CATÓLICA
FACULTY OF BIOTECHNOLOGY
PORTO

PRODUCTION AND CHARACTERIZATION OF BIOACTIVE PEPTIDES FROM BLUE MUSSEL SHELLS

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PRODUCTION AND CHARACTERIZATION OF BIOACTIVE PEPTIDES FROM BLUE MUSSEL SHELLS

Thesis presented to Escola Superior de Biotecnologia of the Universidade Católica Portuguesa to fulfill the requirements of Master of Science degree in Applied Microbiology

By

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Abstract

Mussels, a popular global seafood choice, and the disposal of large amounts of mussel shells raise environmental concerns, prompting the industry to explore two main approaches, extracting calcium carbonate and valorizing the organic matrix composed of proteins, for more sustainable waste utilization. In this study we aimed to extract and characterize bioactive peptides from the blue mussel (*Mytilus edulis*) shells, contributing to an added-value upcycling of this waste stream.

In terms of demineralization, the overall yield of extraction was 84.6%, as for the solubilization step, it was 53.2%, and for the hydrolysis, it obtained a global yield of 59.1%.

The blue mussel shells peptides obtained exhibited antioxidant activities, namely 3.98 ± 0.41 µmol Trolox Equivalent/mg protein for ABTS method (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) and 4.12 ± 0.22 µmol Trolox Equivalent/mg protein for ORAC method (oxygen radical absorbance capacity). The antihypertensive activity, determined by Angiotensin-converting enzyme (ACE) inhibitory activity, resulted in an IC_{50} of 805.7 ± 18.0 µg protein/mL.

The peptide size composition of the obtained fractions characterized through High Pressure Size Exclusion Chromatography (HPSEC) revealed that most of the peptides are in the range between 1 and 3 kDa and under 1 kDa.

Following, for the gastrointestinal simulation, the acquired peptides demonstrated antioxidant properties at the end of digestion, specifically 7.06 ± 1.96 µmol Trolox equivalents/mg of protein using the ABTS method and 4.38 ± 0.36 µmol Trolox equivalents/mg of protein using the ORAC method.

This pioneering study allowed us to obtain a good yield of peptides from the blue mussel shell and demonstrated their bioactive potential.

Keywords: bioactive peptides, enzymatic hydrolysis, blue mussel shell (*Mytilus edulis*), antioxidant activity, antihypertensive activity, antimicrobial activity

Resumo

Os mexilhões, são uma escolha muito popular de frutos do mar e o descarte de grandes quantidades de conchas de mexilhão levanta preocupações ambientais, levando a indústria a explorar duas abordagens principais para o seu reaproveitamento, a extração de carbonato de cálcio e a valorização da sua matriz orgânica, composta por proteínas, para uma utilização mais sustentável dos resíduos. Este estudo tem como objetivo extrair e caracterizar péptidos bioativos das conchas de mexilhão-azul (*Mytilus edulis*).

Para extrair os péptidos bioativos das conchas de mexilhão foram realizadas três etapas, sendo estas, desmineralização, solubilização e hidrólise enzimática. Em termos de desmineralização, o rendimento global de extração foi de 84.6%, para a etapa de solubilização de 53.2% e para a hidrólise obteve-se um rendimento global de 59.1%.

Os péptidos obtidos a partir da concha de mexilhão azul, exibiram atividades antioxidantes, nomeadamente de 3.98 ± 0.41 µmol de equivalente Trolox/mg de proteína para o método de ABTS e 4.12 ± 0.22 µmol de equivalente de Trolox/mg de proteína para o método de ORAC. A atividade anti-hipertensiva, determinada pelo método iACE, resultou em IC_{50} de 805.7 ± 18.0 µg proteína/mL.

A distribuição do tamanho dos péptidos obtidos foi caracterizada por cromatografia de exclusão de tamanho, e revelou que a maioria dos péptidos apresentavam um tamanho entre 1 e 3 kDa e abaixo de 1 kDa.

Após a simulação gastrointestinal, os peptídeos adquiridos demonstraram propriedades antioxidantes, resultado em 7.06 ± 1.96 µmol equivalentes de Trolox/mg de proteína usando o método ABTS e 4.38 ± 0.36 µmol equivalentes de Trolox/mg de proteína usando o método ORAC. Este estudo pioneiro permitiu extrair péptidos bioativos da concha de mexilhão azul e demonstra o seu potencial bioativo.

Palavras-chaves: péptidos bioativos, hidrolise enzimática, concha de mexilhão-azul (*Mytilus edulis*), atividade antioxidante, atividade anti-hipertensiva, atividade antimicrobiana

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This thesis is the result of collective efforts and support from these wonderful individuals and institutions that gave me the strength and tools to make this work and thesis possible. Thank you for being an integral part of my academic and life journeys.

Content

Abbreviations

AA Amino acids

AAPH 2,2′-azobis(2-methylpropionamidine) dihydrochloride

ABTS 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)

Abz Aminobenzoic Acid

ACE Angiotensin-converting enzyme

Ala Alanine

ANOVA Analysis of variance

AUC Area under the curve

BCA Bicinchoninic Acid

BSA Albumin standard

DH Degree of Hydrolysis

DNA Deoxyribonucleic acid

DS Demineralized shells

Gly Glycine

HPSEC High-Performance Size-Exclusion Chromatography

iACE Inhibition of Angiotensin-Converting Enzyme

Ile Isoleucine

MIC Minimum Inhibitory Concentration

MW Molecular Weight

ORAC Oxygen Radical Absorbance Capacity

PBS Phosphate Buffer Saline

Phe Phenylalanine

Pro Proline

RGE Rabbit gastric extract

RNA Ribonucleic acid

ROS Reactive Oxygen Species

SD Standard Deviation

SDGs Sustainable Development Goals

SDS-PAGE Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

SGF Simulated Gastric Fluid

SIF Simulated Intestinal Fluid

SP Solubilized proteins

SSF Simulated Salivary Fluid

TNBS Trinitro-Benzene-Sulfonic Acid

WS Whole shells

Introduction

1. Food waste

The global population is rising at an astronomic pace, currently with about 8.1 billion people and estimated to reach 11 billion by 2100.[1] Therefore, this abrupt growth impacts every aspect of our planet, whether climatic, water resources, erosion, lack of food, among others. All of that is so concerning and dangerous for the planet's future and humankind that in 2015, the United Nations had to create the Sustainable Development Goals (SDGs). In 2019, the United Nations (2021), affirmed that 931 million tons of food were wasted.[2] Food waste in Europe is a significant problem with profound economic, social, and environmental implications. According to the United Nations, only household wastes account for an average of 290 kilograms per capita yearly in Europe. To those numbers, Portugal and Spain contribute with 84 and 77 kilograms per capita each year, respectively.[3] To address this issue, the European government has taken various initiatives to reduce food waste, such as, The European Union's Circular Economy Action Plan, which includes possible changes in the laws to promote sustainable food systems and minimize food waste.[4] Moreover, all those wastes can be reduced or used as by-products, and like that, have a "second life" and maybe even more market value.[5,6] In sum, 61% of the food is wasted in the household, 26% is lost in the food service, and 13% in retail.[3]

Food waste in the sea industry, particularly in fisheries and aquaculture, is a pressing environmental and economic concern. As the global demand for seafood increases, so does the amount of discarded fish and other marine species. Therefore, this wasteful practice poses significant challenges to marine ecosystems and the sustainability of the sea industry. Factors like bycatch contribute to food waste in the seafood industry. Bycatch refers to the accidental capture of non-target species during fishing or the discarding of undersized or unwanted catch to meet regulations or market demands. In seafood processing, unused parts like heads, tails, and shells often become waste, increasing the industry's total food waste. [7]

Mussels are a popular seafood choice worldwide, but their processing and consumption contribute to significant food waste. During mussel processing, non-edible parts like shells, beards, and other components are discarded, making up a substantial portion of this industry's waste. Similarly, mussels failing to meet size or quality standards are often thrown away during various stages, such as harvesting and post-harvesting. Additionally, consumer preferences for specific sizes and appearances can lead to the disposal of otherwise edible mussels. [5,8]

Discarding large mussel shells creates various issues, including economic and nutritional setbacks. Most critically, the typical waste disposal methods are dumping them into the sea, landfills, or incineration, which have environmental drawbacks. Dumping shells into water reduces light penetration and lowers oxygen levels in the sea's upper layers. Landfill disposal can produce harmful gases like methane, hydrogen sulfide, and ammonia.[9] Incineration, while feasible, is costly, consumes significant energy, and generates carbon emissions.[10] In order to find ways to upcycle waste, the industry is focusing on two main approaches for valorizing mussel shells. The first approach targets calcium carbonate, 90 to 99% of the shell's composition. The second approach also focuses on valorizing the shell's organic matrix, primarily composed of proteins.[11]

To sum up, the mussel consumption (*Mytilus edulis* and *Mytilus galloprovincialis*) in 2019 was around 650 000 tons. In the European Union, the total production and importation was about 660 000 tons, which means that at least 1.5% of the product was wasted. Still, those numbers do not consider the waste generated by the shells.[12] Thus, considering that 70 to 80% (dry weight) of the mussel is the shell, it can be affirmed that the actual values of mussel waste are much higher than the ones described by Medina Uzcátegui, *et al.* (2022).[11]

2. Mussel shells matrix

The blue mussel (*Mytilus edulis*) shell matrix is a remarkable biocomposite material with significant interest in various scientific fields. The shell's matrix is mainly composed of calcium carbonate, proteins, and other organic components, which contribute to the structural integrity and resilience of the shells. This matrix is a fascinating example of biomineralization, where living organisms generate minerals within their tissues. The proteins in this matrix are crucial for controlling the formation of calcium carbonate crystals, they serve as templates that guide the deposition of minerals, building a complex yet solid shell structure. The unique layout of these proteins also enhances the shell's mechanical properties, making it both strong and flexible. Those are key factors for the shell to withstand environmental pressures like wave action and predation. Beyond biology, studying the blue mussel shell matrix has inspired researchers to develop new biomaterials, such as, bone grafts and biomedical coatings. In short, the matrix of the mussel shell exemplifies biomineralization, highlighting the essential role of proteins in shaping its complex structure.[13–15]

Concerning the organic matrix in the blue mussel shell, it is present in one of the layers of the shell, called periostracum. This organic matrix contains chitin, acid polysaccharides, glycoproteins, and proteins. These proteins are secreted by the shell-forming cells and are essential for nucleating and controlling the growth of calcium carbonate crystals.[16,17] They function as templates and inhibitors during mineralization, shaping the crystal morphology and preventing uncontrolled mineral growth. Some key proteins can be found in the shell matrix, including MSP-1 (*Mytilus edulis* shell protein 1), L-7, and other acidic proteins that we discovered by Marie *et al.* (2011).[17] As a result, those proteins are challenging to solubilize due to their arrangement and links to the minerals and also because the main present proteins are the conchiolins. Conchiolins are a variety of hydrophobic proteins with a wide range of molecular weights and are rich in glycine and alanine. [18–20]

3. Uses for mussel shells

Blue mussel shells have various practical uses in different industries. Owing to their unique properties and abundance, some of the significant applications of blue mussel shells are in Figure 1.

Blue mussel shells offer a versatile range of applications across various fields. Their high calcium carbonate content, in agriculture and gardening, is an effective soil amendment to raise soil pH, enhance soil structure, and provide essential nutrients like calcium to plants.[21] In wastewater treatment, crushed shells are a sustainable alternative for adsorbing heavy metals and other pollutants, purifying water, and minimizing environmental harm.[22] They also offer a valuable source of calcium for animal nutrition, particularly for poultry and livestock, improving bone development and eggshell quality.[23] The calcium carbonate in these shells presents an eco-friendly substitute for synthetic calcium supplements for human consumption.[24]

They also play a role in bioremediation, providing a substrate for microorganisms, that break down pollutants in contaminated areas, and in aquaculture, these shells serve as a substrate material that supports the growth of beneficial bacteria, aiding in water quality management. [25,26] These by-product can also act as a natural substrate, for cultivating marine organisms, like oysters and sea cucumbers, contributing to sustainable mariculture practices.[25,26] For erosion control and shoreline stabilization, crushed shells can be part of bioengineering solutions, that protect coastlines and promote vegetation growth.[27] Researchers have even explored their potential as additives in cement and concrete production, where their calcium carbonate content

can partially substitute for traditional limestone, thereby reducing carbon dioxide emissions and promoting sustainability in construction materials.[28]

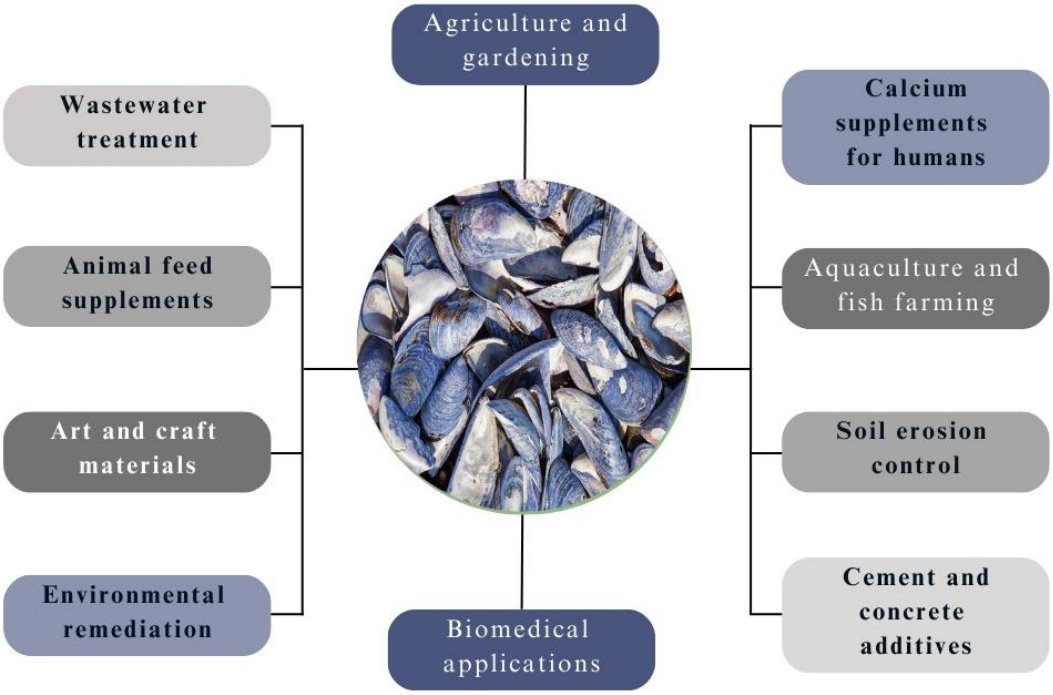

Figure 1 – Some applications of the blue mussel shells by-products.

Although calcium carbonate is the most abundant molecule on the shell and has many applications, compounds, with more value, can be found on the organic matrix, such as, protein and derived bioactive peptides, that could be used in biomedical and nutraceutical applications.[29]

4. Extraction of the bioactive peptides

Demineralization and enzymatic hydrolysis are the most used methods to extract the organic matrix of shells. The existing literature offers limited insights into extracting proteins and peptides from mussel shells. However, we can make supported assumptions based on the similarity of these shells to those of other bivalves. For example, studies have identified four specific types of proteins in Pacific oyster shells: epidermal growth factor (EGF)-like, lactamase-like, sea urchin spicule homolog, and fibronectin domain-containing proteins.[30,31] In blue mussel shells, researchers have detected several uncharacterized proteins, as well as peptides that match Mcal-carbonic anhydrase, a partial putative sequence of Mcal-Fibronectin,

and proteins similar to perlwapin, nacrein, fibronectin, and perlucin.[32] As for the triangle-shell pearl mussel, studies have found a C1q domain-containing (C1qDC) protein.[33] It was also found in the triangle-shell pearl mussel a mature protein of hic7,[19] and various immunomodulatory proteins, including toll-like receptor 4 and lectin, along with small heat shock proteins.[34] These studies have also outlined some of the involved pathways.

4.1. Demineralization with HCl

Demineralization is a crucial step in extracting peptides from blue mussel shells. As mentioned before, the shells of blue mussels are primarily composed of calcium carbonate, which imparts their rigid and robust structure. So, the demineralization involves the removal of the mineral component, leaving behind the organic, protein-rich shell matrix, that is then subjected to further processing for peptide extraction.[13]

During the demineralization process, some solutions can be used, from acid to alkaline or even water. Hydrochloric acid (HCl), is the strongest solution, used to dissolve the calcium carbonate from the shell matrix. The HCl and calcium carbonate reaction breaks down the mineral component, liberating carbon dioxide gas. This reaction also transforms calcium carbonate into calcium chloride, leaving behind a demineralized shell matrix enriched with proteins.[35]

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O$$

The demineralized shell matrix, now devoid of calcium carbonate, allows further processing to extract bioactive peptides from the existing proteins.[35]

5. Enzymatic hydrolysis

Peptides are a specific sequence and arrangement of amino acids, that determine their unique three-dimensional structure and functional properties. The primary structure of a peptide refers to the linear sequence of amino acids, linked by peptide bonds. The arrangement of different amino acids, in this sequence, influences the overall folding and conformation of the peptide chain. The secondary structure of peptides involves interactions between nearby amino acids within the peptide chain. Common secondary structures include alpha-helices and beta-sheets, which result from hydrogen bonding between the backbone atoms of amino acids. The tertiary structure of peptides describes the three-dimensional arrangement of the entire peptide chain, including the folding and bending of the secondary structure. This level of organization is

crucial for determining the peptide's stability and specific biological activity. The quaternary structure of peptides refers to the association of multiple peptide chains to form a larger functional unit. In the context of blue mussel shell proteins, the quaternary structure may involve the assembly of various peptides into complex protein arrangements, that interact with calcium carbonate, to facilitate biomineralization.[16,36]

Enzymatic hydrolysis of proteins in blue mussel shells is critical to release bioactive peptides, with various health-promoting properties. This process employs specific enzymes, such as, Alcalase, to break down proteins into smaller peptide fragments, unlocking their bioactive properties and potential applications. Alcalase comes from *Bacillus licheniformis* and is commercially available. It is primarily used in food matrixes for its proteolytic activity. As a serine endopeptidase, Alcalase targets and cleaves peptide bonds within protein structures, yielding smaller fragments without altering the terminal amino acid sequences. This precise enzymatic reaction generates peptides of varying sequences and lengths, each with unique bioactive properties. Moreover, using Alcalase makes it easier to predict the types of peptides that will be produced.[37,38]

Alcalase activity is tightly controlled by adjusting key parameters such as enzyme concentration, temperature, and reaction time. These adjustments directly affect the enzyme's efficiency and specificity in hydrolysis, enabling the production of various bioactive peptides. Most notably, Alcalase is commonly used to produce antioxidant peptides.[38]

6. Bioactive peptides

As mentioned, bioactive peptides are short amino acid chains with specific physiological effects, and several key factors influence their bioactivity. The structure of bioactive peptides lacks a consensus, but their amino acid sequence is generally considered crucial.[39] Specific sequences can engage with biological targets, like receptors or enzymes, initiating physiological responses.[39] Peptide size also matters; most bioactive peptides have 2 to 20 amino acid residues, facilitating easy absorption and transport in the body.[39] Furthermore, the peptide's three-dimensional structure, including secondary and tertiary formations such as alpha-helices and beta-sheets, is vital for effective interactions with target molecules.[39]

Enzymatic hydrolysis of proteins releases bioactive peptides, as specific enzymes cleave peptide bonds, generating fragments with unique functionalities. Bioactive peptides can exert various beneficial effects on the body, such as antioxidant, antimicrobial, anti-inflammatory,

antihypertensive, and immune-modulatory activities. Their ability to interact with cell surface receptors, triggers intracellular signaling pathways, leading to these responses.[39]

The literature reveals that there are bioactive peptides present in the meat of blue mussels and other mollusks but lacks information on peptides from blue mussel shells. Researchers have identified that blue mussel meat contains bioactive peptides with multiple health benefits, including inhibiting the angiotensin-I-converting enzyme (ACE), liver protection, antioxidant effects, bone growth promotion, and antithrombotic activities.[40–44]

Antioxidant activity can have several mechanisms involving scavenging or neutralizing reactive oxygen species (ROS) and reactive nitrogen species. These mechanisms contribute to the reduction of oxidative stress and prevent cellular damage. The antioxidant peptides can directly interact with ROS, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals. By donating electrons or hydrogen atoms, these peptides neutralize ROS and prevent them from causing oxidative damage to cellular components.[45] Therefore, some peptides can chelate metal ions, such as iron and copper. The peptides can inhibit ROS production and oxidative stress by sequestering these metal ions.[46] Additionally, they can stimulate the production and activity of some antioxidant enzymes, namely, superoxide dismutase, catalase, and glutathione peroxidase, these enzymes play a vital role in the neutralization of ROS.[47] Antioxidant activity can have several mechanisms; therefore, it is assumed that the type, position, and hydrophobicity of AAs (amino acids) within peptides play a significant role in their bioactivity. Many reactive AAs in proteins possess nucleophilic sulfur-containing side chains, such as, taurine, cysteine, and methionine, or aromatic side chains, such as tryptophan, tyrosine, and phenylalanine. The antioxidant capacity of proteins and peptides is influenced by structural properties such as molecular weight (MW), AA structure, sequence, and hydrophobicity. [48]

Some bioactive peptides can inhibit the angiotensin-I-converting enzyme (ACE) by disrupting the renin-angiotensin-aldosterone system, which controls blood pressure and fluid balance. ACE inhibitors target this enzyme and reduce its activity, lowering blood pressure. These inhibitors primarily attach to the enzyme's active site, block its function, and stop the conversion of angiotensin I to angiotensin II.[49,50] Moreover, the effectiveness of peptides in reducing high blood pressure (antihypertensive activity) is influenced by several factors, including their molecular weight (MW), molecular interactions, and chain length. Furthermore, the presence

of specific amino acids, notably proline (Pro) and isoleucine (Ile), plays a crucial role in this activity.[48]

The antimicrobial activity of a peptide can act through various mechanisms that disrupt the integrity and functionality of microbial cells. These mechanisms involve interactions with the cell membrane, intracellular components, and specific microbial enzymes. Many antimicrobial peptides target microbial cell membranes. These peptides can disrupt the structure of the membrane, leading to leakage of cellular contents, loss of ion gradients, and, ultimately, cell death. The peptides can do that, for example, inducing the formation of pores in the microbial membranes, those pores disrupt the membrane integrity and can lead to uncontrolled movement of ions and molecules, causing cell lysis.[51,52] Furthermore, some peptides can penetrate microbial cells and engage with vital intracellular elements like DNA, RNA, enzymes, and proteins, these interactions impair vital cellular functions, such as the activity of microbial enzymes crucial for growth and survival, ultimately leading to metabolic failure or cell death.[53,54] These peptides are characterized by their various molecular sizes, secondary structures, and net charges, which allow for a wide range of mechanisms for inhibiting microorganisms. The distinguishing feature of most antimicrobial peptides is their non-specific action, exerting inhibitory effects through disruptive cytolytic or pore-forming activities. This non-specificity is advantageous because it prevents bacteria from resisting these peptides, rendering them an excellent alternative to traditional antibiotics.[48]

7. Objectives

This thesis aims to valorize the shells of blue mussels, as a by-product, through the extraction and characterization of bioactive peptides.

In order to achieve the main goal of this project, it is necessary to improve the standard extraction of protein from the whole mussel shells, namely demineralization and hydrolysis processes. Additionally, a new extraction step will be implemented, so a solubilization step will be introduced, to enhance the yield of protein and peptide extraction.

Then, after obtaining the optimized fractions, they will be analyzed, in terms of, physicochemical properties, quantifying protein, ashes, and humidity. It will also be determined the quantitative profile of free amino acids and the profile of peptide size. Besides, it is important to evaluate potential bioactivities, such as, antioxidant, antihypertensive and

antimicrobial. Finally, the extracted proteins and peptides will be submitted to a gastrointestinal simulation to evaluate their digestibility.

This project represents a collaborative effort between the Escola Superior de Biotecnologia from Universidade Católica Portuguesa (through its center of Biotecnology and Fine Chemistry - CBQF) and the Derio unit of AZTI's research center in the Basque Country under an Erasmus Master mobility.

Materials and Methods

We divided the practical work into four stages: extracting bioactive peptides, characterizing their physicochemical properties, analyzing their bioactivities, and simulating their gastrointestinal digestion. We employed a three-step process to extract these bioactive peptides: demineralization, solubilization, and enzymatic hydrolysis, as shown in Figure 2.

Figure 2 – General graphical representation of the extraction of bioactive peptides

1. Preparation of the shells

AZTI in Derio, a research center in the Basque Country, provided the blue mussel shells for the study, where the initial stage of the project was executed. We received three distinct types of shell by-products. The first type consisted of shells already cleaned and crushed to the optimal size. The second type included whole shells with mussels still attached, while the third type featured mussels and shells crushed together. We pooled shells from different boxes to create a more varied sample set. We manually removed most mussels from the shells in the second and third types, after that, we rinsed these shells with distilled water to eliminate any remaining mussels and crushed them to the ideal size, as shown in Figure 3. We stored the final samples in sealed bags at -40 ºC.

Figure 3 – Size of the mussel shells used, and the mussel pieces that were removed

2. Demineralization

To begin the extraction of the peptides, it was crucial to remove the most abundant compound on the whole shells (WS), the calcium carbonate. This step is called demineralization and consists of soaking the shells in a solution of HCl 0.6N in a 1:45 (g WS/mL HCl) ratio.[21] In our case, this reaction was held in a 10 L reactor, agitating it at 400 rpm for 24 hours while measuring the pH, hourly, a rising pH indicated the solubilization of calcium carbonate. After 24 hours, we safely discharged the aqueous phase and recovered the demineralized shells (DS). We washed the DS three times using centrifugation with deionized water to normalize the pH. Subsequently, we freeze-dried the DS for storage at -40 ºC. Several batches of demineralized shells were obtained from various batches of whole shells, as shown in Figure 4.

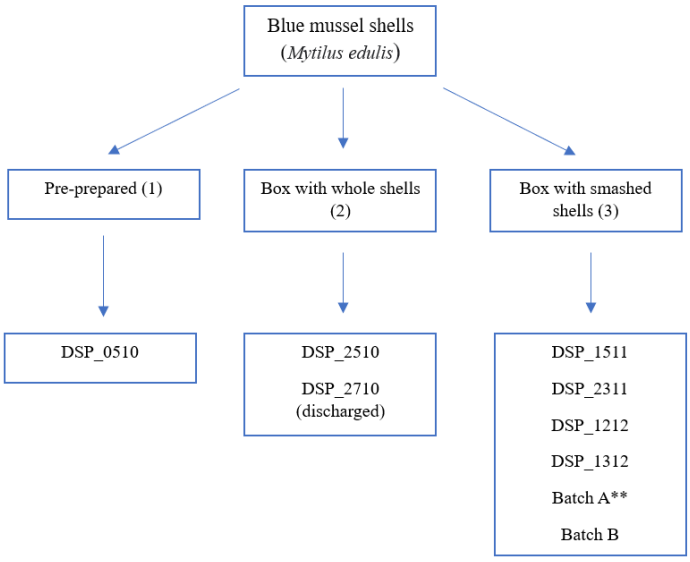

Figure 4 – Representation of the different batches obtained. Note: 3 different types of whole shells were used for the demineralization: (1) shells that were pre-prepared by grinding, (2) shells that were ground from whole shell with the entire mussel, (3) shells that were from a box identified as "concha triturada", that had smashed large pieces with mussel attached. ** Batch A is the combination of batches DSP_2311, DSP_1212, DSP_1312

3. Extraction of soluble protein

Proteins in the DS (demineralized shells) were solubilized using NaOH, enhancing the extraction of soluble proteins and preparing non-soluble protein structures for later enzymatic action. In the study, we treated the freeze-dried DS with a 1:50 ratio of 1%, 2%, and 3% NaOH solutions, and each solution was tested at 60 ºC and room temperature (approximately 23.5ºC), for 4 hours.[55,56] Additionally, the impact of granular versus powdery forms of DS was also assessed. After NaOH treatment, the samples underwent centrifugation at 4500 rpm for 30 minutes. The supernatant was collected and freeze-dried for subsequent analysis. The treatment produced two fractions: a supernatant rich in bioactive peptides and solubilized proteins (SP) and a remaining pellet to be later submitted to hydrolysis.

4. Hydrolysis of the pellet with alcalase

The enzymatic hydrolysis of the remaining pellet was improved using 1% and 3% Alcalase 2.4L (Novozymes). The hydrolysis process lasted 4 hours at 50 ºC and started with an initial pH of 8, which was adjusted periodically. Additionally, it was used a 1:50 ratio, represented as mg of protein per mL of phosphate buffer at 200 mM. The enzyme was inactivated by heating the mixture to 95 ºC for 15 minutes, followed by centrifugation at 4500 rpm for 30 minutes. After centrifugation, the supernatant was collected, and several aliquots were taken for further analysis before freeze-drying. The freeze-dried samples were stored in sealed containers at room temperature for future use.[57,58]

5. Physicochemical characterization

The extraction process yielded four distinct fractions: whole shells, demineralized shells in solid form, and solubilized protein and peptides, along with hydrolyzed peptides in liquid form. Figure 5 presents the physicochemical characterization of the solid fractions for the whole and demineralized shells; protein, ash, and moisture content were measured. Protein content was determined for the liquid fractions using the Thermo Scientific BCA assay kit. The percentage of free amino groups was calculated using the TNBS method, and the peptide profile was analyzed through high-performance size-exclusion chromatography (HPSEC) and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).[59,60]

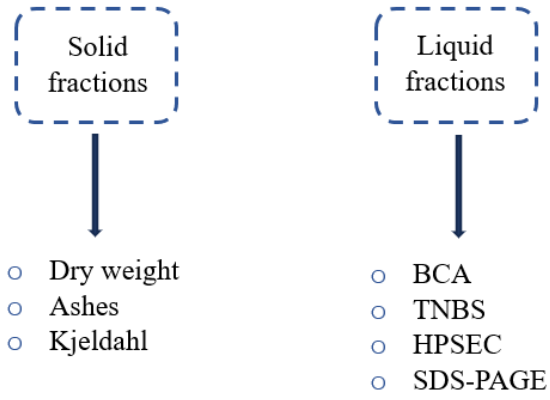

Figure 5 – General graphical representation for the characterization of the different types of fractions.

5.1. Physicochemical characterization of the solid fractions

The moisture content was determined by the method outlined by Li & Mira De Orduña (2010).[61] The oven was preheated to 105 °C, and empty, pre-weighed crucibles were prepared.[61] Samples were then added to these crucibles, and their weight was recorded. The crucibles remained in the oven at 105°C overnight. The next day, the samples were cooled in a desiccator and weighed again. The dry weight was determined by calculating the weight difference before and after oven drying.[61]

To determine the ash content, primarily composed of calcium carbonate, the dried samples in the crucibles were subjected to a temperature ramp in a muffle furnace, following the protocol outlined by Liu (2019).[62] Specifically, the samples were heated at 300 °C for 30 minutes, at 400 °C for 2 hours, at 500 °C for 2 hours, and finally at 600 °C overnight. The following day, the samples were cooled in a desiccator and weighed. The ash content was calculated based on the weight difference before and after the muffle furnace treatment.[62]

At last, the protein content was measured using the Kjeldahl method (total organic nitrogen content).[63] The process started with adding 0.2 g of sample to 1 g of catalyst and 4 mL of H_2SO_4. The mixture was placed in the digestor (that was preheated) at 416 ºC. The digestion tubes were removed and cooled at room temperature when the solution turned transparent green. Subsequently, 20 mL of distilled water was added. Afterward, a distillation process was made, the tube was put in the digestor, and a flask containing 25 mL of boric acid (4%) (with pH indicator) in the collector side of the digestor, and 10 M sodium hydroxide was added until the

flask reaches 150 mL. Finally, a titration step was performed, the flask solution was titrated with 0.1 M hydrochloric acid until the solution turned pink. Then, the equations used to calculate the protein content were the same ones as described in Chang & Zhang (2017).[63,64]

5.2. Physicochemical characterization of the liquid fractions

The characterization of the liquid fractions started by measuring the protein content, in the liquid fractions, using the Thermo Scientific BCA assay kit. Standard solutions, ranging from 5 µg/mL to 250 µg/mL, were prepared from the 2 mg/mL Albumin standard (BSA) provided in the kit. A BCA working reagent, a 50:1 mixture of BCA reagent A and BCA reagent B, was also prepared. Next, 25 µL of the samples and standards were pipetted into a 96-well microplate (Nunc, Denmark). After adding 200 µL of the working reagent, the microplate was incubated at 37 ºC for 30 minutes. Finally, absorbance was measured at a wavelength of 562 nm using a Synergy H1 Multidetector plate reader (BioTek Instruments, Winooski, VT, USA).[65]

The percentage of free amino groups was measured using the TNBS method, with some modifications to the procedure outlined by Spellman et al. (2003).[66] The process began by combining 300 µL of the sample with 2.7 mL of 6 M HCl to determine the maximum degree of hydrolysis. The mixture was sealed in flasks and heated at 105 ºC for 24 hours. After hydrolysis, the mixture was neutralized by adding 2.7 mL of 6 M NaOH. Dilutions were then prepared from both aliquots and fully hydrolyzed samples. For the assay, 50 µL of each dilution was pipetted into a 96-well microplate. The plate received 125 µL of 200 mM sodium phosphate buffer (pH 8.2) and 50 µL of TNBS solution per well. The TNBS solution contained 25 µL of 5% stock TNBS and 4.975 mL of distilled water. After adding all the solutions, the microplate was covered with aluminum foil and incubated at 50 ºC for 1 hour. Absorbance was then read at 340 nm. The calibration involved standards of leucine ranging from 50 mM to 250 mM. To calculate the degree of hydrolysis, the equation below was used,

$$\%\, DH = \frac{Lt - L0}{Lmax - L0} \times 100$$

where the Lt is the value of µmol equivalents of leucine per mL of sample (aliquots), L0 represents the same content, but when the time of hydrolysis is zero. Lastly, Lmax describes the value for the total hydrolysis. [66]

The peptide profile was determined by High Pressure Size Exclusion Chromatography (HPSEC).[59] The method relies on molecule diffusion within the stationary phase's pores. Specifically, smaller molecules diffuse quickly into the pores and elute later, while larger molecules that cannot penetrate the particles elute earlier. The study employed a Waters e2695 separation module system with a UV/Vis photodiode array detector (PDA 190-600 nm) for chromatographic analysis. An Agilent AdvanceBio SEC column (130 Å, 2.7 µm, 7.8 x 300 mm) facilitated the separation process. The chromatographic runs used a 150 mM phosphate buffer (pH 7) containing 0.2 g/L of sodium azide (NaN3) with a 1 mL/min flow rate, for 18 minutes. The Empower 3 Software handled data collection and analysis. Calibration involved standard proteins and peptides like Ovalbumin (44,300 Da), Myoglobin (17,600 Da), Cytochrome C (12,327 Da), Aprotinin (6,511 Da), Neurotensin (1,672 Da), Angiotensin-II (1,040 Da), Tyr-Phe dipeptide (328.4 Da), and L-tryptophan (204 Da). Each sample had a 10 µL injection volume and underwent pre-analysis filtration using PTFE/L 0.22 µm filters.[67]

The Tricine-SDS-PAGE method used by Schägger *et al*. (1987) was adopted for this assay.[68] Stacking and resolving gels (6% and 10%, respectively) were prepared with acrylamide, TRIS buffer, SDS, APS, and TEMED, and the 10 µL of those samples and 5 µL of control marker (Amersham™ ECL™ Rainbow™) were loaded into the gel. The gel was run at 75 V for 5 minutes, then at 150 V for about 45 minutes. The gel was treated with a 10% Trichloroacetic Acid solution, to fix the bands, for an hour. Staining was done using a 0.25% Coomassie blue R-250 solution for about an hour, followed by distaining with a mixture of acetic acid, methanol, and water. The resulting image was captured using ChemiDocTM XRS+ and analyzed using Imaging System Image LabTM Software. [69]

6. Bioactivities

A few bioactivities were evaluated regarding the hydrolysates and the solubilized proteins and peptides, such as, antioxidant, antihypertensive, and antimicrobial activities.

We evaluated the bioactivities of hydrolysates and solubilized proteins and peptides, focusing on their antioxidant, antihypertensive, and antimicrobial properties. Two methods were employed for the antioxidant assessment: ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) and ORAC (Oxygen Radical Absorbance Capacity).

For ABTS, a stock solution was prepared by dissolving 38.4 mg of ABTS in 10 mL of ultrapure water, resulting in a concentration of about 7 mM. Separately, 6.6 mg of potassium persulfate ($K_2O_8S_2$) was also dissolved in 10 mL of ultrapure water. Both solutions were then mixed in a tube shielded with aluminum foil. The stock was diluted with phosphate buffer to prepare the working solution until it reached an absorbance of 0.70 at 734 nm. The antioxidant activity of the target compounds was assessed by preparing dilutions from aliquots and standards. The standards included a 1000 µM Trolox solution, further diluted to concentrations ranging from 25 µM to 175 µM. Next, 20 µL of both standards and sample dilutions were added to a 96-well microplate, followed by 180 µL of the ABTS working solution. The microplate was incubated at room temperature for 6 minutes in the dark. After incubation, absorbance readings were taken using the Synergy H1 Multidetector plate reader at 734 nm. A calibration curve was created by plotting the resulting absorbance values against the standard concentrations. This curve served as a reference for determining the antioxidant activity of the samples. The final antioxidant activity was then ascertained by comparing sample absorbance to this calibration curve, offering key insights into the compounds' effectiveness in neutralizing free radicals and combating oxidative stress.[70,71]

The ORAC assay quantifies the antioxidative capacity against peroxyl radicals. To prepare a calibration curve, firstly a Trolox stock solution was prepared by dissolving 0.0125 g of Trolox in 1 mL of methanol, then the volume was completed until 50 mL with phosphate-buffered saline (PBS). From the previous solution 1 mL was taken, and PBS was added up to 10 mL. Then, a calibration curve was established to standardize the method. This involved diluting 1 mL of Trolox into 10 mL of 75 mM PBS at pH 7.4, resulting in concentrations between 10 µM and 80 µM. For the assay, a 96-well microplate received 20 µL of the standard sample and 120 µL of a prepared Fluorescein solution in each well. The Fluorescein solution was prepared by mixing 10.97 mg of Fluorescein disodium salt with 25 mL of 75 mM PBS (pH 7.4). A working solution was then made by diluting 100 µL of this mixture into 10 mL of PBS, yielding a Fluorescein concentration of 116.66 nM. Initial fluorescence was recorded with a microplate reader set to 485 nm excitation and 520 nm emission. After a 10-minute incubation at 37 °C, each well received 60 µL of a pre-prepared AAPH (2,2'-Azobis(2-methylpropionamidine) dihydrochloride) solution, made by dissolving 130.18 mg of AAPH in 10 mL of PBS. Fluorescence was then monitored every minute for 80 minutes at 37 °C. Data analysis involved computing the area under the curve (AUC) for each sample's fluorescence values over 80 minutes. Finally, the AUC values were normalized to Trolox equivalents (µM) using the

calibration curve, allowing the expression of ORAC values in μmol Trolox Equivalents per mL of sample.[72–74]

Additionally, we analyzed the antihypertensive activity of the peptides using an *in vitro* Angiotensin-Converting Enzyme inhibition (iACE) assay. A 1 U/mL enzyme stock solution was prepared by dissolving commercial ACE (peptidyl-dipeptidase A, EC 3.4.15.1) in a 50% glycerol and ultrapure water mixture. The stock solution was then diluted 1/24 to achieve a working ACE concentration of 42 mU/mL using an enzyme buffer. This buffer was prepared by diluting 0.15 M Tris 0.1 mM $ZnCl_2$ buffer (pH 8.3) 1/10. A substrate solution was also prepared using 3.2872 g of NaCl and 3.6 mg of the substrate (o-Abz-Gly-p-Phe(NO_2)-Pro-OH) in 0.15 M Tris buffer (pH 8.3). Six dilutions were tested, usually ranging from 1/1 to 1/32. In a 96-well microplate, 80 μL of blank (ultrapure water), 40 μL of sample blank, control, and sample were placed. An additional 40 μL of ACE was added to the control and sample wells, and 160 μL of substrate was added to each well. The microplate was incubated at 37 ºC for 30 minutes, and fluorescence was read using a Multidetector plate reader. The iACE assay determined the IC_{50} value, which represents the peptide concentration needed to reduce ACE activity by 50% in the analyzed conditions. Data analysis was performed using the Gen5 software from BioTek Instruments.[75]

At last, the aliquots were analyzed for antimicrobial activity using the Minimum Inhibitory Concentrations (MICs) method, and this assay assesses the ability of compounds to inhibit the growth of the microorganisms. For this assay, the protocol was based on Alves *et al*. (2013) and Wiegand *et al*. (2008).[76,77] So, pathogenic strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella enterica*) were used for the antimicrobial assay. Furthermore, to prepare the assay, stock solutions of the peptides were created by solubilizing the freeze-dried peptides into ultrapure water, creating solutions with an initial concentration of 20 mg/mL, and then the stock solutions were subsequently diluted to generate a range of concentrations. In addition, to cultivate the target microorganisms, they were grown in Mueller-Hinton on plate and then passed to Mueller-Hinton broth, until their concentration was adjusted to a concentration of 0.5 McFarland standard, ensuring uniformity and standardization of the inoculum. Finally, a 96-well microplate was set up to include blank wells for the growth medium control (column 1), positive and negative control antibiotics (column 2), and test peptides at various concentrations (columns 3 to 12). Then, the microbial inoculum was added to all the wells except the blank ones. The microplate was then incubated at 37 °C for 16-20

hours to allow microbial growth and inhibition. After the incubation, the wells were observed for visible growth or inhibition of microbial growth. The MIC was defined as the lowest concentration of the test solution that completely inhibited visible growth compared to the growth medium control.[76,77]

7. Simulation of gastrointestinal digestion

The hydrolysates and solubilized proteins underwent a gastrointestinal simulation using the INFOGEST method by Brodkorb, *et al.* (2019).[78] This simulation obtained five distinct sampling stages: oral, stomach, intestine, and dialysis (permeated and retained). Firstly, the digestion fluids were prepared: SSF, SGF, and SIF, which are a mixture of different substances. The samples were submitted to a simulated saliva with SSF fluid, $CaCl_2$ (0.3 M), and a suspension of α-amylase in ultrapure water. Also, the pH was adjusted to 7, and the reaction was left for 2 minutes. Subsequently, the samples kept going through all the digestion phases. The simulated gastric fluid was a mixture of SGF fluid, $CaCl_2$ (0.3 M), rabbit gastric extract (RGE) with lipase, and pepsin in ultrapure water. The pH of this mixture was adjusted to 3, and this phase was incubated for 2 hours with 150 rpm orbital agitation. Next, the simulated intestinal fluid was prepared by mixing the SIF fluid, bile salts (preferential bovine bile salts), and pancreatin, and afterward, the pH was adjusted to 7. Once again, the reaction was incubated with 45 rpm orbital agitation for another 2 hours. Lastly, to simulate the dialysis, the final content of the digestion was placed in a membrane (3.5 kDa). The membrane was sealed and immersed in 20 mL of ultrapure water, so the flasks were incubated again with agitation overnight. All the steps were taken at 37 ºC. Furthermore, the antioxidant and antihypertensive activities were analyzed for the digestion fractions, and the antimicrobial activity was only analyzed for the retained fraction. [79]

8. Statistical analysis

The collected data underwent statistical analysis through the IBM SPSS Statistics 29.0 software. Various analytical approaches were employed to assess the results from diverse samples and assays.

In the initial step, concerning the data obtained from the demineralization extraction, our focus was solely on testing its normality. This involved the application of the Shapiro-Wilk's test with

a null hypothesis, assuming that mean differences are equal to zero. The significance level for this test was set at 5%.

The solubilization results were divided into two categories: the results from the improvement and the results obtained with the best conditions. For the first one, a univariate analysis variance was applied, followed by Tukey's post hoc test. This analysis considered the variation across different time points as well as the overall differences between the mean values, mean separation was determined using the least significant difference at a 5% probability level. As for the results obtained under the best conditions, they were subjected to a non-parametric test, precisely the independent samples Kruskal-Wallis test. In this case, mean separation was also conducted using the least significant difference at a 5% significance level.

The data resulting from the hydrolysis of the demineralized shells (DS) underwent a one-way ANOVA, followed by a Tukey's post hoc test. This comprehensive analysis considered trends during the various stages of hydrolysis and evaluated the overall differences between the mean release values. Separation of means was determined using the least significant difference at a 5% significance level. For the remaining hydrolysis results, they were divided into three categories: H3%_DS and H3%_P, H3%_A_P and H1%_A_P, H1%_A_P and H1%_B_P, to each set of samples, was applied a T-test. Mean separation was conducted using the least significant difference at a 5% probability level.

As for the data from the ABTS and ORAC assays, the samples from the enzyme inactivation trial were analyzed, in which the ABTS assay was used one-way ANOVA with the post hoc Tukey's test. Then, for the ORAC assay, a non-parametric test analyzed the independent samples Kruskal-Wallis' test. Mean separation was conducted using the least significant difference at a 5% probability level. The subsequent analysis remained consistent for both assays. Univariate analysis of variance was performed to compare the four different types of extracts, followed by Tukey's post hoc test. This analysis considered variations across different time points and the overall differences between the mean values, with mean separation determined using the least significant difference at a 5% significance level. Moreover, to evaluate the differences between the different extractions in the solubilization step and the hydrolysis, a T-test was applied, with a mean separation conducted using the least significant difference at a 5% probability level.

The results for the iACE assay were only applied a T-test, with a mean separation conducted using the least significant difference at a 5% probability level.

The TNBS assay results for the four types of fractions were subjected to analysis using the Kruskal-Wallis test for independent samples, a non-parametric test. Mean separation was carried out using the least significant difference at a 5% probability level. To compare the results from free amino groups between the hydrolysates with 1% and 3% Alcalase, a T-test was used for independent samples, with a mean separation conducted using the least significant difference at a 5% probability level.

Regarding the data obtained from the ABTS and ORAC assays after INFOGEST, a uniform approach was adopted for both assays and various sample types. This analysis encompassed univariate variance analysis, followed by applying Tukey's post hoc test. The analysis considered the variations across different time points and evaluated the overall differences between the mean values. Mean separation was determined using the least significant difference at a 5% significance level.

Finally, regarding the dialysis results, the analysis of ABTS values involved a univariate variance analysis, followed by the application of Tukey's post hoc test. This analysis considered variations across different time points and assessed overall differences between mean values. Mean separation was determined using the least significant difference at a 5% significance level. As for the ORAC results, the analysis employed the Kruskal-Wallis test for independent samples, a non-parametric test. Mean separation for ORAC results was conducted using the least significant difference at a 5% probability level.

Results and Discussion

For all the results presented in this section, there are flowcharts in the Annexes, explaining the course of the reactions, demonstrating the mass balances (based on dry weight), and the statistical analysis.

1. Demineralization

We conducted seven demineralization extractions but only used six for further analysis; we discarded one extraction that failed to meet effective demineralization criteria. We based our definition of successful demineralization on three factors: total weight yield, protein extraction yield, and the physical texture of the demineralized shells (DS), which should ideally appear paper-like post-process.

Figure 6 reveals that the total weight yields, for these extractions, varied between 3.58% and 3.96%, with two outliers at 5.26% and 20.5%. After eliminating these outliers, the average yield stood at 3.76%. Therefore, the outliers are not present in the graph in Figure 6.

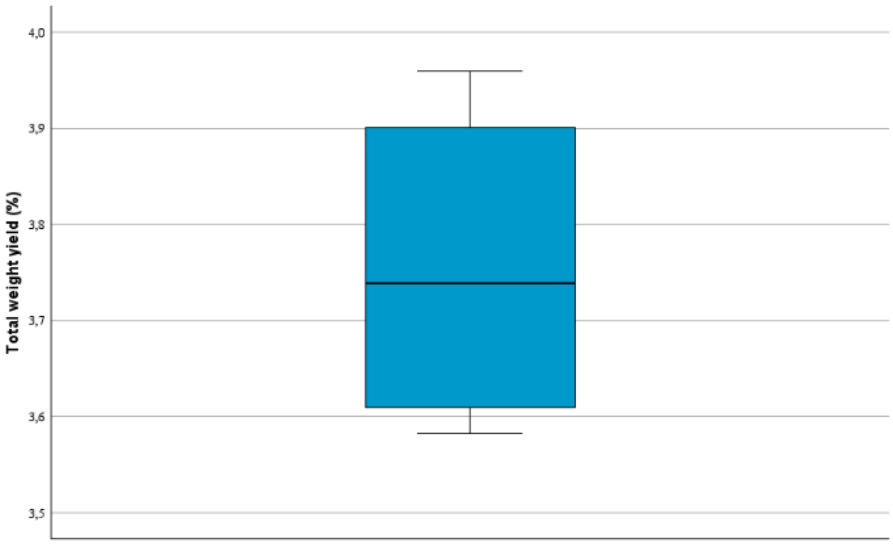

Figure 6 – Total weight yield percentage for the demineralization process, n = 6. Values follow a normal distribution ($p > 0.05$)

As for protein extraction yields, also depicted in Figure 7, the values ranged from 63.5% to 110%, resulting in an average yield of 84.6%. We found no existing literature data on protein extraction yields during demineralization to be compared.

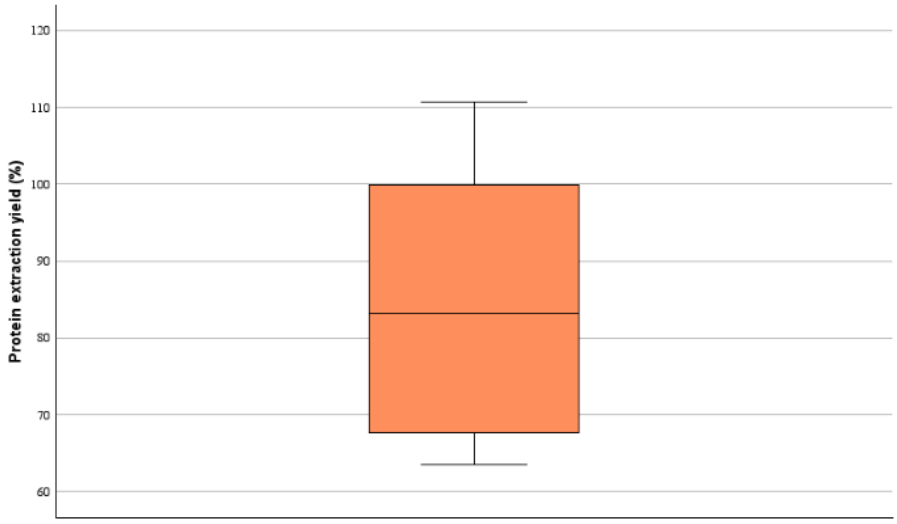

Figure 7 –Protein extraction yield percentage for the demineralization process, n = 6. Values follow a normal distribution ($p > 0.05$)

The total weight yield should be minimal for a demineralization process to succeed. This suggests that most of the weight from the WS (primarily comprising calcium carbonate) was dissolved. Consequently, the remaining weight predominantly represents protein. This assertion is further supported when the high protein extraction yield implies optimal protein recovery from the WS.

2. Solubilization with NaOH

The structure of proteins in blue mussel shells is complex, with various secondary, tertiary, and quaternary structures that play a crucial role in biomineralization. These shell matrix proteins are rich in amino acids with acidic and basic residues, which can lead to electrostatic interactions and hydrogen bonding within the protein structure. Additionally, they may have hydrophobic regions that can cause aggregation and reduce solubility.[14]

An alkaline solution, such as sodium hydroxide (NaOH), is commonly used to solubilize proteins.[80] NaOH is a strong base that dissociates into hydroxide ions (OH^-) in water, these hydroxide ions react with the acidic residues present in the protein structure, breaking the electrostatic interactions and hydrogen bonds.[81] As a result, the proteins unfold and become more soluble in the alkaline environment.[82] Therefore, the solubilization of proteins by NaOH leads to the formation of proteinates, which are water-soluble complexes of proteins and alkali

metal ions, these proteinates can be separated from the demineralized shell matrix, and the resulting solution can then undergo further processing, such as enzymatic hydrolysis, to release and isolate bioactive peptides.[83] Taking that into account, this study proposed for the first time the inclusion of another step between the demineralization and the hydrolysis, following the solubilization previously mentioned for chitin extraction.[56,84]

2.1. Improvement of the solubilization with NaOH

To improve the solubilization conditions, we conducted experiments on demineralized shells (DS) using three different concentrations of sodium hydroxide: 1%, 2%, and 3%. These tests took place at two distinct temperatures: 25 ºC and 60 ºC. Figure 8 shows that the highest yield of 39.6% occurred with a 3% NaOH concentration, while the yields for 1% and 2% concentrations were 23.5% and 32.6%, respectively.

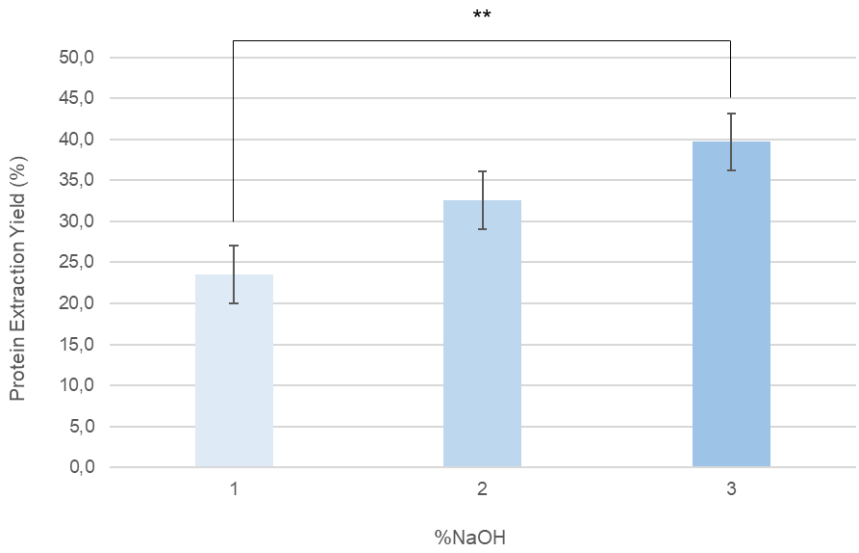

Figure 8 – Average protein extraction yield percentage for the different NaOH concentrations. Data are expressed in mean ± standard error of mean, n = 4. ** $p \leq 0.01$

In Figure 9, we observed two different results according to the experimental temperatures tested. At 60 ºC, we achieved a yield of 53.2%, significantly higher than the yield obtained (10.6%) at 25 ºC. The highest solubilization rate occurred with 3% NaOH at 60 ºC, which we selected as the best condition for solubilizing the subsequently extracted DS.

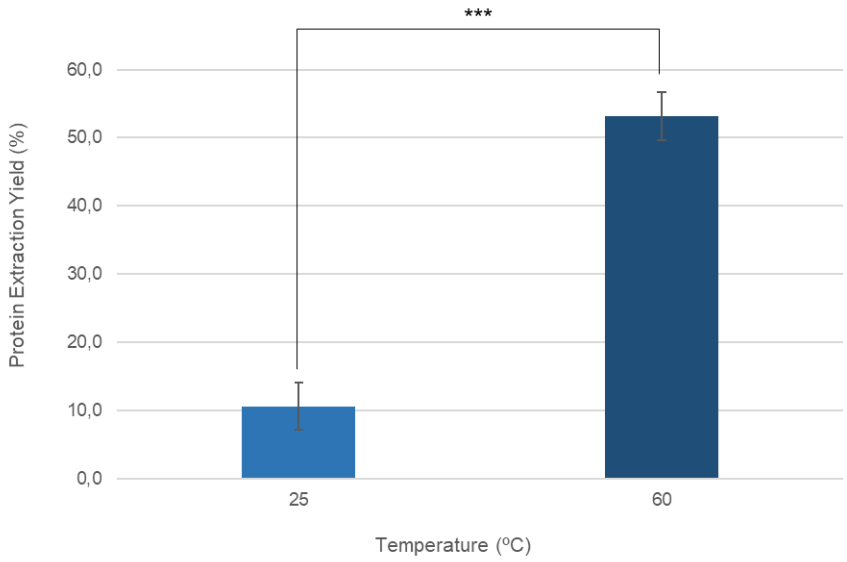

Figure 9 – Average protein extraction yield percentage for the different temperatures. Data are expressed in mean ± standard error of mean, n = 6, *** $p \leq 0.001$

2.2. Solubilization with the best tested conditions

Figure 10 shows the solubilization yields for four samples, although we initially tested five. We excluded one sample, labeled as 3%60º_3 because it was an outlier. This outlier was derived from granular DS, unlike the other samples from powdered DS. This suggests that proteins in powdered DS are more easily solubilized due to their increased surface area in contact with the alkaline solution.

The four samples showed no significant differences, with the highest average yield being 60.0%. This comparison was made using a non-parametric test because the variance's homogeneity requirement for a parametric test (ANOVA) was not filled.

We compared these results using mackerel whole fish as the matrix, as no data exists for blue mussel shells. Álvarez, *et al.* (2018) successfully recovered 74.25% of protein using 0.4 M NaOH.[82]

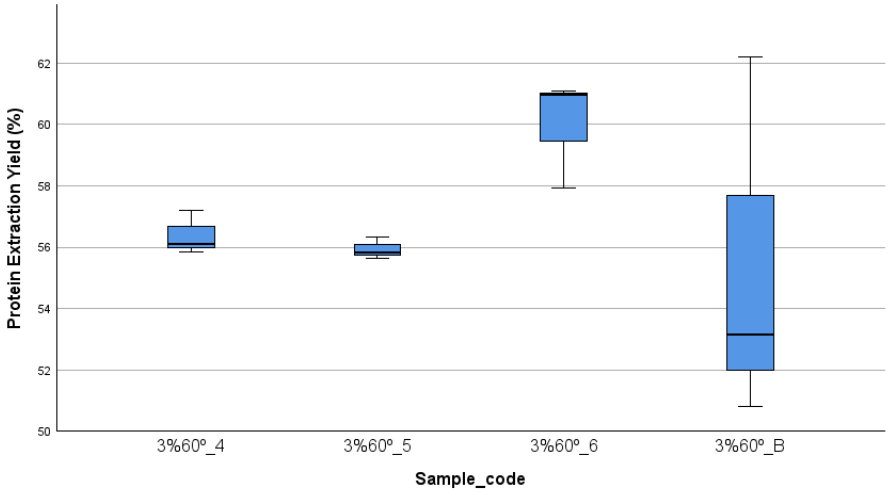

Figure 10 – Protein extraction yield percentage for the samples, using the optimal conditions, n = 3. There are no significant differences ($p > 0.05$). Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC), the different numbers and letters refer to the origin batch of demineralized shells

3. Enzymatic hydrolysis

Enzymatic hydrolysis of proteins in blue mussel shells is critical to extract the insoluble proteins and release bioactive peptides with various health-promoting properties. This reaction is promoted using specific enzymes, which, in the case of this study, the enzyme Alcalase was tested. The hydrolysis allows the breaking down of the proteins into smaller peptide fragments, unlocking their bioactivity and potential applications. Alcalase, derived from *Bacillus licheniformis*, is a commercial enzyme known for its proteolytic activity and is mainly used in food matrixes. It is a serine endopeptidase, which targets the peptide bonds within the protein structure and cleaves them, breaking down the proteins into smaller peptide fragments without affecting the amino acid sequences at the terminal ends. This controlled enzymatic reaction releases peptides with different sequences and lengths, each possessing distinct bioactive properties. Also, it is easier to predict the peptide sequence, which is going to be obtained using this enzyme.[37,38]

All the hydrolysates were obtained using Alcalase 2.4L, and this step was employed for 4 hours at 50ºC, with an initial pH of 8.

We hydrolyzed demineralized shells, using 3% Alcalase, and achieved protein extraction yields ranging from 11.1% to 11.7%, as illustrated in Figure 11. Using DS in powdered form ensured consistent results. In contrast, initial tests with granular DS yielded highly variable data.

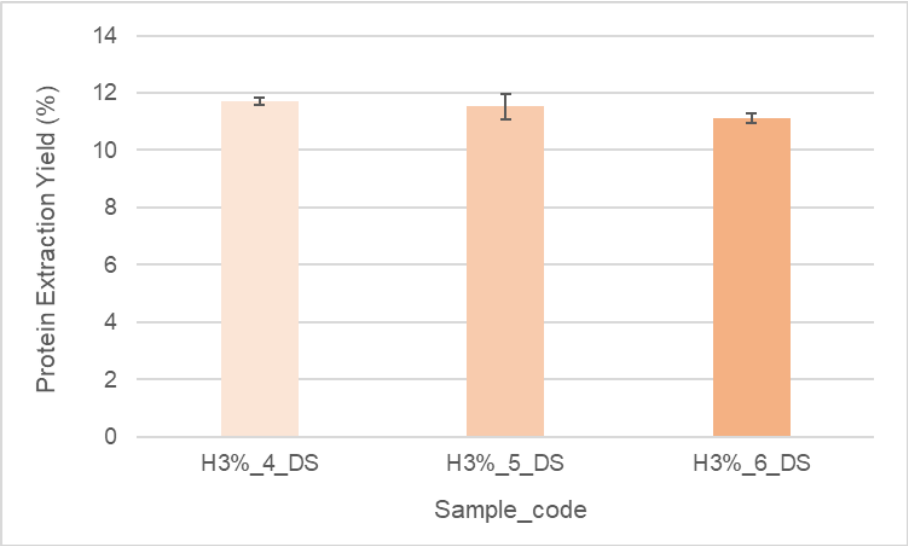

Figure 11 – Protein extraction average yield percentage and, for the hydrolyzed demineralized shells. Data are expressed in mean ± standard deviation, n = 2. There are no significant differences ($p > 0.05$). Samples legend: H3%_DS (hydrolysis, with 3% Alcalase, of the demineralized shells), the different numbers refer to the origin batch of demineralized shells

We implemented a solubilization step on the demineralized shells to enhance the low protein extraction yield. This step produced two components: a liquid fraction and a remaining pellet. We conducted enzymatic hydrolysis tests on both, using 3% Alcalase. The protein extraction yield for the liquid fraction matched the solubilized fraction, as indicated in Figure 10. This result suggests that the hydrolysis process did not alter the protein content.

For a more direct comparison, we also performed hydrolysis on the remaining pellet, labeled as H3%_P, and compared it to the hydrolysis of the DS, labeled as H3%_DS. Figure 12 reveals that the protein extraction yield, from the remaining pellet, was 59.1%, markedly higher than the 11.4% yield from the DS hydrolysates. This confirms that adding a solubilization step significantly improves the protein extraction yield.

Currently, no data exists on the enzymatic hydrolysis of blue mussel shells. Nonetheless, the highest reported hydrolysis rate using Alcalase is 78.2% on hydrolysates from anchovy sprat (*Clupeonella engrauliformis*) .[58]

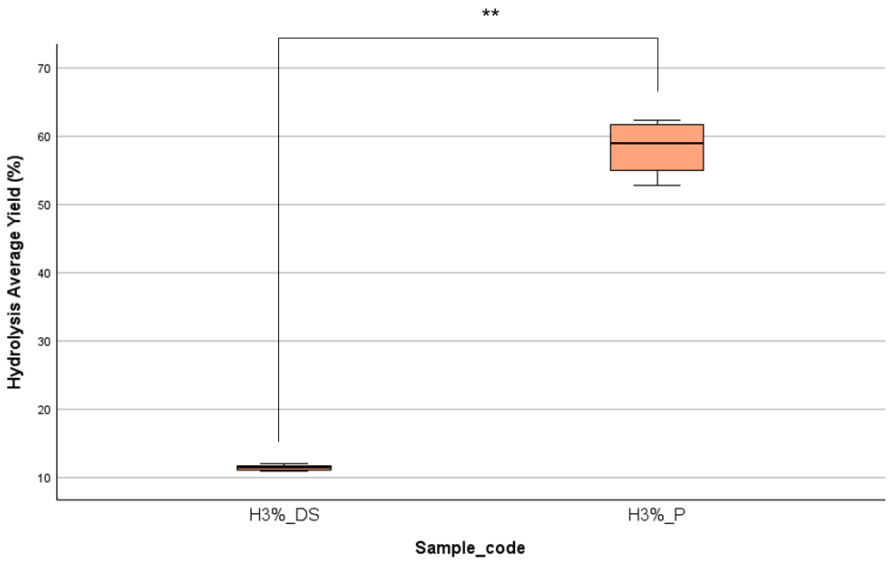

Figure 12 – Protein extraction average yield percentage and, for the hydrolyzed demineralized shells and the hydrolyzed pellet, n = 9 (H3%_DS) and n = 8 (H3%_P). ** $p \leq 0.01$. Samples legend: H3%_DS (hydrolysis, with 3% Alcalase, of the demineralized shells) and H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step)

Initially, hydrolysis was conducted under the most extreme conditions to maximize yield. To assess whether such high enzyme concentration was necessary, we performed additional hydrolysis, on the remaining pellet, using 1% and 3% Alcalase concentrations.

Figure 13 shows that hydrolysis with 3% Alcalase (H3%_A_P) yielded 52.4%, while hydrolysis with 1% Alcalase (H1%_A_P) produced a yield of 51.4%. These results showed no significant difference between the two concentrations. Therefore, we considered 1% Alcalase more efficient as it avoided enzyme wastage.

Figure 14 compares the protein extraction yields from the remaining hydrolyzed pellet, using 1% Alcalase, between two DS extractions, A and B (H1%_A_P and H1%_B_P, respectively). The goal was to investigate whether the timing of DS extraction affects the yield. The sample H1%_A_P yielded 51.4%, while the sample H1%_B_P yielded 24.8%. This difference can be due to the natural heterogeneity of the whole shells or to some difference in the solubilization step, which was not analyzed for batch A.

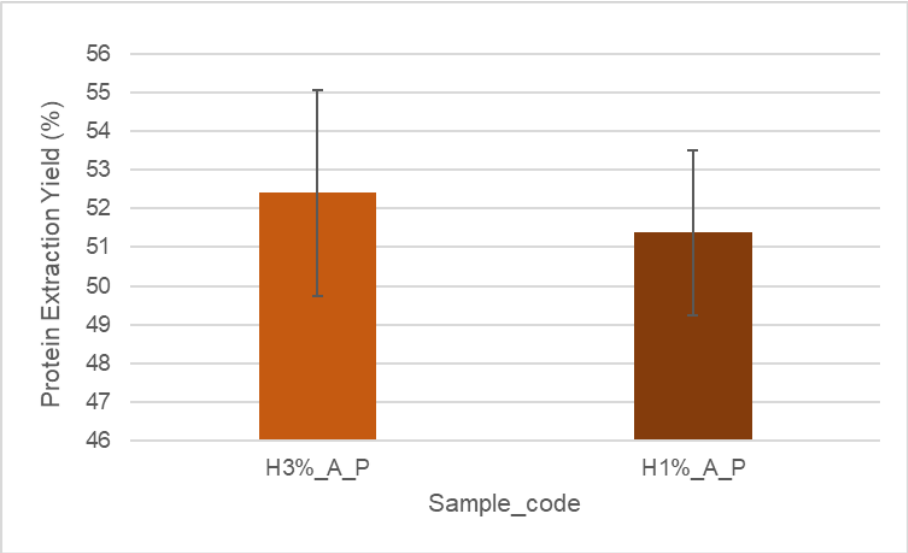

Figure 13 – Protein extraction average yield percentage and, for the hydrolyzed pellet, with 3% and 1% Alcalase. Data are expressed in mean ± standard deviation, n = 3. There are no significant differences ($p > 0.05$). Samples legend: H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step) and H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batch A of demineralized shells

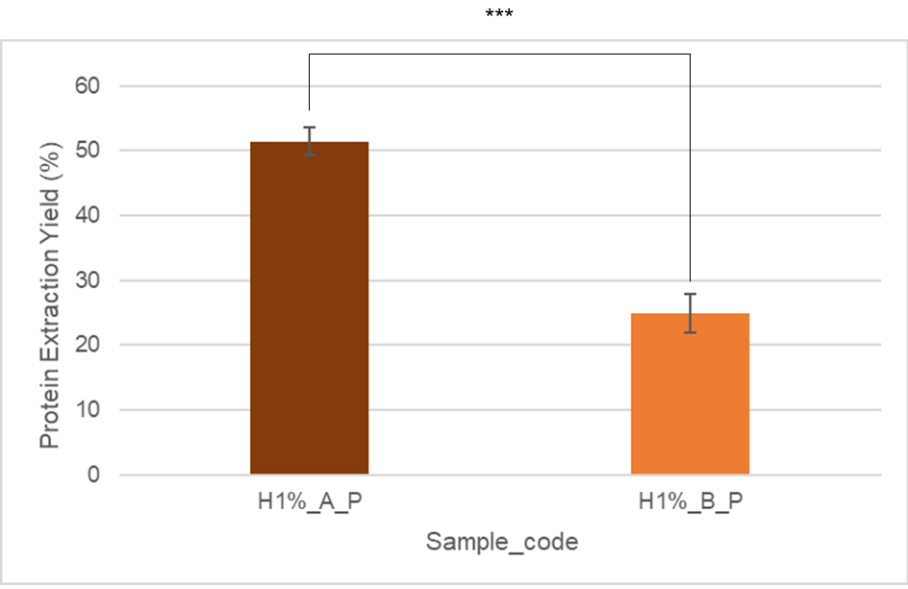

Figure 14 – Protein extraction average yield percentage and, for the hydrolyzed pellet, with 1% Alcalase. Data are expressed in mean ± standard deviation, n = 3. *** $p \leq 0.001$. Samples legend: H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batches A and B of demineralized shells

4. Bioactivities

In this study, the obtained proteins and peptide fractions were analyzed for potential bioactivities, such as antioxidant (ABTS and ORAC assays), antihypertensive (iACE assay), and antimicrobial.

4.1. Antioxidant activity

Multiple tests are preferred because each assay measures a specific type of antioxidant activity under unique conditions rather than assessing total antioxidative activity. This preference arises from the fact that these methods are based on different reaction mechanisms and target different free radicals. ORAC evaluates an antioxidant's capacity to neutralize a free radical ($ROO^{\cdot}$) by donating a hydrogen atom, whereas the ABTS method measures the release of an electron to the ($ROO^{\cdot}$), converting it into an anion (ROO^{-}).[48]

4.1.1. Different methods of inactivating alcalase

In some cases, high temperatures can lead to protein aggregation, creating complex structures that become difficult to reverse. [85,86] To investigate the effects of different enzyme inactivation methods on protein aggregation at high temperatures, three approaches were tested: standard heat treatment at 95 ºC for 15 minutes, nanofiltration using filters smaller than 10 kDa, and a combination of both heat treatment followed by nanofiltration. Figure 15 reveals that nanofiltration (H3%_A_P_F) negatively affected the antioxidant activity, as measured by ABTS, showing lower values than the sample inactivated solely by heat (H3%_A_P). Interestingly, the sample subjected to heat and nanofiltration (H3%_A_P_TF) exhibited higher ABTS values than those treated with nanofiltration alone.

The sample inactivated by heat alone had an antioxidant activity of 0.56 ± 0.13 µmol TE/mg protein, compared to 0.32 ± 0.06 µmol TE/mg protein for the sample, in which the enzyme was inactivated only by nanofiltration. Statistical analysis confirmed a significant difference between the two inactivation methods. This disparity may arise because nanofiltration retained the peptides > 3 kDa, and as we are going to observe further in the peptide size profile, we have bioactive peptides larger than 3 kDa.

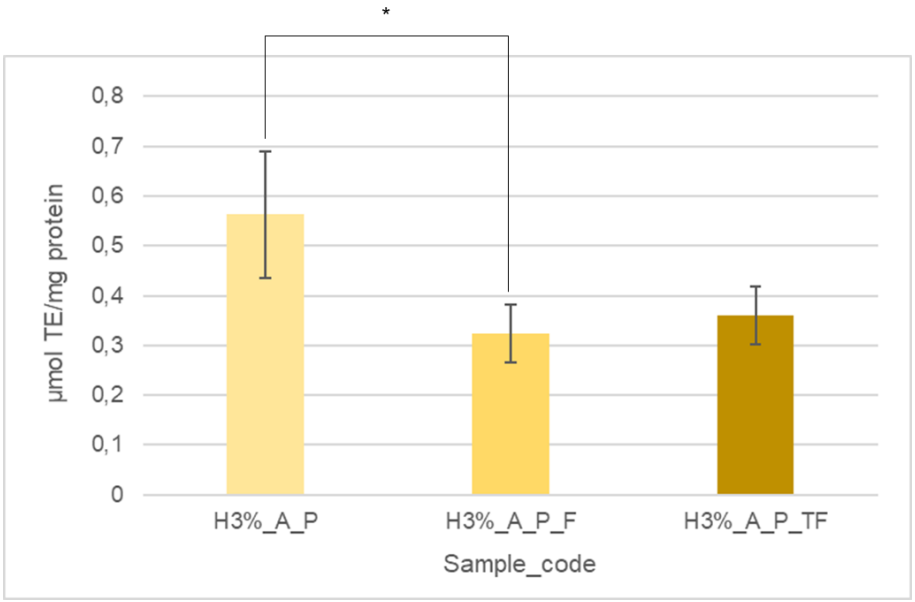

Figure 15 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein for the hydrolyzed demineralized shells. Data are expressed in mean ± standard deviation, n = 3 (H3%_A_P, H3%_A_P_F) and n = 2 (H3%_A_P_TF). * $p \leq 0.05$. Samples legend: H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), H3%_P_F (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step, with the enzyme inactivated by nanfiltration) and H3%_P_TF (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step, with the enzyme inactivated by nanfiltration and temperature), from batch A of demineralized shells

Contrastingly, the ORAC assay (Figure 16) showed that the sample just submitted to temperature condition had an antioxidant activity of 1.16 ± 0.06 TE/mg protein, similar to the sample submitted to a single treatment of nanofiltration had 1.12 ± 0.01 µmol TE/mg protein. No significant differences between the inactivation methods were found in this case, opposing the observations for ABTS.

Furthermore, the results indicate that when the sample is nanofiltrated, it concentrates the sample in small peptides and free amino acids, potentially resulting in efficient hydrogen atom transfer but a loss of electron transfer. This may explain peptides with low ABTS values but with similar ORAC results. Additionally, the composition of amino acids has a significant impact on antioxidant activity, with a higher rate of hydrophobic AAs like Ala being associated with enhanced antioxidant activity.[87] Overall, we considered it preferable to inactivate the enzyme through heat treatment. Otherwise, we lose almost half of the bioactivity, and it is noticeable that inactivating the enzyme with temperature did not create aggregation.

