Effect of Heat-Treatment on the Content and Polysaccharide Composition of Dietary Fiber

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Received July 31, 2001; Accepted December 11, 2001

SDF (soluble dietary fiber) and IDF (insoluble dietary fiber) fractions were extracted from 14 different foods by the modified Prosky method. Ion exchange chromatography with DEAE cellulose was employed to examine each of the fractionated sugars. Aloe fractions were further subjected to gel chromatography to examine the effect of heating on molecular weight. The SDF fractions of anhydrous samples of okra, cabbage, celery, bitter gourd, and carrot were large when autoclaved, while that of aloe was largest when unheated, and those of eggplant, edible burdock, Japanese radish, hijiki, and nameko were large when microwaved. The SDF fractions of moroheiya and okra included acid polysaccharides in large quantities, and acid polysaccharides increased further with autoclaving. The SDF fraction of celery did not show as pronounced a tendency for heat-induced increase of acid sugar. IR analysis confirmed that heating affected the functional groups of this fraction.

Keywords: dietary fiber, autoclave, microwave, acid polysaccharides, IR analysis

We have heretofore focused on soya in our studies of the effect of heating on dietary fiber (DF), whereupon it has been pointed out that different foods are affected differently by heat processing, owing to differences in the DF each food contains (Takeyama et al., 1986, 1991, 1996). We therefore chose to study the effect of heating on the DF content and polysaccharide composition of SDF (water soluble DF) and IDF (water insoluble DF) fractions extracted by the modified Prosky method (Prosky et al., 1988) from 14 different samples.

To study polysaccharide composition, we focused primarily on the SDF fractions of aloe, moroheiya, okra, and celery, tested by ion exchange chromatography and IR analysis, while aloe was subjected to further testing by gel filtration.

Materials and Methods

Samples Samples consisted of 14 different low fat, low protein foods (Table 1). 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 either placed in a commercially available microwave package and intermittently heated in a microwave oven for 8 to 13 min, or placed in a tall beaker, covered by a petri dish, and autoclaved at 121˚C for 30 min. Each of the processed samples was then freeze dried together with the unheated samples, then pulverized and put through a 32-mesh sieve for use in our study. All equipment was soaked overnight in 15% nitric acid solution, then washed with tap water and purified water before use.

Extraction of IDF and SDF fractions by the modified Prosky method Two to 6 g of the samples were subjected to extraction by the modified Prosky method (Prosky et al., 1988) as described in a previous report (Takeyama et al., 2001), followed by dialysis and freeze drying, to obtain IDF and SDF fractions. IDF was filtered by suction with a PYREX 17G3 glass filter and the SDF was filtered by suction using a HARIO 17G5 glass filter (Takeyama et al., 2001).

Fractionation by ion exchange chromatography Of the DF obtained, the SDF fractions of aloe, moroheiya, okra, and celery obtained in above were dissolved, and their polysaccharides fractionated by allowing the solution to flow through a DEAE cellulose (Whatman, DE52 type) column (926x900 mm) using a phosphate buffer (pH 6.0) flowing sequentially from 0.02 M (1.5 l), 0.1 M (1 l), 0.25 M (1.5 l), to 0.5 M (1.25 l). Flow rate was kept at 2 ml/min using a peristaltic pump, and eluate was collected in 50 ml portions using a fraction collector. Each fraction was assessed for total sugar by the phenol sulfuric acid method (Fukui, 1990) and for pectic substance by the carboxyl sulfuric acid method (Fukui, 1990). Each fraction was designated as fraction A1, A2, A3, or A4 to correspond with the order of elution in the buffer liquid.

Fractionation by gel filtration Out of the samples above, aloe alone was subjected to gel filtration using Sepharose 4B (Pharmacia, molecular weight fractionation range 30,000–5,000,000) for fraction A1, and Sephadex G-200 (Pharmacia, molecular weight fractionation range 1,000–200,000) for fractions A2 through A4. A 0.2 m NaCl solution was used as the solvent, and the eluate was collected in 5 ml portions using the fraction collector. Identification of molecular weight was conducted separately using pullulan standard samples (Showa Denko K.K., STANDARD P-2) P-5, -10, -20, -50, -100, -200, -400 and -800 (molecular weight 5,900, 12,200, 22,800, 47,300, 112,000, 212,000, 380,000 and 788,000).
The infrared absorption spectrum was measured using the KBr pellet technique with a Shimadzu IR-460 infrared spectrophotometer. Samples consisted of freeze dried SDF and IDF, vacuum dried immediately prior to analysis.

