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ABSTRACT

Background: Atherosclerosis is the primary cause of cardiovascular diseases (CVDs), contributing to more than 33% of the annual deaths globally. Westernized dietary patterns, a high prevalence (50%) of overweight and obesity, and an increased incidence of glucose intolerance and type-2 diabetes are related to atherosclerosis. However, increased demand for functional foods has boosted the production of different foods to improve people's life quality and decrease the CVDs' risk. Nonetheless, functional foods targeting CVDs are scarce in the marketplace.

Scope and approach: To perform a multidisciplinary and cross-sectoral approach by linking atherosclerosis biomarkers, potential bioactive compounds (e.g., phenolics), and food technology, besides scientific limitations, we propose a practical step-by-step guide to designing functional foods. First, a comprehensive and up-to-date overview of atherosclerosis is provided, focusing on the inflammation markers to counteract its onset and progression. Then, a structure-based–(SBDD) or ligand-based drug design (LBDD) approach is presented, and illustrated by the incorporation of vescalagin, a phenolic compound from jaboticaba seed, into a functional food to mitigate atherosclerosis.

Key findings and conclusions: Tailored functional foods added with phenolic compounds can be designed through computational approaches predicting their bioactivity. Together with chemical analyses, mathematical models can explore a vast array of molecular mechanisms, allowing the discovery of novel bioactive compound sources. Altogether, food science/technology, nutrition, and structure- and ligand-based approaches should be combined to support the design of tailor-made functional foods/nutraceuticals to contribute to public health interventions related to atherosclerosis and other cardiometabolic diseases.

1. Introduction

Overweight and obesity affected over 2 billion people in 2016 and refer to individuals with body mass index over 25 and 30, respectively. This health condition is one risk factor for cardiovascular diseases (CVDs), responsible for over 4 million annual deaths, and has become an epidemic in the 21st century (WHO, 2022). CVDs are responsible for causing approximately 32% of worldwide deaths, whereby over 17.9 million people die each year. Among CVDs, atherosclerosis is the leading cause of death worldwide – in developing and developed countries – from the young to the elderly from all ethnic groups (Libby, 2021).

Atherosclerosis is a lipid-driven chronic inflammation of middle-sized and large arteries, where cytokines and chemokines (family of small cytokines with chemotactic properties) and their receptors participate in the initiation of fatty streaks and the atherosclerotic

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Abbreviations and acronyms

ACS	adult stem cells
ADME	absorption, distribution, metabolism, and excretion
AKT1	serine/threonine-protein kinase
AMI	acute myocardial infarction
AMPK	AMP-activated protein kinase
ASC	apoptosis-associated speck-like protein containing a caspase-1 recruitment domain
CTRP9	C1q/TNF-related protein 9; CVDs, cardiovascular diseases
COX-2	cyclooxygenase-2
EFSA	European Food Safety Authority
ERK1/2	extracellular signal regulated kinase 1/2
FDA	Food and Drug Administration
GHS	globally harmonised system
GRAS	generally recognised safe
HDL-C,	high-density lipoprotein cholesterol
HMG-CoA reductase	3-hydroxy-3-methylglutaryl-CoA reductase
HsCRP	high-sensitivity C-reactive protein
ICAM-1	intercellular adhesion molecule-1
IL-1, IL-6, IL-10, IL-1β	interleukin family
iPSC	induced pluripotent stem cells
JNK	c-Jun N-terminal kinases
LBDD	ligand-based drug design
LDLR	LDL receptor
LOX-1	LDL receptor expression
LOX-1	lectin-like oxidised low-density lipoprotein receptor-1
Lp-PLA2	lipoprotein-associated phospholipase A2
LysoPCs	lysophosphatidylcholines
MAPK1	mitogen-activated protein kinase 1
MCP-1	monocyte chemoattractant protein, MPO myeloperoxidase protein
MSR1	macrophage scavenger receptor type1
NFκβ	nuclear factor kappa B; NIH, National Institutes of Health
NLRP3	nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3
NO	nitric oxide;
NOS	nitric oxide synthases
NOX	NADPH oxidases
Nrf2/ARE	nuclear factor erythroid 2-related factor 2/antioxidant responsive element
oxFA	oxidised fatty acids
ox-LDL,	oxidised LDL;
PON1	paraoxonase 1
QSAR	quantitative structure-activity relationship;
RCT	reverse cholesterol transport
RMSD	root mean score deviation
RNS	reactive nitrogen species
RONS	reactive oxygen/nitrogen species
ROS	oxygen species
SAT	subcutaneous adipose tissue
SBDD	structure-based drug design
SEA	similarity ensemble approach
SMILES	Simplified Molecular Input Line Entry Specification
Src	proto-oncogene tyrosine-protein kinase Src
TLR	toll-like receptors
TNF	tumor necrosis factor
TNF-α,	tumor necrosis factor-α;
TXNIP	thioredoxin interacting protein
VAT	visceral adipose tissue
VCAM-1	vascular cell adhesion molecule
XO	xanthine oxidase

plaque formation (final stage of the disease) (Yan et al., 2021). The consequences of atherosclerotic cardiovascular disease are of significant public health concern: myocardial infarction, ischemic cardiomyopathy, ischemic cerebrovascular disease, stroke, and peripheral arterial disease. These conditions may limit daily routine activities and contribute to morbidity, thus increasing the odds of arthritis, depression, and sadness (Biros et al., 2022).

Therefore, from the public health perspective, it is relevant and timely to study the disease's physiopathology and mechanisms of action of potential natural compounds to counteract the consequences of atherosclerosis and increase individuals' quality of life. Consequently, decreasing incidence and death numbers and reducing costs in the public sector related to chronic treatment. Although the risk of atherosclerosis increases depending on several factors, such as lifestyle (i.e., smoking, alcohol and drug abuse, physical inactivity), eating habits (i.e., high consumption of fats), and pre-existing clinical conditions (i.e., diabetes, hypercholesterolemia, hypertension), there is no single or straightforward treatment, but rather a multifaceted approach is required.

Per se, overweight and obesity stimulate the release of inflammatory mediators, such as tumour necrosis factor-α (TNF-α) and interleukin 6 (IL-6), and reduce adiponectin production, boosting low-grade inflammation and oxidative events in blood and tissues (Ellulu et al., 2017; Tyrrell & Goldstein, 2021). Accordingly, epidemiological evidence connects obesity with sustained overproduction of reactive oxygen species (ROS) that can oxidise biomolecules, structurally modify proteins/genes and decrease the endogenous antioxidant defence. The ROS-induced inflammation triggered by obesity enhances signalling cascades, such as the hypersecretion of pro-inflammatory cytokines that can start and sustain the progression of endothelial/macrovascular dysfunction, the leading cause of cardiovascular diseases. In line with this, trace metal dyshomeostasis has been linked to atherosclerosis (Chowdhury et al., 2018; Moss & Ramji, 2016) since processes such as pro-inflammatory signalling leading to immune cell infiltration, platelet-thrombus formation, nitric oxide (NO) homeostasis, and oxidative stress levels are regulated by essential metals and influenced by non-essential metal contaminants (Chowdhury et al., 2018; Siti et al., 2015).

The secretion regulation of pro-inflammatory cytokines/chemokines, such interleukin family (IL-6, IL-1, IL-1β), TNF-α and alterations in signalling pathways (i.e., level of monocyte chemoattractant protein, MCP-1) employing multifaceted strategies represent a suitable and clinically relevant approach. Indeed, these scientific evidence-based tailored therapies and holistic strategies combining food technologies and health sciences should focus on the development of functional foods, beverages, nutraceuticals, and ingredients that have proven biological efficacy in reducing inflammation pathways and, invariably, the risk of adverse cardiovascular events (Fernandez & Giannarelli, 2022; Siti et al., 2015).

Modern research has focused on using natural products and foods containing bioactive compounds to downregulate pro-inflammatory cytokines and decrease oxidation *in vivo* (do Carmo et al., 2021; Maurer et al., 2020). However, up to now, functional food development has individually focused on the formulation, safety, and efficacy aspects, without designing (and synthesising) toxicologically safe bioactive molecules to be implemented in the personalised functional food design and nutraceuticals.

The current state-of-the-art treatment of CVDs (i.e., atherosclerosis) is based on continuous medications to reduce the serum levels of low-density lipoproteins (LDL) and triglycerides. In addition, nutritional recommendations for a more balanced diet and healthy habits are informed to the patients. In food science and technology, the

development of functional foods relies on adding phytosterols/stanols (e.g., β-sitosterol) and/or polyunsaturated fatty acids (e.g., omega 3) in different products to reduce LDL and triglycerides serum levels. However, only a few options are available on the marketplace, which may limit their applications and general use by consumers globally.

A new strategy should be adopted to formulate functional foods with added bioactive compounds to promote consumer cardiovascular health to move beyond the current state-of-the-art, this work proposes an integrated, multidisciplinary, and cross-functional approach between food and health sciences to counteract the inflammation pathways using tailored functional foods added with phenolic compounds. First, we discuss the main risk factors and overall characteristics of atherosclerosis with emphasis on the inflammation markers, such as pro-and anti-inflammatory interleukins and the key controlling checkpoints of atherosclerosis-associated inflammatory pathways, such as the serine/threonine-protein kinase (AKT1), mitogen-activated protein kinase 1 (MAPK1), proto-oncogene tyrosine-protein kinase Src (Src), nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NRLP3) inflammasome, and nuclear factor kappa B (NFκβ) activation, as well as the activity of enzymes, such as 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA reductase), lecithin-cholesterol acyltransferase, and AMP-activated protein kinase (AMPK). Second, we discuss the applicability of *in silico* three-dimensional (3D) molecular modelling to address the mechanistic understanding and prediction of molecular ligand-binding activity between a phenolic compound and the enzymes linked to atherosclerosis. Third, we bridge the gap between the pro-inflammatory pathophysiology of atherosclerosis, the bioactivities of natural products (i.e., phenolics) and the use of computational approaches to predict their bioactivity. Fourth, the inclusion of bioactive compounds in functional foods is discussed considering the relationship between the atherosclerosis biomarkers, the chemical structure, and phenolic compounds' bioactivities. Finally, we present a practical step-by-step guide that can be adopted in the functional food/nutraceutical design to contribute to public health interventions related to atherosclerosis and other cardiometabolic diseases.

