RESEARCH ARTICLE

Studies on biochemical changes occurs during storage of leaf extract

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ABSTRACT

Leaf extract or leaf juice is the first fraction of green crop fractionation process (GCF) suggested by N.W. Pirie in 1942. During mechanical fractionation, the leaf juice is released due to the pressing of pulp crop. It is a high moisture- high protein product, free from fibrous material. The juice or leaf extract contains proteins, sugars, lipids, salts, vitamins and other soluble components of the cell protoplasm. This fraction is very labile and its composition changes rapidly. Therefore it should be processed for LPC preparation immediately without any delay because as soon as the leaf juice is released, catabolic activities starts and leading to degradation of protein, polysaccharides, pigments and other macromolecules. This results in impaired quality of juice.

Keywords: LPC, green crop fractionation process

INTRODUCTION

Various workers have suggested the use of leaf extract from lucerne and berseem as a liquid feed or milk replacer to pre-ruminant calves (Prasad *et al.* 1977a, 1977b; Mungikar *et al*. 1978). The leaf extract has been used successfully as a supplement in pig diets (Maguire and Brooks, 1972, 1973; Houseman and Connel, 1976; Ohshima and Ueda, 1982). The juice of berseem used successfully for feeding to the calves (Grower *et al.* 1980). The feeding of leaf juice is cheaper than milk in spite of its quality is not so good as suggested by Rangnekar *et al.* (1979). In addition to this, when the juice is to be used as a liquid feed to the animals, it should be stored carefully for further use. If sufficient care is not taken during storage of juice, the protein degradation and destruction of amino acids deteriorates its performance as a cattle feed (Barber *et al.* 1979).

During the present investigation, attempts were made to observe the biochemical changes occurs during storage of leaf extract of five plant species up to 42 hours. During the storage of leaf extract decreased in pH, protein content, total carbohydrates, chlorophylls and yield of LPC was found to be more pronounced. In addition to this, the cytoplasmic proteins were lost during earlier stages of storage.

MATERIAL AND METHOD

During present investigation, The studies on storage of leaf extract was carried out on five different plant species which are *Goniocaulon indicum, Brassica oleracea, Celosia argentea, Vigna trilobata,* and *Digera muricata.* The leaves of these selected plants were collected from the field at vegetative stage (pre-flowering stage). Fresh green foliages brought to the laboratory washed with running, tap and distilled water to remove unwanted debris. Pulped in a mixer and pressed as described in GCF process suggested by Pirie in 1942. Leaf extract (juice) released due to pressing, were stored in plugged conical flask for 0, 6, 12, 18, 24, 30, 36, and 42 hours at room temperature (Minimum 12^0C to 24^0C; Maximum 22^0C to 36^0C) and the samples were removed periodically for chemical analysis. The pH was measured using glass electrode on pH meter. The soluble protein (Bradford, 1976), carbohydrates (Hedge and Hofreiter, 1962), reducing sugar (Somogyi, 1952) and chlorophyll content (Aron, 1949) were quantified. Simultaneously, 100ml leaf extract was taken for the preparation of leaf protein concentrates.

RESULT AND DISCUSSION

Preparation of LPC (Leaf protein concentrates) from leaf juice immediately after its extraction is recommended for maximum recovery of proteins and optimum yield of leaf protein concentrates. If the preparation of LPC is delayed, the enzymes in the juice degrade protein and other nutrients, which results into decrease in protein nitrogen and increase in non-protein nitrogen due to proteolysis (Mungikar *et al*. 1978).

During the present investigation, attempts were made to observe the biochemical changes occur during the storage of leaf extracts at six hours time interval i.e. 0, 6, 12, 18, 24, 30, 36 and 42 hours. The data obtained were statistically analyzed for standard deviation and analysis of variance (ANOVA) following Gomez & Gomez (1976) and Mungikar (2003) and the results were presented in Table No. 1. The significant differences were observed in concentration of soluble protein, carbohydrates, reducing sugar, chlorophyll content and yield of LPC in storage of leaf extract up to 42 hours among the plant species under investigation. The pH of all the extracts decreased due to the storage. Protein content, chlorophyll content and sugar content in leaf extract gradually declined with the storage time. Leaf extract from all the plants showed decreased recovery of leaf protein concentrates as a result of storage for 42 hours.

