Send Orders for Reprints to reprints@benthamscience.net

Recent Patents on Food, Nutrition & Agriculture, 2021, *12*, 58-62

RESEARCH ARTICLE

An *In Vitro* Study on the Effects of Selected Natural Dietary Fiber from Salad Vegetables for Lowering Intestinal Glucose and Lipid Absorption

Pannapa Powthong[1,*], Bajaree Jantrapanukorn[2], Pattra Suntornthiticharoen[1] and Chitradee Luprasong[3]

[1]*Department of Medical Sciences, Faculty of Science, Rangsit University, Pathumthani 12000, Thailand;* [2]*Faculty of Medical Technology, Rangsit University, Pathumthani 12000, Thailand;* [3]*Sun Herb, Thai, Chinese Manufacturing, Rangsit University, Pathumthani 12000, Thailand*

ARTICLE HISTORY

Received: July 01, 2020
Revised: November 30, 2020
Accepted: January 04, 2021

DOI:
10.2174/2212798412666210311163258

Abstract: ***Background:*** Salad vegetables are good sources of dietary fiber and are becoming increasingly popular among consumers. Therefore, these plants have the potential to be developed as functional foods.

***Objective*:** Using an in vitro model, this study investigated the physical properties and intestinal glucose and lipid absorption capacities of dry dietary fiber from vegetables typically consumed in salads (types of lettuce, including red oak, red coral, green oak, butterhead, and cos).

***Method*:** Fiber was prepared from each type of lettuce using an enzymatic method and then characterized. Physical properties, including solubility and water-binding, swelling, cation-exchange, and oil-binding capacities, and antihyperglycemic and antihypercholesterolemic effects of fiber were investigated.

***Results*:** The hydration capacity of total dietary fiber and insoluble fiber from the majority of sources was significantly different from that of cellulose. Adsorption and diffusion of glucose were directly proportional to incubation time, and the diffusion rate was significantly lower in the treatments containing fiber compared to the cellulose control. Fiber from these vegetables also inhibited amylase and alpha-glucosidase activities. Moreover, fiber from all sources exhibited significantly higher sodium cholate and cholesterol-binding capacity compared to cellulose and also retarded pancreatic cholesterol esterase activity in a concentration-dependent manner.

***Conclusion*:** This study demonstrates that natural dietary fiber from salad vegetables can reduce glucose and lipid absorption and breakdown rates, thus preventing increases in postprandial blood glucose and cholesterol levels, which can be beneficial to human health.

Keywords: dietary fiber, salad, intestine, glucose, lipid, absorption.

1. INTRODUCTION

Dietary fiber, including soluble and insoluble fiber, is a diverse group of carbohydrates and lignin that is not hydrolyzed by human enzymes; therefore, it is either resistant to digestion or processed in the small intestine [1]. Soluble fiber includes viscous fiber, such as glucans, fructans (inulin and fructooligosaccharides), gum, pectin, and mucilage, and non-viscous fiber, such as hemicelluloses. Insoluble fiber is found in whole grain, wheat, bran, nuts, seeds, and some fruits and vegetables. Although dietary fiber is indigestible, it can be partly hydrolyzed by bacteria in the colon using colonic enzymes. It has several protective effects against chronic diseases, including reducing the risk of coronary heart diseases. It also lowers the levels of plasma cholesterol and reduces the glycemic response to meals in people with diabetes, and, therefore, decreases the risk of metabolic syndrome, inflammatory bowel syndrome, diverticular disease, obesity, colorectal cancer, and obesity [2-6].

In recent times, eating patterns have changed toward increased consumption of instant, fast, and prepared foods. These foods can lead to nutritional problems because they often provide high amounts of fat, sugar, refined carbohydrates, and energy while being low in dietary fiber. Numerous health organizations have suggested that people should increase the consumption of dietary fiber, with the specific

*Address correspondence to this author at the Department of Medical Sciences, Faculty of Science, Rangsit University, Pathumthani 12000, Thailand; Tel: +008 2 997 2220; E-mail: pannapa.p@rsu.ac.th

2212-7984/21 $65.00+.00

recommendation of 15–38 g/day [7]. High-fiber products such as vegetables and wheat include a number of high-fiber ingredients. Several vegetable products have the potential for use as dietary fiber sources and should thus be investigated.

Lettuce is one of the most popular vegetables whose leaves are widely used to prepare salads. It is a rich source of bioactive compounds and nutrients important for human health and protection against diseases. These healthy properties of lettuce are attributed to a large supply of antioxidant compounds (*e.g.*, vitamins C and E, carotenoids, and polyphenols) as well as fiber content [8–10]. These vegetables are increasingly accepted by consumers because they are healthy and easy to find and prepare for fresh consumption. Therefore, dietary fiber from the lettuce family should be developed to become a functional food. However, information is not available currently about the dietary fiber in these vegetables.

Therefore, the objective of this study was to evaluate and compare the physicochemical properties and in vitro antihyperglycemic and antihypercholesterolemic potential of different fiber-rich meals prepared from types of lettuce consumed as salad vegetables (*e.g.*, red oak, red coral, green oak, butterhead, and cos lettuce). This study may facilitate the improved use of this resource by determining the functionality of dietary fiber and differences between total dietary fiber (TDF) and insoluble dietary fiber (IDF), as well as the potential applications of these vegetables as fiber sources.

2. MATERIALS AND METHODS

2.1. Plant Materials and Preparation of Selected Lettuces Consumed as Salad Vegetables

The vegetables included in this study were types of *Lactuca sativa* var. *crispa* (red oak, red coral, and green oak lettuce), *Lactuca sativa* L. cv. Panama (butterhead lettuce), and *Lactuca sativa longifolia* (cos lettuce) were purchased from a local market. The vegetables were cleaned and dried at 40 °C for 15–20 h, and then ground into powder and defined as TDF containing both soluble and IDF. The standard method of enzymatic extraction of the Association of Official Analytical Chemists [11] was used for extracting insoluble fiber. The vegetable powder was digested with heat-stable alpha-amylase (100 °C, 1 h) and then with protease (60 °C, 1 h), followed by incubation with amyloglucosidase (60 °C, 1 h) to remove protein and starch. The enzyme-treated mixture was then centrifuged at 5,000 rpm for 20 min at 25 °C. The residue recovered in this step, defined as IDF, was washed sequentially with 78% ethanol, 95% ethanol, and acetone. The residue was oven-dried overnight at 60 °C and stored for further analysis. Alpha-cellulose (Cat. No. C-8200; Sigma Aldrich) was used as a control.

2.2. Physicochemical Properties of Selected Lettuces Consumed as Salad Vegetables

2.2.1. Solubility

Solubility was determined according to the method described by Chau, Wang, and Wen [12]. In brief, TDF and IDF were mixed with distilled water (1:10 w/v) and left for 1 h at room temperature. After centrifugation at 1,500 rpm for 10 min, the supernatant was collected and dried at 100 ºC in a hot air oven. The residue was weighed to determine solubility using the formula:

$$\text{Solubility (\%)} = \frac{\text{Weight (g) of supernatant after drying} \times 100}{\text{Weight (g) of sample}}.$$

2.2.2. Swelling Capacity

Swelling capacity was investigated using a modification of Gorecka *et al*.'s [13] and Sowbhaya *et al*.'s [14] method. In brief, 0.5 g of TDF and IDF were allocated into three different 15 ml containers with 1 M phosphate buffer, which maintained the pH value at 6.6, 1.8, and 8.7, and the mixture was allowed to swell for 7 min, 135 min, and 60 min, respectively, to assess experimental pH conditions that may be encountered in specific portions of the human gastrointestinal tract (GIT; i.e., oral cavity: pH 6.6, passage time 7 min; stomach: pH 1.8 and 135 min; and duodenum: pH 8.7 and 60 min). The final volumes were determined and expressed as ml of swollen sample per gram of dry initial sample using the formula:

$$\text{Swelling capacity} = \frac{\text{Volume (ml) of final dietary fiber}}{\text{Weight (g) of initial dietary fiber}}.$$

