method would be acceptble for diagnostic and most experimental work. Isaac and Johnson (1975) and Smith and Schrenk (1972) found no difference between dry ashing and wet ashing for micronutrients in alfalfa, citrus, orchard, pecan, and tomato leaves, and in alfalfa, corn, sorghum, and wheat leaves, respectively. Baker and Smith (1974) also reported no differences for micronutrients in cabbage leaves but reported lower value for Cu in corn cob by dry ashing relative to wet ashing. Our experience has been that for nonleaf samples, such as dried strawberry fruit and wheat flour, lower recoveries occur for Cu by dry ashing. The slightly lower recovery of Cu on a number of crops by method 1 relative to method 3 could be due to retention of this element by Si on the crucible, or by Si in the sample (Allen, 1971). An increase in ashing temperature from 475 to 500 °C may have given slightly higher Cu values because Cu appears to be retained on unoxidized carbonaceous residue (Baker and Smith, 1974). Although temperatures above 500 °C may give better Cu values, significant losses of K and Mn could occur (Gorsuch, 1970; Isaac and Jones, 1972).

The higher values for K by method 1 compared to method 3 on some crops could be partly explained as a result of precipitation of $KClO_4$ when the digest was being diluted in method 3. Apparently the warming of the solution did not quantitatively dissolve the precipitate. Precipitation of K as $KClO_4$ is likely if the K levels are high and the sample weight is also high (Johnson and Ulrich, 1959). There is no obvious explanation for the slightly higher Mg values by method 1. Variation of some replicates for Mg was greater than normal and the possibility of contamination cannot be ruled out.

From this investigation the following can be concluded: (i) A dry ashing procedure which gave comparable results to wet ashing for Fe, Mn, Zn, Cu, Ca, and Mg using atomic absorption and for K using flame emission has been found acceptable for a wide variety of horticultural crops. (ii) No advantage was gained by digesting the ash in 6 N HCl and bringing it to dryness twice; the more simple and rapid method of warming the ash for 0.5 h with 2 N HCl gave comparable results. (iii) Wet ashing using $H_2SO_4 + H_2O_2$ gave a high percentage of unsatisfactory Fe values and occasional unsatisfactory Ca and Zn values. This method, therefore, has a limited application.

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Celery Leaf Juice: Evaluation and Utilization of a Product from Harvest Debris

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Celery trimmings, primarily leaves, from Fraser Valley and Okanagen vegetable packinghouses, which are normally not utilized for human consumption, yielded upon macerating and pressing 70–80% of a dark-green, bitter-tasting juice. Removal of the chloroplast fraction by low centrifugal fields resulted in a clear, yellow, celery-flavored juice, of acceptable taste. This refined celery leaf juice contained 5–6% of total solids, 0.8% of total crude protein, 0.1% of heat-precipitable proteins and amino acids, peptides, and proteins not preciptitated by heat. It also contained the vitamins, niacin, riboflavin, thiamin, and ascorbic acid at 0.45, 0.05, 0.01, and 2 mg/100 g, respectively. The major mineral constituents were Ca, 0.30%; Na, 0.20%; 0.15%; Mg, 0.02%; P, 0.01%; trace amounts of Fe, Cu, Mn, Al, Ni, Cr, Ba, and Sr were also present. Most of the lipid material, which contained some thiodan I, II, and malathion from agricultural practices, was removed from the juice with the chloroplast fraction during centrifugation. Rat feeding trials with the refined celery leaf juice solids (LJS) over a period of 8 weeks showed no growth inhibition or gross abnormalities of the major organs. Taste panels preferred tomato–celery leaf juice blends over commercially available V-8 type vegetable juices.

Relatively few of the plant leaves growing on earth are used directly in the human diet. Edible leafy plants representative of different plant families in the northern hemisphere such as spinach, lettuce, cabbage, asparagus, parsley, onion, and bamboo shoot have been selected over many years for their low bitterness, low fiber content, and for the absence of toxic constituents. The inherent objectionable bitterness and astringent character are major quality aspects which make most of the plant leaves unsuitable for human consumption.

