Determination of Macronutrient Compositions in Selected, Frequently Consumed Cereals Cereal Based Foods, and Pulses Prepared According To Common Culinary Methods in Sri Lanka

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***Abstract*- Cereal grains, legumes and pulses have a vast contribution towards Sri Lankan diet fulfilling the dietary requirements of the community. This research is mainly focusing on determining the macronutrient composition of major cereals, legumes and pulses commonly consumed in local cuisine. White Samba, White Basmati, Red Kekulu and Nadu rice were selected as the main rice varieties and in addition maize, and finger millet flour-based food were analyzed under cereals and related products. Under legume category Red cowpea, green gram, chickpea and vegetable soya bean were taken as the most consumed beans in Sri Lanka while lentils were analyzed as the most preferred pulse in local cuisine. Particularly in Sri Lanka, for every main meal, people consume rice with few curries in which rice accounts roughly two third of the dish. Lentils and soya curry ought to be two of the most popular curries among locals. Moreover, bread has become the second most preferred food next to rice and usually consumed in breakfast or dinner. The determination of macronutrient compositions (dry matter basis) of most consumed cereals and other cereal based products like bread, Pittu, several varieties of cooked rice, are helpful to identify their contribution towards carbohydrate, protein and dietary fiber fulfilment in diet. Every item was cooked according to the common culinary practices. Proximate analysis was carried out according to the AOAC standard guidelines for the determination of moisture, carbohydrate, fat, and protein whereas, insoluble dietary fiber and soluble dietary fiber were determined by enzymatic digestion. Results reveal digestible carbohydrate level range from 64.6±5.3 % to 74.6±7.2 among tested rice varieties while protein content varies from 5.6±2.2 to 8.9±2.6. Insoluble dietary firer was high in boiled Red Kekulu (9.8±0.2) and low in boiled White Basmati. Amongst tested leguminous beans pressure cooked red cowpea had the highest protein level (24.5±2.3%) while pressure cooked green gram had the lowest protein content (20.0±0.6%). Pressure cooked chick pea had higher insoluble dietary content (14.8±1.3%) compared to other beans. Soybean curry () had the highest protein (36.6%) and fat content (8.2%) among all the tested varieties. Pittu and roti varieties made with wheat flour and finger millet flour had considerable higher levels of digestible fiber compared to roti made solely with wheat flour. It can be concluded that typical Sri Lankan diet is rich with fiber and protein while it provides excellent macronutrient profile due to the proper complementary mixing of food ingredients.**

Index Terms- Cereals grains, pulses, Macronutrients, enzymatic digestion.

INTRODUCTION

Food consumption pattern in Sri Lanka has faced dramatic changes over last two decades. It is linked with many factors like income, prices of the food, availability and accessibility, and urbanized lifestyles of people (Herath, R.M et al., 2007). Rice (Oryza sativa L.) is both, the most consumed staple food (Ranawake et al., 2013) and principle cereal grain cultivated in Sri Lanka. In addition to rice there are several other cereals, cereal based foods like bread and pulses which are also very common in Sri Lankan main meals. Whole grains are rich in nutrients and phytochemicals such as antioxidants dietary fibre, vitamins, minerals and fat (Slavin 2003, cited in Hettiarachchi et al., 2001). Rice being the staple food in country, has a great contributon to fulfill the dietery energy, fat and protein requirements of the consumers. For instance, Rice contributes to nearly 50% of the enrgy and 40 % of preotein intake in daily diet (Darandakumbura et al., 2013) Therefore, understanding the nutritional composition rice and other grains associated with main diets is at utmost importance which in turn helps to decide the healthy utilization of grains and pulses in our diet.

Among rice, wheat and pulses, rice is the most common and cheapest source of energy in Sri Lanka. In contrast, Bread has become a staple diet in urban areas. Together they account for 65% of the energy intake and the contribution form rice has been decreased from 80% in 1980 to 46% in 1990 (Herath, R.M et al.,2007). Although usual white bread is categorized under high Glycemic Index (GI) foods, incorporation of lentils can reduce the GI values of meal from high to medium GI category (Hettiaratchi, U.P,K et al, 2009). Yet, the consumption of rice versus wheat flour products like bread is shifting time to time depending on the wheat flour price. Increased Wheat four consumption has been raising lots of health concerns among people. Recent studies implies that there is a definite upward trend in in the prevalence of diabetes mellitus in Sri Lanka while in 1990 prevalence was 2.5% and it has increased up to 14.2 % in males and 13.5% in females by 2005 (Katulanda, P et al., 2006) while urban areas (16.4%) are more prone to diabetes compared to rural areas (8.7%) (Ekanayake, 2010). Moreover, it emphasizes that high prevalence in diabetes is corelated with high consumption of sugar, white rice, wheat flour and lack of exercise (Ekanayake,S 2010., Hettiaratchi., U.P,K et al, 2009,)