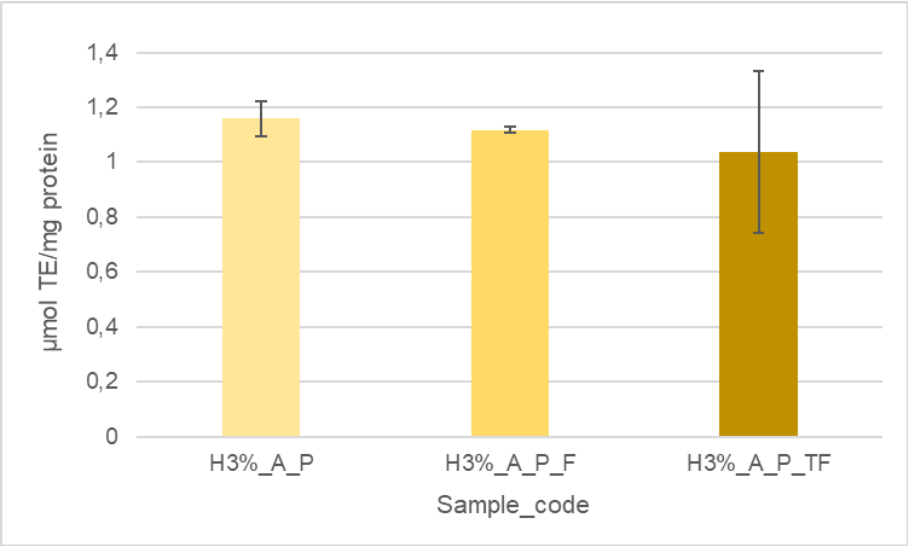

Figure 16 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the hydrolysates on the pellet for different inactivation methods. Data are expressed in mean ± standard deviation, n = 3 (H3%_A_P, H3%_A_P_F) and n = 2 (H3%_A_P_TF). There are no significant differences ($p > 0.05$). Samples legend: H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), H3%_P_F (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step, with the enzyme inactivated by nanfiltration) and H3%_P_TF (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step, with the enzyme inactivated by nanfiltration and temperature), from batch A of demineralized shells

4.1.2. Different obtained fractions

The peptides tested included hydrolyzed peptides from the DS (H3%_DS), solubilized peptides (3%60º), hydrolyzed peptides from the remaining pellet (H3%_P), and hydrolyzed peptides from the liquid fraction (H3%_L).

In the ABTS assay (Figure 17), the solubilized peptides showed the highest antioxidant activity, measuring 3.98 ± 0.41 µmol TE/mg protein. Additionally, we recovered more peptides with antioxidant activity from the remaining pellet after solubilization, measuring 2.34 ± 0.30 µmol TE/mg protein. Moreover, the sample labeled as H3%_DS showed the lowest values for antioxidant activity, measuring at 1.92 ± 0.08 µmol TE/mg protein after hydrolysis. This suggests that the solubilized peptides might have already been at their optimal size for bioactivity. Further hydrolysis appeared to diminish their effectiveness. In comparison with prior research, our samples exhibited promising antioxidant activities. This outcome aligns with the known capabilities of Alcalase for generating high-antioxidant-activity peptides. As Zou, *et al*. (2006) noted, peptides with the highest antioxidant activity typically have a molecular

weight under 1 kDa, which is also true for our samples.[88] When we compared our results to the ABTS assays conducted on hydrolysates, we found that those samples had an antioxidant activity of 3.8 µM TE/mg protein sample and on hydrolysates from Armoured Catfish (*Pterygoplichthys disjunctivus*), that presented a result of 174.68 µmol Trolox equivalent/g fish.[89,90]

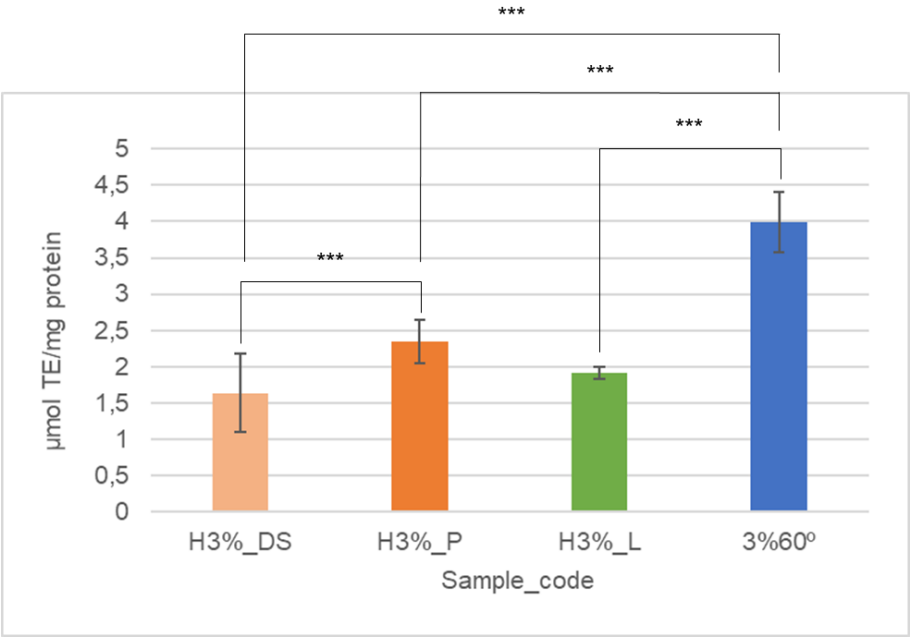

Figure 17 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein, for the solubilization and hydrolyzed samples, with 3% Alcalase. Data are expressed in mean ± standard deviation, n = 9 (H3%_DS, 3%60º, H3%_L) and n = 8 (H3%_P). *** $p \leq 0.001$. Samples legend: H3%_DS (hydrolysis, with 3% Alcalase, of the demineralized shells), H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), H3%_L (hydrolysis, with 3% Alcalase, of the liquid fraction from the solubilization step, with the enzyme inactivated by nanfiltration) and 3%60º (solubilized fraction with 3% NaOH at 60 ºC)

In the ORAC assay (Figure 18), which tests for the inhibition of oxidation caused by peroxyl radicals, the peptides in their soluble form showed the highest activity, registering 2.22 ± 0.08 µmol TE/mg protein. This activity decreased to 1.38 ± 0.04 µmol TE/mg protein when the fraction underwent hydrolysis. When compared to hydrolysates from *Mytilus galloprovincialis* meat, which had an antioxidant activity of 485.63 ± 60.65 µmol TE/g of hydrolysate, the hydrolysates from the *Mytilus edulis* shell demonstrated significantly greater oxidation-inhibiting activity.[91] But, when compared to enzymatic hydrolysates from soy, 3.9 ± 0.1 µmol TE/mg protein, it had similar results. [92]

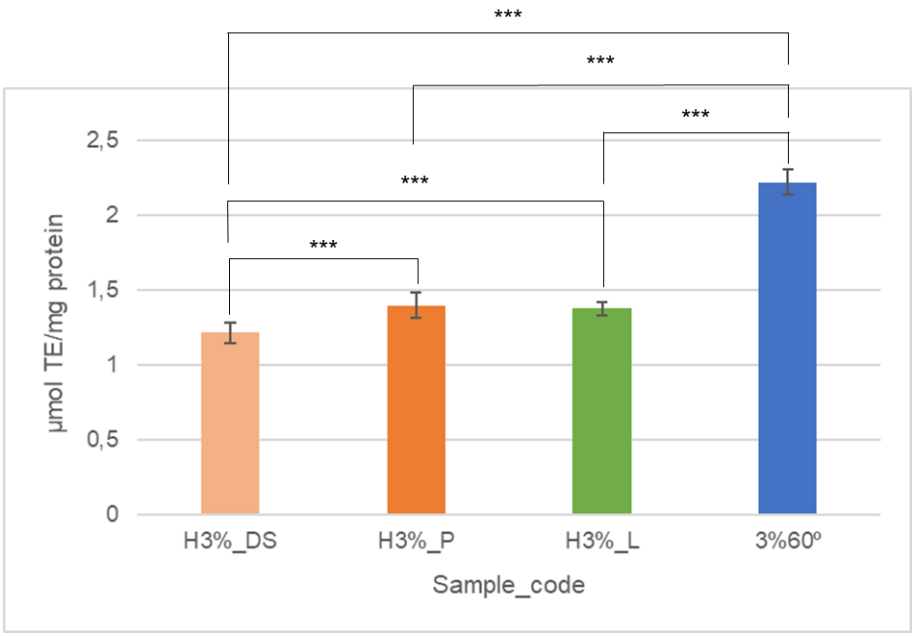

Figure 18 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the solubilization and hydrolyzed samples, with 3% Alcalase. Data are expressed in mean ± standard deviation, n = 9 (H3%_DS, 3%60º, H3%_L) and n = 8 (H3%_P). *** $p \leq 0.001$. Samples legend: H3%_DS (hydrolysis, with 3% Alcalase, of the demineralized shells), H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), H3%_L (hydrolysis, with 3% Alcalase, of the liquid fraction from the solubilization step, with the enzyme inactivated by nanfiltration) and 3%60º (solubilized fraction with 3% NaOH at 60 ºC)

Figures 19 and 20 compare the two assays to evaluate whether the timing of DS extraction during the solubilization step leads to significant differences. For the ABTS assay (Figure 19), we measured an antioxidant activity of 1.54 ± 0.36 µmol µmol TE/mg protein, for 3%60º_A, and 3.24 ± 0.24 µmol µmol TE/mg protein, for 3%60º_B. In the ORAC assay (Figure 20), the antioxidant activity was 1.52 ± 0.10 µmol µmol TE/mg protein, for 3%60º_A, and 1.81 ± 0.15 µmol µmol TE/mg protein, for 3%60º_B. The protein extraction yield was not measured between these two batches for the solubilization step, but it was for the hydrolysis (Figure 14), and it is possible to deduce that H1%_A_P had a higher protein extraction yield, so this difference can be due to the heterogeneity of the whole shells. So, it is important to have a heterogeneous method of preparation of the whole shells, so the batches are more similar, and the smaller the powder particles are turned into is, larger is the contact surface with the sodium hydroxide or Alcalase, promoting a more consistent reaction.

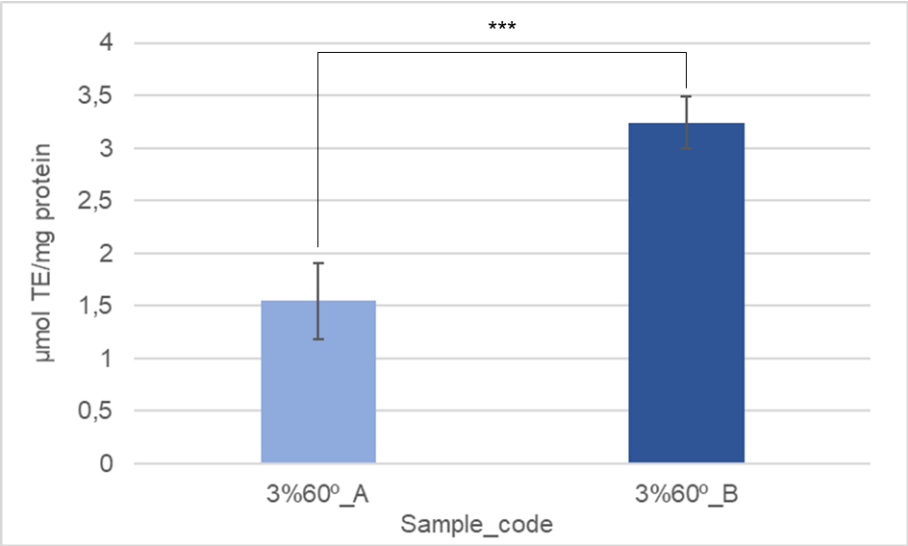

Figure 19 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein for the solubilized sample. Data are expressed in mean ± standard deviation, n = 3. *** $p \leq 0.001$. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC), from batches A and B of demineralized shells

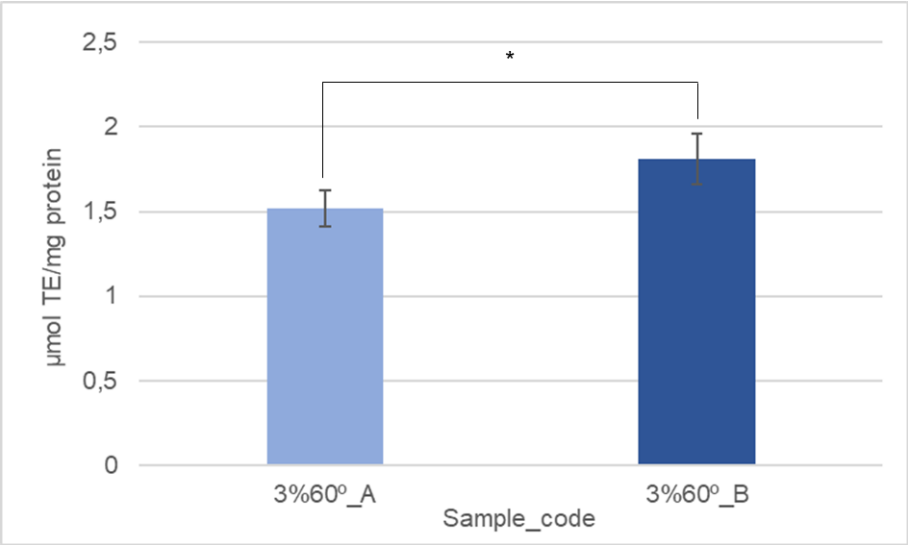

Figure 20 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the solubilized sample. Data are expressed in mean ± standard deviation, n = 3. * $p \leq 0.05$. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC), from batches A and B of demineralized shells

The goal was to evaluate if different pellets used in the solubilization step significantly impact the antioxidant activity. In the ABTS assay (Figure 21), the antioxidant activity for H1%_A_P was 0.20 ± 0.02 µmol TE/mg protein; for H1%_B_P, it was 2.18 ± 0.56 µmol TE/mg protein. For the ORAC assay (Figure 22), the activities were 0.66 ± 0.05 µmol TE/mg protein for

H1%_A_P and 4.12 ± 0.22 µmol TE/mg protein for H1%_B_P. Compared to Figure 14, it is possible to deduce that H1%_A_P had a higher protein extraction yield, so probably, in this case, as there was more protein to extract, the enzyme did not have enough time to break down the proteins or the same justification as the solubilized step.

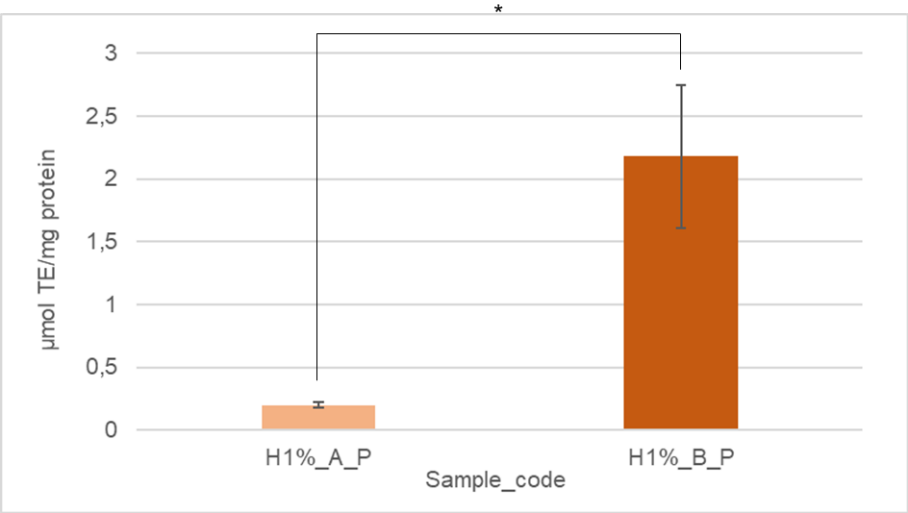

Figure 21 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein for the hydrolyzed sample, with 1% Alcalase. Data are expressed in mean ± standard deviation, n = 3. * $p \leq 0.05$. Samples legend: H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batches A and B of demineralized shells

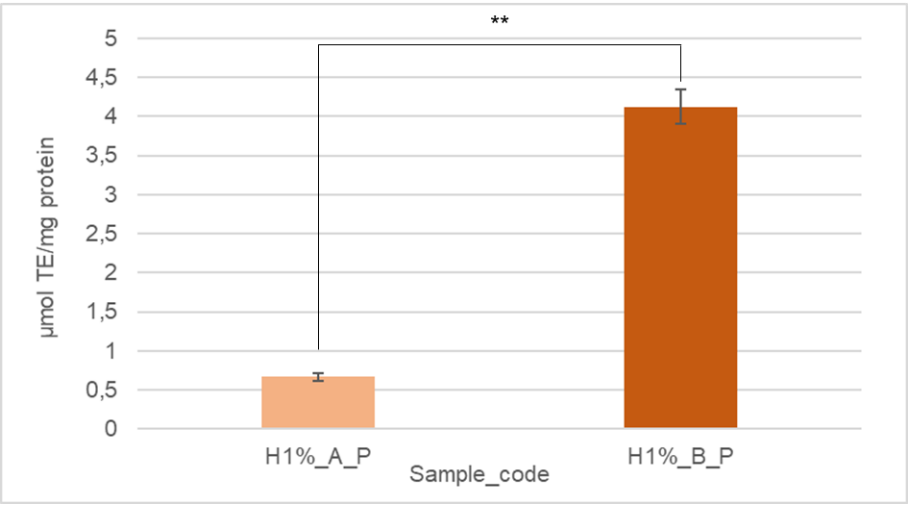

Figure 22 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the hydrolyzed sample, with 1% Alcalase. Data are expressed in mean ± standard deviation, n = 3. ** $p \leq 0.01$. Samples legend: H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batches A and B of demineralized shells

4.2. Antihypertensive activity

Furthermore, the ability of the peptides to inhibit the angiotensin-converting enzyme-I was tested using the iACE assay. For this method, the results were expressed as IC_{50} (µg/mL), which means the amount of peptides needed per milliliter to inhibit 50% of the angiotensin-converting enzyme-I. This bioactivity was tested for two types of fractions, the solubilized and the hydrolyzed peptides, with 1% Alcalase. Each of them was analyzed in two different moments of extraction.

Firstly, in Figure 23, it is possible to observe the results for the solubilized samples, for which obtained an antihypertensive activity of 809.5 ± 75.9 µg protein/mL and 805.7 ± 18.0 µg protein/mL, for 3%60º_ A and 3%60º_B, respectively. For the hydrolyzed samples (Figure 24), the obtained values were 1167.8 ± 174.6 µg protein/mL and 949.6 ± 80.7 µg protein/mL for H1%_A_P and H1%_B_P, respectively. In both cases, significant differences were not found, between the two samples, which again suggests a good reproducibility between the different extractions of DS. Taking into account there are no results for the blue mussel shells, it is necessary to compare with hydrolysates from other matrixes, such as, the Mediterranean fish discards, the antihypertensive activity was 279 and 302 µg/mL, using subtilisin and trypsin, respectively.[93] As the hydrolysates from *Mytilus galloprovincialis* meat presented a value of 1.0 ± 0.56 mg of protein/mL, using the subtilisin protease and 3.7 ± 0.33 mg of protein/mL, using corolase.[91] Lastly, compared with hydrolysates from blue mussel meat, the best results in the study by Dai, *et al.* (2010), were obtained using Alcalase, 66.34 µg protein/mL.[40]

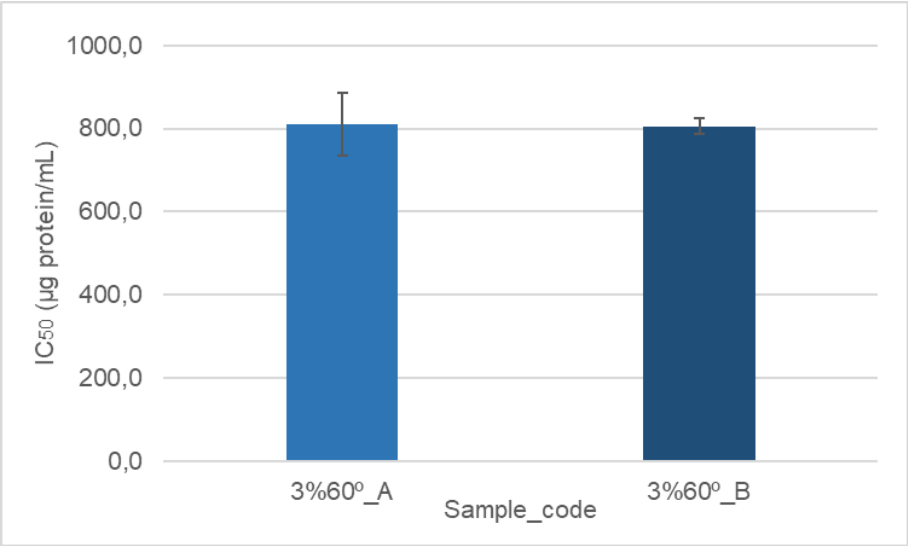

Figure 23 – iACE activity expressed in IC_{50} (µg protein/mL) for the solubilized fraction. Data are expressed in mean ± standard deviation, n = 3. There are no significant differences ($p > 0.05$). Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC), from batches A and B of demineralized shells

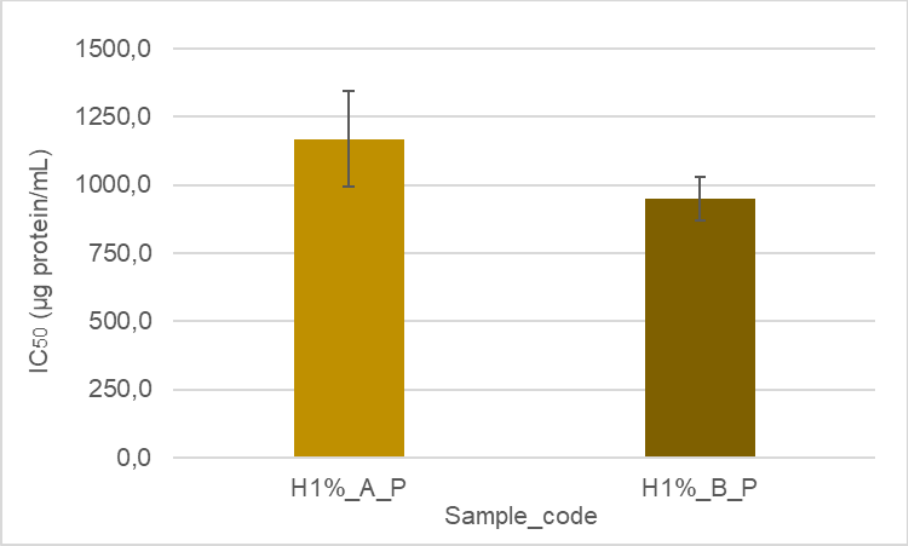

Figure 24 – iACE activity expressed in IC_{50} (µg protein/mL) for the hydrolysis with 1% alcalase. Data are expressed in mean ± standard deviation, n = 3. There are no significant differences ($p > 0.05$). Samples legend: H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batches A and B of demineralized shells

Regarding the comparison between the solubilized and hydrolyzed samples (Figure 25), significant differences were detected between them. Moreover, for this bioactivity, the solubilized fraction presented a higher bioactivity because, in this case, having lower values means less quantity of peptides to inhibit 50% of the enzyme. Therefore, the solubilized condition presented better results.

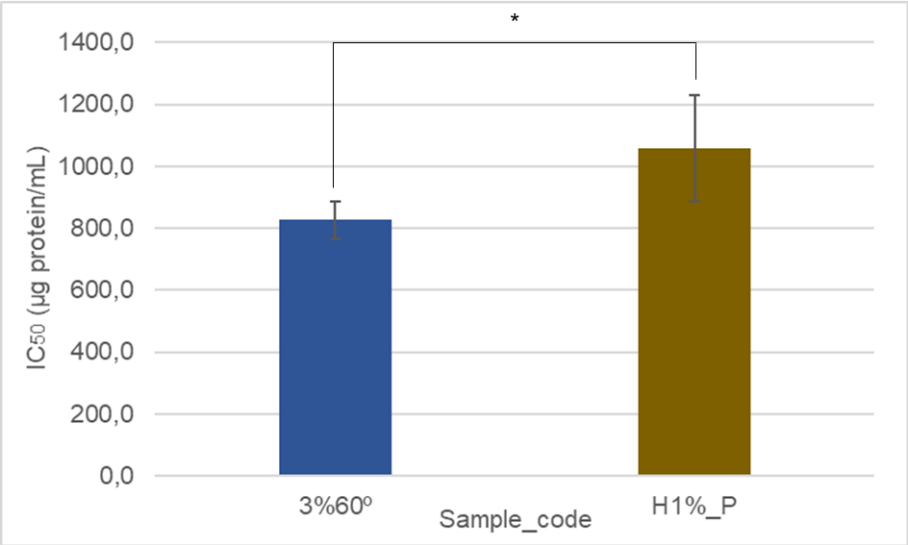

Figure 25 – iACE activity expressed in IC_{50} (µg protein/mL) for the solubilized fraction and for the hydrolysis with 1% alcalase. Data are expressed in mean ± standard deviation, n = 6. * $p \leq 0.05$. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC) and H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step)

4.3. Antimicrobial activity

After analyzing all the bioactivities above, the antimicrobial activity of the peptides was tested from the following conditions: 3%60º (solubilized proteins and peptides) and H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet), using the Minimum Inhibitory Concentration (MIC) method. MIC is the lowest concentration of an antimicrobial substance that inhibits the visible growth of a microorganism, this quantitative method is crucial for determining the susceptibility of bacteria, fungi, or other pathogens to a specific drug. [94] In MIC testing, a range of concentrations of the antimicrobial agent is tested against the target microorganism, the concentration at which no visible growth occurs represents the MIC. [94] This analysis was carried out using the method of MICs with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes,* and *Escherichia coli*.

The results for the antimicrobial activity are shown in Figures 26, 27, and 28, and it was possible to observe that none of them presented an absence of color. If there is coloration, there are viable cells in the wells; therefore, the peptide fraction was not able to completely inhibit the growth of those microorganisms, which means that for the tested microorganisms, they are resistant for the obtained peptides when the concentration is equivalent or lower to 10 mg of protein per mL. Moreover, there is not much information about antimicrobial peptides from mollusk shells. However, for example, Li, *et al.* (2014) attributed the antibacterial capacity of the shells to the conversion of $CaCO_3$ to CaO, which is the defense ability of the shells.[95] Furthermore, Naik and Hayes (2019) affirm that the defense mechanisms against mussels' pathogens come from the lysozymes present in the meat and the antimicrobial peptides rich in cysteine-rich from the hemolymph of the mussels. [20] Another study by Mei, *et al*. (2020) attributes the antimicrobial activity to the melanin in the blue mussel shell.[96]

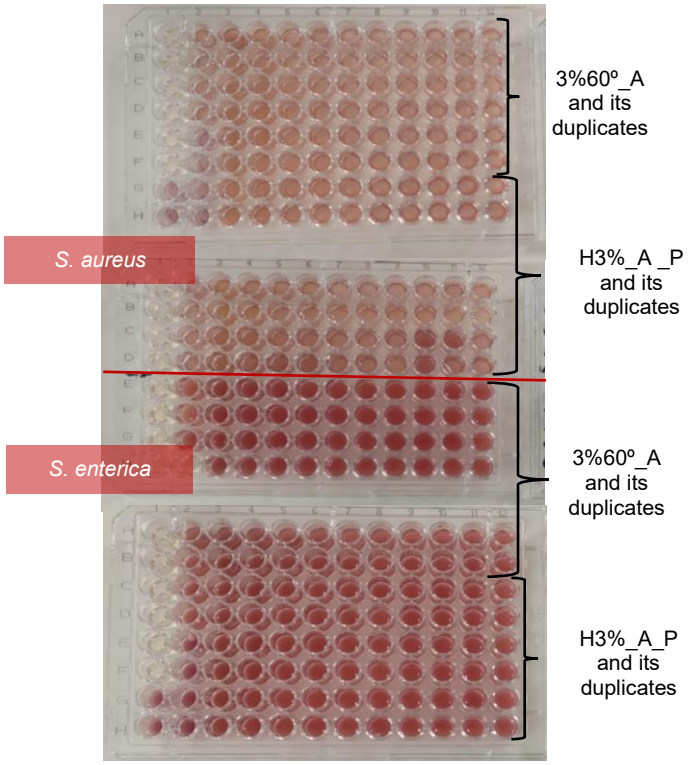

Figure 26 – Results of the MICs assay for the solubilized and hydrolyzed samples, for *Staphylococcus aureus* and *Salmonella enterica*. Samples legend: 3%60º (solubilize fraction with 3% NaOH at 60 ºC) and H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), from batch A of demineralized shells

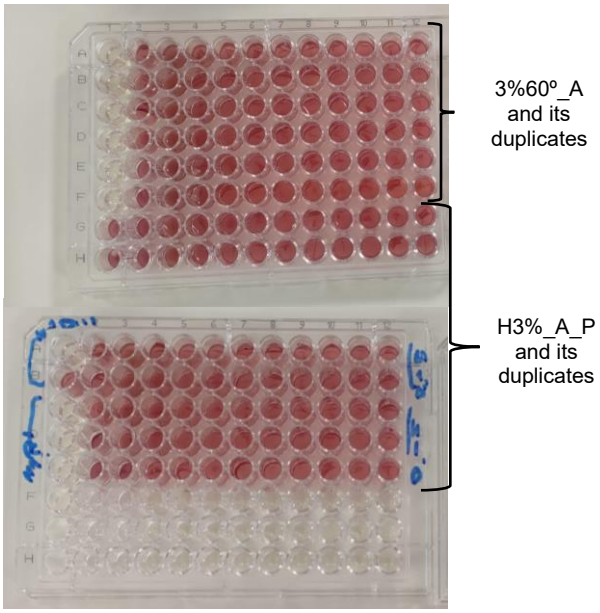

Figure 27 – Results of the MICs assay for the solubilized and hydrolyzed samples, for *Pseudomonas aeruginosa*. Samples legend: 3%60º (solubilize fraction with 3% NaOH at 60 ºC) and H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), from batch A of demineralized shells

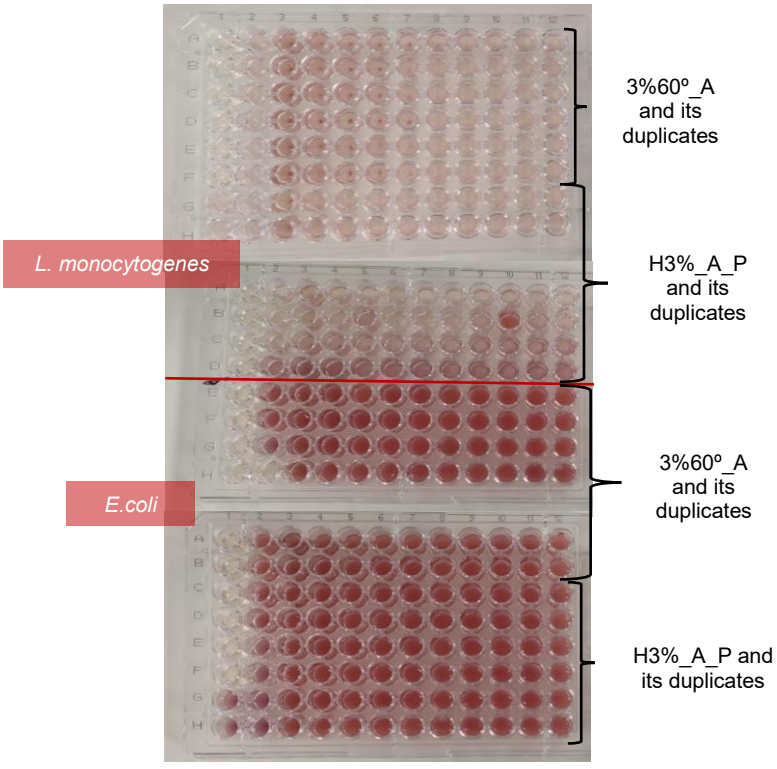

Figure 28 – Results of the MICs assay for the solubilized and hydrolyzed samples for *Listeria monocytogenes* and *Escherichia coli*. Samples legend: 3%60º (solubilize fraction with 3% NaOH at 60 ºC) and H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), from batch A of demineralized shells

5. Peptide content of the obtained fractions

In order to better understand the results obtained for the bioactivities, the percentage of free amino groups of each sample and its peptide size profile were analyzed by HPSEC and SDS-PAGE.

Figure 29 shows the percentages of free amino groups for the four types of fractions, namely the solubilized fraction (3%60º), the hydrolyzed DS (H3%_DS), the hydrolyzed remaining pellet (H3%_P), and the hydrolyzed liquid fraction (H3%_L), the three hydrolysate samples were obtained with 3% Alcalase. Therefore, it is possible to observe that the hydrolysates from the demineralized shells (DS) presented a higher amount of free amino groups, 59.1 ± 13.6%. When compared to the bioactivity results (Figures 17 and 18), it was possible to observe that this was the fraction with the lower performance values. In contrast, the hydrolyzed liquid fraction demonstrated the lowest percentage of free amino groups, 11.8 ± 2.6%, but also did not present favorable values of bioactivity. Considering that this fraction has the same protein as 3%60º but hydrolyzed, it is possible that some aggregation was created by the formation of hydrophobic peptides like this, negatively influencing the measurement by the TNBS assay.[97,98] Moreover, the fraction that presented higher bioactivity was 3%60º and contained 32.7 ± 10.6% of free amino groups. So, it is possible to affirm that it is not favorable to have such a high percentage of free amino groups as H3%_DS because that means the peptides are too small to hold bioactivity. As Lin, *et al.* (2012) mentioned, the peptides with highest antioxidant activity presented a percentage of free amino groups ranging from 26.8 ± 0.2% to 28.1 ± 0.3%, in this case the obtained peptides were from whey protein, using Alcalase.[99] Additionally, for the statistical analysis of these results, a non-parametric test was used and omitted an outlier of 64.7% (H3%_P).

Regarding the percentage of free amino groups, shown in Figure 30, it was possible to observe that there is a significant difference between H3%_P and H1%_P. This indicates that, overall, the hydrolysis with 3% Alcalase was more efficient because it allowed the release of more free amino groups, as expected due to the higher amount of enzyme leading to a stronger break to generate the peptides. In addition, an outlier of 64.7% (H3%_P) was omitted.

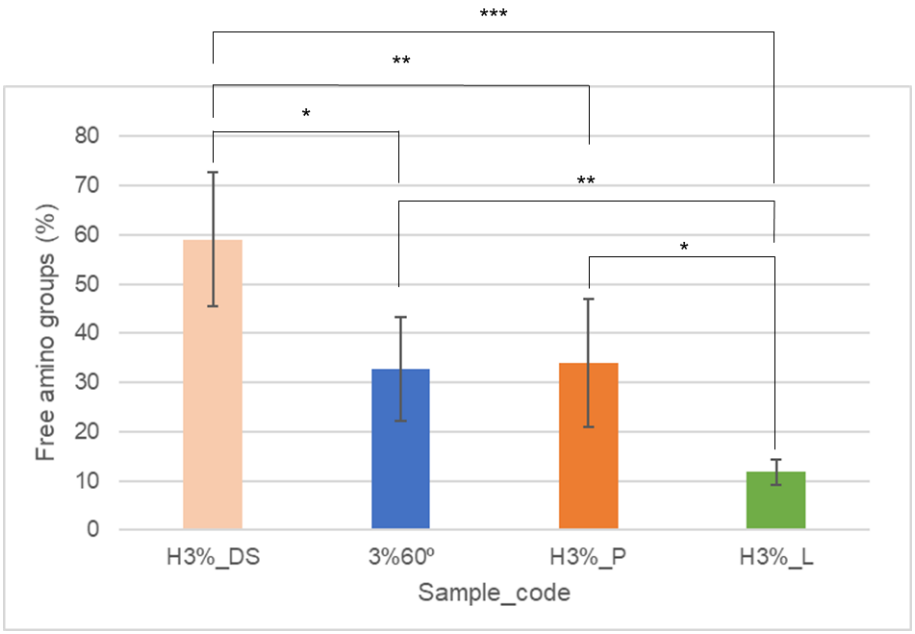

Figure 29 – Percentage of free amino groups, for the solubilization samples and hydrolyzed sample, with 3% Alcalase, by the TNBS assay. Data are expressed in mean ± standard deviation, n = 9 (H3%_DS and 3%60º), n = 8 (H3%_L) and n = 7 ,(H3%_L). * $p \leq 0.05$,** $p \leq 0.01$,*** $p \leq 0.001$. Samples legend: H3%_DS (hydrolysis, with 3% Alcalase, of the demineralized shells), H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step), H3%_L (hydrolysis, with 3% Alcalase, of the liquid fraction from the solubilization step, with the enzyme inactivated by nanfiltration) and 3%60º (solubilized fraction with 3% NaOH at 60 ºC)

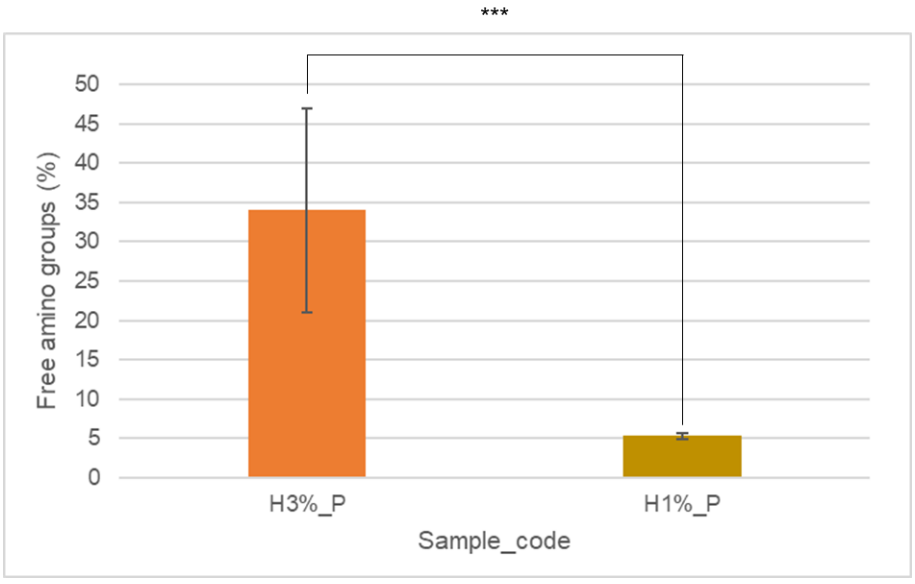

Figure 30 – Percentage of free amino groups, for the solubilization samples, hydrolyzed sample, with 3% and 1% Alcalase, by the TNBS assay. Data are expressed in mean ± standard deviation, n = 7 (H3%_P) and n = 3 (H1%_P). *** $p \leq 0.001$. Samples legend: H3%_P (hydrolysis, with 3% Alcalase, of the remaining pellet from the solubilization step) and H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization

Figures 31 and 32 show the peptide size profiles for the solubilized and hydrolyzed peptides, with 1% Alcalase, respectively. Firstly, Figure 31 demonstrates that both batches of extraction, 3%60º_A, and 3%60º_B had similar peptide' size distribution, having more than half of the peptides <1 kDa and about 30% between 1 kDa and 3 kDa. In Figure 32, there are differences within the H%_A_P and H1%_B_P.

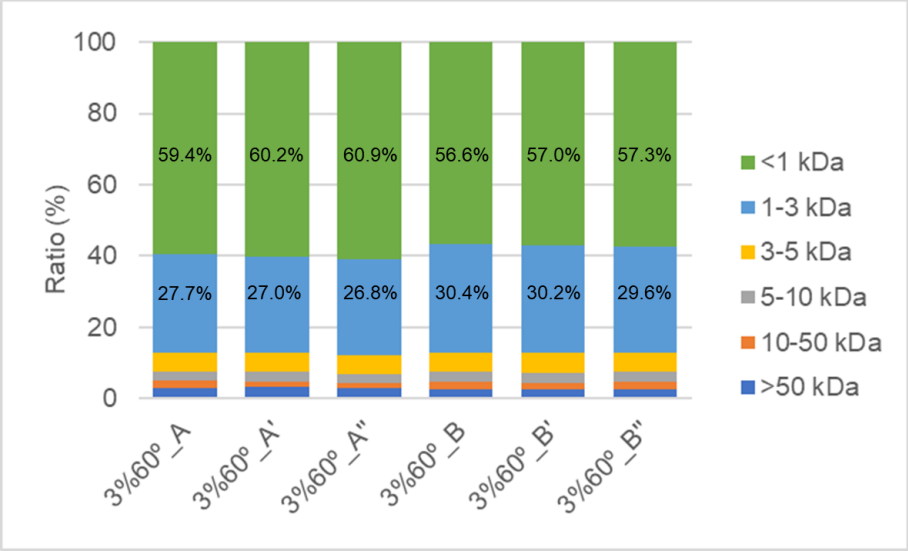

Figure 31 – Peptide size profile of the solubilized fraction. Ratios based on HPSEC areas. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC), from batches A and B of demineralized shells

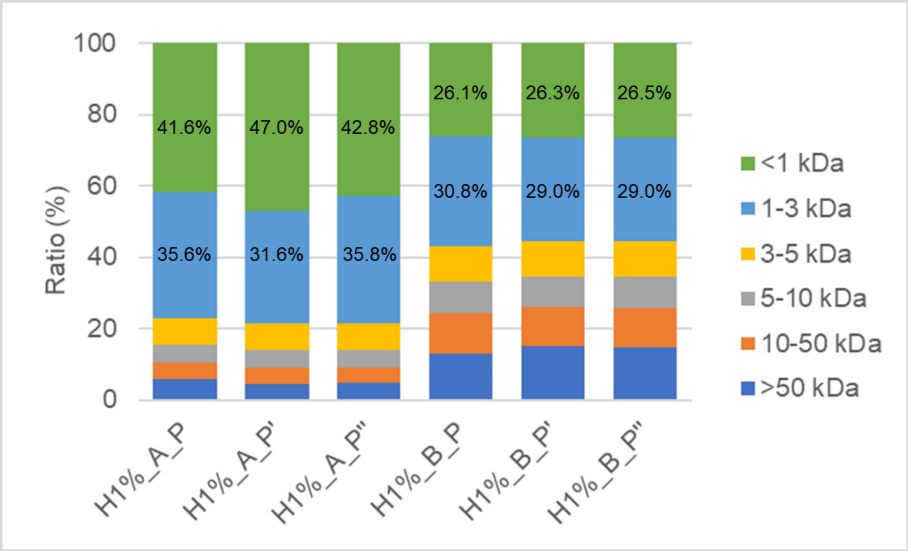

Figure 32 - Peptide size profile of the hydrolyzed peptides with 1% Alcalase. Ratios based on HPSEC areas. Samples legend: H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batches A and B of demineralized shells

Moreover, comparing the fractions 3%60, H1%_A_P, and H1%_B_P (Figure 33), the solubilized fraction presented the smaller peptides, followed by H1%_A_P, H1%_B_P, the most different among them and with the higher percentage of larger peptides. However, when compared to the bioactivities, for the antioxidant activity measured by the ABTS assay, it was 3%60º that presented the highest activity. On the contrary, for the ORAC assay, H1%_B_P had a much higher activity than the rest of the extracted fractions. Regarding antihypertensive activity, even though our peptides did not demonstrate positive results, it was 3%60º that had the higher activity.

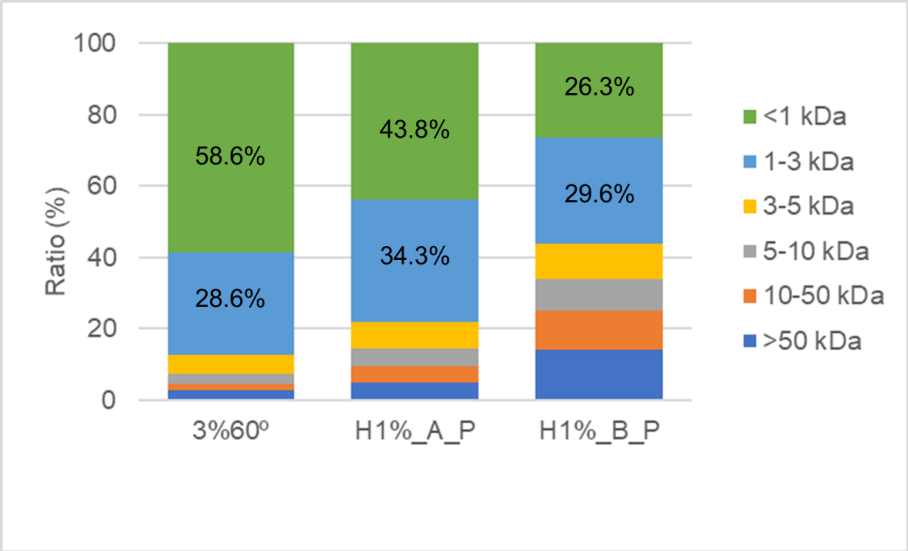

Figure 33 – Average peptide size profile of the solubilized and hydrolyzed peptides with 1% Alcalase. Ratios based on HPSEC areas. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC) and H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batches A and B of demineralized shells

The validation that small peptides were obtained was done using the SDS-PAGE method, shown in Figure 34. It is visible that for the solubilized samples, there is a difference in the intensity of the color between the 3%60º_A and 3%60º_B, which was not confirmed by the peptide profile (Figure 31). Additionally, for both solubilized fractions, it is possible to observe that the color is more intense from the beginning of the column until the mark of 31 kDa. Concerning the hydrolyzed samples, the method was tested for hydrolysates with 3% and 1% Alcalase (H3%_A_P, H1_A_P, and H1%_B_P, respectively), and it is noticeable that the sample

presenting a more intense color is H3%_A_P, which is in agreement with Figure 30, that hydrolysis with 3% Alcalase seems more efficient. It is also remarkable that the sample H1%_B_P and its duplicate seem to have a dispersion of color along the band, as is also shown in the peptide size profile (Figure 33).

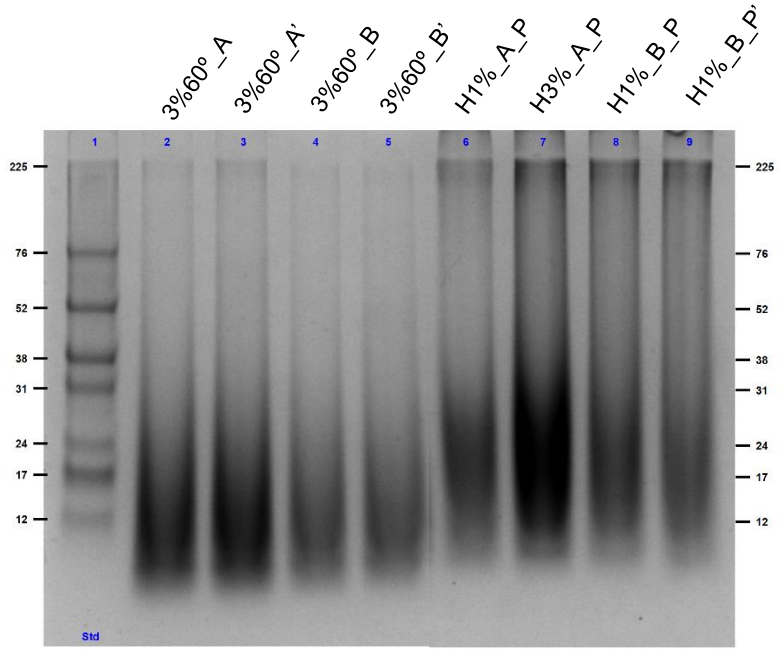

Figure 34 – SDS-PAGE results from the solubilized sample and hydrolysis with 1% and 3% Alcalase samples. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC) and H1%_P (hydrolysis, with 1% Alcalase, of the remaining pellet from the solubilization step), from batches A and B of demineralized shells

In blue mussel meat, other low-weight bioactive peptides were found. For example, Oh, *et al.* (2020) found peptides that can inhibit adipogenesis.[100] Additionally, Qiao, *et al.* (2017) obtained hydrolysates with antithrombotic activity, mainly with peptides <15 kDa.[44] In another study, Jung and Kim (2009) discovered an oligopeptide with anticoagulant capacity extracted from the edible parts of blue mussel (*Mytilus edulis*), with approximately 2.5 kDa of molecular weight.[101] Also, in the blue mussel meat was found an antioxidant peptide with a theoretical mass of 575.3193 Da, in the study carried out by Wang *et al.* (2013).[42]

Mentioning bioactive peptides from other mollusks, Yang, *et al.* (2019) found antioxidant peptides in *Tergillarca granosa*, a marine bivalve, with a molecular weight ranging from 442.48 Da to 754.81 Da.[102] On the pearl oyster (*Pinctada fucata*) shell, Sasaki, *et al*. (2019) found an angiotensin I-converting enzyme inhibiting peptide, with 578.7 Da of molecular weight.[103]

As for peptides found in mollusk shells, there is not much information about the subject, but for the blue mussel shell, there were extracted some novel proteins and peptides by Marie *et al.* (2011), which were analyzed for proteomics and presented theoretical mass from 14 kDa to 36 kDa.[17]

Lastly, it is important to mention that Ghalamara, *et al.* (2022), using cooked sardine, obtained the highest antioxidant activity measured by ORAC in the samples with smaller peptides, which was the opposite of our results, because the sample H1%_B_P was the one with the greater ORAC results and also exhibited a higher amount of larger peptides.[48]

6. INFOGEST

INFOGEST is an international network focused on advancing knowledge about food digestion and its impact on health. By bringing together experts in physiology, enzymology, and microbiology, INFOGEST fosters collaboration to improve our understanding of how different foods are broken down in the digestive system. The network's efforts contribute to the development of standardized methods for assessing food digestibility, influencing nutritional recommendations and food product development.[78]

Regarding the samples taken during INFOGEST, as previously mentioned, five sampling times were obtained: during the oral, gastric, and intestinal phases and the outcomes from dialysis of the permeate and the retained. For the gastrointestinal and dialysis simulations, the solubilized peptides (3%60º_B) were used. Since there was primarily no significant difference between the hydrolysates from the 3% or 1% Alcalase hydrolysis, it was decided to apply INFOGEST to the hydrolysates derived from the 1% Alcalase of the remaining pellet (H1%_B).

6.1. Antioxidant activity

The antioxidant activity was tested for the sample obtained from the oral, gastric, and intestinal phases (O, G, I). Furthermore, the bioactivity was measured using the ABTS and ORAC assays.

Figure 35 presents the results of the ABTS assay for the three phases of the solubilized peptides, and Figure 36 presents the results of the ABTS assay for the hydrolysates. For the solubilized samples, it is possible to observe that there is a significant difference between the antioxidant activity of the peptides in the oral stage and in the intestinal phase. The intestinal phase provided

a higher antioxidant activity of 7.06 ± 1.96 µmol Trolox equivalents per mg of protein and 2.50 ± 1.50 µmol Trolox equivalents per mg of protein for 3%60º_B_I and H1%_B_I, respectively. The studies conducted by Cho (2020) and Zhang, *et al.* (2018) also observed an increase in antioxidant activity with the gastrointestinal simulation.[104,105]

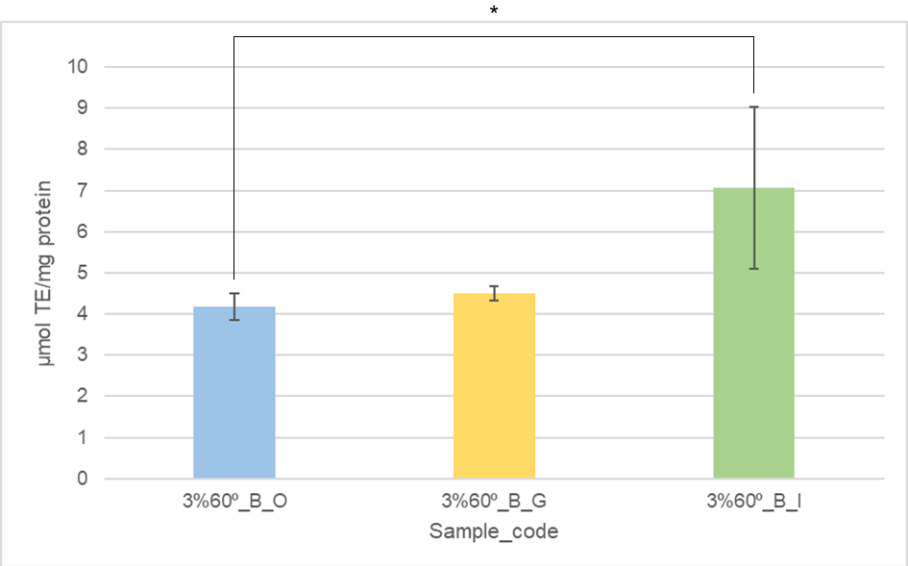

Figure 35 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein for the solubilized sample, after INFOGEST. Data are expressed in mean ± standard deviation, n = 3. * $p \leq 0.05$. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC) and O, G and I, stands for oral, gastric and intestinal phase, respectively

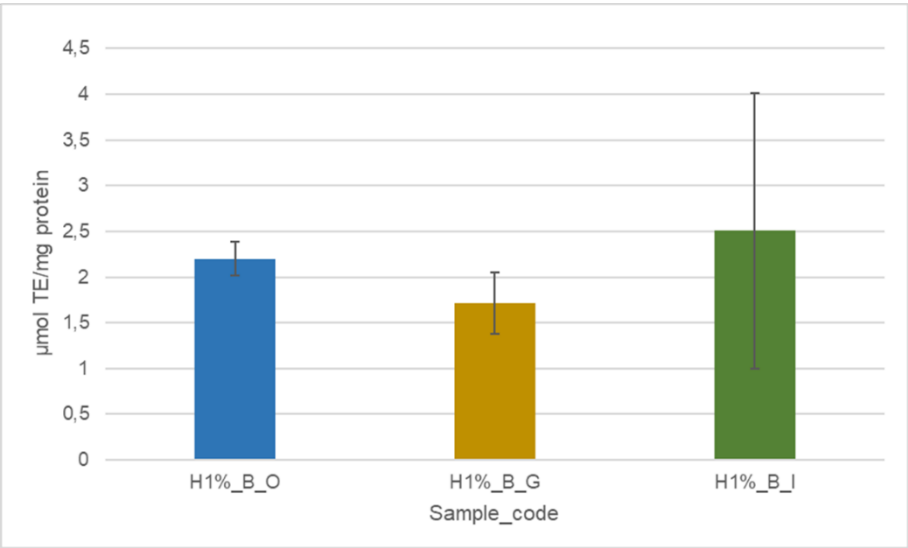

Figure 36 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein for the hydrolyzed remaining pellet, with 1% Alcalase sample, after INFOGEST. Data are expressed in mean ± standard deviation, n = 3. There are no significant differences ($p > 0.05$). Samples legend: H1%_B (hydrolysis, with 1% Alcalase, from the remaining pellet) and O, G and I, stands for oral, gastric and intestinal phase, respectively

Concerning the antioxidant activity of the peptide submitted to the dialysis, simulating the intestine absorption, they were first analyzed through the ABTS assay. Figures 37 and 38 show the results for the solubilized and hydrolyzed fractions, respectively. In both cases, there was no significant difference. However, for the solubilized, the highest value was attributed to 3%60º_B_P, 14.5 ± 3.66 µmol Trolox equivalents per mg of protein, and concerning the hydrolyzed fraction the best phase was H1%_B_P, with antioxidant activity of 4.04 ± 0.34 µmol Trolox equivalents per mg of protein.

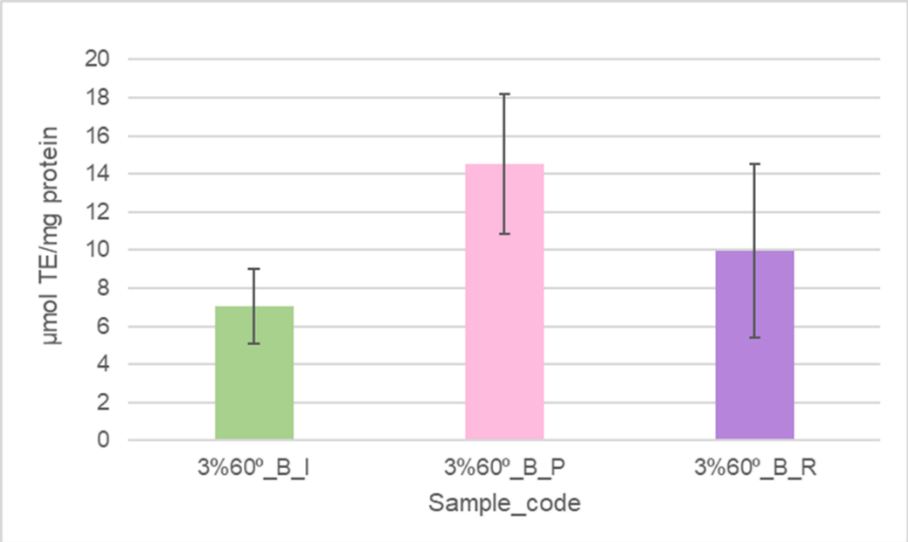

Figure 37 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein for the solubilized sample after dialysis. Data are expressed in mean ± standard deviation, n = 3. There are no significant differences ($p > 0.05$). Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC) and I, P and R, stands for intestinal, permeated and retained phase, respectively

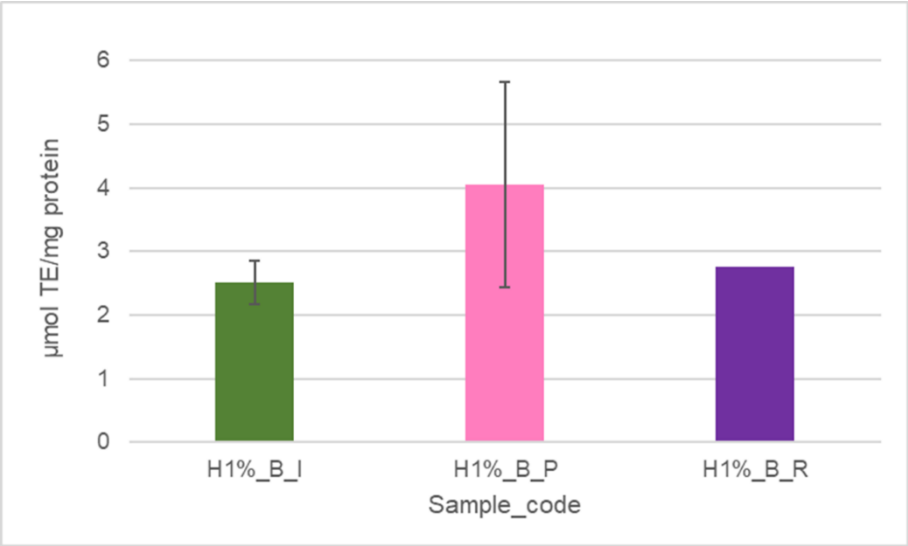

Figure 38 – Antioxidant activity (ABTS) expressed in µmol TE/mg of protein for the hydrolyzed remaining pellet, with 1% Alcalase sample, after dialysis. Data are expressed in mean ± standard deviation, n = 3. There are no significant differences ($p > 0.05$). Samples legend: H1%_B (hydrolysis, with 1% Alcalase, from the remaining pellet) and I, P and R, stands for intestinal, permeated and retained phase, respectively

Figure 39 demonstrates the results of the ORAC assay for the solubilized peptides, and Figure 40 for the hydrolysates across three phases. Notably, for the solubilized peptides the gastric phase (3%60º_B_G) presented the highest antioxidant activity of 4.38 ± 0.36 µmol Trolox equivalents per mg of protein, as for the hydrolyzed peptides it was the intestine phase (H1%_B_I) that exhibited the highest antioxidant activity, registering 3.40 ± 1.68 µmol Trolox equivalents per mg of protein. Although the values for the solubilized samples exhibited significant differences, the values for the hydrolyzed peptides did not show significant differences. Moreover, in Figure 39, maybe the peptides were hydrolyzed, leading to the loss of bioactivity.

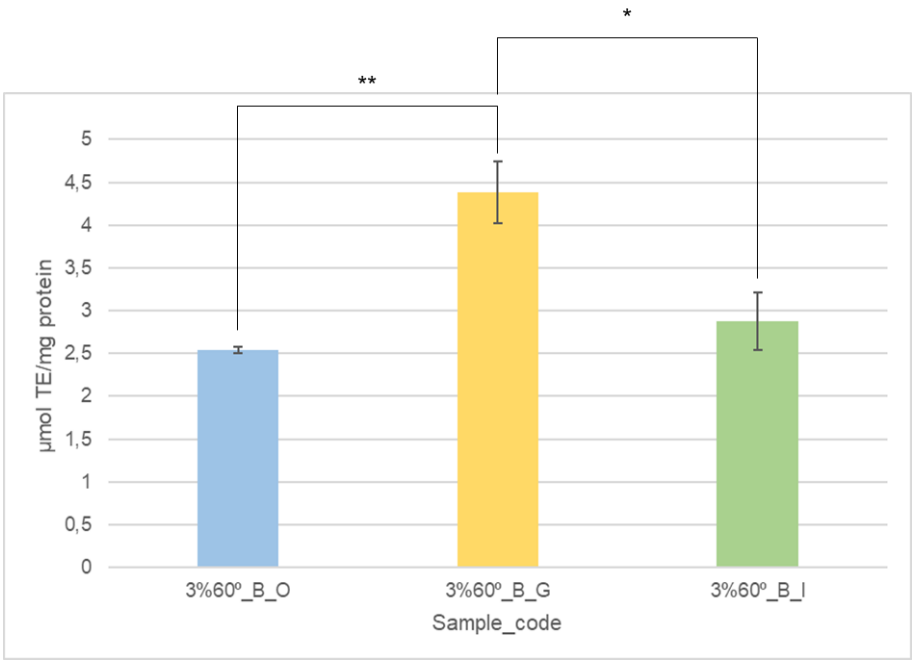

Figure 39 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the solubilized sample, after INFOGEST. Data are expressed in mean ± standard deviation, n = 3 (3%60º_B_I) and n = 2 (3%60º_B_O and 3%60º_B_G). * $p \leq 0.05$, ** $p \leq 0.01$. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC) and O, G and I, stands for oral, gastric and intestinal phase, respectively

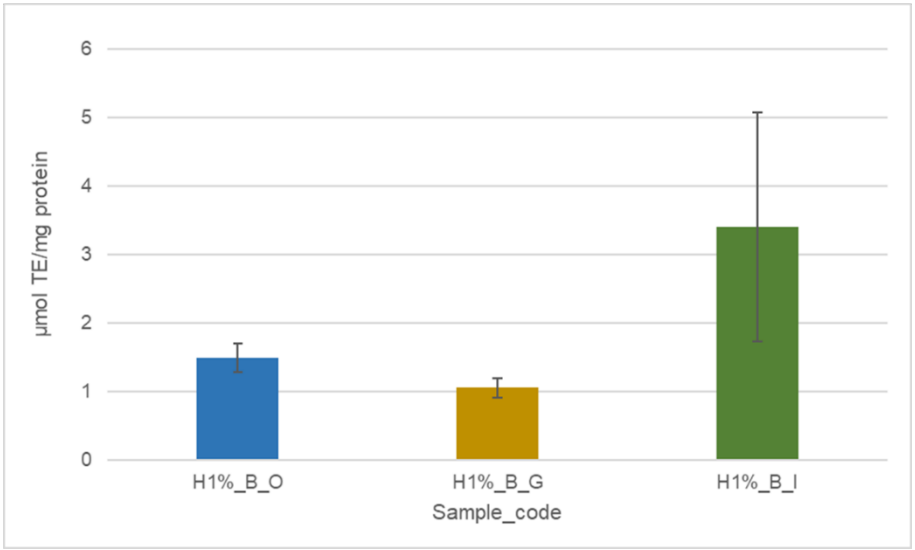

Figure 40 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the hydrolyzed remaining pellet, with 1% Alcalase sample, after INFOGEST. Data are expressed in mean ± standard deviation, n = 3. There are no significant differences ($p > 0.05$). Samples legend: H1%_B (hydrolysis, with 1% Alcalase, from the remaining pellet) and O, G and I, stands for oral, gastric and intestinal phase, respectively

Finally, the peptides from the dialysis process were submitted to the ORAC assay. Figures 41 and 42 show the results from the solubilized and hydrolyzed fractions, respectively. With an antioxidant activity, for the solubilized (3%60º_B_P) of 10.2 ± 6.26 µmol Trolox equivalents per mg of protein, as for the hydrolysate (H1%_B_P), the antioxidant activity was 3.24 ± 1.88 µmol Trolox equivalents per mg of protein. It is possible to observe that in Figure 41, there is a significant difference between the antioxidant activity in the intestinal stage and the permeated phase (for this statistical analysis, a non-parametric test was used). This distinction may arise from the poor inactivation of the enzymes from pancreatin, which carried on the hydrolysis throughout the dialysis simulation. Even if it was not expected, it gives us valuable insight into what actually happens in the human body because, in our system, the enzyme is not naturally inactivated, so it is possible to observe that these peptides would be further hydrolyzed and increase their bioactivity until they can reach the bloodstream.

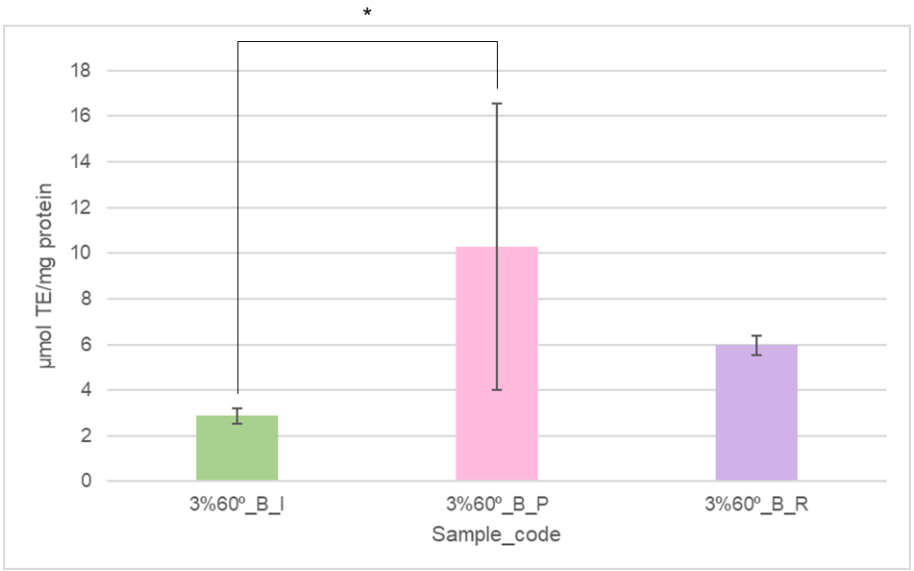

Figure 41 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the solubilized sample after dialysis. Data are expressed in mean ± standard deviation, n = 3. * $p \leq 0.05$. Samples legend: 3%60º (solubilized fraction with 3% NaOH at 60 ºC) and I, P and R, stands for intestinal, permeated and retained phase, respectively

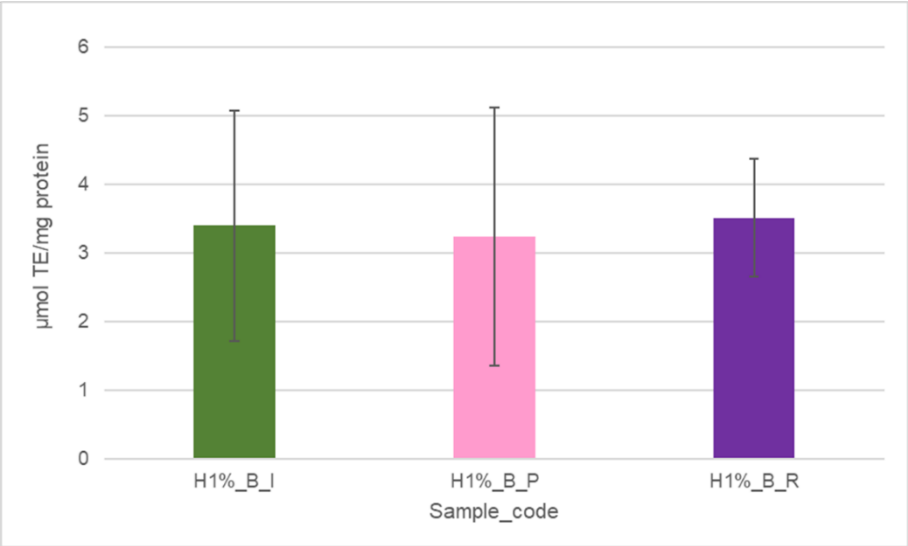

Figure 42 – Antioxidant activity (ORAC) expressed in µmol TE/mg of protein for the hydrolyzed remaining pellet, with 1% Alcalase sample, after dialysis. Data are expressed in mean ± standard deviation, n = 3 (H1%_B_I and H1%_B_R) and n = 2 (H1%_B_P). There are no significant differences ($p > 0.05$). Samples legend: H1%_B (hydrolysis, with 1% Alcalase, from the remaining pellet) and I, P and R, stands for intestinal, permeated and retained phase, respectively

6.2. Antimicrobial activity

The antimicrobial activity was measured in the retentate phase using the minimum inhibitory concentration (MIC) assay. This bioactivity was only applied to the retentate because it is the phase that remains in the intestine, in contact with the microbiota. Because it was to simulate what remained in the intestine, the sample from 3%60º and H3%_P were applied to *Salmonella enterica* and *Escherichia coli*. Figures 43 and 44 show the results of this assay, and it was possible to observe that none of them presented peptides with antimicrobial activity, because it is possible to observe microbial growth in all the wells, with the obtained peptides, which means that for the tested microorganisms, they are resistant for the obtained peptides when the concentration is equivalent or lower to 10 mg of protein per mL.

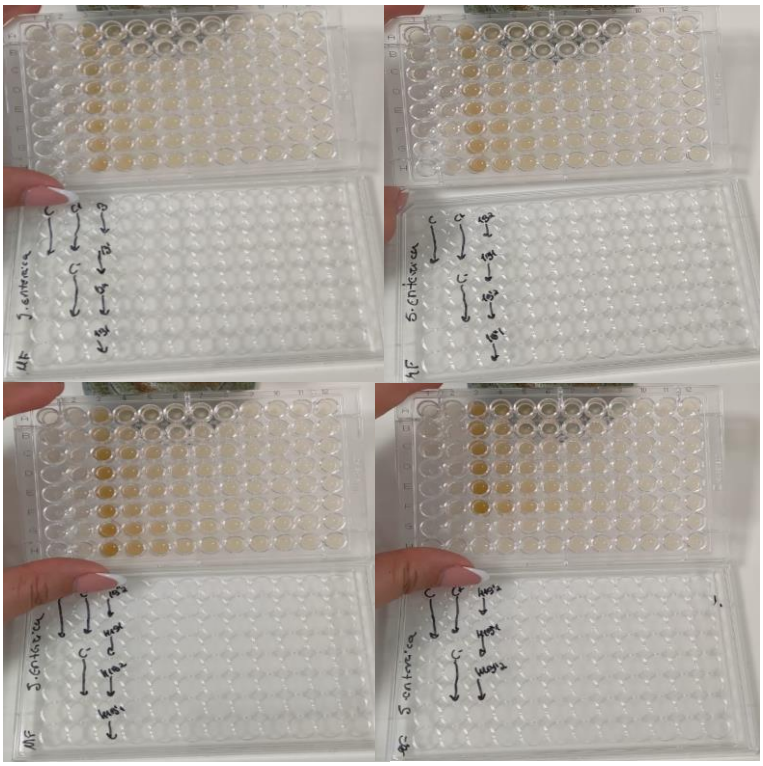

Figure 43 – Results of the MICs assay for the solubilized and hydrolyzed samples, for *Salmonella enterica*

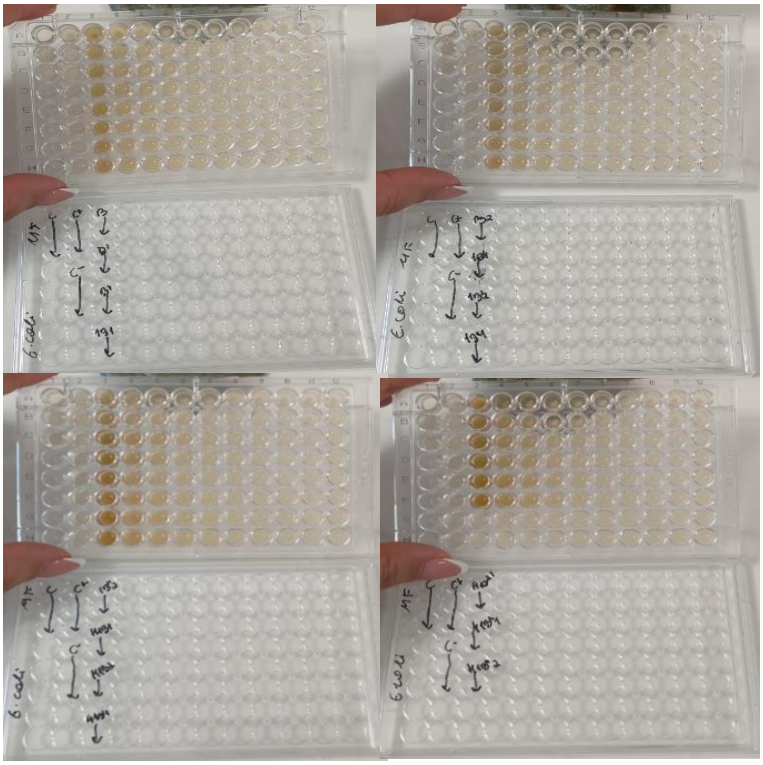

Figure 44 – Results of the MICs assay for the solubilized and hydrolyzed samples, for *Escherichia coli*

Conclusion

The escalating concern surrounding food waste, exacerbated by the increasing global population, demands inventive solutions to manage waste effectively and convert it into valuable products. This study focused on exploring the biotechnological potential of blue mussel shells and sheds light on a pathway to repurpose marine by-products into bioactive peptides with promising applications.

The meticulous extraction processes employed in this study, involving demineralization, solubilization and enzymatic hydrolysis, have uncovered peptides from blue mussel shells exhibiting noteworthy antioxidant activity. Through ORAC and ABTS assays, specific peptides, particularly in their soluble form, demonstrated significant antioxidant capabilities. The study's comparative analysis, contrasting results from blue mussel shells with those from other sources, provided a comprehensive view of the efficacy of these peptides.

Notably, the research delved into the intricacies of the extraction process and revealed that the extraction time during the solubilization step had minimal impact on antioxidant activity. This insight simplifies the extraction protocol, ensuring a more straightforward standardization and replication process. Additionally, the exploration of hydrolysis methods, considering the potential benefits of serial hydrolysis with 1% Alcalase versus a single hydrolysis with 3% Alcalase, suggests the need for further investigation. The use of different enzymes, including plant-sourced ones like cardosin, bromelain, and papain, could enhance sustainability and broaden the scope of research.

It is worth noting that the solubilized fraction demonstrated the highest bioactive potential. Extracting a greater amount of peptides from the shells is advantageous, leading to the application of hydrolysis to the remaining pellet. However, even without this step, the solubilized peptides exhibited promising antioxidant activity, eliminating the necessity for additional procedures. Regarding the antihypertensive and antimicrobial activities, no promising results were observed when Alcalase was employed for hydrolysis.

Moreover, concerning the distribution of peptide sizes, it was noted that for peptides to exhibit bioactivity, they should be predominantly small, particularly below 3 kDa. Additionally, in terms of free amino group content, it was observed that an ideal percentage for higher bioactivity is around 35%.

