RESEARCH ARTICLE

Exploitation of green biomass for the preparation of leaf protein concentrates

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ABSTRACT

The available food resources are always remaining to be constrained for the ever increasing population. Hence the non-conventional food resourced is to be considered in the coming year. The present study includesgreen foliages from 7 plants to prepare leaf protein concentrate viz., Berseem (*Trifoliuma lexandrium*L.), *Alysicarpus vaginalis* L. var. stocksii., *Alternanthera paronychioides* St. Hil., Cabbage (*Brassica oleracea* L. var. capitata), Radish (*Raphanus sativus*L.), Adulsa (*Adhatoda vasica* Nees.), and Bauchi (*Psoralia corylifolia* L.). The selected plants were fractionated and subjected to prepare LPC as suggested by Pirie (1971). The maximum yield of juice and fiber are reported in green foliages of *Brassica oleracea* L. (550 ml/Kg) and *Alysicarpus vaginalis* L.(540 g/Kg) respectively. Maximum dry weight of juice and DPJ was reported in green foliage of *Adhatoda vasica* Nees. (120 and 70.22 g respectively) Maximum dry weight of fiber was reported in green foliage of *Alysicarpus vaginalis* L. (305 g). Maximum dry weight of LPC was reported in green foliages of *Psoralia corylifolia* L. (80.52 g).

Keywords: leaf protein concentrates, non-conventional food resources, DPJ, LPC, fiber, biomass.

INTRODUCTION

In our country huge amount of green biomass are available in the form of weeds, fodder crops and by-products of the vegetable material. The utilization of this material can be enhanced for the benefit of human being by the extracting available protein in this green biomass. To extract proteins from green leaves numerous technologies have been developed over last 50 years. For this purpose, a technique of fractionation has been proposed by Pirie from United Kingdom. This technique of fractionation recommended by Pirie (1942) has now become popular as "Green Crop Fractionation (GCF)" which involves the separation of proteins from the indigestible fibrous material of leaves.

The process of GCF is basically consists of mechanical operations of grinding and pressing, which enable the fresh green leafy foliages to be separated into two fractions; a protein rich juice and the pressed crop residue

(PCR). For this purpose the crop is macerated with IBP pulper (Davys and Pirie, 1969a)or with simple grinder mixer and then pressed with IBP press (Davys *et al*.,1969b) or by hand pressing method. After thorough maceration of the crop, almost all cells in it are damaged and the proteins get liberated in the juice expressed after pressing the pulp. After releasing the juice a fibrous material left behind called as pressed crop which is also nutritionally sufficient to the cattle. The juice or leaf extract contain, along with the proteins, sugars, lipids, vitamins and other soluble components of the cell protoplasm. When this fraction is either heated or acidified, a green curd is produced due to the precipitation of protein. This protein rich curd is referred as leaf protein concentrates (LPC). The LPC can be separated from remaining part of the juice, known as deproteinized juice (DPJ) by filtration through a simple cotton or muslin cloth. Thus, the process of GCF results into four fractions i.e. Leaf extract or juice, Pressed crop residue (PCR) or Fiber, Leaf protein concentrate (LPC), Deproteinized juice (DPJ). All these four products can be used in different ways as suggested by several workers.

First suggestion on the use of protein extracted from leaves was made by Ereky (1927). Slade (1937) argued that the use of protein from grass was more economical than meat obtained after feeding the grass to animals. Chibnall (1939) and his co-workers provided information on the quantity and properties of protein in leaves.

Pirie (1942) put forth the real potential of leaf protein (LP) and its use as human food in during II World War. After two decades of war several workers took interest in leaf protein (Davies *et al*., 1952; Carpenter *et al*., 1954; Mcdonald, 1954; Tilley *et al*., 1954; Anandaswamy and Date, 1956; Cowlishaw *et al.,* 1956; Raymond and Harris, 1957; Guha, 1960; and Chayen *et al.,* 1961).

