

Mineral Composition of Leaf Protein Concentrate Prepared from Refuse Tea

G A A R Perera, I S B Abeysinghe, L S K Hettiarachchi, and

R G A Wijayawardhana

(Tea Research Institute of Sri Lanka Talawakelle, Sri Lanka)

ABSTRACT

Mineral composition of Leaf Protein Concentrate and refuse tea were analyzed with the objectives of identifying the changes in mineral composition during the preparation of Leaf Protein Concentrate and also to determine the nutritional significance of Leaf Protein Concentrate prepared from refuse tea. P and Al contents were determined by standard colorimetric methods using the Vanadate-Molibdate reagent and Aluminon reagent, respectively. All other mineral contents were determined by atomic absorption spectrophotometry. Purified Leaf Protein Concentrate was a rich source of macro minerals such as Ca (12118 ppm), Mg (736 ppm), K (2720 ppm), Na (2903 ppm) and P (3530 ppm). It was also rich in trace minerals such as Zn (51 ppm), Cu (108 ppm) and Mn (284 ppm). Although refuse tea was rich in Fe (281 ppm) it decreased to a non detectable level during the preparation of Leaf Protein Concentrate. To avoid high Al content in purified Leaf Protein Concentrate (663 ppm) it is necessary to use instruments made of stainless steel.

Key words: Atomic Absorption spectrophotometry, black tea, leaf protein concentrate, minerals, protein, refuse- tea

INTRODUCTION

Black tea is produced from the young and tender shoots of the tea plant (*Camellia sinensis* L. O. Kuntze). The rejected fractions, during cleaning, sorting and grading in black tea manufacture, are pooled to form a portion collectively called refuse tea (RT) or waste tea. About 3-6% of the tea harvest is discarded as RT in the manufacture of black tea (Keegal, 1983). Refuse tea consists largely of stalks, fibre, fluff (fine, lighter weight dust like particles) and clumped tender particles. The made tea left after brewing is called spent tea or infused tea.

Refuse tea as well as infused (spent) tea contain 20-30% of crude protein (Wickremasinghe, 1978). Ananthasubramaniam and Menachery (1977) also reported that spent tea contained 28.7% of crude protein.

With the objective of utilizing protein present in RT as human food or animal feed, Perera *et al.* (2007) optimized conditions for preparing Leaf Protein Concentrate (LPC) from RT.

The purified LPC obtained from RT contained 42.8% crude protein, 0.9% crude fibre, 8.1% polyphenol, 5.1% ash and 1.6% ether extract (Perera *et al.*, 2007). This LPC prepared from RT seems to be a promising source of protein for human and/or animals.

Some minerals are essential for optimum growth and performance of both human and animals because they play important roles as, structural components of connective tissues (Ca), constituents of enzymes involved in metabolism (Zn), component of blood haemoglobin (Fe), enzyme activators (Mg, Mn, Cu) and osmotic pressure regulators (Na, K) (Corah, 1996; Potter and Hotchkiss, 1996; Ahmed *et al.*, 2000; Hostetler *et al.*, 2003; Cabrera *et al.*, 2004; Rude *et al.*, 2005). Mineral requirement for human varies with the age, physiological status *etc* (Potter and Hotchkiss, 1996). Animals also differ in their mineral requirements depending on the species, breed, strain, age and physiological status (Wiener, 1979; Ahmed *et al.*, 2000).

Some minerals could become toxic when excessive amount are ingested (e.g. Al causes Alzheimer's disease) (International Occupational Safety and Health Centre, 1999). Even essential minerals may cause acute or chronic toxicity when they are ingested in large quantities (Corah, 1996; Aschner and Aschner, 2005).

Mineral composition of tea shoot varies with variety, season, maturity, management practices, and soil mineral composition (Hasselo, 1965; Kalita, 1993; Hettiarachchi *et al.*, 1997; Ravichandran, 1998). Refuse tea contains P (2100 ppm), K (20000 ppm), Mg (1680 ppm), Ca (3500 ppm), Cu (22 ppm), Mn (146 ppm), Zn (34 ppm) and Fe (188 ppm) (Ananthacumaraswamy *et al.*, 1990).

Information on the mineral composition of RT and LPC prepared from RT is essential to prevent mineral deficiencies and toxicities in human and/or animals when LPC is used as animal feed or human food.

The aim of the present study was to determine the contents of important minerals in RT and to determine the changes of the content of these minerals in the preparation and purification of LPC.

