SRYAHWA PUBLICATIONS

Research Journal of Food and Nutrition
ISSN: 2637-5583 | Volume 6, Issue 1, 2023
https://doi.org/10.22259/2637-5583.0601001

RESEARCH ARTICLE

Undaria Pinnatifida (Wakame) Moderates Postprandial Glycaemia in Healthy Young Japanese Women

Rieko Mitamura[1], Keiko Yoshinaga[2]

[1]*Faculty of Human Life Sciences, Fuji Women's University, Hokkaido, Japan.*
[2]*Food Planning Development Department, Riken Vitamin Co., Ltd., Tokyo, Japan.*

Received: 16 February 2023 Accepted: 08 March 2023 Published: 27 March 2023

Corresponding Author: Rieko Mitamura, Faculty of Human Life Sciences, Fuji Women's University, Hanakawa Minami 4-5, Ishikari, Hokkaido, 061-3204, Japan.

Abstract

Background and Objectives: Brown seaweeds, such as *Undaria pinnatifida* (wakame), contain viscous dietary fiber sodium alginate, which can affect postprandial blood glucose concentrations. This study aimed to investigate the effects of wakame ingestion on postprandial blood glucose concentrations.

Methods and Study Design: In this randomized controlled cross-over study, healthy young women were recruited.The blood glucose concentrations were measured by self-monitoring at 0 min, and 15, 30, 45, 60, 90 and 120 min after consumed 150 g of rice with or without wakame (1.8 g or 4.0 g dried wakame).Glucose release rate of wakame extracts was measures using a cell-free artificial digestion test.

Results: The blood glucose concentrationat 15 min was significantly lower after consuming rice with 1.8 g of wakame than that of control (5.66±0.13vs 5.99±0.11 mmol/L, p=0.021, mean±SEM). Moreover, the blood glucose concentrations at 15 min (5.27±0.13 vs 5.63±0.12 mmol/L, p<0.001), 30 min (7.45±0.16 vs 7.95±0.18 mmol/L, p=0.002), and 45 min (7.75±0.22 vs 8.23±0.24 mmol/L, p=0.018) in consuming 4.0 g of wakame were all significantly lower than those of control. In the artificial digestion test, the glucose concentrations at 20 min and glucose release rate of all wakame extracts were significantly lower than those of the control (p<0.001). The soluble fraction, which contained sodium alginate, significantly inhibited the release of glucose.

Conclusions: These results suggest that the highly viscous soluble fraction of wakame might increases the viscosity of the gastrointestinal contents and delays glucose absorption.

Keywords: Undaria pinnatifida, wakame, postprandial blood glucose, soluble dietary fiber, glucose release rate

1. Introduction

The traditional Japanese diet is rich in plant-based foods that are often high in dietary fiber, which is not digestible in the upper gastrointestinal tract and has numerous health benefits.[1]Dietary fiber is a heterogeneous food compound with a variety of structures and physical properties. Water-soluble viscous fibers such as β-glucan, guar gum, and alginate, may improve the glycaemic response.[2] High viscosity induces delayed gastric emptying and absorption of glucose from the lumen ofthe small intestine.[2-4]Inhibitors of key enzymes such as α-amylase and α-glucosidase decrease the rate of glucose absorption from the small intestine. Someof these inhibitors are derived from seaweeds.[5,6]One meta-analysis reported that dietary fiber improved

Citation: Rieko Mitamura. *Undaria Pinnatifida* (Wakame) Moderates Postprandial Glycaemia in Healthy Young Japanese Women. Research Journal of Food and Nutrition. 2023;6(1): 01-07.

fasting blood glucose concentrations in patients with type 2 diabetes.[7]However, the reported effects on fasting blood glucose concentrations have differed depending on the type of dietary fiber.

Seaweeds are a good source of water-soluble viscous dietary fibers, which may influence glucose uptake. Wakame, (i.e., the Japanese name for brown seaweed *Undaria pinnatifida*), is an edible seaweed that is popular in Japan. Alginates are present in brown seaweeds, generally as sodium and calcium salts. Sodium alginate forms a gel within the gastrointestinal tract, and may suppress both appetite and glucose absorption by reducing gastric emptying.[2, 8-10]Some studies have shown that ingestion of seaweed or seaweed extract influences postprandial glycaemic control, which is related to slower gastric emptying and delayed glucose absorption.[11-14]However, different effects have been reported based on different seaweed species, locations, and extraction methods.

