Note

Aluminium Binding Amount of Dietary Fiber Extracted from 14 Kinds of Food

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We studied the impact of heating on the aluminium ion ($\text{Al}(\text{NO}_3)_3$) binding amount of IDF (insoluble dietary fiber) and SDF (soluble dietary fiber) fractions extracted by the modified Prosky method from 14 kinds of food after different heat treatments. Al binding of unheated IDF was high in edible burdock, carrot, cabbage and hijiki. Heating marginally raised the Al binding amount of aloe and okra, but the other samples showed a tendency for the Al binding amount to fall when heated. Al binding of unheated SDF was extremely high in aloe, which has a high mucilage content, followed by okra and eggplant. Whereas non-mucilaginous foods had lower binding capacities when heated, the binding capacities of such viscous foods as aloe, okra, and moroheiya were increased by microwaving.

Keywords: dietary fiber, Al binding, heating

Dietary fiber (DF) is known to possess such physiological actions as normalization of the intestinal environment and inhibition of sugar absorption (Hanai *et al.*, 1997; Anderson *et al.*, 1995; Salmeron *et al.*, 1997; Jenkins *et al.*, 1995; Davidson & McDonald, 1998). DF is also comprised of acidic polysaccharides that have a polar group, and acts to bind with, adsorb and promote to excretion a variety of metal ions by intermolecular force, chemical and physical factors that stem from its polysaccharide structure (Ou *et al.*, 1999; Schrijver & Conrad, 1992). DF is usually ingested from heated foods. At the same time, the polysaccharide structure of DF has been shown in experiments by the authors (Takeyama *et al.*, 2002) to be affected both physically and chemically by heating.

SDF (soluble dietary fiber) and IDF (insoluble dietary fiber) fractions were extracted via modified Prosky method (Prosky *et al.*, 1988) from 14 different heated samples, and the influence of heating on the metal binding ability that is a function of DF was determined.

Aluminium nitrate ($\text{Al}(\text{NO}_3)_3$) was used as the metal ion. Aluminium (Al) is known to accumulate in the brain and may cause neurotoxic injury by long-term exposure (Sarin *et al.*, 1997; Oshiro *et al.*, 1998; Gonda & Lehotzky, 1996; Bielarczyk *et al.*, 1998; Gandolfi *et al.*, 1998).

Materials and Methods

Materials We used 14 different samples in our study: aloe, okra, moroheiya, cabbage, celery, eggplant, bitter gourd, edible burdock, carrot, Japanese radish, hijiki, shiitake, nameko and common mushroom. Each sample was washed and wiped dry, whereupon the edible portion was either grated, using a plastic or ceramic grater, or minced with a ceramic knife. A portion of each sample was intermittently heated in a microwave oven for 13 min, or autoclaved at 121°C for 30 min. The processed samples were then freeze dried together with the unheated sam-

ples, then pulverized and put through a 32-mesh sieve for use in the study. All equipment was soaked overnight in 15% nitric acid solution, then washed with tap water or purified water before use.

Extraction of IDF and SDF by the modified Prosky method Two to 6 grams of the samples obtained mentioned above were subjected to extraction by the modified Prosky method (Prosky *et al.*, 1988) as described in a previous report (Takeyama *et al.*, 2002), followed by dialysis and freeze drying, to obtain IDF and SDF fractions.

Measurement of binding amount of Al with SDF To 2 ml of solution containing 20 µg of Al ion ($\text{Al}(\text{NO}_3)_3$), 10 ml of solution containing 10–20 mg of SDF fraction and 3 ml of water were added to a 50 ml measuring flask (in pairs), and allowed to shake at room temperature for 1 h. Twenty-five milliliters of 0.3% sodium mercaptoacetate solution was added to one measuring flask and 25 ml of 0.04% EDTA solution was added to another. Five milliliters of 0.075% eriochrome cyanine R solution and 5 ml of 4.15 M ammonium acetate buffer (pH 6.0) were then added to both flasks and the flasks filled with distilled water. These were then measured at 535 nm by absorption photometry using eriochrome cyanine R (Muki Oyo Hishoku Bunseki Hensyu Iinkai, 1973). The remaining quantity of Al ion was subtracted from 20 µg to calculate the binding amount.

Measurement of binding amount of Al with IDF Fifteen to 30 mg of IDF sample was measured into a conical beaker, to which 3 ml of purified water was added; mixing with a glass stick followed, 5 ml of 4 µg/ml Al standard solution was added and the mixture was allowed to shake at room temperature for 1 h. Using an Advantec Toyo No. 5C filter paper, the mixture was filtered by suction into a 50 ml measuring flask, whereupon the residue on the filter was washed thoroughly with 5 ml of 0.1 M HCl and water, then brought to a constant volume. The Al ion in the filtrate was analyzed with chrome azurol S (Muki Oyo Hishoku Bunseki Hensyu Iinkai, 1973). Ten milliliters of this solution, 0.6 ml of 0.5 M HCl solution, 10 ml of 0.02% Chromazurol S, 0.05% CTMAC (cetyl trimethyl ammonium chloride)

solution and 5 ml of 4 M ammonium acetate buffer (pH 6.3) were added to measuring flask which was then filled to 50 ml (pH 5.9). Al ion was analyzed at 620 nm using a blank sample, and the quantity of bound Al was calculated from the quantity of residual Al ion in the same manner as used in SDF.