In Sri Lanka, both traditional and improved high yielding rice varieties are being cultivated. According Rajapakse,R.M.T et al(2000), there had been thousands of traditional rice varieties and until 1950's they were the only rice varieties available for local consumption. Traditional rice varieties tend to digest slowly and gives better satiety for an extended time compared to improved varieties (Pathiraje et al., 2010). Physiochemical properties such as grain color, grain size, grain shape and gelatinization characteristics influence the consumer preference. Besides these physiochemical properties nutritional attributes also tend to have a great effect on the consumers preference (Abeysekera et al., 2017). According to a research done in Kandy district reveals that majority of the consumers preferred red parboiled rice while consumers in southern coastal areas also preferred raw red rice varieties. People in urban areas preferred parboiled white rice. Hence, it shows that there is a regional difference in the consumption of rice.

Rice varieties vary significantly in their nutritional aspects. Among different varieties, red rice is believed to possess better nutritional qualities, as such, some physiological aspects like glycemic index are believed to be lower in red rice compared to white rice. According to a research done by Hettiarachchi et al., (2001) suggests that red parboiled varieties have significantly lower glycemic index than white raw rice. He further states that red parboiled varieties and Bg 350 can be recommended for diabetic patients due to their slow digestion of dietary carbohydrates. Hence, color of the rice variety is not yet considered as a good predictor to determine the glycemic index of rice varieties (Hettiarachchi et al., 2001, Pathiraje et al., 2010). Glycemic index of red rice varieties varies from 56- 73 whereas, in white rice it ranges from 62-68 (Hettiarachchi et al.,2001) Furthermore, some of the red rice varieties supposed to have medicinal and favorable antioxidant properties (Rajapakse,R.M.T et al., 2000) while parboiled red rice varieties have significantly higher levels of non- starch polysaccharides (fiber) (Hettiarachchi et al., 2001) compared to white rice.

Among the Sri Lankan foods studied boiled legumes (chickpea, cowpea, mung beans), roti varieties also play a major role in local diet. According to studies consumption of pulses shows a negative trend over time while cereal consumption is growing at an average annual rate of 3.37 %. It was estimated that pulses consumption was at level of 21 g per person per day had been reduced by 4.76% in 2007(Sri Lanka Pulses consumption, 1992-2007 - knoema.com, 2011) But there are less number of studies done to identify the nutritional composition and their contribution towards the health and other aspects of local population.

Hence, people are deviating from traditional dietary patterns rapidly (Jayasinghe et al., 2015), it is of utmost importance to imply their nutritional value experimentally. Many previous research has showed that red rice is more health beneficial compared to white rice and benefits of leguminous grains cultivated traditionally in the country are highly nourishes. Yet, we haven't analyzed them after cooling, except in porridge making (Senadheera., 2016); making it difficult to find the actual nutrient gains. Despite of these efforts nutritional based evaluation of cooked grains and pulses, has been infrequently tested with respected to the Sri Lankan consumption patterns and also very few research has been made for the comprehensive evaluation of the macronutrient composition of these foods. Therefore, this research is trying to find possible nutritive variations in accordance to various cereals and grains commonly consumed in Sri Lanka.

METHODOLOGY

Determination of moisture content (AOAC 1984 a)
Three replicates of each food sample (1 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.

% Moisture content = (W2 - W1) × 100 / W
W: Weight of the sample
W2: Weight of crucible + sample before drying
W1: Weight of crucible + sample after drying
Determination of the digestible carbohydrate content (Modified method of Holm et al. 1986)

Preparation of reagents

- Sodium acetate (NaOAc) buffer (pH = 4.75)
  Sodium acetate (1.1361g) was dissolved in 90 mL of distilled water and pH was adjusted to 4.75 with 1M acetic acid. Volume was made to 100 mL.
- Acetic acid (1M)
  A volume of 5.72 mL from the acetic acid solution (specific density = 1.05 g/mL) was dissolved in distilled water and volume was adjusted to 100 mL.