With the storage of juice the soluble protein of *Goniocaulon indicum* leaf extract decreased from 20.39mg/g to 19.80mg/g, 31.18mg/g to 29.53mg/g in *Brassica oleracea*, 13.38mg/g to 12.25mg/g in *Celosia argentea*, 27.19mg/g to 25.77mg/g in *Vigna trilobata* and 18.92mg/g to 18.05mg/g in *Digera muricata*. Similarly, the decreasing values for carbohydrates, reducing sugar and chlorophyll contents were also observed during the storage of leaf extract. With the storage of juice the yield of wet LPC per 100ml of juice decreased gradually from 4.38g to 3.75g at the end of 42 hours in *Goniocaulon indicum* leaf extract. Similar decreasing trend was observed for other leaf extract. The initial value for LPC of *Brassica oleracea* is 9.29g which was decreased to 8.78g at the end of 42hours, 6.48g to 5.88g in *Celosia argentea* LPC whereas 7.51g to 6.86g in *Vigna trilobata* and 5.54g to 4.98g in *Digera muricata* LPC.

In general, during the storage of leaf extract up to 42 hours, the maximum decreased in pH was found in *Celosia argentea* i.e. 1.03 and minimum in *Goniocaulon indicum* i.e. 0.83. The maximum loss of soluble protein and carbohydrate content was found in *Brassica oleracea* (1.52mg/g) and in *Celosia argentea* (3.32mg/g) respectively while minimum loss of protein and carbohydrate was found in *Digera muricata* i.e. 0.87mg/g and 2.23mg/g respectively. The maximum reducing sugar was lost in *Vigna trilobata* (0.84mg/g) leaf extract while minimum loss was observed in *Celosia argentea* (0.41mg/g). The maximum loss in yield of LPC was observed from *Vigna trilobata* leaf extract (0.65g/100ml juice) and minimum loss was found in *Brassica oleracea* leaf extract (0.51g/100ml juice) during the storage of leaf extract of total 42 hours.

The results obtained during the present study were comparable with the results reported by Reddy and Mungikar (1988). If the juice is kept at room temperature, protein breakdown takes place which results in to low recovery of LPC from the stored juice. In the stored leaf extract, microbial activity may results into changes in carbohydrates fraction. The nutrient loss in juice during storage can be corrected

Table 1: To study the Biochemical changes occurs during storage of juice (Leaf Extract) of various plants.

Name of plant	Name of Constituents	Storage period (hours)								Mean	C.D. (5%)	C.D. (1%)	C.V. (%)
		0	6	12	18	24	30	36	42				
Goniocaulon indicum (Klein ex willd). C.B. C.L.	PH	7.29	7.18	7.08	6.98	6.86	6.76	6.63	6.46	6.91	0.03	0.04	0.32
	Soluble Protein (mg/gm)	20.39	20.15	20.05	19.95	19.77	19.56	19.39	19.17	19.80	0.05	0.08	0.21
	Carbohydrates (mg/gm)	34.85	34.82	34.74	34.53	34.32	33.68	32.53	32.17	33.95	0.42	0.64	0.93
	Reducing Sugar (mg/gm)	3.42	3.55	3.72	3.18	3.06	2.99	2.93	2.84	3.21	0.04	0.07	1.02
	Chlorophyll Content (mg/gm)	1.18	1.16	1.12	1.07	1.00	0.97	0.93	0.88	1.04	0.02	0.03	1.45
	Yield of LPC(gm)/100ml Juice	4.38	4.30	4.24	4.16	4.09	3.98	3.91	3.75	4.10	-	-	-
Brassica oleracea var. *Botrytis* L.	PH	5.49	5.35	5.20	5.12	5.01	4.88	4.72	4.49	5.03	0.02	0.03	0.33
	Soluble Protein (mg/gm)	31.18	31.05	30.28	30.15	30.03	29.83	29.66	29.53	30.21	0.35	0.52	0.86
	Carbohydrates (mg/gm)	19.83	19.82	19.76	19.53	19.25	18.68	17.73	17.13	18.97	0.46	0.70	1.82
	Reducing Sugar (mg/gm)	2.20	2.28	2.37	2.10	1.99	1.90	1.81	1.74	2.05	0.06	0.09	2.15
	Chlorophyll Content (mg/gm)	0.91	0.87	0.81	0.77	0.74	0.70	0.65	0.62	0.76	0.02	0.02	1.58
	Yield of LPC(gm)/100ml Juice	9.29	9.21	9.17	9.11	9.02	8.96	8.88	8.78	9.05	-	-	-
Celosia argentea L.	PH	7.43	7.16	7.11	7.00	6.89	6.78	6.65	6.46	6.93	0.06	0.09	0.64
	Soluble Protein (mg/gm)	13.38	13.15	13.05	12.97	12.85	12.69	12.48	12.25	12.85	0.05	0.07	0.28
	Carbohydrates (mg/gm)	64.90	64.83	64.78	64.59	64.39	63.09	62.12	61.58	63.78	0.55	0.84	0.65
	Reducing Sugar (mg/gm)	2.08	2.25	2.35	1.98	1.91	1.83	1.73	1.67	1.98	0.05	0.07	1.81
	Chlorophyll Content (mg/gm)	0.55	0.51	0.46	0.42	0.39	0.36	0.33	0.31	0.42	0.02	0.02	2.92
	Yield of LPC(gm)/100ml Juice	6.48	6.39	6.34	6.27	6.18	6.10	5.99	5.88	6.20	-	-	-