To the best of the authors' knowledge, an integrated approach has not been applied to creating a workflow dedicated to developing functional foods and nutraceuticals. However, by adopting structure- and ligand-based approaches, some advantages will be evident for food and pharma industries: saving of time and resources, reduced use of chemical reagents and solvents, reduced use of animals, increased accuracy to observe positive effects on inflammatory and oxidative markers due to the use of *in silico* simulations.

2. Counteracting atherosclerosis by regulating inflammation and oxidative stress biomarkers

2.1. Atherosclerosis: pathophysiology, biomarkers, and potential natural therapeutic candidates

Atherosclerosis can be defined by specific immunological, cellular, and physicochemical events as an inflammatory disease characterised by the build-up of modified fatty deposits in the wall of large- and medium-sized arteries. These complex mechanisms surpass the simplistic model that describes atheroma as a passive deposition of lipid debris on the artery wall (Rocha & Libby, 2009). Beyond these cellular processes, the vessel wall is exposed to hemodynamic shear stress from the frictional force induced by the blood flow against the endothelium lining of the vessel wall, as well as wall strain (developed due to blood pressure), which are known to provoke endothelial injury and, consequently, atherogenesis (Ilias et al., 2021).

Regardless of the initial trigger factors, evidence shows that inflammation in the early phase of atherosclerosis is thought to be a consequence of endothelial injury, which subsequently induces the expression of adhesion molecules, such as vascular cell adhesion molecule (VCAM-1). VCAM-1 can retain migrating inflammatory cells along the arterial wall. Monocytes differentiate into macrophages and phagocytose the LDL particles to form foam cells, the hallmark of atherosclerotic lesions (Libby, 2021). Foam cells contribute to the accumulation of the atheroma in the plaque and secrete proinflammatory cytokines, such as IL-1β. Together with inflammatory cells, these cytokines enhance the development of early atherosclerotic plaques called fatty streaks. Infiltration of more inflammatory cells and smooth muscle cells attracted by the cytokines leads to the formation of a fibrous cap over the lipid core, neovascularisation, haemorrhage in the plaque, activation of matrix metalloproteases, and the degradation of collagen fibres in the fibrous cap (Silvis et al., 2021). Although the plaque development is asymptomatic, until the plaque ruptures, platelet aggregation rapidly occurs, hindering blood flow through the artery, resulting in a coronary event (Moss & Ramji, 2016). The initiation of these complex events is associated with risk factors attributed to sex, age, family history of premature cardiovascular diseases, cholesterol, increased blood pressure, cigarette smoking, the lack of essential metals or the presence of non-essential, potentially toxic metals, and obesity/overweight, among others (Ilias et al., 2021). Despite effective interventions for controlling LDL, blood pressure, and other traditional risk factors, a considerable residual risk remains for atherosclerotic cardiovascular disease (Libby, 2021). Indeed, despite improved lifestyle and medical treatment strategies, major cardiovascular events still occur in a substantial proportion of the population.

This issue is commonly described as the problem of "residual risk". Such residual risk for developing a cardiovascular event may reflect aspects of atherogenesis, such as specific inflammatory pathways, which are not targeted by the current treatment strategies (Ridker, 2017).

The roles of different cytokines and chemokines in atherosclerosis have been extensively reviewed and can be generally classified as either pro-inflammatory or anti-inflammatory. For example, TNF-α causes overexpression of oxidised LDL receptor (LOX-1) and is associated with increases in NFκβ signalling and NFκβ activation-dependent adhesion molecules such as VCAM-1 and ICAM-1 expression (Siti et al., 2015). These pathways and other atherosclerosis biomarkers are presented in Fig. 1.

In this sense, LOX-1 expression is up-regulated after exposure to several proinflammatory cytokines, increasing cell foam formation. Moreover, the inflammatory cytokines, importantly TNF-α and C-reactive protein at the same time, will activate NADPH oxidases (NOX) that, as previously explained, produce superoxide anion, considered one of the primary sources of ROS (Siti et al., 2015).

Particular attention should be given to MAPK, a family of serine/threonine kinases (STK). It is an essential mediator of the inflammatory signalling cascade by activating the cytokine (TNFα, IL-1, IL-6) production, cyclooxygenase-2 (COX-2), and metalloproteinases during acute coronary syndrome. MAPK family is categorised into four distinguished classes: extracellular signal-related kinases 1 and 2 (ERK-1/2), c-Jun N-terminal kinases (JNK-1/2/3), p38 proteins (α, β, γ, δ), and ERK5, that become activated in response to stress mediators, such as hypertension, oxidised LDL (oxLDL), vascular injury, and ischemia (Gluba-Brzózka et al., 2021). In general, phytopigments inhibit p38 and JNK (related to cell proliferation, apoptosis and inflammation) and activate ERK1/2 (Varghese et al., 2022). ERK1/2 plays a central role in cell proliferation control, inducing positive cell cycle regulators and inactivating antiproliferative genes to promote cell growth (Meloche & Pouysségur, 2007). The p38 is involved in inflammatory signalling and activated in response to various intracellular and extracellular stimuli, including oxidative stress, cytokines, and growth factors, which are abundantly present in atherosclerotic and aortic valve sclerotic lesions (Reustle & Torzewski, 2018). Among bioactive compounds, luteolin inhibits apoptosis and promotes healing from myocardial infarction via activation of ERK1/2 and downregulation of p38 and JNK (Varghese et al., 2022), while curcumin protects vascular smooth muscle cells from LPS induced inflammatory damage by blocking of NFκβ and the JNK

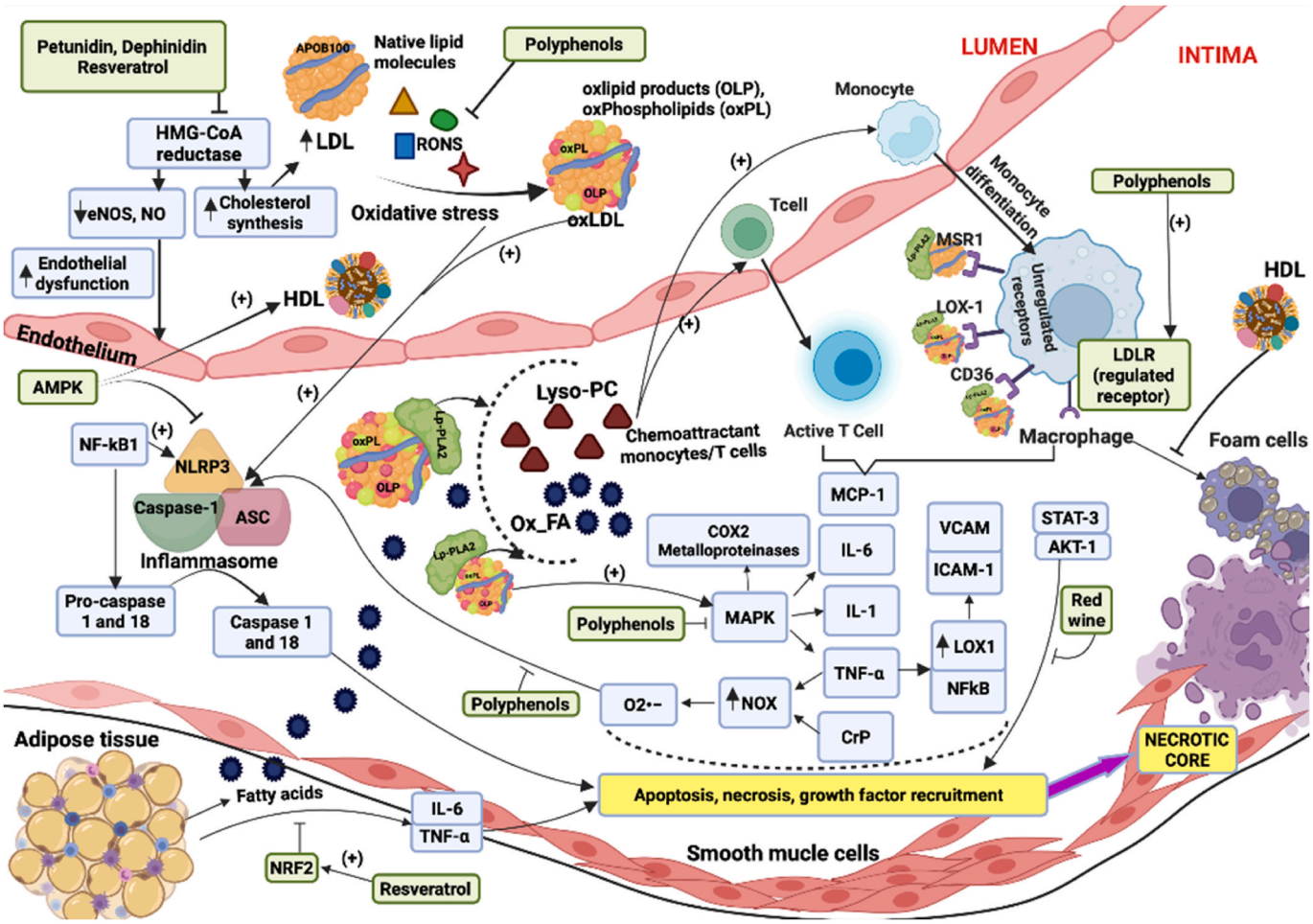

Fig. 1. Atherosclerosis disease progression and the role of critical cytokines (blue bokes), blood cells (monocytes and T cells), lipoproteins (HDL and LDL), oxidation cascade (e.g., oxFatty acids) and adipose tissue orchestrating the apoptosis/necrosis, which are in the centre of necrotic core formation (yellow boxes). These pathways are inhibited (red boxes) by bioactive phenolic compounds to open new strategies for discovering how to use food as therapy and aid the identification of food bioactive compounds with direct therapeutic impact. ↑, increase; ↓decrease. (For interpretation of the abbreviations in this figure legend, the reader is referred to the Abbreviations and Acronyms section). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

signalling pathway (Ruan et al., 2022).

