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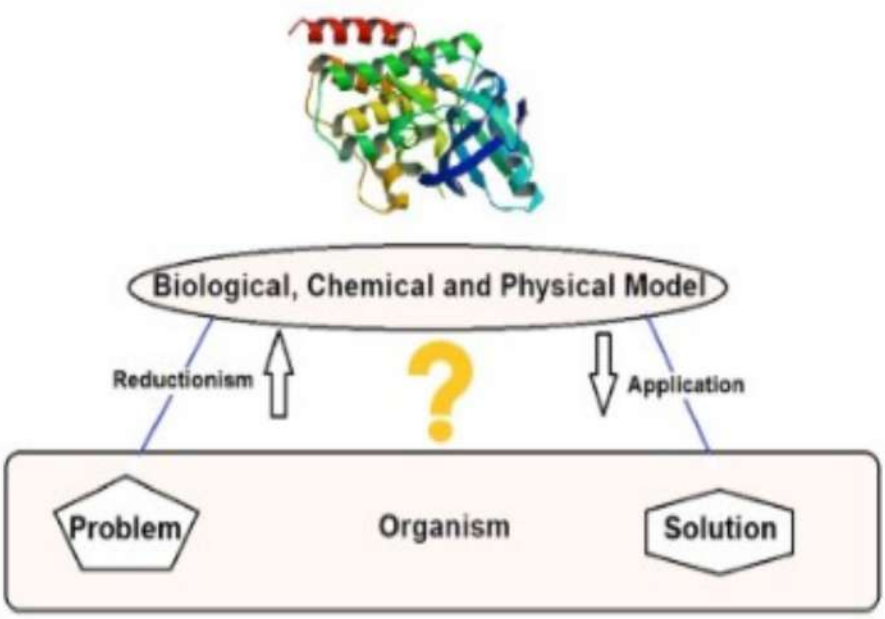
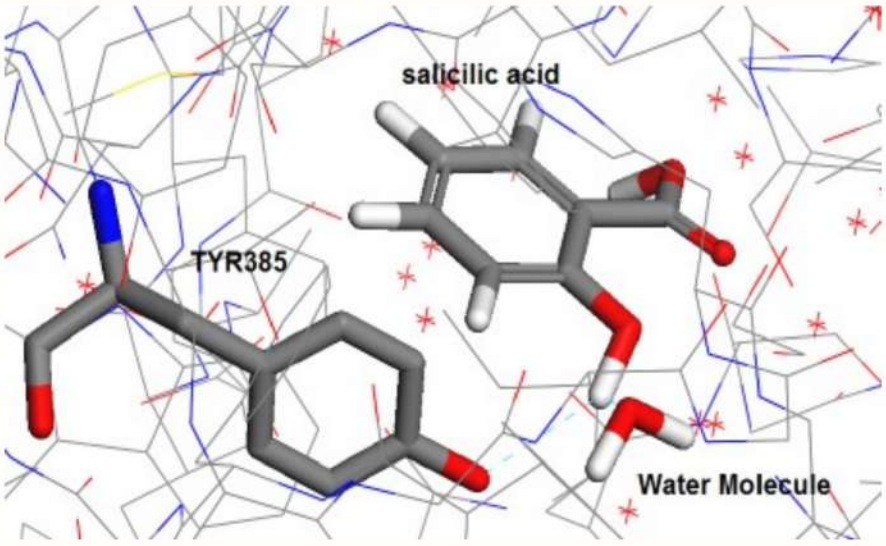


Systems biology

A criticism of the reductionist and holistic vision in the planning of drugs in biological, chemical and physical level

Magnesium

Ecofriendly and low-cost sample preparation methods for magnesium determination in beer



Thermal desorption

Geographical chemical variability and processing oxidation of volatile compounds of *Casearia sylvestris* leaves

Bioenergy

Artificial intelligence method developed for classifying raw sugarcane in the presence of the solid impurity

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Editorial

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Editorial

Wishing all readers better times, the Editor together with the Eclet. Quim. J. team, proudly present the third edition of this year, with the certainty that the authors and readers of this issue will be satisfied with the scientific findings here reported.

Opening this issue, a review article presents an interesting discussion about two philosophical premises of science applied in the understanding of diseases and in the planning of drugs. The first premise is the reductionism which is the predominant way in modern science and considers that a problem can be reduced to the sum of its individual parts. Thus, diseases can be understood as the metabolic action of few enzymes, drugs can be planned through the mimicry of a specific enzymatic substrate. The second premise is the holistic view of the phenomenon which must be understood as the whole. Then, drug design would be seen from a viewpoint of a network of proteins and not from a single enzymatic target. Nowadays, a holistic view combined with methodological reductionism is used to develop new potential drugs. In the sequence, it is presented an interesting methodology to determine magnesium in beer based on ultrasound-assisted extraction and direct analysis by flame atomic absorption spectrometry. According to the authors, this methodology was demonstrated to be suitable for quality control routines of beer samples in the industry. Follow, it is described unknown volatile chemicals present in the *in natura* leaves of two *Casearia sylvestris* populations from Atlantic Forest and Cerrado compared to the composition of dried leaf essential oil. All compounds identified were Sesquiterpenes with the main components being (*E*)-caryophyllene, bicyclogermacrene, *β*-elemene, spathulenol, and caryophyllene oxide. The sesquiterpene hydrocarbon content increased and the oxygenated sesquiterpene content decreased going from the *in natura* leaves to the dried leaf essential oil and the volatile chemical composition was different between the two studied populations. Completes this issue, the description of an important methodology based on artificial intelligence that can be used to evaluate a big issue in biorefineries, that is solid impurity in raw sugarcane, which requires a high-frequency, low-cost, and noninvasive method. The methodology has low-computational cost and a simple setup for image acquisition method could screen solid impurity in sugarcane shipments, standing out as a promising application.

The Editor and the team of Eclet. Chem. J. are immensely grateful to the authors and reviewers' dedication, who spared no effort for the successful completion of this issue.

Assis Vicente Benedetti
Editor-in-Chief of EQJ

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SUMMARY

Eclética Química Journal

Review article

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| Vol. 46 | n. 3 | 2021 |

A criticism of the reductionist and holistic vision in the planning of drugs in biological, chemical and physical level

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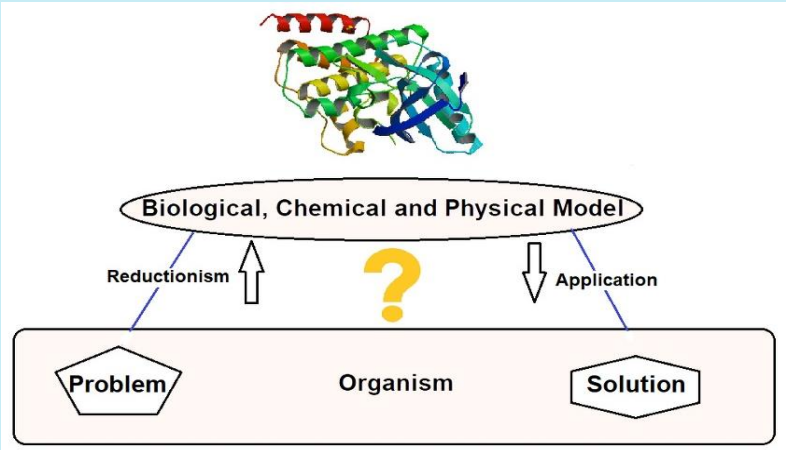
5. systems biology

ABSTRACT: In this work, two philosophical premises of science applied in the understanding of diseases and in the planning of drugs were studied. The first premise is reductionism. This idea is present in modern science when a problem can be reduced to the sum of its individual parts. Diseases can be understood as the metabolic action of few enzymes. Drugs can be planned through the mimicry of a specific enzymatic substrate. Biological molecules can be explained by the quantum theory applied to atoms and molecules. This idea has been the predominant way in modern science. On the other hand, there is a holistic view of the phenomenon. In this holistic view, the phenomenon must be understood as the whole. Drug design should be thought from a network of proteins, not just from a single enzymatic target. There is in fact a slight advantage in the reductionist method, because this philosophical view simplifies the problem. Today, a holistic view combined with methodological reductionism is used to develop new potential drugs.



CONTENTS

1. Introduction

Discussion between reductionist and holistic methods have been used to understand the nature of matter. Democritus and Leucippus, the "creators" of the atom idea, were the first to establish a reductionist explanation of matter assuming the existence of atoms. On the other hand, Aristotle admitted holistic explanations. There are three types of reductionism. Ontological reductionism is justified on the assumption that behavior of the whole can be explained by the sum of the individual properties of the components. Methodological reductionism is based on the idea that scientific explanation must be reduced to the smallest elements. The explanation of a particular phenomenon can be made in terms of the most fundamental constituents of this phenomenon. The methodological reductionism is very clear in some scientific discussion above molecular drug planning, because several biochemical processes are described by molecular and atom association. Researchers have employed quantum mechanics to describe biochemical phenomena. The fundamental constituents of matter are used to develop new drugs. Lastly, there is the epistemological reductionism, which a theory takes the form of a deductive argument where the premises are the primary theory and the conclusion is the secondary theory. The theories of biology can be derived as special cases of the laws and theories of the physical sciences (Zalta et al., 2017). Holistic view contrasts with the reductionist premise by assuming that the explanation of the phenomenon must necessarily describe the whole. In this case, the interactive aspects of the individual components are considered. In this paper, the reductionist and holistic assumptions are discussed to comprehend diseases and drug design. This discussion is interesting because it can be used to trace the scientific pathways to be used in the study of a particular scientific phenomenon.

Nowadays, the properties of atoms can be obtained from the quantum mechanics equations. The fundamental particles, that are explained by quantum mechanics, can be used to describe the chemical properties of atoms and molecules. The biomolecules that are the components of life can be explained through physical and chemical phenomena. Finally, biology becomes nothing more than a cluster of atoms that explain the phenomenon of life as a whole. This idea was advocated by Schrödinger (1946). The reductionism gains a new perspective by quantum theory. Individual molecules can be used to explain diseases as a whole. Defective genes may be the cause of cancer. A drug can be designed due to the idea that a specific enzyme interacts with a biological receptor. This molecular interaction controls a complex biological response. Currently, reductionism is the theory most employed to understand the existence of diseases and design new drugs for treating illness.

In opposition to reductionism, the holistic vision assumes that diseases must be comprehended by individual components interactions. In order to develop new drugs, the researcher must study the biological system as a whole. A molecule does not interact with a single enzyme because it can have a different effect from what was planned. Biochemical mechanisms should be understood based on the interactions between the biomolecules as a whole and not as a summation of individual properties. Thus, it is important that the researcher gives special attention to these visions in order to find a way to understand the biological mechanisms and rules that govern life.

In this manuscript, the discussion of reductionism is performed at the biological, chemical, and physical levels to understand the development of drugs and diseases. After the previous discussion about reductionism paradigms applied to drug discovery, the holistic idea is discussed in order to understand the same problems using the biology of systems. Methodological reductionism and holism are not entirely opposite to each other. Each approach has limitations.

2. Methodology

The purpose of this manuscript is to show how ontological reductionism brings difficulties to understand diseases and design new drugs. Holistic approach derived from the biology of the system is presented in the manuscript. This discussion extends at a biological, chemical, and physical level. The old and current literature were also evaluated to show the two premises adopted in the study and development of drugs. Finally, methodological reductionism is used to build holistic approaches, showing the complementarity of both approaches.

3. Drug design

The discussion on the use of proteins in drug design began more than 35 years ago with the emergence of information on the three-dimensional (3D) structure of globin and polypeptide hormones (Schechter, 2008). The protein structures are used as biological targets, virtual screening and fragment screening.

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The development of structural genomics provided more 3D structures that can be determined by gene sequences. These methodologies were particularly important for the development of computational methods, which can help to identify the sites involved in the intermolecular interaction with the inhibitors.

First, it is possible to use silicon approaches (virtual screening and redesign) to select a subset of samples from a large compound file. A new drug can also be designed from previously identified ligands. Secondly, molecular modeling can be used to study the interaction between a possible biological target and the protein receptor. The evaluation of the activity of a possible drug can be made by the similarity to the original substrate, through the interactions performed by the possible drug, or using a multilinear regression to perform a study of structure and activity relationship. Molecular modeling uses molecular structure and electronic calculations to study conformational changes and molecular interactions between an enzyme and a potential drug candidate. The electronic structure calculations are based on methodologies derivative from quantum mechanics approximations. Now, there is prior information (docking, quantum chemistry simulations, Quantitative structure–activity relationship [QSAR]) for a rational organic synthesis of the new drug. The synthesized drug will be tested *in vitro* and *in vivo*. The success of these tests may or may not lead to a new drug. Briefly, rational drug design is preceded by the choice of a biological target. This biological target can be known or created by sequencing the amino acids. The elucidation of molecular structure will allow the comprehension of the interaction between the biological target with the substrates. Subsequently, the electronic structure and molecular mechanical calculations will be used to understand the enzymatic active site. Molecular docking is used to generate a set of conformations inside the catalytic site. The comprehension about the catalytic site will identify which amino acids interact with the possible drug. In this stage, the theoretical approaches will help to establish the most important hydrogen bonds, pi stacking interactions, ion interaction and induced dipole interactions. The bind energy can be calculated using classical force fields or quantum mechanics/molecular mechanics (QM/MM) approaches.

Next step is to start the synthesis of the drug candidate. Then, this molecule may be assayed on *in vitro* enzymes, for example. This is a brief summary of the well-known "rational" design of new drugs. In the manuscript, it will be shown that the idea behind the rational design of drugs starts from ontological reductionism, which does not cover the whole problem to be solved.

4. The origin of biological reductionism

The discussion about reductionism in different meanings can be observed in the declarations below.

Crick (1966) claims that "The ultimate aim of the modern movement in biology is to explain all biology in terms of physics and chemistry". This approach epitomizes the reductionist mindset that has permeated molecular biology for half a century. The most extreme manifestation of the reductionist view is the belief that is held by some neuroscientists that consciousness and mental states can be reduced to chemical reactions that occur in the brain.

The epistemological reductionism can be observed in the words of Crick (1966). The domain of biology is reduced to chemical reactions. It could be possible to predict biological phenomena due to specific chemical reactions. On the other hand, Paul Nurse criticized the reductionism defended by Crick and other scientists.

Nurse (1997) begins his writing in nature with a reductionist question:

"If we had knowledge of all the molecular reactions that take place within the cell, would we know the cell?" The article comes up with the following answer: "Explanations in science must always have some elements of reductionism, but descriptions of increasing detail may only provide a delusion of understanding; overenthusiastic pursuit of reductionism can limit discovery and also has ideological implications in defining what is considered to be the best science in terms of publication and financial support..." However, there is a real problem to define what is an enough explanation.

