scientific reports

Check for updates

OPEN

Effects of incorporating green leafy vegetables with meals on starch and lipid digestibility under simulated gastrointestinal digestion

Thanaporn Kaewpradup[1], Kritmongkhon Kamonsuwan[1], Charoonsri Chusak[1], Mario Siervo[2,3,4] & Sirichai Adisakwattana[1]✉

Green leafy vegetables (GLV) are known for their cardiovascular health benefits. However, the effects of their serving size on delaying carbohydrate and lipid digestion remain unclear. This study investigated the impact of varying MyPlate-recommended GLV serving sizes on the digestibility of carbohydrates and lipids and antioxidant activity during in vitro gastrointestinal digestion. Eight GLV including *Asteraceae* (cos, green oak, red oak, loose-leaf) and *Brassicaceae* (cabbage, cauliflower, broccoli, Chinese cabbage) vegetables were incorporated into mixed meals at 0.5, 1.0, and 1.5 times the MyPlate recommendation. The results showed that the total phenolic content (TPC) ranged from 5.77 to 9.46 mg GAE/g extract. Nitrate accumulation exhibited a higher content in *Asteraceae* (590.90-1155.04 mg NO_3-NE/g extract) than in *Brassicaceae* families (244.96–726.20 mg NO_3-NE/g extract). Incorporating ≥ 1 serving of all GLV significantly decreased rapidly and slowly digestible starch fractions, while undigestible starch significantly increased, resulting in delaying glucose release. Antioxidant activity was significantly enhanced with ≥ 1 serving and free fatty acid concentrations decreased with higher vegetable servings. Post-digestion nitrate concentrations ranged from 127.3 to 188.5 µg NO_3-N/mL, positively correlating with GLV serving size. These effects were dose-dependent and varied across species. These findings suggest that incorporating GLV at or above the MyPlate recommendation may have protective effects on cardiovascular health.

Keywords Green leafy vegetables, Recommendation, Digestion, Carbohydrate digestibility, Lipid digestibility

Abbreviations

GLV	Green Leafy Vegetables
TPC	Total Phenolic Content
AUC	Area Under the Curve
RDS	Rapidly Digestible Starch
SDS	Slowly Digestible Starch
US	Undigestible Starch
FFA	Free Fatty Acids
NCDs	Non-Communicable Diseases
NO	Nitric Oxide

[1]Phytochemical and Functional Food Research Unit for Clinical Nutrition, Department of Nutrition and Dietetics, Faculty of Allied Health Science, Chulalongkorn University, Bangkok 10330, Thailand. [2]Faculty of Health Sciences, School of Population Health, Curtin University, Perth, WA 6102, Australia. [3]Curtin Dementia Centre of Excellence, Enable Institute, Curtin University, Perth, WA 6102, Australia. [4]Vascular and Metabolic Disorders Group, Curtin Health Innovation Research Institute (CHIRI), Curtin University, Perth, WA 6102, Australia. ✉email: sirichai.a@chula.ac.th

Cardiovascular diseases (CVDs) are the most prevalent non-communicable diseases leading to causes of death and disability worldwide, representing a significant global health challenge as they account for 20.5 million global deaths[1]. Excessive consumption of diets, including fried foods, added fats, organ meats, processed meats, and sugar-sweetened beverages has been associated with impaired vascular function, mediated through hypercholesterolemia, hypertension, and diabetes[2]. Lifestyle interventions focused on reducing blood glucose, lipid, and blood pressure have demonstrated efficacy in mitigating the risk of developing cardiovascular disease risk factors[3].

In recent years, the World Health Organization (WHO) has recommended a daily intake of at least 400 g of fruits and vegetables to promote overall health[4]. Numerous studies have consistently highlighted that increased fruit and vegetable consumption correlates with reduced inflammation and lower susceptibility to chronic diseases, including diabetes, coronary heart disease, stroke, cardiovascular diseases, cancer, and all-cause mortality[5]. Research shows that consuming up to 800 g of fruits and vegetables daily can reduce the incidence of coronary heart disease by 20% and cardiovascular mortality by 28%, demonstrating the importance of meeting dietary guidelines[6]. Additionally, plant-based diets such as the Mediterranean diet and the DASH (Dietary Approaches to Stop Hypertension) diet, which promote the consumption of fruits, nuts, vegetables, legumes, and lean or low-fat animal proteins, have demonstrated a positive association with reduced CVD mortality[7,8].

Green leafy vegetables (GLV), primarily from the *Asteraceae* and *Brassicaceae* families, such as lettuce, cabbage, and broccoli, are staple foods in European and Asian diets, featuring prominently in diverse local cuisines[9]. They can be consumed fresh in salads, blended into smoothies, or lightly cooked in various dishes, offering substantial nutritional benefits[10]. GLV is not only a source of fiber but also rich in bioactive compounds, such as phenolics, nitrates, and phytosterols[11]. Studies have linked GLV consumption with reduced cardiovascular risk factors, such as decreased oxidative stress, regulated postprandial blood glucose, and modulated lipid metabolism[12]. Furthermore, dietary fiber in GLV inhibits carbohydrate-digesting enzyme activity and slows glucose absorption, leading to lower postprandial glucose levels[13,14]. GLV also contains phytosterols, plant-derived compounds structurally similar to cholesterol, that can lower blood lipids in rats and humans by competing with cholesterol for absorption[11,15,16]. Moreover, GLV is rich in nitrates, which can be converted to nitric oxide via the non-enzymatic synthetic pathway[17], promoting vasodilation, improving endothelial function, and lowering blood pressure[18,19], thereby reducing cardiovascular risks. Despite these known benefits, GLV intake is often insufficient globally, with 75% of countries reporting inadequate consumption levels[20].

The USDA's MyPlate guideline provides a practical tool promoting healthy eating by recommending a 2:1:1 plate ratio, with half allocated to fruits and vegetables and half to grains and proteins. Previous studies examining dietary patterns that promote GLV intake including the DASH diet, the Mediterranean diet, and plant-based diets show cardiovascular benefits but lack detailed insights into GLV serving size-dependent health effects[8,21]. MyPlate's portion guidance provides practical, quantifiable vegetable serving recommendations that can be readily implemented in daily meals. While this model encourages vegetable consumption, many struggle to meet the recommended amounts. Furthermore, the guideline lacks detailed insights into the physiological impacts or specific health benefits of recommended portions. Additionally, it provides limited insights into how specific GLV servings impact carbohydrate and lipid digestion. While in vitro gastrointestinal digestion models offer valuable insights into food component behavior under simulated physiological conditions[22], data on GLV with complex meals remains limited. This study addresses this gap through in vitro experiments, evaluating how different types and quantities of GLV influence these digestive processes in alignment with the MyPlate pattern.

