

RESEARCH ARTICLE

Impact of select Polyphenols on α -amylase Activity under *In vitro* Conditions

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ABSTRACT:

Background: Flavonoids have been extensively researched for their ability to modulate enzyme activity, particularly in relation to controlling blood sugar and carbohydrates metabolism. However, there is limited information on the tissue-specific effects of structurally diverse flavonoids on α -amylase activity. This study aimed to investigate and compare rutin, quercetin, luteolin, chrysosplenetin, and thameflavide effects on α -amylase activity in various parts of the digestive system. **Methods:** We used homogenates from pancreatic tissue, parotid gland, intestinal mucosa, and chyme during *in vitro* assays. Flavonoids were tested at different levels and α -amylase activity was measured using a spectrophotometer. The Student-Fisher t-test was used to run a statistical analysis to determine dose and tissue-dependent effects. **Results:** Rutin, quercetin, luteolin, and chrysosplenetin significantly and dose-dependently inhibited the α -amylase activity. The parotid gland and intestinal mucosa were more sensitive ($p < 0.05$). Luteolin had the strongest inhibitory impact, perhaps because of its hydroxylation patterns. On the other hand, thameflavide had a distinct influence on α -amylase activity, particularly in the salivary and intestinal compartments suggesting allosteric activation pathways. The varied modulatory profiles were correlated with flavonoid structure-function relationships, where the distribution of hydroxyl and methoxyl groups determines the enzyme binding and outcomes. **Conclusion:** The results showed that some flavonoids might be used as natural α -amylase inhibitors to regulate blood sugar levels and treat metabolic disorders. They also showed that thameflavide could help those who need better carbohydrate digestion. These findings revealed that flavonoids might be added to nutraceutical formulations in an organized way. Furthermore, *in vivo* and clinical studies are needed to determine their safety and efficacy as therapeutic agents.

KEYWORDS: Flavonoids, α -Amylase, Rutin, Quercetin, Luteolin, Chrysosplenetin, Thameflavide, Enzyme Modulation, Glycemic Control, Digestive Enzymes.

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INTRODUCTION:

In recent years, the amount of environmental xenobiotics, including heavy metal salts, nitrates and nitrites, other mineral fertilizers, insecticides, phenolic compounds and food additives has been increasing. These substances enter the digestive tract through contaminated drinking water and food products, interacting with digestive with digestive enzymes in saliva, bile, gastric, pancreatic and intestinal juices. The

efficiency of the final stages of nutrient hydrolysis and absorption depends on the intensity of initial stages of hydrolysis; therefore, changes in enzyme activity particularly α -amylase, are crucial for understanding the effects of these substances¹. Due to the high sensitivity of α -amylase towards various internal and external environmental factors, we considered to examine the impact of foreign substances entering the digestive tract on α -amylase activity as a model for these effects². α -Amylase also known as α -1,4-glucan-4-glucanohydrolase classified under E.C. 3.2.1.1, belonging to glycosyl hydrolase family 13, plays a critical role in catalyzing the hydrolysis of α -(1,4)-glycosidic linkages in starch^{3,4}. This enzyme is responsible for breakdown of complex carbohydrates composed of multiple glucose units. The amylase enzyme family, consisting of 30 distinct glycoside hydrolases, can be differentiated by their specific biochemical characteristics⁵. Specifically, α -amylase (1,4- α -D-glucan-4-glucanohydrolases) facilitates, random non-selective endo-type cleavage of α -(1,4)-glycosidic bonds in amylose and amylopectin. According to Wild et al., in 1954 this amylase produces “limit dextrins” from amylopectin and “maltose, maltotriose and higher oligosaccharides” from amylose⁶. The mechanism of starch metabolism in humans begins with the activity of human salivary α -amylase. Suppression in enzymatic activity of human salivary α -amylase offers simple opportunities to alleviate postprandial hyperglycemia by regulating starch catabolism¹. The cleavage of bonds can occur between any glucose residues, with the resulting monosaccharide residues taking the α -monomer configuration. Amylase has weakly acidic bonds and is activated in the presence of Ca^{2+} and Cl^- ions⁷. Amylase activity is measured for diagnostic purposes, particularly to identify glycogenolysis, pancreatitis or diabetes mellitus⁸. There are two isozymes of pancreatic amylase, each about 56 kDa in size and 496 amino acids long. Two genes for pancreatic amylase exist on chromosome 1 (AMY2A and AMY2B)⁹. X-ray structural studies revealed that the enzyme molecule contains one calcium ion and two chloride ions (Payan, Qian, 2003). In the enzyme’s catalytic center, the Asp-300 amino acid residue binds to the substrate, altering its conformational state¹⁰. Given the lack of literature on the dose-dependent effects of phytopreparations on α -amylase activity *in vitro*, we aimed to investigate their effects on α -amylase-secreting glands in growing rats.

MATERIALS AND METHODS:

Experimental Animals:

In this research study, adult white outbred rats, having weight of $200 \pm 20\text{g}$ on average, were used. These animals were kept housed under controlled vivarium conditions in plastic cages. They had free access to water and a standard grain-based diet and were

maintained a 12h natural light-dark cycle. Environmental parameters were kept stable, with an ambient room temperature of $22\text{--}24^\circ\text{C}$ and relative humidity of 40–60%.

Five animals were housed per cage ($50 \times 30 \times 28\text{cm}^3$). All experimental procedures strictly followed the guidelines of the ethical committee on the use and care of laboratory animals at the faculty of biology, national university of Uzbekistan.

Experimental design:

Tissue homogenates from rats were used to evaluate the *in vitro* effects of selected flavonoids on α -amylase activity. The study included five experimental conditions, each tested in six replicates ($n = 6$):

Control group ($\leq 0.1\%$ DMSO)– pancreatic, parotid, intestinal mucosa, and chyme homogenates incubated with vehicle solution only.

Rutin groups – homogenates incubated with rutin at 10, 20, 30, 40, and $50\mu\text{g/L}$ concentrations.

Quercetin groups – homogenates incubated with quercetin at 10, 20, 30, 40, and $50\mu\text{g/L}$ concentrations.

Thamiflaside groups – homogenates incubated with thamiflaside at 10, 20, 30, 40, and $50\mu\text{g/L}$ concentrations.

Luteolin groups – homogenates incubated with thamiflaside at 10, 20, 30, 40, and $50\mu\text{g/L}$ concentrations.

Chrysosplenetin groups – homogenates incubated with thamiflaside at 10, 20, 30, 40, and $50\mu\text{g/L}$ concentrations.

Each concentration for all flavonoids and tissue types were assayed in six independent replicates ($n = 6$) to ensure the statistical reliability.