There are many philosophers who believe that there is a reduction from causality at the macro level to causality at the micro level. Menzies (1988) calls this idea causal reductionism. Reducing causal relationships from macro to micro levels presupposes some way to correlate the events that have causation effect at different levels.

Ontological reductionism analyzes the whole in parts and decides the associations between the parts. This approach accepts that only the molecules supply a comprehension of the entire system.

Currently, it is evident that the specificity of a complex biological activity does not emerge from the properties of the individual molecules. This molecular

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structure constantly works in numerous distinctive forms (Nicholson, 2019; Pierce Junior et al., 1960). There are yet studies that lead to a holistic view for the treatment of certain types of disease (Birkbak and McGranahan, 2020; Nicholson, 2019; Pierce Junior et al., 1960). The cancer treatment is a particularly interesting case. Cancer is a disease of multifactorial origin that is treated in the context of that the problem originated from somatic mutations in cells. Barry Pierce and his colleagues showed the differentiation of malignant neoplastic cells into benign cell types (Lok, 2006; Pierce Junior et al., 1960). These researchers refuted the initial dogma "once a cancer cell, always a cancer cell". The microenvironment and normal tissue architectures may restrict tumor development, but otherwise may also promote and induce cancer (Hagios et al., 1998; NCI, 2018). A brief survey on the page of the National Cancer Institute in the United States (Manley et al., 2002) shows that many of the drugs employed for cancer treatment assume that the problem is restricted to cellular communication. A methodological reductionism will be applied to develop drugs against cancer. Many of the drugs are inhibitors of the enzyme tyrosine kinase (Jemal et al., 2010; Shah et al., 2020). The treatment of a disease begins with the initial idea that the interaction of a molecule with an enzyme will give responses for a multifactorial disease. There is a clear limitation of the complexity of the problem. This limitation has an approach based on ontological reductionism (when the disease is based on the idea that the constituents can explain the whole) at the same time will apply methodological reductionism to understand the disease (due to technological limitations, a simple model is chosen to start the study about the disease).

Until 1990, mortality rates had expanded a lot. After the 90s, a gradual decrease in the number of cases was observed, mainly due to cancer prevention through tobacco control and other healthy behaviors (Chabner and Roberts Junior, 2005). However, patients with strong tumors do not react to any drugs (Chabner and Roberts Junior, 2005; Ecker, 2015). In any case, the persistence of cancer stem cells and unfavorable medicate impacts still restrain their capacity to stabilize or cure malignant invasion in the long run (Cardoso, 2020; Chabner and Roberts Junior, 2005; Ecker, 2015). The mortality rates had a modest decrease in recent years, but the cancer remains a major cause of death within the industrialized world. The reasons are complex: insufficient tumor models used within the different cancer screening programs; critical long-term harmfulness of anticancer drugs; reaction rates in patients due to sedate affectability; rapid evolution of aggressive drug resistant cells due to high mutation rates and selective pressure, resulting in transient treatment responses (Johnson, 2013; Lok, 2006; Ponting and Russell, 2002; Zhong and Virshup, 2020).

Methodological reductionism approaches to biomedical experiments have provided significant insight into the predominant regions associated with specific functions. Such discoveries, in turn, led to significant applications. For example, antibody fragments such as antigen binding fragment (Fab), single chain variable fragment (scFv) or fragment chain (Fc) are widely used as screening reagents and as therapeutic potentials (Ahn et al., 2006; Van Regenmortel, 2004). Structural refinement protein domain classifications and functional predictions are additionally used for therapeutic purposes (Krakauer et al., 2017). However, the reductionist approach, while accessible and incredibly useful, ignores the broader framework of interregional communications and their possible cooperative effects (Krakauer et al., 2017) that would be useful for further analysis. Given the advances in technology that have led to advanced experimental and computational techniques in recent years, the subsequent level of scientific advances may require proteins to be analyzed in the most holistic way. These approaches are already present in various specialties with these efforts to jointly generate ideas derived from reductionist investigation (Albergante et al., 2016; Bieber, 2015; Fardet, 2014; Phua et al., 2019). According to Van Regenmortel (2004), it is important to revisit biological systems completely as a whole (O'Sullivan et al., 2018; Soto and Sonnenschein, 2005). In this line of thinking, while limitations on looking at whole systems are always present, it is possible, however, to already be reaching a saturation point for scientific advances within the reductionist approach. Recent literature has suggested an analysis of proteins in their entirety (when possible) (van Ommen et al., 2008).

The reductionist nutritional approach thrived using the tools of analytical chemistry and experimental biology in nutrition. Recent advances in high-yield organic molecules complex structure (OMIC) technology, computational and statistical tools have led scientists to explore current challenges. These tools provide global measurements reporting the diversity of individuals and complex interactions between vitamins and human bodies (Davis and Hord, 2005; Omenn et al., 1996; Weinstock, 2012). High-performance omics technology includes genomics, transcriptomics, proteomics, metabolomics and metagenomics. Nutrigenomics, or genomics in nutrition, refer to the study of how genes and dietary components interact to

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change the phenotype (Blumberg, 1994; Chirita-Emandi and Niculescu, 2020). The three genomic categories in which knowledge is critically necessary are human genomics, plant and animal genomics, and microbial genomics (Davis and Hord, 2005). Nutrigenomics explain how the response to food components depends on an individual's genetic background (nutrigenetics), nutrient-induced changes in DNA methylation, chromatin changes (nutritional epigenetics), nutrient-induced changes in gene expression (nutritional transcriptomics) and proteins (nutritional proteomics) (Blumberg, 1994). Metabolomics is one of the newest omics and has been defined as a comprehensive analysis of changes in many low molecular weight compounds and their fluxes through human metabolism in response to dietary treatments (Davis and Hord, 2005).

Metagenomics studies the combined genome of microbial communities using next generation of DNA sequencing, which explains differences in community structure between sampling sites, individuals, and between healthy versus disease states (Rietjens et al., 2002). A good example of limitation related to the application of reductionism in nutritional research can be found in prospective studies that investigate the effects of the intake of isolated antioxidant vitamin intake and cancer development (Fardet and Rock, 2014; Peterson, 2008). The results showed controversies. Although antioxidants become pro-oxidants after exerting their antioxidant effect in *in vitro* systems, pro-oxidant formation is rapidly attenuated by recyclable chain reactions involving glutathione in the human body (Fojo, 2008). However, at a very high dose, antioxidant vitamins can lead to toxic pro-oxidant actions, indicating the absence of linear cause and effect association.

The vitamins may not have the same activity in crude and characteristic foods (Fardet and Rock, 2014). There is a demand that the potential for nourishment depends on both supplement composition and nourishment structure properties, frequently driving to conflicting discoveries. Subsequently, it was expected that a more comprehensive vision of antioxidant potential would approach a few different angles of cancer prevention agents with approaches based on synergistic, adversarial, or multi-component and multi-target additives.

Examples of reductionism at the biological level and its implications are common. Here, diseases and health problems are reduced to biological molecules, such as DNA, RNA, and specific enzymes. It is necessary to know biological targets for the synthesis of new drugs. There is yet a technological obstacle that prevents the study of the system as a whole. In this way, methodological reductionism must be comprehended as a step to reach ontological holism. However, a new type of reductionism is noted when scientists reduce the problem to simple molecules.

5. Chemical reductionism

Modern chemistry was born with Lavoisier's quantitative studies. On the other hand, biology has been developed since classical antiquity. Aristotle had manuscripts classifying the living beings. Nowadays there is a particular branch or knowledge shared by chemistry and biology. These sciences use methodologies derivative from biochemistry. The studies in both disciplines start by creating rigorous tests to study molecules, genes or proteins. However, the targets are remarkably diverse in both disciplines. Biological systems use the comparison between two systems to understand connections, uncovering tall arrangements in organized structures and modeling complexity. On the other hand, biochemistry is ruled by the attitude of matching pairing individual compounds and molecular targets. As a result, efficient biochemistry ponders are frequently centered on query databases to recognize interactions between particular molecules and a single target.

In general, the salicylic acid is described as a cyclooxygenase (COX) (Fig. 1) inhibitor, atorvastatin as a 3-hydroxy-3-methylglutaryl (HMG) coenzyme A reductase inhibitor and lithium as a glycogen synthase kinase 3β (GSK3β) inhibitor.

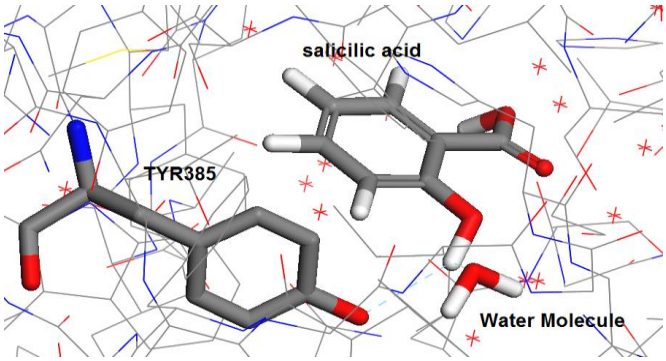

Figure 1. Salicylic acid interacting with cyclooxygenase site. Structure obtained from 5F1A (The Crystal Structure of Salicylate Bound to Human Cyclooxygenase-2/Protein Data Bank).

These descriptors are useful and carry some information, but, in reality, these small molecules do much more than inhibit a single enzyme. The benefits and negative effects on humans are not fully explained

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by their actions on a single enzyme. It is important to invoke a holism view of the system. Selective kinase inhibitors in clinical oncology were the main goal of research a decade ago. However, many experts now believe that kinase inhibitors may be advantageous in treating cancer due to the multi target reached by these kinds of molecules. However, the rational drug design continues to follow the idea that "Cell life is closely linked to a large number of specific and selective interactions between bio macromolecules" (Cheng et al., 2012; Cavasotto et al., 2018). Macromolecular modeling by docking studies provides drug-receptor interaction. In this approach, the drug structure is designed based on its 3D adjustment to the receptor site structure (Aucar and Cavasotto, 2020). A clear example is the inhibitors of enzyme kinase. Kinases are enzymes associated with the biochemical process known as signal transduction. In this process, the enzyme kinase catalyzes phosphorylation reactions of other enzymes. This biochemical process triggers a cascade of chemical signals that are converted into physical responses by the cell. In this case, the target protein is chosen for the first step of rational drug design. At this stage, the drug is designed in order to mimic the structure of the adenosine triphosphate (ATP) molecule. The premise is that the drug must have ATP-common pharmacophoric groups in order to inhibit enzyme activity through the induced docking model proposed by Koshland (1958). In this model, the substrate is able to adapt to changes in enzyme conformation. This idea permeates the construction of new drugs aimed at cancer treatment (Akhtar et al., 2019; Müller et al., 2019). However, these molecules act in different enzyme targets. Dasatinib is an example of a drug that interacts with several kinases. Dasatinib is a targeted therapy medication used to treat certain cases of chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia. Dasatinib is a potent bioavailable oral inhibitor of several kinases, including breakpoint cluster region protein in ABL genes (BCR-ABL) (Fig. 2), proto-oncogene tyrosine-protein kinase SRC, c-KIT and platelet-derived growth factor receptor beta (PDGFR-β) (Lombardo et al., 2004). It has been discovered by synthesizing and testing a series of thiazole-based compounds with activity against SRC and ABL kinases (Lombardo et al., 2004).

However, the dasatinib interaction is not limited to kinases. The i*n vivo* pharmacokinetic study shows that pretreatment with dasatinib monohydrate decreased the blood level of CsA (cyclosporine) in rats, perhaps due to induction of cytochrome P450 3A4 (CYP3A2) isoenzymes (Abdelgalil et al., 2019). The cycle-oxygenase inhibitor aspirin also acts on different enzyme targets. Aspirin blocks the formation of metastatic intravascular niches by inhibition of platelet-derived COX-1/thromboxane A2 (Ramasarma, 1994). The intrinsic properties of a protein allow catalysis of many reactions.

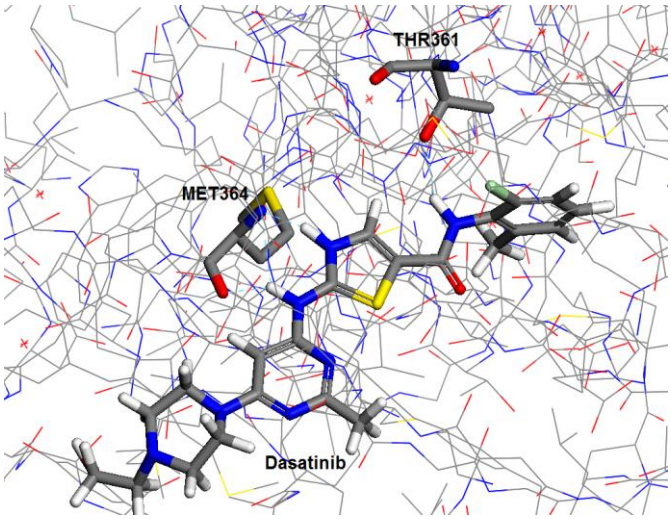

Figure 2. Dasatinib interacting with ABL2/ARG kinase. Crystal structure of ABL2/ARG kinase in complex with dasatinib obtained from protein data bank code 4XLI.

Ramasarma (1994) describes a list of more than fifty proteins with the capacity to know several kinds of molecules. These elective capacities incorporate a set of activities. These biomolecules act as enzymes, particle carriers and inhibit different cellular processes. It was discovered that intensive information of the physical and chemical properties of a protein will not give data about what it does. The question about what chemistry can contribute to the whole biological process is not so clear. Reductionism would explain which proteins produce effects on the biological system. This idea was created by Pigliucci and Kaplan (2010). It is exceptionally common to say that a protein is an esterase, kinase, etc., which appears as its primary function. The protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) plays an important role in glyceraldehyde 3-phosphate catalysis. Glyceraldehyde 3-phosphate dehydrogenase is a bio molecule with a specific function. This enzyme does not require a specific molecular structure, but an environment in which glyceraldehyde-3-phosphate is changed. Chemistry alone cannot tell that a specific protein is GAPDH. This bio molecule will only perform the specific activity if it has an environment that

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incorporates other components that together make its work particular.

While chemistry concerns molecular structures, biochemistry will work with these particles inside a system. The exclusive study about the molecule will not offer a complete comprehension about the function of biomolecules (Alm and Arkin, 2003). Genes that influence natural product fly memory arrangement encode cyclic adenosine monophosphate (cAMP) proteins within the signaling pathway are not a particular memory. It is the cell compartment and the environment in which a cAMP is discharged that permits a hereditary item to have an impact. Natural specificity comes from the way that these components gather and work together.