2.2.3. Water-Binding Capacity (WBC)

WBC was determined using a modification of the method described by Gorecka *et al*. [13] and Sowbhaya *et al*. [14] by adding 0.5 g of TDF or IDF into different 15 ml containers with 1 M phosphate buffer, which maintained the pH at 6.6, 1.8, and 8.7, and the mixture was allowed to swell in the water bath and was shaken at 37 °C for 7 min, 135 min, and 60 min, respectively, to assess experimental pH conditions that may be encountered in specific portions of the human GIT. The mixture was subjected to centrifugation at 3,000 rpm for 15 min, and the supernatants were carefully removed. The tubes were slanted for 30 min to drain off the excess water. The wet mass in each tube was removed, weighed, and dried to achieve a constant weight (± 0.05 mg) at 100 °C. The data obtained were expressed as a gram of water-bound per gram of dry initial sample using the formula:

$$\text{Waterbinding capacity} = \frac{\text{Weight (g) of water-bound dietary fiber}}{\text{Weight (g) of initial dietary fiber}}.$$

2.2.4. Cation Exchange Capacity (CEC)

Cation exchange capacity was determined using the method by Gorecka *et al*. [13] under the same conditions as for WBC. Three different cation exchange capacities were investigated by treating the fiber samples with excess 2M HCl for 48 hours. The wet residue was filtered, and 15 ml of 5% NaCl in buffer, maintaining the pH at 6.6, 1.8, and 8.7, was added to each tube. The samples were incubated and shaken at 37 °C for 7 min, 135 min, and 60 min, respectively. The process of water removal and drying was the same as for WBC. The results were expressed as meq per gram of dry initial sample using the formula:

$$\text{Cation exchange capacity} = \frac{\text{Weight (g) of waterbound dietary fiber}}{\text{Weight (g) of initial dietary fiber}}.$$

2.2.5. Oil-Binding Capacity

The method for measuring binding capacity in oil was modified from that of Sangnark and Noomhorm [15]. In brief, 0.5 g of TDF or IDF was added to 15 ml of different oils (*e.g.*, sunflower oil, rice bran oil, palm oil, soybean oil, and lard). The samples were incubated in a shaker water bath for 1 h at 37 °C. Each tube was subjected to centrifugation at 3,000 rpm for 15 min, and the supernatants were carefully removed to drain off the excess oil. The data obtained were expressed as a gram of oil bound per one gram of dry initial sample using the formula:

$$\text{Oilbinding capacity} = \frac{\text{Weight (g) of oil-bound dietary fiber}}{\text{Weight (g) of initial dietary fiber}}.$$

2.3. Effects of Dietary Fiber on Antihyperglycemic Activity

2.3.1. Determination of Glucose Adsorption Capacity (GAC)

The glucose adsorption capacity was determined according to the method described by Ou *et al*. [16] with some modifications. First, 0.5 grams of TDF or IDF was added to containers with 15 ml of different glucose solutions (5, 10, 20, 50, and 100 mM/L) and incubated in a shaker water bath at 37 °C for 6 hours. After centrifugation at 5,000 rpm for 20 min, the glucose content in the supernatant was measured using the glucose oxidase method to estimate the amount of glucose adsorbed by the fiber sample. Each concentration of the glucose solution was replicated without the addition of fiber as a control. Glucose binding was determined using the formula:

$$\text{Glucose binding} = \frac{(\text{G1h} - \text{G6h})}{\text{Weight of the sample}},$$

where G1h was the initial glucose concentration, and G6h was the final glucose concentration after 6 h of incubation.

2.3.2. Glucose Diffusion and Glucose Dialysis Retardation Index (GDRI)

The glucose diffusion assay was modified from the method of Ou *et al.* [16]. In brief, 0.1 and 0.5 g of TDF or IDF was added to a dialysis tube with a molecular weight cutoff value of 12,000 D. Then, 5 ml of a series of different glucose concentrations (5, 10, 20, and 100 mM) was mixed with the fiber in each dialysis tube. The mixture was then dialyzed against 40 ml of distilled water and incubated in a shaker water bath at 37 °C for 3 h to mimic intestinal peristalsis. The amount of glucose in the dialysate was measured at regular time intervals (10, 30, 60, and 120 min) during incubation using a glucose oxidase–peroxidase method by means of spectrophotometry. A control test was performed without the addition of fiber. The glucose content in the dialysate was measured, and GDRI was calculated using the formula:

$$\text{GDRI} = \frac{100 - (\text{Glucose content in dialysate with fiber} \times 100)}{\text{Glucose content in dialysate of control}}.$$

2.3.4 Analysis of Alpha-Amylase Activity

The amylase inhibitory effect (%) was determined using 0.5 g of TDF or IDF incubated in a shaker water bath at 37 °C for 1 h with a mixture of 0.4% v/v alpha-amylase in 10 ml of potato starch solution (4% w/v). Starch digestion was halted by the addition of 0.5 ml of 50% acetic acid. Each tube was subjected to centrifugation at 3500 rpm for 15 min followed by measurement of the final glucose content in the mixture using the glucose oxidase–peroxidase method. The amylase inhibitory effect (%) was defined as the decrease in OD compared to the control (without fiber) using the formula:

$$\text{Amylase inhibitory effect (\%)} = \frac{(\text{OD control} - \text{OD test}) \times 100}{\text{OD control}}.$$

2.3.4. Alpha-Glucosidase Inhibition

The working substrate for alpha-glucosidase inhibition was prepared by mixing 1 mM p-nitrophenyl α-D-glucopyranoside with 0.1 M sodium phosphate buffer (pH 6.9). The initial concentration of α-glucosidase was 1 U/ml. Then, 0.1, 0.5, and 1g of TDF or IDF was added to tubes containing 3,000 µl of α-glucosidase. The mixture was then incubated in a shaker water bath at 37 °C and 120 rpm for 10 min, followed by the addition of 1,500 µl of p-nitrophenyl α-D-glucopyranoside. The tubes were then incubated again in the shaker water bath at 37 °C and 120 rpm for 20 min. The reaction was halted by adding 6,000 µl of 1 M Na_2CO_3 followed by centrifugation at 3,000 rpm for 10 min. The supernatant was analyzed for α-glucosidase activity by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm. The results were expressed as a percentage of the blank control, as follows:

$$\text{\% alpha-glucosidase inhibition} = \frac{(\text{OD control} - \text{OD test}) \times 100}{\text{OD control}}.$$

2.4. Effects of Dietary Fiber on Antihypercholesterolemic Activity

2.4.1 Sodium Cholate Binding

Sodium cholate binding was determined according to the method described by Zhang *et al*. [17], with slight modifications. First, 0.5 g of TDF or IDF was incubated with 15 ml of sodium cholate at a concentration of 0.25 mg/mL in phosphate buffer (pH 7.0). The mixture was then incubated in a shaker water bath at 37 °C at 120 rpm for 3h. Then, 50 µl of the supernatant was transferred to the polypropylene tube, 800 µl of concentrated sulfuric acid was added, and mixed well using a vortex mixer and left for 10 min at RT. The amount of sodium cholate was determined via spectrometry at 389 nm. Sodium cholate binding was calculated using the formula:

$$\text{Sodium cholate binding} = \frac{(\text{C before} - \text{C after}) \times \text{volume of solution}}{\text{Weight of the sample}},$$

where C before was the initial concentration of sodium cholate and C after was the final concentration of sodium cholate after 3 h of incubation.

2.4.2. Binding of Cholesterol in the Yolk Sac

The solubility of fiber in cholesterol in yolk sacs was determined with a method modified from that of Matsuoka *et al.,* [18]. In brief, 0.5 g of TDF or IDF was mixed with 15 ml of dilute 1:50 and 1:100 egg yolk sacs in distilled water. The mixture was then incubated at RT for 3 h. Then, 10 µl of the supernatant was used to determine the remaining cholesterol using cholesterol oxidase techniques. Cholesterol binding was calculated using the formula:

$$\text{Cholesterol binding} = \frac{(\text{Cho before} - \text{Cho after}) \times \text{volume of solution}}{\text{Weight of the sample}},$$

where Cho before was the initial concentration of cholesterol and Cho after was the final concentration of cholesterol after 3 h of incubation.