Preparation of Biologically Active Material:

Prior to experimentation, animals were humanely euthanized by decapitation between 08:00 and 10:00 AM to minimize circadian variation in enzyme activity. For the collection of parotid glands, the scalp was carefully opened to avoid tissue damage. Three parotid glands were gently dissected free from surrounding connective tissue, weighed, and homogenized under chilled conditions using an HG-15A homogenizer. The homogenate was diluted with distilled water at ratio of 1:500. Subsequently, the abdominal cavity was opened to isolate the pancreas, which was carefully cleared of adipose tissue and weighed. The pancreatic tissue was minced with sterile scissors, placed in chilled test tubes, and diluted to ratio of 1:10 with ice-cold physiological saline. Homogenization was performed using a glass homogenizer placed in an ice bath to maintain low temperature, operating at 200–300rpm for 60 seconds. The final homogenate was further diluted to a ratio of 1:40,000. For enzymatic assays, 0.5mL of pancreatic

homogenate was incubated with 0.5mL of test solutions containing graded concentrations (10, 20, 30, 40, and 50µg/L) of rutin, quercetin, or thamiflaside. Optimal pH, temperature, and substrate concentrations were carefully maintained to ensure consistent enzymatic hydrolysis. The reaction mixtures were incubated in a thermostat at 37–38°C to mimic physiological conditions.

Determination of α -Amylase Activity in Pancreatic Homogenates:

The activity of α -amylase in rat blood plasma and pancreatic homogenates was assessed using a kinetic colorimetric assay with the chromogenic substrate 2-chloro-4-nitrophenyl- α -maltotrioside (CNP3) (Human Diagnostics, Germany). This method enables direct quantification of α -amylase activity without the need for auxiliary enzymes. During enzymatic hydrolysis, CNP3 is cleaved, releasing 2-chloro-4-nitrophenol (CNP), which produces a measurable enhancement in absorbance at 405nm. Pancreatic α -amylase activity was determined using a semi-automated biochemical analyzer (Rayto RT 1904C, China). The assay was performed in a reaction mixture containing a MES buffer (2-(N-morpholinoethanesulfonic acid), pH 6.0, 100mmol/L), CNP3 (2.25mmol/L), sodium chloride (350 mmol/L), calcium acetate (6mmol/L), potassium thiocyanate (900mmol/L), and sodium nitrite (0.95g/L) (Foo et al., 1998). For *in vitro* experiments, three flavonoids—rutin, quercetin, and thamiflaside—were tested. These compounds were obtained from the Institute of Chemistry of Plant Substances, Academy of Sciences of Uzbekistan, and dissolved in distilled water or DMSO to prepare working solutions at 10, 20, 30, 40, and 50 µg/L. After incubation with tissue homogenates, the absorbance of the resulting supernatants was measured at 405 nm using the same biochemical analyzer.

All enzymatic measurements were performed using a Rayto RT 1904C semi-automated biochemical analyzer (China), calibrated prior to each experimental run.

Used flavonoids:

The flavonoid compounds used in this study (rutin, quercetin, thamiflaside, luteolin, and chrysosplenetin) were obtained from the Laboratory of Terpenoids and Phenolic Compounds, Institute of Chemistry of Plant Substances, Academy of Sciences of Uzbekistan. All compounds had a purity of >98% as confirmed by HPLC analysis.

For *in vitro* experiments, each flavonoid was freshly dissolved in distilled water containing $\leq 0.1\%$ DMSO to prepare working solutions at the desired concentrations. The same solvent without flavonoids served as the vehicle control for untreated samples.

Rutin:

Rutin, also referred to as vitamin P, rutoside, quercetin-3-O-rutinoside or sophorin is a flavonol glycoside commonly found in wide range of plants including buckwheat, tea, passion flower and apples. It is considered an important nutritional component of foods due to its potential health benefits^{11,12}. The name “Rutin” originates from the plant *Ruta graveolens*, which also contain this compound (as presented Figure 1). Chemically, rutin is a glycoside consisting of disaccharide rutinose and flavonol aglycone quercetin. As an anti-oxidant, it is found in fruit and vegetable products of various plant species including: Solanaceae, Polygonaceae, Celastraceae, Rosaceae, Asparagaceae, Amaranthaceae, Capparaceae, Asteraceae, Chenopodiaceae and Lamiaceae^{13,14,15,16}. Its structure features multiple phenolic hydroxyl groups, particularly catechol groups, which enhance its absorption in the small intestine and its ability to bind with endogenous proteins. This enables its transport through the intestinal walls in to the bloodstream, facilitating distribution to different tissues in throughout body¹⁷. Due to its pharmacological potential, rutin is extremely relevant to the scientific community. Its’ cardiovascular, hepatoprotective, anti-cancer, anti-inflammatory, anti-diabetic and neuroprotective properties were explored as available in literature^{18,19,20,21,22}. The anti-proliferative and anti-metastatic properties of rutin have been demonstrated in a variety of cancer cells. The migration and proliferation of human lung and colon cancer cell lines have been shown to be inhibited by this natural product²³.

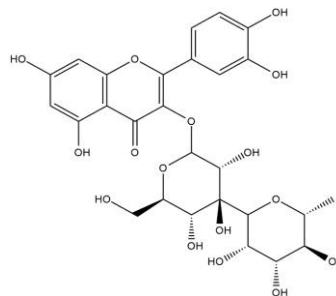


Figure 1. Chemical structure of rutin¹¹.

Quercetin's The quercetin has the chemical structure 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, with a molecular formula of $C_{15}H_{10}O_7$, a molecular weight 302.24g/mol and a melting point of approximately 316°C as illustrated in Figure 2. It contains five hydroxyl moieties^{24,25}. In nature, quercetin is predominantly found in glycosylated forms, where sugar such as glucose, rhamnose, or rutinose is mainly attached to the C-3 position. It rarely found in its free aglycone form^{26,27}. The anti-oxidant activity of this compound is largely due to its unique chemical structure²⁸. The presence of an *ortho*-dihydroxy

(catechol) group along with hydroxyl groups at positions 3 and 5 that are conjugated with a 4-oxo group, enhance its stability and biological efficacy²⁹. These functional groups allow quercetin to donate protons to free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), leading to the formation of stable quinone intermediates. However, the hydrogen donating ability is reduced in certain quercetin derivatives, particularly, those glycosylated at the C3 and C4OH positions. Additionally, compared to its aglycone form, quercetin's C3OH derivatives have maximum reducing potential³⁰.³¹ Moreover, quercetin also has maximum polarity to pass through the phospholipid bilayers in the epithelium's cell walls, making it impossible to absorb through a passive mechanism. But the intestinal microbiota and the metabolic enzymes secreted by body, enable quercetin to go through chemical changes that make it absorbable in the gastrointestinal system³².

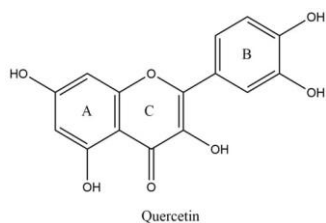


Figure 2. The structural formula of quercetin²⁶.