MATERIALS AND METHODS

Sample preparation

A representative sample of RT was collected from St Coombs tea factory, Tea Research Institute, Talawakelle, Sri Lanka and medium size particle of refuse tea (RTm) (which passes through No 4 mesh: aperture size 4.3 mm) was used to prepare LPC. Procedure of preparing LPC includes, extraction of protein from RTm with a 0.55 *N* NaOH (1:6 solid to solvent ratio), precipitation of extracted protein by adjusting the pH to 4.4 with 100% trichloro acetic acid (TCA, w/v) and recovery of crude protein precipitate (LPC-1) by centrifugation at 12000 rpm for 30 minutes. Purification of LPC was carried out as explained below.

A portion of the LPC-1 was mixed with boiled distilled water (at 1:6 ratio of LPC: boiled distilled water) using a top drive macerator (Ultra-Turrax, USA). The slurry was centrifuged at 12000 rpm for 30 minutes and the supernatant was decanted. A portion of the precipitate (LPC-2) was subjected to the above purification procedure using 70% methanol instead of boiled distilled water. After decanting the supernatant, precipitate (LPC-3) was obtained.

Determination of mineral composition

Samples of RTm, LPC-1, LPC-2 and LPC-3 were subjected to dry ashing (AOAC 1999). Ca, Mg, Mn, Fe, Zn, Cu and Na were determined according to AOAC method (1999) using an Atomic Absorption spectrophotometer (GBC Avanta-p). Na was determined in emission mode while the other elements were determined in absorption mode. Air-acetylene flame was used in the determination of Mg, Mn, Fe, Zn, Cu, and Na where as Nitrous oxide-acetylene flame was used in the determination of Ca.

P and Al contents were determined by standard colorimetric methods using the Vanadate-Molibdate (Bhargava and Ragupathi, 1998) and Aluminon reagents (Chenery, 1948), respectively.

Statistical analysis

Data were subjected to analysis of variance followed by Duncan grouping ($p < 0.05$) using the computer software of SAS 8.1-2000 (SAS 8.1, 2000).

Chemicals

All chemicals used were of analytical grade and the standard solutions were supplied by the BDH.

RESULTS AND DISCUSSION

Macro mineral and Al contents are presented in Table 1 while micro mineral contents are presented in Table 2.

Results showed that RTm was a rich source of macro and trace minerals (Table 1). Mineral contents reported in this study were similar to the values reported for refuse tea by Ananthacumaraswamy *et al.* (1990).

Ca is the most abundant mineral present in the LPC-3 (12118 ppm) (Table 1). Ca content increased significantly at each stage of purification. Owing to very low solubility of Ca in water (Ravichandran and Parthiban, 1998), it was not removed with the purification treatments and concentrated in LPC, resulting in significantly higher levels of Ca in LPC-3 (12118 ppm) as compared to LPC-1(790 ppm) and LPC-2 (8397 ppm).

Due to high solubility of Mg in water (Ravichandran and Parthiban, 1998), purification of LPC-1 with boiled distilled water resulted in significantly low amount of Mg in LPC-2 (600 ppm) as compared to LPC-1 (1142 ppm). Mg content in the LPC-3 (736 ppm) was significantly higher as compared to that of LPC-2 (600 ppm) due to concentration during purification of LPC-2.

P content decreased significantly from 3818 ppm in LPC-1 to 2956 ppm in LPC-2 after purification of LPC-1 with boiled distilled water. Purification of LPC-2 with 70% methanol resulted in significantly higher level of P in LPC-3 (3530 ppm) compared to LPC-2. P is the second most abundant mineral present in the LPC-3.

Na content of RTm was found to be low (160 ppm). Tea shoot generally contains low level of Na (160-190 ppm) and the mineral content of green leaf does not change during the manufacture (small differences may be observed due to differences in geographical location and genetic nature) (Ravichandran and Parthiban, 1998). However, LPC-1 and LPC-2 contained significantly higher level of Na (Table 1) with comparison to RTm due to formation of NaCl during the neutralization of NaOH extract with TCA (Wu and Stringfellow, 1980). Owing to high solubility of NaCl in water, consecutive purification treatments resulted in LPC-3 with significantly low level of Na content (2903 ppm) as compared to LPC-1 and LPC-2. It may be possible to reduce the Na content further to a desirable level by purifying with boiled water.

RTm used for the preparation of LPC contained very high amounts of K (21215 ppm). K is the most abundant mineral present in tea shoot, black tea and RT (Ravichandran

and Parthiban, 1998; Ananthacumaraswamy *et al.*, 1990).

Though the K content of LPCs decreased significantly at each stage of purification it was the third most abundant mineral present in LPC-3. Increased intake of K, Ca, and Mg helps to reduce the blood pressure (Karppanen *et al.*, 2005). Therefore, purified LPC with its high level of K, Ca, and Mg would have health benefits if the Na content lowered to a desirable level.

