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REVIEW ARTICLE

Investigation of the potential of phytochemicals derived from citrus peels to inhibit digestive enzymes: an overture to the management of lifestyle diseases

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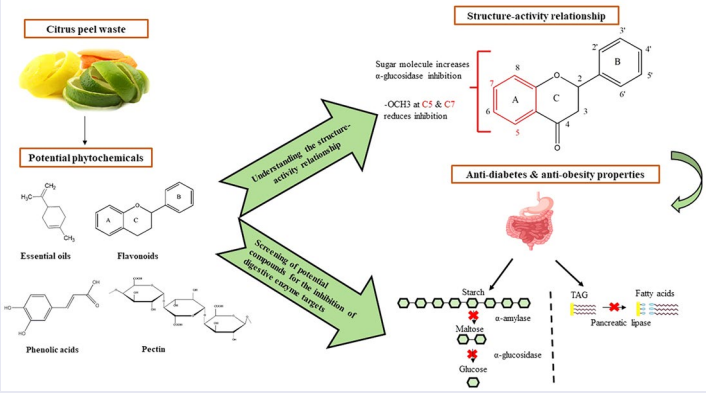
ABSTRACT

The food industry relies on citrus fruits for juice, canned fruit, and jam, creating significant waste from peels, seeds, and pomace. This waste contains valuable phytochemicals like carotenoids, essential oils, (poly)phenols, pectin, and vitamins, which can be used as nutraceuticals or key ingredients in functional foods for managing diabetes and obesity. Repurposing citrus peel waste offers an excellent opportunity to advance biorefineries and the bioeconomy. Compounds derived from citrus have attracted attention for their potential therapeutic effects on diabetes and obesity, and their effectiveness depends on various mechanisms. This review summarises citrus-derived phytochemicals that inhibit α-glucosidase and pancreatic lipase *in vitro*, highlighting their potential as anti-diabetic and anti-obesity compounds. We also discuss progress in using molecular docking screening against key drug targets linked to type II diabetes and obesity. This review explores novel citrus phytochemicals for the development of nutraceuticals and functional food ingredients with enhanced health benefits.

GRAPHICAL ABSTRACT



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Citrus peels; diabetes mellitus; obesity; phytochemicals; valorisation and computational techniques

1. Introduction

Obesity is a multifaceted disorder characterised by excessive dysregulation of body weight due to modification of adipose tissue[1]. Excess energy is stored in the adipose tissue when the body has a long-term positive energy balance and caloric intake exceeds energy use[2]. Obesity has far-reaching economic, social, psychological, and medical implications[3]. As obesity rates increase, so do healthcare costs,

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including the incidence of illness and death[4]. The World Obesity Federation estimates that over one billion people will be obese by 2030[5].

As obesity is primarily caused by excess energy, drugs that reduce energy imbalance are of research interest. The currently used anti-obesity drugs act through various mechanisms of action, including reducing food intake (appetite suppressants/satiety inducers), increasing energy expenditure (mitochondrial uncouplers), promoting fat mobilisation (leptin analogues/agonists, GLP-1 agonists) and decreasing digestion/absorption of food (pancreatic lipase inhibitors)[6,7].

This review discusses pancreatic lipase inhibitors, which slow down the hydrolysis of triacylglycerols (TAG) into fatty acids (FA) in the small intestine (as depicted in Figure 1). This inhibition prevents the absorption of high-calorie macromolecules into the body, leading to their excretion. As lipids are calorie-dense macromolecules, reducing their absorption results in a negative energy balance, resulting in weight loss. Weight loss reduces the risk of obesity-associated comorbidities such as coronary heart disease, non-alcoholic fatty liver, hypertension, and type II diabetes (T2D)[8].

T2D is a metabolic disease in which tissues resist insulin exposure[9]. Insulin is produced and secreted by pancreatic β-cells in response to elevated blood glucose levels[10]. The pathology of T2D is not fully understood, but it is associated with β-cell dysfunction, an immobile lifestyle, lipotoxicity, metabolic inflexibility and unhealthy diet[11–13]. Furthermore, T2D is associated with complications such as cardiovascular disease (CVD), neuropathy, nephropathy, non-alcoholic fatty liver disease (NAFLD), stroke and retinopathy[14–16]. The incidence and prevalence of diabetes continue to rise as reported by the Federation[17], with over 530 million people diagnosed with diabetes in 2021 and an estimated 780 million people living with diabetes by 2045. Therefore, effective treatment of T2D is crucial in alleviating the disease.

One approach to treating T2D is to limit glucose production and absorption into the bloodstream. In the human body, glucose originates from the breakdown of dietary carbohydrates[15]. Starch, one of the major dietary carbohydrates, is subjected to salivary and pancreatic α-amylases that cleave it to produce maltodextrins[18]. Maltodextrins are broken down into glucose by α-glucosidase in the small intestinal brush border[14]. Sodium–glucose co-transporter 1 (SGLT1) then transports glucose from the small intestine into epithelial cells, where it is transported into the bloodstream by glucose transporter 2 (GLUT2)[19–21]. Citrus peel-derived phytochemicals may be utilised as digestive enzyme inhibitors to slow down the breakdown of dietary carbohydrates by amylolytic enzymes, such as α-amylase and α-glucosidase, thereby leading to lower blood glucose levels. These phytochemicals may also assist in inhibiting pancreatic lipase, which is involved in breaking down triacylglycerols (TAGs) into fatty acids (FAs), thereby promoting better nutrition and health (Figure 1)[22].

Citrus fruits are among the most widely produced, processed and consumed in the world[23]. Well-known citrus fruit cultivars are bergamot (*Citrus bergamia* Risso & Poiteau), grapefruit (*Citrus paradisi* Macfad.), orange (*Citrus sinensis* (L.) Osbeck), lemon (*Citrus limon* (L.) Osbeck), lime (*Citrus aurantiifolia* (Christm.)

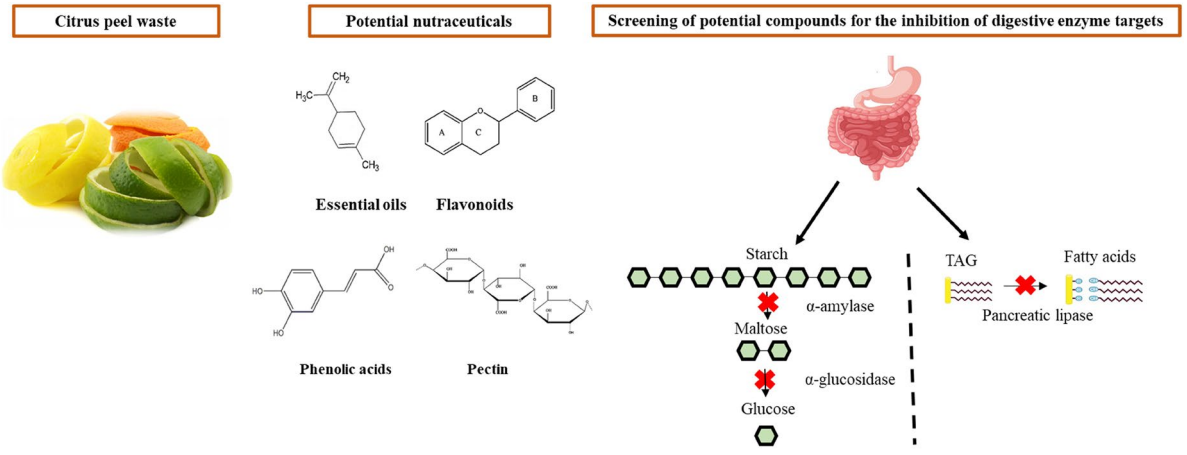

Figure 1. Diagram illustrating citrus peels that contain potential nutraceuticals, which can serve as drug targets for digestive enzymes. These nutraceuticals may be utilised to influence the breakdown of starch by amylolytic enzymes, specifically α-amylase and α-glucosidase, resulting in the production of glucose. Additionally, they can aid in the breakdown of triacylglycerols (TAGs) by pancreatic lipase into fatty acids (FAs). The figure was created in www.biorender.com.

Swingle), mandarin (*Citrus reticulata* Blanco), and pomelo (*Citrus maxima* (Burm.) Merr.), which belong to the Rutaceae family (Figure 2)[23]. These citrus varieties contain various phytochemicals such as essential oils, pigments (carotenoids), and (poly)phenols[24]. Since citrus peels (CP) contain various bioactive compounds that have the potential for medicinal use, their valorisation can play a crucial role in the ecological management of waste and the bio-based economy[25].