Gastrointestinal simulation (INFOGEST) introduced real-world applicability, demonstrating that certain peptides retained or even enhanced their bioactivity post-simulation, emphasizing their robustness and potential efficacy when consumed.

Challenges were encountered, particularly in antimicrobial assays, revealing limitations in the peptides' ability to combat pathogens like *Salmonella enterica* and *Escherichia coli*.

This thesis contributes significantly to biotechnological research, unveiling the untapped potential of marine by-products and providing a blueprint for their extraction and utilization. The collaboration between the Portuguese Catholic University (CBQF) research center and the Derio unit of AZTI's research center exemplifies the universal nature of the issue and the collaborative spirit required to address it.

In conclusion, while the journey to transform marine by-products into a solution for various health and nutritional challenges is lengthy and challenging, this research marks a decisive step forward. The positive and challenging findings pave the way for future endeavours, urging the scientific community to view our oceans with renewed interest and hope.

Moreover, for upcoming research, we suggest conducting a thorough proteomic analysis of the fractions to identify specific peptides, providing a more comprehensive understanding of the product's potential. Additionally, further investigations should be carried out to optimize the steps of solubilization and hydrolysis. It is also essential to consider whether the resources invested in extracting these peptides justify the outcomes.

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Annexes

1. Demineralization

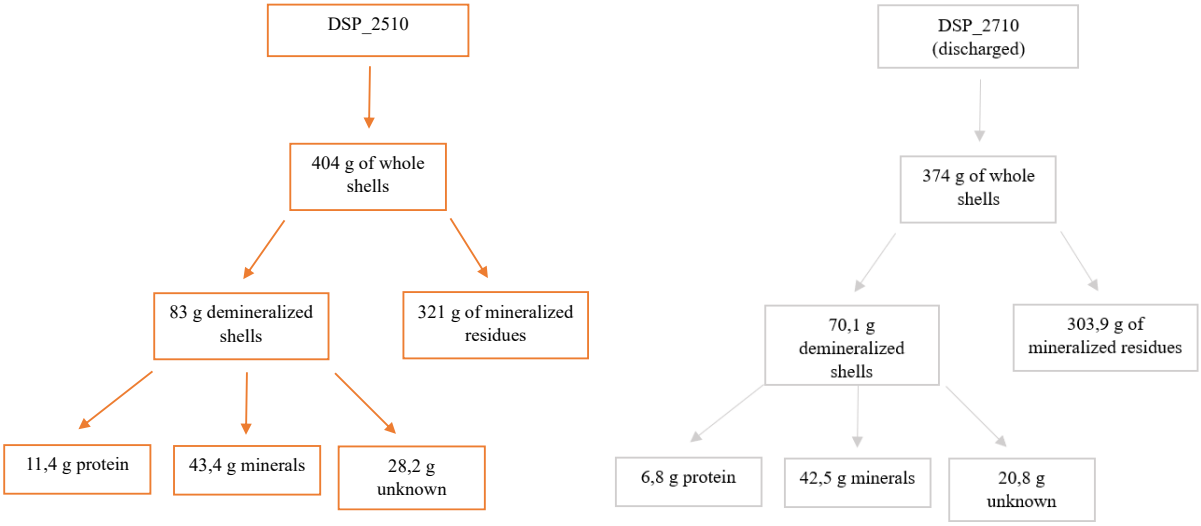


Figure A1 – Resume of the mass balances for the demineralization process for batches 1 (DSP_2510) and 2 (DSP_2710)

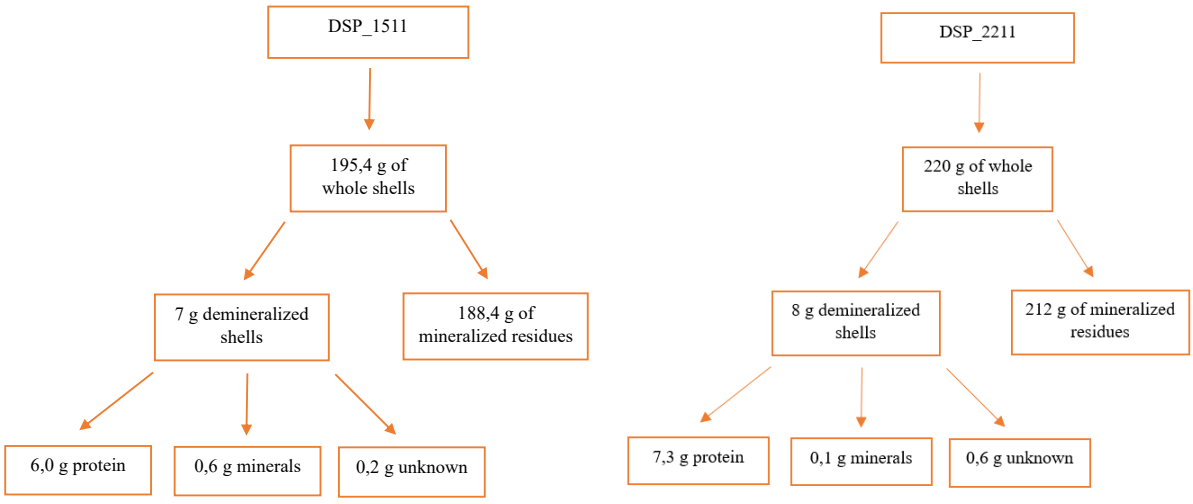


Figure A2 – Resume of the mass balances for the demineralization process for batches 3 (DSP_1511) and 4 (DSP_2211)

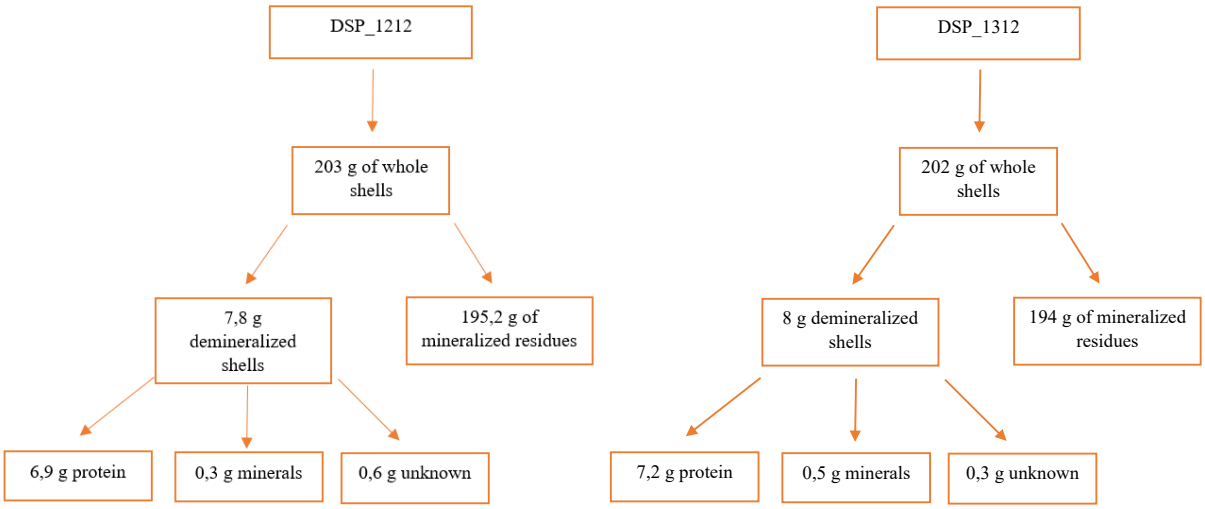


Figure A3 – Resume of the mass balances for the demineralization process for batches 5 (DSP_1212) and 6 (DSP_1312)

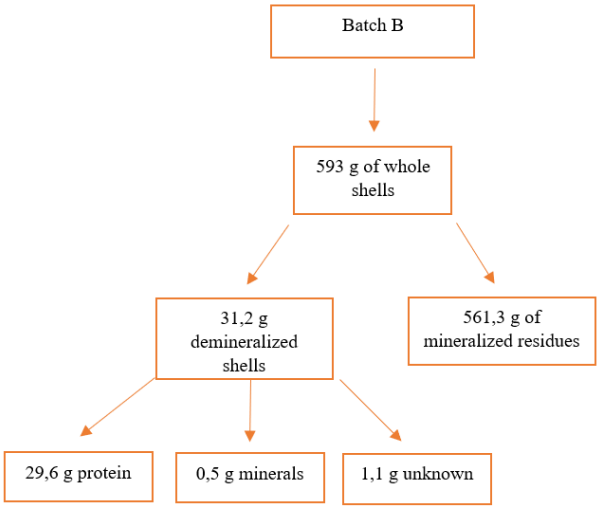


Figure A4 – Resume of the mass balances for the demineralization process for batch B

2. Solubilization

2.1. Solubilization trials

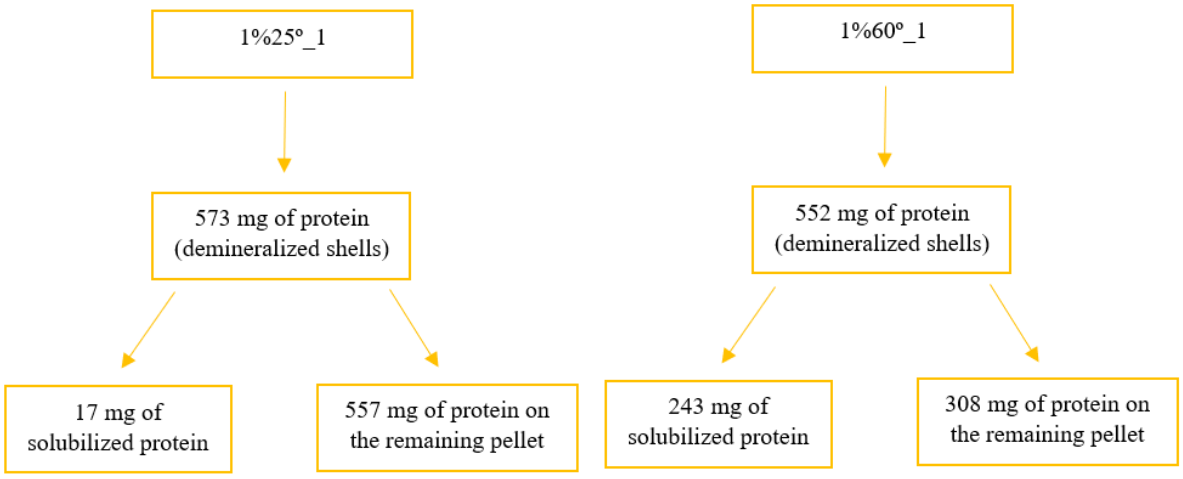


Figure A5 – Resume of the mass balances, for the optimization of the solubilization process, with 1% NaOH at 25ºC and 60ºC

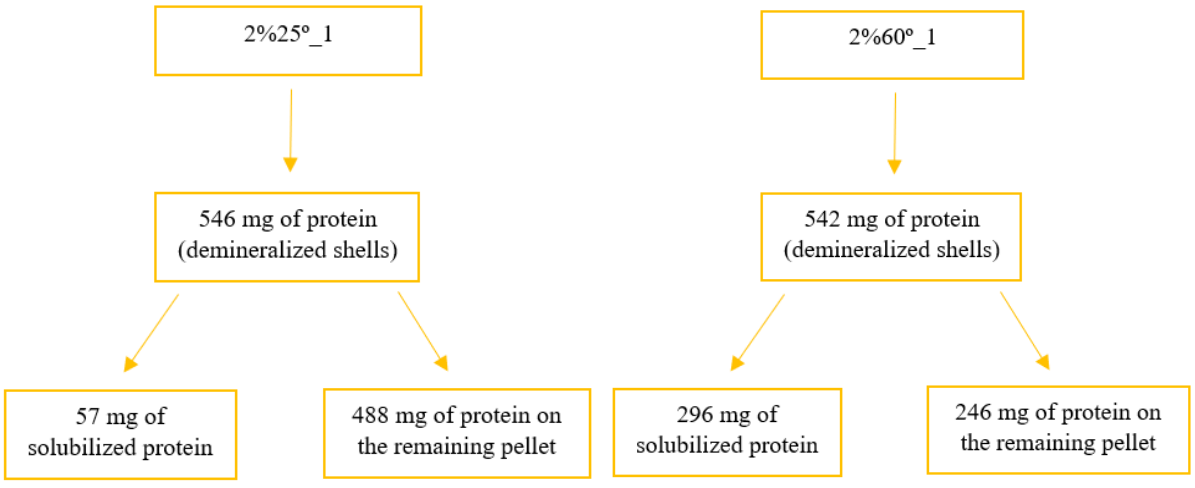


Figure A6 – Resume of the mass balances, for the optimization of the solubilization process, with 2% NaOH at 25ºC and 60ºC

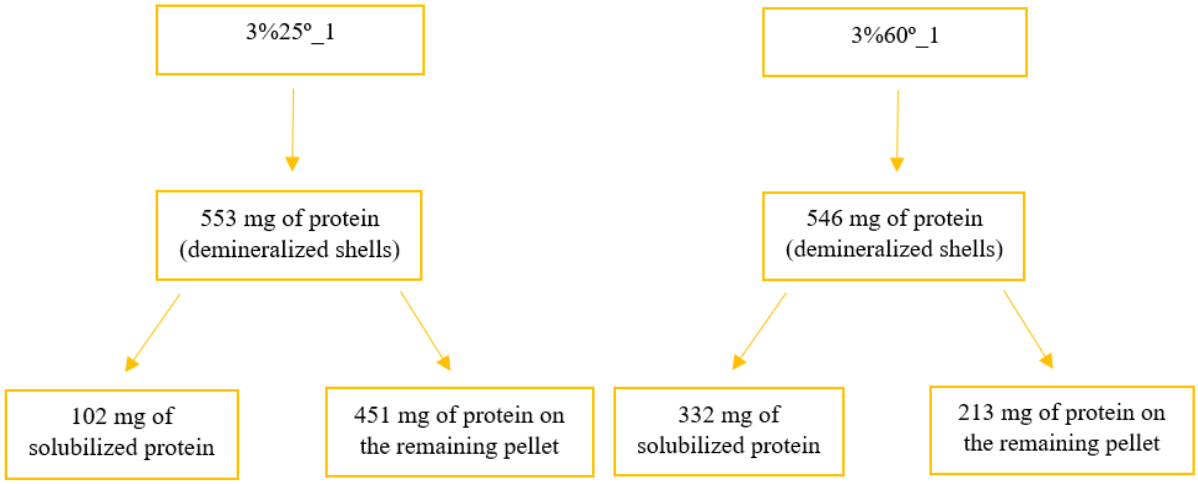


Figure A7 – Resume of the mass balances, for the optimization of the solubilization process, with 3% NaOH at 25ºC and 60ºC

2.2. Solubilization with the best condition

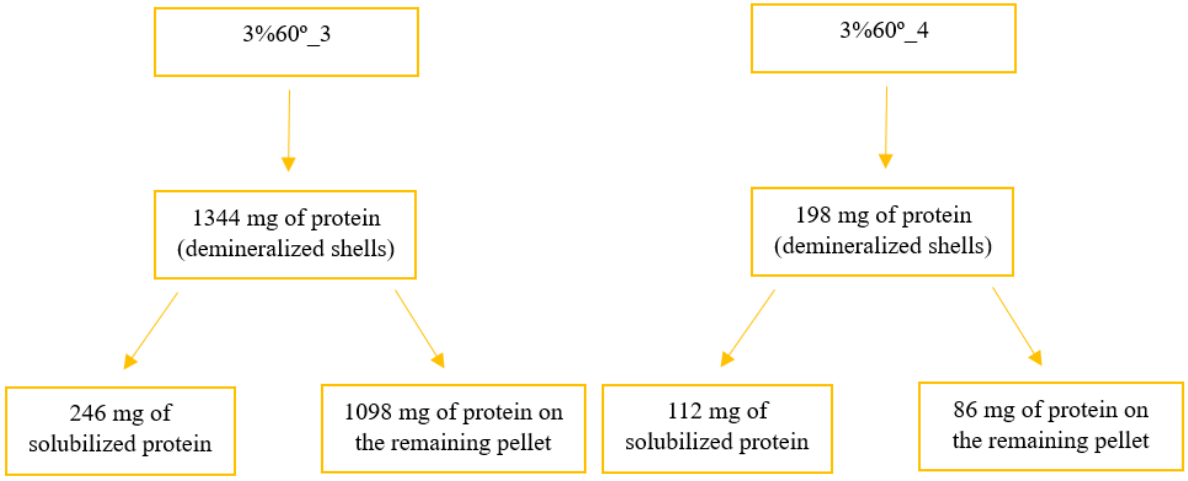


Figure A8 – Resume of the mass balances, for the solubilization process, with 3% NaOH at 60ºC, for batches 3 and 4 of DS

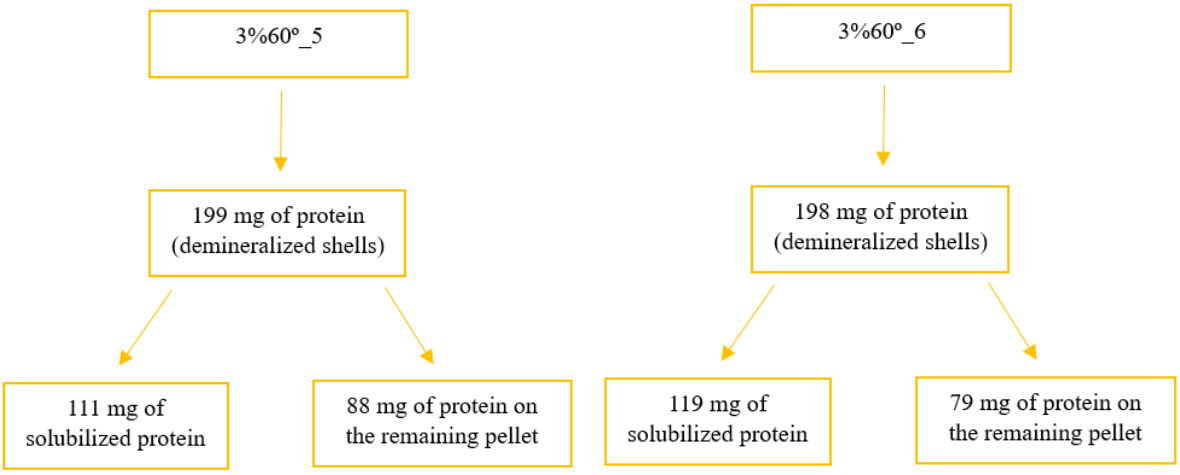


Figure A9 – Resume of the mass balances, for the solubilization process, with 3% NaOH at 60ºC, for batches 5 and 6 of DS

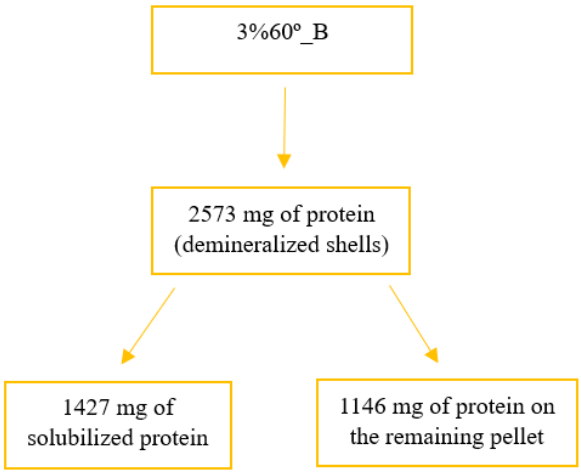


Figure A10 – Resume of the mass balances, for the solubilization process, with 3% NaOH at 60ºC, for batch B of DS

3. Hydrolysis

3.1. Hydrolysis of the demineralized shells

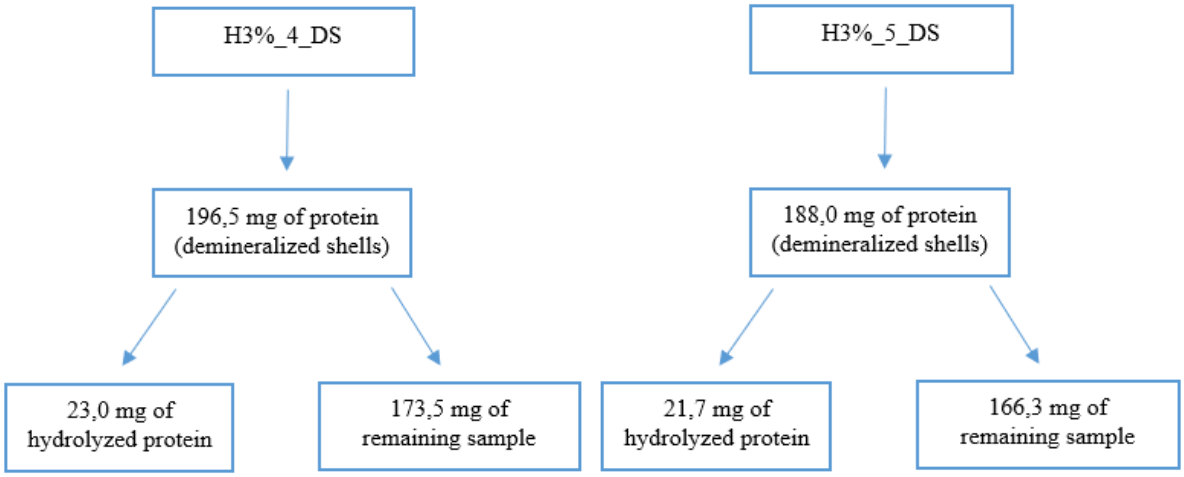


Figure A11 – Resume of the mass balances, for the hydrolysis process of the demineralized shells, with 3% Alcalase, for batches 4 and 5

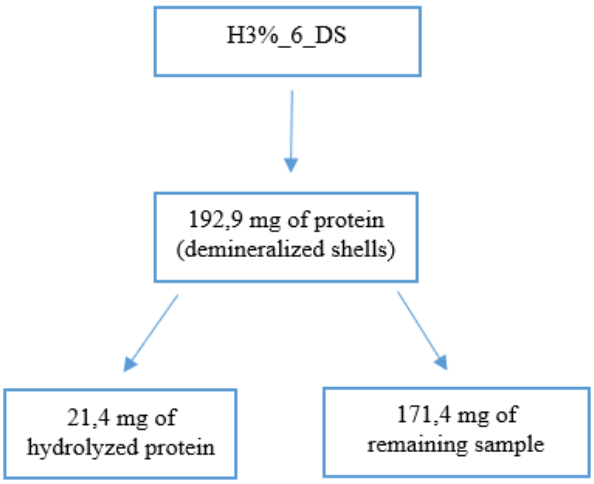


Figure A12 – Resume of the mass balances, for the hydrolysis process of the DS, with 3% Alcalase, for batch B

3.2. Hydrolysis of the remaining pellet

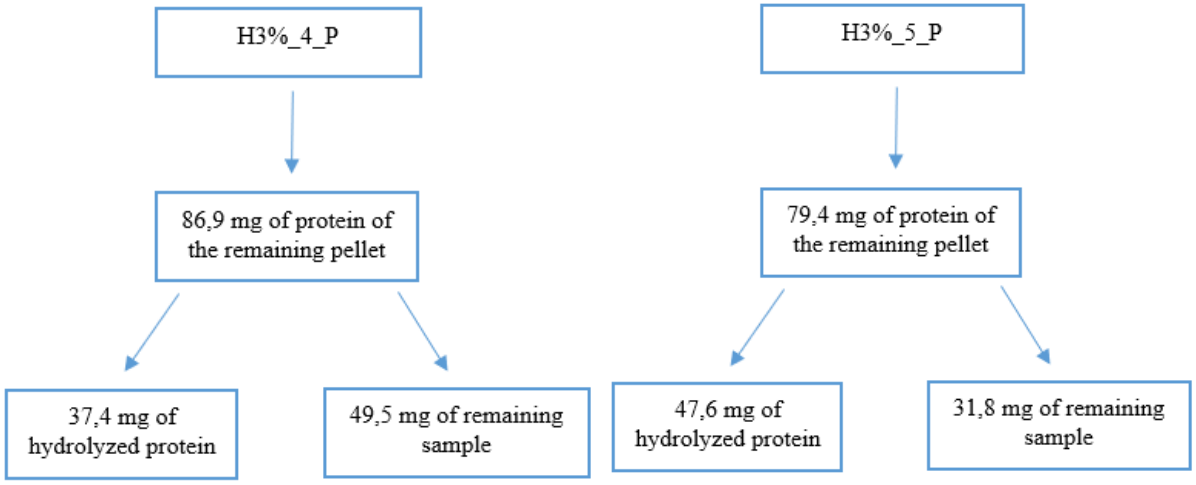


Figure A13 – Resume of the mass balances, for the hydrolysis process of the remaining pellet, with 3% Alcalase, for batches 4 and 5

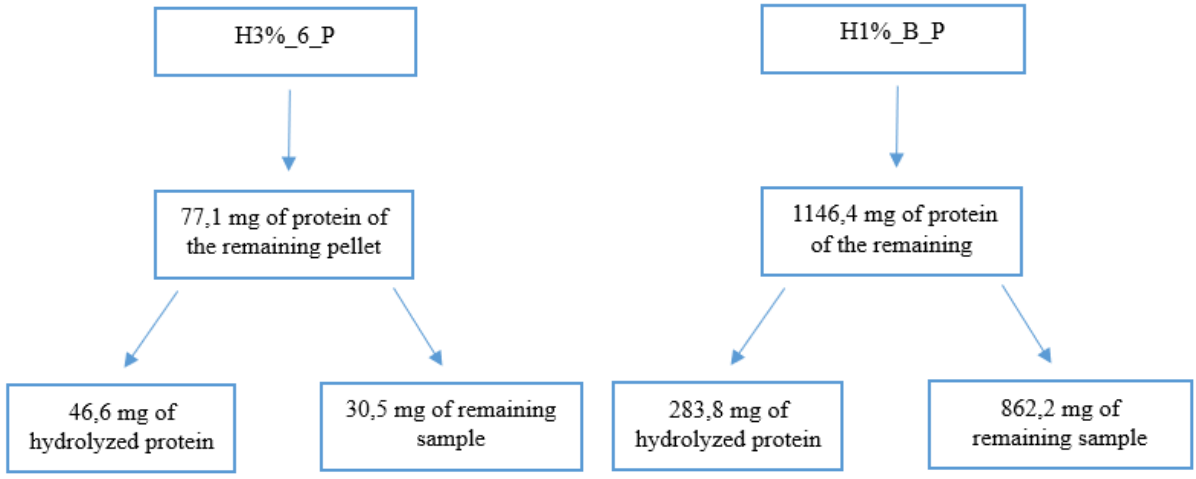


Figure A14 – Resume of the mass balances, for the hydrolysis process of the remaining pellet, with 3% Alcalase, for batches 6 and B

4. Global extraction

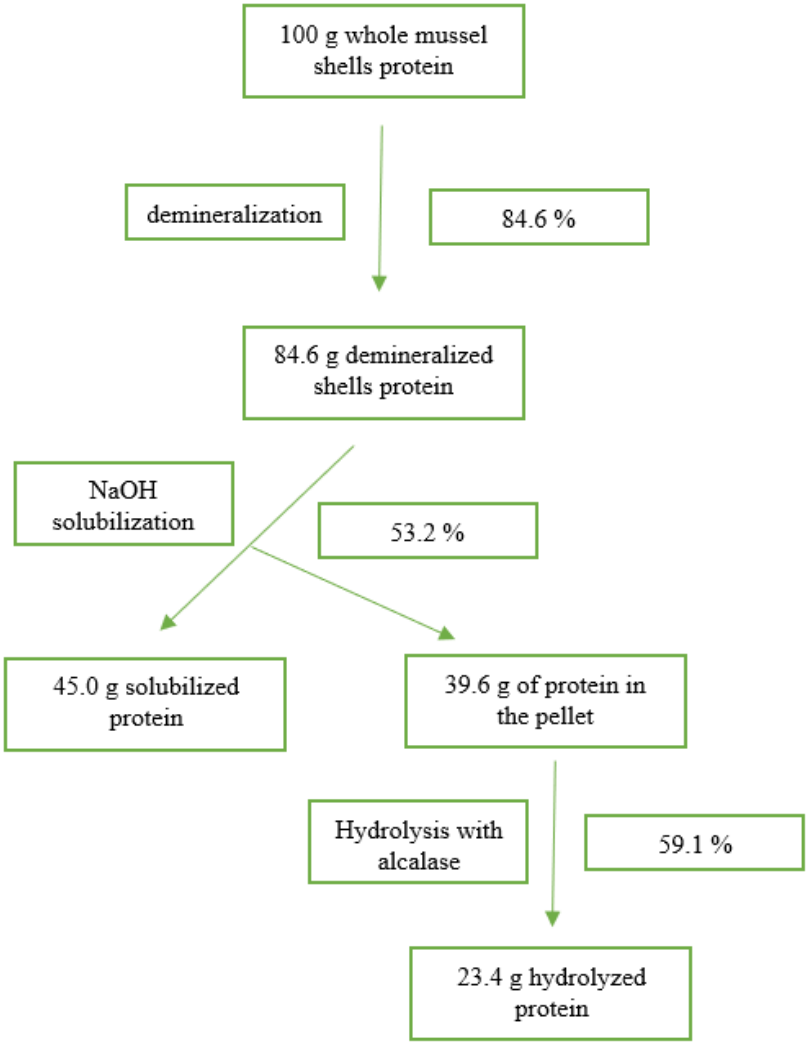


Figure A14 – Resume of the mass balances, with the mean of all the obtained values, for the global process

5. Statistical analysis

5.1. Demineralization

- **Explore**

Observations

Output Created		29-SEP-2023 11:02:54
Comments		
Entry	Active dataset	DataSet5
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	7
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing. System default and user-defined values for factors are treated as valid data.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Weight_yield Protein_yield/PLOT BOXPLOT STEMLEAF NPPLOT SPREADLEVEL/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING REPORT/NOTOTAL.
Resources	Processor time	00:00:00,22
	Elapsed time	00:00:00,73

Warnings

Level vs. scatter plots were requested, but no factor variables were specified. Level vs. scatter plots will not be produced.

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percentage	N	Percentage	N	Percentage
Weight_yield	6	85,7%	1	14,3%	7	100,0%
Protein_yield	6	85,7%	1	14,3%	7	100,0%

Descriptive

			Statistic	Std. Error
Weight_yield	Mean		6,80433	2,759681
	95% Confidence Interval for Mean	Lower Bound	-,28965	
		Upper Bound	13,89832	
	5% Trimmed Mean		6,21998	
	Median		3,90100	
	Variance		45,695	
	Std. Deviation		6,759811	
	Minimum		3,582	
	Maximum		20,545	
	Range		16,963	
	Interquartile Range		5,460	
	Skewness		2,405	,845
	Kurtosis		5,820	1,741
Protein_yield	Mean		84,64767	7,928407
	95% Confidence Interval for Mean	Lower Bound	64,26705	
		Upper Bound	105,02829	
	5% Trimmed Mean		84,37752	
	Median		83,10800	
	Variance		377,158	
	Std. Deviation		19,420553	
	Minimum		63,478	
	Maximum		110,680	
	Range		47,202	
	Interquartile Range		35,964	
	Skewness		,232	,845
	Kurtosis		-2,175	1,741

M Estimators

	Huber's Estimator M[a]	Tukey biweighting[b]	Hmpel's Estimator M[c]	Andrews Wave[d]
Weight_yield	3,95023	3,75470	3,84485	3,75470
Protein_yield	83,74313	84,16029	84,64767	84,15935

Note: a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

			Case number	Value
Weight_yield	Upper	1	1	20,545
		2	6	5,261
		3	5	3,960
	Lower	1	2	3,582
		2	3	3,636
		3	4	3,842
Protein_yield	Upper	1	1	110,680
		2	6	99,865
		3	2	93,939
	Lower	1	3	63,478
		2	4	67,647
		3	5	72,277

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

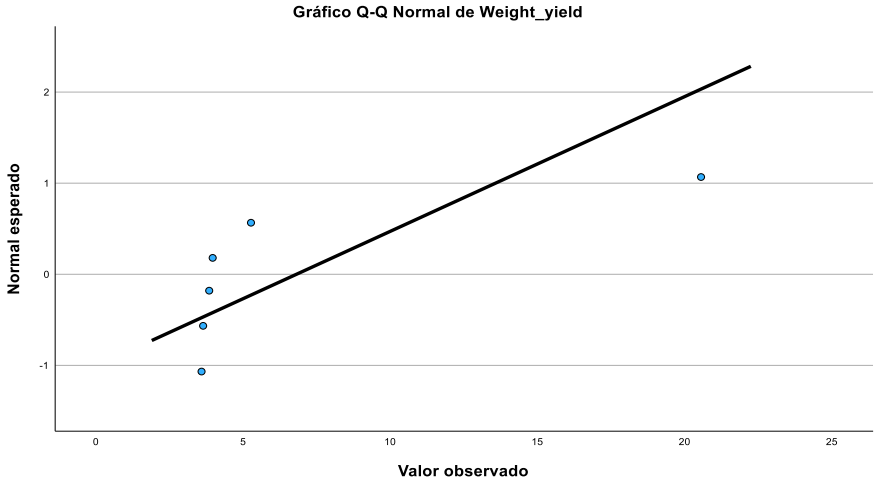
Normality Tests

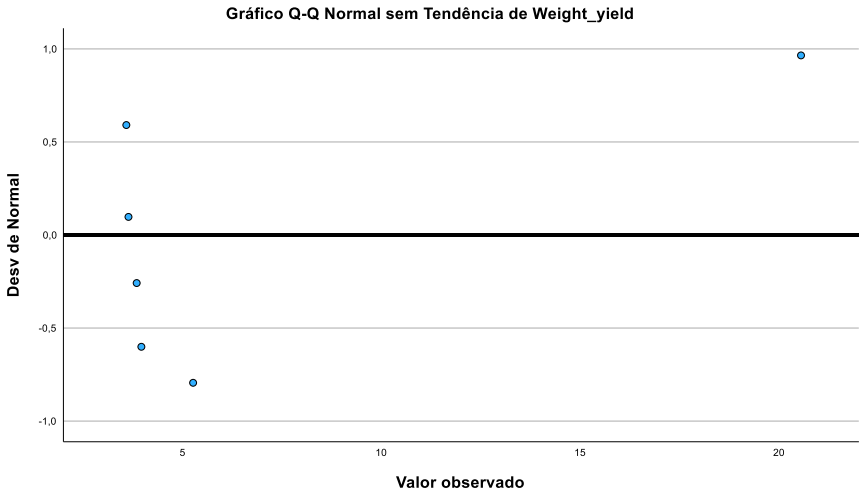
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistics	gl	Sig.	Statistics	gl	Sig.
Weight_yield	,424	6	,001	,566	6	<.001
Protein_yield	,238	6	,200*	,905	6	,403

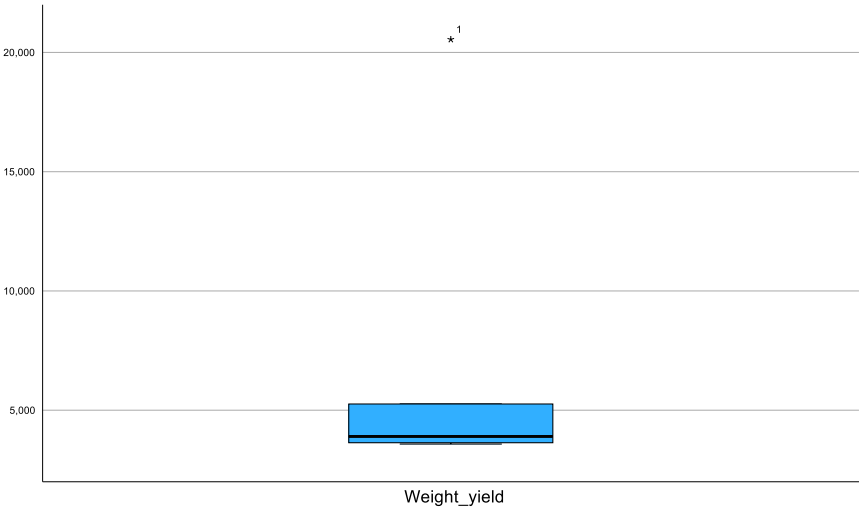
*. This is a lower bound of true significance.

a. Lilliefors Significance Correlation

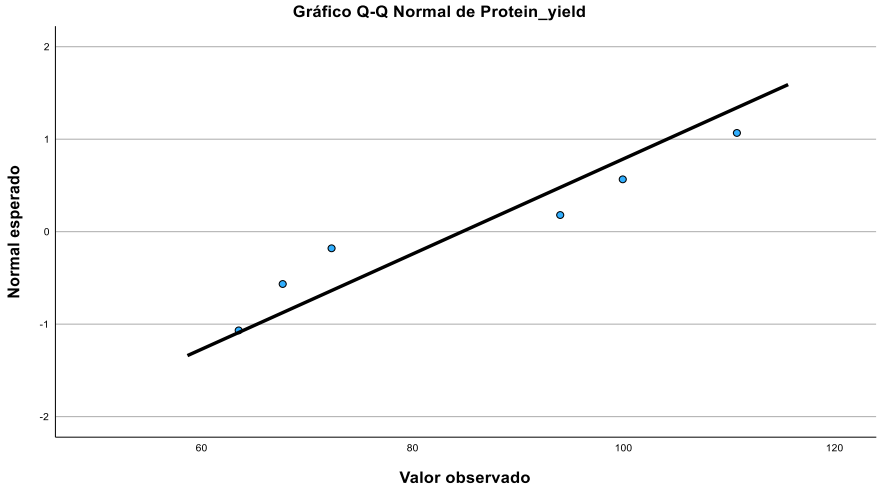
- **Weight_yield Root & Leaf Frequency**







- **Protein_yield Root & Leaf Frequency**



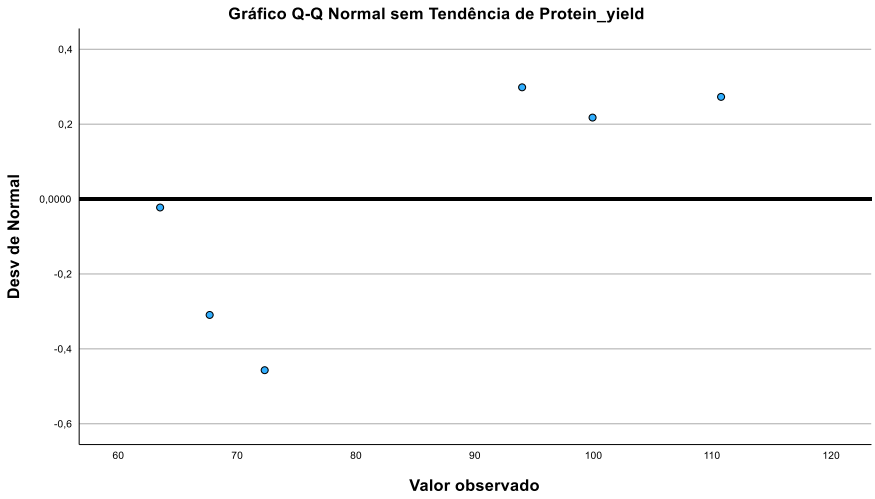
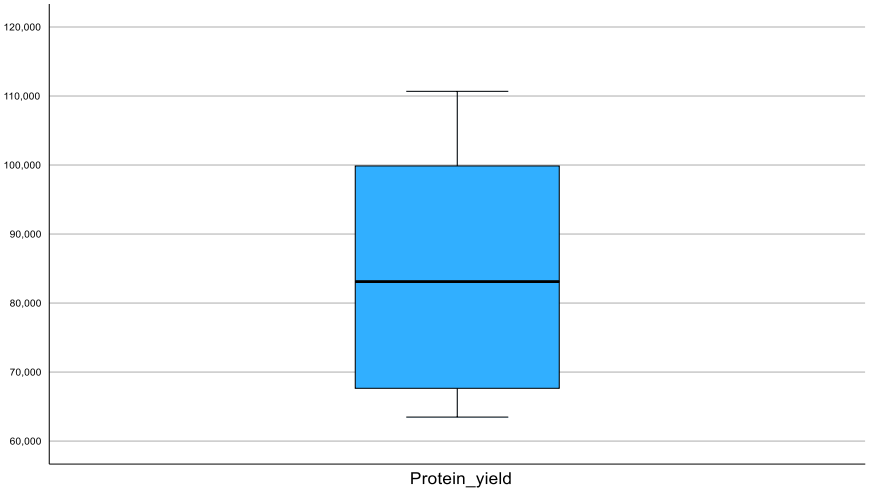


Gráfico Q-Q Normal sem Tendência de Protein_yield
Desv de Normal
0,4
0,2
0,0000
-0,2
-0,4
-0,6
60
70
80
90
100
110
120
Valor observado


120,000
110,000
100,000
90,000
80,000
70,000
60,000
Protein_yield

- **Explore**

Observations

Output Created		29-SEP-2023 11:05:47
Comments		
Entry	Active dataset	DataSet5
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	7
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing. System default and user-defined values for factors are treated as valid data.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Weight_yield/PLOT BOXPLOT STEMLEAF NPPLOT SPREADLEVEL/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVE EXTREME/ CINTERVAL 95/MISSING REPORT/NOTOTAL.
Resources	Processor time	00:00:00,16
	Elapsed time	00:00:00,36

Warnings

Level vs. scatter plots were requested, but no factor variables were specified. Level vs. scatter plots will not be produced.

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percentage	N	Percentage	N	Percentage
Weight_yield	5	71,4%	2	28,6%	7	100,0%

Descriptive

			Statistic	Std. Error
Weight_yield	Mean		4,05620	,308878
	95% Confidence Interval for Mean	Lower Bound	3,19862	
		Upper Bound	4,91378	
	5% Trimmed Mean		4,01561	
	Median		3,84200	
	Variance		,477	
	Std. Deviation		,690672	
	Minimum		3,582	
	Maximum		5,261	
	Range		1,679	
	Interquartile Range		1,002	
	Skewness		1,969	,913
	Kurtosis		4,035	2,000

M Estimators

	Huber's Estimator		Hmpel's Estimator	
	M[a]	Tukey biweighting[b]	M[c]	Andrews Wave[d]
Weight_yield	3,82479	3,75448	3,77791	3,75449

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

			Case number	Value
Weight_yield	Upper	1	6	5,261
		2	5	3,960
	Lower	1	2	3,582
		2	3	3,636

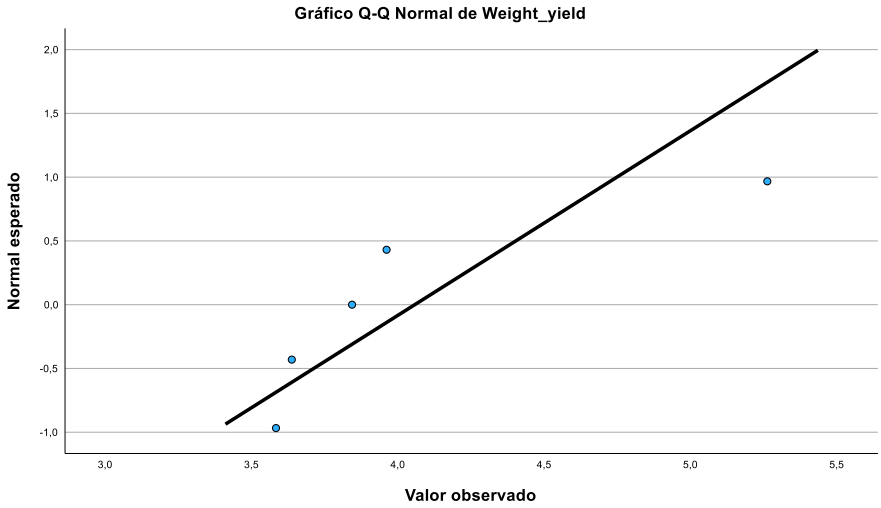
a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

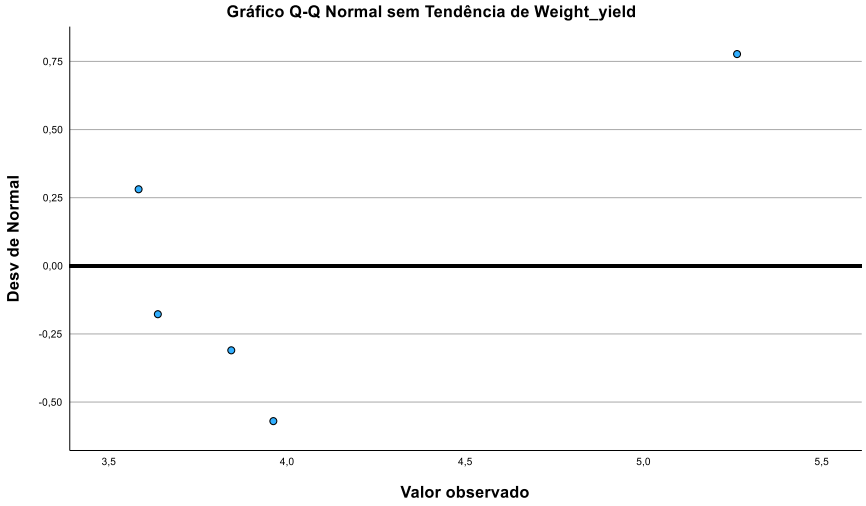
Normality Tests

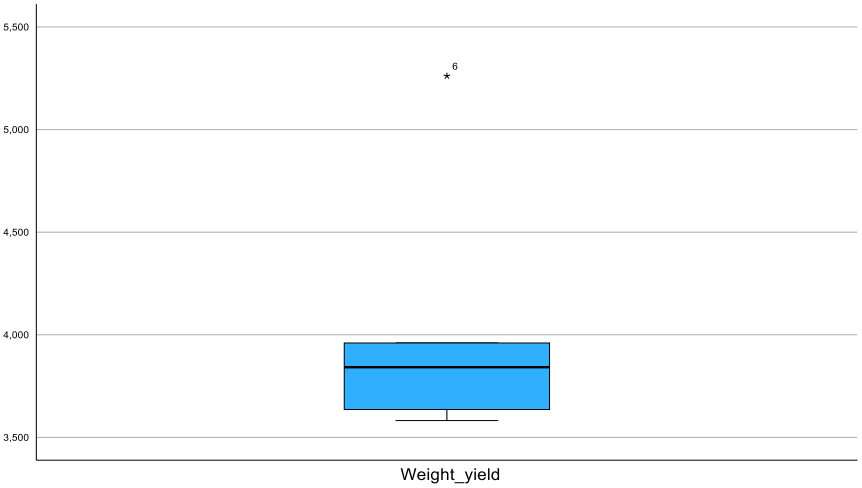
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistics	gl	Sig.	Statistics	gl	Sig.
Weight_yield	,355	5	,038	,747	5	,028

a. Lilliefors Significance Correlation

- **Weight_yield Root & Leaf Frequency**







- **Explore**

Observations

Output Created		29-SEP-2023 11:07:02
Comments		
Entry	Active dataset	DataSet5
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	7
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing. System default and user-defined values for factors are treated as valid data.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Weight_yield/PLOT BOXPLOT STEMLEAF NPPLOT SPREADLEVEL/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING REPORT/NOTOTAL.
Resources	Processor time	00:00:00,50
	Elapsed time	00:00:00,40

Warnings

Level vs. scatter plots were requested, but no factor variables were specified. Level vs. scatter plots will not be produced.

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percentage	N	Percentage	N	Percentage
Weight_yield	4	57,1%	3	42,9%	7	100,0%

Descriptive

			Statistic	Std. Error
Weight_yield	Mean		3,75500	,088357
	95% Confidence Interval for Mean	Lower Bound	3,47381	
		Upper Bound	4,03619	
	5% Trimmed Mean		3,75322	
	Median		3,73900	
	Variance		,031	
	Std. Deviation		,176714	
	Minimum		3,582	
	Maximum		3,960	
	Range		,378	
	Interquartile Range		,335	
	Skewness		,291	1,014
	Kurtosis		-3,520	2,619

M Estimators

	Huber's Estimator		Hmpel's Estimator	
	M[a]	Tukey biweighting[b]	M[c]	Andrews Wave[d]
Weight_yield	3,74351	3,74749	3,75500	3,74747

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

			Case number	Value
Weight_yield	Upper	1	5	3,960
		2	4	3,842
	Lower	1	2	3,582
		2	3	3,636

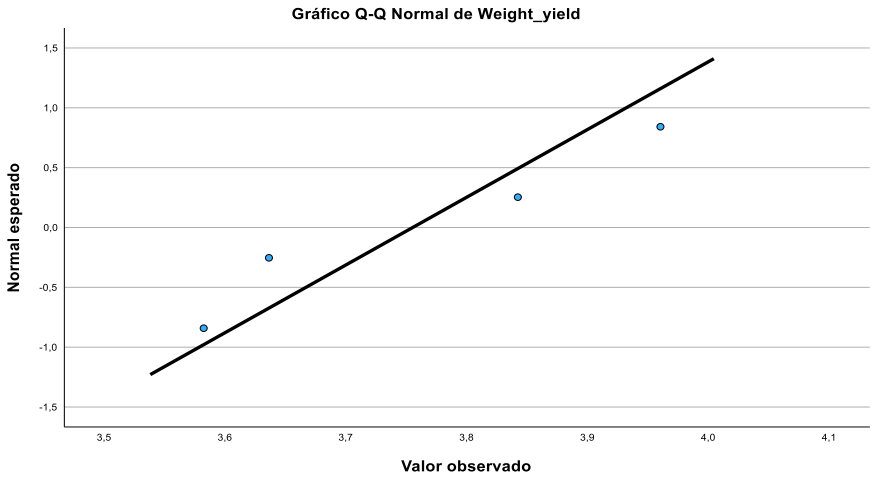
a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

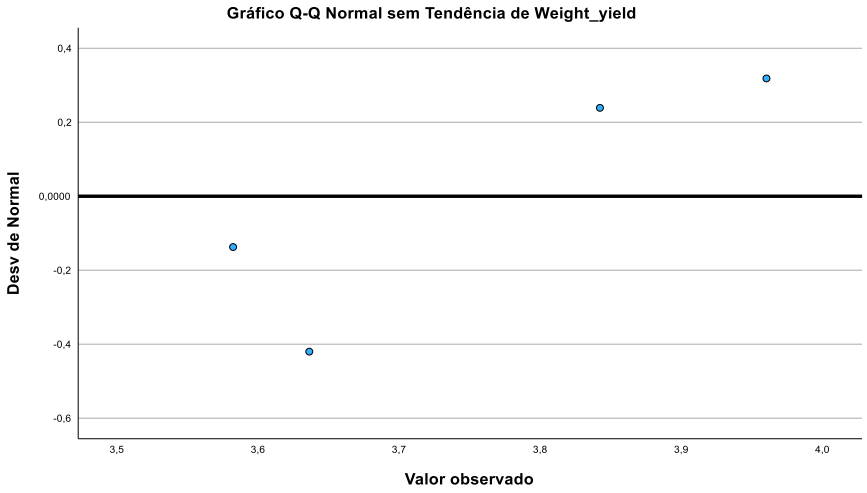
Normality Tests

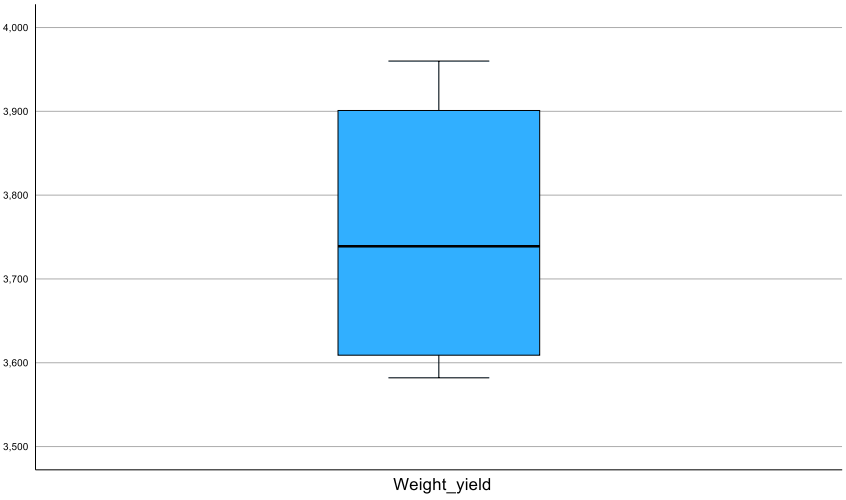
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistics	gl	Sig.	Statistics	gl	Sig.
Weight_yield	,250	4	.	,923	4	,554

a. Lilliefors Significance Correlation

- **Weight_yield Root & Leaf Frequency**







5.2. Solubilization

- **Univariate Analysis of Variance**

Factors Between Subjects

		N
NaOH	1,00	4
	2,00	4
	3,00	4
Temperature	25,00	6
	60,00	6

Descriptive Statistics

Dependent variable: Yield_solubilization

NaOH	Temperature	Average	Pattern Statistics	N
1,00	25,00	2,92650	1,014698	2
	60,00	44,10300	5,320271	2
	Total	23,51475	23,978039	4
2,00	25,00	10,52150	,709228	2
	60,00	54,58000	8,259007	2
	Total	32,55075	25,883493	4
3,00	25,00	18,45850	,149200	2
	60,00	60,90500	7,051269	2
	Total	39,68175	24,842491	4
Total	25,00	10,63550	6,969030	6
	60,00	53,19600	9,319769	6
	Total	31,91575	23,570621	12

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
Yield_solubilization	Based on Mean	3001601156523383500000000000000,000	5	6	<.001
	Based on Median	3001601156523383500000000000000,000	5	6	<.001
	Based on Median and with adjusted df	3001601156523383500000000000000,000	5	1,008	<.001
	Based on trimmed mean	5735401466368101000000000000000,000	5	6	<.001

Note: Tests the null hypothesis that the variance of the error of the dependent variable is equal between groups.[a,b] a. Dependent variable: Yield_solubilization, b. Design: Intercept + NaOH + Temperature + NaOH * Temperature

- **Tests for Heteroscedasticity**

White Test for Heteroscedasticity[a,b,c]

Chi-square	df	Sig.
12,000	5	,035

a. Dependent variable: Yield_solubilization. b. It tests the null hypothesis that the variation of errors does not depend on the values of the independent variables. c. Design: Intercept + NaOH + Temperature + NaOH * Temperature

Modified Breusch-Pagan Test for Heteroscedasticity [a,b,c]

Chi-square	df	Sig.
10,170	1	,001

a. Dependent variable: Yield_solubilization. b. It tests the null hypothesis that the variation of errors does not depend on the values of the independent variables c. Predicted values from the design: Intercept + NaOH + Temperature + NaOH * Temperature.

Breusch-Pagan test for heteroscedasticity [a,b,c]

Chi-square	Df	Sig.
5,988	1	,014

a. Dependent variable: Yield_solubilization. b. It tests the null hypothesis that the variation of errors does not depend on the values of the independent variables. c. Predicted values from the design: Intercept + NaOH + Temperature + NaOH * Temperature

F-Test for Heteroscedasticity [a,b,c]

With	df1	df2	Sig.
55,564	1	10	<.001

a. Dependent variable: Yield_solubilization. b. It tests the null hypothesis that the variation of errors does not depend on the values of the independent variables. c. Predicted values from the design: Intercept + NaOH + Temperature + NaOH * Temperature

Tests of effects between subjects

Dependent variable: Yield_solubilization

Origin	Type III Sum of Squares	df	Medium Square	With	Sig.
Corrected model	5963,524a	5	1192,705	48,421	<.001
Intercept	12223,381	1	12223,381	496,241	<.001
NaOH	525,163	2	262,582	10,660	,011
Temperature	5434,188	1	5434,188	220,615	<.001
NaOH * Temperature	4,172	2	2,086	,085	,920
Pattern	147,792	6	24,632		
Total	18334,697	12			
Corrected total	6111,316	11			

Tests of effects between subjects

Dependent variable: Yield_solubilization

Origin	Square Partial ETA	Noncent. Parameter	Observed power[b]
Corrected model	,976	242,105	1,000
Intercept	,988	496,241	1,000
NaOH	,780	21,320	,889
Temperature	,974	220,615	1,000
NaOH * Temperature	,027	,169	,058
Pattern			
Total			
Corrected total			

Note: a. R Squared = .976 (Adjusted R Squared = .956). b. Calculated using alpha = .05

Parameter Estimates

Dependent variable: Yield_solubilization

Parameter	B	Pattern Statistics	t	Sig.	95% Confidence Interval Lower Limit
Intercept	60,905	3,509	17,355	<.001	52,318
[NaOH=1.00]	-16,802	4,963	-3,385	,015	-28,946
[NaOH=2.00]	-6,325	4,963	-1,274	,250	-18,469
[NaOH=3.00]	0a	.	.	.	.
[Temperature=25.00]	-42,447	4,963	-8,552	<.001	-54,591
[Temperature=60.00]	0[a]	.	.	.	.
[NaOH=1.00] * [Temperature=25.00]	1,270	7,019	,181	,862	-15,904
[NaOH=1.00] * [Temperature=60.00]	0[a]	.	.	.	.
[NaOH=2.00] * [Temperature=25.00]	-1,612	7,019	-,230	,826	-18,786
[NaOH=2.00] * [Temperature=60.00]	0[a]	.	.	.	.
[NaOH=3.00] * [Temperature=25.00]	0[a]	.	.	.	.
[NaOH=3.00] * [Temperature=60.00]	0[a]	.	.	.	.

Parameter Estimates

Dependent variable: Yield_solubilization

Parameter	95% Confidence Interval Upper limit	Square Partial ETA	Noncent. Parameter	Observed power[b]
Intercept	69,492	,980	17,355	1,000
[NaOH=1.00]	-4,658	,656	3,385	,804
[NaOH=2.00]	5,819	,213	1,274	,190
[NaOH=3.00]	.	.	.	.
[Temperature=25.00]	-30,302	,924	8,552	1,000
[Temperature=60.00]	.	.	.	.
[NaOH=1.00] * [Temperature=25.00]	18,444	,005	,181	,053
[NaOH=1.00] * [Temperature=60.00]	.	.	.	.
[NaOH=2.00] * [Temperature=25.00]	15,562	,009	,230	,054
[NaOH=2.00] * [Temperature=60.00]	.	.	.	.
[NaOH=3.00] * [Temperature=25.00]	.	.	.	.
[NaOH=3.00] * [Temperature=60.00]	.	.	.	.

a. This parameter is set to zero because it is redundant. b. Calculated using alpha = .05

Lack of fit testing

Dependent variable: Yield_solubilization

Origin	Sum of Squares	df	Medium Square	With	Sig.	Square Partial ETA
Lack of Fit	,000	0	.	.	.	,000
Pure error	147,792	6	24,632			

Lack of fit testing

Dependent variable: Yield_solubilization

Origin	Noncent. Parameter	Observed power[a]
Lack of Fit	,000	.
Pure error		

a. Calculated using alpha = .05

- **After-Testing**
  NaOH

Multiple Comparisons

Dependent variable: Yield_solubilization

Tukey HSD

(I) NaOH	(J) NaOH	Mean difference (I-J)	Pattern Statistics	Sig.	95% Confidence Interval	
					Lower Limit	Upper limit
1,00	2,00	-9,03600	3,509413	,093	-19,80384	1,73184
	3,00	-16,16700*	3,509413	,009	-26,93484	-5,39916
2,00	1,00	9,03600	3,509413	,093	-1,73184	19,80384
	3,00	-7,13100	3,509413	,185	-17,89884	3,63684
3,00	1,00	16,16700*	3,509413	,009	5,39916	26,93484
	2,00	7,13100	3,509413	,185	-3,63684	17,89884

Based on observed averages. The error term is Mean Square (Error) = 24.632. *. The average difference is significant at the .05 level.

- **Homogeneous subsets**

Yield_solubilization

Tukey HSD [a,b]

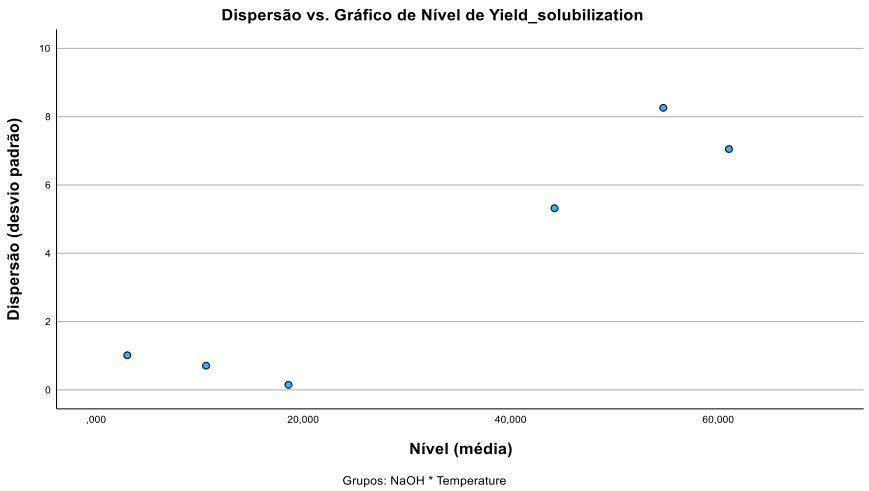
NaOH	N	Subset 1	Subset 2
1,00	4	23,51475	
2,00	4	32,55075	32,55075
3,00	4		39,68175
Sig.		,093	,185

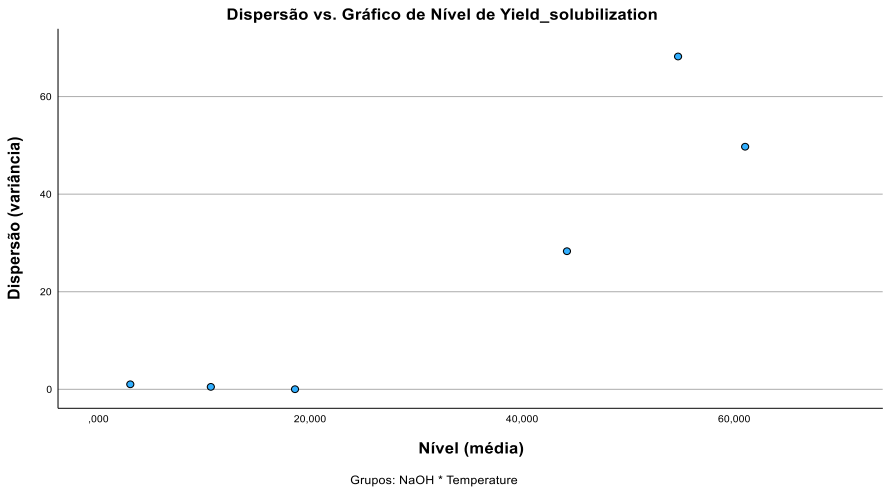
Averages are displayed for the groups in subsets homogêneos.Com based on observed averages. The error term is Mean Square (Error) = 24.632.

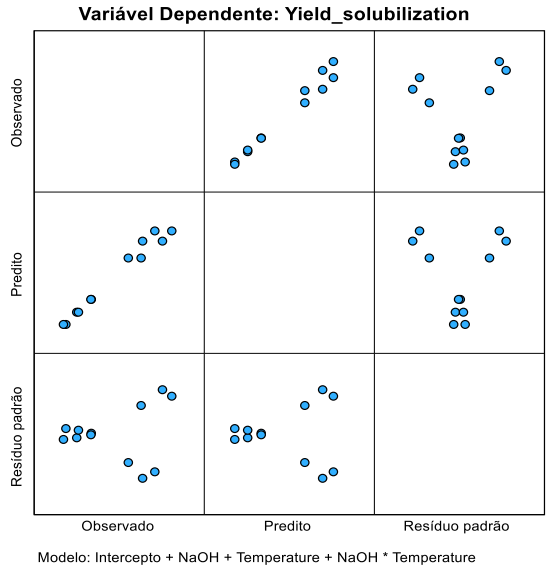
a. Uses the Harmonic Mean Sample Size = 4,000.

b. Alpha = .05.

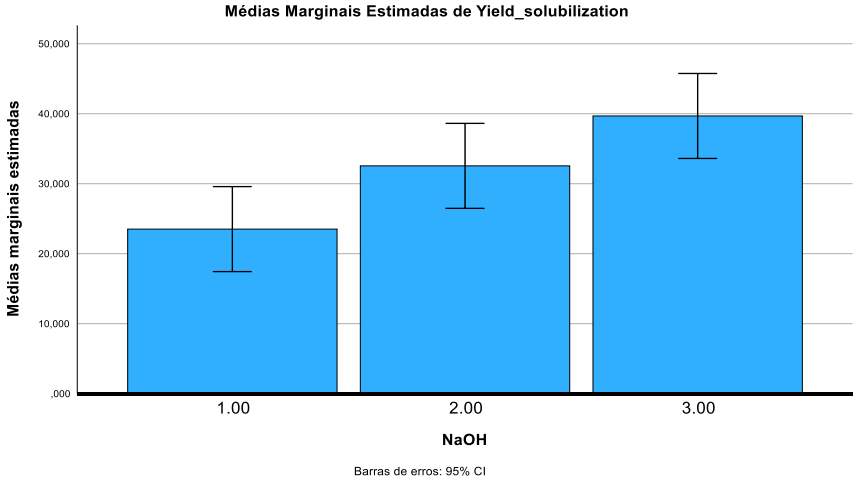
- **Level Chart vs. Scatter**

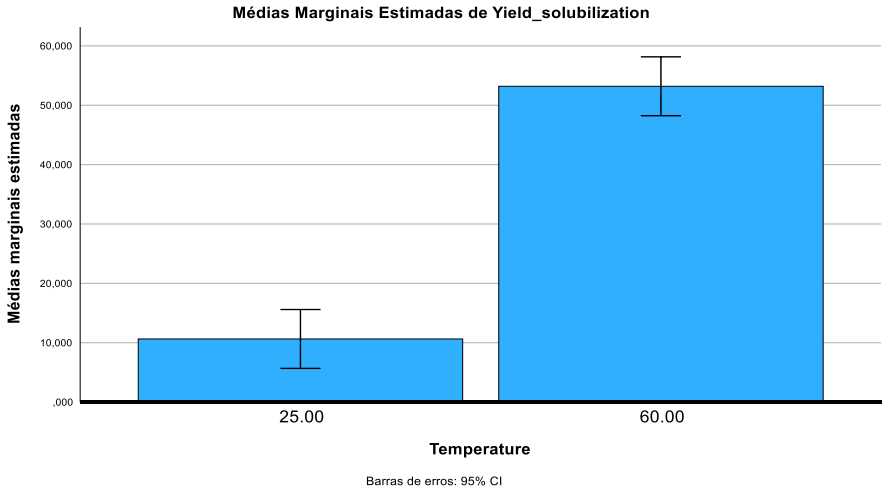






- **Profile Charts**





- **One-way ANOVA**

Observations

Output Created		29-SEP-2023 09:05:13
Comments		
Entry	Active dataset	DataSet2
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	26
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY Yield_solubilization_2 BY Sample_code/ES=OVERALL/STATISTICS DESCRIPTIVES EFFECTS HOMOGENEITY WELCH/PLOT MEANS/MISSING ANALYSIS/CRITERIA=CILEVEL(0.95)/POSTHOC=TUKEY ALPHA(0.05).
Resources	Processor time	00:00:00,00
	Elapsed time	00:00:00,15

Descriptive

Yield_solubilization_2

		N	Average	Standard deviation	Standard Error	95% Confidence Interval to Mean Lower Limit
	3%60º_3	2	18,34100	1,921916	1,359000	1,07327
	3%60º_4	3	56,39633	,714732	,412651	54,62084
	3%60º_5	3	55,93900	,358275	,206850	55,04900
	3%60º_6	3	59,99833	1,795816	1,036815	55,53728
	3%60º_B	3	55,40267	6,009746	3,469728	40,47363
	Total	14	51,42079	14,347342	3,834488	43,13688
Model	Fixed Effects			3,048791	,814824	49,57753
	Random Effects				6,889254	32,29315

Descriptive

Yield_solubilization_2

		95% Confidence Interval to Mean Upper limit	Minimal	Maximum	Variance between components
	3%60º_3	35,60873	16,982	19,700	
	3%60º_4	58,17183	55,857	57,207	
	3%60º_5	56,82900	55,635	56,334	
	3%60º_6	64,45939	57,926	61,098	
	3%60º_B	70,33170	50,828	62,209	
	Total	59,70469	16,982	62,209	
Model	Fixed Effects	53,26405			
	Random Effects	70,54842			229,309592

Variance homogeneity tests

		Levene's Statistics	df1	df2
Yield_solubilization_2	Based on average	6,890	4	9
	Based on median	1,080	4	9
	Based on median and adjusted gl	1,080	4	2,606
	Based on trimmed average	6,049	4	9

Variance homogeneity tests

		Sig.
Yield_solubilization_2	Based on average	,008
	Based on median	,421
	Based on median and adjusted gl	,507
	Based on trimmed average	,012

ANOVA

Yield_solubilization_2

	Sum of Squares	df	Medium Square	With	Sig.
Between Groups	2592,345	4	648,086	69,723	<.001
In groups	83,656	9	9,295		
Total	2676,001	13			

ANOVAEffect Sizes [a]

			95% Confidence Interval	
		Point Estimation	Inferior	Superior
Yield_solubilization_2	Square Eta	,969	,844	,977
	Square Epsilon	,955	,774	,967
	Fixed Effect of Square Omega	,952	,761	,964
	Square Omega Random Effect	,831	,444	,872

a. Eta square and Epsilon square are estimated based on the fixed-effect model.

Robust Average Equality Tests

Yield_solubilization_2

	Statistics[a]	df1	df2	Sig.
Welch	123,333	4	3,495	<.001

a. F distributed asymptotically.

- **After-Testing**

Multiple Comparisons

Dependent variable: Yield_solubilization_2

Tukey HSD

(I) Sample_code	(J) Sample_code	Mean difference (I-J)	Standard Error	Sig.	95% Confidence Interval Lower Limit
3%60º_3	3%60º_4	-38,055333*	2,783153	<.001	-47,41390
	3%60º_5	-37,598000*	2,783153	<.001	-46,95657
	3%60º_6	-41,657333*	2,783153	<.001	-51,01590
	3%60º_B	-37,061667*	2,783153	<.001	-46,42024
3%60º_4	3%60º_3	38,055333*	2,783153	<.001	28,69676
	3%60º_5	,457333	2,489328	1,000	-7,91323
	3%60º_6	-3,602000	2,489328	,616	-11,97256
	3%60º_B	,993667	2,489328	,994	-7,37689
3%60º_5	3%60º_3	37,598000*	2,783153	<.001	28,23943
	3%60º_4	-,457333	2,489328	1,000	-8,82789
	3%60º_6	-4,059333	2,489328	,515	-12,42989
	3%60º_B	,536333	2,489328	,999	-7,83423
3%60º_6	3%60º_3	41,657333*	2,783153	<.001	32,29876
	3%60º_4	3,602000	2,489328	,616	-4,76856
	3%60º_5	4,059333	2,489328	,515	-4,31123
	3%60º_B	4,595667	2,489328	,406	-3,77489
3%60º_B	3%60º_3	37,061667	2,783153	<.001	27,70310
	3%60º_4	-,993667	2,489328	,994	-9,36423
	3%60º_5	-,536333	2,489328	,999	-8,90689
	3%60º_6	-4,595667	2,489328	,406	-12,96623

Multiple Comparisons

Dependent variable: Yield_solubilization_2

Tukey HSD

(I) Sample_code	(J) Sample_code	95% Confidence Interval Upper limit
3%60º_3	3%60º_4	-28,69676
	3%60º_5	-28,23943
	3%60º_6	-32,29876
	3%60º_B	-27,70310
3%60º_4	3%60º_3	47,41390
	3%60º_5	8,82789
	3%60º_6	4,76856
	3%60º_B	9,36423
3%60º_5	3%60º_3	46,95657
	3%60º_4	7,91323
	3%60º_6	4,31123
	3%60º_B	8,90689
3%60º_6	3%60º_3	51,01590
	3%60º_4	11,97256
	3%60º_5	12,42989
	3%60º_B	12,96623
3%60º_B	3%60º_3	46,42024
	3%60º_4	7,37689
	3%60º_5	7,83423
	3%60º_6	3,77489

*. The average difference is significant at the 0.05 level.

- **Homogeneous subsets**

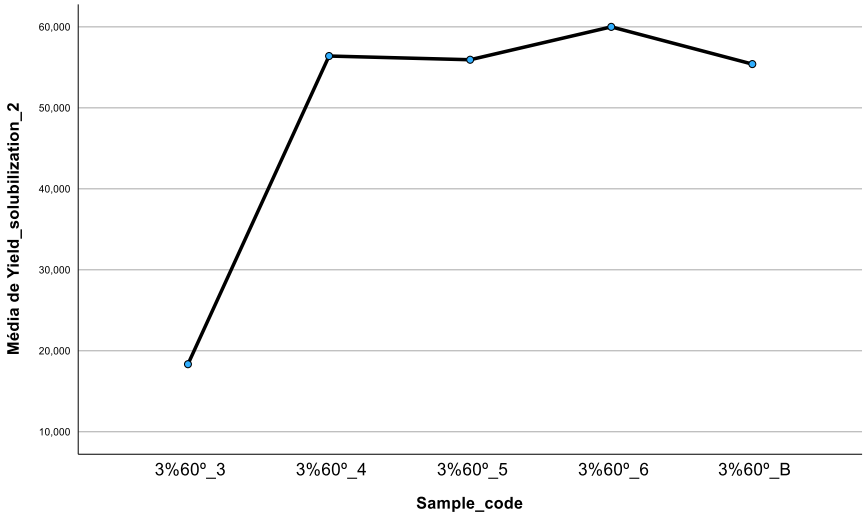
Yield_solubilization_2

Tukey HSD [a,b]

Sample_code	N	Subset to alpha = 0.05 1	2
3%60º_3	2	18,34100	
3%60º_B	3		55,40267
3%60º_5	3		55,93900
3%60º_4	3		56,39633
3%60º_6	3		59,99833
Sig.		1,000	,448

The averages for the groups in homogeneous subsets are displayed. a. Uses the Harmonic Mean Sample Size = 2.727. b. Group sizes are uneven. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

- **Averages Charts**



- **Non-parametric tests**

Observations

	Output Created	29-SEP-2023 09:06:28
	Comments	
Entry	Active dataset	DataSet2
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	26
	Syntax	NPTESTS/INDEPENDENT TEST (Yield_solubilization_2) GROUP (Sample_code)/MISSING SCOPE=ANALYSIS USERMISSING=EXCLUDE/CRITERIA ALPHA=0.05 CILEVEL=95.
Resources	Processor time	00:00:00,19
	Elapsed time	00:00:00,52

Hypothesis Testing Summarization

	Null hypothesis	Test	Sig.[a,b]
1	The distribution of Yield_solubilization_2 is equal in the categories of Sample_code.	Independent Kruskal-Wallis Test Specimens	,097

Hypothesis Testing Summarization

	Decision
1	Retain the null hypothesis.

a. The significance level is .050. b. Asymptotic significance is displayed-

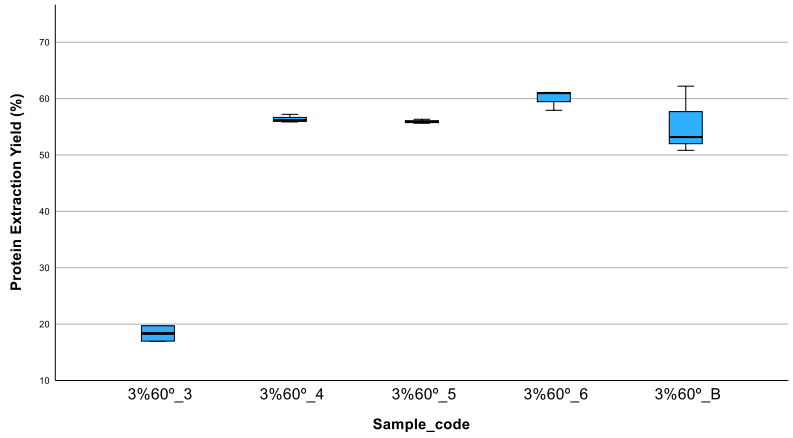
- **Independent Kruskal-Wallis Test Specimens**

Yield_solubilization_2 between Sample_code

Kruskal-Wallis Test Summary Independent Samples

Total N	14
Test Statistics	7,867[a]
Degree of Freedom	4
Asymptotic signal (two-sided test)	,097

a. The test statistic is adjusted for draws.