Our previous clinical trialsindicated that, compared to rice intake alone, blood glucose and insulin concentrations in the early postprandial stage were effectively reduced when wakame was consumed before rice.[15]That study demonstrated that the acute consumption of wakame containing 1.4 g of fiber reduced glycaemic and insulinemic responses; however,the effect of consuming wakame along with rice, and the underlying mechanism behind the effect, are still unclear. Therefore, we aimed to investigate the effects of ingesting wakame with rice on the postprandial blood glucose response. Although the cell wall of wakame contains fucoidan, alginate, and cellulose, viscous dietary fibers such as alginate, which is particularly abundant in wakame,may represent an important tool for controlling postprandial blood glucose.[16]This study conducted both clinical trials to determine the acute dose-response effects of wakame on postprandial blood glucose concentrations among young Japanese women, and *in vitro* cell-free studies to determine the inhibitory activity of wakame extracton the glucose release rate and α-glucosidase activity.

2. Methods

2.1 Subject of Clinical Trial Study

Healthy young Japanese women were recruited from April 21, 2016 to Jun 26, 2019.The exclusion criteria were as follows: fasting blood glucose concentrations >6.9 mmol/L, excessive alcohol intake, possible allergy to seaweed, use of any medicine for any clinical treatment of disease, and taking oral drugs or supplements that could affect blood glucose concentrations.This study was conducted in accordance with the Declaration of Helsinki, and with the approval of the Fuji Women's University Ethics Committee (registration; April 19, 2016 and May30, 2018).The protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000047854). Written consent was obtained from all subjects after explanation of the study purpose, design, and risks of the study. All subjects finished the study.

2.2 Clinical Trial Study Method

An open-label, randomised, two-period, crossover design was used. Subjects were randomly allocated (1:1) to one of two sequences using a computer-generated randomisation list.The subjects consumed 150 g of boiled white rice(Sato Foods Co., Ltd., Niigata, Japan) and wakame soup or soup without wakame at breakfast. The wakame soup was a commercial product (WAKAME SOUP) manufactured by Riken Food Co., Ltd., (Tokyo, Japan). This soup contained 1.8 g of dried wakame, 0.7 g of fiber, 0.9 g of protein, 0.7 g of lipids, and 2.2 g of carbohydrates.The soup without wakame was made using the same soup, only omitting the wakame. Each subject attended two breakfast sessions, which were set at least two days apart from each other. In the second study, the subjects ingested 150 g of boiled white rice, with or without a wakame salad at breakfast. We used 4.0 g of dried wakame (FUERU WAKAME-CHAN® Sanriku), manufactured by Riken Food Co., Ltd., by soaking it in water, draining it, and squeezing out excess water. The 4.0 g of dried wakame contained 1.4 g of fiber, 0.8 g of protein, 0.2 g of lipids, and1.6 g of carbohydrates. All meals were prepared and served by the research group at Fuji Women's University. In all studies, breakfast stared at 9:00 am and all food had to be finished in15 min. Blood glucose concentrations were measuredat 0, 15, 30, 45, 60, 90, and 120 min after consuming the test meal using a simple blood glucose meter (Freestyle-freedom-lite, Abbott Japan LLC, Tokyo, Japan). Incremental area-under-the-curve for glucose (IAUC) was calculated.