In the same context of inflammatory signalling, emerging evidence has been spurring a considerable evolution related to the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome and its downstream cytokines, IL-1β and IL-18. Inflammasomes are intracellular protein complexes that respond to many pathologies, containing NLRP3, ASC (apoptosis-associated speck-like protein containing a caspase-1 recruitment domain), and caspase-1 (H. Zhang, Gong, et al., 2019). The formation of NLRP3 inflammasomes typically requires a priming step. The activated NFκβ drives increased expression of NLRP3 and pro-interleukin-1, and pro-interleukin-18, whose subsequent activation in NLRP3/ASC/caspase-1 complex is typically triggered by oxidative stress. The assembly of this complex leads to the activation of caspase-1, that further cleaves the immature pro-inflammatory cytokines (pro-IL-1β and pro-IL-18), resulting in their active forms and inflammation responses (Swanson et al., 2019; H. Zhang, Gong, et al., 2019). This brief sketch of inflammasome activation enables us to deduce that specific measures would tend to block inflammasome formation, such as the inhibition of NFκβ activation; the thioredoxin and thioredoxin reductase overexpression; TXNIP down-regulation; inhibition of the generation of NOX-mediated superoxide, and boosting hydrogen peroxide catabolism, by increasing the expression of related enzymes. Specific antioxidant nutraceuticals have the potential to accomplish these aims and reach the clinical potential for suppressing the contribution of NLRP3 inflammasomes to several inflammation-linked pathologies in which such inflammasomes play a critical regulatory role (McCarty et al., 2021).

2.1.1. Reactive oxygen/nitrogen species (RONS), atherosclerosis, and natural compounds: building a multidisciplinary and cross-sectorial approach

Several studies report that the risk factors for atherosclerosis are closely related to increased RONS production by endothelial cells, smooth muscle cells, and the adventitial cells (Santhakumar et al., 2018). Oxidative/Nitrosative stress is an imbalance between endogenous antioxidants and RONS, with the predominance of the latter (Mladěnka et al., 2010). ROS include free radicals such as $O^{2\bullet-}$ (superoxide) and $OH^{\bullet}$ (hydroxyl), and non-radicals such as H_2O_2 (hydrogen peroxide) (Siti et al., 2015), while reactive nitrogen species (RNS) refer to various nitrogenous products from nitric oxide synthases (NOS), such as nitric oxide (NO), nitrogen dioxide (NO_2), nitroxyl anion (NO^-), nitrite (NO^{2-}) and, $ONOO^{\bullet-}$ (peroxynitrite). Oxidative/Nitrosative stress drives endothelial dysfunction and LDL oxidation, followed by an inflammatory response on the vasculature, which is considered the first step of atherosclerosis (Santhakumar et al., 2018). RONS also participate in acute myocardial infarction (AMI) and its expected consequences, such as heart failure and arrhythmias. In the ischemic phase of AMI

platelet aggregation, several processes play essential roles: activation of neutrophils, increase in cellular free redox-active iron, and transformation of xanthine dehydrogenase into ROS producing xanthine oxidase (XO) (Mladěnka et al., 2010).

The presence of non-essential metals, for example, Cd, Pb, Hg, or the absence of essential metals such as Zn, increases oxidative stress by generating an imbalance between the production and detoxification of ROS (Kataba et al., 2021), which is mediated by enzymes that are dependent on essential metals. In line with this, Cd concentrations correlate positively with the degree of atherosclerosis, while the crucial metals Cu, Co, and Zn levels show a negative correlation (Solenkova et al., 2014). The effect of metals on ROS generation may explain why exposure to As, Pb, Cd, and Cu is directly associated with an increased risk of CVD incidence and mortality (Chowdhury et al., 2018).

Physiologically, the antioxidant defence systems include endogenous enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase, non-enzyme molecules including albumin, bilirubin, and glutathione, the molecular regulatory mechanism by Nrf2/ARE-mediated antioxidant gene expression as well as micronutrients such as trace metals, and vitamins (Siti et al., 2015). Although the recent consensus suggests that oxidative stress is not a significant driver of atherosclerosis (Libby, 2021; Tyrrell & Goldstein, 2021), its role seems to be more complex, involving both the hemodynamic (vasoconstrictive) and structural (vascular remodelling) aspects in the initiation and progression of atherosclerosis (Mladěnka et al., 2010). The precise mechanisms underlying this phenomenon remain unclear, and the gaps in our understanding regarding oxidant-antioxidant status matter leave plenty of room for discovery (Siti et al., 2015; Tyrrell & Goldstein, 2021). Deciphering the mechanisms by which inflammatory and oxidant-antioxidant status can promote atherosclerotic cardiovascular disease will be fundamental for developing novel therapies (including functional foods and nutraceuticals) to reduce the burden of atherosclerosis.

Although the bioavailability of polyphenols remains controversial, its oral intake has been pointed out as an intervention therapy for cardiovascular disease by exhibiting promising results, with the possibility of combining this bioactive compound with drugs to reduce atherosclerotic progression in at-risk populations by multiple synergistic mechanisms (Santana et al., 2022; Santhakumar et al., 2018). From a mechanistic perspective, polyphenols exert anti- or pro-oxidant activity by modulating protein activity, interacting with digestive enzymes, and modulating gut microbiota growth (do Valle et al., 2021).

Apart from the direct antioxidant properties, which include direct ROS scavenging activity and transient metal chelation, it is essential to highlight that polyphenols present many other significant effects, such as a) direct interaction and inhibition of XO, NOX, and lipoxygenases enzymes; b) decreased platelet aggregation and leukocyte adhesion and c) vasodilatory properties. For these properties, a catholic B-ring from the flavonoid structure (Fig. 2) is necessary for scavenging activity; hydroxyl groups in an *ortho* position, the 3-hydroxy-4-keto group, or the 5-hydroxy-4-keto group enable iron chelation; planar confirmation with the 4-keto group and 2,3-double bond is essential for inhibition of leukocyte adhesion, and platelet aggregation; specific hydroxy-methoxy *ortho* conformation in B ring is necessary for the inhibition of NOX; and the 4-keto group is a requisite for vasodilatory action (Mladěnka et al., 2010).

For this purpose, some flavonoids act in the hydrophobic core of the membrane, where they may establish the fundamental mechanisms that include cellular interaction and signal transduction, which drive the prevention of access to oxidants and the protection of cell structure and function. In addition, the interaction of polyphenols with NOS activity may modulate NO production. For example, quercetin, silibinin, and luteolin can inhibit the activity of XO, considered a critical source of free radicals (Hussain et al., 2016). The metal-chelating properties of polyphenols at physiological pH may further contribute to re-balancing essential trace metals by scavenging toxic heavy metals such as Hg (Girard et al., 2018). Thus, besides reducing the effects of metals resulting in an increase in ROS, normalisation of Zn levels, for example, will also minimise pro-inflammatory signalling (Gammoh & Rink, 2017).

In order to deeply understand polyphenol molecular mechanism, Santana et al. (2022) randomised clinical trials assessed the resveratrol effect on 12 biomarkers observing that: a) the lipid-improving effect of resveratrol could be due to its downregulation of the hepatic enzyme HMG-CoA reductase and the increment the expression of LDL receptors contributed to both reducing LDL concentration in the circulation and increasing LDL excretion from the enterohepatic cycle; b) the beneficial effect of resveratrol on systolic blood pressure may involve the increase of NO concentration by stimulating endothelial NOS, via AMPK-activation upon phosphorylation; c) Resveratrol's antioxidant