Results and discussion

Nitrate, phenolics, and antioxidant activity in green leafy vegetables

The nitrate accumulation represented in Table 1 revealed that loose-leaf lettuce accumulated the highest nitrate levels among the varieties tested, with significantly decreasing levels observed in cos, red oak, and green oak lettuces, respectively. These findings align with previous studies establishing that lettuces belonging to the *Asteraceae* family are well-known as high nitrate-accumulating vegetables[23]. In contrast, vegetables from the

Type	TPC mg GAE/g extract	FRAP mmol $FeSO_4E$/g extract	Nitrate mg NO_3-NE/g extract
Asteraceae vegetables			
Cos	6.21 ± 0.13^{c}	82.85 ± 0.46^{c}	1155.04 ± 2.17^{d}
Green oak	6.63 ± 0.01^{b}	85.15 ± 0.99^{c}	590.90 ± 4.67^{c}
Red oak	9.21 ± 0.08^{a}	208.79 ± 0.78^{b}	804.30 ± 3.95^{b}
Loose leaf	9.46 ± 0.05^{a}	240.32 ± 0.84^{a}	1510.45 ± 5.03^{a}
Brassicaceae vegetables			
Cabbage	5.77 ± 0.05^{d}	64.90 ± 0.75^{c}	572.75 ± 1.26^{b}
Cauliflower	7.76 ± 0.07^{b}	100.48 ± 0.94^{b}	538.88 ± 3.85^{c}
Broccoli	8.39 ± 0.01^{a}	134.25 ± 0.67^{a}	244.96 ± 4.12^{d}
Chinese cabbage	6.47 ± 0.09^{c}	102.70 ± 0.75^{b}	726.20 ± 3.65^{a}

Table 1. Nitrate content, total phenolic content, and antioxidant activity of green leafy vegetables. Data are presented as mean ± S.E.M. ($n = 3$). Values with different superscript letters indicate significant differences ($p < 0.05$).

Brassicaceae family, recognized for their cruciferous properties, typically exhibit lower nitrate levels. However, Chinese cabbage displayed a significantly higher nitrate content than other *Brassicaceae* vegetables tested. This observation aligns with previous studies indicating that Chinese cabbage can accumulate higher nitrate levels than other cruciferous vegetables, attributable to variations in nitrate accumulation across different plant tissues[24,25].

The TPC analysis revealed distinct patterns between the *Asteraceae* and *Brassicaceae* families. For the *Asteraceae* group, TPC values ranged from 6.21 to 9.46 mg GAE/g extract, indicating a higher concentration of phenolic compounds relative to the *Brassicaceae* group, which exhibited TPC values ranging from 5.77 to 8.39 mg GAE/g extract. Within the *Asteraceae* family, lettuce varieties, particularly loose-leaf and red oak lettuces, demonstrated significantly elevated TPC levels[26]. This phenomenon is likely attributed to the higher abundance of predominant phenolic acids found in the tissues of Lollo rosso and red lettuce compared to green lettuce varieties, as supported by previous studies[27,28]. Among the *Brassicaceae* vegetables, broccoli showed the highest TPC[27,28], which can be attributed to its rich diversity of flavonoid compounds and their derivatives, significantly contributing to its phenolic content[29].

FRAP analysis indicated significant variation in antioxidant activity among *Asteraceae* (82.85–240.32 mmol $FeSO_4E$/g extract) and *Brassicaceae* vegetables (64.90–134.25 mmol $FeSO_4E$/g extract). Notably, loose-leaf lettuce and broccoli stood out within their respective families, exhibiting the highest FRAP values. These findings suggest a potential correlation between the elevated TPC observed in our study and the enhanced FRAP values exhibited by these vegetables. This highlights the importance of cultivar selection within vegetable groups, as it can significantly influence their overall antioxidant potential[26].

In vitro starch digestibility and starch fraction

Figure 1 presents a photograph of a mixed meal illustrating various vegetable serving sizes. In vitro digestion mimicking the conditions of the small intestine (Fig. 2A-B), demonstrated a rapid rise in glucose release from all vegetables within 20 min, with a minimal further increase at 30 min. Interestingly, the incorporation of varying amounts (0.5, 1.0, or 1.5 servings) of lettuce varieties (cos, green oak, red oak, and loose-leaf) or *Brassicaceae* vegetables (cabbage, cauliflower, broccoli, and Chinese cabbage) into the meal significantly reduced glucose release compared to the meal alone. This effect was found to be dose-dependent, with the most substantial reduction in glucose release observed with the addition of 1.5 servings of vegetables in both groups. The AUC data presented in Table 2 supports this finding, revealing that for the *Asteraceae* group (lettuce varieties), the AUC for glucose release ranged from 56.7 to 83.3 mg glucose×min/g sample. Similarly, for the *Brassicaceae* group, the AUC ranged from 61.7 to 76.3 mg glucose×min/g sample, indicating lower overall glucose release compared to the meal alone, with greater vegetable amounts resulting in significantly lower glucose levels.

The observed reduction in glucose release can be attributed to the increased fiber content from added vegetables. Dietary fiber can slow digestive processes by enhancing meal viscosity, resulting in a slower rate of gastric emptying[30]. Furthermore, dietary fibers can directly bind to α-amylase, thereby hindering the breakdown of carbohydrates in the digestive tract[31]. Previous research has shown that lactucaxanthin, a key carotenoid in lettuce, significantly inhibits α-amylase and α-glucosidase activity[32]. Collectively, these non-competitive inhibitions contribute to delayed glucose release observed in our study. The dose-dependent decrease in glucose release aligns with previous findings that higher vegetable intake significantly greater reduction in glycemic response[33].

The starch fraction analysis presented in Table 2 demonstrates that increased vegetable incorporation resulted in reduced starch fractions, corresponding with the observed decrease in glucose release. Compared to the control meal, incorporating ≥ 1 serving of any *Asteraceae* vegetable (lettuce varieties) significantly decreased the fractions of RDS and SDS. This trend was consistent across all vegetables except for a single serving of green oak and loose-leaf lettuce, suggesting potential cultivar-specific variations in starch composition. Conversely, there was a notable increase in the US fraction with ≥ 1 serving additions. Similar patterns were observed for the *Brassicaceae* group (cabbage, cauliflower, broccoli, and Chinese cabbage), where both 1.0 and 1.5 serving additions led to significant reductions in RDS and SDS fractions compared to the control, accompanied by an increase in the US. Interestingly, no significant difference was noted with a 0.5 serving addition in either group, indicating a dose-dependent effect of vegetable incorporation on starch digestibility.

The higher US content associated with vegetable consumption likely contributes to the observed lower glucose release. Dietary fiber in vegetables interacts with starch granules through mechanisms like enlargement, overlapping, crystallinity stabilization, and coating effects[23]. These interactions hinder starch gelatinization, a crucial step in enzymatic breakdown[25]. Moreover, certain vegetables, particularly those in the *Brassicaceae* family, contain α-amylase inhibitors that further inhibit starch breakdown in the small intestine, resulting in lower starch digestibility by promoting the escape of undigested starch from intestinal digestion[34]. For instance, phenolic compounds and proteins presented in vegetables can physically block α-amylase and other digestive enzymes, thereby reducing the overall rate of starch digestion[35]. The observed increase in phenolic content with vegetable consumption suggests a potential synergistic effect between dietary fiber and phenolics in restricting enzyme access to starch molecules[36,37].

