicates within itself; allowing the development of new tools and methodologies for the evaluation of neuropsychiatric and neurodegenerative diseases, design of different neurobiological models that accurately show how the disease starts anddevelops, and develop new treatments or preventive methodologies to improve well-beingand health.

As reported earlier, there is a lot of investment in the knowledge of the gut-brain connection, and the interconnection of the fields of biotechnology and psychology are showing a great input in increasing knowledge. Similarly, understanding how the foods/products we consume impact the gut microbiota and the consequences on the brain has proven to be a very attractive area of interest for human physical and mental health.

3. Methodology

As previously mentioned, this internship was placed in two centers of investigation, CBQF and HNL. In each laboratory, different methodologies of investigation were used. The combination of different methodologies allows the development of interdisciplinary investigations, which are very present in these research centers. Also addressed were transversal methodologies in research at the level of scientific communication.

3.1. Research methodologies in the CBQF

Most of the CBQF tasks involve the following and develop of protocols. I became familiar with several protocols that exist in the literature, used for the study of the gut microbiota (Andrade et al., 2020; Ribeiro et al., 2021; Gullon et al., 2015 and Pimentel et al, 2015). During this internship, beyond the contact with several protocols (mentioned above), I was able to administer them and conceive new protocols (Annex I and II) related with the evaluation of the gut microbiota.

3.1.1. Human faeces samples processing

The human faeces samples treatment protocol is developed by a set of steps. The first stage begins with the collection of human faeces samples from the participants (two grams by participant). The eligible criteria for recruitment were age between 18-65 years, with no pathologies associated and no restrictive diet. After recruitment and before sampling donation, participants were presented with an informed consent form, where it assured data protection and that they could leave the experiment any time. This form mustbe always signed before starting the study. After sample collection the individual fecal inoculum was prepared by diluting the individual faeces to 100 g/L in reduced physiological salt in anaerobic workstation. Sampleswere incubated for 48 h at 37 °C, inside an anaerobic workstation.
We used sample collected by 5 participants and the processing results in 110 samples. The following were created 2 controls (positive and negative), and to each of the sample were given 5 oils separately, replicated in the 0h, 12h, 24h and 48h. The negative and positive control had, respectively, fecal inoculum only and fructooligosaccharides.
Subsequently, 50 mL of basal medium for fermentation was added to glass bottles with pH value adjusted to 6.8 using hydrogen chloride (1 M), and the solution bubbled

with nitrogen until a yellowish color was obtained. Approximately 2% (v/v) of individual fecalinoculum was added to the previously prepared bottles containing basal medium and samples were incubated for 48 h at 37 °C, inside an anaerobic workstation.

Aliquots were collected of each of 110 sampling point, on 0, 12, 24 and 48h. At each sample was added 1mL of tampon, then vortex them and centrifuged at 4000 rpm for 10 min. The supernatant was discarded and 180 μL of lysozyme solution was added to the samples, being incubated for 2h at 37ºC. Samples were centrifuged again at a 4000 rpm for10 minutes. The resulting supernatants are to be used to evaluate the production of SCFAs and the pellet was used to extract genomic DNA.

3.1.2. **Techniques used in the Sample processing**

3.1.2.1. **DNA extraction**

The DNA was extracted through NZY Tissue gDNA isolation kit (NZYTech, Lisbon, Portugal). The resulting pellets were washed with the TE buffer, vortexed, centrifuged, and the supernatant was discarded. Subsequently, 180 μL of the lysozyme was added, and the solution was incubated at 37ºC, for 2 hours. After the centrifugation, both the pellet and thesupernatant were kept. The next steps were done accordingly to the manufacture's manual. The DNA concentration was assessed through a microplate photometer.

3.1.2.2. **Short-chain fatty acids**

Short-chain and branched-chain fatty analysis were determined in supernatants resulted from the fecal fermentation, which were filtered through a 0.22 μm syringe filterto be analyzed through HPLC. The analyses were performed using a HPLC coupled to infrared and ultraviolet at 55ºC and 35 mmol L-1 of sulfuric acid as mobile phase (flow rate:

0.5 mL/min). The identification and quantification were achieved by comparison of the relative retention times of sample peaks with standards and using a calibration curve in therange of concentrations of 0.2–2.0 mg mL-1. The main SCFAs evaluated were acetate, propionate, butyrate, lactate.