2.4.3. Pancreatic Cholesterol Esterase Inhibition

Pancreatic cholesterol esterase inhibition was determined via the addition of 0.5 g TDF or IDF (stirred well) into a mixture of 5.16 mM taurocholic acid, 0.2 mM *p*-NPB, 100 mM sodium phosphate buffer, and 100 mM NaCl (pH 7.0). Porcine pancreatic cholesterol esterase (1 mg/mL) was added to this mixture, which was then incubated at 25 °C for 5 min. The supernatant was analyzed for pancreatic cholesterol esterase activity by measuring OD at 405 nm; simvastatin was used as a positive control. Analysis of cholesterol esterase inhibition (%) was calculated compared to the control (without fiber) using the formula:

$$\%\ \text{Cholesterol esterase inhibition} = \frac{(\text{OD control} - \text{OD test}) \times 100}{\text{OD control}}.$$

2.5. Statistical Analysis

Each measurement was made in triplicate. The results are expressed as mean ± SD. Data were statistically analyzed by one-way analysis of variance (ANOVA) for significance ($p \leq 0.05$) and the Tukey's test was used for multiple means comparisons at the 95% confidence level. SPSS (version 17) was used for all statistical analyses.

3. RESULTS

The selected lettuces consumed as salad vegetables can be considered appropriate sources of dietary fiber. Physicochemical properties, including water solubility and swelling, water-binding, cation-exchange, and oil-binding capacities, of each dietary fiber are presented in Tables **1** and **2**. When fiber was in the insoluble form, the oil-binding and swelling capacity increased, although water-binding capacity decreased. However, water solubility and cation-exchange capacity varied depending on the type of fiber. Water solubility was highest for red oak TDF (23.00 ± 0.01%), and swelling capacity was highest under oral cavity and duodenum conditions for red coral IDF (8.11 ± 0.31and 6.58 ± 0.47 ml/g, respectively) and under stomach conditions for red oak IDF (9.23 ± 0.04 ml/g). Water-binding capacity under oral cavity conditions was highest for cos lettuce TDF (11.14 ± 1.22 g/g) and under stomach and duodenum conditions for red coral TDF (8.53 ± 1.56 and 8.97 ± 0.34 g/g, respectively), whereas cation-exchange capacity under oral cavity and stomach conditions were highest for green oak IDF (8.98 ± 0.08 and 6.48 ± 0.01 g/g, respectively) and under duodenum conditions for cos lettuce IDF (7.46 ± 0.00 g/g). The oil-binding capacity of red oak IDF was highest for sunflower oil (9.81 ± 0.01 g/g), rice bran oil (9.75 ± 0.07 g/g), lard (7.91 ± 0.31 g/g), and palm oil (9.03 ± 0.14 g/g), whereas cos lettuce IDF showed the highest biding capacity in the case of soybean oil (7.67 ± 0.74 g/g). The majority of fiber samples from the lettuces studied showed significantly higher ($p < 0.05$) swelling, water-binding, and cation-exchange capacities and water solubility than cellulose**.** The oil-binding capacities of the fiber from the selected lettuces were lower than that of cellulose.

Table 1. Solubility and swelling capacity of total dietary fiber (TDF) and insoluble dietary fiber (IDF) from various types of lettuce were determined under experimental pH conditions that may be encountered in specific portions of the human gastrointestinal tract (i.e., oral cavity: pH 6.6, passage time 7 min; stomach: pH 1.8 and 135 min; and duodenum: pH 8.7 and 60 min). Values are expressed as mean ± SD, n = 3. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*$p < 0.05$).

Treatment	Type of fiber	Solubility (%)	Swelling capacity (ml/g)		
			Oral Cavity pH 6.6 Incubation Time 7 min	Stomach pH 1.8 Incubation Time 135 min	Duodenum pH 8.7 Incubation Time 60 min
1.	Red oak total dietary fiber	23.00 ± 0.01*	3.23 ± 0.05	3.91 ± 0.01	3.67 ± 0.03*
	insoluble fiber	19.00 ± 0.01*	5.34 ± 0.01	9.23 ± 0.04*	4.92 ± 0.12*
2.	Red coral total dietary fiber	7.50 ± 0.71*	3.61 ± 0.01	4.07± 0.54*	3.57 ± 0.37*
	insoluble fiber	6.00 ± 0.83*	8.11 ± 0.31*	5.50 ± 0.01*	6.58 ± 0.47*
3.	Green oak total dietary fiber	6.00 ± 0.00*	2.93 ± 0.12	3.6 ± 0.45	2.41 ± 0.14
	insoluble fiber	7.50 ± 0.71*	5.30 ± 0.28*	6.79 ± 0.13*	5.17 ± 0.03*
4.	Cos total dietary fiber	2.00 ± 0.00*	4.00 ± 0.86	4.31 ± 0.75	2.78 ± 0.01
	insoluble fiber	8.50 ± 0.71*	6.15 ± 0.66*	6.23 ±0.35*	4.90 ± 0.68*
5.	Butterhead total dietary fiber	15.00 ± 0.01*	3.37 ± 0.05	4.35 ± 0.01	2.70 ± 0.04
	insoluble fiber	17.00 ± 0.01*	6.05 ± 0.07*	6.19 ± 0.01*	4.85 ± 0.22*
Control	Cellulose	1.25± 0.03	3.53 ± 0.17	2.47 ± 001	2.87 ± 0.01

Table 2. Water-binding, cation-exchange, and oil-binding capacities of total dietary fiber (TDF) and insoluble fiber (IDF) from different types of lettuce under simulated conditions that may be encountered in specific parts of the human gastrointesti-

nal tract (i.e., oral cavity: pH 6.6, passage time 7 min; stomach: pH 1.8 and 135 min; and duodenum: pH 8.7 and 60 min). The results are shown as mean ± SD. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*$p < 0.05$).

Treatment	Type of Fiber	Water-binding capacity (g/g)			Cation-exchange capacity (g/g)			Oil-binding capacity (g/g)				
		Oral cavity pH 6.6 incubation time 7 min	Stomach pH 1.8 incubation time 135 min	Duodenum pH 8.7 incubation time 60 min	Oral cavity pH 6.6 incubation time 7 min	Stomach pH 1.8 incubation time 135 min	Duodenum pH 8.7 incubation time 60 min	Sunflower oil	Rice bran oil	Lard	Soybean oil	Palm oil
1.	Red oak total dietary fiber	7.40 ± 0.11*	3.71 ± 0.01	4.07 ± 0.04*	3.34 ± 0.01*	3.92 ± 0.01*	4.27 ± 0.01*	5.08 ± 0.04	5.37 ± 0.03	4.50 ± 0.16	5.00 ± 0.03	5.62 ± 0.04
	insoluble fiber	2.93 ± 0.04*	6.02± 0.03*	6.81 ± 0.20*	5.77 ± 0.03*	4.73 ± 0.02*	5.49 ± 0.04*	9.81 ± 0.01*	9.75 ± 0.07*	7.91 ± 0.31	7.12 ± 0.04	9.03 ± 0.14
2.	Red coral total dietary fiber	9.26 ± 1.96*	8.53 ± 1.56*	8.97 ± 0.34*	5.36 ± 0.08*	5.62 ± 0.03*	5.74 ± 0.01*	3.99 ± 0.01	3.98 ± 0.01	4.06 ± 0.01	3.97 ± 0.42	4.20 ± 0.23
	insoluble fiber	4.69 ± 0.08*	2.88 ± 0.03	3.26 ± 0.17*	3.44 ± 0.01*	4.80 ± 0.76*	5.38 ± 0.40*	6.77 ± 0.37	6.65 ± 0.83	7.20 ± 0.34	6.87 ± 0.33	5.40 ± 0.54
3.	Green oak total dietary fiber	10.30 ± 1.12*	5.49 ± 1.32*	2.79 ± 0.12	5.13 ± 0.06*	4.85 ± 0.06*	5.40 ± 0.23*	4.48 ± 0.01	3.66 ± 0.01	4.66 ± 0.01	4.62 ± 0.01	3.78 ± 0.11
	insoluble fiber	5.69 ± 0.35*	5.84 ± 0.11*	4.48 ± 0.28*	8.98 ± 0.08*	6.48 ± 0.01*	7.15 ± 0.09*	5.60 ± 0.81	5.84 ± 0.58	5.79± 0.33	6.10 ± 0.74	6.20 ± 0.47
4.	Cos total dietary fiber	11.14 ± 1.22*	7.66 ± 0.13*	4.97 ± 0.16*	4.44 ± 0.02*	6.20 ± 0.11*	5.92 ± 0.00*	3.54 ± 0.01	3.78 ± 0.01	3.52 ± 0.01	3.87 ± 0.01	4.47 ± 0.14
	insoluble fiber	2.60 ± 0.15*	2.60 ± 0.40	6.17 ± 1.08*	5.64± 0.00*	5.50± 0.09*	7.46 ± 0.00*	7.32 ± 0.53	6.78 ± 0.81	6.99 ± 0.52	7.67 ± 0.74	7.17 ± 0.65
5.	Butterhead total dietary fiber	6.52 ± 0.11*	3.24 ± 0.02	6.43 ± 0.10*	4.27 ± 0.01*	3.24 ± 0.01*	3.52 ± 0.01*	6.35 ± 0.01	6.13 ± 0.04	5.34 ± 0.06	5.46 ± 0.04	5.96 ± 0.25
	insoluble fiber	3.96 ± 0.11*	2.46 ± 0.09	2.52 ± 0.02	3.73 ± 0.01*	3.70 ± 0.01*	4.45 ± 0.01*	7.61 ± 0.04	6.70 ± 0.63	6.86 ± 0.24	7.41 ± 0.16	7.65 ± 0.42
Control	Cellulose	1.67 ± 0.01	1.46 ± 0.02	1.65 ± 0.01	1.95 ± 0.01	1.85 ± 0.01	1.86 ± 0.01	9.11 ± 0.02	8.81 ± 0.16	8.81 ± 0.16	9.57 ± 0.06	9.66 ± 0.06