Procedure:
Six samples (0.5 g) from each food item, in their wet weights, were added to six, clean and previously dried 100 mL beakers respectively. Distilled water (15 mL) was added to each sample. Mixtures were stirred using the magnetic stirrer (Mfd. By Remi equipments) followed by addition of 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, solutions were transferred to clean, dry 50 mL volumetric flasks respectively. Then, 2 mL of the Sodium acetate buffer was added to six test tubes, followed by the addition of 1 mL of the sample. Amyloglucosidase (EC 3.2.1.3 Sigma-Aldrich, USA) 11.5 µL (10.808 units) was added to each test tube and mixed well using the magnetic stirrer. All six test tubes were incubated at 60ºC water bath, mouths covered with aluminum foil, shaken every five minutes. Contents of test tubes were allowed to cool and added to 10 mL volumetric flasks. Volume was made up to 10 mL by adding distilled water. Each sample (10 µL) was mixed with the reagent (1mL) from glucose oxidase kit respectively. Test tubes were incubated (37ºC, 10 min.) and absorbance measured at 500 nm against a reagent blank.

$$\% \text{ Digestible Starch} = \frac{\text{Absorbance of the sample} \times 50 \times 10 \times 0.9 \times 100}{\text{Absorbance of the standard} \times W}$$

W: Weight of the sample in milligrams

Determination of fat content (Croon et al., 1980)
Preparation of reagents

- HCl solution 7.7 M
  Conc. HCl (67.28 mL, Assay min. 35.4% AR PS Park scientific Ltd, UK) was dissolved in 100 mL of distilled water.

Procedure:
Six samples (2.0 g) from each food item, in their wet weights were measured in to six clean and dry Majonnier flasks respectively and moistened with of 95% ethanol (2 mL). Acid digestion with 10 mL of 7.7 M HCl at 75ºC 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 and stoppered with water soaked stoppers. The solution mixture was inverted, opening the mouth by removing stopper in time intervals to release the gas produced. When the gas production stopped, 25 mL of pet ether (EC N o. 265-151-9 VWR International Ltd., England) was added to Majonnier flasks and

mixed by inverting the flasks for about 20 times, with the releasing of gas at intervals. Flasks were kept still until separation of layers was clearly visible. Upper layers were transferred to six, labeled, previously dried and weighed 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 at each time were separated to relevant conical flasks respectively. Ether contents in conical flasks were allowed to be evaporated, by keeping them inside the fume hood for several days and kept in desiccators until constant weights were obtained.

$$\%\ \text{Fat content} = \frac{W_2 - W_1 \times 100}{W}$$

W2: Weight of the conical flask + fat
W1: Weight of the empty conical flask
W: Weight of the sample
Determination of dietary fibre (Asp et al., 1983)

Preparation of reagents

- HCl (0.2M)
  A volume of 3.4 mL Conc. HCl (Assay min. 35.4% AR PS Park Scientific Ltd, UK) was added to 200 mL of distilled water and mixed well.
- Phosphate buffer
  Anhydrous NaHPO4 2.42 g (VWR International Ltd. England) was mixed with 0.35 g of Na2HPO4 (VWR International Ltd. England) and was diluted to 200 mL by adding distilled water.
  Insoluble dietary fibre (Asp et al., 1983)

Procedure:
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 µL α amylase in a boiling water bath at 100ºC for 25 minutes was carried on followed by the addition of 20 mL of 0.2M cold HCl (4ºC), after when samples were cooled up to the room temperature. 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 40ºC in a shaking water bath (EYELA Uni Thermo Shaker NTS-1300) for an hour. After allowing cooling to the room temperature, 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 40ºC 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, labeled accordingly for the determination of soluble dietary fibre (3.2.1.5.2). 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) respectively. Finally, crucibles were dried at 105ºC until a constant weight was observed followed by incineration at 550ºC for 5 hours.

$$\%\ \text{IDF} = \frac{W1 - W2 \times 100}{\text{Weight of the sample}}$$

W1: Weight of the crucible after drying at 105ºC
W2: Weight of the crucible after incinerating at 550ºC

Soluble dietary fibre
Absolute ethanol was added to six filtrates separated in the above procedure until each of their EtOH concentration was 76%. Samples covered by Aluminium foils were subjected to incubation at 60ºC for an hour. Samples were filtered using No.4 filtration crucibles by applying suction force, followed by washing with two 15 mL portions of ethanol and two 15 mL portions of acetone respectively. Crucibles were dried at 105ºC until a constant weight was observed followed by incineration at 550ºC for 5 hours.

$$\%\ \text{SDF} = \frac{W1 - W2 \times 100}{\text{Weight of the sample}}$$

- W1: Weight after drying at 105ºC
- W2: Weight after incinerating at 550ºC

Determination of ash (AOAC 1984 b)
Flour sample (1.0 g) was measured to a clean, dry, pre-weighed crucible and was kept in the muffle furnace (FORNS HOBERSAL) at 550ºC for 5 hours. Six replicates were done for each variety. Final weights were obtained.

