Universidade de Lisboa

Faculdade de Farmácia

DISSERTATION

The biological effect of raspberry (*Rubus idaeus L.)* in oxidative and inflammatory stresses

Cátia Sofia Feitor da Costa

Dissertation supervised by Professor Maria Eduardo Costa Morgado Figueira

Master Degree in Biopharmaceutical Sciences

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Abstract

Chronic Non-communicable Diseases (NCDs) have become the largest contributor to mortality and disability worldwide, globally representing the largest cause of death in people of productive age. Among the main diseases associated with this phenomenon are cardiovascular diseases (30%), cancer (13%), chronic respiratory diseases (7%) and diabetes (2%). Behavioral risk factors associated with NCDs include a diet rich in saturated fat and poor in fresh fruit and vegetables, sedentary lifestyle, smoking and excess alcohol.

A diet rich in saturated fats and sugars leads to sharp postprandial peaks of glucose and blood lipids, a factor that generates an excess of reactive oxygen species, inducing oxidative stress and inflammation, which can alter the metabolic state of various tissues. The internal strategies to fight oxidation are done by endogenous enzymes, however, when protection is not guaranteed by these antioxidants due to the exaggerated formation of reactive oxygen species, it is necessary to ingest exogenous antioxidants through the diet in order to help reduce this imbalance.

Fruits and vegetables are rich sources of nutrients that contain phytochemicals (also known as bioactive compounds), which are recognized for their nutraceutical effects and health benefits. Phytochemicals are compounds such as Vit C, Vit E and phenolic compounds present in fruits and vegetables that can promote health and well-being and may be associated with the reduction of risk factors for the development of chronic diseases. Phenolic compounds represent the most important class of secondary metabolites and perform a wide range of biological functions in plants. Because they are present in fruits, they are also considered important bioactive compounds in human nutrition and are closely related to the reduction of oxidative and inflammatory stresses.

Red raspberries have been described for their unique composition of nutrients and phytochemicals and their potential role in mitigating the risk of diseases. Some animal and human studies have recognized raspberry as having many health benefits, since their consumption was associated with a reduced risk of developing various chronic diseases. This review intends to gather all the studies carried out in the last two decades in *Rubus idaeus L.* focused on the importance of bioactive compounds present in raspberries and their preventive and / or reducing action on oxidative and inflammatory stresses.

Keywords: *Rubus idaeus L.,* biological activity, bioactive compounds, oxidative stress, inflammatory stress.

Resumo

As Doenças Crónicas Não Transmissíveis (NCDs) tornaram-se o maior contribuidor para a mortalidade e a incapacidade, e representam globalmente a maior causa de morte em pessoas em idade produtiva. Entre as principais doenças associadas a esse fenómeno estão as doenças cardiovasculares (30%), o cancro (13%), as doenças respiratórias crónicas (7%) e diabetes (2%). Os fatores de risco comportamentais associados às NCDs incluem uma dieta rica em gordura saturada e pobre em frutas e vegetais frescos, um estilo de vida sedentário, tabagismo e excesso de álcool.

Uma dieta rica em gorduras saturadas e açúcares leva a picos pós-prandiais agudos de glicose e lípidos no sangue, um fator que gera um excesso de espécies reativas de oxigênio, induzindo stress oxidativo e inflamação, que podem alterar o estado metabólico de vários tecidos. As estratégias internas de combate à oxidação são feitas por enzimas endógenas, porém, quando a proteção não é garantida por antioxidantes devido à formação exagerada de espécies reativas de oxigênio, é necessário que seja feita uma ingestão de antioxidantes exógenos por meio da dieta para ajudar a reduzir este desequilíbrio.

As frutas e os vegetais são fontes ricas em nutrientes que contêm fitoquímicos (também conhecidos como compostos bioativos), que são reconhecidos pelos seus efeitos nutracêuticos e benefícios à saúde. Os fitoquímicos são compostos como Vit C, Vit E e compostos fenólicos que podem promover saúde e bem-estar e podem estar associados à redução de fatores de risco para o desenvolvimento de doenças crónicas. Os compostos fenólicos representam a classe mais importante de metabólitos secundários e desempenham uma ampla gama de funções biológicas nas plantas. Por estarem presentes nas frutas, também são considerados importantes compostos bioativos na nutrição humana e estão intimamente relacionados à redução do stress oxidativo e inflamatório.

As framboesas vermelhas têm sido descritas pela sua composição única em nutrientes e fitoquímicos e pelo seu potencial papel na mitigação do risco de certas doenças. Alguns estudos em animais e humanos reconheceram que a framboesa tem muitos benefícios para a saúde. O seu consumo foi associado a um risco reduzido de desenvolvimento de várias doenças crónicas. Esta revisão pretende reunir todos os estudos realizados nas últimas duas décadas em *Rubus idaeus L.* dando foco à importância dos compostos bioativos presentes nas framboesas e à sua ação preventiva e / ou redutora no stress oxidativo e inflamatório.

Palavras-chave: *Rubus idaeus L.*, atividade biológica, compostos bioativos, stress oxidativo, stress inflamatório.

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To you all, from the heart, a big thank you.

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1. Introduction

1.1 Brief General Introduction

The food we eat can determine the course of not only our life but also our society, and the irrefutable proof of this statement are the many diseases associated with poor dieting.

Non-communicable diseases (NCDs) result from an interaction between genetics, lifestyle, and environmental factors (1). The behavioural risk factors associated with NCDs include a diet rich in saturated fat and sugar, poor in fresh fruit and vegetables, sedentary lifestyle, smoking, and excess alcohol. Having a metabolic disease also greatly increases the risk of developing a NCDs because these individuals have abdominal obesity, hyperglycaemia, metabolic disorder of glucose, hypertension, and heterogeneous dyslipidaemia (2)(3).

Vascular homeostasis is altered during the postprandial state, which is the period following a meal. Inadequate or excessive nutrient consumption leads to oxidative and inflammatory stresses, which may alter the metabolic status of various tissues. Several foods and consumption patterns have been associated with various cancers and approximately 30–35% of those cases are correlated with over nutrition or malnutrition. Indeed, postprandial hyperlipidaemia and hyperglycaemia, or the so-called postprandial dysfunction in the body, are gradually gaining vital consideration as major risk factors for metabolic syndrome (abdominal obesity, hyperglycaemia, hypertension, and heterogeneous dyslipidaemia) (4)(5) as are some diseases as diabetes mellitus, cardiovascular diseases, and cancer (6).

In recent years, chronic non-communicable diseases (NCDs) have become the largest contributor to mortality and disability worldwide, globally representing the single largest cause of death in people of working age. Among the main diseases associated with this mortality are cardiovascular diseases (30%), cancer (13%), chronic respiratory diseases (7%) and diabetes (2%) (7)(8).

Due to their long duration and slow progression, NCDs impose a heavy burden on national health systems, both financially and structurally. These diseases have a huge negative impact on national gross domestic product, directly causing a decrease in national economic development, due to the incapacity they cause on the patient’s daily work and life. As well as on their families in the form of days missing work to offer informal care, which results in losses of production and productivity (9).

A fundamental strategy that can be used to control oxidative stress and exaggerated and persistent inflammation may be the adaptation of our diet to ingest a higher content of fruits and vegetables, since these foods are associated with lower oxidative stress and inflammation (10). Dietary antioxidants such as vitamin E, vitamin C, carotenoids, some minerals (Zn, Mn, Cu, Se) and polyphenols (flavonoids, phenolic acids, stilbenes, lignans) help endogenous antioxidants maintain internal redox homeostasis and may have a major impact on the contribution to the prevention and treatment of some chronic diseases (11)(12)(13).

1.2 Oxidative stress

Reactive oxygen species (ROS) are free radicals associated with the oxygen atom (O) or their equivalents and have stronger reactivity with other molecules, rather than with O_2. As a product of oxidation processes, reactive species can occur internally, due to enzymatic and non-enzymatic factors, or appear as a consequence of external factors, such as exposure to X-rays, ozone, smoking, air pollutants and products industrial chemicals. Enzymatic and non-enzymatic reactions that occur naturally in the body are the main internal sources of free radical formation; the enzymatic reactions responsible for the formation of free radicals are those that are involved in the respiratory chain, in phagocytosis, in the synthesis of prostaglandins and in the cytochrome P-450 system. While non-enzymatic reactions are formed through oxygen reactions with organic compounds or ionizing reactions. Generally, involved as a product of cellular metabolism and ionizing radiation are the following four species: superoxide anion (O^-_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen ($^1O_2$). The most important free radicals involved in many disease states are oxygen derivatives, particularly superoxide anion and the hydroxyl radical (14)(15).

Living organisms have a reduction-oxidation system that is necessary to maintain the balance between the generated free radicals and the antioxidant system, however when excessive reactive oxygen species (ROS) are formed, oxidative stress arises, resulting in DNA damage, lipid peroxidation, oxidation of amino acids causing changes in its structure and functions and inflammatory responses (15)(16). The internal strategies to fight oxidation is done by endogenous enzymes (Figure 1), they are part of this group: superoxide dismutase, catalase, glutathione peroxidase or non-enzymatic compounds such as uric acid, bilirubin, albumin and metallothioneins. However, when protection is not guaranteed by endogenous antioxidants, it is necessary to ingest exogenous antioxidants through the diet. These exogenous antioxidants may come from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but they can also be synthetic compounds. Among the most important exogenous antioxidants are vitamin E, vitamin C, β-carotene, vitamin D, vitamin K_3 and phenolic compounds. These are typically found in plant sources such as dark-coloured vegetables, citrus fruits, legumes, nuts, grains, seeds, and oils (15)(17)(18).

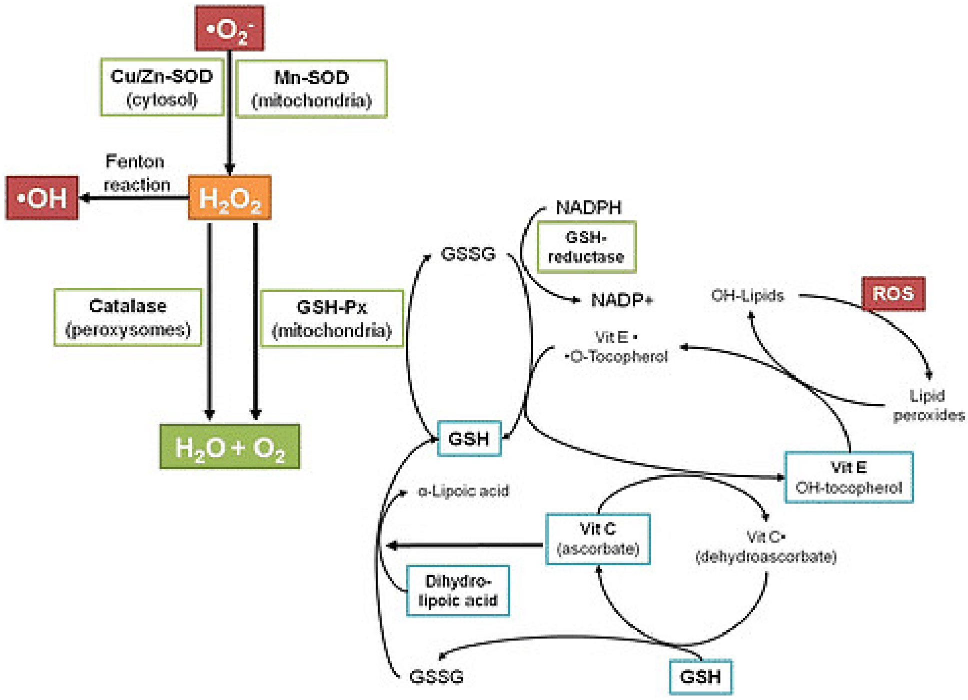

Figure 1. Antioxidant defences in the organism.