The demand for natural and plant-based therapies is increasing, and CPs are an accessible and cost-effective source of potential bioactive compounds[26]. This perfectly aligns with consumer preferences for natural remedies over synthetic drugs. CPs have the potential to revolutionise preventive healthcare strategies. Regularly consuming citrus-derived products can significantly reduce the risk of obesity and diabetes-related complications. Embracing this byproduct of the citrus industry promotes environmental sustainability and provides substantial health benefits. This review focuses on studies that have evaluated *in vitro* bioactivity, particularly amylolytic enzyme and pancreatic lipase inhibition, of citrus-derived phytochemicals for potential application in treating obesity and diabetes.

2. Methodology

The literature source approach applied to this systematic review used electronic databases such as Google Scholar, PubMed, Elsevier, and Web of Science to search for reviews and *in vitro* experimental studies focusing on CP bioactive compounds, their nutraceutical properties, and health benefits. Some of the keywords used in the search were "obesity", "diabetes mellitus", "citrus peels", "α-amylase", "α-glucosidase", "pancreatic lipase", "citrus-derived nutraceuticals (carotenoids, essential oils, flavonoids, (poly)phenols, and pectin)", "*in silico* techniques", and "valorisation". The inclusion criteria comprised a literature search for the valorisation of CPs, bioactive compounds extracted from CP, health-benefit properties, virtual screening of the compounds on T2D and obesity targets, and only full-text English articles published between 2013 and 2025 were considered. However, several articles ($n = 12$) that were not published between 2013 and 2025 were considered in the literature search. The literature search excluded articles focusing on studies evaluating the cytotoxicity and *in vivo* and human studies of citrus peel-derived nutraceuticals.

3. Different classes of major compounds found in citrus peels

Phytochemicals present in CPs, such as essential oils, carotenoids and phenolics, are useful in various industries, including food and cosmetics and are beneficial in combating lifestyle diseases[27]. This review

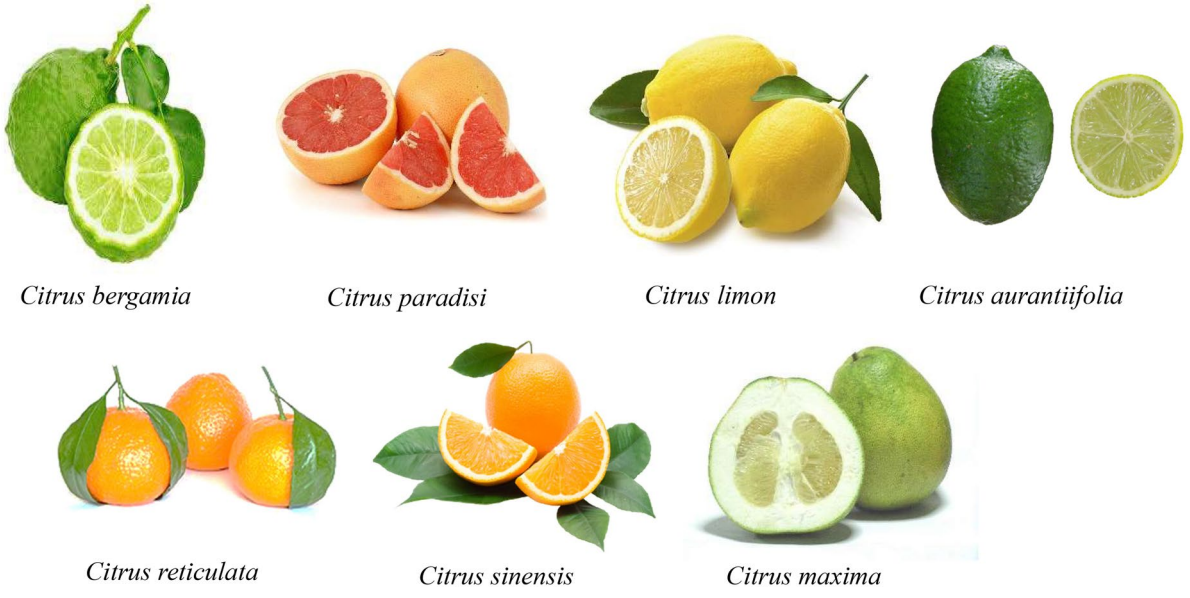

Figure 2. Citrus fruit cultivars of bergamot (*Citrus bergamia* Risso & Poiteau)[1], grapefruit (*Citrus paradisi* Macfad.)[2], orange (*Citrus sinensis* (L.) Osbeck)[3], lemon (*Citrus limon* (L.) Osbeck)[4], lime (*Citrus aurantiifolia* (Christm.) Swingle)[5], mandarin (*Citrus reticulata* Blanco)[6], and pomelo (*Citrus maxima* (Burm.) Merr.)[7] belonging to the Rutaceae family.

provides an overview of four major classes of citrus-derived phytochemicals, including carotenoids, essential oils, (poly)phenols, (phenolic acids and flavonoids) and pectin with their proposed health benefits, particularly in combating diabetes and obesity, based on bioactivities reported from *in vitro* studies.

3.1. Carotenoids

Carotenoids are a class of compounds with an isoprenoid basic structure (Figure 3a) that are produced by organisms such as bacteria, fungi, and plants[25]. Two groups of carotenoids, namely carotenes, consisting of hydrocarbons and xanthophylls, contain one or more oxygen atoms in their structure[28]. The most abundant carotenoids in citrus fruits include α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin[29]. Carotenoids are widely known for their pigmentation (yellow, orange, and red) and antioxidant activity, as well as for ornamentation, photoprotection, and regulation of the immune system[30]. Total carotenoid content has been estimated to be as high as 359.3 ± 3.5 mg/100 g of fresh weight in *Citrus sinensis*[31].

3.2. Essential oils

Essential oils are a mixture of volatile compounds typically derived from terpenes, a class of chemicals made up of isoprenes, which are five-carbon units[32]. The most abundant terpenes found in essential oils are monoterpenes (Figure 3b) and sesquiterpenes (Figure 3c), which are composed of 10 carbons (two isoprenes) and 15 carbons (three isoprenes), respectively[33]. Up to 5% of the fresh weight of citrus peels contains essential oils, which are mainly found in the oil glands of the flavedo[34,35]. CP essential oils are in high demand worldwide because of their promising benefits in the food, perfume, cosmetic and medical industries[36]. They have a wide range of pharmaceutical importance, including antioxidant, anti-inflammatory, anti-microbial, anti-diabetic and anti-cancer effects and can be used as suitable substitutes for chemical preservatives in the food industry[35,37].

3.3. (Poly)phenols

3.3.1. Flavonoids

Flavonoids consist of a 15-carbon skeleton with two six-carbon phenyl rings linked by a heterocyclic ring with an embedded oxygen, as shown in Figure 3d.[25] Flavonoids can be classified into different groups,

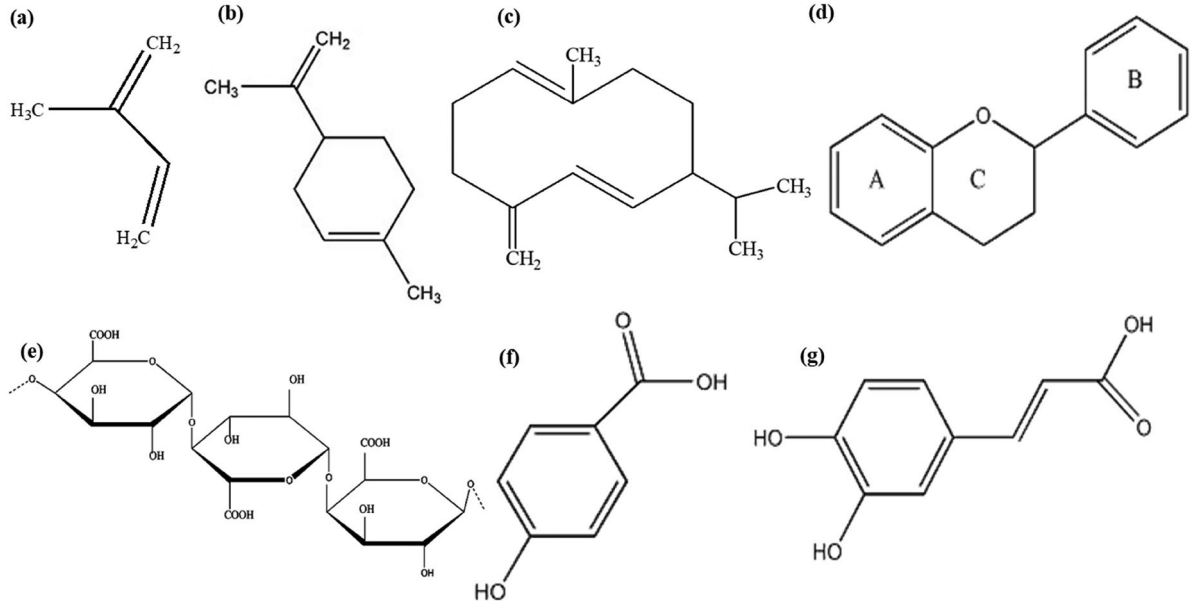

Figure 3. Basic structures of different classes of compounds in citrus peel. **(a)** Isoprenoid, **(b)** monoterpenes, **(c)** sesquiterpenes, **(d)** flavonoid, **(e)** pectin, **(f)** hydroxybenzoic acid and **(g)** hydroxycinnamic acid. The structures were constructed using ChemDraw.

including flavones, flavonols, flavanones, flavanonols, flavonols (flavan-3-ols), isoflavones, chalcones, anthocyanins and catechins, based on the substituent configuration of the heterocyclic ring[38]. Citrus contains anthocyanins, flavones, flavonols, polymethoxylated flavones (PMFs) and flavanone[25,39].