3.1. Effect on Glucose Adsorption

A series of different glucose concentrations (5–100 mmol/L) were used to study the glucose adsorption capacity of the lettuces consumed as salad vegetables. As seen in Fig. (**1**), when a low glucose concentration was used (5 and 10 mmol/L), the glucose adsorption capacity of dietary fiber (8.75 ± 0.02 and 17.29 ± 0.06, respectively) was lower than that of cellulose. At 5 and 10 mmol/L of glucose concentration, green oak TDF (1.50 ± 0.24 and 6.69 ± 0.02 mmol/g, respectively) had the highest adsorption capacity. However, when a high glucose concentration (20 and 50 mmol/L) was used, the adsorption capacity of dietary fiber was higher than that of cellulose. The glucose adsorption capacities of red coral TDF and green oak IDF at different glucose concentrations (33.56 ± 0.98 and 37.59 ± 0.39 mmol/g) were significantly higher ($p < 0.05$) compared to that of cellulose (15.25 ± 1.37 and 28.40 ± 1.18, respectively). At high glucose concentrations (100mmol/L), the highest adsorption capacity of green oak TDF (40.08 ± 1.77) was lower than that of cellulose (44.38 ± 1.18).

3.2. Effect on Glucose Diffusion

In this study, the effect of lettuce dietary fiber on glucose diffusion was compared to that of the control from 30 min to 180 min. All types of fiber significantly decreased ($p < 0.05$) the diffusion of glucose across the dialysis membrane when compared to the control (see Table **3**). The dialysate glucose content for lettuce fiber and cellulose ranged from 1.73 ± 0.31 to 4.70 ± 0.06 μmol at 30 min and from 3.01 ± 0.00 to 9.60 ± 0.08 μmol at 180 min. Glucose diffusion was lowest for green oak IDF at 30 and 60 min (1.77 ± 0.03 and 2.18 ± 0.11μmol, respectively) compared to the control and the other fiber. After incubation for 180 min, the glucose content in the dialysate was significantly reduced ($p < 0.05$) by all types of fiber tested compared to the control; however, the reduction was highest for green oak IDF (3.39 ± 0.07 μmol). Moreover, glucose diffusion was low for the total fiber of each type of lettuce compared to IDF.

3.3. Effect on the Glucose Dialysis Retardation Index (GDRI)

The effects of lettuce dietary fiber on GDRI over time are reported in Table **3**. The maximum GDRI was reached after 30 min for all types of fiber tested, and the values were reduced over time. At 30 min, the GDRI of all lettuces studied was 80.76 ± 0.97%. Furthermore, cos lettuce TDF had the highest GDRI value (80.76 ± 0.97%) among the lettuce fiber studied, which was significantly higher ($p < 0.05$) compared to that of cellulose (41.78 ± 0.09%) at 30 min. A similar trend was observed at 60, 120, and 180 min of incubation.

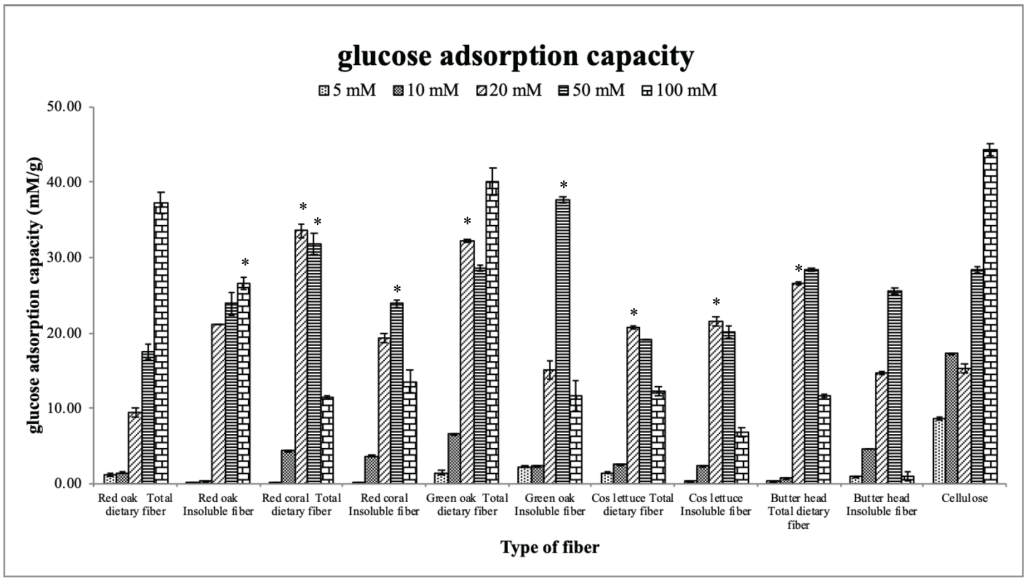

Fig. (1). Glucose adsorption capacities (GAC) of total dietary fiber (TDF) and insoluble fiber (IDF) from various types of lettuce were determined for different glucose concentrations (5, 10, 20, 50, and 100 mmol/L). Values are expressed as mean ± SD, n = 3. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*$p < 0.05$).

Table 3. Relationships between glucose diffusion, glucose dialysis retardation index (GDRI) (5, 10, 20, and 100 mmol/L) and time of incubation (30, 60, 120, and 180 mins) of total dietary fiber (TDF) and insoluble fiber (IDF) from various types of lettuce. Values are expressed as mean ± SD, n =3. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*p<0.05).