Table 1: Continued…

Name of plant	Name of Constituents	Storage period (hours)								Mean	C.D. (5%)	C.D. (1%)	C.V. (%)
Vigna trilobata (L.) Verde.	PH	6.32	6.16	6.08	6.19	5.76	5.61	5.42	5.29	5.85	0.05	0.08	0.69
	Soluble Protein (mg/gm)	27.19	27.05	26.62	26.47	26.30	26.08	25.90	25.77	26.42	0.36	0.54	1.02
	Carbohydrates (mg/gm)	25.50	25.47	25.41	25.00	25.12	24.11	24.03	22.20	24.60	0.76	1.15	2.31
	Reducing Sugar (mg/gm)	3.75	3.87	3.95	3.32	3.15	3.07	2.98	2.91	3.37	0.07	0.11	1.62
	Chlorophyll Content (mg/gm)	1.24	1.21	1.18	1.14	1.07	1.00	0.95	0.89	1.08	0.02	0.03	1.41
	Yield of LPC(gm)/100ml Juice	7.51	7.40	7.32	7.24	7.13	7.02	6.95	6.86	7.18	-	-	-
Digera muricata (L.) Mart.	PH	5.95	5.61	5.51	5.41	5.25	5.16	5.08	4.98	5.37	0.03	0.05	0.47
	Soluble Protein (mg/gm)	18.92	18.70	18.62	18.55	18.43	18.31	18.15	18.05	18.47	0.07	0.11	0.29
	Carbohydrates (mg/gm)	11.06	10.98	10.91	10.78	10.50	9.86	9.08	8.83	10.25	0.30	0.46	2.21
	Reducing Sugar (mg/gm)	1.60	1.73	1.88	1.25	1.17	1.09	0.99	0.92	1.33	0.06	0.09	3.46
	Chlorophyll Content (mg/gm)	1.19	1.17	1.12	1.07	1.03	0.97	0.93	0.86	1.04	0.02	0.03	1.52
	Yield of LPC(gm)/100ml Juice	5.54	5.49	5.41	5.38	5.26	5.15	5.05	4.98	5.28	-	-	-

by decreased in the pH. Mainderkar (1990) recommended the use of silage additives to prevent the loss of nutrient in the juice during storage while Bhande (1989) suggested heating of the juice prior to storage to minimize nutrient loss. Similar results were also reported by Gogle (2000) however, the values reported by these workers were different but the trend was similar as found in the present study. The variation exhibit among the species is the characteristics of plant species.

CONCLUSION

For the maximum recovery of leaf protein or for gaining advantages of green crop fractionation process, the leaf extract (Juice) should be processed for LPC production immediately after extraction without any delay.

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