Thamiflaside The aerial portion of *Thalictrum minus* (family Ranunculaceae) yielded the flavonoid diglycoside. Apigenin 7-O- α -L-2''-methoxyrhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside is identified as the glycoside's chemical structure. Its molecular weight is 591.0g/mol, its melting point is in between 264 and 266 °C, and its molecular formula is C₂₈H₃₁O₁₄ (Figure 3). Along with apigenin, thamiflaside also contains residues of rhamnopyranoside and glucopyranoside. Often sold as apigenin aglycone, thamiflaside is a glycosidic compound that is bound to the C-7 carbon by glucose, rhamnose, and seven hydroxyl groups of derivatives. Furthermore, the potent anti-oxidant properties of thamiflaside are due to its distinct chemical structure. The methoxyl group is present at the C-2'' position of the rhamnopyranose residue, and the structural ones are the hydroxyl groups, which gives thamiflaside stability and anti-oxidant nature³³.

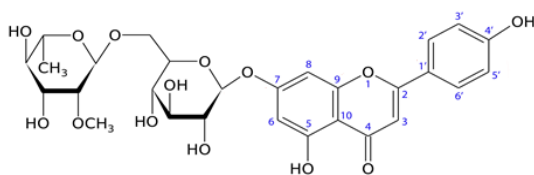


Figure 3. New chemical structure of Thamiflaside³³.

Luteolin, chemically known as 3',4',5,7-tetrahydroxyflavone or 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one, is a naturally occurring flavonoid widely distributed in fruits, vegetables, and medicinal herbs (Figure 4). As a member of the flavone subclass, luteolin is present in both aglycone and glycosylated forms, contributing significantly to their diverse biological and pharmacological activities³⁴. Luteolin contains four hydroxyl groups at positions 3', 4', 5, and 7, which are responsible for its potent anti-oxidant and free radical scavenging properties. In human body, luteolin acts as an anti-inflammatory agent, immune modulator, and cytoprotective molecule, with growing evidence supporting its beneficial effects in metabolic, autoimmune, digestive, and oncological disorders³⁵. Luteolin has been extensively studied for its anti-inflammatory activity, both *in vitro* and *in vivo*. It inhibits the production of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and interleukin-1 β (IL-1 β) in lipopolysaccharide (LPS)-stimulated macrophages. Furthermore, it deregulates cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), thereby reducing nitric oxide (NO) production and suppressing chronic inflammatory responses. In autoimmune thyroiditis (AIT), chronic inflammation is driven by the excessive activation of the NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway. NF- κ B promotes Th1/Th17-mediated immune responses, enhancing lymphocyte infiltration and thyroid follicular destruction. Luteolin inhibits NF- κ B signaling by preventing the degradation of I κ B α , leading to a marked reduction in inflammatory mediator synthesis and suppression of tertiary lymphoid structure formation within the thyroid³⁶.

Additionally, luteolin interferes with the STAT3 (Signal Transducer and Activator of Transcription 3) pathway, which is crucial for Th17 cell differentiation. By blocking STAT3 phosphorylation, luteolin decreases Th17 cell populations and restores regulatory T cell (Treg) balance, thereby attenuating autoantibody production and limiting autoimmune tissue damage³⁷. In autoimmune and metabolic disorders, excessive production of reactive oxygen species (ROS) and hydrogen peroxide (H₂O₂) contributes to oxidative stress, enhancing the immunogenicity of thyroid autoantigens such as thyroid peroxidase (TPO) and thyroglobulin (TG). Luteolin activates the Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway, which induces the expression of anti-oxidant enzymes including heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and catalase. Through this Nrf2-mediated anti-oxidant defense, luteolin reduces oxidative stress, decreases antigen presentation, and limits aberrant immune activation³⁸. Moreover, luteolin

deregulates chemokines such as CXCL13 and lymphotoxin- β , both of which drive ectopic lymphoid tissue formation in autoimmune diseases³⁹. Beyond its activity in immune-mediated disorders, luteolin has been shown to exert significant effects on pancreatic function and digestive health. In the endocrine pancreas, luteolin protects pancreatic β -cells from apoptosis induced by high glucose, uric acid, and endoplasmic reticulum stress^{35, 40}. It regulates the HNF4 α /Drak2 signaling axis, enhances autophagy, and preserves glucose-stimulated insulin secretion. These protective effects suggest a therapeutic potential for luteolin in preserving β -cell mass and function in both type 1 and type 2 diabetes mellitus⁴¹. In the exocrine pancreas, luteolin has demonstrated significant protective effects in experimental models of severe acute pancreatitis (SAP). In cerulein + LPS-induced pancreatitis, luteolin reduced pancreatic edema, acinar cell necrosis, and inflammatory cell infiltration. It was achieved by upregulating the anti-oxidant enzyme HO-1, increasing IL-10 production, and inhibiting NF- κ B signaling, thereby attenuating oxidative stress and suppressing inflammatory cascades^{42, 43}.

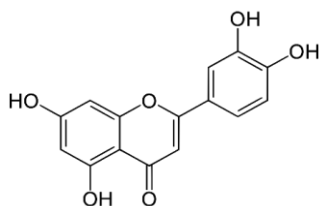


Figure 4. New chemical structure of Luteolin³⁵.

Collectively, luteolin is a multitargeted bioactive flavonoid that not only modulates immune responses and oxidative stress but also exerts protective effects on pancreatic β -cells, supports exocrine pancreatic function, regulates digestive enzyme activity, and improves overall metabolic homeostasis. These pleiotropic properties highlight its therapeutic potential for managing diabetes, pancreatitis, digestive dysfunction, autoimmune thyroiditis, and cancer.

Chrysosplenetin (also referred to as Chrysosplenetin) is an O-methylated flavonol naturally occurring in various plant species (figure 5). In our study, it was isolated from the aerial parts of *Pulicaria salviifolia* (family Asteraceae)⁴⁴. Chemically, chrysosplenetin is identified as 4',5-dihydroxy-3,3',6,7-tetramethoxyflavone (also described as 5,4'-dihydroxy-3,6,7,3'-tetramethoxyflavone). Its structure is characterized by hydroxyl groups at positions 4' and 5 and methoxy groups at positions 3, 3', 6, and 7 on the flavonol backbone. Chrysosplenetin has a molecular formula of C₁₉H₁₈O₈, a molecular weight of 374.34 g/mol, and a melting point ranging between 180 and 183 °C⁴⁵.

Chrysosplenetin exhibits multifaceted biological properties, including anti-inflammatory, anti-oxidant, pharmacokinetic modulatory, osteoprotective, and potential anti-tumor activities. It inhibits the activity of inflammatory mediators such as neutrophil elastase and suppresses the NF- κ B signaling pathway, leading to reduced production of pro-inflammatory cytokines, including TNF- α , IL-6, and IL-1 β . Moreover, chrysosplenetin has been shown to attenuate inflammatory marker expression in lipopolysaccharide (LPS)-stimulated macrophages, thereby contributing to the regulation of chronic inflammatory processes⁴⁶.

From a pharmacokinetic perspective, chrysosplenetin is of particular interest due to its ability to inhibit P-glycoprotein (P-gp) and CYP3A enzymes, resulting in improved bioavailability of co-administered drugs such as artemisinin. This interaction highlights its potential in enhancing the efficacy of anti-malarial therapies⁴⁵.

