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Short Communication

Determination of Macronutrient Compositions in Selected, Frequently Consumed Leafy Vegetables, Prepared According to Common Culinary Methods in Sri Lanka

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Abstract

Information regarding realistic macronutrient gains by consuming cooked Sri Lankan leafy vegetables are rare. Some available information often overestimates available carbohydrate contents and under-estimates dietary fibre contents, as a result of not using in-vitro digestion models prior to proximate analysis. In aim to address this issue, nine most frequently consumed leafy vegetables types in Sri Lanka were cooked and analysed for their moisture, digestible carbohydrate, protein, fat, soluble fibre and insoluble fibre contents. All vegetables were prepared in most frequently practiced culinary methods by the local community such as salads, tempered with oils, or as curries/gravies. Dry weights of all macronutrients were determined using six replicates to maximize the accuracy of results.

Majority of the leafy vegetable types selected elicited substantial amounts of dietary fibre. The highest content of insoluble dietary fibre was present in *Centella asiatica* (centella) leaves salad (51.0±3.4%), whereas highest percentage of soluble dietary fibre was in *Heracleum sphondylium* (hogweed) leaves prepared as a curry (16.4±1.7%). *H. sphondylium* curry (20.7±1.3%) and *Sesbania grandiflora* (Hummingbird) leaves salad (20.7±0.9%) resulted in greatest amounts of digestible carbohydrates. The highest fat content (12.6±0.5%) was in *Ipomoea aquatic* (water spinach) since it was tempered with coconut oil as it is the mostly practiced local culinary method for *Ipomoea*. *Spinacia oleracea* (spinach) curry elicited the highest protein content (4.8±0.9%) among all leafy vegetables.

Keywords: Dietary fibre, leafy vegetables, digestible carbohydrates, *Centella asiatica*, *Heracleum sphondylium*, *Sesbania grandiflora*

1. Introduction

The diversity of leafy vegetables grown in Sri Lanka is high due to its tropical climate and soil fertility. The preparation methods by locals are diverse. Realistic gains of nutrients by consuming prepared leafy vegetable are rare to find, making it difficult to understand the varietal differences of nutrients as well as the impact by cooking / preparation on the total nutrient content (Jayasinghe et al., 2015).

Misleading of the dietary fibre content present in leafy vegetables as carbohydrates is often when carbohydrate amount is being calculated as the 'remainder' after analysing proximate compositions of

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other nutrients (Thadani et al., 2010). Thus, over-estimation of carbohydrates in leafy vegetables and under-estimation of dietary fibre content happens frequently. Such results have become obstacles when searching literature to conduct glycaemic studies (Samarasinghe et al., 2018).

The available fat content of a prepared leafy vegetable may vary greatly according to the preparation method (Aletor et al., 2002). The levels of fat absorbance to different leafy vegetables during tempering or frying greatly vary as well. Further, it is a common practice to incorporate scraped coconut when preparing leafy vegetable salads in Sri Lanka, which again increase their fat content significantly (Senadheera, 2016). Hence, it is important to know realistic available fat content in cooked/prepared leafy vegetables.

All of the stated requirements of macronutrient composition analysis were expected to be fulfilled by this study. The results of this study will contribute to the national/international food composition databases.

2. Materials and methods

2.1 Determination of moisture content

Three replicates of each food sample (1.0 g) were measured (Sartorius BP 110 S max 110 g) in to dry, cool and weighed porcelain crucibles respectively and dried at 105° C (Memmet) until constant weight (AOAC 993.19 and 993.21).

$$\% \text{Moisture} = [(W2 - W1) \times 100] \div W \quad (1)$$

Where: W=Weight of the sample

W2=Weight of crucible+sample before drying

W1=Weight of crucible+sample after drying

2.2 Determination of the digestible carbohydrate content

Six samples (0.5 g) from each food item, were digested using 200 µL (3979 units) of α-amylase (Sigma-Aldrich, USA) in a boiling water bath (OLS 200) for 25 minutes. When samples were cooled to the room temperature, they were added with Amyloglucosidase (EC 3.2.1.3 Sigma-Aldrich, USA) 11.5 µL (10.808 units) in a Sodium Acetate buffer medium. All six test tubes were incubated at 60° C water bath. Digested samples were tested for their glucose content by glucose oxidase kit respectively. Absorbance was measured at 500 nm against a reagent blank (Holm et al., 1986).

$$\% \text{DigestibleStarch} = (\text{Absorbance Sample} \times 50 \times 10 \times 0.9 \times 100) \div (\text{Abs. Standard} \times W) \quad (2)$$