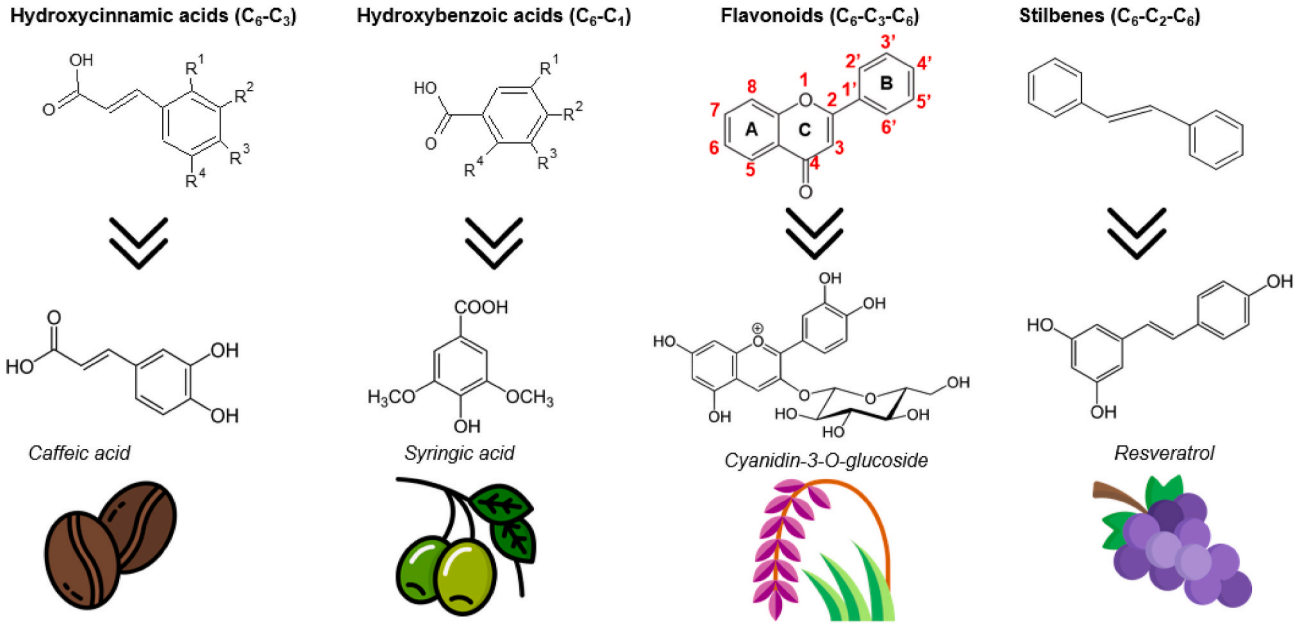

Fig. 2. Chemical structure, examples, and food sources of some phenolic subclasses (phenolic acids, stilbenes, and flavonoids).

capacity attenuates vascular oxidative stress and prevents NO breakdown, mediating its vasodilatory property; d) resveratrol regulates critical players in the inflammatory cascade, including molecular-targets such as NFκβ, toll-like receptors (TLR) and Nrf2, suppressing pro-inflammatory cytokine production, such as IL-6, TNF-α and high-sensitivity C-reactive protein (hsCRP); and, at the end, e) the interventions last about 2 months, with the amount of dosage of 500 mg/day is more often applied in the clinical assays.

Appropriate NO levels modulate the vascular tone through its capacity to reverse the acetylcholine's constrictive effects, thereby favouring vasorelaxation, and maintaining the balance of endothelium-derived contracting factors, such as endothelin-1 and thromboxane A2. Alternatively, decreased NO bioavailability has been related to atherothrombosis processes, given the NO antithrombotic, antiapoptotic, anti-inflammatory, and antioxidant effects (Santhakumar et al., 2018). This same effect is achieved by several plant metabolites that inhibit the enzyme's activity (Bahrami et al., 2020; Kozuka et al., 2020; Ruscica et al., 2021).

2.1.2. Obesity and plasma biomarkers related to lipid metabolism and endothelial dysfunction: choosing possible targets for tailoring functional foods

Obesity is a pro-inflammatory condition with a link between the adipose tissue and immune system that contributes to changes in secretory functions of adipose tissue involving both adipokines (cytokines) and chemokines. Some adipokines (e.g. adiponectin and omentin) have a protective role in atherosclerosis development and are reduced with increased adiposity and have proinflammatory effects (e.g., leptin) (Yan et al., 2021; Zhang, Gong, et al., 2019). C1q/TNF-related protein 9 (CTRP9) is a newly identified paralog of adiponectin with anti-inflammation activities. CTRP9 links the AMPK and NLRP3 pathways by activating the AMPK that enhances glucose uptake, fat metabolism, autophagy, and NO production and suppresses the actions of the NLRP3 inflammasome. In this sense, CTRP9 has been suggested to exert an atheroprotective effect via the AMPK pathway (H. Zhang, Gong, et al., 2019). Moreover, increased body adiposity levels change the secretory pattern and plasmatic levels of such biomarkers, resulting in metabolic disorders, such as insulin resistance and atherosclerosis (Santana et al., 2022; T. Zhang, Gong, et al., 2019).

Indeed, the expanded visceral adipose tissue (VAT) becomes inflamed due to the macrophage infiltration into hypertrophied adipocytes, leading to increased production of pro-inflammatory mediators (such as TNF-α and IL-6) and reduced production of a protective adipokine, adiponectin. Adiponectin is an anti-atherogenic, anti-diabetic, and anti-inflammatory protein that is abundantly released from subcutaneous adipose tissue (SAT) and VAT. However, excess VAT is associated with decreased adiponectin production in whole-body adipose tissues, probably because of the increased secretion of inflammatory adipokines. Moreover, increased influx of free fatty acids into the liver has been shown to induce enhanced gene transcription of hepatic acyl-coenzyme A synthetase, one of the limiting enzymes of triglyceride synthesis, as well as of microsomal triglyceride transfer protein (a rate-limiting protein involved in the secretion of VLDLs), contributing to the development of hypertriglyceridemia (Neeland et al., 2019).

HMG-CoA reductase is another biomarker associated with the lipid pathway, the liver's key enzyme of cholesterol synthesis. Statins are inhibitors of HMG-CoA reductase and represent a family of cholesterol-lowering drugs. They are widely used in medical practice for patients at increased risk of atherosclerotic cardiovascular disease. Through inhibiting HMG-CoA reductase and preventing L-mevalonic acid synthesis, these inhibitors reduce the generation of subsequent isoprenoid elements of this pathway, besides over-expression and activation of endothelial NOS and NO production (Bahrami et al., 2020). Using an *in vitro* method to analyse potential HMG-CoA reductase inhibitors from Aronia berry juice, petunidin-glycoside-arabinoside and delphinidin-arabinoside, two major anthocyanins, were able to reduce 57% of the activity (Kozuka et al., 2020).

The accumulation of fatty deposits and the oxidation of plasma LDL in the intima of the artery is another relevant step in the progression of atherosclerosis (Santhakumar et al., 2018). The immune response operates during atherogenesis, targeting candidate antigens forms of LDL such as oxLDL (Ilias et al., 2021; Libby, 2021). Oxidised phospholipids in LDL are hydrolysed by lipoprotein-associated phospholipase A2 (Lp-PLA2) to generate lysophosphatidylcholines (lysoPCs) and oxidised nonesterified fatty acids. LysoPCs are a potent chemoattractant for T cells and monocytes. The local inflammation is triggered by the influx of monocytes that differentiate into macrophages, associated with an increased expression of scavenger receptors on their macrophages' surface. Differently, the uptake of LDL via the LDL receptor (LDLR), which is controlled by a negative feedback loop, the oxLDL uptake is unregulated via scavenger receptors, such as macrophage scavenger receptor type1 (MSR1) and CD36 (Moss & Ramji, 2016). The central ox-LDL receptor of endothelial cells is LOX-1. LOX-1 has pro-inflammatory potential in atherogenesis, and is up-regulated after exposure to several proinflammatory and proatherogenic stimuli. On the other hand, the suppression of the NFκβ inflammatory signalling pathway, is shown to downregulate LOX-1 receptor expression, subsequently reducing the scavenging of ox-LDL and thus the formation of foam cells (Siti et al., 2015). Moreover, pro-inflammatory cytokines can induce foam cell formation by altering the expression of critical genes implicated in cholesterol metabolism and transport regulation, such as ABCA1, ACAT1, APOE, and MSR1. Foam cells subsequently accumulate, forming an initial lesion that matures into an atherosclerotic plaque (Moss & Ramji, 2016). T cells are also recruited to the forming lesion parallel with macrophages, mainly of the proinflammatory T-helper (Th) subtype, producing inflammatory cytokines (Gisterå et al., 2018). Bearing it in mind, LDL per se appears to be a relatively weak stimulus to innate immune activation (Libby, 2021). Overall, this promotes endothelial dysfunction inducing the release of arachidonic acid and inflammatory cytokines, such as TNF-α, IL-1, and IL-6, and inducing apoptosis of endothelial and vascular smooth muscle cells, in addition to stimulating the MCP-1 release from macrophages (Siti et al., 2015).

Regarding HDL, this lipoprotein has multiple atheroprotective effects beyond of reverse cholesterol transport (RCT) pathway linked to inflammation, oxidative stress, and endothelial cell maintenance, which are related to HDL-associated proteins that bind to HDL, such as myeloperoxidase (MPO) and paraoxonase 1 (PON1). Other proteins contribute to cholesterol efflux and scavenger receptors, whereas others are involved in HDL's maturation and hepatic uptake, such as LCAT (Ma et al., 2017). Adipose tissue can maintain normal cardiovascular function at average body weight by regulating HDL-C levels and HDL function. In contrast, in obese individuals, adipose tissue morphology has been changed, leading to impaired regulatory function and eventually accelerating the occurrence of cardiovascular disease (T. Zhang, Gong, et al., 2019). Ma et al. (2017) pointed out that HDL's anti-atherogenic properties *in vitro* are enhanced by the pharmacological AMPK activation, which represents one of the critical mechanisms by which AMPK activation attenuates atherosclerosis.