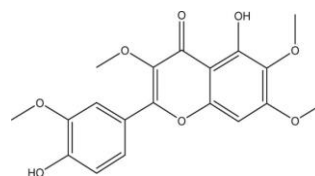


Figure 5. Chemical structure of Chrysosplenetin⁴⁵.

Chrysosplenetin also promotes osteoblast differentiation and bone formation by activating the Wnt/ β -catenin signaling pathway, increasing the expression of osteogenic genes such as RUNX2, COL1, and BGLAP, and preventing bone loss in models of estrogen deficiency. Notably, these osteoprotective effects occur without inducing significant toxicity, suggesting a potential role in maintaining bone health⁴⁷.

Another promising aspect of chrysosplenetin is its anti-tumor and anti-metastatic activity. It suppresses tumor growth by inducing apoptosis, regulating cell cycle progression, and inhibiting angiogenesis. These effects are mediated through the down regulation of Akt, PLK-1, and cyclins, alongside the upregulation of pro-apoptotic proteins⁴⁸. Furthermore, computational modeling has demonstrated chrysosplenetin's ability to bind with main protease (Mpro) of SARS-CoV-2, suggesting potential anti-viral properties^{49,50}.

By combining pharmacokinetic modulation, anti-inflammatory activity, and osteoprotective effects, chrysosplenetin represents a multi-target bioactive molecule of considerable scientific and clinical interest. However, its effects on pancreatic beta-cells function and exocrine pancreatic activity have not yet been investigated, highlighting an important gap for future research.

Table 1 :The *In Vitro* Effect of Rutin on α -Amylase Activity in the Digestive System (mean $\pm$ s.d., n = 6)

	Control	Rutin doses				
		10 μ g/L	20 μ g/L	30 μ g/L	40 μ g/L	50 μ g/L
Pancreas U/L P	233.89 $\pm$ 8.11	195.25 $\pm$ 9.13 <0.01	181.47 $\pm$ 7.26 <0.001	191.35 $\pm$ 7.27 >0.002	194.26 $\pm$ 7.11 >0.002	205.36 $\pm$ 6.64 <0.02
Parotid gland U/L P	9.91 $\pm$ 0.94	8.87 $\pm$ 0.45 >0.30	8.46 $\pm$ 0.76 <0.30	8.01 $\pm$ 0.93 <0.20	8.11 $\pm$ 1.14 <0.30	8.14 $\pm$ 0.96 >0.20
Intestinal mucose U/L P	82.47 $\pm$ 6.23	70.23 $\pm$ 5.78 >0.40	65.46 $\pm$ 4.97 <0.02	64.38 $\pm$ 4.45 <0.01	66.39 $\pm$ 6.13 <0.001	67.06 $\pm$ 4.86 <0.01
Chymus U/L P	6.52 $\pm$ 0.76	5.58 $\pm$ 0.49 <0.30	5.41 $\pm$ 0.45 <0.20	5.34 $\pm$ 0.63 >0.30	5.53 $\pm$ 1.59 <0.50	5.19 $\pm$ 0.73 <0.20

RESULTS:

The Effect of Rutin on α -Amylase Activity.

Under *in vitro* conditions, rutin demonstrated a clear dose-dependent inhibitory effect on α -amylase activity within different compartments of the digestive system. In pancreatic homogenates, the control group showed an enzymatic activity of 233.89 $\pm$ 8.11 U/L. Treatment with 10 μ g/L rutin reduced α -amylase activity to 195.25 $\pm$ 9.33 U/L, corresponding to a 16.5 % $\pm$ 2.3 decrease compared to the control. The maximal inhibition in pancreatic tissue was observed at 20 μ g/L, where the enzyme activity decreased to 181.47 $\pm$ 7.26 U/L, equivalent to a 22.4 % $\pm$ 1.8 reduction (p <0.05).

At 30 and 40 μ g/L, the reduction remained significant, reaching 18.2 % $\pm$ 2.1 and 16.9 % $\pm$ 1.9 respectively, while at the highest concentration of 50 μ g/L, the inhibitory effect slightly declined to 12.2 % $\pm$ 1.6 but remained statistically significant (p <0.02), suggesting a U-shaped dose-response profile. In parotid gland homogenates, baseline activity was 9.91 $\pm$ 0.49 U/L. Rutin treatment resulted in a modest, non-significant reduction: 10 μ g/L lowered activity by 10.5 % $\pm$ 1.7, while 20, 30, 40, and 50 μ g/L produced decreases of 14.6 % $\pm$ 2.0, 19.2 % $\pm$ 2.5, 18.2 % $\pm$ 2.3, and 17.9 % $\pm$ 2.1 respectively (p >0.20 for all doses), indicating that salivary α -amylase is less sensitive to rutin under the tested conditions. A more pronounced inhibition was observed in intestinal mucosa. The control group exhibited α -amylase activity of 82.47 $\pm$ 6.73 U/L, whereas exposure to 10 μ g/L rutin reduced it to 70.23 $\pm$ 5.75 U/L (–14.8 % $\pm$ 1.9, p <0.05). Increasing the dose to 20 and 30 μ g/L further suppressed enzyme activity to 65.46 $\pm$ 4.71 U/L (–20.6 % $\pm$ 2.1) and 64.38 $\pm$ 4.45 U/L (–21.9 % $\pm$ 2.0), both highly significant (p <0.01). At 40 μ g/L, the inhibition slightly declined to 19.5 % $\pm$ 2.3, and at 50 μ g/L it remained at 18.7 % $\pm$ 2.2 (p <0.01), confirming that intestinal α -amylase is strongly susceptible to rutin-mediated inhibition (Table 1). In intestinal chyme, baseline α -amylase activity was 6.52 $\pm$ 0.67 U/L. Rutin administration led to mild, non-significant reductions: 10 μ g/L decreased activity by 14.4 % $\pm$ 1.6, 20 μ g/L by 19.2 % $\pm$ 1.8, 30 μ g/L by 17.0 % $\pm$ 1.9, 40 μ g/L by 15.2 % $\pm$ 2.1, and 50 μ g/L by 20.4 % $\pm$ 2.4 (p >0.20 for all doses). Taken together,

these findings indicate that rutin exerts a compartment-specific and dose-dependent inhibitory effect on α -amylase activity, with the most pronounced and statistically significant effects observed in the pancreas and intestinal mucosa. The observed suppression of α -amylase might be attributed to the ability of rutin's hydroxyl groups to interact with the catalytic residues of the enzyme through hydrogen bonding, thereby reducing substrate binding and catalytic turnover. In addition, phenolic compounds like rutin may alter the conformational stability of α -amylase, leading to decreased enzymatic activity. The less pronounced effect in salivary and chyme fractions suggests tissue-specific sensitivity, possibly related to differences in enzyme isoforms and local microenvironmental conditions.