Results and Discussion

The Al binding capacities of IDF are shown in Fig. 1. With IDF extracted from unheated samples, high levels of Al binding were seen for edible burdock, carrot, cabbage, and hijiki. Edible burdock in particular had a high rate of Al binding, approximately 2 mg per gram of dried IDF fraction.

The DF amounts were shown in the previous report (Takeyama *et al.*, 2002). These amounts of unheated IDF extracted from edible burdock, carrot, cabbage and hijiki were also higher than their heated samples.

A report by Yoshida and Saito (1985) describes an especially high hemicellulose content of raw carrot and raw eggplant, and high hemicellulose and lignin contents of raw edible burdock. In fact, xylan, which included uronic acid or protein, was shown to have Al binding ability (Fukushima & Tanimura, 1995). In short, the Al binding amount of xylan is believed to be greatly influenced by its uronic acid content. It is conceivable that the ratio of hemicellulose in DF and its structure might be closely related to Al binding.

The absorption amount of Al by unheated hijiki DF was 1070 $\mu\text{g/g}$, and autoclaved and microwaved hijiki DF were absorbed each 781 $\mu\text{g/g}$ and 807 $\mu\text{g/g}$. Hijiki is a kind of brown alga; the dried polysaccharides of brown alga are 10–47% of alginic acid, whose primary constituents are mannuronic acid and gluronic acid (Maeda & Nishizawa, 1974). It can be surmised that the $-\text{COOH}$ of alginic acid has high activity and so had an

impact on Al binding. It should be noted that, because alginic acid is readily depolymerized when heated in an acidic solution, it is possible that the autoclaving caused it to transfer into the water soluble fraction.

With heating, Al binding amount of the IDF fraction rose slightly in fractions extracted from autoclaved and microwaved aloe and okra, while in other samples, the Al binding amount of IDF fractions decreased after heat processing. In particular, the Al binding capacities of IDF fractions extracted from autoclaved and microwaved edible burdock and carrot showed marked decreases.

The amounts of unheated and heated DF (IDF and SDF) for edible burdock and carrot were shown in a previous report (Takeyama *et al.*, 2002); the particularly high hemicellulose content is very likely to have become soluble with heating and spilled over into the SDF fraction (about 10–30% of IDF), and this tendency was also true for soybean (Takeyama *et al.*, 1996). With microwaved okra, microwaved common mushroom and microwaved bitter gourd, the IDF fractions (35.1, 27.6 and 32.1%) increased over their non-heated counterparts (30.3, 24.8 and 29.8%) (Takeyama *et al.*, 2002), and a tendency was seen for a greater increase in Al binding amount for those foods that showed substantial increases. The increase of IDF is believed to be attributable to a part of the soluble portion having become insoluble.

Meanwhile, SDF fractions contain large quantities of water soluble hemicellulose, pectin, vegetable gum, and mucopolysaccharides. As shown in Fig. 2, the Al binding capacities of the SDF fractions extracted from the 14 different foods showed high values when unheated for aloe, okra and eggplant. Aloe in particular had extremely high values. As the SDF of aloe contains an especially high amount of galactan and mucopolysaccharides

Fig. 1. Absorption amount of Al ion by IDF.

Fig. 2. Absorption amount of Al ion by SDF.

(Dweck, 1999; Yasuda *et al.*, 1999), it is conceivable that the galacturonic acid attached to the galactan and the mucilage primarily consisting of mucopolysaccharides formed chemical and / or physical bonds with Al.

The mucilage of okra is composed of such substances as pectin, galactan, and araban (Egami *et al.*, 1969; Miyazaki, 1990). It is conceivable that the Al binding amount was enhanced due to the particularly high content of acidic sugars, which have cationic exchange properties, in pectin and hemicellulose. However, moroheiya, which also had a high ratio of acidic polysaccharide (ca 65% of DF) (Otani *et al.*, 1995), had a lower Al binding ability compared to okra. These differences are believed to be responsible for the sugar composition of DF, which is mainly comprised of glucuronic acid in moroheiya (ca 43%) but galacturonic acid in okra (ca 33%).

The Al binding amount for SDF fractions extracted from heat processed samples decreased in over half the samples, but it increased in microwaved aloe, okra, moroheiya, nameko, and hijiki, as well as in autoclaved and microwaved shiitake. While autoclaving had the effect of decreasing Al binding amount for almost all other samples, the Al binding quantity of shiitake went up to approximately twice its unheated values. Considering that shiitake contains a large amount of free amino acids such as glutamic acid, which may react with saccharides to form melanoidin (Kurasawa *et al.*, 1982, 1991), the increase of Al binding may be partially caused by the formation of melanoidin, which is reported to have metal binding activity (Horikoshi & Gomyo, 1976).

The IDF of hijiki has a high alginic acid content, which depolymerizes with autoclaving (Tsuji & Mori, 1998). However, microwaving does not cause as much depolymerization as autoclaving, and it is highly probable that the alginic acid remained

within the SDF fraction. It is conceivable that for this reason the Al binding amount became particularly high with microwaving. Nevertheless, further examination is required on the details.

Based on the above experiments, we found that Al binding amount with DF changed remarkably by heating. But the effects of heating could not be simplified, and the variation in ranges differed in each food. Especially, mucilaginous and non-mucilaginous foods showed quite opposite results. The DF was affected by heating, and we found the same true in Al binding as shown in many other reports.

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