In vitro total phenolic content and antioxidant activity

As presented in Supplementary Fig. S1, the TPC values during the intestinal phase of in vitro digestion remained stable in both *Asteraceae* and *Brassicaceae* vegetables. The incorporation of all amounts (0.5, 1.0, and 1.5 servings) of vegetables into the meal a slight increase in TPC compared to the meal alone. A dose-response relationship was observed, with the highest TPC occurring at 1.5 servings of vegetable incorporation across all varieties in both families. The AUC of the TPC values for all vegetables, shown in Supplementary Fig. S3, further supports the increased total phenolic content during simulated digestion when incorporating vegetables into the meal even

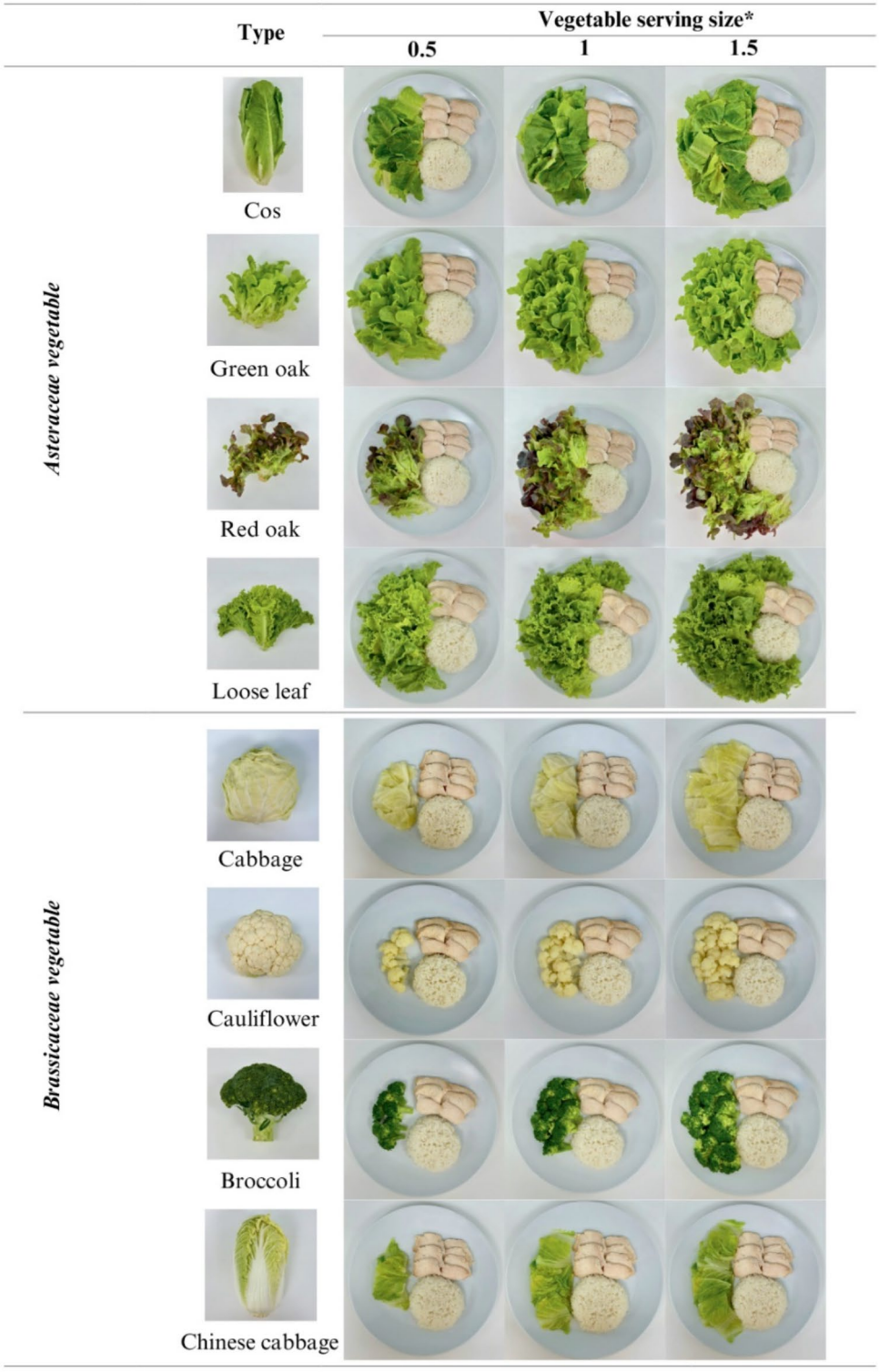

Fig. 1. Plate photograph showcasing various vegetable serving sizes incorporated into a mixed meal. *The plate portion reflects the ratio of vegetables, rice, and meat according to the MyPlate recommended vegetable portion.

though the differences were not statistically significant. This finding aligns with previous studies demonstrating that GLV, such as lettuce and cruciferous vegetables, contain high amounts of phenolic compounds with high bioaccessibility after simulated gastrointestinal digestion[29,38]. This enhanced digestibility of polyphenols can be attributed to the combined effects of acidic pH, enzymatic activity, and prolonged digestive exposure time, which facilitate the release of polyphenols bound within the food matrix[39].

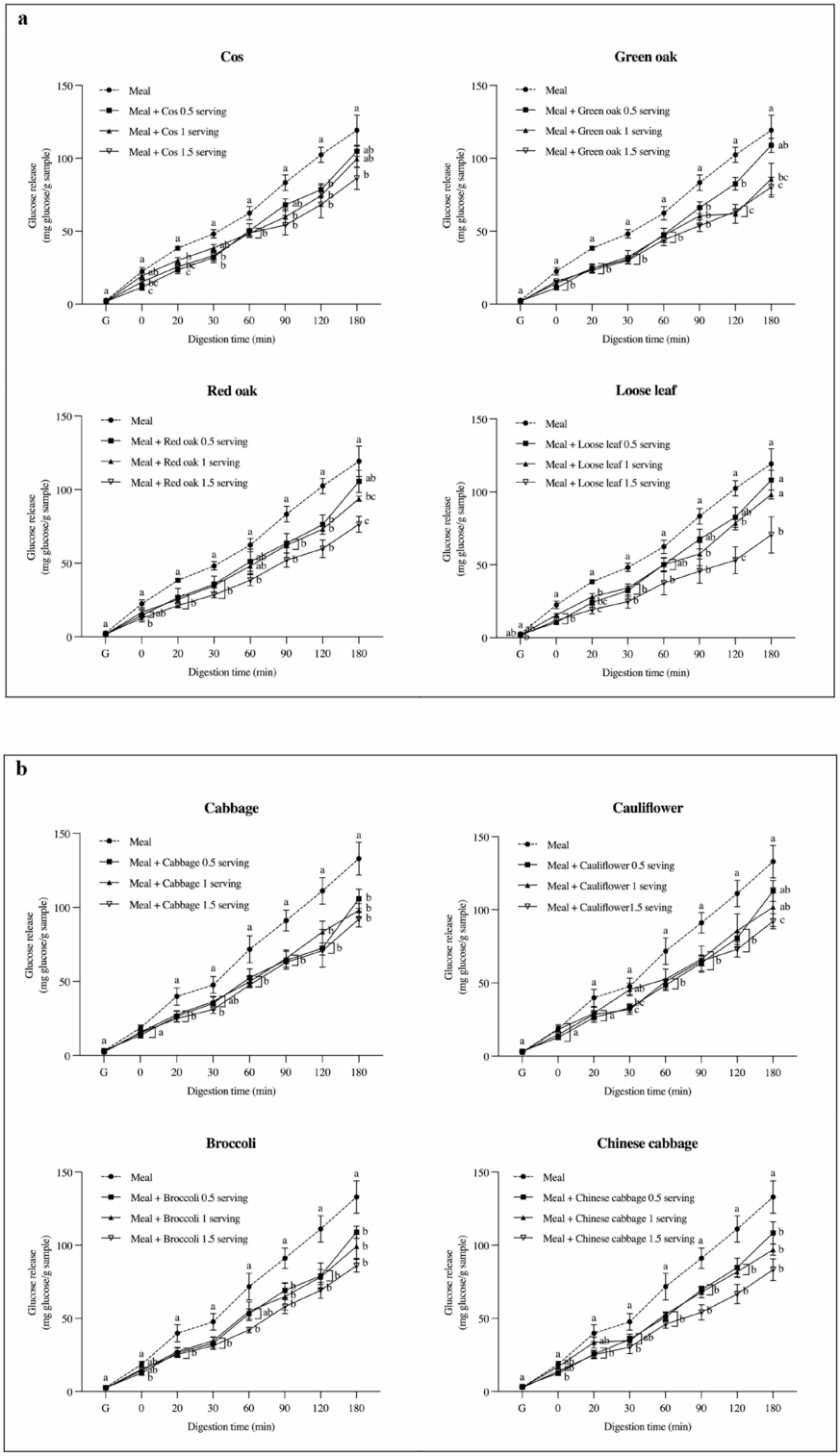

Fig. 2. Glucose concentration during in vitro digestion of (**a**) *Asteraceae* families; (**b**) *Brassicaceae* families. The results are presented as mean ± SEM ($n = 4$). Different superscript letters represented a significant difference ($p < 0.05$).