Byers (1961) evaluated the extractability of leaf protein from leaves of 60 tropical spp. growing in Ghana. His results showed that some common weeds offered promising results, yielding more protein than the leaves of many crops with good quality. Devi *et al*. (1964) studied the isolation and composition of leaf protein from certain species of Indian flora and reported that some of the proteins isolated are potentially useful for supplementation of cereal diets deficient in lysine and methionine. Carlsson and Clarke (1983) studied the suitability of *Atriplexhortensis* L. as a source of leaf protein concentrate and showed that it could be utilized for the preparation of LPC as it was found superior to *Spinaceaoleracea* L. The cultivation of berseem for the measurement of LP shown that it could give a yield of 700kg per hectare extractable protein in 140 days (Mungikar, 1974; Tekale, 1975; Mungikar *et al*., 1978; Mungikar *et al*., 1976b; Patil and Mungikar, 1992). The yield of extractable protein from leaves taken at the time of harvest of the edible part (root, tuber) from brassicas, beet root, turnip and radish ranged between 76 to 171kg/ha (Tekale, 1975; Deshmukh*et al*., 1974; Tekale and Joshi,1976; Giri and Nagpal, 1984; Giri *et al*., 1983). Leafy vegetables were also found suitable for producing good quality of leaf protein concentrates (Mungikar and Ajaykumar, 1995).

MATERIALS AND METHODS

Selection of plants: For the present work green foliages from 7 plants were selected to prepare leaf protein concentrate viz., Berseem (*Trifolium alexandrium*L.), *Alysicarpus vaginalis* L. var. stocksii*., Alternanthera paronychioides* St. Hil., Cabbage (*Brassica oleracea* L. var. capitata), Radish (*Raphanus sativus* L.), Adulsa (*Adhato davasica* Nees.), and Bauchi (*Psoralia corylifolia* L.). These plant material were authentified at Department of Botany, RTM Nagpur University, Nagpur. These plants were collected from different places. Berseem was collected from Walu Sangopan Kendra, Nagpur. Bauchi was collected from Krishi Vidyapith Campus, Nagpur. Cabbage and Radish were collected from local vegetable market. *Alternanthera*, *Alysicarpus,* and *Adhatoda* were collected from RTM Nagpur University campus, Nagpur.

Preparation of leaf protein concentrates: The selected plants were fractionated and subjected to prepare LPC as suggested by Pirie (1971). These plants were first washed well with water and pulped with grinder mixer. The juice was then expressed by hand pressing method. The amount of juice and fiber obtained per Kg of green foliages was recorded. The juice obtained was employed for the preparation of LPC. LPC was prepared by heat coagulation method.

Heat coagulation method: For this purpose, a sample of 100 ml juice was slowly added to 20 ml boiling water with continuous stirring, as a result proteins in juice coagulated resulting into green colour curd called as leaf protein concentrate (LPC). During whole process the temperature was maintained at 95°C. This

heated juice was then filtered through preweighed Whatmann filter paper No.1. During filtration the yellowish filtrate was obtained which is called as deproteinized leaf juice (DPJ) or whey. The green coloured curd (LPC) along with filter paper and DPJ was dried at 55°C in hot air oven. The amount of the dried LPC and DPJ was recorded per Kg of fresh green foliages.

RESULT AND DISCUSSION

I. Fresh weight of fiber and amount of juice from different plants obtained during green crop fractionation.

The yield of juice and fibers of various plants obtained during GCF are summarized and shown in table 1 and fig. 1a, 1b respectively. The maximum yield of juice and fiber are reported in green foliages of *Brassica oleracea*L. (550 ml/Kg) and *Alysicarpus vaginalis* L.(540 g/Kg) respectively, whereas, the minimum yield of juice and fiber are reported in green foliages of *Adhatoda vasica* Nees. (217 ml/Kg) and *Brassica oleracea* L. (250 g/Kg) respectively.

During present investigation 7 plants were considered for fractionation. From these, two are vegetable crops (radish and cabbage), a fodder crop (berseem) and four wild plants (*Adhatoda vasica* Nees., *Psoralia corylifolia* L., *Alysicarpus vaginalis* L., and *Alternanthera paronychioides* St. Hil.). The green foliages (leaves and tender stem) of these plants were employed for the preparation of LPC. Though the foliages of these wild plants are not used as source of vegetables, however, the young shoot part of *Psoralia corylifolia* L., *Alysicarpus vaginalis* L., and *Alternanthera paronychioides* St. Hil. were generally consumed by animals and *Adhatoda vasica* Nees.is well known for its medicinal value. A large variation in both juice as well as fiber content was observed due to their time ofharvesting and vegetative growth. The coefficient of variation of the obtained values of juice and fiber was 35.60 and 30.81% respectively.