(Kurutas 2016)

Under normal conditions about 1% of reactive oxygen species daily escapes the control of the endogenous antioxidant defences and contribute to oxidative damage of surrounding tissues. Under stress there is a high ROS production and endogenous protection is not enough and leads to the worsening of more than one hundred pathological conditions (19). Therefore, it is probably crucial to try to keep the ROS balance as levelled as possible, ideally by taking antioxidants in order to prolong, if not prevent, the onset of many disorders (20). Indeed, antioxidant supplementation has been shown to attenuate endogenous antioxidant depletion, alleviating associated oxidative damage in some clinical research (21).

Oxidative stress can cause inflammation and the inflammatory process itself causes the release of reactive oxygen species and this condition can lead to many chronic diseases, including diabetes and diabetic complications, hypertension and cardiovascular diseases, neurodegenerative diseases, alcoholic liver disease, chronic kidney disease, cancer and aging (22).

1.3 Inflammatory stress

Inflammation is a crucial process of innate immunity and is one of the most important physiological processes in the body. Is initiated as a protective response to challenges with pathogens or foreign bodies, or injury, experienced by host tissues and it is regarded as a complex reaction in vascularized connective tissue in response to stimuli (23). During these inflammatory events, there is a change in vascular permeability, recruitment and accumulation of leukocytes and inflammatory markers (24). At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function that result from cell responses to infection or injury (25).

Inflammation is characterized by a sequence of events comprising an induction phase, which leads to the peak of inflammation and is gradually followed by a resolution phase (Figure 2). The induction phase of inflammation is designed to allow fast and robust immune activation that is required for effective host defence and the resolution phase is essential to curtail inflammation and restore tissue homeostasis once the danger signal has been eliminated (25)(26). During inflammation, the tissue-resident cells of the innate immune system detect the damaging insult and alarm circulating neutrophils that migrate to the inflamed tissue, promote recruitment of inflammatory monocytes, and potentiate the pro-inflammatory environment allowing to appropriately deal with the inflammation. The elimination of apoptotic neutrophils indicates a shift from a pro-inflammatory macrophage to an anti-inflammatory phenotype, which is a prerequisite for macrophage to exit through the lymphatic vessels favouring return to tissue homeostasis (27).

The inflammatory response triggers transcriptional activation of numerous genes such as nuclear factor (NF-Kb), activator protein (AP)-1, peroxisome proliferator activated receptors (PPARs) and nuclear factor erythroid 2-related factor 2 (Nrf2), whose DNA-binding capacity is modified by signal transduction pathways, including mitogen activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI3K)/RAC-alpha serine/threonine-protein kinase (Akt) and ubiquitin-proteasome system (28). In turn these genes regulate several physiological functions such as exposure to bacterial pathogens that induce the expression of inflammatory cytokines, including tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), as well as chemokines such as chemokine ligand 2 (motif CC) (CCL2, also known as monocyte chemotactic protein 1, (MCP-1) and chemokine ligand 8 (motif CXC) (CXCL8, also known as IL -8). In the case of viral infections, they induce the expression of type 1 interferons and parasitic worms' infections or exposure to allergens are associated with the expression of histamine, IL-4, IL-5 and IL-13 (29).

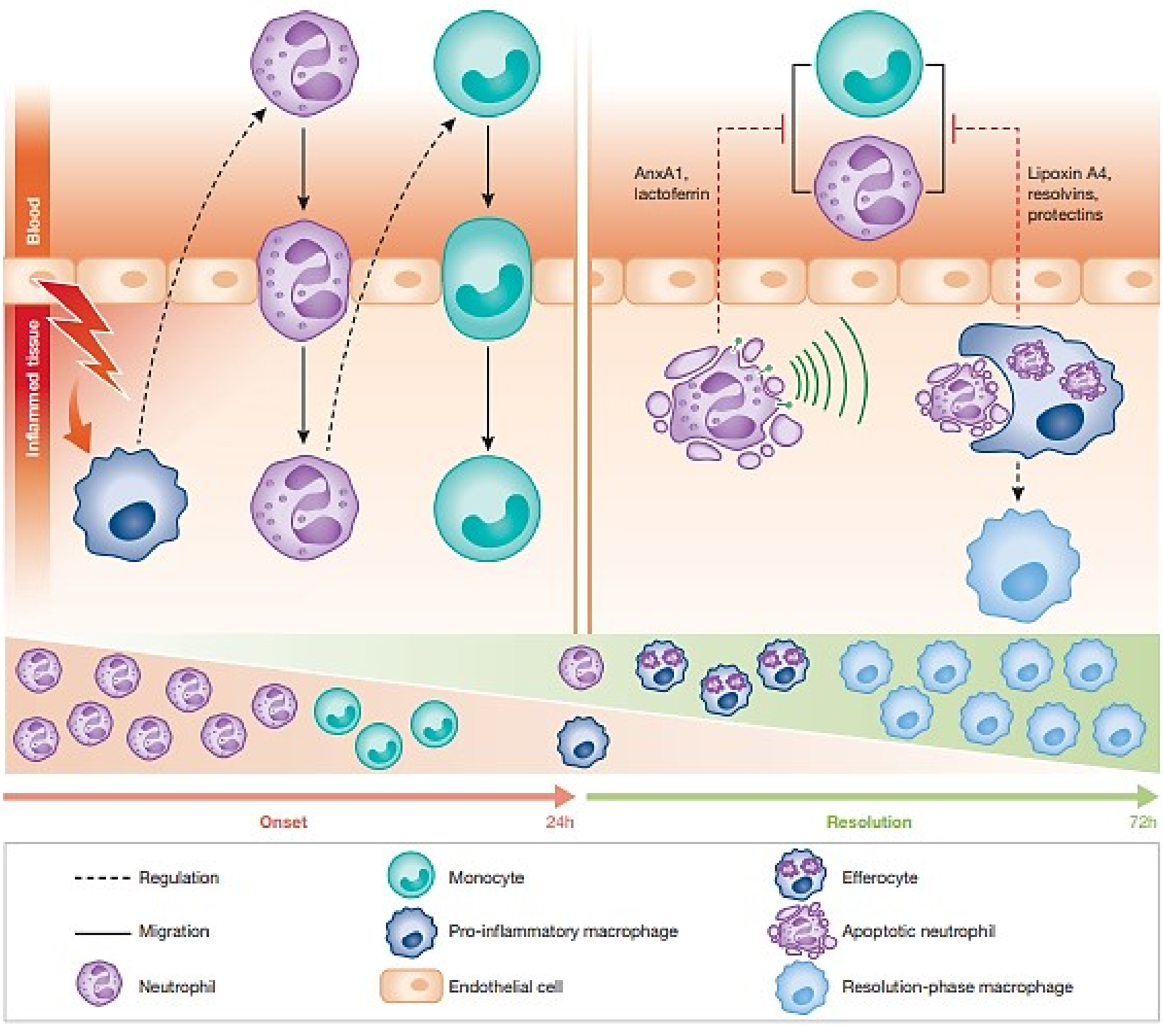

Figure 2. Cellular interplay during inflammation

(Ortega-Gómez, Perretti & Soehnlein. O, 2013)

A diet rich in saturated fats and sugars causes a greater inflammatory response, and if this intake occurs regularly and for a long time, it can induce tissue damage and oxidative stress by releasing many enzymes (neutral proteases, elastase, collagenase, acid hydrolases, phosphatases, lipases, etc.), reactive species (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, etc.), and chemical mediators (eicosanoids, complement components, cytokines, chemokines, nitric oxide, etc) (30).

The ideal inflammatory response to the organism is rapid and destructive (when necessary), yet specific and self-limiting (26). Inadequate or insufficient resolution of inflammation can lead to chronic inflammation, excessive tissue damage, and dysregulation of tissue healing. In fact, signs of persistent unresolved inflammation are not only typical of classical inflammatory diseases but also an underlying feature of a variety of human conditions not previously thought to have an inflammatory component, including Alzheimer's disease, atherosclerosis, cardiovascular disease, and cancer (27)(31).

In resume, inflammation involving the innate and adaptive immune systems is a normal response to infection. However, when allowed to continue unchecked, inflammation may result in many chronic diseases that is why it is so important the search for new and safe agents capable of blocking inflammatory reactions is essential.

It is known that the dietary intake of phenolic compounds (PC) play a critical role in the physiological or pathological response to tissue inflammation and oxidative stress. In fact, dietary PC have a well-recognized role in reducing the risk of chronic diseases, the increase of healthy life years and the promotion of active healthy aging (32)(33).

1.4 Phenolic compounds or polyphenols

The underlying mechanisms that lead to a reduction of human diseases through the consumption of fruits and vegetables are still being intensively studied; however, risk factor reduction by maintaining or re-establishing normal cellular and tissue function is critical to this end. Some fruits and vegetables, that are rich in antioxidants, influence the cellular processes that affect pathophysiological (risk) factors may decrease the likelihood of developing chronic diseases. Recent hypotheses have focused on testing health-promoting attributes of fruits and vegetables (34). It is known that antioxidants of natural origin, which can be extracted from plants, food by-products and other natural sources, should preferably be ingested since synthetic antioxidants are supposed to promote carcinogenic activity (35).

Antioxidants can be classified, according to their origin into endogenous, those which are synthesized in the cell, or naturals, when they are part of the food, such as vitamin C, vitamin E, carotenoids, phenolic compounds, and oligoelements like selenium and zinc (36).

Polyphenols are described as secondary metabolites of plants, involved in the defence of ultraviolet radiation or in aggression by pathogens, which are naturally found in fruits, vegetables, cereals, and drinks. These compounds belong to the largest categories of phytochemicals that provide many functions for plants but are also beneficial when consumed by humans and/or animals. In fruits and fruit juices, phenolics can contribute to bitterness and astringency due to the interaction between procyanidin and glycoprotein in saliva. These compounds can also affect colour and flavour between wines due to the reaction between oxygen and phenolic compounds and are critical for the maturation, preservation and aging of the wine (37)(38). The most recent epidemiological studies and associated meta-analysis strongly suggested that polyphenols have various biological properties, such as anti-proliferative, anti-diabetic, anticancer, anti-microbial, anti-inflammatory, antiviral and antioxidant contributing to a wide variety of beneficial effects on human health (39)(40)(41).

Phenolic compounds belong to a wide and heterogeneous group of chemical components that possess one or more aromatic rings with a conjugated aromatic system and one or more hydroxyl groups. Can occur in free and conjugated forms with sugars, acids, and other biomolecules as water-soluble (phenolic acids, flavonoids and quinones) or water-insoluble compounds (condensed tannins). The various main classes of natural polyphenols are shown in Figure 3, but they can simply be classified as flavonoids and non-flavonoids. These compounds have relevant *in vitro* and *in vivo* antioxidant activities because they tend to donate an electron or a hydrogen atom to a free radical and convert it into an inoffensive molecule (42).