**Results and Discussion**

**Changes of dietary fiber fractions by heat-treatment**

Changes in combined SDF and IDF fractions (TDF fraction)

The amounts of IDF, SDF and TDF are shown in Table 1. The TDF fraction within the anhydrous sample decreased upon heating in aloe, moroheiya, cabbage and eggplant, and increased upon heating in bitter gourd and nameko. Microwaved hijiki showed virtually similar values to their unheated counterparts, while values fell markedly with autoclaving. Microwaved okra, common mushroom and shiitake had higher values compared to unheated samples. With celery and Japanese radish no major differences were found between the TDF fraction yields of unheated, autoclaved, and microwaved samples, while the SDF and IDF yields of these foods were affected by heating.

**Changes in SDF fractions**

The SDF fractions within anhydrous sample in okra, moroheiya, celery, bitter gourd, and carrot were largest when autoclaved, while aloe SDF was largest when unheated, and the SDF of eggplant, edible burdock, Japanese radish, hijiki, and nameko largest when microwaved. Okra, moroheiya, celery, bitter gourd, and carrot had smaller SDF fractions when microwaved than when autoclaved, while eggplant, edible burdock, Japanese radish, hijiki, and nameko had larger SDF fractions when microwaved than when autoclaved. With shiitake and common mushroom, there was virtually no difference between heated and unheated samples.

**Fruits and vegetables contain insoluble pectin which does not occur in isolation; rather, insoluble pectin (protopectin) binds with cellulose to constitute the cell membrane. A characteristic of protopectin it changes to soluble in hot water under high pressure (Takase & Teramoto, 1989). Thus protopectin, when autoclaved, detaches from cellulose, and is further hydrolyzed to cause a shift to the water soluble fraction (Nakahama, 1976). This conceivably causes the increase in the SDF fractions of okra, cabbage,
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celery, bitter gourd, and carrot. In general, heat processing causes
the solubilization of insoluble hemicellulose and pectic sub-
stances, leading to a transfer to the soluble fraction and an
increase in SDF content (Schrumpf & Charley, 1975; Brandt et
al., 1984; Lintas & Cappelloni, 1988; Penner & Kim, 1991; Doi
& Tsuji, 1997).

Meanwhile, aloe showed higher SDF value when unheated.
The neutral sugar level of aloe is higher than its acid and amino
sugar levels. Yasuda et al. (1999) have reported that aloe frac-
tions extracted with hot water have a particularly high sugar con-
tent, which exceeds 60% in the mesophyll. Further, the sugar
components are prominently used to form glucomannan consist-
ing of glucose and mannose combined in a ratio of 1 : 22
(Dweck, 1999). In the case of aloe, it is conceivable that this glu-
comannan was hydrolyzed by heating, and that a portion of the
same was degraded to such an extent that it was no longer classi-
fiable as a polysaccharide. Additionally, aloe differs from the typ-
ical non-mucilaginous sample in that it contains the plant
mucilage mucopolysaccharide (the mucilage glycoprotein)
(Dweck, 1999; Harada & Misaki, 1974; Yagi, 1994; Asaoka,
1986). It is conceivable that a portion of the mucilage was lost
due to heating.

When unheated and microwaved samples were compared,
SDF fractions were found to be large in microwaved eggplant,
edible burdock, Japanese radish, and hijiki. Koshijima (1994) has
reported that insoluble hemicellulose is broken down and solubi-
lized by microwaves. It is likely that a portion of insoluble hemi-
cellulose was solubilized by the application of microwaves in our
study as well.

Changes in IDF fraction The IDF of the following 6 foods
decreased with heating compared to when left unheated: cab-
bage, eggplant, edible burdock, carrot, Japanese radish, and hiji-
ki. The IDF of okra and moroheiya decreased with autoclaving
and increased when microwaved. By contrast, autoclaving re-
sulted in the highest IDF values for aloe and nameko. Autoclav-
ing yielded values roughly similar or slightly higher than those
for unheated bitter gourd, shiitake, and common mushroom,
while microwaving resulted in high values for these foods.

