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A new vision for agri-food waste from bell pepper (*Capsicum annuum* L.) production: a potential source of bioactive compounds

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Abstract

Agri-food waste and loss has become a pressing and urgent problem with notable environmental and economic consequences. Among various sectors, the fruit and vegetable industry, particularly the bell pepper industry, stands out as a major contributor to food waste and by-products. This industry generates substantial amounts waste, which presents an opportunity to showcase the potential of the circular economy through valorization of bio-waste.

Over the past two decades, there has been a concerted effort to study the biochemical composition of various natural matrices, aiming to discover new functional compounds. Among these, the bell pepper has emerged as a particularly interesting vegetable, in terms of its nutritional value and its biochemical characteristics. However, a significant quantity of waste is generated during production, which is considered an excellent source of bioactive compounds of high interest. These waste fruits and their by-products, namely stalks and seeds, contain a wide range of phytochemicals, including capsaicinoids, phenolic compounds, carotenoids, and vitamins, which exhibit important biological activities such as antioxidant, antimicrobial, and anti-inflammatory. These bioactive compounds hold promise for utilization in food, cosmetic and pharmaceutical industries. Therefore, the valorization of agri-food waste has become an area of great interest in biotechnology and innovation, with beneficial effects not only for the environment but also for the economy and the population.

In this sense, the present study aimed to evaluate the proximal and biochemical composition of waste-green, orange, and red fruits of *Capsicum annuum* L. Additionally, the bioactive potential of the fruit, stalk, and seed hydroethanolic extracts obtained from the waste-colored bell peppers was equally assessed.

Moisture, ash, protein, fat, carbohydrate, and energy contents were determined following official AOAC analysis methodologies. The quantification of free sugars was performed using a HPLC-RI system, organic acids by UHPLC-DAD, fatty acids by GC-FID and volatile organic compounds by a GC-MSD system. The phenolic composition of waste-colored bell peppers was determined by HPLC-DAD-ESI/MS. Furthermore, different biological activities (antioxidant, cytotoxicity, antimicrobial) were analyzed through different *in vitro* assays.

Overall, the proximal and chemical assessment revealed that carbohydrates were the predominant macronutrient in the analyzed samples, with fructose standing out as the main free sugar in two different waste-colored bell peppers (orange and red), whilst glucose was the main

present free sugar in green bell peppers. Four organic acids were identified in the samples, of which oxalic acid was presented greater content in orange and red bell peppers, whilst malic acid was the most abundant in green bell peppers. Regarding fatty acids, fifteen compounds in total were identified being palmitic acid, linoleic acid, and α-linolenic acid present in major quantities, verifying the prevalence as well of PUFAs. In the assessment of phenolics, twenty-two compounds were identified in waste-colored bell peppers including seven phenolic acids, fourteen flavonoids and one stilbene. Moreover, the analysis of volatile organic compounds revealed the presence of thirty-four compounds, including eight heterocyclic compounds, seven acids, six esters, three alcohols, two amines, two alkanes, one acetal, one aldehyde, one ether, one glyceride, one ketone, one ketose.

Concerning the bioactive properties assessment, the extracts of stalks, fruits, and seeds revealed antioxidant capacity, with extracts from waste-green bell peppers presenting the best result (0.36±0.01 mg/mL, 0.44±0.02 mg/mL, and 0.21±0.01 mg/mL, respectively). All the extracts demonstrated no cytotoxicity activity at the maximum concentration tested (GI_{50}>400μg/mL). Fruit extracts demonstrated superior antibacterial activity when compared to extracts of stalks and seeds with best results identified against food contaminants *Y. enterocolitica*, *B. cereus*, *S. aureus* (MIC = 5 mg/mL), and clinical isolates *E. faecalis* and *MRSA* (MIC = 5 mg/mL). Furthermore, all the extracts revealed no bactericidal effects for any bacteria up to the maximum concentration tested (MBC>10 mg/mL). In the antifungal activity assessment, the fungal strain *A. brasiliensis* was the most susceptible to the tested samples, especially against the extracts of the fruits, with waste-red bell pepper demonstrating superior inhibitory effects (MIC = 1.25 mg/mL). Moreover, all the extracts showed no fungicidal effects for both fungal strains up to the maximum concentration tested (MFC>10 mg/mL).

In general, this study aimed to valorize waste-colored bell peppers as natural source of bioactive compounds that can be exploited by different industries, through their conversion into value-added products, contributing simultaneously to the implementation of circular economy guidelines.

Keywords: agri-food waste, bell peppers, bioactive compounds, circular bioeconomy.

Resumo

O desperdício e as perdas de alimentos na indústria agrícola tornaram-se um problema premente e urgente, com consequências ambientais e económicos notáveis. Entre vários setores, a indústria agroalimentar, em particular a indústria do pimento, destaca-se como um dos principais contribuidores para a geração de subprodutos agroalimentares. Esta indústria gera quantidades substanciais de resíduos proporcionando assim, uma excelente oportunidade para demonstrar o potencial da bioeconomia circular, através da valorização de biorresíduos.

Ao longo das últimas duas décadas, tem havido um esforço concertado para estudar a composição bioquímica de várias matrizes naturais, com o objetivo de descobrir novos compostos funcionais. Entre estes, o pimento surgiu como um vegetal particularmente interessante, tanto em termos do seu valor nutricional bem como das suas características bioquímicas. No entanto, durante a sua produção, é gerada uma quantidade significativa de biorresíduos, que são considerados uma excelente fonte de compostos bioativos de alto interesse. Estes biorresíduos (fruto e seus subprodutos) contêm uma ampla gama de fitoquímicos, incluindo compostos fenólicos, capsaicinoides, carotenoides e vitaminas, que exibem importantes atividades antioxidantes, antimicrobianas anti-inflamatórias, entre outras. Estes compostos bioativos são altamente promissores para a sua utilização em diversos setores como a indústria alimentar e farmacêutica. Assim, a valorização do desperdício no setor alimentar tornou-se uma área de grande interesse no âmbito da biotecnologia e inovação, com efeitos benéficos não somente para o ambiente, mas também para a economia e a população.

Nesse sentido, o presente estudo teve como principal objetivo avaliar a composição proximal e bioquímica de pimentos laranja, vermelho e verde (*Capsicum annuum* L.) que foram desperdiçados. Suplementarmente, também foi objeto de estudo o potencial bioativo dos extratos hidroetanólicos obtidos dos diferentes pimentos desperdiçados, incluindo os talos e as sementes.

Os teores de humidade, cinzas, proteínas, gorduras, hidratos de carbono e energia foram determinados seguindo metodologias oficiais de análise de alimentos AOAC. A quantificação dos açúcares livres foi realizada utilizando o sistema HPLC-RI, ácidos orgânicos por UHPLC-DAD, ácidos gordos por GC-FID, e compostos orgânicos voláteis por GC-MSD. A composição fenólica das amostras foi determinada por HPLC-DAD-ESI/MS. Além disso, diferentes atividades biológicas (antioxidante, citotoxicidade e antimicrobiana) foram analisadas através de diferentes ensaios *in vitro*.

A composição proximal e química revelou que os hidratos de carbono foram o macronutriente predominante nas amostras analisadas, destacando-se a frutose como o principal açúcar livre em pimentos laranja e vermelho, por outro lado a glucose foi o principal açúcar livre presente nas amostras de pimentos verdes. Quatro ácidos orgânicos foram identificados nas amostras sendo o ácido oxálico o mais abundante em pimentos laranja e vermelho; por outro lado verificou-se a predominância do ácido málico em pimentos verdes. Quanto aos ácidos gordos, foram identificados quinze no total sendo os ácidos palmítico, linoleico e α-linolénico presentes em maiores quantidades nas amostras, verificando a prevalência de ácidos polinsaturados. Na avaliação dos compostos fenólicos, um total de vinte e dois substâncias foram identificados nas amostras, incluindo sete ácidos fenólicos, catorze flavonoides e um estilbenos. A caracterização de compostos voláteis dos pimentos desperdiçados revelou a presença de trinta e quatro substâncias incluindo oito compostos heterocíclicos, sete ácidos, seis esteres, três álcoois, duas aminas, dois alcanos, um acetal, um aldeído, um éter, um glicérido, uma acetona e uma cetose.

Relativamente à avaliação das propriedades bioativas, os extratos hidroetanólicos dos frutos, talos e sementes dos pimentos desperdiçados revelaram capacidade antioxidante, sendo o pimento verde aquele que apresentou o melhor resultado (0.36±0.01 mg/mL, 0.44±0.02 mg/mL, e 0.21±0.01 mg/mL, respetivamente). Suplementarmente, todos os extratos demonstraram ausência de atividade citotóxica. Ainda, os extratos dos frutos também demonstraram uma superior capacidade antibacteriana em relação aos extratos dos talos e das sementes, com melhores resultados identificados contra contaminantes alimentares *Y. enterocolitica, B. cereus, S. aureus* (MIC = 5 mg/mL), e isolados clínicos *E. faecalis* and *MRSA* (MIC = 5 mg/mL). Além disso, todos os extratos demonstraram não ser bactericidas para nenhuma bactéria testada até à concentração máxima testada (MBC>10 mg/mL). Na avaliação da atividade antifúngica, a linhagem fúngica *A. brasiliensis* revelou-se ser a mais suscetível às amostras testadas, especialmente nos extratos dos frutos, sendo o pimento verde aquele que demonstrou efeito inibitório superior (MIC = 1.25 mg/mL). Além disso, nenhum extrato demonstrou capacidade fungicida para ambos os fungos até à concentração máxima testada (MFC>10 mg/mL).

De uma forma geral, este estudo teve como objetivo valorizar os pimentos coloridos como fonte natural de compostos bioativos que podem ser explorados por diferentes indústrias através da sua conversão em produtos de valor acrescentado, contribuindo simultaneamente para a implementação de diretrizes da bioeconomia circular.

Palavras-chave: desperdício alimentar, pimentos, compostos bioativos, bioeconomia circular.

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List of abbreviations

ABTS: 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid)

AMD: Aged-related macular degeneration

ANOVA: Analysis of variance

AOAC: Association of Official Analytical Chemists

ATCC: American Type Culture Collection

BHT: Butylhydroxytoluene

CFU: Colony Forming Unit

DAD: Diode array detector

DHA: Docosahexaenoic acid

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DRI: Dietary Reference Intake

dw: dry weight

EC_{50}: Extract concentration corresponding to 50% of the antioxidant activity

EFSA: European Food Safety Authority

ESI: Electrospray ionization

EU: European Union

FAME: Fatty acid methyl esters

FAO: United Nations Food and Agriculture Organization

FBS: Fetal bovine serum

FDA: U.S. Food and Drug Administration

FID: Flame ionization detector

FP: Fluorescence detector

FRAP: Ferric reducing antioxidant power

fw: fresh weight

GAE: Gallic Acid Equivalent

GC: Gas chromatography

GHG: Greenhouse gas

GI_{50}: Concentration that inhibits 50% of cell growth

HPLC: High-performance liquid chromatography

HSD: Tukey's honestly significant difference

INT: *p*-Iodonitrotetrazolium chloride

IS: Internal standard

LOX: Soyal lipoxygenase enzyme

MBC: Minimum bactericidal concentration

MDA–TBA: Malondialdehyde – thiobarbituric acid

MEB: Malt Extract Broth

MHB: Mueller-Hinton agar

MIC: Minimum inhibitory concentration

MRSA: Methicillin-resistant *Staphylococcus aureus*

MS: Mass spectrometer

MSD: Mass selective detector

MUFA: Monounsaturated fatty acids

ORAC: Oxygen radical antioxidant activity

PDA: Photo-diode array detector

PUFA: Polyunsaturated fatty acids

RI: Refraction index detector

ROS: Reactive oxygen species

Rt: Retention time

SD: Standard deviation

SDG: Sustainable development goals

SFA: Saturated fatty acids

SRB: Sulforhodamine B

TBARS: Thiobarbituric acid reactive substances

TCA: Trichloroacetic acid

TE: Trolox equivalents

TSB: Tryptic soy broth

UHPLC: Ultra-high-performance liquid chromatography

UK: United Kingdom

USA: United States of America

UV: Ultraviolet Radiation

VERO: Non-tumor African green monkey kidney cell line

VOCs: Volatile organic compounds

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1. Introduction

Food loss and waste continue to be a global issue and a pressing challenge in the quest for efficient and sustainable agri-food systems, as well as in the pursuit of food security and nutrition for all. A significant portion of food, regardless of its plant or animal origin, is not consumed by humans or animals and is instead discarded as waste, even though it possesses nutritional value [1]. It is estimated that approximately one third of all food produced worldwide is lost or wasted annually [2]. The volume was observed to be as high as 1.3-1.4 billion tonnes and is expected to rise as high as 2.6 billion tonnes by 2025 [3]. Worldwide, food loss and waste primarily occur in fresh fruits and vegetables which is mainly associated with post-harvest processing operations and consumer behavior [4]. This waste can also serve as a valuable source of bioactive compounds with industrial applications (**Figure 1**). Thus, preventing food loss and waste can have a favorable impact on the environment, economy, and food security of the world's population, contributing to the circular economy. In addition to prevention, valorising these food wastes is a technologically viable strategy as it involves using them as natural bioactive ingredients to generate, for example, new functional products by different industries as an alternative to synthetic compounds [5].

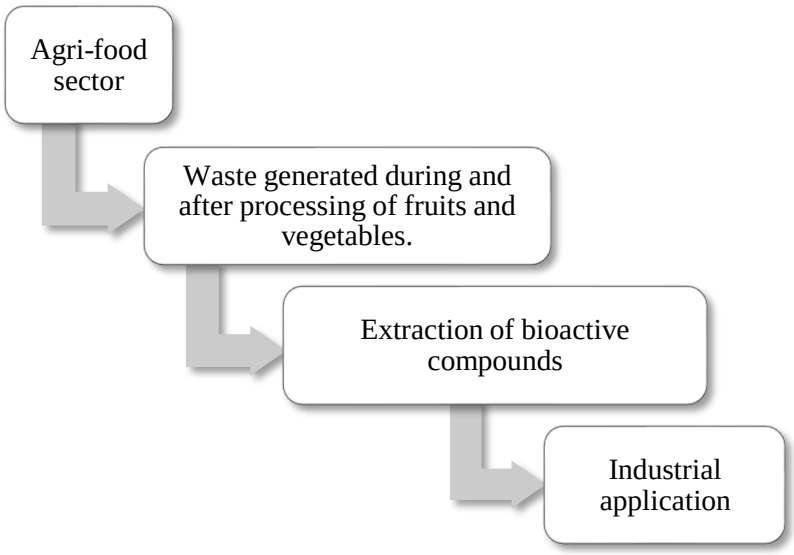

Figure 1: Preferable outcome for fruit and vegetable wastes.

1.1. Food loss and waste in agri-food industry: framing the issues

The agri-food sector has always been one of the main industries around the world and a primarily economic activity on which societies rely for their overall development [6]. Modern advances in biotechnological techniques and industrialization not only have enable scientists to directly modify the DNA of crops, achieving significant improvements, but also have facilitated the commercialization and large-scale intensification of agriculture. However, these major advances also lead to the generation of substantial amounts of agri-food loss and waste, posing a significant threat to the environmental balance [7].

Food loss and waste refer to the decrease in the quantity or quality of edible portions harvested or produced for human consumption along the food supply chain [8]. Waste occurring early in the supply chain during production, processing, or transportation is usually defined as food loss, whereas food waste includes the actions of food retailers and consumers [9]. The increasing generation of loss and waste in the agri-food industry has motivated nations to move towards a more circular bioeconomy, as the availability of natural resources has become limited [10]. Reducing food loss and waste is now a priority program area in the United Nations Food and Agriculture Organization (FAO) Strategic Framework 2022-31, in support of the 2030 Agenda for Sustainable Development [11]. According to the FAO, the agri-food sector generates more than 40 percent of the loss and waste from fruit and vegetables produced worldwide each year (**Figure 2**).

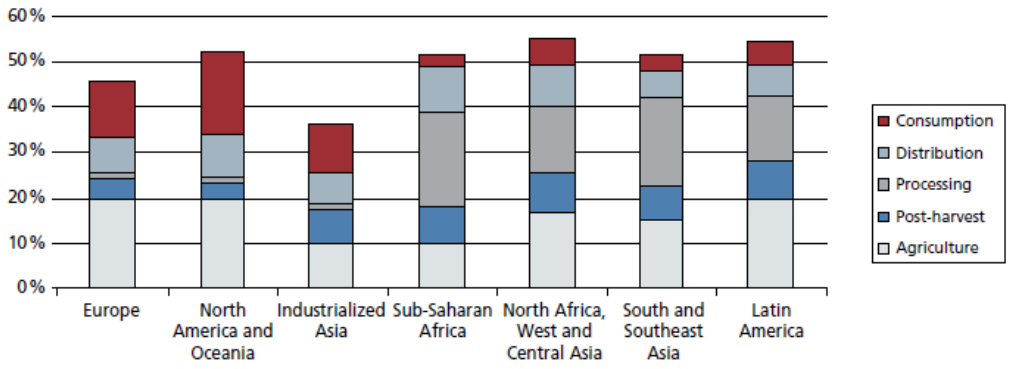

Figure 2: Global panorama of fruit and vegetables losses and waste in agri-food industry [12].

The high-added-value compounds contained in food loss and waste from fruit and vegetables can offer a wide spectrum of possibilities for their reuse and valorization, contributing to the circular bioeconomy [13]. Fresh fruit and vegetables are a highly hydrated, perishable, and fragile product at every step of the supply chain, from harvesting, grading, processing, and transportation to households, which generates significant amounts of waste [14]. The loss and waste of fruits and vegetables are equally high in both industrialized and developing countries. In medium- and high-income countries food is wasted to a high extent at retail and consumer levels, whereas in low-income countries food is mostly lost during the production-to-processing stages of the food supply chain [12]. Nowadays, the European Union is committed to achieving the global Sustainable Development Goal (SDG) Target 12.3, which aims to halve *per* capita food waste at the retail and consumer level by 2030 and reduce not only agri-food but all food losses along the food production and supply chains [15]. The main causes and drivers of food loss and waste in agri-food industry are associated with damage caused by improper transport and storage of products, losses in processing or contamination, and improper packaging [16]. The limited shelf life and the necessity to meet rigorous quality and aesthetic standards regarding color, shape, size, and appearance are the main causes of food waste at the retail stage [17]. Consumer practices can have a significant impact on the environment, thus awareness for a responsible behavior and conscious effort towards reducing food waste are absolutely crucial for mitigating the environmental impacts of food waste [18]. Currently, the world is facing various global crises, including the Russia-Ukraine war and the resulting high global prices for energy, fertilizer, and food. Additionally, the aftershocks of COVID-19 have all contributed to a deterioration in global food security and nutrition [19].