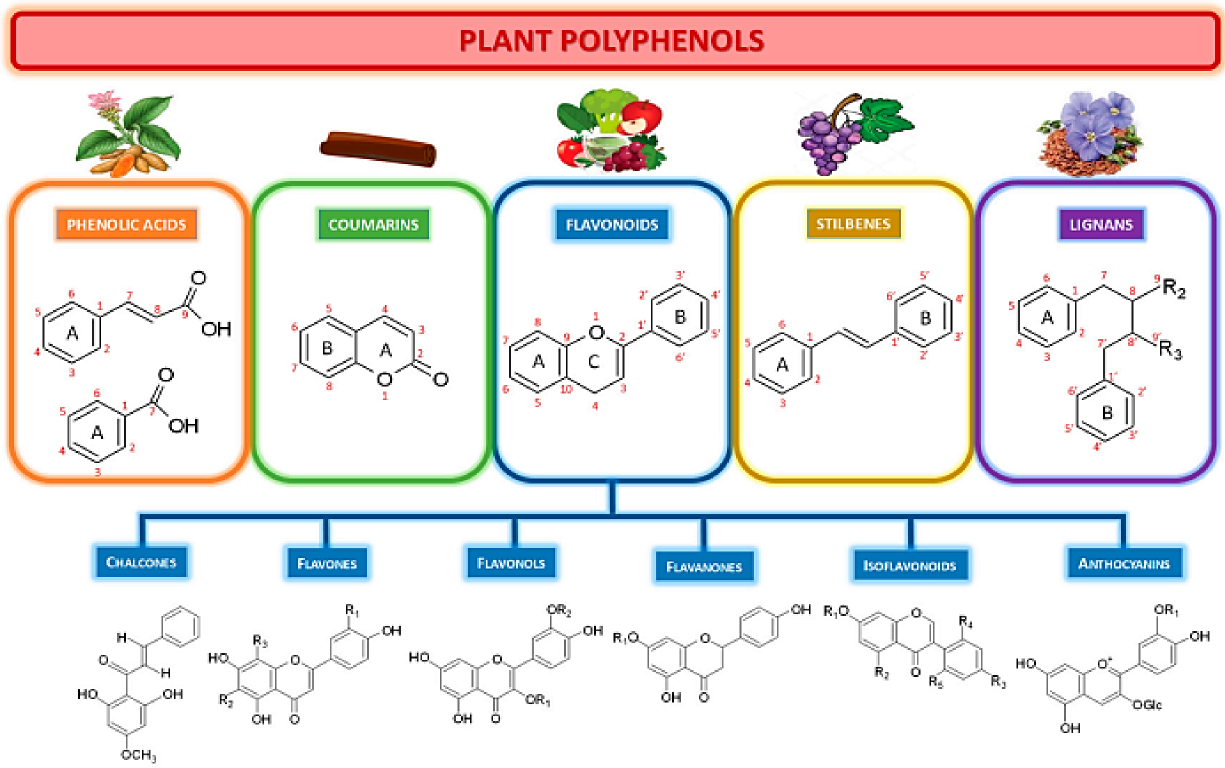

Figure 3. Polyphenols classification

(Hano C, Tungmunnithum D., 2020)

Flavonoids encompasses most antioxidants from fruits and vegetables, such as quercetin, kaempferol, isorhamnetin, fisetin, genistein. This group are most commonly divided into six subclasses, based on the connection position of the B and C rings as well as the degree of saturation, oxidation, and hydroxylation of the C ring, as flavonols, flavones, flavanones, flavan-3-ols (or catechins), isoflavones, and a anthocyanins (43)(44). Flavonoids are associated with a broad spectrum of health-promoting effects and are indispensable component in a variety of nutraceutical, pharmaceutical, medicinal, and cosmetic applications. This is because of their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions(45). They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase (33).

Phenolic acids (PA) are found abundantly in foods and divided into two classes: derivatives of benzoic acid and derivatives of cinnamic acid. The hydroxycinnamic acids are more common than hydroxybenzoic acids and consist chiefly of p-coumaric, caffeic, ferulic and sinapic acids. Phenolic acids are directly involved in the response of plants to different types of stress: they contribute to healing by lignification of damaged areas possess antimicrobial properties, and their concentrations may increase after infection (46). In humans, extensive research on PAs strongly suggests that consumption of these compounds hold promise to offer protection against various ailments and reduce the risk of life-threatening diseases such as HIV, diabetes, CVDs, and cancer (47).

Natural stilbenes are an important group of non-flavonoid phytochemicals characterized by the presence of a 1, 2-diphenylethylene nucleus. There are more than 400 natural stilbenes, however they are present in a limited and heterogeneous group of plant families since the key enzyme involved in stilbene biosynthesis, stilbene synthase, is not ubiquitously expressed (39). Stilbenes have an extraordinary potential for the prevention and treatment of different diseases, including cancer, due to their antioxidant, cell death activation, and anti-inflammatory properties which associate with low toxicity under *in vivo* conditions (48).

Lignans are diphenolic compounds that contain a 2, 3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues. They are found in the highest concentrations in flax and sesame seeds and in lower concentrations in grains, other seeds, fruits, and vegetables. Due to their various bioactive properties, dietary intake of lignan-rich foods may prevent certain types of cancers (e.g., breast cancer in post-menopausal women and colon cancer). Regarding chronic lifestyle-related diseases, some studies indicate that lignan intake is associated with a lower risk of developing cardiovascular disease (41).

Coumarin (1,2-benzopyrone or 2H-1-benzopyran-2-one) and coumarin derivatives are natural compounds that are widely available in plants as a heteroside or free form Coumarin and its derivatives are frequently found in the seeds, roots and leaves of many plant species belonging to families (especially Rutaceae and Apiaceae) in the Dicotyledonae class of the division of Spermatophyta (49). In recent years these compounds have become important due to their various biological activities, the most studied pharmacological activities in coumarins are antibacterial, antitubercular, antifungal, antiviral, antimutagenic, antioxidant, scavenging of reactive oxygen species (ROS), anti-inflammatory, antithrombotic, anticancer, anticoagulant activities, and cyclooxygenase, lipooxygenase, cholinesterase (ChE) and monoamine oxidase (MAO) inhibitory activities, CNS stimulant, vasodilator, and cytotoxic effects(50).

The relationship between diet and health has increased consumer demand for more information on diet rich in nutraceuticals, which include fruits and vegetables as their bioactive constituents (51). The search for the understanding of how wild fruits, such as raspberry *(R. idaeus L.)* can be one of these healthy growth compounds has been growing since they contain a wide variety of bioactive compounds.

This study aimed to investigate whether the phenolic compounds present in red raspberries reduce the oxidative and inflammatory stresses in healthy human volunteers caused by the ingestion of a meal rich in fats and sugar. The trial proposed to measure oxidation and inflammation markers before and after the volunteers ingested a very caloric meal, rich in sugar and fat, in order to accentuate oxidative and inflammatory stress. They would then be asked to take raspberry daily for three weeks. After this period, the study would be repeated to verify the effect of phenolic compounds and Vit C of raspberry. The pandemic made this project impossible, and we had to modify the objective of the thesis for a bibliographic review.

The present work intends to compile and present the latest studies carried out in *Rubus Idaeus L.*, in which the objective was to evaluate the antioxidant and anti-inflammatory potential of the compounds present in the fruit and, consequently, the benefits of the fruit in preventing and / or delaying some diseases.

2. Methodology

Pubmed was the biggest source of information to write this review to locate various online databases in order to find academic articles. Punctually *Google Scholar*™ was also used. These online databases included ERIC (Education Resources Information Center), SciELO (Scientific Electronic Library Online), ScienceDirect and Nature.

A variety of keyword descriptors were used to search the online databases mentioned above. Keyword descriptors included: raspberry, Rubus idaeus L. biological effects of raspberry, oxidative stress, inflammatory stress, berries, antioxidant effect of raspberry *in vitro* and *in vivo* studies, antioxidant effect of raspberry in humans, anti-inflammatory effect of raspberry in vitro and in vivo studies, anti-inflammatory effect of raspberry on humans, natural compounds, non-communicable diseases, phenolic compounds, bioactive compounds.

The initial resources were selected by reviewing the abstracts of the article and then determining whether the content was relevant to the keywords. The resource evaluation was based on: relevance to the theme, and the year of publication from 2000 to the most current year. The citation index search technique was also used to examine the frequency of citations for the author's work in other publications.

3. Raspberry (*Rubus idaeus L.*)

Food is an essential condition for maintaining life, which is why the right nutrients must be ingested in the appropriate quantity and variety; otherwise, the body does not perform its functions properly and therefore cannot prevent diseases caused by poor diet. Diet plays a key role in human health and it is already widely recognized that the food we eat daily are responsible for some of the diseases that come up over the years. Intake of plant foods, fruits and vegetables are strongly correlated with a high consumption of bioactive compounds (BAC) such as vitamins, minerals, folate, dietary fibre, plant sterols, carotenoids, and various phytochemicals that plays a crucial role preventing a wide range of diseases as well as all the other general health benefits they provide (52). In the last few years, some authors have concentrated on testing health-promoting attributes of fruits and vegetables, such as polyphenols, carotenoids and other phytochemicals with biological activity (35).

Berries are usually sold in their fresh or processed form and are consumed by humans worldwide. The most common berries include blackberry (*Rubus sp.*), currant (*Ribes nigrum*), blueberry (*Vaccinium corymbosum*), raspberry (*Rubus idaeus*) and strawberry (Fragaria x ananassa) and the main group of phytochemicals present in them are polyphenols, comprising flavonoids (anthocyanins, flavonols, flavones, flavanols, flavanones and isoflavonoids), stilbenes, tannins and phenolic acids (53).

Rubus idaeus L. (raspberry, also called red raspberry or, occasionally, European raspberry to differentiate it from other raspberries), is a fruit grown all over the world, and has received a lot of attention for its culinary versatility and its health benefits, mainly due to its bioactive activity (54). This increase is due to the growing consumer interest in health and well-being and in parallel to the increase in research publications and media communications that describe the unique composition of nutrients and phytochemicals of red raspberries and their potential role in mitigating the risk of diseases (55). Research areas of interest include the bioavailability of specific components of red raspberries, the characterization of their metabolites, effects on oxidative stress and inflammation markers and evaluation of their antimicrobial properties. Much of the work in these areas has been conducted in *in vitro* cell culture systems, although studies in animals and humans have steadily increased (54)(56).

3.1 Nutritional characterization

The nutritional composition of raspberries may differ considering factors such as harvest, processing, and storage. These fruits should be consumed quickly, as they last for a noticeably short time after being picked; therefore, one of the ways to preserve it is through freezing. The main forms of processed red raspberries available are: individual quick freeze (IQF) (sweetened or unsweetened), juice concentrates (single or concentrated potencies) and puree (seeded or seedless) (54).

Red raspberries are among the highest whole food sources of dietary fibber, providing 6.5 g/100 g of fresh weight, which on a calorie basis is 12.5 g/100 kcal. They also contain vitamin C, magnesium, and a variety of other nutrients, such as potassium, vitamin K, calcium, and iron (Table 1).