From a physiological perspective, this inhibition of α -amylase activity has important implications for carbohydrate digestion and postprandial glycemic control. By slowing the breakdown of complex carbohydrates in the upper gastrointestinal tract, rutin might attenuate the rate of glucose absorption, reduce postprandial hyperglycemia, and improve overall glycemic homeostasis. Such effects align with the therapeutic potential of dietary flavonoids in managing type 2 diabetes mellitus and metabolic syndrome. Furthermore, the inhibitory properties of rutin on intestinal α -amylase support its role as a functional nutraceutical for modulating digestive enzyme activity and delaying starch hydrolysis, thereby lowering the glycemic index of carbohydrate-rich meals.

Quercetin's Impact on α -Amylase Activity.

Various doses of quercetin demonstrated differing effects on α -amylase enzyme activity under *in vitro* conditions. This analysis revealed that quercetin exerts a clear dose-dependent inhibitory effect on α -amylase activity across different compartments of the digestive system. In pancreatic homogenates, the control group demonstrated baseline enzyme activity of 228.46 $\pm$ 6.73 U/L. Treatment with 10 μ g/L quercetin reduced α -amylase activity to 218.39 $\pm$ 13.11 U/L, corresponding to a modest decrease of 4.4 % $\pm$ 1.3, which was not statistically significant (p >0.50). At 20 μ g/L, pancreatic α -amylase activity dropped more

significantly to 205.13 ± 9.56 U/L, representing a $10.2\% \pm 1.7$ reduction ($p < 0.05$). Increasing the dose to 30 and $40\mu\text{g/L}$ caused stronger inhibition, with enzyme activity decreasing to 195.72 ± 8.34 U/L ($-14.3\% \pm 1.9$, $p < 0.01$) and 198.38 ± 9.59 U/L ($-13.1\% \pm 1.8$, $p < 0.02$), respectively. At the highest concentration of $50\mu\text{g/L}$, pancreatic enzyme activity partially recovered to 201.76 ± 16.55 U/L ($-11.7\% \pm 2.0$, $p > 0.20$), suggesting a biphasic response. In the parotid gland homogenate, baseline activity was 10.13 ± 0.53 U/L. Quercetin at $10\mu\text{g/L}$ reduced salivary α -amylase by $12.9\% \pm 1.6$, at $20\mu\text{g/L}$ by $16.5\% \pm 1.9$, at $30\mu\text{g/L}$ by $19.8\% \pm 2.1$, and at $40\mu\text{g/L}$ by $25.4\% \pm 2.3$ ($p < 0.01$). At $50\mu\text{g/L}$, the inhibition decreased to $14.4\% \pm 2.0$ but remained statistically significant ($p < 0.02$), indicating that quercetin affects salivary α -amylase more effectively than pancreatic isoforms within the tested range. A more robust inhibitory effect was seen in the intestinal mucosa homogenate. The control group showed α -amylase activity of 81.35 ± 5.44 U/L. Upon quercetin treatment, enzyme activity was reduced to 69.56 ± 4.49 U/L at $10\mu\text{g/L}$ ($-11.5\% \pm 1.7$, $p < 0.05$), 63.26 ± 5.75 U/L at $20\mu\text{g/L}$ ($-19.5\% \pm 2.0$, $p < 0.01$), 65.07 ± 5.12 U/L at $30\mu\text{g/L}$ ($-22.9\% \pm 2.3$, $p < 0.01$), 63.46 ± 4.64 U/L at $40\mu\text{g/L}$ ($-19.2\% \pm 2.1$, $p < 0.05$), and 68.52 ± 3.61 U/L at $50\mu\text{g/L}$ ($-12.8\% \pm 1.9$, $p > 0.10$). These findings highlight that intestinal mucosa α -amylase is particularly sensitive to quercetin, with peak inhibition achieved at intermediate concentrations. In intestinal chyme, the baseline enzyme activity was 7.28 ± 0.54 U/L. Quercetin administration at 10, 20, 30, 40, and $50\mu\text{g/L}$ suppressed α -amylase activity to 6.36 ± 0.51 U/L ($-12.6\% \pm 1.4$, $p > 0.20$), 6.31 ± 0.15 U/L ($-13.3\% \pm 1.6$, $p > 0.20$), 6.11 ± 0.46 U/L ($-16.1\% \pm 1.8$, $p < 0.10$), 6.03 ± 0.62 U/L ($-17.2\% \pm 1.9$, $p < 0.10$), and 6.09 ± 0.12 U/L ($-16.4\% \pm 1.8$, $p < 0.05$), showing a moderate but consistent inhibition (Table 2).

Collectively, these results demonstrate that quercetin suppresses α -amylase activity in a dose- and tissue-dependent manner, with the intestinal mucosa and parotid gland showing the strongest responses. The mechanism of inhibition is likely related to quercetin's phenolic hydroxyl groups, which can interact with the catalytic residues of α -amylase through hydrogen bonding and π - π stacking, thereby blocking substrate access to the active site. Additionally, quercetin may induce conformational changes that destabilize the enzyme structure, further reducing its catalytic efficiency. From a physiological perspective, quercetin-mediated α -amylase inhibition delays starch hydrolysis,

thereby reducing the postprandial rise in blood glucose. This supports the potential of quercetin-rich diets or supplements as nutraceutical agents for glycemic control in type 2 diabetes and obesity.

When directly compared with rutin, quercetin displayed slightly stronger inhibitory effects in the intestinal mucosa and salivary glands, while rutin showed more pronounced suppression in pancreatic homogenates. Both flavonoids exhibited U-shaped dose-response patterns, with maximal inhibition observed at intermediate concentrations (20 – $30\mu\text{g/L}$). These differences may stem from subtle structural variations in their hydroxylation and glycosylation patterns, which influence their binding affinity to different α -amylase isoforms. Together, rutin and quercetin represent complementary dietary flavonoids that can modulate carbohydrate digestion and helps to manage postprandial glycemia through multiple enzymatic targets.