No.	Type of Fiber	Glucose Diffusion (Glucose Dialysis Retardation Index [GDRI])			
		30 mins	60 mins	120 mins	180 mins
1.	Red oak total dietary fiber	3.48 ± 0.11 (74.04 ± 1.48) *	4.52 ± 0.08 (67.62 ± 0.96) *	5.47 ± 0.03 (66.13 ± 0.11) *	6.18 ± 0.03 (64.32 ± 0.23) *
	insoluble fiber	1.73 ± 0.31 (36.80 ± 6.96)	1.87 ± 0.06 (27.89 ± 0.95)	4.11 ± 0.11 (49.70 ± 1.18) *	4.50 ± 0.11 (46.89 ± 0.78) *
2.	Red coral total dietary fiber	1.79 ± 0.00 (38.02 ± 0.46)	2.44 ± 0.08(36.42 ± 1.09)	2.93 ± 0.00 (35.42 ± 0.12)	3.34 ± 0.03 (34.80 ± 0.57)
	insoluble fiber	3.44 ± 0.00 (73.21 ± 0.88) *	4.23 ± 0.00 (63.21 ± 0.27) *	4.84 ± 0.03 (58.51 ± 0.54) *	5.49 ± 0.00 (57.15 ± 0.46) *
3.	Green oak total dietary fiber	2.83 ± 0.47 (60.16 ± 9.35) *	3.81 ± 0.08 (57.03 ± 1.49)	4.58 ± 0.00 (55.42 ± 0.19) *	4.98 ± 0.00 (51.82 ± 0.42) *
	insoluble fiber	1.77 ± 0.03 (37.60 ± 1.05)	2.18 ± 0.11 (32.60 ± 1.80)	2.59 ± 0.14 (31.37 ± 1.79)	3.01 ± 0.00 (31.31 ± 0.25)
4.	Cos total dietary fiber	3.80 ± 0.00 (80.76 ± 0.97) *	4.54 ± 0.06 (67.92 ± 1.12) *	5.76 ± 0.00 (69.70 ± 0.24) *	6.53 ± 0.03 (68.02 ± 0.84) *
	insoluble fiber	1.98 ± 0.00 (42.21 ± 0.51)	2.55 ± 0.03 (38.19 ± 0.58)	2.99 ± 0.08 (36.13 ± 1.13)	3.39 ± 0.07 (35.31 ± 1.01)
5.	Butter head total dietary fiber	2.34 ± 0.06 (49.76 ± 1.78)	3.13 ± 0.00 (46.72 ± 0.20)	3.42 ± 0.14 (41.37 ± 1.83)	3.81 ± 0.14 (39.71 ± 1.13)
	insoluble fiber	2.85 ± 0.00 (60.64 ± 0.73) *	3.93 ± 0.03 (58.79 ± 0.17)	4.70 ± 0.00 (56.85 ± 0.19) *	5.23 ± 0.14 (54.48 ± 1.01) *
control	Cellulose	1.96 ± 0.03 (41.78 ± 0.09)	2.69 ± 0.00 (40.25 ± 0.17)	3.22 ± 0.03 (38.99 ± 0.47)	3.76 ± 0.11 (39.11 ± 1.48)
	Only glucose in dialysis tube	4.70 ± 0.06	6.69 ± 0.03	8.27 ± 0.03	9.60 ± 0.08

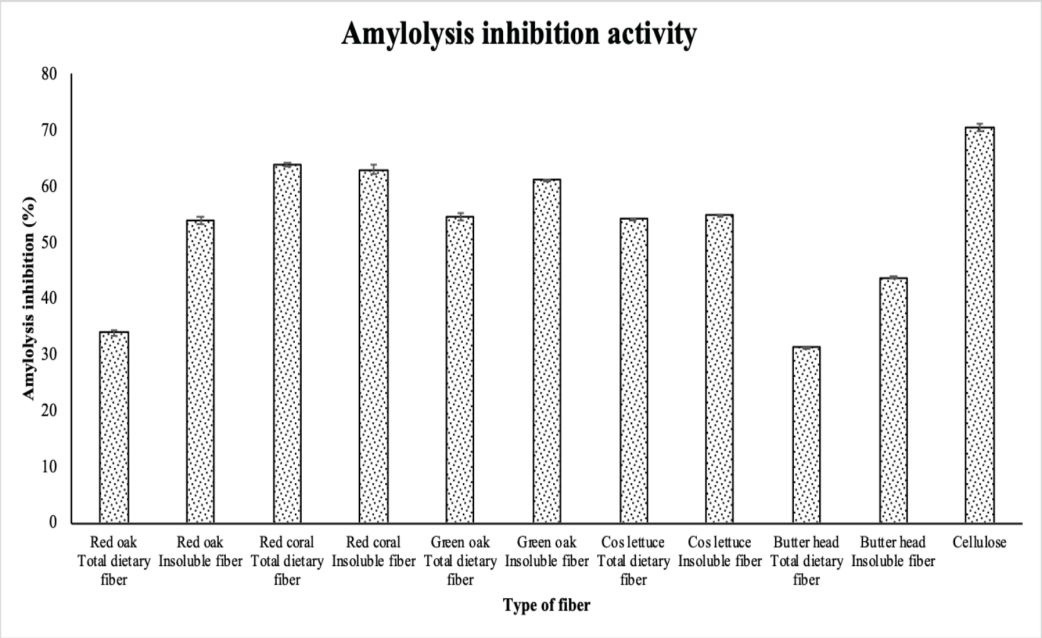

Fig. (2). Inhibition of amylolysis (%) by total dietary fiber (TDF) and insoluble fiber (IDF) from different types of lettuce. Values are shown as mean ± SD, n = 3.

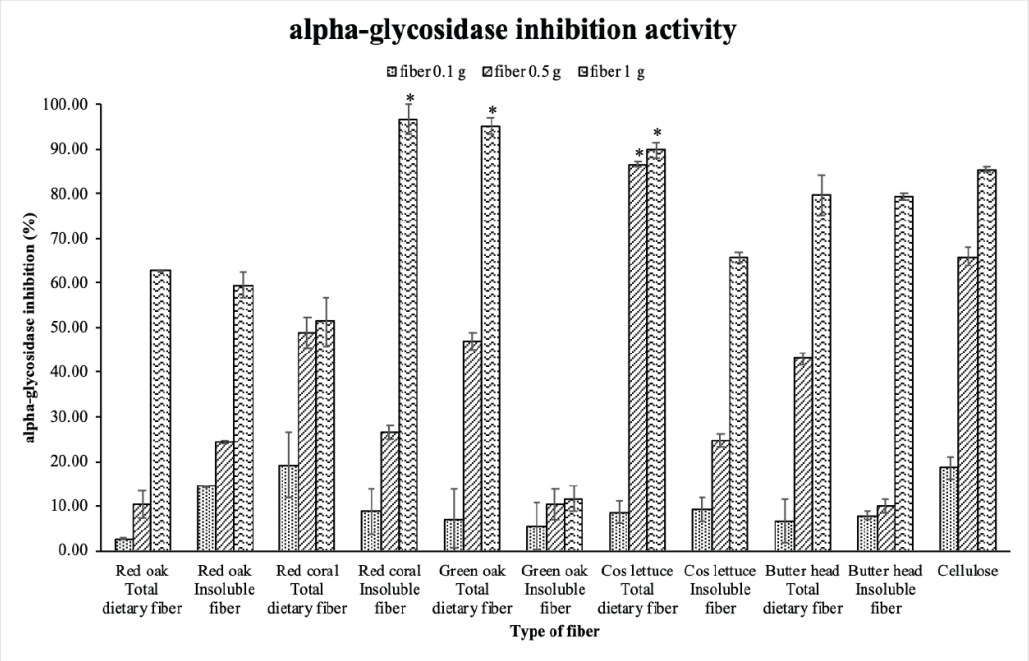

Fig. (3). The percentage inhibition of alpha-glycosidase by total dietary fiber (TDF) and insoluble fiber (IDF) from different types of lettuce at varying fiber concentrations (0.1, 0.5, and 1 g). Values are shown as mean ± SD, n = 3. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*$p < 0.05$).

3.4. Effect on Alpha-amylase Activity

The effect of fiber on alpha-amylase activity is shown as enzyme inhibitory activity (%) in Fig. (**2**). The influence of lettuce dietary fiber on alpha-amylase activity was similar to that of cellulose (70.48 ± 0.45%). Red coral TDF exhibited a stronger effect in reducing alpha-amylase activity (by 63.83 ± 0.23%). These results showed that lettuces consumed as salad vegetables can decrease amylase activity.

3.5. Effect on Alpha-glucosidase Inhibition

The effect of fiber on alpha-glucosidase is shown as enzyme inhibitory activity (%) in Fig. (**3**). Alpha-glucosidase activity was also influenced by dietary fiber. Moreover, the degree of inhibition was dependent on the amount of fiber. At 0.1 g of fiber, red coral TDF had a stronger effect on alpha-glucosidase activity and reduced it by 19.16 ± 7.44%, which was similar to the effect of cellulose (18.5 ± 0.00%).