Table 1. Nutritional Characterization of *Rubus idaeus L.* (Adapted from Burton-Freeman *et al*. 2016)

Component	Raw	Frozen and unsweetened	Frozen and sweetened	Canned in syrup
Water, g/100 g	85.75	85.75	72.75	75.33
Energy, kcal/100 g	52	52	103	91
Protein, g/100 g	1.20	1.2	0.7	0.83
Total lipid (fat), g/100 g	0.65	0.65	0.16	0.12
Ash, g/100 g	0.46	—	0.24	—
Carbohydrate (by difference), g/100 g	11.94	11.94	26.16	23.36
Total dietary fiber, g/100 g	6.5	6.5	4.4	3.3
Total sugar, g/100 g	4.42	4.42	21.76	20.06
Calcium, mg/100 g	25	25	15	11
Iron, mg/100 g	0.69	0.69	0.65	0.42
Magnesium, mg/100 g	22	22	13	12
Phosphorus, mg/100 g	29	29	17	9
Potassium, mg/100 g	151	151	114	94
Sodium, mg/100 g	1	1	1	3
Zinc, mg/100 g	0.42	0.42	0.18	0.16
Copper, mg/100 g	0.090	—	0.105	—
Manganese, mg/100 g	0.670	—	0.65	—
Selenium, µg/100 g	0.2	—	0.3	—
Vitamin C (total ascorbic acid), mg/100 g	26.2	26.2	16.5	8.7
Thiamin, mg/100 g	0.032	0.032	0.019	0.02
Riboflavin, mg/100 g	0.038	0.038	0.045	0.031
Niacin, mg/100 g	0.598	0.598	0.23	0.443
Pantothenic acid, mg/100 g	0.329	—	0.15	—
Vitamin B-6, mg/100 g	0.055	0.055	0.034	0.042
Total folate, µg/100 g	21	21	26	11
Total choline, mg/100 g	12.3	—	10.2	—
Vitamin A, µg RAE/100 g	2	2	3	2
β-Carotene, µg/100 g	12	—	21	—
α-Carotene, µg/100 g	16	—	29	—
Vitamin A, IU/100 g	33	33	60	33
Lutein + zeaxanthin, µg/100 g	136	—	113	—
Vitamin E (α-tocopherol), mg/100 g	0.87	0.87	0.72	0.59
Vitamin K (phylloquinone), µg/100 g	7.8	7.8	6.5	5.2
Anthocyanidins, mg/100 g				
Cyanidin	—	—	22.60	—
Delphinidin	—	—	0.02	—
Pelargonidin	—	—	1.60	—
Flavones, mg/100 g				
Apigenin	—	—	0.01	—
Luteolin	—	—	0.02	—
Flavonols, mg/100 g				
Kaempferol	—	—	0.01	—
Myricetin	—	—	0.03	—
Quercetin	—	—	1.10	—

3.2 Bioactive compounds present in Raspberry

The relevant bioactive compounds in berries are vitamins like Vit C, Vit D and E and phenolic compounds that include flavonoids, such as anthocyanins (i.e., cyanidin glucosides and pelargonidin glucosides), flavonols (quercetin, kaempferol, myricetin), flavanols (catechins and epicatechin). Furthermore, phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) and hydrolysable tannins, such as ellagitannins that act as important BAC (Figure 4). These components, either individually or combined, are mainly responsible for berry health benefits and are also associated with their biological properties (53).

Raspberries (*Rubus idaeus L.)* have a high free radical scavenging capacity and are rich in both vitamin C and total phenolics. Among the approximately 50 phenolic compounds found in red raspberries, the major phenolic antioxidants found is cyanidin-3-sophoroside with smaller quantities of other anthocyanins, including cyanidin-3- (2G-glucosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside, pelargonidin-3-(2G-glucosylrutinoside), and pelargonidin-3-glucoside (2-4). Some varieties of raspberries contain extremely high levels of ellagitannins, which on hydrolysis release ellagic acid (57).

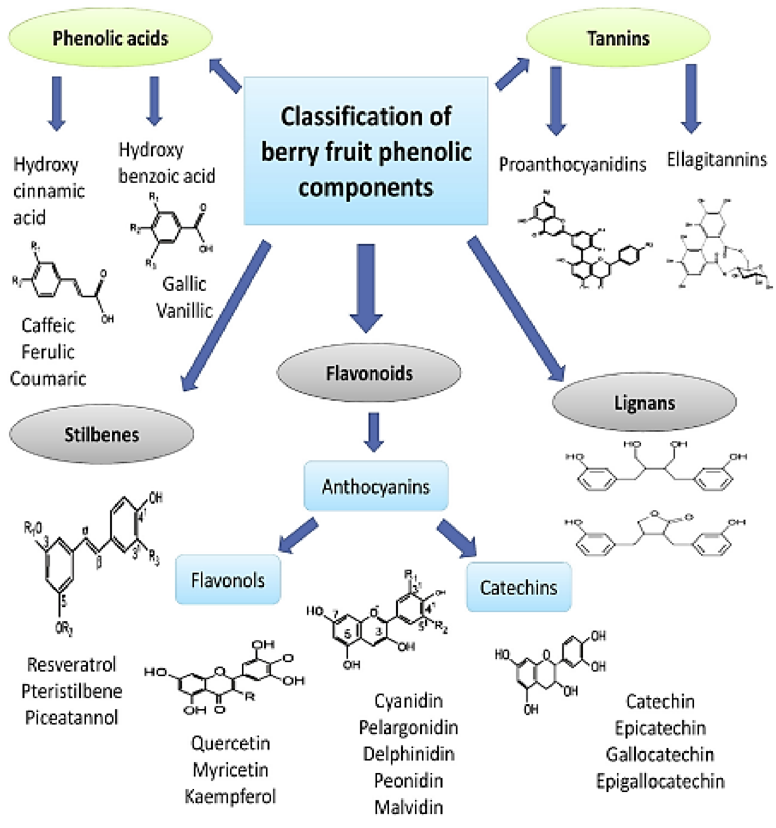

Figure 4. Bioactive components in berries. (*Nile, S. H. et al*. 2014)

Anthocyanins are pigments belonging to the group of flavonoids and are the main group of polyphenols present in *Rubus idaeus L.* contributing ~25% to its antioxidant capacity(53). Anthocyanins are responsible for a wide variety of colours of fruits, flowers and leaves ranging from red-orange, to bright red, purple and blue. These compounds have antioxidant properties and are mainly responsible for the elimination of free radicals, chelation of transition metal ions (copper, iron), inhibition of enzymes involved in the formation of reactive oxygen species (ROS), induction of endogenous antioxidant enzymes and prevention of lipid peroxidation (58). It is known that antioxidants of natural origin, which can be extracted from plants, food by-products and other natural sources, should preferably be ingested since synthetic antioxidants are supposed to promote carcinogenic activity (34).

Tannins play an essential role in shaping the sensory properties of fruit and fruit products, they are responsible for the tart taste and for changes in the colour of fruit and fruit juice. The tart taste results from the interactions among tannins, the proteins of mucous membranes, and gustatory receptors. As enzyme inhibitors, tannins decrease the nutritive value of some plant products. In fruits rich in anthocyanins, tannins stabilize anthocyanins by binding to them to form copolymers. Most fruits contain condensed tannins, despite hydrolysable tannins (derivatives of gallic and ellagic acids) are less frequently encountered, these compounds have been found in strawberries, raspberries, and blackberries (55).

Ellagitannins, the other major group of polyphenols in red raspberries, are categorized as hydrolysable tannins and are esters of hexahydroxydiphenoyl group that consists of either a glucose or quinic acid core. The main raspberry ellagitannins are sanguiin H-6 and lambertianin C and it is because of the complex structure of ellagitannins, their content in red raspberries is typically determined by after being hydrolysed to ellagic acid. In raspberries, free ellagic acid comprises only a minor part of the total ellagic acid pool and ellagitannins are the primary source of ellagic acid released by acid hydrolysis. However, many other ellagic acid-containing compounds also are present in raspberries. There is a particular interest in ellagic acid because of evidence of its potential chemopreventive, anti-inflammatory, antioxidant, and antibacterial effects (64-67).

Apart from anthocyanins and ellagitannins, other phenolic compounds are also present in red raspberries such as hydroxycinnamic acids (caffeic, p-coumaric, and ferulic acids), hydroxybenzoic acids (ellagic and p-hydroxybenzoic acids) and flavonols in free and conjugated form (quercetin and kaempferol) (54).

Raspberry is also rich in vitamin C, this vitamin can also be found in citrus fruits, berries, tomatoes, potatoes, and green leafy vegetables. Humans are unable to synthesize it, so it is strictly obtained by eating fruits and vegetables in the diet. Vitamin C, also called ascorbic acid, is water-soluble, antioxidant and an essential cofactor for collagen biosynthesis, carnitine and catecholamine metabolism and dietary iron absorption. As an antioxidant, it protects the body from various deleterious effects of free radicals, pollutants, and toxins (59). More recently, several studies have shown that vitamin C plays an important role in vascular function (60).

4. Biological activity

Metabolism is divided into two main categories in living organisms: Primary and Secondary metabolism. Primary metabolites are microbial products made by mostly microbial fermentation which include biological molecules, i.e., nucleic acids, fats, carbohydrates, and proteins, which are essential for the survival and well-being of the body and exert biological effects on the body or cell (61). Secondary metabolism involves the biosynthesis of secondary metabolites (natural products) that exert biological effect within the organism and in other organisms. Biological processes responsible to produce secondary metabolites include photosynthesis, glycolysis, and Kreb cycle (62).

In recent years, *in vitro* and *in vivo studies* have shown that the phenolic compounds of red raspberry have a wide variety of biological activities (63).

4.1 Antioxidant activity of Raspberry

Antioxidants are mainly free radical scavengers that act through different mechanisms and in different compartments: 1) they directly neutralise free radicals, 2) they reduce the peroxide concentrations and repair oxidized membranes, 3) they quench iron to decrease reactive oxygen species production, 4) via lipid metabolism, short-chain free fatty acids and cholesteryl esters neutralise reactive oxygen species (64).

Although some scientists argue that in many instances oxidative stress is not the primary cause of disease and that the formation of radicals is secondary to tissue damage by disease, there is some evidence linking oxidative stress with several chronic diseases (14).

4.1.1 *In vitro* studies and *in vivo* studies

Table. 2 Potential antioxidant effect of *Rubus idaeus L.* on health: *in vitro* and *in vivo* studies. Studies published and cited in PubMed between 2006 and 2020.

Reference	Type of study	Year	Brief description of the study	Effects
Raudone (65)	*in vitro*	2014	Direct measurement of hydrogen peroxide using a J774 macrophage culture stimulated by arachidonic acid and phorbol-12-myristate- 13 acetate	ROS downregulation
Garcia (66)	*in vitro*	2017	SKN-MC cells were pre-treated for 24h with either the original extract or the human digestion (GIB) fraction at the range of physiologically relevant concentrations (0 to 5μmol L-1)	Protection against oxidative stress by either the original extract or the GIB fraction raspberry extracts
Kowalska (67)	*in vitro*	2019	Use of raspberry fruit extract over 24hours to investigate the potential in reducing ROS production in hypertrophied 3T3-L1 adipocytes	Reduction of ROS production
Gao, W. (68)	*in vitro*	2018	Normal Human Dermal Fibroblasts (NHDF) treated with *Rubus idaeus L.* extract to study the potentially effective in preventing UVB induced skin photoaging	Reduction of ROS production
Noratto, G. D (69)	*in vivo*	2017	Red raspberry fruit intake was investigated on obese diabetic (db/db) to examine if the fruit protect against diabetes-induced oxidative stress.	Raspberry intake protected db/db mice against oxidative stress associated with type 2 diabetes.