PROCESS OF GREEN CROP FRACTIONATION (GCF)

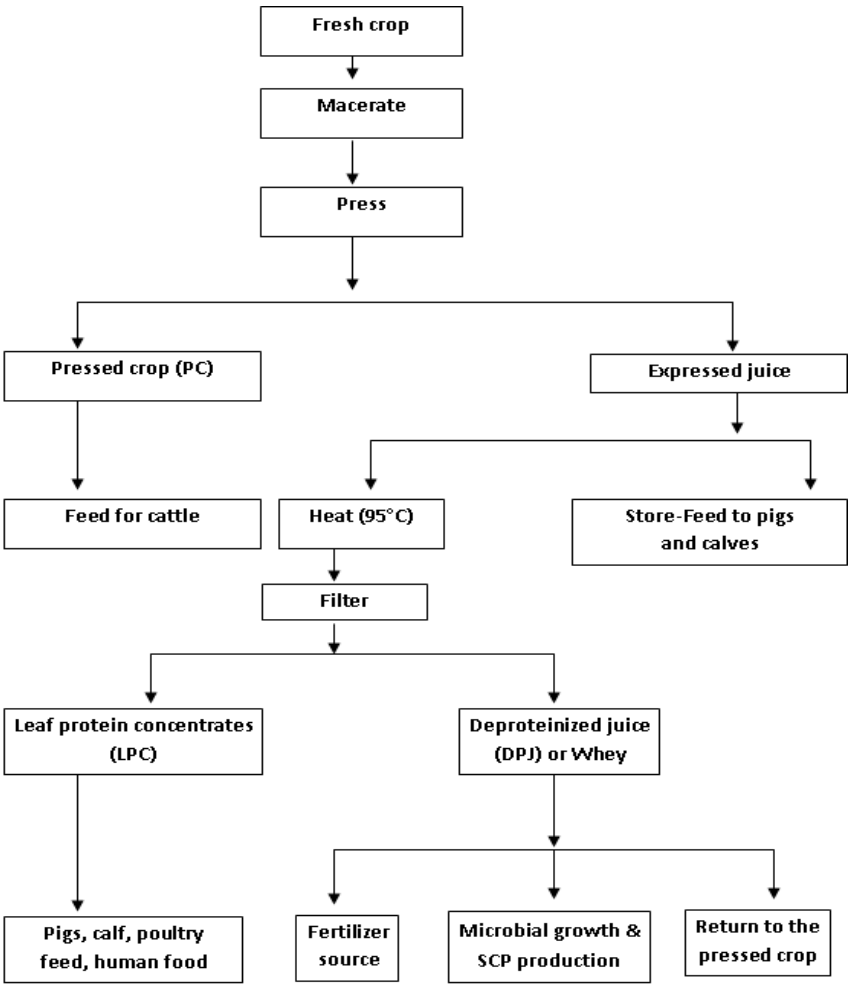

Table 1: Fresh weight of fiber and amount of juice from various plants obtained during green crop fractionation.

Sr. No.	Name of the plant's	Yield of wet fractions of plant materials.	
		Juice (ml / Kg)	Fiber (g / Kg)
1	*Alysicarpus vaginalis* L.	265	540
2	*Trifolium alexandrium* L.	390	354
3	*Alternanthera paronychioides*St. Hil.	407	343
4	*Raphanus sativus* L.	520	258
5	*Brassica oleracea* L.	550	250
6	*Psoralia corylifolia* L.	252	494
7	*Adhatoda vasica* Nees.	217	508
-	Mean	372	392
-	Std. Deviation	132	121
-	Std. Error	50.00	45.70
-	Coefficient of variation	35.60%	30.81%

Table 2: Dry weight of various fractions of plants obtained during green crop fractionation.

Sr. No.	Name of the plant's	Dry weight of fractions, g / Kg of fresh green foliages.			
		Juice	**Fiber**	**LPC**	**DPJ**
1	*Alysicarpus vaginalis* L.	118.00	305.00	71.15	38.95
2	*Trifolium alexandrium* L.	62.75	88.00	31.64	23.36
3	*Alternanthera paronychioides* St. Hil.	115.00	94.74	72.41	31.42
4	*Raphanus sativus* L.	53.40	80.00	20.83	17.83
5	*Brassica oleracea* L.	62.23	68.34	21.51	36.33
6	*Psoralia corylifolia* L.	117.24	160.30	80.52	30.53
7	*Adhatoda vasica* Nees.	120.00	175.00	42.42	70.22
	Mean	92.70	139.00	48.60	35.50
	Std. Deviation	31.20	84.00	25.60	16.90
	Std. Error	11.80	31.80	9.67	6.40
	Coefficient of variation	33.71%	60.53%	52.58%	47.65%

The fresh green foliages from all these plants were fractionated for the measurement of the yield of juice (fresh and dried), fiber (fresh and dried), LPC (dried) and DPJ (dried) per unit weight of foliage. The fractionation of foliages was undertaken using mechanical mixer for pulping and hand pressing method was employed for expressing juice. To justify the usefulness of wild plants in green crop fractionation process, the yields of the wild plants have been compared with *Brassica oleracea*L. since this plant have been extensively utilized for the green crop fractionation process by various workers.