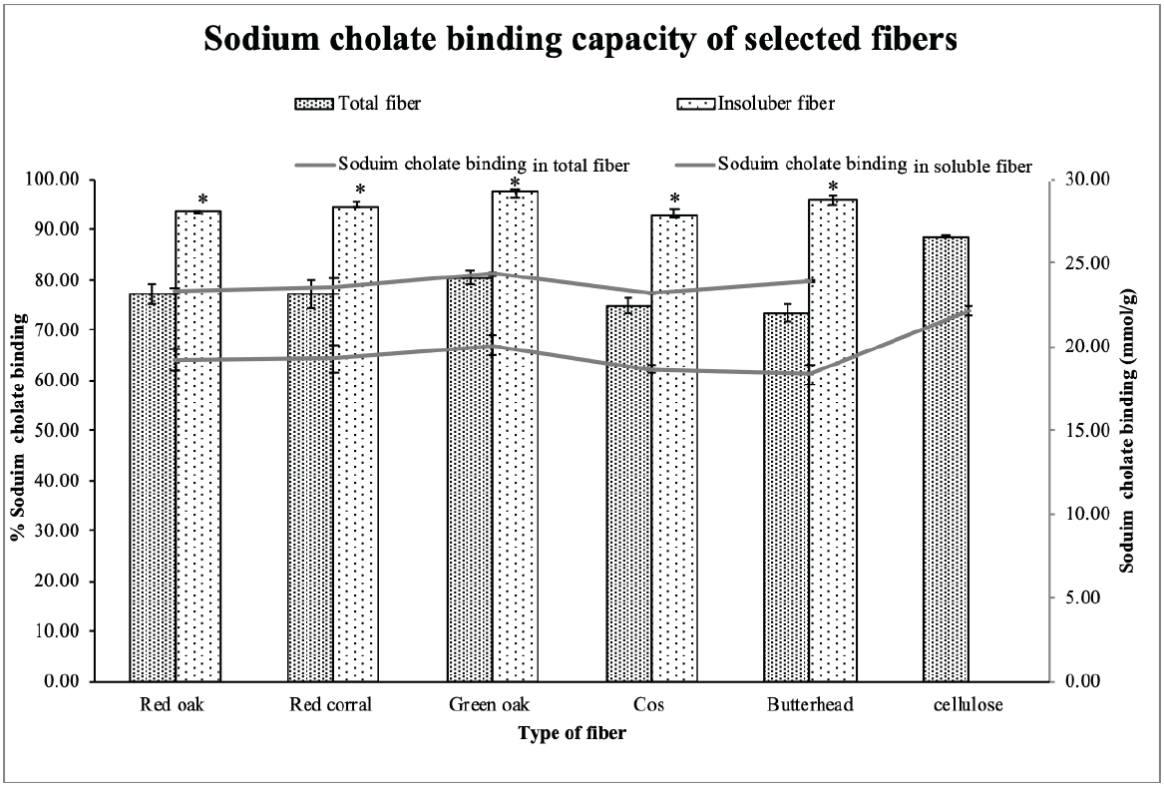

Fig. (4). Sodium cholate adsorption and percentage of sodium cholate binding by total dietary fiber (TDF) and insoluble fiber (IDF) from different types of lettuce. Values are expressed as mean ± SD, n = 3. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*$p < 0.05$).

On increasing the concentration of fiber to 0.5 and 1 g, cos lettuce TDF and red coral IDF significantly reduced ($p < 0.05$) alpha-glucosidase activity (86.75 ± 0.62% and 96.72 ± 3.35%, respectively) compared to cellulose (66.02 ± 0.00% and 85.62 ± 0.08%, respectively).

3.6. Capacity to Bind Sodium Cholate

The percentage of sodium cholate binding to vegetable dietary fiber (mmol per g fiber) is presented in Fig. (**4**). All tested IDFs were capable of interacting with sodium cholate with varying degrees of binding higher than that of total fiber. The percentage of sodium cholate bound to fiber ranged from 73.44 ± 1.72% to 97.59 ± 0.46% (18.34 ± 0.61 to 24.37 ± 0.12 mmol/g). Green oak IDF was the most effective, with a significantly higher ($p < 0.01$) sodium cholate binding capacity of 97.59 ± 0.46% (24.37 ± 0.12 mmol/g) compared to the other fiber sources. Furthermore, butterhead IDF, red coral IDF, and red oak IDF also exhibited comparatively higher sodium cholate adsorption values of 95.87 ± 0.94%, 94.48 ± 1.03%, and 93.62 ± 0.18%, respectively (95.87 ± 0.94, 23.59 ± 0.48, 41.18 ± 3.02, and 40.26 ± 0.91 mmol/g, respectively).

3.7. Capacity to Bind Cholesterol in Yolk Sac

The percentage of cholesterol binding to vegetable dietary fiber and cholesterol bound in terms of mg per g of fiber are presented in Table **4**. Most of the tested IDFs were capable of binding cholesterol at varying degrees higher than that of total fiber in a dilution-dependent manner. The percentage of cholesterol bound to fiber ranged from 73.49 ± 0.13 to 76.40 ± 0.03% (2957.81 ± 5.30 to 3074.99 ± 1.33 mg/g). Among the types of fiber, butterhead TDF was the most effective, with the highest cholesterol-binding capacity at 76.40 ± 0.03% (3074.99 ± 1.33 mg/g) compared to that of cellulose (73.49 ± 0.13%; 2957.81 ± 5.30 mg/g). Furthermore, red coral IDF, butterhead IDF, and green oak IDF also exhibited comparatively higher adsorption of cholesterol at 75.91 ± 0.40, 75.77 ± 0.33, and 75.15 ± 0.03%, respectively (3055.3 ± 15.91, 3049.68 ± 13.26, and 3024.37 ± 1.33 mg/g, respectively).

3.8. Effect of Fiber on Cholesterol Esterase Inhibition

The effect of fiber on cholesterol esterase is presented as enzyme inhibitory activity (%) in Fig. (**5**). The activity of cholesterol esterase was also influenced by the type of vegetable dietary fiber. Moreover, the degree of inhibition was dependent on the amount of fiber present. At 0.1 g of fiber, butterhead IDF reduced cholesterol esterase activity (by 74.60 ± 1.23%) more strongly than did cellulose (by 53.64 ± 9.47%). On increasing the amount of fiber to 0.5 g, cos lettuce IDF reduced cholesterol esterase activity (80.71 ± 3.94%) more strongly than did cellulose (81.92 ± 0.12%).

4. DISCUSSION

The results of this study demonstrated that each type of dietary fiber had its unique physicochemical characteristics depending on its form; fiber obtained with enzymes and thermal treatment exhibited elevated physicochemical properties. Enzyme digestion and heating may potentially modify the structural characteristics of the fiber, thereby facilitating its water uptake [19].

Table 4. Cholesterol adsorption and percentage of cholesterol binding at varying concentrations (dilution 1:50 and 1:100) by total dietary fiber (TDF) and insoluble fiber (IDF) from different types of lettuce. Values are expressed as mean ± SD, n = 3. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*$p < 0.05$).