6. Physical reductionism

The early twentieth century had a significant transformation in the context of atomic structure. The old Greeks tried to explain the existence of atoms from philosophical central conjectures. Dalton later differentiated atoms with the help of the balance. However, the atoms remained nondivisible, as that of the Greeks (Democritus and Leucippus). Thomson used the cathode ray tube to discover electrons. Rutherford bombardments a gold plate to discover the existence of the nucleus. Planck begins quantum mechanics studying the heat from materials at high temperatures. Einstein theorizes the corpuscular nature using the photoelectric effect. Schrödinger (1926) developed a wave equation whose solutions are the quantum numbers. At this point, the reductionism of chemistry to physics began, as Dirac (1929) points out:

"The general theory of quantum mechanics is now almost complete, the imperfections that still remain being in connection with the exact fitting in of the theory with relativity ideas. These give rise to difficulties only when high-speed particles are involved, and are therefore of no importance in the consideration of atomic and molecular structure and ordinary chemical reactions, in which it is, indeed, usually sufficiently accurate if one neglects relativity variation of mass with velocity and assumes only Coulomb forces between the various electrons and atomic nuclei. The underlying physical laws necessary for the mathematical theory of a large part of physics and the whole of chemistry are thus completely known, and the difficulty is only that the exact application of these laws leads to equations much too complicated to be soluble."

This is a clear epistemological reductionism defended by Dirac. It is not only a methodological approach, but a complete explanation of chemistry using the quantum mechanics equations. However, it will be shown that the presumptions made by Dirac are not entirely correct. Although a methodological reductionism can be used to simulate the atoms and molecules.

Physical-level reductionism is visible in biomolecule modeling for new drug planning purposes. The behavior of biological molecules is studied from quantum approaches, semi-empirical and classical models. In this case, the molecules and their properties are explained by quantum mechanics and their approximations. In general, the coordinates of atoms are given. From these coordinates, atomic number, mass, among other properties, are extracted chemical and biological properties of interest. The heart of quantum theory for atoms and molecules is the resolution of the Schrodinger equation. However, this equation has no analytical solution for atoms with more than one electron. Approaches are required to solve this problem. One of the first approximations to facilitate the resolution of the Schrödinger equation is the Born–Oppenheimer approximation. In this approach, it is assumed that the motion of the atomic nucleus and electrons can be separated (Born and Oppenheimer, 1927). Chemical structure cannot be found in pure quantum mechanics applied to a chemical system. It is imposed by the Born–Oppenheimer approach (Woolley, 1978). New approaches were developed to explain the chemical bond originating quantum chemistry methodologies

The introduction of the electron spin and the Pauli exclusion principle were used to explain the formation of a chemical bond. The linear combination of atomic orbitals (LCAO) and valency theory were used to describe the chemical bond. However, there is the problem associated with the Schrodinger equation solution to many electrons. Several approaches were used to solve this limitation. The first, already presented, is that of Born–Oppenheimer. The other approximations are dependent on the method used. It is important to note that neither approach solves the Schrödinger equation analytically. Hartree's (1928a, b) approach, for example, considers the interaction of an electron with the average field generated by the other electrons. Jordan and Fock (1930) introduced the antisymmetric fermion product in Hartree's method. Finally, Roothaan (1951) added the linear combination of atomic orbitals to the method. Hartree's initial approximation contains a limit in the system energy, which will always be greater than that obtained by the exact solution of the Schrödinger equation. For Woolley (1978), the concept of molecular structure is

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absent at the actual quantum level. Electronic structure calculations are based on approximations.

Löwdin (1955) denominated the difference between exact energy for that calculated from the *ab-initio* approach as correlation energy. The lowest and most accurate energy would be found by overcoming the Born–Oppenheimer approximation and by inclusion of relativistic corrections. Post Hartree–Fock approaches named coupled cluster use iterative single, double and triple perturbative excitations (CCSD(T)) that are able to perform calculations with a precision of ± 5 kJ mol^{-1} for small and medium molecules (Kümmel, 2002; Shavitt and Bartlett, 2009). There is still the problem with computational time. Robust calculations, such as those derived from Møller–Plesset perturbation theory (MP2 and MP3 methods) and those approaches that use double and triple excitations are computationally unviable for the treatment of biomolecules. The classic force fields are employed to study biological molecules. In this method, quantum mechanics is replaced by classical mechanics. Electrons are forgotten and chemical bonds are treated as spring-mass systems. Interactions are described by electrostatic and van der Waals equations. This method is the molecular mechanics (Kümmel, 2002; Leach, 2001) that allows the study of optimization and dynamics of proteins and other biological molecules. Systematic validations based on quantum mechanics have been performed in order to standardize the molecular force fields (van der Spoel, 2021).

Rational drug design at the molecular level is generally based on X-ray diffraction. In this technique, the position of atoms in the crystal is determined to obtain the three-dimensional structure of the biomolecule. This structure can be studied as a whole with classical mechanics. There is also the possibility of temporal evolution of the system using classical force fields associated with the integration of Newton's equations step by step in time. This technique is known as molecular dynamics. The longest molecular dynamic ever performed was on the order of 10^{-6} s. The problem is that some biochemical mechanisms, such as protein folding, are in the temporal interstice of 10^{-3} s. In the study of structure and activity relationship (SAR), the activity of a set of known drugs can be correlated with a series of their physicochemical properties. The idea behind this technique is to construct a multilinear regression curve that relates known drug activity to a particular universe of physicochemical properties. The graph allows the inferences of the activity of a new drug before the synthesis.

Fundamental presumption for QSAR is that comparable molecules have comparable activities. This rule is called the structure-activity relationship. The fundamental issue is how to characterize a little distinction at the molecule level, since each sort of movement and response capacity, biotransformation capacity, solvency, target movement, and so on, may depend on another distinction. Great examples have been given in the literature (Brown, 2012; Patani and LaVoie, 1996).

The speculations depend on a limited amount of chemical information. The rule of acceptance must be regarded to maintain a strategic distance from over adjusted theories and to determine over adjusted elucidations in basic molecular information. The reality is that not all comparative molecules have comparative activities.

It was shown several reductionism kinds. Dirac (1929) defends a complete epistemological reductionism, which quantum mechanics premises is the primary theory and the conclusion is chemistry. On the other hand, Crick (1966) argues that biology can be reduced to chemical reactions. Both these propositions were criticized, showing arguments against this kind of reductionism. It is important to declare that biology developed itself without the knowledge of chemistry. There is not a causal dependence between chemistry, biology and physics.

However, methodological reductionism is applied nowadays to begin the studies in drug design. It will show the problems associated with methodological reductionism. The holistic approaches that can be used to work with drug design will be described as an alternative to approaches merely reductionist.

7. The problem in design of new drugs

The number of modern drugs approved by Food and Drug Administration (FDA) diminished over the last years. The number of approved drugs reduced from 10 to 20 during the year of 2002. This decline has been held in spite of proceeded industry mergers, acquisitions and yearly investing over US$ 30 billion. Some commentators qualify this declination due to organization causes, such as wasteful venture administration, expanded prerequisites, a decrease in clinical science that bargains with entirety living beings, an overemphasis on technology-driven inquire about, and a need of eagerness to do so, center on items that are not anticipated to create deals of at slightest US$ 0.5 to 1.0 billion per year (Drews, 2003; Fojo, 2008; Gershell and Atkins, 2003; Kubinyi, 2003; Miska, 2003). Furthermore, these results show that

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methodologies based on tall throughput screening, combinatorial chemistry, genomics, proteomics, and bioinformatics are not bringing the modern conquests that were predicted (Glassman and Sun, 2004; Kubinyi, 2003; Miska, 2003).

Information about human genome arrangements and different pathogens led to a set number of modern drug targets (Drews, 2003). In addition, a few biotechnology enterprises fizzled to correspond to the perspectives in order to establish quality treatment, investigating antisense innovation and cancer immunizations. A common issue with numerous of these advancements is that the potential for dangers and undesirable side impacts tend to be ignored, as was the case with gene therapy (Glassman and Sun, 2004).

However, there is a probable reason for these disappointments: most of these approaches have been guided by supreme reductionism. As a result, the complexity of organic frameworks, whole life forms and patients tends to be underestimated (Horrobin, 2001). Illnesses results from the interaction of numerous genes and sometimes it is conceivable to know all the genes and hereditary included in a specific biological function.

Another field of biomedical science strongly influenced by reductionist theory is the so-called vaccine design, which is based on the premise that the concepts of drug design based on molecular structure can be applied to vaccines (Van Regenmortel, 2001; 2021). However, this approach neglects that the relationship between a drug and its receptor or molecular target is exceptionally particular, whereas the relationship between an antigen and a counter acting agent is much less limited. The binding site of an immunoglobulin molecule comprises approximately 50 hypervariable residues that together constitute the complementarity determining regions (CDRs). Hypervariable residues together make the CDRs. In general, around 10–15 of these residues have an interest in interaction with a specific epitope, but the total complement of all 50 hypervariable residues does not constitute a real binding site for any epitope. This implies that approximately 35 of the CDRs residues can possibly bind to other epitopes that are small or no likeness to the previous. This complexity clarifies the broad multi-specificity of immunoglobulins and the numerous distinctive paratopes or bind sites on each molecule. The capacity of an immunoglobulin molecule to bind to different antigenic structures is improved by the impressive adaptability of CDRs. The molecule of immunoglobulin has different conformations with several binding sites (Bosshard, 2001; Goh et al., 2004; James et al., 2003). In in the 2000s, rational drug design has become a necessity in the advancement of antibody research contradicting observational data (Van Regenmortel, 2000). The term rational drug design suggests that this investigation uses molecular information and structural knowledge, whereas the term design shows that the developed products are predictable. Rational drug design is considered the more logical approach than experimental "trial-and-error" screening and molecular selection.

The conviction that a molecular design plan will be effective for the improvement of unused antibodies is ordinary of the reductionist approach. Ontological reductionism expects that a biological phenomenon can be decreased to the chemistry level. However, there are numerous reasons that show that reductionist approaches to antibody advancement are impossible to succeed. First, the antigenic determinants or epitopes of an infectious agent are rising substances that are characterized by their particular antibody partners and exist as it were within the setting of the immune system. Epitopes and paratopes are not intrinsic characteristics of an antigen. Immunoglobulin individually cannot be recognized autonomously by a binding reaction. Second, the idea behind the antibodies that essentially bind to the pathogen have small consideration in immunization advancement. Antibodies that have a functional activity are required; specifically, the capacity to neutralize the infectious agents *in vivo*. Human capacity to anticipate protein function is constrained. The ability to predict neutralizing action of an antibody by chemical structure is for all intents and purposes inexistent (Van Regenmortel, 2000; 2002). Immunization and protective resistance have meaning at the level of the entire living being: particles, tissues and organs cannot be vaccinated. Immunization takes place in the organic domain and cannot be decreased to the level of chemistry. Third, in spite of an exceptional worldwide request for investigation efforts using reductionist approaches, no immunodeficiency infection antibody is in sight (Burton and Moore, 1998). Reductionist approach on HIV immunization advancement continues to be advocated (Burton et al., 2004), although there is no evidence that it will be efficient. This approach includes deciding the atomic structure of monoclonal antibodies to HIV antigens using X-ray crystallography to illustrate the structure of HIV epitopes. The basis for these studies is the suspicion that information of the structure of epitopes that are recognized by neutralizing antibodies will offer assistance to plan a viable antibody against HIV. Crystallographic X-ray investigation of HIV-

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neutralizing antibodies may describe the structure of epitopes inside the molecular pockets, but it does not tell how to utilize immunization and initiate antibodies with the same specificity (Van Regenmortel, 2002; Burton and Moore, 1998). The structures of epitopes and paratopes that are displayed in a complex describe the last conformation of an energetic handle by the alteration in the somatic change. It is not conceivable that the conformation of the epitope on the immunogen is eventually responsible for the appearance of neutralizing antibodies.

8. Organicism as a counterproposal in cancer treatment

The progress in molecular science over the past three decades has cleared the way for a hereditary cancer hypothesis, the somatic mutation theory (SMT) (Boveri, 1929; Weinberg, 1998). This hypothesis qualifies as a "standard hypothesis" because it has collected most of the financing for cancer investigation. This hypothesis assumes that cancer was generated from genetic breakdown at the cellular level.

This model of cancer can be followed back to the work of Boveri (1929). Nowadays, it is ordinarily defended by Weinberg (1998). This reductionist approach was explicitly exposed in Weinberg's book One Renegade Cell, that emphasizes that the basic part of a single mutated cell will originate from cancer (Weinberg, 1998). One alternative comes from traditional biology and emphasizes that the living being must be studied as a whole. The central point of this research is the work of Waddington (1935). According to the tissue organizational field theory (TOFT), the causes of cancer should be investigated not at the hereditary level, but at the tissue level. The TOFT claims that cancer starts from a disturbance of tissue organization. This theory has been examined by Dolberg and Bissell (1984) and characterizes the conceptual system with an organicist point of view.

The SMT approach, whereby the cause of cancer must be inquired about at the hereditary level, has regularly been qualified as an illustration of reductionism, and more accurately as hereditary reductionism. The ideas behind SMT inside hereditary qualities based on reductionist paradigm really rose within the 1970s. At the time, an impressive number of carcinogenic chemicals could cause hereditary changes.

Afterward, it was found that a few of the so-called tumor infections (also called quality transformers or oncogenes) could lead to the advancement of tumors that carry hereditary transformations in tainted cells. At this point, the hereditary cause of cancer changed from exogenous to endogenous and a few endogenous oncogenes were distinguished as transformed shapes of ordinary cell qualities.

Later researches showed the true complexity of cancer. It was found that a few cases of carcinogenesis could be understood as a multistep to prepare numerous oncogenes, as well as possibly one or more anti-oncogenes or tumor silencers. Around 100 oncogenes and 15 anti-oncogenes have been recognized (Hanahan and Weinberg, 2000). The cell cycle clock was considered critical, as were the characteristics of the cell life. The explanation described by Weinberg has been amplified beyond intracellular hereditary causes. Nowadays, there are numerous biomolecular pathways, including signals of communication between cells. In carcinogenesis, the cells acquire six particular capacities: specification, "self-sufficiency" in development signals, cold-heartedness inhibitory development signals (antigrowth), avoidance of modified cell passing (apoptosis), replicative potential boundless, maintained angiogenesis in tissue (Hanahan and Weinberg, 2000).