2.2. Natural compounds to mitigate atherosclerosis

Statins and ezetimibe are the classical drugs used to manage atherogenic plaques in traditional medicine by decreasing the serum LDL and total levels. However, considering more holistic and nature-based lifestyles, natural products are pursued to replace synthetic cholesterol synthesis/absorption inhibitors that have been widely adopted. The National Center for Complementary and Integrative Health, from the U.S. National Institutes of Health (NIH), suggests the consumption of plant-based stanols and sterols marketed as nutraceuticals (i.e., pills and tablets) or added into functional foods (i.e., yoghurts, cream cheese, and spreads), soy (i.e., containing isoflavones), flaxseed (i.e., containing lignans), green tea (i.e., containing flavanols), oats and

oat bran (i.e., containing dietary fibres, such as β-glucans), and red yeast rice (i.e., containing monacolin K) (NCCIH & NIH, 2019). However, experimental, clinical, and epidemiological studies have pointed out that bioactive compounds, especially phenolics, can avoid/retard LDL oxidation, enhance the activity of endogenous antioxidant enzymes (e.g. catalase, superoxide dismutase, and glutathione peroxidase), scavenge RONS, reduce platelet aggregation, and mitigate inflammation by suppressing/decreasing the release of pro-inflammatory cytokine in tissues (Khalil et al., 2021; Santhakumar et al., 2018; Xue et al., 2021).

In this scenario, obtaining naturally derived compounds to formulate functional foods, beverages, and ingredients, will play a decisive role in human nutrition by delivering health benefits and fulfilling basic nutritional requirements. Indeed, evidence is mounting that greater consumption of potentially functional foods can significantly reduce morbidity/mortality associated with cardiovascular diseases by modulating specific biomarkers (Bitok & Sabaté, 2018; Fedacko et al., 2019).

Furthermore, functional foods allow the distribution of naturally derived bioactive compounds that would not be possible to be accessed by the general population. Additionally, gaps in knowledge concerning underutilised food and its by-products should be addressed to promote and expand their use in food applications, promoting the value chains to propel the ones to broader commercial markets and food environments (Granato et al., 2017; Pfukwa et al., 2020).

Herein, we highlight that bioprospection could provide novel bioactive compounds in food applications, allowing the exploration and increasing the value of a wide range of foods or their by-products, with potential application in antioxidants extraction or other health bioactive benefits (Pfukwa et al., 2020). In this view, a strong alliance between science, technology, and health is the best way to understand and create functional foods. However, one should bear in mind that functional foods require *in silico*, *in vitro*, *in vivo* (animals), and human interventions to ensure clinical efficacy and, thus, support any health claims (Granato et al., 2020).

For example, Sinaga et al. (2021) studied the effects of supplementation of *Rhodomyrtus tomentosa* (rose myrtle) fruit juice, a rich source of piceatannol and flavonoids, in hypercholesterolemia and atherosclerosis in male albino rats fed with a high-fat high cholesterol diet. They found that the consumption of this phenolic-rich fruit juice was associated with a decreased triacylglycerides, LDL, and total cholesterol in serum while preventing the thickening of the blood vessel wall, deposition of lipid formation and foam cells in the tunica intima of the aorta and coronary arteries. Similarly, Che Idris et al. (2014) found that extracts of oil palm fruit, which are also sources of phenolic acids (protocatechuic, *p*-hydroxybenzoic, and caffeoyl shikimic acids), increased the plasma antioxidant capacity and reduced the development of fatty streaks, fatty plaque, and fibrous plaque in male New Zealand White rabbits fed a high-fat atherogenic diet. These two examples show the significant association between the consumption of phenolics and the decreased risk factors for atherosclerosis. It is well established that phenolics can reduce the onset and progression of atherosclerosis by modulating the secretion of pro and anti-inflammatory cytokines in different tissues, especially in coronary vessels (Vázquez-Agell et al., 2013; Zhu et al., 2013). Indeed, cytokines play a significant role in mediating the inflammatory response in atherosclerosis. In atherosclerosis, low-grade chronic inflammation involves activating the vascular endothelium and a concomitant increase in adhesion of mononuclear cells and platelets to the injured endothelial layer (Santhakumar et al., 2018). Pro-inflammatory cytokines, such as TNF-α and IL-1β, commonly found in atherosclerotic lesions, can induce chemotactic factors and other cytokines that contribute to cell adhesion molecules, which is the early stage and a critical stage of atherosclerosis (Al-Sharea et al., 2018). Thus, regulating pro-inflammatory cytokine secretion by a sustained consumption of bioactive compounds is of pivotal public health importance. It may significantly decrease low-grade inflammation and retard plaque formation in coronary vessels.

2.3. The usefulness of computational chemistry for the design and development of functional foods

The assessment of bioactivity and the health-claim formulations of potentially functional foods and ingredients should be based on *in vitro*/*in vivo* and human testing. For the development of these products, the current food science and technology approach relies on the extraction of bioactive compounds, characterisation of bioactive-rich extracts in terms of chemical composition and bioactivities, and application in food models. Although this approach is valuable, its application in functional food design is technologically limited, with plenty of room for a non-success rate; the potential functional food may not display bioactivity either *in vitro* or *in vivo* (Granato et al., 2017, 2020). To overcome this limitation and increase the odds of success, *in silico* methods can be used, which has put them in the spotlight on food development (Peredo-Lovillo et al., 2022).

Herein, we seek to provide the reader with the necessary *in silico* tools to begin designing food models with health benefits. A case study was applied based on the relationship between target proteins (MAPK1 as atherosclerosis biomarker) and the atomic level's interest polyphenol (vescalagin). The polyphenol vescalagin from jabuticaba seeds was selected by presenting antioxidant and antineoplastic effects (do Carmo et al., 2021; Fidelis et al., 2021). To demonstrate how the *in silico* framework can systematically suggest the therapeutic effects of selected polyphenols, here we focus on presenting the underpinning steps and necessary software for computational simulations.

In silico approach is usually referred to as any experimentation performed by or on a computer, and is in line with the more commonly known biological terms *in vivo* and *in vitro* (Ekins et al., 2007). *In silico* approaches primarily aim to investigate the relationship between chemicals and molecular targets and/or metabolic pathways related to their effects before experimentation (Daina et al., 2017). For example, molecular docking allows the study of compound behaviour within the binding site of the target enzymes and enables a structure-based virtual screening to identify new bioactive compounds (Cheurfa et al., 2019).

In this sense, structure-based drug design (SBDD) or ligand-based drug design (LBDD) (Lima et al., 2016) approaches can be employed to develop potential functional foods. SBDD calculates the interaction between a molecular target (usually a protein or DNA/RNA) and a bioactive compound (Pinzi & Rastelli, 2019). Molecular docking is one of the most critical methods employed in SBDD strategies. It predicts the binding mode of a given ligand and its respective binding affinity to the studied biomarker, such as a protein. It combines algorithms to predict a pose, that is, a conformation and orientation of a compound structure inside a binding pocket of a molecular target (Torres et al., 2019).

The second strategy, LBDD, relies only on the chemical structure of the bioactive compounds. In this sense, an activity (e.g. inhibition of an enzyme) could be predicted using methods that compare its structure with other compounds with known biological properties (usually mentioned as a dataset). Here one can cite: (i) molecular similarity, that is, a method to calculate and quantify how two or more compounds are alikeness (Maggiora et al., 2014), (ii) quantitative structure-activity relationship (QSAR) models, which aim to predict a numeric activity value or to classify compounds between active/inactive (Gramatica, 2020); and (iii) pharmacophore modelling, which consists in the determination of specific functions related to a given biological activity, and their comparison with a compound of interest (Lu et al., 2018).

In summary, both strategies could be employed in the food chemistry scenario to support the functionalisation of food ingredients. Combining two or more computational tools (e.g. SBDD and LBDD) can improve their reliability (Vázquez et al., 2020). Indeed, LBDD strategies could be employed before docking studies to guide the selection of molecular targets in a disease, such as atherosclerosis.

With one or more bioactive compounds available, some *in silico* approaches could be employed to study biochemical processes that may affect health or present a therapeutic potential, for example, the use of

functional foods and their bioactive compounds against atherosclerosis and their biomarkers. In summary, a few steps can be taken in *in silico* studies: (i) select a target (biomarker) crystal structure available; (ii) analyse binding interactions with the target's co-crystallized ligand (bioactive compound) by redocking; (iii) evaluate other ligands potential interactions with the target by molecular docking; (iv) predict other potential targets related to these ligands; (v) predict the toxicity and pharmacokinetic properties of the ligands; and (vi) evaluate selected ligands *in vitro*. Herein, we described these computational steps applying to vescalagin from jaboticaba's seed (do Carmo et al., 2021; Fidelis et al., 2021) to explore the potential anti-atherosclerosis mechanism (Fig. 3).

As the first step, enzyme structures with suitable quality must be selected for computational studies; in our study case, MAPK1 enzyme was selected. For this, the search in the Protein Data Bank (PDB) could be conducted considering three criteria for selecting appropriate structures for study analysis: i) resolution values preferably <2.0 Å; ii) presence of co-crystallized ligands in the complex; iii) absence of mutations; and iv) absence of unmodelled regions. The PyMOL software (v0.99c) (DeLano, 2006) can analyse results and generate figures (including those presented in this review). Here, a representative protein structure of the (MAPK1), one of the main pathways associated with atherosclerosis, was selected for the exemplification analyses (PDB ID: 4QTE) with vescalagin.

Towards the second step, molecular docking analyses can start with the redocking of the co-crystallized inhibitor, which is the ligand present in the experimentally determined 3D structure of the studied protein, MAPK1 in this case, as a validation procedure of docking protocol. Besides our case study, we can prepare any target structure by i) removing water molecules in the structure; ii) adding hydrogens atoms; iii) extracting the co-crystallized ligand and other substructures; and iv) calculating atomic partial charges with default parameters. The redocking is an essential step in accessing the reliability of the docking algorithm to predict experimental binding poses. The parameters of the programs, such as Surflex-Dock (Jain, 2003), including scoring function, the flexibility of residues and atoms, and the number of poses, should be previously tested and evaluated to reproduce better and represent the experimental binding mode of co-crystallized ligands (Vallone et al., 2018).

