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Total dietary fibre content of some green and root vegetables obtained at different ethanol concentrations*

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The effect of reducing ethanol concentration for the precipitation of soluble dietary fibre in the Association of Official Analytical Chemists (AOAC, 1990, 1992, *Official Methods of Analysis*) enzymatic–gravimetric method 985·29 for total dietary fibre (TDF) in food was investigated. Alcohol concentration was decreased from 76% to 41% and 56% in determining TDF of raw collard and mustard greens, sweet potato leaves and storage roots, and sugar beet leaves and roots. TDF contents of the vegetables at the reduced ethanol concentrations were generally not different from those at the AOAC recommended volume ($P < 0.05$). When there was a significant difference, the TDF means for the same vegetable varied within the range of the standard deviations. Reduction of ethanol volume can lower analysis cost, lessen environmental organic solvent contamination and shorten filtration time.

INTRODUCTION

The Association of Official Analytical Chemists (AOAC) enzymatic–gravimetric methods for total dietary fibre (TDF) (AOAC, 1990, 1992) are rapid, simple and suitable for routine testing, and are recommended for nutrition labeling of food packages in the USA (Lee & Prosky, 1992; Mongeau & Brassard, 1986). Method 985·29 was modified (method 991·43) by replacing phosphate buffer with MES–Tris buffer, eliminating one pH adjustment and reducing total mixture volume (Lee *et al.*, 1992). A simplified method using starch gelatinisation and digestion with amyloglucosidase only provided comparable values for 12 food samples (Li & Andrews, 1988), and for several canned legumes but not for cooked beans due to incomplete starch removal (Li & Cardozo, 1993). Soluble dietary fibre (SDF) was precipitated in these methods by adding four volumes of 95% ethanol. Li and Cardozo (1992) also used a similar ethanol volume in their nonenzymatic–gravimetric procedure for fruits and vegetables.

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During TDF determinations of several sweet potato leaves, high residue ash concentrations (>30%), suggesting salt coprecipitation, were observed. Coprecipitation of oligosaccharides or Maillard reaction products (Wolters *et al.*, 1992), reagents such as buffer salts (Lee & Hicks, 1990), soluble sugars (Theander & Westerlund, 1986; Marlett & Navis, 1988; Marlett, 1990), starch hydrolysis products (Marlett, 1990) and condensed tannins (Saura-Calixto, 1988) occur during ethanol precipitation, probably contributing to errors in the TDF method. Mañas and Saura-Calixto (1993) obtained incomplete or greater than 100% recovery of alcohol precipitated pectin depending on the added organic acid.

Using less alcohol volumes to precipitate SDF may decrease coprecipitation. This study was conducted to determine whether reducing final ethanol concentrations from 76% to 41% and 56% will give similar TDF values for some green and root vegetables. Samples analyzed were raw collard and mustard greens, sweet potato leaves and storage roots, and sugar beet leaves and storage root.

MATERIALS AND METHODS

Materials

Collard (*Brassica oleracea* L.) and mustard (*Brassica juncea* (L.) Coss.) greens were purchased from a local

store in Tuskegee, AL (USA). Their outer damaged and/or slightly yellow leaves were discarded. Three cultivars (Georgia Jet, Jewel and TU-82-155) of sweet potato (*Ipomoea batatas* (L.) Lam.) greens were harvested from greenhouse beds in the George Washington Carver Agricultural Experiment Station. Only the leaves with their petioles in a 20 cm tip, or the unopened leaves and the first eight opened leaves with their petioles from the growing stem ends were used. Sweet potato storage roots, variety TU-82-155, were obtained from greenhouse plants grown by nutrient film technique (NFT) (Mortley *et al.*, 1991; Bonsi *et al.*, 1992). Sugar beet (*Beta vulgaris* L. cv. Great Western Sugar) leaves and storage roots were harvested from greenhouse plants grown by the same technique. Samples for the two trials were separately collected.

The greens were cut into small pieces with *c.* 5 mm lengths after washing and drying with paper towels. The storage roots were diced into *c.* 5 mm cubes after washing and peeling. Portions (5 g) were taken for dry matter determination (105°C for at least 4 h). The rest of the greens were dried in an oven at 50°C for *c.* 24 h while the remaining storage roots were freeze-dried to prevent browning reactions. The dried samples were ground to less than 0·1 mm size and stored in air-tight bags. All samples were not defatted before TDF determination since the crude fat content obtained by Sohxlet method was less than 5% (dry basis).