Insoluble pectin is fractionated into the IDF fraction. However,
when heated under conditions of high moisture content, it is pos-
sible that a portion of insoluble pectin is hydrolyzed together
with insoluble hemicellulose and becomes soluble, or is de-
graded to such an extent that it is no longer classifiable as a
polysaccharide. This may be the reason for the autoclaved IDF
fractions of all samples, with the exception of aloe, bitter gourd,
and the common mushroom, being smaller than their unheated
counterparts.

Meanwhile, shiitake, nameko, and common mushroom con-
tain chitin, which is a protein complex, in their cell walls
(Karasawa et al., 1991; Spiller, 2001). The strong crystalline
structure of chitin, and its consequent insolubility in dilute acid
or dilute alkaline, is the likely cause of the IDF of the above
foods being resistant to the effect of heating. Microwaving
increased the IDF of shiitake and common mushroom; this is
believed to be due to the formation of melanoidin (Karasawa et
al., 1982) and other such products of reactions between carbohy-
drates and proteins or amino acids contained in the mushrooms.
In contrast to the nameko, the shiitake and common mushroom
have no sliminess, so that transpiration brought about by micro-
waving is unimpaired and chemical reactions more easily occur.
On the other hand, because the pectin content of the slimy por-
tion of the nameko is high, much of this portion may become

Fig. 1. Chromatograms of Aloe, Okra, Moroheiya, Celery SDF on DEAE-cellulose. ●, Sugar, △, Pectic substances. *, Buffer (M), concentration of phosphate buffer (M).
soluble, yielding a greater proportion of SDF. The increased IDF of microwaved okra and moroheiya could be attributable to insolubilization chemical reactions brought about by the marked transpiration at the time of heat processing in other substances.

As outlined above, it was demonstrated that microwaving and autoclaving resulted in changes between the heated and unheated DF fractions of each food. It was also observed that there were marked property differences in DF extraction between mucilaginous and non-mucilaginous foods. We therefore chose to examine these physicochemical qualities by DEAE cellulose column chromatography, gel chromatography, and IR analysis.

Fractionation by ion exchange chromatography and gel filtration

We examined the effect of heating on the polysaccharide composition of foods by subjecting the SDF fractions of the aloe, okra, moroheiya, and celery to ion exchange chromatography using DEAE cellulose. Molecular weight of fractions thus obtained was assessed by gel filtration, with results for both tests shown in Fig. 1.

The SDF fractions of unheated, microwaved, and autoclaved aloe all demonstrated a high peak in the A1 fraction. The A1 fraction is classified as a neutral polysaccharide, as it is a polysaccharide that elutes at the most dilute phosphate buffer concentration of 0.02 M. There was also a peak observed at the A3 fraction, indicating the presence of acid polysaccharide, albeit in lower concentrations than the A1 fraction. The A1 fraction was largest in unheated samples, followed by microwaved samples; the autoclaved samples had the lowest peaks. By contrast, the A3 fractions were smallest in unheated samples, and largest in autoclaved samples. These phenomena suggest that heating increased acid sugars; however, the peak observed in the A4 fraction was very small.

The SDF fractions of moroheiya showed higher peaks in the A3 and A4 fractions than in the A1 and A2 fractions, suggesting a high acid polysaccharide content. These peaks were extremely small in the unheated sample, followed by the microwaved sample; autoclaving resulted in the highest peaks. Furthermore, ratio of the elution of A3 to A4 are 3:2 in case of unheated, 2:3 of microwaved, and 1:1 of autoclaved, and the A4 fraction increased by heating. In ion exchange chromatography, the weaker the polysaccharide’s affinity for the ion exchanger, the faster it elutes (Matsuda, 1987); it follows, therefore, that the A4 fraction has a stronger ionic affinity than the A3 fraction. It is thus thought that the acid sugar levels in moroheiya were further increased by heating. While the SDF fractions of okra also showed higher peaks in the A3 and A4 fractions compared to the A1 and A2 fractions, the A3 fraction had higher peaks than the A4 fraction. This likewise suggests a high acid sugar content, although that of okra is slightly different from that of moroheiya. For both A3 and A4 fractions, autoclaving resulted in the highest peaks, followed by microwaving; unheated samples had the lowest peaks, indicating that acid sugars increased with heating. On the other hand, the unheated SDF fraction of celery demonstrated 4 small peaks ranging from neutral sugars to acid sugars. While microwaving caused a marginal decrease in the peaks in the A1 and A2 fractions, the peak at the A3 fraction showed a marginal rise. Autoclaving resulted in a further decrease of A1 and A2 fractions, but unlike the profiles of the mucilaginous aloe and okra, there was a slight indication of a decrease on the acid sugar side.