Fig. 3. Alternate steps for an *in silico* approach. A computational approach study of biochemical processes such as molecular mechanisms and their modulation may involve six usually sequential steps: (1) the selection of a target structure (e.g. MAPK1), followed by its (2) binding interactions analysis with its co-crystallized ligand (e.g. VTX-11e, a novel ERK1/2 inhibitor) and (3) other bioactive compounds (e.g. vescalagin), as well as (4) prediction of other targets (e.g. AKT1) and (5) ligands toxicity or kinetics (e.g. cardiotoxicity and mutagenicity), and lastly (6) an evaluation *in vitro* or *in vivo*. In addition, any of the steps can be followed systematically or be reworked/disregarded as needed. Images were generated with PyMOL v0.99c (DeLano, 2006) and from free vectors available online at Vecteezy: https://www.vecteezy.com (accessed on March 02, 2022).

It is essential to mention that each docking program (e.g. GOLD, AutoDock, AutoDock Vina, and Glide) employs different algorithms and scoring functions and different accuracies for specific systems (Z. Wang et al., 2016). This has implications for the choice of docking programs/protocols according to the particular targets and protein families (Cross et al., 2009) and also be influenced by the resolution of a target's crystal (Agrawal et al., 2019). It is interesting to test and compare different docking programs and protocols to find the most similar binding pose compared to the experimental data (Castro-Alvarez et al., 2017). After assessing a successful redocking protocol, a docking with a different bioactive compound, the third step can be performed. This phase results in predicted binding modes (poses); in our example, the aim is to understand how vescalagin interacts with MAPK1 (Fig. 4).

Our results from the docking procedure suggest that vescalagin could interact with MAPK1 due to the similar binding mode and score compared to the crystallographic ligand (Fig. 4) and be further experimentally evaluated. Particular attention was given to MAPK1, as it becomes activated in response to stress mediators related to atherosclerosis, such as hypertension, ischemia, and vascular injury (Gluba-Brzózka et al., 2021).

The fourth step relies on the prediction of potential targets related to vescalagin, which can be based on biological information of the ligand, and predicted using webservers, such as the Similarity Ensemble Approach (SEA) (Keiser et al., 2009) (https://sea.bkslab.org/), SuperPred (Dunkel et al., 2008) (https://prediction.charite.de/index.php), and the SwissTargetPrediction (Daina et al., 2017) (http://www.swisstargetprediction.ch/). Bioactive compounds are submitted to the webservers in SMILES (Simplified Molecular Input Line Entry Specification) format, returning data from predicted targets that could bind to it.

Briefly, the SEA analyses are based on the chemical similarity between different chemical structures with a given ligand by Tanimoto combo (Tc). Herein, the squalene monooxygenase (enzyme related to the cholesterol biosynthetic pathway as of HMG-CoA), IL-6, and prothrombin (precursor of thrombin which plays a vital role in the pathogenesis of atherosclerosis) were predicted as potential targets, whose statistical significance was attained - *p*-value was lower than 0.005 in all cases.

SuperPred is based on machine learning, using a linear logistic regression model to evaluate and score potential targets and predict some macromolecules (probability in parenthesis) directly or indirectly associated with vascular dysfunctions and coagulation related to atherosclerosis, such as the coagulation factor XIII (81.16%), plasminogen activator inhibitor-1 (68.28%), dual specificity mitogen-activated protein kinase 2 (53.39%), and a tissue-type plasminogen activator (50.17%). Lastly, SwissTargetPrediction estimates potential targets from a compound assumed as bioactive, employing a combination of 2D and 3D similarities on different proteins from three different species (*Homo sapiens*, *Mus musculus* and *Rattus norvegicus*). These combinations can be of interest for further investigations *in vivo* and*in vitro* models. The thrombin and coagulation factor X, a target related to atherosclerosis, was predicted with the highest probability. Furthermore, other approaches may consider target prediction methods that use molecular similarity (Serafim et al., 2019), machine learning (Veríssimo et al., 2019), and other ligand-based approaches (Asse Junior et al., 2020) and can also be considered to be used aiming to improve the reliability of predictions.

The fifth step considers the prediction of pharmacokinetics and

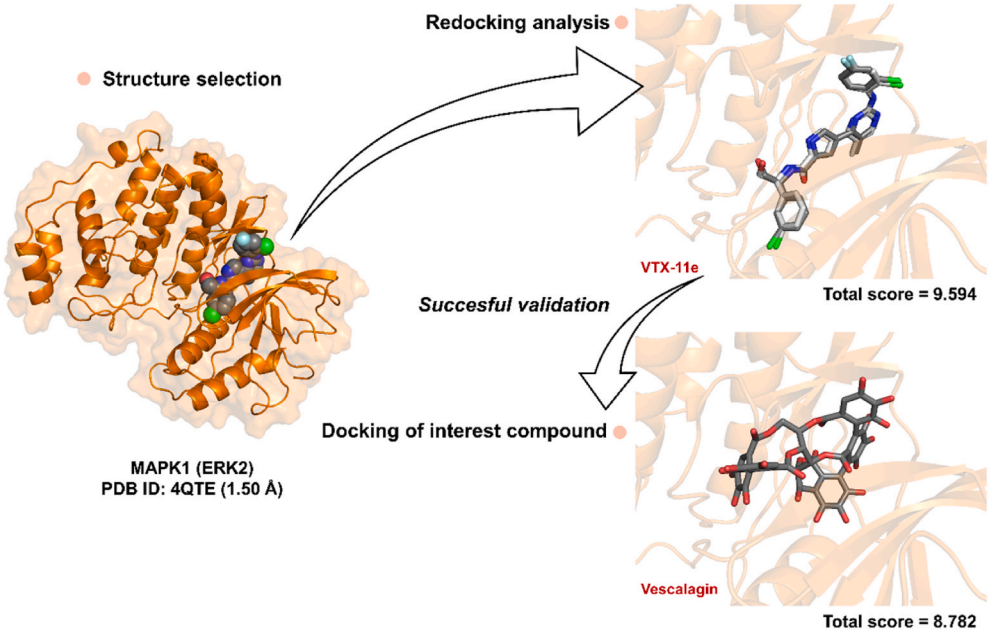

Fig. 4. The *in silico* pipeline of a docking approach regarding any atherosclerosis target, such as the MAPK1. After the MAPK1 crystal structure selection (PDB ID: 4QTE (Chaikuad et al., 2014), a redocking analysis of its co-crystallized ligand (VTX-11e (Arnov et al., 2009), the experimental binding mode in dark grey and docking pose in light grey) is performed with a docking program (e.g. Surflex-Dock), resulting in a total score (9.594). If successful, the analyses follow the molecular docking of specific bioactive compounds (e.g. vescalagin), which may result in a similar total score (8.782) and an interesting similar binding mode to the same pocket as VTX-11e.

toxicological parameters, for example, using admetSAR (Yang et al., 2019) (http://lmmd.ecust.edu.cn/admetsar2/), ProTox-II (Banerjee et al., 2018) (http://tox.charite.de/protox_II/), and Pred-hERG 4.2 (Braga et al., 2015) (http://predherg.labmol.com.br/) webservers. The compounds are also submitted to the webservers in SMILES format, returning prediction data from compounds already described with one or more properties associated with specific pharmacokinetic or toxicity profiles.

Briefly, Pred-hERG 4.2 predicts a ligand to bind or inhibit potassium channels, the human ether-a-go-go-related gene (hERG), in cardiac cells (Braga et al., 2015). In this case, vescalagin was predicted to have low cardiotoxicity with 60% confidence but, unfortunately, is considered out of the model's applicability domain. In other words, it is different from compounds used to generate the predictions, and this result is unreliable.

ProTox-II predictions for vescalagin indicated no or low hepatotoxicity, mutagenicity, immunotoxicity, and other adverse outcomes. It also predicted the lethal dose of 50% (LD_{50}) as harmful if swallowed ($2000 < LD_{50} \leq 5000$ mg/kg), according to the globally harmonised system (GHS) (Occupational Safety and Health Administration). A low toxicity profile could favour the potential oral bioavailability of a bioactive compound (e.g. vescalagin) (Fidelis et al., 2020), for example, with functional foods, such as the incorporation of jaboticaba seed extracts in yoghurts (Fidelis et al., 2021) and other dietary sources (Inada et al., 2015). Lastly, admetSAR shows vescalagin predictions for human intestinal absorption and oral bioavailability of 98.83% and 58.57%, respectively, which are somewhat desired for potential drugs and potential bioactive compounds (Yang et al., 2019), as well as functional food.

Specifically, it is recommended that more than one algorithm is employed to increase the reliability of the overall prediction and avoid erroneous conclusions (Pantaleão et al., 2022). Finally, these tools and available data may support and direct the sixth and last step, suggesting initial analyses *in vitro* and following assays *in vivo*. These steps help target-specific studies, such as docking, and potentially predict and explore different targets related to a disease and its modulation. The approach may aid in synthesising novel bioactive compounds and their kinetics as potential inhibitors, leading to intervention studies to unveil new therapeutic options against atherosclerosis.