Supplementary Fig. S2 illustrates the antioxidant activity through the FRAP values during the intestinal phase of in vitro digestion. The antioxidant activity of the meal remained stable from 0 to 180 min of intestinal digestion. Incorporation of any serving size of both *Asteraceae* and *Brassicaceae* vegetables into the meal showed a positive trend in antioxidant activity compared to the meal alone, supported by the AUC of the FRAP values depicted in Supplementary Fig. S4. Incorporating 1.5 servings of either *Asteraceae* or *Brassicaceae* vegetables

Type	Sample	Vegetable portion	AUC for glucose (mg glucose × min/g sample)	Starch fraction			Type	Sample	Vegetable portion	AUC for glucose (mg glucose × min/g sample)	Starch fraction		
				%RDS	%SDS	%US					%RDS	%SDS	%US
Asteraceae family	Meal	N.A.	100.0 ± 6.9^{a}	4.5 ± 0.6^{a}	18.0 ± 1.5^{a}	77.6 ± 1.9^{a}	*Brassicaceae* family	Meal	N.A.	100.0 ± 8.8^{a}	6.8 ± 1.5^{a}	22.9 ± 2.0^{a}	70.3 ± 2.5^{a}
	MC	0.5	81.4 ± 4.6^{b}	3.9 ± 0.3^{ab}	17.9 ± 0.8^{a}	78.2 ± 1.1^{a}		MCB	0.5	71.6 ± 7.5^{b}	4.7 ± 0.8^{ab}	17.2 ± 3.9^{ab}	78.1 ± 4.3^{ab}
		1.0	79.0 ± 4.7^{b}	2.7 ± 0.5^{b}	11.1 ± 1.9^{b}	86.2 ± 2.2^{b}			1.0	73.4 ± 5.4^{b}	3.3 ± 0.1^{b}	15.3 ± 1.4^{b}	81.4 ± 1.6^{b}
		1.5	71.5 ± 6.7^{b}	2.5 ± 0.6^{b}	10.4 ± 2.1^{b}	87.0 ± 2.5^{b}			1.5	66.7 ± 2.5^{b}	2.7 ± 0.5^{b}	13.0 ± 0.4^{b}	84.3 ± 0.8^{b}
	MG	0.5	82.5 ± 4.5^{b}	4.1 ± 0.7^{a}	17.6 ± 0.5^{a}	78.3 ± 1.1^{a}		MCL	0.5	73.2 ± 5.0^{b}	5.4 ± 0.7^{ab}	21.5 ± 1.5^{a}	73.1 ± 1.4^{a}
		1.0	69.9 ± 6.3^{b}	2.8 ± 0.7^{ab}	10.2 ± 1.2^{b}	87.1 ± 1.7^{b}			1.0	76.9 ± 9.9^{b}	3.0 ± 0.5^{b}	15.6 ± 2.1^{b}	81.4 ± 2.3^{b}
		1.5	66.8 ± 2.0^{b}	1.9 ± 0.6^{b}	9.9 ± 0.7^{b}	88.2 ± 0.5^{b}			1.5	68.8 ± 3.4^{b}	3.6 ± 0.4^{b}	11.4 ± 1.3^{b}	85.1 ± 0.9^{b}
	MR	0.5	81.1 ± 8.1^{b}	3.8 ± 0.8^{ab}	15.8 ± 1.8^{ab}	80.4 ± 2.0^{ab}		MB	0.5	74.8 ± 5.6^{b}	5.4 ± 0.6^{ab}	20.5 ± 2.5^{a}	74.2 ± 2.9^{a}
		1.0	76.5 ± 2.3^{b}	2.4 ± 0.5^{b}	12.0 ± 0.9^{bc}	85.6 ± 0.9^{bc}			1.0	72.6 ± 6.5^{b}	3.5 ± 0.6^{b}	13.9 ± 0.7^{b}	82.6 ± 1.2^{b}
		1.5	62.6 ± 5.0^{b}	2.3 ± 0.3^{b}	10.6 ± 1.6^{c}	87.1 ± 1.8^{c}			1.5	63.1 ± 3.8^{b}	2.6 ± 0.4^{b}	11.2 ± 1.4^{b}	86.2 ± 1.3^{b}
	ML	0.5	83.3 ± 6.8^{a}	4.1 ± 0.7^{a}	17.8 ± 1.2^{a}	78.0 ± 1.8^{a}		MCCB	0.5	76.3 ± 4.4^{b}	5.0 ± 0.5^{ab}	22.9 ± 1.8^{a}	72.1 ± 2.2^{a}
		1.0	78.7 ± 4.2^{ab}	3.1 ± 0.5^{ab}	12.8 ± 1.0^{b}	84.1 ± 1.2^{b}			1.0	74.2 ± 3.2^{b}	4.4 ± 0.8^{ab}	12.7 ± 0.2^{b}	83.0 ± 0.9^{b}
		1.5	56.7 ± 9.8^{b}	1.8 ± 0.4^{b}	8.3 ± 1.6^{c}	89.8 ± 1.8^{c}			1.5	61.7 ± 5.6^{b}	2.8 ± 0.3^{b}	10.4 ± 1.0^{b}	86.7 ± 1.3^{b}

Table 2. Glucose release and starch fraction after in vitro digestion. Data are presented as mean ± S.E.M., ($n = 4$). Values with different superscript letters within each column indicate significant differences ($p < 0.05$). N.A.: not analyzed, MC: meal + cos, MG: meal + green oak, MR: meal + red oak, ML: meal + loose leaf, MCB: meal + cabbage, MCL: meal + cauliflower, MB: meal + broccoli, MCCB: meal + Chinese cabbage, AUC: area under the curve, %RDS: the percentage of rapidly digestible starch, %SDS: the percentage of slowly digestible starch, %US: the percentage of undigestible starch.