Citrus-derived flavonoids exist in glycoside or aglycone forms, with the aglycone not occurring naturally but linked to the glycosides through a sugar moiety[40]. Flavonoid *O*-glycosides have attracted more interest than flavonoid C-glycosides. Flavonoid *C*-glycosides have extensive health benefits[41]. Flavonoids exhibit a wide range of biological activities, including hepatoprotective, anti-inflammatory, anti-cancer, antioxidant, anti-bacterial and anti-allergic activities[42]. The total flavonoid content of citrus peels has been reported to be as high as 49.2 ± 1.33 mg/g of peel biomass in rutin equivalents[43].

3.3.2. Phenolic acids

Phenolics contain a single phenol group that may be substituted with various functional groups, such as one or more hydroxyl groups or a carboxylic acid group (phenolic acids)[44]. These compounds act as protective secondary metabolites in plants and are typically found in the vacuoles and cell walls[45]. Phenolics are low-weight polar molecules that can be extracted in polar solvents and easily separated from the plant matrix. The most abundant and commonly extracted phenolic acids can be grouped into hydroxybenzoic acids (Figure 3f) and hydroxycinnamic acids (Figure 3g)[46]. The CP contains a wide range of (poly)phenolics including caffeic acid, chlorogenic acid, gallic acid, hesperidin, naringin, narirutin, nobiletin, neohesperidin, ρ-coumaric acid and sinapic acid[47].

These citrus peel-derived compounds, including pectin, (poly)phenols, carotenoids and essential oils, have been well studied for their pharmacological activities, including anti-inflammatory, antioxidant, anti-bacterial and cytotoxic activities[25,42]. Studies on the ability of citrus peel-derived (poly)phenols to inhibit digestive enzymes *in vitro* remain limited. The next section provides a summary of some of the major bioactive compounds identified from different citrus sources using *in vitro* studies and computational screening to identify potential nutraceuticals that can target specific proteins as disease targets. This review further highlights the significant role of the structural features of these bioactive compounds on their efficacy.

3.4. Pectin

Pectin polymers consist of alternating α-1,4-linked galacturonic acids and α-1,2-linked rhamnose residues in the backbone (Figure 3e), branched by β-1,4-linked D-galactose and α-1,5-linked L-arabinose residues[48,49]. Pectin is found in the primary cell walls of plants and is abundant in most vegetables and fruits[50]. Pectin has been extracted from various citrus processed by-products, such as orange, lemon, mandarin peels and orange pulp. Several studies have been conducted to investigate the biological activities of pectin, and have shown it to have anti-atherosclerosis[25], anti-diabetic properties[34] and prebiotic effects[24]. Pectin content has previously been reported to be as high as 26.79% (w/w dry weight)[51].

4. The mechanism of action of amylolytic enzymes and pancreatic lipase against their respective substrates

It is essential to understand how the substrates interact with amylolytic and lipolytic enzymes prior to examining the inhibitory effects of potential phytochemicals against these enzymes. α-Amylase breaks down starch, specifically targeting α-(1,4) glycosidic linkages, releasing maltotriose and maltose from amylose, as well as glucose, maltose, and limit dextrins from amylopectin[52,53]. The catalytic triad of α-amylase consists of two aspartate (Asp) residues and one glutamate (Glu) residue that aid in starch hydrolysis. In *Sus scrofa* (porcine) α-amylase (PDB ID: 1DHK), these essential residues are Asp195, Glu223, and Asp300[54,55]. Asp195 functions as a nucleophile that attacks the anomeric carbon of starch, leading to the formation of a covalent β-glycosyl-α-amylase intermediate. Concurrently, Glu223 and Asp300 play a role in stabilising this intermediate. Subsequently, Glu223 facilitates the activation of a water molecule, which assists in the deglycosylation of α-amylase, leading to the release of hydrolysed starch while preserving the anomeric configuration[56].

α-Glucosidase hydrolyses α-1,4-glucosidic linkages in disaccharides and oligosaccharides, releasing glucose into the intestinal lumen, where it is then absorbed into the bloodstream[57]. In *Saccharomyces cerevisiae* α-glucosidase (PDB ID: 3AJ7), Glu277 serves as an acid/base catalyst, whereas Asp352 functions as a nucleophile. These amino acids are essential for the double displacement mechanism by initially forming a covalent intermediate between the enzyme and the oligosaccharide[58]. Glu277 aids in cleaving the attached oligosaccharide using water, while maintaining the stereochemistry of the anomeric carbon. This process results in the release of a glucose molecule from the non-reducing end of the oligosaccharide[58].

Pancreatic lipase hydrolyses dietary triglycerides (TGs) into monoglycerides and free fatty acids through a serine hydrolase-type catalytic process[59]. The catalytic triad of *Sus scrofa* (porcine) pancreatic lipase (PDB ID: 1ETH) consists of Ser153, Asp177, and His264[60]. The interaction between lipids and water induces alterations in the lipase structure, particularly through the relocation of its lid domain[61]. This phenomenon, referred to as interfacial activation, enables substrates to reach the active site. Ser153 functions as the nucleophile, targeting the carbonyl carbon of the triglyceride (TG) ester bond, creating an acyl–lipase intermediate. His264 acts as a general acid/base, helping to break the ester bond. Asp177 assists His264 by keeping a positive charge, which is crucial for the proton transfer needed for bond cleavage. Furthermore, His264 triggers a water molecule to attack the acyl–lipase intermediate, leading to the liberation of the free fatty acid and the restoration of the enzyme's active site[60].

5. Virtual screening of citrus peel phytochemicals against T2D and obesity enzyme targets: amylolytic enzymes and pancreatic lipase

In the field of drug discovery, computational techniques are commonly used to screen for potential compounds that target specific proteins as disease targets. These techniques involve the use of computer-aided methods to filter small groups of predicted compounds from compound libraries, which are then tested experimentally for biological activity[62]. Additionally, computational techniques have been used to aid in the design of novel compounds by allowing researchers to evaluate how structurally altering known compounds would affect their affinity of the derivatives for the target protein, potentially enhancing their biological activity[63].

Computational techniques include quantitative structure–property relationship (QSPR) and quantitative structure–activity relationship (QSAR) methods to help correlate the molecular descriptors and their biological activities. QSPR refers to the prediction of the chemical properties of desired compounds, whereas QSAR models biological activities[62,64].

Virtual screening identifies molecules with high binding affinities for specific protein targets and associates molecular descriptors with biological activities[65,66]. Virtual screening aims to predict outcomes and reduce the need for multiple *in vitro* screenings. This process involves obtaining the 3D structures of molecules and target proteins, preparing the target proteins by removing water and other small molecules, and performing molecular docking using software such as Maestro Glide, Gold, MOE Dock, and AutoDock[67]. The prediction is evaluated using scoring functions that correlate with binding affinity, and the software ranks the binding results and can hypothesise how the binding affinity is related to *in vitro* inhibition[65].

The docking score is influenced by the binding interactions between the ligand and the binding site of the target proteins, which include non-covalent interactions, such as hydrogen bonds and hydrophobic forces, as illustrated in Figure 4. According to a study by Du et al.[68] the inhibition and substrate binding of pancreatic lipase depend on amino acid residues Ser153, Asp177, and His264 located at the catalytic site. Inhibition of α-glucosidase involves the participation of several residues at the active site, including Lys156, Glu276, Phe303, His351, and Arg312, as reported by Rahim et al.[69] and Mehmood et al.[70] Additionally, Gln63, Asp197, His201, Glu233, Asp300, and Asn301 are key residues that actively participate in pancreatic α-amylase inhibition[54,55].