In this sense, it is more necessary than ever to make progress on food loss and waste, particularly from fruit and vegetables, as they can constitute an economically and ecologically important source for nutritional and bioactive compounds that can be valorized instead of being discarded as waste in landfills.

1.1.1. Impacts of food loss and waste

Food loss and waste have significant effects on the sustainability of food systems, impacting the economy, environment, food security and nutrition. The annual market value of food lost or wasted globally is estimated to be around 400 billion euros [20]. FAO estimates that this lost and wasted food could feed 1.26 billion hungry people every year [21].

The economic impact of food loss and waste can manifest at a national level, resulting in a reduced gross domestic product for the agriculture sector. The economic costs of food loss and waste are also felt by households, which spend money on food that is eventually wasted, as well as by businesses along the food supply chain [22]. Concerning the environment, food loss and waste contribute to greenhouse gas (GHG) emissions and represent a waste of resources used in food production, such as land, water, and energy. Food loss and waste is responsible for an estimated 8 to 10 percent of annual GHG emissions and consumes one-quarter of the freshwater used by agriculture each year [23]. Additionally, the production of food that is eventually lost or wasted use a significant land area and contributes to the degradation of natural ecosystems and the loss of biodiversity [24]. Furthermore, food loss and waste can also impact food security and nutrition through quality and nutrient losses along food supply chains, thereby reducing the global and local availability of food. Nevertheless, the world population is growing rapidly. It is projected to reach 9.8 billion by 2050, being expected to result in a significant increase in the global demand for food [25], which therefore implies a transition to a sustainable food system associated with nutrient recovery from wasted utilization and sources of food/feed, in order to reduce the shortage of world food supplies while ensuring food security and nutrition in the future.

Reducing food loss and waste is widely seen as a viable strategy to reduce production costs and increase the efficiency of the food system, improve food security and nutrition, and contribute to environmental sustainability [26]. In addition, reducing food loss and waste also alleviates ethical and moral concerns that some people regarding the occurrence of loss and waste while millions suffer from hunger and malnutrition, with adverse effects on the environment and the survival of future generations [27].

1.2. Bell peppers (*Capsicum annuum* L.)

1.2.1. Description of bell peppers

Bell peppers belong to the Solanaceae family, which includes a great number of important crops for human diet and economic interest, such as tomato (*Lycopersicum esculentum*), potato (*Solanum tuberosum*), and eggplant (*Solanum melongena*) [28]. The Solanaceae family comprises more than 90 genera and 2000 species, with approximately 30 species included in the genus *Capsicum*. Generally, pepper fruits are consumed raw, fermented, as a colorant (paprika) or in powdered form as a spice. They range from sweet, large, and thick varieties like bell peppers to thin and hot varieties like cayenne [29]. The principal cultivated varieties include both hot and sweet pepper fruits, such as *C. annuum*, *C. baccatum*, *C. frutescens*, *C. pubescens*, and *C. chinense*, with this last species regarded as the spiciest (most pungent) (**Figure 3**).

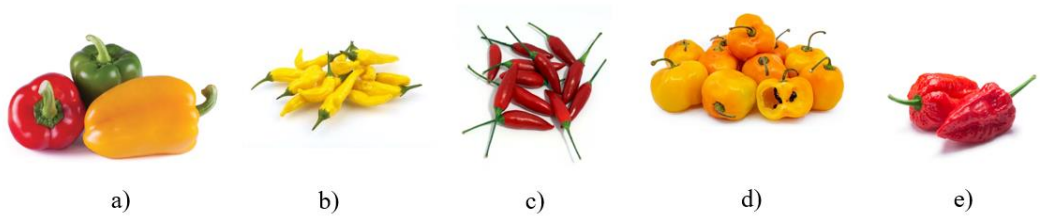

Figure 3: *Capsicum* fruits and its major species; a) *C. annuum* (Sweet bell pepper), b) *C. baccatum* (Aji pepper), c) *C. frutescens* (Bird's eye chili), d) *C. pubescens* (Rocoto pepper), e) *C. chinense* (Bhut jolokia Pepper) [30].

C. annuum is the scientific binomial species name of bell peppers, characterized by their smooth and glossy exterior [31]. The most common colors of bell pepper are green, yellow, orange, and red, corresponding to distinct stages of maturation and capacities to synthetize carotenoids or chlorophylls [32]. Other exotic colors include purple, brown, white, and black, depending on the variety [33]. Although the species name means 'annual' (from Latin *annus* "year"), the plant is not an annual but rather frost tender. In the absence of winter frosts, it can survive several seasons and grow into a large, shrubby perennial herb [34]. Bell peppers are classically considered non-starchy vegetables, and their botanical definition classifies them as a fruit since they contain seeds [35]. It is hypothesized that the center of origin of *Capsicum* peppers is Bolivia, while

non-pungent pepper varieties are said to have originated from Central America and southern Mexico [36]. Today, bell pepper production occurs all over the world and has significantly increased in the past few years. Regarding the nutritional aspect, bell peppers contain high levels of water and carbohydrates, adequate content of dietary fiber, with a low protein and fat content which makes them a low-calorie food (**Table 1**).

Table 1: Principal phytonutrient content by 100g of edible part of fresh bell pepper (*C. annuum* var. *annuum*).

Component	Value
Energy (Kcal/KJ)	27/111
Moisture (g)	92.8
Carbohydrates (g)	2.7
Dietary fiber (g)	2
Protein (g)	1.6
Total fat (g)	0.6
Ash (g)	0.4
Vitamins (mg)	
Niacin	0.6
Folate total	0.028
Vitamin A	0.217
Vitamin C	90
Vitamin E	0.8
Vitamin B6	0.31
Minerals (mg)	
Sodium, Na	4
Potassium, K	120
Calcium, Ca	9
Magnesium, Mg	10
Phosphorus, P	24
Iron, Fe	0.6

Source: Food Composition Table – National Health Institute Dr. Ricardo Jorge (2023) [37].

The nutritional content of bell peppers has important implications for consumers' health, and it has been reported that it directly depends on the color fruit's, cultivated variety, growing conditions, postharvest processing, and developmental stage of the fruit [38].

1.2.2. Bell pepper industry

The bell pepper industry is well-suited to demonstrate the potential of the circular bioeconomy, as it produces enormous quantities of waste. Bell pepper is one of the most widely cultivated vegetable crops in the world [39], primarily in tropical and subtropical countries, but also in temperate climates in greenhouses [40]. Since 2000, the world production and consumption of *Capsicum* peppers has been consistently increasing. According to FAO, the global production of pepper fruits in 2022 reached over 35 million tonnes harvested on almost 2 million hectares of land (**Figure 4**).

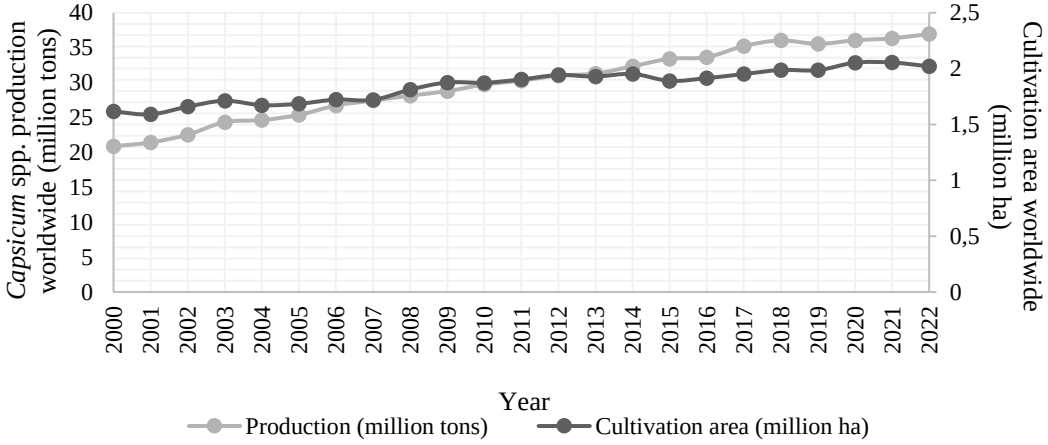

Figure 4: Pepper fruits production and cultivation area worldwide from 2000 until 2022 [41].

China was the largest producer in 2022 with almost 17 million tonnes of pepper fruits, followed by Mexico, Indonesia, Turkey, and Spain (**Figure 5**).

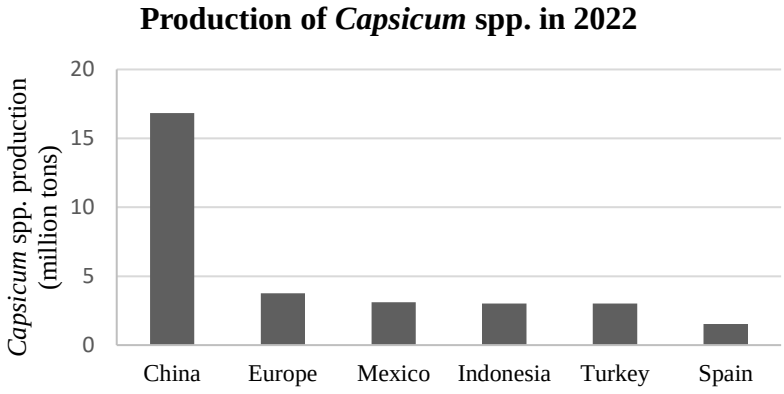

Figure 5: Production of pepper fruits in Europe and 5 most producing countries in the world [41].

In addition to pepper fruits, this industry generates considerable amount of bio-waste, reflected in almost 46 percent of loss and waste [42], which at the end of the fruit production cycle is composed into organic fertilizer, disposed of in landfills, or incinerated with no economic return to the farmer. The loss and waste of bell peppers can have a negative impact on the sustainability of the food industry, resulting in environmental and economic consequences. However, they can also represent an economic and renewable biomass that, in the context of the circular bioeconomy, can be recovered high-value nutritional and bioactive compounds with potential to be exploited as active ingredients in diverse range of products, therefore contributing to the sustainability of the supply chain [43].

1.2.3. Bioactive compounds present in bell peppers

Bell pepper is highly popular and consumed worldwide, due to its nutritional value, exotic colors, and flavor [44]. However, significant amounts of pepper fruits are lost or wasted. These bell peppers that are wasted could represent a desirable source of raw material to obtain valuable bioactive compounds. Furthermore, due to the current eco-friendly behavior of consumers and industries, there is a growing interest in searching for bioactive compounds as natural ingredients for industrial applications. Bioactive compounds are defined as "essential and non-essential compounds" (*e.g.*, vitamins or polyphenols) that occur in nature, part of the food chain, which can demonstrate beneficial and/or toxic effects on human health [45]. Among the several bioactive compounds found in bell peppers capsaicinoids, phenolic compounds, carotenoids (provitamin A) and vitamins (C and E) stand out [46].

1.2.3.1. Capsaicinoids and capsinoids

Capsaicinoids are one of the most abundant natural compounds present in pepper fruits. They are alkaloid substances produced only in the *Capsicum* genus which can produce pungency (spiciness) [47] being considered to play a major role in the defense mechanisms of plants [48]. Some of the genes involved in the biosynthesis of capsaicinoids have been characterized in both pungent and non-pungent cultivars. The pun1 gene is a key regulator in the capsaicinoid pathway and controls the accumulation of capsaicinoids [49]. The characteristic of pungency, as well as their bioactivity, is a

result of the molecular structure, which allows them to interact differently with nervous receptors and cellular membrane [50]. Approximately 90 percent of capsaicinoids are located in the pericarp (fruit), which comprises 40 percent of the pepper fruits, while the remaining 10 percent is found in the seeds [51]. Their content in pepper fruit varieties has been reported to be largely influenced by the genotype, fertilization practices, environmental growing conditions, and fruit maturity [52]. The main capsaicinoids present in peppers are capsaicin and dihydrocapsaicin **(Figure 6)**.

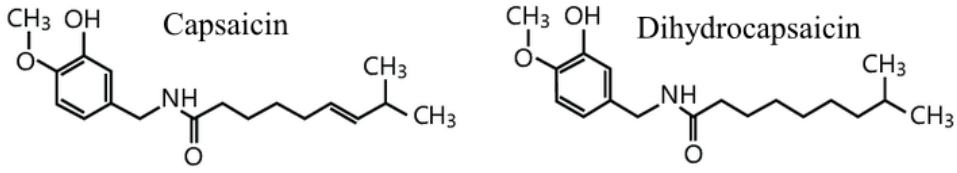

Figure 6: Chemical structures of main capsaicinoids found in pepper fruits [53].

It has been estimated that capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide) account for more than 80 percent of the total capsaicinoids, and their quantities in bell pepper fruits determine the level of pungency [54]. For decades, capsaicinoids have been extensively explored by industry due to their bioactivity and pungency properties. Recent studies have demonstrated the antioxidant, anticarcinogenic, anti-inflammatory, and thermogenic properties of capsaicinoids [55]. The particular thermogenic effect of capsaicin have been reported as a promoter of weight loss, making it an important addition in treating obesity [56]. Additionally, a similar group of compounds named capsinoids **(figure 7)** is also synthesized exclusively within the *Capsicum* genus in both non-pungent and pungent cultivars. Capsinoids are generally present in a relatively low concentration in pepper fruits and have the beneficial effects of capsaicinoids but without the pungency [57]. Their pungent characteristic is due to their slightly different structure which stimulates receptors in the intestine but not in the mouth. This valuable attribute could be presented as a promising alternative for those who abstain from foods containing capsaicinoids due to their pungency [58].

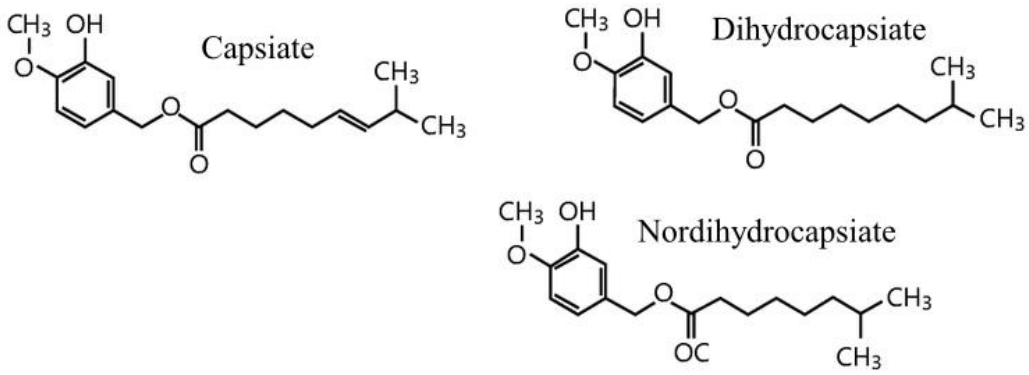

Figure 7: Chemical structures of capsinoids found in pepper fruits [53].

1.2.3.2. Phenolic compounds

Bell peppers have been reported as being highly rich in phenolic compounds [59]. Phenolic compounds originate from plant secondary metabolism and are normally involved in defense mechanisms as a result of their adaptation to the environment [60] during stress conditions such as wounding, infection, or exposure to UV radiation [61]. In relation to their chemical structure, phenolic compounds contain at least one phenol group which is composed of an aromatic ring with one or more hydroxyl groups [62]. Based on the existing number of phenol units, phenolic compounds can either be classified as simple phenols (only one phenol unit) or polyphenols. Polyphenols consist of two or more phenolic groups, up to polymeric structures. They often have one hydroxyl group attached to the molecule's various benzene rings. Moreover, phenolic compounds are further divided into different classes according to their chemical structure and number of carbons. For example, phenolic acids have a C6-C1 basic skeleton while flavonoids have a C6-C3-C6 structure [63].

The main classes of phenolic compounds found in bell peppers are polyphenols, including phenolic acids and flavonoids such as luteolin, quercetin and anthocyanins. In most cases, the biological effects of polyphenols have been attributed to their antioxidant capacity [64]. Polyphenols play a key role in strengthening the immune system as well, regulating gene expression, cell signaling, and hormone metabolism [65]. As a result, they have important health-beneficial effects, particularly in the prevention of numerous pathologies, including cardiovascular diseases, cancer, and neurodegenerative disorders

[66]. Furthermore, the relevance of polyphenols in food products is widely known as they have a strong influence on color and taste properties [67].

1.2.3.3. Carotenoids

Carotenoids are natural and lipophilic pigments widely found in plants, responsible for the exotic colors ranging from yellow through orange to red in all pepper fruits from the *Capsicum* genus [68]. Bell peppers are one of the richest sources of carotenoids and their distinct colors are due to the different carotenoid profiles. The profile and concentration of carotenoids can differ among species, depending on factors such as growing conditions, stage of maturity and processing practices [69]. A total of four genes (y, c_1, c_2, cl) and more than 20 different carotenoids have been identified as responsible for the color of mature and fully ripened fruits in all species of the genus *Capsicum* [70]. As a result of carotenoids metabolism and their accumulation in the chromoplasts, the green color of the fruit, due to the presence of chlorophyll, changes to yellow/orange, having these varieties of violaxanthin, lutein, and β-carotene as a major carotenoid content within the fruit. The red pigments, capsanthin and capsorubin, are produced at the end of the biosynthetic pathway and therefore only accumulated in red ripe pepper fruits [71] (**Figure 8**). The important beneficial aspects of bell peppers are attributed, in part, to their carotenoid content. In addition to being responsible for the color, carotenoids can function as antioxidants, playing a key role in various biological and health-related activities [30]. Moreover, the consumption of carotenoids has been related to the improvement of cognitive function, cardiovascular health, and the prevention of certain types of cancer. Among them, α-carotene, β-carotene, and β-cryptoxanthin identified as precursors of vitamin A, capable of producing retinol and retinoic acid which has been reported to have important role on immune cells and inflammatory intestinal diseases [72]. Other dietary carotenoids mentioned, such as lutein and zeaxanthin, have the ability to reach the human retina and absorb specific wavelengths of light. This can help protect the eyes and play a crucial role in preventing age-related macular degeneration (AMD) and other ocular diseases, including cataracts [30].