Some of the tools, software, and servers mentioned earlier are standard options for various *in silico* approaches, including their application to "functionalise" foods. As discussed, some methods are related to a protein target's structure or to a bioactive compound's chemical structure. For instance, molecular docking approaches may incrementally increase accuracy, being a fast and inexpensive *in silico* method based on the structural and physical approaches. In addition, each docking program's algorithms to predict binding poses are unique (Koutsoukas et al., 2011). For example, the Surflex-Dock combines a scoring function with an incremental search engine that considers fragments from the bioactive molecule in several layers, thus rapidly generating poses for chemical structures (Jain, 2003). The results, such as those shown in Fig. 4, may suggest the reliability of a ligand-binding mode simulation compared with its crystallographic experimental results (e.g., redocking). There are also systematic or non-deterministic dockings, classified as exhaustive, incremental, and conformational methods (Guedes et al., 2014). Herein Glide can be highlighted by assessesing the conformation and position of a ligand in space, followed by an energy optimisation of this chemical structure, thus generating various putative poses (Friesner et al., 2004). Lastly, there are stochastic dockings, which randomly change all ligands' conformations, and thus produce a great diversity of poses, followed by probabilistic criteria to define the most likely putative binding mode (Guedes et al., 2014), such as GOLD (Verdonk et al., 2003).

Additionally, ligands' binding interactions make it possible to predict a bioactive compound's potential toxicity and pharmacokinetic parameters, which can increase the chances of success of bioactive compounds in initial design or repurposing processes (Pantaleão et al., 2022). Here, among the web servers (admetSAR, Pred-hERG 4.2 and ProTox-II), many can be the desired parameters, such as an interaction to a single cell receptor and the kinetics related to absorption, distribution, metabolism, excretion, and toxicity (ADMET), which corresponds to the bioavailability of a given compound, important for functional foods (Fidelis et al., 2021). The methods usually consider structure-activity relationships, such as QSAR (admetSAR, Pred-hERG 4.2 and ProTox-II), and can be compared and potentially corroborated. Interestingly, there are methods such as pkCSM (Pires et al., 2015) that also employ graph-based modelling, a graphical representation of chemical structures together with the experimental data and machine learning, to predict pharmacokinetic and toxicity properties.

Lastly, protein structures can also be considered to analyse their binding to a single potential ligand, prospectively searching for other potential targets or designing novel ligands. SEA and

SwissTargetPrediction consider 2D and 3D similarities for filtering available targets, while SuperPred can employ machine learning algorithms to compare a ligand with many different structures from a database, narrowing the results. A few web servers may be an alternative to these bioactive compounds' similarity analyses, such as FATCAT (Ye & Godzik, 2004) and FATCAT 2.0 (Li et al., 2020), which developed algorithms for protein structure comparison that automatically identifies rearrangements in between two or more protein, being able to search in a database for structurally similar proteins. Ultimately, those tools could be employed to guide the selection of proteins to be evaluated by docking or corroborate docking results. Here we briefly consider the tools assessed in this work and highlight a few other available examples (Table 1).

2.4. Functional foods design to mitigate atherosclerosis: assembling the puzzle between chemistry, food technology, and health sciences

Functional foods do not have a legal definition using the European Food Safety Authority (EFSA) regulations. Still, they can be scientifically defined as "foods that have a potentially positive effect on health beyond basic nutrition, helping promote optimal health conditions and reducing the risk of non-communicable diseases" (Granato et al., 2017). Therefore, applied research in food science and technology has devoted attention to the development of food models/prototypes that are "functionalised" by the addition of bioactive compounds, namely phenolic acids, flavonoids and proanthocyanidins, carotenoids, functional lipids, terpenoids, and peptides (Bucalossi et al., 2020; Cândido et al., 2018; de Souza Mesquita et al., 2020).

Despite the myriad of examples from the literature, the exact effect of food bioactive compounds remains unclear. There are still inconsistent results on cardiovascular-related biomarkers, mainly because of the population's variable dosage, intervention time, and baseline characteristics (Cheurfa et al., 2019; Diker & Kutluay, 2021; Santana et al., 2022). In this sense, Granato (2022) highlighted the need to develop functional foods aimed to lower inflammation and RONS cascades and, ultimately, decrease the odds of CVDs.

Moreover, the leader bioactive compounds chosen in food nutritional/chemical studies represent only a fraction of the more than 26,000 distinct compounds with documented effects on health, representing a tiny part of the number of secondary metabolites estimated to exceed 49,000. These numbers highlight how food matrices are still unexplored with an incomplete assessment of the true complexity of natural compounds we consume. The invisibility of these compounds in experimental, clinical, epidemiological, and demographic studies—turns them into the virtual "dark matter" of nutrition research (Barabási et al., 2020). In this sense, unveiling this dark nutritional matter by exploring mathematical and chemical techniques could open new strategies for discovering the vast array of bioactive compounds and manufacturing functional food with direct therapeutic impact (Barabási et al., 2020; de Noronha et al., 2022; Granato et al., 2017).

For producing functional foods added with bioactive components not naturally present in the food (Fig. 5), traditionally, two possibilities arise i) use of the bioactive-rich materials (i.e., plants, fungi, micro/macroalgae) directly in the food in a liquid or solid form and ii) use of crude extracts containing bioactive compounds added into food matrices (i.e., meat products and analogues, dairy foods, oleogels/hydrogels, juices, and bakery foods) to produce different potential functional food models.

The bioactivities usually include the antioxidant capacity in different chemical and physiologically relevant media (Chen et al., 2019), initial toxicity screening (Adouni et al., 2022; de Moura et al., 2022), and inhibition of digestive and hypertension-related enzymes (Pasini Deolindo et al., 2019). In the past, assays using recombinant enzymes, liver microsomes, and two-dimensional (2D) cell cultures, such as primary hepatocytes and Caco-2 cells, have been used to evaluate absorption, distribution, metabolism, and excretion (ADME), biological effects, and to assess the safety and kinetics of novel bioactive compounds (Wang et al., 2021). However, in recent years, significant advancements have been made in intestinal tissue engineering. Adult stem cells (ACS) induced pluripotent stem cells (iPSC) and the frequently used immortalised cell lines provided valuable insights into understanding ADME

Table 1
Free, academic, and commercial tools (software and servers) available for the computational analysis of targets, ligands, and potential interactions.

Method	Software/ Server	Availability	References
Molecular docking	Surflex-Dock	https://www.biopharmics.com/	Jain (2003)
	GOLD	https://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/	Verdonk et al., (2003)
	Glide	https://www.schrodinger.com/products/glide	Friesner et al., (2004)
	AutoDock	https://autodock.scripps.edu/	Goodsell et al. (1996)
	AutoDock Vina	https://vina.scripps.edu/	Trott & Olson (2009)
	DockThor	https://dockthor.lncc.br/v2/	de Magalhães et al. (2014)
	FlexX	https://www.biosolveit.de/	Kramer et al. (1999)
	DOCK 3.7	https://dock.compbio.ucsf.edu/DOCK3.7/	Coleman et al. (2013)
	Dock6	https://dock.compbio.ucsf.edu/DOCK_6/index.htm	Lang et al. (2009)
	OEDocking/ FRED	https://www.eyesopen.com/oedocking	McGann (2011)
	Molegro Virutal Docker/ MolDock	http://molexus.io/molegro-virtual-docker/	Thomsen & Christensen (2006)
Targets'prediction	SEA	https://sea.bkslab.org/	Keiser et al., (2009)
	SuperPred	https://prediction.charite.de/	Dunkel et al., (2008)
	Swiss Target Predicion	http://www.swisstargetprediction.ch/	Daina et al. (2017)
	GUSAR	http://way2drug.com/Gusar/index.html	Lagunin et al. (2011)
	PASS	http://way2drug.com/PassOnline/index.php	Geronikaki et al. (2008)
	Reverse Screen 3D	http://www.modelling.leeds.ac.uk/ReverseScreen3D	Kinnings & Jackson (2011)
	FATCAT (2.0)	https://fatcat.godziklab.org/	Li et al., (2020)
ADMET parameters calculation	ProTox-II	https://tox-new.charite.de/protox_II/	Banerjee et al., (2018)
	Pred-hERG 4.2	http://predherg.labmol.com.br/	Braga et al., (2015)
	admetSAR	http://lmmd.ecust.edu.cn/admetsar2	Yang et al., (2019)
	PreADMET	https://preadmet.webservice.bmdrc.org/	Lee et al. (2002)
	ADMETlab	https://admetmesh.scbdd.com/	Dong et al. (2018)
	SwissADME	http://www.swissadme.ch/	Daina et al. (2017)
	DrugMint	http://crdd.osdd.net/oscadd/drugmint/	Dhanda et al. (2013)
	FAF-Drugs4	https://fafdrugs4.rpbs.univ-paris-diderot.fr/	Lagorce et al. (2017)
	NERDD	https://nerdd.univie.ac.at/	Stork et al. (2019)
	pkCSM	http://biosig.unimelb.edu.au/pkcsm/	Pires et al., (2015)