Kshatriya, D. (70)	*in vivo*	2019	Male C57BL/6J mice were placed on a high-fat diet (45% fat) for 4 weeks and received a daily oral dose of four different extracts	The raspberry extract high reduced body weight gain (approximately 5%-9%) and white adipose mass (approximately 20%) and the hepatic gene expression of heme oxygenase-1 and lipoprotein lipase was upregulated.
Song, B. (71)	*in vivo*	2020	3 different C. elegans were insulted by paraquat has been used to induce oxidative stress.	Raspberry could ameliorate oxidative stress in C. elegans via the SKN-1/Nrf2 pathway by reduction of the levels of ROS

The abundance of natural antioxidant compounds in raspberry is an argument that encourages the consumption of this fruit. Several studies conducted between 2006 and 2020 argue that the intake *Rubus idaeus L.* can contribute to the decrease of oxidative stress in *in vitro* studies and *in vivo* studies. (table. 1)

The chronic oxidative stress leads to a generation of great amounts of ROS and activation of pro-inflammatory pathways. This stress can also lead to the activation of a variety of transcription factors including nuclear factor kappa light chain enhancer of activated B cells (NF-κB), AP-1activator protein 1, p53, HIF-hypoxia-inducible factor 1α, peroxisome proliferator-activated receptor γ (PPAR-γ), β-catenin/Wnt, and NF-E2-related factor (Nrf2). On the other hand, the activation of these transcription factors can be responsible to the expression of over 500 different genes (72).

The NF-E2- p45-related factor 2 (Nrf2) binds with small Maf proteins to the antioxidant response element (ARE) in the regulatory regions of target genes, while Keap1 (Kelch ECH associating protein 1) is a repressor protein that binds to Nrf2 and promotes its degradation by the ubiquitin proteasome pathway. The Nrf2-Keap1 signalling pathway (Figure 5) in basal conditions are described by the Nrf2-bounding Keap1 resulting in rapid Nrf2 degradation. A small proportion of Nrf2 escapes the inhibitory complex and accumulates in the nucleus to mediate basal ARE-dependent gene expression, thereby maintaining the cellular homeostasis. Under stress conditions, inducers modify the Keap1 cysteines leading to the disruption of binding with Nrf2, the newly synthesized Nrf2 proteins bypass Keap1 and translocate into the nucleus, bind to the Antioxidant Response Element (ARE) and drive the expression of Nrf2 target genes such NAD(P)H: quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HMOX1), glutamate-cysteine ligase (GCL) and glutathione S transferases (GSTs). The redox-sensitive signalling system Keap1/Nrf2/ARE plays a key role in the maintenance of cellular homeostasis under stress, inflammatory, carcinogenic, and pro-apoptotic conditions, reason why its consider as a pharmacological target (73)(74).

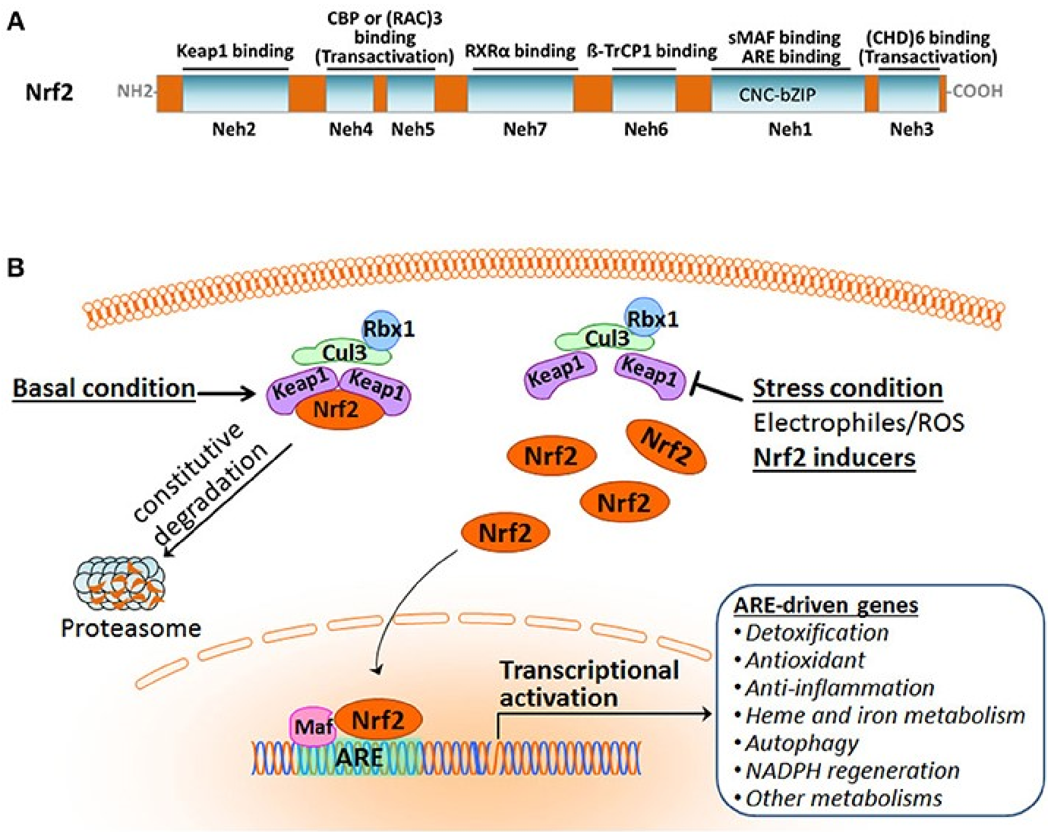

Figure 5. The Nrf2-Keap1 signalling pathway

(Liu L, Locascio LM, Doré S., 2019)

Nrf2 is critical in generation of antioxidant cofactors and controls nicotinamide adenine dinucleotide phosphate (NADPH) production, which is an essential cofactor for many drug-metabolizing enzymes and antioxidants, by regulating key NADPH-generating enzymes, such as glucose-6-phosphate dehydrogenase (G6pd), 6-phosphogluconate dehydrogenase (Pgd), isocitrate dehydrogenase 1 (Idh1), and malic enzyme 1(75). The NADPH oxidases (NOX) enzymes are multi-subunit protein complexes that are membrane-bound proteins, and their main function is to transfer electrons across the plasma membrane to molecular oxygen—which results in the generation of the superoxide anion and subsequently reactive oxygen species (ROS) (76).

Raudone and the co-authors used a culture of J774 macrophages stimulated by arachidonic acid and phorbol-12-myristate-13 acetate to quantify the production of ROS and observed a negative regulation of ROS, suggesting that the antioxidant effects mediated by the raspberry extract may be due to the regulation of NADPH oxidase activity (65). Another study by Kowalska used raspberry extracts over 24 hours to investigate the potential for reducing ROS production in hypertrophied 3T3-L1 adipocytes (mouse cell line), found that the extract in concentrations of 5, 10 and 20mg / ml reduced the ROS production by 11.7 ($p > 0.05$), 19.3 ($p < 0.05$) and 19.9% ($p < 0.05$), respectively. The viability of adipocytes was maintained, indicating that their inhibitory effect on the generation of intracellular ROS was not due to cytotoxicity. The authors believe that the decrease in intracellular ROS generation in hypertrophied adipocytes was due to increased expression of the antioxidant defence enzymes SOD, catalase and GPx, and inhibition of an oxidizing enzyme NADPH oxidase 4 (67). In a study made with normal Human Dermal Fibroblasts (NHDF) treated with *Rubus idaeus L.* extract, to study the potentially effective in preventing UVB induced skin photoaging, was seen that cells treated with raspberry (100 μg/ml) reduced UVB-induced ROS production and the authors could conclude that raspberry accelerated procollagen type I synthesis by activating the transforming growth factor-β/Smad pathway and enhanced the expression of cytoprotective antioxidants such as heme oxygenase-1 and NHD(P)H quinone oxidoreductase 1 by promoting nuclear factor E2- related factor 2 nuclear transfer (68).

To provide health benefits, the compounds of interest must be released from the food matrix postingestion and become bioaccessible in the gastrointestinal tract. Bioavailability can be defined as a fraction of ingested compounds that reach the circulatory system and specific organs to exert biological effects in human health. Overall, it is a result of the absorption, distribution, metabolism, and excretion of ingested compounds. The frequently used *in vitro* gastrointestinal digestion models use enzymes, bile salts, and sometimes mucin or other biological molecules to assess the bioaccessible compound, to mimic the chemical changes that occur during human digestion (77)(78). A study by Garcia et al. used a neuronal cell line SK-N-MC manipulated with hydrogen peroxide (H_2O_2) to mimic chronic oxidative stress, this cell line was treated with raspberry extracts digested in vitro to simulate the chemical changes that occur during human digestion (GIB) and with the original raspberry extracts. They saw that both the original extract and the extracts of the raspberry GIB fraction in lower concentration (1.25 μg GAE mL-1) can induce protection against oxidative stress. Although the production of basal ROS was significantly reduced by the original extract to a concentration of 2.5 μg GAE mL-1, this effect was not observed with the GIB fraction suggesting the loss of the ability to eliminate (poly) phenols after digestion. Authors recorded that only 19.2% of total phenols were recovered in the post-digestion GIB fraction. They conclude that raspberry polyphenols may present a dietary route to the retardation or amelioration of neurodegenerative related dysfunctions (66).

Noratto GD, Chew BP and Atienza LM. investigated red raspberry daily intake on obese diabetic (db/db) mice for 8 weeks to examine if the fruit protect against diabetes-induced oxidative stress. The animals were fed with freeze dried raspberry daily intake was ∼0.8 g per animal per day, this raspberry dose translated for a human adult of 60 kg is equivalent to 112.2 g freeze dried raspberry per day. They observed a protection against oxidative stress associated with type 2 diabetes and conclude that their results support other *in vitro* studies reporting that raspberry polyphenolic fractions reduced oxidative stress through decreasing lipid peroxidation, DNA damage, ROS generation and induction of antioxidant enzymes activities (69).

A study by Kshatriya D., *et al*. explored the daily intake of four different extracts: vehicle (VEH; 50% propylene glycol, 40% water and 10% dimethyl sulfoxide), low raspberry extract (REL; 0.2 g / kg), high raspberry extract (REH; 2 g / kg), or raspberry ketone (RK; 0.2 g / kg) in male C57BL / 6J mice that were placed on a high-fat (45% fat) diet for 4 weeks. The results showed that REH and RK reduced body weight gain (approximately 5% -9%) and white fat (approximately 20%) compared to VEH. REH animals showed increased expression of Hmox1, which may play a role in antioxidant mechanisms, but the animals treated with RK showed no increase in Hmox1 expression. This further suggests that a raspberry extract enriched with polyphenol and raspberry ketone affects different metabolic pathways (70).