Numerous phytochemicals found in CPs, such as chlorogenic acid, hesperidin, naringenin, and quercetin, exhibited higher binding affinities than acarbose and orlistat against the respective protein targets of these FDA-approved drugs when assessed with AutoDock Vina v1.1.2 (Table 1). Nabil-Adam et al.[71] also reported this trend and observed a strong binding affinity for chlorogenic acid at the active sites of α-amylase and lipase. The authors suggested that the binding affinity is strongly influenced by the

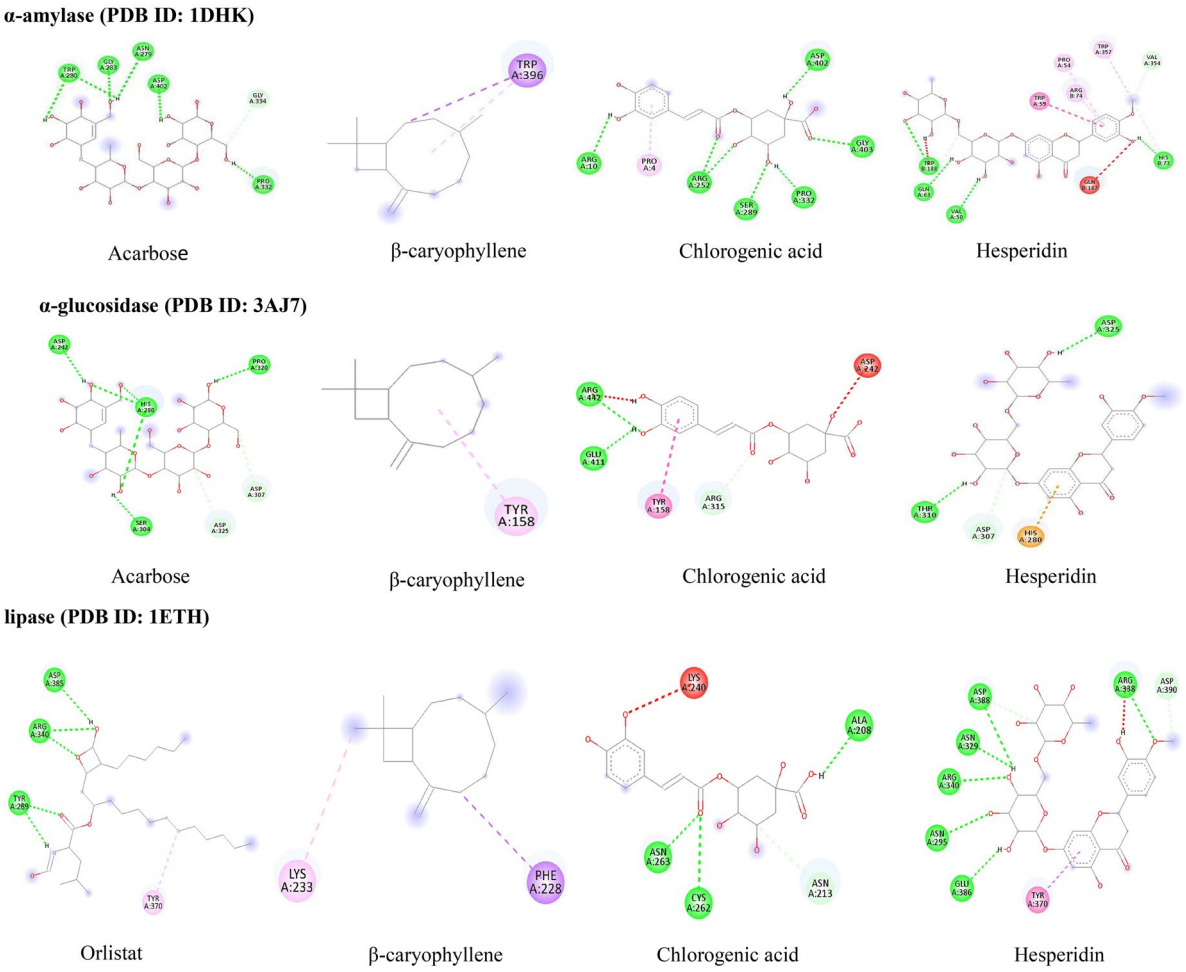

Figure 4. Binding interaction network of the best-docked compounds in the active sites of α-amylase (PDB ID: 1DHK) and α-glucosidase (PDB ID: 3AJ7), as reported in Table 1. Green dashed lines indicate hydrogen bonds, while pink and purple dashed lines indicate π–π hydrophobic interactions. Red dashed lines represent unfavourable donor–donor or acceptor–acceptor interactions.

Binding interaction network of the best-docked compounds in the active site of pancreatic lipase (PDB ID: 1ETH) as reported in Table 1. Green dashed lines indicate hydrogen bonds, while pink and purple dashed lines indicate π–π hydrophobic interactions. Red dashed lines represent unfavourable donor–donor or acceptor–acceptor interactions

number of hydrogen bonds and hydrophobic forces between the compound and the protein amino acid residues lining its active site[71].

Table 1 shows some of the major CP compounds, with their respective quantities, and their reported inhibition constants (K_i), modes of inhibition and their docking scores against protein targets associated with T2D and obesity. In this review, AutoDock Vina v1.1.2 with default parameters was used to obtain the binding affinities of selected compounds with inhibition constant (K_i) values reported in the literature against the protein targets: *Sus scrofa* α-amylase (1DHK), *Saccharomyces cerevisiae* α-glucosidase (3AJ7), and *S. scrofa* pancreatic lipase (1ETH), as presented in Table 1[72,73]. Figure 3 shows the binding interaction network of the best-docked compounds in the active sites of protein targets. Acarbose and orlistat docking scores were determined in this study and included as positive controls for inhibitors of amylolytic enzymes and pancreatic lipase, respectively.

The docking score is influenced by the binding interactions between the ligand and the binding site of the target proteins, which include non-covalent interactions, such as hydrogen bonds and hydrophobic forces, as illustrated in Figure 4. According to a study by Du et al.[68] the inhibition and substrate binding of pancreatic lipase depend on amino acid residues Ser153, Asp177, and His264 located at the catalytic site. Inhibition of α-glucosidase involves the participation of several residues at the active site, including Lys156, Glu276, Phe303, His351, and Arg312, as reported by Rahim et al.[69] and Mehmood

Table 1. Major citrus peel phytochemicals with docking scores against *S. scrofa* α-amylase (1DHK), *S. cerevisiae* α-glucosidase (3AJ7), and *S. scrofa* pancreatic lipase (1ETH) as drug targets.

Compound	Chemical class	Quantity (dry weight basis)	Drug target	Docking score (kcal/mol)	K_i **value** (mM)	Mode of inhibition	References
Caffeic acid	Phenolic	503.36 µg/g	Lipase	−6.6	0.307	Uncompetitive	Nyambe-Silavwe and Williamson[74]
			α-Amylase	−7.0	0.028	Competitive	
			α-Glucosidase	−7.0	0.989	Competitive	
β-Caryophyllene	Essential oil	0.0048 % (w/w)	Lipase	−7.8	–		Mahnashi et al.[75], Yang et al.[66], Nodola et al.[76]
			α-Amylase	−6.5	–		
			α-Glucosidase	−7.6	–		
Chlorogenic acid	Phenolic	1442.19 µg/g	Lipase	−8.5	–	–	Cardullo et al.[77], Nayak et al.[78], Zheng et al.[79]
			α-Amylase	−7.3	0.246	Mixed type	
			α-Glucosidase	−9.0	0.203	Competitive	
ρ-Coumaric acid	Phenolic	171.47 µg/g	Lipase	−6.8	0.206	Uncompetitive	Martinez-Gonzalez et al.[80], Nabil-Adam et al.[71], McMillan et al.[81], Nayak et al.[78]
			α-Amylase	−6.4	2.46	Uncompetitive	
			α-Glucosidase	−7.0	9.58	Competitive	
Gallic acid	Phenolic	210.65 µg/g	Lipase	−6.0	44.6	–	Nyambe-Silavwe and Williamson[74], Behera et al.[82], Kokila et al.[83], Wang et al.[84]
			α-Amylase	−6.4	–	–	
			α-Glucosidase	−5.5	0.18	–	
Hesperidin	Flavonoid	20.7 ± 0.38 mg/g	Lipase	−9.4	0.270	Competitive	Luo et al.[85], Kaliaperumal et al.[86], Cardullo et al.[87]
			α-Amylase	−9.2	0.041	–	
			α-Glucosidase	−10.4		Uncompetitive	
Limonene	Essential oil	0.58% (w/w)	Lipase	−6.9	–	–	Yang et al.[66], Tshiyoyo et al.[88], Nodola et al.[76]
			α-Amylase	−5.5	0.604	–	
			α-Glucosidase	−6.3	–	Competitive	
Naringenin	Flavonoid	0.36 ± 0.004 mg/g DW	Lipase	−10.2	–	–	Priscilla et al.[89], Wang et al.[84]
			α-Amylase	−7.3	–	–	
			α-Glucosidase	−8.6	0.384	Competitive	
α-Pinene	Essential oil	0.0032% (w/w)	Lipase	−5.7	–	–	Nodola et al.[76]
			α-Amylase	−5.4	–	–	
			α-Glucosidase	−5.6	–	–	
β-Pinene	Essential oil	0.035% (w/w)	Lipase	−5.8	–		Nodola et al.[76]
			α-Amylase	−5.3	–		
			α-Glucosidase	−5.8	–		
Quercetin	Flavonoid	0.14 ± 0.002 mg/g	Lipase	−9.9	0.012	Competitive	Martinez-Gonzalez et al.[80], Tshiyoyo et al.[88], Wang et al.[43]
			α-Amylase	−8.5	0.465	Competitive	
			α-Glucosidase	−8.4	0.045	Mixed type	
Acarbose	aminocyclitol glycoside	NA	α-Amylase	−7.5	0.0038	Competitive	Tolmie et al.[14]
			α-Glucosidase	−8.4	0.170	Mixed type	
Orlistat	carboxylic ester	NA	Lipase	−7.3	0.017	Mixed type	Martinez-Gonzalez et al.[80]