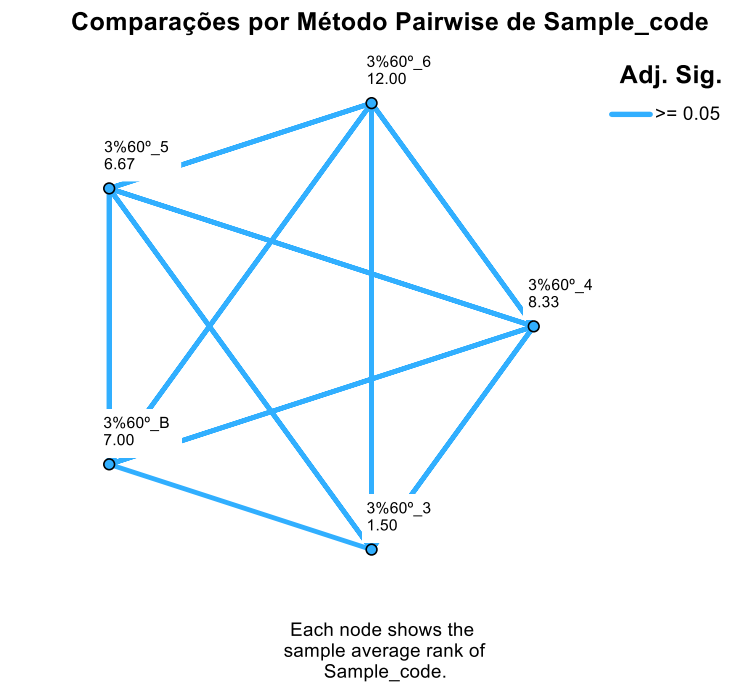


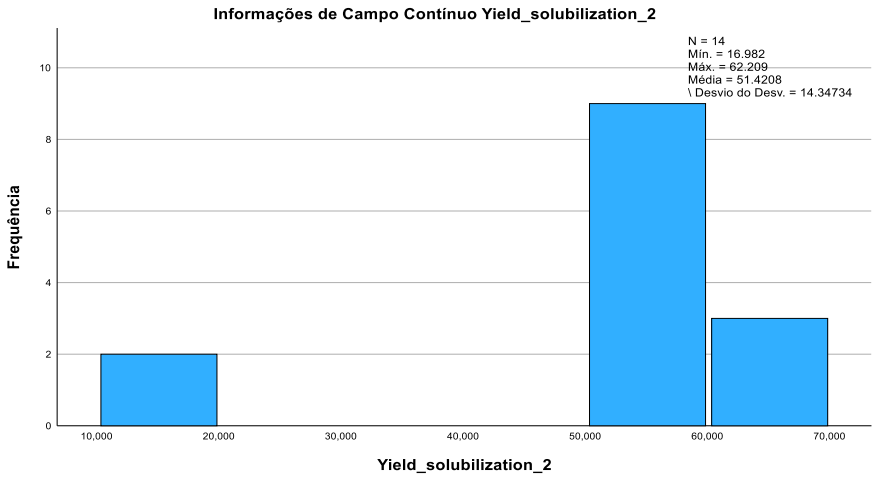
Pairwise Method Comparisons of Sample_code

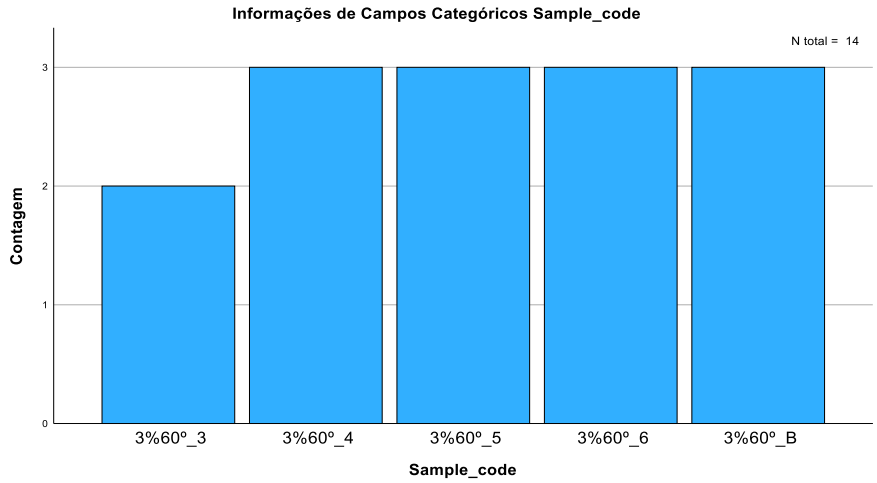
Sample 1-Sample 2	Test Statistics	Standard Error	Pattern Statistics	Sig.	adj. Sig.[a]
3%60°_3-3%60°_5	-5,167	3,819	-1,353	,176	1,000
3%60°_3-3%60°_B	-5,500	3,819	-1,440	,150	1,000
3%60°_3-3%60°_4	-6,833	3,819	-1,789	,074	,736
3%60°_3-3%60°_6	-10,500	3,819	-2,750	,006	,060
3%60°_5-3%60°_B	-,333	3,416	-,098	,922	1,000
3%60°_5-3%60°_4	1,667	3,416	,488	,626	1,000
3%60°_5-3%60°_6	-5,333	3,416	-1,561	,118	1,000
3%60°_B-3%60°_4	1,333	3,416	,390	,696	1,000
3%60°_B-3%60°_6	5,000	3,416	1,464	,143	1,000
3%60°_4-3%60°_6	-3,667	3,416	-1,073	,283	1,000

Each row tests the null hypothesis where the Sample 1 and Sample 2 distributions are equal. Asymptotic significances (2-sided test) are displayed. The significance level is .050.

a. Significance values were adjusted by Bonferroni correction for several tests.


Comparações por Método Pairwise de Sample_code
3%60º_6
12.00
Adj. Sig.
>= 0.05
3%60º_5
6.67
3%60º_4
8.33
3%60º_B
7.00
3%60º_3
1.50
Each node shows the sample average rank of Sample_code.


Informações de Campo Contínuo Yield_solubilization_2
N = 14
Mín. = 16.982
Máx. = 62.209
Média = 51.4208
\ Desvio do Desv. = 14.34734
Frequência
0
2
4
6
8
10
10,000
20,000
30,000
40,000
50,000
60,000
70,000
Yield_solubilization_2


Informações de Campos Categóricos Sample_code
N total = 14
Contagem
0
1
2
3
3%60º_3
3%60º_4
3%60º_5
3%60º_6
3%60º_B
Sample_code

- **One-way ANOVA**

Observations

Output Created		29-SEP-2023 09:10:41
Comments		
Entry	Active dataset	DataSet2
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	26
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY Yield_solubilization_3 BY Sample_code/ES=OVERALL/STATISTICS DESCRIPTIVES EFFECTS HOMOGENEITY WELCH/PLOT MEANS /MISSING ANALYSIS/CRITERIA=CILEVEL(0.95)/POSTHOC=TUKEY ALPHA(0.05).
Resources	Processor time	00:00:00,13
	Elapsed time	00:00:00,15

Descriptive

Yield_solubilization_3

						95% Confidence Interval to Mean
		N	Average	Standard deviation	Standard Error	Lower Limit
	3%60º_4	3	56,3963	,71473	,41265	54,6208
	3%60º_5	3	55,9390	,35828	,20685	55,0490
	3%60º_6	3	59,9983	1,79582	1,03682	55,5373
	3%60º_B	3	55,4027	6,00975	3,46973	40,4736
	Total	12	56,9341	3,28917	,94950	54,8442
Model	Fixed Effects			3,16153	,91266	54,8295
	Random Effects				1,04140	53,6199

Descriptive

Yield_solubilization_3

			95% Confidence Interval to Mean			
			Upper limit	Minimal	Maximum	Variance between components
	3%60º_4		58,1718	55,86	57,21	
	3%60º_5		56,8290	55,64	56,33	
	3%60º_6		64,4594	57,93	61,10	
	3%60º_B		70,3317	50,83	62,21	
	Total		59,0239	50,83	62,21	
Model	Fixed Effects		59,0387			
	Random Effects		60,2483			1,00631

Variance homogeneity tests

		Levene's Statistics	df1	df2
Yield_solubilization_3	Based on average	8,124	3	8
	Based on median	1,280	3	8
	Based on median and adjusted gl	1,280	3	2,606
	Based on trimmed average	7,136	3	8

Variance homogeneity tests

		Sig.
Yield_solubilization_3	Based on average	,008
	Based on median	,345
	Based on median and adjusted gl	,437
	Based on trimmed average	,012

ANOVA

Yield_solubilization_3

	Sum of Squares	df	Medium Square	With	Sig.
Between Groups	39,043	3	13,014	1,302	,339
In groups	79,962	8	9,995		
Total	119,005	11			

ANOVA effect sizes [a,b]

			95% Confidence Interval	
		Point Estimation	Inferior	Superior
Yield_solubilization_3	Square Eta	,328	,000	,541
	Square Epsilon	,076	-,375	,369
	Fixed Effect of Square Omega	,070	-,333	,349
	Square Omega Random Effect	,025	-,091	,152

a. Eta square and Epsilon square are estimated based on the fixed-effect model. b. Negative but less biased estimates are maintained, not rounded to zero.

Robust Average Equality Tests

Yield_solubilization_3

	Statistics[a]	df1	df2	Sig.
Welch	3,754	3	3,795	,123

a. F distributed asymptotically.

- **After-Testing**

Multiple comparision

dependent variable: Yield_solubilization_3

Tukey HSD

(I) Sample_code	(J) Sample_code	Mean Difference (I-J)	Standard Error	Sig.	Confidence interval 95% Inferior Limit
3%60º_4	3%60º_5	,45733	2,58138	,998	-7,8092
	3%60º_6	-3,60200	2,58138	,535	-11,8685
	3%60º_B	,99367	2,58138	,979	-7,2728
3%60º_5	3%60º_4	-,45733	2,58138	,998	-8,7238
	3%60º_6	-4,05933	2,58138	,443	-12,3258
	3%60º_B	,53633	2,58138	,997	-7,7302
3%60º_6	3%60º_4	3,60200	2,58138	,535	-4,6645
	3%60º_5	4,05933	2,58138	,443	-4,2072
	3%60º_B	4,59567	2,58138	,348	-3,6708
3%60º_B	3%60º_4	-,99367	2,58138	,979	-9,2602
	3%60º_5	-,53633	2,58138	,997	-8,8028
	3%60º_6	-4,59567	2,58138	,348	-12,8622

Multiple comparision

dependent variable: Yield_solubilization_3

Tukey HSD

(I) Sample_code	(J) Sample_code	Confidence interval 95% Superior Limit
3%60º_4	3%60º_5	8,7238
	3%60º_6	4,6645
	3%60º_B	9,2602
3%60º_5	3%60º_4	7,8092
	3%60º_6	4,2072
	3%60º_B	8,8028
3%60º_6	3%60º_4	11,8685
	3%60º_5	12,3258
	3%60º_B	12,8622
3%60º_B	3%60º_4	7,2728
	3%60º_5	7,7302
	3%60º_6	3,6708

- **Homogeneous subsets**

Yield_solubilization_3

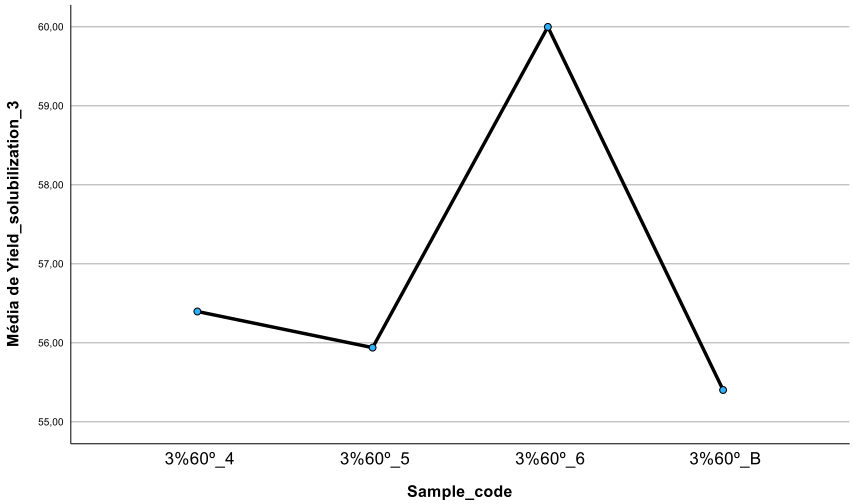
Tukey HSD[a]

Sample_code	N	Subset to alpha = 0.05
		1
3%60º_B	3	55,4027
3%60º_5	3	55,9390
3%60º_4	3	56,3963
3%60º_6	3	59,9983
Sig.		,348

The averages for the groups in homogeneous subsets are displayed.

a. Uses the Harmonic Mean Sample Size = 3,000.

- **Averages Charts**



- **Non-parametric tests**

Observations

	Output Created	29-SEP-2023 09:17:11
	Comments	
Entry	Active dataset	DataSet2
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	26
	Syntax	NPTESTS/INDEPENDENT TEST (Yield_solubilization_3) GROUP (Sample_code)/MISSING SCOPE=ANALYSIS USERMISSING=EXCLUDE/CRITERIA ALPHA=0.05 CILEVEL=95.
Resources	Processor time	00:00:00,22
	Elapsed time	00:00:00,56

Hypothesis Testing Summarization

	Null hypothesis	Test	Sig.[a,b]
1	The distribution of Yield_solubilization_3 is equal in the Sample_code categories.	Independent Kruskal-Wallis Test Specimens	,248

Hypothesis Testing Summarization

	Decision
1	Retain the null hypothesis.

a. The significance level is .050. b. Asymptotic significance is displayed.

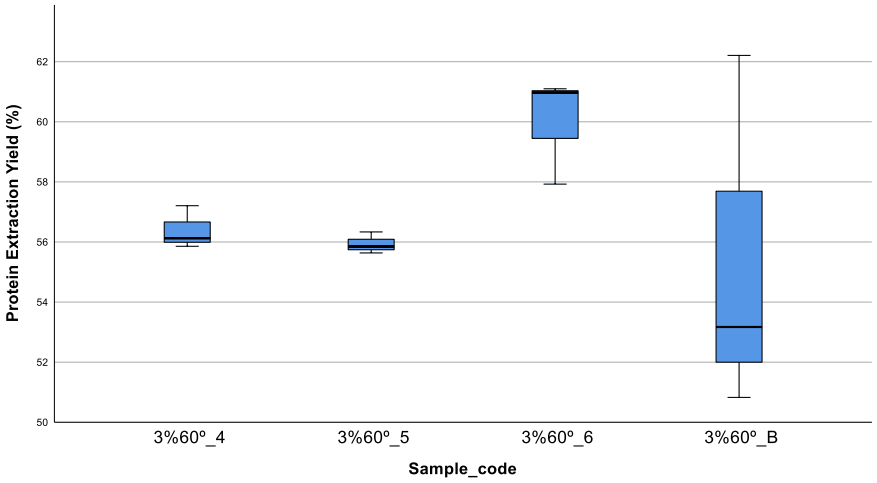
- **Independent Kruskal-Wallis Test Specimens**

Yield_solubilization_3 between Sample_code

Kruskal-Wallis Test Summary Independent Samples

Total N	12
Test Statistics	4,128[a]
Degree of Freedom	3
Asymptotic signal (two-sided test)	,248

a. The test statistic is adjusted for draws.



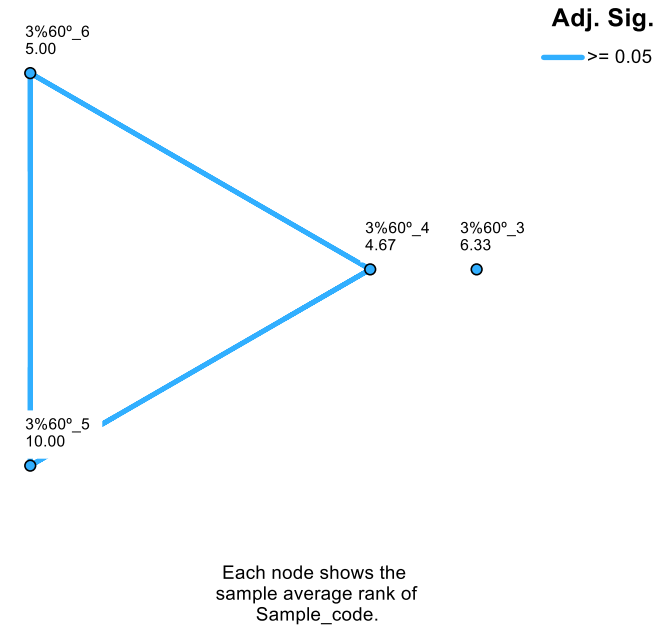
Pairwise Method Comparisons of Sample_code

Sample 1-Sample 2	Test Statistics	Standard Error	Pattern Statistics	Sig.	adj. Sig.[a]
3%60º_5-3%60º_B	-,333	2,944	-,113	,910	1,000
3%60º_5-3%60º_4	1,667	2,944	,566	,571	1,000
3%60º_5-3%60º_6	-5,333	2,944	-1,812	,070	,420
3%60º_B-3%60º_4	1,333	2,944	,453	,651	1,000
3%60º_B-3%60º_6	5,000	2,944	1,698	,089	,537
3%60º_4-3%60º_6	-3,667	2,944	-1,246	,213	1,000

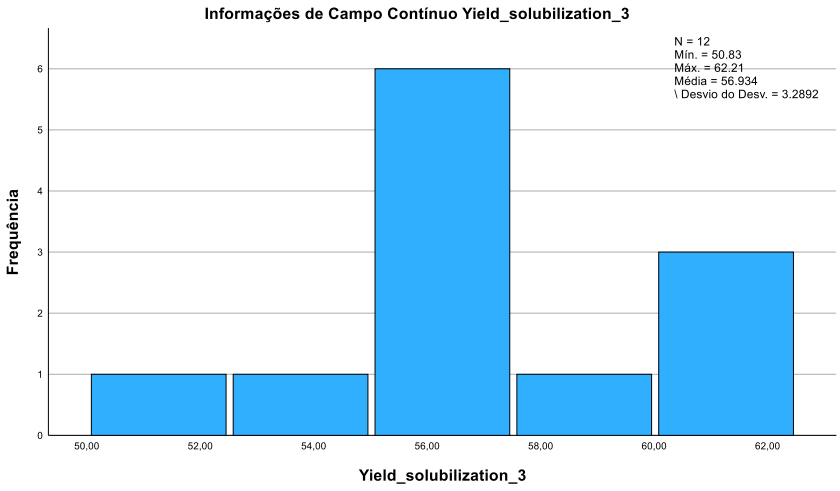
Each row tests the null hypothesis where the Sample 1 and Sample 2 distributions are equal. Asymptotic significances (2-sided test) are displayed. The significance level is .050.

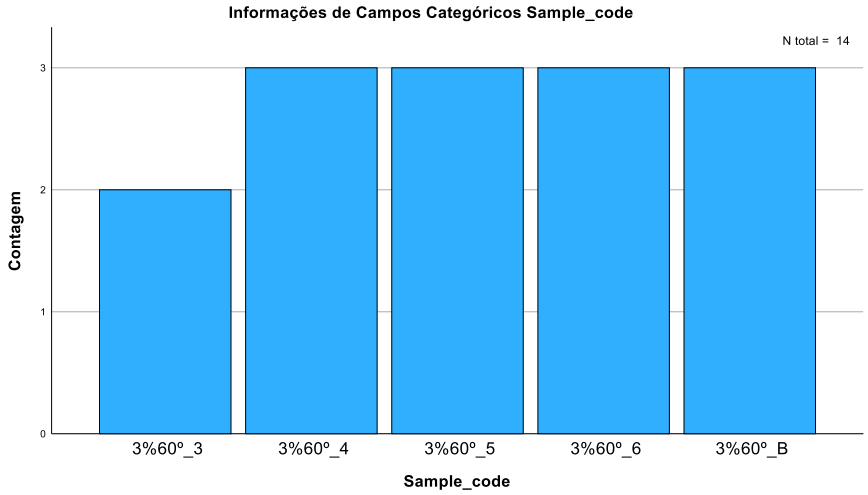
a. Significance values were adjusted by Bonferroni correction for several tests.

Comparações por Método Pairwise de Sample_code



Each node shows the sample average rank of Sample_code.


Informações de Campo Contínuo Yield_solubilization_3
N = 12
Mín. = 50.83
Máx. = 62.21
Média = 56.934
\ Desvio do Desv. = 3.2892
Frequência
0
1
2
3
4
5
6
50,00
52,00
54,00
56,00
58,00
60,00
62,00
Yield_solubilization_3


Informações de Campos Categóricos Sample_code
N total = 14
Contagem
0
1
2
3
3%60º_3
3%60º_4
3%60º_5
3%60º_6
3%60º_B
Sample_code

5.3. Hydrolysis

- **One-way ANOVA**

Observations

Output Created		29-SEP-2023 05:35:58
Comments		
Entry	Data	C:\Users\ezerc\Google Drive\Work\Postdoc\Orientations\MSc\Marisa Ferreira\SPSS\H3_DS.sav
	Active dataset	DataSet0
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	9
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY Yield BY Sample_code/ES=OVERALL/STATISTICS DESCRIPTIVES EFFECTS HOMOGENEITY WELCH/PLOT MEANS/MISSING ANALYSIS /CRITERIA=CILEVEL(0.95)/POSTHOC=TUKEY C ALPHA(0.05).
Resources	Processor time	00:00:00,11
	Elapsed time	00:00:00,21

Descriptive

Yield

		N	Average	Standard deviation	Standard Error	95% Confidence Interval to Mean Lower Limit
	H3%_4_DS	3	11,733	,1528	,0882	11,354
	H3%_5_DS	3	11,500	,5568	,3215	10,117
	H3%_6_DS	3	11,100	,2000	,1155	10,603
	Total	9	11,444	,4126	,1375	11,127
Model	Fixed Effects			,3528	,1176	11,157
	Random Effects				,1849	10,649

Descriptive

Yield

			95% Confidence Interval to Mean			
			Upper limit	Minimal	Maximum	Variance between components
	H3%_4_DS	12,113	11,6	11,9		
	H3%_5_DS	12,883	10,9	12,0		
	H3%_6_DS	11,597	10,9	11,3		
	Total	11,762	10,9	12,0		
Model	Fixed Effects	11,732				
	Random Effects	12,240				,0611

Variance homogeneity tests

		Levene's Statistics	df1	df2	Sig.
Yield	Based on average	2,639	2	6	,151
	Based on median	1,295	2	6	,341
	Based on median and adjusted gl	1,295	2	2,778	,400
	Based on trimmed average	2,539	2	6	,159

ANOVA

Yield

	Sum of Squares	df	Medium Square	With	Sig.
Between Groups	,616	2	,308	2,473	,165
In groups	,747	6	,124		
Total	1,362	8			

ANOVA effect sizes[a,b]

			95% Confidence Interval	
		Point Estimation	Inferior	Superior
Yield	Square Eta	,452	,000	,676
	Square Epsilon	,269	-,333	,567
	Fixed Effect of Square Omega	,247	-,286	,538
	Square Omega Random Effect	,141	-,125	,368

a. Eta square and Epsilon square are estimated based on the fixed-effect model. b. Negative but less biased estimates are maintained, not rounded to zero.

Robust Average Equality Tests

Yield

	Statistics[a]	df1	df2	Sig.
Welch	8,009	2	3,581	,048

a. F distributed asymptotically.

- **After-Testing**

Multiple comparisons

Dependent variable: Yield

	(I) Sample_code	(J) Sample_code	Average Difference (I-J)	Standard Error	Sig.
Tukey HSD	H3%_4_DS	H3%_5_DS	,2333	,2880	,711
		H3%_6_DS	,6333	,2880	,150
	H3%_5_DS	H3%_4_DS	-,2333	,2880	,711
		H3%_6_DS	,4000	,2880	,404
	H3%_6_DS	H3%_4_DS	-,6333	,2880	,150
		H3%_5_DS	-,4000	,2880	,404
Dunnett C	H3%_4_DS	H3%_5_DS	,2333	,3333	
		H3%_6_DS	,6333	,1453	
	H3%_5_DS	H3%_4_DS	-,2333	,3333	
		H3%_6_DS	,4000	,3416	
	H3%_6_DS	H3%_4_DS	-,6333	,1453	
		H3%_5_DS	-,4000	,3416	

Multiple comparisons

Dependent variable: Yield

	(I) Sample_code	(J) Sample_code	Confidence Interval95% Lower Limit	Superior Limit
Tukey HSD	H3%_4_DS	H3%_5_DS	-,650	1,117
		H3%_6_DS	-,250	1,517
	H3%_5_DS	H3%_4_DS	-1,117	,650
		H3%_6_DS	-,484	1,284
	H3%_6_DS	H3%_4_DS	-1,517	,250
		H3%_5_DS	-1,284	,484
Dunnett C	H3%_4_DS	H3%_5_DS	-1,730	2,197
		H3%_6_DS	-,223	1,489
	H3%_5_DS	H3%_4_DS	-2,197	1,730
		H3%_6_DS	-1,612	2,412
	H3%_6_DS	H3%_4_DS	-1,489	,223
		H3%_5_DS	-2,412	1,612

- **Homogeneous subsets**

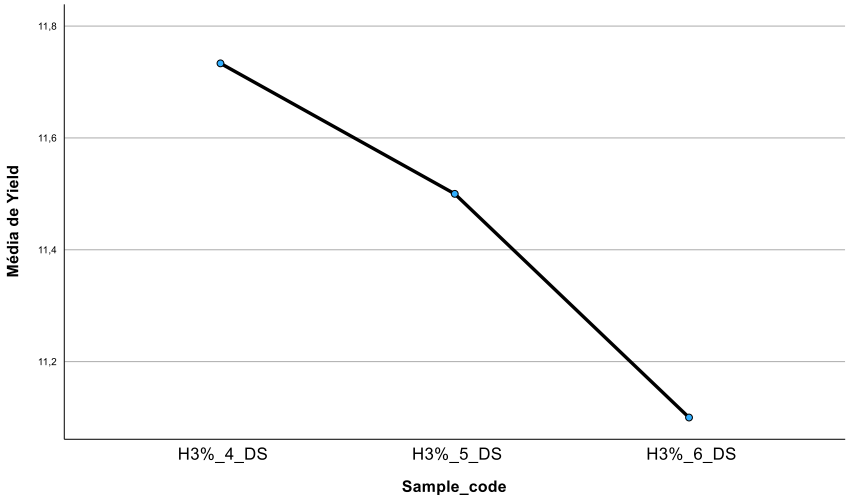
Yield

	Sample_code	N	Subset to alpha = 0.05 1
Tukey HSD[a]	H3%_6_DS	3	11,100
	H3%_5_DS	3	11,500
	H3%_4_DS	3	11,733
	Sig.		,150

The averages for the groups in homogeneous subsets are displayed.

a. Uses the Harmonic Mean Sample Size = 3,000.

- **Averages Charts**



- **Explore**

Observations

Output Created		29-SEP-2023 09:49:50
Comments		
Entry	Active dataset	DataSet3
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	18
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Yield BY Sample/PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING LISTWISE /NOTOTAL.
Resources	Processor time	00:00:00,37
	Elapsed time	00:00:00,83

Case Processing Summary

		Cases					
		Valid		Silent		Total	
	Sample	N	Percentage	N	Percentage	N	Percentage
Yield	H3%_DS	9	100,0%	0	0,0%	9	100,0%
	H3%_P	8	88,9%	1	11,1%	9	100,0%

Descriptive

	Sample			Statistics	Pattern Statistics
Yield	H3%_DS	Average		11,44322	,133735
		95% Confidence Interval to Mean	Lower Limit	11,13483	
			Upper limit	11,75162	
		5% of average trimmed		11,43936	
		Median		11,55600	
		Variance		,161	
		Standard Error		,401206	
		Minimal		10,915	
		Maximum		12,041	
		Amplitude		1,126	
		Interquartile range		,755	
		Asymmetry		-,052	,717
		Kurtosis		-1,254	1,400
	H3%_P	Average		55,85213	3,430938
		95% Confidence Interval to Mean	Lower Limit	47,73925	
			Upper limit	63,96500	
		5% of average trimmed		56,76092	
		Median		58,92700	
		Variance		94,171	
		Standard Error		9,704159	
		Minimal		33,084	
		Maximum		62,262	
		Amplitude		29,178	
		Interquartile range		7,853	
		Asymmetry		-2,312	,752
		Kurtosis		5,660	1,481

M Estimators

	Sample	Hubera Estimator M	Tukeyb bi-weighting	Hampelc Estimator M	Andrewsd Wave
Yield	H3%_DS	11,45807	11,45647	11,44197	11,45652
	H3%_P	58,88673	59,43451	59,33697	59,43293

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

		Sample		Case number	Value
Yield	H3%_DS	Upper	1	4	12,041
			2	3	11,858
			3	2	11,682
			4	5	11,574
		Lower	1	8	10,915
			2	6	10,934
			3	9	11,095
			4	7	11,334
	H3%_P	Upper	1	16	62,262
			2	14	61,880
			3	17	61,628
			4	15	59,330
		Lower	1	10	33,084
			2	12	52,873
			3	18	57,236
			4	13	58,524

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

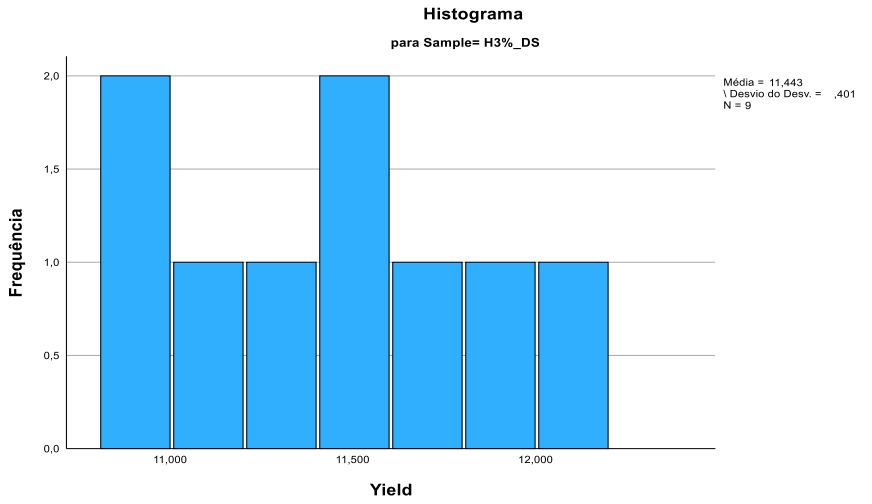
Normality Tests

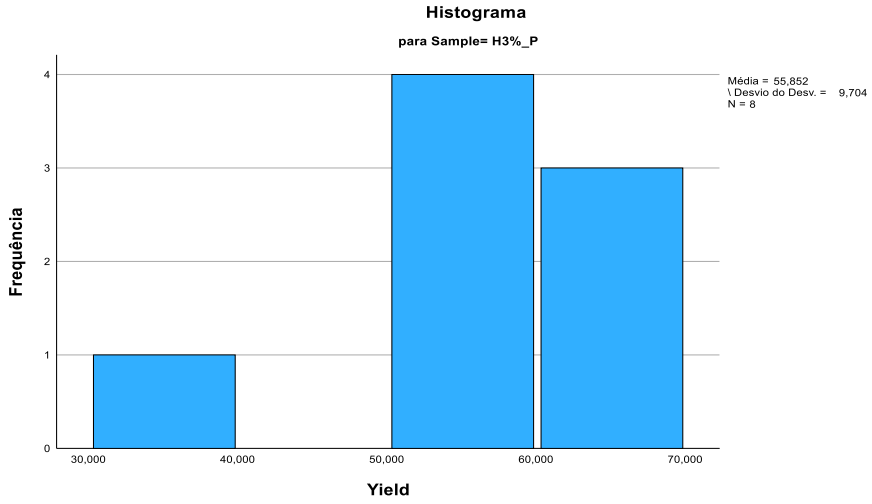
		Kolmogorov-Smirnova			Shapiro-Wilk		
	Sample	Statistics	gl	Sig.	Statistics	gl	Sig.
Yield	H3%_DS	,166	9	,200*	,942	9	,607
	H3%_P	,307	8	,026	,689	8	,002

*. This is a lower bound of true significance.

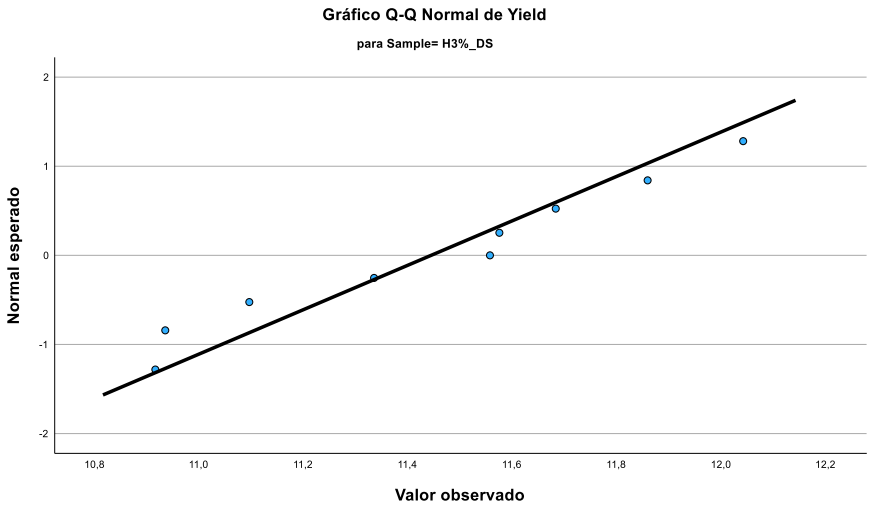
a. Lilliefors Significance Correlation

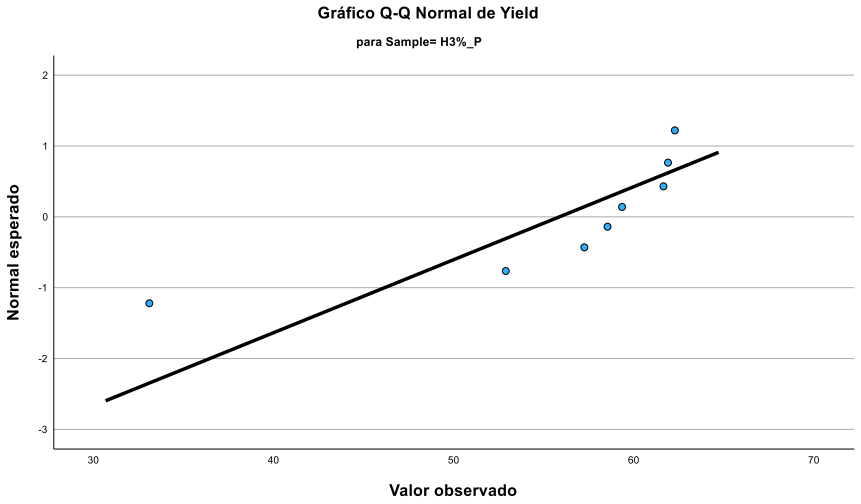
- **Histograms**
  Yield



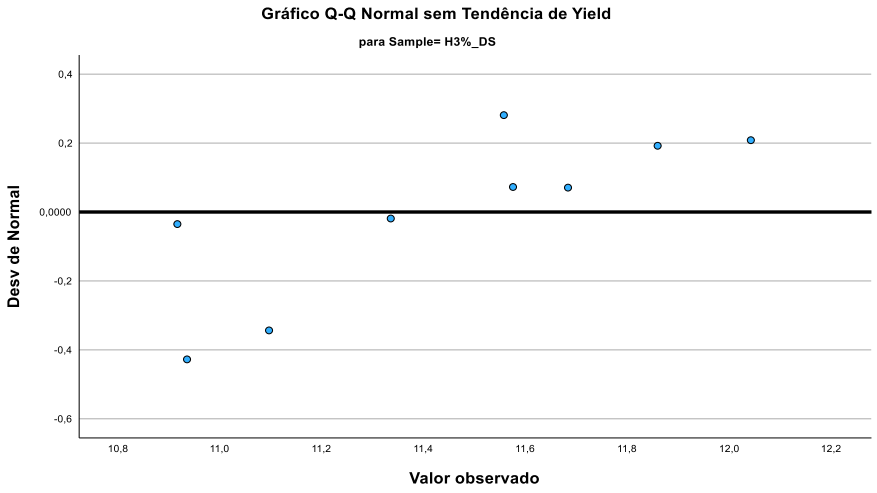


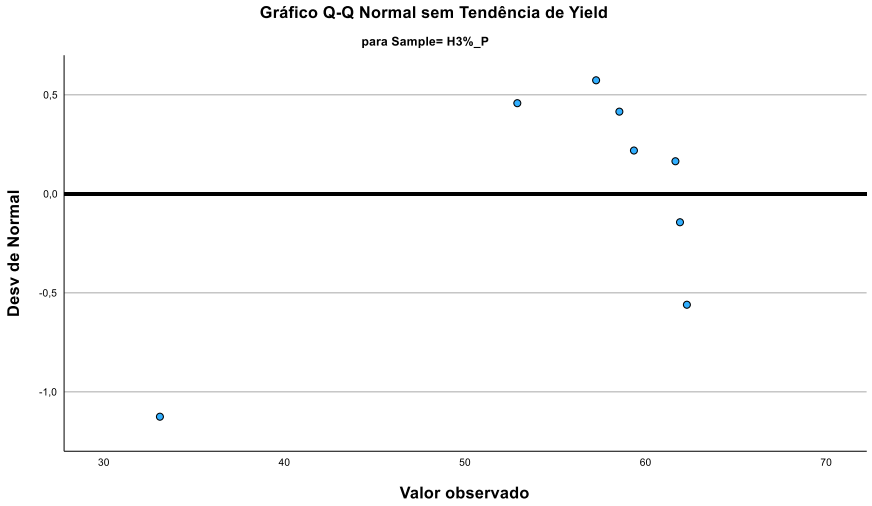
- **Normal Q-Q Chart**

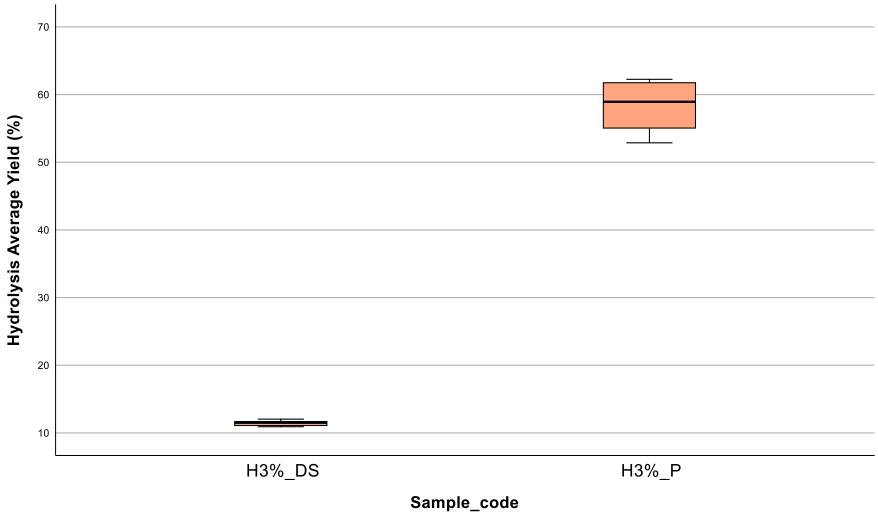




- **Normal Q-Q Chart No Trend**







- **T-Test**

Observations

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Comments		
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	N rows in job data file	45
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample(1 2)/MISSING=ANALYSIS/VARIABLES=Yield/ES DISPLAY(TRUE)/CRITERIA=CI(.95).
Resources	Processor time	00:00:00,00
	Elapsed time	00:00:00,00

Group Statistics

	Sample	N	Average	Standard deviation	Standard mean error
Yield	H3%_DS	9	11,44322	,401206	,133735
	H3%_P	7	59,10471	3,335465	1,260687

Independent samples test

		Levene's test for equality of variances		t-test for Equality of Means	
		With	Sig.	t	df
Yield	Equal variances assumed	10,607	,006	-42,900	14
	Equal variances not assumed			-37,595	6,135

Independent samples test

		t-test for Equality of Means			
		Significance			Standard difference
		Unilateral p	Bilateral p	Average difference	error
Yield	Equal variances assumed	<.001	<.001	-47,661492	1,110982
	Equal variances not assumed	<.001	<.001	-47,661492	1,267761

Independent samples test

		t-test for Equality of Means	
		95% Difference Confidence Intreval	
		Inferior	Superior
Yield	Equal variances assumed	-50,044311	-45,278673
	Equal variances not assumed	-50,747094	-44,575890

Independent Sample Effect Sizes

				95% Confidence Interval	
		Standard	Point Estimation	Inferior	Superior
Yield	Cohen's d	2,204536	-21,620	-29,586	-13,641
	Hedges Correction	2,332132	-20,437	-27,968	-12,894
	Glass Delta	3,335465	-14,289	-22,229	-6,416

a. The denominator used in estimating effect sizes. Cohen's d uses the clustered standard deviation. Hedges correction uses the clustered standard deviation in addition to a correction factor. The Glass delta uses the sample standard deviation of the control group (i.e., the second) group.

- **Teste-T**

Observations

Output Created		29-SEP-2023 10:22:58
Comments		
Entrance	Active dataset	DataSet3
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	Weighting	<none>
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	N rows in job data file	45
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample(3 4)/MISSING=ANALYSIS /VARIABLES=Yield/ES DISPLAY(TRUE)/CRITERIA=CI(. 95).
Resources	Processor time	00:00:00,02
	Elapsed time	00:00:00,00

Group Statistics

	Sample	N	Average	Standard deviation	Standard mean error
Yield	H3%_A_P	3	52,39600	2,671718	1,542517
	H1%_A_P	3	51,36900	2,115926	1,221630

Independent Samples Test

		Levene's test for equality of variances		t-test for Equality of Means	
		With	Sig.	t	df
Yield	Equal variances assumed	,231	,656	,522	4
	Equal variances not assumed			,522	3,801

Independent Samples Test

		t-test for Equality of Means			
		Significance			Standard difference
		Unilateral p	Bilateral p	Average difference	error
Yield	Equal variances assumed	,315	,629	1,027000	1,967673
	Equal variances not assumed	,315	,631	1,027000	1,967673

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Inferior	Superior
Yield	Equal variances assumed	-4,436137	6,490137
	Equal variances not assumed	-4,551093	6,605093

Independent Sample Effect Sizes

				95% Confidence Interval	
		Standard	Point Estimation	Inferior	Superior
Yield	Cohen's d	2,409898	,426	-1,224	2,027
	Hedges Correction	3,020359	,340	-,977	1,617
	Glass Delta	2,115926	,485	-1,225	2,095

a. The denominator used in estimating effect sizes. Cohen's d uses the clustered standard deviation. Hedges correction uses the clustered standard deviation in addition to a correction factor. The Glass delta uses the sample standard deviation of the control group (i.e., the second) group.

- **T-test**

Observations

Output Created		29-SEP-2023 10:24:10
Comments		
Entrance	Active dataset	DataSet3
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	45
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample(4 5)/MISSING=ANALYSIS/VARIAB LES=Yield/ES DISPLAY(TRUE)/CRITERIA=CI(. 95).
Resources	Processor time	00:00:00,00
	Elapsed time	00:00:00,00

Group Statistics

	Sample	N	Average	Standard deviation	Standard mean error
Yield	H1%_A_P	3	51,36900	2,115926	1,221630
	H1%_B_P	3	24,89367	2,937010	1,695684

Independent Samples Test

		Levene's test for equality of variances		t-test for Equality of Means	
		With	Sig.	t	df
Yield	Equal variances assumed	,556	,497	12,668	4
	Equal variances not assumed			12,668	3,636

Independent Samples Test

		t-test for Equality of Means			
		Significance			Standard difference
		Unilateral p	Bilateral p	Average difference	error
Yield	Equal variances assumed	<.001	<.001	26,475333	2,089910
	Equal variances not assumed	<.001	<.001	26,475333	2,089910

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Inferior	Superior
Yield	Equal variances assumed	20,672813	32,277854
	Equal variances not assumed	20,436026	32,514640

Independent Sample Effect Sizes

				95% Confidence Interval	
		Standardizer	Point Estimation	Inferior	Superior
Yield	Cohen's d	2,559607	10,344	3,370	17,420
	Hedges Correction	3,207991	8,253	2,689	13,899
	Glass Delta	2,937010	9,014	1,198	17,436

a. The denominator used in estimating effect sizes. Cohen's d uses the clustered standard deviation. Hedges correction uses the clustered standard deviation in addition to a correction factor. The Glass delta uses the sample standard deviation of the control group (i.e., the second) group.

5.4. Antioxidant activity

- **Univariate Analysis of Variance**

Notes

Output Created		29-SEP-2023 17:10:54
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\SPSS_mean_ORAC_ABTS.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	108
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA ABTS_1 BY Sample_1 /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Sample_1(TUKEY) /PLOT=PROFILE(Sample_1) TYPE=BAR ERRORBAR=CI MEANREFERENCE=NO /PRINT ETASQ DESCRIPTIVE PARAMETER HOMOGENEITY OPOWER /PLOT=RESIDUALS /CRITERIA=ALPHA(.05) /DESIGN=Sample_1.
Resources	Processor Time	00:00:00,80
	Elapsed Time	00:00:00,37

Between-Subjects Factors

		Value Label	N
Sample_1	1	H3%_DS	9
	2	3%60º	9
	3	H3%_P	8
	4	H3%_L	9

Descriptive Statistics

Dependent Variable: ABTS_1

Sample_1	Mean	Std. Deviation	N
H3%_DS	1,64184	,540077	9
3%60º	3,98498	,412704	9
H3%_P	2,34024	,299333	8
H3%_L	1,91503	,077957	9
Total	2,47425	1,001570	35

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
ABTS_1	Based on Mean	1,840	3	31	,160
	Based on Median	1,187	3	31	,331
	Based on Median and with adjusted df	1,187	3	15,959	,346
	Based on trimmed mean	1,555	3	31	,220

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a,b]

a. Dependent variable: ABTS_1. b. Design: Intercept + Sample_1

Tests of Between-Subjects Effects

Dependent Variable: ABTS_1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	29,735[a]	3	9,912	70,281	<.001	,872
Intercept	213,067	1	213,067	1 510,810	<.001	,980
Sample_1	29,735	3	9,912	70,281	<.001	,872
Error	4,372	31	,141			
Total	248,373	35				
Corrected Total	34,107	34				

Tests of Between-Subjects Effects

Dependent Variable: ABTS_1

Source	Noncent. Parameter	Observed Power[b]
Corrected Model	210,844	1,000
Intercept	1 510,810	1,000
Sample_1	210,844	1,000
Error		
Total		
Corrected Total		

a. R Squared = ,872 (Adjusted R Squared = ,859). b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: ABTS_1

					95% Confidence Interval	
Parameter	B	Std. Error	t	Sig.	Lower Bound	Upper Bound
Intercept	1,915	,125	15,298	<.001	1,660	2,170
[Sample_1=1]	-,273	,177	-1,543	,133	-,634	,088
[Sample_1=2]	2,070	,177	11,693	<.001	1,709	2,431
[Sample_1=3]	,425	,182	2,330	,026	,053	,797
[Sample_1=4]	0[a]	.	.	.	.	.

Parameter Estimates

Dependent Variable: ABTS_1

Parameter	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Intercept	,883	15,298	1,000
[Sample_1=1]	,071	1,543	,321
[Sample_1=2]	,815	11,693	1,000
[Sample_1=3]	,149	2,330	,617
[Sample_1=4]	.	.	.

a. This parameter is set to zero because it is redundant. b. Computed using alpha = .05

- **Post Hoc Tests**

Multiple Comparisons

Dependent Variable: ABTS_1

Tukey HSD

(I) Sample_1	(J) Sample_1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
H3%_DS	3%60º	-2,34313*	,177030	<.001	-2,82361	-1,86266
	H3%_P	-,69839*	,182478	,003	-1,19365	-,20313
	H3%_L	-,27319	,177030	,425	-,75366	,20728
3%60º	H3%_DS	2,34313*	,177030	<.001	1,86266	2,82361
	H3%_P	1,64474*	,182478	<.001	1,14948	2,14000
	H3%_L	2,06994*	,177030	<.001	1,58947	2,55042
H3%_P	H3%_DS	,69839*	,182478	,003	,20313	1,19365
	3%60º	-1,64474*	,182478	<.001	-2,14000	-1,14948
	H3%_L	,42520	,182478	,113	-,07006	,92046
H3%_L	H3%_DS	,27319	,177030	,425	-,20728	,75366
	3%60º	-2,06994*	,177030	<.001	-2,55042	-1,58947
	H3%_P	-,42520	,182478	,113	-,92046	,07006

Based on observed means. The error term is Mean Square(Error) = ,141. *. The mean difference is significant at the .05 level.

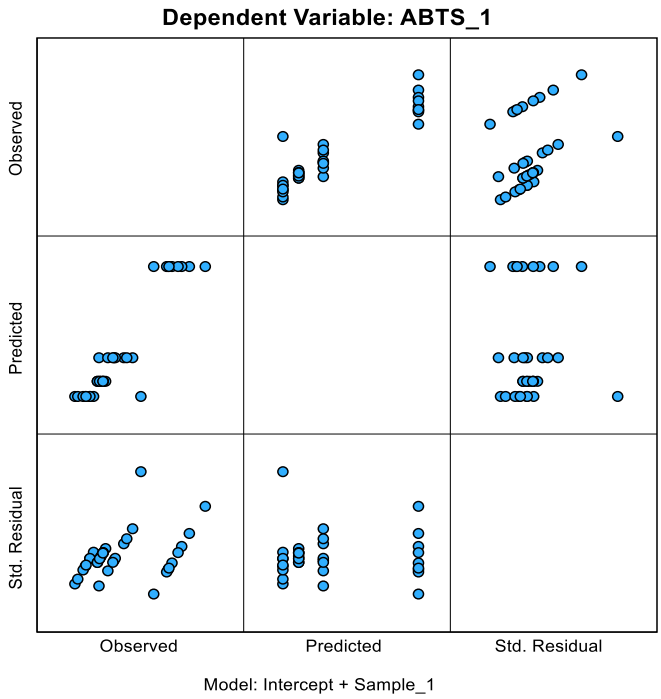
- **Homogeneous Subsets**

ABTS_1

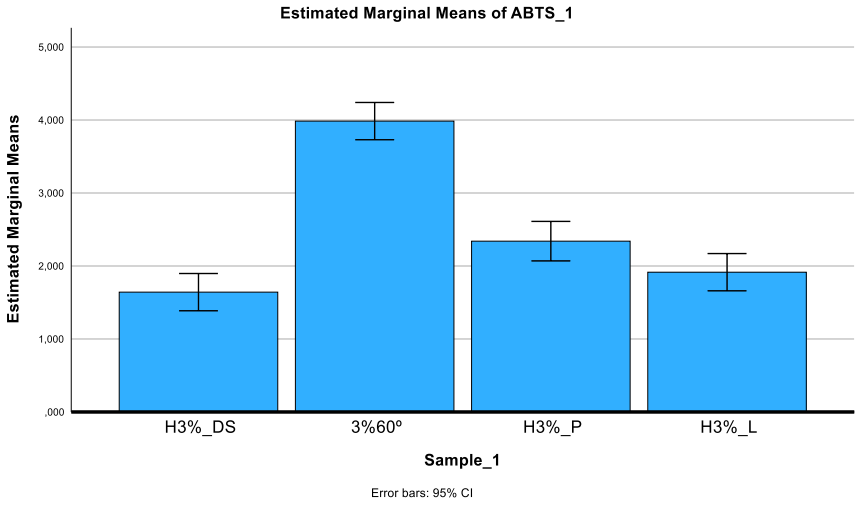
Tukey HSD[a,b,c]

Sample_1	N	Subset 1	Subset 2	Subset 3
H3%_DS	9	1,64184		
H3%_L	9	1,91503	1,91503	
H3%_P	8		2,34024	
3%60º	9			3,98498
Sig.		,438	,105	1,000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = ,141. a. Uses Harmonic Mean Sample Size = 8,727. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed. c. Alpha = .05.



- **Profile Plots**



- **Univariate Analysis of Variance**

Notes

Output Created		29-SEP-2023 17:11:26
Comments		
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	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	108
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA ORAC_1 BY Sample_1 /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Sample_1(TUKEY) /PLOT=PROFILE(Sample_1) TYPE=BAR ERRORBAR=CI MEANREFERENCE=NO /PRINT ETASQ DESCRIPTIVE PARAMETER HOMOGENEITY OPOWER /PLOT=RESIDUALS /CRITERIA=ALPHA(.05) /DESIGN=Sample_1.
Resources	Processor Time	00:00:00,36
	Elapsed Time	00:00:00,31

Between-Subjects Factors

		Value Label	N
Sample_1	1	H3%_DS	9
	2	3%60º	9
	3	H3%_P	8
	4	H3%_L	9

Descriptive Statistics

Dependent Variable: ORAC_1

Sample_1	Mean	Std. Deviation	N
H3%_DS	1,21778	,070848	9
3%60º	2,22111	,085505	9
H3%_P	1,39875	,085597	8
H3%_L	1,37667	,047170	9
Total	1,55800	,408216	35

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
ORAC_1	Based on Mean	,965	3	31	,422
	Based on Median	,552	3	31	,651
	Based on Median and with adjusted df	,552	3	26,088	,651
	Based on trimmed mean	,935	3	31	,436

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a,b]

a. Dependent variable: ORAC_1. b. Design: Intercept + Sample_1

Tests of Between-Subjects Effects

Dependent Variable: ORAC_1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5,498[a]	3	1,833	338,713	<.001
Intercept	84,257	1	84,257	15 572,189	<.001
Sample_1	5,498	3	1,833	338,713	<.001
Error	,168	31	,005		
Total	90,624	35			
Corrected Total	5,666	34			

Tests of Between-Subjects Effects

Dependent Variable: ORAC_1

Source	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	,970	1 016,138	1,000
Intercept	,998	15 572,189	1,000
Sample_1	,970	1 016,138	1,000
Error			
Total			
Corrected Total			

a. R Squared = ,970 (Adjusted R Squared = ,968). b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: ORAC_1

					95% Confidence Interval	
Parameter	B	Std. Error	t	Sig.	Lower Bound	Upper Bound
Intercept	1,377	,025	56,147	<.001	1,327	1,427
[Sample_1=1]	-,159	,035	-4,582	<.001	-,230	-,088
[Sample_1=2]	,844	,035	24,353	<.001	,774	,915
[Sample_1=3]	,022	,036	,618	,541	-,051	,095
[Sample_1=4]	0[a]	.	.	.	.	.

Parameter Estimates

Dependent Variable: ORAC_1

Parameter	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Intercept	,990	56,147	1,000
[Sample_1=1]	,404	4,582	,993
[Sample_1=2]	,950	24,353	1,000
[Sample_1=3]	,012	,618	,092
[Sample_1=4]	.	.	.

a. This parameter is set to zero because it is redundant. b. Computed using alpha = .05

- **Post Hoc Tests**

Multiple Comparisons

Dependent Variable: ORAC_1

Tukey HSD

(I) Sample_1	(J) Sample_1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
H3%_DS	3%60º	-1,00333*	,034675	<.001	-1,09744	-,90922
	H3%_P	-,18097*	,035743	<.001	-,27798	-,08396
	H3%_L	-,15889*	,034675	<.001	-,25300	-,06478
3%60º	H3%_DS	1,00333*	,034675	<.001	,90922	1,09744
	H3%_P	,82236*	,035743	<.001	,72535	,91937
	H3%_L	,84444*	,034675	<.001	,75033	,93856
H3%_P	H3%_DS	,18097*	,035743	<.001	,08396	,27798
	3%60º	-,82236*	,035743	<.001	-,91937	-,72535
	H3%_L	,02208	,035743	,926	-,07492	,11909
H3%_L	H3%_DS	,15889*	,034675	<.001	,06478	,25300
	3%60º	-,84444*	,034675	<.001	-,93856	-,75033
	H3%_P	-,02208	,035743	,926	-,11909	,07492

Based on observed means. The error term is Mean Square(Error) = ,005. *. The mean difference is significant at the .05 level.

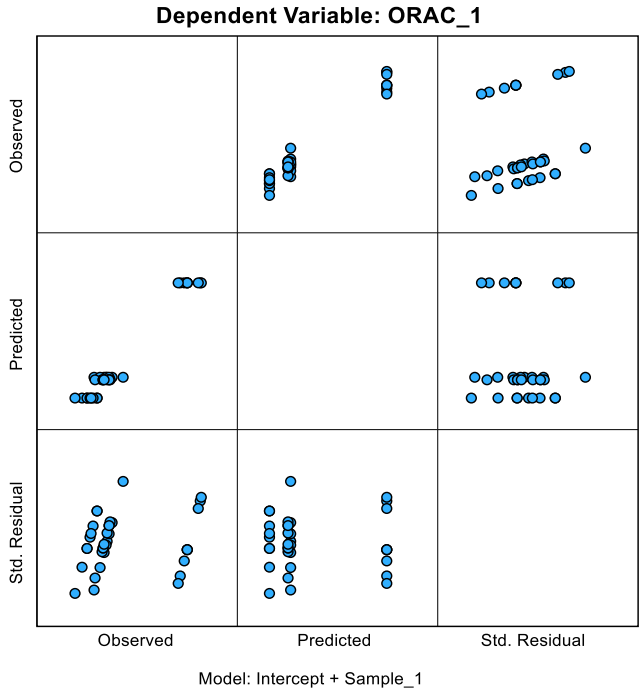
- **Homogeneous Subsets**

ORAC_1

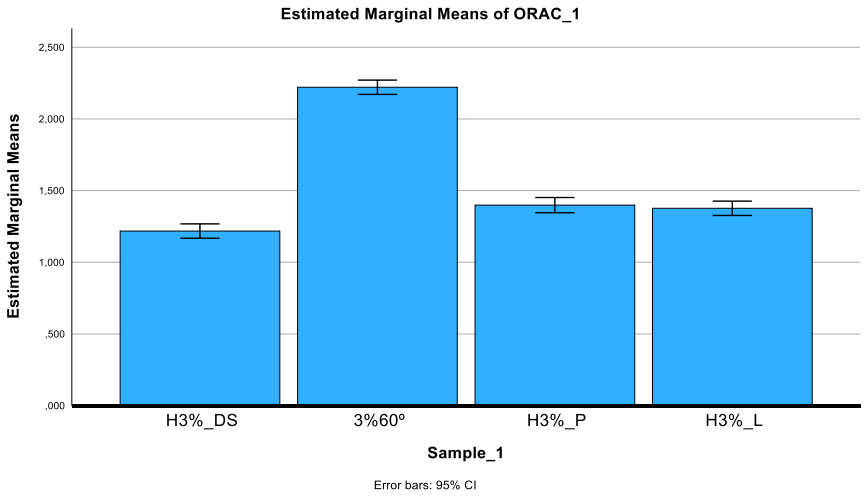
Tukey HSD[a,b,c]

Sample_1	N	Subset 1	2	3
H3%_DS	9	1,21778		
H3%_L	9		1,37667	
H3%_P	8		1,39875	
3%60º	9			2,22111
Sig.		1,000	,923	1,000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = ,005. a. Uses Harmonic Mean Sample Size = 8,727. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed. c. Alpha = .05.



- **Profile Plots**



- **One-way ANOVA**

Notes

Output Created		29-SEP-2023 17:13:04
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\SPSS_mean_ORAC_ABTS.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	108
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY ABTS_2 ORAC_2 BY Sample_2 /ES=OVERALL /STATISTICS DESCRIPTIVES EFFECTS HOMOGENEITY WELCH /PLOT MEANS /MISSING ANALYSIS /CRITERIA=CILEVEL(0.95) /POSTHOC=TUKEY ALPHA(0.05).
Resources	Processor Time	00:00:00,17
	Elapsed Time	00:00:00,27

Descriptives

			N	Mean	Std. Deviation	Std. Error
ABTS_2		H3%_A_P	3	,56233	,119069	,068744
		H3%_A_P_F	3	,32400	,056930	,032868
		H3%_A_P_TF	2	,36050	,057276	,040500
		Total	8	,42250	,138137	,048839
	Model	Fixed Effects			,087312	,030870
		Random Effects				,078770
ORAC_2		H3%_A_P	3	1,16000	,060828	,035119
		H3%_A_P_F	3	1,12000	,010000	,005774
		H3%_A_P_TF	2	1,04000	,296985	,210000
		Total	8	1,11500	,127167	,044960
	Model	Fixed Effects			,138420	,048939
		Random Effects				,048939[a]

Descriptives

			95% Confidence Interval for Mean			
			Lower Bound	Upper Bound	Minimum	Maximum
ABTS_2		H3%_A_P	,26655	,85812	,471	,697
		H3%_A_P_F	,18258	,46542	,283	,389
		H3%_A_P_TF	-,15410	,87510	,320	,401
		Total	,30701	,53799	,283	,697
	Model	Fixed Effects	,34315	,50185		
		Random Effects	,08358	,76142		
ORAC_2		H3%_A_P	1,00890	1,31110	1,120	1,230
		H3%_A_P_F	1,09516	1,14484	1,110	1,130
		H3%_A_P_TF	-1,62830	3,70830	,830	1,250
		Total	1,00869	1,22131	,830	1,250
	Model	Fixed Effects	,98920	1,24080		
		Random Effects	,90443[a]	1,32557[a]		

Descriptives

			Between-Component Variance
ABTS_2	H3%_A_P		
	H3%_A_P_F		
	H3%_A_P_TF		
	Total		
	Model	Fixed Effects	
		Random Effects	,015278
ORAC_2	H3%_A_P		
	H3%_A_P_F		
	H3%_A_P_TF		
	Total		
	Model	Fixed Effects	
		Random Effects	-,003985

a. Warning: Between-component variance is negative. It was replaced by 0.0 in computing this random effects measure.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ABTS_2	Between Groups	,095	2	,048	6,261	,044
	Within Groups	,038	5	,008		
	Total	,134	7			
ORAC_2	Between Groups	,017	2	,009	,454	,659
	Within Groups	,096	5	,019		
	Total	,113	7			

ANOVA Effect Sizes[a,b]

			95% Confidence Interval	
		Point Estimate	Lower	Upper
ABTS_2	Eta-squared	,715	,000	,831
	Epsilon-squared	,600	-,400	,763
	Omega-squared Fixed-effect	,568	-,333	,738
	Omega-squared Random-effect	,397	-,143	,585
ORAC_2	Eta-squared	,154	,000	,464
	Epsilon-squared	-,185	-,400	,250
	Omega-squared Fixed-effect	-,158	-,333	,226
	Omega-squared Random-effect	-,073	-,143	,127

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model. b. Negative but less biased estimates are retained, not rounded to zero.

Robust Tests of Equality of Means

		Statistic[a]	df1	df2	Sig.
ABTS_2	Welch	3,986	2	2,897	,147
ORAC_2	Welch	,516	2	1,810	,665

a. Asymptotically F distributed.

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
ABTS_2	Based on Mean	2,117	2	5	,216
	Based on Median	,319	2	5	,741
	Based on Median and with adjusted df	,319	2	2,985	,749
	Based on trimmed mean	1,858	2	5	,249
ORAC_2	Based on Mean	141,473	2	5	<.001
	Based on Median	22,237	2	5	,003
	Based on Median and with adjusted df	22,237	2	2,044	,041
	Based on trimmed mean	120,641	2	5	<.001

- **Post Hoc Tests**

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Sample_2	(J) Sample_2	Mean Difference (I-J)	Std. Error	Sig.
ABTS_2	H3%_A_P	H3%_A_P_F	,238333*	,071290	,045
		H3%_A_P_TF	,201833	,079705	,112
	H3%_A_P_F	H3%_A_P	-,238333*	,071290	,045
		H3%_A_P_TF	-,036500	,079705	,893
	H3%_A_P_TF	H3%_A_P	-,201833	,079705	,112
		H3%_A_P_F	,036500	,079705	,893
ORAC_2	H3%_A_P	H3%_A_P_F	,040000	,113019	,934
		H3%_A_P_TF	,120000	,126359	,636
	H3%_A_P_F	H3%_A_P	-,040000	,113019	,934
		H3%_A_P_TF	,080000	,126359	,809
	H3%_A_P_TF	H3%_A_P	-,120000	,126359	,636
		H3%_A_P_F	-,080000	,126359	,809

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Sample_2	(J) Sample_2	95% Confidence Interval	
			Lower Bound	Upper Bound
ABTS_2	H3%_A_P	H3%_A_P_F	,00636	,47031
		H3%_A_P_TF	-,05752	,46119
	H3%_A_P_F	H3%_A_P	-,47031	-,00636
		H3%_A_P_TF	-,29585	,22285
	H3%_A_P_TF	H3%_A_P	-,46119	,05752
		H3%_A_P_F	-,22285	,29585
ORAC_2	H3%_A_P	H3%_A_P_F	-,32775	,40775
		H3%_A_P_TF	-,29116	,53116
	H3%_A_P_F	H3%_A_P	-,40775	,32775
		H3%_A_P_TF	-,33116	,49116
	H3%_A_P_TF	H3%_A_P	-,53116	,29116
		H3%_A_P_F	-,49116	,33116

*. The mean difference is significant at the 0.05 level.

- **Homogeneous Subsets**

ABTS_2

Tukey HSD[a,b]

Sample_2	N	Subset for alpha = 0.05
		1
H3%_A_P_F	3	,32400
H3%_A_P_TF	2	,36050
H3%_A_P	3	,56233
Sig.		,059

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 2,571. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed

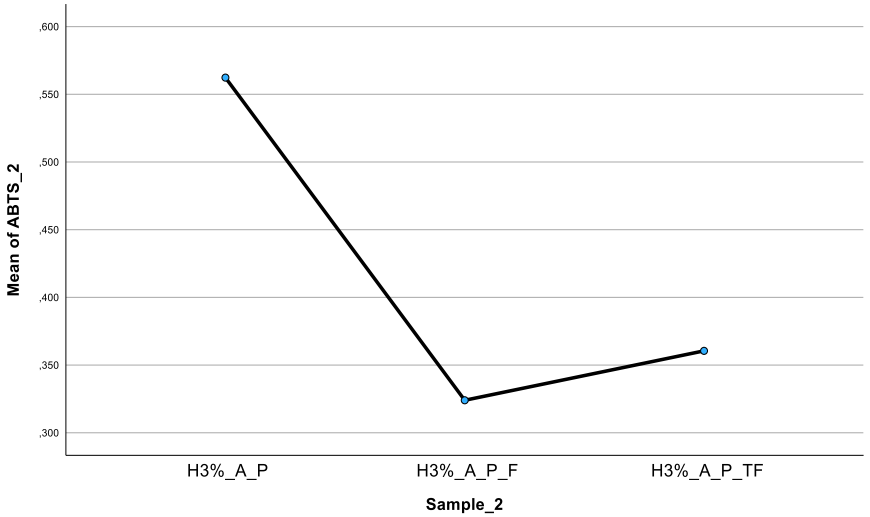
ORAC_2

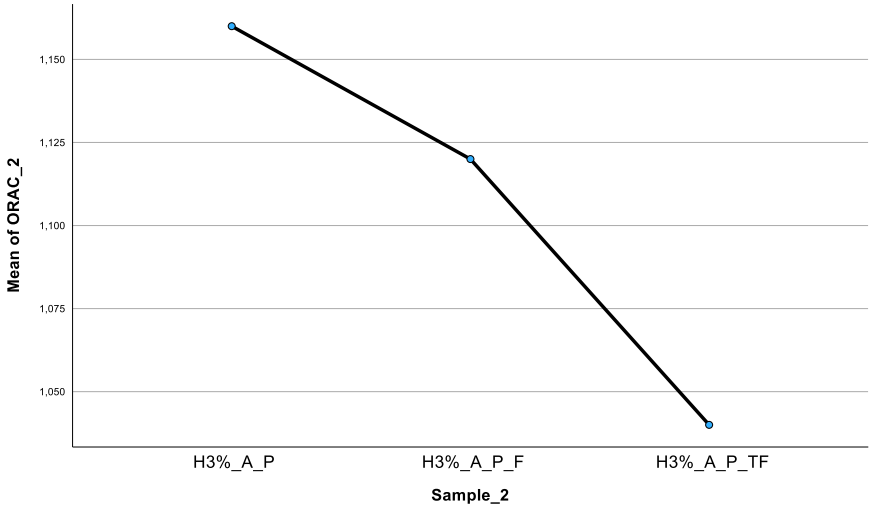
Tukey HSD[a,b]

Sample_2	N	Subset for alpha = 0.05
		1
H3%_A_P_TF	2	1,04000
H3%_A_P_F	3	1,12000
H3%_A_P	3	1,16000
Sig.		,618

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 2,571. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

- **Means Plots**


,600
,550
,500
,450
,400
,350
,300
Mean of ABTS_2
H3%_A_P
H3%_A_P_F
H3%_A_P_TF
Sample_2


1,150
1,125
1,100
1,075
1,050
Mean of ORAC_2
H3%_A_P
H3%_A_P_F
H3%_A_P_TF
Sample_2

- **Nonparametric Tests**

Notes

Output Created		29-SEP-2023 17:18:03
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\SPSS_mean_ORAC_ABTS.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	108
	Syntax	NPTESTS /INDEPENDENT TEST (ORAC_2) GROUP (Sample_2) /MISSING SCOPE=ANALYSIS USERMISSING=EXCLUDE /CRITERIA ALPHA=0.05 CILEVEL=95.
Resources	Processor Time	00:00:00,64
	Elapsed Time	00:00:00,91

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]
1	The distribution of ORAC_2 is the same across categories of Sample_2.	Independent-Samples Kruskal-Wallis Test	,701

Hypothesis Test Summary

	Decision
1	Retain the null hypothesis.

a. The significance level is ,050. b. Asymptotic significance is displayed.

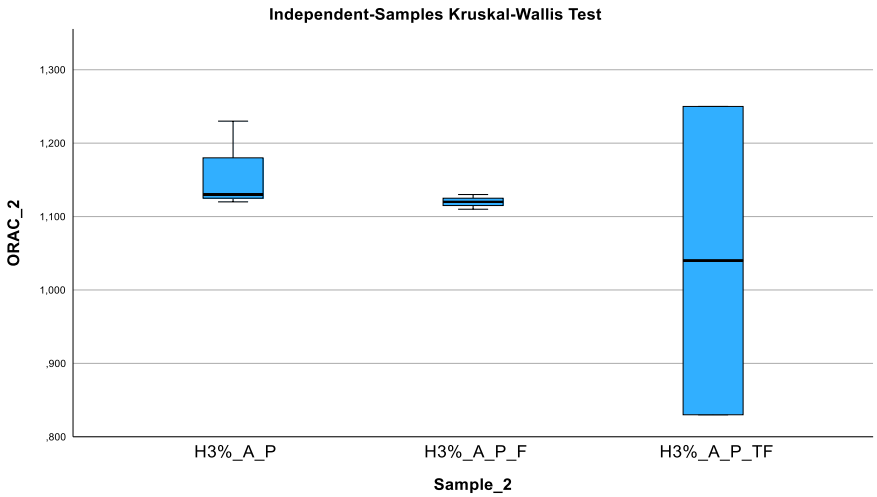
- **Independent-Samples Kruskal-Wallis Test**

ORAC_2 across Sample_2

Independent-Samples Kruskal-Wallis Test Summary

Total N	8
Test Statistic	,711[a]
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	,701

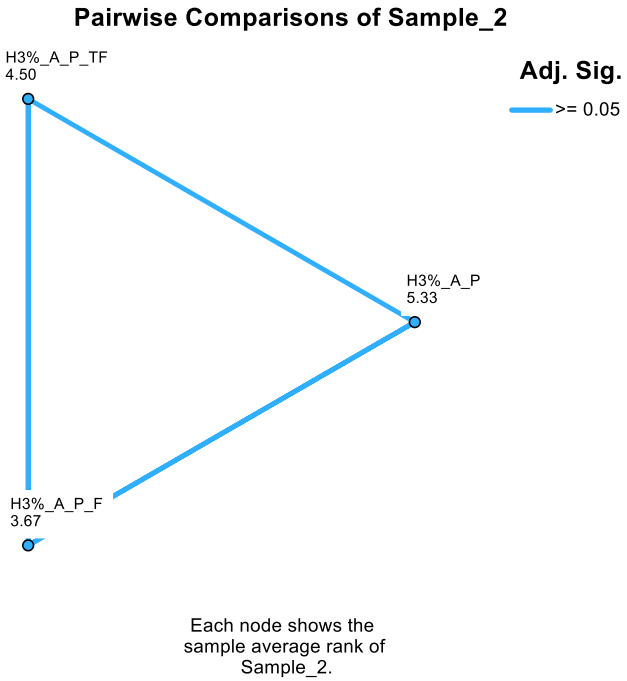
a. The test statistic is adjusted for ties.