Type	Sample	Vegetable portion	Intestinal phase (I180 min)		Type	Sample	Vegetable portion	Intestinal phase (I180 min)	
			Free fatty acid (μmol/mL)	Nitrate (μg NO_3-N/mL)				Free fatty acid (μmol/mL)	Nitrate (μg NO_3-N/mL)
Asteraceae family	Meal	N.A.	162.7 ± 3.2^{a}	103.7 ± 7.8^{a}	*Brassicaceae* family	Meal	N.A.	156.0 ± 4.4^{a}	99.4 ± 3.9^{a}
	MC	0.5	146.3 ± 5.6^{bc}	158.2 ± 4.2^{bc}		MCB	0.5	131.2 ± 8.9^{bc}	146.6 ± 1.3^{cd}
		10	135.6 ± 5.3^{bcde}	166.5 ± 0.5^{cd}			1.0	121.9 ± 5.7^{bc}	158.9 ± 2.0^{de}
		1.5	128.6 ± 0.5^{de}	182.7 ± 4.7^{ef}			1.5	120.4 ± 0.3^{bc}	163.2 ± 6.1^{e}
	MG	0.5	140.8 ± 7.5^{bcd}	161.1 ± 4.2^{bcd}		MCL	0.5	137.2 ± 6.7^{b}	127.3 ± 6.4^{b}
		1.0	130.8 ± 8.2^{bcde}	164.8 ± 1.9^{cd}			1.0	128.3 ± 8.1^{bc}	148.1 ± 2.6^{cd}
		1.5	126.0 ± 4.0^{de}	172.9 ± 1.9^{de}			1.5	127.7 ± 0.4^{bc}	162.8 ± 3.3^{e}
	MR	0.5	136.2 ± 5.6^{bcde}	152.2 ± 3.6^{b}		MB	0.5	132.1 ± 2.8^{bc}	146.5 ± 3.8^{cd}
		1.0	132.7 ± 5.9^{bcde}	169.4 ± 4.2^{cd}			1.0	129.9 ± 5.9^{bc}	148.1 ± 4.2^{cd}
		1.5	126.6 ± 6.6^{de}	182.5 ± 3.8^{ef}			1.5	117.8 ± 1.5^{c}	154.2 ± 3.2^{de}
	ML	0.5	148.0 ± 0.7^{ab}	162.0 ± 1.3^{bcd}		MCCB	0.5	127.7 ± 1.2^{bc}	138.8 ± 6.2^{bc}
		1.0	129.0 ± 4.6^{cde}	166.2 ± 4.3^{cd}			1.0	120.9 ± 4.9^{bc}	148.5 ± 4.2^{cd}
		1.5	118.5 ± 4.9^{e}	188.5 ± 2.0^{ef}			1.5	115.6 ± 3.1^{c}	150.6 ± 0.5^{cde}

Table 3. Free fatty acid and nitrate content after 180 min of in vitro digestion. Data are presented as mean ± S.E.M., ($n = 3$). Values with different superscript letters within each column indicate significant differences ($p < 0.05$). N.A.: not analyzed, MC: meal + cos, MG: meal + green oak, MR: meal + red oak, ML: meal + loose leaf, MCB: meal + cabbage, MCL: meal + cauliflower, MB: meal + broccoli, MCCB: meal + Chinese cabbage.

resulted in the highest FRAP value. Notably, all *Asteraceae* vegetables significantly increased antioxidant activity when ≥ 1 serving was incorporated. This effect was similarly observed with cauliflower among the *Brassicaceae* vegetables. However, other *Brassicaceae* vegetables exhibited higher antioxidant activity with no statistically significant difference.

The enhanced antioxidant activity observed when incorporating various vegetable serving sizes into the meal is likely related to the phenolic content found in our study. Although the total phenolic content did not differ significantly during simulated digestion, the antioxidant activity was notably elevated. This finding can be attributed to the diversity of phytonutrients naturally occurring in GLV. Previous studies have reported that carotenoids and flavonoids are important phytonutrients detected in lettuce and Brassica vegetables, respectively, leading to the generation of esters and secondary compounds that can increase antioxidant activity[40,41]. Additionally, other bioactive compounds in lettuce, such as ascorbic acid and cysteine, can promote antioxidant activity by inhibiting phenolic oxidation[42]. Furthermore, previous studies have indicated that lipophilic antioxidants, such as aglycone forms, are easily absorbed with increasing pH during digestion, promoting polyphenol polymerization and bioavailability, which potentially enhance radical scavenging activity in GLV[40–42]. Therefore, these synergistic effects contribute to maintaining and enhancing the antioxidant activity in lettuce, potentially improving its nutritional value and cardiovascular protection[40,43].

In vitro lipid digestibility

The concentrations of FFA after 180 min of in vitro digestion, demonstrating the effect of incorporating various vegetables into the meal are shown in Table 3. The *Asteraceae* family (cos, green oak, red oak, loose leaf) showed free fatty acid content ranging from 118.5 to 148.0 μmol/mL, while the *Brassicaceae* family (cabbage, cauliflower, broccoli, Chinese cabbage) ranged from 115.6 to 137.2 μmol/mL. These results demonstrate significant variation in free fatty acid content among different vegetable varieties. Notably, all tested servings (0.5, 1.0, 1.5) from both families significantly reduced free fatty acid content compared to the control meal.

The observed reduction in FFA concentrations was found when increased vegetable serving sizes. This reduction aligns with the inhibitory effects of vegetable bioactive compounds on pancreatic lipase, a key enzyme involved in the hydrolysis of triglycerides[44]. Phenolic compounds such as anthocyanin, catechin, and tannins have demonstrated inhibitory effects on pancreatic lipase[45,46]. Additionally, sulforaphane, prevalent in cruciferous vegetables, can inhibit endothelial lipase expression by suppressing NF-κB in endothelial cells[47]. An inverse relationship between polyphenol content and FFA concentration was observed with increasing vegetable portions. Moreover, dietary fiber in vegetables can bind bile acids, reducing their availability for fat emulsification and subsequently decreasing triglyceride digestibility by lipolytic enzymes[48]. This finding is consistent with previous research demonstrating high bile acid binding capacity in green leafy vegetables, particularly within the *Brassicaceae* family[49]. This mechanism impacts lipid metabolism and has broader implications for non-communicable diseases (NCDs), potentially enhancing cardiovascular health by reducing the risk of atherosclerosis and hypertension[43,50]. These findings suggest that incorporating vegetables into the diet may serve as a strategic approach to improving lipid metabolism and promoting cardiovascular health.

Nitrate content

Table 3 presents nitrate concentrations after 180 min of in vitro digestion. The *Asteraceae* and *Brassicaceae* families exhibited higher nitrate content than the control meal, with concentrations ranging from 152.2

to 188.5 µg NO_3-N/mL and 127.3–163.2 µg NO_3-N/mL, respectively. All vegetable types and serving sizes significantly increased nitrate concentrations compared to the meal alone. A dose-dependent increase in nitrate concentration was observed with increasing vegetable portions (1.5 > 1.0 > 0.5 portions) across all vegetable types.

The elevated nitrate content in vegetables compared to the control meal aligns with previous studies demonstrating higher nitrate accumulation in leafy greens versus root vegetables[23]. Notably, our findings indicate that increasing vegetable serving sizes results in highly preserved post-digestion nitrate content, potentially promoting nitric oxide (NO) formation through the nitrate-nitrite-NO pathway[51]. This process facilitates the formation of NO, a potent vasodilator that helps to improve endothelial function and lower blood pressure[51]. Ingested nitrate is converted into nitrite by salivary bacteria and further forms NO by reducing nitrite under gastric conditions in the stomach[52]. A previous study determined the effect of consuming a nitrate-rich meal containing spinach, which improved arterial stiffness and increased large artery compliance acutely in healthy men and women[53]. Additionally, consuming cruciferous vegetables significantly reduced systolic blood pressure compared to root and squash vegetable groups, suggesting potential cardiovascular benefits[54,55]. These findings suggest that increasing vegetable consumption, particularly from nitrate-rich sources, may contribute to enhanced cardiovascular health.