$$\%\ \text{Ash content} = \frac{W2 - W1 \times 100}{W}$$

W: Weight of the sample
W1: Weight of the crucible + ash
W2: Weight of the crucible
Determination of protein content (Kjeldhal method)

Preparation of reagents

- Sodium sulphate
  Sodium hydroxide (VWR International Ltd. England) 50 g and 8 g of sodium thiosulphate (VWR International Ltd. England) were dissolved in distilled water and the volume was topped up to 100 mL.
- Kjeldhal indicator
  Two parts (50 mL) of 2% alcoholic methyl red solution (VWR International Ltd. England) was mixed with one part (25 mL) of 0.2% alcoholic methylene blue solution (Park Scientific Ltd, UK).
- Boric acid (5%)
  Boric acid 5 g was dissolved in distilled water and the volume was increased to 100 mL.

Procedure:
Homogenous mixtures of food items were transferred to the Kjeldhal titration flask followed by a Kjeldhal catalyst tablet and 2 mL of Conc. Sulphuric acid. Six such replicates were made and flasks were connected to the fume trap and digested until colourless solutions were obtained. The digested solution was transferred to the semi micro Kjeldhal distillation apparatus, which has been previously conditioned by passing steam through for several minutes. The 5% boric acid solution was mixed with few drops of the indicator and clamped at the end of the digestion apparatus. Sodium sulphate solution 8 mL was added to the flask followed by passing steam through until about 15 mL of distillate was received. Tritration was carried out with the standard HCL solution until the pink colour of the end point was reached.

$$\% \text{Nitrogen} = \frac{(\text{Sample titre} - \text{blank titre}) \times \text{Molarity of HCl} \times 14 \times 100}{\text{Weight of the sample} \times 1000}$$

% Protein = % Nitrogen × 4.39*

Results

Table 1: Macronutrient compositions of cereals, cereal based foods, and pulses prepared according to the most common culinary methods

Food item	Moisture % (wb)	Mineral Ash %(dw)	Carbohyd-rate % (dw)	Insolubldietary fibre % (dw)	Soluble dietary fibre % (dw)	Fat % (dw)	Protein % (dw)
Boiled White Samba rice	70.1±2.5	1.8±0.2	74.6±7.2	7.6±0.1	5.0±0.2	2.4±0.7	8.5±2.3
Boiled Red Kekulu	72.2±0.9	2.3±0.6	72.5±6.0	9.8±0.2	6.1±0.4	2.3±0.5	6.7±1.7
Boiled Nadu rice	67.4±2.0	0.8±0.2	64.6±5.3	8.6±0.7	7.2±0.5	1.8±0.7	5.6±2.2
Boiled White Basmati	71.6±3.5	1.0±0.4	74.5±3.7	6.6±1.1	4.8±0.8	3.1±0.8	8.9±2.6
Pressure cooked Chick pea	63.4±1.2	3.0±0.6	62.1±2.1	14.8±1.3	6.9±0.2	2.2±0.1	22.4±1.1
Pressure cooked Green gram	64±3.2	3.4±0.6	65.9±2.21	7.0±0.7	5.3±0.1	2.6±0.8	20.0±0.6
Pressure cooked Red Cowpea	67.8±4.2	3.0±0.7	64.6±2.3	7.8±0.3	5.8±0.2	4.4±0.8	24.5±2.3
Boiled Maize	67.6±2.5	2.5±0.6	64.6±4.2	9.6±0.7	4.0±0.2	5.4±0.7	4.2±0.3
Wheat bread	12.1±2.5	1.8±0.6	77.6±4.3	3.6±0.3	2.7±0.5	6.9±1.5	12.2±2.0
Pittu (wheat flour)	50.5±2.3	1.2±0.6	69.2±5.0	8.9±1.1	6.1±0.4	6.8±0.4	8.2±0.6
Pittu(wheat+Kurakkan flour)3:1	46.5±3.5	2.2±0.5	61.9±4.6	14.5±0.3	8.8±0.5	6.3±0.9	4.1±0.6
Roti (wheat flour)	26.5±1.5	2.1±0.5	57.8±4.3	8.5±0.3	6.0±0.5	7.1±0.5	12.7±0.6
Roti(wheat+Kurakkan flour)3:1	25.9±3.6	2.8±0.8	52.8±4.7	15.2±0.3	7.9±0.5	7.0±0.6	10.2±0.3
Lentil curry	85.3±4.5	2.6±0.2	54.2±4.8	8.6±0.2	5.8±0.8	4.6±0.2	14.4±0.2
Soy curry	86.7±3.3	2.8±0.7	34.6±3.6	9.3±0.7	6.7±0.4	8.2±1.2	36.6±1.9

DISCUSSION

In this research, 15 different cereal and grain-based food items were tested in their cooked form for proximate composition. These items are either consumed as sole foods (boiled chick pea, boiled cowpea, boiled green gram) or incorporated as curries (lentil, soy) with bread or rice (ex: bread - lentil meal) in in usual consumption pattern in Sri

Lanka. Other than that, boiled maize is often used as a snack and is not commonly used in daily consumption.