Treatment	Type of fiber	Cholesterol bound (% cholesterol binding)	
		Dilution 1:100 Control =134.16 ± 0.13 mg/dl	Dilution 1:50 Control = 147.66 ± 0.31 mg/dl
1.	Red oak total dietary fiber insoluble fiber	2992.49 ± 1.33 (74.35 ± 0.03) 2998.12 ± 19.89 (74.49 ± 0.49) *	3163.59 ± 7.29 (71.42 ± 0.16) 3402.65 ± 3.31 (76.81 ± 0.07) *
2.	Red coral total dietary fiber insoluble fiber	2991.56 ± 2.65 (74.33 ± 0.07) 3055.31 ± 15.91 (75.91 ± 0.40) *	3202.96 ± 1.99 (72.31 ± 0.04) * 3345.46 ± 0.66 (75.52 ± 0.01) *
3.	Green oak total dietary fiber insoluble fiber	2974.68 ± 13.26 (73.91 ± 0.33) 3024.37 ± 1.33 (75.15 ± 0.03) *	3354.84 ± 4.64 (75.74 ± 0.01) * 3444.84 ± 4.64 (77.77 ± 0.01) *
4.	Cos total dietary fiber insoluble fiber	3000.93 ± 2.65 (74.56 ± 0.07) * 3046.87 ± 11.93 (75.70 ± 0.30) *	3261.09 ± 0.66 (73.62 ± 0.01) * 2167.02 ± 33. 81 (48.92 ± 0.76) *
5.	Butterhead total dietary fiber insoluble fiber	3074.99 ± 1.33 (76.40 ± 0.03) * 3049.68 ± 13.26 (75.77 ± 0.33) *	3376.40 ± 3.31 (76.22 ± 0.07) * 2927.34 ± 77.56 (66.08 ± 1.75) *
control	Cellulose	2957.81 ± 5.30 (73.49 ± 0.13)	3908.90 ± 23.20 (69.96 ± 0.52)

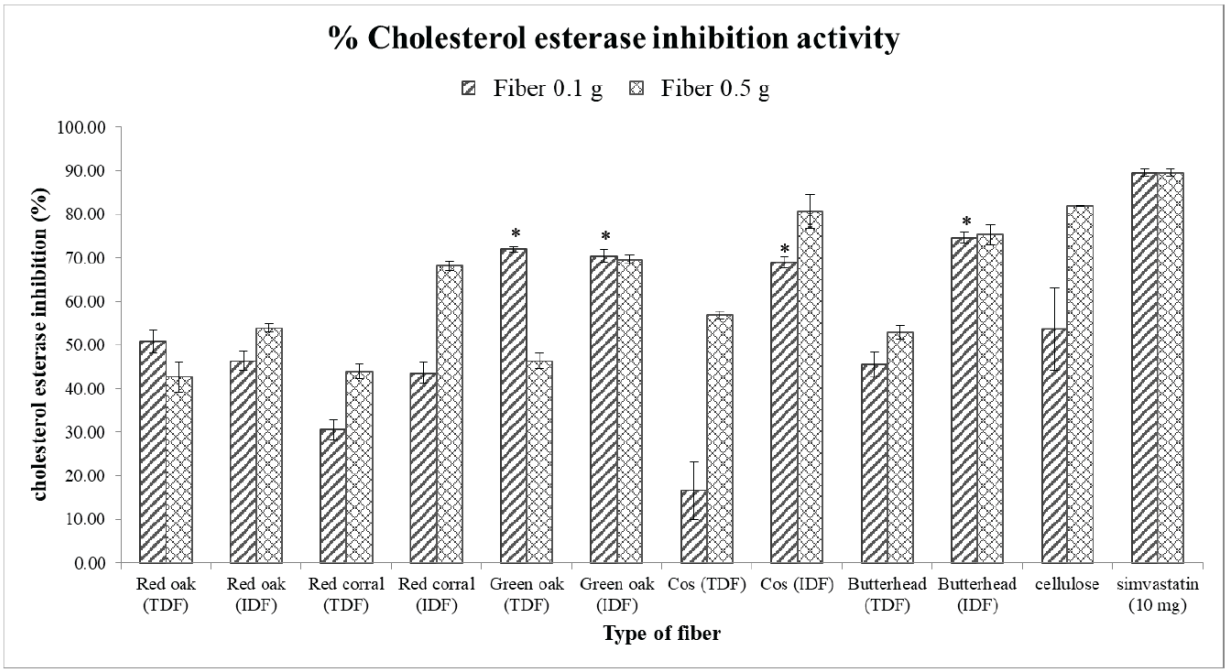

Fig. (5). Percentage inhibition of cholesterol esterase activity by total dietary fiber (TDF) and insoluble fiber (IDF) from different types of lettuce at varying fiber concentrations (0.1 and 0.5 g). Values are expressed as mean ± SD, n = 3. The significance of differences from the control (cellulose) was determined by ANOVA followed by Dunnett's test (*$p < 0.05$).

Excessive sugar intake may lead to a greater risk of diabetes. However, fiber taken with meals may help reduce the absorption of sugar in the digestive tract. The effects of salad vegetable fiber on glucose adsorption in this study indicate that fiber could effectively bind to glucose at high glucose concentrations but does not disturb glucose absorption at lower concentrations, thereby reducing the amount of accessible glucose in the small intestine. The glucose adsorption capacity of the fiber is directly related to the available glucose concentration. Chau *et al*. [20] reported a similar ability of IDF-rich fractions from *Averrhoa carambola* to bind glucose and further decrease available glucose. Thus, insoluble vegetable fiber may be beneficial with respect to reducing the amount of accessible glucose in the small intestine.

This study found that lettuce dietary fiber efficiently decreased glucose diffusion across a dialysis membrane with different degrees of diffusion, thereby controlling the glucose level, depending on the duration of incubation and fiber source. Ahmed *et al*. [21] reported that IDFs obtained from wheat and psyllium husk have the ability to bind glucose up to 0.75 ± 0.03 and 0.59 ± 0.03, respectively.

GDRI is useful in vitro index for predicting the ability of fiber to delay glucose absorption in the GIT [16, 22]. The results for GDRI showed that vegetable dietary fiber from different sources exhibited high retardation index values and possibly aided in antihyperglycemic effects. Moreover, fiber from lettuces consumed as salad vegetables may also decrease alpha-glucosidase activity.

Cholesterol is another risk factor for obesity and chronic diseases associated with obesity. Excessive cholesterol has also been found to be associated with coronary risk factors. Many studies have suggested that a high-fiber diet can help reduce the risks of obesity and cardiovascular diseases. The observed variation in enzyme inhibition among the types of fiber studied demonstrates that inhibition depends on the source of vegetable fiber. This effect may be due to several possible factors, such as fiber concentration, the presence of an inhibitor in fiber, encapsulation of starch and enzymes by fiber, and direct adsorption of enzyme on fiber, which reduce the accessibility of starch, subsequently leading to decreased amylase activity [22, 23]. This differential reduction of enzyme activity could be due to compositional differences in IDFs from different sources.

The percentage of sodium cholate binding to lettuce dietary fiber varied. Binding is the result of hydrophobic interaction between IDF and sodium cholate [24]. Palanuvej and coworker [25] identified the binding capacities of IDFs from various seaweeds (7–27 mmol/g), which are comparatively lower than the capacities of the vegetable fiber investigated in this study (40.26–50.55 mmol/g). This result supports the notion that the lettuces studied have good antihypercholesterolemic effects. Furthermore, fiber from the lettuce varieties strongly reduced cholesterol esterase activity, indicating that the vegetable fiber studied has a positive effect. This result was similar to that reported by Mäkynen and coworker [26], who demonstrated that soluble fiber from *Abelmoschus esculentus* inhibited pancreatic cholesterol esterase at IC50 = 3.17 mg/ml, with the inhibitory effect evident in a dose-dependent manner.

In vivo evidence shows that dietary fiber can reduce glucose and lipid metabolism. Bennekum and coworker [27] demonstrated in vivo properties of insoluble dietary fiber with moderate to poor bile acid-binding capacities. The action mechanism can contribute to its ability to suppress the intake of energy. Both satiation (*i.e*., reduction in the amount of a meal) and satiety (*i.e*., inhibition of the need for another meal) can be caused by dietary fiber [28]. Both effects can reduce the intake of some cholesterol from the diet. Moreover, some phytochemicals, such as antioxidants (vitamins C, E, and carotenoids), in lettuce increase the total cholesterol end-product excretion and improve antioxidant status [29].