Each of these capabilities may be obtained by distinctive molecular pathways: self-sufficiency in development signals coming about in independent cell development and multiplication that may be searched by the modification of extracellular development signals, transcellular transducers of such signals or circuits, which decipher these signals into activity. These six capabilities would indeed be empowered by a seventh characteristic, the genomic precariousness, in some cases it is known as "more prominent variability". The definition of the SMT has been reformulated in terms of "heterotypic intuitive" between early tumor cells and their ordinary neighbors. (Hanahan and Weinberg, 2000). Hereditary reductionism, which describes the origin of cancer by a cell mutation, has been adjusted to cover the complexity of carcinogenesis and the variety of atomic pathways that lead to tumor cell multiplication. Although SMT presents a clear limitation to explain the cancer origin, the paradigm centered on molecular substances remains the most fundamental explanation.

Cancer approach proposed by Sonnenschein and Soto (2020) and their group is based on tissue investigation. The TOFT finds an explanation of cancer in terms of disturbance of tissue organization as opposition to the expression of a defective gene. The TOFT is based on two fundamental presumptions: (i) expansion is the default state of all cells, and (ii) carcinogens act initially due to distribution of the

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interaction that happens between cells within the stroma and parenchyma of an organ.

For TOFT researchers, the SMT program works with a reductionist problem, because it looks for carcinogenesis caused at the genes and molecular components level of cells instead of the total level of tissues: cancer is seen as an intracellular problem caused by mutations within the DNA of the cancer cell. The basic level that carcinogenesis causes ought to be explored is the tissue level, because this level is used in biopsy that can give an authoritative conclusion of cancer (Soto and Sonnenschein, 2005). Sonnenschein and Soto (2020) characterize hereditary reductionism as: a great number of biologists demand that cancer clarifications should be analyzed in gene or gene product level. This reductionist-genetic predicts that everything in biology can be diminished to genes since the genome is the store of transmissible information.

The approach taken by SMT is exceptionally prohibitive because it limits the issue in terms of genes and molecular expressions. It has indeed condemned the cancer investigation. For this reason, this theory should be eliminated or supplanted. In other words, the SMT approach is criticized to reduce the cancer to genes and particles inside cells. This approach will inhibit the exam of other potential causes, such as tissue organization. As a substitute for hereditary reductionism, TOFT proposes an approach based on organicism: cancer is seen as an issue comparable to histogenesis or organogenesis and is hence a developmental science. Organicists "select to work" at the level of organization at which the phenomenon is observed. The TOFT cautiously wanders into lower levels of organization, slowly moving through the different various levels in which the phenomenon is observed.

8.1 Systems biology

The objective of systems biology is to consider the physiological and conditions of administrative arrangement levels, signaling pathways, cells, tissue, organs, and inevitably the whole life form (Berg, 2014). It is a holistic view based in some methodological reductionism to compute the properties that will integrate the whole. The structure of science comprises a number of approaches and models that help within the consideration of the organic complexity of different illnesses. System biology combines an expansive sum of reductionist genomic information, proteomic and metabolomic tests to produce an organized data set for considering a disease.

System biology points to the complex behavior of organic frameworks that develop from personal framework components and intelligence between them (Sobie et al., 2011). Hence, a fundamental highlight of system biology is that the interaction between all framework components is examined instead of the characteristics of each individual component. It gives a few approaches to creating forecasts that can be tested experimentally. System biology depends on the combination of test ponders, that creates information concerning cellular components of a framework as well as approaches that help within the examination of different information sets. Two major computational approaches are utilized in system biology, specifically, data oriented (top-down approach) and hypothesis driven (bottom-up approach) (Faratian et al., 20093) to create new treatments.

8.2 Predicting drug-target interactions (DTI)

Drugs often bind to more than one biomolecular target. It is fundamental to get the polypharmacology of a medicament. Exploratory approaches to identify DTI are costly, difficult, and time expending. Hence, computational approaches are broadly used for DTI expectation. Computational approaches characterize each target by a set of known connection covers, searching for chemically organized drugs anticipating modern DTIs (Keiser et al. 2009). Associations between drugs and targets can be anticipated based on the chemical structure of the drug and protein sequence (Li et al., 2015; 2017). Drug-target interactions can moreover be distinguished based on similitudes of side impacts, i.e., drugs with comparable side impacts tend to be associated with the same biological targets (Berg, 2014; Campillos et al., 2008). The combination of pharmacogenomic likenesses and side impacts are used in DTI (Li et al., 2017). In addition, a comparison of three administered induction strategies to anticipate DTI is the induction likeness, target-based likeness deduction, and network-b.

Complex diseases are controlled by the interconnected systems of numerous pathways related to cell expansion, attack and medicate resistance (Ryall and Tan, 2015). In this way, it is troublesome to create modern treatments against complex infections. Drug-target interactions approach uses a combination of medicating treatments that at the same time balance numerous targets and may have more advantages than employing a single drug (Jia et al., 2009). This approach gives strong evidence for anticipating treatments of an infection. A few network-based approaches have been employed to anticipate drug

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combinations for cancer treatment. In this calculation the kinase inhibition profile and the region with tumor are used to develop a set of drugs as well as to anticipate tumor sensitivities for new drugs or drug combinations (Pal and Berlow, 2012). Vital kinases for mesenchymal and epithelial cell migration were anticipated, employing a joint approach to strong cancer drug combinations. This pooled approach uses flexible net regularization with mRNA expression profiling and a huge set of kinase inhibition.

9. Concluding remarks

Today, there is a clear application of methodological reductionism to develop new drugs although there is a grasp that holism must be reached to understand the full biological process. On the other hand, holistic approaches like system biology use some reductionist methodologies to build a whole vision of the organism. Nowadays, a balancing of methodological reductionist approach and the holistic view is combined to produce new drugs and understand new diseases.

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Ecofriendly and low-cost sample preparation methods for magnesium determination in beer

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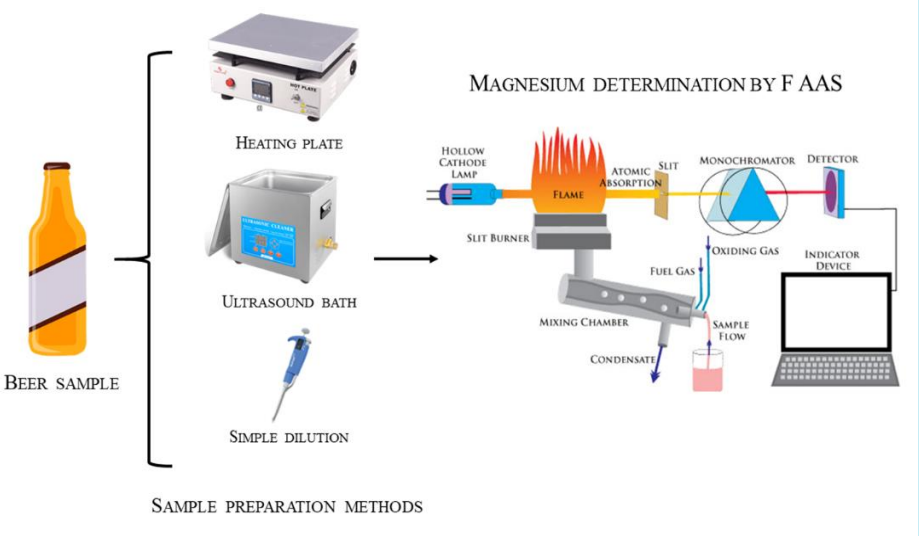
ABSTRACT: Ultrasound-assisted extraction and direct analysis were compared with total digestion for magnesium determination in beer samples by flame atomic absorption spectrometry. The method for total digestion used concentrated nitric acid under plate heating. In optimized instrumental conditions, validation of the analytical method was promoted, with good linear range (0.06 to 0.5 mg L^{-1}), low limits of detection and quantification (0.04 and 0.12 µg g^{-1}, respectively), good precision, relative standard deviation (RSD) < 3.4%, and accuracy (recovery levels of 91.5 to 99.0%). The characteristic concentration (C0) was 9 µg L^{-1}. The extraction procedure was performed in a 1:1 nitric acid solution for 55 min in an ultrasonic bath at 60 °C, while the direct analysis involved a dilution of the samples in a 2% v/v nitric acid solution. The different sample preparation methods were applied to 13 beer samples and at a 95% confidence level, no significant differences were observed. Thus, direct analysis proved to be more suitable for quality control routines of beer samples in the industry.



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1. Introduction

Beer is one of the most popular drinks worldwide and the third most consumed, after water and tea (Pai et al., 2015; Sampaolesi et al., 2019). Historical reports date back to their production by Sumerians and Assyrians around 8,000 years ago (Rosa and Afonso, 2015), while the Egyptians were responsible for spreading their production among the eastern people in the Mediterranean basin and from there to the rest of Europe (Ferreira et al., 2011). Beer is traditionally produced with malted barley because of its high enzymatic content, which allows for the rapid conversion of starch into fermentable sugars that give rise to alcohol, carbon dioxide and flavor compounds during yeast fermentation (*Saccharomyces cerevisiae*) (Omari et al., 2020). In addition, hops are used in the fermentation process to add a characteristic bitterness and distinct aroma to the beer (Kishimoto et al., 2020).

The composition of beer varies according to style, however, the presence of minerals, such as Ca, K, Mg, P and Zn (Rosa and Afonso, 2015; Sleiman et al., 2010), has already been reported, which can correspond to up to 10% of the recommended daily intake values. Moderate beer consumption is associated with several benefits, ranging from diuretic properties and antioxidant action to positive effects against several cardiovascular risk factors, including an increase in high density lipoprotein (HDL) cholesterol and a lower risk of ischemic stroke (Arranz et al., 2012; Gaetano et al., 2016; Lordan et al., 2019). These factors may be related to the presence of moderate levels of magnesium in beer, influencing the quality of the drink. Magnesium is an essential micronutrient associated with more than 300 enzymatic processes in the body (Rosanoff, 2013). In beer, when associated with calcium it helps in the kinetics of the isomerization reaction of α-acids in cis and trans-iso-α-acids, constituents responsible for the bitterness of beer (Wietstock et al., 2015).

Different methods of analysis have been used to determine magnesium in several types of samples, including colorimetry (Shishov et al., 2019), liquid chromatography (Paull et al., 1997), electrochemistry (Akhter et al., 2020), capillary electrophoresis (Sako et al., 2018), flame atomic absorption spectrometry (F AAS) and graphite furnace atomic absorption spectrometry (GF AAS) (Santos et al., 2019; Seeger et al., 2019), inductively coupled plasma optical emission spectrometry (ICP OES) (Souza et al., 2019) and inductively coupled plasma mass spectrometry (ICP-MS) (Moreda-Piñeiro et al., 2018). The methods recommended by the American Society of Brewing Chemists for determining magnesium in beer samples are based on the spectrophotometry, F AAS, GF AAS and ICP OES techniques (ASBC). Among these, the one with the lowest cost and most adequate is the F AAS, as it presents good selectivity, precision, robustness, high analytical frequency associated with the low cost of acquisition and maintenance of equipment and analysis (Khajeh and Sanchooli, 2010; Pohl and Sergiel, 2010).

The optimization of sample preparation conditions, which is a critical step that involves from simple dilution to partial or total solubilization, are essential for the development of analysis methods (Santos et al., 2019). Currently, the main objective is focused on obtaining the best results in the shortest time, with minimum error, low consumption of reagents and minimum generation of residues, the latter two topics being associated with green chemistry. However, the methods commonly used for this purpose are digestion using concentrated acids under heating (heating plate and microwave oven) and alkaline solubilization, both methods require massive volume of reagent, high energy consumption and generate toxic waste (Mketo et al., 2016). Excellent alternatives to this problem are the use of ultrasound-assisted extraction (UAE) and direct analysis, as demonstrated by the scientific literature (Adolfo et al., 2020; Ferreira et al., 2014; Oliveira et al., 2017; Santos et al., 2018; Szymczycha-Madeja et al., 2013; Welna et al., 2014).

Thus, the present work aims to develop and compare different methods of preparing samples by digestion in heating plate, UAE and simple dilution to evaluation of magnesium levels in beer samples by F AAS.

2. Experimental

2.1 Materials, reagents and samples

All measurements were performed on a flame atomic absorption spectrometer (Thermo Scientific, model SOLAAR Serie M5; USA). A magnesium hollow cathode lamp (Photron Lamps; USA) was used, operating with a maximum current of 4 mA and a wavelength of 285.2 nm. The acid digestion was performed on a heating plate (Warmnest, model DB-IVA). The extraction was performed in an ultrasonic bath with power 220 W and frequency of 40 kHz, temperature control (30 to 60 °C) and volume 9.5 L (Unique, model USC-2800A). All reagents used were of analytical grade. The solutions were prepared using deionized water with resistivity of at least 18.2 MΩ cm

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(Elga Purelab Option-Q, model LA611; UK). Nitric acid (Sciavicco Comércio e Indústria Ltda, 65% v/v) was used to prepare samples and solutions. The analytical curve was prepared from a stock solution of Mg 1000 mg L^{-1} (Vetec Química Fina Ltda). All glassware used was cleaned in at 10% (v/v) nitric acid bath for at least 24 h, then washed with deionized water at least three times and dried at room temperature. The 13 samples of beer of different brands were purchased on the local market, named A01 to A13.

2.2 Sample preparation

Initially, the samples were subjected to a degassing process through sonication in an ultrasonic bath for 15 min–to remove CO_2 (Blanco et al., 2010). The acid digestion of the samples was performed on a heating plate using beakers and watch glasses as a reflux system. The procedure was carried out in the exhaust hood. Initially, about 500 mg of degassed sample was weighed, then 10.0 mL of concentrated HNO_3 was added. After this stage, the mixture was taken to a heating plate at 90 °C until the release of nitrous vapors ceases.

For the extraction procedure in ultrasonic bath, 500 mg of the sample and 5.0 mL of diluted HNO_3 solution (1:1) were added in a polypropylene tube. The tubes were positioned under the support and sonicated for 55 min at a controlled temperature of 60 °C. All samples were transferred to volumetric flasks of 25.0 mL and completed volume with deionized water.

For direct analysis, 125 mg of sample was weighed and diluted to 25.00 mL in a volumetric flask with HNO_3 solution (2% v/v).

2.3 Instrumental conditions optimization of F AAS

Instrumental optimization was performed according to the recommendation of the equipment manual. The gas flow was evaluated from 1.0 to 1.3 L min^{-1}, the spectral bandpass from 0.1 to 1.0 nm and the burner height from 6.8 to 7.2 mm. The optimization studies were performed in a univariate manner and all measurements were performed in triplicate.