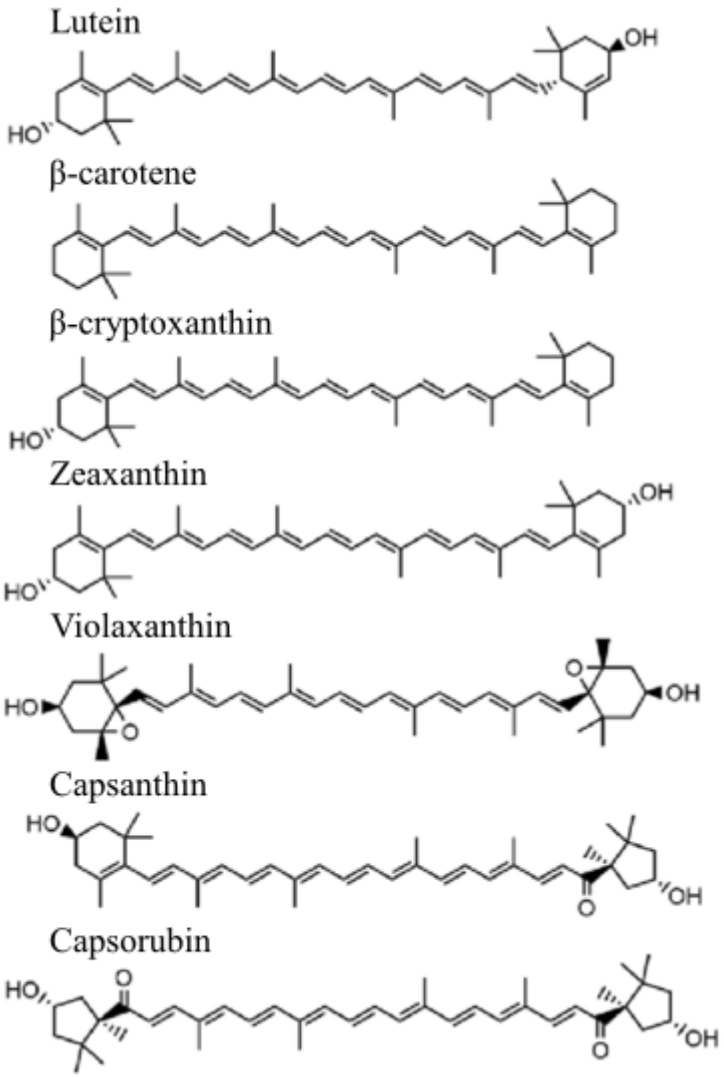

Figure 8: Chemical structure of main carotenoids found in pepper fruits [73].

1.2.3.4. Vitamins

Vitamins are a group of nutrients that are required for the normal functioning of the human body. They comprise natural compounds with diverse chemical structures, classified together based on their physiological activity. Thirteen substances or group of substances have been recognized as vitamins, which are categorized into two groups according to their solubility: fat-soluble vitamins, which includes vitamins A, E and K and water-soluble vitamins, which include vitamin C and niacin [74]. Bell peppers are excellent sources of vitamins, containing high levels of vitamin C (L-ascorbic acid), provitamin A (β-carotene as a precursor), and vitamin E (tocopherols) (**Table 1**).

Vitamin studies on pepper fruits have been conducted for a long time. In fact, Albert Szent-Gyorgyi, the recipient of the Nobel Prize in Medicine (1937), discovered bell peppers as a rich source of vitamin C (L-ascorbic acid) while he was conducting a series of early experiments on citrus plants [75]. Indeed, vitamin C has been reported to be present in some pepper varieties at twice the concentration as in tomatoes, apples, or oranges per gram of fruit weight [76]. Furthermore, according to European Food Safety Authority (EFSA), one medium-sized green bell pepper fruit (100g) provide approximately 82 percent of the Dietary Reference Intake (DRI) for vitamin C, which is 110 mg/day for men and 95 mg/day for women. They also provide around 10 percent of the DRI for vitamin A and vitamin E [77]. Vitamin A is an essential nutrient required to maintain healthy eyesight and a functional immune system. As described before, carotenoids, mainly β-carotene and β-crytoxanthin, are responsible for the vitamin A biological functions. They are metabolized intracellularly to retinol and retinoic acid, the active forms of vitamin A [78]. Vitamin E is a generic term that refers to the tocopherols, e.g. α-, β-, σ-, γ-tocopherol. Tocopherols can play a key role in protecting approximately 80 diseases, by scavenging free radicals, preventing cancer, anemia, diabetes and cardiovascular diseases, inhibition of oxidation of low-density lipoproteins, disorders of the skin, eye lungs and other constituents of the lipid-rich body [79]. In general, the levels of vitamins present in bell pepper fruits have been reported of being dependent on several factors such as genotype (variety), maturity stage, harvesting time, postharvest handling/processing, and storage conditions [80].

1.2.3.5. Fatty acids

Fatty acids mainly occur in plants bound form, esterified to glycerol as fats or lipids [81]. In *Capsicum* genus, fatty acids constitute a small portion of the edible portion however they make part of important metabolic and structural role in pepper fruits including maintaining the stability of the color [82]. They are characterized by long chains of carbon atoms topped off with a carboxyl group. The length of the chain can vary, although most are between 14 and 20 carbons. In higher order plants and animals, fatty acids can be found in major species with a chain length between 16 and 18 carbons [83]. Furthermore, they can be classified as saturated or unsaturated and both of them have been shown to exist in *Capsicum* fruits. In a fatty acid chain, if there are only single bonds

between neighboring carbons in the hydrocarbon chain, the fatty acid is saturated. Saturated fatty acids are saturated with hydrogen. In other words, the number of hydrogens atoms attached to the carbon skeleton is maximized [84]. Stearic acid is an example of a usual saturated fatty acid found in pepper fruits (**Figure 9**).

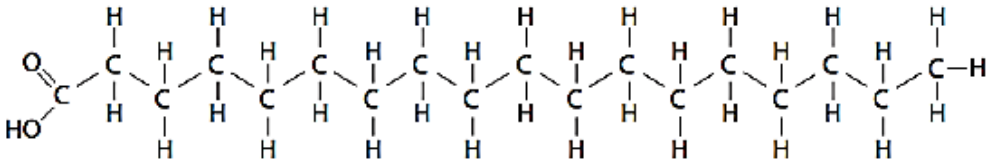

Figure 9: Steric acid, a common saturated fatty acid found in bell peppers [83].

When the hydrocarbon chain contains a double bond, the fatty acid is unsaturated. Oleic acid is an example of a usual unsaturated fatty acid found in *Capsicum* fruits. (**Figure 10**).

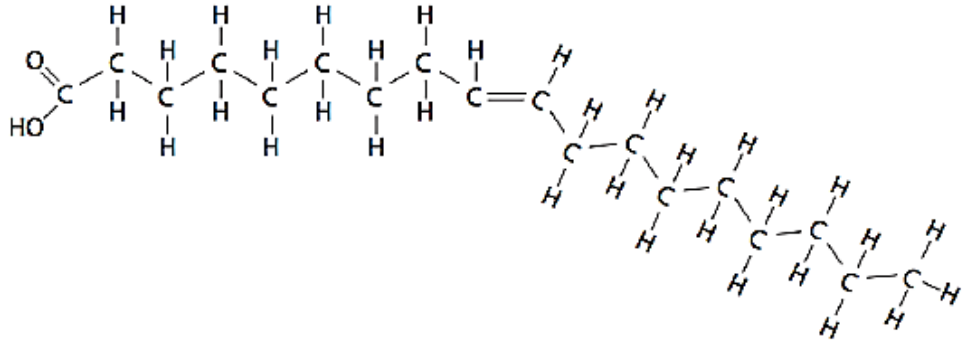

Figure 10: Oleic acid, a common unsaturated fatty acid found in bell peppers [83].

Fatty acids can accumulate both in the pericarp and seed of pepper fruits [85]. There is limited information available about fatty acid composition from different colored bell peppers. Genotypes, growing conditions and location have been reported to have great influence on the fatty acid composition and oil accumulation in pepper fruits [86].

1.2.3.6. Minerals

Minerals are naturally occurring inorganic substances that help the human body develop and function normally. They are essential for good health [87]. The most common essential minerals found in peppers, ordered from higher to lower content, are potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na) and iron (Fe). Their contents in fresh bell peppers are shown in **Table 1**. In addition, other minerals

such as zinc (Zn), manganese (Mn), boron (B), copper (Cu) and selenium (Se) have been also identified in bell peppers. The concentrations of these vital elements in bell peppers can vary greatly as they are influenced by numerous agricultural practices such as types of fertilizer, soil, use of herbicides and pesticides, as well the phenotype and maturity stage of the fruit [88]. Previous studies suggested that agricultural practices (organic *vs.* conventional farming) may influence the mineral contents of peppers, with organic farming increasing mineral contents [89]. Although a healthy diet requires small quantities of minerals, they play a vital role in several metabolic processes essential for maintaining good health and preventing diseases. Deficiency in minerals can have harmful effects on the normal biochemical functioning of the human body. Minerals hold various roles, including prevention of hypertension, wound healing, normal functioning of the nervous system, as well as antioxidant and immunomodulatory functions [90].

1.2.3.7. Volatile organic compounds

Bell pepper fruits have a considerable amount of volatile organic compounds (VOCs) in their composition. VOCs are organic chemical compounds that have high vapor pressure and low water solubility [91], meaning that under normal conditions they are gaseous or can vaporize and enter the atmosphere [92]. According to their structural characteristics, VOCs can be divided into fatty derivatives, terpene derivatives, aromatic derivatives, as well as nitrogen-containing and oxygen-containing heterocyclic compounds [93]. The fraction of VOCs present in pepper fruits has been reported to be rich and diverse, comprising over 125 substances [94], including main compounds classified as esters, terpenes, alcohols, ketones, alkenes, and aldehydes [95]. There is a close relationship between the fruit aroma and its volatile compounds [96]. VOCs can be emitted constitutively or in response to a variety of stimuli in plants, thus playing a key role in defining the sensory characteristics of fruits and vegetables by affecting their flavor and fragrance [97]. It is well known that VOCs have several biological activities which can have industrial applications such as food additives, oil for aromatherapy, commercial chemicals for many food products, soaps, and perfumes [98]. In general, VOCs content in bell pepper fruits have been reported to be influenced by geographical origin, processing, cultivar, production methods, storage time, and the maturation stage [99].

1.2.3.8. Other phytochemicals in bell pepper fruits, seeds, and leaves

Other substances have been identified in bell peppers extracts from pulp, seeds, and leaves. Phytochemicals, such as colneleic acid, capsoside A, gingerglycolipid A, blumenol C glucoside, glycerolipids and glycerophospholipids, sphingomyelin, and ceramindes, have been qualitatively identified in bell pepper fruits. Additionally glycosides, saponins, terpenoids, phenols, and alkaloids have been also identified in bell pepper fruits [100]. These compounds could exert interesting biological activities. Previous studies on bell pepper seeds reported that they contain sterols, terpenoids, and fatty acids, which could exert antioxidant properties [101]. Furthermore, fat-soluble vitamins such as vitamins A, B1 and B2 has been found in bell pepper leaves. In addition, it has been reported that pepper foliage contains beneficial bioactive compounds such as phenols, flavonoids, alkaloids, among others [102]. Moreover, a recent study has demonstrated that peptides extracted from leaves of bell pepper crops showed antibacterial and antifungal activities, and exhibited exciting potential for biotechnological use [103].

1.2.4. Biological activities of bell pepper extracts

As discussed in the previous sections, bell pepper fruits including their seeds and stalks contain bioactive compounds that are associated with different biological activities for industrial applications (**Figure 11**).

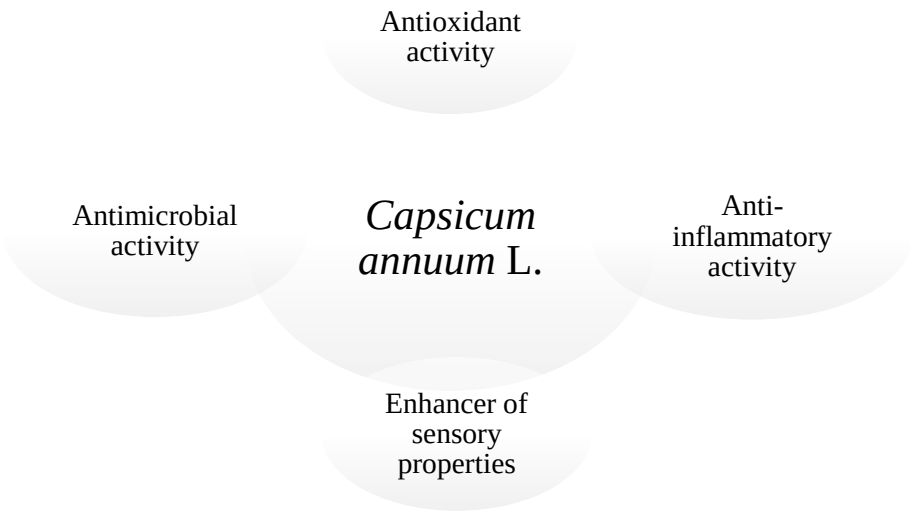

Figure 11: Schematic description of biological activities of bell peppers.

1.2.4.1. Antioxidant activity

Bell pepper fruits contain an extensive range of secondary metabolites with renowned antioxidant properties. The antioxidant activity of any fruit or vegetable is determined by different bioactive compounds, which exhibit various mechanisms of action to inhibit free radicals [104]. Antioxidants inhibit or delay the chain reactions of oxidation in other molecules, which are associated with the formation of free radicals that can be harmful to human cells [105]. Thus, antioxidants play a crucial role in protecting against various non-communicable diseases, such as several cancers, heart diseases, and diabetes, which are responsible for 74 percent of all deaths worldwide [106].

The biological effects of carotenoids, capsaicinoids, vitamins (C and E) and phenolic compounds, found in bell pepper fruits have been correlated to their antioxidant capacity [107]. This attribute has been associated with presence of a system of conjugated double bonds in their structure, which enables them to scavenge ROS and neutralize free radicals [108], which consequently has an important role in the prevention of various degenerative diseases, coronary heart disease, and certain types of cancer such as gastrointestinal, lung, prostate, and breast cancers [109]. Furthermore, antioxidants have been of great interest to researchers for many years due to their health-promoting capabilities. Their extensive applications in the food industry are well-documented, offering an excellent opportunity for food processors to extract and quantify them from various foods [110]. Previous studies reported that the use of bell pepper as an antioxidant ingredient in foods could significantly reduce cholesterol decomposition and the degradation of docosahexaenoic (DHA) acid during cooking. This helps prevent the production of toxic oxidation products, which can lead to off-flavors, deterioration of food, and increased risk of coronary heart disease and cancer [111]. Based on the above, the significant antioxidant potential of pepper fruits enables their application as natural antioxidants in dietary supplements or natural ingredients as substitutes of artificial additives.

1.2.4.2. Antimicrobial activity

Bell pepper extracts have shown to possess antimicrobial activity against foodborne and clinical pathogens. They contain antimicrobial compounds that inhibit

microbial growth, making them potentially suitable for industrial applications [112]. Studies have been reported that bioactive compounds isolated from bell pepper seeds and fresh fruits have different effects on microbial species. They demonstrated potent antimicrobial capacity against bacteria and fungi. These compounds include capsaicinoids, which have been reported to have antiviral effects as well. For instance, capsaicinoid investigations on pepper fruits have shown that capsaicin can function as an inhibitor of virus entry in different cell lines [113]. Furthermore, capsinoids, which are analogs of capsaicinoids, have also been reported to exhibit interesting antimicrobial activity [114]. Nowadays, there is an interest in discovering new natural bioactive compounds able to solve problems of antibiotic resistant bacteria [115]. Therefore, these findings can contribute to future developments in new therapeutic substances against pathogenic species, providing a more efficient response at lower concentrations of antimicrobial agents [116]. Moreover, phenolic compounds present in bell peppers and their derivates have been also reported to shown antibacterial and antiviral effects against pathogenic microorganisms [117]. The antimicrobial properties of polyphenols extracted from bell pepper are of extreme interest to be exploited as natural preservatives in the food industry, as substitutes of synthetic preservatives.

1.2.4.3. Anti-inflammatory properties

Inflammation is a biological defense response triggered by a host of factors such as infection, injury, and toxic compounds and acts by removing injurious stimuli and initiating the healing process [118]. It is a defense mechanism vital to health. Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases [119].

Bell peppers can display anti-inflammatory properties in animal assays, which are closely associated with the prevention of diverse pathologies [120]. Several studies have reported the anti-inflammatory activity of bioactive compounds sourced from bell peppers, including polyphenols, flavonoids, tocopherols, capsaicinoids, and capsinoids. The anti-inflammatory property of *Capsicum* peppers is mediated by the inhibition of the

Soyal lipoxygenase (LOX) enzyme. The role of the LOX enzyme is to catalyze the oxygenation of polyunsaturated fatty acids (PUFA), and oxygenated lipids initiate subsequent biological reactions, such as the inflammatory response [121]. Different varieties in the genus *Capsicum*, including bell peppers, have been reported to demonstrate varying degrees of LOX inhibition [122]. The anti-inflammatory properties of pepper fruits are mostly due to their capsaicin content, which is well-documented [123]. In conclusion, *Capsicum* peppers show potential anti-inflammatory compounds which could be tested as drug candidates against oxidative and inflammation-related pathological processes.