Al content of RTm was 663 ppm. Tea plant is known to be an Al accumulator (Coriat and Gillard, 1986; Ananthacumaraswamy and Sivapalan, 1990) and Al content in the flush increases progressively from bud to mature leaf from 50 to 1500 ppm (Chenery, 1955). High Al level in diets has link with Alzheimer's disease (International Occupational Safety and Health Centre, 1999). Though black tea contains high level of Al only a very low level of Al (2-6 ppm) is extracted in to tea brew (Flaten and Odegard, 1988; Ananthacumaraswamy and Sivapalan, 1990). LPC-1 contained significantly higher level of Al (969 ppm) as compared to that of RTm and it increased significantly with the purification treatments due to concentration. Use of NaOH and hydraulic press made of Al in the extraction of protein from the RTm may have resulted in LPCs with high Al contents. Therefore it may be necessary to use a hydraulic press made of stainless steel in order to reduce the Al content in LPCs.

Zn content of LPCs reduced significantly after purification with boiled distilled water and increased significantly after purification with 70% methanol. Cu content significantly increased after each stage of purification whereas Fe content significantly decreased. Purification of LPC with boiled distilled water did not affect significantly on Mn content but purification with 70% methanol caused significant increase in Mn content. Recommended concentrations of Cu, Mn and Zn in a mineral supplement for grazing cattle are 10, 50, and 40 ppm respectively (Corah, 1996). Cu, Mn and Zn were present in purified LPC (LPC-3) in quantities of 108, 284 and 51 ppm, respectively. Therefore purified LPC prepared from RT is a potential rich source of essential trace minerals such as Cu, Mn and Zn.

CONCLUSIONS

Purified Leaf Protein Concentrate (LPC-3) is a promising source of macro-minerals such as Ca, P, Mg, Na and K and trace minerals such as Mn, Zn, and Cu. Introduction of an additional purification step is necessary to reduce the Na content. Al content in LPCs could be reduced by using hydraulic press made of Stainless steel. Lack of Fe in LPC-3 should be taken in to consideration when formulating animal feed/ human food.

REFERENCES

- Ahmed M M M, Siham A K and Barri M E S 2000 Macro mineral profile in the plasma of Nubian goats as affected by the physiological state. *Small Ruminant Research* 38, 249-254.
- Ananthacumaraswamy S, Balakrishnan T and Wijetunga W M S 1990 Macro and micro nutrients in Sri Lankan black tea and tea brew. *Sri Lanka J. Tea Sci.* 59(1), 49-58.
- Ananthacumaraswamy S and Sivapalan P 1990 Aluminium in black tea. *Sri Lanka J. Tea Sci.* 59(1), 4-8.
- Ananthasubramaniam C R and Menachery M 1977 Nutritive value of tea (*Camellia sinensis*, Linn) waste for cattle. *Kerala J. Vet. Sci.* 8(1), 37-40.
- Official Methods of Analysis 1999, 16th edn. Association of Official Analytical Chemist, Washington. DC
- Aschner J L and Aschner M 2005 Nutritional aspects of manganese homeostasis. *Molecular Aspects of Medicine* 26(4-5), 353-362.
- Bhargava B S, Raghupathi H B 1998 Analysis of plant materials for macro and micro nutrients. *In* Methods of Analysis of Soils, Plants, Waters and Fertilizers. Ed. HLS Tandon. pp 49-82, Fertilizer Development and Consultation Organization, New Delhi, India.
- Cabrera N W G, Coffey K P, Coblenz W K, Sarbrough D A, Turner J E, Kegley E B, Johnson Z B, Kellogg D W, Gunsaulis J L and Daniels M B 2004 In situ solubility of selected macro minerals from common bermudagrass fertilized with different nitrogen rates and harvested on two dates. *Anim. Feed Sci. Technol.* 3 (1-4), 203-221.
- Chenery E M 1948 Thioglycolic acid as an inhibitor for iron in the colorimetric determination of aluminium by means of 'Aluminon'. *Analyst* 73, 501-503.
- Chenery E M 1955 A preliminary study of aluminium and the tea bush. *Plant and soil* 6(2), 174-200.
- Corah L 1996 Trace mineral requirement of grazing cattle. *Anim. Feed Sci. Technol.* 59, 61-70.
- Coriat A M and Gillard 1986 Beware the cup that cheer. *Nature* 321, 570-575.
- Flaten T P and Odegard M 1988 Tea, aluminium and alzheimer's disease. *Fd. Chem. Toxic.* 26, 959-960.
- Hasselo H N 1965 The nitrogen, potassium, phosphorus, calcium, magnesium, sodium, manganese, iron, copper, boron, zinc, molybdenum, and aluminium contents of tea leaves of increasing age. *Tea Quarterly* 36, 122 - 137

Hettiarachchi L S K, Balasingham A, Ananthacumaraswamy S, Gunaratna G P, and Warnasiri H A P 1997 Mineral composition in relation to leaf maturity from 2000, 3000 and 4000 clonal series: Leaf analysis as a guide in tea crop nutrition. *S. L. Tea Sci* 65 (1/2), 11 - 33.