2.4 Figures of merit

All analytical parameters were validated according to the Resolution RDC No. 166 of July 24, 2017, of the National Health Surveillance Agency (Anvisa), which provides for the validation of methods analytical and other measures (Anvisa, 2017). The linearity of the analytical curve, homoscedasticity and normality of the data were verified using the analysis of variance tests (ANOVA), Cochran and Shapiro–Wilk, respectively, at a 95% confidence level. The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated by multiplying the value of the standard deviation (10 measurements of absorbance signal of the blank digestions on a heating plate) by 3.3 (LOD) or 10 (LOQ), then the result was divided by the slope of the analytical curve. The limits obtained by the previous calculation were corrected by a factor where they were multiplied by the final volume of the analysis solutions (0.025 L) and divided by the mass of samples (approximately 0.5 g), obtaining LOD and LOQ in µg g^{-1}, as already demonstrated by Mimura et al. (2016).

Regarding the method detectability, sensitivity was evaluated by calculating the characteristic concentration (C_0) (Welz and Sperling, 1999). The precision of the method was verified using the relative standard deviation (RSD). Spike tests were also performed in order to evaluate the accuracy. These were performed at three levels of concentration. The analytes were added, as solutions, to the samples immediately after weighing. For the first level, the concentrations of Mg in the samples were 10 mg L^{-1}. For the second level, the concentrations of Mg in the samples were 20 mg L^{-1}. For the third level, the concentrations were 30 mg L^{-1}. Blank samples were also evaluated at a concentration level of 10 mg L^{-1}. After sample preparation, dilution processes were necessary for magnesium quantification in the concentration range of the analytical curve.

3. Results and discussion

3.1 Instrumental conditions optimization

The development of methods for determining mineral elements by F AAS requires a step of optimizing instrumental conditions. Generally, it is recommended to evaluate parameters such as gas flow, burner height and spectral bandpass. Magnesium, as well as other alkaline-earth metals, is more sensitive to slightly reducing flames, where maximum temperatures are obtained from 2100 to 2200 °C (Welz and Sperling, 1999). With the increase in the flow of acetylene gas, the flame increases its reducing power, which directly influences to obtain flames with milder temperatures and lower gradients. Thus, it is expected

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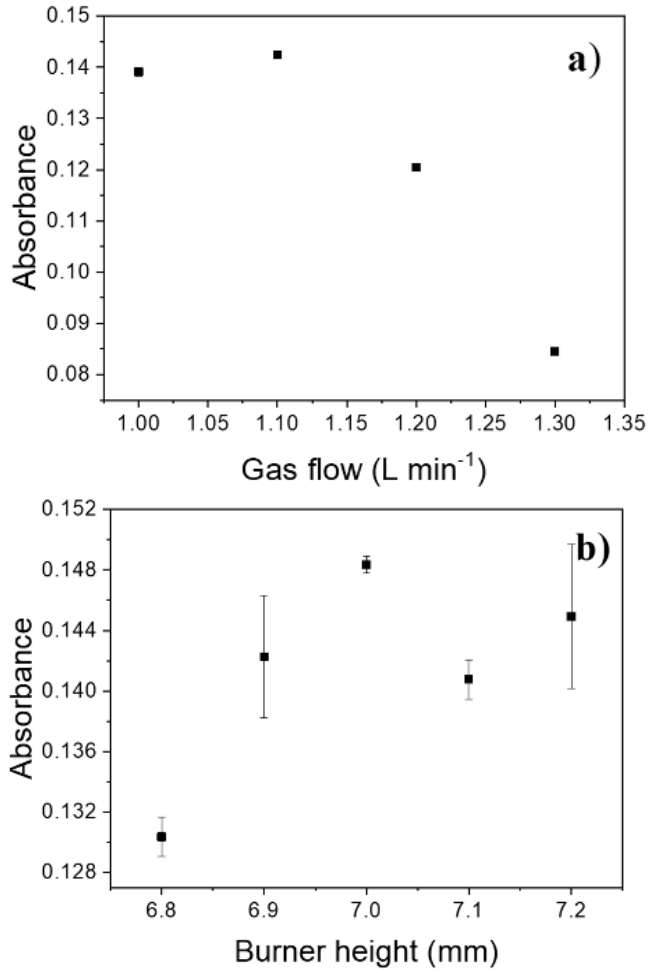
to reduce the absorbance signal, as shown in Fig. 1a. The adjustment of the burner height (observation height), associated with analytical sensitivity, is important for the residence time of the metal in the fundamental state in the gas phase. In this sense, as evidenced by Fig. 1b, the positioning of the burner influenced the absorption conditions of the radiation beam that passes through the flame. There was an increase in analytical signal up to 7.0 mm, from this value less analytical sensitivity and considerable loss of precision was observed, showing a significant fluctuation of the atomic absorption signals. In atomic absorption spectrometry (AAS), the monochromator has the exclusive task of separating the analytical line from other emission lines from the source. The saturation of the detector can often be observed from certain values of the spectral bandpass, as shown in Fig. 1c, where from 0.2 nm the measured absorbance remains practically constant. However, in very narrow bandpass, the small amount of radiation reaching the detector also compromises sensitivity. Table 1 shows the experimental conditions optimized for the analyses.



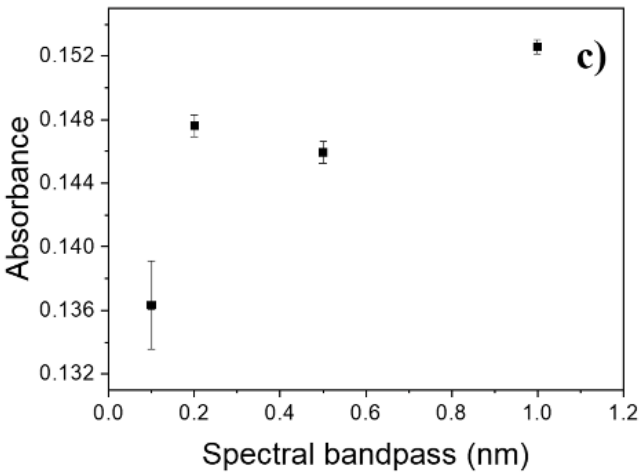


Figure 1. Optimization curves for instrumental conditions related to the magnesium determination in beer samples by flame atomic absorption spectrometry. **a)** gas flow; **b)** observation height; and **c)** spectral bandpass. Conditions: the optimizations were performed using a standard solution with a concentration of 0.3 mg L^{-1}, according to instructions in the equipment manual. Optimal gas flow 1.1 L min^{-1}; burner height 7.0 mm; spectral bandpass 0.2 nm.

Table 1. Instrumental conditions for magnesium determination in beer samples by F AAS.

Parameters	Conditions
Gas flow (L min^{-1})	1.1
Spectral bandpass (nm)	0.2
Burner height (mm)	7.0
Wavelength (nm)	285.2
Gas mixture	Air/acetylene

3.2 Figures of merit

Linearity was evaluated through ANOVA for an external analytical calibration curve with the concentration ranging from 0.06 to 0.5 mg L^{-1}, constructed under previously optimized conditions. The analytical curve showed good linearity, with a linear correlation coefficient (R) greater than 0.99, as shown in Fig. 2. The regression showed no lack of fit for $\alpha = 0.05$ ($F_{calc} = 0.617 < F_{tab} = 3.26$), and it was highly significant and useful for prediction purposes. The homoscedasticity and normality of the data were verified using the Cochran ($C_{calc} = 0.606 < C_{tab} = 0.616$) and Shapiro–Wilk ($W_{calc} = 0.938 > W_{tab} = 0.897$) tests, respectively, at a 95% confidence level. The test results indicated that the data were distributed homogeneously and according to a normal function.

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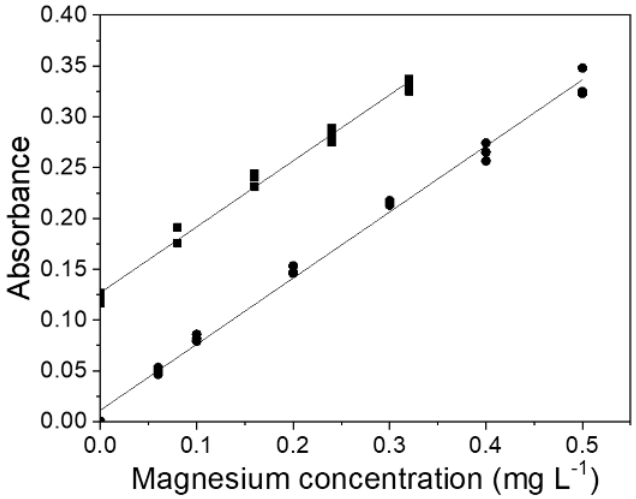

Figure 2. External calibration curve (●) (y = 0.650x + 0.011, R^2 = 0.99) and standard addition curve (■) (y = 0.649x + 0.127, R^2 = 0.99) for determining Mg in beer samples.

The matrix effect in determinations using F AAS can be evaluated by comparing the external calibration with the standard addition curves. Figure 2 shows the calibration curves for beer samples with concentration ranging from 0.06 to 0.50 mg L^{-1} for external calibration curve and from 0.08 to 0.32 mg L^{-1} for standard addition curve. The concentration variances determined by both methods, Tab. 2, were compared using the F test (Fisher–Snedecor). The calculated F value (6.45) was greater than the critical F value (3.44), which indicates that the calculation for comparing the means must be performed using the t-test with ungrouped variances. The t-test was calculated at a 95% confidence level and, since the calculated t-value (0.93) was less than the critical t-value (2.23), it can be said that there is no significant evidence of differences between the different calibration methods, indicating that the established method presents satisfactory selectivity, with no matrix effect on the determinations.

Table 2. Magnesium concentrations in beer samples obtained by F AAS using an external calibration curve and standard addition curve. Concentration ± standard deviation (sd), n = 3.

Samples	Magnesium concentration (µg L^{-1})	
	External calibration curve	Standard addition curve
1	172 ± 1	193 ± 3
2	156 ± 1	143 ± 2
3	178 ± 6	195 ± 9

The spike tests, carried out at three concentration levels, allowed to evaluate the accuracy of the method. It should be noted that the standard solution was added to the samples prior to the digestion process and the blank sample was also prepared at a concentration level for control. The recovery percentages were 98.0 ± 0.1, 99.0 ± 2.0 and 99.0 ± 3.0% for levels 1, 2 and 3, respectively; while for the blank sample, the percentage of recovery was 91.5 ± 0.5–%. These recovery values close to 100% indicate that the method, digestion in heating plate, presented good accuracy for magnesium determination in beer samples.

The precision of the measurements was also evaluated using repeatability tests (n = 10). For beer samples, this value was 3.4%. Considering the concentration level in the reading solution (approximately 0.24 mg L^{-1}), it can be inferred that the measurements were highly precise. The LOD and LOQ calculated for the method were 0.04 and 0.12 µg g^{-1}, respectively. The analytical sensitivity expressed as the characteristic concentration (C_0) was 9 µg L^{-1}. The value found is close to values in the literature, such as that obtained by Ieggli et al. (2010; 2011). for chocolate samples (19 µg L^{-1}) and emulsified egg samples (14 µg L^{-1}).

3.3 Application and comparison of methods

After validation of the method involving total digestion, the UAE method and dilutions for direct analysis were performed. The results, shown in Tab. 3, were compared using the IBM SPSS Statistics 21 software. The first assumption evaluated was normality through the Shapiro–Wilk test and the results obtained indicate that the residues showed normal distribution, since the significance presented a value higher than the p-value (0.05). The homoscedasticity was evaluated by Levene test and similarly it was found that showed significant p-value greater than the value (0.05). The independence was evaluated using the Durbin–Watson (DW) test, to test for the presence of autocorrelation in the errors of a regression model and since the calculated DW value is within the critical range (dU < DW < 4 dU, that is, 1.38 < DW < 2.62), it can be said that, at a 95% confidence level, the residues are independent. Thus, regression analysis of the methods (ANOVA, single factor) was performed and it was found that at a 95% confidence level the calculated F value (0.050) is less than the critical F value (3.259) and, therefore, it can be said that the results are statistically equivalent.

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Table 3. Results for magnesium determination in mg g^{-1} by F AAS in beer samples using different sample preparation techniques. Concentration ± sd, n = 3.

Samples	Heating plate digestion (µg g^{-1})	Ultrasound-assisted extraction (µg g^{-1})	Direct analysis (µg g^{-1})
A01	113 ± 3	101 ± 8	114 ± 3
A02	82 ± 4	93 ± 5	89 ± 3
A03	71 ± 4	74 ± 5	70 ± 5
A04	74 ± 1	77 ± 2	86 ± 5
A05	72 ± 7	69 ± 1	88 ± 6
A06	70 ± 7	72 ± 3	65 ± 8
A07	46 ± 3	44 ± 2	49 ± 7
A08	62 ± 4	55 ± 1	54 ± 3
A09	60 ± 2	58 ± 5	67 ± 1
A10	68 ± 5	69 ± 4	65 ± 5
A11	85 ± 4	85 ± 2	85 ± 2
A12	81 ± 4	78 ± 6	71 ± 2
A13	92 ± 6	93 ± 7	90 ± 1

ANOVA: p-value = 0.952, F = 0.050
Residual normality test (Shapiro–Wilk test): p-value = 0.517
Residual homoscedasticity test (Levene test): p-value = 0.780
Residual independence test (Durbin–Watson test): p-value = 1.747

Both methods of sample preparation proved to be adequate for the evaluation of magnesium levels in beer samples. Highlighting in relation to the procedures recommended by the American Society of Brewing Chemists, demand for lower consumption of concentrated acid reagents, time and analytical cost, providing even less operational risk to the analyst (ASBC). Thus, among the methods evaluated, direct analysis is shown to be the best cost-effective alternative for monitoring magnesium in beers.

The magnesium levels in beer samples ranged from 44 to 114 µg g^{-1} and their source may be related to the malt used in the production process, as reported by Omari et al. (2020) and Styburski et al. (2018). Similar results are described in the scientific literature involving different sample preparation methods and instrumental techniques. Leão et al. (2018) used a mixture of nitric acid and hydrogen peroxide for digestion in digesting block with determination by microwave-induced plasma optical emission spectrometry (MIP-OES), with magnesium levels ranging from 46 to 97 µg g^{-1} (Leão et al., 2018). On the other hand, Marcano et al. (2010) used direct analysis associated with ICP OES, with magnesium levels ranging from 29 to 85 µg g^{-1}. In general, the method developed in the present work has more attractive characteristics, either due to the simplicity of sample preparation or the instrumental and operational cost of the analysis technique.

4. Conclusion

The sample preparation methods developed proved to be adequate for the magnesium determination in beer samples by flame atomic absorption spectrometry. The detection and quantification limits were 0.04 and 0.12 µg g^{-1}, respectively, making it possible to determine the levels of this micronutrient in 13 samples, with adequate precision and accuracy. It is noteworthy that the observed levels are in accordance with other works reported.