1.2.4.4. Enhancer of sensory properties

As previously mentioned, dried and ground red bell pepper (paprika) is a widely known worldwide spice used to improve the color, flavor, and add pungency to many foods and dishes. Paprika oleoresin have been also used in food products as a substitute for ground paprika due to its concentrated color and flavor [124]. Paprika extract (E 160c) is recognized as a food additive (natural dye) within the European Union [125]. The main quality attribute of this ingredient is the intensity of its red color, which influences both consumer acceptance and commercial value [126]. The use of bell pepper derivatives as coloring enhancers is widespread, and many products are available. It is usually added to meat products to improve their color and flavor [127]. The characteristics of bell pepper flavor are a complex trait influenced by environmental factors during growth and postharvest processing [128]. Previous studies reported that bell pepper powder can effectively inhibit oxidative reactions and extend the shelf life of processed products [129]. Furthermore, it was found that bell pepper powder could have a role in satiation at the end of the meal. Capsaicin has an important role in the enhancement of sensory properties. The consumption of food with added capsaicin, such as a soup with the addition of pungent pepper, may alter appetite sensations and sensory-specific desires, resulting in significantly higher satiation at the end of the meal and one-hour post-intake, hence influencing eating behavior [130].

1.2.5. Industrial applications of bioactive compounds from bell peppers

The benefits resulting from the use of natural compounds rich in bioactive substances have promoted the growing interest of different industries such as food, cosmetic and pharmaceutical [131]. Nowadays, consumers are showing greater interest in healthier and more innovative products whose ingredients promote general well-being, including disease prevention [132]. Regarding these demands, industries have opted for the use of plant-derived compounds that present bioactivities, which can result in the generation of new functional products with positive effects on human health. In this sense, bell peppers emerge as an important source of bioactive compounds for industrial applications such as preservatives, additives, or technological ingredients with antioxidant, anti-inflammatory, and antimicrobial functionalities.

Capsaicin is the most well-known and commonly used bioactive compound extracted from bell pepper fruits. Due to its anti-inflammatory properties previously mentioned, capsaicin are being used as a drug for topical treatment of musculoskeletal and neuropathic pain disorders, with the EU and FDA approvement since 2009 [133]. The use of natural ingredients instead of synthetic preservatives could enhance the health properties of cosmetic and pharmaceutical products, avoiding the contact allergies caused as side effects. Capsaicin also is commercially available as liquid oleoresin which contains other natural antioxidants. *Capsicum* oleoresin has been used as a cosmetic colorants in bath oils, shower gels, shampoo, soaps and many beaty products including eye make-up and lipsticks [134]. This oleoresin has been used in the food industry as well, as a coloring and flavoring agent in a wide number of processed food products, including cheeses, meats, mortadella, sausages, soups, sauces, snacks, condiments, sweets, and even alcoholic beverages [135]. The meat industry is, in particular, the one that utilizes these capsaicinoids the most due to both their ability to confer the characteristic sensory profile related to spicy foods as well as their reported antimicrobial properties against gram-negative and gram-positive bacteria. This justifies the extensive use of these bioactive compounds in the manufacture of meat pastes, such as sausages and chorizo, to improve their color and flavor [136].

Carotenoids from bell peppers have also been extracted and used as natural additives in several industries, primarily to impart color into products. As previously

mentioned, carotenoids in bell peppers are recognized for their antioxidant, anticancer, anti-inflammatory, and immunomodulatory properties. They are being utilized as natural colorants and therapeutic compounds (UV protectors) in pharmacy and cosmetic products [137]. In the food industry, carotenoids are being used as natural colorants as well, to mitigate the incidence of digestive problems, allergies, insomnia, hyperactivity, and cancer in humans that are associated with the use of synthetic colorants [114]. Moreover, flours from pepper fruits have recently been used as a complement to fruit and vegetable flours intended for breading or seasoning different foods, providing them with flavor and color while also improving their stability and antioxidant capacity. Eventually, *Capsicum* flours can be considered as a potential ingredient with functional properties given by the type of bioactive compounds it contains, which increase both the sensory and nutritional value of the final product [138].

2. Objectives

This work was carried out to valorize waste-green, orange, and red fruits of *C. annuum* including their seeds and stalks at the post-harvesting stage. In this sense, the specific objectives were:

- Proximate composition assessment (fat, ash, protein, carbohydrate, and energetic value) and biochemical characterization (free sugars, organic acids, tocopherols, fatty acids, pigments, phenolic compounds, and volatile compounds) of the waste-colored bell peppers fruits.
- Evaluation of bioactive properties (antioxidant, cytotoxic, and antimicrobial activities) of the waste-colored bell pepper fruits including their seeds and stalks hydroethanolic extracts.

3. Materials and methods

3.1. Samples preparation

The agri-food waste of *Capsicum annuum var. grossum* production was supplied by a local producer in Bragança, Portugal. The fruits of orange, red and green waste bell peppers, including their seeds and stalks, were carefully separated, frozen and lyophilized.

3.2. Standards and reagents

High-performance liquid chromatography (HPLC) grade n-hexane, 99% acetonitrile and 99.98% ethyl acetate solvents were purchased from Fisher Scientific (Lisbon, Portugal) and analytical grade methanol solvent was purchased from Pronalab (Lisbon, Portugal). The water was treated prior to use by the Milli-Q-Water purification system (TGI Pure Water Systems, Greenville, South Carolina, USA). The standard blend with 37 fatty acid methyl esters (FAME) (standard 47885-U) was purchased from Sigma (St. Louis, Missouri, USA), as well as other individual fatty acid isomers, sugar standards, organic acid standards. Fetal bovine serum (FBS), *L*-glutamine, Hank's saline solution (HBSS), and DMEM medium (medium for animal cells (Dulbecco Modified Eagle)) were purchased from Hyclone (Logan, Utah, USA). Acetic acid, ellipticin, sulforodamine B (SRB), trichloroacetic acid (TCA) and Tris were supplied by Sigma-Aldrich (St. Louis, Missouri, USA). Mueller-Hinton agar (MHB) was obtained from Biolab® (Hungary). The compound *p*-Iodonitrotetrazolium chloride (INT) was purchased from Panreac Applichem (Barcelona, Spain).

3.3. Proximate composition analysis

Samples of waste-orange, red and green bell pepper fruits were stored at deep freezing conditions and later lyophilized, to avoid the degradation of bioactive compounds, before the chemical composition analysis. The determination of the proximate composition (g/100 g of dried weight (dw)) and energetic values was carried out according to the procedures described by the Association of Official Analytical Chemists (AOAC) [139]. Crude protein content (*N* x 6.25) was quantified by the macro-Kjeldahl method using an automatic distillation and titration unit (model Pro-Nitro-A, JP

Selecta, Barcelona, Spain); the crude fat content was estimated using a *Soxhlet* apparatus (Behr Labor Technik, Dussedolf, Germany) by extraction with petroleum ether; the ash content was evaluated by incinerating the samples at 550±15 ºC in a muffle. The total carbohydrate content was evaluated by the difference according to **Equation 1**, and the energetic value was calculated according to the Regulation (EU) No. 1169/2011 [140] as shown in **Equation 2**.

$$\text{Carbohydrates} = 100 - (\text{g fat} + \text{g ash} + \text{g protein})$$

Equation 1: Total carbohydrate calculation.

$$\text{Energy}\left(\frac{\text{kcal}}{100\text{ g of dw}}\right) = 4 \times (\text{g proteins} + \text{g carbohydrates}) + 9 \times \text{fat}$$

Equation 2: Energetic value determination.

3.4. Hydrophilic compounds analysis

3.4.1. Free sugars profile

The free sugars were identified by HPLC system coupled with a refraction index (RI) detector (Knauer, Smartline system 1000, Berlin, Germany), as previously described by Spréa et al. [141]. Waste-colored bell pepper fruits were subjected to an extraction procedure in a water bath (80 °C; 30 min) with ethanol (80%; 40 mL), with the addition of melezitose (5 mg/mL) as internal standard (IS) (Sigma-Aldrich, St. Louis, Missouri, USA). After centrifugation, the supernatant was filtered, concentrated under reduced pressure (60 °C); and defatted with ethyl ether (10 mL; three times). Thereafter, extracts were centrifuged and concentrated (40 °C), re-dissolved in water (5 mL final volume) and filtered for injection (0.2 µm nylon filters from Whatman) in a HPLC-RI system. After separation, the free sugar compounds were identified by comparison with standards of their relative retention (Rt), while quantification was made by the IS, with calibration curves constructed with standards. Raw data were processed through the Clarity 2.4 software package (DataApex, Prague, Czech Republic) and expressed in g per 100 g of dw.

3.4.2. Organic acids profile

The organic acids profile was characterized following a procedure earlier described and optimized by the authors Pereira et al. [142]. Waste-colored bell pepper samples (1g) were subjected to an extraction with meta-phosphoric acid (25 mL; 25 °C; 150 rpm; 45 min) and filtered through Whatman No. 4 paper and 0.2 µm nylon filters before injection. It was used ultra-high-performance liquid chromatography coupled with a diode array detector (UHPLC–DAD, Shimadzu 20A series UHPLC, Shimadzu Corporation, Kyoto, Japan) to detect the absorption in the UV to VIS region. Detection was performed by a photo-diode array detector (PDA), using 280 nm as the preferable wavelengths and 215 nm for measuring ascorbic acid.

The identification was carried out by comparing the chromatograms obtained for the analyzed samples with those obtained using commercial standards. For quantification, standard compounds were used to construct calibration curves, and the peak areas were compared. The organic acids content was expressed in g per 100 g of dw.

3.5. Lipophilic compounds analysis

3.5.1. Fatty acids profile

The fatty acid methyl esters (FAME) were characterized after transesterification of the lipid fraction obtained after Soxhlet extraction were subjected to a methylation process with methanol/sulphuric acid/toluene (2:1:1 (v/v/v); 5 mL) in a water bath (12 h; 50 °C; 160 rpm). Deionized water (3 mL) and diethyl ether (3 mL) were added to obtain phase separation and recover the FAME phase. A YOUNG IN Chromass 6500 Gas Chromatography (GC) System (YL Instruments, Anyang, Korea) equipped with a split/splitless injector, a flame ionization detector (FID), and a Zebron-Fame column (30 m × 0.25 mm × 0.20 µm, Phenomenex, Lisbon, Portugal) was used in the analysis. The elution and operation conditions were previously described by Spréa et al. [141]. The identification and quantification were determined by comparing the relative retention times of the FAME peaks of the samples with commercial standards (FAMEs, reference standard mixture 37, Sigma-Aldrich, St. Louis, Missouri, USA). The results recorded were processed using CSW 4.0 Software (Informer Technologies, Inc., Solihull, UK). The results were expressed in the relative percentage of each fatty acid compound.

3.5.2. Tocopherols profile

Tocopherol content was determined following a procedure previously described by the authors Spréa et al. [141]. Butylhydroxytoluene (BHT; 10 mg/mL; 100 μL) and internal standard (IS; tocol; 50 μg/mL; 400 μL) solutions, prepared with hexane, were added to the waste-colored bell peppers fruits samples. The extraction procedure consisted of successively adding methanol (4 mL), hexane (4 mL), and saturated sodium chloride aqueous solution (2 mL). The extract was then centrifuged and the clear upper layer containing tocopherols was collected to a vial, protected from light exposure. The described extraction procedure was repeated twice, with hexane, and the combined extracts were dried under nitrogen stream. To perform the analysis, the dried bell pepper extracts were re-dissolved in n-hexane (2 mL), passed through a sodium sulphate anhydrous micro-column, and filtered (0.2 μm nylon filters from Whatman) to a dark injection vial. A HPLC system coupled to a fluorescence detector (FP-2020; Jasco, Tokyo, Japan) programmed for excitation at 290 nm and emission at 330 nm was used. The identification was achieved by chromatographic comparison with authentic standards and the quantification was based on the fluorescence signal response of each standard, using the internal standard (IS; tocol; 50 mg/mL); Matreya, Pleasant Gap, Pennsylvania, USA) method and calibration curves constructed with commercial standards. The results were expressed in mg per 100 g of dw.

3.5.3. Pigments content

The content of pigments was evaluated using a method described by the authors Petropoulos et al. [143]. Briefly, the samples (500 mg) were vigorously shaken with 10 mL of acetone/hexane mixture (4:6, v/v) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance was measured at 453, 505, 645 and 663 nm, and the contents of carotenoids (β-carotene and lycopene) and chlorophyll *a* and chlorophyll *b* were obtained with the following equations, and expressed in mg per 100 g of dw:

$$\text{β-carotene}\left(\frac{\text{mg}}{100\ \text{mL}}\right) = 0.216 \times \text{A663} - 1.220 \times \text{A645} - 0.304 \times \text{A505} + 0.452 \times \text{A453}$$

Equation 3: β-carotene content calculation.

$$\text{Lycopene}\left(\frac{\text{mg}}{100\ \text{mL}}\right) = -0.0458 \times \text{A663} + 0.204 \times \text{A645} - 0.304 \times \text{A505} + 0.452 \times \text{A453}$$

Equation 4: Lycopene content calculation.

$$\text{Chlorophyll } a\ \left(\frac{\text{mg}}{100\ \text{mL}}\right) = 0.999 \times \text{A663} - 0.0989 \times \text{A645}$$

Equation 5: Chlorophyll *a* content calculation.

$$\text{Chlorophyll } b\ \left(\frac{\text{mg}}{100\ \text{mL}}\right) = -0.328 \times \text{A663} + 1.77 \times \text{A645}$$

Equation 6: Chlorophyll *b* content calculation.

3.6. Volatile organic compounds analysis

Waste-colored bell pepper fruits (100 mg) were extracted with ethanol (80%, 1 mL) in an ultrasonic bath for 1 h. After centrifugation for 10 min at 1500 rpm, the supernatants were filtered through 0,2 pm cellulose filters (Agilent Technologies, Santa Clara, California, USA) and store at 4ºC until use. All the analyses were performed in triplicates.

Agilent 8890 gas chromatography (GC) System with mass selective detector (MSD-5977B; Agilent Technologies, USA) was used for profiling volatile compounds in samples, following the methodology described by the authors Aničić et al. [144]. HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness) (Agilent Technologies, USA) is used for chromatographic separations and Helium (99.999%, The Linde Group, Ireland) is used as a carrier gas at a flow rate of 1.6 ml min^{-1}. The temperature of the column was programmed linearly from 40 to 300 °C at a rate of 20 °C min^{-1} and then held isothermally at 240 °C for 10 minutes. The detector temperature was set to 270 °C and the transfer line temperature was heated at 280 °C. Electron Ionization (EI) source set to 280 °C and the mass spectra were collected in positive EI mode (+70 eV). In a *split* mode (20:1), an ethanol extract (1 μL) were injected with a *split* flow of 24 mL/min. The analyses were carried out in SCAN mode, with the compounds being tracked between 45 and 500 amu. Compounds of the reaction mixtures were identified by comparison of their mass spectra and retention times with the NIST05 library. Amounts were expressed as their relative content percentage (%) within the sample.

3.7. Preparation of the hydroethanolic extracts

The hydroethanolic extracts from waste-colored bell pepper fruits including their seeds and stalks, were prepared by mixing 1 g of the sample with 30 mL of ethanol:water mixture (80:20, v/v) at room temperature during 1h, followed by filtration through a Whatman filter paper No. 4. The remain residue was re-extracted with an additional portion of ethanol:water mixture and the combined extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and stored in the fridge until further analysis.

3.8. Phenolic compounds characterization

The hydroethanolic extracts prepared above of the selected waste bell pepper fruits including their stalks and seeds were dissolved in 20% aqueous ethanol at 5 mg/mL and filtered through a 0.22-µm disposable LC filter disk. Chromatographic analysis were performed in a Dionex Ultimate 3000 HPLC (ThermoScientific, San Jose, California, USA) system equipped with a diode array detector coupled to an electrospray ionization mass detector (LC-DAD-ESI/MS^n), a quaternary pump, an auto-sampler (kept at 5ºC), a degasser and an automated thermostatted column compartment. Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2C18 (3 µm, 4.6 mm × 150 mm, Waters, Mil-ford, Massachusetts, USA) column thermostatted at 35ºC.

The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% B (5 min), 15% B to 20% B (5 min), 20–25% B (10 min), 25–35% B (10 min), 35–50% B (10 min), and re-equilibration of the column, using a flow rate of 0.5 mL/min. Double online detection was carried out in the DAD using 280 and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet.

MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, California, USA) equipped with an ESI source. Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 5 kV, a source temperature of 325ºC, a capillary voltage of -20 V. The tube lens offset was kept at a voltage of −66 V. The full scan covered the mass range from m/z

100–1500. The collision energy used was 35 (arbitrary units). Data acquisition was carried out with Xcalibur® data system (ThermoFinnigan, San Jose, California, USA).

The phenolic compounds were identified by comparing their retention times, UV--vis and mass spectra with those obtained from standard compounds, when available. Otherwise, compounds were tentatively identified by comparing the obtained information with available data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of the most similar available standard. The results were expressed as mg/g of extract.

3.9. Assessment of antioxidant and cytotoxic activities

3.9.1. Assessment of the capacity to inhibit the formation of thiobarbituric acid reactive substances (TBARS)

The hydroethanolic extracts obtained from waste bell pepper fruits including their stalks and seeds were re-dissolved in water and subjected to dilutions from 10 to 0.1562 mg/mL. The lipid peroxidation inhibition in porcine brain cell homogenates was evaluated by the decrease in TBARS as described by Spréa et al. [141]; the color intensity of malondialdehyde – thiobarbituric acid (MDA–TBA) was measured at 532 nm. The inhibition ratio (%) was calculated using the formula shown in **Equation 7**, where A and B correspond to the absorbance of the control and extract sample, respectively. Trolox (Sigma-Aldrich, St. Louis, Missouri, USA) was used as positive control, a water-soluble analog of vitamin E widely used as an antioxidant in biochemical assays. The results were expressed in EC_{50} values (mg/mL, sample concentration providing 50% of antioxidant activity).

$$\text{Inhibition ratio (\%)} = \frac{\mathrm{A} - \mathrm{B}}{A} \times 100$$

Equation 7: Inhibition ratio calculation in TBARS assay

3.9.2. Cytotoxicity activity

The cytotoxicity effects of the extracts were evaluated by the sulforhodamine B assay against non-tumor African green monkey kidney cell line (VERO), seeded in 96-well plates, with a final density of 1.0×10^4 cell/ well, and fixation was allowed for 24h. Afterwards, the cells were tested with different concentrations of the extracts, ranging from 400 to 6.5 µg/mL, and incubated for 48 h, as described by Spréa et al. [141]. Ellipticine (Sigma-Aldrich, St. Louis, Missouri, USA) was used as a positive control. Results were expressed as GI_{50} values, relative to the extract concentration (µg/mL) that caused 50% of cell growth inhibition.