(–) indicates that the mode of inhibition was not determined. The docking scores of α-pinene, β-pinene, acarbose, chlorogenic acid, and orlistat were determined using Autodock Vina v1.12.

et al.[70] Additionally, Gln63, Asp197, His201, Glu233, Asp300, and Asn301 are key residues that actively participate in pancreatic α-amylase inhibition[54,55].

Understanding the correlation between *in silico* and *in vitro* bioactivity results is crucial; a more negative docking score could translate to higher *in vitro* inhibitory activity of a compound against the protein target. The inhibition constant (K_i) values can be used to compare the inhibitory activity of phytochemicals; it represents the concentration of an inhibitor required to reduce the maximal rate of an enzyme-catalysed reaction by half[14]. A lower K_i value suggests a higher binding affinity of the inhibitor to the target protein[14]. Currently, only a few studies have demonstrated a correlation between compounds with strong binding affinities according to docking studies and lower K_i values against targeted enzymes (α-amylase, α-glucosidase, and lipase).

As shown in Table 1, the K_i values and modes of inhibition for β-caryophyllene, chlorogenic acid, α-pinene, gallic acid, naringenin and β-pinene remained undetermined. This highlights an area of potential research that could yield valuable insights about the bioactivities of these compounds. More studies should focus on linking *the in silico* and *in vitro* results to better understand the inhibitory activity of compounds against targeted enzymes. This will provide insights into the inhibitory mechanisms of amylolytic and lipase enzymes by these compounds.

6. Structure–activity relationship (SAR) of citrus peel compounds towards amylolytic enzymes and pancreatic lipase

The structural features of compounds play a significant role in their bioactivity. The chemical class of compounds and the different configurations in the molecular structure within the chemical class influence the binding affinity and, in turn, their bioactivity towards protein targets. For example, (poly)phenols have been shown to exhibit stronger binding affinities than other classes of compounds, such as essential oils, towards amylolytic enzymes and pancreatic lipase (Table 1). This is in line with Huang et al. (90) who reported a relationship between the (poly)phenol content of CP and the inhibition of pancreatic lipase, with hesperidin being the main inhibitor. The authors also reported a strong binding affinity of hesperidin (−9.7 kcal/mol) for pancreatic lipase *in silico*. Differences in configurations of the molecular structure within the same class include hydroxylation, carbonyl groups, glycosylation, methylation, methoxylation and galloylation[91].

6.1. Carotenoids

Several studies have identified carotenoid extracts from natural sources as potent inhibitors of metabolic enzymes, including α-amylase, α-glucosidase, and pancreatic lipase[92]. Nowicka et al.[93] reported that α-carotene and β-carotene exhibited a potential inhibitory effect against α-amylase, α-glucosidase and pancreatic lipase. Another study on peach fruits showed high potential for the inhibition of pancreatic lipase, indicating a potential anti-obesity effect; the fruit consisted of polyphenols, anthocyanins, and carotenoids, with β-carotene being the dominant carotenoid[93]. Most studies have been conducted on carotenoid extracts from non-citrus sources, and most have investigated crude extracts containing numerous phytochemicals rather than isolated compounds.

Carotenoid compounds are characterised by some structural features that influence their biological properties and activities; structural features such as their long chains conjugated double bond (polyene), presence of different functional groups, oxygenated derivatives (xanthophylls) and terminal ring structures[94]. Only a few studies have reported the inhibitory effects of isolated carotenoid compounds, including fucoxanthin and α-tocopherol, on pancreatic lipase and α-glucosidase[95,96].

Carotenoids are widely recognised for their antioxidant properties and their potential anti-diabetic and anti-obesity activities[93,97,98]. The antioxidant potential of carotenoids has been well studied, but little is known about the SAR of carotenoids in diabetes and obesity. Therefore, further research is needed to identify key structural configuration drivers conferring bioactivities of carotenoids, particularly those extracted from citrus fruits.

6.2. Essential oils

Previous studies have reported the binding affinity of various essential oils, such as terpenes, limonene, terpinene, and pinene, to α-amylase, α-glucosidase, and pancreatic lipase, with varying affinities (Table 1). This difference between these various essential oils against the protein targets may be attributed to their different chemical structures. For instance, β-caryophyllene has been reported to inhibit α-amylase more than other essential oils, such as limonene, because of the two methyl moieties and the oxygen atom attached to the (S)-2-methyl oxirane moiety[75]. β-Caryophyllene forms five hydrogen bonds with the α-amylase active site residues Asp 197, Glu 233, and Asp 300. In addition, it forms seven hydrogen bonds and one H–pi interaction with Glu 276, Phe 177, Arg 312, Asp 349, Arg 439, Gln 350, and Asp 408 active site residues of α-glucosidase, giving it high anti-diabetic potential[75]. Valdes et al.[99] investigated the SAR of terpenes, a division of essential oils, for their *in vitro* inhibition of α-glucosidase. The authors reported that the addition of and acetylation of hydroxyl groups enhances the α-glucosidase inhibitory effects of terpenes. Another example is the hydroxylation of ρ-cymene, which leads to the synthesis of thymol; the latter exhibits a better α-glucosidase inhibitory and antioxidant effect than its precursor molecule[100]. In the current review, the *in silico* data presented in Table 1 show that hydroxylated γ-terpinene (4-terpineol) has a stronger binding affinity than γ-terpinene, confirming the influence of hydroxylation on the binding affinity of

compounds. Mahnashi et al.[75] reported that the modification of β-caryophyllene to β-caryophyllene epoxide improved the binding affinity of the compound at the active site of α-amylase and α-glucosidase. Another study by Tshiyoyo et al.[88] investigated the inhibition of α-amylase and α-glucosidase by a selection of citrus peel-derived essential oils. Despite finding little to no inhibition against α-amylase, the authors reported more potent inhibition by two cyclic terpenes, valencene and carveol, suggesting that the ring form may influence the activity of essential oils more than linear terpenes[88]. However, in the same study, geraniol, a linear terpene, exhibited more potent inhibition than limonene, a cyclic terpene. This may be due to the hydroxyl group present in geraniol and absent in limonene. Studies focusing on the inhibition of pancreatic lipase by citrus peel-derived essential oils are limited in the current literature.

Most studies have investigated the amylolytic enzymes and pancreatic lipase inhibition activities of essential oils as extracts[101,102]; only a few studies, as reported above, have shown the inhibitory activity of pure compounds. Therefore, further research is needed to determine the bioactivity of pure chemical compounds within this class and to analyse the SAR of essential oils as amylolytic enzymes and pancreatic lipase inhibitors.