3.1.2.3 Fatty acids profile

For evaluation and comparison of total fatty acids profile of the samples we used the GC-FID. This has been the preferential method of fatty acids analysis because it is a very sensitive method that can identify and quantify these compounds. Because fatty acids are non-polar molecules and have high boiling points, which are difficult to evaporate causing the GC column to plug upon injection, a derivatization process is needed. This derivatization,i.e., esterification of fatty acids into fatty acid methyl esters is performed using an alkylation derivatization reagent and causes neutralization of the polar carboxyl functional groups making them volatile and FID detectable. This then allows column chemistry to perform separations by boiling point elution, and by degree of unsaturation, position of unsaturation, and even the cis vs. trans configuration of unsaturation. After the derivatization process the samples were introduced into the GC detector, where the both the GC instrument and canisters containing air, nitrogen and hydrogen were already turned on. The samples were then injected into the inlet, connected to the column, through a septum which enables the injection of the sample mixture without losing the mobile phase.The sample was then transferred to the analytical column where the components were separated by their different interactions with the stationary phase. The molecules detectedwere the ones that elute from the column and were analyzed through GC predefinedcomputerized program. The analytical conditions were flame ionization detector temperature 275ºC, injector temperature 250ºC, oven at 150ºC and column flow at 1mL/min.

3.1.2.4. Identification of the gut microbial strains

The strains present in the microbiome of the 110 samples were identified by qPCR technique. The most common strains in the microbiota, such as *Lactobacillus* spp., *Clostridium leptum*, *Bacteroidetes*, *Bifidobacterium spp*. and *Firmicutes* primers were bought,and instructions of the manufacturers were followed. The general analytical conditions of qPCR were hot start at 95 ºC for 10 min followed by 45 cycles of denaturation (95 °C for 10s), annealing and extension (72 °C for 15 s). As a control of PCR quality, a melting curve analysis was made for each PCR, by temperatures ranging from 60 to 97 ºC, with an increase of 0.1 °C per 0.01 min. Standard curves were generated using serial dilutions of bacterial gDNA standards of Lactobacillus gasseri, Bacteroides vulgatus, Clostridium leptum and Bifidobacterium longum subsp. Infantis (DSMZ, Braunschweig, Germany).

3.2. HNL methodologies

The methodology applied during my internship in HNL had as an objective to understand the acquisition, processing, and analysis of neuropsychophysiological data.

Research methodologies acquired during the internship at HNL were diversified. It was used the EDA, RA, ECG and EEG tools. These techniques were learned and executed during 2 research studies that were taking place at HNL. The neurophysiological techniques learned were measured by an easy-to-use, versatile, and scalable software Bluetooth device that allows to collect real-time different biosignals.

3.2.1. **Electrodermal activity**

EDA indicates the electrical properties of the skin, which are associated with sympathetic activation that increases sweat activity, increasing skin conductance, associated with fight or flight response. It tends to be lower with deep stages of sleep and higher with the person being awake, being a marker for stress (Anusha et al., 2022).

This technique is performed through the strategic placement of electrodes. The EDA was measured using the BioSignals Plux device and software and the electrodes were placed as instructed in the manual of the manufacturer: positive electrode in the index finger and negative electrode in the middle finger (Figure 1).

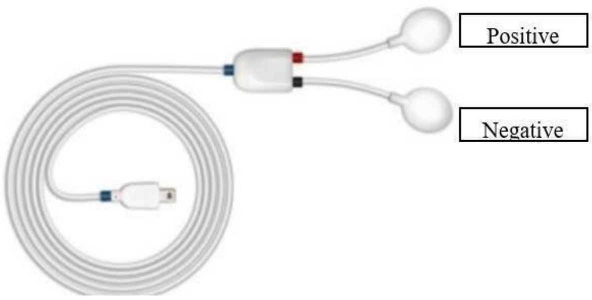

Figure 1. Composition of EDA electrodes.