3.10. Antimicrobial activity

3.10.1. Antibacterial activity

The antibacterial activity was evaluated by the by the broth microdilution method coupled to the rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay. The extracts were tested against five Gram-negative bacteria, namely, *Enterobacter cloacae* (ATCC 49741), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica subsp* (ATCC 13076), *Yersinia enterocolitica* (ATCC 8610) and three Gram-positive bacteria, namely *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19111) and *Staphylococcus aureus* (ATCC 25923). These bacterial strains (food contaminants) were purchase at Frilabo, Porto, Portugal. The bacteria were incubated at 37 ºC, in an appropriate fresh medium, for 24 h before analysis to maintain the exponential growth phase.

Moreover, the extracts were also tested against clinical isolates microorganisms obtained from patients hospitalized in various departments at the Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal). Five Gram-negative bacteria *Escherichia coli* (VRU12881), *Proteus mirabilis* (VRU17684), *Klebsiella pneumoniae* (VRI17214), *Pseudomonas aeruginosa* (VRU14123) and *Morganella morganii* (VRU14272), and three Gram-positive bacteria *Enterococcus faecalis* (VRU14041), *Listeria monocytogenes* (VRU17684), and methicillin-resistant *Staphylococcus aureus* (MRSA; VRI17654) were tested.

The positive controls were prepared with TSB (tryptic soy broth) and each inoculum, as well as another with medium, antibiotics, and bacteria. Ampicillin and streptomycin were used for all ATCC bacteria tested, and methicillin was also used for *Staphylococcus aureus*. For clinical isolates, ampicillin was used for all tested bacteria, while vancomycin was used for Gram-positive, and imipenem was used for Gram-negative bacteria.

3.10.1.1. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was determined by the colorimetric microbial viability based on the reduction of the INT colorant (0.2 mg/mL; Panreac AppliChem, Barcelona, Spain). Firstly, the samples were dissolved in 5% (v/v) Dimethyl sulfoxide (DMSO) and 95% of autoclaved distilled water to give a final concentration of 20 mg/ mL for the stock solution. Then, 90 µL of this concentration was added in the first well (96-well microplate) in duplicate with 100 µL of TSB. In the remaining wells 90 µL of TSB medium were added. Afterwards, the samples were serially diluted to obtain the concentration ranges (10 to 0.03125 mg/mL). To finish, 10 µL of inoculum (standardized at 1.5×10^6 Colony Forming Unit (CFU)/mL) was added at all the well assuring the presence of 1.5×10^5 CFU. The microplates were incubated at 37°C for 24 h. The MIC of samples was detected following addition (40 µL) of 0.2 mg/mL INT and incubation at 37°C for 30 min. MIC was defined as the lowest concentration that inhibits the visible bacterial growth determinate by changing the coloration from yellow to pink if the microorganisms are viable. For the determination of MBC, 10 µL of liquid from each well that showed no change in color was plated on solid medium, Blood agar (7% sheep blood), and incubated at 37°C for 24 h. The lowest concentration that yielded no growth determines the MBC. MBC was defined as the lowest concentration required to kill bacteria. The results of MIC and MBC were expressed in mg per mL of the aqueous solutions of the lyophilized waste bell pepper samples.

3.10.2. Antifungal activity

Antifungal activity was performed according to the methodology described by Heleno et al. [145]. Two fungal strains, namely *Aspergillus brasiliensis* (ATCC 16404) and *Aspergillus fumigatus* (ATCC 204305) were used. The organisms were obtained from Frilabo, Porto, Portugal. The micromycetes were maintained on malt agar and the cultures stored at 4 °C and were further placed in new medium and incubated at 25ºC for 72h. In order to investigate the antifungal activity, the fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL per well. The samples were first of all dissolved in 5% (v/v) dimethyl sulfoxide (DMSO) and 95% of autoclaved distilled water to give a final concentration of 10 mg/mL for the stock solution. Afterwards, 90 µL of this concentration was added in the first well (96-well microplate) in duplicate with 100 µL of malt Extract Broth (MEB). The microplates were incubated for 72 h at 28ºC. The lowest concentration with no visible growth (using a binocular microscope) were defined as the MIC. The minimum fungicidal concentrations (MFCs) were determined by serial sub-cultivation of 2 µL in microtiter plates containing 100 pl of malt broth per well and further by incubation for 72 h at 28ºC. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. Commercial fungicide ketoconazole (Frilabo, Porto, Portugal), was used as positive control. The results of MFC were expressed as mg per mL of the aqueous solutions of the lyophilized waste bell pepper samples.

3.11. Statistical analysis

All assays were performed in triplicate (n = 3) and the values were expressed as mean ± standard deviation (SD). Statistical tests were performed with a significance level of 5%, using the statistical software SPSS (IBM SPSS Statistics for Windows, Version 23.0; IBM Corp., Armonk, New York, USA). The differences between the samples were evaluated by unidirectional analysis of variance (ANOVA) and the means were compared according to the Tukey's HSD test (p = 0.05). A Student's t-test was used when only two samples were assessed.

4. Results and discussion

4.1. Proximate composition

Since the main objective of this study was to evaluate the potential of bell peppers residues (*C. annuum* var. *grossum*) as a source of valuable nutritional compounds which could be used as natural ingredients in different industries, a complete evaluation of the nutritional profile of the waste-colored bell pepper fruits was performed, and the results are shown in **Table 2**. The results showed statistically significant differences ($p < 0.05$) among most of the analyzed components.

Waste-colored bell pepper fruits hold high water content, with waste-green bell peppers presenting the highest content (92.5±0.5 % fw), followed by orange (90.7±0.5% fw) and red (89.4±0.9% fw) fruits. Ozgur et al. [146] also indicated a similar observation, reporting moisture contents in fresh green and red peppers fruits of 94.5±0.1 g/100 g and 92.4±0.5 g/100 g, respectively.

Carbohydrates were the main present macronutrients in all waste-colored bell pepper fruits, with waste-red bell peppers holding the highest content (88.20±0.06 g/100g dw), followed by the green (87.4±0.8 g/100g dw) and orange (86.7±0.3 g/100g) varieties. According to Olatunji et al. [147], in their study on the nutritional characteristics of different varieties of mature pepper fruits, *C. annuum* var. *grossum* presented lower average carbohydrates content (46.22% dw) compared to the present study. However, Cisternas-Jamet et al. [148] reported similar carbohydrate average concentrations in red (88.36±0.58 g/100g dw) and green (90.64±0.50 g/100g dw) bell pepper. These differences in results could be related to the cultivation region and pepper fruit genotype, as suggested by Kim et at. [149] in their study on the effect of genetic and environmental factors on the nutritional characteristics of different varieties of red pepper fruits (*C. annuum*). Moreover, it has been reported that growing conditions, postharvest processing, and the maturity stage of the fruit have an influence on the macronutrient contents of bell pepper fruits [38].

Regarding protein content, waste-orange bell pepper registered the highest content (11.8±0.3 g/100g dw), followed by green (11±1 g/100g dw) and red (9.9±0.1 g/100g dw) varieties. These results are in line with Olatunji et al. [147], who reported similar average crude protein content (14.87%) in mature *C. annuum* var. *grossum* fruits. In addition, Kim

et al. [149] also recorded similar protein average contents (11.9%-14% dw) in red bell pepper fruits.

Regarding ash content, waste-red bell pepper presented the highest amount (11.2±0.8 mg/100g dw), followed by orange (10.2±0.4 mg/100g dw) and green (10±1 mg/100g dw) varieties. Olatunji et al. [147] reported higher average contents of total ash (7.63% dw) in mature *C. annuum* var. *grossum* fruits. Similarly, Cisternas-Jamet et al. [148] reported higher total ash average concentrations in red (9.5±0.6 g/100g) and green (8.1±0.5 g/100g) bell peppers when compared to the present study. Environmental and genetic factors could the main contributors to this discrepancy in results.

Regarding fat content, the results indicated that waste-red bell peppers presented the highest average content (1.88±0.02 g/100g dw), followed by green (1.81±0.05 g/100g dw) and orange (1.51±0.09 g/100g dw) fruits. By contrast, Olatunji et al. [147] reported higher total fat average contents (5.76%) in mature *C. annuum* var. *grossum* fruits. However, the study performed by Cisternas-Jamet et al. [148] reported similar fat average contents in red (1.93±0.02 g/100g dw) and green (0.98±0.02 g/100g dw) bell peppers compared to the present study. Following this trend, El-Ghorab et al. [150] reported similar fat average content (1.28±0.10 g/100g dw) in the red "No.1" cultivar of Pakistani bell pepper fruits.

Lastly, waste-red bell peppers presented the highest energy value (409.35±0.07 kcal/100g dw), followed by green (409±0.2 kcal/100g dw) and orange (407.5±0.3 kcal/100g dw) fruits. Olatunji et al. [147] reported lower energy values (296.17 kcal/100g dw) in mature *C. annuum* var. *grossum* fruits when compared to the present study. Following this trend, Kefale et al. [151] also indicated lower energy value (249±5 kcal/100g dw) in red bell peppers of *C. annuum* when compared with the present study. As already mentioned, the nutritional composition of bell pepper fruits can vary according to the variety, ripening stage, growing conditions, cultivation region, postharvest processing and storage conditions [110], which hence could explain the differences in phytochemical results obtained in the present study with the referred other authors.

Table 2: Proximate composition of waste-colored bell peppers fruits.

Proximate composition	Waste-colored bell peppers		
	Orange	Red	Green
Moisture (%, fw)	90.7±0.5b	89.4±0.9bc	92.5±0.5a
Fat (g/100g dw)	1.51±0.09c	1.88±0.02a	1.81±0.05ab
Proteins (g/100g dw)	11.8±0.3a	9.9±0.1bc	11±1ab
Ash (mg/100g dw)	10.2±0.4ab	11.2±0.8a	10±1bc
Carbohydrates (g/100g dw)	86.7±0.3bc	88.20±0.06a	87.4±0.8ab
Energy (kcal/100g dw)	407.5±0.3bc	409.35±0.07a	409.0±0.2ab

Results are expressed mean ± standard deviation (n = 3). fw – fresh weight. dw – dried weight. Statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters.

4.2. Hydrophilic compounds

4.2.1. Free sugars

Soluble sugars are important components of plant foods being responsible for flavor attributes that enhance the acceptability of this type of products [152]. The identified free sugars of waste-colored bell pepper samples are present in **Table 3**. Fructose, glucose, sucrose, and trehalose were identified in the all of the analyzed waste-colored bell pepper fruits, with statistically significant differences ($p < 0.05$) being detected among all the identified free sugars.

Fructose was the main free sugar in waste-orange and red bell pepper fruits (21.1±0.7 g/100g dw and 17.1±0.9 g/100g dw, respectively) followed by glucose (15.0±0.7 g/100g dw and 14.2±0.7 g/100g dw, respectively), sucrose (2.0±0.4 g/100g dw and 2.1±0.1 g/100g dw, respectively), and trehalose (0.63±0.04 g/100g dw and 0.47±0.07 g/100g dw, respectively). Differently, waste-green bell peppers presented glucose as the main free sugar (10.03±0.07 g/100g dw), followed by fructose (8.25±0.02 g/100g dw), sucrose (1.31±0.03 g/100g dw) and trehalose (0.32±0.01 g/100g dw). Overall, waste-orange bell peppers showed greater concentrations of total free sugars (39±2 g/100g dw), followed by red bell peppers (34±2 g/100g dw) and in last green bell peppers (19.0±0.1 g/100g dw). These results are in line with Zamljen et al. [153] who investigated metabolic variation among different peppers fruits cultivars from *Capsicum* spp. genus, reporting similar average contents of fructose (14.7 to 17.9 g/100g dw), glucose (14.4 to 20.2 g/100g dw), and sucrose (1.73 to 2.71 g/100g dw) of red *C. annuum* fruits. However, Kim

et al. [154] reported higher average contents of fructose (24.15±0.82 g/100g dw and 16.48±0.19 g/100g dw), glucose (21.52±0.74 g/100g dw and 18.08±0.33 g/100g dw) and sucrose (1.39±0.09 g/100g dw and 7.16±0.26 g/100g dw) in red and green pepper fruits (respectively) when compared with the present study. In general, sugars in fruits increase with ripening until they reach their maturity [155]. These differences in results may be due to the bell pepper genotype tested, geographical situation, and/or fruit ripeness. Moreover, agricultural practices and environmental conditions while growing bell peppers can also have an influence on their content of free sugars [149].

Table 3: Free sugars of waste-colored bell peppers fruits.

Free sugars (g/100g dw)	Waste-colored bell peppers		
	Orange	Red	Green
Fructose	21.1±0.7a	17.1±0.9b	8.25±0.02c
Glucose	15.0±0.7a	14.2±0.7b	10.03±0.07c
Sucrose	2.0±0.4ab	2.1±0.1a	1.31±0.03c
Trehalose	0.63±0.04a	0.47±0.07b	0.32±0.01c
Total	39±2a	34±2b	19.91±0.10c

Results are expressed mean ± standard deviation (n = 3). dw – dried weight. Statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters.

4.2.2. Organic acids

Organic acids play a vital role in maintaining the nutritional value and sensory quality of foods and are also an important class of food additives, including their use as preservatives, acidity regulators, antioxidants, with a wide range of applications [156]. Data on the identified organic acids in waste-colored bell pepper samples are present in **Table 4**. Oxalic, malic, shikimic, and ascorbic acids were identified in the all of the analyzed waste-colored bell pepper fruits, with statistically significant differences ($p < 0.05$) being detected among all the identified compounds.

Oxalic acid was the most abundant organic acid occurring in waste-orange and red bell peppers (6.07±0.01 g/100g dw and 5.68±0.01 g/100g dw, respectively), followed by malic acid (5.99±0.01 g/100g dw and 5.22±0.02 g/100g dw, respectively). Regarding waste-green bell peppers, malic acid was the main compound identified (8.29±0.02 g/100g dw), followed by oxalic acid (5.42±0.03 g/100g dw). Ascorbic acid comes next, being identified in waste-green bell peppers with major content (1.63±0.01 g/100g dw),

followed by red (1.46±0.01 g/100g dw) and orange (1.40±0.01 g/100g dw) fruits. Lastly, shikimic acid presented the lowest average concentrations in waste-colored bell pepper samples, with the green variety presenting the highest content (1.27±0.06 g/100g dw), followed by red (0.95±0.01 g/100g dw) and orange (0.78±0.02 g/100g dw) bell pepper fruits. Overall, waste-green bell pepper fruits presented the highest content of total organic acids (16.62±0.08 g/100g dw), followed by orange (14.24±0.01 g/100g dw) and red (13.32±0.04 g/100g dw) bell pepper fruits. Comparing the obtained results with other authors, such as Kye et al. [157], who studied the phytochemical profile of different fruit colors and shapes of *C. annuum*, the reported average concentrations of oxalic (1.40±0.03 g/100g dw and 1.99±0.02 g/100g dw) and malic (3.94±0.13 g/100g dw and 3.89±0.13 g/100g dw) in orange and red "conical type" pepper fruits (respectively) probe to be relatively lower when compared with the present study. In addition, these authors also indicated higher average concentrations of ascorbic acid in the same two bell peppers with the orange "conical type" variety presenting the highest content (13.6±0.4 g/100g dw and 10.4±0.7 g/100g dw). Similarly, Ozgur et al. [146] previous mentioned study also identified red pepper fruits with the highest ascorbic acid average content (0.108±0.006 g/100g dw) followed by green pepper fruits (0.088±0.002 g/100g dw), however these results prove to be relatively lower when compared with the present study. It has been reported that the content of organic acids in pepper fruits are influenced by the growing region, climate and species, as well as their concentration in pepper fruits is highly dependent on the stage and part of ripening [156].

Table 4: Organic acids of waste-colored bell peppers fruits.

Organic acids (g/100g dw)	**Waste-colored bell peppers**		
	Orange	**Red**	**Green**
Oxalic acid	6.07±0.01a	5.68±0.01b	5.42±0.03c
Malic acid	5.99±0.01b	5.22±0.02c	8.29±0.02a
Shikinic acid	0.78±0.02c	0.95±0.01b	1.27±0.06a
Ascorbic acid	1.40±0.01c	1.46±0.01bc	1.63±0.01a
Total	14.24±0.01b	13.32±0.04c	16.62±0.08a

Results are expressed mean ± standard deviation (n = 3). dw – dried weight. Statistically significant differences (p < 0.05) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD) and are indicated by different letters.

4.3. Lipophilic compounds

4.3.1. Fatty acids

Lipids and fatty acids compose a small portion of the edible part of peppers. However, they play important roles in the structure of the pepper fruits, which is expected, considering several bioactive compounds, vitamins and carotenoids are liposoluble [149]. A total of fifteen fatty acids were identified in waste-colored bell pepper fruits and the results are present in **Table 5**, with statistically significative differences ($p < 0.05$) being detected between the different classes of fatty acids in all of the analyzed samples.