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Experiments to obtain from leaves nutrient preparations, particularly proteins, suitable for nonruminants were begun about 40–50 years ago (Pirie, 1971). In recent years, processing of alfalfa has received considerable attention. Removal of the high lipid content chloroplast fraction from alfalfa press juice was not possible by low-speed centrifugation and only partially successful at very high centrifugal forces (de Fremery et al., 1973). Developmental efforts, to extract nutrients present in leaves for human consumption, continued therefore to be centered on separating protein fractions from the press juices of green leaves by heat, solvent, or acid precipitation (Pirie, 1971; Bickoff and Kohler, 1974; Huang et al., 1971). However, in these commonly employed techniques, nutrients such as vitamins and minerals, soluble proteins, peptides, and amino acids were lost or returned to the animal feed fraction.

Celery leaves and trimmings accumulate during harvest at an average of 26 tons/acre or about 3,800 tons/year in the Fraser Valley (Schierbeck, 1973). The present studies, into a more extensive utilization of the leafy material particularly from celery, but also some other plants, were therefore undertaken with emphasis on plant leaf juices from which the chloroplast fraction can be easily removed by low-speed centrifugation, which is not possible in the case of alfalfa press juice.

EXPERIMENTAL SECTION

Juicing Operation. Fresh or frozen leafy material and trimmings (50 kg) from Utah 15 and 52–70 celery, *Apium graveolens* L. varieties, were macerated in a hammer mill at 0–10 °C, with the addition of 0.01 to 0.02% of ascorbic acid. Throughout the operation the starting material as well as the product were kept as close to 0 °C as possible to reduce the rate of browning and degradation of nutrients.

Pressing the macerate on a rack and cloth press about 17 MPa yielded a dark-green juice. The green chloroplast fraction was removed from the juice by centrifugation at 0 °C in a bucket-type or angle-head centrifuge at an average centrifugal force of about 2500*g* for 10 min and the clear, light-yellow supernatant liquid, the juice, was readily decanted from the dark-green, bitter-tasting, firm chloroplast pellet.

Freeze-drying of the centrifuged leaf juice was carried out in a Virtis pilot plant freeze-dryer with initial conditions of −30 °C and 1.32 to 0.67 cPa of pressure. After 1 h the conditions were changed to 20 °C shelf temperature while the pressure adjusted itself to about 0.4 cPa. Under these conditions the juice remained frozen, and after 24 h of freeze-drying, the final dried leaf juice solids (LJS) with the entrapped flavor substances were obtained. Other umbelliferous plant leaves such as parsley and carrot, when treated as described above for the celery material, yielded similar juices and leaf juice solids with flavors characteristic of the plants used.

Chemical Analyses. After ashing of the samples, the mineral constituents of the LJS were analyzed by an emission spectrograph. Amino acid analysis was carried out with a Beckman Amino Acid Analyzer on the total LJS and on the heat-precipitated proteins. Acid hydrolyses of samples containing about 5.0 mg of protein were carried out with 6 N HCl (1.0 mL/2-mg sample) at 110 °C for 22 and 48 h. The hydrolysis tubes were evacuated prior to being sealed. The humin formed during hydrolysis was filtered off after the tubes had again been opened and the filtrate was then taken to dryness. The dry matter was redissolved in distilled water and taken to dryness two more times before being dissolved in 7.5 mL of 2.0 M sodium citrate buffer, pH 2.2, for analysis.

Vitamin analyses were carried out according to the procedures described in Methods of Vitamin Assay (Association of Vitamin Chemists, 1966). Riboflavin and thiamin were determined by the fluorometric and thiochrome methods, respectively, niacin by a bacteriological method with *Lactobacillus arabinosus*, and ascorbic acid by the dichlorophenolindophenol titration.

Established procedures (McLeod et al., 1973) were used for pesticide residue extraction, cleanup, gas–liquid chromatographic detection, and in the case of endosulfan, heptachlor epoxide, dieldrin, and related dichlorodiphenyltrichloroethane (*p,p'*-DDT) derivatives such as *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD confirmatory analysis following procedures of oxidation, hydrolysis, acetylation, or silylation etc. was carried out.