3.2.2. **Respiratory activity**

RA indicates how many breaths an individual has per minute while at rest. According to research, is intimately correlated with emotional expression and its intensity, where the individual breathing frequency increases with individual anxiety (Jerath et al., 2015; Masoaka et al., 2001). To measure the breathing rate, the subject needs to be laid down or sit comfortably. Next, a respiration belt (Figure 2) is placed, not loosely at the point of complete exhalation around the torso. The subject is asked to breathe deeply, and the belt must be put accordingly to maximize sensitivity. This type of measure is applied inpsychological studies to evaluate the state of relaxation or anxiety of the individual.The RA was measured through Biopac System belt placed around the torso and software.

Figure 2. Biopac Belt.

3.2.3. **Electrocardiogram**

ECG is an instrument that measures the heart's electrical activity and is an effective and reliable source of information for human emotional response and recognition structures (Nita et al., 2022). This measurement is possible using polar and reference electrodes, attached on the human chest, that collect the cardiac electrical activity and rhythm at each heartbeat. The ECG is divided in P wave (depolarization), PR interval (time between onset of atrial depolarization and onset of ventricular depolarization), QRS complex (depolarization of ventricles), QT interval (time interval between onset of ventricular depolarization and end of ventricular repolarization), ST segment, T wave (time interval between onset of ventricular depolarization and end of ventricular repolarization) and U wave (relaxation of ventricles). The signal is digitalized via analogue to digital converter using the software of the company of which the electrodes and the device associated are made (Cascino & Shea, 2021). This protocol consists in the placing of three electrodes, two polarized (positive and negative) and one reference pole (Figure 3).

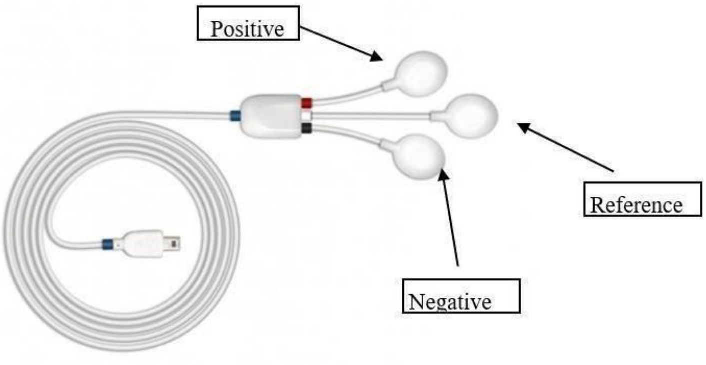

Figure 3. ECG electrodes: positive electrode (red); black electrode is the negative one; white electrode is the reference one.

It should be noted that the placement of the electrodes occurs in the left side of the upper chest but can be placed slightly differently, whether the individual is female or male (Figure 4). In the female body, the electrodes must be above the breast to form a scalene triangle, to preserve the accuracy of the activity monitored and the privacy and respect for the participant body. In the male body the electrodes must adopt the position of an isosceles triangle.

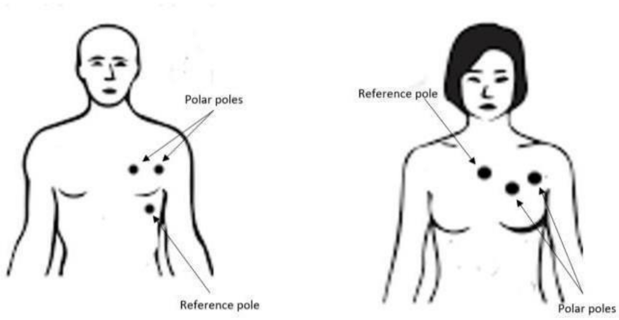

Figure 4-Position of the electrodes in the (A) male body, and (B) female body.

ECG electrodes came from Biosignals Plux, and the signals were digitalized via analogue to digital converter, controlled and recorded through OpenSignals Revolution, the software associated that manages and records the biofeedback signals (Figure 5).