2.3 Preparation of Wakame Extracts in Vitro Cell-Free Experiments

The alginate extraction process from wakame is based

on the conversion of alginic acid from the cell wall into alginate salts.[17, 18]Dried wakame, manufactured by Riken Food Co., Ltd. (Tokyo, Japan), was extraction in ethanol (20% w/w) and powdered, which yielded a low-sodium wakame powder. Low-sodium and low-fat wakame powder was prepared from dry wakame powder using acetone extraction. The soluble and insoluble fractions were obtained from the low-sodium and low-fat wakame powder as follows: the powdered samplewith 0.4%w/w trisodium citrate dihydrate was stirred for 60 min. After centrifugation (Avanti HP-20XP, Beckman Coulter Co., Ltd., Tokyo, Japan) for 20 min at 12,000×g, the supernatants were washed with ethanol and acetone, and dried to obtain the water-soluble fraction containing alginate. After solubilizing the alginic acid in the wakame powder, the precipitate was used as an insoluble fraction. Extraction yieldswere as follow; 75% low-sodium powder, 68% low-sodium and low-fat powder, 22% insoluble fraction, and 35% soluble fraction. Commercial alginate (Kikkoman BiochemifaCompany, Japan) was used as a reference.

2.4 Measurement of Glucose Release Rate

The glucose release rate was determined using *in vitro* cell-free artificial digestion. Cooked white rice was used as a control. This rice was digested by several enzymes, so the free glucose concentration could be measured. The wakame extracts were added to the cooked rice, to final concentration of 6 g/L. Briefly, 0.3%pepsin with 1N hydrochloric acid was added to simulate gastric digestion for 30 min, and 0.3% pancreatin with 0.5% invertase were added to simulate intraluminal digestion for either 20 min or 16 hours. The supernatant obtained from each sample was analysed using the crude enzyme α-glucosidase obtained from rat intestinal acetone powder (Sigma-Aldrich, St Louis, MO,USA). The glucose concentration of each sample was measured using a commercial kit (glucose C-II test Wako, FUJIFILM Wako Pure Chemical Co., Osaka, Japan). The glucose release rate was calculated as follows: % glucose release = (20 min glucose concentration / 16 h glucose concentration) ×100.

2.5 Measurement of α-Glucosidase Activity

Measurement of α-glucosidase was determined by glucose production from amaltose solution. The crude α-glucosidase enzyme was obtained from rat intestinal acetone powder (Sigma-Aldrich, St Louis, MO,USA).The maltose solution with added wakame extracts at a final concentration of 3g/L, were added to the α-glucosidase and incubated at 37 °C for 40 min. For the positive control, 1 mg/mL of acarbose was used instead of wakame extract. The amount of glucose was measured in the same manner as the glucose-release rate.

Analytical grade chemical reagents were used in the *in vitro* experiments. Ethanol, acetone, trisodium citrate dihydrate, hydrochloric acid, sodium carbonate, pepsin, pancreatin, maltose, and acarbose were obtained from FUJIFILM Wako Pure Chemical Co., (Osaka, Japan).

2.6 Statistical Analysis

Data are shown as the means ± standard error of the mean (SEM). Statistical differences were determined using Wilcoxon signed-rank test for all glycaemic parametersin the clinical trial study, and by Duncan's multiple range test for the *in vitro* study. All analyses were performed with SPSS Statistics version 25 software (IBM Corp., Armonk, NY, USA).Statistically significant differences were determined at $p<0.05$.

3. Result

3.1 Clinical Trial Study

All subjects completed the study. Treatment compliance was complete and no side effects were reported from the test meal during the study. The first study included 12 healthy women aged 24.8 ±2.22 years with body mass index of 19.4±0.50 kg/m^2. The subjects ingested 150 g of white rice with wakame soup, which contained 0.7 g of fiber, or soup without wakame (control). The blood glucose profiles and IAUC are shown in Figure 1. Compared to the control group, ingestion of wakame soup resulted in significantly lower (5.66 ± 0.13 vs 5.99 ± 0.11 mmol/L, $p=0.021$) postprandial glucose concentrations after 15 min. In the second study, the subjects were 16 healthy women, aged 21.9 ± 0.68 years with body mass index of 20.2 ± 0.36 kg/m^2, who ingested white rice with or without wakame salad which contained 1.4 g of fiber. The blood glucose profiles, shown in Figure 2, at 15 min (5.27 ± 0.13 vs 5.63 ± 0.12 mmol/L, $p<0.001$), 30 min (7.45 ± 0.16 vs 7.95 ± 0.18 mmol/L, $p=0.002$), and 45 min (7.75 ± 0.22 vs 8.23 ± 0.24 mmol/L, $p=0.018$) in the group that consumed wakame salad were all significantly lower than those of the control group.The IAUC did not change in either of these studies.