6.3. (Poly)phenols

6.3.1. Flavonoids

Duan et al.[103] found that hydroxylated polymethoxyflavone compounds in CP have better amylolytic enzymes and pancreatic lipase activities. In contrast, Sarian et al.[104] found the presence of a double bond of C-2–C-3 and a carbonyl group C-4 to be essential characteristics for the α-glucosidase activity of flavonoids. Additionally, *in silico* data showed that hesperidin has a stronger binding affinity than naringenin for α-amylase and α-glucosidase (Table 1). This may be due to the differentiation of glycosylation and methylation of the structure of hesperidin from that of naringenin. According to Zhang et al.[105] a methyl group at the C4′ position of the flavanone ring B positively affects its inhibitory activity against α-glucosidase. Additionally, the inhibitory effect of flavonoids against α-glucosidase increases when the sugar molecule is substituted on ring A[105]. Moreover, Proença et al.[106] found that the presence of $-OCH_3$ groups at the C-5 and C-7 positions of ring A reduced the α-amylase inhibitory potential of flavonoids. This reduction is more significant when –OH groups at the C3′ and C5′ positions of ring B are replaced by $-OCH_3$ groups found that hydroxylated polymethoxyflavone compounds in CP have better amylolytic enzymes and pancreatic lipase activities. In contrast, Sarian et al.[104] found the presence of a double bond of C-2–C-3 and a carbonyl group C-4 to be essential characteristics for the α-glucosidase activity of flavonoids. Additionally, in silico data showed that hesperidin has a stronger binding affinity than naringenin for α-amylase and α-glucosidase (Table 1). This may be due to the differentiation of glycosylation and methylation of the structure of hesperidin from that of naringenin.

6.3.2. Phenolics

Guan et al.[107] analysed the SAR of phenolics using benzoic acid as the parent structure. This study found that the presence of the –OH group on C-2 of the benzene ring was positively correlated with α-amylase inhibitory activity. Conversely, the –OH group on C-5 and the $-OCH_3$ group at C-2 of the benzene group negatively affect the inhibitory activity of phenolics. Furthermore, phenolics with more than one –OH group have shown strong inhibitory activity against α-glucosidase owing to the promotion of strong hydrogen bonds with the enzyme[108]. Aleixandre et al.[108] reported stronger α-amylase and α-glucosidase inhibitory activity of chlorogenic acid when compared to ρ-coumaric acid; these results correlated with the *in silico* data reported in Table 1, where chlorogenic acid exhibited stronger binding affinity than ρ-coumaric acid. Phenolic compounds that possess significant hydrogen bond donors, hydrogen bond acceptors, and aromatic rings can form strong bindings with pancreatic lipase. This interaction results in effective inhibition of the enzyme[109]. Compounds that possess significant hydrogen bond donors, hydrogen bond acceptors, and aromatic rings can effectively bind to pancreatic lipase. This strong interaction leads to efficient inhibition of the enzyme's activity[109]. Chlorogenic acid is formed when caffeic acid is combined with quinic acid in an esterified form[110]. Chlorogenic acid contains more functional groups and hydrogen bond donors than caffeic acid. This difference may lead to stronger inhibition of pancreatic lipase[109].

6.4. Pectin

The effects of pectin on the regulation and digestion of starch and lipids have been studied extensively. Obtaining the structural details of pectin samples is crucial for analysing structural relationships. These include molecular weight, sugar linkages, side-chain composition, and the extent of acetylation, methylation, and esterification. Comparing the functions of structurally distinct pectins is necessary to understand their SAR[111].

Pectin extracted from lemons has a high concentration of eriocitrin and has been reported to be a more potent inhibitor of α-glucosidase than other citrus species[112]. Additionally, molecular docking studies revealed that the eriocitrin B ring's 3′,4′ dihydroxyl groups interact with the α-glucosidase active site residues, demonstrating a high binding affinity[34]. Wang et al.[113] studied how pectin extracted from grapefruit using ultrasound-assisted and conventional heating methods inhibited pancreatic lipase. They found that using ultrasound, pectin extracted had a stronger inhibitory effect on lipase than conventionally extracted pectin. The researchers attributed this inhibitory potential to the viscosity, solubility, and structure of pectin, which depends on the degree of methylation and acetylation[113,114].

A study by Espinal-Ruiz et al.[114] compared the impact of medium-methoxylated pectin (52% mol/mol) from banana passion fruit to commercial citrus pectin with high (71% mol/mol) and low (30% mol/mol) methoxylation degrees. The results indicated that as pectin methoxylation increased, the speed and amount of lipid digestion decreased. A higher methyl content results in more hydrophobic groups that attract bile salts through hydrophobic interactions, preventing them from interacting with the surfaces of the fat droplets. Furthermore, an increase in the molecular weight of pectin samples may increase the viscosity of gastrointestinal phases, limiting the ability of lipids and pancreatic lipase, and preventing the substrate and catalyst from interacting[114].

In a study conducted by Liang et al.[115], the impact of citrus pectin's molecular weight on the inhibition of α-glucosidase was examined. The researchers used pectin extracted from navel orange peels and found it to be more effective than pectin extracted from unripe fruit pomace of Rubus Hu, as reported by Chen et al.[44] Additionally, the pectin from navel orange peels demonstrated a mixed-type non-competitive inhibition of α-glucosidase[115]. Morales-Contreras et al.[116] investigated the effect of pectin esterification on pancreatic lipase inhibition; the authors observed that a higher degree of esterification reduced enzyme activity. Bai et al.[111] examined the relationship between the structure and properties of pectin and its ability to inhibit porcine pancreatic α-amylase, highlighting the significance of the degree of esterification of pectin on enzyme inhibition.

7. *In vitro* bioactivity of citrus compounds against amylolytic enzymes and pancreatic lipase

7.1. Amylolytic enzyme inhibitory properties of citrus peel-derived phytochemicals

Several studies have demonstrated the inhibition of amylolytic enzymes by CP-derived essential oils as a promising alternative approach to T2D management. For example, Dang et al.[117] reported that CP essential oils inhibited yeast α-glucosidase and showed a synergistic effect when combined with acarbose. Similarly, Radünz et al.[102] sweet orange essential oils strongly inhibited α-glucosidase, with thymol, D-limonene, and 4-terpineol being the major components. Oboh et al.[118] revealed that essential oils from orange (*C. sinensis*) and lemon (*C. limon*) peels inhibited Hog pancreatic α-amylase and α-glucosidase. Heydari Koochi et al.[101] also reported on the promising anti-diabetic potential of CP essential oils. The authors found that the different oils inhibited α-amylase and α-glucosidase in different ways, including non-competitive, uncompetitive, and mixed inhibition[101].

A comparative study by Rangarajan et al.[119] tested the hydroalcoholic extracts of lemon and orange peels for their ability to inhibit α-amylase and α-glucosidase; the authors reported more potent inhibition of both enzymes for lemon peel extract compared to orange peel[119]. A recent study by Ashmawy et al.[120] investigated α-amylase and α-glucosidase inhibition by essential oils from *Citrus aurantium* (Bitter orange) using three different extraction methods: steam distillation, microwave-assisted distillation and

hydrodistillation. The authors reported inhibitory activity for all extracts, with the hydrodistillation extract displaying more potent activity due to the unique presence of oxygenated monoterpenes such as linalool and α-terpineol acetate[120]. Another study by Elhawary et al.[121] used the same three extraction methods to obtain essential oils from *Citrus aurantium* leaves. Their results showed that the essential oil extracted by steam distillation presented the most potent α-amylase inhibition, while the microwave distillation showed the highest α-glucosidase inhibition, followed by hydrodistillation[121]. These results suggest that the citrus cultivar, citrus parts (peels, roots, pomaces) and extraction techniques influence the enzyme inhibition potency of citrus essential oils.

Flavonoids have also shown promise in controlling T2D and metabolic syndrome by inhibiting α-glucosidase and lowering blood lipid levels[122]. For instance, Priscilla et al.[89] studied the effects of naringenin on yeast and mammalian α-glucosidase inhibition, revealing an inhibitory concentration (IC_{50}) value of 6.51 and 384 µM, respectively, against these proteins (Table 2). Shen et al.[123] investigated flavonoids, such as hesperidin, narirutin, nobiletin and neohesperidin, for their inhibitory activity against pancreatic α-amylase and α-glucosidase. Nobiletin showed better α-amylase inhibition at 160 µM compared to the other studied flavonoids. All four compounds evaluated in this study demonstrated weak inhibition (below 20%) against α-glucosidase activity (Table 2). Furthermore, Zhang et al.[105] studied hesperidin, naringin and nobiletin isolated from *C. sinensis* for their α-glucosidase inhibition activity. Hesperidin, naringin and nobiletin displayed IC_{50} values of 26.18, 6.51 and >155.32 µM, respectively, against α-glucosidase.

Liang et al.[115] reported IC_{50} values of 1.182 and 2.524 mg/mL for pectin of normal and infected navel orange, respectively, against α-glucosidase. Additionally, Bai et al.[111] investigated six commercial pectins (one from citrus peels) on porcine pancreatic α-amylase inhibition activity. High methoxyl pectin (CP) and low methoxyl pectin (PGA) displayed opposing effects on porcine pancreatic amylase activity, with CP causing a notable increase and PGA leading to a significant decrease. Inhibition kinetics further indicated that PGA was a non-competitive inhibitor of amylase and the interaction between PGA and amylase was almost certainly mediated by electrostatic interaction[111].