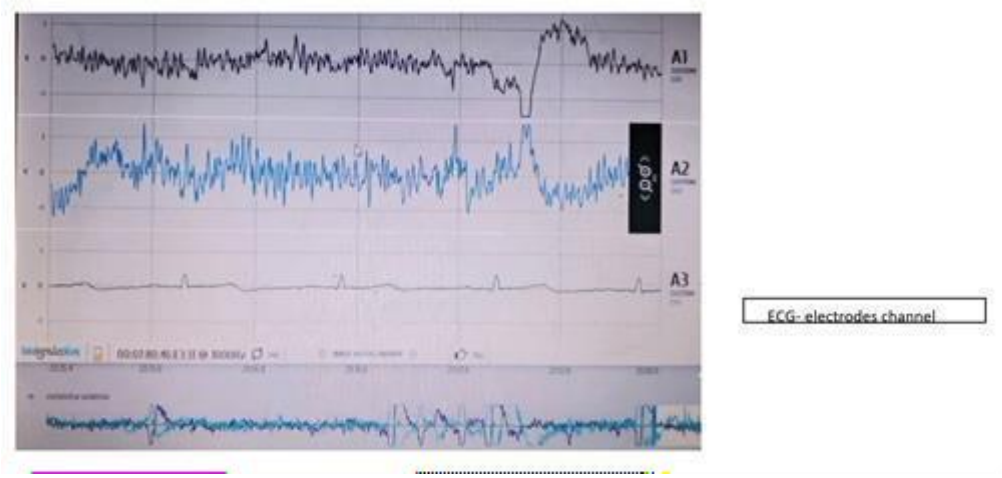

Figure 5– Example of a recorded ECG biofeedback signals.

3.2.4. **Electroencephalogram**

The EEG is painless test used to monitor brain activity through electrodes trying to understand the asymmetries between the brain hemispheres, slow activity, and unusual brain wave's patterns (Wu et al., 2022). There are four types of brain waves: delta, theta, alpha and beta (Farnsworth, 2019). Delta waves tend to be more prominent frontally in adults and posteriorly in children, being associated with a deeper state o sleep. Theta waveshave a higher frequency than delta waves and it is associated with sleep and dreams, beingunusual its presence in awakened adults. Alpha waves have an intermediary frequency andare associated with relaxing and daydreaming mechanisms. Beta waves have a higher frequency and tend to be associated with alert, energetic, arousal and anxious behavior being more perceptible frontally.

The electrodes can be distributed using a CAP, to firm the electrodes in place, to measure all lobes' activities or directly in the scalp and in the tempora, with a conductive gel or paste that maximizes skin contact and minimizes resistance recording throughout the skin (McGill, 2019). However due to the absence of a CAP we used three electrode EEG detector. Each device, like the ECG, has two polar electrodes and one reference electrode (Figure 6).

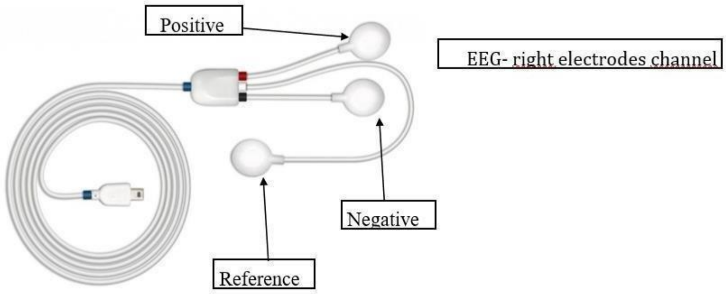

Figure 6- EEG 3 electrodes device: two polar poles, the red electrode (positive) and the black electrode (negative); and the white pole is the reference pole.

Each device, like the ECG, has a proper placement to obtain more accurate data. In this study right and left polarized electrodes were displayed in the correspondent side of forehead and tempora and the reference electrodes were put behind and below of the correspondent ear. Since it was used to measure beta waves, usually exhibited frontally,the study was not compromised (Figure 7).

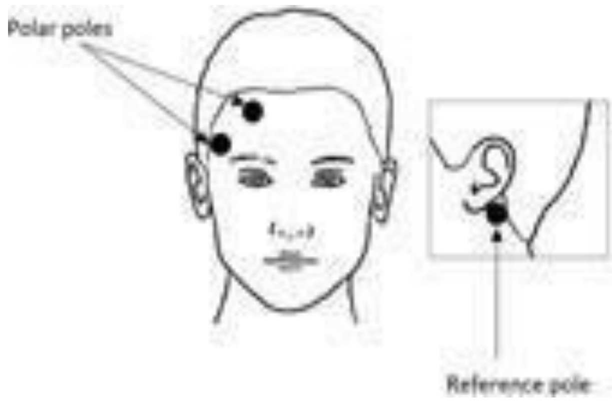

Figure 7- Placement of the 3 electrodes EEG.