Where: W=Weight of the sample in milligrams

2.3 Determination of fat content

Six samples (2.0 g) from each food item, were moistened with of 95% ethanol (2 mL). Acid digestion with 10 mL of 7.7 M HCl at 75° C for an hour was followed by the addition of 95% ethanol. Peroxide free diethyl ether 25 mL (EC No.200-467-2 VWR International Ltd, England) was added to Majonnier flasks. The solution mixture was continuously inverted, opening in time intervals to release the gas produced. When the gas production stopped, 25 mL of pet ether (EC No. 265-151-9 VWR International Ltd., England) was added and the process was continued. After keeping still, upper layers were transferred to conical flasks. Contents remaining in each Majonneir flasks were washed with 30 mL diethyl ether-pet ether mixtures of 1:1 ratio twice and upper layers separated again were added to conical flasks. Ether contents in flasks were allowed to be evaporated (Croon et al., 1980).

$$\% \text{ Fat} = [(W2 - W1) \div W] \times 100 \quad (3)$$

Where: W2=Weight of the conical flask+fat
 W1=Weight of the empty conical flask
 W=Weight of the sample

2.4 Insoluble dietary fibre determination

Six samples from each food item were homogenized at 17300 rpm for 2 min (ULTRA-TURRAX[®]) in 25 mL of phosphate buffer (pH 6.2). Digestion with 100 μ L α amylase in a boiling water bath at 100° C for 25 minutes was carried on followed by the addition of 20 mL of 0.2M cold HCl (4° C). The pH was adjusted to 1.5 (Thermo Electron Corporation Orion 410A+) and mixtures were digested with 0.1 g of pepsin (Fisons, England) at 40° C in a shaking water bath (EYELA Uni Thermo Shaker NTS-1300) for an hour. After cooling, pH was adjusted to 6.8. Pancreatin 0.1 g (EC No 232-468-9 Sigma-Aldrich, USA) was added to each sample and heated in 40° C for an hour with continuous shaking. The pH was adjusted to 4.5 and sample mixtures were filtered using No.02 porosity crucibles. Filtrates were put in separate conical flasks. Residues were washed with two 15 mL portions of 95% Ethanol and two 15 mL portions of Acetone (EC No 200-662-2-Sigma-Aldrich, USA). Finally, crucibles were dried at 105° C until a constant weight was observed followed by incineration at 550° C for 5 hours (Asp et al., 1983).

$$\% \text{ IDF} = (W1 - W2 \times 100) \div \text{Weight of Sample} \quad (4)$$

Where: W1=Weight of the crucible after drying at 105° C
 W2=Weight of the crucible after incinerating at 550° C

2.5 Soluble dietary fibre determination

Absolute ethanol was added to six filtrates separated in the above procedure until EtOH concentration was 76%. Samples were filtered using No.4 filtration crucibles and washed with two 15 mL portions of ethanol and acetone respectively. Crucibles were dried at 105° C until a constant weight was observed followed by incineration at 550° C for 5 hours (Asp et al., 1983).

$$\% \text{ SDF} = (W1 - W2 \times 100) \div \text{Weight of Sample} \quad (5)$$

Where: W1=Weight after drying at 105° C
 W2=Weight after incinerating at 550° C

2.6 Determination of ash

Flour sample (1.0 g) were kept in the muffle furnace (FORNS HOBERSAL) at 550° C for 5 hours. Six replicates were done for each variety. Final weights were obtained (AOAC 999.10).

$$\% \text{ Ash Content} = (W2 - W1 \times 100) \div W \quad (6)$$

Where: W=Weight of the sample
 W1=Weight of the crucible+ash
 W2=Weight of the crucible

2.7 Determination of protein content Kjeldhal method

Homogenous mixtures of food items digested using catalyst tablet (Cu) and 2 mL of Conc. Sulphuric acid. Six such replicates were made and flasks were connected to the fume trap and digested. The 5% boric acid solution was mixed with the indicator and clamped to the apparatus end. Sodium sulphate solution 8 mL was added to the flask followed by passing steam through until about 15 mL of

distillate was received. Titration was carried out with the standard HCL solution until the pink colour of the end point was reached (Bradstreet, 1954).

$$\% \text{ Nitrogen} = \left[(\text{Sample titre} - \text{Blank titre}) \times \text{Molarity of HCl} \times 14 \times 100 \right] \div (W \times 100) \quad (7)$$

Where: W=Weight of Sample

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 4.39 * \quad (8)$$

*Factor is highly specified for leafy/non leafy vegetables

3. Results

Results indicate about a greater impact by the cooking method on macronutrient composition due to incorporation of additional culinary ingredients. Moisture contents of the leafy vegetables were diminished significantly when tempered. Tempered *Ipomoea aquatica* contained only 54.8% of moisture (Table 1) compared to all other leafy vegetables prepared in different ways to that of *Ipomea*.

When prepared as curries, high absorption of fat from coconut milk have increased the fat content as well as in salads, where scraped coconut is being used for salad preparation. All leafy vegetables elicited insoluble fibre contents higher than 34%, where *Allium porum* resulted in highest value of 51.0±3.4%. All selected leafy vegetables were having considerable amounts of soluble fibre, where the highest amount (16.4±1.7%) was found in *Heracleum sphondylium* prepared as a curry (Table 1).