Materials and methods

Materials

Fresh *Asteraceae* (cos, green oak, red oak, and loose leaf) and *Brassicaceae* vegetables (cabbage, cauliflower, broccoli, and Chinese cabbage) were purchased from a local supermarket, in Thailand. Folin-Ciocalteu reagent was purchased from Merck (KGaA, Darmstadt, Germany). TPTZ (2,4,6-tripyridyl-s-triazine), α-amylase Type VI-B from porcine pancreas (15.8 U/mg), porcine bile extract, pepsin from porcine gastric mucosa powder (250 U/mg), and pancreatin from porcine pancreas (4×U.S. Pharmacopeia (USP) specifications) were obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA). Amyloglucosidase (3260 U/mL) was purchased from Megazyme International Ireland Ltd. (Bray, Ireland). The glucose oxidase kit (Glucose liquicolor®) was purchased from Human (Human Diagnostics, Wiesbaden, Germany). The free fatty acid (FFA) content assay kit was purchased from Solarbio (Beijing, China). All other chemical reagents were analytical grade.

Preparation of vegetables

Fresh vegetables were sanitized by soaking in 0.075% (w/v) of sodium bicarbonate solution for 15 min and rinsed through the water for 2 min. Then, cut into small pieces approximately 0.3 × 0.3 cm. The *Brassicaceae* vegetables were boiled for 3 min after the washing process. All samples were immediately used for further analysis. Fresh vegetables were frozen in liquid nitrogen and ground into powder. The powder was then extracted with 70% ethanol in a ratio of (1:1.5 w/v) overnight at pH 3.2 adjusted with formic acid. The solution was filtered using Whatman filter paper and evaporated to dryness under reduced pressure (43–45 °C) using a rotary evaporator. The extracted sample was stored at −20 °C. Additionally, 10 mg of the extracted sample was reconstituted in 1 mL of 70% ethanol. Then the supernatant after centrifugation at 7826 x g for 5 min was used for subsequent analysis.

Test meal preparation

The test meal was prepared by combining cooked Thai jasmine rice, chicken, soybean oil, and vegetable samples. The ingredients were freshly prepared before the experiment. White rice was cooked using a rice cooker. The raw chicken was boiled until fully cooked. After cooking, the rice and chicken were separately minced into small pieces. A mixed meal was then prepared by combining 1 g of cooked white rice, 0.6 g of cooked chicken, and 0.09 g of soybean oil in a centrifuge tube for simulated in vitro gastrointestinal digestion.

Vegetable samples from the *Asteraceae* and *Brassicaceae* families were incorporated into the mixed meal in three portion sizes–0.5, 1.0, and 1.5 times the MyPlate-recommended vegetable serving size for a 2000 kcal/day diet. The vegetable quantities were calculated based on an 80 g standard serving size, with adjustments by a factor of 100 to align with in vitro digestion conditions. Thus, 0.4 g, 0.8 g, and 1.2 g of finely chopped vegetables were added to the test meals, representing 0.5, 1.0, and 1.5 servings, respectively. The accuracy of these calculations was verified by three registered dietitians.

A control meal, identical in composition but without vegetables, was prepared for comparative analysis. The inclusion of both fresh and cooked vegetable samples followed previously outlined preparation protocols, ensuring consistency across experimental conditions. These test meals were immediately subjected to simulated digestion to minimize potential nutrient degradation. All meal was freshly prepared in triplicate.

Total phenolic content (TPC)

The amount of TPC was determined using the Folin-Ciocalteu method with minor modifications[56]. In brief, 10 mg of vegetable extract was dissolved in 70% ethanol. After centrifugation for 5 min at 5652 x g, 50 µL of the supernatant was mixed with 10% (v/v) Folin-Ciocalteu reagent and incubated at room temperature for 5 min followed by the addition of 50 µL of 10% (w/v) Na_2CO_3. Then, the mixture was left in the dark for 30 min. The absorbance was measured at 760 nm. The results were expressed as mg GAE/g extract.

Nitrate content

The nitrate content was performed according to the previous protocol with minor modifications[57]. The salicylic solution was freshly prepared with 5% (w/v) of salicylic acid in 98% H_2SO_4. Briefly, all samples were centrifuged at 1413 x g for 5 min. 10 mL of supernatant was mixed with 30 mL of salicylic solution, followed by adding 760 mL of 2 M NaOH after incubation for 20 min at room temperature. After that, the mixture was incubated at

room temperature for 5 min and measured the absorbance at 410 nm. KNO_3 was used to prepare the standard curve (0–12.5 µg NO_3-N in a 0.25 mL aliquot). The result was calculated as mg NO_3-NE/g extract.

Ferric reducing antioxidant power (FRAP)

The antioxidant capacity was measured using the ferric reducing antioxidant power (FRAP) assay, with minor modifications according to the previous study[58]. After dissolved and centrifuged, the supernatant of the vegetable extract mixture was diluted with 70% ethanol (1:2). The FRAP reagent was freshly made by mixing 0.3 M acetate buffer, pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3$ in a ratio of 10:1:1. 10 µL of diluted sample was mixed with 90 µL of the stock reagent for 30 min under the dark condition. The absorbance was read at 590 nm. Distilled water was used as a control. The result was calculated from the $FeSO_4$ standard curve (0–100 µmol/L) and expressed as mmol of $FeSO_4$E/g extract.

In vitro gastrointestinal digestion

The in vitro gastrointestinal digestion procedure was adapted from the previous method[59]. In the oral phase, the sample was incubated at 37 °C in a shaking water bath at 100 rpm with 1 mL of α-amylase (250 U/mL in 0.2 M carbonate buffer, pH 7) for 15–20 s. Thereafter, the gastric phase was initiated by adding 5 mL of porcine pepsin solution (3200 U/mL in 0.02 N HCl, pH 2) and incubated at 37 °C for 30 min. After that, gastric digesta was neutralized with 5 mL of 0.02 N NaOH and 25 mL of 0.2 M sodium acetate buffer, pH 6. Then, 5 mL of bile solution (10% bile extract in MQ water) was added followed by the addition of 5 mL of pancreatin (2 mg/mL) and amyloglucosidase (28 U/mL) in 0.2 M sodium acetate buffer, pH 6 and incubated at 37 °C in a shaking water bath. The aliquot sample was collected at 0, 20, 30, 60, 90, 120, and 180 min and immediately heated at 100 °C for 10 min. After heating, the sample was centrifuged at 5652 x g at 4 °C for 20 min and stored at −20 °C for further analysis. The gastric and intestinal digesta were determined the release of total phenolic content and antioxidant activity using the Folin-Ciocalteu assay and FRAP assay procedures described above. Nitrate content was determined after 180 min in the intestinal phase.

Starch digestibility

Digestibility of starch was conducted following a previously established method, with minor modification[59]. The digesta obtained from in vitro digestion process was utilized to quantify the amount of glucose released using a glucose oxidase-peroxidase (GOPOD) kit. The results were reported as a mg glucose/g sample. The trapezoidal rule was used to determine the area under the curve (AUC). The starch fraction was represented as the percentage of rapidly digestible starch (RDS), slowly digestible starch (SDS), and undigestible starch using Eqs. (1, 2, 3)[60].

$$\%\mathrm{RDS} = (\mathrm{G}_{20} - \mathrm{G}_0)\,/\mathrm{TS} \times\ 0.9 \times\ 100 \tag{1}$$

$$\%\mathrm{SDS} = (\mathrm{G}_{120} - \mathrm{G}_{20})\,/\mathrm{TS} \times\ 0.9 \times\ 100 \tag{2}$$

$$\%\mathrm{Undigestible\ starch} = (\mathrm{TS} - \mathrm{RDS} - \mathrm{SDS})\,/\mathrm{TS} \tag{3}$$

where G_0 represents the glucose quantity released at 0 min; G_{20} is the amount of glucose released after 20 min; G_{120} is the amount of glucose released after 120 min, and TS is the total starch presented in the sample.