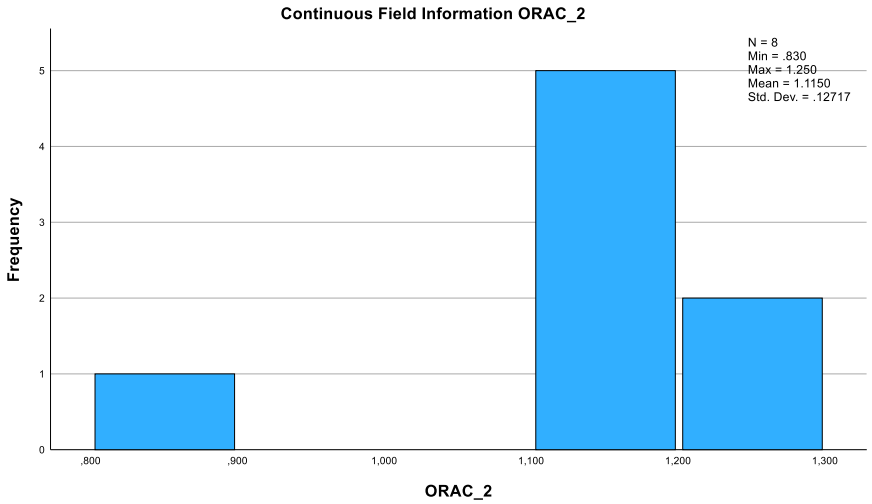


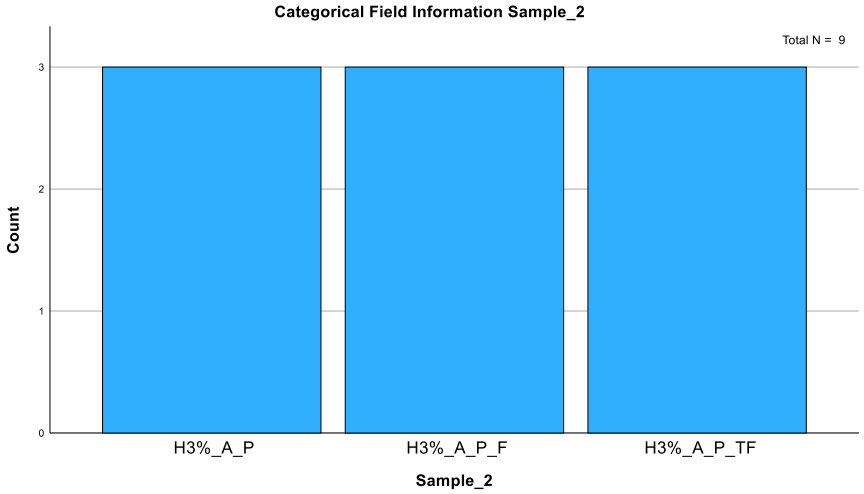
Pairwise Comparisons of Sample_2

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
H3%_A_P_F-H3%_A_P_TF	-,833	2,209	-,377	,706	1,000
H3%_A_P_F-H3%_A_P	1,667	1,976	,843	,399	1,000
H3%_A_P_TF-H3%_A_P	,833	2,209	,377	,706	1,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is ,050. a. Significance values have been adjusted by the Bonferroni correction for multiple tests.




Continuous Field Information ORAC_2
N = 8
Min = .830
Max = 1.250
Mean = 1.1150
Std. Dev. = .12717
Frequency
0
1
2
3
4
5
,800
,900
1,000
1,100
1,200
1,300
ORAC_2


Categorical Field Information Sample_2
Total N = 9
Count
0
1
2
3
H3%_A_P
H3%_A_P_F
H3%_A_P_TF
Sample_2

- **T-Test**

Notes

Output Created		29-SEP-2023 17:18:49
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\SPSS_mean_ORAC_ABTS.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	108
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample_3(1 2) /MISSING=ANALYSIS /VARIABLES=ABTS_3 ORAC_3 /ES DISPLAY(TRUE) /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,01

Group Statistics

	Sample_3	N	Mean	Std. Deviation	Std. Error Mean
ABTS_3	3%60º_A	3	1,54500	,358967	,207249
	3%60º_B	3	3,24267	,244590	,141214
ORAC_3	3%60º_A	3	1,51667	,109697	,063333
	3%60º_B	3	1,81000	,150997	,087178

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
ABTS_3	Equal variances assumed	1,068	,360	-6,769	4
	Equal variances not assumed			-6,769	3,528
ORAC_3	Equal variances assumed	,235	,653	-2,722	4
	Equal variances not assumed			-2,722	3,651

Independent Samples Test

		t-test for Equality of Means			
		Significance			Std. Error
		One-Sided p	Two-Sided p	Mean Difference	Difference
ABTS_3	Equal variances assumed	,001	,002	-1,697667	,250786
	Equal variances not assumed	,002	,004	-1,697667	,250786
ORAC_3	Equal variances assumed	,026	,053	-,293333	,107755
	Equal variances not assumed	,029	,058	-,293333	,107755

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
ABTS_3	Equal variances assumed	-2,393961	-1,001372
	Equal variances not assumed	-2,432306	-,963027
ORAC_3	Equal variances assumed	-,592509	,005842
	Equal variances not assumed	-,604125	,017459

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
ABTS_3	Cohen's d	,307149	-5,527	-9,511	-1,513
	Hedges' correction	,384955	-4,410	-7,589	-1,207
	Glass's delta	,244590	-6,941	-13,489	-,796
ORAC_3	Cohen's d	,131972	-2,223	-4,340	,024
	Hedges' correction	,165403	-1,773	-3,463	,019
	Glass's delta	,150997	-1,943	-4,251	,503

- **T-Test**

Notes

Output Created		29-SEP-2023 17:20:38
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\SPSS_mean_ORAC_ABTS.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	108
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample_3(3 4) /MISSING=ANALYSIS /VARIABLES=ABTS_3 ORAC_3 /ES DISPLAY(TRUE) /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,01

Group Statistics

	Sample_3	N	Mean	Std. Deviation	Std. Error Mean
ABTS_3	H1%_A_P	3	,19767	,023180	,013383
	H1%_B_P	3	2,18067	,569861	,329009
ORAC_3	H1%_A_P	3	,66333	,050332	,029059
	H1%_B_P	3	4,12000	,217025	,125300

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
ABTS_3	Equal variances assumed	14,497	,019	-6,022	4
	Equal variances not assumed			-6,022	2,007
ORAC_3	Equal variances assumed	8,495	,043	-26,874	4
	Equal variances not assumed			-26,874	2,215

Independent Samples Test

		t-test for Equality of Means			
		Significance			Std. Error
		One-Sided p	Two-Sided p	Mean Difference	Difference
ABTS_3	Equal variances assumed	,002	,004	-1,983000	,329281
	Equal variances not assumed	,013	,026	-1,983000	,329281
ORAC_3	Equal variances assumed	<.001	<.001	-3,456667	,128625
	Equal variances not assumed	<.001	<.001	-3,456667	,128625

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
ABTS_3	Equal variances assumed	-2,897232	-1,068768
	Equal variances not assumed	-3,395315	-,570685
ORAC_3	Equal variances assumed	-3,813787	-3,099546
	Equal variances not assumed	-3,961766	-2,951567

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
ABTS_3	Cohen's d	,403286	-4,917	-8,526	-1,258
	Hedges' correction	,505444	-3,923	-6,803	-1,003
	Glass's delta	,569861	-3,480	-6,992	,009
ORAC_3	Cohen's d	,157533	-21,942	-36,698	-7,527
	Hedges' correction	,197438	-17,508	-29,281	-6,005
	Glass's delta	,217025	-15,927	-30,660	-2,406

a. The denominator used in estimating the effect sizes. Cohen's d uses the pooled standard deviation. Hedges' correction uses the pooled standard deviation, plus a correction factor. Glass's delta uses the sample standard deviation of the control (i.e., the second) group.

5.5. Anti-hypertensive activity

- **Explore**

Observations

Output Created		29-SEP-2023 10:48:37
Comments		
Entry	Active dataset	DataSet4
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	12
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=IC50 BY Sample/PLOT BOXPLOT STEMLEAF NPPLOT/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING LISTWISE/NOTOTAL.
Resources	Processor time	00:00:00,52
	Elapsed time	00:00:01,03

Case Processing Summary

		Cases					
		Valid		Silent		Total	
	Sample	N	Percentage	N	Percentage	N	Percentage
IC50	3%60º_A	3	100,0%	0	0,0%	3	100,0%
	3%60º_B	3	100,0%	0	0,0%	3	100,0%
	H1%_A_P	3	100,0%	0	0,0%	3	100,0%
	H1%_B_P	3	100,0%	0	0,0%	3	100,0%

Descriptive

	Sample		Statistics	Pattern Statistics
IC50	3%60º_A	Average	849,50300	50,624894
	95% Confidence Interval to Mean	Lower Limit	631,68166	
		Upper limit	1067,32434	
	5% of average trimmed		.	
	Median		863,18600	
	Variance		7688,640	
	Standard Error		87,684888	
	Minimal		755,781	
	Maximum		929,542	
	Amplitude		173,761	
	Interquartile range		.	
	Asymmetry		-,685	1,225
	Kurtosis		.	.
	3%60º_B	Average	803,20233	7,734162
	95% Confidence Interval to Mean	Lower Limit	769,92492	
		Upper limit	836,47975	
	5% of average trimmed		.	
	Median		798,29000	
	Variance		179,452	
	Standard Error		13,395961	
	Minimal		792,956	
	Maximum		818,361	
	Amplitude		25,405	
	Interquartile range		.	
	Asymmetry		1,428	1,225
	Kurtosis		.	.
	H1%_A_P	Average	1167,81867	100,823182
	95% Confidence Interval to Mean	Lower Limit	734,01153	
		Upper limit	1601,62581	
	5% of average trimmed		.	
	Median		1204,46700	
	Variance		30495,942	
	Standard Error		174,630874	
	Minimal		977,772	
	Maximum		1321,217	
	Amplitude		343,445	
	Interquartile range		.	

		Asymmetry		-,903	1,225
		Kurtosis		.	.
	H1%_B_P	Average		949,64633	46,590361
		95% Confidence Interval to Mean	Lower Limit	749,18419	
			Upper limit	1150,10848	
		5% of average trimmed		.	
		Median		911,97600	
		Variance		6511,985	
		Standard Error		80,696872	
		Minimal		894,673	
		Maximum		1042,290	
		Amplitude		147,617	
		Interquartile range		.	
		Asymmetry		1,643	1,225
		Kurtosis		.	.

M Estimators

	Sample	Hubera Estimator M	Tukeyb bi-weighting	Hampelc Estimator M	Andrewsd Wave
IC50	3%60º_A	852,95484	852,83563	849,50300	852,85941
	3%60º_B	799,05755	797,14577	799,56650	797,04995
	H1%_A_P	1186,04475	1180,26889	1167,81867	1180,29779
	H1%_B_P	914,66942	903,34308	905,17663	903,34366

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

		Sample		Case number	Value
IC50	3%60º_A	Upper	1	1	929,542
		Lower	1	2	755,781
	3%60º_B	Upper	1	6	818,361
		Lower	1	5	792,956
	H1%_A_P	Upper	1	7	1321,217
		Lower	1	8	977,772
	H1%_B_P	Upper	1	12	1042,290
		Lower	1	10	894,673

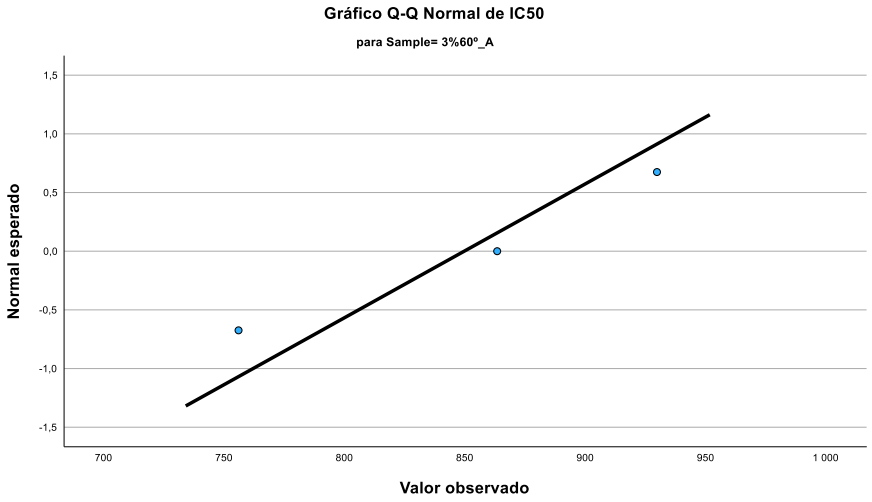
a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

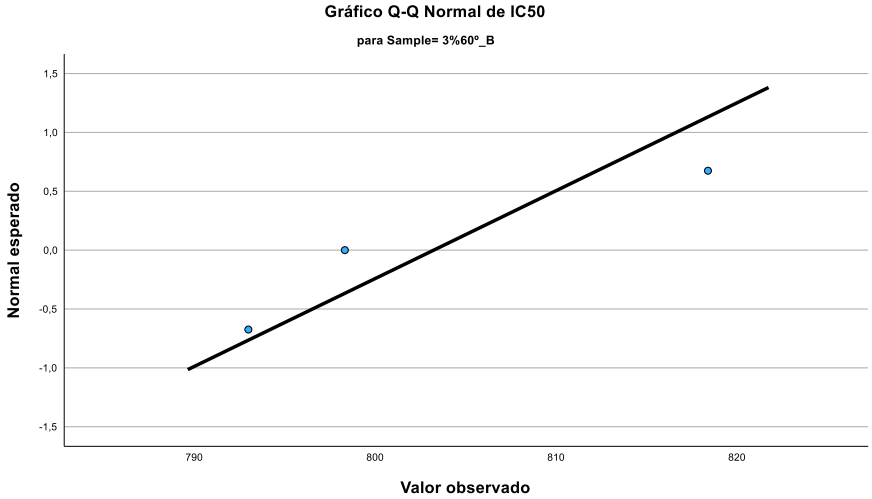
Normality Tests

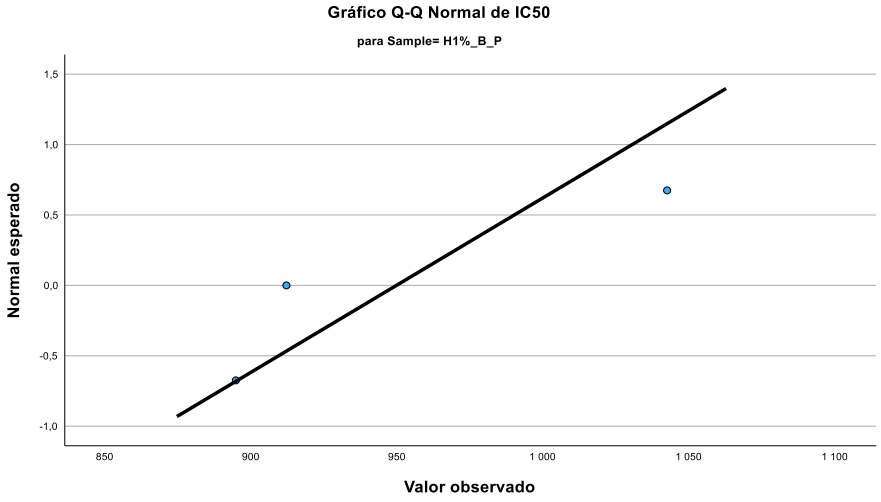
		Kolmogorov-Smirnova			Shapiro-Wilk		
	Sample	Statistics	gl	Sig.	Statistics	gl	Sig.
IC50	3%60º_A	,229	3	.	,982	3	,741
	3%60º_B	,310	3	.	,899	3	,383
	H1%_A_P	,250	3	.	,967	3	,651
	H1%_B_P	,346	3	.	,837	3	,205

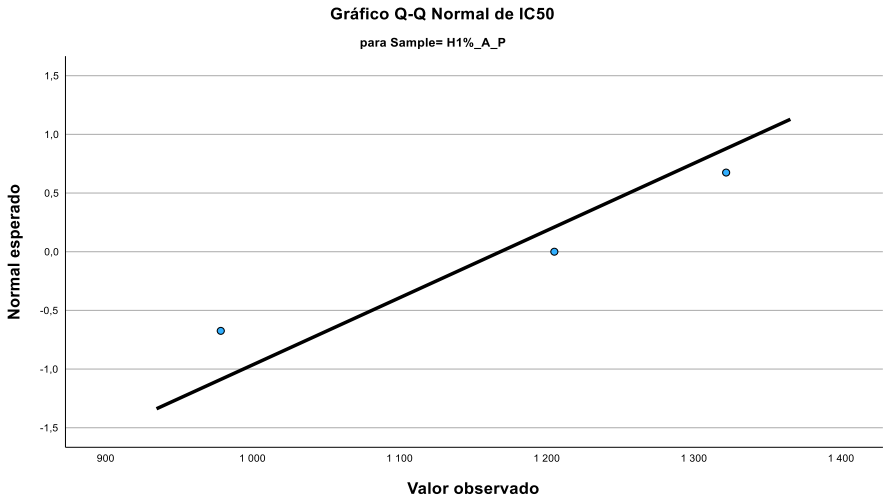
a. Lilliefors Significance Correlation

- **Normal Q-Q Chart**

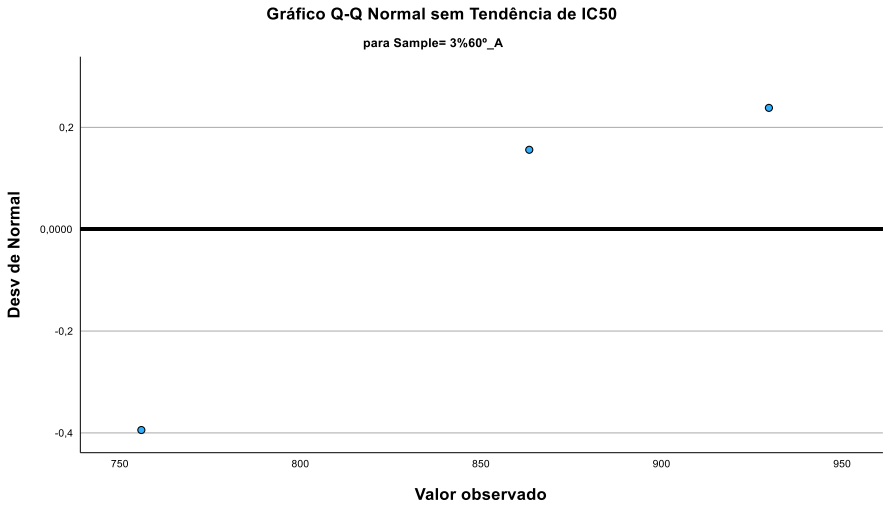


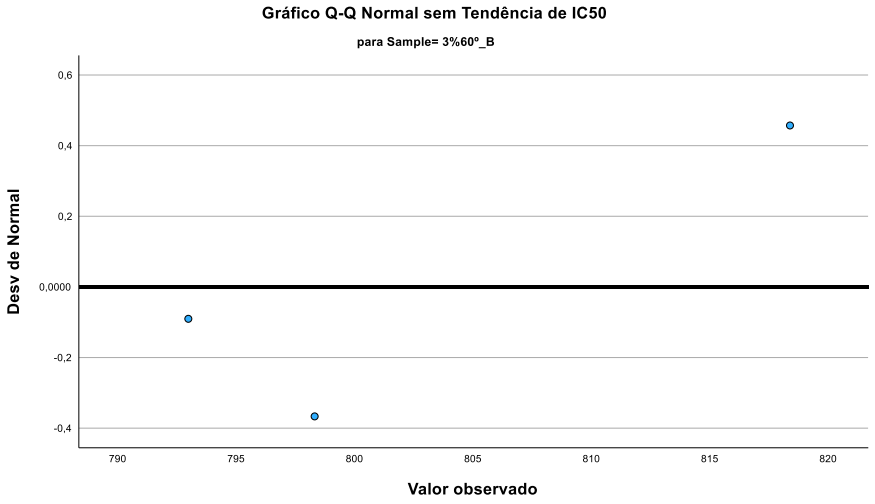






- **Normal Q-Q Chart No Trend**





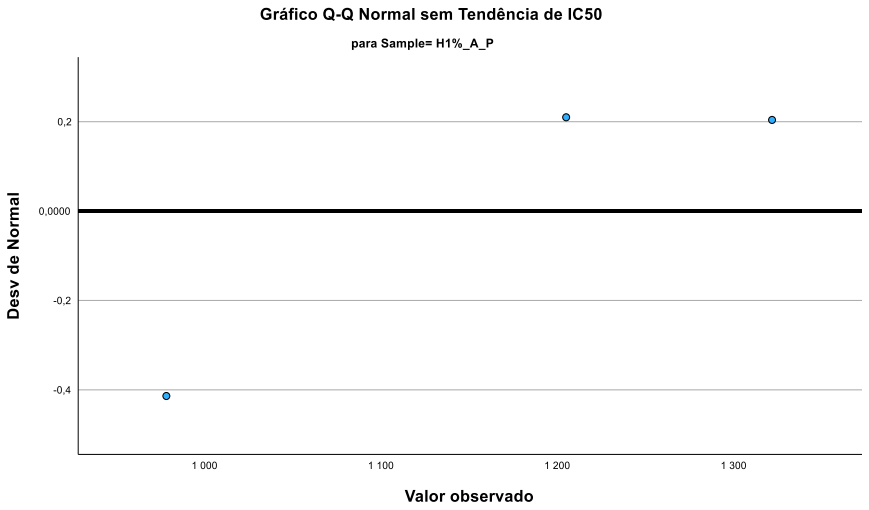


Gráfico Q-Q Normal sem Tendência de IC50
para Sample= H1%_A_P
Desv de Normal
0,2
0,0000
-0,2
-0,4
1 000
1 100
1 200
1 300
Valor observado

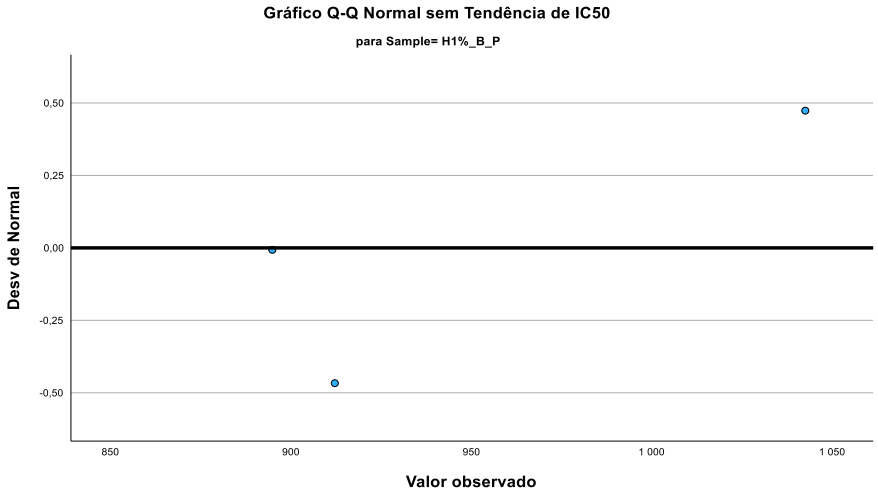
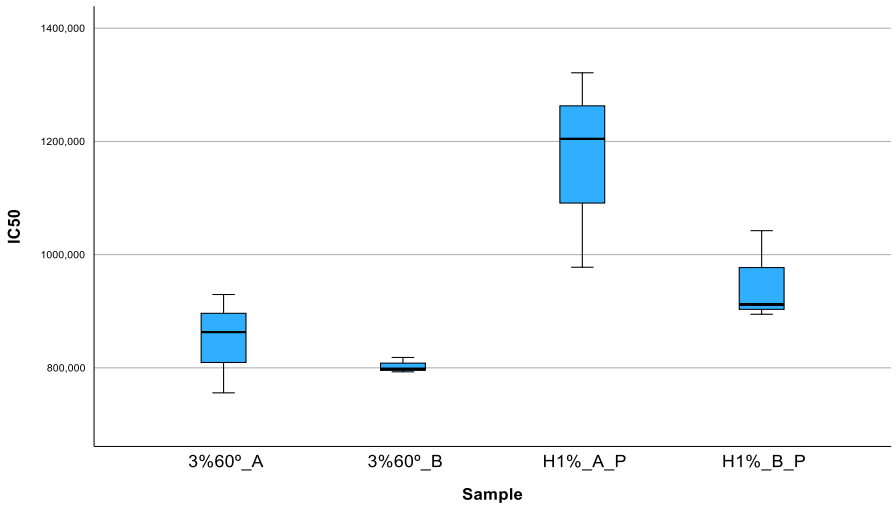


Gráfico Q-Q Normal sem Tendência de IC50
para Sample= H1%_B_P
Desv de Normal
0,50
0,25
0,00
-0,25
-0,50
850
900
950
1 000
1 050
Valor observado


IC50
1400,000
1200,000
1000,000
800,000
3%60º_A
3%60º_B
H1%_A_P
H1%_B_P
Sample

- **T-Test**

Observations

Output Created		29-SEP-2023 10:50:02
Comments		
Entry	Active dataset	DataSet4
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	12
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample(1 2)/MISSING=ANALYSIS/VARIABLES=IC50/ES DISPLAY(TRUE)/CRITERIA=CI(.95).
Resources	Processor time	00:00:00,00
	Elapsed time	00:00:00,01

Group Stats

	Sample	N	Average	Standard deviation	Standard Mean Error
IC50	3%60º_A	3	849,50300	87,684888	50,624894
	3%60º_B	3	803,20233	13,395961	7,734162

Independent Sample Testing

		Levene's test for equality of variances		t-test for Equality of Means	
		With	Sig.	t	df
IC50	Equal variances assumed	4,427	,103	,904	4
	Equal variances not assumed			,904	2,093

Independent Sample Testing

		t-test for Equality of Means			
		Significance			Standard
		Unilateral p	Bilateral p	Mean difference	Difference Error
IC50	Equal variances assumed	,209	,417	46,300667	51,212275
	Equal variances not assumed	,229	,458	46,300667	51,212275

Independent Sample Testing

		t-test for Equality of Means	
		95% Confidence Interval of Difference	
		Inferior	Superior
IC50	Equal variances assumed	-95,887404	188,488738
	Equal variances not assumed	-164,898178	257,499512

Independent Sample Effect Sizes

		Standardizer	Point Estimation	95% Confidence Interval Inferior	Superior
IC50	Cohen's d	62,721971	,738	-,977	2,373
	Hedges Correction	78,610334	,589	-,779	1,893
	Glass Delta	13,395961	3,456	-,016	6,949

a. The denominator used in estimating effect sizes. Cohen's d uses the clustered standard deviation. Hedges correction uses the clustered standard deviation in addition to a correction factor. The Glass delta uses the sample standard deviation of the control group (i.e., the second) group.

- **Teste-T**

Observations

Output Created		29-SEP-2023 10:50:38
Comments		
Entrance	Active dataset	DataSet4
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	12
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample(3 4)/MISSING=ANALYSIS/VARIABLES=IC50/ES DISPLAY(TRUE)/CRITERIA=CI(.95).
Resources	Processor time	00:00:00,00
	Elapsed time	00:00:00,00

Group Stats

	Sample	N	Average	Standard deviation	Standard Mean Error
IC50	H1%_A_P	3	1167,81867	174,630874	100,823182
	H1%_B_P	3	949,64633	80,696872	46,590361

Independent Sample Testing

		Levene's test for equality of variances		t-test for Equality of Means	
		With	Sig.	t	df
IC50	Equal variances assumed	1,755	,256	1,964	4
	Equal variances not assumed			1,964	2,817

Independent Sample Testing

		t-test for Equality of Means			
		Significance			Standard
		Unilateral p	Bilateral p	Mean difference	Difference Error
IC50	Equal variances assumed	,060	,121	218,172333	111,067438
	Equal variances not assumed	,075	,150	218,172333	111,067438

Independent Sample Testing

		t-test for Equality of Means	
		95% Confidence Interval of Difference	
		Inferior	Superior
IC50	Equal variances assumed	-90,200312	526,544978
	Equal variances not assumed	-148,654130	584,998797

Independent Sample Effect Sizes

				95% Confidence Interval	
		Standardizer	Point Estimation	Inferior	Superior
IC50	Cohen's d	136,029275	1,604	-,386	3,467
	Hedges Correction	170,487414	1,280	-,308	2,766
	Glass Delta	80,696872	2,704	-,239	5,582

a. The denominator used in estimating effect sizes. Cohen's d uses the clustered standard deviation. Hedges correction uses the clustered standard deviation in addition to a correction factor. The Glass delta uses the sample standard deviation of the control group (i.e., the second) group.

- **Teste-T**

Observations

Output Created		29-SEP-2023 10:52:11
Comments		
Entrance	Active dataset	DataSet4
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	12
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	The statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Sample_bis(12)/MISSING=ANALYSIS/VARIABLES=IC50/ES DISPLAY(TRUE)/CRITERIA=CI(.95).
Resources	Processor time	00:00:00,00
	Elapsed time	00:00:00,00

Group Stats

	Sample_bis	N	Average	Standard deviation	Standard Mean Error
IC50	3%60º	6	826,35267	61,565917	25,134180
	H1%_P	6	1058,73250	170,537155	69,621502

Independent Sample Testing

		Levene's test for equality of variances		t-test for Equality of Means	
		With	Sig.	t	df
IC50	Equal variances assumed	5,964	,035	-3,139	10
	Equal variances not assumed			-3,139	6,282

Independent Sample Testing

		t-test for Equality of Means			
		Significance			Standard
		Unilateral p	Bilateral p	Mean difference	Difference Error
IC50	Equal variances assumed	,005	,011	-232,379833	74,019460
	Equal variances not assumed	,009	,019	-232,379833	74,019460

Independent Sample Testing

		t-test for Equality of Means	
		95% Confidence Interval of Difference	
		Inferior	Superior
IC50	Equal variances assumed	-397,305469	-67,454198
	Equal variances not assumed	-411,549318	-53,210348

Independent Sample Effect Sizes

		Standardizer	Point Estimation	95% Confidence Interval	
				Inferior	Superior
IC50	Cohen's d	128,205466	-1,813	-3,159	-,406
	Hedges Correction	138,939124	-1,673	-2,915	-,375
	Glass Delta	170,537155	-1,363	-2,719	,077

a. The denominator used in estimating effect sizes. Cohen's d uses the clustered standard deviation. Hedges correction uses the clustered standard deviation in addition to a correction factor. The Glass delta uses the sample standard deviation of the control group (i.e., the second) group.

5.6. Free amino groups

- Explore

Observations

Output Created		10-NOV-2023 15:50:15
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\OKS\OKS1.sav
	Active dataset	DataSet1
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	36
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Freeaminogroups BY TNBS1/PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING LISTWISE/NOTOTAL.
Resources	Processor time	00:00:02,31
	Elapsed time	00:00:01,75

Case Processing Summary

		Cases				
		Valid		Silent		Total
	TNBS1	N	Percentage	N	Percentage	N
Free amino groups (%)	H3%_DS	9	100,0%	0	0,0%	9
	3%60º	9	100,0%	0	0,0%	9
	H3%_P	8	88,9%	1	11,1%	9
	H3%_L	8	88,9%	1	11,1%	9

Case Processing Summary

		Cases	
		Total	
	TNBS1	Percentage	
Free amino groups (%)	H3%_DS	100,0%	
	3%60º	100,0%	

	H3%_P	100,0%
	H3%_L	100,0%

Descriptive

	TNBS1			Statistics	Pattern Statistics
Free amino groups (%)	H3%_DS	Average		59,067	4,5220
		95% Confidence Interval to Mean	Lower Limit	48,639	
			Upper limit	69,494	
		5% of average trimmed		58,757	
		Median		58,700	
		Variance		184,033	
		Standard Error		13,5659	
		Minimal		39,8	
		Maximum		83,9	
		Amplitude		44,1	
		Interquartile range		20,3	
		Asymmetry		,438	,717
		Kurtosis		,191	1,400
	3%60º	Average		32,689	3,5531
		95% Confidence Interval to Mean	Lower Limit	24,495	
			Upper limit	40,882	
		5% of average trimmed		32,627	
		Median		33,000	
		Variance		113,624	
		Standard Error		10,6594	
		Minimal		19,3	
		Maximum		47,2	
		Amplitude		27,9	
		Interquartile range		22,3	
		Asymmetry		-,141	,717
		Kurtosis		-1,536	1,400
	H3%_P	Average		34,025	4,5931
		95% Confidence Interval to Mean	Lower Limit	23,164	
			Upper limit	44,886	
		5% of average trimmed		32,961	
		Median		30,600	
		Variance		168,771	
		Standard Error		12,9912	
		Minimal		22,5	

		Maximum		64,7	
		Amplitude		42,2	
		Interquartile range		7,5	
		Asymmetry		2,316	,752
		Kurtosis		6,027	1,481
	H3%_L	Average		11,825	,9147
		95% Confidence Interval to Mean	Lower Limit	9,662	
			Upper limit	13,988	
		5% of average trimmed		11,906	
		Median		13,400	
		Variance		6,694	
		Standard Error		2,5872	
		Minimal		7,9	
		Maximum		14,3	
		Amplitude		6,4	
		Interquartile range		4,7	
		Asymmetry		-,769	,752
		Kurtosis		-1,544	1,481

M Estimators

	TNBS1	Huber's M estimator[at]	Tukey biweighting[b]	Hampel M estimator[c]
Free amino groups (%)	H3%_DS	58,488	57,035	58,259
	3%60º	32,689	32,801	32,689
	H3%_P	30,884	30,001	29,893
	H3%_L	13,123	13,623	13,565

M Estimators

	TNBS1	Andrews Wave[d]
Free amino groups (%)	H3%_DS	56,716
	3%60º	32,803
	H3%_P	29,999
	H3%_L	13,623

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500.
d. The weighting constant is 1.340*pi.

Extreme Values[a]

	TNBS1			Case number	Value
Free amino groups (%)	H3%_DS	Upper	1	4	83,9
			2	9	72,0
			3	6	65,3
			4	8	59,1
		Lower	1	5	39,8
			2	1	43,9
			3	3	52,8
			4	7	56,1
	3%60º	Upper	1	13	47,2
			2	15	44,1
			3	14	41,1
			4	12	35,9
		Lower	1	16	19,3
			2	17	19,7
			3	18	20,9
			4	11	33,0b
	H3%_P	Upper	1	19	64,7
			2	24	35,1
			3	23	32,8
			4	21	30,8
		Lower	1	25	22,5
			2	26	26,0
			3	27	29,9
			4	22	30,4
	H3%_L	Upper	1	31	14,3
			2	29	13,6
			3	28	13,5
			4	30	13,5
		Lower	1	36	7,9
			2	34	8,5
			3	35	10,0
			4	33	13,3

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed. b. Only a partial list of cases with a value of 33.0 is shown in the table of lower extremes.

Normality Tests

		Kolmogorov-Smirnova			Shapiro-Wilk	
	TNBS1	Statistics	gl	Sig.	Statistics	gl
Free amino groups (%)	H3%_DS	,166	9	,200*	,972	9
	3%60º	,199	9	,200*	,901	9
	H3%_P	,342	8	,006	,709	8
	H3%_L	,341	8	,007	,797	8

Normality Tests

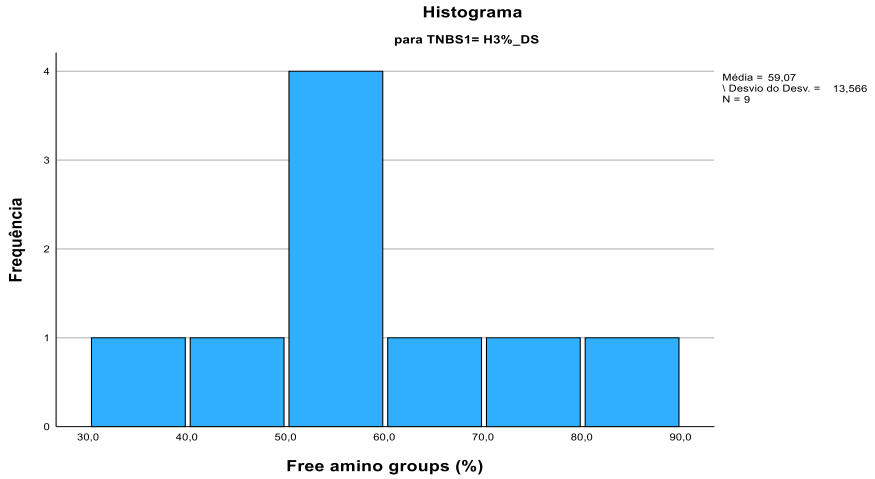
		Shapiro-Wilk
	TNBS1	Sig.
Free amino groups (%)	H3%_DS	,914
	3%60º	,257

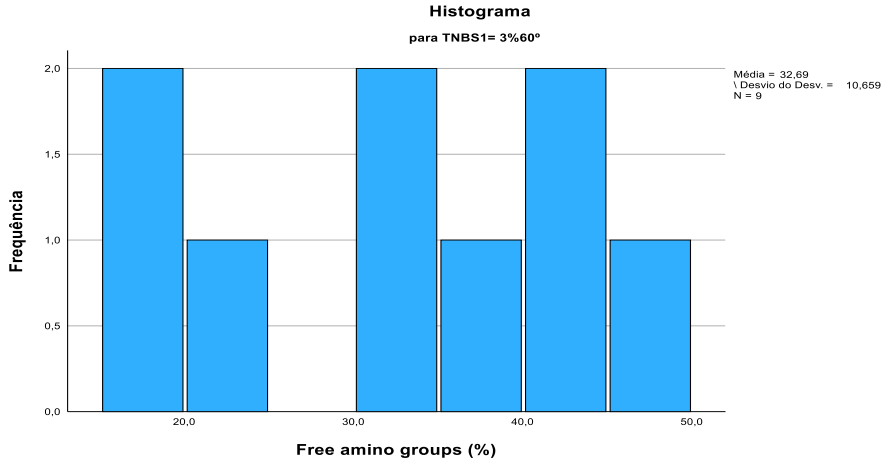
	H3%_P	,003
	H3%_L	,026

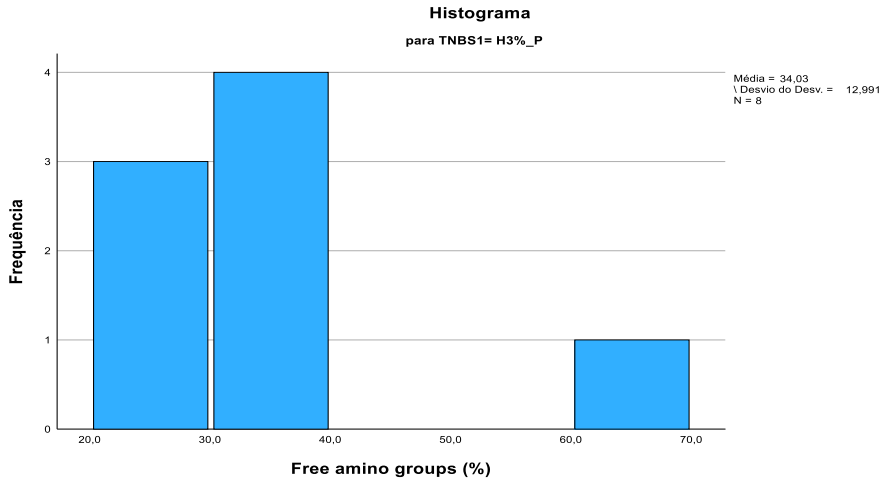
*. This is a lower bound of true significance.

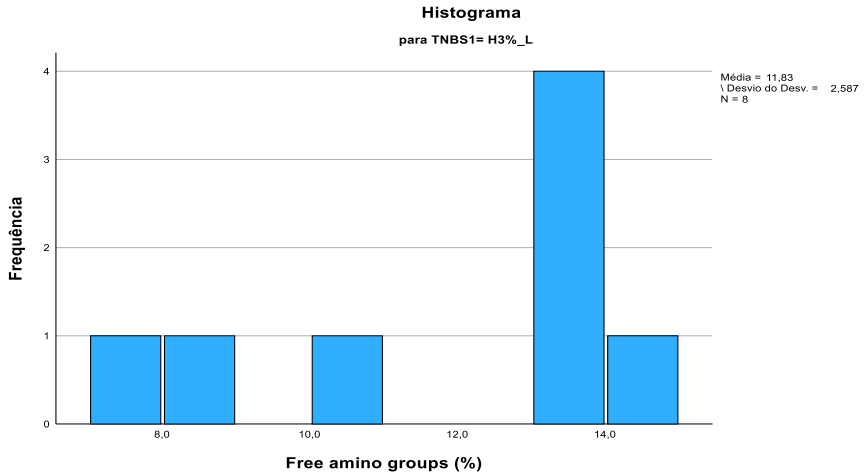
a. Lilliefors Significance Correlation

- Histograms

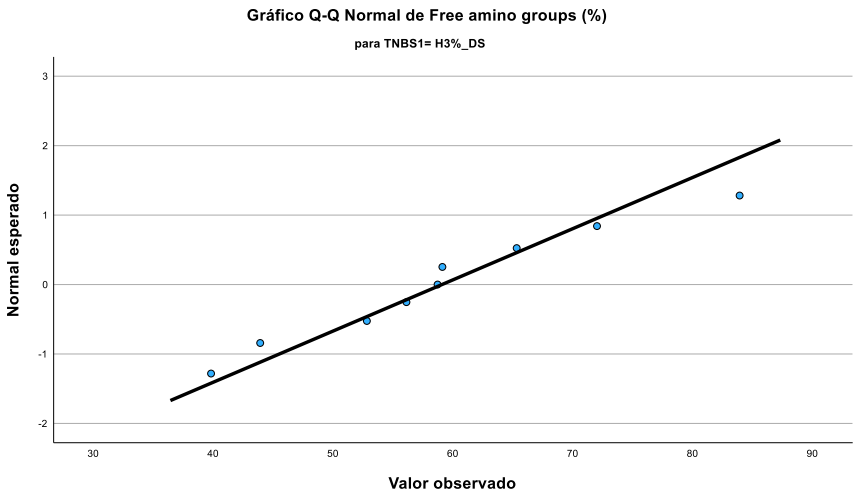








- Normal Q-Q Chart



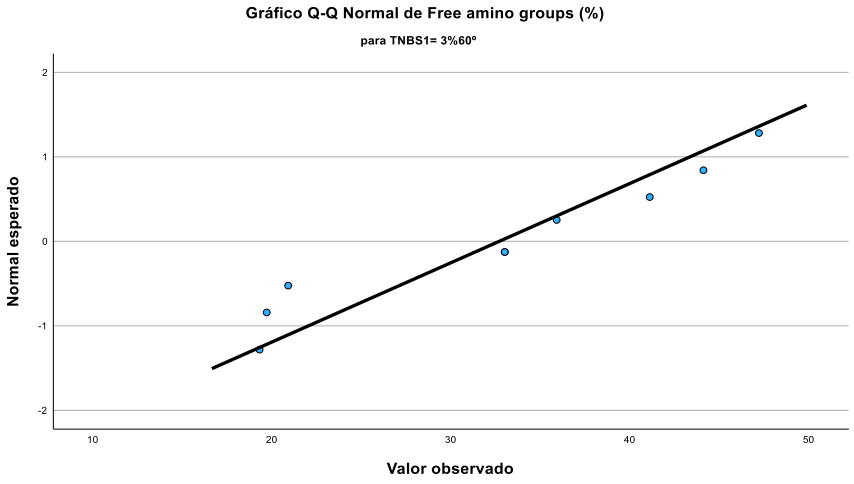


Gráfico Q-Q Normal de Free amino groups (%)
para TNBS1= 3%60°
Normal esperado
Valor observado
2
1
0
-1
-2
10
20
30
40
50

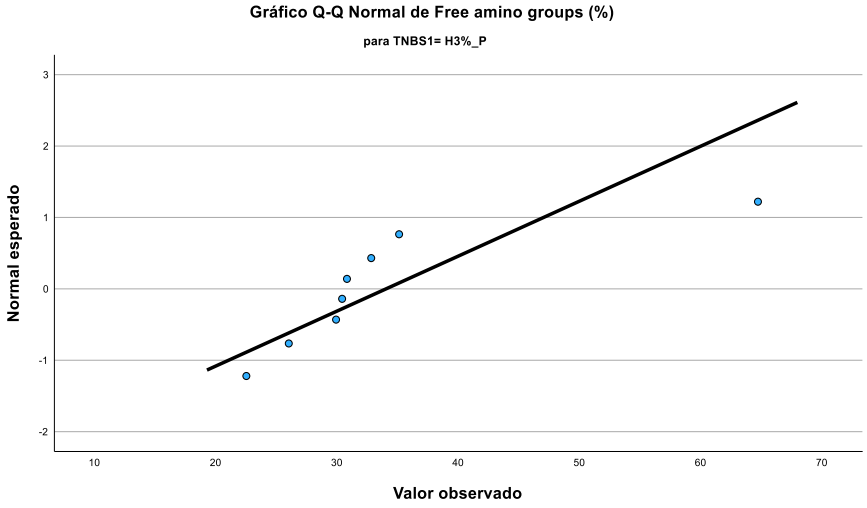


Gráfico Q-Q Normal de Free amino groups (%)
para TNBS1= H3%_P
Normal esperado
Valor observado
3
2
1
0
-1
-2
10
20
30
40
50
60
70

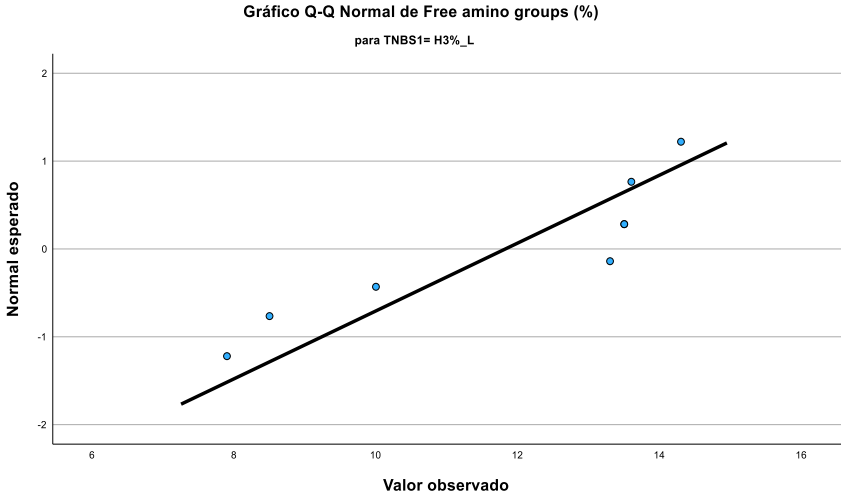
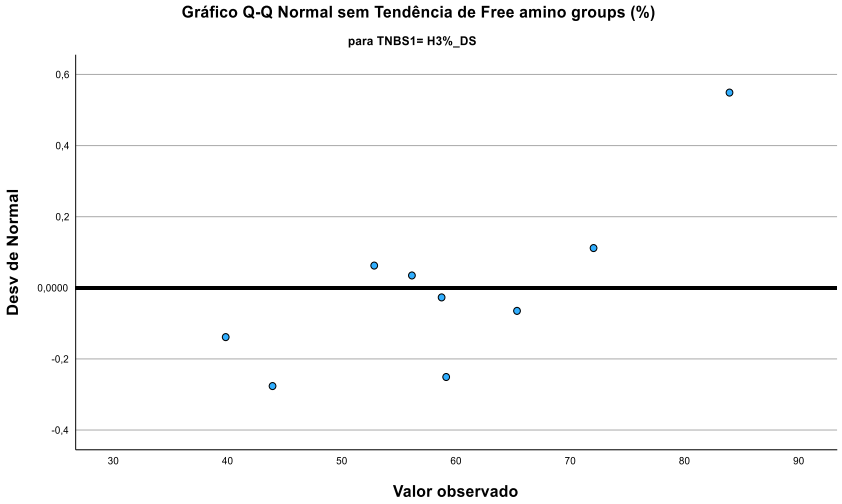
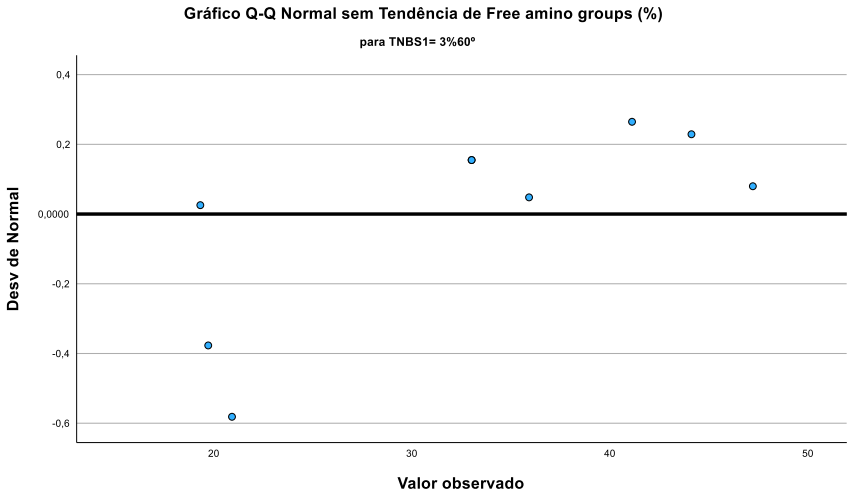
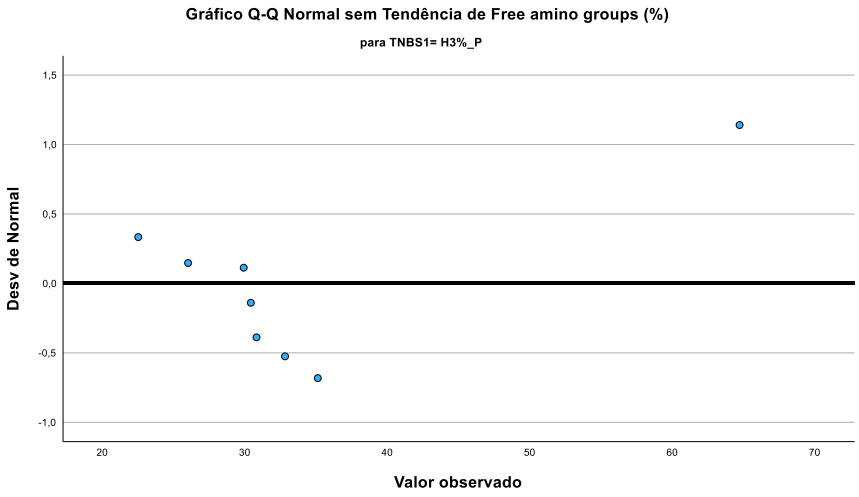


Gráfico Q-Q Normal de Free amino groups (%)
para TNBS1= H3%_L
Normal esperado
Valor observado
2
1
0
-1
-2
6
8
10
12
14
16

- Normal Q-Q Chart No Trend







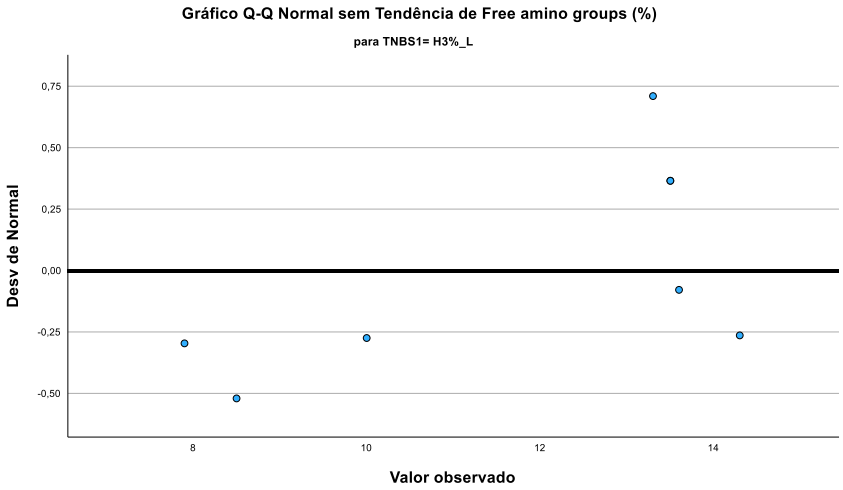
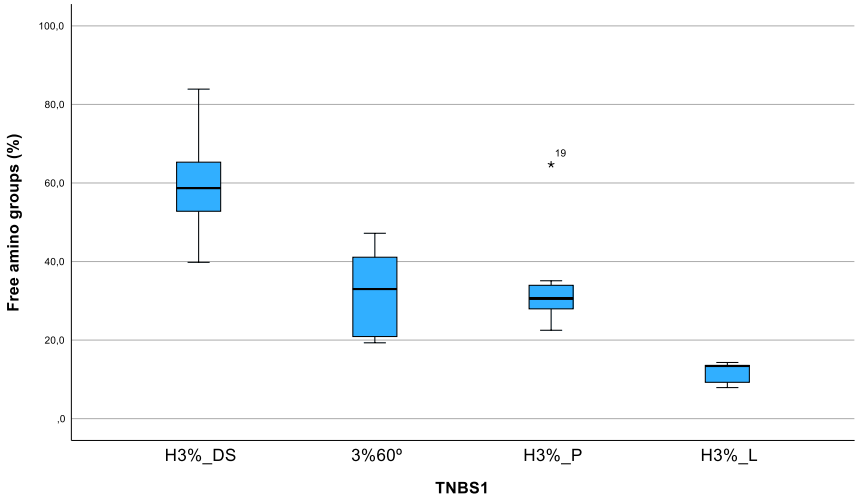


Gráfico Q-Q Normal sem Tendência de Free amino groups (%)
para TNBS1= H3%_L
Desv de Normal
0,75
0,50
0,25
0,00
-0,25
-0,50
8
10
12
14
Valor observado


Free amino groups (%)
100,0
80,0
60,0
40,0
20,0
,0
19
H3%_DS
3%60º
H3%_P
H3%_L
TNBS1

- Explore

Observations

Output Created		10-NOV-2023 15:51:16
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\OKS\OKS1.sav
	Active dataset	DataSet1
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	35
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Freeaminogroups BY TNBS1/PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING LISTWISE/NOTOTAL.
Resources	Processor time	00:00:00,91
	Elapsed time	00:00:01,18

Case Processing Summary

		Cases				
		Valid		Silent		Total
	TNBS1	N	Percentage	N	Percentage	N
Free amino groups (%)	H3%_DS	9	100,0%	0	0,0%	9
	3%60º	9	100,0%	0	0,0%	9
	H3%_P	7	87,5%	1	12,5%	8
	H3%_L	8	88,9%	1	11,1%	9

Case Processing Summary

		Cases
		Total
	TNBS1	Percentage
Free amino groups (%)	H3%_DS	100,0%
	3%60º	100,0%
	H3%_P	100,0%
	H3%_L	100,0%

Descriptive

	TNBS1			Statistics	Pattern Statistics
Free amino groups (%)	H3%_DS	Average		59,067	4,5220
		95% Confidence Interval to Mean	Lower Limit	48,639	
			Upper limit	69,494	
		5% of average trimmed		58,757	
		Median		58,700	
		Variance		184,033	
		Standard Error		13,5659	
		Minimal		39,8	
		Maximum		83,9	
		Amplitude		44,1	
		Interquartile range		20,3	
		Asymmetry		,438	,717
		Kurtosis		,191	1,400
	3%60º	Average		32,689	3,5531
		95% Confidence Interval to Mean	Lower Limit	24,495	
			Upper limit	40,882	
		5% of average trimmed		32,627	
		Median		33,000	
		Variance		113,624	
		Standard Error		10,6594	
		Minimal		19,3	
		Maximum		47,2	
		Amplitude		27,9	
		Interquartile range		22,3	
		Asymmetry		-,141	,717
		Kurtosis		-1,536	1,400
	H3%_P	Average		29,643	1,5888
		95% Confidence Interval to Mean	Lower Limit	25,755	
			Upper limit	33,530	
		5% of average trimmed		29,737	
		Median		30,400	
		Variance		17,670	
		Standard Error		4,2035	
		Minimal		22,5	
		Maximum		35,1	
		Amplitude		12,6	
		Interquartile range		6,8	

		Asymmetry		-,681	,794
		Kurtosis		,231	1,587
	H3%_L	Average		11,825	,9147
		95% Confidence Interval to Mean	Lower Limit	9,662	
			Upper limit	13,988	
		5% of average trimmed		11,906	
		Median		13,400	
		Variance		6,694	
		Standard Error		2,5872	
		Minimal		7,9	
		Maximum		14,3	
		Amplitude		6,4	
		Interquartile range		4,7	
		Asymmetry		-,769	,752
		Kurtosis		-1,544	1,481

M Estimators

	TNBS1	Huber's M estimator[at]	Tukey biweighting[b]	Hampel M estimator[c]
Free amino groups (%)	H3%_DS	58,488	57,035	58,259
	3%60º	32,689	32,801	32,689
	H3%_P	30,194	30,419	30,015
	H3%_L	13,123	13,623	13,565

M Estimators

	TNBS1	Andrews Wave[d]
Free amino groups (%)	H3%_DS	56,716
	3%60º	32,803
	H3%_P	30,417
	H3%_L	13,623

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500.
d. The weighting constant is 1.340*pi.

Extreme Values[a]

	TNBS1			Case number	Value
Free amino groups (%)	H3%_DS	Upper	1	4	83,9
			2	9	72,0
			3	6	65,3
			4	8	59,1
		Lower	1	5	39,8
			2	1	43,9
			3	3	52,8
			4	7	56,1
	3%60º	Upper	1	13	47,2
			2	15	44,1
			3	14	41,1
			4	12	35,9
		Lower	1	16	19,3
			2	17	19,7
			3	18	20,9
			4	11	33,0b
	H3%_P	Upper	1	23	35,1
			2	22	32,8
			3	20	30,8
		Lower	1	24	22,5
			2	25	26,0
			3	26	29,9
	H3%_L	Upper	1	30	14,3
			2	28	13,6
			3	27	13,5
			4	29	13,5
		Lower	1	35	7,9
			2	33	8,5
			3	34	10,0
			4	32	13,3

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

b. Only a partial list of cases with a value of 33.0 is shown in the table of lower extremes.

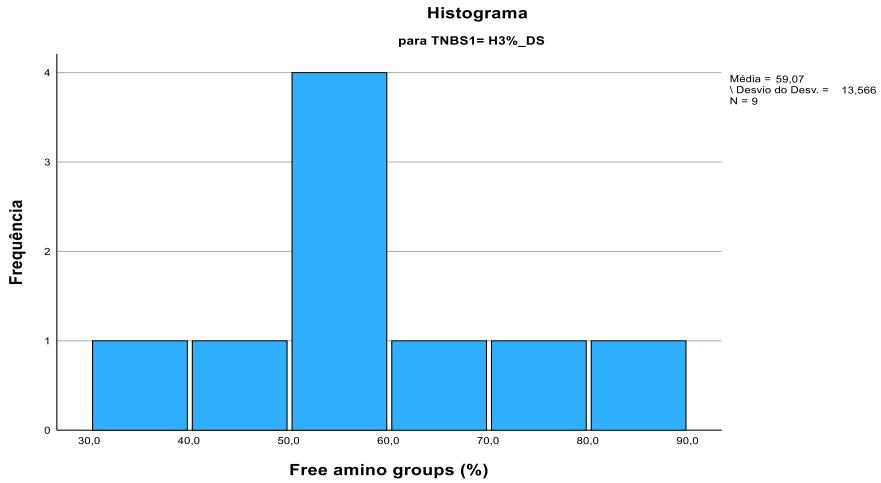
Normality Tests

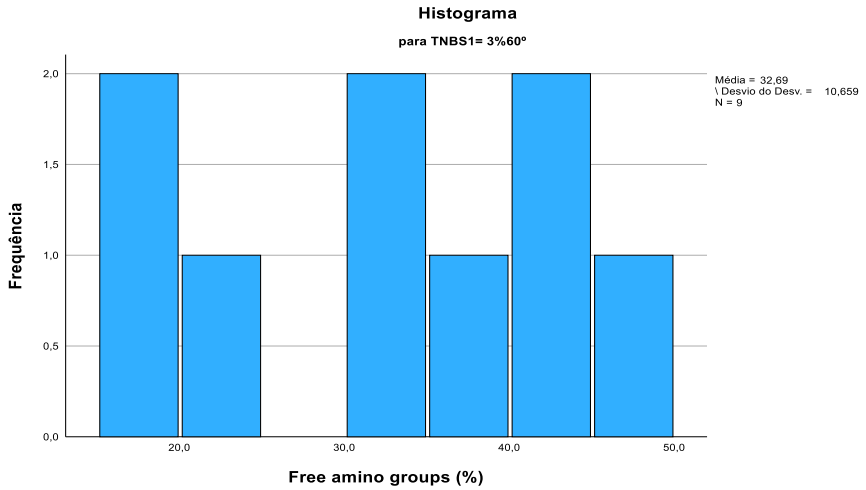
		Kolmogorov-Smirnova			Shapiro-Wilk	
	TNBS1	Statistics	gl	Sig.	Statistics	gl
Free amino groups (%)	H3%_DS	,166	9	,200*	,972	9
	3%60º	,199	9	,200*	,901	9
	H3%_P	,239	7	,200*	,951	7
	H3%_L	,341	8	,007	,797	8

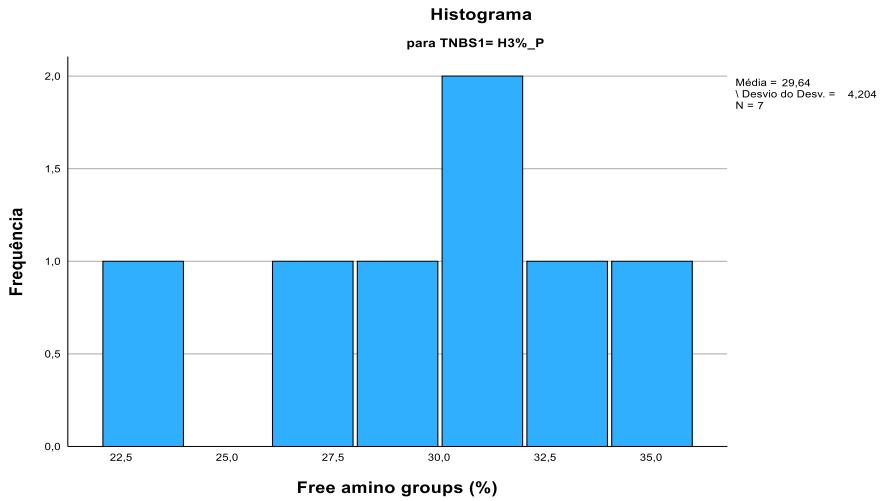
Normality Tests

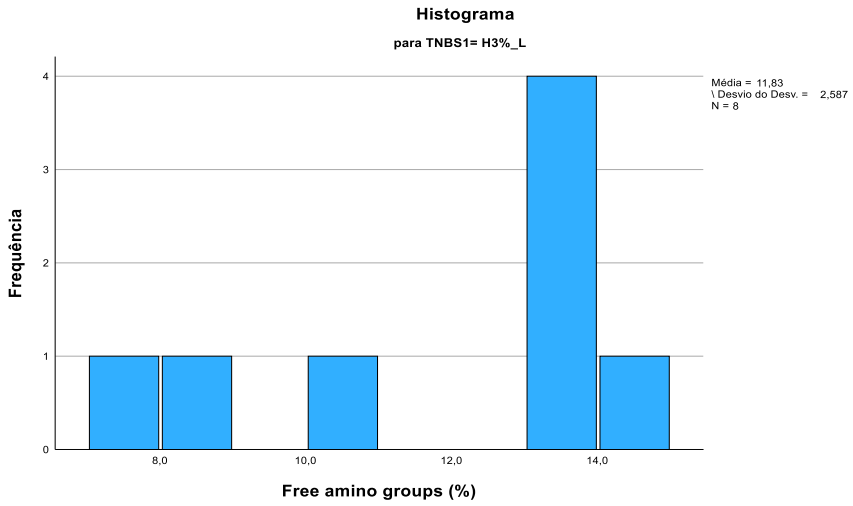
		Shapiro-Wilk
	TNBS1	Sig.
Free amino groups (%)	H3%_DS	,914
	3%60º	,257
	H3%_P	,735
	H3%_L	,026

- Histograms

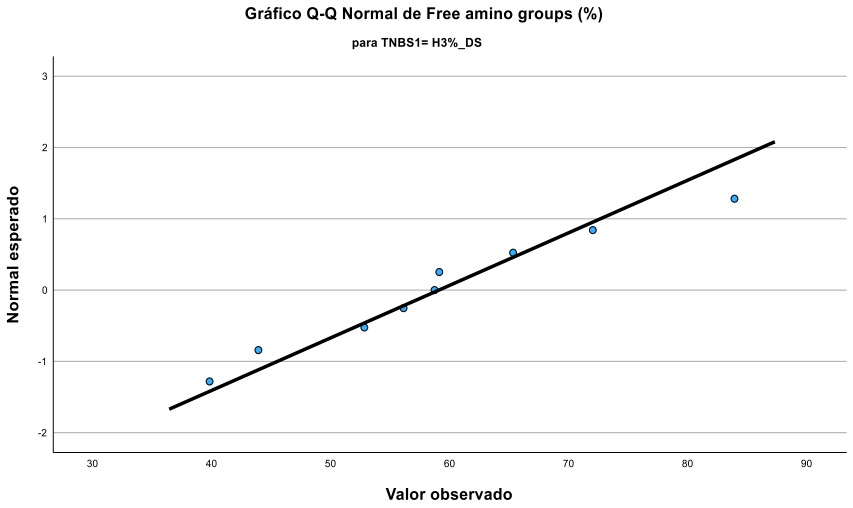

Histograma
para TNBS1= H3%_DS
Média = 59,07
\ Desvio do Desv. = 13,566
N = 9
Frequência
Free amino groups (%)

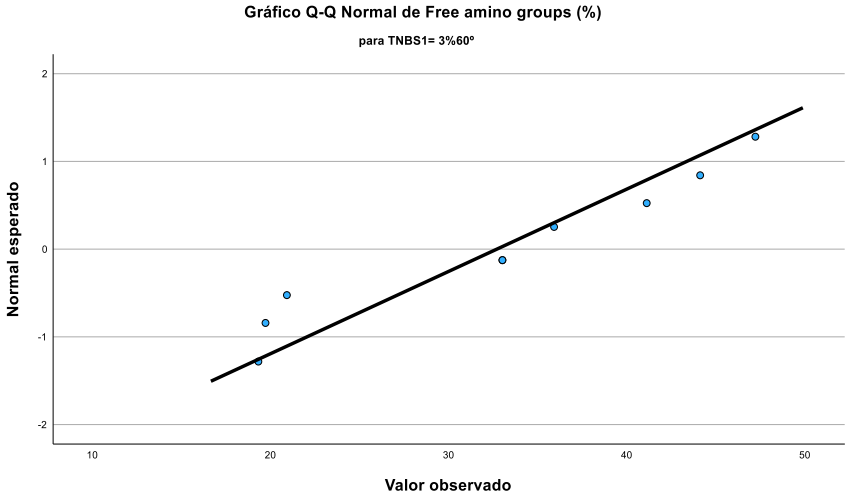

Histograma
para TNBS1= 3%60º
Média = 32,69
\ Desvio do Desv. = 10,659
N = 9
Frequência
Free amino groups (%)

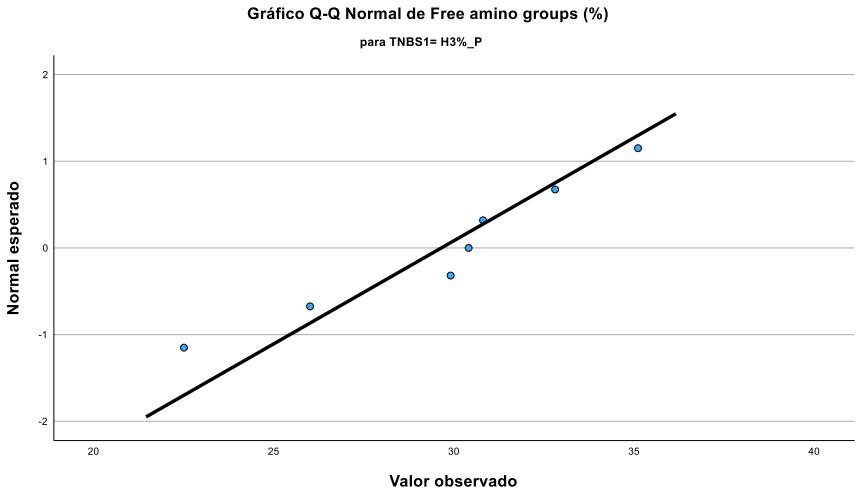

Histograma
para TNBS1= H3%_P
Média = 29,64
\ Desvio do Desv. = 4,204
N = 7
Frequência
Free amino groups (%)

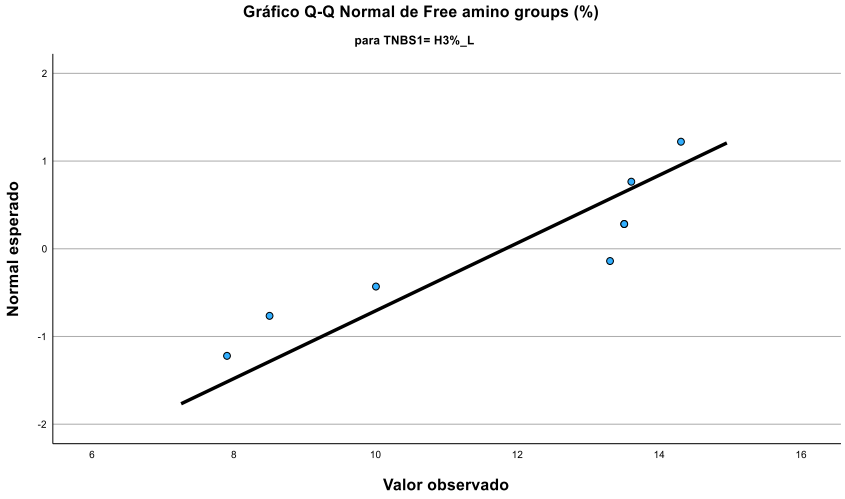


- Normal Q-Q Chart

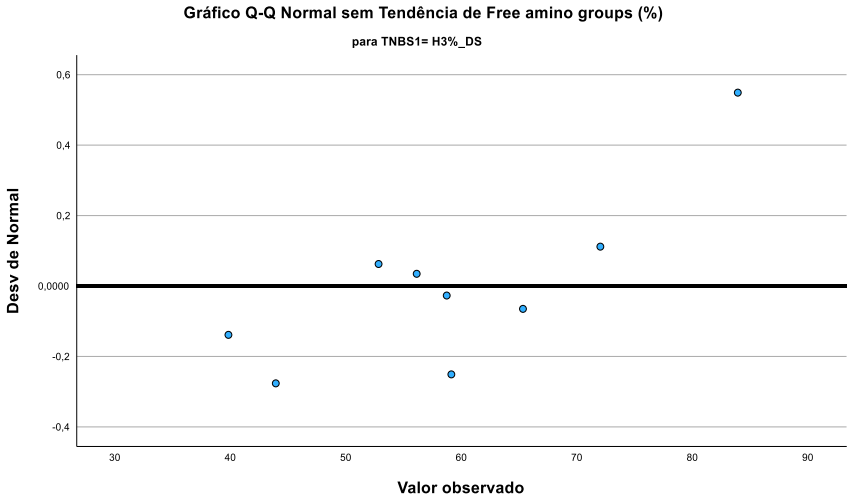








- Normal Q-Q Chart No Trend



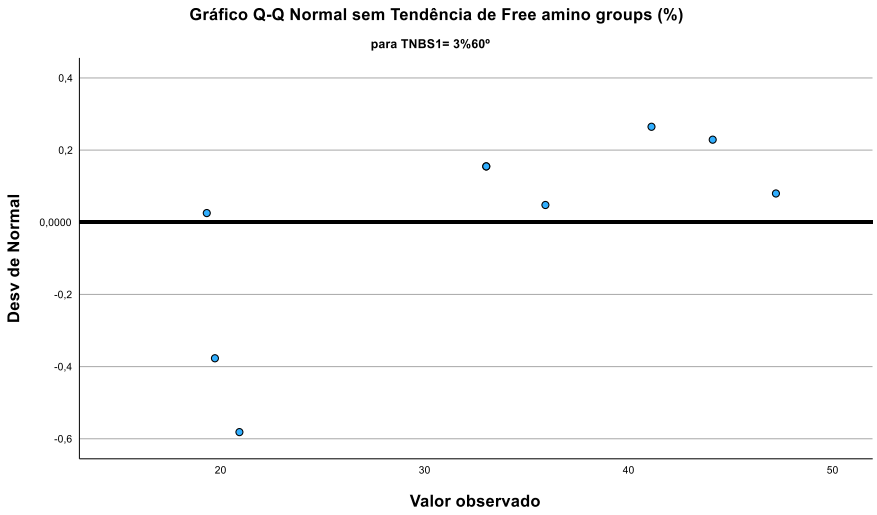


Gráfico Q-Q Normal sem Tendência de Free amino groups (%)
para TNBS1= 3%60º
Desv de Normal
0,4
0,2
0,0000
-0,2
-0,4
-0,6
20
30
40
50
Valor observado

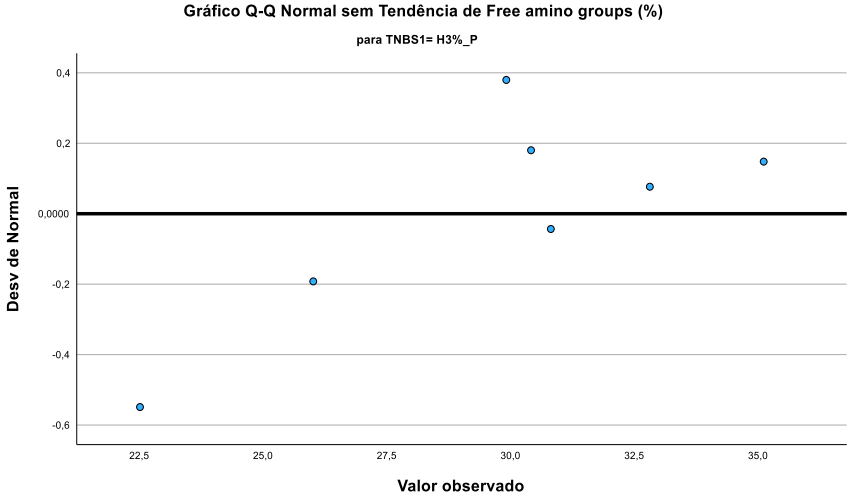


Gráfico Q-Q Normal sem Tendência de Free amino groups (%)
para TNBS1= H3%_P
Desv de Normal
0,4
0,2
0,0000
-0,2
-0,4
-0,6
22,5
25,0
27,5
30,0
32,5
35,0
Valor observado

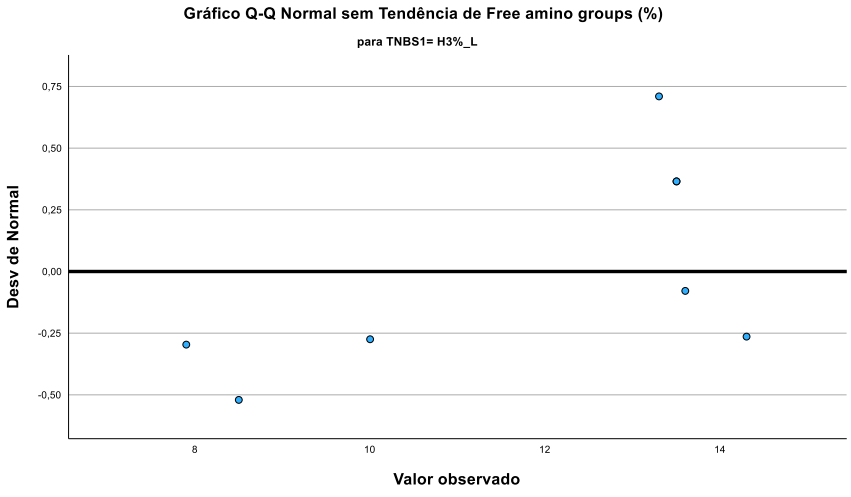
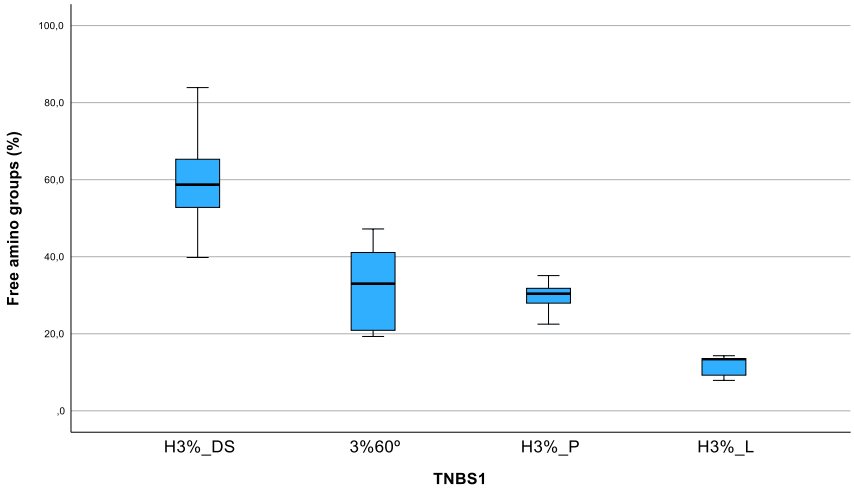


Gráfico Q-Q Normal sem Tendência de Free amino groups (%)
para TNBS1= H3%_L
Desv de Normal
0,75
0,50
0,25
0,00
-0,25
-0,50
8
10
12
14
Valor observado



- Univariate Analysis of Variance

Observations

Output Created		10-NOV-2023 15:52:14
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\OKS\OKS1.sav
	Active dataset	DataSet1
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	35
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Freeaminogroups BY TNBS1 /METHOD=SSTYPE(3)/INTERCEPT=INCLUDE/POSTHOC=TNBS1(TUKEY)/PLOT=PROFILE(TNBS1) TYPE=LINE ERRORBAR=NO MEANREFERENCE=NO YAXIS=AUTO /PRINT DESCRIPTIVE HOMOGENEITY/CRITERIA=ALPHA(.05)/DESIGN=TNBS1.
Resources	Processor time	00:00:00,06
	Elapsed time	00:00:00,10

Factors Between Subjects

		Value Label	N
TNBS1	1,00	H3%_DS	9
	2,00	3%60º	9
	3,00	H3%_P	7
	4,00	H3%_L	8

Descriptive Statistics

Dependent variable: Free amino groups (%)

TNBS1	Average	Pattern Statistics	N
H3%_DS	59,067	13,5659	9
3%60º	32,689	10,6594	9
H3%_P	29,643	4,2035	7
H3%_L	11,825	2,5872	8
Total	34,179	19,5818	33

Equality of variances test of Levene's error[a,b]

		Levene's Statistics	df1	df2
Free amino groups (%)	Based on average	3,733	3	29
	Based on median	3,714	3	29
	Based on median and adjusted gl	3,714	3	16,709
	Based on trimmed average	3,725	3	29

Equality of variances test of Levene's error[a,b]

		Sig.
Free amino groups (%)	Based on average	,022
	Based on median	,022
	Based on median and adjusted gl	,032
	Based on trimmed average	,022

Tests the null hypothesis that the variance of the error of the dependent variable is equal between groups.[a,b] a. Dependent variable: Free amino groups (%).b. Design: Interception + TNBS1

Tests of effects between subjects

Dependent variable: Free amino groups (%)

Origin	Type III Sum of Squares	df	Medium Square	With	Sig.
Corrected model	9736,194a	3	3245,398	37,140	<,001
Intercept	36215,517	1	36215,517	414,444	<,001
TNBS1	9736,194	3	3245,398	37,140	<,001
Pattern	2534,121	29	87,383		
Total	50820,570	33			
Corrected total	12270,315	32			

a. R Squared = .793 (Adjusted R Squared = .772)

- Posterior Testes

Multiple Comparisons

Dependent variable: Free amino groups (%)
Tukey HSD

(I) TNBS1	(J) TNBS1	Mean difference (I-J)	Pattern Statistics	Sig.	95% Confidence Interval	
					Lower Limit	Upper limit
H3%_DS	3%60º	26,378*	4,4066	<,001	14,372	38,384
	H3%_P	29,424*	4,7109	<,001	16,589	42,259
	H3%_L	47,242*	4,5423	<,001	34,866	59,617
3%60º	H3%_DS	-26,378*	4,4066	<,001	-38,384	-14,372
	H3%_P	3,046	4,7109	,916	-9,789	15,881
	H3%_L	20,864*	4,5423	<,001	8,488	33,239
H3%_P	H3%_DS	-29,424*	4,7109	<,001	-42,259	-16,589
	3%60º	-3,046	4,7109	,916	-15,881	9,789
	H3%_L	17,818*	4,8380	,005	4,637	30,999
H3%_L	H3%_DS	-47,242*	4,5423	<,001	-59,617	-34,866
	3%60º	-20,864*	4,5423	<,001	-33,239	-8,488
	H3%_P	-17,818*	4,8380	,005	-30,999	-4,637

Based on observed averages. The error term is Mean Square (Error) = 87.383.