The method involving direct analysis proved to be promising, since it requires minimal handling, reducing the risk of analyte losses and/or contamination, with low demand for samples, reagents and expensive instrumentation. Thus, it is shown as a viable alternative for implementation in routine analyzes for the quality control of beers in the industry.

Authors' contribution

Conceptualization: Lisboa, T. P.; Matos, M. A. C.
Data curation: Lisboa, T. P.; Lemos, L. C. S.; Calzavara, F. C.; Barbosa, B. S.; Baia, G. E.
Formal Analysis: Lisboa, T. P.; Lemos, L. C. S.; Calzavara, F. C.; Barbosa, B. S.; Baia, G. E.
Funding acquisition: Matos, M. A. C.; Silva, J. C. J.; Oliveira, M. A. L.
Investigation: Lisboa, T. P.; Lemos, L. C. S.; Calzavara, F. C.; Barbosa, B. S.; Baia, G. E.; Farias, D. M.
Methodology: Lisboa, T. P.

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Software: Not applicable.
Supervision: Lisboa, T. P.; Faria, L. V.; Matos, M. A. C.; Silva, J. C. J.; Oliveira, M. A.
Validation: Lisboa, T. P.; Faria, L. V.
Visualization: Lisboa, T. P.; Faria, L. V.
Writing – original draft: Lisboa, T. P.; Faria, L. V.
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Data availability statement

All data sets were generated or analyzed in the current study.

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Geographical chemical variability and processing oxidation of volatile compounds of *Casearia sylvestris* leaves

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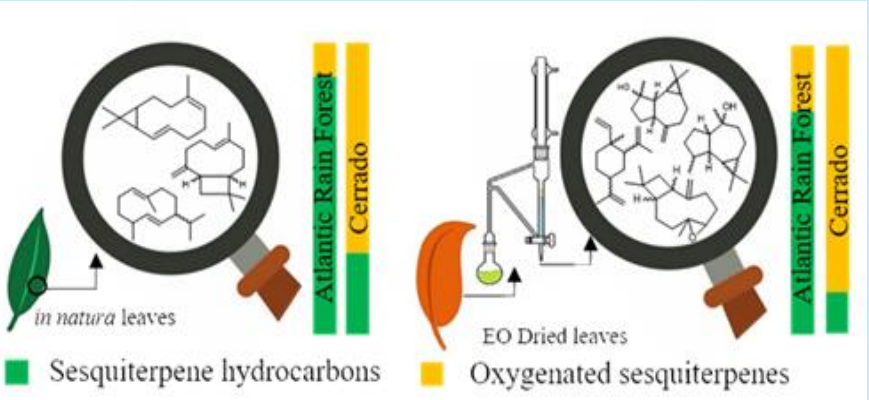
ABSTRACT: The *Casearia sylvestris* Sw. dried leaf essential oil (EO) contains sesquiterpenes as the main components. However, the volatile components in the *in natura* leaves remain unknown. This study compares the volatile chemicals in the *in natura* leaves and dried leaf EO of two *C. sylvestris* populations from Atlantic Forest and Cerrado. The volatile compounds were directly analysed by thermal desorption (TD) coupled to gas chromatography mass spectrometry (GC-MS); the dried leaf EO composition was determined by GC-MS. All the identified compounds were sesquiterpenes, and the major components were (*E*)-caryophyllene, bicyclogermacrene, *β*-elemene, spathulenol, and caryophyllene oxide. In both populations, the sesquiterpene hydrocarbon content and the oxygenated sesquiterpene content respectively decreased and increased on going from the *in natura* leaves to the dried leaf essential oil, indicating that drying and/or hydrodistillation modified the volatile chemical composition by generating oxidation artifacts. Results suggested that (*E*)-caryophyllene and bicyclogermacrene may be oxidized during the process to yield caryophyllene oxide and spathulenol, respectively. The two *C. sylvestris* populations also differed in terms of volatile chemical composition.



Comparative study on volatile compounds of the *in natura* leaves (TD-CG-MS) and dried leaf essential oil (GC-MS) from two *Casearia sylvestris* populations.

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1. Introduction

Casearia sylvestris Swartz (Salicaceae) is an important medicinal plant which is employed throughout Latin America (Xia et al., 2015). The essential oil (EO) from *C. sylvestris* leaves displays several biological and pharmacological activities, including antimicrobial, antileishmanial, antitumor, antiulcerogenic, and anti-inflammatory actions. Typically, the sesquiterpenes are the only compounds that are detected in this EO, where (*E*)-caryophyllene, germacrene D, *α*-zingiberene, bicyclogermacrene, *δ*-cadinene, and spathulenol predominate (Bou et al., 2013; Carvalho et al., 2018; Moreira et al., 2019; Spósito et al., 2019).

Artifacts generated during leaf drying and hydrodistillation may modify the leaf volatile chemical composition through chemical reactions, such as oxidation, rearrangement, ring-opening, and cyclization. Thus, leaves (*in natura*) and their respective EO have distinct volatile chemical profiles (Touaibia et al., 2019). Thermal desorption can be used to identify volatile components in plants without altering their original composition because this technique does not require leaf drying or hydrodistillation or the use of solvents (Arbulu et al., 2013).

The well-established application of *C. sylvestris* in Brazilian folk medicine and the pharmacological actions of its EO highlight that it can potentially be employed as the basis of medicines (Xia et al., 2015). However, the use of EOs as pharmaceutical raw material demands chemical standardization because genetic, environmental, and processing factors and storage may affect the EO chemical composition (Gobbo-Neto and Lopes, 2007; Kiazolu et al., 2016). Therefore, this study evaluates how drying and hydrodistillation (processing factors), as well as the geographical origin (environmental factor), impact the *C. sylvestris* var. *sylvestris* leaf volatile chemical composition by comparing the chemical composition of the volatile fraction of *in natura* leaves and dried leaf EO obtained from two *C. sylvestris* var. *sylvestris* populations collected from different Brazilian biomes (Atlantic Forest and Cerrado).

2. Experimental

2.1 Plant material

Casearia sylvestris Swartz var. *sylvestris* leaves were collected from 10 specimens of each of the two populations, designated CB and SA, in December 2016. The CB population: Carlos Botelho State Park, São Miguel Arcanjo, São Paulo, Brazil (24°3’42”8–24°3’84”0 S, 47°59’45”4–47º59’80”5 W); Atlantic Rain Forest biome. The SA population: School of Agriculture, Botucatu (22°50’22”5–22°50’94”8 S, 48°25’50”6–48°25’63”7 W); Cerrado biome. The specimens were identified by Dr. Luis V. S. Sacramento from the School of Pharmaceutical Sciences, Unesp, and the voucher specimens were deposited at the Herbarium “D. Bento Pickel” under the codes CB 51.816–51.826 and SA 301–310. This study was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge of Brazil (SisGen) under No. AEFB157.

2.2 Essential oil extraction

The leaves of each specimen were separately dried in an oven with air circulation at 40 °C for 3 days. The dried leaves (30 g) of each specimen were separately extracted by hydrodistillation in a clevenger-type apparatus for 4 h (Anvisa, 2010). The EO yield of the CB and SA populations was 1.3 ± 0.2 and 0.9 ± 0.3% (v/w), respectively.

2.3 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography mass spectrometry analyses were performed on a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler and fitted with a Rtx-5MS capillary column (5% diphenyl and 95% polydimethylsiloxane, 30 m × 0.25 mm, 0.25-µm film thickness). Helium (99.9999%) was used as the carrier gas (1.0 mL min^{-1}). The samples were prepared by mixing the EO obtained from the 10 specimens of a given population, CB or SA. Next, 1.0 µL of the sample (0.3 µL mL^{-1}, hexane) was injected and analyzed in the split mode (1:10). The injector and the ion source temperature were 240 and 280 °C, respectively; the oven temperature was programmed to rise from 60 to 250 °C (3 °C min^{-1}, 80 min). The electron ionization mass spectra were obtained at 70 eV and recorded with a scan interval of 0.5 s for masses ranging from 40 to 600 Da.

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2.4 Thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS)

The *C. sylvestris* leaves (0.5 mm^2) were inserted into a glass tube (0.63 × 8.89 cm, Supelco) with glass wool around it. The volatile components were concentrated (5 min) on a Shimadzu TD-20 (Shimadzu Corporation, Kyoto, Japan) thermal desorption system fitted to a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system by using the chromatographic conditions described above.

2.5 Essential oil chemical identification

The EO components were identified on the basis of the linear retention indices relative to a homologous series of *n*-alkanes (C_8-C_{40} Sigma-Aldrich) (Adams, 2007; Van Den Dool and Kratz, 1963) and the retention times of authentic (*E*)-caryophyllene, *α*-humulene and caryophyllene oxide standards (Sigma-Aldrich). The acquired spectra were computer-matched with reference spectra of the mass spectral libraries (NIST 08, WILEY 7 and FFNSC 1.2), and the fragmentation were compared to the fragmentation patterns (Kiazolu et al., 2016).

3. Results and discussion

Sesquiterpenes were the only compounds in the *in natura* leaf volatile fraction (Tab. 1, Figs. 1 and 2). These results agreed with the results of most studies on *C. sylvestris* EO. On the other hand (Sousa et al., 2007), monoterpenes and phenylpropanoids were identified as minor *C. sylvestris* EO components. According to literature data, sesquiterpene hydrocarbons predominate in *C. sylvestris* fresh leaf and dried leaf EO (Bou et al., 2013; Moreira et al., 2019; Spósito et al., 2019). However, the dried leaf EO of the populations CB and SA showed higher content of oxygenated sesquiterpenes.

Table 1. Volatile chemical composition of *C. sylvestris* var. *sylvestris in natura* leaves and dried leaf EO as determined by GC-MS analyses. The EO from the CB or SA population consisted of the EO that was extracted from10 specimens of each population.

Components population	CB *in natura* leaves (%)	CB dried leaf EO (%)	SA *in natura* leaves (%)	SA dried leaf EO (%)
α-copaene	-	0.2	0.7	0.6
δ-elemene	3.6	-	5.9	0.2
β-elemene	1.6	12.2	2.8	1.7
β-boubornene	-	0.2	-	-
γ-gurjunene	-	0.9	-	-
(*E*)-caryophyllene[1]	32.5	0.9	1.9	-
β-copaene	0.7	-	-	-
γ-elemene	0.7	-	-	-
aromadendrene	5.6	1.2	3.1	0.9
α-humulene[1]	3.6	0.2	-	0.7
α-gurjunene	0.5	-	-	-
9-*epi*-(*E*)-caryophyllene	-	0.2	-	0.3
γ-muurolene	0.5	-	-	0.5
α-muurolene	-	0.2	-	0.3
germacrene D	7.0	-	5.5	-
β-selinene	-	4.3	5.2	2.2
γ-patchoulene	-	0.9	-	-
(*Z*)-calamenene	-	-	-	4.6
bicyclogermacrene	22.1	-	32.8	-
γ-cadinene	0.4	0.6	-	-
δ-cadinene	0.8	-	-	-
germacrene B	-	1.7	-	-
Sesquiterpene hydrocarbons	**79.0**	**23.7**	**57.9**	**12.0**
silphyperfol-5-en-3-ol A	-	2.3	-	-
globulol	-	-	-	10.5
ledol	-	0.9	-	2.7
palustrol	-	1.9	-	1.4

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spathulenol	8.7	16.7	16.7	30.0
viridiflorol	1.5	3.1	-	3.3
caryophyllene oxide[a]	1.7	21.6	-	-
humulene epoxide II	-	3.6	-	9.3
bulnesol	-	4.4	-	-
α-muurolol	-	0.5	-	-
cubenol	-	0.3	-	1.4
(*Z*)-cadin-4-en-7-ol	-	0.6	-	2.7
(*Z*)-*α*-santalol	-	2.5	-	-
Oxygenated sesquiterpenes	**10.4**	**58.4**	**16.7**	**61.3**
Identified compounds	**89.4**	**82.1**	**74.6**	**73.3**

[a]Compounds identified by comparison with the retention times of authentic standards.

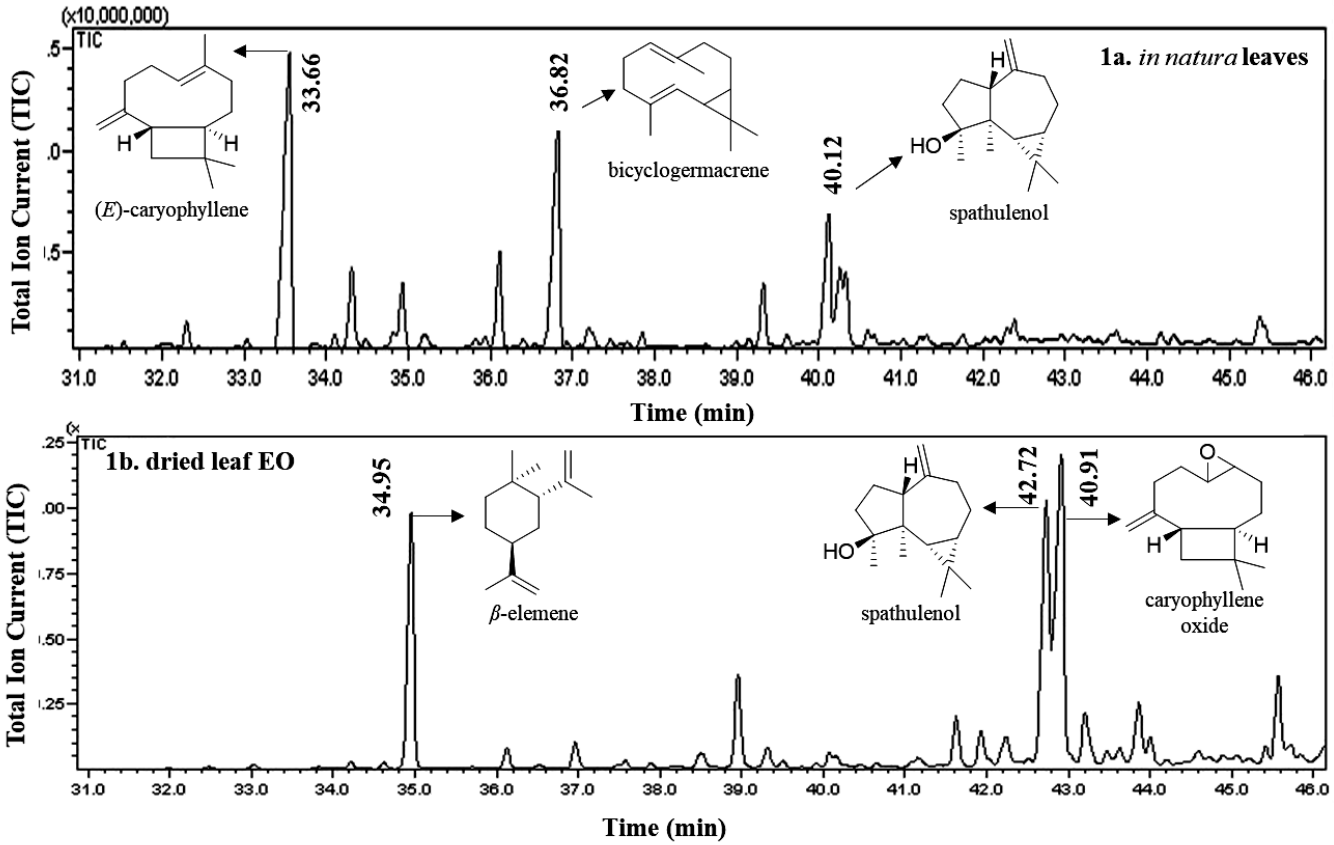

Figure 1. Essential oil expanded chromatograms (31–46 min) of CB population (each sample represents the EO mixture of the leaves of 10 specimens): volatile components in the *in natura* leaves **(a)** and dried leaf EO **(b)**. Chromatographic conditions: capillary column Rtx5-MS (30 m × 0.25 mm i.d., 0.25-µm film thickness), 60–310 °C, 3 °C min^{-1}, for 80 min; injector temperature = 250 °C; split mode 1:10.