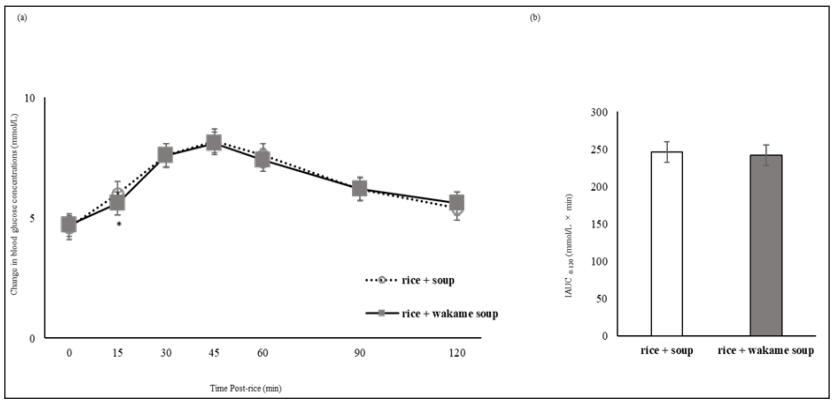

Figure 1. *Reduction of postprandial blood glucose response in healthy young women after consuming rice with or without wakame soup (the first study) (a). Change in blood glucose concentration from baseline over 120 min. (b). The incremental area under the blood concentration-time curve for glycaemia over 0 to 120 min. Values are shown as means ± standard error of the mean. Significant difference by inter-group comparison: *p<0.05 (Wilcoxon signed-rank test).*

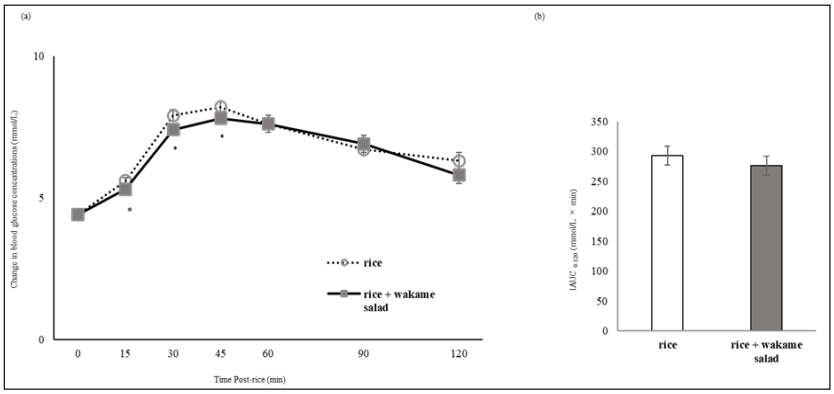

Figure 2. *Reduction of postprandial blood glucose response in healthy young women after consuming rice with or without wakame salad (the second study) (a). Change in blood glucose concentration from baseline over 120 min. (b). The incremental area under the blood concentration-time curve for glycaemia over 0 to 120 min. Values are shown as means ± standard error of the mean. Significant difference by inter-group comparison: *p<0.05 (Wilcoxon signed-rank test).*

3.2 Measurement of Glucose Release Rate and α-Glucosidase Activity

As shown in Table 1, wakame extracts and commercial sodium alginate lowered glucose concentrations at 20 min and glucose release rate compared to the control. In particular, the soluble fraction showed stronger inhibition of glucose release than the other fractions. The water-soluble fraction was presumed to contain sodium alginate, and its viscosity was measured using commercial alginate as a reference. It was confirmed that the viscosity of the soluble fraction (86.3 mPa/s, 0.2%) was much higher than that of commercial alginate (8.50mPa/s, 0.2%). However, inhibition of α-glucosidase activity was not observed in any of the wakame extracts (data not shown).