TDF determination

The AOAC enzymatic–gravimetric method 985·29 (AOAC, 1990) was used with some modifications. Sample size was reduced to 0·5 g and reagent volumes were halved. Incubation with heat-stable α-amylase (Sigma Chemical Co., St. Louis, MO, USA) in boiling water bath was extended to 30 min. The cooled assay solution pH was adjusted to 7·5 with 0·5 M NaOH and checked with pHydrion paper, pH range 6·0–8·0 (Micro Essential Laboratory, Brooklyn, NY, USA). The pH of the suspension after protease (Sigma) incubation was adjusted to 4·5 with 0·5 M HCl and checked with pHydrion paper, pH range 3·0–5·5. Using pHydrion paper rather than a pH meter reduced assay volume change due to electrode rinsing. Total assay volume after amyloglucosidase (Sigma) digestion was about 30 ml. Volumes of analytical grade 90% ethanol used for precipitation were 25, 50 and 160 ml, corresponding to final concentrations of 41%, 56% and 76% (v/v).

The residue and Celite were removed from the crucible, ground and mixed well, and only portions (25 mg) were used for micro-Kjeldahl N determination. Correction for Celite weight was made by dividing %N for residue plus Celite by the ratio residue weight/residue plus Celite weight.

Statistical analysis

TDF in the vegetables was analyzed with six replicates in Trial 1 and four replicates in Trial 2. Means, standard deviations (SD) and errors of the mean (SE), and statistical significance of the differences among the means were obtained by analysis of variance using Statview 4·01 (Abacus Concepts, Inc., Berkeley, CA, USA).

RESULTS AND DISCUSSION

TDF values for uncooked sweet potato leaves and storage roots at 41%, 56% and 76% final ethanol concentrations are shown in Table 1. In Trial 1, only the unopened and open leaves with their petioles from a 20 cm stem were used. Since more mature leaves are also consumed in countries where the leaves are eaten as greens, the unopened and the first eight open leaves with petioles from the growing end were used in Trial 2. In many Asian urban markets, the stems sold are generally at least 40 cm long. TDF levels at different alcohol concentrations were not different in at least one trial for each sample. When there was a significant difference, the means ± SD overlapped.

Table 1. Total dietary fibre content of sweet potato leaves and storage roots (% fresh weight) at different final ethanol concentrations[a,b]

Vegetable	Trial	% Dry matter	Reduced ethanol		AOAC method
			41%	56%	76%
Leaves[c]					
Jewel	1	10·33	3·72 ± 0·26a	3·63 ± 0·07a	3·30 ± 0·33b
	2	16·34	6·53 ± 0·48a	6·35 ± 0·11a	6·09 ± 0·24a
Georgia Jet	1	10·15	3·81 ± 0·15a	3·60 ± 0·14a	3·81 ± 0·60a
	2	19·15	7·22 ± 0·27a	7·25 ± 0·26a	7·24 ± 0·56a
TU-82-155	1	9·37	3·48 ± 0·16a	3·02 ± 0·06b	3·40 ± 0·23a
	2	15·20	5·10 ± 0·23a	5·16 ± 0·51a	5·39 ± 0·20a
Storage root					
TU-82-155	1	16·79	2·08 ± 0·23b	2·30 ± 0·16a	1·87 ± 0·12c
	2	16·38	2·64 ± 0·15a	2·42 ± 0·28a	2·56 ± 0·10a

[a]Mean ± SD for six replicates in Trial 1 and four replicates in Trial 2.
[b]Means with different following letters for the same vegetable and trial are significantly different at $P < 0·05$.
[c]Trial 1 used leaves from a 20 cm stem while Trial 2 used the unopened and first eight open leaves from the growing end, the stem length depending on the distance between internodes.