The Effect of Thamiflaside on α -Amylase Activity:

Thamiflaside demonstrated a clear dose-dependent stimulatory effect on α -amylase activity across all digestive tissues. In pancreatic homogenates, the most prominent activation occurred at $20\mu\text{g/L}$ ($+16.4\% \pm 2.1$), followed by $10\mu\text{g/L}$ ($+13.5\% \pm 1.8$) and $30\mu\text{g/L}$ ($+14.4\% \pm 2.0$). At higher concentrations of 40 and $50\mu\text{g/L}$, the stimulation declined but remained above baseline ($+8.8\% \pm 1.6$ and $+7.0\% \pm 1.5$, respectively), indicating a bell-shaped response profile. In parotid gland homogenates, the relative increase was stronger, with $+20.6\% \pm 2.3$ at $10\mu\text{g/L}$, peaking at $+30.3\% \pm 2.5$ at $30\mu\text{g/L}$, followed by moderate reductions at $40\mu\text{g/L}$ ($+17.5\% \pm 2.0$) and $50\mu\text{g/L}$ ($+21.0\% \pm 2.1$). Intestinal mucosa homogenates displayed a progressive stimulation, reaching $+21.7\% \pm 2.4$ at $30\mu\text{g/L}$, with lower but significant effects at $10\mu\text{g/L}$ ($+8.1\% \pm 1.9$) and $20\mu\text{g/L}$ ($+12.7\% \pm 2.1$). At 40 and $50\mu\text{g/L}$, activity remained elevated ($+16.6\% \pm 2.2$ and $+15.0\% \pm 1.8$, respectively). Intestinal chyme homogenates exhibited a milder but consistent stimulation, ranging from $+17.4\% \pm 1.7$ at $10\mu\text{g/L}$ to $+5.3\% \pm 1.5$ at $50\mu\text{g/L}$, suggesting a gradual decline in stimulation with higher concentrations (Table 3). Collectively, thamiflaside promoted α -amylase activity most effectively at low-to-moderate concentrations (20 – $30\mu\text{g/L}$), with the strongest effects observed in parotid glands and intestinal mucosa, while chyme homogenates showed a weaker response.

Table 2: Quercetin's *In Vitro* Impact on the Digestive System's α -Amylase Activity. (mean $\pm$ s.d., n = 6)

	Control	Quercetin doses				
		10 μ g/L	20 μ g/L	30 μ g/L	40 μ g/L	50 μ g/L
Pancreas U/L P	228.46 $\pm$ 6.73	218.39 $\pm$ 13.11 >0.50	205.13 $\pm$ 9.56 <0.05	195.72 $\pm$ 8.34 <0.01	198.38 $\pm$ 9.59 <0.02	201.76 $\pm$ 16.55 >0.20
Parotid gland U/L P	10.13 $\pm$ 0.53	8.82 $\pm$ 0.78 >0.20	8.46 $\pm$ 0.66 >0.05	8.12 $\pm$ 0.43 >0.01	7.56 $\pm$ 1.13 <0.01	8.67 $\pm$ 0.73 <0.02
Intestinal mucose U/L P	81.35 $\pm$ 5.04	69.56 $\pm$ 4.49 >0.20	63.26 $\pm$ 5.75 <0.05	60.56 $\pm$ 7.12 >0.05	63.46 $\pm$ 4.64 >0.05	68.52 $\pm$ 3.61 >0.10
Chymus U/L P	7.28 $\pm$ 0.54	6.36 $\pm$ 0.51 >0.20	6.31 $\pm$ 0.15 >0.10	6.11 $\pm$ 0.46 >0.10	6.03 $\pm$ 0.62 <0.10	6.09 $\pm$ 0.12 <0.05

Table 3: The *In Vitro* Effect of Thamiflaside on α -Amylase Activity in the Digestive System (mean $\pm$ s.d., n = 6)

	Control	Thamiflaside doses				
		10 μ g/L	20 μ g/L	30 μ g/L	40 μ g/L	50 μ g/L
Pancreas U/L P	225.86 $\pm$ 9.09	256.23 $\pm$ 13.39 >0.10	262.83 $\pm$ 11.65 >0.02	258.36 $\pm$ 14.37 <0.10	245.66 $\pm$ 11.61 <0.20	241.76 $\pm$ 8.76 <0.20
Parotid gland U/L P	11.47 $\pm$ 0.19	13.83 $\pm$ 0.66 >0.20	14.38 $\pm$ 0.87 >0.05	14.94 $\pm$ 1.67 >0.01	13.48 $\pm$ 1.64 <0.01	13.88 $\pm$ 1.97 <0.02
Intestinal mucose U/L P	81.79 $\pm$ 4.51	88.38 $\pm$ 6.16 >0.20	92.17 $\pm$ 5.49 <0.05	99.56 $\pm$ 7.12 >0.05	95.34 $\pm$ 3.73 >0.05	94.1 $\pm$ 3.43 >0.10
Chymus U/L P	9.14 $\pm$ 0.83	10.73 $\pm$ 1.13 >0.20	10.31 $\pm$ 1.02 >0.10	10.11 $\pm$ 0.98 >0.10	9.91 $\pm$ 0.52 <0.10	9.62 $\pm$ 0.57 <0.05

The stimulatory mechanism of thamiflaside on α -amylase likely involves multiple molecular interactions distinct from the rutin and quercetin. Thamiflaside is a flavonoid diglycoside containing multiple hydroxyl and methoxyl groups, which may interact allosterically with the enzyme, enhancing its conformational flexibility and substrate affinity rather than blocking the catalytic site. By stabilizing the active conformation of α -amylase, thamiflaside may increase the turnover rate of starch hydrolysis. Additionally, its glycosylated structure can mimic carbohydrate substrates and induce a positive conformational shift in the enzyme, improving catalytic efficiency. It is also possible that thamiflaside modulates the microenvironment of the enzyme, such as maintaining essential calcium ion binding or optimizing hydrophobic interactions required for catalytic stability. This stimulatory profile contrasts with rutin and quercetin, which inhibit α -amylase by engaging the active site and forming hydrogen bonds that restrict substrate access. Thus, thamiflaside acts as a positive modulator, enhancing enzyme activity in tissues where carbohydrate digestion requires augmentation. Thamiflaside demonstrated a clear dose-dependent stimulatory effect on α -amylase activity across all digestive tissues, with maximal activation observed at 20–30 μ g/L. This enhancement was most pronounced in the parotid gland and intestinal mucosa, whereas chyme homogenates exhibited a milder response. Such a stimulatory profile suggests that thamiflaside acts as a pro-digestive flavonoid with distinct molecular mechanisms compared to the inhibitory effects of rutin and quercetin. From a mechanistic perspective, thamiflaside, a diglycosylated flavonoid with multiple hydroxyl and methoxyl groups, likely interacts with secondary allosteric sites on the α -amylase surface

through hydrogen bonding and π - π stacking, thereby stabilizing the enzyme's active conformation and increasing its substrate affinity. Additionally, thamiflaside may enhance the binding of essential Ca^{2+} and Cl^- ions required for α -amylase catalytic stability. This allosteric modulation contrasts with rutin and quercetin, which inhibit the enzyme by occupying the catalytic site and restricting substrate access. At the tissue level, thamiflaside may stimulate α -amylase synthesis in pancreatic acinar cells, enhance secretion from serous cells of the parotid gland, and upregulate digestive enzyme activity in the intestinal mucosa. This effect may involve modulation of cAMP/PKA signaling or Ca^{2+} -dependent pathways within secretory cells, as flavonoids are known to interact with GPCRs and ion channels. By enhancing enzymatic turnover, thamiflaside promotes more efficient hydrolysis of complex polysaccharides, facilitating carbohydrate digestion. Clinically, α -amylase stimulation may be beneficial in conditions associated with reduced digestive enzyme secretion. Thamiflaside could have therapeutic potential in exocrine pancreatic insufficiency (e.g., chronic pancreatitis, cystic fibrosis), malabsorption syndromes, and postoperative digestive enzyme deficiency (e.g., after gastric or intestinal resections). Moreover, its stimulation of parotid α -amylase suggests potential usefulness in conditions with impaired salivary secretion, such as xerostomia, where the initial phase of starch digestion is compromised. At the receptor and signaling level, thamiflaside may modulate NF- κ B and MAPK pathways, enhancing transcriptional regulation of digestive enzyme synthesis. Additionally, its glycosylated moiety may mimic carbohydrate substrates, inducing conformational shifts that facilitate the transition of α -amylase from a semi-active to a fully

active state, thereby accelerating enzyme–substrate complex formation and catalytic turnover.