Table 1. *Glucose release rate of wakame extracts in a cell-free artificial digestion test (n=8)*

	Glucose (mmol/L) 20 min	Glucose (mmol/L) < 16 hours	Glucose release rate (%)
Rice (control)	3.25 ± 0.06 a	4.74 ± 0.03	68.5 ± 1.18 a
Rice + low-sodium powder	2.59 ± 0.08 b	4.68 ± 0.10	55.3 ± 0.96 b
Rice + low-sodium and low-fat powder	2.58 ± 0.05 b	4.86 ± 0.08	53.3 ± 1.08 b
Rice + insoluble fraction	2.67 ± 0.07 b	4.83 ± 0.10	55.4 ± 0.65 b
Rice + soluble fraction	2.12 ± 0.07 c	4.70 ± 0.02	45.1 ± 1.49 c
Rice + commercial alginate	2.58 ± 0.03 b	4.90 ± 0.09	52.8 ± 1.70 b

Values are shown as means ± standard error of the mean. The glucose release rate was calculated as follows: % glucose release = (20 min glucose concentration / 16 h glucose concentration) ×100.

Different superscript letters are significantly different using Duncan's multiple range test at $p<0.05$

4. Discussion

This clinical study showed that the ingestion of wakame combined with 150 g of white rice significantly reduced the postprandial blood glucose response in the early phase (Figure 1&2). The *in vitro* study also showed that wakame extract, which contains sodium alginate, slowed the glucose release rate (Table 1). In particular, the soluble fraction showed stronger inhibition of glucose release than the other fractions. The soluble fraction has a much higher viscosity than commercial alginate, and this high viscosity may be what inhibits glucose release. Soluble viscous fibers generally affect carbohydrate metabolism in the intestine via delays in gastric emptying.[2-4]Alginate derived from wakame may slow down glucose absorption by increase digestive fluid viscosity and forming a gel within the gastrointestinal tract, thus decreasing the gastric emptying rate. Georg *et al* reported that an alginate-based beverage that contained 15.0 g of alginate per 500 ml reduced the blood glucose response in healthy subjects.[8]Compared to that study, the concentration of alginate in wakame is low, and the effects of wakame consumption on postprandial glycemia in our test subjects was limited to the early phase in our studies. Our previous study demonstrated that in the early postprandial stage, insulin concentration was significantly lower following wakame intake than following intake of rice alone.[15]In addition, this study demonstrated that ingestion of wakame in a dose dependent manner significantly reduced postprandial blood glucose levels. Slower gastric emptying and inhibited glucose diffusion are contributory factors that may explain lower glucose and insulin concentrations in the postprandial early stage following ingestion of wakame. On the other hand, in our *in vitro* study, wakame extracts did not inhibit α-glucosidase and did not change glucose concentrations at 16 h compared to controls, which may explain why IAUC was not affected in our clinical study. Phenolic compounds and fucoidans are characterised as α-glucosidase inhibitors.[6,12,19]However, compared with other seaweeds, wakame has low polyphenols and fucoidans; therefore, its α-glucosidase inhibitory activity is likely weak.

One prospective cohort study showed that Japanese dietary patterns, which are highly correlated with seaweed, soybean products, fish, vegetables, fruit, and green tea consumption, are associated with a low risk of cardiovascular disease mortality.[20]However, recently, there have been changes in the diet of the Japanese population. The intake of plant-based foods has declined, and the Western-style diet, which is rich in lipids, has increased.[21]Obesity, metabolic syndrome, and type 2 diabetes have become major health problems in Japan, owing of this dietary trend. Seaweeds are an important component of the daily diet in Japan, and there is substantial evidence regarding the health benefits of seaweed-derived food products, including a decreased risk of diabetes mellitus.[22-24]In a previous report, we demonstrated that wakame ingestion reduces postprandial glucose and insulin concentrations in healthy adults. In the early postprandial stage (0–30 min), blood glucose and insulin levels were significantly lower following wakame intake compared to after rice intake alone.[15]The brown algae of seaweed are rich in soluble viscous fibers such as alginates. Torsdottir *et al.*[25]showed that acute ingestion of 5 g sodium alginate with a lipid meal reduced postprandial glucose and insulin concentrations in patients with type 2 diabetes, and that this correlated with delayed gastric emptying on scintigraphy. Our results are also consistent with those of previous studies, and show that wakame may represent a functional food with specific anti-hyperglycaemic properties in humans.