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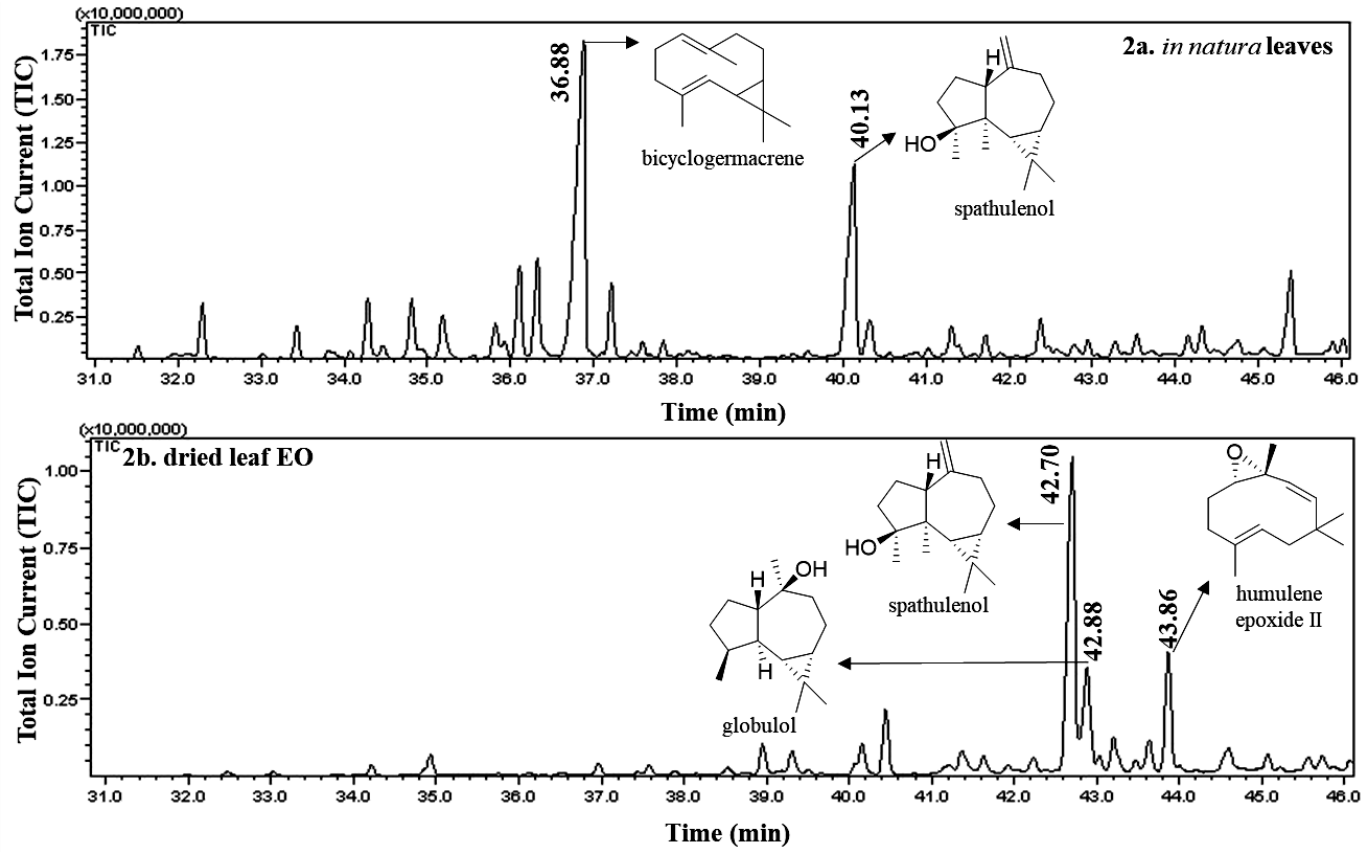

Figure 2. Essential oil expanded chromatograms (31–46 min) of SA population (each sample represents the EO mixture of the leaves of 10 specimens): volatile components in the *in natura* leaves **(a)** and dried leaf EO **(b)**. Chromatographic conditions: capillary column Rtx5-MS (30 m × 0.25 mm i.d., 0.25-µm film thickness), 60–310 °C, 3 °C min^{-1}, for 80 min; injector temperature = 250 °C; split mode 1:10.

The sesquiterpene hydrocarbon content in the CB population *in natura* leaves and dried leaf EO was 79.0 and 23.7%, respectively, while the oxygenated sesquiterpene content in the CB population *in natura* leaves and dried leaf EO was 10.4 and 58.4%, respectively. Data for the SA population revealed the same trend: the sesquiterpene hydrocarbon content in the *in natura* leaves and dried leaf EO was 57.9 and 16.7%, respectively, whereas the oxygenated sesquiterpene content was 12.0 and 61.3%, respectively (Tab. 1). The oxygenated sesquiterpene content increased after leaf drying and hydrodistillation, probably because oxidation reactions converted sesquiterpene hydrocarbons into oxygenated sesquiterpenes (Gopalakrishnan, 1994; Touaibia et al., 2019).

The major volatile constituents in the CB population (Fig. 3) *in natura* leaves were (*E*)-caryophyllene (32.5%) and bicyclogermacrene (22.1%), whilst *β*-elemene (12.2%), spathulenol (16.7%) and caryophyllene oxide (21.6%) were the main components in the dried leaf EO. In the SA population, the main components (Fig. 3) in the *in natura* leaves were bicyclogermacrene (32.8%) and spathulenol (16.7%), whereas spathulenol (30.0%) was predominant in the dried leaf EO. According to the literature, the main *C. sylvestris* leaf EO components are (*E*)-caryophyllene, *α*-zingiberene, germacrene D, bicyclogermacrene, *δ*-cadinene, and spathulenol (Bou et al., 2013; Carvalho et al., 2018; Moreira et al., 2019; Spósito et al., 2019), which partially match the major compounds that were identified in the CB and SA populations.

Here, the sesquiterpenes 9-*epi*-(*E*)-caryophyllene, silphyperfol-5-en-3-ol A, (*Z*)-cadin-4-en-7-ol, and (*Z*)-*α*-santalol have been identified in the *C. sylvestris* leaves for the first time (Tab. 1); silphyperfol-5-en-3-ol A and (*Z*)-*α*-santalol were detected in the CB population only.

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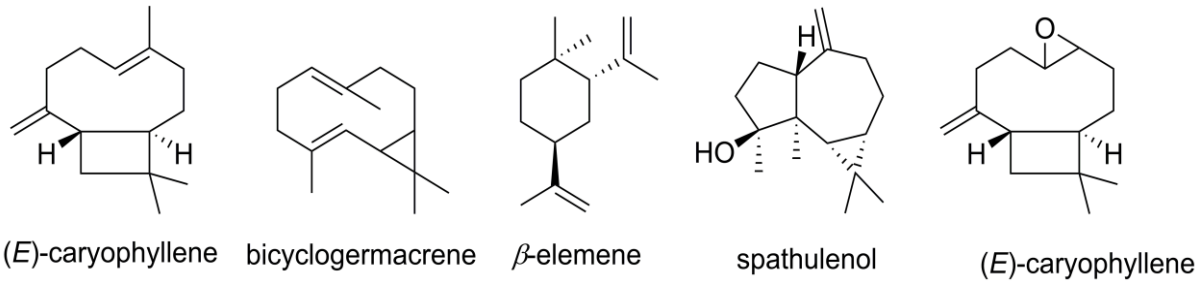

Figure 3. Chemical structures of the major components in *C. sylvestris* leaf volatiles.

In the CB population, the (*E*)-caryophyllene content decreased from 32.5% in the *in natura* leaves to 0.9% in the dried leaf EO, while the caryophyllene oxide content increased from 1.7 to 21.6%. According to data literature (Sköld et al., 2006), during (*E*)-caryophyllene (standard) exposure to air, this compound underwent total degradation, to generate caryophyllene oxide through hydroperoxide reactions. However, the conversion into caryophyllene oxide was about 40%, which led to the conclusion that drying and hydrodistillation may produce chemical oxidation reactions that convert (*E*)-caryophyllene into caryophyllene oxide.

The bicyclogermacrene content in the CB population decreased from 22.1% in the *in natura* leaves to not detected in the dried leaf EO, while the spathulenol and viridiflorol contents increased from 8.7 and 1.5% in the *in natura* leaves to 16.7 and 3.1% in the dried leaf EO, respectively. In the SA population, the bicyclogermacrene content decreased from 32.8% in the *in natura* leaves to not detected in the dried leaf EO, whilst the spathulenol, viridiflorol, and globulol contents increased from 16.7%, not detected, and not detected in the *in natura* leaves to 30.0, 3.3, and 10.5% in the dried leaf EO, respectively. These results indicated that bicyclogermacrene may have been oxidized and converted into spathulenol, viridiflorol, and globulol during drying and hydrodistillation (Nascimento et al., 2018; Njoroge et al., 1996; 2003; Telascrea et al., 2008; Toyota et al., 1996).

4. Conclusion

In summary, these results demonstrated qualitative and quantitative chemical variability between the *C. sylvestris* var. *sylvestris* populations from Cerrado and Atlantic Forest and generation of oxygenated degradation products from sesquiterpene hydrocarbons during drying and/or hydrodistillation. This study also reinforced the potential of thermal desorption to determine the chemical composition of volatile compounds *in natura*.

Authors' contribution

Conceptualization: Carvalho, F. A.; Crotti, A. E. M.; Santos, A. G.
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Formal Analysis: Carvalho, F. A.; Oda, F. B.; Crevelin, E. J.; Crotti, A. E. M.; Santos, A. G.
Funding acquisition: Santos, A. G.
Investigation: Carvalho, F. A.; Oda, F. B.; Crevelin, E. J.
Methodology: Carvalho, F. A.; Santos, A. G.
Project administration: Santos, A. G.
Resources: Not applicable.
Software: Not applicable.
Supervision: Crotti, A. E. M.; Santos, A. G.
Validation: Not applicable.
Visualization: Santos, A. G.
Writing – original draft: Carvalho, F. A.; Oda, F. B.
Writing – review & editing: Crevelin, E. J.; Crotti, A. E. M.; Santos, A. G.

Data availability statement

The data will be available upon request.

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Artificial intelligence method developed for classifying raw sugarcane in the presence of the solid impurity

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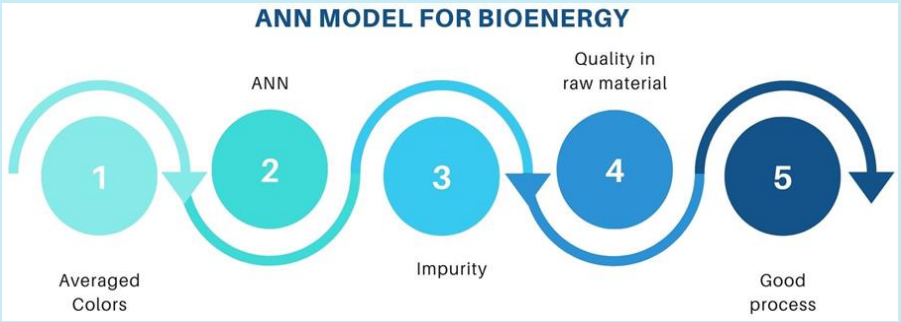
ABSTRACT: An investigation dedicated to evaluating a big issue in biorefineries, solid impurity in raw sugarcane, is presented. This relevant industrial sector requests a high-frequency, low-cost, and noninvasive method. Then, the developed method uses the averaged color values of ten color-scale descriptors: R (red), G (green), B (blue), their relative colors (r, g, and b), H (hue), S (saturation), V (value) and L (luminosity) from digital images acquired from 146 solid mixtures among sugarcane stalks and solid impurity — vegetal parts (green and dry leaves) and soil. The solid mixture of samples was prepared considering desirable and undesirable scenarios for the solid impurity amounts. The outstanding result was revealed by an artificial neural network (ANN), achieving 100% of accurate classifications for two ranges of raw sugarcane in the samples: from 90 to 100 wt% and from 41 to 87 wt%. Low-computational cost and a simple setup for image acquisition method could screen solid impurity in sugarcane shipments as a promising application.



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1. Introduction

Image and color information has played an important role in analytical chemistry and can help solve many issues, mainly because of its versatility and availability of many low-cost devices for *in loco* or laboratory analysis (Capitán-Vallvey et al., 2007; Diniz, 2020; Pereira and Bueno, 2007; Pereira et al., 2011).

Our research group has developed analytical methods to evaluate raw sugarcane to help the mills or biorefineries manufacturing process of this material routinely monitored as a consignment for payment purposes. The quality of raw sugarcane influences the manufacturing process, directly compromising two essential commodities — sugar and ethanol (Andrade et al., 2018; Guedes and Pereira, 2018; 2019; Guedes et al., 2020; Romera et al., 2016).

Solid impurity in raw sugarcane is defined as the plant presence (tops, green, brown, and dry leaves) and the soil (Eggleston et al., 2010). This issue is impacted by the type of harvesting process, as harvesting green or burnt cane. In specific, harvesting green increases the quantity of solid impurity in raw sugarcane, as reported in technical notes and scientific literature (Lisboa et al., 2018; Norris et al., 2015).