None of the leafy vegetables elicited considerable amounts of proteins. The highest value (4.8±0.9%) was found in *Spinacia oleracea* curry. The highest digestible carbohydrate content was elicited by *Sesbania grandiflora* salad, although it was just 20.7±0.9% (Table 1).

Table 1: Macronutrient compositions of leafy vegetables prepared according to most common culinary methods.

Food item	Moisture% (wb)	Mineral Ash% (dw)	Carbohy- drate% (dw)	Insoluble dietary fibre% (dw)	Soluble dietary fibre% (dw)	Fat% (dw)	Protein% (dw)
<i>Centella asiatica</i> (Centella/Gotukola) salad	74.8±3.7	6.5±1.1	18.6±2.2	49.3±2.3	15.7±1.2	5.6±0.5	0.6±0.1
<i>Sesbania grandiflora</i> (Hummingbird/Kathurumurunga) salad	73.7±3.6	7.3±2.7	20.7±0.9	47.0±3.8	13.6±0.6	4.8±1.5	2.1±0.6
<i>Ipomoea aquatic</i> (Water spinach/Kankun) tempered	54.8±2.9	5.5±1.5	18.0±1.6	49.3±3.8	15.7±0.8	12.6±0.5	1.6±0.1
<i>Allium porum</i> (Leek) curry	76.6±3.0	8.0±1.3	18.6±1.7	51.0±3.4	10.3±0.7	7.3±0.7	3.6±0.3
<i>Lasia spinosa</i> (Lasia/Kohila) Leaves and shoots salad	72.5±4.2	7.1±1.6	16.7±1.4	38.1±4.2	16.0±2.4	6.5±0.8	1.8±0.5
<i>Spinacia oleracea</i> (Spinach) curry	74.6±3.0	6.5±1.5	19.7±0.9	34.5±2.2	14.4±0.7	9.5±1.0	4.8±0.9
<i>Alternanthera sessilis</i> (Dwarf copperleaf/Mukunuwenna) salad	70.5±4.1	8.0±1.0	15.7±2.0	49.3±3.6	11.8±0.9	3.5±0.6	2.0±0.3
<i>Amaranthus viridis</i> (Green Amaranth/Koora thampala) curry	76.4±3.8	7.2±1.3	18.5±0.7	36.5±4.2	12.5±2.7	8.3±1.1	3.3±0.1
<i>Heracleum sphondylium</i> (Hogweed/Saarana) curry	78.6±2.0	5.0±2.3	20.5±1.3	38.6±3.2	16.4±1.7	8.3±1.0	1.0±0.2

wb: wet weight basis, dw: dry weight basis

4. Discussion

High contents of soluble and insoluble fibre are often falsely interpreted as carbohydrates, when in-vitro enzyme digestion mechanisms are not followed (Thadini et al., 2010). Often in similar studies, the ‘remainder’; after determination of other macronutrients are considered as the carbohydrate content. That may lead in to a false understanding as most of the soluble and insoluble fibre are embedded in the remainder. Therefore, South Asian communities often under-estimate the value of tropical leafy vegetables as a rich source of dietary fibre.

A typical local diet is expected to be rich in both soluble and insoluble dietary fibre. Lifestyle plus attitude changes combined with unawareness have refrained local community from utilizing the most of fibre rich food items, resulting rather low portion sizes of fruits and vegetables that are rich in dietary fibre. This reveals the impact on increased rates of bowel disorders, hemorrhoids and colon cancers (Bingham et al., 2003) amongst people with lower daily fibre intakes. Even in the social segments considered to have higher literacy levels; such as managerial level employees, the fibre intake levels were very poor due to lack of available data and awareness.

According to a previous dietary survey conducted in Sri Lanka, when individuals have been questioned about rich dietary fibre sources available for consumption, nearly 82% of individuals have answered by giving names of ‘papaya’ (*Carica papaya*) and Kohila (*Lasia spinosa*) only (Jayasinghe and Ranaweera, 2015). *Lasia spinosa* being very popular among locals as a rich source of insoluble fibre, is often consumed to prevent constipation. Although this trend gains many health benefits, the accumulation of heavy metals is much higher in *Lasia* than in other local leafy vegetable types (Kananke et al., 2016).

Incorporation of coconut milk seemed to have increased the protein content of leafy vegetables when they are prepared as curries/gravies. The significant contributions to the fat and protein contents of prepared leafy vegetables are assumed to be by the addition of coconut/palm oil and scraped coconut (Senadheera, 2016).

Locals often tend to consume leafy vegetables with scraped coconut when prepared as salads, and sometimes they use coconut milk to make curries. Addition of local spices and condiments such as garlic, onions, chilli and curry leaves is practiced irrespective of preparation methods. These culinary practices may add significant amounts of vitamins, minerals and phytochemicals to leafy vegetable side dishes (Jayasinghe et al., 2015). Hence, their micronutrient as well as antioxidant contents needs to be investigated further.

5. Conclusion

The selected nine types of Sri Lankan leafy vegetables, when prepared according to mostly practiced culinary methods; are excellent sources of dietary fibre and not remarkable sources of other macronutrients.

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