Linoleic (C18:2n6c), palmitic (C16:0), and α-linolenic (C18:3n3) acids were the major fatty acids present in the waste-colored bell pepper fruit samples, with linoleic acid standing out as the one present in higher average concentrations (44.3±0.01%, 43.9±0.1%, 42.8±0.95% for waste-red, green, and orange bell pepper fruits, respectively). Palmitic acid comes next, with the waste-orange bell pepper fruits presenting the highest average content (21.0±0.1%), followed by red (20.61±0.01%) and green (18.4±0.5%) varieties. The α-linolenic acid, in turn, was mostly identified in waste-green pepper fruits (19.4±0.2), followed by the orange (17.0±0.5%) and red (14.88±0.01%) varieties. These results are in line with other authors. For instance, Saini et al. [86] reported similar average concentrations of linoleic (36.74% and 57.83%), palmitic (21.22% and 14.82%), and linolenic (21.64% and 17.20%) acids in the orange and red fruits between six colored sweet peppers (*C. annuum* L.). Following this trend, Martinez et al. [158] who investigated the profile of fatty acids in three *C. annuum* varieties also reported similar average concentrations of linoleic (41.47±0.09% dw and 39.79±0.36%), palmitic (17.09±0.14% and 19.56±0.23%), and linolenic (17.08±0.03% and 21.69±0.19%) acids in red and green fruits of "Arnoia" peppers respectively, when compared with the present study. Moreover, regarding their classification, it is important to emphasize that polyunsaturated fatty acids (PUFAs) were found in higher proportions in all of the analyzed waste-colored bell pepper fruits, with the green variety presenting the highest content (63.65±0.03%), followed by orange (60±1%) and red (59.4±0.1%) fruits. Saturated fatty acids appear next, where waste-orange bell pepper fruit exhibited the major concentration (37±1%), followed by the green (34.9±0.4%) and red (34.3±0.1) varieties, and finally, monounsaturated fatty acids (MUFA), which are only present in

much lower concentrations in all bell pepper fruits. These observations are in line with previous mentioned Saini et al. [86] and Martinez et al. [158] studies, in which PUFA were also identified by the as the predominant class, and MUFA as the least identified class of fatty acids in *C. annuum* fruits. Although bell pepper fruits are not a major source of lipids in food, their high contents of polyunsaturated fatty acids (linoleic and linolenic acid) may have positive implications for human health, as these fatty acids are responsible for reducing the frequency of cardiovascular disease and type 2 diabetes [159].

Table 5: Fatty acids composition of waste-colored bell peppers fruits.

Fatty acids	**Waste-colored bell peppers**		
(relative percentage %)	**Orange**	**Red**	**Green**
Lauric (C12:0)	0.51±0.01	0.84±0.01	0.116±0.004
Myristic (C14:0)	2.1±0.1	2.80±0.01	1.41±0.01
Pentadecylic (C15:0)	0.175±0.004	0.15±0.01	0.16±0.01
Palmitic (C16:0)	21.0±0.1	20.61±0.01	18.4±0.5
Palmitoleic (C16:1)	0.68±0.01	1.81±0.01	0.52±0.02
Margaric (C17:0)	0.36±0.03	0.28±0.01	0.48±0.01
Stearic (C18:0)	5.6±0.1	5.20±0.01	4.9±0.1
Oleic (C18:1n9c)	2.4±0.2	4.50±0.01	1.19±0.01
Linoleic (C18:2n6c)	42.8±0.9	44.3±0.01	43.9±0.1
α-linolenic (C18:3n3)	17.0±0.5	14.88±0.01	19.4±0.2
Arachidic (C20:0)	1.09±0.08	1.94±0.01	2.88±0.08
Eicosadienoic (C20:2)	0.41±0.01	0.237±0.001	0.36±0.01
Behenic (C22:0)	2.1±0.7	1.40±0.01	2.18±0.03
Tricosylic (C23:0)	0.74±0.02	0.307±0.001	0.56±0.01
Lignoceric (C24:0)	3.07±0.05	0.73±0.01	3.86±0.07
SFA	37±1a	34.3±0.1bc	34.9±0.4b
MUFA	3.1±0.2b	6.31±0.01a	1.71±0.01c
PUFA	60±1b	59.4±0.1bc	63.65±0.03a

Results are expressed mean ± standard deviation (n = 3). nd – not detected. SFA – Saturated fatty acids. MUFA – Monounsaturated fatty acids. PUFA – Polyunsaturated fatty acids. Statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters.

4.3.2. Tocopherols

The analysis of tocopherols indicated the presence of four isoforms, α-, β-, γ-, and δ-tocopherols, in waste-colored bell peppers in the present study (**Table 6**), showing statistically significant differences ($p < 0.05$) among the different varieties.

The α-tocopherol was the most prevalent isoform in all samples, in which waste-orange bell pepper fruits showed the highest average concentration (11.4±0.3 mg/100g dw), followed by the red (9.45±0.09 mg/100g dw) and green (7.24±0.01 mg/100g dw) varieties. This tocopherol isoform is regarded as the one who exhibits the highest vitamin E and antioxidant activities among tocopherols [160]. Similarly, β-tocopherol was mostly identified in waste-orange fruits (1.21±0.02 mg/100g dw), followed by red (0.76±0.02 mg/100g dw) and green (0.40±0.01 mg/100g dw) bell pepper fruits. Regarding γ-tocopherol, waste-red bell peppers showed the highest amount (0.48±0.01 mg/100g dw), followed by orange (0.44±0.01 mg/100g dw) and green (0.39±0.01 mg/100g dw) varieties. Lastly, the average concentrations for δ-tocopherol were the lowest, accounting the waste-red bell pepper fruits with 0.19±0.01 mg/100g dw, orange fruits with 0.12±0.01 mg/100g dw and green fruits with 0.06±0.01 mg/100g dw. Moreover, it is important to emphasize that waste-orange bell peppers presented the highest content of total tocopherols (13.1±0.4 mg/100g dw), followed by red (10.88±0.07 mg/100g dw) and green (8.10±0.01 mg/100g dw) varieties. Comparing these results with other authors, Kye et al. [157] reported average concentrations of α-tocopherol (13.6±0.4 mg/100g), β-tocopherol (1.14±0.02 mg/100g dw), γ-tocopherol (0.818±0.002 mg/100g dw), and δ-tocopherol (1.41±0.01 mg/100g dw) in orange pepper fruits prove to be slightly higher when compared with the present study. In addition, these authors also indicated higher average concentrations of α-tocopherol (16±2 mg/100g), β-tocopherol (1.42±0.07 mg/100g dw), γ-tocopherol (0.836±0.008 mg/100g dw), and δ-tocopherol (1.38±0.06 mg/100g dw) in red pepper fruits, denoting as well from their results that this variety presented the highest content of total tocopherols, as by contrast with the finding in the present study. In a study conducted by Bhandari et al. [161] red pepper fruits showed slightly higher values of α-tocopherol (17.48 mg/100g dw), β-tocopherol (3.6 mg/100g dw), γ-tocopherol (4.53 mg/100g), and δ-tocopherol (2.7 mg/100g dw) than in our study. These differences in the results could be related to physicochemical characteristics of the waste-colored bell pepper used as well as several environmental and genetic factors since tocopherol content in pepper fruits has been reported to be influenced by the genotype, maturity stage, harvesting time, postharvest handling/processing, and storage conditions [80].

Table 6: Tocopherols composition of waste-colored bell peppers fruits.

Tocopherols (mg/100g dw)	Waste colored bell peppers		
	Orange	Red	Green
α-tocopherol	11.4±0.3a	9.45±0.09b	7.24±0.01c
β-tocopherol	1.21±0.02a	0.76±0.02b	0.40±0.01c
γ-tocopherol	0.44±0.01ab	0.48±0.01a	0.39±0.01c
δ-tocopherol	0.12±0.01b	0.19±0.01a	0.06±0.01c
Total	13.1±0.4a	10.88±0.07b	8.10±0.01c

Results are expressed mean ± standard deviation (n = 3). dw – dry weight. Statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters.

4.3.3. Pigments

The analysis of pigments in the waste-colored bell pepper fruits allowed the identification of four equivalents: β-carotene, lycopene, chlorophyll *a*, and chlorophyll *b* (**Table 7**). Statistically significant differences ($p < 0.05$) were detected among the different varieties.

β-carotene stood out as the most prevalent carotenoid in all waste-colored bell pepper samples. This fat-soluble pigment is well known for having several important biological functions and great commercial value due to its uses in food, pharmaceutical products, cosmetics and textiles [162]. Waste-orange bell pepper fruits presented the highest β-carotene average concentration (44.9±0.3 mg/100g dw), followed by the red (16±4 mg/100g dw) and green (5.8±0.3 mg/100g dw) varieties. According to Kye et al. [157] the β-carotene average concentrations reported in orange (3.14±0.26 mg/100g dw) and red pepper fruits (4.46±0.35 mg/100g dw) prove to be relatively lower when compared with the present study. Similarly, Guil-Guerrero et al [163] also reported lower β-carotene contents in orange (1.88±2.9 mg/100g dw), red (ranging from 9.45±1.17 mg/100g dw to 13.48±1.53 mg/100g dw) and green (ranging from 0.02±0.02 mg/100g dw to 2.8±0.3 mg/100g dw) varieties, when compared with the present study.

As for lycopene, the highest average concentration was identified in waste-red (7.5±0.1 mg/100g dw) followed by waste-green (0.46±0.06 mg/100g dw) bell pepper fruits, however it was not detected in waste-orange bell pepper fruits. Ozgur et al. [146] previously mentioned higher average lycopene contents in red (12.39±0.02 mg/100g dw) and green (17.16±0.01 mg/100g dw) pepper samples. In addition, by contrast with the

present study, Razola-Díaz et al. [164] detected lycopene contents in orange bell pepper fruits (*C. annuum* var. Kioto) from two different companies ranging between 0.026-0.082 mg/100g dw.

Lastly, for both chlorophyll *a* and chlorophyll *b*, waste-green bell pepper fruits showed the highest average concentration (15.4±0.8 mg/100g dw and 15.4±0.8 mg/100g dw, respectively) followed by red (0.45±0.03 mg/100g dw and 1.2±0.7 mg/100g dw, respectively) and orange (0.22±0.09 mg/100g dw and 0.61±0.01 mg/100g dw, respectively) fruits. Żurawik et al. [165] study on antioxidant properties of Polish and Bulgarian pepper cultivars (*C. annuum*), reported higher average concentrations for both chlorophyll *a* (ranging from 5.25-29.25 mg/100g dw and 0.73-1.83 mg/100g dw) and chlorophyll *b* (ranging from 2.3-17.5 mg/100g dw and 0.78-2.64 mg/100g dw) in green and colored bell pepper fruits (respectively) when compared with results in the present study. The profile and concentration of carotenoids differ among species, depending on factors such as growing conditions, maturity stage and processing practices [69], which could hence explain the differences observed concerning the results obtained in the present study with other authors referred.

Table 7: Pigments composition of waste-colored bell peppers fruits.

Pigments (mg/100g dw)	Waste-colored bell peppers		
	Orange	**Red**	**Green**
β-carotene	44.9±0.3a	16±4b	5.8±0.3c
Lycopene*	nd	7.5±0.1	0.46±0.06
Chlorophyll *a*	0.22±0.09c	0.45±0.03b	15.4±0.8a
Chlorophyll *b*	0.61±0.01c	1.2±0.7b	5.6±0.7a

Results are expressed mean ± standard deviation (n = 3). nd – not detected. Statistically significant differences (p < 0.05) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD), and are indicated by different letters. * Significant differences (p < 0.001) between the two samples were assessed by a Student's *t*-test.

4.4. Phenolic compounds

The tentative identification of the phenolic compounds found in the waste-colored bell peppers and their by-products hydroethanolic extracts, as well as the retention times (Rt), maximum absorbance (λ_{max}), pseudomolecular ion ([M-H]-), main ion fragments (MS^2), and quantification of each compound are presented in **Table 8**. Individual phenolic compounds were tentatively identified according to the data presented and in comparison

with available standard compounds and/or existing literature. Among the twenty-two compounds spotted, seven were phenolic acids, fourteen were flavonoids, and one was a stilbene. Regarding phenolic acids and considering the parameters below, the compounds were tentatively identified by comparing their retention times and UV spectrum with available standards as vanillic acid hexoside (**Peak 1**; λ_{max}, 287; [M-H$^-$] at *m/z* 329), syringic acid hexoside (**Peak 2**; λ_{max}, 285; [M-H]$^-$at *m/z* 359), hydroxysinaptic acid hexoside (**Peak 3**; λ_{max}, 281; [M-H$^-$] at *m/z* 403), *p*-coumaric acid hexoside (**Peak 4**; λ_{max}, 291; [M-H$^-$] at *m/z* 325), syringic acid rhamnoside (**Peak 6**; λ_{max}, 281; [M-H$^-$] at *m/z* 361), ferulic acid hexoside (**Peak 7**; λ_{max}, 330; [M-H$^-$] at *m/z* 355), and finally synaptic acid hexoside (**Peak 8**; λ_{max}, 330; [M-H$^-$] at *m/z* 385).

The flavonoids group appear as the most abundant in the prepared waste-colored bell pepper extracts, among which stands out luteolin derivatives, present in greater number than the other identified flavonoids, followed by apigenin and quercetin derivatives in their glycosidic and isomeric forms. Thus, our results allowed the tentative identification of luteolin-6,8-di-C-glucoside (**peak 5;** λ_{max}, 330; [M-H$^-$] at *m/z* 609), apigenin-6,8-di-C-glucoside (**peak 9;** λ_{max}, 339; [M-H$^-$] at *m/z* 593), quercetin-glucopyranoside-rhamnopyranoside **(peak 10;** λ_{max}, 339; [M-H$^-$] at *m/z* 609), luteolin-C-pentosyl-C-hexoside Isomer I and II **(peaks 11** and **12;** λ_{max}, 330; [M-H$^-$] at *m/z* 579), apigenin-6-C-glucoside-8-C-arabinose (schaftoside and isoschaftoside) (**peaks 13** and **14;** λ_{max}, 335; [M-H$^-$] at *m/z* 563), dimethylluteolin-apioside Isomer I and II **(peaks 15** and **16;** λ_{max}, 330 and 331, respectively; [M-H$^-$] at *m/z* 447), apigenin-6-C-β-D-glucopyranoside-8-C-α-L-arabinopyranoside **(peak 17**; λ_{max}, 332; [M-H$^-$] at *m/z* 563), luteolin-*O*-(apiosyl)-hexoside **(peak 19**; λ_{max}, 345; [M-H$^-$] at *m/z* 579), luteolin-O-(apiosyl-acetyl)-hexoside **(peak 20;** λ_{max}, 345; [M-H$^-$] at *m/z* 621), quercetin-3-O.rhamnoside **(peak 21;** λ_{max}, 341; [M-H$^-$] at *m/z* 447), and luteolin-*O*-(apiosyl-malonyl)-hexoside **(peak 22;** λ_{max}, 349; [M-H$^-$] at *m/z* 665) by comparing their retention times and UV spectrum with available standards. Icariside E5 (**peak 17**; λ_{max}, 259; [M-H$^-$] at *m/z* 521) was the only stilbene identified in all of the analyzed parts of waste-colored bell peppers.

The quantitative data of the phenolic compounds tentatively identified in waste-colored bell pepper stalks, fruits, and seeds hydroethanolic extracts (**Table 7**) shows a dissimilar profile of the identified compounds between each part and varieties, with

waste-orange bell pepper stalks presenting the highest total phenolics (3.05±0.02 mg/g) among all the analyzed samples. Also, the results showed a higher prevalence of luteolin-*O*-(apiosyl)-hexoside (0.502 to 0.72 mg/g extract) and luteolin-*O*-(apiosyl-malonyl)-hexoside (0.485 to 0.741 mg/g extract) in the stalks and fruits, while in seeds icariside E5 (0.391 to 0.436 mg/g extract) prevailed. Overall, the seeds from waste-colored bell pepper presented the smallest amount of total phenolic compounds. Abdalla et al. [166] also investigated the phenolic profile of fresh yellow bell, red bell, and Balady green pepper fruits (*C. annuum*) and their results showed some similarities with those from our study, since they also identified phenolic acids and flavonoids as the main classes of phenolic compounds in bell pepper fruits. However, these authors found hesperidin as the predominant phenolic compound in both red and green varieties (1513.13 and 1065.65 μg/g extract, respectively), followed by other compounds in high or moderate concentrations, such as catechin, pyrogallol, luteolin-7-glucose, P-OH-benzoic acid, apigenin-6-rhamnose-8-glucose, rutin, naringin, quercetin, ellagic acid, apigenin-6-arbinose-8-galactose, salicylic, gallic and benzoic acids. Also, in addition to a greater variety of phenolic acids being detected in this study, simple phenols and free flavonoids were also identified in the three varieties analyzed. These differences, in addition to being related to genetic, soil and climate factors, among others, can also be attributed to the fact that, in our study, bell pepper samples already considered waste were used, and are therefore in an advanced state of maturation. Shaha et al. [167] suggested that the accumulation of polyphenols in different peppers is predominantly influenced by the state of maturation and, therefore, this could be the main factor responsible not only for the phenolic profile identified but also for the reduced amounts of these detected in our study. Likewise, Razola-Diaz et al. [168] studied the phenolic profile of whole yellow bell pepper, including the edible portion, seeds, and peduncles, identifying 38 compounds, counting phenolic acids and flavonoids. Of these, phenolic acids predominate, constituting 66-93% of the total phenolic profile, where feruloyl glucose prevailed, followed by sinapic acid-*O*-hexoside and vanillic acid 1-*O*-D-glucopyranosylester. Regarding flavonoids, kaempferol-3-(3”-acetyl-alpha-L-arabinopyranosyl)-glucoside was mostly present, followed by also high amounts of quercetin, luteolin, and apigenin derivatives. Once again, these results may be related, in this case, to the variety and soil-climatic factors, but also to the state of maturation of the samples under investigation.

Table 8: Retention time (Rt), maximum absorption wavelengths in the visible region (λmax), mass spectral data, relative abundances of fragment ions, attempted identification, and quantification (mg/g extract) of phenolic compounds found in waste-colored bell pepper stalks, fruits, and seeds hydroethanolic extracts (mean ± SD, n=3).