*. The average difference is significant at the .05 level.

- Homogeneous subsets

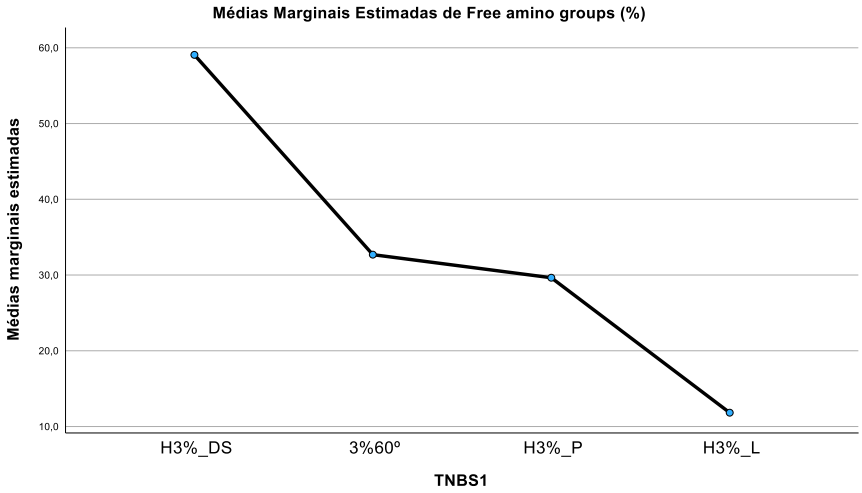
Free amino groups (%)

Tukey HSD[a,b,c]

TNBS1	N	Subset		
		1	2	3
H3%_L	8	11,825		
H3%_P	7		29,643	
3%60º	9		32,689	
H3%_DS	9			59,067
Say.		1,000	,912	1,000

Averages are displayed for the groups in subsets homogêneos.Com based on observed averages. The error term is Mean Square (Error) = 87.383. a. Uses the Harmonic Mean Sample Size = 8.162. b. Group sizes are uneven. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed. c. alpha = ,05.

- Profile Charts



- Non-parametric tests

Observations

Output Created		10-NOV-2023 15:52:45
Comments		
Entrance	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\OKS\OKS1.sav
	Active dataset	DataSet1
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	35
Syntax		NPTESTS/INDEPENDENT TEST (Freeaminogroups) GROUP (TNBS1)/MISSING SCOPE=ANALYSIS USERMISSING=EXCLUDE/CRITERIA ALPHA=0.05 CILEVEL=95.
Resources	Processor time	00:00:00,39
	Elapsed time	00:00:00,68

Hypothesis Testing Summarization

	Null hypothesis	Test	Sig.[a,b]
1	The distribution of Free amino groups (%) is the same in the TNBS1 categories.	Independent Kruskal-Wallis Test Specimens	<,001

Hypothesis Testing Summarization

	Decision
1	Reject the null hypothesis.

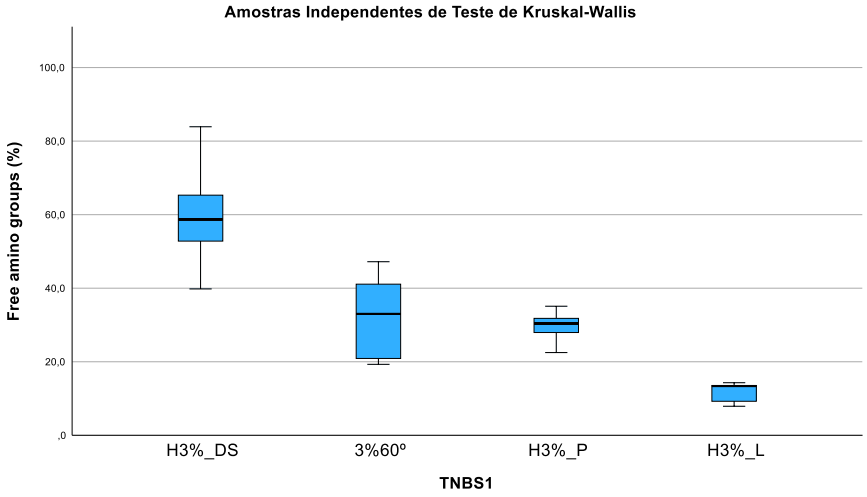
a. The significance level is .050. b. Asymptotic significance is displayed.

- Independent Kruskal-Wallis Test Specimens

Kruskal-Wallis Test Summary Independent Samples

N total	33
Test Statistics	26,301[a]
Degree of Freedom	3
Asymptotic signal (two-sided test)	<,001

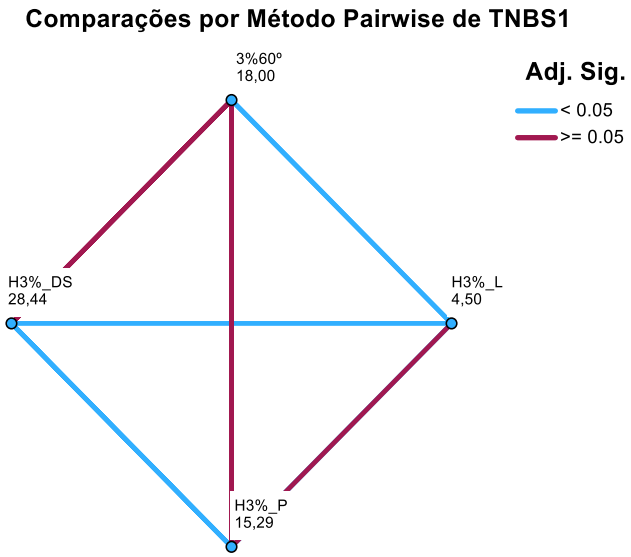
a. The test statistic is adjusted for draws.

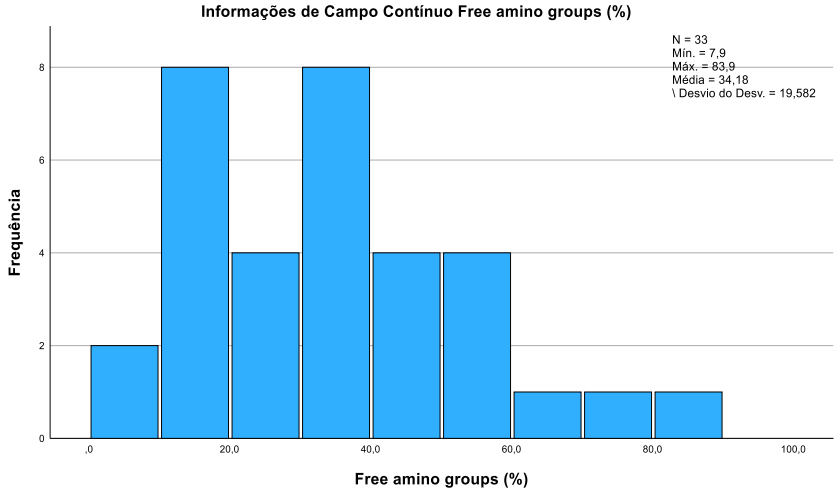


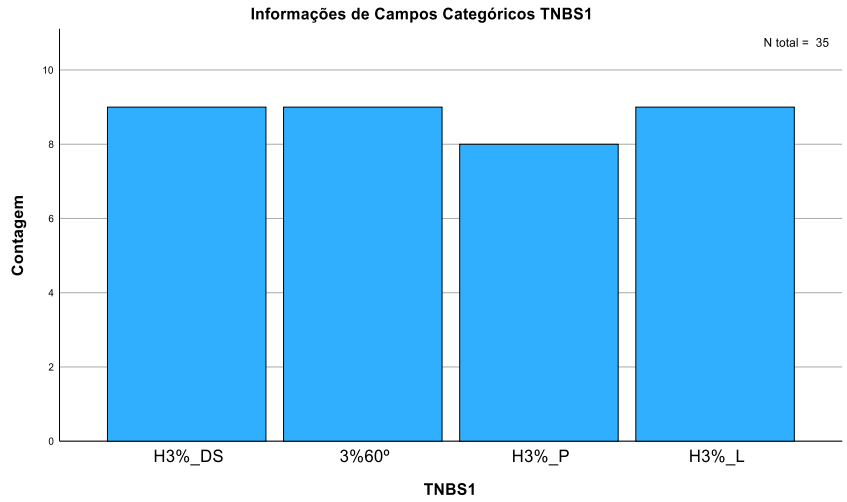
Pairwise Method Comparisons of TNBS1

Sample 1-Sample 2	Test Statistics	Standard Error	Pattern Statistics	Sig.	Adj. Sig.[a]
H3%_L-H3%_P	10,786	5,004	2,156	,031	,187
H3%_L-3%60º	13,500	4,698	2,874	,004	,024
H3%_L-H3%_DS	23,944	4,698	5,097	<,001	,000
H3%_P-3%60º	2,714	4,872	,557	,577	1,000
H3%_P-H3%_DS	13,159	4,872	2,701	,007	,042
3%60º-H3%_DS	10,444	4,558	2,292	,022	,132

Each row tests the null hypothesis where the Sample 1 and Sample 2 distributions are equal. Asymptotic significances (2-sided test) are displayed. The significance level is .050. a. Significance values were adjusted by Bonferroni correction for several tests.


Comparações por Método Pairwise de TNBS1
3%60º
18,00
Adj. Sig.
< 0.05
>= 0.05
H3%_DS
28,44
H3%_L
4,50
H3%_P
15,29
Each node shows the sample average rank of TNBS1.


Informações de Campo Contínuo Free amino groups (%)
N = 33
Mín. = 7,9
Máx. = 83,9
Média = 34,18
\ Desvio do Desv. = 19,582
Frequência
0
2
4
6
8
,0
20,0
40,0
60,0
80,0
100,0
Free amino groups (%)



- Explore

Notes

Output Created		12-NOV-2023 15:54:08
Comments		
Input	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\TNBS\TNBS2.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values for dependent variables are treated as missing.
	Cases Used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Freeaminogroups BY TNBS2 /PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT /COMPARE GROUPS /MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVES EXTREME /CINTERVAL 95 /MISSING LISTWISE /NOTOTAL.
Resources	Processor Time	00:00:01,20
	Elapsed Time	00:00:01,09

Case Processing Summary

		Cases				
		Valid		Missing		Total
	TNBS2	N	Percent	N	Percent	N
Free amino groups (%)	H3%_P	8	88,9%	1	11,1%	9
	H1%_P	3	100,0%	0	0,0%	3

Case Processing Summary

		Cases
		Total
	TNBS2	Percent
Free amino groups (%)	H3%_P	100,0%
	H1%_P	100,0%

Descriptives

	TNBS2			Statistic	Std. Error
Free amino groups (%)	H3%_P	Mean		34,025	4,5931
		95% Confidence Interval for Mean	Lower Bound	23,164	
			Upper Bound	44,886	
		5% Trimmed Mean		32,961	
		Median		30,600	
		Variance		168,771	
		Std. Deviation		12,9912	
		Minimum		22,5	
		Maximum		64,7	
		Range		42,2	
		Interquartile Range		7,5	
		Skewness		2,316	,752
		Kurtosis		6,027	1,481
	H1%_P	Mean		5,367	,2186
		95% Confidence Interval for Mean	Lower Bound	4,426	
			Upper Bound	6,307	
		5% Trimmed Mean		.	
		Median		5,200	
		Variance		,143	
		Std. Deviation		,3786	
		Minimum		5,1	
		Maximum		5,8	
		Range		,7	
		Interquartile Range		.	
		Skewness		1,597	1,225
		Kurtosis		.	.

M-Estimators

	TNBS2	Huber's M-Estimator[a]	Tukey's Biweight[b]	Hampel's M-Estimator[c]	Andrews' Wave[d]
Free amino groups (%)	H3%_P	30,884	30,001	29,893	29,999
	H1%_P	5,215	5,150	5,192	5,150

a. The weighting constant is 1,339. b. The weighting constant is 4,685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1,340*pi.

Extreme Values[a]

	TNBS2			Case Number	Value
Free amino groups (%)	H3%_P	Highest	1	1	64,7
			2	6	35,1
			3	5	32,8
			4	3	30,8
		Lowest	1	7	22,5
			2	8	26,0
			3	9	29,9
			4	4	30,4
	H1%_P	Highest	1	12	5,8
		Lowest	1	11	5,1

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes is displayed.

Tests of Normality

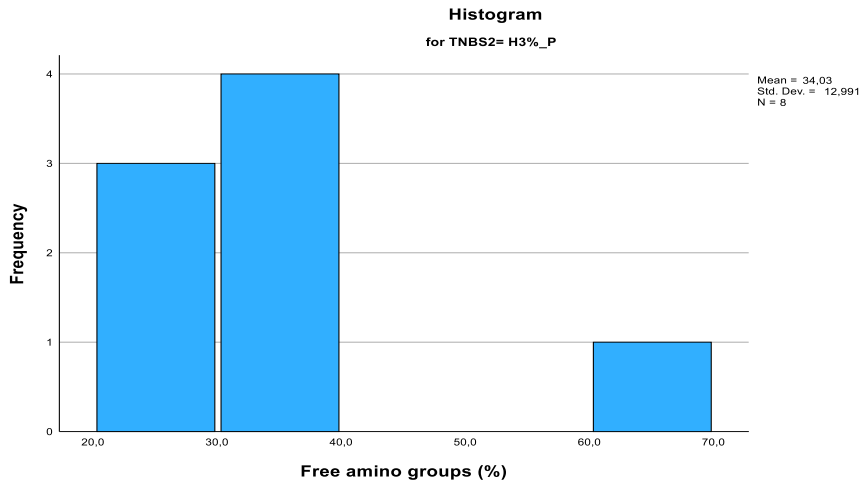
		Kolmogorov-Smirnov[a]			Shapiro-Wilk	
	TNBS2	Statistic	df	Sig.	Statistic	df
Free amino groups (%)	H3%_P	,342	8	,006	,709	8
	H1%_P	,337	3	.	,855	3

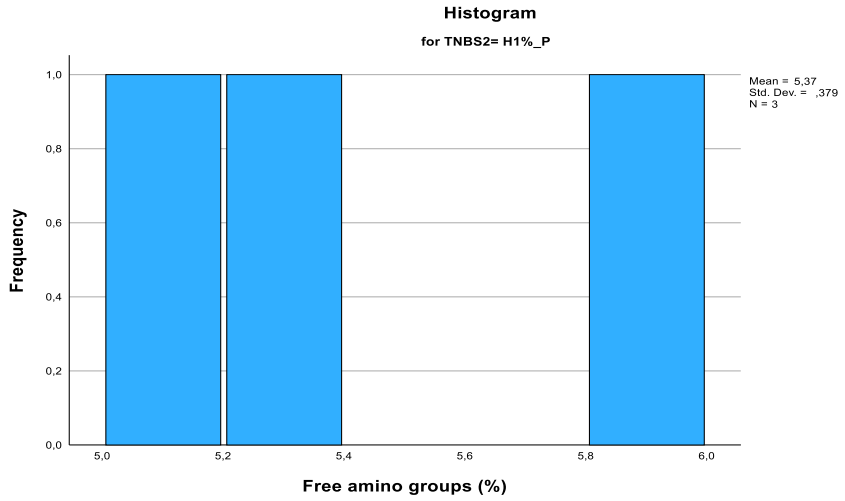
Tests of Normality

		Shapiro-Wilk[a]
	TNBS2	Sig.
Free amino groups (%)	H3%_P	,003
	H1%_P	,253

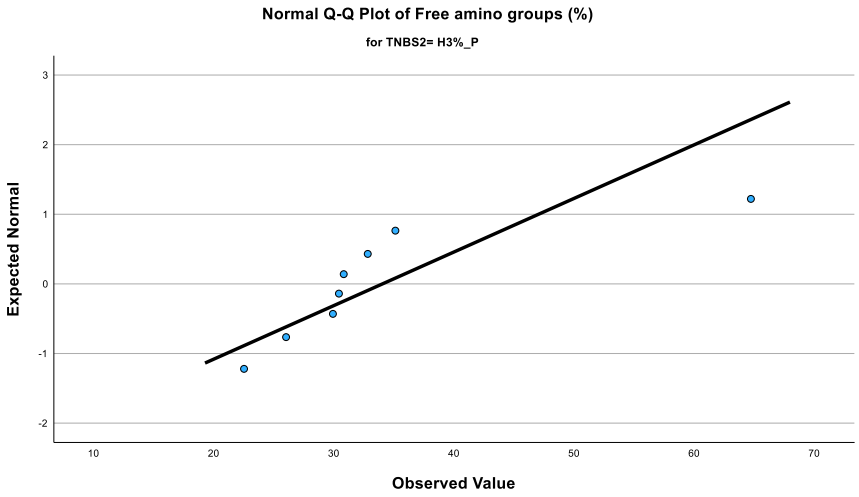
a. Lilliefors Significance Correction

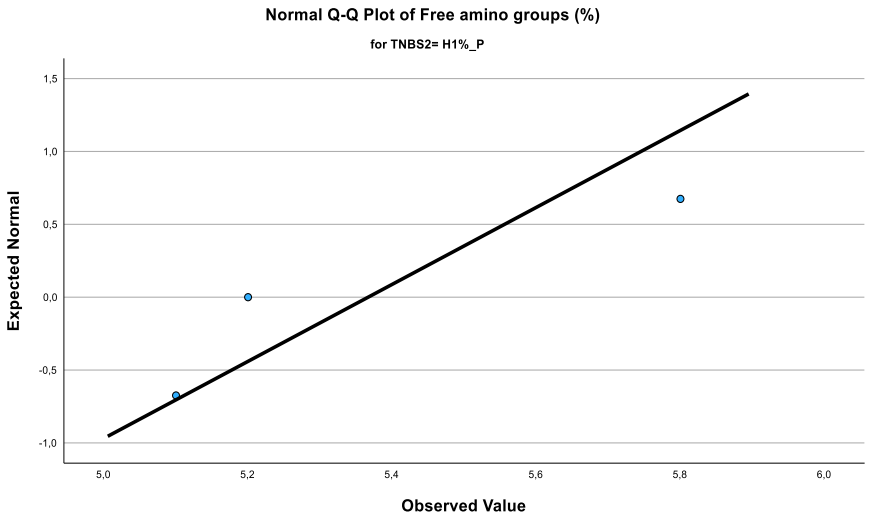
- Histograms



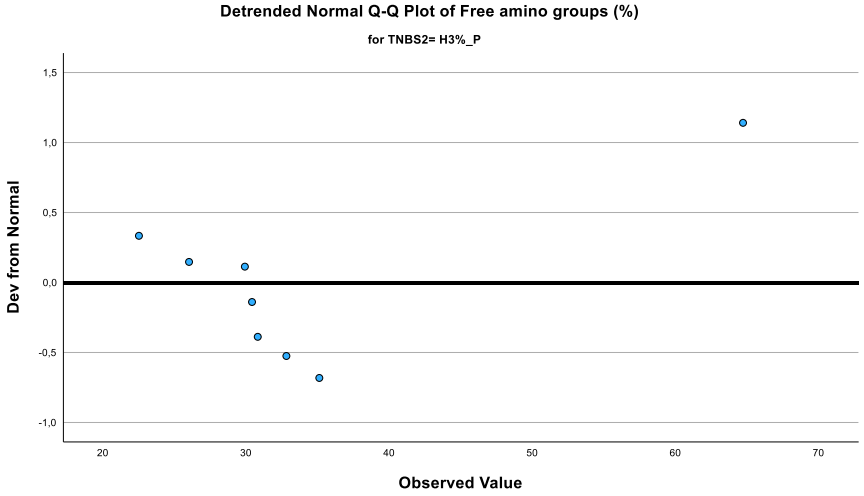


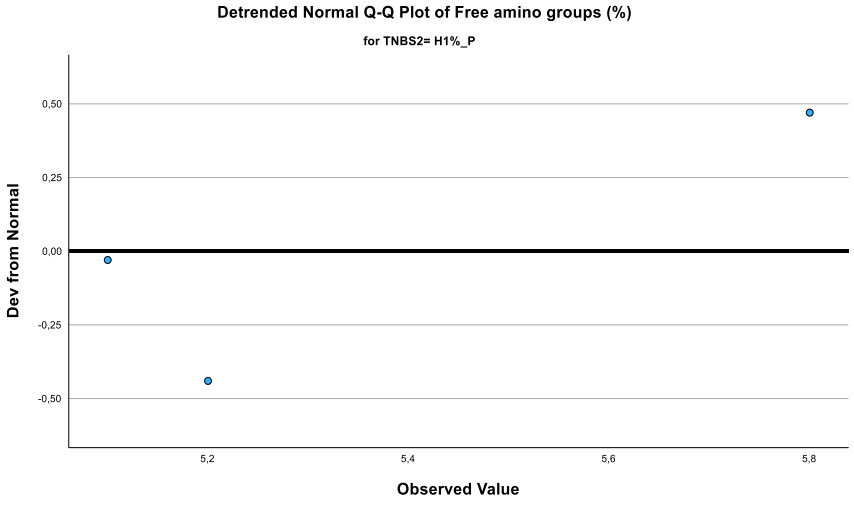
- Normal Q-Q Plots

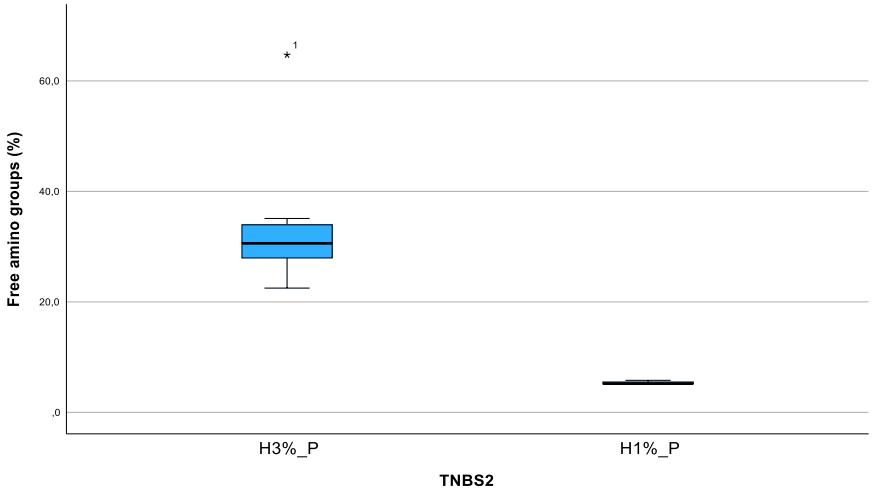




- Detrended Normal Q-Q Plots







- Explore

Notes

Output Created		12-NOV-2023 15:54:50
Comments		
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	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values for dependent variables are treated as missing.
	Cases Used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=Freeaminogroups BY TNBS2 /PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT /COMPARE GROUPS /MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVES EXTREME /CINTERVAL 95 /MISSING LISTWISE /NOTOTAL.
Resources	Processor Time	00:00:02,00
	Elapsed Time	00:00:00,82

Case Processing Summary

		Cases				
		Valid		Missing		Total
	TNBS2	N	Percent	N	Percent	N
Free amino groups (%)	H3%_P	7	77,8%	2	22,2%	9
	H1%_P	3	100,0%	0	0,0%	3

Case Processing Summary

		Cases
		Total
	TNBS2	Percent
Free amino groups (%)	H3%_P	100,0%
	H1%_P	100,0%

Descriptives

	TNBS2			Statistic	Std. Error
Free amino groups (%)	H3%_P	Mean		29,643	1,5888
		95% Confidence Interval for Mean	Lower Bound	25,755	
			Upper Bound	33,530	
		5% Trimmed Mean		29,737	
		Median		30,400	
		Variance		17,670	
		Std. Deviation		4,2035	
		Minimum		22,5	
		Maximum		35,1	
		Range		12,6	
		Interquartile Range		6,8	
		Skewness		-,681	,794
		Kurtosis		,231	1,587
	H1%_P	Mean		5,367	,2186
		95% Confidence Interval for Mean	Lower Bound	4,426	
			Upper Bound	6,307	
		5% Trimmed Mean		.	
		Median		5,200	
		Variance		,143	
		Std. Deviation		,3786	
		Minimum		5,1	
		Maximum		5,8	
		Range		,7	
		Interquartile Range		.	
		Skewness		1,597	1,225
		Kurtosis		.	.

M-Estimators

	TNBS2	Huber's M-Estimator[a]	Tukey's Biweight[b]	Hampel's M-Estimator[c]	Andrews' Wave[d]
Free amino groups (%)	H3%_P	30,194	30,419	30,015	30,417
	H1%_P	5,215	5,150	5,192	5,150

a. The weighting constant is 1,339. b. The weighting constant is 4,685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1,340*pi.

Extreme Values[a]

	TNBS2			Case Number	Value
Free amino groups (%)	H3%_P	Highest	1	6	35,1
			2	5	32,8
			3	3	30,8
		Lowest	1	7	22,5
			2	8	26,0
			3	9	29,9
	H1%_P	Highest	1	12	5,8
		Lowest	1	11	5,1

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes is displayed.

Tests of Normality

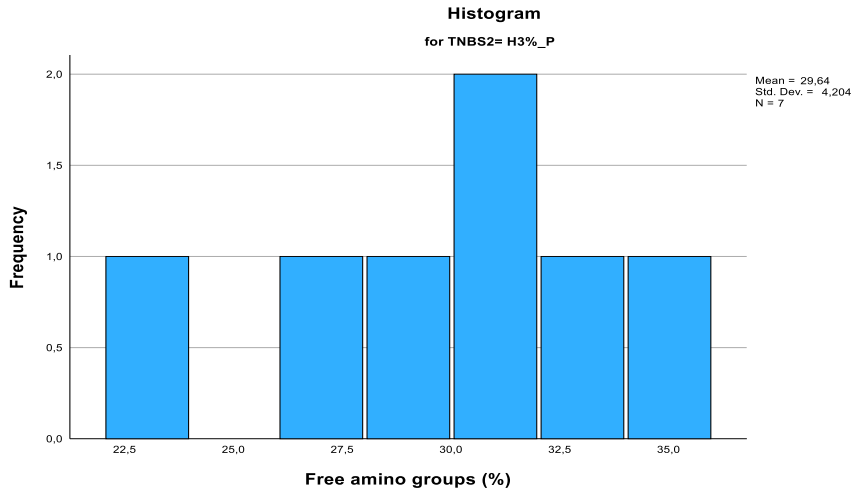
		Kolmogorov-Smirnov[a]			Shapiro-Wilk	
	TNBS2	Statistic	df	Sig.	Statistic	df
Free amino groups (%)	H3%_P	,239	7	,200*	,951	7
	H1%_P	,337	3	.	,855	3

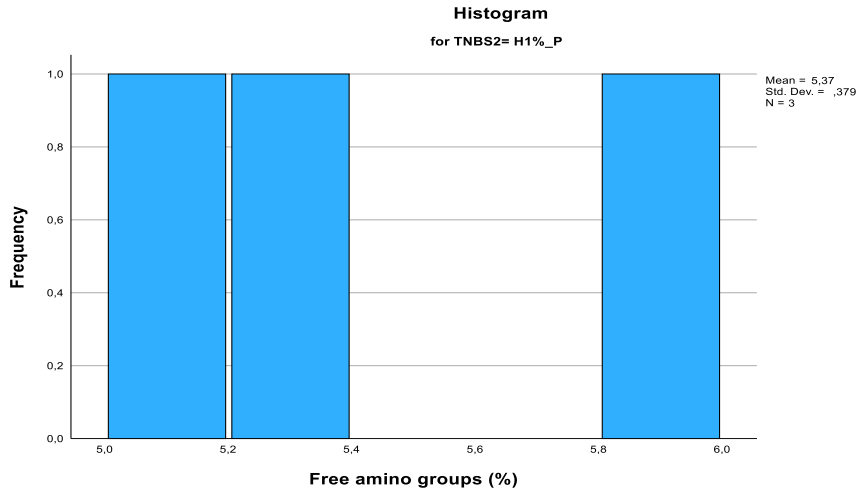
Tests of Normality

		Shapiro-Wilk[a]
	TNBS2	Sig.
Free amino groups (%)	H3%_P	,735
	H1%_P	,253

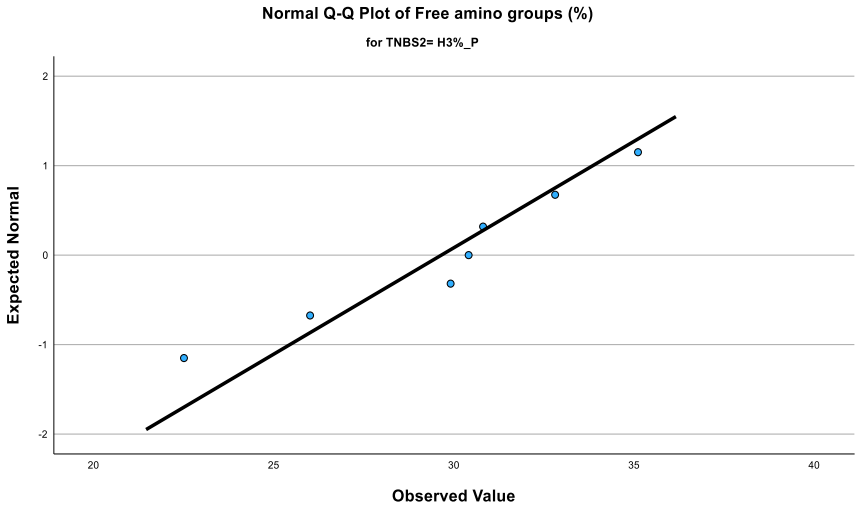
*. This is a lower bound of the true significance. a. Lilliefors Significance Correction

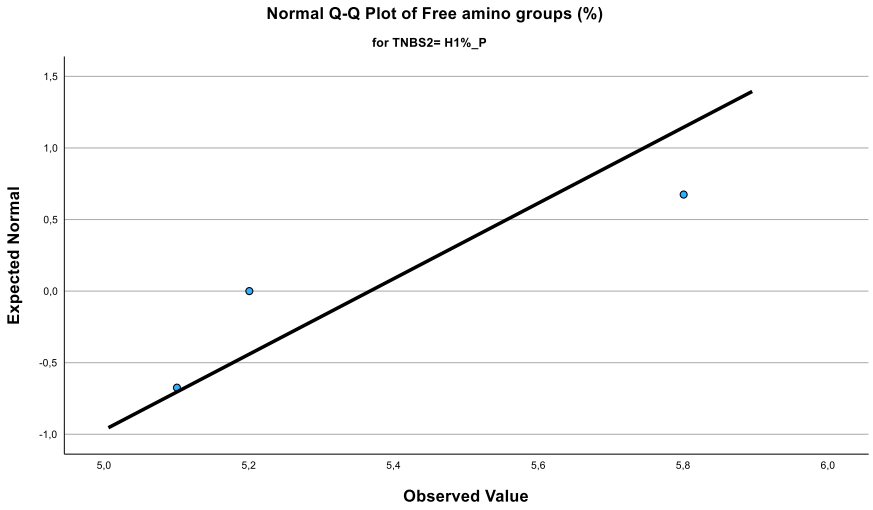
- **Histograms**



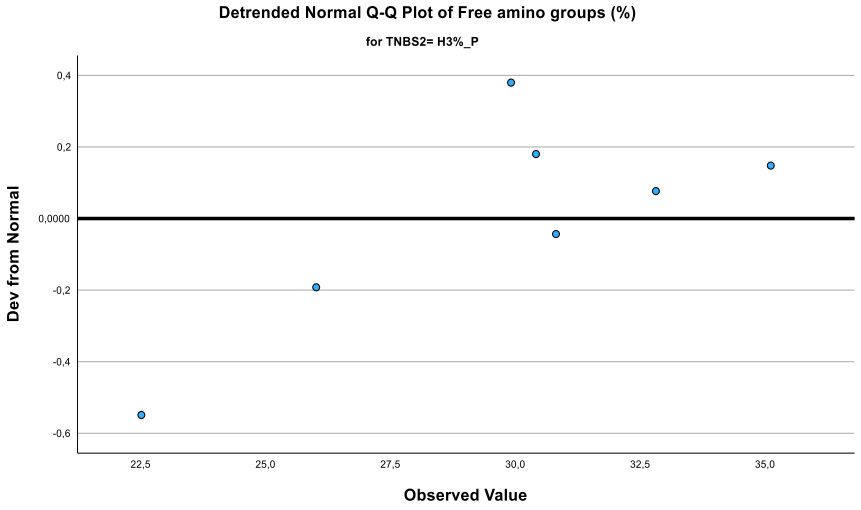


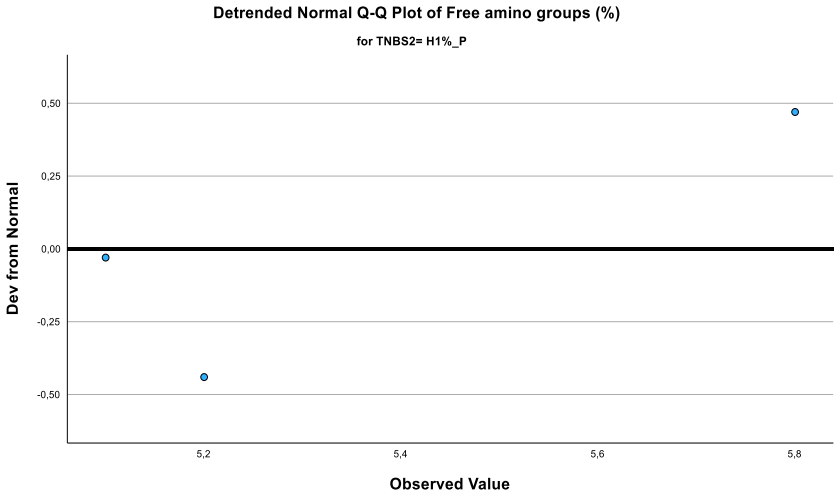
- Normal Q-Q Plots

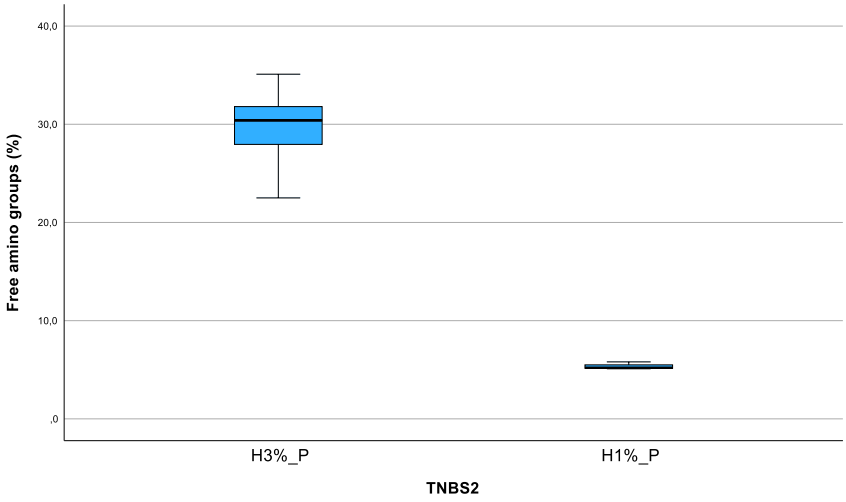




- Detrended Normal Q-Q Plots







- T-Test

Notes

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Comments		
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	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=TNBS2(2 3) /MISSING=ANALYSIS /VARIABLES=Freeaminogroups /ES DISPLAY(TRUE) /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,02
	Elapsed Time	00:00:00,00

Group Statistics

	TNBS2	N	Mean	Std. Deviation	Std. Error Mean
Free amino groups (%)	H3%_P	7	29,643	4,2035	1,5888
	H1%_P	3	5,367	,3786	,2186

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means
		F	Sig.	t
Free amino groups (%)	Equal variances assumed	3,311	,106	9,651
	Equal variances not assumed			15,137

Independent Samples Test

		t-test for Equality of Means		
			Significance	
		df	One-Sided p	Two-Sided p
Free amino groups (%)	Equal variances assumed	8	<,001	<,001
	Equal variances not assumed	6,223	<,001	<,001

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Free amino groups (%)	Equal variances assumed	24,2762	2,5155
	Equal variances not assumed	24,2762	1,6037

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Free amino groups (%)	Equal variances assumed	18,4755	30,0769
	Equal variances not assumed	20,3857	28,1666

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
Free amino groups (%)	Cohen's d	3,6453	6,660	3,165	10,106
	Hedges' correction	4,0382	6,012	2,857	9,123
	Glass's delta	,3786	64,122	10,181	123,168

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control (i.e., the second) group.

5.7. Antioxidant activity – INFOGEST

- **Explore**

Notes

Output Created		29-SEP-2023 12:39:45
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\ABTS_INFOGEST_SPSS.sav
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	Filter	<none>
	Weight	<none>
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	N of Rows in Working Data File	18
Missing Value Handling	Definition of Missing	User-defined missing values for dependent variables are treated as missing.
	Cases Used	Statistics are based on cases with no missing values for any dependent variable or factor used.
	Syntax	EXAMINE VARIABLES=ABTS_3p60º_B BY SGI_phase /PLOT BOXPLOT STEMLEAF NPPLOT /COMPARE GROUPS /MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVES EXTREME /CINTERVAL 95 /MISSING LISTWISE /NOTOTAL.
Resources	Processor Time	00:00:01,39
	Elapsed Time	00:00:00,94

Case Processing Summary

		Cases					
		Valid		Missing		Total	
	SGI_phase	N	Percent	N	Percent	N	Percent
ABTS_3%60º_B	Oral	3	100,0%	0	0,0%	3	100,0%
	Gastric	3	100,0%	0	0,0%	3	100,0%
	Intestinal	3	100,0%	0	0,0%	3	100,0%

Descriptives

	SGI_phase			Statistic	Std. Error
ABTS_3%60º_B	Oral	Mean		4,18267	,135487
		95% Confidence Interval for Mean	Lower Bound	3,59971	
			Upper Bound	4,76562	
		5% Trimmed Mean		.	
		Median		4,19300	
		Variance		,055	
		Std. Deviation		,234671	
		Minimum		3,943	
		Maximum		4,412	
		Range		,469	
		Interquartile Range		.	
		Skewness		-,198	1,225
		Kurtosis		.	.
	Gastric	Mean		4,59267	,124756
		95% Confidence Interval for Mean	Lower Bound	4,05588	
			Upper Bound	5,12945	
		5% Trimmed Mean		.	
		Median		4,61100	
		Variance		,047	
		Std. Deviation		,216084	
		Minimum		4,368	
		Maximum		4,799	
		Range		,431	
		Interquartile Range		.	
		Skewness		-,379	1,225
		Kurtosis		.	.
	Intestinal	Mean		7,06067	1,135631
		95% Confidence Interval for Mean	Lower Bound	2,17444	
			Upper Bound	11,94689	
		5% Trimmed Mean		.	
		Median		6,51100	
		Variance		3,869	
		Std. Deviation		1,966970	
		Minimum		5,427	
		Maximum		9,244	
		Range		3,817	
		Interquartile Range		.	

	Skewness	1,159	1,225
	Kurtosis	.	.

M-Estimators

	SGI_phase	Huber's M-Estimator[a]	Tukey's Biweight[b]	Hampel's M-Estimator[c]	Andrews' Wave[d]
ABTS_3%60º_B	Oral	4,18267	4,18524	4,18267	4,18526
	Gastric	4,59267	4,59792	4,59267	4,59796
	Intestinal	6,68629	6,76536	6,88310	6,76757

a. The weighting constant is 1,339. b. The weighting constant is 4,685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1,340*pi.

Extreme Values[a]

	SGI_phase			Case Number	Value
ABTS_3%60º_B	Oral	Highest	1	3	4,412
		Lowest	1	2	3,943
	Gastric	Highest	1	4	4,799
		Lowest	1	5	4,368
	Intestinal	Highest	1	7	9,244
		Lowest	1	9	5,427

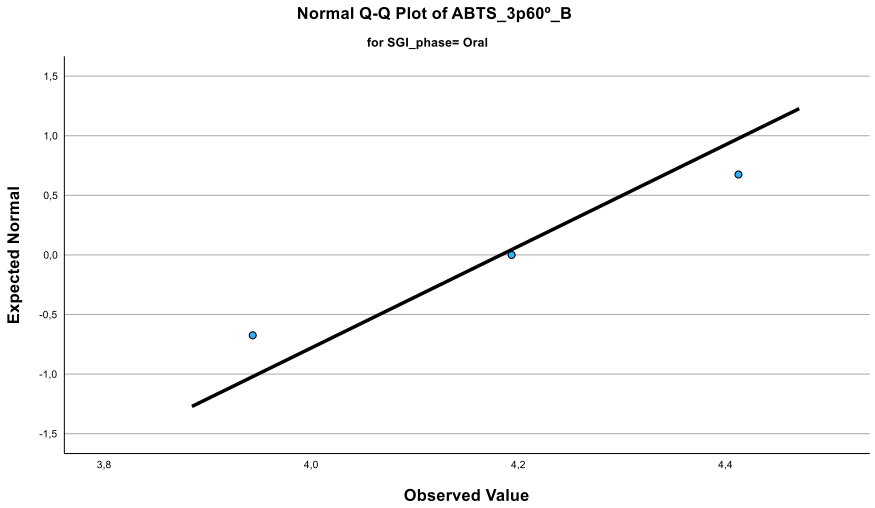
a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes is displayed.

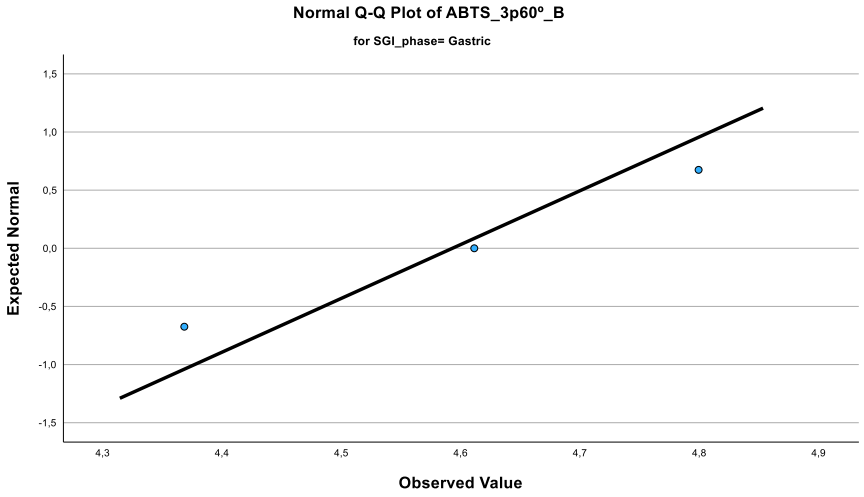
Tests of Normality

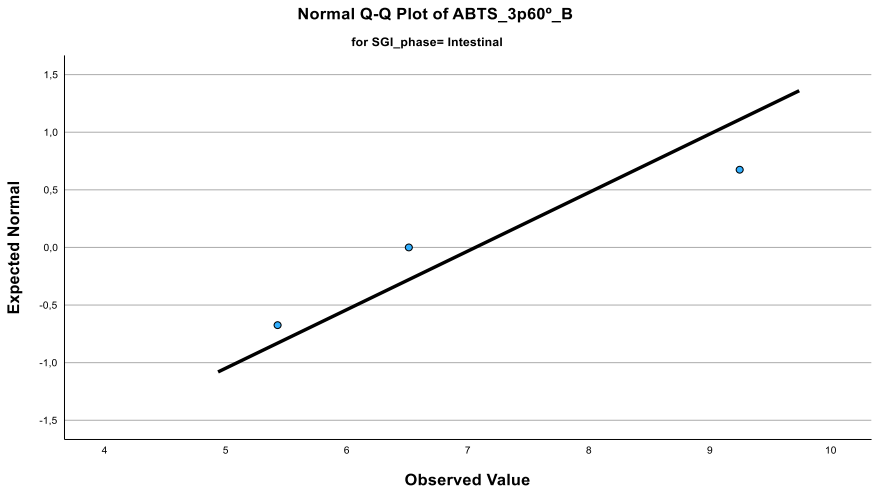
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	SGI_phase	Statistic	df	Sig.	Statistic	df	Sig.
ABTS_3%60º_B	Oral	,184	3	.	,999	3	,927
	Gastric	,200	3	.	,995	3	,860
	Intestinal	,277	3	.	,941	3	,533

a. Lilliefors Significance Correction

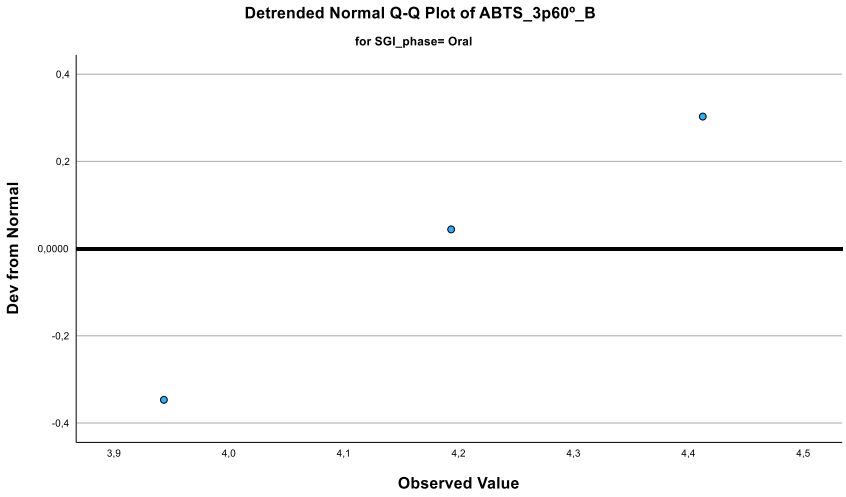
- **Normal Q-Q Plots**

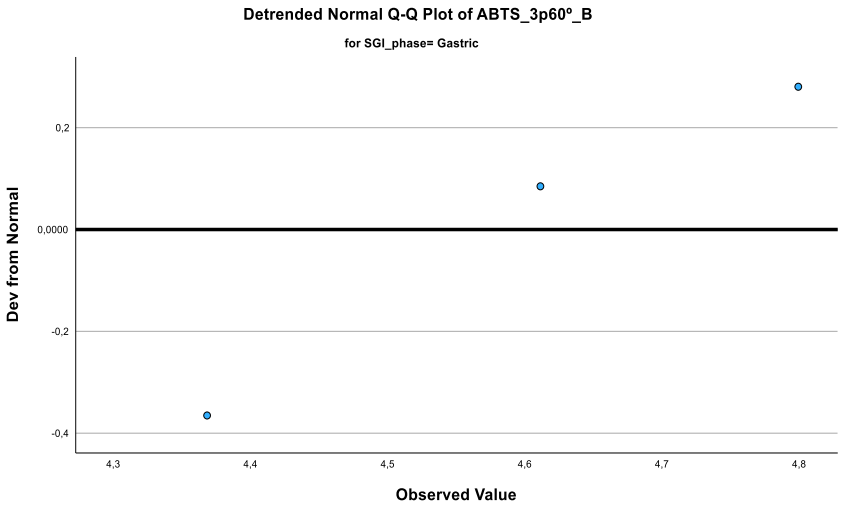


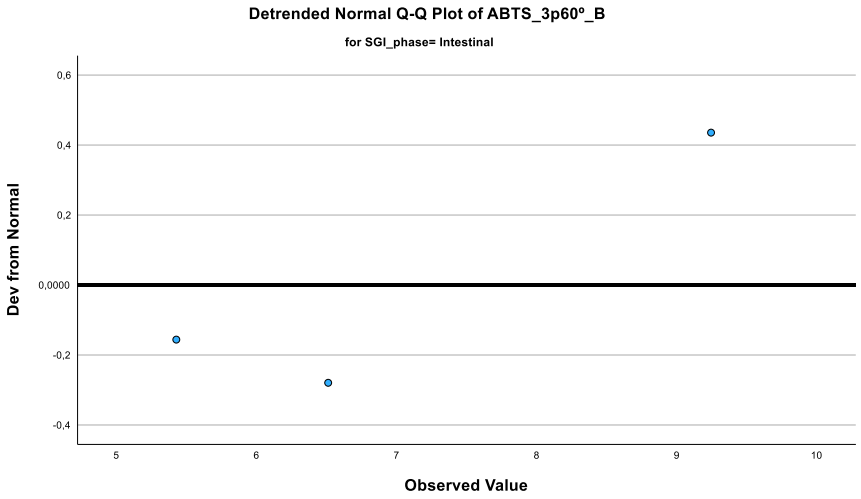


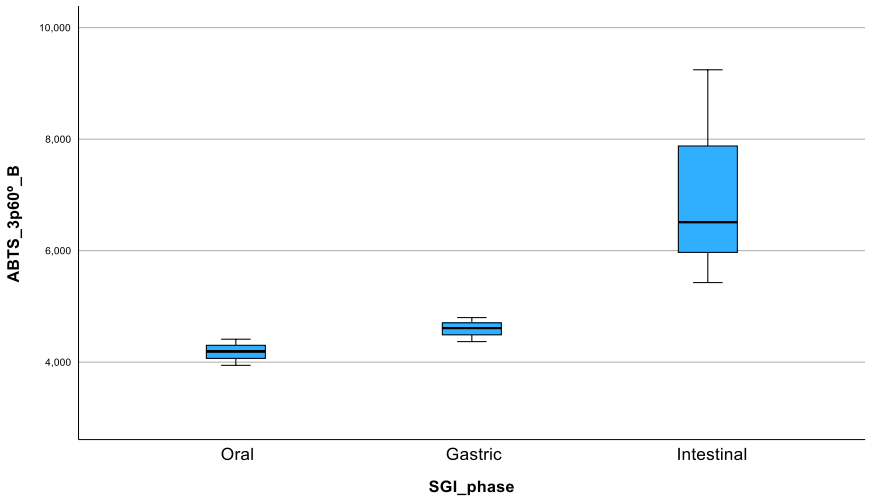


- **Detrended Normal Q-Q Plots**









- **Explore**

Notes

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	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	18
Missing Value Handling	Definition of Missing	User-defined missing values for dependent variables are treated as missing.
	Cases Used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=ABTS_H1p_B BY SGI_phase /PLOT BOXPLOT STEMLEAF NPPLOT /COMPARE GROUPS /MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVES EXTREME /CINTERVAL 95 /MISSING LISTWISE /NOTOTAL.
Resources	Processor Time	00:00:00,70
	Elapsed Time	00:00:00,90

Case Processing Summary

		Cases					
		Valid		Missing		Total	
	SGI_phase	N	Percent	N	Percent	N	Percent
ABTS_H1%_B	Oral	3	100,0%	0	0,0%	3	100,0%
	Gastric	3	100,0%	0	0,0%	3	100,0%
	Intestinal	3	100,0%	0	0,0%	3	100,0%

Descriptives

	SGI_phase			Statistic	Std. Error
ABTS_H1%_B	Oral	Mean		2,25567	,091923
		95% Confidence Interval for Mean	Lower Bound	1,86016	
			Upper Bound	2,65118	
		5% Trimmed Mean		.	
		Median		2,32900	
		Variance		,025	
		Std. Deviation		,159215	
		Minimum		2,073	
		Maximum		2,365	
		Range		,292	
		Interquartile Range		.	
		Skewness		-1,633	1,225
		Kurtosis		.	.
	Gastric	Mean		1,75067	,143237
		95% Confidence Interval for Mean	Lower Bound	1,13437	
			Upper Bound	2,36696	
		5% Trimmed Mean		.	
		Median		1,82100	
		Variance		,062	
		Std. Deviation		,248093	
		Minimum		1,475	
		Maximum		1,956	
		Range		,481	
		Interquartile Range		.	
		Skewness		-1,173	1,225
		Kurtosis		.	.
	Intestinal	Mean		2,50733	,870280
		95% Confidence Interval for Mean	Lower Bound	-1,23718	
			Upper Bound	6,25185	
		5% Trimmed Mean		.	

	Median	1,86800	
	Variance	2,272	
	Std. Deviation	1,507370	
	Minimum	1,425	
	Maximum	4,229	
	Range	2,804	
	Interquartile Range	.	
	Skewness	1,565	1,225
	Kurtosis	.	.

M-Estimators

	SGI_phase	Huber's M-Estimator[a]	Tukey's Biweight[b]	Hampel's M-Estimator[c]	Andrews' Wave[d]
ABTS_H1%_B	Oral	2,32342	2,34696	2,33836	2,34696
	Gastric	1,79914	1,79339	1,77693	1,79306
	Intestinal	1,94213	1,64652	1,88221	1,64652

a. The weighting constant is 1,339. b. The weighting constant is 4,685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1,340*pi.

Extreme Values[a]

				Case Number	Value
ABTS_H1%_B	Oral	Highest	1	1	2,365
		Lowest	1	2	2,073
	Gastric	Highest	1	4	1,956
		Lowest	1	6	1,475
	Intestinal	Highest	1	9	4,229
		Lowest	1	7	1,425

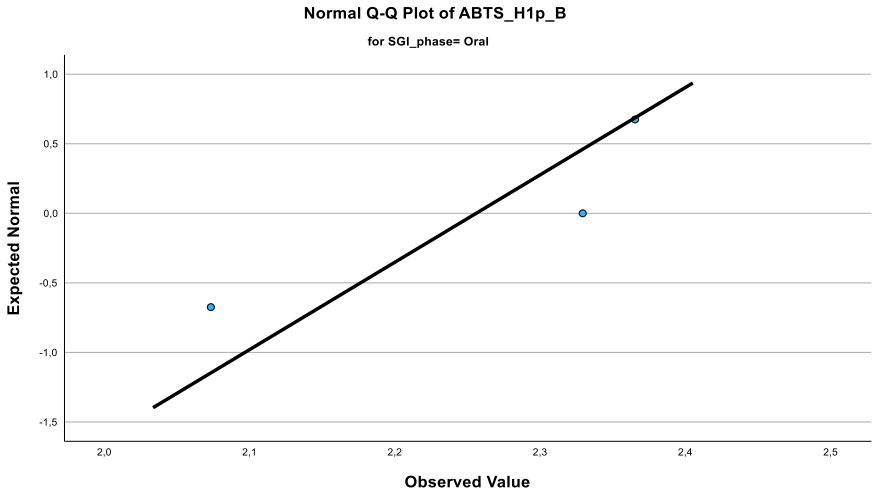
a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes is displayed.

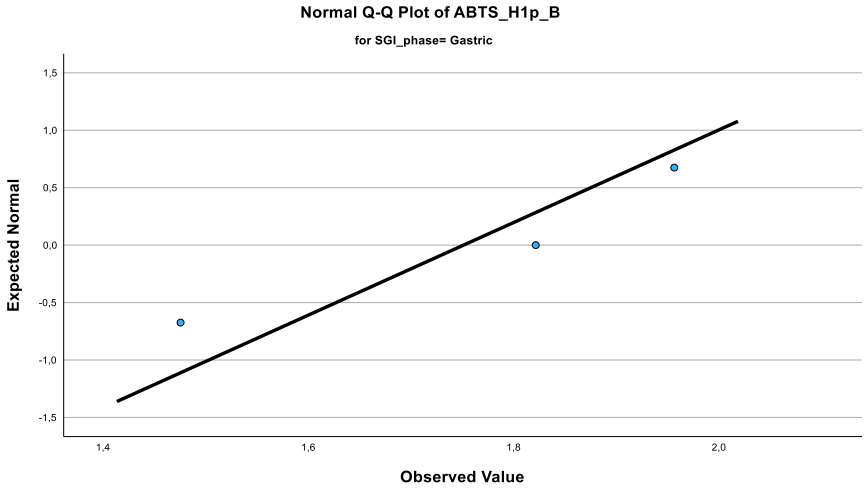
Tests of Normality

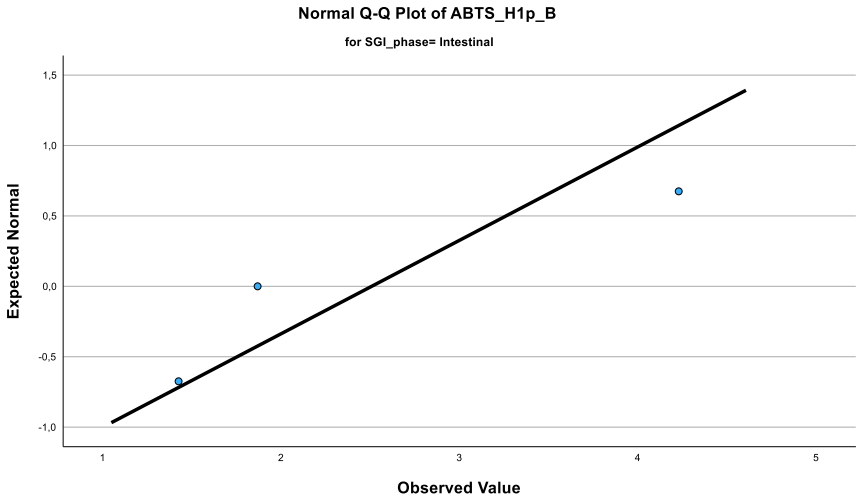
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	SGI_phase	Statistic	df	Sig.	Statistic	df	Sig.
ABTS_H1%_B	Oral	,344	3	.	,841	3	,216
	Gastric	,278	3	.	,940	3	,526
	Intestinal	,331	3	.	,865	3	,282

a. Lilliefors Significance Correction

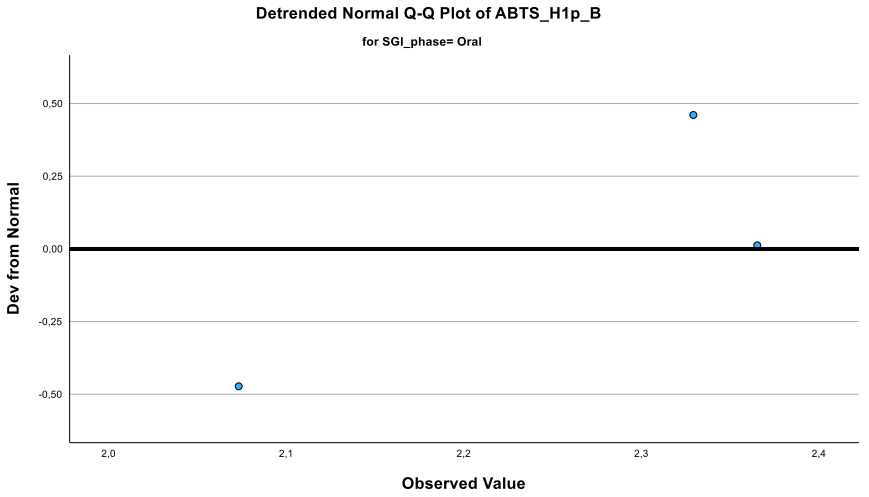
- **Normal Q-Q Plots**

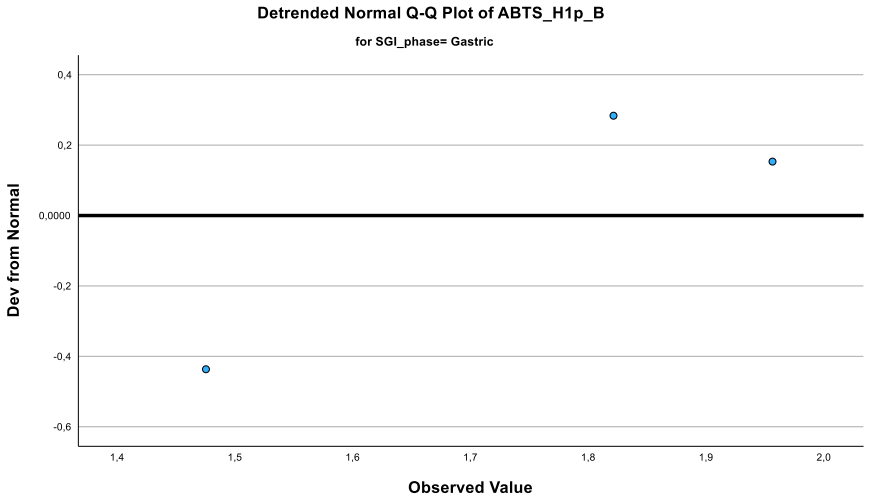


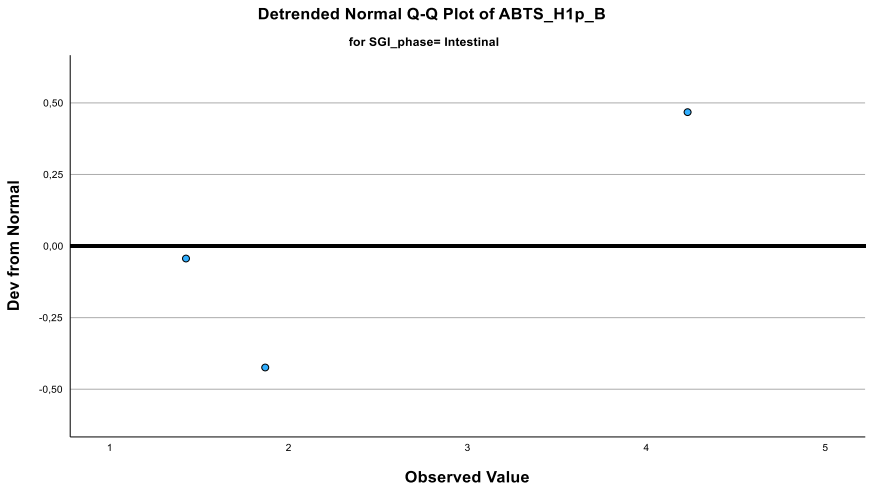


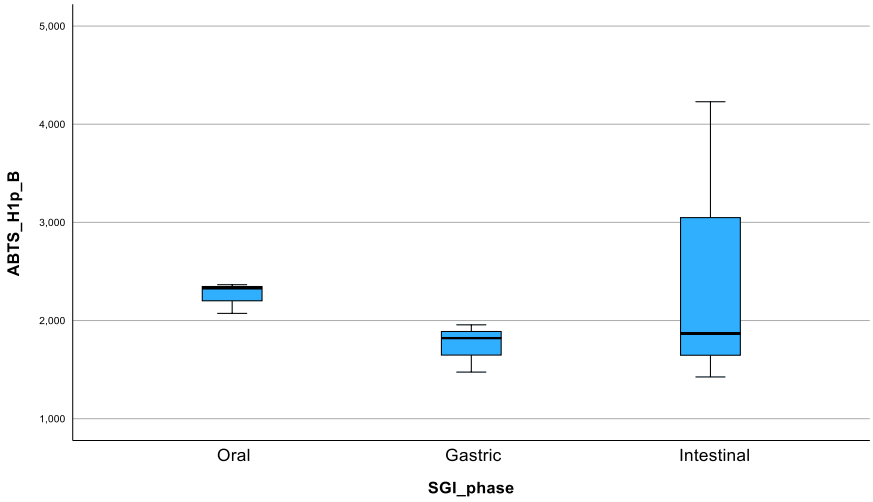


- **Detrended Normal Q-Q Plots**









- **Univariate Analysis of Variance**

Notes

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	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
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Between-Subjects Factors

		Value Label	N
SGI_phase	1	Oral	3
	2	Gastric	3
	3	Intestinal	3

Descriptive Statistics

Dependent Variable: ABTS_3p60º_B

SGI_phase	Mean	Std. Deviation	N
Oral	4,18267	,234671	3
Gastric	4,59267	,216084	3
Intestinal	7,06067	1,966970	3
Total	5,27867	1,676435	9

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
ABTS_3%60º_B	Based on Mean	7,042	2	6	,027
	Based on Median	1,959	2	6	,221
	Based on Median and with adjusted df	1,959	2	2,074	,333
	Based on trimmed mean	6,520	2	6	,031

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a,b] a. Dependent variable: ABTS_3p60º_B. b. Design: Intercept + SGI_phase

Tests of Between-Subjects Effects

Dependent Variable: ABTS_3p60º_B

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	14,542[a]	2	7,271	5,493	,044	,647
Intercept	250,779	1	250,779	189,470	<.001	,969
SGI_phase	14,542	2	7,271	5,493	,044	,647
Error	7,941	6	1,324			
Total	273,262	9				
Corrected Total	22,483	8				

Tests of Between-Subjects Effects

Dependent Variable: ABTS_3p60º_B

Source	Noncent. Parameter	Observed Power[b]
Corrected Model	10,987	,621
Intercept	189,470	1,000
SGI_phase	10,987	,621
Error		
Total		
Corrected Total		

a. R Squared = ,647 (Adjusted R Squared = ,529). b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: ABTS_3%60º_B

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	7,061	,664	10,630	<.001	5,435	8,686
[SGI_phase=1]	-2,878	,939	-3,064	,022	-5,177	-,579
[SGI_phase=2]	-2,468	,939	-2,627	,039	-4,767	-,169
[SGI_phase=3]	0[a]	.	.	.	.	.

Parameter Estimates

Dependent Variable: ABTS_3%60º_B

Parameter	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Intercept	,950	10,630	1,000
[SGI_phase=1]	,610	3,064	,724
[SGI_phase=2]	,535	2,627	,595
[SGI_phase=3]	.	.	.

a. This parameter is set to zero because it is redundant. b. Computed using alpha = .05

- **Post Hoc Tests**

Multiple Comparisons

Dependent Variable: ABTS_3p60º_B

Tukey HSD

(I) SGI_phase	(J) SGI_phase	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Oral	Gastric	-,41000	,939354	,902	-3,29220	2,47220
	Intestinal	-2,87800	,939354	,050	-5,76020	,00420
Gastric	Oral	,41000	,939354	,902	-2,47220	3,29220
	Intestinal	-2,46800	,939354	,087	-5,35020	,41420
Intestinal	Oral	2,87800	,939354	,050	-,00420	5,76020
	Gastric	2,46800	,939354	,087	-,41420	5,35020

Based on observed means.

The error term is Mean Square(Error) = 1,324.

- **Homogeneous Subsets**

ABTS_3%60º_B

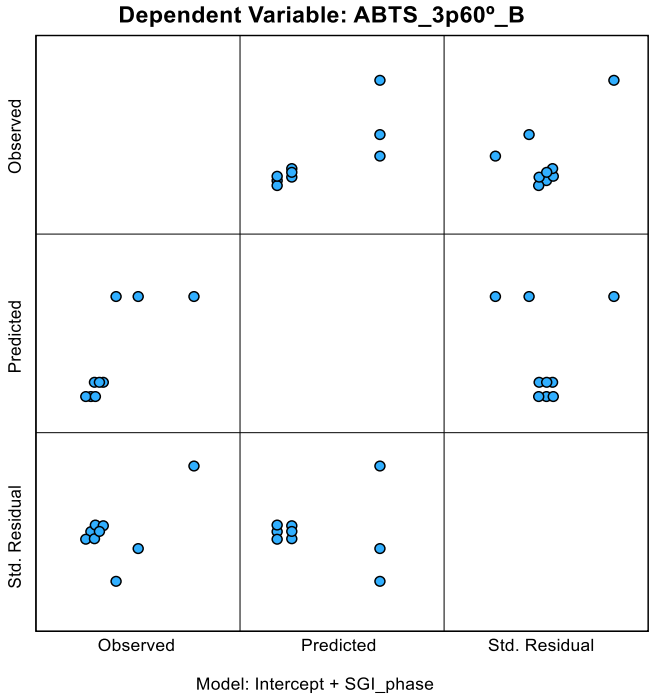
Tukey HSD[a,b]

SGI_phase	N	Subset 1
Oral	3	4,18267
Gastric	3	4,59267
Intestinal	3	7,06067
Sig.		,050

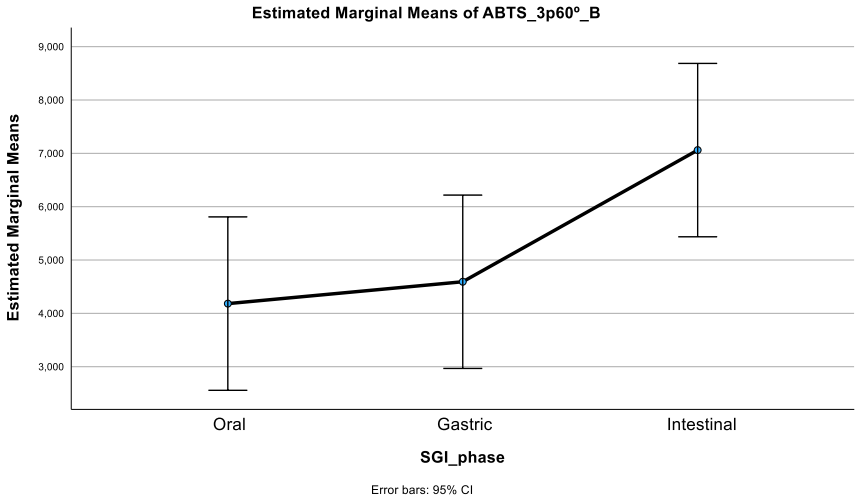
Means for groups in homogeneous subsets are displayed. Based on observed means.

The error term is Mean Square(Error) =

1,324. a. Uses Harmonic Mean Sample Size = 3,000. b. Alpha = .05.