EEG electrodes came from Biosignals Plux, and the signals were digitalized via analogue to digital converter, controlled and recorded through OpenSignals Revolution, the software associated that manages and records the biofeedback signals (Figure 8).

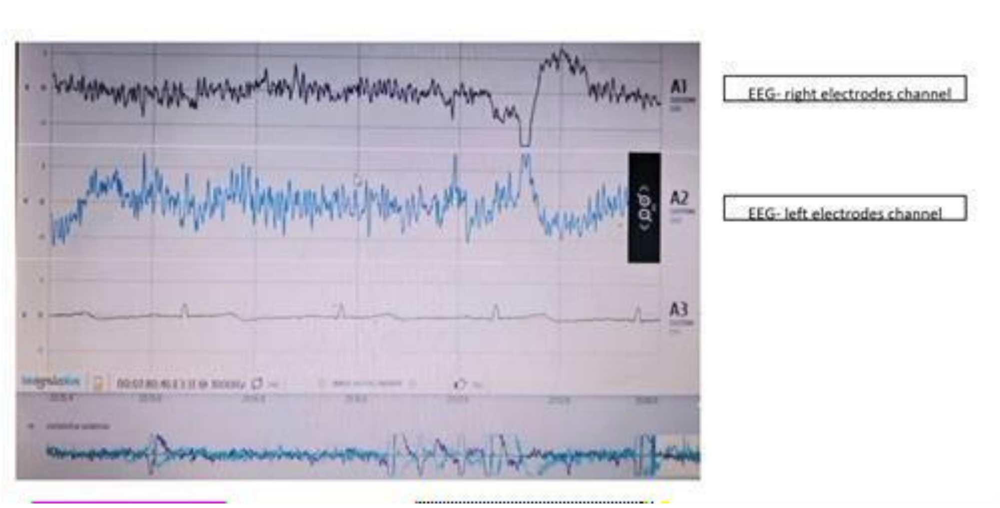

Figure 8- Example of the recorded EEG biofeedback signals.

3.3. Transversal methodologies of Investigation

This internship allowed the development of transversal competencies to any investigation, like collection and selection of scientific relevant information, through consultation of different databases and reading and interpretation/analysis of different articles. Practical application of the knowledge acquired through the design and writing of scientific articles, both review and research articles. The development of transversal methodologies also included the planning and participation of scientific events and investigation meetings, to enhance my knowledge and use of scientific language.

4. Results and Discussion

4.1. Activities in the CBQF

In the CBQF laboratory I learned basic laboratory practice procedures, as well as different research and analysis methodologies.

Regarding basic laboratory practices, it is important to know the safety rules, such as the use of personal protective equipment, hygiene rules, correct use of equipment, correct handling of the different laboratory instruments (materials, reagents), and others. Before each experimental activity is carried out, it should be planned, the respective protocol selected, and carefully analyzed before executing it. The accuracy and precision are very important to guarantee the correct data obtained.

In addition to the development of laboratory techniques, theory, and practical knowledgel acquired in managing certain protocols, I also developed practical research skills. I performed and followed several protocols, always under supervision, as mentioned in the methodology section.

I also had the opportunity to design two protocols, based on the knowledge acquired and the literature consulted (Annex I and II). These new protocols could be used in a future research study, about Kombucha and mental health, that is also being planned at HNL. The first protocol (Annex I) is about the ideal Kombucha's tea production, it was created based on different studies to develop the "perfect Kombucha" considering the fundamental parameters reported inthe literature. The second protocol (Annex II) refers to the analysis of the human gut microbiota. This protocol was based on the protocols followed in the CBQF laboratory within the view of the Kombucha and Mental Health thematic and can be improved if combined with neurophysiologicalmeasures learned in the HNL. A crucial part of investigation is the application of the informed consent form, allowing us to assure the participant that the data collected from her or him will be protected by professional secrecy.

4.2. Activities in the HNL

During this internship in the HNL, I acquired technical skills through contact with different neurophysiological measures, by participating in the data collection (under supervision) of two studies that were occurring in HNL laboratory.

The first study conducted consisted in a little wine test with six participants where the goal was to understand if the intake of wine is somehow correlated with relaxation or anxiety. In this case it was used the EDA and RA measurement to understand what emotional respond there was towards the different wines presented.