Peaks	Rt (min)	λmax (*nm*)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative Identification	Stalk			Fruits			Seeds		
						Orange	Red	Green	Orange	Red	Green	Orange	Red	Green
1	5.19	287	329	167(100)	Vanillic acid hexoside	0.209±0.002	0.206±0.002	0.224±0.005	0.105±0.001	tr	0.103±0.001	0.147±0.001	0.161±0.001	0.130±0.001
2	5.53	285	359	239(31),197(100)	Syringic acid hexoside	0.015±0.001	0.014±0.001	0.014±0.001	0.014±0.001	0.014±0.001	nd	nd	nd	nd
3	6.08	281	403	385(100),223(21),208(9)	Hydroxysinapic acid hexoside	nd	nd	nd	0.013±0.001	nd	nd	nd	nd	nd
4	6.73	291	325	163(100)	p-coumaric acid hexoside	nd	nd	nd	0.008±0.001	nd	0.015±0.001	0.003±0.001	0.002±0.001	0.001±0.001
5	7.33	330	609	591(3),519(22),489(100),399(32),369(39)	Luteolin-6,8-di-*C*-glucoside (Lucenin 2)	0.058±0.001	0.052±0.001	0.087±0.001	0.044±0.001	0.047±0.001	0.053±0.001	nd	nd	nd
6	8.45	281	361	343(31),197(100)	Syringic acid rhamnoside	nd	nd	nd	0.013±0.001	nd	0.014±0.002	nd	nd	nd
7	9.13	330	355	193(100)	Ferulic acid hexoside	0.030±0.001	0.030±0.001	0.030±0.001	0.030±0.001	0.030±0.001	0.030±0.001	0.020±0.001	0.030±0.001	0.030±0.001
8	9.68	330	385	205(100),163(11)	Sinapic acid hexoside	0.013±0.001	nd	nd	0.014±0.001	nd	0.013±0.001	nd	nd	0.013±0.001
9	10.19	339	593	473(100),383(36),353(11)	Apigenin-6,8-di-*C*-glucoside (Vicenin-2)	0.055±0.001	0.056±0.001	0.096±0.001	0.042±0.001	0.050±0.001	nd	nd	nd	nd
10	10.63	339	609	463(100),447(23),301(14),179(10),151(5)	Quercetin-glucopyranoside-rhamnopyranoside	0.464±0.002	0.462±0.001	0.464±0.001	0.461±0.001	0.461±0.001	0.462±0.001	nd	nd	nd
11	11.49	330	579	459(100),369(35),341(12),313(11)	Luteolin-*C*-pentosyl-*C*-hexoside Isomer 1	0.056±0.001	0.05±0.001	0.075±0.001	0.042±0.001	0.044±0.001	nd	nd	nd	nd
12	12.82	330	579	459(100),369(35),341(12),313(11)	Luteolin-*C*-pentosyl-*C*-hexoside Isomer 2	0.054±0.001	0.049±0.001	0.064±0.001	0.043±0.001	0.044±0.001	nd	0.131±0.002	0.108±0.001	0.100±0.001
13	13.21	335	563	473(100),353(13)	Apigenin-6-*C*-glucoside-8-*C*-arabinoside (Schaftoside)	0.044±0.001	0.042±0.001	0.048±0.001	nd	0.042±0.001	nd	0.085±0.001	0.125±0.001	0.111±0.001
14	14.12	335	563	473(100),353(10)	Apigenin-6-*C*-glucoside-8-*C*-arabinoside (Isoschaftoside)	nd	0.043±0.001	0.050±0.001	nd	0.043±0.001	nd	nd	nd	nd
15	14.73	330	447	327(100),299(33),284(21),269(9)	Dimethylluteolin-apioside Isomer I	0.057±0.001	0.048±0.001	0.07±0.01	0.043±0.001	0.044±0.001	nd	nd	nd	nd
16	15.03	331	447	327(100),299(31),284(10)	Dimethylluteolin-apioside Isomer II	0.059±0.001	0.046±0.001	0.071±0.001	0.044±0.001	0.044±0.001	0.048±0.001	nd	nd	nd
17	15.56	332	563	443(100),383(15),353(21)	Apigenin-6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside	0.049±0.001	0.045±0.001	0.058±0.001	0.042±0.001	0.043±0.001	nd	nd	nd	nd
18	16.51	259	521	359(1009,329(23),299(33),283(3)	Icariside E5	0.114±0.001	0.086±0.001	0.058±0.001	0.468±0.001	0.055±0.001	0.056±0.001	0.398±0.003	0.436±0.006	0.391±0.004
19	18.75	345	579	447(100),285(23),241(15),175(5)	Luteolin-*O*-(apiosyl)-hexoside	0.669±0.003	0.502±0.001	0.72±0.001	0.468±0.001	0.466±0.001	0.442±0.001	nd	nd	nd
20	21.74	345	621	579(100),447(33),285(21)	Luteolin-*O*-(apiosyl-acetyl)hexoside	0.438±0.001	0.435±0.001	nd	nd	0.435±0.001	nd	nd	nd	nd
21	22.79	341	447	301(100),151(13)	Quercetin-3-*O*-rhamnoside	nd	nd	nd	0.464±0.001	0.472±0.001	0.461±0.001	nd	nd	nd
22	23.26	349	665	489(100),447(23),285(8)	Luteolin-*O*-(apiosyl-malonyl)hexoside	0.667±0.006	0.485±0.001	0.741±0.003	0.452±0.001	0.485±0.001	0.435±0.001	nd	nd	nd
					Total Phenolic Acids	0.266±0.002a	0.249±0.002b	0.269±0.005a	0.197±0.004c	0.044±0.001g	0.174±0.001f	0.177±0.001e	0.193±0.001c,d	0.174±0.001f
					Total Flavonoids	2.67±0.01a,b	2.315±0.004e	2.55±0.02c	2.14±0.01f	2.72±0.01a	1.90±0.01h	0.216±0.002f	0.233±0.003d	0.211±0.001f,g
					Total Stilbene	0.114±0.01d	0.086±0.001e	0.057±0.001f	0.468±0.001b	0.055±0.001f	0.056±0.001f	0.398±0.003c	0.86±0.01a	0.391±0.004c
					Total Phenolic compounds	3.05±0.02a	2.65±0.01d	2.873±0.02c	2.81±0.01b	2.82±0.01b	2.13±0.001e	0.79±0.01e	0.86±0.01	0.776±0.004e,f

All compounds were quantified using standards, namely vanillic acid (1), syringic acid (2 and 6), sinapic acid (3 and 8), p-coumaric acid (4), luteolin-6-C-glucoside (5, 9, 11-17), ferulic acid (7), quercetin-3-O-glucoside (10, 21), resveratrol (17), and apigenin-7-O-glucoside (19-20, 22). The results are presented as mean ± SD. Different letters present statistically significant differences ($p < 0.05$) according to *t*-students test.

4.5. Volatile organic compounds

The tentative identification of the volatile organic compounds (VOCs) in the waste-colored bell peppers hydroethanolic extracts, as well as the retention times (Rt) and quantification of each compound are presented in **Table 9**. Individual VOCs were tentatively identified according to the data presented and by comparison with available standard compounds and/or existing literature. Thirty-four VOCs were identified in the samples, including eight heterocyclic compounds, seven acids, six esters, three alcohols, two amines, two alkanes, one acetal, one aldehyde, one ether, one glyceride, one ketone, one ketose.

The quantitative data of the VOCs tentatively identified in waste-colored bell pepper fruits shows a dissimilar profile of the identified compounds between each variety, which can be attributed to the biochemical characteristics of the matrix, specifically varying stages of maturation. The main volatile compounds identified in waste-red bell pepper fruits were the dihydroxyacetone (9.49%), glyceraldehyde (6.20%), 4,5,7-trihydroxyoct-2-enoic acid (5.52%), methyl pyruvate (5.46%), and methyl 2-[(2-methoxy-2-oxoethyl)amino] acetate (5.46%). Waste-orange bell pepper fruits, in turn, presented dihydroxyacetone (7.86%) as the major volatile compound, followed by methyl pyruvate (6.93%), 4,5,7-trihydroxyoct-2-enoic acid (5.52%), and methyl 2-[(2-methoxy-2-oxoethyl)amino]acetate (5.46%). Finally, in waste-green bell pepper fruits, glyceraldehyde (8.94%), methyl pyruvate (7.14%), thymine (4.65%), and n-(ethoxycarbonyl)-glycine ethyl ester (4.29%) were the main volatile organic compounds recorded. Forero et al. [94] investigated the VOCs content in two ripening stages (green and red fruits) of chile pepper (*C. annuum* var. *glabriusculum*), with results obtained agreeing with the present study, since they also identified esters (67.67% and 51%), aldehydes and ketones (8.08% and 13%), alcohols (2.66% and 4.5%), and acids (0.89% and 2.2%) in both pepper fruits (respectively). However these authors indicated that compounds such as hexyl isopentanoate, hexyl 2-methylbutanoate, limonene, hexyl isohexanoate, (E)-2-hexenal, isopentyl isopentanoate, and (Z)-3-hexenyl isopentanoate, were the major constituents in both red and green varieties. Another study developed by Korkmaz et al. [169] identified a total of 136 volatile compounds in full ripe pepper fruit (*C. annuum* cv. *Inan3363*) submitted to a sun drying process. The volatiles recorded mainly belonged to the class of terpenoids, aldehydes, alcohols, ketones, esters, and acids, with α-limonene (13.16%), acetic acid (12.67%), n-butyl isobutyrate (7.45%), methyl

caproate (3.97%), p-cymene (3.79%), β-myrecene (3.38%), 3-hydroxy-2-butanone (3.14%), isobutyl butyrate (3.07%), 2-methylpropanal (2.60%), α-terpinene (2.35%), isovaleric acid (2.14%), 1,8-cineole (2.13%), and ethanol (2.09%) classified as major compounds found in the pepper fruits. Qiu et al. [170] suggested that types and contents of volatile substances can vary according to the cultivated species, as well as growing location and development stage of the fruit. Furthermore, VOCs are closely related to aroma, an important feature of bell pepper fruits' flavor and quality. Zhang et al. [95] study on wrinkled green bell peppers identified 2-isobutyl-methoxypyrazine (an heterocyclic compound) as major contributor to aroma among the 19 pepper varieties. In the present study heterocyclic compounds prevailed between the waste-bell pepper samples, being detected 1.2-Dihydro-3H-1.2.4-triazol-3-one, 3-Methyl-2.5-piperazinedione, 2.3-Dihydro-3.5-dihydroxy-6-methyl-4H-pyran-4-one, (S)-5-Hydroxymethyl-2[5H]-furanone, (S)-(+)-2'.3'-Dideoxyribonolactone, 2-Methyl-3-propyloxaziridine, and 2.4(3H.5H)-Furandione. Heterocyclic compounds have been identified as an important volatile components of many fruits, as the odor strength and complexity of these compounds makes them desirable as flavoring ingredients [171].

Table 9: Volatile compounds found in waste-colored bell pepper fruits hydroethanolic extracts (mean ± SD, n=3).

No.	Retention Time	Compound Name	Class	Molecular Formula	Relative percentage (%)		
					Orange	Red	Green
1	4.75	1.1-Dimethoxy-propane	Acetal	C5H12O2	3.20	4.13	3.67
2	4.98	Glyceraldehyde	Aldehyde	C3H6O3	nd	6.20	8.94
3	5.72	Methyl pyruvate	Ester	C4H6O3	6.93	5.46	7.14
4	5.86	2-Hydroxyethyl acetate	Alcohol	C4H8O3	nd	nd	2.07
5	5.89	Dihydroxyacetone	Ketose	C3H6O3	7.86	9.49	5.10
6	5.94	N-Ethyl-4-methyl-2-pentanamine	Amine	C8H19N	nd	nd	2.66
7	6.04	(S)-1.3-Butanediol	Alcohol	C4H10O2	7.86	nd	nd
8	6.26	1.2-Cyclopentanedione	Ketone	C5H6O2	2.37	3.07	2.94
9	6.97	2-Hydroxy-γ-butyrolactone	Ester	C4H6O3	2.01	nd	2.94
10	6.97	2-Propen-1-ol	Alcohol	C3H6O	nd	2.85	nd
11	7.56	1.2-Dihydro-3H-1.2.4-triazol-3-one	HC	C2H3N3O	nd	nd	2.18
12	7.58	3-Methyl-2.5-piperazinedione	HC	C5H8N2O2	nd	nd	2.18
13	7.76	Thymine	HC	C5H6N2O2	3.39	4.07	4.65
14	8.39	2.3-Dihydro-3.5-dihydroxy-6-methyl-4H-pyran-4-one	HC	C6H8O4	nd	2.61	2.51
15	8.61	(S)-5-Hydroxymethyl-2[5H]-furanone	HC	C5H6O3	nd	2.35	2.18
16	8.72	(S)-(+)-2'.3'-Dideoxyribonolactone	HC	C5H8O3	nd	2.08	nd
17	8.89	N-Vinylacetamide	Acid	C4H7NO	nd	2.51	nd
18	8.91	N-Acetyl-cyclobutylamine	Amine	C6H11NO	2.94	nd	nd
19	9.13	Glycerol α-monoacetate	Glyceride	C5H10O4	3.61	nd	nd
20	10.59	2-Oxo-butanoic acid	Acid	C4H6O3	4.38	nd	nd
21	10.59	2-Methyl-3-propyloxaziridine	HC	C5H11NO	4.38	nd	nd
22	10.76	2.4(3H.5H)-Furandione	HC	C4H4O3	nd	nd	2.02
23	10.96	Methyl (E.4S)-4-amino-5-methylhex-2-enoate	Ester	C7H13NO2	2.49	nd	nd
24	10.96	(3-methyl-4-oxobut-2-enyl) acetate	Ester	C7H10O3	2.50	nd	nd
25	11.46	Hexadecane	Alkane	C16H34	nd	nd	2.28
26	11.48	N-(ethoxycarbonyl)-glycine ethyl ester	Ester	C7H13NO4	nd	nd	4.29
27	11.52	2.5-Diethoxy-3-methyl-3-vinylhexane	Alkane	C13H26O2	nd	3.42	nd
28	11.52	Dipropyl ether	Ether	C6H14O	nd	2.41	nd
29	11.61	4.5.7-trihydroxyoct-2-enoic acid	Acid	C8H14O5	5.52	nd	nd
30	11.62	methyl 2-[(2-methoxy-2-oxoethyl)amino]acetate	Ester	C6H11NO4	5.46	nd	nd
31	11.69	3-Deoxy-D-mannonic acid	Acid	C6H12O6	nd	2.41	nd
32	11.69	N.N-Dimethylformamide	Acid	C3H7NO	nd	2.26	nd
33	14.27	(Z.Z)-9.12-Octadecadienoic acid	Acid	C18H32O2	2.35	2.26	nd
34	14.30	(Z.Z.Z)-9.12.15-Octadecatrienoic acid	Acid	C18H30O2	2.21	2.41	2.06

Volatile organic compounds identified in the samples considering relative content above 2.00 %; RT – retention time; HC – heterocyclic compound; nd – not detected.

4.6. Antioxidant and cytotoxicity activities

Data regarding the antioxidant and cytotoxic activities of the hydroethanolic extracts prepared from the waste-colored bell pepper fruits, stalks, and seeds are present in **Table 8**. The results obtained indicate that the extracts of the different parts of the waste-colored bell peppers performed different ranges of antioxidant capacity, with statistically significative differences ($p < 0.05$) being detected among them.

Regarding the extract of the stalks, waste-green bell peppers presented the lowest EC_{50} values (0.36±0.01 mg/mL), indicative of its higher antioxidant activity, followed by the red (0.37±0.01 mg/mL) and orange (0.49±0.02 mg/mL) varieties. Regarding bell pepper fruits' extract, waste-green bell peppers also presented the lowest EC_{50} values (0.44±0.02 mg/mL), followed by the red (0.95±0.02 mg/mL) and orange (1.85±0.05 mg/mL) bell pepper extracts. Lastly, seed extracts from waste-green bell peppers stood out with the lowest EC_{50} values (0.21±0.01 mg/mL), followed by the red (0.86±0.05 mg/mL) and orange (0.89±0.02 mg/mL) varieties. Through these results, it becomes obvious that all parts of the waste-green bell pepper variety performed the best antioxidant activity among all varieties. This observation is also shared by an earlier report study from Thupairo et al. [172], who investigated the effect of solvent extraction (hexane, ethyl acetate, and aqueous-ethanol) on the antioxidant properties (DPPH, FRAP, and ORAC tests) of colored bell peppers (green, red, orange, and yellow). They indicated that the highest antioxidant activity was observed in green bell pepper by all three investigated methods (25.15, 30.15, and 61.96 µmol TE/g, respectively), followed by the red (23.79, 28.12, and 54.81 µmol TE/g, respectively), orange (22.20, 25.20, and 51.12 µmol TE/g, respectively), and yellow (18.63, 19.87, and 51.65 µmol TE/g, respectively) pepper fruits. Authors attributed this behavior to the higher ascorbic acid and phenolic content in green and red bell peppers, especially by *p*-coumaric and ferulic acids. Chávez-Mendoza et al. [173], on the other hand, demonstrated that the ethanolic extract of red bell pepper exhibit slightly higher antioxidant capacity (79.65%) than the green (78%), and orange (70%) fruits while performing DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity assay. Nevertheless, Martí et al. [174] did not find significant changes in the total antioxidant activity between green and red peppers of different cultivars, while performing the ABTS ((ferrylmyoglobin/2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid)) methodology. Differences in results might be partially explained to the methodology assessed in the evaluation of the antioxidant activity, since different

methods evaluate different types of free radicals and mechanisms of action in living organisms, having great dependency on the *Capsicum* cultivar under study as well [175]. Overall, stalk and seed extracts from waste-colored bell peppers showed slightly higher antioxidant potential than the fruit extracts. This observation is also shared by Chen et al. [176] who reported extracts from stalks of red pepper (*C. annuum*) having stronger antioxidant activity than extracts from their fruits, this being evaluated using the superoxide dismutase (SOD) inhibition activity methodology. These authors attributed this behavior with their higher concentrations of phenolic compounds such as chlorogenic acid and *p*-coumaric acid in bell pepper stalks. In addition, Sousa-Sora et al. [177] while investigating the antioxidant activity of bell pepper fruits and seeds using DPPH, ABTS, and FRAP methods indicated that seed extracts exhibited the highest antioxidant capacity (11.32, 89.25, and 9.94 µ-mol TE/g, respectively) than the pulp extracts (2.28, 17.17, and 3.99 µ-mol TE/g, respectively), associating such event with the total phenolic content of seeds (409 mg GAE/g) and pulp (119 mg GAE/g).

Concerning the evaluation of *in vitro* cytotoxicity activity, the highest tested concentration of the extracts prepared from stalks, fruits and seeds of waste bell peppers did not show growth inhibition of non-tumor African green monkey kidney cell line (VERO), which demonstrates the absence of toxicity. This points to a low potential of causing damage in non-tumor cells hence their safe use for human.

Table 10: Antioxidant and hepatotoxicity activities of waste-colored bell peppers fruits including their seeds and stalks hydroethanolic extracts.