The effect of wakame consumption on postprandial glycemia may to be due to alginate in the water-soluble fraction. However, it is still unclear whether wakame actually delays the gastric emptying rate. Further *in vitro* Caco cell studies and/or *in vivo* studies will be needed to determine the physiological mechanisms of wakame.

Our findings showed that ingestion of wakame with white rice effectively reduced postprandial glucose concentrations in healthy young women. This effect was observed in a dose dependent manner. The viscosity of water-soluble wakame extract may reduce postprandial glucose concentrations by inhibiting the release rate of glucose.

Acknowledgements

We thank all of the volunteers who participated in this study. We also would like to thank Editage (www.editage.com) for English language editing.

Author Disclosures

RM has no conflicts of interest to declare, KY is an employee of Riken Vitamin Co., Ltd.

5. References

1. Suzuki N, Goto Y, Ota H, Kito K, Mano F, Joo E, Ikeda K, Inagaki N, Nakayama T. Characteristics

of the Japanese diet described in epidemiologic publication: A qualitative systematic review.*J Nutr Sci Vitaminol (Tokyo)*. 2018;64(2):129-137. doi: 10.3177/jnsv.64.129.

2. Müller M, Canfora EE, Blaak EE.Gastrointestinal Transit Time, Glucose Homeostasis and Metabolic Health: Modulation by Dietary Fibers. *Nutrients.* 2018;10(3):275. doi: 10.3390/nu10030275.

3. Yu K, Ke MY, Li WH, Zhang SQ, Fang XC.The impact of soluble dietary fibre on gastric emptying, postprandial bold glucose and insulin in patients with type 2 diabetes. *Asia Pac J Clin Nutr.* 2014;23(2):210-8. doi: 10.6133/apjcn.2014.23.2.01.

4. Jenkins DJ, Wolever TM, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Metz GL, Alberti KG.Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br Med J.* 1978;1(6124):1392-4. doi: 10.1136/bmj.1.6124.1392.

5. Okimura T, Jiang Z, Liang Y, Yamaguchi K, Oda T.Suppressive effect of ascophyllan HS on postprandial blood sugar level through the inhibition of α-glucosidase and stimulation of glucagon-like peptide-1 (GLP-1) secretion. *Int J BiolMacromol.*2019;125:453-8. doi: 10.1016/j.ijbiomac.2018.12.084.

6. Attjioui M, Ryan S, Ristic AK, Higgins T, Goñi O, Gibney ER, Tierney J, O'Connell S. Comparison of edible brown algae extracts for the inhibition of intestinal carbohydrate digestive enzymes involved in glucose release from the diet. *J Nutr Sci.* 2021;10:e5. doi: 10.1017/jns.2020.56.

7. Post RE, Mainous AG 3rd, King DE, Simpson KN.Dietary fiber for the treatment of type 2 diabetes mellitus: A meta-analysis. *J Am Board Fam Med.* 2012;25(1):16-23. doi: 10.3122/jabfm.2012.01.110148.

8. Georg Jensen M, Kristensen M, Belza A, Knudsen JC, Astrup A. Acute effect of alginate-based preload on satiety feelings, energy intake, and gastric emptying rate in healthy subjects. *Obesity (Silver Spring).* 2012;20(9):1851-8. doi: 10.1038/oby.2011.232.

9. Gabbia D, De Martin S. Brown seaweeds for the management of metabolic syndrome and associated diseases. *Molecules.* 2020;25(18):4182. doi:10.3390/molecules25184182.

10. Xing M, Cao Q, Wang Y, Xiao H, Zhao J, Zhang Q, Ji A, Song S. Advances in research on the bioactivity of alginate oligosaccharides. *Mar Drugs.* 2020;18(3):144. doi: 10.3390/md18030144.

11. Tanemura Y, Yamanaka-Okumura H, Sakuma M, Nii Y, Taketani Y, Takeda E.Effects of the intake of Undaria pinnatifida (Wakame) and its sporophylls (Mekabu) on postprandial glucose and insulin metabolism. *J Med Invest.* 2014;61(3-4):291-7. doi: 10.2152/jmi.61.291.