Rat Feeding Studies. Two test groups of Sprague Dawley rats, each group consisting of five males (45–48 g) and five females (47–53 g), whose feed consisted of a basal diet into which increasing amounts of LJS were mixed, so that the average weekly expected intake would be about 5.0 g of LJS/kg of bodyweight. The control groups consisted also of male (43–48 g) and female (47–52 g) weanling rats but were fed a basal diet only. The feeding studies were continued for a total of 8 weeks and weight gain and feed consumption were recorded on a weekly basis.

Taste Panels. To achieve optimum levels of the tomato–celery juice mixes, ten experienced panelists evaluated the juices for bitterness (very bitter, not objectionable and no bitterness at all) as well as for celery flavor (good, some off-flavor, no celery flavor). Preference tests with commericially available V-8 type juices and 20–40% celery leaf juice: tomato juice mixtures were carried out and statistical analysis of the ranked results was made with the Multiple Comparison Difference and Duncan's Multiple Range Tests.

RESULTS AND DISCUSSION

Hydraulic pressing of celery harvest debris yielded 70–80% of a dark-green, bitter-tasting juice. After centrifugation at an average centrifugal force of 2500*g*, a clear-yellow, celery-flavored juice of acceptable taste containing 5–6% total solids was obtained, which was readily decanted from the dark-green exceedingly bitter-tasting, firm chloroplast pellet. Under identical conditions, alfalfa yielded only 45–50% of press juice and during centrifugation most of its chloroplast fraction remained suspended in solution while a small sediment of it was sufficiently fluid to mix readily with the supernate during decantation. Vegetable leaf juices of the kind made from celery press juice could also be prepared from leaves such as parsley, dill, carrot, lettuce, and cabbage. However, the distinct flavors associated with juices from umbelliferous plant leaves made them preferable additions in the preparation of vegetable drinks, particularly when mixed at levels of 20 to 40% with tomato juice prepared from tomato concentrate. Taste panel evaluations showed that the tomato–celery leaf juice blends were preferred over commercially available tomato juice based vegetable V-8 type drinks. Statistical analysis of the preference for a blend containing 20% refined celery leaf juice (A) over samples of V-8 type vegetable juice (B) and 40% celery leaf juice (C) was significant at the 5% level. In these tests A was found to be significantly different from B and C, but B was not significantly different from C.

While tomato juice is rather thick bodied or viscous, celery leaf juice has very little body, and the mixed product at leaf juice proportions greater than 20% therefore tends

Table I. Proximate Gross Chemical Composition of Different Celery Leaf Fractions from Fraser Valley Harvest Debris

	% on fresh basis		% on dry basis		
	Crude protein[a]	Moisture	Crude protein[a]	Ash	Lipid
Leaves	2.7-2.9	90-91	26-29	12	1.3-2.5
Press cake	7.5-8.5	70-75	29-31		2.3
Leaf juice (LJS)[b]	0.7	94-95	13.5-14.0	28-30	0.05
"Chloroplasts"[c]	36	10	42		25.4
Petioles	1.2	92	12		

[a] Crude protein = (% N × 6.25). [b] Leaf juice solids (LJS) are obtained after freeze-drying of the centrifuged leaf juice. [c] The chloroplast fraction is present in the press juice at about 1-3% level.

to attain a relatively thin appearance. This problem can be overcome by thickening the mixture slightly with carboxymethyl cellulose or by using celery juice and water for the reconstitution of tomato paste. A 20% tomato–celery blend usually was at pH 4.30–4.36 and required no acidification for processing.

Freeze-drying of the leaf juices produced hygroscopic powders, the leaf juice solids (LJS), which retained the vegetable flavors originally present in the juices. Upon resuspending the LJS in water the flavors were again released and could be distilled from the solution. This behavior made the LJS suitable as a nutritive, dry mix flavor additive for water-based products.