One of the last *in vivo* studies made in 2020 was conducted by Song B. and the co-authors used three different *C. elegans* strains: Bristol N2 (wild-type), LG333 [skn1(zu135) IV.] and LG326 [skn-1(zu169) IV.] that were manipulated by paraquat to induce oxidative stress. The purpose of using three different lines was because each one of the mutants create a premature stop codon, allowing to study downstream genes involved in oxidation. After nematodes were treated with different concentrations of raspberry extracts for 5 days, the levels of ROS were significantly reduced in a dose-dependent manner. The result suggested that raspberry extract could regulate the SKN-1 transcription factor to activate genes downstream, thereby mediating oxidative stress and longevity in *C. elegans* (71).

4.1.2. Human studies

In vitro and *in vivo* studies are important to elucidate the mechanism of action of polyphenols. In any case, the role of human intervention studies in calculating bioavailability and understanding the metabolic fate of ingested compounds is especially important (79). There are not many studies in the literature on the effects of *Rubus idaeus L.* on oxidative stress markers in humans, and none have tested red raspberries exclusively (table.3).

Table. 3 Potential antioxidant effect of juices rich in raspberry-containing polyphenols on human health. Studies published and cited in PubMed between 2004 and 2020.

Reference	Year	Brief description of the study	Effects
Morillas-Ruiz J. (80)	2005	Randomized control trial tested the consumption of mixed berry juice in thirty sportsmen trained cyclists using an exercise model to induce oxidative stress	Attenuation of oxidative changes
Carmen Ramirez-Tortosa M. (81)	2004	Randomized control trial tested the consumption of mixed berry juice in elderly people for 2 weeks	No relevant changes
Groh, I. A. M. (82)	2020	Study of the impact of consumption of three different anthocyanin-rich beverages (beverage 1–3) in healthy volunteers on the transcription of Nrf2 in peripheral blood lymphocytes (PBL) was investigated and compared with oxidative DNA damage	Bilberry extract modulates the transcription of Nrf2- regulated genes in peripheral blood lymphocytes (PBL) accompanied by decreased DNA damage

Long duration and high intensity exercise may contribute to a rise in ROS production. A randomized control trial tested the consumption of mixed berry juice contained black grape (81 g/l), raspberry (93 g/l) and red currant (39 g/l), in thirty sportsmen trained cyclists using an exercise model to induce oxidative stress. The study suggests that antioxidant ingestion prior and during the exercise significantly attenuates oxidative changes (80).

Carmen Ramirez-Tortosa M. *et al.* investigated the effect of eating a 200 g pot a day (composed of concentrated grape, cherry, blackberry, blackcurrant and raspberry juices) for a period of 2 weeks in a group of elderly people. On days 1 and 15, blood samples were collected to analyse the total antioxidant capacity, biochemical parameters, antioxidant vitamins, low-density lipoprotein (LDL) peroxidation and DNA damage in peripheral blood lymphocytes. The study's conclusion is that a 2-week intervention does not give enough time to find changes in the antioxidant and oxidative state, perhaps due to the high state of oxidative stress during aging (81).

A recent study investigated the impact of consuming three different anthocyanin-rich drinks: Drink 1 (red fruit juice), 2 (juice with grape skin extract) and 3 (blueberry juice) on the transcription of Nrf2 and genes dependent on Nrf2 in healthy volunteers. They saw that all beverages affected the transcription of Nrf2, HO-1 and NQO-1 in different ways. The consumption of red fruit juice significantly reduced total DNA strand breaks, suggesting antioxidant and protective DNA effects, although levels of transcription of Nrf2-dependent genes have reached baseline and the amount of basic DNA strand breaks (damage without oxidative DNA strand breaks) has remained unchanged. In contrast, a drink prepared with grape skin extract significantly increased the basic and total DNA strand breaks. The authors underline the need for further investigation regarding the composition, safety, and consumer acceptance of the respective products to exclude undesirable adverse effects (82).

Although no conclusions can be drawn relative to raspberries, the trials suggest that polyphenol-rich juices may be useful in re-establishing homeostasis or protecting cells/cell components from being damaged during stress situations.

4.2 Anti-inflammatory activity of Raspberry

The major therapeutic actions of NSAIDs (Non-Steroidal Anti-Inflammatory Drug) are primarily enacted by their ability to block certain prostaglandins (PGs) synthesis through the cyclooxygenase enzymes (COX-1 and COX-2) inhibition. COX-1 is expressed in normal cells and its inhibition plays a major role in the undesired side effects such as Gastro Intestinal (GI) and renal toxicity. While COX-2 is induced in inflammatory cells and its inhibition most likely represents the desired effect of NSAIDs' anti-inflammatory, antipyretic and analgesic response.

The search for new alternatives to interfere with key players in inflammation has been growing (83), most research in pharmacology has focused on finding new compounds and raspberry has been the subject of some studies.

4.2.1 *In vitro* studies *and in vivo* studies

Table. 4 Potential anti-inflammatory effect of raspberry *in vitro* and *in vivo* studies. Studies published and cited in PubMed between 2006 and 2020.

Reference	Year	Type of study	Experiment	Effects
Sangiovanni, E. (84)	2013	*in vitro*	Gastric cell line AGS stimulated by TNF-a and IL-1b were treated by ellagitannin enriched extracts (ETs) for evaluating the effect on NF-kB driven transcription, nuclear translocation and IL-8 secretion	Inhibition of the translocation and driven-transcription of NF-kB and the release of IL-8.
Li, L. (85)	2014	*in vitro*	Evaluation of the anti-inflammatory activities in lipopolysaccharide (LPS)/IFN-γ-activated RAW264.7 macrophages	RR-ARFs attenuate LPS/IFN-γ-induced inflammatory responses in RAW264.7 cells by blocking activation of NF-κB and MAPK/JNK
Gao, W. (68)	*2018*	*in vitro*	Normal Human Dermal Fibroblasts (NHDF) treated with *Rubus idaeus L.* (RI) extract to study the potentially effective in reducing the inflammatory factors production	RI notably reduced UVB pro-inflammatory mediators production, and significantly suppressed UVB-induced activation of mitogen-activated protein kinase (MAPK)
Garcia (66)	*2017*	*in vitro*	N9 murine microglial cell line were pre-treated with LPS as an inflammatory insult and with either the original extract or the human digestion (GIB) fraction	Inhibition of microglial proinflammatory activation

Szymanowska, U., (86)	2018	*in vitro*	The anti-inflammatory potential of crude extracts (CE), anthocyanin-rich fractions (ARF), and phenolic fractions (PF) from raspberry (R) and raspberry juice (J) was evaluated in J.45 and HL60 cell lines	Inhibition of lipoxygenase (LOX) and cyclooxygenase-2 (COX-2) activities.
Kowalska (67)	2019	*in vitro*	Use of raspberry fruit extract to investigate the anti-inflammatory activity in hypertrophied 3T3-L1 adipocytes.	Raspberry exhibited a high anti-inflammatory potential.
Jean-Gilles, D (87)	2011	*in vivo*	Study of raspberry anti-inflammatory effect in an antigen-induced arthritis rat model	Significant inhibition of inflammation
Figueira, M. E., (88)	2014	*in vivo*	Evaluation of anti-inflammatory properties of raspberries in two experimental models of inflammation	Reduction of clinical signs of arthritis
Mohamed, M. T (89)	2020	*in vivo*	Evaluation of the possible protective effects of raspberry ketones (RKs) against lung toxicity induced by Cyclophosphamide (CP) in rats	Amelioration of pulmonary injury
Sangiovanni, E. (84)	2013	*in vivo*	Evaluation of the effect of ellagitannins (ETs) in a rat model of gastric lesions induced by ethanol.	Blackberries and raspberries ETS induce protective effects on gastric lesions
Li (85)	2014	*in vivo*	Study of anti-inflammatory effect in a mouse colitis model treated with RR-ARFs to evaluate	RR-ARFs reduced the severity of colitis

The human body, when exposed to foreign organisms or tissue damage, reacts to maintain homeostasis, and this role is played by the innate immune system and the immune and adaptive system. The innate immune system constitutes the first line of host defence during infection and therefore plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens. On the other hand, the adaptive immune system is responsible for elimination of pathogens in the late phase of infection and in the generation of immunological memory. The innate immune cells that reside in tissues such as macrophages, fibroblasts, mast cells and dendritic cells, as well as circulating leukocytes, including monocytes and neutrophils, recognize the invasion of pathogens or cellular damage with intracellular or surface-expressed pattern recognition receptors (PRRs). These receptors are capable of recognize molecular patterns associated with pathogens (PAMPs), such as microbial nucleic acids, lipoproteins and carbohydrates, or damage-associated molecular patterns (DAMPs) released from injured cells (90)(91).

Upon PAMPs or DAMPs recognitions, PRRs trigger cascades of intracellular signalling in order to fight the infection culminating in the expression of a variety of pro-inflammatory molecules and antimicrobial responses by activating a complex intracellular signalling pathways, including adaptor molecules, kinases, and transcription factors. PRR-induced signal transduction pathways ultimately result in the activation of gene expression and synthesis of a broad range of molecules, including cytokines, chemokines, cell adhesion molecules, and immunoreceptors, which represent an important link to the adaptive immune response (Figure 6) (92)(93).

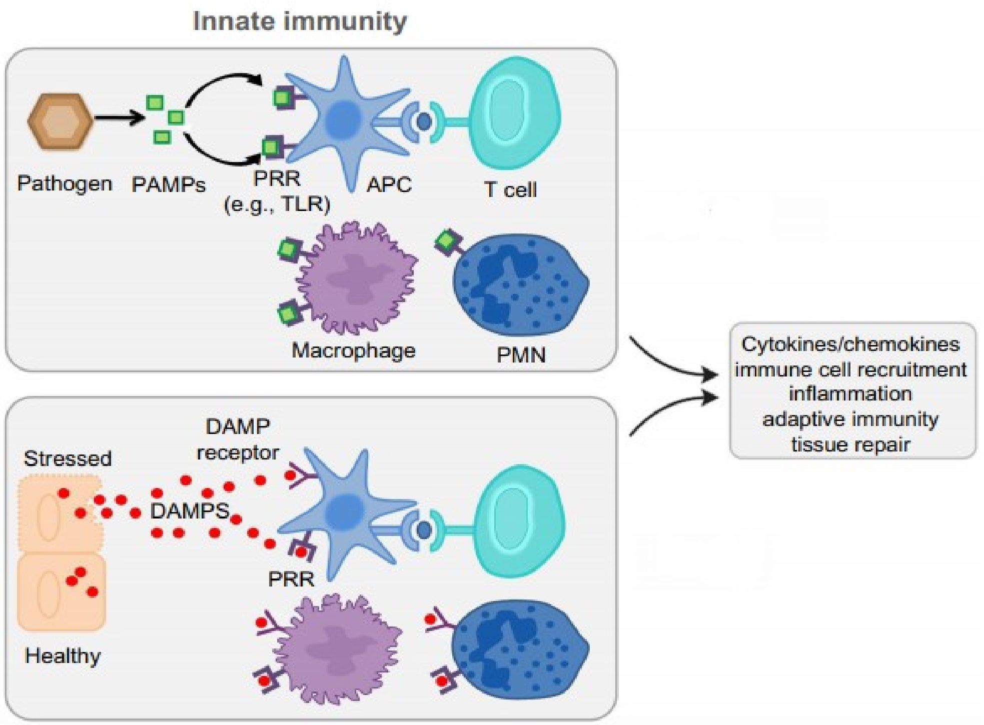

Figure 6. PAMPs and DAMPs in the inflammatory response

(Valles, P. *et al.* 2014)

Most of the knowledge of signalling inflammation was gained from studying members of the IL-1 and TNF receptor families. The best characterized class of recognized pattern receptors (PRRs) are Toll-like receptors (TLRs) which belong to the IL-1R family. These are associated with the plasma and endosomal membrane and constitute the organism's first line of defence against invading microbial pathogens, tissue damage or cancer, playing an important role in the innate and subsequent immune response to the adaptive immune response. On the other hand, IL-1 and TNFα represent the archetypal pro-inflammatory cytokines that are rapidly released on tissue injury or infection (93).