- **Profile Plots**



- **Univariate Analysis of Variance**

Notes

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Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA ABTS_H1p_B BY SGI_phase /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=SGI_phase(TUKEY) /PLOT=PROFILE(SGI_phase) TYPE=LINE ERRORBAR=CI MEANREFERENCE=NO YAXIS=AUTO /PRINT ETASQ DESCRIPTIVE PARAMETER HOMOGENEITY OPOWER /PLOT=RESIDUALS /CRITERIA=ALPHA(.05) /DESIGN=SGI_phase.
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Between-Subjects Factors

		Value Label	N
SGI_phase	1	Oral	3
	2	Gastric	3
	3	Intestinal	3

Descriptive Statistics

Dependent Variable: ABTS_H1p_B

SGI_phase	Mean	Std. Deviation	N
Oral	2,25567	,159215	3
Gastric	1,75067	,248093	3
Intestinal	2,50733	1,507370	3
Total	2,17122	,837335	9

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
ABTS_H1p_B	Based on Mean	9,607	2	6	,013
	Based on Median	1,205	2	6	,363
	Based on Median and with adjusted df	1,205	2	2,127	,447
	Based on trimmed mean	8,244	2	6	,019

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a,b]

a. Dependent variable: ABTS_H1%_B. b. Design: Intercept + SGI_phase

Tests of Between-Subjects Effects

Dependent Variable: ABTS_H1%_B

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	,891[a]	2	,445	,566	,595	,159
Intercept	42,428	1	42,428	53,955	<.001	,900
SGI_phase	,891	2	,445	,566	,595	,159
Error	4,718	6	,786			
Total	48,037	9				
Corrected Total	5,609	8				

Tests of Between-Subjects Effects

Dependent Variable: ABTS_H1%_B

Source	Noncent. Parameter	Observed Power[b]
Corrected Model	1,133	,107
Intercept	53,955	1,000
SGI_phase	1,133	,107
Error		
Total		
Corrected Total		

a. R Squared = ,159 (Adjusted R Squared = -,122). b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: ABTS_H1%_B

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	2,507	,512	4,897	,003	1,255	3,760
[SGI_phase=1]	-,252	,724	-,348	,740	-2,023	1,520
[SGI_phase=2]	-,757	,724	-1,045	,336	-2,528	1,015
[SGI_phase=3]	0[a]	.	.	.	.	.

Parameter Estimates

Dependent Variable: ABTS_H1%_B

Parameter	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Intercept	,800	4,897	,981
[SGI_phase=1]	,020	,348	,060
[SGI_phase=2]	,154	1,045	,144
[SGI_phase=3]	.	.	.

a. This parameter is set to zero because it is redundant. b. Computed using alpha = .05

- **Post Hoc Tests**

Multiple Comparisons

Dependent Variable: ABTS_H1%_B

Tukey HSD

(I) SGI_phase	(J) SGI_phase	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Oral	Gastric	,50500	,724042	,774	-1,71656	2,72656
	Intestinal	-,25167	,724042	,936	-2,47323	1,96989
Gastric	Oral	-,50500	,724042	,774	-2,72656	1,71656
	Intestinal	-,75667	,724042	,578	-2,97823	1,46489
Intestinal	Oral	,25167	,724042	,936	-1,96989	2,47323
	Gastric	,75667	,724042	,578	-1,46489	2,97823

Based on observed means.
The error term is Mean Square(Error) = ,786.

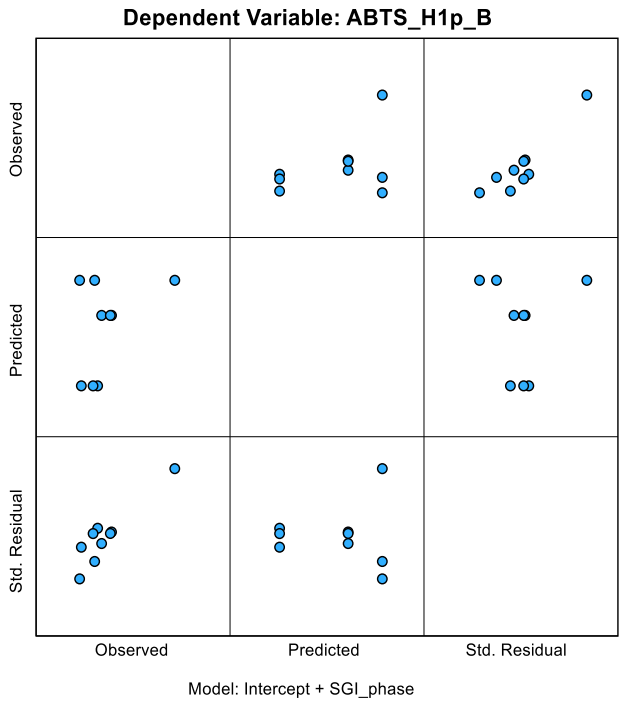
- **Homogeneous Subsets**

ABTS_H1%_B

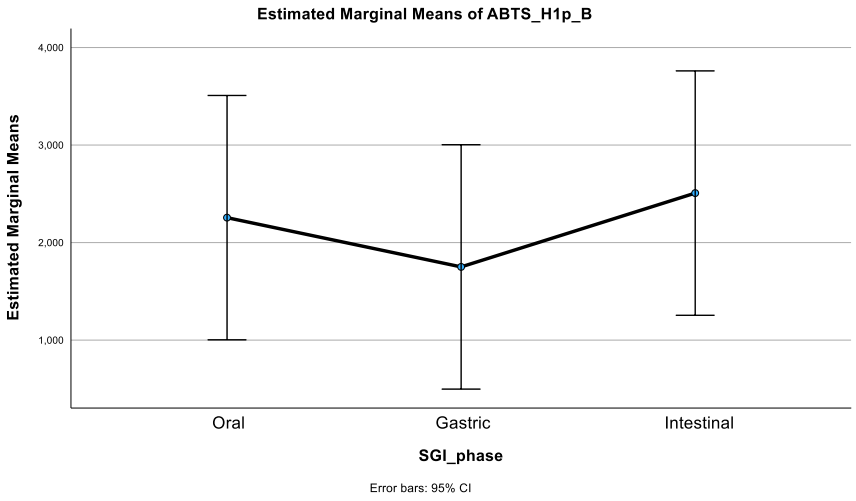
Tukey HSD[a,b]

SGI_phase	N	Subset 1
Gastric	3	1,75067
Oral	3	2,25567
Intestinal	3	2,50733
Sig.		,578

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = ,786. a. Uses Harmonic Mean Sample Size = 3,000. b. Alpha = .05.



- **Profile Plots**



- **Explore**

Notes

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Missing Value Handling	Definition of Missing	User-defined missing values for dependent variables are treated as missing.
	Cases Used	Statistics are based on cases with no missing values for any dependent variable or factor used.
	Syntax	EXAMINE VARIABLES=ORAC_3p60º_B BY SGI_phase /PLOT BOXPLOT STEMLEAF NPPLOT /COMPARE GROUPS /MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVES EXTREME /CINTERVAL 95 /MISSING LISTWISE /NOTOTAL.
Resources	Processor Time	00:00:00,31
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Case Processing Summary

		Cases					
		Valid		Missing		Total	
	SGI_phase	N	Percent	N	Percent	N	Percent
ORAC_3%60º_B	Oral	2	66,7%	1	33,3%	3	100,0%
	Gastric	2	66,7%	1	33,3%	3	100,0%
	Intestinal	3	100,0%	0	0,0%	3	100,0%

Descriptives

	SGI_phase			Statistic	Std. Error
ORAC_3%60º_B	Oral	Mean		2,54050	,028500
		95% Confidence Interval for Mean	Lower Bound	2,17837	
			Upper Bound	2,90263	
		5% Trimmed Mean		.	
		Median		2,54050	
		Variance		,002	
		Std. Deviation		,040305	
		Minimum		2,512	
		Maximum		2,569	
		Range		,057	
		Interquartile Range		.	
		Skewness		.	.
		Kurtosis		.	.
	Gastric	Mean		4,38700	,256000
		95% Confidence Interval for Mean	Lower Bound	1,13421	
			Upper Bound	7,63979	
		5% Trimmed Mean		.	
		Median		4,38700	
		Variance		,131	
		Std. Deviation		,362039	
		Minimum		4,131	
		Maximum		4,643	
		Range		,512	
		Interquartile Range		.	
		Skewness		.	.
		Kurtosis		.	.
	Intestinal	Mean		2,87900	,193578
		95% Confidence Interval for Mean	Lower Bound	2,04610	
			Upper Bound	3,71190	
		5% Trimmed Mean		.	
		Median		2,86300	
		Variance		,112	
		Std. Deviation		,335286	
		Minimum		2,552	
		Maximum		3,222	
		Range		,670	
		Interquartile Range		.	

	Skewness	,214	1,225
	Kurtosis	.	.

M-Estimators

	SGI_phase	Huber's M-Estimator[a]	Tukey's Biweight[b]	Hampel's M-Estimator[c]	Andrews' Wave[d]
ORAC_3%60º_B	Oral	2,54050	2,54050	2,54050	2,54050
	Gastric	4,38700	4,38700	4,38700	4,38700
	Intestinal	2,87900	2,87497	2,87900	2,87493

a. The weighting constant is 1,339. b. The weighting constant is 4,685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1,340*pi.

Extreme Values[a]

	SGI_phase			Case Number	Value
ORAC_3%60º_B	Oral	Highest	1	3	2,569
		Lowest	1	2	2,512
	Gastric	Highest	1	4	4,643
		Lowest	1	5	4,131
	Intestinal	Highest	1	7	3,222
		Lowest	1	9	2,552

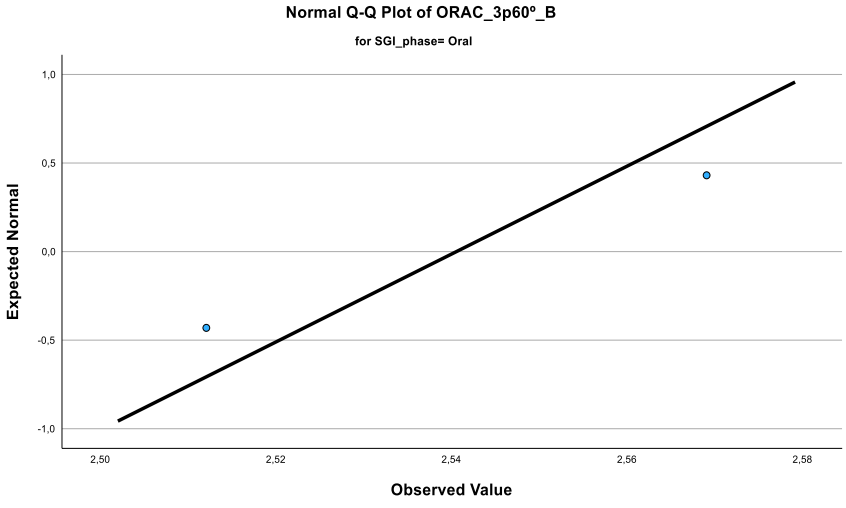
a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes is displayed.

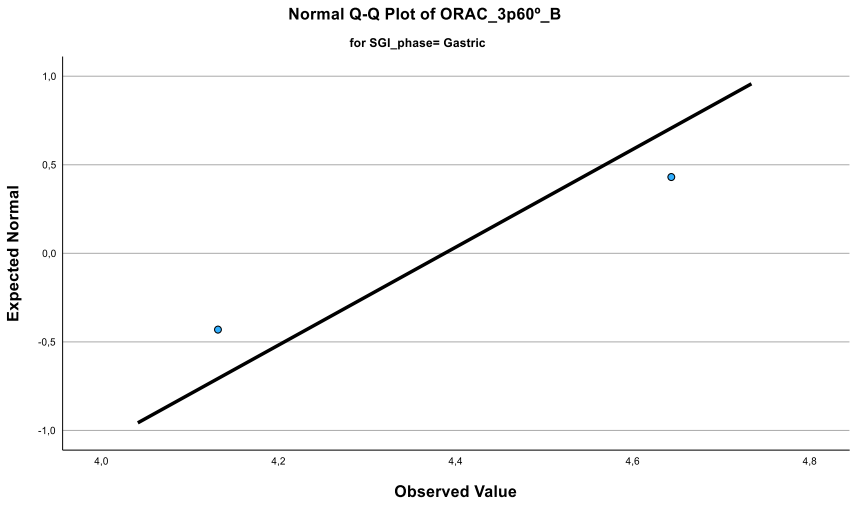
Tests of Normality

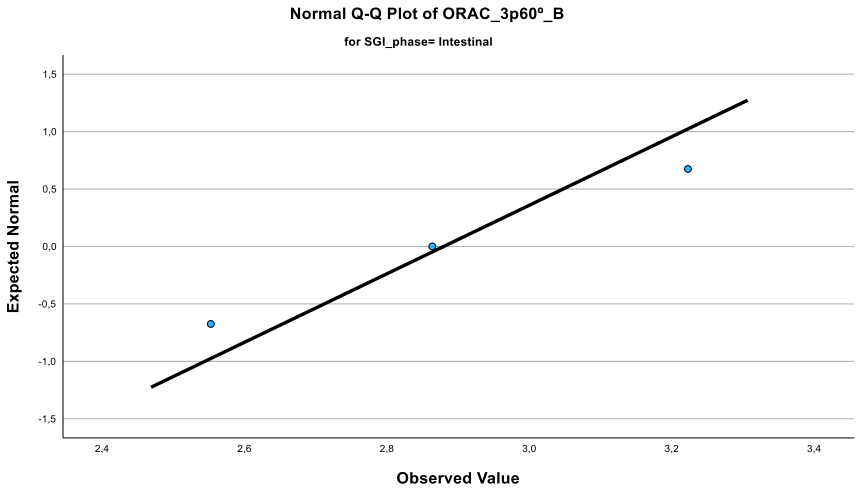
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	SGI_phase	Statistic	df	Sig.	Statistic	df	Sig.
ORAC_3%60º_B	Oral	,260	2	.			
	Gastric	,260	2	.			
	Intestinal	,186	3	.	,998	3	,921

a. Lilliefors Significance Correction

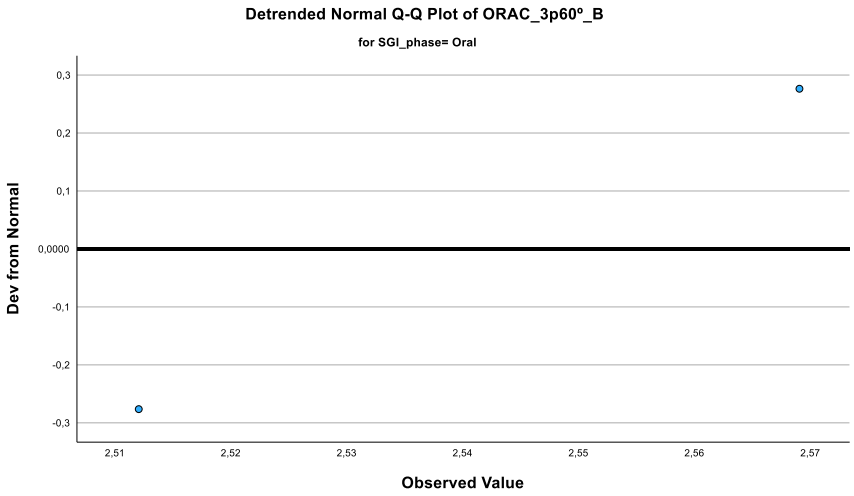
- **Normal Q-Q Plots**

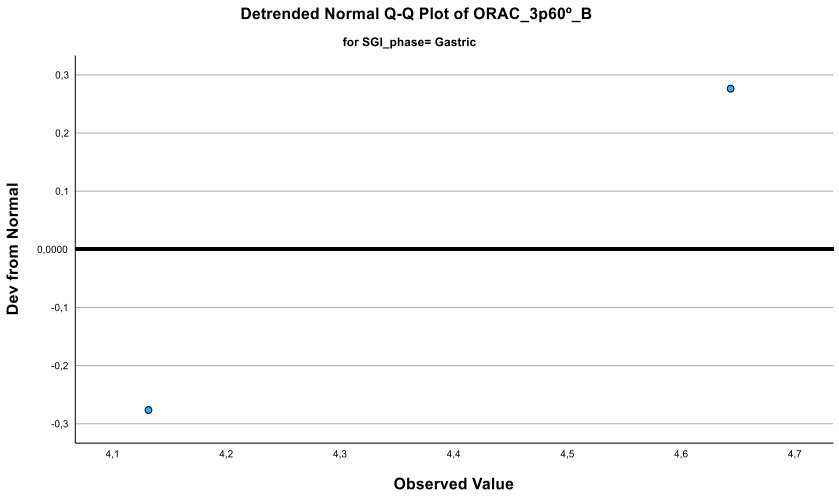






- **Detrended Normal Q-Q Plots**




Detrended Normal Q-Q Plot of ORAC_3p60º_B
for SGI_phase= Gastric
Dev from Normal
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0,1
0,0000
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-0,2
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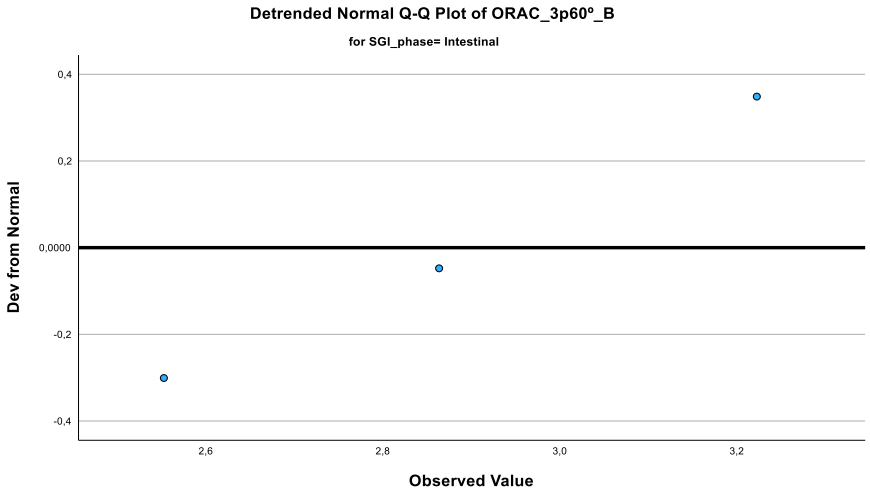
4,1
4,2
4,3
4,4
4,5
4,6
4,7
Observed Value


Detrended Normal Q-Q Plot of ORAC_3p60º_B
for SGI_phase= Intestinal
Dev from Normal
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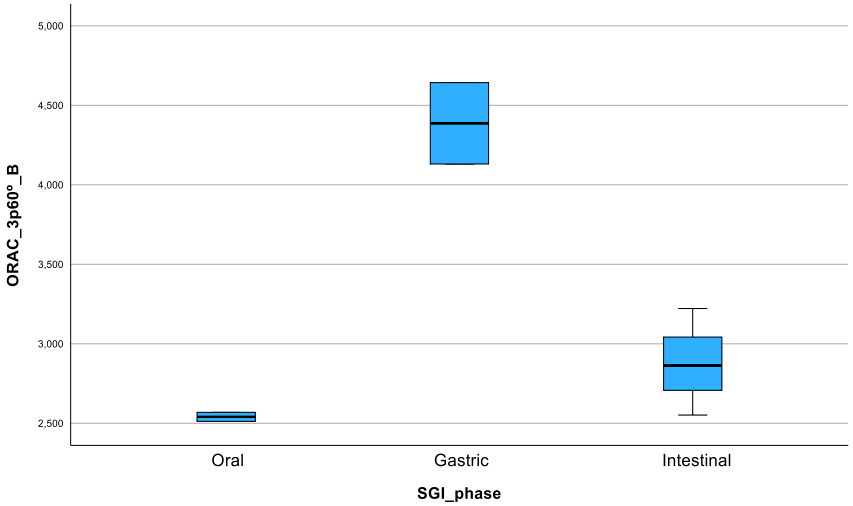
2,6
2,8
3,0
3,2
Observed Value


ORAC_3p60º_B
5,000
4,500
4,000
3,500
3,000
2,500
Oral
Gastric
Intestinal
SGI_phase

- **Explore**

Notes

Output Created		29-SEP-2023 12:50:05
Comments		
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	Filter	<none>
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	Split File	<none>
	N of Rows in Working Data File	18
Missing Value Handling	Definition of Missing	User-defined missing values for dependent variables are treated as missing.
	Cases Used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=ORAC_H1p_B BY SGI_phase /PLOT BOXPLOT STEMLEAF NPPLOT /COMPARE GROUPS /MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685) /STATISTICS DESCRIPTIVES EXTREME /CINTERVAL 95 /MISSING LISTWISE /NOTOTAL.
Resources	Processor Time	00:00:00,31
	Elapsed Time	00:00:00,91

Case Processing Summary

		Cases					
		Valid		Missing		Total	
	SGI_phase	N	Percent	N	Percent	N	Percent
ORAC_H1%_B	Oral	3	100,0%	0	0,0%	3	100,0%
	Gastric	3	100,0%	0	0,0%	3	100,0%
	Intestinal	3	100,0%	0	0,0%	3	100,0%

Descriptives

		SGI_phase		Statistic	Std. Error
ORAC_H1%_B	Oral	Mean		1,47933	,084743
		95% Confidence Interval for Mean	Lower Bound	1,11471	
			Upper Bound	1,84395	
		5% Trimmed Mean		.	
		Median		1,45700	
		Variance		,022	
		Std. Deviation		,146780	
		Minimum		1,345	
		Maximum		1,636	
		Range		,291	
		Interquartile Range		.	
		Skewness		,669	1,225
		Kurtosis		.	.
	Gastric	Mean		1,04700	,059102
		95% Confidence Interval for Mean	Lower Bound	,79271	
			Upper Bound	1,30129	
		5% Trimmed Mean		.	
		Median		1,03700	
		Variance		,010	
		Std. Deviation		,102367	
		Minimum		,950	
		Maximum		1,154	
		Range		,204	
		Interquartile Range		.	
		Skewness		,435	1,225
		Kurtosis		.	.
	Intestinal	Mean		3,39600	,968240
		95% Confidence Interval for Mean	Lower Bound	-,77000	
			Upper Bound	7,56200	
		5% Trimmed Mean		.	
		Median		3,47900	
		Variance		2,812	
		Std. Deviation		1,677041	
		Minimum		1,679	
		Maximum		5,030	
		Range		3,351	
		Interquartile Range		.	

	Skewness	-,222	1,225
	Kurtosis	.	.

M-Estimators

	SGI_phase	Huber's M-Estimator[a]	Tukey's Biweight[b]	Hampel's M-Estimator[c]	Andrews' Wave[d]
ORAC_H1%_B	Oral	1,47424	1,47401	1,47933	1,47397
	Gastric	1,04700	1,04520	1,04700	1,04518
	Intestinal	3,39600	3,40809	3,39600	3,40824

a. The weighting constant is 1,339. b. The weighting constant is 4,685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1,340*pi.

Extreme Values[a]

	SGI_phase			Case Number	Value
ORAC_H1%_B	Oral	Highest	1	3	1,636
		Lowest	1	2	1,345
	Gastric	Highest	1	4	1,154
		Lowest	1	6	,950
	Intestinal	Highest	1	9	5,030
		Lowest	1	7	1,679

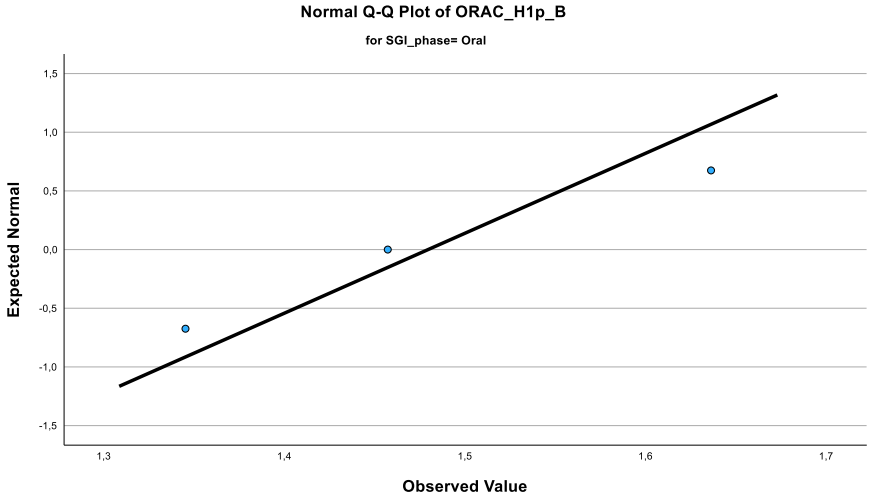
a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes is displayed.

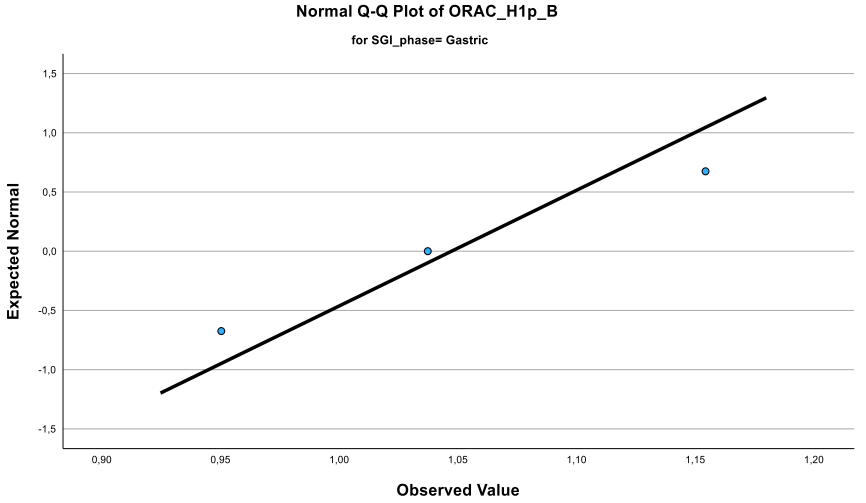
Tests of Normality

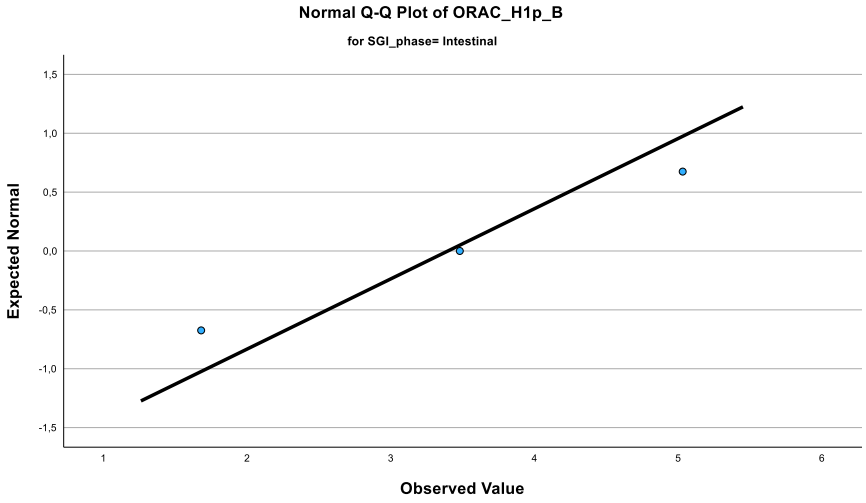
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	SGI_phase	Statistic	df	Sig.	Statistic	df	Sig.
ORAC_H1%_B	Oral	,227	3	.	,983	3	,748
	Gastric	,206	3	.	,993	3	,838
	Intestinal	,186	3	.	,998	3	,918

a. Lilliefors Significance Correction

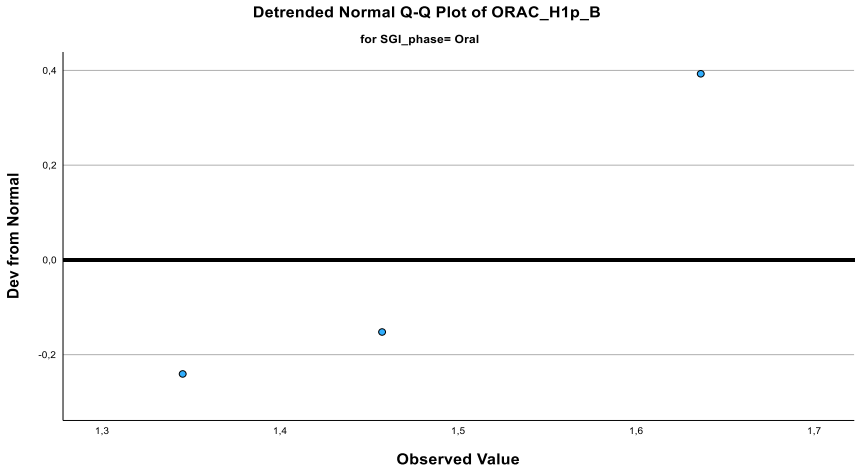
- **Normal Q-Q Plots**

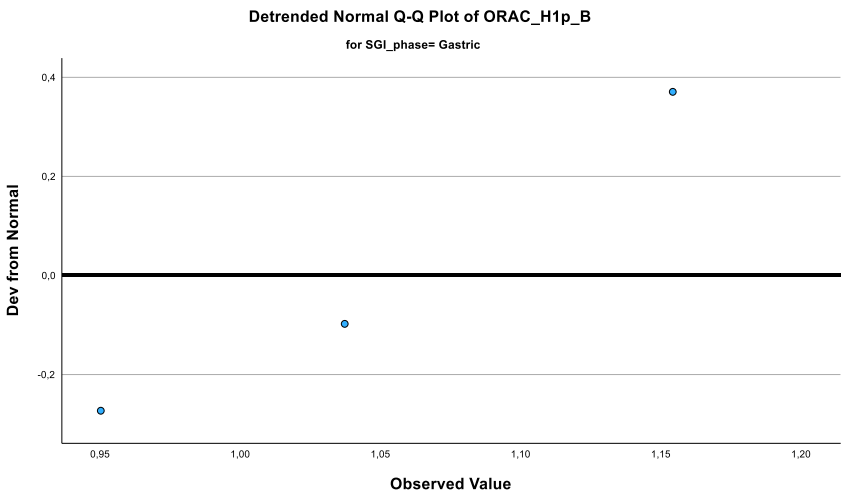


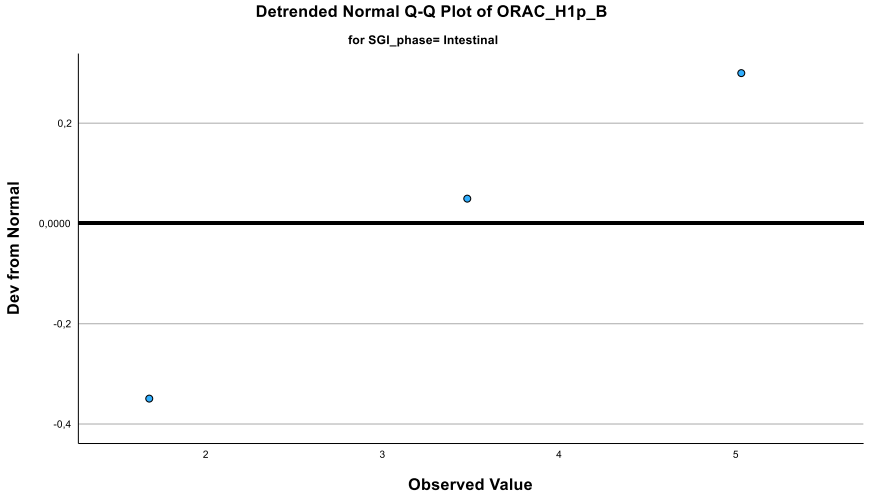


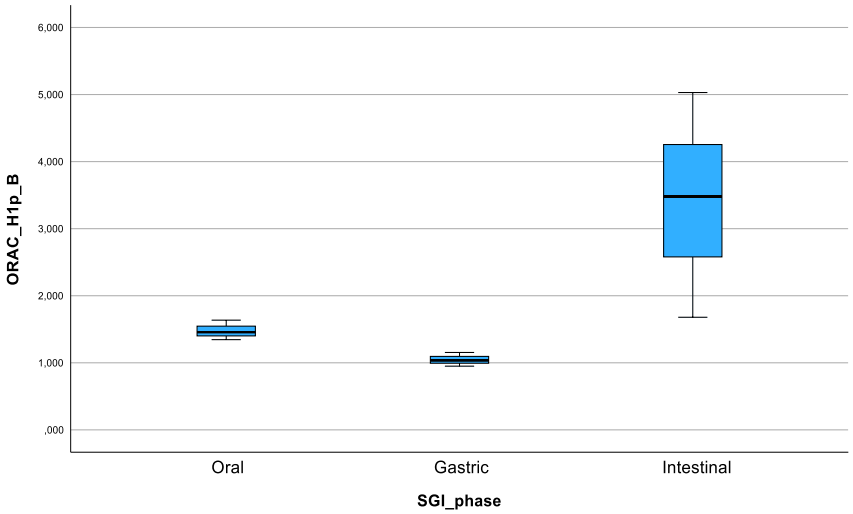


- **Detrended Normal Q-Q Plots**




Detrended Normal Q-Q Plot of ORAC_H1p_B
for SGI_phase= Gastric
Dev from Normal
0,4
0,2
0,0
-0,2
0,95
1,00
1,05
1,10
1,15
1,20
Observed Value


Detrended Normal Q-Q Plot of ORAC_H1p_B
for SGI_phase= Intestinal
Dev from Normal
0,2
0,0000
-0,2
-0,4
2
3
4
5
Observed Value


ORAC_H1p_B
6,000
5,000
4,000
3,000
2,000
1,000
,000
Oral
Gastric
Intestinal
SGI_phase

- **Univariate Analysis of Variance**

Notes

Output Created		29-SEP-2023 12:50:48
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\ABTS_INFOGEST_SPSS.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	18
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA ORAC_3p60º_B BY SGI_phase /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=SGI_phase(TUKEY) /PLOT=PROFILE(SGI_phase) TYPE=LINE ERRORBAR=CI MEANREFERENCE=NO YAXIS=AUTO /PRINT ETASQ DESCRIPTIVE PARAMETER HOMOGENEITY OPOWER /PLOT=RESIDUALS /CRITERIA=ALPHA(.05) /DESIGN=SGI_phase.
Resources	Processor Time	00:00:00,13
	Elapsed Time	00:00:00,34

Between-Subjects Factors

		Value Label	N
SGI_phase	1	Oral	2
	2	Gastric	2
	3	Intestinal	3

Descriptive Statistics

Dependent Variable: ORAC_3%60º_B

SGI_phase	Mean	Std. Deviation	N
Oral	2,54050	,040305	2
Gastric	4,38700	,362039	2
Intestinal	2,87900	,335286	3
Total	3,21314	,851789	7

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
ORAC_3%60º_B	Based on Mean	1,900	2	4	,263
	Based on Median	1,659	2	4	,299
	Based on Median and with adjusted df	1,659	2	2,000	,376
	Based on trimmed mean	1,886	2	4	,265

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a,b] a. Dependent variable: ORAC_3p60º_B. b. Design: Intercept + SGI_phase

Tests of Between-Subjects Effects

Dependent Variable: ORAC_3%60º_B

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3,996[a]	2	1,998	22,352	,007	,918
Intercept	72,126	1	72,126	806,931	<.001	,995
SGI_phase	3,996	2	1,998	22,352	,007	,918
Error	,358	4	,089			
Total	76,623	7				
Corrected Total	4,353	6				

Tests of Between-Subjects Effects

Dependent Variable: ORAC_3%60º_B

Source	Noncent. Parameter	Observed Power[b]
Corrected Model	44,704	,973
Intercept	806,931	1,000
SGI_phase	44,704	,973
Error		
Total		
Corrected Total		

a. R Squared = ,918 (Adjusted R Squared = ,877). b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: ORAC_3%60º_B

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	2,879	,173	16,679	<.001	2,400	3,358
[SGI_phase=1]	-,339	,273	-1,240	,283	-1,096	,419
[SGI_phase=2]	1,508	,273	5,525	,005	,750	2,266
[SGI_phase=3]	0[a]	.	.	.	.	.

Parameter Estimates

Dependent Variable: ORAC_3%60º_B

Parameter	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Intercept	,986	16,679	1,000
[SGI_phase=1]	,278	1,240	,162
[SGI_phase=2]	,884	5,525	,980
[SGI_phase=3]	.	.	.

a. This parameter is set to zero because it is redundant.

b. Computed using alpha = .05

- **Post Hoc Tests**

Multiple Comparisons

Dependent Variable: ORAC_3%60º_B

Tukey HSD

(I) SGI_phase	(J) SGI_phase	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Oral	Gastric	-1,84650*	,298969	,008	-2,91202	-,78098
	Intestinal	-,33850	,272920	,494	-1,31119	,63419
Gastric	Oral	1,84650*	,298969	,008	,78098	2,91202
	Intestinal	1,50800*	,272920	,011	,53531	2,48069
Intestinal	Oral	,33850	,272920	,494	-,63419	1,31119
	Gastric	-1,50800*	,272920	,011	-2,48069	-,53531

Based on observed means. The error term is Mean Square(Error) = ,089. *. The mean difference is significant at the .05 level.

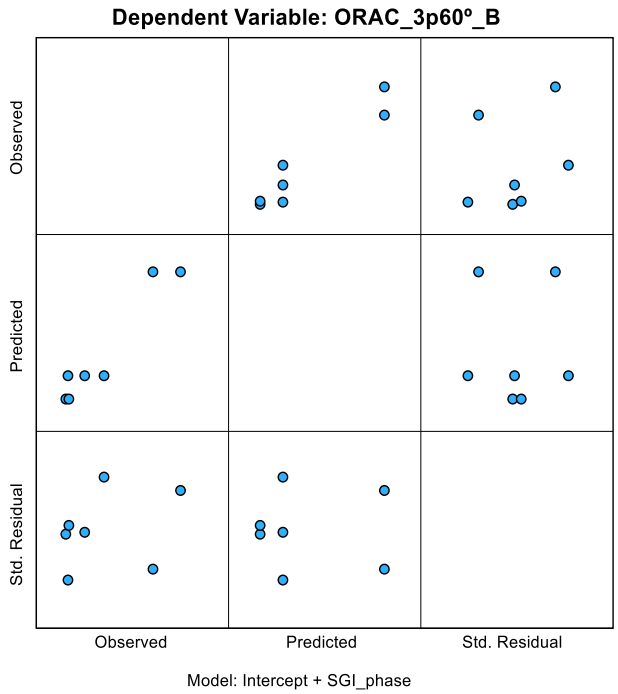
- **Homogeneous Subsets**

ORAC_3%60º_B

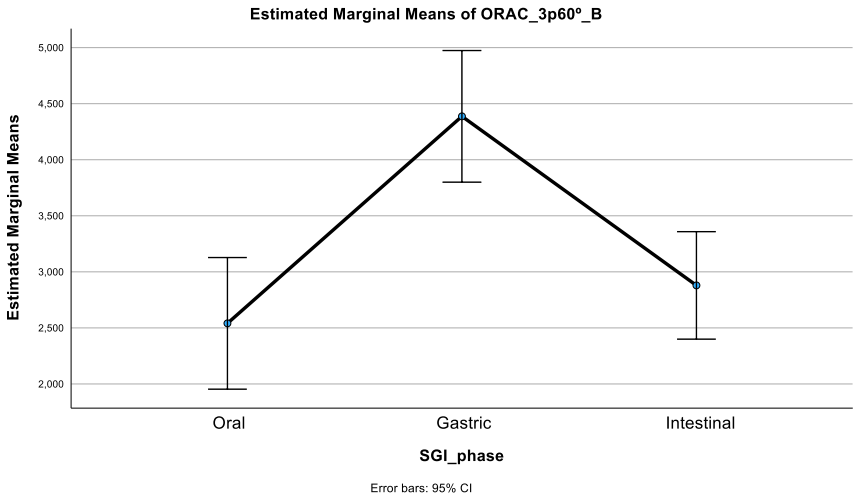
Tukey HSD[a,b,c]

SGI_phase	N	Subset 1	Subset 2
Oral	2	2,54050	
Intestinal	3	2,87900	
Gastric	2		4,38700
Sig.		,513	1,000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = ,089. a. Uses Harmonic Mean Sample Size = 2,250. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed. c. Alpha = .05.



- **Profile Plots**



- **Univariate Analysis of Variance**

Notes

Output Created		29-SEP-2023 12:51:13
Comments		
Input	Data	C:\Users\ezerc\Google Drive\Trabajo\Posdoc\Orientaciones\MSc\Marisa Ferreira\SPSS\ABTS_INFOGEST_SPSS.sav
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	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	18
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
	Syntax	UNIANOVA ORAC_H1p_B BY SGI_phase /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=SGI_phase(TUKEY) /PLOT=PROFILE(SGI_phase) TYPE=LINE ERRORBAR=CI MEANREFERENCE=NO YAXIS=AUTO /PRINT ETASQ DESCRIPTIVE PARAMETER HOMOGENEITY OPOWER /PLOT=RESIDUALS /CRITERIA=ALPHA(.05) /DESIGN=SGI_phase.
Resources	Processor Time	00:00:00,11
	Elapsed Time	00:00:00,29

Between-Subjects Factors

		Value Label	N
SGI_phase	1	Oral	3
	2	Gastric	3
	3	Intestinal	3

Descriptive Statistics

Dependent Variable: ORAC_H1%_B

SGI_phase	Mean	Std. Deviation	N
Oral	1,47933	,146780	3
Gastric	1,04700	,102367	3
Intestinal	3,39600	1,677041	3
Total	1,97411	1,372375	9

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
ORAC_H1%_B	Based on Mean	3,921	2	6	,081
	Based on Median	3,335	2	6	,106
	Based on Median and with adjusted df	3,335	2	2,050	,227
	Based on trimmed mean	3,887	2	6	,083

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a,b] a. Dependent variable: ORAC_H1%_B. b. Design: Intercept + SGI_phase

Tests of Between-Subjects Effects

Dependent Variable: ORAC_H1%_B

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	9,378[a]	2	4,689	4,946	,054	,622
Intercept	35,074	1	35,074	36,992	<.001	,860
SGI_phase	9,378	2	4,689	4,946	,054	,622
Error	5,689	6	,948			
Total	50,141	9				
Corrected Total	15,067	8				

Tests of Between-Subjects Effects

Dependent Variable: ORAC_H1%_B

Source	Noncent. Parameter	Observed Power[b]
Corrected Model	9,891	,575
Intercept	36,992	,999
SGI_phase	9,891	,575
Error		
Total		
Corrected Total		

a. R Squared = ,622 (Adjusted R Squared = ,497)

b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: ORAC_H1%_B

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	3,396	,562	6,041	<.001	2,020	4,772
[SGI_phase=1]	-1,917	,795	-2,411	,053	-3,862	,029
[SGI_phase=2]	-2,349	,795	-2,955	,025	-4,294	-,404
[SGI_phase=3]	0[a]	.	.	.	.	.

Parameter Estimates

Dependent Variable: ORAC_H1%_B

Parameter	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Intercept	,859	6,041	,998
[SGI_phase=1]	,492	2,411	,525
[SGI_phase=2]	,593	2,955	,694
[SGI_phase=3]	.	.	.

a. This parameter is set to zero because it is redundant.

b. Computed using alpha = .05

- **Post Hoc Tests**

Multiple Comparisons

Dependent Variable: ORAC_H1%_B

Tukey HSD

(I) SGI_phase	(J) SGI_phase	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Oral	Gastric	,43233	,795053	,853	-2,00711	2,87177
	Intestinal	-1,91667	,795053	,114	-4,35611	,52277
Gastric	Oral	-,43233	,795053	,853	-2,87177	2,00711
	Intestinal	-2,34900	,795053	,058	-4,78844	,09044
Intestinal	Oral	1,91667	,795053	,114	-,52277	4,35611
	Gastric	2,34900	,795053	,058	-,09044	4,78844

Based on observed means.
The error term is Mean Square(Error) = ,948.

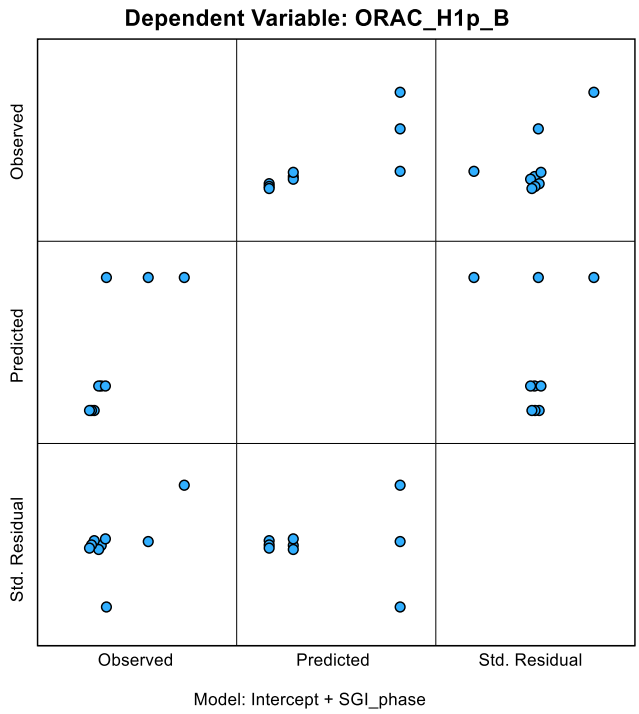
- **Homogeneous Subsets**

ORAC_H1%_B

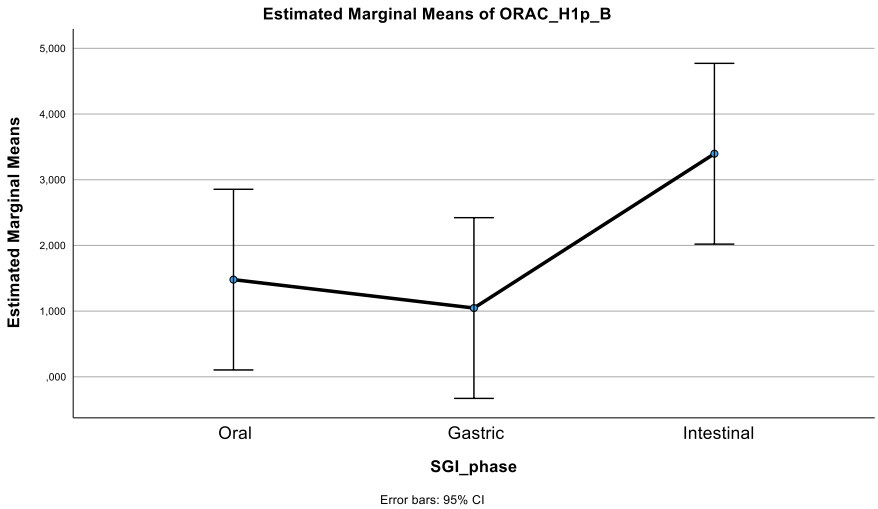
Tukey HSD[a,b]

SGI_phase	N	Subset 1
Gastric	3	1,04700
Oral	3	1,47933
Intestinal	3	3,39600
Sig.		,058

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = ,948. a. Uses Harmonic Mean Sample Size = 3,000. b. Alpha = .05.



- **Profile Plots**



5.8. Antioxidant activity – Dialysis

- Explore

Observations

Output Created		09-NOV-2023 18:42:10
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\INFOGEST\DIALISIS_ABTS.sav
	Active dataset	DataSet2
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=umolequivalentestroloxmgproteina BY ABTS_dialisis_solu/PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT /COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING LISTWISE/NOTOTAL.
Resources	Processor time	00:00:01,19
	Elapsed time	00:00:00,90

Case Processing Summary

		Cases		
		Valid		Silent
	ABTS_dialisis_solu	N	Percentage	N
Umol Trolox Equivalents/mg Protein	3%60º_I	3	100,0%	0
	3%60º_P	3	100,0%	0
	3%60º_R	3	100,0%	0

Case Processing Summary

		Cases Silent		Total	
	ABTS_dialisis_solu	Percentage	N	Percentage
Umol Trolox Equivalents/mg Protein	3%60º_I	0,0%	3	100,0%
	3%60º_P	0,0%	3	100,0%
	3%60º_R	0,0%	3	100,0%

Descriptive

	ABTS_dialisis_solu			Statistics
Umol Trolox Equivalents/mg Protein	3%60th_I	Average		7,0600
		95% Confidence Interval to Mean	Lower Limit	2,1820
			Upper limit	11,9380
		5% of average trimmed		.
		Median		6,5100
		Variance		3,856
		Standard Error		1,96364
		Minimal		5,43
		Maximum		9,24
		Amplitude		3,81
		Interquartile range		.
		Asymmetry		1,162
		Kurtosis		.
	3%60th_P	Average		14,5233
		95% Confidence Interval to Mean	Lower Limit	5,4391
			Upper limit	23,6076
		5% of average trimmed		.
		Median		15,0800
		Variance		13,373
		Standard Error		3,65692
		Minimal		10,62
		Maximum		17,87
		Amplitude		7,25
		Interquartile range		.
		Asymmetry		-,669
		Kurtosis		.
	3%60th_R	Average		9,9400
		95% Confidence Interval to Mean	Lower Limit	-1,3933
			Upper limit	21,2733
		5% of average trimmed		.
		Median		9,4200
		Variance		20,814
		Standard Error		4,56228
		Minimum		5,66
		Maximum		14,74
		Amplitude		9,08
		Interquartile range		.
		Asymmetry		,506
		Kurtosis		.

Descriptive

	ABTS_dialisis_solu			Pattern Statistics
Umol Trolox Equivalents/mg Protein	3%60º_I	Average		1,13371
		95% Confidence Interval to Mean	Lower Limit	
			Upper limit	
		5% of average trimmed		
		Median		
		Variance		
		Standard Error		
		Minimum		
		Maximum		
		Amplitude		
		Interquartile range		
		Asymmetry		1,225
		Kurtosis		.
	3%60º_P	Average		2,11132
		95% Confidence Interval to Mean	Lower Limit	
			Upper limit	
		5% of average trimmed		
		Median		
		Variance		
		Standard Error		
		Minimum		
		Maximum		
		Amplitude		
		Interquartile range		
		Asymmetry		1,225
		Kurtosis		.
	3%60º_R	Average		2,63403
		95% Confidence Interval to Mean	Lower Limit	
			Upper limit	
		5% of average trimmed		
		Median		
		Variance		
		Standard Error		
		Minimum		
		Maximum		
		Amplitude		
		Interquartile range		

		Asymmetry	1,225
		Kurtosis	.

M Estimators

	ABTS_dialisis_solu	Hubera Estimator M	Tukeyb bi-weighting	Hampelc M Estimator
Umol Trolox Equivalents/mg Protein	3%60º_I	6,6847	6,7626	6,8808
	3%60º_P	14,6209	14,6401	14,5233
	3%60º_R	9,9400	9,8503	9,9400

M Estimators

	ABTS_dialisis_solu	Andrewsd Wave
Umol Trolox Equivalents/mg Protein	3%60º_I	6,7649
	3%60º_P	14,6410
	3%60º_R	9,8494

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

	ABTS_dialisis_solu			Case number	Value
Umol Trolox Equivalents/mg Protein	3%60º_I	Upper	1	1	9,24
		Lower	1	3	5,43
	3%60º_P	Upper	1	4	17,87
		Lower	1	5	10,62
	3%60º_R	Upper	1	7	14,74
		Lower	1	9	5,66

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

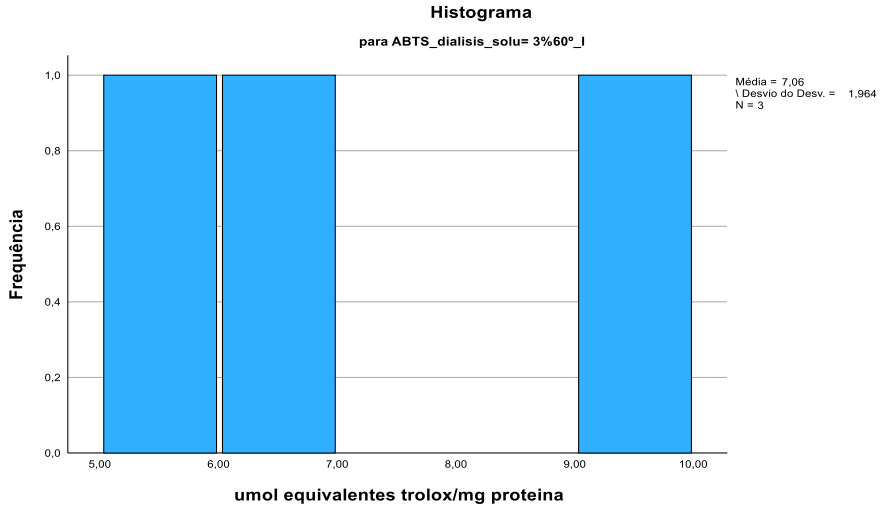
Normality Tests

		Kolmogorov-Smirnova			Shapiro-Wilk
	ABTS_dialisis_solu	Statistics	gl	Sig.	Statistics
Umol Trolox Equivalents/mg Protein	3%60º_I	,277	3	.	,941
	3%60º_P	,227	3	.	,983
	3%60º_R	,212	3	.	,990

Normality Tests

		Shapiro-Wilk	
	ABTS_dialisis_solu	Gl	Sig.
Umol Trolox Equivalents/mg Protein	3%60º_I	3	,532
	3%60º_P	3	,747
	3%60º_R	3	,811

- Histograms


Histograma
para ABTS_dialisis_solu= 3%60º_I
Média = 7,06
\ Desvio do Desv. = 1,964
N = 3
Frequência
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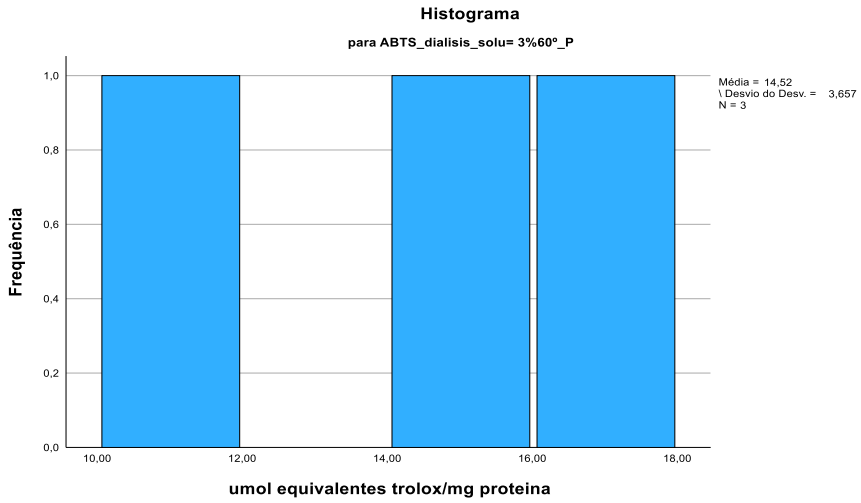
0,8
0,6
0,4
0,2
0,0
5,00
6,00
7,00
8,00
9,00
10,00
umol equivalentes trolox/mg proteina


Histograma
para ABTS_dialisis_solu= 3%60º_P
Média = 14,52
\ Desvio do Desv. = 3,657
N = 3
Frequência
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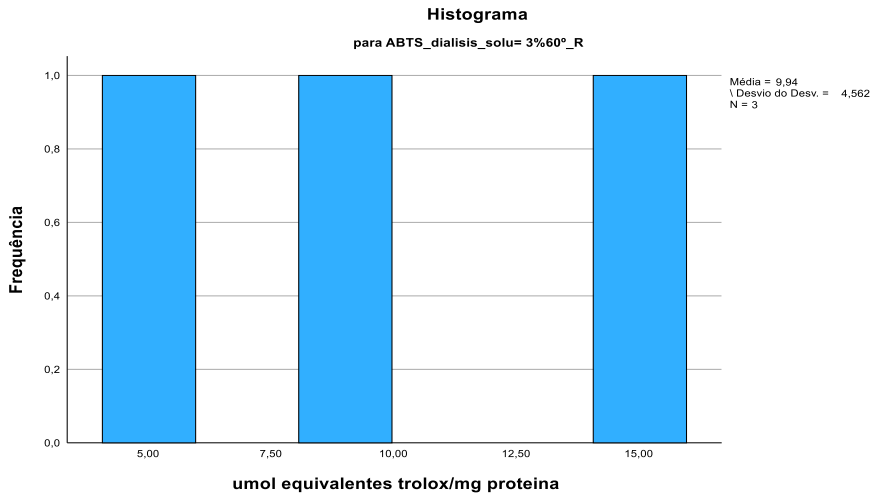
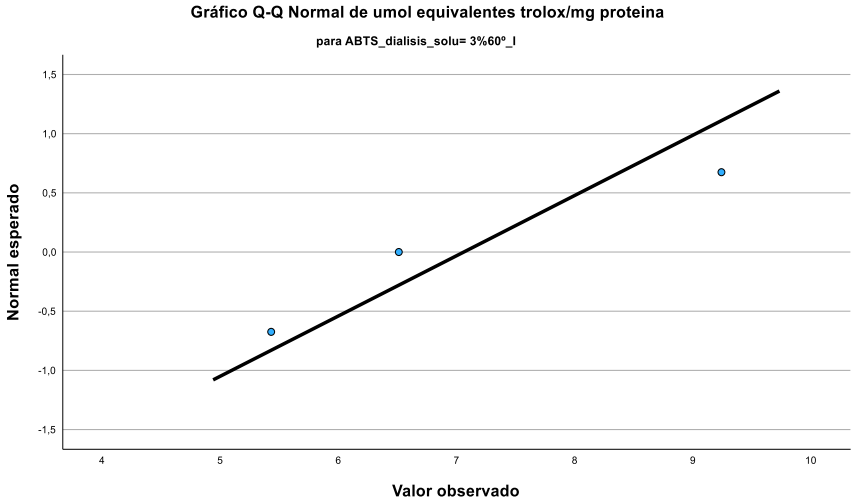
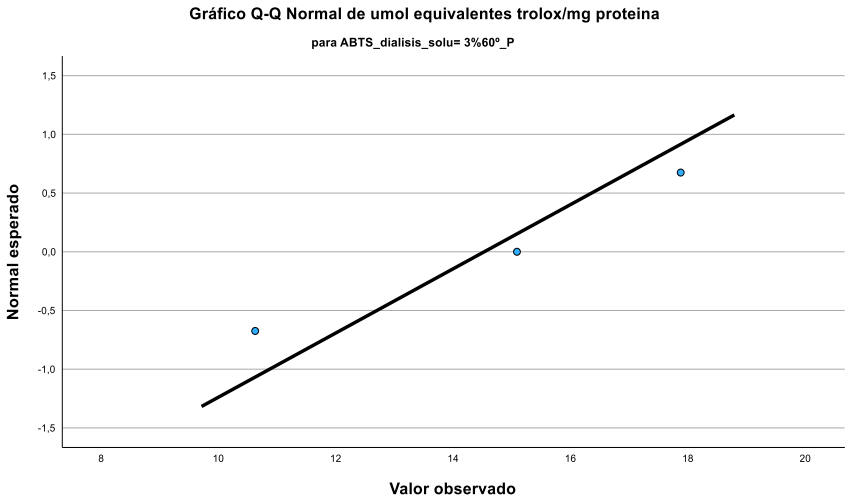
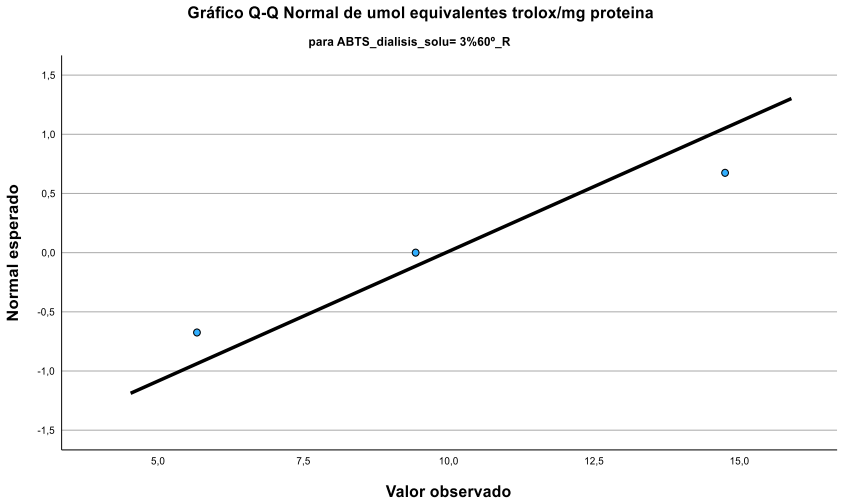
0,4
0,2
0,0
10,00
12,00
14,00
16,00
18,00
umol equivalentes trolox/mg proteina


Histograma
para ABTS_dialisis_solu= 3%60º_R
Média = 9,94
\ Desvio do Desv. = 4,562
N = 3
Frequência
1,0
0,8
0,6
0,4
0,2
0,0
5,00
7,50
10,00
12,50
15,00
umol equivalentes trolox/mg proteina

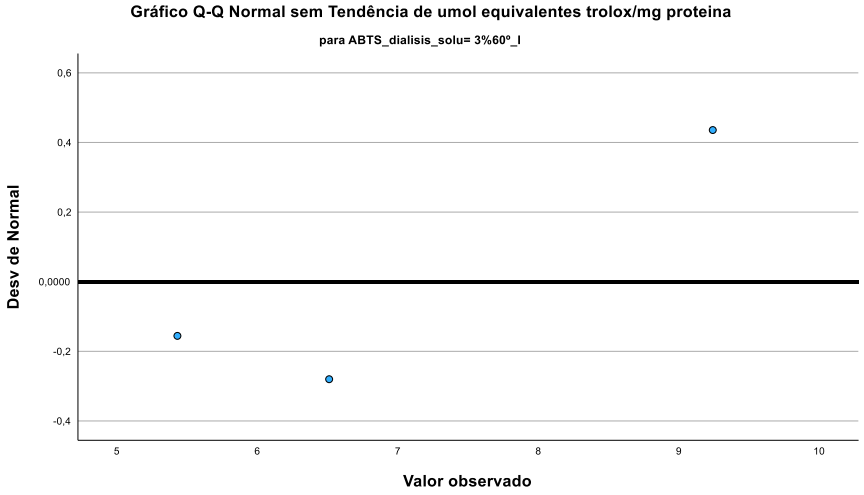
- Normal Q-Q Chart

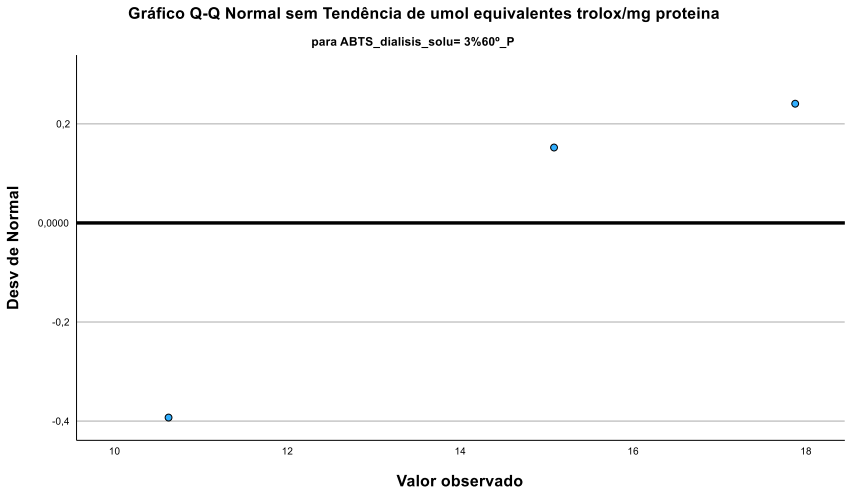


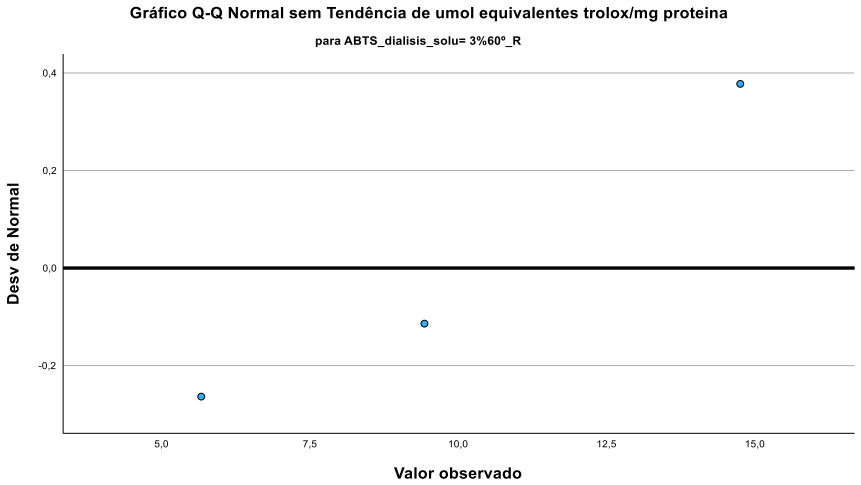


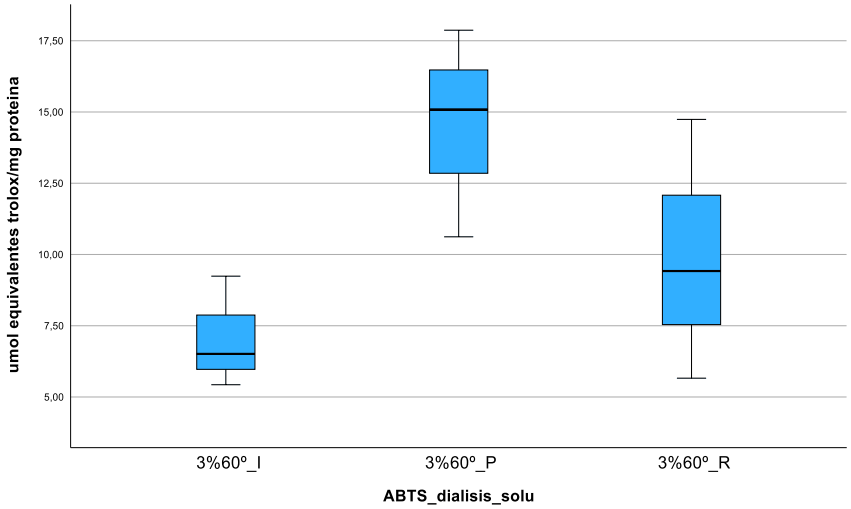


- Normal Q-Q Chart No Trend









- Univariate Analysis of Variance

Observations

Output Created		09-NOV-2023 18:43:02
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\INFOGEST\DIALISIS_ABTS.sav
	Active dataset	DataSet2
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA umolequivalentestroloxmgproteina BY ABTS_dialisis_solu/METHOD=SSTYPE(3)/INTERCEPT=INCLUDE/POSTHOC=ABTS_dialisis_solu(TUKEY)/PLOT=PROFILE(ABTS_dialisis_solu) TYPE=LINE ERRORBAR=NO MEANREFERENCE=NO YAXIS=AUTO/PRINT DESCRIPTIVE HOMOGENEITY/CRITERIA=ALPHA(.05)/DESIGN=ABTS_dialisis_solu.
Resources	Processor time	00:00:00,00
	Elapsed time	00:00:00,11

Factors Between Subjects

		Value Label	N
ABTS_dialisis_solu	1,00	3%60º_I	3
	2,00	3%60º_P	3
	3,00	3%60º_R	3

Descriptive Statistics

Dependent variable: umol trolox/mg protein equivalents

ABTS_dialisis_solu	Average	Pattern Statistics	N
3%60º_I	7,0600	1,96364	3
3%60º_P	14,5233	3,65692	3
3%60º_R	9,9400	4,56228	3
Total	10,5078	4,48733	9

Levenea's error variance equality test,b

		Levene's Statistics	df1	df2
Umol Trolox Equivalents/mg Protein	Based on average	,758	2	6
	Based on median	,496	2	6
	Based on median and adjusted gl	,496	2	4,894
	Based on trimmed average	,741	2	6

Levenea's error variance equality test,b

		Sig.
Umol Trolox Equivalents/mg Protein	Based on average	,509
	Based on median	,632
	Based on median and adjusted gl	,637
	Based on trimmed average	,516

Tests the null hypothesis that the variance of the error of the dependent variable is equal between groups.[a,b] a. Dependent variable: umol trolox/mg protein equivalents. b. Design: Intercept + ABTS_dialisis_solu

Tests of effects between subjects

Dependent variable: umol trolox/mg protein equivalents

Origin	Type III Sum of Squares	df	Medium Square	With	Sig.
Corrected model	85,003[a]	2	42,501	3,352	,105
Intercept	993,721	1	993,721	78,362	<,001
ABTS_dialisis_solu	85,003	2	42,501	3,352	,105
Pattern	76,087	6	12,681		
Total	1154,810	9			
Corrected total	161,089	8			

a. R Squared = .528 (Adjusted R Squared = .370)

- Posterior Testes

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents
Tukey HSD

(I) ABTS_dialisis_solu	(J) ABTS_dialisis_solu	Mean difference (I-J)	Pattern Statistics	Sig.
3%60º_I	3%60º_P	-7,4633	2,90759	,094
	3%60º_R	-2,8800	2,90759	,609
3%60º_P	3%60º_I	7,4633	2,90759	,094
	3%60º_R	4,5833	2,90759	,325
3%60º_R	3%60º_I	2,8800	2,90759	,609
	3%60º_P	-4,5833	2,90759	,325

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents
Tukey HSD

		95% Confidence Interval	
(I) ABTS_dialisis_solu	(J) ABTS_dialisis_solu	Lower Limit	Upper limit
3%60º_I	3%60º_P	-16,3846	1,4579
	3%60º_R	-11,8013	6,0413
3%60º_P	3%60º_I	-1,4579	16,3846
	3%60º_R	-4,3379	13,5046
3%60º_R	3%60º_I	-6,0413	11,8013
	3%60º_P	-13,5046	4,3379

Based on observed averages. The error term is Mean Square (Error) = 12.681.

- Homogeneous subsets

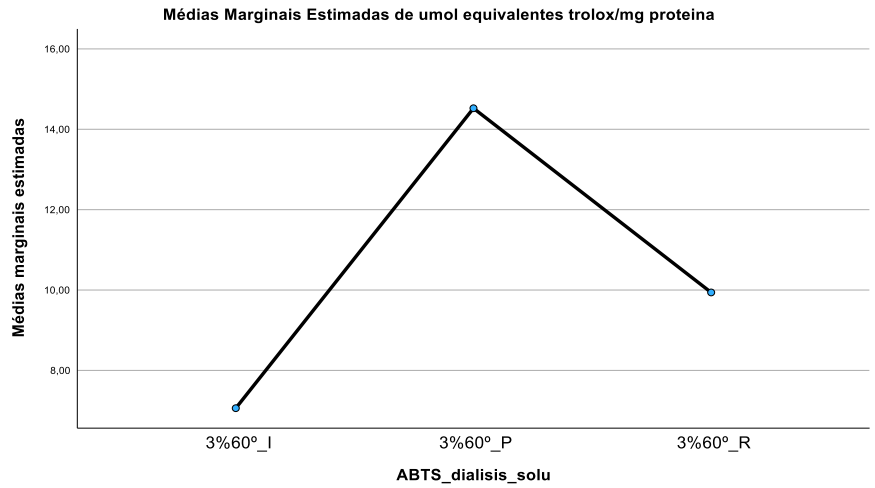
Umol Trolox Equivalents/mg Protein

Tukey HSD a,b

		Subset
ABTS_dialisis_solu	N	1
3%60º_I	3	7,0600
3%60º_R	3	9,9400
3%60º_P	3	14,5233
Sig.		,094

Averages are displayed for the groups in subsets homogêneos.Com based on observed averages. The error term is Mean Square (Error) = 12.681. a. Uses the Harmonic Mean Sample Size = 3,000. b. Alpha = .05.

- Profile Charts



- Explore

Observations

Output Created		09-NOV-2023 18:44:00
Comments		
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	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=umolequivalentestroloxmgproteina BY ABTS_dialisis_hidro/PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING

		LISTWISE/NOTOTAL.
Resources	Processor time	00:00:00,55
	Elapsed time	00:00:00,89

Case Processing Summary

		Cases			
		Valid		Silent	
	ABTS_dialisis_hidro	N	Percentage	N	
Umol Trolox Equivalents/mg Protein	H1%_I	3	100,0%	0	
	H1%_P	2	66,7%	1	
	H1%_R	3	100,0%	0	

Case Processing Summary

		Cases Silent			
			Total		
	ABTS_dialisis_hidro	Percentage	N	Percentage	
Umol Trolox Equivalents/mg Protein	H1%_I	0,0%	3	100,0%	
	H1%_P	33,3%	3	100,0%	
	H1%_R	0,0%	3	100,0%	

Descriptive

	ABTS_dialisis_hidro			Statistics	Pattern Statistics
Umol Trolox Equivalents/mg Protein	H1%_I	Average		2,5100	,86933
		95% Confidence Interval to Mean	Lower Limit	-1,2304	
			Upper limit	6,2504	
		5% of average trimmed		.	
		Median		1,8700	
		Variance		2,267	
		Standard Error		1,50572	
		Minimal		1,43	
		Maximum		4,23	
		Amplitude		2,80	
		Interquartile range		.	
		Asymmetry		1,567	1,225
		Kurtosis		.	.
	H1%_P	Average		4,0500	,24000
		95% Confidence Interval to Mean	Lower Limit	1,0005	
			Upper limit	7,0995	
		5% of average trimmed		.	
		Median		4,0500	
		Variance		,115	
		Standard Error		,33941	

		Minimal		3,81	
		Maximum		4,29	
		Amplitude		,48	
		Interquartile range		.	
		Asymmetry		.	.
		Kurtosis		.	.
	H1%_R	Average		2,7600	,92803
		95% Confidence Interval to Mean	Lower Limit	-1,2330	
			Upper limit	6,7530	
		5% of average trimmed		.	
		Median		2,2900	
		Variance		2,584	
		Standard Error		1,60739	
		Minimal		1,44	
		Maximum		4,55	
		Amplitude		3,11	
		Interquartile range		.	
		Asymmetry		1,203	1,225
		Kurtosis		.	.