12. Schultz Moreira AR, Garcimartín A, Bastida S, Jiménez-Escrig A, Rupérez P, Green BD, Rafferty E, Sánchez-Muniz FJ, Benedí J.Effect of Undaria Pinnatifida, Himanthalia elongate and Porphyraumbilicalison in vitro α-glucosidase activity and glucose diffusion. *Nutr Hosp.* 2014;29(6):1434-46. doi: 10.3305/nh.2014.29.6.7381.

13. Kato T, Idota Y, Shiragami K, Koike M, Nishibori F, Tomokane Met al. Rondomized, double-blind, crossover clinical trial of the effect of calcium alginate in noodles on postprandial blood glucose level. *Biol Pharm Bull.* 2018;41(9):1367-71. doi: 10.1248/bpb.b18-00156.

14. Kim MS, Kim JY, Choi WH, Lee SS.Effects of seaweed supplementation on blood glucose concentration, lipid profile, and antioxidant enzyme activities in patients with type 2 diabetes mellitus. *Nutr Res Pract.* 2008;2(2):62-7. doi: 10.4162/nrp.2008.2.2.62.

15. Yoshinaga K, Mitamura R.Effects of Undaria pinnatifida (Wakame) on postprandial glycemia and insulin levels in humans: a randomized crossover trial. *Plant Foods Hum Nutr.* 2019;74(4):461-7. doi: 10.1007/s11130-019-00763-5.

16. Paxman JR, Richardson JC, Dettmar PW, Corfe BM. Alginate reduces the increased uptake of cholesterol and glucose in overweight male subjects: a pilot study. *Nutr Res.* 2008;28(8):501-5. doi: 10.1016/j.nutres.2008.05.008.

17. Montes L, Gisbert M, Hinojosa I, Sineiro J, Moreira R.Impact of drying on the sodium alginate obtained after polyphenols ultrasound-assisted extraction from Ascophyllum nodosum seaweeds. *CarbohydrPolym.*2021;272:118455. doi: 10.1016/j.carbpol.2021.118455.

18. Flórez-Fernández N, Domínguez H, Torres MD.Functional features of alginates recovered from Himanthalia elongate using subcritical water extraction. *Molecules.* 2021;26(16):4726. doi: 10.3390/molecules26164726.

19. Nowicka P, Wojdyło A, Laskowski P.Inhibitory potential against digestive enzymes linked to obesity and type 2 diabetes and content of bioactive compounds in 20 cultivars of the peach fruit grown in

Poland. *Plant Foods Hum Nutr.* 2018;73(4):314-20. doi: 10.1007/s11130-018-0688-8.

20. Shimazu T, Kuriyama S, Hozawa A, Ohmori K, Sato Y, Nakaya N, Nishino Y, Tsubono Y, Tsuji I.Dietary patterns and cardiovascular disease mortality in Japan: a prospective cohort study. *Int J Epidemiol.* 2007;36(3):600-9. doi: 10.1093/ije/dym005.

21. Murakami K, Livingstone MBE, Sasaki S.Thirteen-Year Trends in Dietary Patterns among Japanese Adults in the National Health and Nutrition Survey 2003–2015: Continuous Westernization of the Japanese Diet. *Nutrients.* 2018;10(8):994. doi: 10.3390/nu10080994.

22. Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire ME, Brawley SH. Algae as nutritional and functional food sources: revisiting our understanding. *J ApplPhycol.* 2017;29(2):949-82. doi: 10.1007/s10811-016-0974-5.

23. Lee HJ, Kim HC, Vitek L, Nam CM.Algae consumption and risk of type 2 diabetes: Korean National Health and Nutrition Examination Survey in 2005. *J Nutr Sci Vitaminol (Tokyo).* 2010;56(1):13-8. doi: 10.3177/jnsv.56.13.

24. Maeda H, Yamamoto R, Hirao K, Tochikubo O.Effects of agar (kanten) diet on obese patients with impaired glucose tolerance and type 2 diabetes. *Diabetes ObesMetab.* 2005;7(1):40-6. doi: 10.1111/j.1463-1326.2004.00370.x.

25. Torsdottir I, Alpsten M, Holm G, Sandberg AS, Tölli J.A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. *J Nutr.* 1991;121(6):795-9. doi: 10.1093/jn/121.6.795.