Food Chemistry 192 (2016) 1109–1115

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Anti-glycation properties of the aqueous extract solutions of dried algae products and effect of lactic acid fermentation on the properties

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ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form 23 June 2015
Accepted 17 July 2015
Available online 18 July 2015

Keywords:
Edible algae
Anti-glycation
Antioxidant
Lactobacillus plantarum

ABSTRACT

The antioxidant and anti-glycation properties in aqueous extract solutions (AESs) of 11 dried algae products were investigated. AESs of brown algae *Ecklonia kurome* (kurome) and *Ecklonia stolonifera* (tsuruarame) showed a strong DPPH radical-scavenging capacity and Fe-reducing power with high total phenolic compound content. On the other hand, superoxide anion radical-scavenging capacities of *Porphyra* sp. (iwanori, red alga), sporophyll of *Undaria pinnatifida* (mekabu, brown alga), and *Gelidiaceae* sp. (tengusa, red alga) were also high. Anti-glycation activities in BSA-fructose and BSA-methylglyoxal glycation were also high in kurome, while iwanori showed high activity. Results of the BSA-fructose model agreed with those of superoxide anion radical-scavenging. On the other hand, those of the BSA-methylglyoxal model agreed with those of the phenolic content, DPPH radical-scavenging capacity, and Fe-reducing power. Anti-glycation activities of iwanori, *U. pinnatifida* (wakame), and mekabu in the BSA-fructose model were clearly increased by fermentation with *Lactobacillus plantarum* AN6.

1. Introduction

Since ancient times, the inhabitants of coastal regions of Far Eastern countries, such as Korea and Japan, have discovered and collected edible algae from beach cast (Ikehara & Hayashida, 2003). Particularly in the Noto peninsula coastal region, which faces the middle of the East Sea (the Sea of Japan), 100 or more species of algae can be collected, of which approximately 40 are edible (Kuda & Ikemori, 2009). the Ministry of the Environment, Government of Japan, defines a *Satoumi* as a coastal area where biological productivity and biodiversity have increased as a consequence of human activity (Berque & Matsuda, 2013). The traditional eating habit of various algae is considered one of the features of the Noto *Satoumi* region. It is known that these algae, particularly the brown algae *Ecklonia cava, Ecklonia stolonifera,* and *Ecklonia kurome,* contain notable bioactive compounds, such as high content of phlorotannins (algal polyphenols), that have antiviral, antibacterial, and antioxidative properties (Kuda, Kunii, Goto, Suzuki, & Yano, 2007; Kwon et al., 2013).

In recent years, anti-glycation properties have attracted attention as having food function (Deetae, Parichanon, Trakunleewatthana, Chanseetis, & Lertsiri, 2012; Ho, Wu, Lin, & Tang, 2010). Glycation is a non-enzymatic reaction of reducing sugars with amino acids and/or proteins in processed food and *in vivo* (Anguizola et al., 2013). Advanced glycation end products (AGEs), such as carboxylmethyl lysine and carboxylethyl lysine are generated after various intermediates such as glyoxal, methylglyoxal (MGO), and 3-deoxyglucosone (Nemet, Varga-Defterdarovic, & Turk, 2006). AGE formation is irreversible. AGEs are thought to induce diabetes and other diabetes- and ageing-related illnesses such as retinopathy, cataracts, arteriosclerosis, and renal dysfunction (Semba, Nicklett, & Ferrucci, 2010). Because there are oxidation reactions in several parts in the glycation reactions for AGE generation, antioxidants are considered inhibitory materials for medicines and treatment diets that prevent AGE formation (Nagai, Mori, Yamamoto, Kaji, & Yonei, 2010). In the case of food materials, various foodstuffs are reported to be anti-glycative materials with antioxidant properties (Deetae et al., 2012; Ho et al., 2010). As mentioned above, some edible macroalgae have strong antioxidant activities; however, their anti-glycation properties have yet to be elucidated.

On the other hand, some lactic acid bacteria (LAB) including probiotics also have antioxidant and anti-inflammatory activities *in vitro* and in mice (Kanno, Kuda, An, Takahashi, & Kimura, 2012; Kuda, Kawahara, Nemoto, Takahashi, & Kimura, 2014). Some LABs can increase the antioxidant capacities of vegetables, milk, and soy milk during the fermentation (Kanno et al., 2012;

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http://dx.doi.org/10.1016/j.foodchem.2015.07.073
0308-8146/

Kawahara et al., 2015; Kuda, Kaneko, Yano, & Mori, 2010). The anti-glycation properties of the probiotics *Lactobacillus acidophilus* and *Bifidobacterium infantis* were recently reported (Stancu, Sanda, Rogoz, & Sima, 2012). Furthermore, one study reported that the prebiotics inulin and oligofructose ameliorated the AGE-related pathology of adult volunteers with pre-diabetes (Kellow, Coughlan, Savige, & Reid, 2014).

In this study, to clarify the anti-glycation effect of traditional edible algae, we determined the inhibitory effect of aqueous extract solutions of dried algae products obtained from the Noto *Satoumi* region on glycation in bovine serum albumin-fructose (BSA-Fru), BSA-methylglyoxal (BSA-MGO), and lysine-glucose (Maillard reaction) models. Furthermore, to examine the additive or synergistic effect of the food materials and LAB or lactic acid fermentation, the effect of the *Lactobacillus plantarum* isolated from the Noto *Satoumi* region on the anti-glycation capacities of the selected algae samples was also investigated.