EDA gives biofeedback reflecting the output of integrated attentional, affective, and motivational processes within the CNS acting on the body due to application of differentstimuli. Due to privacy and comfortability issues electrodes are applied in the fingertips. The electrodes can be applied in a skin surface but mainly where is more notable the sweat production, like palm of the hand, sole of the feet, toes, and fingertips. The RA gives anoutput of the emotional state of the individual by evaluating and quantify the respiratory frequency. This device must be correctly placed around the torso and any type of noise should be avoided so that accurate data is collected. With the Biopac System software the signal must be close to 0 volts to the biosignals be precise. When rested, individuals have a normal RA which reveals to be a normal and constant breathingfrequency. Alterations in the physical and or mental state of the individual reveals to have an altered RA with variations of the breathing frequency to higher or lower.

The other conducted research consisted in evaluating the human response to certain stimuli came from three games, through neurophysiological measures, in 100

participants. The two main techniques here applied were the EEG and ECG. Of the games selected, two are very well established in the literature as one being a game stress inducer,Tetris, and the other being a game stress reductor, Bejewelled. The other game is Energy, by Infinity Games, which is the one we were trying to evaluate and compare with the stressor and stress reducer games, to determine what is it effect on stressed behavior. Thefirst moment of this study refers to the informed consent, sociodemographic questionnaire and the three games tutorials. The second moment consisted in placing the EEG and ECG electrodes, measure baseline data, application of the TSST (1, 2, 3) and application of the games. Both TSSTs and games had a duration of 10 minutes. After applying the EEG and ECG electrodes, baseline, i.e., the electrical signal from the sensor when no variable is present, must be conducted, to then compared it with the signal displayed after the stimulus is applied and the study continued sequentially as mentioned above.

As for the EEG it is very important that the EEG electrodes be symmetrically spread so that the brain activity between the two hemispheres can be accurately measured. Here, we were evaluating mainly the alpha and beta waves since anxiety is usually correlated withthe overpower of beta waves on alpha waves. To have a better comparison and analysis of the activity of both hemispheres, the EEG electrodes must be on consecutive channels in the BioSignals Plux. The ECG electrodes have also a standard protocol, as mentioned above. Thisnot only ensures an accurate trace of the activity but also the comparability of different records. To perform these assays is necessary to be careful and thoughtful in the management of the device and application of the respective electrodes. All electrodes mustbe correctly applied, on a clean surface, otherwise, there will be a non-accurate register, due to the noise associated.

More than the technical skills development, there was the enhancement of personal competences towards approaching the participant. A researcher must study how will the contact with the participant be, i.e., how will identified him- or herself to the participants and how will establish an empathic relation with them throughout the study. It is essential that before every study starts, an informed consent form be displayed where it assures dataprotection and that the participant can quit the study at any time.

4.3. Other scientific activities

4.3.1. Scientific Writing

Beyond laboratory skills, this internship allowed me to grow as a researcher by gaining writing aptitudes towards scientific articles. This this task was concluded with the

participation in the elaboration of three articles (two reviews articles and one research article).

All articles were constructed under the same guidelines, research, and careful reading of the information on the subject available in the literature. First, research using different databases was conducted, accordingly to each thematic portrayed in the articles and, secondly, articles were written based on the different information gathered.

I started by participating in the writing of a review article, because when you start a research process it is fundamental to know and understand the theme state of the art. Thefirst review article intitled "Kombucha: perceptions and future prospects" involved intensive research on Kombucha legislation and Kombucha production guidelines. This is an interesting theme because there is a lack of legislation reported in the literature, associated with not only probiotics in general, but mainly with Kombucha, sparked the development of this article. It is important to draw attention for this thematic because the consumption of this product is increasing and there are no regulations for its production that could endanger the health and safety of consumers. Being my first scientific publication(Annex III) demanded a careful dedication and the need for guidance, so this process was developed in 2 phases. In the first phase, we used scientific database such as Scopus and Web of Science and selected descriptors like "Kombucha", "composition", "probiotics" and "legislation". The following were established exclusion and inclusion criteria and analyzed the 200 articles found. To collect as much information as possible about the content we wanted to evaluate, we also consulted government and legal information on the theme. On the second phase, the relevant information was selected, and a carefully structured article was designed to be interesting, rich in content and innovative. The article was then submitted and accepted in an international scientific journal, *Foods* 2022.