The gross composition of the different celery leaf fractions is presented in Table I. Comparing the crude protein extracted from the leaves into the refined leaf juice or LJS, with the total protein of the press cake and of the leaf juice or LJS, the protein obtained for human consumption was about 20–25%. While the bulk of the lipid material was also retained in the press cake, most of the lipids of the leaf juice (or LJS) remained in the sedimented chloroplast fraction. Representative analyses of lipid fractions from the celery leaves harvested in 1974 in the Fraser Valley for pesticide residues were as follows: thiodan I and II were found at levels of 454.9 and 377.7 parts per million, respectively, malathion at 261.2 ppm while heptachlor epoxide was present at 2.4 ppm and dieldrin at 0.5 ppm. The DDT (1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane) derivatives *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE were present at 3.3, 1.0, 0.7, and 0.7 ppm. The low level of lipids in the final products had two advantages. Firstly, problems from oxidative storage deterioration in the juice and the LJS were reduced, and secondly, the level of residual pesticides, associated primarily with the lipid fraction, was also reduced considerably.

The above levels of pesticide residues converted to the residue content of fresh celery, according to Health and Welfare standards, would still be in the allowable region for human consumption. However, this situation does point out that the production of by-products from cultivated crops must be approached with extreme caution so as not to concentrate undesirable residues in the final product.

The possible presence of toxic material such as alkaloids, enzyme inhibitors etc. in the leafy material was investigated by submitting the refined LJS, prepared from the centrifuged celery leaf juice, to animal feeding tests. During the 8-week study, the test animals, males and females alike, consumed an average of 5.43 g of LJS (kg of bodyweight)$^{-1}$ week^{-1} without showing any significant differences in weight gain from the control groups. At the end of the feeding experiments, the average weight gain of the males and females in the control group was 267.8 and 154.4 g, respectively, while the animals in the test groups gained 259.0 and 145.8 g. Futhermore, since post-mortem examinations revealed no gross abnormalities in any tissues or organs, signs of toxicity in the LJS were not present under the conditions of the test. Extrapolated to human condition the test was equivalent to the consumption of about 12 × 100 mL of centrifuged celery leaf juice per day for 8 weeks.

Table II. Amino Acid Composition (g of Amino Acids/100 g of Recovered Amino Acids) of the Different Celery Leaf Fractions Compared with Alfalfa

	Celery leaf juice solids (LJS),[a]	Heat-precipitated proteins	
		Celery	Alfalfa[b]
Lys	3.93	7.63	6.3
His	1.30	1.90	2.1
NH_3	9.38	0.81	
Arg	1.19	4.33	5.8
$CySO_3H$	2.06		
Asp	28.24	11.94	10.2
Thr	3.66	6.15	5.1
Ser	3.88	5.84	4.3
Glu	19.56	11.22	11.4
Pro	4.69	4.82	4.8
Cys		0.82	0.6
Gly	2.69	5.32	5.7
Ala	4.13	5.97	6.4
Val	5.09	6.17	6.3
Met		1.98	1.9
Ile	3.21	5.06	6.6
Leu	3.88	9.41	9.6
Tyr		4.94	4.5
Phe	3.12	5.68	6.4
Try	*c*	*c*	1.9
	100.01	99.99	99.9

[a] (LJS) are the solids obtained from the press juice after removal of a green, bitter-tasting chloroplast fraction. [b] Data according to Gerloff et al. (1965). [c] Analysis not carried out.