Hostetler C E, Kincaid R L and Mirando M A 2003 The role of essential trace elements in embryonic and foetal development in livestock. *The Veterinary Journal* 166, 125-139.

International Occupational Safety and Information Centre 1999 Metals in basis of chemical safety. International Labour Organization, Geneva.

Kalita J N and Mahanta P K R 1993 Analysis of mineral composition of some Assam and Darjeeling black tea. *J. Sci. Food Agri.* 62,105-109.

Karppanen H, Karppanen P and Mervaala E 2005 Why and how to implement sodium, potassium, calcium and magnesium changes in food items and diets. *Journal of Human Hypertension* 19, 10-19.

Keegal E L 1983 Tea manufacture in Ceylon. Monograph on tea production in Ceylon 04, 2nd edn. Tea Research Institute of Sri Lanka, Talawalelle, Sri Lanka. pp 104.

Perera G A A R, Abeysinghe I S B and Jayaweera C D 2007 Optimization of conditions for preparing leaf protein concentrates from refuse tea. *J. Food Sci. Technol.* 44 (4), 413-416.

Potter N N and Hotchkiss J H 1996. *Food Science* 5th edn. CBS publishers and distributors, 4596/1A,11 Darya Ganji, New Delhi, India. pp 60-67.

Ravichandran R and Parthiban R 1998 Macro and micro nutrients contents in black tea and their solubility in tea infusion. *J. Food Sci. Technol.* 35 (4), 361-364.

Rude R K, Gruber H E, Norton H J, Wei L Y, Frusto A and Kilburn J 2005 Dietary magnesium reduction to 25% of nutrient requirement disrupts bone and mineral metabolism in the rat. *Bone* 37(2), 211-219.

SAS (8.1) 2000. *SAS User's Guide*. SAS Institute Inc, Cary, N.C., USA

Wiener G 1979 Review of genetic aspects of mineral metabolism with particular reference to copper in sheep. *Livestock Production Science* 6(3), 223-232.

Wickremasinghe R L 1978 Facets of tea research in practice. Monograph on tea production in Sri Lanka 07, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka. pp 48.

Wu Y V and Stringfellow A C 1980 Protein isolate from alkaline extraction of air-classified high-protein soft wheat flour. *J. Food Sci.* 45, 1383-1386.

Table 1. Macro mineral and aluminium content

Sample	Mineral (ppm)					
	Ca	Mg	Na	K	P	Al
RTm	6543±85 ^d	2267±62 ^a	160±7 ^d	21215±11 ^a	3307±85 ^c	663±77 ^d
LPC-1	790±56 ^c	1142±85 ^b	35262±189 ^a	8770±0 ^b	3818±3 ^a	969±71 ^c
LPC-2	8397±41 ^b	600±44 ^d	5718±51 ^b	3298±1 ^c	2956±31 ^d	1123±7 ^b
LPC-3	12118±14 ^a	736±37 ^c	2903±35 ^c	2720±6 ^d	3530±74 ^b	1500±16 ^a
	CV=0.46	CV=3.66	CV=0.65	CV=0.05	CV=1.24	CV=3.59

(n=2)

RTm-Medium size particle of refuse tea; LPC- Leaf protein concentrate

CV- Coefficient of variance

Figures with different superscripts in a column differ significantly (≤ 0.05)**Table 2. Trace mineral content**

Sample	Mineral (ppm)			
	Zn	Cu	Fe	Mn
RTm	40.89±1.88 ^c	33.64±0.65 ^d	281.39±5.83 ^b	266.48±2.39 ^b
LPC-1	47.53±0.15 ^b	68.33±2.10 ^c	414.53±11.18 ^a	237.43±0.40 ^c
LPC-2	43.46±1.91 ^c	87.43±0.76 ^b	226.78±2.13 ^c	229.44±11.08 ^c
LPC-3	51.37±1.21 ^a	108.40±0.23 ^a	ND	283.78±0.15 ^a
	CV=2.32	CV=1.13	CV=2.00	CV=1.61

(n=2)

ND- Not Detected

CV- Coefficient of variance

RTm-Medium size particle of refuse tea; LPC- Leaf protein concentrate

Figures with different superscripts in a column differ significantly (≤ 0.05)