Bell pepper extracts	Stalk			Fruits			Seeds			Positive controls
	Orange	Red	Green	Orange	Red	Green	Orange	Red	Green	
Antioxidant activity										**Trolox**
TBARS (EC_{50}, mg/mL)	0.49±0.02e	0.37±0.01g	0.36±0.01gh	1.85±0.05a	0.95±0.02b	0.44±0.02f	0.89±0.02c	0.86±0.05d	0.21±0.01i	0.0054±0.0003
Cytotoxic activity										**Ellipticine**
VERO (GI_{50}, µg/mL)	>400	>400	>400	>400	>400	>400	>400	>400	>400	0.64±0.05

Results are expressed mean ± SD (n = 3). Statistically significant differences ($p < 0.05$) between samples were assessed by a one-way ANOVA, using Tukey's significant difference (HSD) and are indicated by different letters. EC_{50} values (TBARS): extract concentration corresponding to a 50% of antioxidant activity, GI_{50} values (VERO): extract concentration (µg/mL) that caused 50% of cell growth inhibition.

4.7. Antimicrobial activities

4.7.1. Antibacterial activity

The antibacterial activity of the hydroethanolic extracts prepared from waste-colored bell peppers' fruits and their byproducts were evaluated using microorganisms of different profiles (Gram-positive and Gram-negative bacteria) categorized as food contaminants and clinical isolates. The results obtained from the evaluation are present in **Table 11** and **Table 12** respectively. The results were expressed as the minimal inhibitory (MIC) and bactericidal (MBC) concentrations.

The antibacterial results from the assessment against food contaminants indicated that extracts of the fruits stood out, demonstrating inhibitory capacity against the majority of the bacterial strains. Only the bacterial strains *E. cloacae* and *P. aeruginosa* demonstrated to be less susceptible to the extracts of the fruits, being required higher concentrations to inhibit their growth (MIC>10). The best antibacterial results were verified against *Y. enterocolitica* (MIC = 5 mg/mL, in all samples), *B. cereus* (MIC = 5 mg/mL, for orange and green bell peppers), and *S. aureus* (MIC = 5 mg/mL, in all samples). Following, the extracts of the stalks demonstrated inhibitory capacity against the bacterial strains *Y. enterocolitica*, *B. cereus*, *S. aureus*, in which they all revealed to be more susceptible waste-orange and red bell peppers (MIC = 10 mg/mL), and *L. monocytogenes* with all waste-colored bell peppers exhibiting great inhibitory potential (MIC = 5 mg/mL). Lastly, the extracts of the seeds revealed antibacterial activity against four bacterial strains namely *Y. enterocolitica* with waste-red bell peppers being more active (MIC = 2.5 mg/mL), *B. cereus* which demonstrated to be more susceptible to waste-orange bell peppers (MIC = 10 mg/mL), *L. monocytogenes* which demonstrated to be more susceptible to waste-red and green bell peppers (MIC = 10 mg/mL), and *S. aureus* which demonstrated to be more susceptible to waste-orange (MIC = 5 mg/mL) and red (MIC = 10 mg/mL) bell peppers. Moreover, it is convenient to refer that all the extracts from the waste bell pepper fruits and their byproducts proved to be not bactericidal for any bacteria up to the maximum concentration tested (10 mg/mL), and results obtained from all extracts did not show superior antibacterial activity when compared to positive controls streptomicin, methicillin, and ampicillin.

The antibacterial results from the assessment against clinical isolates indicated superior inhibitory capacity of extracts of the fruits when compared with extracts of the stalks and seeds. The best results were spotted against *E. faecalis* and *MRSA*, with waste-

orange and red bell peppers exhibiting the same value for minimal inhibitory concentration (MIC = 5 mg/mL). However, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* bacterial strains demonstrated to be less susceptible to the extracts of the fruits (MIC>10). Following next, the extracts of the stalks revealed antibacterial activity against four bacterial strains. The best results were verified against *L. monocytogenes*, with waste-orange and red bell pepper exhibiting the same minimal inhibitory concentration (MIC = 5 mg/mL), and with waste-orange bell peppers tested against *E. faecalis* (MIC = 5mg/mL). Furthermore, *M. morganii* demonstrated to be more susceptible to stalk extracts of waste-orange bell peppers, and *MRSA* to stalk extracts of waste-red bell peppers. Lastly, the extracts of the seeds revealed antibacterial activity also against four bacterial strains namely *M. morganii* who demonstrated to be more susceptible to waste-orange bell peppers, *E. faecalis* who demonstrated to be more susceptible to the waste-green bell peppers, *L. monocytogenes* with waste-red and green bell peppers being more active, and *MRSA* with waste-orange and red bell peppers being more active. In addition, it is convenient to refer that the stalk extract of waste-orange bell peppers, the extract of the fruits of waste-orange and red bell peppers, and the extract of the seeds of waste-orange bell peppers demonstrated superior inhibitory effects against *M. morganii* when compared to the positive control Ampicillin. However, when tested against the rest of the clinical bacterial isolate strains (*E. coli*, *P. mirabilis*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, *L. monocytogenes* and *MRSA)*, the extracts did not show superior antibacterial activity compared to positive controls (ampicillin, imipenem and vancomycin), also demonstrating to be not bactericidal for any clinical bacterial isolate strain up to the maximum concentration tested (10 mg/mL).

Other authors also investigated the antibacterial activity of extracts obtained from different varieties of *C. annuum*. For instance, Loizzo et al. [178] approached the effects of different varieties of *C. annuum* on foodborne pathogenic organisms reporting that all pepper extracts used inhibited the growth of *S. aureus* and *Listeria monocytogenes*, with *Effix* and *Fantasia* red varieties of peppers fruits being considered the most effective varieties in terms of antimicrobial activity exhibiting MIC between 12.5 and 25.0 mg/mL. Following this trend, Akkoç et al. [179] indicated from their work varying degrees of antimicrobial activity of different red bell peppers, with Kahta bell pepper variety presenting the lowest MIC values against the bacterial strain *S. aureus*. In addition, Ekom et al. [180] study on the antibacterial and therapeutic properties of the *C. annuum* L. fruit extract in an infected wound in a rat model, reported that methanolic extract of the bell

pepper fruits inhibited the growth of all bacteria tested, in which the lowest MIC value of 64 µg/mL was recorded against the clinical isolate of *S. aureus 18*. These authors suggested that *C. annuum* fruits could be used in the treatment of wounds and infectious diseases, explaining that the antibacterial behavior of pepper fruit extracts was linked to its total phenolic, flavonoid, and tannin contents, that can act by inhibiting biofilm formation, ATPases/H+ proton pumps, and dehydrogenase activity, as well as altering the bacterial cell membrane through the leakage of nucleic acids, reducing sugars and total proteins.

Table 11: Antimicrobial activity from waste-colored bell pepper fruits including their seeds and stalks hydroethanolic extracts against food contaminants isolates strains.

	Gram-negative bacteria					Gram-positive bacteria		
Food contaminants	*Enterobacter cloacae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Yersinia enterocolitica*	*Bacillus cereus*	*Listeria monocytogenes*	*Staphylococcus aureus*
	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
Stalks								
Orange	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	10/>10	5/>10	10/>10
Red	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	10/>10	5/>10	10/>10
Green	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	5/>10	>10/>10
Fruits								
Orange	>10/>10	10/>10	>10/>10	10/>10	5/>10	5/>10	10/>10	5/>10
Red	>10/>10	10/>10	>10/>10	10/>10	5/>10	10/>10	10/>10	5/>10
Green	>10/>10	10/>10	>10/>10	10/>10	5/>10	5/>10	10/>10	5/>10
Seeds								
Orange	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	10/>10	>10/>10	5/>10
Red	>10/>10	>10/>10	>10/>10	>10/>10	2.5/>10	>10/>10	10/>10	10/>10
Green	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	>10/>10	10/>10	>10/>10
Positive controls								
Streptomicin (1mg/mL)	0.007/0.007	0.01/0.01	0.06/0.06	0.007/0.007	0.007/0.007	0.007/0.007	0.007/0.007	0.007/0.007
Methicilin (1mg/mL)	n.t./n.t	n.t./n.t	n.t./n.t	n.t./n.t	n.t./n.t	n.t./n.t	n.t./n.t	0.007/0.007
Ampicillin (10mg/mL)	0.15/0.15	0.15/0.15	0.63/0.63	0.15/0.15	0.15/0.15	n.t./n.t	0.15/0.15	0.15/0.15

MIC values correspond to the minimal extract concentration that inhibits the bacterial growth for the maximum tested concentration of 10 mg/mL; MBC - minimal bactericidal concentration; n.t. – not tested.

Table 12: Antimicrobial activity from waste-colored bell pepper fruits including their seeds and stalks hydroethanolic extracts against clinical bacterial isolates strains.

Clinical isolates	Gram-negative bacteria					Gram-positive bacteria		
	Escherichia coli	*Klebsiella pneumoniae*	*Morganella morganii*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*	*Listeria monocytogenes*	*MRSA*
	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
Stalks								
Orange	>10/>10	>10/>10	10/>10	>10/>10	>10/>10	5/>10	5/>10	>10/>10
Red	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	5/>10	10/>10
Green	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	10/>10	>10/>10
Fruits								
Orange	10/>10	>10/>10	10/>10	>10/>10	>10/>10	5/>10	10/>10	5/>10
Red	>10/>10	>10/>10	10/>10	>10/>10	>10/>10	5/>10	10/>10	5/>10
Green	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	10/>10	10/>10
Seeds								
Orange	>10/>10	>10/>10	10/>10	>10/>10	>10/>10	>10/>10	>10/>10	10/>10
Red	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	10/>10
Green	>10/>10	>10/>10	>10/>10	>10/>10	>10/>10	10/>10	10/>10	>10/>10
Positive controls								
Ampicillin (10mg/mL)	<0.15/<0.15	10/>10	>10/>10	<015/<015	>10/>10	<015/<015	<015/<015	<015/<015
Imipenem (1mg/mL)	<0.0078/<0.0078	<0.0078/<0.0078	<0.0078/<0.0078	<0.0078/<0.0078	0.5/1	n.t./n.t.	<0.0078/<0.0078	n.t./n.t.
Vancomycin (1mg/mL)	n.t./n.t.	n.t./n.t.	n.t./n.t.	n.t./n.t.	n.t./n.t.	<0.0078/<0.0078	n.t./n.t.	0.25/0.5

MIC values correspond to the minimal extract concentration that inhibits the bacterial growth for the maximum tested concentration of 10 mg/mL; MBC - minimal bactericide concentration; MRSA – Methicillin-resistant *Staphylococcus aureus*; n.t. – not tested.

4.7.2. Antifungal activity

The antifungal activity was evaluated against two fungi strains, namely *A. brasiliensis* and *A. fumigatus*, with obtained results presented in **Table 13**. Overall, hydroethanolic extracts of waste-colored bell pepper fruits and their byproducts, namely stalks and seeds, revealed antifungal activity against both of the fungi strains.

A. brasiliensis revealed to be the most susceptible to the tested samples, especially in the extracts of the fruits, with waste-red bell pepper demonstrating superior inhibitory effects (MIC = 1.25 mg/mL) than orange (MIC = 2.5 mg/mL) and green (MIC = 5 mg/mL) varieties. Regarding extracts of the stalks, waste-red bell peppers also presented the best antifungal activity (MIC = 2.5 mg/mL), followed by orange and green varieties, which presented the same minimal inhibitory concentration (MIC = 5 mg/mL). Lastly, seed extracts of waste-red and green bell peppers exhibited the best antifungal activity (MIC = 2.5 mg/mL), followed by waste-orange bell peppers (MIC = 5 mg/mL).

Antifungal results against *A. fumigatus* indicated that all extracts revealed inhibitory activity against it (MIC = 10 mg/mL). Moreover, all the extracts proved to be not fungicidal for both fungal strains up to the maximum concentration tested (10 mg/mL), as well as they did not demonstrate better antifungal activity when compared to the tested positive control Ketoconazole.

These results are in accordance with other authors. For instance, previous study conducted by Añibarro-Ortega et al. [180] reported remarkable antifungal activity of the extracts from Holland and Italian bell pepper varieties. These authors indicated that bell pepper fruits exhibited inhibitory effects of against *A. fumigatus* (MIC = 1.54 and MIC = 1.52, for Holland and Italian varieties, respectively), stating as well that the extracts exhibited better inhibitory effects against the majority of the other fungal strains tested compared to the positive controls. In another study, López-Muñoz et al. [181] reported that ethanolic fruit extracts from red bell peppers (*C. annuum*) exhibited antifungal activity against *F. andiyasi* and *Cochliobolus* spp., associating with the phenolic content in the extract and their ability to alter the fungal cell wall, leading to cell death. Furthermore, Hajji-Hedf et al. [182] reported that *C. annuum* seed extracts showed antifungal potential in which significantly inhibited the growth rate of *Botrytis cinera* Pers., the causal agent of gray mold disease in tomato crops.

Table 13: Antifungal activity from waste-colored bell pepper fruits including their seeds and stalks hydroethanolic extracts.

Fungal strains	***A. brasiliensis***	***A. fumigatus***
	MIC/MFC	MIC/MFC
Stalks		
Orange	5/>10	10/>10
Red	2.5/>10	10/>10
Green	5/>10	10/>10
Fruits		
Orange	2.5/>10	10/>10
Red	1.25/>10	10/>10
Green	5/>10	10/>10
Seeds		
Orange	5/>10	10/>10
Red	2.5/>10	10/>10
Green	2.5/>10	10/>10
Ketoconazole	0.06/0.125	0.5/1

MIC – Minimum inhibitory concentration; MFC – Minimum fungicidal concentration.

5. Conclusions and future prospects

The increasing waste and loss generation in the agri-food industry and the limited availability of natural resources have motivated the scientific community to investigate possibilities to recover valuable bioactive compounds from agri-food residues to minimize their negative impact on the planet. The valorization of food waste for edible purposes and pharmaceutical applications is an area of opportunity for researchers due to the significant content of bioactive compounds present, which can be reused and add value thereby reducing the generation of wasted food. In addition, the growing market of functional ingredients has emerged in response to the current social awareness about consuming natural ingredients instead of synthetic substitutes. In this sense, a complete proximal and biochemical characterization of waste-orange, red, and green bell pepper fruits were assessed. The bell pepper waste consists of all the fruit discarded when it doesn't meet the necessary marketing standards to proceed to the market such as its shape, color, or size. Discarding these bio-wastes leads to the loss of their commercial and nutritional value, contributing to the degradation of the environment through the mismanagement of practices when dealing with wastes.

The proximal and biochemical characterization of the waste-colored bell pepper fruits, as well as the bioactive assessment of the fruits including their seeds and stalks, was intended to promote its valorization.

In general, waste-colored bell peppers showed a promising proximal and chemical composition. They presented four free sugars in their composition, with fructose being the main free sugar in waste-orange and red bell peppers, while waste-green bell peppers presented glucose as the main free sugar in their composition. Four organic acids were identified in the samples with oxalic acid being the most present in waste-orange and red bell peppers, whilst waste-green bell peppers presented malic acid as the main organic acid in their composition. Regarding fatty acids, fifteen were identified with palmitic acid, linoleic acid and α-linolenic acid present in major quantities, verifying the prevalence of PUFAs. The analysis of tocopherols showed the presence of four isoforms, being the *α*-tocopherol the most abundant in the samples. Pigments' analysis revealed the presence of four equivalents in the samples, with β-carotene being prevalent in orange and red bell peppers, and chlorophyll *a* being prevalent in green bell peppers. In the assessment of phenolic compounds, twenty-two substances were identified in waste-colored bell peppers including seven phenolic acids, fourteen flavonoids and one stilbene. The VOCs' characterization revealed the presence of thirty-four compounds, including

eight heterocyclic compounds, seven acids, six esters, three alcohols, two amines, two alkanes, one acetal, one aldehyde, one ether, one glyceride, one ketone, one ketose.

Concerning bioactive properties assessment, hydroethanolic extracts from waste-colored bell pepper stalks, fruits, and seeds revealed antioxidant capacity, with extracts from waste-green bell peppers presenting the best result (0.36±0.01 mg/mL, 0.44±0.02 mg/mL, and 0.21±0.01 mg/mL, respectively). All the extracts demonstrated an absence of cytotoxicity at the maximum concentration tested (GI_{50}>400µg/mL). Fruit extracts demonstrated superior antibacterial activity when compared to extracts of stalks and seeds with best results identified against food contaminants *Y. enterocolitica*, *B. cereus*, *S. aureus* (MIC = 5 mg/mL), and clinical isolates *E. faecalis* and *MRSA* (MIC = 5 mg/mL). Furthermore, all the extracts proved to be not bactericidal for any bacteria tested up to the maximum concentration tested (MBC>10 mg/mL). In the antifungal activity assessment, the fungal strain *A.brasiliensis* revealed to be the most susceptible to the tested samples, especially against the extracts of the fruits, with waste-red bell pepper demonstrating superior inhibitory effects (MIC = 1.25 mg/mL). Moreover, all the extracts proved to be not fungicidal for both fungal strains up to the maximum concentration tested (MFC>10 mg/mL).

Thus, this study found that waste bell peppers could be considered a great source of bioactive compounds to be exploited on an industrial scale. Due to their antioxidant and antimicrobial potential, waste bell peppers in combination with other natural ingredients could be explored as natural preservatives, since the food industry is looking even more toward naturally occurring antimicrobials agents to control the foodborne pathogens. Furthermore, pharmaceutical and cosmetic industries could make use of important bioactivities presented by extracts from bell peppers for the development of new functional products. Most studies have been carried out in *vitro*, so *in vivo* investigations are also necessary to demonstrate the effectiveness of the bioactive compounds, in addition to guaranteeing their safety and understanding their mechanisms of action. As an additional prospect, it would also be interesting to evaluate in depth the interaction of environmental factors that may have a significant impact on chemical composition and bioactive properties of bell peppers and the bioavailability of compounds.

Lastly, as a final note, this study emphasized the importance of the use of waste generated from the agri-food sector to extract bioactive compounds with several health benefits that can be incorporated into food, cosmetic or pharmaceutical products, eventually solving a

global waste problem that weights on global economy, environment, food security and malnutrition. The recovery of bio-waste in the industrial field will considerably promote the circular bioeconomy, contribute to a sustainable food system and increase global availability of food and therapeutic products.

6. References

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