Next we used gel filtration to determine the molecular weight of each of the aloe SDF fractions A1 to A4 that was fractionated by ion exchange chromatography. Results are shown in Figs. 2 and 3. The molecular weight of each peak was determined using pullulan samples of known molecular weights standard.
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The neutral sugar A1 fraction of aloe was assessed with a Sepharose 4B, whereupon peaks were detected at No.23 and 61 for unheated sample. The peak at No. 23 appeared at a position before the pullulan P-800, which suggests that its molecular weight is larger than 788,000. The peak at No. 61 was suggested to be a molecular weight larger than 47,300. Autoclaved samples showed peaks at No. 32 through 42, and No. 66 through 82. The former peak indicated a molecular weight of roughly 788,000 and the latter suggested a molecular weight smaller than 47,300. We were thus able to detect a heating-induced decrease in molecular weight for the A1 fraction. The A2 and subsequent fractions of aloe were assessed with a Sephadex G-200, whereupon peaks were observed at No. 21 to 22 and No. 32 through 43 in the unheated sample. The former peak suggested a molecular weight larger than 112,000, while the latter suggested a molecular weight of roughly 48,000. While a microwaved sample showed peaks at positions similar to the unheated counterpart, a gentle slope followed the 48,000 peak, indicating a higher degree of degradation compared to the unheated sample. Autoclaving resulted in a large, gentle peak from No. 20 to the vicinity of No. 70, suggesting a molecular weight of roughly 112,000 to 5900, with a degree of degradation even further pronounced than that of the unheated sample. The A3 fraction, unheated and microwaved samples both showed peaks above 112,000 molecular weight. The microwaved sample alone demonstrated a small peak in the vicinity of 22,800, with another small peak observed below 5900, indicating a further degree of degradation compared to the unheated sample. In the autoclaved sample, the 112,000 peak was exceedingly low, and the presence of a gentle, large peak of molecular weight 110,000–5000, centering around 22,800, and another small peak following that, indicated an even more pronounced degree of degradation. While no marked differences were observed between unheated, microwaved, and autoclaved samples of the A4 fractions, the autoclaved sample had a slightly larger peak in the vicinity of the low molecular weight section of 48,000–22,800 compared to the others.

The above results allowed us to confirm that heating causes a decrease in molecular weight, with such tendency being more pronounced with autoclaving than heating by microwave. Marry et al. (2000) have reported that the cell wall of the sugar beet undergoes a decrease in molecular weight when subjected to heat processing. A similar result was observed for aloe in our study.

**IR analysis** IR spectra are shown in Fig. 4. While with aloe, there was virtually no observable difference between the unheated and microwaved samples in absorption near 1730 cm\(^{-1}\), thought to be ester-type or free carboxylic acid-derived, this absorption was decreased in the autoclaved sample. Absorption near 1640 cm\(^{-1}\), believed to be of salt-type carboxyl group, was higher in the heated samples than in the unheated sample. In addition to the NH group 1560–1500 cm\(^{-1}\) absorption was increased by microwaved. With the SDF fraction of microwaved moroheiya and okra, absorption near 1730 cm\(^{-1}\) increased in the microwaved sample only, albeit marginally, compared to unheated and autoclaved samples of the same, while absorption near 1640 cm\(^{-1}\) decreased in the microwaved sample only, and in the case of autoclaved okra, absorption of 1640 cm\(^{-1}\) increased. In the case of SDF fraction of autoclaved celery, showed marked decrease in vibrations 2500–4000 cm\(^{-1}\) derived from -COOH and -OH groups. It was observed that the functional groups were readily affected by heating.
A portion of this study was funded by a 2000–2001 Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science. The authors wish to express their gratitude.

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