In summary, thamiflaside represents a unique bioactive flavonoid capable of enhancing α -amylase activity and improving carbohydrate digestion. Unlike rutin and quercetin, which act as natural α -amylase inhibitors for glycemic control, thamiflaside could serve as a functional pro-digestive agent, supporting carbohydrate metabolism in conditions of enzyme deficiency or malabsorption. These findings provide a scientific basis for further investigation of thamiflaside as a therapeutic or nutraceutical compound for digestive support.

The Effect of Luteolin on α -Amylase Activity:

Luteolin's *in vitro* impact on α -amylase activity demonstrates a clear dose-dependent inhibitory effect across all studied digestive compartments (pancreas, parotid gland, intestinal mucosa, and chyme).

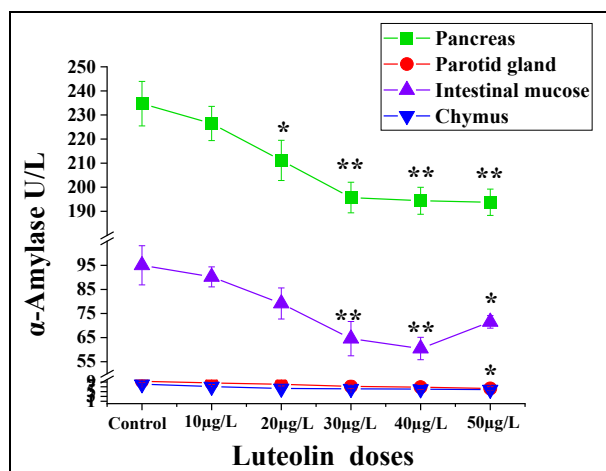


Figure 6. The *In Vitro* Effect of Luteolin on α -Amylase Activity in the Digestive System (mean $\pm$ s.d., $n = 6$)

Compared to control values, α -amylase activity in pancreatic homogenates decreased progressively: at 10 μ g/L by 3.5% $\pm$ 1.4, at 20 μ g/L by 10.1% $\pm$ 2.0, at 30 μ g/L by 16.6% $\pm$ 2.1, at 40 μ g/L by 17.2% $\pm$ 1.9, and at 50 μ g/L by 17.5% $\pm$ 1.8. In the parotid gland, luteolin reduced enzyme activity by 7.7% $\pm$ 1.3 at 10 μ g/L, 13.6% $\pm$ 1.5 at 20 μ g/L, 22.7% $\pm$ 1.7 at 30 μ g/L, 26.5% $\pm$ 2.0 at 40 μ g/L, and 32.0% $\pm$ 2.2 at 50 μ g/L, showing a more pronounced inhibition in salivary amylase compared to the pancreas.

In the intestinal mucosa, α -amylase activity declined by 5.1% $\pm$ 1.4 at 10 μ g/L, 16.7% $\pm$ 2.1 at 20 μ g/L, 32.1% $\pm$ 2.4 at 30 μ g/L, 36.3% $\pm$ 2.5 at 40 μ g/L, and 24.7% $\pm$ 2.3 at 50 μ g/L, indicating a biphasic response with peak inhibition at 40 μ g/L. In chyme homogenates, luteolin reduced α -amylase activity by 12.6% $\pm$ 1.5 at 10 μ g/L, 22.4% $\pm$ 1.7 at 20 μ g/L, 23.9% $\pm$ 1.8 at 30 μ g/L, 25.7%

$\pm$ 2.0 at 40 μ g/L, and 28.4% $\pm$ 2.1 at 50 μ g/L, demonstrating a cumulative inhibitory trend (figure 6).

Mechanistically, luteolin likely inhibits α -amylase by forming hydrogen bonds between its hydroxyl groups (especially at C-3', C-4') and the catalytic residues of the enzyme (Asp-197, Glu-233, Asp-300), thus blocking substrate access. Additionally, π - π stacking interactions between luteolin's planar flavone structure and aromatic residues in the enzyme's active site (like Trp-58 and Tyr-62) may further stabilize the inhibitor-enzyme complex, reducing catalytic efficiency. Luteolin's capacity to induce conformational shifts and destabilize α -amylase's active conformation could also contribute to the observed suppression. Moreover, luteolin's anti-oxidant property may reduce oxidative activation of digestive enzymes, adding another layer of enzymatic regulation. The tissue-specific differences observed suggest that luteolin's inhibitory effects are more potent in the parotid gland and intestinal mucosa, potentially due to differences in enzyme isoforms or microenvironmental factors such as pH and ionic strength that affect inhibitor binding affinity.

In conclusion, luteolin demonstrates potent α -amylase inhibitory effects in a dose-dependent manner, with notable tissue-specific sensitivity. These findings highlight luteolin's potential in managing conditions associated with excessive carbohydrate hydrolysis and postprandial hyperglycemia, particularly in type 2 diabetes mellitus and metabolic syndrome. Additionally, luteolin's regulatory effect on digestive enzymes could be beneficial in therapeutic diets aimed at modulating glycemic index and managing obesity. Furthermore, its anti-oxidant and anti-inflammatory properties broaden its therapeutic relevance in diseases where oxidative stress and digestive inefficiencies coexist, such as pancreatitis and irritable bowel syndrome.

The Effect of Chrysosplenetin on α -Amylase Activity:

In vitro analysis revealed that chrysosplenetin exerts a significant dose-dependent inhibitory effect on α -amylase activity across all digestive tissues evaluated (pancreas, parotid gland, intestinal mucosa, and chyme). In pancreatic homogenates, enzymatic activity decreased progressively from 226.75 $\pm$ 8.14 U/L (Control) to 203.76 $\pm$ 5.45 U/L at 50 μ g/L, reflecting a 10.14% $\pm$ 2.40% reduction ($p = 0.00018$). The inhibitory effect became statistically significant from 30 μ g/L ($p = 0.011$), indicating a dose-threshold effect.

In the parotid gland, α -amylase activity declined from 11.47 $\pm$ 0.53 U/L in the control to 7.31 $\pm$ 0.73 U/L at 50 μ g/L (–36.27% $\pm$ 6.36%, $p < 0.000001$). The reduction was significant even at the lowest tested concentration of 10 μ g/L ($p = 0.0039$), suggesting high

sensitivity of salivary α -amylase isoforms to chrysosplenetin.