For instance, classifying solid impurity in raw sugarcane can be performed with chemometric techniques, such as soft independent modeling of class analogy (SIMCA), partial least squares discriminant analysis (PLS-DA), and k-nearest neighbors (kNN) by using the conversion of digital images in color histograms (Guedes and Pereira, 2019). The content of raw sugarcane between 85 and 100 wt% was accurately classified. According to approximately 0.97 of receiver operating characteristic (ROC) area curves for sensibility and specificity using PLS-DA and 1 for SIMCA and kNN. Although these results were promising, the average color values were also tested with no successful results.

In this sense, it is possible to develop a faster computational method using another strategy as the artificial neural network (ANN) model and the averaged color values. The advantage of the averaged color values from images is that ten color-scale represent the average of the color interval with originally 256 intensities/variables, as follows: R (red), G (green), B (blue), their relative colors (r, g, and b), H (hue), S (saturation), V (value) and L (luminosity), which means less running time for computational tests.

The solid impurity in raw sugarcane was successfully estimated using the ANN model for color image data since the data showed no-linear nature. The parameters computed for the ANN model were very promising, the relative errors were 3%, and the data were highly correlated, with the reference values achieving 0.98 for the training set (Guedes et al., 2020).

Artificial neural network methods include accurate results, ease of implementation, low computational cost, speed in obtaining results, and the ability to learn through a set of examples and provide consistent responses to new data (Braga et al., 2000; Guedes et al., 2020; Santos et al., 2019).

Therefore, the main goal was to classify raw sugarcane in the presence of solid impurity using the ANN method, as the last part of series of investigations dedicated to this critical issue for sugar mills and biorefineries.

2. Experimental

2.1 Samples and image acquisition

Among sugarcane stalks, vegetal plant parts, and soil, one-hundred forty-six solid mixtures were prepared to acquire digital images, as shown in Fig. 1. Each one was placed in a paper tray (26.5 × 21.5 cm) into a laboratory-made setup (Guedes and Pereira, 2019) composed of a black box, a digital camera Nikon (COOLPIX S3500, Tokyo, Japan) 20.1-megapixel resolution. The images with a 1600 × 1200-pixel size (width × height) and 300 × 300 dpi (dots per inch) resolution were recorded with the tray in a horizontal position. The camera's focal distance was 10 mm, with a maximum aperture of 3.5, and the region of interest (ROI) corresponded to 100% of the original image. During the acquisition of the images, the camera software automatic adjustments were intentionally disabled. Five images were acquired per sample, and the samples were shaken after each image recording to mimic natural conditions at shipments. The same images were converted into colors using an 'imread' function in MATLAB R2020a (MathWorks, Natick, MA, USA). Afterward, the images were converted into color histograms using another function, 'imhist' in MATLAB. The average color values, which were comprised of ten color-scale descriptors: R (red), G (green), B (blue), their relative colors (r, g, and b), H (hue), S (saturation), V (value) and L (luminosity), using a laboratory-made MATLAB code was available in the study of Camargo et al. (2018).

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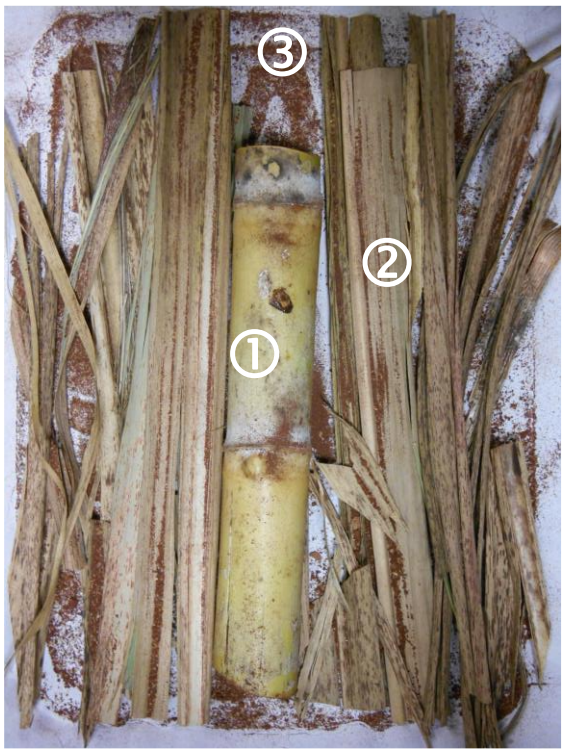

Figure 1. Solid mixture sample of sugarcane stalks (85 wt%) denoted as number 1, among vegetal parts (10 wt%) as 2, and soil (5 wt%) as 3.

2.2 Neural model

The development of the neural models was performed using the scaled conjugate gradient backpropagation algorithm (*traincsg*). For this, the MATLAB R2018a software was used, with the 'NNStart' tool available in the software, choosing the pattern recognition app button in which the ANN input layer (with ten neurons representing the ten inputs: R, G, B, H, S, V, r, g, b, and L, number of intermediate layers and an output layer with two neurons were set manually. The number of neurons in the intermediate layer was defined by trial and error to achieve the best classification of raw sugarcane content in solid mixtures.

The classification was based on the content of raw sugarcane in wt% denoted as number 1, in the presence of different proportions among green and dry leaves, as number 2, and soil denoted as 3 in Fig. 1. The following division was made: 90–100 wt% designated as class 1 — appropriate (given by binary code 1 0), representing 36 samples; while 41–87 wt% was class 2 — inappropriate (provided by binary code 0 1), representing 110 samples.

The 146 samples were randomly divided using the 'dividerand' function, available in MATLAB, into three sets: 70% (102 samples) for training; 15% (22 samples) for validation, to verify that the network is generalizing the information and to interrupt the training before overfitting occurs; the remaining 15% (22 samples) were for the test, independent of the generalization of the neural model.

3. Results and discussion

Figure 2a shows a treemap chart for the color descriptors magnitudes of the digital images. Red, G, and B had interval values: 104-172, 92–159, and 79–159. In the case of L, the counts were from 275 to 483. The relative colors of RGB, represented as r, g, and b, varied from 0.2 to 0.4, H 0.1–0.7, S 0.1–0.4, and V ranged from 0.4 to 0.7 (see the highlighted tiny area by the purple arrow). The representative images for the ideal scenario mean 100% content of sugarcane stalk, as shown in Fig. 2b, and other different situations with more or less solid impurities, far away from biorefineries need, shown in Fig. 2c, d, and e.

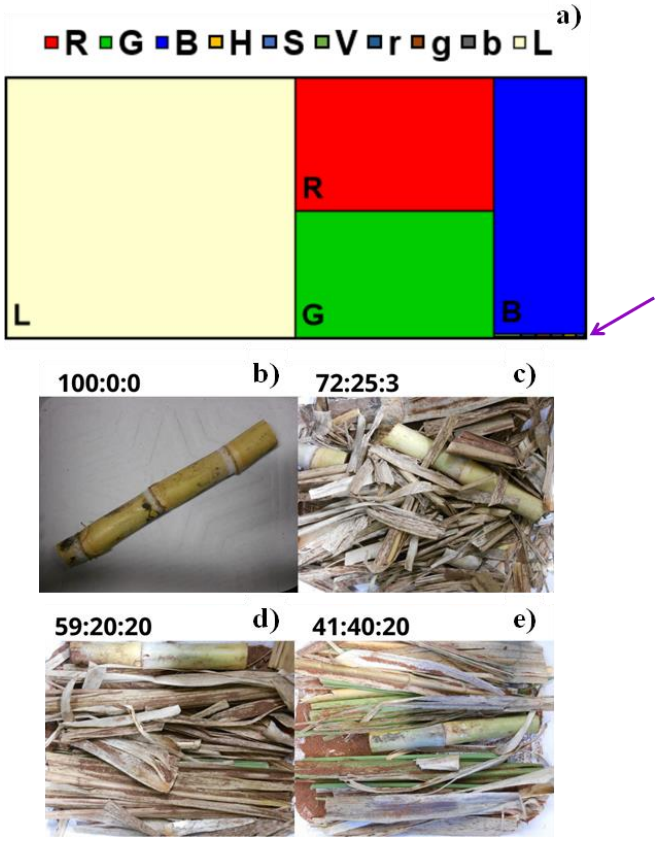

Figure 2. (a) Treemap chart of ten color-scale descriptors: R (red), G (green), B (blue), their relative colors (r, g, and b), H (hue), S (saturation), V (value) and L (luminosity) and **(b-e)** Solid mixture samples of sugarcane stalks among vegetal parts, and soil proportions, respectively. Note in the treemap chart

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that 'HSV' and 'rgb' are in a tiny area below the right side highlighted by the purple arrow, above B color.

Several architectures were tested to develop the model, varying the number of neurons in the intermediate layer. An artificial neural network with six neurons in the intermediate layer registered the best result with a cross-entropy error of 0.0062 for the validation set. The cross-entropy can measure a classification performance, for which inputs are probability values between 0 and 1. It was observed that ANN could not generalize the problem and classify the samples correctly by increasing the number of neurons in the intermediate layer.

For the training set, the correct classifications of 28 samples of class 1 and 74 samples of class 2 were practicable. For the validation set, no misclassifications for all samples were a remarkable result. Finally, for the test set, all samples were classified as members of their classes. Therefore, all 146 samples achieved a 100% accuracy rate, as shown by the confusion matrices for the training, validation, test sets, and an all-confusion matrix in Fig. 3. Table 1 shows the responses obtained and expected by the ANN for the test set, with five samples of class 1 — appropriate (90–100 wt% of raw sugarcane) and 17 samples of class 2 — inappropriate (41–87 wt% of raw sugarcane).

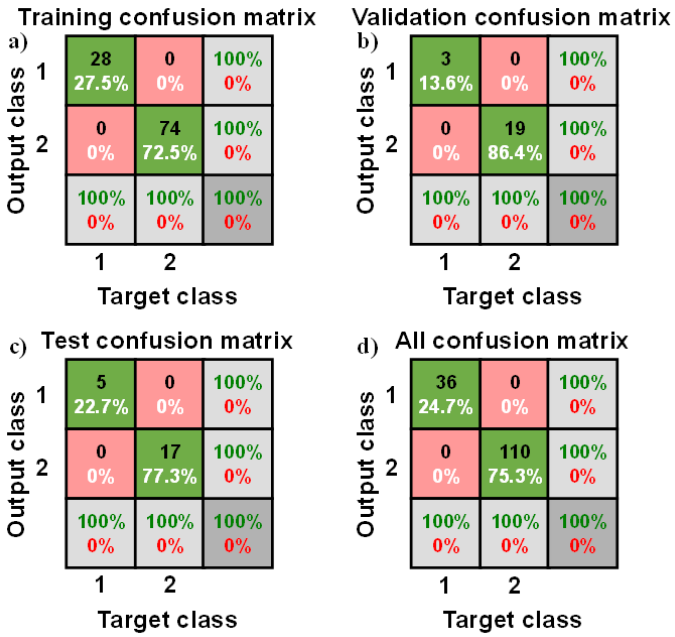

Figure 3. Confusion matrices for sets of training, validation, test, and all results of raw sugarcane content classification in the presence of impurity using an ANN model.

Table 1. Responses were obtained and expected using ANN to classify the raw sugarcane content (wt%) for the test set.

Sample	ANN outputs		Class obtained by the ANN	Targets		Expected class
2	0.0001	0.9999	2	0	1	2
3	0.0006	0.9994	2	0	1	2
16	0.1604	0.8396	2	0	1	2
21	0.0000	1.0000	2	0	1	2
25	0.0000	1.0000	2	0	1	2
28	0.0001	0.9999	2	0	1	2
29	0.0000	1.0000	2	0	1	2
37	0.0000	1.0000	2	0	1	2
51	0.0000	1.0000	2	0	1	2
54	0.0000	1.0000	2	0	1	2
65	0.0001	0.9999	2	0	1	2
68	0.0000	1.0000	2	0	1	2
73	0.0191	0.9809	2	0	1	2
82	0.0001	0.9999	2	0	1	2
86	0.0007	0.9993	2	0	1	2
92	0.0097	0.9903	2	0	1	2
110	0.3197	0.6803	2	0	1	2
111	0.9876	0.0124	1	1	0	1
113	0.9904	0.0096	1	1	0	1
115	0.9993	0.0007	1	1	0	1
133	0.9968	0.0032	1	1	0	1
135	0.9998	0.0002	1	1	0	1

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Two other ANN models were also investigated: (i) a model for classifying samples based on the maximum desirable content of vegetal impurity (8 wt%), the range from 0 to 8 wt% designated as class 1 (with binary code 1 0), representing 50 samples, and between 9 and 40 wt% as class 2 (with binary code 0 1), comprising 96 samples; (ii) a model for classification based on the maximum tolerate content of the soil as an impurity (3 wt%), ranging from 0 to 3 was class 1 (with binary code 1 0), with 40 samples and from 4 to 20 wt% as class 2 (with binary code 0 1), a total of 106 samples.

In the ANN model for classifying vegetal impurity content, the best result was 12 neurons in the intermediate layer. The training set was observed among 35 samples of class 1; the neural model misclassified two samples as class 2; among 67 samples of class 2, only one sample was misclassified; that is, the percentage of accurate classifications for the training set was 97.1%. For the validation set, it was observed that the nine samples of class 1 and the 13 samples of class 2 were 100% accurately classified. Finally, for the test set, it was observed that among six samples of class 1, only one sample was misclassified as class 2, and among the 16 samples of class 2, there was one misclassification, that is, the percentage of accurate classifications for the test set was 90.9%. Therefore, for all 146 samples, five were misclassified, representing an average rate of 96.6%.

For classifying the soil content as a solid impurity, 18 neurons in the intermediate layer were the best result. For the training and test sets, eight and three misclassifications were verified, respectively. Therefore, of the 146 samples, 11 were misclassified, representing a 92.5% average rate of accurate classifications.

Thus, the remarkable ANN result is for raw sugarcane content, considering the lowest cross-entropy error, was achieved in this model (0.0062). In contrast, the best models for vegetal parts and soil contents resulted in 0.0160 and 0.0482, respectively.

4. Conclusion

The outstanding result using the ANN method and averaged color values from digital images achieved the lowest cross-entropy errors and 100% of accurate classifications for the content of raw sugarcane, considering the presence of two different types of solid impurity — vegetal plant parts of plants and soil.

Additionally, the ANN running takes a few seconds, and the system of a digital image is an easy-to-use system that can be carried out in any location. Thus, the method can be implemented in sugar cane mills as a screening method of raw sugarcane shipments in the presence of solid impurity as vegetal parts of the plant itself and soil.

Authors' contribution

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Data availability statement

The data will be available upon request.

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