Lipid digestibility

Lipid digestibility was evaluated by quantifying free fatty acids (FFA) using a commercial assay kit with minor modifications. Briefly, digesta samples were extracted with the extract solution (1:10 v/v), centrifuged at 3617 x g for 10 min at 4 °C, and the supernatant was analyzed. Results were expressed as µmol/mL.

Statistical analysis

The results were reported as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to determine the mean difference among groups. Statistical analyses were performed using IBM SPSS Statistics version 28.0.0. Graphs were generated with GraphPad PRISM version 10.1.1. Statistical significance was established at $p < 0.05$.

Conclusion

GLV are rich sources of antioxidants and nitrates, with significant variability among types. This study demonstrates that incorporating GLV into meals, particularly at 1.5 times the MyPlate serving size, significantly delays carbohydrate digestion and decreases lipid digestion while enhancing antioxidant activity. While these effects are dose-dependent, the challenges of consuming larger GLV portions must be acknowledged. Notably, incorporating GLV at the standard serving size of the MyPlate recommendation also yields significant positive effects. Our findings support the MyPlate recommendation as an effective and practical strategy for promoting healthy dietary patterns and potentially reducing cardiovascular health risks. The observed variations among GLV species underscore the importance of diverse vegetable consumption for maximizing health benefits. Further research is needed to explore mixed vegetable consumption and develop targeted dietary strategies to increase GLV intake, ultimately enhancing overall cardiovascular risk prevention.

Data availability

"The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."

Received: 10 December 2024; Accepted: 6 February 2025
Published online: 12 February 2025

References

1. World Heart Report. : Confronting the world's number one killer, World Heart Federation, Geneva, Switzerland, 2023. (2023).
2. Satija, A. et al. Healthful and unhealthful plant-based diets and the risk of coronary heart disease in U.S. adults. *J. Am. Coll. Cardiol.* **70**, 411–422 (2017).
3. Arnett, D. K. et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. *Circulation* **140**, e596–e646 (2019).
4. Diet nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser* 916, i-viii, 1-149, (2003).
5. Wallace, T. C. et al. Fruits, vegetables, and health: a comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. *Crit. Rev. Food Sci. Nutr.* **60**, 2174–2211 (2020).
6. Aune, D. et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. *Int. J. Epidemiol.* **46**, 1029–1056 (2017).
7. Tharrey, M. et al. Patterns of plant and animal protein intake are strongly associated with cardiovascular mortality: the Adventist Health Study-2 cohort. *Int. J. Epidemiol.* **47**, 1603–1612 (2018).
8. Bricarello, P. Effects of the Dietary Approach to stop hypertension (DASH) diet on blood pressure, overweight and obesity in adolescents: a systematic review. *Clin. Nutr. ESPEN.* **28**, 1–11 (2018).
9. Randhawa, M. A., Khan, A. A., Javed, M. S. & Sajid, M. W. in *In Handbook of Fertility*. 205–220 (eds Watson, R. R.) (Academic, 2015).
10. Singh, A. et al. Emergence of microgreens as a valuable food, current understanding of their market and consumer perception: a review. *Food Chemistry: X*. **23**, 101527 (2024).
11. Saini, R. K. et al. Profiling of nutritionally vital bioactive compounds in emerging green leafy vegetables: a comparative study. *Foods* **11**, 3867 (2022).
12. Schoeneck, M. & Iggman, D. The effects of foods on LDL cholesterol levels: a systematic review of the accumulated evidence from systematic reviews and meta-analyses of randomized controlled trials. *Nutr. Metab. Cardiovasc. Dis.* **31**, 1325–1338 (2021).
13. Zhang, H., Sun, S. & Ai, L. Physical barrier effects of dietary fibers on lowering starch digestibility. *Curr. Opin. Food Sci.* **48**, 100940 (2022).
14. Weickert, M. O. & Pfeiffer, A. F. H. Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. *J. Nutr.* **148**, 7–12 (2018).
15. Johnson, M., McElhenney, W. H. & Egnin, M. Influence of green leafy vegetables in diets with an elevated omega-6:Omega-3 fatty acid ratio on rat blood pressure, plasma lipids, antioxidant status and markers of inflammation. *Nutrients* **11**, 301 (2019).
16. Meslier, V. et al. Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut* **69**, 1258–1268 (2020).
17. Olas, B. The cardioprotective role of nitrate-rich vegetables. *Foods* **13**, 691 (2024).
18. Kumar, G., Dey, S. K. & Kundu, S. Functional implications of vascular endothelium in regulation of endothelial nitric oxide synthesis to control blood pressure and cardiac functions. *Life Sci.* **259**, 118377 (2020).
19. Daiber, A. et al. New therapeutic implications of endothelial nitric oxide synthase (Enos) function/dysfunction in cardiovascular disease. *Int. J. Mol. Sci.* **20**, 187 (2019).
20. Smith, L. et al. Fruit and vegetable intake and non-communicable diseases among adults aged ≥ 50 years in low- and middle-income countries. *J. Nutr. Health Aging.* **26**, 1003–1009 (2022).
21. Chen, Y. Y. et al. Healthy dietary patterns and risk of cardiovascular disease in US Hispanics/Latinos: the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *Am. J. Clin. Nutr.* **116**, 920–927 (2022).
22. Ketnawa, S., Suwannachot, J. & Ogawa, Y. In vitro gastrointestinal digestion of crisphead lettuce: changes in bioactive compounds and antioxidant potential. *Food Chem.* **311**, 125885 (2020).
23. Luetic, S. et al. Leafy vegetable nitrite and nitrate content: potential health effects. *Foods* **12**, 1655 (2023).
24. Colla, G., Kim, H. J., Kyriacou, M. C. & Rouphael, Y. Nitrate in fruits and vegetables. *Sci. Hort.* **237**, 221–238 (2018).
25. Hord, N. G., Tang, Y. & Bryan, N. S. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am. J. Clin. Nutr.* **90**, 1–10 (2009).
26. Deng, G. F. et al. Antioxidant capacities and total phenolic contents of 56 vegetables. *J. Funct. Foods.* **5**, 260–266 (2013).
27. Kim, M. J., Moon, Y., Tou, J. C., Mou, B. & Waterland, N. L. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L). *J. Food Compos. Anal.* **49**, 19–34 (2016).
28. Ferreres, F., Gil, M. I., Castañer, M. & Tomás-Barberán, F. A. Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. *J. Agric. Food Chem.* **45**, 4249–4254 (1997).
29. Li, Z. et al. Profiling of phenolic compounds and antioxidant activity of 12 cruciferous vegetables. *Molecules* **23**, 1139 (2018).
30. Marciani, L. et al. Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *Am. J. Physiol. Gastrointest. Liver Physiol.* **280**, G1227–1233 (2001).
31. Goff, H. D., Repin, N., Fabek, H., Khoury, E., Gidley, M. & D. & J. Dietary fibre for glycaemia control: towards a mechanistic understanding. *Bioactive Carbohydr. Diet. Fibre.* **14**, 39–53 (2018).
32. Gopal, S. S. et al. Lactucaxanthin - a potential anti-diabetic carotenoid from lettuce (*Lactuca sativa*) inhibits alpha-amylase and alpha-glucosidase activity *in vitro* and in diabetic rats. *Food Funct.* **8**, 1124–1131 (2017).
33. Kondo, S., Suzuki, A., Kurokawa, M. & Hasumi, K. Intake of kale suppresses postprandial increases in plasma glucose: a randomized, double-blind, placebo-controlled, crossover study. *Biomed. Rep.* **5**, 553–558 (2016).
34. Podsedek, A., Majewska, I. & Kucharska, A. Z. Inhibitory potential of red cabbage against digestive enzymes linked to obesity and type 2 diabetes. *J. Agric. Food Chem.* **65**, 7192–7199 (2017).
35. Ajayi, I. O., Otemuyiwa, I. O., Adeyanju, A. A. & Falade, O. S. Vegetable polyphenols inhibit starch digestibility and phenolic availability from composite carbohydrate foods *in-vitro*. *J. Agric. Food Res.* **3**, 100116 (2021).
36. Xiong, M. et al. The difference among structure, physicochemical and functional properties of dietary fiber extracted from triticale and hull-less barley. *LWT* **154**, 112771 (2022).
37. Zheng, S. et al. Exploring the synergistic benefits of insoluble dietary fiber and bound phenolics: unveiling the role of bound phenolics in enhancing bioactivities of insoluble dietary fiber. *Trends Food Sci. Technol.* **149**, 104554 (2024).
38. Lafarga, T., Villaro, S., Rivera, A., Bobo, G. & Aguilo-Aguayo, I. Bioaccessibility of polyphenols and antioxidant capacity of fresh or minimally processed modern or traditional lettuce (*Lactuca sativa* L.) varieties. *J. Food Sci. Technol.* **57**, 754–763 (2020).
39. Rodríguez-Roque, M. J., Rojas-Graü, M. A. & Elez-Martínez, P. Martín-Belloso, O. Soymilk phenolic compounds, isoflavones and antioxidant activity as affected by *in vitro* gastrointestinal digestion. *Food Chem.* **136**, 206–212 (2013).
40. Shi, M. et al. Phytochemicals, nutrition, metabolism, bioavailability, and health benefits in lettuce-a comprehensive review. *Antioxid. (Basel)* **11**, 1158 (2022).
41. Thilavech, T., Marnpae, M., Makynen, K. & Adisakwattana, S. Phytochemical composition, antiglycation, antioxidant activity and methylglyoxal-trapping action of brassica vegetables. *Plant. Foods Hum. Nutr.* **76**, 340–346 (2021).
42. Altunkaya, A. & Gökmen, V. Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). *Food Chem.* **107**, 1173–1179 (2008).