Table 2. Total dietary fibre content of collard, mustard and sugar beet (% fresh weight) at different final ethanol concentrations[a,b]

Vegetable	Trial	% Dry matter	Reduced ethanol		AOAC method
			41%	56%	76%
Collard greens	1	13·62	4·63 ± 0·31a	3·95 ± 0·16b	ND[c]
	2	14·72	4·06 ± 0·11a	3·94 ± 0·26a	4·27 ± 0·11a
Mustard greens	1	9·48	2·64 ± 0·24b	2·65 ± 0·05b	2·92 ± 0·23a
	2	10·46	3·26 ± 0·32a	2·65 ± 0·25b	2·65 ± 0·38b
Sugar beet					
Leaves	1	13·55	3·12 ± 0·18b	3·81 ± 0·42a	3·51 ± 1·05b
	2	13·80	3·63 ± 0·58a	3·80 ± 0·49a	4·33 ± 0·06a
Root	1	19·43	2·69 ± 0·16a	2·96 ± 0·21a	2·79 ± 0·40a
	2	19·06	2·95 ± 0·15b	2·79 ± 0·13c	3·94 ± 0·11a

[a]Mean ± SD for six replicates in Trial 1 and four replicates in Trial 2.
[b]Means with different following letters for the same vegetable and trial are significantly different at $P < 0.05$.
[c]Not determined.

Table 3. Ash and protein concentrations in sweet potato leaf residues precipitated by different volumes of ethanol[a,b]

Variety	Ethanol concentration (%)	Ash (%)	Protein (%)
Jewel	41	20·96 ± 2·03b	19·32 ± 1·29a
	56	15·02 ± 1·66c	17·75 ± 0·56b
	76	48·83 ± 3·16a	14·97 ± 0·85c
Georgia Jet	41	23·01 ± 1·01b	17·04 ± 2·39a
	56	17·66 ± 1·89c	17·77 ± 0·76a
	76	50·86 ± 6·88a	11·71 ± 0·69b
TU-82-155	41	10·58 ± 0·65c	22·88 ± 1·02a
	56	14·89 ± 0·41b	20·87 ± 2·14b
	76	35·50 ± 1·56a	15·88 ± 1·33c

[a]Mean ± SD for three replicates for ash and six replicates for protein.
[b]Means with different letters in the same column for each variety are significantly different at $P < 0.001$.

TDF contents for raw collard and mustard greens, sugar beet leaves and storage roots (Table 2) were also not different in some trials. Where there was a significant difference, the means ± SD usually overlapped. Similar to the results in Table 1, the means also did not always increase with more alcohol.

After sequential digestion with α-amylase, protease and amyloglucosidase, the residues were corrected for ash and protein. Percent ash and protein for the sweet potato leaves in Trial 1 are in Table 3. Protein content decreased as more alcohol was used for precipitation. Percent ash at 56% ethanol was lower than the value at 41% for Jewel and Georgia Jet probably due to the greater dilution of the salt concentration in the larger final suspension volume at the higher ethanol concentration. This trend was observed in most of the trials. However, ash concentration increased considerably at 76% ethanol due to the decreased solubility of the salts at this alcohol level. Marlett and Navis (1988) obtained 46·0–61·0% ash in the blank residues at 76% alcohol. Thus, the amount of salt that coprecipitates with SDF vary and partly depends on alcohol concentration.

SDF values determined by the enzymatic–chemical Englyst method (Englyst *et al.*, 1982; Englyst & Cummings, 1988) were higher than those obtained by AOAC method 991·43 (AOAC, 1992) possibly due to incomplete precipitation even at 76% ethanol (Larm *et al.*, 1975; Englyst *et al.*, 1982; Amado & Neukom, 1985). However, Mañas and Saura-Calixto (1993) obtained incomplete or more than 100% recovery of pectin with ethanol due to coprecipitation with added organic acids. The amount of SDF precipitated with ethanol may be influenced by other components in the assay suspension and not by alcohol concentration only.

Reported SDF and TDF values for the vegetables tested in this study are limited, and had been calculated from cellulose, lignin, hemicellulose and non-starch polysaccharide contents determined mainly by Southgate's method (Candlish *et al.*, 1987; Dreher, 1987; Anuradha & Prakash, 1989). The SDF content as percent of TDF ranged from 13·1% for corn to 51·8% for potatoes and averaged 32·3% for 15 vegetables investigated by Anderson and Bridges (1988). In uncooked green and root vegetables, possible error in TDF due to incomplete precipitation of SDF at lower alcohol concentrations may be within the variability of the recommended AOAC method as shown in Tables 1 and 2. Thus, reduced alcohol volumes for TDF determination in green vegetables and some storage roots may be used. In this study, 41% ethanol appears to be sufficient for the vegetables analyzed. Decreasing the amount of alcohol for precipitation reduces analysis cost, filtration time and environmental organic solvent contamination.

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