M Estimators

	ABTS_dialisis_hidro	Hubera Estimator M	Tukeyb bi-weighting	Hampelc M Estimator
Umol Trolox Equivalents/mg Protein	H1%_I	1,9436	1,6500	1,8791
	H1%_P	4,0500	4,0500	4,0500
	H1%_R	2,4328	2,4805	2,5861

M Estimators

	ABTS_dialisis_hidro	Andrewsd Wave
Umol Trolox Equivalents/mg Protein	H1%_I	1,6500
	H1%_P	4,0500
	H1%_R	2,4833

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

	ABTS_dialisis_hidro			Case number	Value
Umol Trolox Equivalents/mg Protein	H1%_I	Upper	1	12	4,23
		Lower	1	10	1,43
	H1%_P	Upper	1	13	4,29
		Lower	1	14	3,81
	H1%_R	Upper	1	16	4,55
		Lower	1	17	1,44

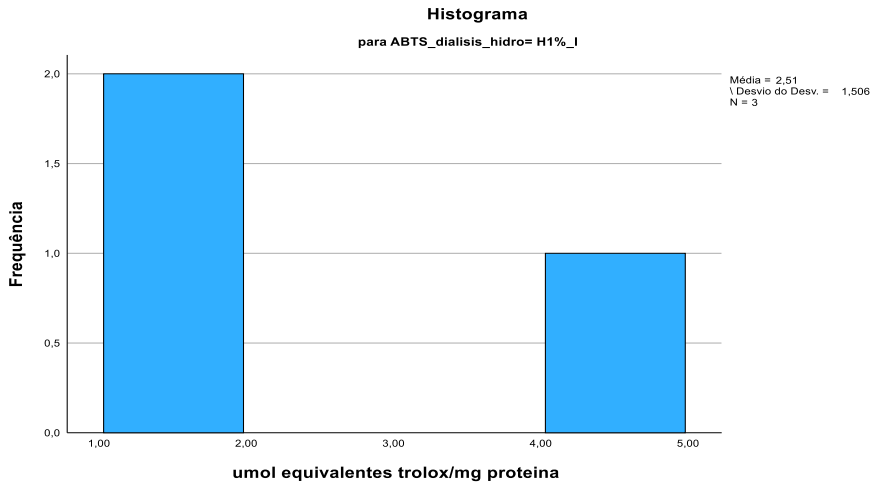
Normality Tests

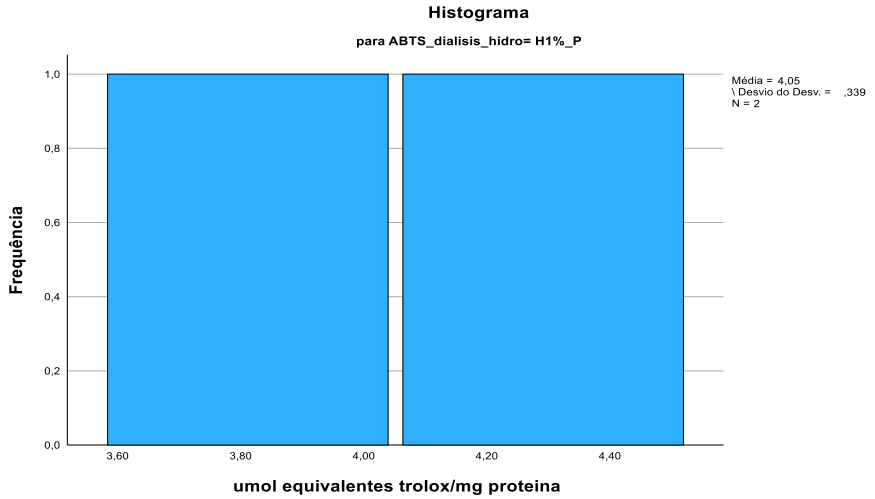
		Kolmogorov-Smirnova			Shapiro-Wilk
	ABTS_dialisis_hidro	Statistics	gl	Sig.	Statistics
Umol Trolox Equivalents/mg Protein	H1%_I	,331	3	.	,865
	H1%_P	,260	2	.	
	H1%_R	,282	3	.	,936

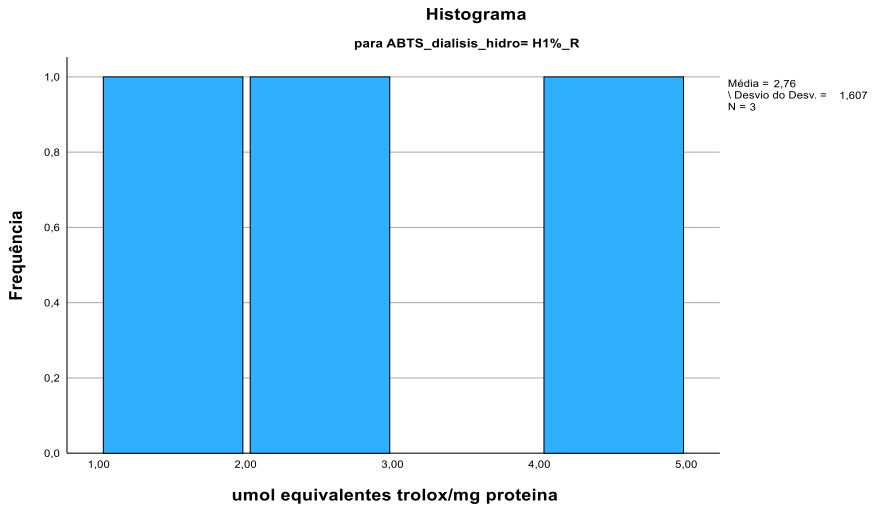
Normality Tests

		Shapiro-Wilk	
	ABTS_dialisis_hidro	gl	Sig.
Umol Trolox Equivalents/mg Protein	H1%_I	3	,280
	H1%_P		
	H1%_R	3	,511

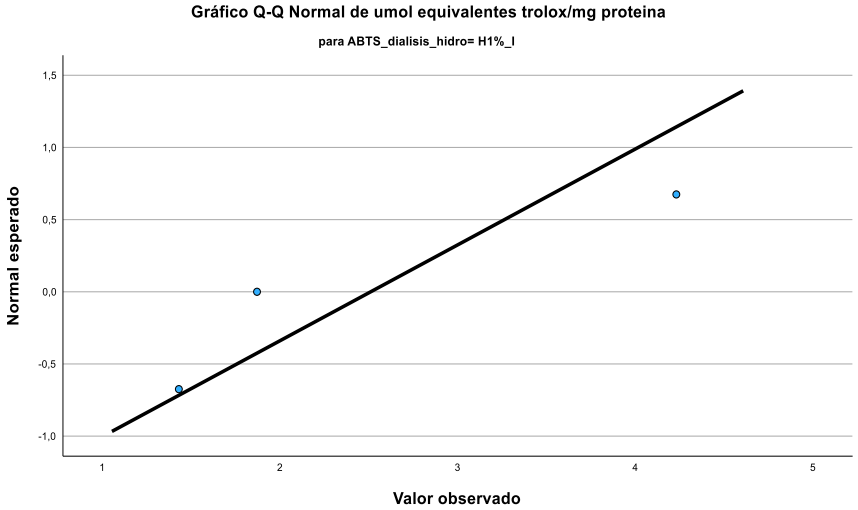
- Histograms

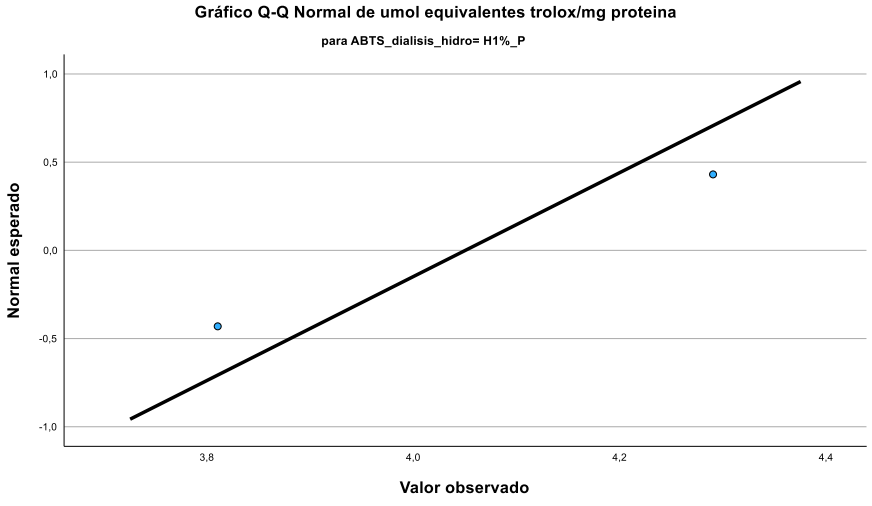


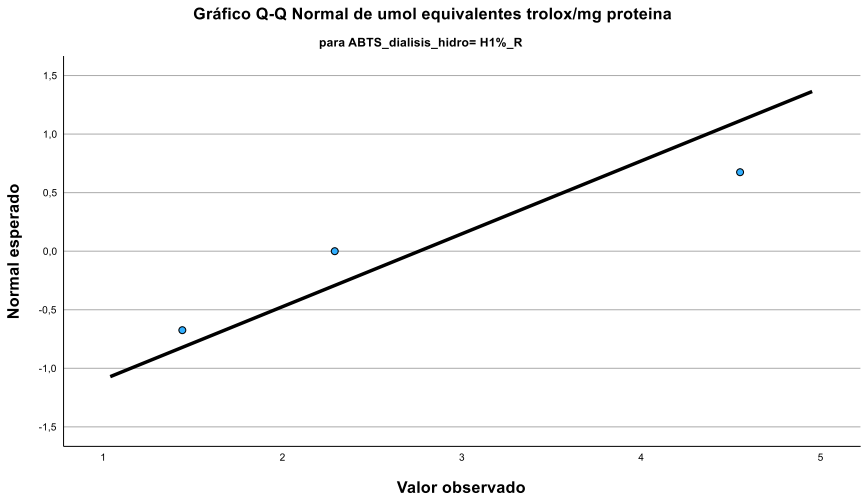




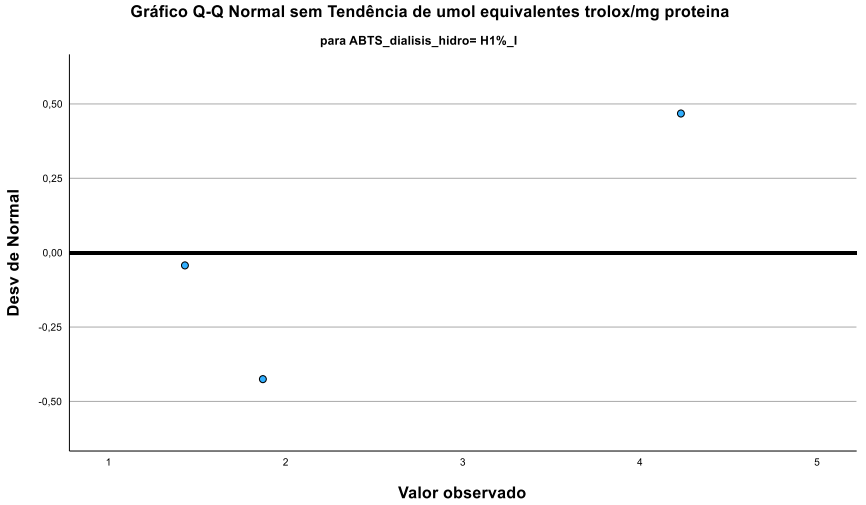
- Normal Q-Q Chart

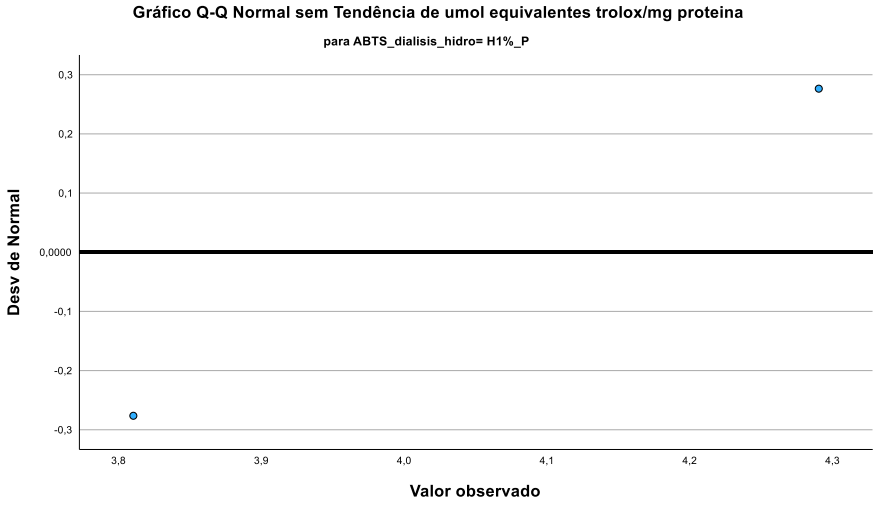






- Normal Q-Q Chart No Trend





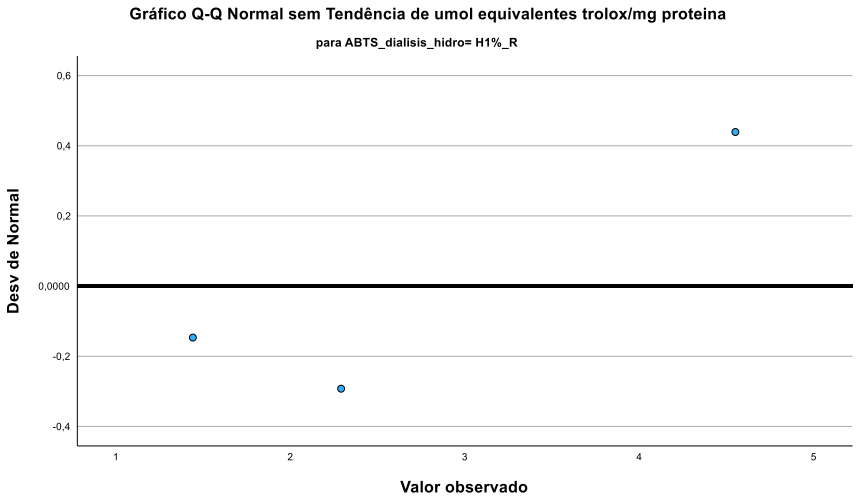
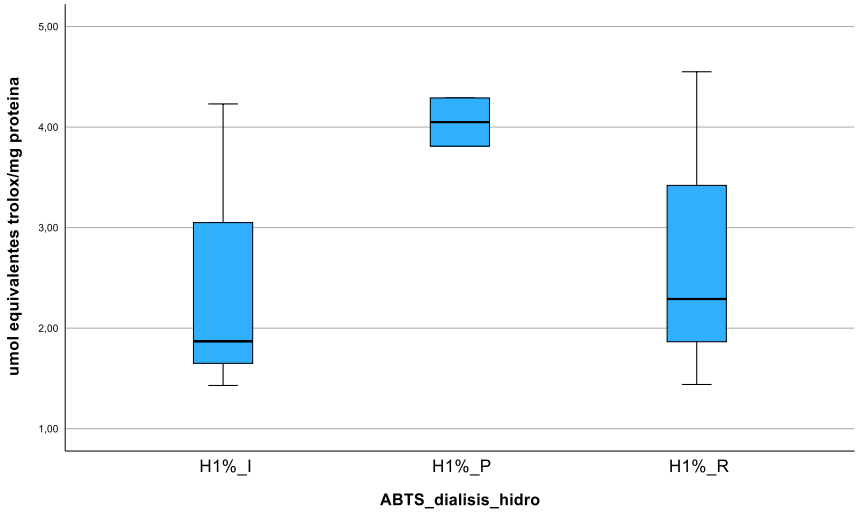


Gráfico Q-Q Normal sem Tendência de umol equivalentes trolox/mg proteina
para ABTS_dialisis_hidro= H1%_R
Desv de Normal
0,6
0,4
0,2
0,0000
-0,2
-0,4
1
2
3
4
5
Valor observado


umol equivalentes trolox/mg proteina
5,00
4,00
3,00
2,00
1,00
H1%_I
H1%_P
H1%_R
ABTS_dialisis_hidro

- Univariate Analysis of Variance

Observations

Output Created		09-NOV-2023 18:46:18
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\INFOGEST\DIALISIS_ABTS.sav
	Active dataset	DataSet2
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA umolequivalentestroloxmgproteina BY ABTS_dialisis_hidro/METHOD=SSTYPE(3)/INTERCEPT=INCLUDE/POSTHOC=ABTS_dialisis_hidro(TUKEY)/PLOT=PROFILE(ABTS_dialisis_hidro) TYPE=LINE ERRORBAR=NO MEANREFERENCE=NO YAXIS=AUTO/PRINT DESCRIPTIVE HOMOGENEITY/CRITERIA=ALPHA(.05)/DESIGN=ABTS_dialisis_hidro.
Resources	Processor time	00:00:00,02
	Elapsed time	00:00:00,10

Factors Between Subjects

		Value Label	N
ABTS_dialisis_hidro	4,00	H1%_I	3
	5,00	H1%_P	2
	6,00	H1%_R	3

Descriptive Statistics

Dependent variable: umol trolox/mg protein equivalents

ABTS_dialisis_hidro	Average	Pattern Statistics	N
H1%_I	2,5100	1,50572	3
H1%_P	4,0500	,33941	2
H1%_R	2,7600	1,60739	3
Total	2,9888	1,35826	8

Levenea's error variance equality test,[b]

		Levene's Statistics	df1	df2
Umol Trolox Equivalents/mg Protein	Based on average	2,189	2	5
	Based on median	,369	2	5
	Based on median and adjusted gl	,369	2	3,965
	Based on trimmed average	1,947	2	5

Levenea's error variance equality test,[b]

		Sig.
Umol Trolox Equivalents/mg Protein	Based on average	,208
	Based on median	,709
	Based on median and adjusted gl	,713
	Based on trimmed average	,237

Tests the null hypothesis that the variance of the error of the dependent variable is equal between groups.[a,b] a. Dependent variable: umol trolox/mg protein equivalents. b. Design: Intercept + ABTS_dialisis_hidro

Tests of effects between subjects

Dependent variable: umol trolox/mg protein equivalents

Origin	Type III Sum of Squares	df	Medium Square	With	Sig.
Corrected model	3,097a	2	1,549	,789	,504
Intercept	74,453	1	74,453	37,921	,002
ABTS_dialisis_hidro	3,097	2	1,549	,789	,504
Pattern	9,817	5	1,963		
Total	84,375	8			
Corrected total	12,914	7			

a. R Squared = .240 (R Squared = -.064)

- Posterior Testes

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents

Tukey HSD

(I) ABTS_dialisis_hidro	(J) ABTS_dialisis_hidro	Mean difference (I-J)	Pattern Statistics	Sig.
H1%_I	H1%_P	-1,5400	1,27913	,501
	H1%_R	-,2500	1,14409	,974
H1%_P	H1%_I	1,5400	1,27913	,501
	H1%_R	1,2900	1,27913	,604
H1%_R	H1%_I	,2500	1,14409	,974
	H1%_P	-1,2900	1,27913	,604

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents

Tukey HSD

(I) ABTS_dialisis_hidro	(J) ABTS_dialisis_hidro	95% Confidence Interval	
		Lower Limit	Upper limit
H1%_I	H1%_P	-5,7022	2,6222
	H1%_R	-3,9728	3,4728
H1%_P	H1%_I	-2,6222	5,7022
	H1%_R	-2,8722	5,4522
H1%_R	H1%_I	-3,4728	3,9728
	H1%_P	-5,4522	2,8722

- Homogeneous subsets

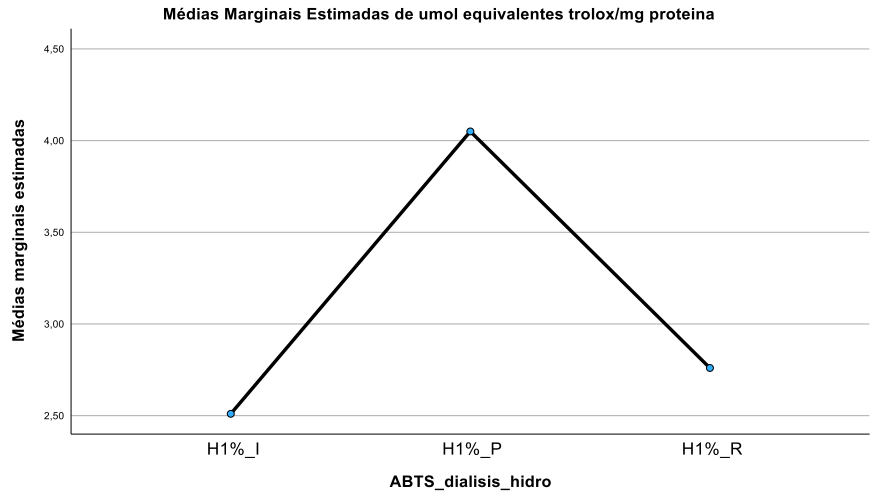
Umol Trolox Equivalents/mg Protein

Tukey HSDa,b,c

ABTS_dialisis_hidro	N	Subset 1
H1%_I	3	2,5100
H1%_R	3	2,7600
H1%_P	2	4,0500
Say.		,480

Averages are displayed for the groups in subsets homogêneos.Com based on observed averages. The error term is Mean Square (Error) = 1.963. a. Uses Harmonic Mean Sample Size = 2.571. b. Group sizes are uneven. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed..c. alpha = ,05.

- Profile Charts



- Explore

Observations

Output Created		09-NOV-2023 18:52:00
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\INFOGEST\DIALISIS_ORAC.sav
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	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=umolequivalentestroloxmgproteina BY ORAC_dialisis_solu/PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT /COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING LISTWISE/NOTOTAL.
Resources	Processor time	00:00:00,61
	Elapsed time	00:00:00,87

Case Processing Summary

		Cases		
		Valid		Silent
	ORAC_dialisis_solu	N	Percentage	N
Umol Trolox Equivalents/mg Protein	3%60º_I	3	100,0%	0
	3%60º_P	3	100,0%	0
	3%60º_R	3	100,0%	0

Case Processing Summary

		Cases			
		Silent		Total	
	ORAC_dialisis_solu	Percentage	N	Percentage	
Umol Trolox Equivalents/mg Protein	3%60º_I	0,0%	3	100,0%	
	3%60º_P	0,0%	3	100,0%	
	3%60º_R	0,0%	3	100,0%	

Descriptive

	ORAC_dialisis_solu			Statistics
Umol Trolox Equivalents/mg Protein	3%60º_I	Average		2,8767
		95% Confidence Interval to Mean	Lower Limit	2,0437
			Upper limit	3,7096
		5% of average trimmed		.
		Median		2,8600
		Variance		,112
		Standard Error		,33531
		Minimal		2,55
		Maximum		3,22
		Amplitude		,67
		Interquartile range		.
		Asymmetry		,223
		Kurtosis		.
	3%60º_P	Average		10,2700
		95% Confidence Interval to Mean	Lower Limit	-5,2935
			Upper limit	25,8335
		5% of average trimmed		.
		Median		8,8600
		Variance		39,252
		Standard Error		6,26515
		Minimal		4,83
		Maximum		17,12
		Amplitude		12,29
		Interquartile range		.
		Asymmetry		,961
		Kurtosis		.
	3%60º_R	Average		5,9600
		95% Confidence Interval to Mean	Lower Limit	4,8988
			Upper limit	7,0212
		5% of average trimmed		.
		Median		5,9100
		Variance		,183
		Standard Error		,42720
		Minimum		5,56
		Maximum		6,41
		Amplitude		,85
		Interquartile range		.
		Asymmetry		,519
		Kurtosis		.

Descriptive

	ORAC_dialisis_solu			Pattern Statistics
Umol Trolox Equivalents/mg Protein	3%60-_I	Average		,19359
		95% Confidence Interval to Mean	Lower Limit	
			Upper limit	
		5% of average trimmed		
		Median		
		Variance		
		Standard Error		
		Minimum		
		Maximum		
		Amplitude		
		Interquartile range		
		Asymmetry		1,225
		Kurtosis		.
	3%60º_P	Average		3,61719
		95% Confidence Interval to Mean	Lower Limit	
			Upper limit	
		5% of average trimmed		
		Median		
		Variance		
		Standard Error		
		Minimum		
		Maximum		
		Amplitude		
		Interquartile range		
		Asymmetry		1,225
		Kurtosis		.
	3%60º_R	Average		,24664
		95% Confidence Interval to Mean	Lower Limit	
			Upper limit	
		5% of average trimmed		
		Median		
		Variance		
		Standard Error		
		Minimum		
		Maximum		
		Amplitude		
		Interquartile range		

		Asymmetry	1,225
		Kurtosis	.

M Estimators

	ORAC_dialisis_solu	Huber's M estimator[at]	Tukey biweighting[b]	Hampel M estimator[c]
Umol Trolox Equivalents/mg Protein	3%60º_I	2,8767	2,8724	2,8767
	3%60º_P	9,5317	9,8167	10,2560
	3%60º_R	5,9600	5,9501	5,9600

M Estimators

	ORAC_dialisis_solu	Andrews Wave[d]
Umol Trolox Equivalents/mg Protein	3%60º_I	2,8724
	3%60º_P	9,8158
	3%60º_R	5,9500

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

	ORAC_dialisis_solu			Case number	Value
Umol Trolox Equivalents/mg Protein	3%60º_I	Upper	1	1	3,22
		Lower	1	3	2,55
	3%60º_P	Upper	1	6	17,12
		Lower	1	5	4,83
	3%60º_R	Upper	1	8	6,41
		Lower	1	9	5,56

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

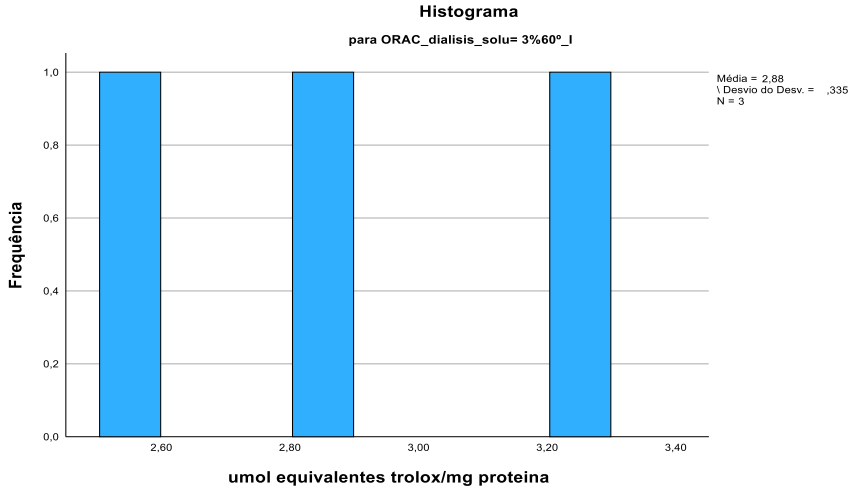
Normality Tests

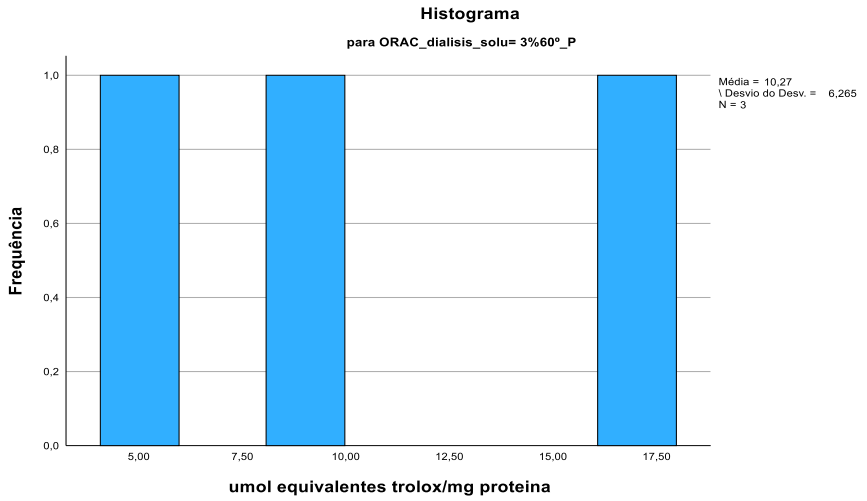
		Kolmogorov-Smirnova			Shapiro-Wilk
	ORAC_dialisis_solu	Statistics	gl	Sig.	Statistics
Umol Trolox Equivalents/mg Protein	3%60º_I	,186	3	.	,998
	3%60º_P	,256	3	.	,962
	3%60º_R	,213	3	.	,990

Normality Tests

		Shapiro-Wilk	
	ORAC_dialisis_solu	Gl	Sig.
Umol Trolox Equivalents/mg Protein	3%60º_I	3	,918
	3%60º_P	3	,625
	3%60º_R	3	,806

- Histograms


Histograma
para ORAC_dialisis_solu= 3%60º_I
Média = 2,88
\ Desvio do Desv. = ,335
N = 3
Frequência
1,0
0,8
0,6
0,4
0,2
0,0
2,60
2,80
3,00
3,20
3,40
umol equivalentes trolox/mg proteina


Histograma
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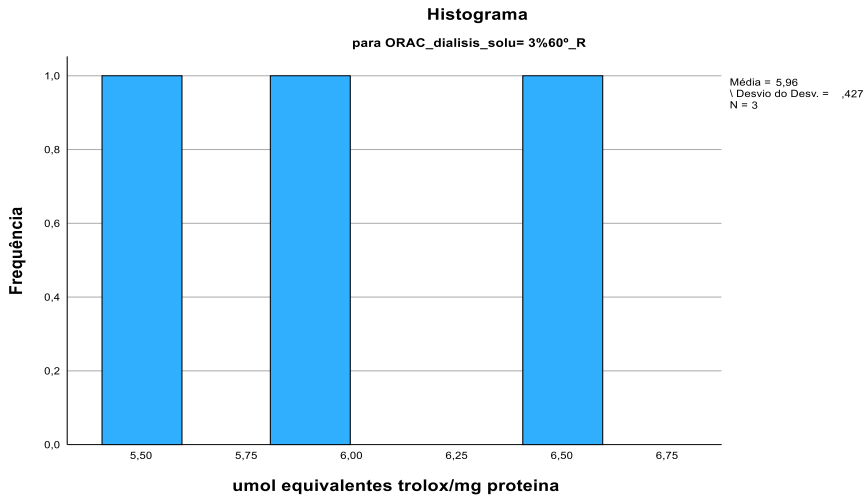
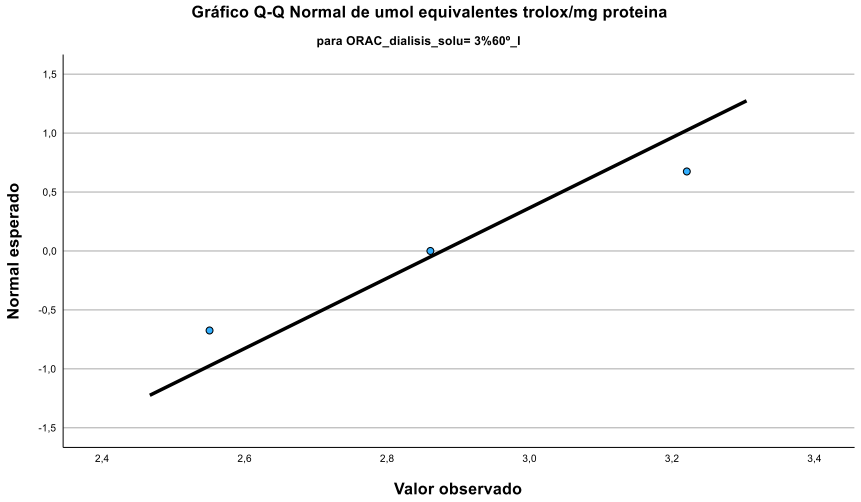
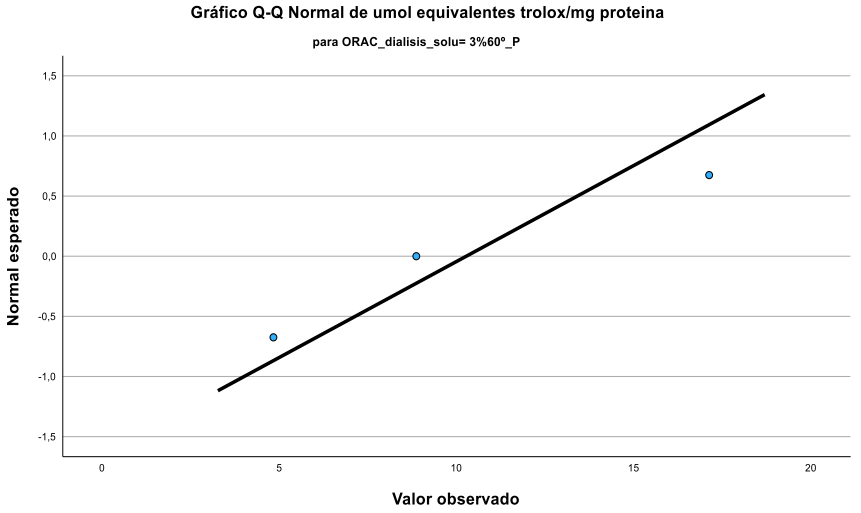
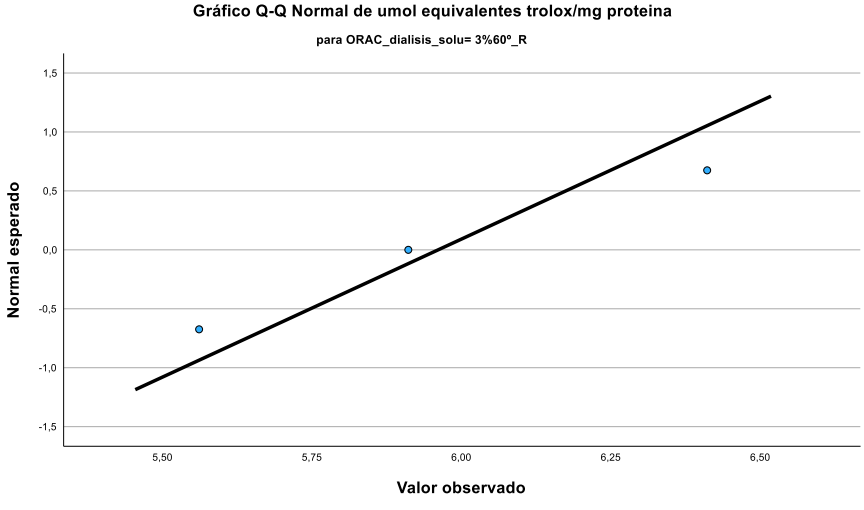
Média = 10,27
\ Desvio do Desv. = 6,265
N = 3
Frequência
1,0
0,8
0,6
0,4
0,2
0,0
5,00
7,50
10,00
12,50
15,00
17,50
umol equivalentes trolox/mg proteina


Histograma
para ORAC_dialisis_solu= 3%60º_R
Média = 5,96
\ Desvio do Desv. = ,427
N = 3
Frequência
1,0
0,8
0,6
0,4
0,2
0,0
5,50
5,75
6,00
6,25
6,50
6,75
umol equivalentes trolox/mg proteina

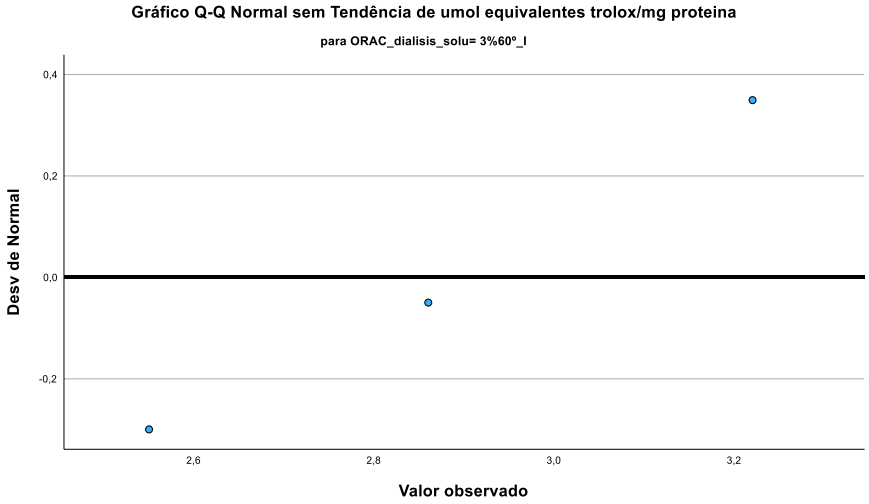
- Normal Q-Q Chart

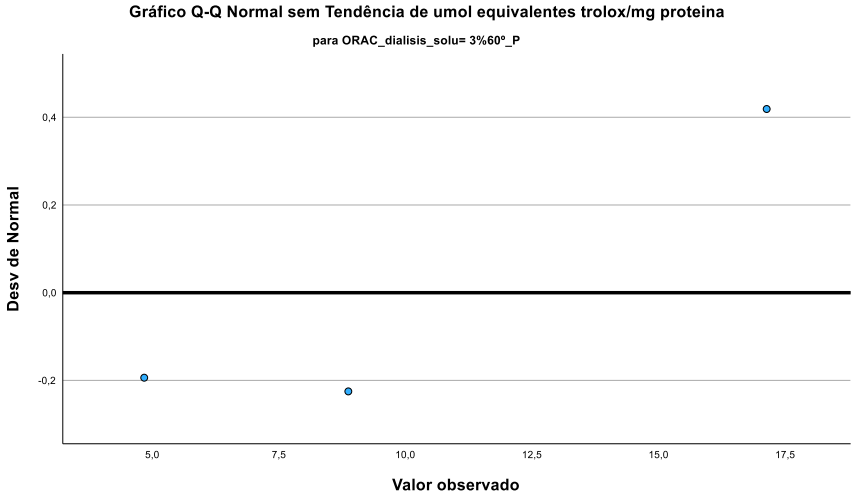


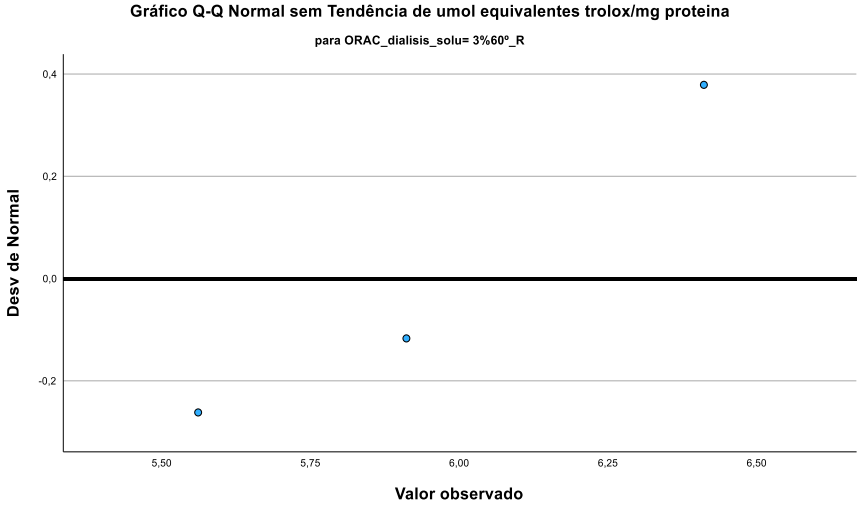


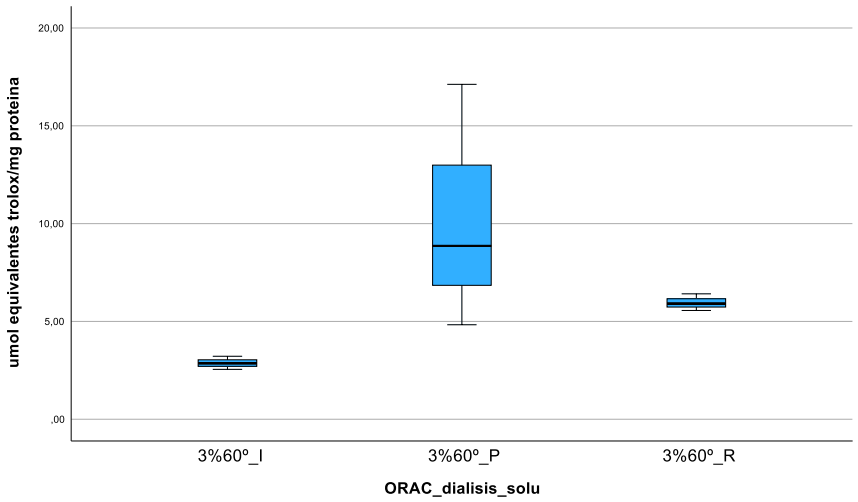


- Normal Q-Q Chart No Trend









- Univariate Analysis of Variance

Observations

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Comments		
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	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA umolequivalentestroloxmgproteina BY ORAC_dialisis_solu/METHOD=SSTYPE(3)/INTERCEPT=INCLUDE/POSTHOC=ORAC_dialisis_solu(TUKEY)/PLOT=PROFILE(ORAC_dialisis_solu) TYPE=LINE ERRORBAR=NO MEANREFERENCE=NO YAXIS=AUTO/PRINT DESCRIPTIVE HOMOGENEITY/CRITERIA=ALPHA(.05)/DESIGN=ORAC_dialisis_solu.
Resources	Processor time	00:00:00,02
	Elapsed time	00:00:00,11

Factors Between Subjects

		Value Label	N
ORAC_dialisis_solu	1,00	3%60º_I	3
	2,00	3%60º_P	3
	3,00	3%60º_R	3

Descriptive Statistics

Dependent variable: umol trolox/mg protein equivalents

ORAC_dialisis_solu	Average	Pattern Statistics	N
3%60º_I	2,8767	,33531	3
3%60º_P	10,2700	6,26515	3
3%60º_R	5,9600	,42720	3
Total	6,3689	4,49776	9

Equality of variances test of Levene's error[a,b]

		Levene's Statistics	df1	df2
Umol Trolox Equivalents/mg Protein	Based on average	6,898	2	6
	Based on median	2,582	2	6
	Based on median and adjusted gl	2,582	2	2,024
	Based on trimmed average	6,519	2	6

Equality of variances test of Levene's error[a,b]

		Sig.
Umol Trolox Equivalents/mg Protein	Based on average	,028
	Based on median	,155
	Based on median and adjusted gl	,277
	Based on trimmed average	,031

Tests the null hypothesis that the variance of the error of the dependent variable is equal between groups.[a,b] a. Dependent variable: umol trolox/mg protein equivalents. b. Design: Intercept + ORAC_dialisis_solu

Tests of effects between subjects

Dependent variable: umol trolox/mg protein equivalents

Origin	Type III Sum of Squares	df	Medium Square	With	Sig.
Corrected model	82,744a	2	41,372	3,138	,117
Intercept	365,065	1	365,065	27,693	,002
ORAC_dialisis_solu	82,744	2	41,372	3,138	,117
Pattern	79,094	6	13,182		
Total	526,903	9			
Corrected total	161,838	8			

a. R Squared = .511 (Adjusted R Squared = .348)

- Posterior Testes

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents
Tukey HSD

(I) ORAC_dialisis_solu	(J) ORAC_dialisis_solu	Mean difference (I-J)	Pattern Statistics	Sig.
3%60º_I	3%60º_P	-7,3933	2,96449	,103
	3%60º_R	-3,0833	2,96449	,581
3%60º_P	3%60º_I	7,3933	2,96449	,103
	3%60º_R	4,3100	2,96449	,375
3%60º_R	3%60º_I	3,0833	2,96449	,581
	3%60º_P	-4,3100	2,96449	,375

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents
Tukey HSD

		95% Confidence Interval	
(I) ORAC_dialisis_solu	(J) ORAC_dialisis_solu	Lower Limit	Upper limit
3%60º_I	3%60º_P	-16,4892	1,7026
	3%60º_R	-12,1792	6,0126
3%60º_P	3%60º_I	-1,7026	16,4892
	3%60º_R	-4,7859	13,4059
3%60º_R	3%60º_I	-6,0126	12,1792
	3%60º_P	-13,4059	4,7859

Based on observed averages. The error term is Mean Square (Error) = 13.182.

- Homogeneous subsets

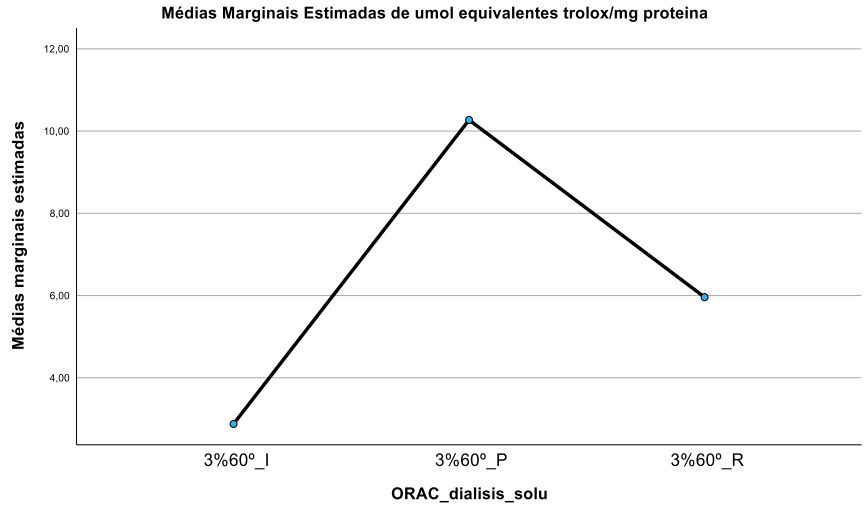
Umol Trolox Equivalents/mg Protein

Tukey HSD[a,b]

		Subset
ORAC_dialisis_solu	N	1
3%60th_I	3	2,8767
3%60th_R	3	5,9600
3%60th_P	3	10,2700
Sig.		,103

Averages are displayed for the groups in subsets homogêneos.Com based on observed averages. The error term is Mean Square (Error) = 13.182. a. Uses the Harmonic Mean Sample Size = 3,000. b. Alpha = .05.

- Profile Charts



- Non-parametric tests

Observations

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Resources	Processor time	00:00:00,61
	Elapsed time	00:00:00,68

Hypothesis Testing Summarization

	Null hypothesis	Test	Sig.[a,b]
1	The distribution of trolox/mg protein equivalents is equal in the ORAC_dialisis_solu categories.	Independent Kruskal-Wallis Test Specimens	,061

Hypothesis Testing Summarization

	Decision
1	Retain the null hypothesis.

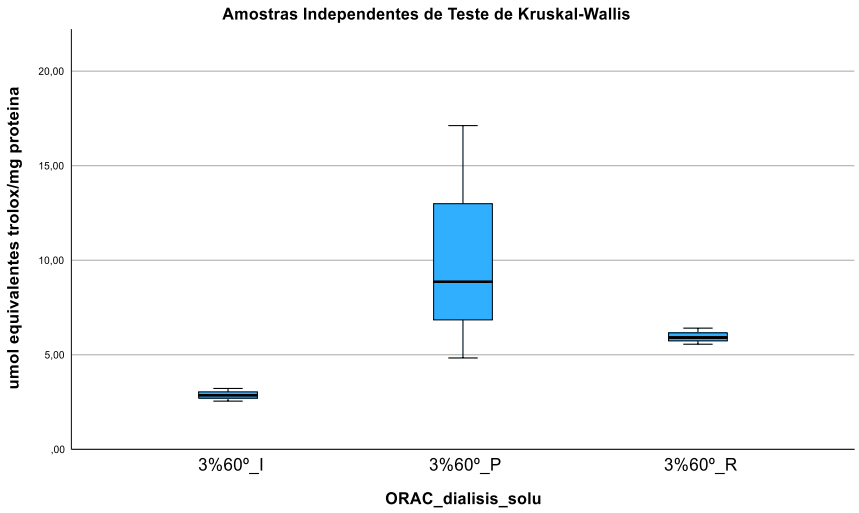
a. The significance level is .050. b. Asymptotic significance is displayed

- Independent Kruskal-Wallis Test Specimens

Kruskal-Wallis Test Summary Independent Samples

Total N	9
Test Statistics	5,600[a]
Degree of Freedom	2
Asymptotic signal (two-sided test)	,061

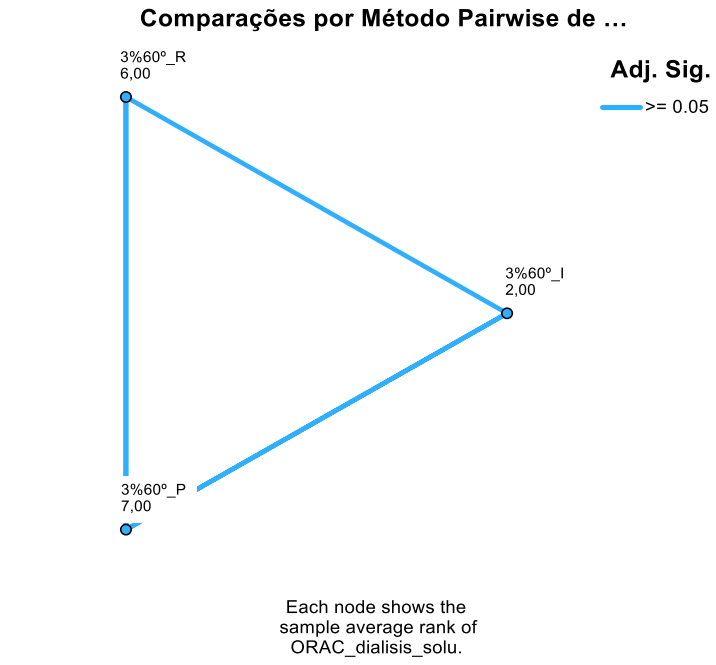
a. The test statistic is adjusted for draws.

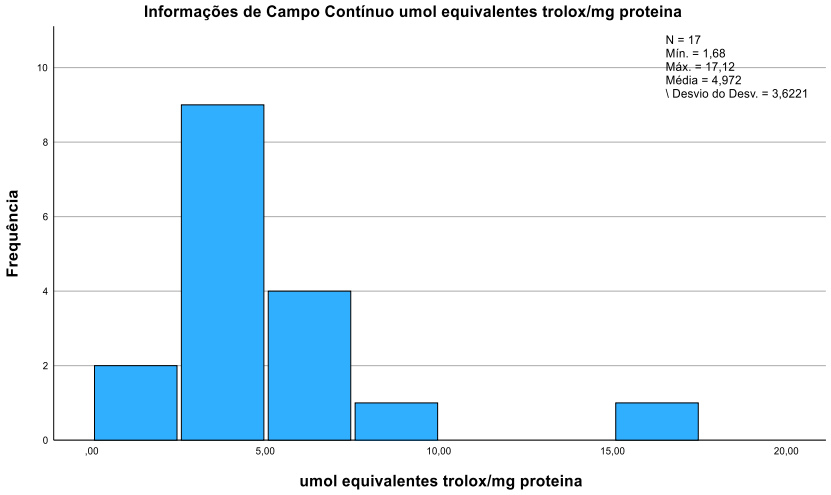


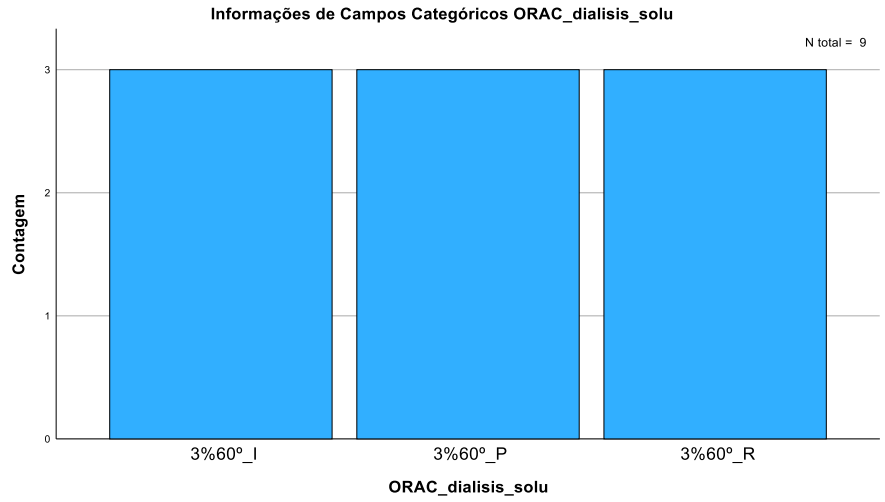
Pairwise Method Comparisons of ORAC_dialisis_solu

Sample 1-Sample 2	Test Statistics	Standard Error	Pattern Statistics	Sig.	Adj. Sig.[a]
3%60º_I-3%60º_R	-4,000	2,236	-1,789	,074	,221
3%60º_I-3%60º_P	-5,000	2,236	-2,236	,025	,076
3%60º_R-3%60º_P	1,000	2,236	,447	,655	1,000

Each row tests the null hypothesis where the Sample 1 and Sample 2 distributions are equal. Asymptotic significances (2-sided test) are displayed. The significance level is .050. a. Significance values were adjusted by Bonferroni correction for several tests.


Comparações por Método Pairwise de ...
3%60º_R
6,00
Adj. Sig.
>= 0.05
3%60º_I
2,00
3%60º_P
7,00
Each node shows the sample average rank of ORAC_dialisis_solu.


Informações de Campo Contínuo umol equivalentes trolox/mg proteina
N = 17
Mín. = 1,68
Máx. = 17,12
Média = 4,972
\ Desvio do Desv. = 3,6221
Frequência
0
2
4
6
8
10
,00
5,00
10,00
15,00
20,00
umol equivalentes trolox/mg proteina



- Explore

Observations

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Comments		
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	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Handling of missing values	Definition of omission	User-defined missing values for dependent variables are treated as missing.
	Cases used	Statistics are based on cases with no missing values for any dependent variable or factor used.
Syntax		EXAMINE VARIABLES=umolequivalentestroloxmgproteina BY ORAC_dialisis_hidro/PLOT BOXPLOT STEMLEAF HISTOGRAM NPPLOT/COMPARE GROUPS/MESTIMATORS HUBER(1.339) ANDREW(1.34) HAMPEL(1.7,3.4,8.5) TUKEY(4.685)/STATISTICS DESCRIPTIVES EXTREME/CINTERVAL 95/MISSING LISTWISE/NOTOTAL.
Resources	Processor time	00:00:00,64
	Elapsed time	00:00:00,95

Case Processing Summary

		Cases			
		Valid		Silent	
	ORAC_dialisis_hidro	N	Percentage	N	
Umol Trolox Equivalents/mg Protein	H1%_I	3	100,0%	0	
	H1%_P	2	66,7%	1	
	H1%_R	3	100,0%	0	

Case Processing Summary

		Cases			
		Silent		Total	
	ORAC_dialisis_hidro	Percentage		N	Percentage
Umol Trolox Equivalents/mg Protein	H1%_I	0,0%		3	100,0%
	H1%_P	33,3%		3	100,0%
	H1%_R	0,0%		3	100,0%

Descriptive

	ORAC_dialisis_hidro			Statistics	Pattern Statistics
Umol Trolox Equivalents/mg Protein	H1%_I	Average		3,3967	,96796
		95% Confidence Interval to Mean	Lower Limit	-,7681	
			Upper limit	7,5615	
		5% of average trimmed		.	
		Median		3,4800	
		Variance		2,811	
		Standard Error		1,67655	
		Minimal		1,68	
		Maximum		5,03	
		Amplitude		3,35	
		Interquartile range		.	
		Asymmetry		-,223	1,225
		Kurtosis		.	.
	H1%_P	Average		3,2350	1,33500
		95% Confidence Interval to Mean	Lower Limit	-13,7278	
			Upper limit	20,1978	
		5% of average trimmed		.	
		Median		3,2350	
		Variance		3,564	
		Standard Error		1,88798	
		Minimal		1,90	
		Maximum		4,57	
		Amplitude		2,67	

		Interquartile range		.	
		Asymmetry		.	.
		Kurtosis		.	.
	H1%_R	Average		3,5133	,49465
		95% Confidence Interval to Mean	Lower Limit	1,3850	
			Upper limit	5,6416	
		5% of average trimmed		.	
		Median		3,4500	
		Variance		,734	
		Standard Error		,85676	
		Minimal		2,69	
		Maximum		4,40	
		Amplitude		1,71	
		Interquartile range		.	
		Asymmetry		,331	1,225
		Kurtosis		.	.

M Estimators

	ORAC_dialisis_hidro	Huber's M estimator[at]	Tukey biweighting[b]	Hampel M estimator[c]
Umol Trolox Equivalents/mg Protein	H1%_I	3,3967	3,4088	3,3967
	H1%_P	3,2350	3,2350	3,2350
	H1%_R	3,5133	3,5031	3,5133

M Estimators

	ORAC_dialisis_hidro	Andrews Wave[d]
Umol Trolox Equivalents/mg Protein	H1%_I	3,4090
	H1%_P	3,2350
	H1%_R	3,5030

a. The weighting constant is 1.339. b. The weighting constant is 4.685. c. The weighting constants are 1,700, 3,400, and 8,500. d. The weighting constant is 1.340*pi.

Extreme Values[a]

	ORAC_dialisis_hidro			Case number	Value
Umol Trolox Equivalents/mg Protein	H1%_I	Upper	1	12	5,03
		Lower	1	10	1,68
	H1%_P	Upper	1	13	4,57
		Lower	1	14	1,90
	H1%_R	Upper	1	16	4,40
		Lower	1	17	2,69

a. The requested number of extreme values exceeds the number of data points. A smaller number of extremes are displayed.

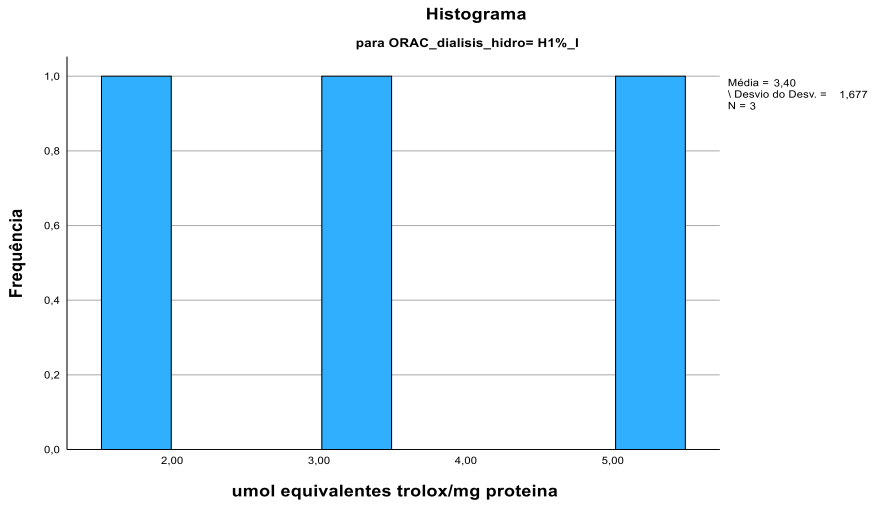
Normality Tests

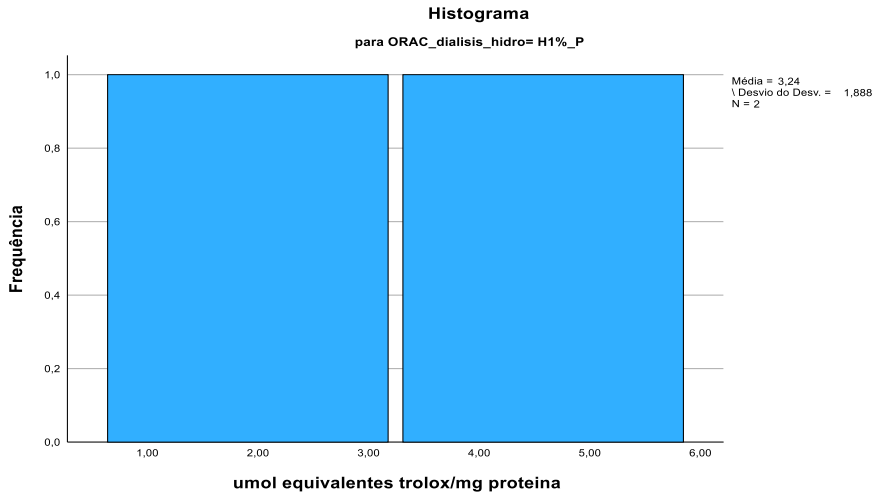
		Kolmogorov-Smirnova			Shapiro-Wilk
	ORAC_dialisis_hidro	Statistics	gl	Sig.	Statistics
Umol Trolox Equivalents/mg Protein	H1%_I	,186	3	.	,998
	H1%_P	,260	2	.	
	H1%_R	,196	3	.	,996

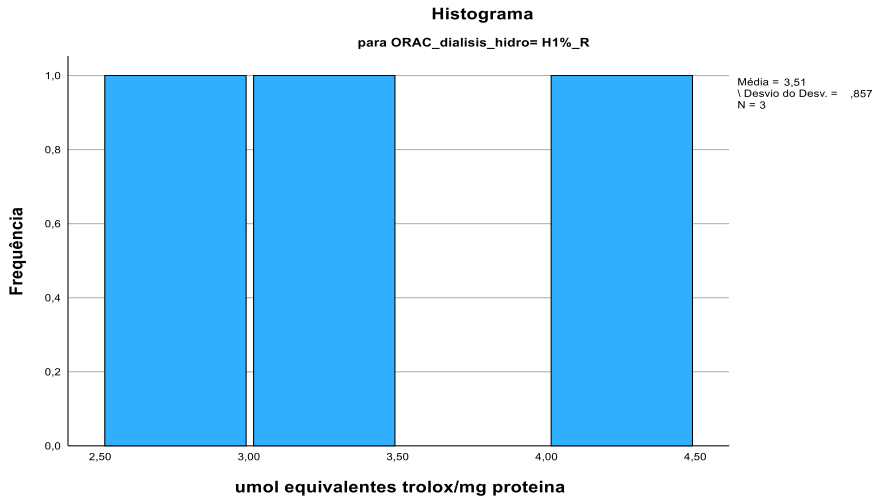
Normality Tests

		Shapiro-Wilk	
	ORAC_dialisis_hidro	gl	Sig.
Umol Trolox Equivalents/mg Protein	H1%_I	3	,918
	H1%_P		
	H1%_R	3	,878

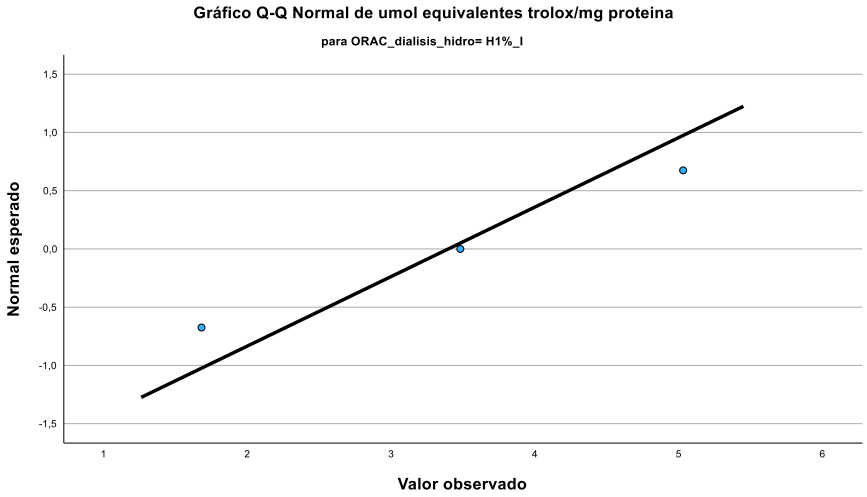
- Histograms

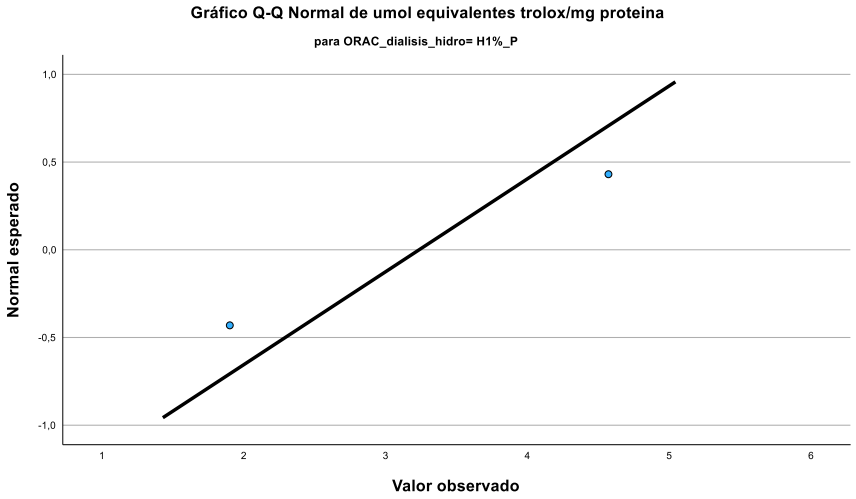


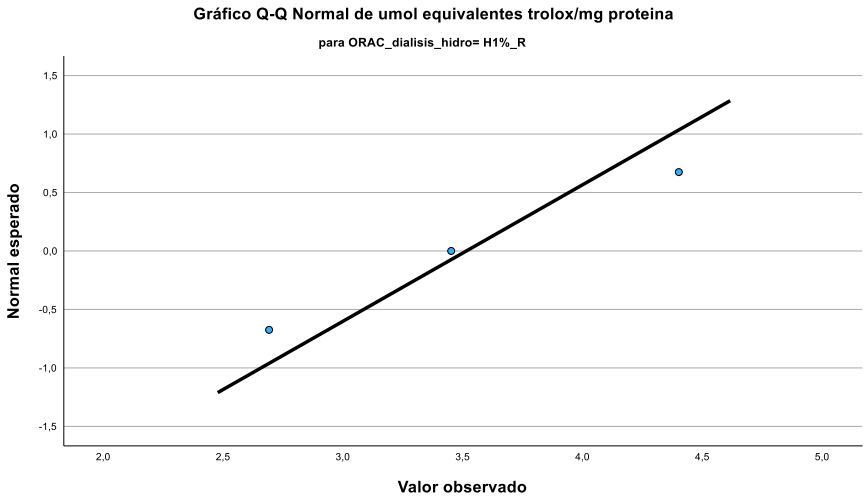




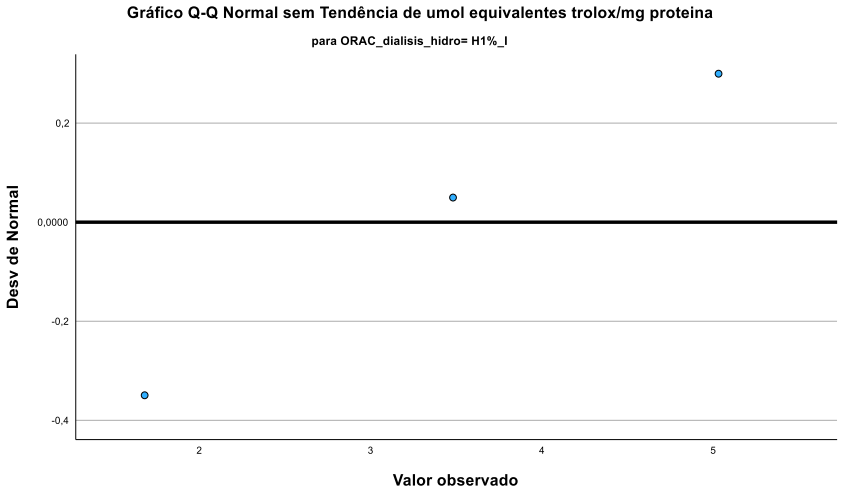
- Normal Q-Q Chart

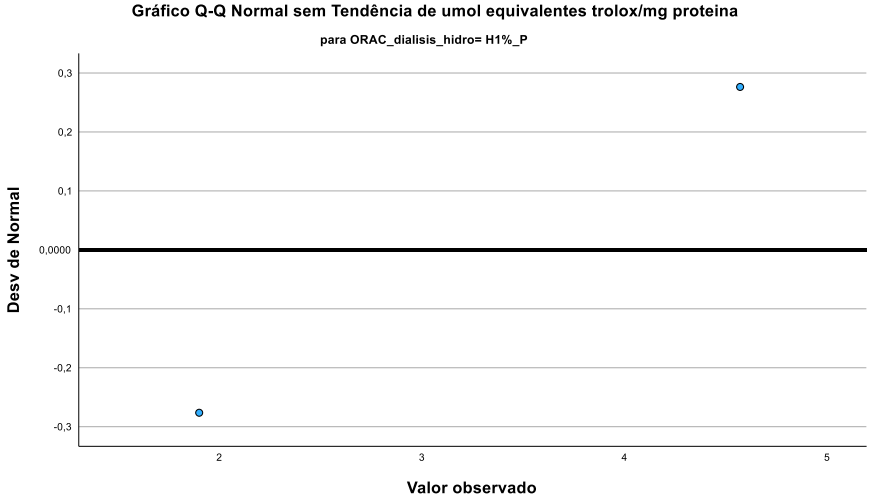






- Normal Q-Q Chart No Trend





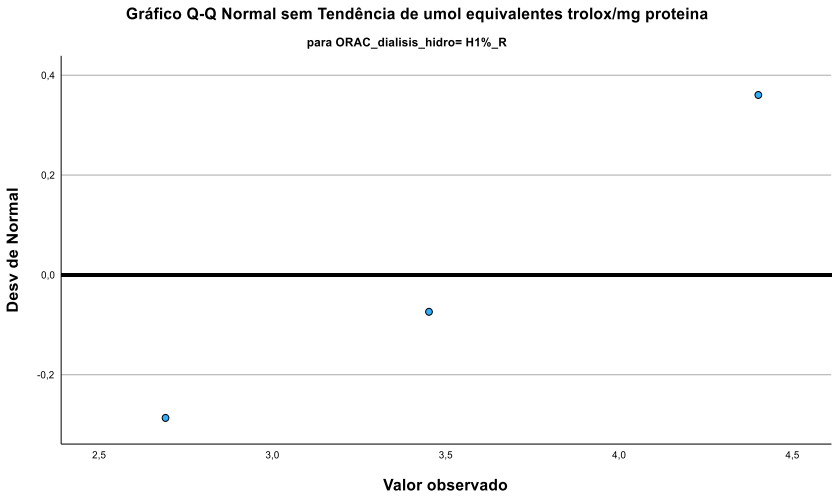
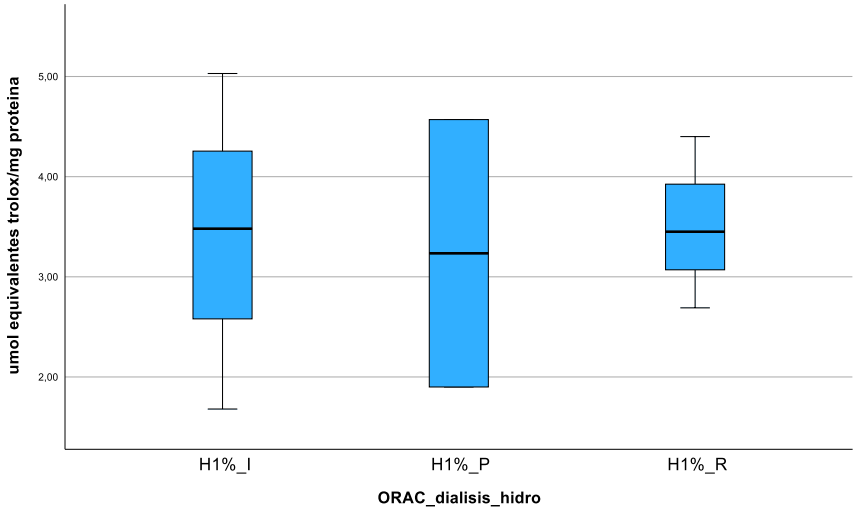


Gráfico Q-Q Normal sem Tendência de umol equivalentes trolox/mg proteina
para ORAC_dialisis_hidro= H1%_R
Desv de Normal
0,4
0,2
0,0
-0,2
2,5
3,0
3,5
4,0
4,5
Valor observado


umol equivalentes trolox/mg proteina
5,00
4,00
3,00
2,00
H1%_I
H1%_P
H1%_R
ORAC_dialisis_hidro

- Univariate Analysis of Variance

Observations

Output Created		09-NOV-2023 19:01:55
Comments		
Entry	Data	C:\Users\maris\OneDrive - ucp.pt\Uni\MSMicr\Tese\WaSeaBi\Writing\SPSS\INFOGEST\DIALISIS_ORAC.sav
	Active dataset	DataSet5
	Filter	<none>
	Weighting	<none>
	Split File	<none>
	N rows in job data file	19
Missing value treatment	Definition of omission	User-defined missing values are treated as missing.
	Cases used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA umolequivalentestroloxmgprotein a BY ORAC_dialisis_hidro/METHOD=SSTYPE(3)/INTERCEPT=INCLUDE/POSTHOC=ORAC_dialisis_hidro(TUKEY)/PLOT=PROFILE(ORAC_dialisis_hidro) TYPE=LINE ERRORBAR=NO MEANREFERENCE=NO YAXIS=AUTO/PRINT DESCRIPTIVE HOMOGENEITY/CRITERIA=ALPHA(.05)/DESIGN=ORAC_dialisis_hidro.
Resources	Processor time	00:00:00,05
	Elapsed time	00:00:00,10

Factors Between Subjects

		Value Label	N
ORAC_dialisis_hidro	4,00	H1%_I	3
	5,00	H1%_P	2
	6,00	H1%_R	3

Descriptive Statistics

Dependent variable: umol trolox/mg protein equivalents

ORAC_dialisis_hidro	Average	Pattern Statistics	N
H1%_I	3,3967	1,67655	3
H1%_P	3,2350	1,88798	2
H1%_R	3,5133	,85676	3
Total	3,4000	1,23908	8

Equality of variances test of Levene's error[a,b]

		Levene's Statistics	df1	df2
Umol Trolox Equivalents/mg Protein	Based on average	,931	2	5
	Based on median	,842	2	5
	Based on median and adjusted gl	,842	2	2,993
	Based on trimmed average	,926	2	5

Equality of variances test of Levene's error[a,b]

		Sig.
Umol Trolox Equivalents/mg Protein	Based on average	,453
	Based on median	,484
	Based on median and adjusted gl	,513
	Based on trimmed average	,455

Tests the null hypothesis that the variance of the error of the dependent variable is equal between groups.[a,b] a. Dependent variable: umol trolox/mg protein equivalents. b. Design: Intercept + ORAC_dialisis_hidro

Tests of effects between subjects

Dependent variable: umol trolox/mg protein equivalents

Origin	Type III Sum of Squares	df	Medium Square	With	Sig.
Corrected model	,093a	2	,047	,022	,979
Intercept	88,218	1	88,218	41,401	,001
ORAC_dialisis_hidro	,093	2	,047	,022	,979
Pattern	10,654	5	2,131		
Total	103,227	8			
Corrected total	10,747	7			

a. R Squared = .009 (Adjusted R Squared = -.388)

- Posterior Testes

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents

Tukey HSD

(I) ORAC_dialisis_hidro	(J) ORAC_dialisis_hidro	Mean difference (I-J)	Pattern Statistics	Sig.
H1%_I	H1%_P	,1617	1,33255	,992
	H1%_R	-,1167	1,19187	,995
H1%_P	H1%_I	-,1617	1,33255	,992
	H1%_R	-,2783	1,33255	,976
H1%_R	H1%_I	,1167	1,19187	,995
	H1%_P	,2783	1,33255	,976

Multiple Comparisons

Dependent variable: umol trolox/mg protein equivalents

Tukey HSD

(I) ORAC_dialisis_hidro	(J) ORAC_dialisis_hidro	95% Confidence Interval	
		Lower Limit	Upper limit
H1%_I	H1%_P	-4,1743	4,4977
	H1%_R	-3,9949	3,7616
H1%_P	H1%_I	-4,4977	4,1743
	H1%_R	-4,6143	4,0577
H1%_R	H1%_I	-3,7616	3,9949
	H1%_P	-4,0577	4,6143

Based on observed averages. The error term is Mean Square (Error) = 2.131

- Homogeneous subsets

Umol Trolox Equivalents/mg Protein

Tukey HSD[a,b,c]

ORAC_dialisis_hidro	N	Subset 1
H1%_P	2	3,2350
H1%_I	3	3,3967
H1%_R	3	3,5133
Say.		,975

Averages are displayed for the groups in subsets homogêneos.Com based on observed averages. The error term is Mean Square (Error) = 2.131. a. Uses Harmonic Mean Sample Size = 2.571. b. Group sizes are uneven. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed. c. alpha = ,05.

- Profile Charts