The phenolics, chlorogenic acid and caffeic acid, were shown to be weak amylolytic enzyme inhibitors with IC_{50} values of 3.9 and 5.55 µM (human α-amylase) and 3.19 and 0.31 µM (rat intestinal maltase), respectively, compared to acarbose[74]. Caffeic acid showed less than 20% inhibition of α-amylase at 1 mM and no inhibition of maltase[108]. The inhibition of α-glucosidase by phenolic acids shows promise, as caffeic acid showed an IC_{50} value significantly different from that of acarbose but caused higher maximum inhibition at lower concentrations[108]. These studies have also shown that the number of hydroxyl groups in phenolic acids correlates positively with inhibitory effects.

However, no phenolic has been reported to be a better amylolytic inhibitor than acarbose. Nonetheless, further research is needed to explore the potential synergistic effects of combining (poly)phenols with acarbose for T2D and obesity treatment. Acarbose has many side effects that might be mitigated by these natural compounds. There are also no studies showing combination therapies, with the possibility of synergistic effects between (poly)phenols and acarbose, which requires further exploration. These phenolic compounds are produced in varying amounts in different citrus species and can be extracted to various degrees depending on the method employed[40].

7.2. Pancreatic lipase inhibitory properties of citrus peel phytochemicals

Pancreatic lipase is the enzyme responsible for digesting 50–70% of triglycerides into monoacylglycerides and free fatty acids (FFA), which are then absorbed into the body. Inhibiting pancreatic lipase is a major target in combating obesity, as it reduces fat absorption and overall energy intake[124]. Inhibition of pancreatic lipase by CP essential oils has not yet been extensively described in the literature. However, studies on essential oils of other plants, such as basil (*Ocimum basilicum*), have reported the anti-lipase activity of essential oil extracts[125]. Some studies focused on the lipolytic (fat-breaking) effects of citrus peel oils from different cultivars, which play a crucial role in lipid metabolism and obesity management, but it is distinct from direct lipase inhibition[126]. A study by Choi et al.[127] reported that three different cultivars showed potent lipolytic effects, with Natsudaidai extract (a type of mandarin orange) having the highest effect, followed by Yuzu (*Citrus junos sieb.*) and lemon (*Citrus limon*); where the authors suggested that oils rich in γ-terpinene, p-cymene, and limonene exhibited strong lipolytic activity[127].

Table 2. The inhibitory effects of common citrus-derived phytochemicals on amylolytic enzymes and pancreatic lipase are reported, including their inhibitory concentration (IC_{50}) and inhibition constant (K_i) values.

		Anti-obesity effect	Anti-diabetic effect		References
Phytochemical	Citrus source	Porcine pancreatic lipase	Hog pancreatic α-amylase	*Saccharomyces cerevisiae* α-glucosidase	Capetti et al.[134]
α-Pinene	*C. reticulata; C. sinensis*	–	7.71 mM*	–	Capetti et al.[134]
α-Terpineol	*C. limon; C. paradisi*	–	5.43 mM*	–	Capetti et al.[134]
β-Pinene	*C. reticulata; C. sinensis; C. paradisi; C. limon*	–	8.59 mM*	–	Martinez-Gonzalez et al.[80], Behera et al.[82], Liu et al.[109]
ρ-Coumaric acid	*C. limon*	0.170 mM*; 0.206 mM#; 1282.28 µM*	52.4 mM*	6.20 mM*	Nyambe-Silavwe and Williamson[74], Liu et al.[109]
Caffeic acid	*C. paradisi ; C. sinensis*	370 µM #; 139.31 µM*	5.55 µM*	0.31 µM*	Tshiyoyo et al.[88]
Carveol	*C. sinensis; C. reticulata*	–	–	0.54 mg/mL*; 0.53 mg/mL#	Nyambe-Silavwe and Williamson[74]
Chlorogenic acid	*C. sinensis*	0.402 mM*	3.9 µM*	3.19 µM*	Nyambe-Silavwe and Williamson[74], Behera et al.[82], Liu et al.[109]
Gallic acid	*C. sinensis*	387.2 µM*; 44.61 µM #; 261.57 µM*	4.35 µM*	1.44 µM*	Tshiyoyo et al.[88]
Geraniol	*C. sinensis; C. reticulata*	–	–	0.71 mg/mL*; 0.56 mg/mL#	Huang et al.[90], Zhang et al.[105]
Hesperidin	*C. sinensis*	0.946 µM*	–	26.18 µM*	Tan et al.[135]
Limonene	*C. reticulata; C. sinensis; C. paradisi;*	–	>10 mM*; 1.51 mg/mL*; 1.10 mg/mL#	>10 mM*	Priscilla et al.[89]
Naringenin	*C. paradisi*	–	–	6.51 µM*; 384 µM#	Shen et al.[123], Zeng et al.[128]
Nobiletin	*C. reticuluta*	65.31 µM*	160 µM*	>155.32 µM*	Karamać and Amarowicz[136]
Sinapic acid	*C. paradisi*	273 mM*	8.92 mM*	1.48 mM*	Liang et al.[115]
Pectin (Normal and infected)	*C. sinensis*	–	–	1.182 mg/mL*; 2.52 mg/mL*	Wang et al.[113]
Pectin	*C. paradisi*	1.75 mg/mL*; 2.00 mg/mL*	–	–	Martinez-Gonzalez et al.[80], Tshiyoyo et al.[15]
Quercetin	*C. sinensis*	6.1 µM*; 12.0 µM#	0.465 mM#	0.045 mM#	Tshiyoyo et al.[88]
Valencene	*C. sinensis; C. reticulata*	–	0.65 mg/mL*; 0.33 mg/mL#	–	Tolmie et al.[14]
Acarbose			3.8 µM#	170 µM#	Martinez-Gonzalez et al.[80]
Orlistat		0.017 mM#			Capetti et al.[134]

*Indicates inhibitory concentration (IC_{50}) value.
#Indicates inhibition constant (K_i) value and (–) indicates no inhibition.

Six bioactive flavonoids (3,5,6,7,8,3′,4′-heptamethoxyflavone, didymin, hesperidin, narirutin, nobiletin and tangeretin) from *C. reticulata* were evaluated for their inhibition of pancreatic lipase[128]. All six compounds had IC_{50} values ranging from 26.28 to 688.25 µg/mL against porcine pancreatic lipase (PPL). Nobiletin showed a stronger inhibitory effect than the lipase inhibitor, orlistat[128]. Martinez-Gonzalez et al.[80] have also studied the pancreatic lipase activity of quercetin identified from *Capsicum frutescens* (hot pepper), which has IC_{50} and K_i values of 6.1 µM and 12.0 µM, respectively. Huang et al.[90] have reported that hesperidin in *C. sinensis* has an IC_{50} value of 0.946 µM against pancreatic lipase. Meanwhile, carotenoids have also been shown to have promising activity in inhibiting pancreatic lipase.

Pectin extracted from fresh grapefruit peels (*C. paradisi*) by ultrasound-assisted extraction and conventional heating was evaluated for its inhibition capacity against pancreatic lipase. The inhibitory activity of ultrasound-extracted pectin against lipase (IC_{50} = 1.75 mg/mL) was stronger than that of conventional heating-extracted pectin (IC_{50} > 2 mg/mL)[113], as shown in Table 2. In another study, citrus pectin inhibited pancreatic lipase activity in a dose-dependent manner, with 70% inhibition at 5 mg/ml[129]. The presence of dietary fibre in the gastrointestinal tract, exemplified by pectin, has shown the potential to decrease caloric intake and reduce the risk of cardiovascular diseases[114]. It has been shown that citrus pectin waste can be used to develop functional prebiotic foods with enhanced bio-functionality. For example, citrus waste from fruit processing can be used to extract pectin, which is hydrolysed into pectic oligosaccharides (POS). POS can be studied for its various biological activities[24,130,131].

A study was conducted to evaluate the impact of pectin on lipase, α-amylase, alkaline phosphatase and protease enzymatic activities. The results indicated that pectin polysaccharides (PPs) were non-competitive inhibitors of these enzymes. The inhibitory effect became more pronounced with higher concentrations and molecular weights of PPs, with lipase being the most susceptible to inhibition[114].

Phenolics are well reported for their pancreatic lipase inhibition action; for example, several studies showed that most phenolic compounds inhibit pancreatic lipase, but not more than orlistat. Caffeic acid, ρ-coumaric acid and gallic acid showed similar inhibition at concentrations 400 times higher than orlistat[80,82]. Liu et al.[109] conducted a computational and *in vitro* study predicting the effects of gallic acid, ferulic acid, ρ-coumaric acid and caffeic acid on pancreatic lipase, and reported IC_{50} values of 261.57 µM, 1129.36 µM, 1282.28 µM, and 139.31 µM, respectively.