There are at least two separate pathways for NF-kB activation: The "canonical" pathway that is triggered by microbial products and pro-inflammatory cytokines such as TNFα and IL-1 and an "alternative" NF-kB pathway that is activated by TNF-family cytokines (94). Sangiovanni, E. and co-authors studied the effect of an enriched ellagitannin extract (ETs) prepared from Rubus fruticosus L. (ETblack) and Rubus idaeus L. (ETrasp) on an AGS gastric cell line stimulated by TNF-α and IL-1b to investigate a) inhibition of NF-kB translocation and targeted transcription activity; b) the effect on IL-8 release in the gastric epithelial cell line (AGS) stimulated with TNF- α, IL-1ß, H2O2 and ethanol. They observed the inhibition of translocation and directed transcription of NF-kB and the release of IL-8, with the concentration required to obtain 50% of this inhibition ranging between 1 and 2 mg / mL of extract. In addition, they found that both ETs can function as binding inhibitors at the TNF-a (TNFR1) and IL-1ß (IL1R1) receptor sites. The authors conclude that the compounds can act at different levels in the complex regulatory process of the NF-kB pathway and suggest that this question should be answered in future studies(84).

One of the key signalling routes responsible for intracellular signal transduction of inflammation is the mitogen-activated protein kinase (MAPK). MAPKs, which belong to a large family of serine/threonine kinases, constitute major inflammatory signalling pathways from the cell surface to the nucleus. There are three well-characterized subfamilies of MAPKs: the extracellular signal-regulated kinases (ERK), the c-Jun NH_2-terminal kinases (JNK) also known as stress-activated protein kinase (SAPK), and the p38 family of kinases (p38 MAPKs). ERK activation is considered essential for entry into cell cycle, the activation of the JNK pathway is associated with programmed cell death or apoptosis and the p38 MAPKs regulate the expression of many cytokines and have an important role in activation of immune response. Big mitogen-activated protein kinase 1 (BMK1), also known as extracellular signal–regulated kinase 5 (ERK5), is a newly identified member of the mitogen-activated protein (MAP) kinase family and is suggested that BMK1 plays an important role in the pathogenesis of cardiovascular disease (95)(96). Li *et al.* evaluated the anti-inflammatory activities of crude extracts (CEs), anthocyanin-rich fractions (ARFs), and des-anthocyanin fractions (DAFs) in lipopolysaccharide (LPS)/IFN-γ-activated RAW264.7 macrophages and they observed that ARFs from red raspberries (RR-ARFs) exhibited the highest efficiency in suppressing NO synthesis. The anti-inflammatory properties were also demonstrated by reducing the expression levels of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin-1 beta (IL-1β) and IL-6 in RAW264.7 cells. The luciferase reporter assay demonstrated that the activities of NF-κB and AP-1 signalling pathways were significantly suppressed by RR-ARFs.

In conclusion, RR-ARFs attenuate LPS/IFN-γ-induced inflammatory responses in RAW264.7 cells by blocking activation of NF-κB and MAPK/JNK. The administration of RR-ARFs significantly inhibited p65 phosphorylation and its nuclear translocation as well as the activation of IκB kinase (IKK), IκBα and JNK, thereby suppressing the expression of pro-inflammatory genes such as iNOS, COX-2, IL-1β, and IL-6 (Figure 7) (85).

Gao and the co-authors investigated the anti-inflammatory potential of an extract of *Rubus idaeus L.* in normal human dermal fibroblasts (NHDF) and they detected that RI notably reduced UVB-induced MMPs secretion and pro-inflammatory mediators production, and significantly suppressed UVB-induced activation of MAPK, nuclear factor-κβ, as well as activator protein 1 (68).

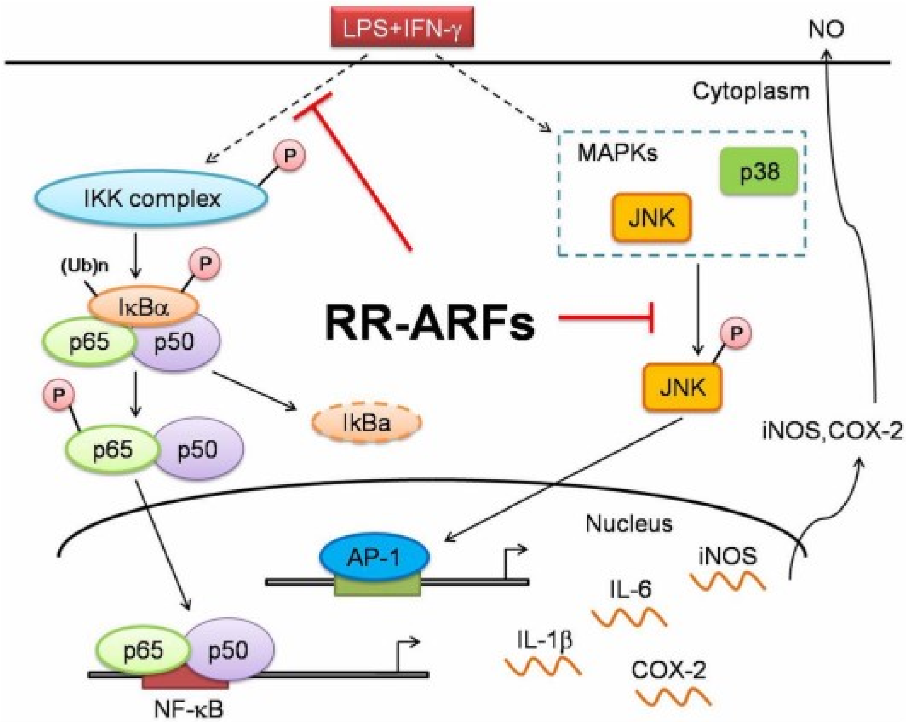

Figure 7. Schematic model showing role of RR-ARFs in inflammatory signaling pathways.

(Li. L, *et al.* 2014*)*

After exposure to pro-inflammatory cytokines IFN-γ, TNF-α and cellular or bacterial debris, resident microglia or macrophages infiltrate the circulation become polarized towards a pro-inflammatory phenotype (M1) and act on the first line defence of the innate immune system. These cells then produce pro-inflammatory cytokines (TNF-α, interleukin (IL) -1β, IL-12), present antigen and express high levels of inducible NO (iNOS) to produce NO in order to kill the foreign aggressor and proceed with an adaptive immune response (97). Garcia and his team studied a N9 murine microglial cell line were pre-treated with LPS as an inflammatory insult and with either the original extract or the human digestion (GIB) fraction at the range of physiologically relevant concentrations (0 to 5µmol L^{-1}). They found that the digested raspberry metabolites exhibited anti-inflammatory activity not only by the reduction of Iba1 expression, a microglia activation marker, but also by inhibiting the release of nitric oxide (NO) and TNF-α, two specific markers of the microglial classical pro-inflammatory activation (M1) (66).

Kowalska *at al.* used raspberry fruit extract over 24 hours to investigate the anti-inflammatory in hypertrophied 3T3-L1 adipocytes cell line and they observed that raspberry exhibited a high anti-inflammatory potential by down-regulation the expression of pro-inflammatory mediators such IL-6, TNF-α, IL-1β, the monocyte chemoattractant protein-1 (MCP-1) and leptin, and counteracted the decrease in adiponectin and IL-10 expression. The authors conclude that raspberry fruit could be potentially valuable dietary ingredient towards mitigating adverse metabolic consequences of fat cell hypertrophy to reduce the risk of metabolic disorders (67).

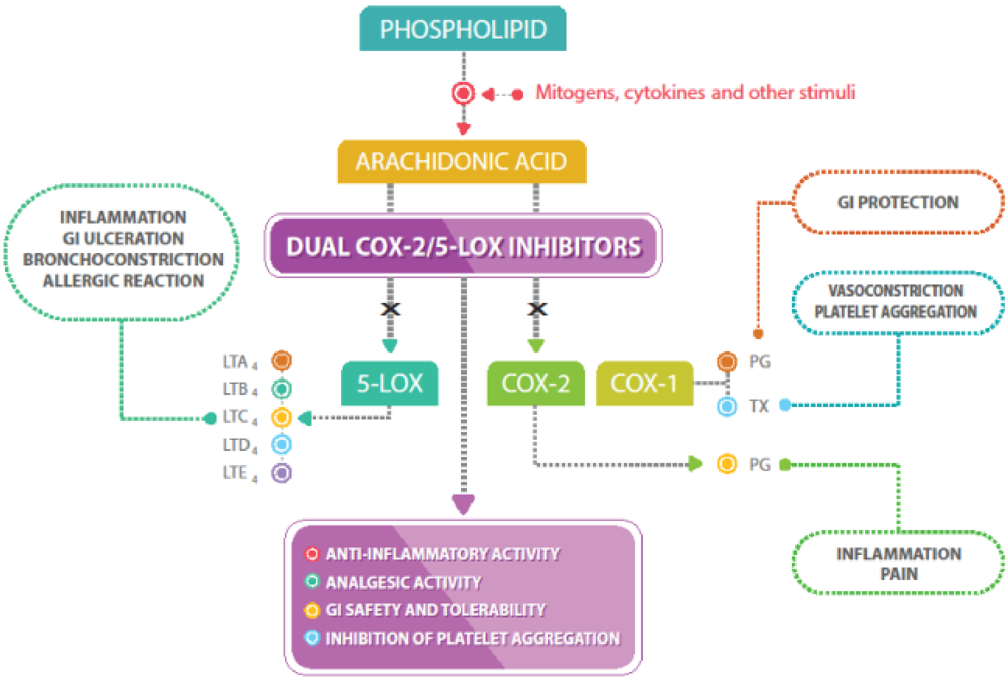

Figure 8. Concept of dual COX-2/5-LOX

(P JJ, Manju SL, Ethiraj KR, Elias G., 2018)

Szymanowska, U. and co-authors studied the anti-inflammatory potential of crude extracts (CE), anthocyanin-rich fractions (ARF), and phenolic fractions (PF) from raspberry (R) and raspberry juice (J) in HL-60 Human Caucasian promyelocytic leukemia and J.45 Human acute T cell leukemia. The anti-inflammatory activity was demonstrated by inhibitory effect on lipoxygenase (LOX) and cyclooxygenase-2 (COX-2) activity *in vitro*. The authors conclude that bioactive compounds, of the different fractions of raspberry, act via different mechanisms. And suggests more research in order to determinate the mechanisms of action and to analyse the type of interactions between individual raspberry antioxidants (synergism, antagonism or no interactions) (86). The expression of COX-2 and 5-LOX is selectively induced by pro-inflammatory cytokines at the site of inflammation and therefore the inhibition of the enzymes cyclooxygenase-2 and 5-lipoxygenase of the arachidonic pathway can be a strong therapeutic justification for the development of new potent agents (Figure 8). This aspect has encouraged the search for dual COX-2/5-LOX inhibitors to achieve excellent clinical results (98)(99).