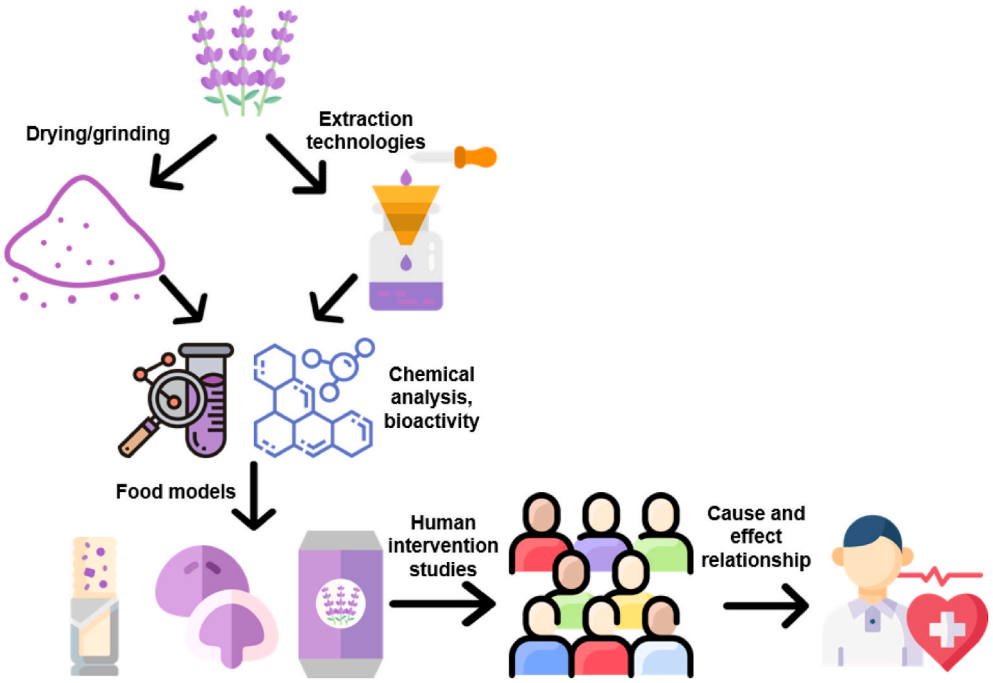

Fig. 5. Illustration pinpoints the traditional way to "functionalised" food products by adding bioactive compounds from generally recognised safe (GRAS) sources.

and the safety and efficacy of compounds. In the future, 3D organoid culture models that are organlike structures generated from ASCs or iPSCs will undoubtedly be employed more frequently to overcome the limitations of 2D cell cultures (Malijauskaite et al., 2021). Their advantage lies in the ability to self-organize and the presence of various organ-specific cell types. Therefore, these organoids more closely model the physiological characteristics of native tissue. Organ-specific organoids such as intestinal organoids, brain organoids, liver organoids, and kidney organoids (Wang et al., 2021) can be maintained in culture longer than cells in 2D monolayers and retain their microenvironmental cues. However, most organoids still lack immune cells, vasculature, neural networks, musculature, and the critical microbiome constituents

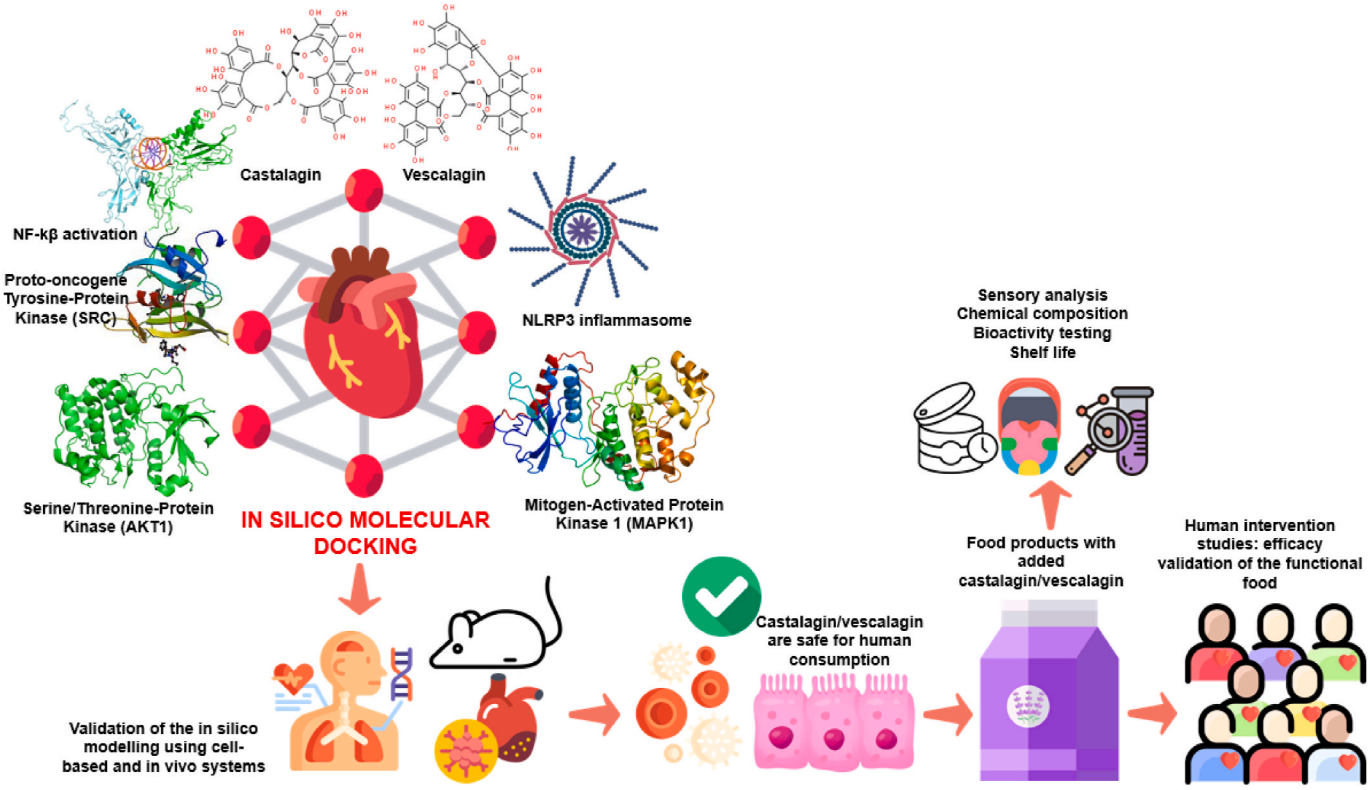

Fig. 6. Integrative strategy to "functionalised" food products by adding bioactive compounds that have been shown to positively downregulate pro-inflammatory and pro-oxidant proteins and enzymes associated with atherosclerosis.

of many organs' *in vivo* anatomy. Nevertheless, their human origin and the option to generate these biomimetic organ systems from patient cells make them prime non-animal models for testing safety and efficacy. However, most *in vitro* studies are still based on "classic" 2D systems such as cancer cell lines (Espín et al., 2007).

When the food model is produced and has demonstrated potential to be a functional food for a specific health condition, such as atherosclerosis and type-2 diabetes, its efficacy must be formally assessed using detailed designs (Brown et al., 2018) to establish the cause-and-effect relationship between the consumption of the potential food and the beneficial physiological effect in the targeted population bearing the health condition. This condition is one of the criteria established by the Food and Drug Administration (FDA) and the European Commission (EC 1924/2006) that EFSA demands to confer any health claim to potential functional foods in the European Union. For this purpose, double-blinded, randomised, placebo-controlled interventions are the golden standards.

Targeting the "functionalisation" of foods by a tailored way to deliver bioactive compounds to either mitigate the harmful effects of atherosclerosis or decrease the onset of the low-grade inflammation and oxidative pathways that lead to increased risks of atherosclerosis, some essential steps should be considered (Fig. 6): i) select the biomarkers of atherosclerosis that have a relevant impact on clinical outcomes; ii) select some bioactive compounds (i.e., castalagin and vescalagin) that will be studied for their effects on essential biomarkers using *in silico* computational modelling; iii) interpret the outputs of the *in silico* studies and decide or not to consider the use of the selected bioactive compounds to be added in food models; iv) validate the findings of the *in silico* modelling using proper cell-based systems and, preferably, *in vitro* protocols; v) assure the bioactive compounds are toxicologically safe to human consumption in doses usually found in foods; vi) manufacture the food that will be added with the bioactive compounds and use proper technological processes that will not degrade the bioactive; vii) undergo sensory, bioactivity, chemical and stability testing of the food model to assure the functional effects will remain insignificantly affected during storage; and viii) validate the efficacy of the food added with bioactive compounds using a human intervention study, preferably using a randomised, double-blind placebo-controlled trial.

3. Conclusions and perspectives

The development of functional foods to counteract atherosclerosis is a timely and ambitious scientific and technological trend. Considering the consumer's needs and the current state-of-the-art in food science/technology, nutrition, and pharmacology, we proposed their integration to identify phenolic compounds that may interact with atherosclerosis-associated biomarkers, such as oxidative and pro-inflammatory signalling agents. The regulation of oxidative stress and the secretion of pro-inflammatory biomarkers represent a suitable and clinically approach necessary to identify bioactive compounds and tailor functional foods to counteract the CVDs.

To meet the market and scientific demands, this work is a pioneer in providing a practical guide to developing personalised functional foods. This guideline is exemplified using vescalagin to demonstrate the selection of the biomarkers, the application of *in silico* computational modelling for establishing interactions with critical biomarkers, and the validation of the *in silico* results using *in vitro/vivo* protocols. Accordingly, *in silico* computational modelling may become a complementary and informative strategy in the food/pharma industry as the mechanisms of antiatherogenic potential can be explored when or how a bioactive compound (e.g., phenolics) interacts with central biomarkers related to atherosclerosis (e.g., MAPK1). Adopting this integrated approach may bring about ground-breaking advances and innovative food products and ingredients to be used in food/pharma processing, ultimately generating positive public health outcomes in the medium and long term.

Author contributions

D.G., L.A.: conceptualization, writing - review & editing; writing - original draft. M.S.M.S., V.G.M.: formal analysis, writing - review & editing; writing - original draft. A.M.G.: writing - review & editing; writing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

Data will be made available on request.

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