The result obtained showed that the extractability of juice and fiber varied from species to species. The results also showed that the quantity of juice is inversely proportional to the quantity of fiber; if the one fraction was more than other would be low or vice versa. As far as yield of expressed juice is concerned, all the wild plants have relatively low extractability rate than that of *Brassica oleracea*L. (table 1). The variation in extractability of juice might be due to nature of the foliages and their moisture content.

Patil and Salve (2000) has been reported the yield of juice for radish as 584ml/ Kg, (fresh wt. basis) respectively. Gogle (2000) mentioned the yield of juice from cabbage and radish as 628 and 711ml/Kg fresh material, respectively. However, in present study, the yield of juice from cabbage and radish was found 550

and 520ml/Kg respectively. This variation in extractability of juice might be due to equipment used for expressing the juice, stage of harvesting and also because of difference in region. In present study the juice was expressed by hand press method.

Yield of fiber from all wild plants are comparatively higher than that of *Brassica oleracea*L. (table 1). Bhande and Mungikar (1990) reported the yield of fiber from lucerne as 426g/Kg (fresh wt. basis), whereas in present study it ranged from 250 to 540g/Kg, fresh material. The yields of fiber from various crops are depends upon their maturity level and also it varied from specie to species.

II. Dry weight of various fractions of plants obtained during green crop fractionation (g/Kg, fresh green foliages).

The results obtained for the various dried fractions are represented and illustrated in table 2 and fig. 2 respectively. Maximum dry weight of juice and DPJ was reported in green foliage of *Adhatoda vasica* Nees. (120 and 70.22 g respectively) and minimum in green foliage of *Raphanus sativus* L. (53.4 and 17.83g respectively). Maximum dry weight of fiber was reported in green foliage of *Alysicarpus vaginalis* L. (305 g) and minimum in *Brassica oleracea* L. (68.34 g). Maximum dry weight of LPC was reported in green foliages of *Psoralia corylifolia* L. (80.52 g) and minimum in *Raphanus sativus* L. (20.83 g).

The yield of various fractions from wild plants was compared with *Brassica oleracea*L. to check their usefulness in green crop fractionation process. The present results revealed that the dry weight of juice, fiber and LPC of all wild plants are relatively higher than that of *Brassica oleracea*L. The higher yield of LPC in wild plants may be attributed to high crude protein content in the juice extracted from their foliages. In general, leguminous plants gave better yield of LPC. However, the yield of DPJ from *Alysicarpus vaginalis* L. and *Adhatoda vasica* Nees. was found higher than that of *Brassica oleracea*L., but it was lower in *Alternanthera paronychioides* St. Hil. and *Psoralia corylifolia* L.

Several workers have been reported the yield of LPC from radish as 40.9 g/Kg (Patil and Salve, 2000), 23.77 g/Kg (Gogle, 2000) and 20.86 g/Kg (Madhekar, 2008). Gogle (2000) have been reported the yield of LPC from cabbage as 43.94 g/Kg. While, in present investigation radish and cabbage showed LPC yield as 20.83 and 21.51 g/Kg respectively. This variation in LPC yield might be due to equipment, regional difference and the duration of processing. Singh (1969) suggested that a plant can be selected for the preparation of LPC, when the yield of dry LPC exceed 10 g/Kg fresh green foliage and the resulting LPC contains more than 5% nitrogen. In this view all plants species which is chosen in present study could be considered suitable for the preparation of LPC.

CONCLUSION

The present results revealed that the yield of expressed juice from all the wild plants was relatively low as compared to *Brassica oleracea*L.; among all these wild plants *Adhatoda vasica* Nees. yielded minimum amount of juice. However all wild plants show comparatively more yield of fiber than that of *Brassica oleracea*L.

Similarly the dry weight of juice, fiber, LPC and DPJ of all wild plants are relatively higher than that of *Brassica oleracea*L. except *Alternanthera paronychioides* St. Hil. and *Psoralia corylifolia* L. which showed lower yield of dry DPJ.

Thus from the present study it is concluded that the green biomass can be successfully used for the extraction of protein and the preparation of leaf protein concentrate. However, the studies regarding its nutritional aspects are to be considered in future.

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