It is important to consider other factors such as antioxidant properties, gastrointestinal stability, cytotoxicity, and their effects on the microbiome. As mentioned above, combination studies should be considered to study any possible synergistic effects between (poly)phenols and orlistat to mitigate side effects and produce a more efficient treatment. The metabolic pathways by which these phytochemicals are produced should be further explored. Furthermore, *Citrus depressa extracts* (shiikuwasa) are traditionally used to treat obesity and dyslipidaemia, as these citrus species contain more coumarins and phenolic acids that have not yet been detailed in the literature, and these groups of compounds can be considered an unexplored niche that still needs to be explored to its full potential.

To date, numerous studies have focused on CP extracts for their inhibitory properties against amylolytic and pancreatic lipase enzymes; however, there are limited studies assessing the inhibition of these enzymes by isolated and pure phytochemicals. This calls for more studies to be carried out using these isolated and pure phytochemicals to better elucidate SAR for each chemical class of CP-derived phytochemicals. This review has, nonetheless, highlighted that several *in silico* studies have investigated the SAR of CP-derived compounds and attempted to decipher how their structural modifications influence bioactivity against the protein targets: amylolytic enzymes and pancreatic lipase[132].

It is worth noting that most *in vitro* studies have used IC_{50} values rather than K_i values to report or compare the inhibitory effects of phytochemicals against the protein targets: amylolytic enzymes and pancreatic lipase. The issue with this is the fact that the IC_{50} is useful for the comparison of results within a measurement series, but not with other values obtained using different assays, while K_i is an *in vitro* constant that best describes binding affinity[133]. Therefore, further investigations are required to record more *in vitro* parameters of these compounds; to confirm their bioactivity against T2D and obesity targets; and to further analyse the relationship between structures and bioactivities. Further, there is a need for standardisation of assay conditions and harmonisation of reporting standards.

7.3. Digestive enzyme inhibitory properties of citrus peel phytochemicals

Digestive enzymes, such as pepsin, trypsin and protease in the gastrointestinal tract, can be inhibited by citrus peel phytochemicals, leading to unwanted cross-reactions that can cause nutritional deficiencies[137]. Grapefruit extracts have been previously reported as good inhibitors of trypsin and pepsin[138]. However, there are no previous studies reported on the inhibitory properties of carotenoids and essential oils against trypsin or pepsin. Low-methoxy pectin and flavonoids (rutin, hesperidin, quercetin and naringenin) have exhibited inhibitory effects against trypsin and pepsin[139–141].

Beneficial cross reactions of citrus peel phytochemicals can include the inhibition of dipeptidyl peptidase 4 (DPP4), a well-known target for diabetes and obesity treatment[142]. DPP4 inhibitors function by augmenting the concentration of specific hormonal agents known as incretins, which play a crucial role in stimulating the pancreatic secretion of insulin in response to high glucose levels. This mechanism ultimately contributes to the reduction of blood glucose concentrations. In the same vein, narirutin has shown interactions with DPP4, indicating possible inhibition[143].

8. Study limitations: *in silico* and *in vitro* studies

There is a lack of standardisation of extraction methods, as different extraction techniques can yield varying concentrations and profiles of phytochemicals, leading to inconsistencies in bioactivity results

across different studies conducted with these differently acquired CP extracts from the same citrus cultivar. Hence, ensuring the purity and concentration of extracted compounds can be challenging, impacting reproducibility in studies. Many studies report inhibitory concentrations against these enzyme targets without including the compounds' mode of inhibition (kinetic studies). Understanding the mode of inhibition is essential for gaining a comprehensive understanding of the compounds' effectiveness.

Reported research studies use human, rat or porcine-derived digestive enzymes to screen for the potential bioactivity of the CP extracts. However, this can lead to variations in the reported efficacy of the said phytochemical due to subtle differences in structural and biochemical properties of the various protein homologues used in these studies. This calls for standardised *in vitro* inhibition assays against these digestive enzymes to expedite the discovery of potential lead compounds for treating diabetes and obesity. The bioavailability of the compounds will also play a role in the efficacy of inhibition. This depends on how the compounds are prepared, whether in an extract or as a pure compound.

Lastly, *in vitro* and *in silico* reports of a phytochemical should be correlated in the same studies. Correlating *in vitro* (laboratory experiments) and *in silico* (computer-simulated models) data enhances the predictive accuracy regarding how a phytochemical will behave *in vivo* (within a living organism). *In silico* models can simulate physiological conditions and predict pharmacokinetic parameters like absorption, distribution, metabolism, and excretion (ADME) based on *in vitro* data. This synergy allows researchers to better forecast the clinical outcomes of phytochemicals, thereby improving the reliability of their findings and reducing the likelihood of unexpected results in clinical trials.[144]

9. Conclusion and future perspectives

This review has highlighted a gap in the potential utility of CP-derived phytochemicals, which have not yet been reviewed for their role as amylolytic enzymes and pancreatic lipase inhibitors, potentially serving as lead anti-diabetic and anti-obesity agents (Figure 5). The review emphasises the importance of reporting *in vitro* studies using K_i values. This standardisation will facilitate comparisons of the inhibitory effects of phytochemicals on specific protein targets, namely amylolytic enzymes and pancreatic lipase. Investigating the structure-activity relationships (SAR) of these CP-derived phytochemicals (essential oils,

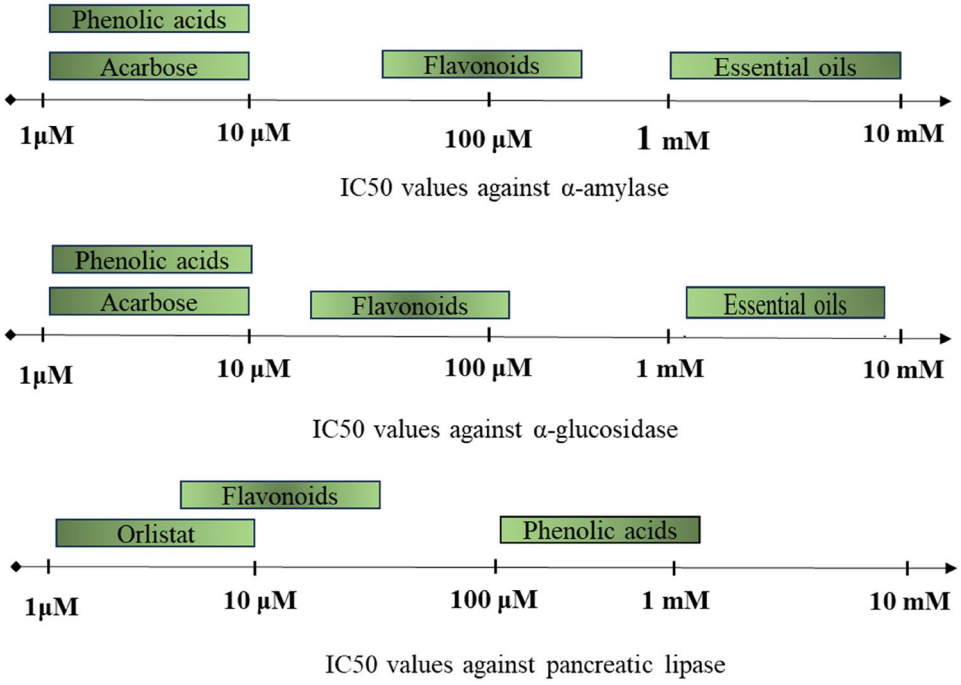

Figure 5. Summary of the range IC_{50} values of CP-derived phytochemicals, including essential oils, flavonoids, and phenolic acids, as well as acarbose and orlistat (drugs), against the amylolytic enzymes and pancreatic lipase. The figure was created in www.biorender.com.

carotenoids, (poly)phenols and pectin) is critical to advancing our understanding of their inhibitory potential. Researchers can identify key structural features that enhance enzyme inhibition by focusing on the molecular interactions between phytochemicals and enzymes like amylase and lipase.

For instance, changes in the number and position of functional groups on a phenolic compound could dramatically alter its ability to bind to the enzyme's active site. These potential bioactive compounds could mitigate the side effects of currently used drugs such as acarbose, therefore, warranting their further investigation through *in vivo* models and pre-clinical studies. Furthermore, understanding the molecular mechanisms of the compounds in targeting these enzymes is crucial, and clinical studies in humans are necessary to confirm their health benefits. Finally, exploring the synergistic effects between CP-derived phytochemicals and existing drugs is also recommended, particularly for alleviating side effects and improving the efficacy of existing drugs.

Notes

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Data availability statement

All the data supporting the literature search findings for this systematic review are included.

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