Jean-Gilles, D. and the co-authors studied the anti-inflammatory effect of raspberry in an antigen-induced arthritis rat model. The rats were fed with raspberry at 30 mg/kg (low dose) and at 120 mg/kg (high dose) for 30 days. They observed that the raspberry-treated animals at the highest dose showed a lower incidence and severity of arthritis compared to control animals. Also, histological analyses revealed significant inhibition of inflammation, pannus formation, cartilage damage, and bone resorption. The authors conclude that red raspberry polyphenols may afford cartilage protection and/or modulate the onset and severity of arthritis (87). Another *in vivo* study made from Figueira, M. E. *et al.* evaluated anti-inflammatory properties of raspberries in two experimental models of inflammation: carrageenan-induced paw edema and collagen-induced arthritis that were tested for 35 days and fed with a raspberry dose of 15 mg kg^{-1} which is equivalent to daily consumption in humans of 150 g. The raspberry extract showed pharmacological activity and was able to significantly reduce the development of clinical signs of arthritis. The authors found a reduction in the degree of bone resorption, soft tissue edema and formation of osteophytes, avoiding joint destruction in the treated animals (88).

Mohamed, M. T. and his team evaluated the possible protective effects of raspberry ketones (RKs) against lung toxicity induced by Cyclophosphamide CP in rats. RKs prevented the activation of NF-KB by blocking the translocation of NF-KB into the nucleus by IkB degeneration, improved the histological structures of lung tissues and reduced thickened alveolar septa, pulmonary congestion, and pulmonary parenchymal inflammatory cell infiltration following CP administration. The authors conclude that RKs were shown to be a promising candidate in ameliorating pulmonary injury induced by CP and attribute the protective effect of RKs to its antioxidant activity (89).

Sangiovanni, E. and co-authors used a rat model of gastric lesions induced by ethanol to evaluate the effect of ellagitannins (ETs). Rats were treated orally for ten days with 20 mg/kg/day of ETs. They observed a reduction of the release of CINC-1, the rat homologue of human IL-8, this effect was obtained at a dose of ETs comparable with the amount consumed in a portion of berries of 125 g. Since the IL-8 expression and secretion in gastric epithelial cells are mainly regulated by redox sensitive transcription of NF-kB, it was assumed that the suppression of NF-kB mediated cell signalling could be a mechanism for inhibition of the inflammatory process by ET. This study reports for the first time that ETs from blackberries and raspberries are able to protect the stomach against the gastric lesions caused by ethanol (62). Another study made by Li *et al.* studied the anti-inflammatory effect of RR-ARFs in a mouse colitis, BALB/c mice of 6- to 8-weeks were injected with 20 mg/kg RR-ARFs for 10 days and they found that RR-ARFs reduced the severity of colitis. These findings suggested that RR-ARFs could be potentially developed as a potentially anti-inflammatory agent for clinical and agriculture applications (84).

4.2.2 Human Studies

Table. 5 Potential anti-inflammatory effect of raspberry polyphenols on human health. Studies published and cited in PubMed between 2004 and 2020.

Reference	Year	Brief description of the study	Effects
Freese R. (100)	2004	Study of the effects of dietary intake of low and high levels of vegetables, berries, including red raspberry and apple on platelet function and inflammatory markers	No significant differences were found between the 4 diets evaluated and the effects on platelets or inflammation markers
Almendingen K. (99)	2005	Study of the modulation of COX-2 by the intake of 2 different diets with fruits (including *Rubus idaeus L.*) and vegetables over 8 weeks	No differences were found in the expression of COX-2
Schell, J. (101)	2019	Effect of raspberry intake on inflammation biomarkers in obese adults with type 2 diabetes	Possible attenuation of lower postprandial hyperglycaemia and inflammation
Xiao, D. (102)	2019	Investigation of the effects of red raspberry intake on meal-induced postprandial metabolic responses in individuals who have overweight or obesity with prediabetes and insulin resistance (PreDM-IR) over three separate days	Improvements in postprandial insulin responses

Cardiovascular diseases are the leading cause of death despite changes in lifestyle and the use of preventative pharmacological approaches. Most cardiovascular diseases can be prevented by addressing behavioural risk factors - such as tobacco use, unhealthy diets and obesity, lack of physical activity and harmful use of alcohol (103). Endothelial function and inflammation are firmly associated with atherosclerosis and thrombosis and may be affected by dietary components. Freese R. and co-authors carried out a highly controlled dietary intervention with healthy volunteers to study how 4 different diets, which differ markedly in the amounts of vegetables and fruit, affect several markers associated with atherosclerosis or thrombosis. They didn't find significant differences between the 4 diets evaluated on platelets or inflammation markers (100).

The study by Almendingen K. *et al.* was carried out with the objective of investigating whether the increase in the intake of mixed fruits and vegetables (FVs) would influence the expression of COX2 in cells of human peripheral blood. Blood cells were selected because peripheral blood monocytes are considered highly inducible, that is, there is a greater probability of detecting the minimal change in the expression of COX-2. The authors found no difference in the expression of COX-2, concluding that further studies on the factors that regulate and are regulated by COX-2 should be performed, first suggesting the evaluation of the correspondence between the expression of COX-2 mRNA and pathway products. COX, like prostaglandins. They also point out that the natural polymorphisms of the promoters and coding regions of COX-2 can contribute to functional variations and response to different diets (101).

" What is a healthy diet? Many doctors have no answer to this quite common question asked by patients. Much remains to be learned about the role of specific nutrients in decreasing the risk of chronic diseases, a large body of evidence supports the usefulness of healthy eating patterns that emphasize whole foods, legumes, vegetables and fruits, and that limit refined, red starches meat, whole dairy products and foods and drinks rich in added sugars (104). Epidemiological studies suggest that several dietary patterns have been favourably associated with the prevention of obesity and type 2 diabetes (105).

Globally, Type 2 Diabetes mellitus (T2DM) is at present one of the most common diseases and its levels are progressively on the rise. It has been evaluated that around 366 million people worldwide or 8.3% in the age group of 20-79 years had T2DM in 2011. This figure is expected to rise to 552 million (9.9%) by 2030 (105). Schell, J. and his co-authors studied the effect of daily raspberry intake on postprandial fasting glucose for 4 weeks in obese adults with type 2 diabetes. They observed that raspberries lowered postprandial blood glucose at 2 and 4 h versus control condition, and tended to lower serum triglycerides (TGs), especially after 4 h. Also, the raspberry supplementation was associated with a significantly lower serum-IL-6 and TNF-α, and a trend toward lower systolic blood pressure. On the other hand, fasting serum glucose and TGs were not affected by raspberries at 4 weeks. The authors conclude that their observations on the anti-inflammatory effects of red raspberries, especially on systemic IL-6 and TNF-α in adults with T2D can be explained by mechanistic studies previously reported (91, 92) and point out that these findings provide further evidence on the benefits of red raspberries in improving postprandial excursions in blood glucose and inflammation that contribute to the burden of atherosclerotic CVD in diabetes (101). Another study done by Xiao, D. *et al.* investigate the effects of raspberry intake in individuals who have overweight or obesity with prediabetes and insulin resistance (PreDM-IR) over three separate days. Participants received 0 g of frozen red raspberries (Control), 125 g of frozen red raspberries (RR-125) or 250 g of frozen red raspberries (RR-250), with a challenge breakfast meal (high in carbohydrates / moderate fat). The results showed a significant reduction in the glucose peak in 2 hours in PreDM-IR in the participants who received 250g of raspberries compared to the control meal without raspberries, suggesting a improvement in postprandial insulin responses. On the other hand, no significant effect was observed with raspberry intake on postprandial inflammation and oxidative stress markers. The authors conclude that raspberries observed in their study have clinical relevance and provide additional opportunities to address risk factors of diabetes and cardiovascular diseases through diet (102).

5. Conclusion

Raspberries (*Rubus idaeus L.)* are rich in both vitamin C and total phenolics. Among the approximately 50 phenolic compounds found in red raspberries, the major phenolic antioxidants found are anthocyanins and ellagitannins. The other phenolic compounds that are also present in small quantities in red raspberries are hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids), hydroxybenzoic acids (ellagic and *p*-hydroxybenzoic acids), flavonols in free and conjugated form (quercetin and kaempferol), and condensed tannins.

In vitro studies shown strong antioxidant power mediated by raspberry extract that may be due to the regulation of NADPH oxidase activity which is controlled by the redox-sensitive signalling system Keap1/Nrf2/ARE. One of the *in vivo* studies already carried out suggests that the antioxidant effect of raspberry polyphenolic fractions could regulate the SKN-1 transcription factor to activate genes downstream, thereby mediating oxidative stress. Another of the studies suggests that raspberry extract enriched with polyphenol and raspberry ketone affects different metabolic pathways. Regarding human studies, no conclusions can be drawn because no one used exclusively raspberries, however, a recent study showed that 3 different anthocyanin-rich drinks affected the transcription of Nrf2, HO-1 and NQO-1 genes in different ways.

Regarding the anti-inflammatory effect, *in vitro and in vivo* studies shown that ETs present in raspberries can act at different levels in the complex regulatory process of the NF-kB pathway. While other *in vitro* and in vivo studies in humans testing raspberry extracts have shown anti-inflammatory based in the same mechanism: blocking p65 phosphorylation pathway as well as the activation of IκB kinase (IKK), IκBα and JNK, thereby suppressing the expression of pro-inflammatory genes such as iNOS, COX-2, IL-1β, and IL-6. Also, one of the *in vitro* studies shown anti-inflammatory activity by the inhibitory effect on lipoxygenase (LOX) and cyclooxygenase-2 (COX-2) whereas a study in humans did not detect any change in the expression of COX-2.

Based on the *in vivo* and in humans studies reported in this review, raspberries seem to be important in preventing diseases like diabetes and CVD diseases due to the benefits of red raspberries in improving postprandial excursions in blood glucose and inflammation. A raspberry extract was able to significantly reduce the development of clinical signs of arthritis due the significant inhibition of inflammation.

The mechanisms of action of the bioactive compounds present in *Rubus idaeus L.* can be diverse, but the antioxidant and anti-inflammatory effects demonstrated by its phenolic compounds clearly interfere with the cell signalling pathways in *in vitro* and *in vivo* studies already carried out. Studies regarding the effect of raspberry intake on humans are still rare and opinions about its beneficial effect are still widely divided. In conclusion, more research is needed to explore whether raspberries provide so many benefits to human health. This issue will be critical for future research priorities that can offer numerous opportunities for raspberries to be used in the food industry, medicine and provide a wide range of benefits not only to our general health and quality of life but also be impactful in reducing how this phenomenon harms the economy of the developed countries.

Future work

This bibliographic review demonstrates that few studies were performed in humans and proves the oxidative and inflammatory potential of red raspberry in some *in vitro*, *in vivo* studies in humans. That said, we would like, as soon as possible, put into practice the primary objective of this master's thesis which aimed to test whether daily intake of raspberry would be able to decrease oxidative stress markers and inflammation in humans when exposed to caloric and sugar-rich diets and fats and how these results could help in the prevention or delay of some chronic diseases

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