2. Materials and methods

2.1. Chemicals

(+)-Catechin, Folin–Ciocalteu's phenol reagent, the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical phenazine methosulphate (PMS), 3-(2-pyridyl)-5,6-di(*p*-sulfophenyl)1,2,4-triazine disodium salt (ferrozine), β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium salt (NBT), and MGO were purchased from Sigma–Aldrich (St. Louis, MO). Phloroglucinol dihydrate (PG), potassium ferricyanide, trichloroacetic acid (TCA), BSA, lysine, D-glucose (Glu), and D-fructose (Fru) were purchased from Wako Chemicals (Osaka, Japan), while 1,10-phenanthroline was purchased from Nacalai Tesque (Kyoto, Japan). The other reagents were of analytical grade.

2.2. Preparation of aqueous extract solutions from dried algae solutions

A total of 11 dried products (Table 1) of five species of Phaeophyta, *E. stolonifera* (tsuruarame), *E. kurome* (kurome), *Undaria pinnatifida* (wakame) (the frond part), mekabu (sporophyll of wakame), and *Chorda filum* (tsurumo), as well as three species of Rhodophyta, *Gelidiaceae* sp. (tengusa), *Campylaephora hypnaeoides* (ego), and *Porphyra* sp. (iwanori), were purchased from retail shops in the Noto peninsula region.

Table 1
Dried algal samples used in this study.

Scientific name	Market name (Japanese)	Harvest place (city)	Abbreviation
Pahaeophyta			
Ecklonia stronifera	Kajime	Wajima	E1
E. kurome	Kajime	Suzu	E2
Undaria pinnatifida (frond part)	Wakame	Wajima	Up1
U. pinnatifida (frond part)	Wakame	Suzu	Up2
U. pinnatifida (sporophyll part)	Mekabu	Wajima	Up'1
U. pinnatifida (sporophyll part)	Mekabu	Suzu	Up'2
Chorda filum	Tsurumo	Wajima	Cf
Rhodophyta			
Gelidiaceae sp.	Tengusa	Wajima	G1
Gelidiaceae sp.	Tengusa	Suzu	G2
Campylaephora hypnaeoides	Ego	Wajima	Ch
Porphyra	Iwanori	Suzu	P

The dried samples were milled using a blender (Oster 16 Speed Blender; Osaka Chemical Co., Osaka, Japan) and sieved through 1-mm^2 mesh. The algae powder (5 g) was added to 200 mL of distilled water and heated at 105 °C for 15 min using an autoclave. After cooling with tap water, the algae suspension was centrifuged at 3000×*g* for 10 min at 4 °C. The collected supernatant was used in the algal aqueous extract solutions (AESs) and stored at −20 °C.

2.3. Noto Satoumi lactic acid bacteria (LAB) strains

Two Noto Satoumi LAB strains *Lb. plantarum* AN6 and *Lactococcus lactis* subsp. *lactis* Noto-SU1 were used in this study. *Lb. plantarum* AN6 was isolated from *aji-narezushi*, a fermented horse mackerel with cooked rice made in the Noto *Satoumi* region (Kuda, Yazaki, Ono, Takahashi, & Kimura, 2013). *Lc. lactis* Noto-SU1 was isolated, in present study, from algal beach cast on a coast and was selected as an acid- and bile-resistant strain. These LAB strains were pre-cultured at 30 °C for 48 h with de Man, Rogosa, and Sharpe (MRS) broth (Oxoid; Basingstoke, UK).

2.4. Total phenolic content and antioxidant properties of the AESs

2.4.1. Phenolic compounds

Total phenolic content as polyphenol content level was determined as described previously (Kuda & Ikemori, 2009) with slight modifications. Briefly, 0.03 mL of a diluted sample solution and 0.06 mL of 10% Folin–Ciocalteu solution were placed in a 96-well microplate. After 3 min, 0.12 mL of 10% sodium carbonate was added. The mixture was allowed to stand for 60 min at ambient temperature and the absorbance was measured at 750 nm using a grating microplate reader (SH-1000 Lab; Corona Electric, Hitachinaka; Ibaraki, Japan). The phenolic content is expressed as PG equivalents PGEq/mL.

The experiments to determine phenolic content, antioxidant properties, and anti-glycation properties were conducted in triplicate.

2.4.2. DPPH radical-scavenging capacity

DPPH radical-scavenging capacity was determined as described previously (Kuda & Yano, 2009) with slight modification. Briefly, sample diluted solution (0.1 mL) and ethanol (0.1 mL) were put into a 96-well microplate, and absorbance at 517 nm (Abs1) was measured using the microplate-reader (SH-1000 Lab). Next, 1 mmol/l DPPH radical in was added and incubated at 37 °C for 30 min and the absorbance (Abs2) was measured again. The DPPH radical-scavenging capacity was calculated using the following formula:

$$\text{Radical scavenging capacity}\ (\%) = (1 - (\text{Abs2 of sample} - \text{Abs1 of sample})/(\text{Abs2 of control} - \text{Abs1 of control}) \times 100$$

2.4.3. Superoxide anion radical-scavenging activity

Superoxide anion radical-scavenging activity was measured using a non-enzymatic method (Kuda & Ikemori, 2009). The sample solution (0.1 mL) was treated with 0.05 mL of 250 mmol/l phosphate buffer (pH 7.2), 2 mmol/l NADH (0.025 mL), and 0.5 mmol/l NBT (0.025 mL), while absorbance at 560 nm was measured as a blank value. After a 5-min incubation at ambient temperature with 0.025 mL of 0.03 mmol/l PMS, the absorbance was measured again. The radical-scavenging capacity was calculated using the above formula.