Rice and bread being the most common sources of carbohydrate, their carb level can give a broad idea about the carbohydrate intake of Sri Lankan community. According to the data obtained, white bread has the highest carbohydrate level (77.6±4.3%) followed by boiled white samba (74.6±7.2) and boiled white basmati (74.5±3.7). Among tested rice varieties boiled Nadu rice has the lowest carbohydrate level (64.6±5.3%). According to previous studies digestible carbohydrate content of different rice varieties ranged from 74 to 84 g%. but Nadu rice has low digestible carbohydrate level maybe because it contains more resistant starch. Hence, there are no sufficient evidences about Nadu rice to prove that fact from previous studies and therefore, need further research. Moreover, glycaemic index of white rice ranged from 62 to 68., while the glycaemic indices of varieties of red rice varied from 56 to 73 (Hettiarachchi et al., 2001). According to Darandakumbura et al (2013), cooking method does not impart any significant effect either on rice composition or GI. Therefore, the reason for low digestible carbohydrate level in Nadu rice maybe a genetically inherent quality of the variety.

Protein content has varied from 5.6±2.2% to 8.9±2.6%. however, according to the FAO report (1993) protein content of rice can vary from 6- 15%. Protein content is largely affected environmental conditions, cropping season, rate and time of nitrogen fertilization, water management, cropping practices etc. (..)

Although usual white bread is considered as high GI food (Hettiaratchi et al., 2009) when it is consumed with protein rich curry such as lentils, it significantly reduces the GI values and meal comprising both bread and lentils then can be categorized under medium GI food (Hettiaratchi et al., 2009). Research proves that GI has a significant negative correlation with both fat and protein (Jenkins et al., 1981, Hettiaratchi et al., 2009).

Legumes include peas, beans, lentils, peanuts, and other podded plants that are used as food (Messina, 1999). They are more popular among Asian countries as they are economical source of protein. In Sri Lanka legumes like dhal, soybean are often consumed as a combination with cereals as they complement each other to provide a complete amino acid profile. Mung Kiribath (meal comprising rice and green gram), rice with dhal curry are such examples which are very common in Sri Lankan cuisine. Dhal is cooked with coconut milk until it reaches soft consistency and then seasoned with spices and condiments. Other legumes like mung bean, green gram and cow pea are boiled and directly consumed with grated coconut. However, the effect of different cooking patterns on the nutritional composition is poorly documented. According to the literature protein content of beans varies from 20- 30 %. Macronutrient composition of selected legume beans are shown in Table 1. Their protein content has been ranged from 22.4±1.1 to 24.5±2.3. Although beans are high in protein, the quality of protein is not estimated properly. However, it is emphasized that due to lack of sulfur containing amino acid, these proteins may have low digestibility and poor protein efficiency ratio (Messina, 1999).

Soya bean is especially popular among low and middle-income families as a substitute for meat and fish as an inexpensive source of protein. Vegetable soy bean is more commonly available in preprocessed form (Textured vegetable protein(TVP)) and generally known as soya meat among consumers. it is consumed as a curry often with rice. Like other legumes soybeans are rich in variety of nutrients. Besides its high protein value (36.6±1.9%) it is also high in dietary fibre, variety of micronutrients and phytochemicals. According to the data (Table1) soya curry contains little more fat (8.2±1.2 %) than usual, is due to the addition of coconut milk during cooking.

Both pittu and rotti made with finger millet (Kurakkan) flour (wheat+Kurakkan flour 3:1) has the highest content of insoluble and soluble dietary fibre, 14.5±0.3/ 8.8±0.5, 15.2±0.3/ 7.9±0.5 respectively

Insoluble dietary fiber was high in roti (15.2±0.3%) in which wheat flour and Kurakkan flour (Eleusine coracana) had been incorporated to 3:1 ratio while the roti made with wheat flour has 8.5±0.3% of insoluble fiber while Wheat bread has the lowest (3.6±0.3%) amount. It reveals that 3:1 addition of Kurrakkan has dramatically increased the insoluble dietary fibre % in Kurakkan roti.

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