CONCLUSION

The results of this study showed that the salad lettuce fiber studied has good physical properties and could effectively adsorb glucose, retard glucose diffusion, and inhibit alpha-amylase and alpha-glucosidase activity. It also binds sodium cholate and cholesterol and inhibits cholesterol esterase. Notably, green oak lettuce fiber exhibited the highest antihyperglycemic effect followed by red coral lettuce and cos lettuce fiber. In the case of antihypercholesterolemic activity, green oak lettuce fiber showed the highest sodium cholate-binding capacity, while butterhead fiber had the highest cholesterol-binding capacity and inhibited cholesterol esterase. Therefore, this study suggests that natural dietary fiber from all salad vegetables may be promising and could help to avoid increased postprandial blood glucose and cholesterol levels. Dietary fiber can be important for enhancing certain physiological effects (*e.g.*, antihyperglycemic and antihypercholesterolemic effects) and can be beneficial to human health.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

The study was funded by the Research Institute of Rangsit University for the award of a research grant (number 73/2557).

CONFLICT OF INTEREST

None.

ACKNOWLEDGMENTS

The author would like to express her sincere gratitude to the Research Institute of Rangsit University for the award of a research grant (number 73/2557), and to P. Powthong for providing additional funding. The authors would also like to extend their gratitude to Sun Herb at Thai Chinese Manufacturing, RSU, for providing natural dietary fiber. The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

[1] Eastwood MA, Kay RM. An hypothesis for the action of dietary fiber along the gastrointestinal tract. Am J Clin Nutr 1979; 32(2): 364-7. http://dx.doi.org/10.1093/ajcn/32.2.364 PMID: 420131

[2] Marlett JA. Dietary fiber and cardiovascular disease.Handbook of Dietary Fiber. 1st ed.. New York, U.S.A.: Baker and Taylor Books 2001; pp. 17-30.

[3] Park Y, Hunter DJ, Spiegelman D, *et al*. Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. JAMA 2005; 294(22): 2849-57. http://dx.doi.org/10.1001/jama.294.22.2849 PMID: 16352792

[4] Crowe FL, Balkwill A, Cairns BJ, *et al*. Source of dietary fibre and diverticular disease incidence: a prospective study of UK women. Gut 2014; 63(9): 1450-6. http://dx.doi.org/10.1136/gutjnl-2013-304644 PMID: 24385599

[5] Cicero AF, Colletti A. Role of phytochemicals in the management of metabolic syndrome. Phytomedicine 2016; 23(11): 1134-44. http://dx.doi.org/10.1016/j.phymed.2015.11.009 PMID: 26778479

[6] Adams S, Che D, Qin G, Rui H, Sello CT, Hailong J. Interactions of Dietary Fibre with Nutritional Components on Gut Microbial Composition, Function and Health in Monogastrics. Curr Protein Pept Sci 2018; 19(10): 1011-23. http://dx.doi.org/10.2174/1389203719666180508111843 PMID: 29745332

[7] Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Protein, and Aminoacids. Washington, DC, USA: National Academy of Sciences 2002.

[8] Serafini M, Bugianesi R, Salucci M, Azzini E, Raguzzini A, Maiani G. Effect of acute ingestion of fresh and stored lettuce (*Lactuca sativa*) on plasma total antioxidant capacity and antioxidant levels in human subjects. Br J Nutr 2002; 88(6): 615-23. http://dx.doi.org/10.1079/BJN2002722 PMID: 12493083

[9] Nicolle C, Cardinault N, Gueux E, *et al*. Health effect of vegetable-based diet: lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. Clin Nutr 2004; 23(4): 605-14. http://dx.doi.org/10.1016/j.clnu.2003.10.009 PMID: 15297097

[10] Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, Gil MI, Ferreres F. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 2008; 108(3): 1028-38. http://dx.doi.org/10.1016/j.foodchem.2007.11.032 PMID: 26065768

[11] AOAC. Official Method of Analysis. 15th. Washington, DC., USA: Association of Official Analytical Chemists (AOAC) 1990.

[12] Chau CF, Wang YT, Wen YL. Different micronization methods significantly improve the functionality of carrot insoluble fiber. Food Chem 2007; 100: 1402-8. http://dx.doi.org/10.1016/j.foodchem.2005.11.034

[13] Górecka D, Lampart-Szczapa E, Janitz W, Sokolowska B. Composition of fractional and functional properties of dietary fiber of lupines (L. luteus and L. albus). Nahrung 2000; 44(4): 229-32. http://dx.doi.org/10.1002/1521-3803(20000701)44:4<229::AID-FOOD229>3.0.CO;2-I PMID: 10996894

[14] Sowbhaya HB, Sampathu SR, Krishnamurthy N. Evaluation of size reduction on the yield and quality of celery seed oil. J Food Eng 2007; 80: 1255-60. http://dx.doi.org/10.1016/j.jfoodeng.2006.09.019

[15] Sangnark A, Noomhorm A. Effect of particle sizes on functional properties of dietary fiber prepared from sugarcane bagasse. Food Chem 2003; 80: 221-9. http://dx.doi.org/10.1016/S0308-8146(02)00257-1

[16] Ou S, Kwok K, Li Y, Fu L. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. J Agric Food Chem 2001; 49(2): 1026-9. http://dx.doi.org/10.1021/jf000574n PMID: 11262066

[17] Zhang N, Huang C, Ou S. In vitro binding capacities of three dietary fibers and their mixture for four toxic elements, cholesterol, and bile acid. J Hazard Mater 2011; 186(1): 236-9. http://dx.doi.org/10.1016/j.jhazmat.2010.10.120 PMID: 21095057

[18] Matsuoka K, Kuranaga Y, Moroi Y. Solubilization of cholesterol and polycyclic aromatic compounds into sodium bile salt micelles (part 2). Biochim Biophys Acta 2002; 1580(2-3): 200-14. http://dx.doi.org/10.1016/S1388-1981(01)00204-9 PMID: 11880244

[19] Figuerola F, Hurtado ML, Estévez AM, Chiffelle I, Asenjo G. Fiber concentrates from apple pomace and citrus peel as potential source for food enrichment. Food Chem 2005; 91: 395-401. http://dx.doi.org/10.1016/j.foodchem.2004.04.036

[20] Chau CF, Huang YL, Lee MH. *In vitro* hypoglycemic effects of different insoluble fiber-rich fractions prepared from the peel of Citrus sinensis L. cv. Liucheng. J Agric Food Chem 2003; 51(22): 6623-6. http://dx.doi.org/10.1021/jf034449y PMID: 14558787

[21] Ahmed F, Sairam S, Urooj A. In vitro hypoglycemic effects of selected dietary fiber sources. J Food Sci Technol 2011; 48(3): 285-9. http://dx.doi.org/10.1007/s13197-010-0153-7 PMID: 23572748

[22] Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology 2006; 130(5): 1480-91. http://dx.doi.org/10.1053/j.gastro.2005.11.061 PMID: 16678561

[23] Bisoi PC, Sahoo G, Mishra SK, Das C, Das KL. Hypoglycemic Effects of Insoluble Fiber Rich Fraction of Different Cereals and Millets. J Food Process Technol 2012; 3(7): 1-11.

[24] Yuanita L. Suyono and Sanjaya IM. The binding of cholic acid by hemi cellulose and pectin of yard-Long bean. J Food Science & Engineering 2011; 1(5): 348-53. [Vignasesquipedalis (L.) Fruhw].

[25] Palanuvej C, Hokputsa S, Tunsaringkarn T. *In Vitro* Glucose Entrapment and Alpha-Glucosidase Inhibition of Mucilaginous Substances from Selected Thai Medicinal Plants. Sci Pharm 2009; 77: 837-49. http://dx.doi.org/10.3797/scipharm.0907-17

[26] Mäkynen K, Ngamukote S, Adisakwattana S, *et al*. The okra skin extract and its cholesterol-lowering activities in vitro study. The FASEB Journal 2014; 28(1)

[27] van Bennekum AM, Nguyen DV, Schulthess G, Hauser H, Phillips MC. Mechanisms of cholesterol-lowering effects of dietary insoluble fibres: relationships with intestinal and hepatic cholesterol parameters. Br J Nutr 2005; 94(3): 331-7. http://dx.doi.org/10.1079/BJN20051498 PMID: 16176602

[28] Burton-Freeman B. Dietary fiber and energy regulation. J Nutr 2000; 130(2S)(Suppl.): 272S-5S. http://dx.doi.org/10.1093/jn/130.2.272S PMID: 10721886