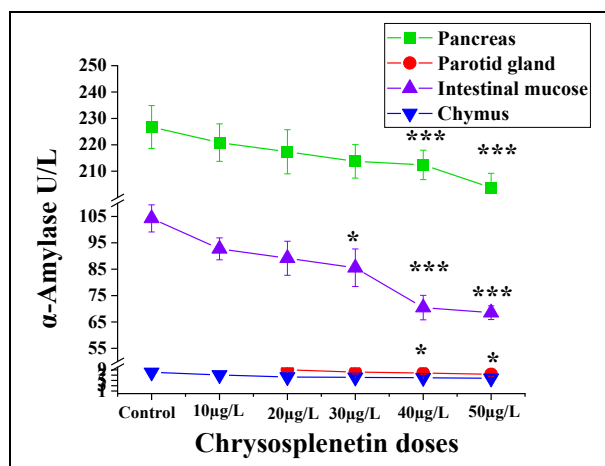


Figure 7. The *In Vitro* Effect of Chrysosplenetin on α -Amylase Activity in the Digestive System (mean $\pm$ s.d., n = 6)

The intestinal mucosa exhibited a pronounced inhibitory response, with enzyme activity decreasing from 104.27 ± 8.14 U/L (Control) to 68.52 ± 2.61 U/L ($-34.28\% \pm 2.50\%$, $p = 0.000001$) at $50\mu\text{g/L}$. Statistically significant inhibition was observed from $10\mu\text{g/L}$ ($p = 0.011$), intensifying with increasing concentrations.

Similarly, chyme α -amylase activity was reduced from 8.1 ± 0.36 U/L (Control) to 5.81 ± 0.12 U/L at $50\mu\text{g/L}$, corresponding to a $28.36\% \pm 1.47\%$ decline ($p = 0.0000004$). Significant inhibition was recorded from $10\mu\text{g/L}$ ($p = 0.0012$), highlighting chyme as a sensitive compartment for chrysosplenetin-mediated α -amylase inhibition (figure 7).

The present study demonstrates that chrysosplenetin, an O-methylated flavonol, significantly inhibits α -amylase activity in a dose- and tissue-dependent manner. The pancreatic, salivary (parotid), intestinal mucosal, and chyme homogenates all showed progressive declines in enzymatic activity, with the parotid gland exhibiting the highest sensitivity. Chrysosplenetin likely exerts its inhibitory effect by interacting with catalytic residues in the α -amylase active site. Its hydroxyl and methoxy functional groups can form hydrogen bonds and π - π stacking interactions with key amino acids (Asp-197, Glu-233, Asp-300), thereby obstructing substrate binding. Additionally, chrysosplenetin may induce conformational alterations that destabilize the enzyme's active conformation, reducing catalytic efficiency. Given the inhibitory magnitude, chrysosplenetin exhibits potential as a natural α -amylase inhibitor, akin to known flavonoids such as quercetin and rutin. Its capacity to modulate digestive enzyme activity positions it as a candidate for managing postprandial hyperglycemia and metabolic disorders where delayed carbohydrate digestion is therapeutically beneficial.

Future *in vivo* studies and clinical trials are warranted to validate these *in vitro* findings and to explore chrysosplenetin's therapeutic potential as a nutraceutical or adjunct pharmacological agent in metabolic disorders.

DISCUSSION:

Our findings provide compelling evidence that selected flavonoids, particularly rutin, quercetin, luteolin, chrysosplenetin, and thamiflaside, exert significant modulatory effects on α -amylase activity across distinct compartments of the digestive system, in a dose- and tissue-dependent manner. These findings align with previous research indicating the inhibitory potential of flavonoids on digestive enzymes, notably through mixed-type of inhibition mechanisms (Proença et al., 2019; Kashtoh & Baek 2023). Rutin and quercetin demonstrated potent inhibition of α -amylase activity, with significant reductions observed across pancreatic, parotid, intestinal mucosal, and chyme homogenates. Luteolin exhibited even stronger inhibitory effects, corroborating findings by Janeček et al. (2014), which emphasized the influence of hydroxylation patterns on enzyme binding affinity. Chrysosplenetin, an O-methylated flavonol, showed a dose-dependent inhibition profile, with maximum sensitivity observed in the parotid gland and intestinal mucosa at lower concentrations ($10\mu\text{g/L}$). Mechanistically, these flavonoids likely exert their effects via hydrogen bonding and π - π stacking interactions with the catalytic residues of α -amylase (Asp-197, Glu-233, Asp-300), leading to conformational changes that impede substrate binding and catalytic efficiency (Henrissat, 1991; Kashtoh & Baek, 2023). The observed dose-threshold effects, particularly for pancreatic inhibition, suggest a binding saturation dynamic inherent to flavonoid-enzyme interactions (Dubey et al., 2017). In contrast, thamiflaside exhibited a unique stimulatory effect on α -amylase activity, particularly in the parotid gland and intestinal mucosa. This suggests potential allosteric activation mechanisms distinct from the inhibitory pathways of other flavonoids. Such a profile positions thamiflaside as a strong candidate for therapeutic interventions in digestive enzyme insufficiency disorders, including exocrine pancreatic insufficiency and malabsorption syndromes. The isoform-specific interactions of these flavonoids underscore the importance of structural-functional relationships, where the number and position of hydroxyl and methoxyl groups dictate the modulatory capacity on α -amylase activity. This aligns with broader pharmacological evidence highlighting the role of flavonoid structural motifs in enzyme specificity (Lebelo et al., 2021). From a pharmacokinetic perspective, these flavonoids, particularly chrysosplenetin and quercetin, exhibit moderate bioavailability with extensive hepatic metabolism and biliary excretion, supporting their

primary activity within the gastrointestinal tract. Moreover, their ability to modulate drug efflux transporters (P-gp) and CYP3A4 enzymes (Chen et al., 2022) suggests a potential role in enhancing the bioavailability of co-administered drugs.

CONCLUSION:

This study elucidates the modulatory effects of selected flavonoids on α -amylase activity *in vitro*, revealing distinct inhibitory and stimulatory profiles that are dependent on compound structure and tissue specificity. Rutin, quercetin, luteolin, and chrysosplenetin exhibited potent α -amylase inhibitory activities, with the parotid gland and intestinal mucosa displaying the highest sensitivity. Conversely, thamiplaside demonstrated a stimulatory effect, indicating its potential utility in enhancing carbohydrate digestion in conditions of enzyme insufficiency.

The differential modulatory profiles observed highlight the critical role of flavonoid structural characteristics, such as hydroxylation and methoxylation patterns, in determining their binding affinity and functional effects on digestive enzymes. These insights paves the way for the strategic incorporation of such bioactive compounds into functional foods and nutraceutical formulations aimed at managing metabolic disorders.

Furthermore, *in vivo* studies and clinical trials are essential to validate these findings, elucidate pharmacokinetic behaviors, and establish optimal dosing regimens to ensure the safe and effective therapeutic applications of these flavonoids.

CONFLICT OF INTEREST:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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