The second review article entitled "Kombucha and mental health" was born from the need to know more about this product and its impact on mental health. This article emerged from the need to find scientific evidence about the relationship between Kombucha and mental health. This relationship is often reported in the literature but is not supported by scientific information. After consulting again scientific databases and using specific descriptors such as "Kombucha" AND ("mental health" OR "mental diseases" OR "mental disorders" OR "neurodegenerative diseases" OR "depression" OR" parkinson" OR "alzheimer" OR "neurodevelopmental disorders") information was collected and carefully analyzed. It was constructed a mini-review article since we concluded that there are few studies (5 studies) with scientific evidence on this topic. However, we consider it pertinentto write the article to draw attention to this problem and the need for more research on it.

Finally, skills in writing a research paper were acquired. This type of article requires a review of the literature, but other skills to be worked at the level of reporting the results obtained, analysis of data collected, and discussion of them. Here HNL data collected was used. The participation in the realization of this article allowed me to acquire other writing skills. This article entitled "Health beliefs and popular perspectives about Kombucha in college students", is still under construction, and aims to address the perception of Portuguese consumers about the consumption of Kombucha. Through the writing of this article, we realized that a new investigation should be carried out. It will be interesting to know and compare the perception of consumers about the consumption of Kombucha in Portugal (where consumption is still recent) and Brazil (where consumption has existed for some time). In this way, it was also possible for me to participate in the planning of this new studythat will emerge. We are currently building an evaluation element, a questionnaire that will allow us to collect data on the consumption of these products in two different populations. This instrument of evaluation is being developed because it does not exist a tool that assesses how the consumption of the same product, in this case Kombucha, works and maydiffer in different populations and countries.

4.3.2. **Event Planning**

In addition to the written skills, it was possible for me to acquire skills in organizing and participating in scientific events. Throughout the Week of March 28th and April 1st, I participated in the International Psychology Week, promoted by FEP-UCP. In this event, I was able to be in contact with different researchers, from different parts of the world, like Italy, Romania, UK, and USA. These researchers from different fields allowed me to gain and develop different skills not only related to Psychology but also personal interaction abilities. I also had the opportunity to attend and participate in the Open Week at Escola Superior de Biotecnologia – UCP which occurred from April 20th to April 22nd with the theme "Biotechnology and Sustainability". This event was directed to the community, mainly high school communities.

Participating in these 2 different events allowed me to know all the work that must be done in developing these experiences and allowed me to grow personally and as a futureresearcher. The researcher's work involves not only the construction of scientific knowledgebut also the communication of the results obtained to different audiences. This communication can be done by oral communications or by written communications, such as scientific or opinion publications. In scientific communication, it is very important to adapt the language to the audience where the message is to be transmitted, such as the scientific community, the public, and the academic community, among others.

4.3.3. **HNL Meetings**

Throughout the internship year, I was allowed to participate in the HNL research meeting that is constituted by diversified team, university professors, researchers of different fields and students. In these meetings were addressed several topics related with neuroscience: ideas, articles discussion, projects development and assignments presentation. This allowed to create a more curious and thorough thinking about all different thematic that involve the brain and how it can help us to deconstruct the human neurologic system so that there was a deeper knowledge about diverse variables and factorsthat impact brain and mental health.

5. Conclusion

The choice of a multidisciplinary internship had as a central objective the interconnection between the biotechnology and psychology fields, knowing and applying different techniques/methodologies individually and subsequently understand how combining them creates a more precise and wide-ranging study.

Throughout this internship all objectives outlined for each task were fulfilled and I was able to obtain and improve scientific competencies, including laboratorial skills due toassays conducted in the CBQF and HNL, as well as scientific researchers, as designing protocols and writing scientific publication. The focus of my internship allowed me to grow in an area that I am excited about, learning more about Kombucha and the impact of microbiota on brain activity.

Even though it was an exhaustive year, it was very gratifying to participate in this interdisciplinary project because it allowed me not only the improvement and consolidate biochemical and biotechnological skills, but to combine them with different psychological skills, and to develop transversal investigation skills to communicate science through different approaches, whether orally or written.

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