The vitamin content of the LJS or the vegetable leaf juice compared to the fresh vegetable portion (Watt and Merrill, 1963) was very similar, except for the lower ascorbic acid concentration. Obviously, in the process of preparation the water-soluble vitamins were extracted from the leaves into the juices and concentrated into the solids. In the respective celery or parsley LJS the analyses per 100 g of sample were 8.69 and 9.33 mg of niacin, 0.99 and 0.91 mg of riboflavin, 0.15 and 0.12 mg of thiamin, and 30 and 35 mg of ascorbic acid. Minerals contained in the LJS were primarily Ca 4.27–5.70%, Na 1.42–4.27%, K 1.42–2.85%, Mg 0.28–0.86%, P 0.14–0.43%, Mn 0.009–0.026%, and Sr 0.006–0.020%. The high values of Ca, Na, K, Mg, P, and Sr were obtained from the LJS prepared from relatively fresh celery leaf samples harvested in the Okanagan Valley (Oliver). The respective LJS also had a 1.9% higher total ash content than the samples from the Fraser Valley. Trace elements reported for all samples were Si, Al, Fe, V, Cu, Ti, Ni, Cr, Ba while the Okanagan samples contained Mo in addition to the other elements. Compared to the mineral content of the edible stalk portion reported (Watt and Merrill, 1963), the celery leaf juice contained about eight times more Ca, two times more Fe, about the same amounts of Na and P, while the K levels were from 10 to 50% lower.

The amino acid composition of the heat precipitable proteins, present in celery leaf juice at a level of about 0.1%, is shown in Table II and compares well with that

of alfalfa (Gerloff et al., 1965; Smith, 1966). Those amino acids present in somewhat greater amounts in the celery protein were lysine, aspartic acid, threonine, and serine. Protein content of leaf protein concentrates can vary from as much as 32 to 84% (Gerloff et al., 1965). The higher percentage of protein in these present preparations (85%) was obtained by washing the precipitated protein fraction three times with water and three times with ethanol, thus extracting extraneous material such as carbohydrates and phenolic material, presumably chlorogenic acid, whose presence has also been shown in alfalfa protein concentrate (Free and Satterlee, 1975). The formation of acid-stable products from the reaction between amines and phenolics or carbohydrates is well documented (Van Sumere et al., 1975; Reynolds, 1965) and could lead to some reduction in the amino acid content of the protein. The LJS besides containing 13.5–14.0% crude protein also contained about 50% of carbohydrate-like material as well as 30% of mineral oxides or ash. During the amino acid analysis of the LJS only about 50% of the Kjeldahl nitrogen present in the sample before hydrolysis was recovered in the form of amino acid nitrogen, including ammonia. Most of the losses occurred presumably with the relatively large amounts of humin filtered from these hydrolysates. The results on the LJS fraction are therefore considered preliminary. However, interesting in this analysis of the total amino acids is the high content of aspartic and glutamic acids and the apparent oxidation of methionine and of cysteine to cysteic acid. Analysis for tryptophan was not carried out.

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Quantitative Determination by GLC of Phenolic Acids as Ethyl Derivatives in Cereal Straws

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A new method was used to analyze barley, oat, wheat, rye, and rice straws for phenolic acids. The acids were extracted with aqueous alkali. The phenolic acid were recovered from this extract and alkylated with ethyl iodide and identified and quantified by GLC and GLC–MS. Eight phenolic acids, *p*-hydroxybenzoic, vanillic, *cis*-*p*-coumaric, syringic, *trans*-*p*-coumaric, *cis*-ferulic, *trans*-ferulic, and *trans*-sinapic acids, were identified in all straws. *trans*-*p*-Coumaric acid and *trans*-ferulic acid were the dominant acids in the investigated straws.

Benzoic acids and cinnamic acids are widely distributed in plants (Ribéreau-Gayon, 1972). The cinnamic acids are found in various combined forms, for example, as glycosides, sugar esters (Ribéreau-Gayon, 1972), and as esters linked to carbohydrates in the cell walls (Hartley, 1973; Hartley et al., 1973; Harris and Hartley, 1976). Phenolic acids have been analyzed in 80% ethanol extracts and in the extraction residues from mature oats, wheat, sorghum, and corn residues by paper chromatography (Guenzi and McCalla, 1966) and in rice straw by GLC (Kuwatsuka and Shindo, 1973). Diferulic acids together with monomeric phenolic acids have been reported from cell walls of *Lolium multiflorum* (Hartley and Jones, 1976).

trans-Cinnamic acids are known to be partially convertable to their cis analogues by UV light, the conversion being maximized between pH 5.0 and 7.0 (Neish, 1961;

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