43. Zhang, X. et al. Cruciferous vegetable consumption is associated with a reduced risk of total and cardiovascular disease mortality. *Am. J. Clin. Nutr.* **94**, 240–246 (2011).
44. Sultana, R. et al. Inhibitory activities of polyphenolic extracts of Bangladeshi vegetables against α-amylase, α-glucosidase, pancreatic lipase, renin, and angiotensin-converting enzyme. *Foods* **9**, 844 (2020).
45. Fabroni, S., Ballistreri, G., Amenta, M., Romeo, F. V. & Rapisarda, P. Screening of the anthocyanin profile and *in vitro* pancreatic lipase inhibition by anthocyanin-containing extracts of fruits, vegetables, legumes and cereals. *J. Sci. Food Agric.* **96**, 4713–4723 (2016).
46. Sergent, T., Vanderstraeten, J., Winand, J., Beguin, P. & Schneider, Y. J. Phenolic compounds and plant extracts as potential natural anti-obesity substances. *Food Chem.* **135**, 68–73 (2012).
47. Kivelä, A. M. et al. Sulforaphane inhibits endothelial lipase expression through NF-κB in endothelial cells. *Atherosclerosis* **213**, 122–128 (2010).
48. Zhang, N., Huang, C. & Ou, S. Vitro *vitro* binding capacities of three dietary fibers and their mixture for four toxic elements, cholesterol, and bile acid. *J. Hazard. Mater.* **186**, 236–239 (2011).
49. Yang, I. F., Jayaprakasha, G. K. & Patil, B. S. *In vitro* bile acid binding capacities of red leaf lettuce and cruciferous vegetables. *J. Agric. Food Chem.* **65**, 8054–8062 (2017).
50. Okyere, J., Ayebeng, C., Owusu, B. A. & Dickson, K. S. Fruits and vegetable consumption, and its association with hypertension among women in Ghana: a cross-sectional study. *Public. Health Nutr.* **27**, e19 (2023).
51. Lidder, S. & Webb, A. J. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. *Br. J. Clin. Pharmacol.* **75**, 677–696 (2013).
52. Lundberg, J. O., Weitzberg, E., Cole, J. A. & Benjamin, N. Nitrate, bacteria and human health. *Nat. Rev. Microbiol.* **2**, 593–602 (2004).
53. Liu, A. H. et al. Effects of a nitrate-rich meal on arterial stiffness and blood pressure in healthy volunteers. *Nitric Oxide*. **35**, 123–130 (2013).
54. Connolly, E. L. et al. Cruciferous vegetables lower blood pressure in adults with mildly elevated blood pressure in a randomized, controlled, crossover trial: the VEgetableS for vaScular hEaLth (VESSEL) study. *BMC Med.* **22**, 353 (2024).
55. Sundqvist, M. L. et al. A randomized clinical trial of the effects of leafy green vegetables and inorganic nitrate on blood pressure. *Am. J. Clin. Nutr.* **111**, 749–756 (2020).
56. Chu, Y. F., Sun, J., Wu, X. & Liu, R. H. Antioxidant and antiproliferative activities of common vegetables. *J. Agric. Food Chem.* **50**, 6910–6916 (2002).
57. Cataldo, D. A., Haroon, M., Schrader, L. E. & Youngs, V. L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* **6**, 71–80 (1975).
58. Pasukamonset, P., Kwon, O. & Adisakwattana, S. Alginate-based encapsulation of polyphenols from *Clitoria ternatea* petal flower extract enhances stability and biological activity under simulated gastrointestinal conditions. *Food Hydrocoll.* **61**, 772–779 (2016).
59. Suklaew, P. O., Chusak, C. & Adisakwattana, S. Physicochemical and functional characteristics of rd43 rice flour and its food application. *Foods* **9**, 1912 (2020).
60. Englyst, H. N., Kingman, S. M. & Cummings, J. H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* **46** (Suppl 2), S33–50 (1992).

Acknowledgements

We would like to thank The Second Century Fund (C2F), Chulalongkorn University.

Author contributions

T.K.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization. K.K.: Investigation. C.C.: Investigation, Resources, Data curation, Writing - Review & Editing, Visualization, Supervision. M.S.: Writing - Review & Editing, Supervision. S.A.: Conceptualization, Methodology, Writing - Review & Editing, Visualization, Supervision.

Funding

This research was supported by The Second Century Fund Office (C2F) and the 90th Anniversary of Chulalongkorn University Scholarship (Ratchadaphiseksomphot Endowment Fund; Grant Number GCUGR1125672068D).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-89573-1.

Correspondence and requests for materials should be addressed to S.A.