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Short Communication

EFFECT OF EXTRACTION TECHNIQUES ON THE EXTRACTION OF PROTEIN FROM TRIFOLIUM ALEXANDRINUM

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Extraction of proteins from leaves has been studied by various workers.[1-4] Byers and Sturrock[5] made a comparative study on the extractability of proteins from various leaves. Chayen *et al.*[6] described impulse rendering process to separate proteins from plants. More recently Davys and Pirie[7,8] developed a machine to extract leaf proteins on pilot scale. Knuckle *et al.*[9,10] extracted protein from leaves using sugarcane rollers.

In the present studies International Biological Programme (IBP) pulper, crypto meat mincer AB-12-74793 (power operated), hand-operated and power-driven sugarcane crushers were used to disintegerate the leaves of *Trifolium alexandrinum*. Juice was extracted by pressing the pulp with a belt press, IBP press and by hands. Proteinous nitrogen (PN) was precipitated with an equal volume of 20% solution of trichloroacetic acid (TCA) and separated by centrifugation at 300 rev/min. The preciptate was analysed for PN and the supernatent for non-proteinous nitrogen (NPN). Nitrogen was determined by a micro-kjeldahl method using $CuSO_4$–K_2SO_4–SeO_2 (1:9:0.02) mixture.[11]

Results and Discussion

Effect of Extraction Techniques on the Extractability of Proteins. Extraction techniques greatly affected the extractability of proteins. It was maximum (60.2%) when the crop was pulped with IBP pulper and the juice was extracted by hand pressing (Table 1). 57.8% proteins were extracted with IBP press and 51.7% with the belt press. The increase in the extraction of proteins by hand pressing seems to be due the higher pressure applied by hands, which resulted in the maximum extraction of chloroplastic as well as cytoplasmic proteins. The decrease in the extractability in case of IBP press or the belt press seems to be due the formation of fibrous cake which caused lesser filtration and greater reabsorption of the chloroplastic proteins. These results agree with the findings of Davys and Pirie.[12]

Extractability of protein was low when crypto meat mincer, power-operated sugarcane crusher or hand operated sugarcane crusher was used for pulping the crop. In case of cyrpto meat mincer it was 50% with hand pressing, 50% with IBP press 43.6% with the belt press. With power operated sugarcane crusher the extractability was 24% in March with hand pressing, 21.0% with IBP press and 20% with belt press. Similarly in case of hand operated sugarcane crusher the extractability varied from 15.5–16% (Table 1). Low extraction with crypto meat mincer, power-operated or hand operated sugarcane crusher appeared to be due to the incomplete rupturing of the cell, and disintegration of the plant tissues which resulted in retention of chloroplastic proteins by the fibrous matter.

Although the extractability of protein with hand pressing and IBP press was higher than that of belt pressing but pressure applied by hand may vary from person to person. Moreover, it seemed unlikely that this technique would be applied for bulk production of leaf protein concentrate. Further, proteins present in the juice extracted by hand pressing or with IBP press were hydrolysed to a greater extent by the proteolytic enzymes present in it due to greater time consumed during these operations. The hydrolysis of protein resulted in a decrease in the PN which was accompanied by an increase in the NPN contents of the juice (Table 2). Hydrolysis of proteins by proteolytic enzymes has also been reported.[13-18]

Extractability of protein also varied considerably. with temperature. Maximum extractability, i.e. 60% was observed during winter (January) which decreased to 35% during May (Fig. 1). The decrease in the extraction rate of protein during these days appeared to be due to decrease in moisture con-

TABLE 1. EFFECT OF EXTRACTION TECHNIQUES ON THE EXTRACTION OF PROTEIN FROM *Trifolium alexandrinum*.

Pulping machines	Extractability of protein (%)*					
	January			March		
	Belt pressing	Hand pressing	IBP pressing	Belt pressing	Hand pressing	IBP pressing
IBP pulper	51·7	60·2	57·8	44·73	50·50	50·00
Crypto meat mincer	43·6	50·0	50·0	34·00	44·10	40·00
Power operated sugarcane crusher	—	—	—	20·00	24·00	21·00
Hand-operated sugarcane crusher	—	—	—	15·50	16·00	15·50

*Extracted protein-N expressed as a% of the total N of the leaf.

TABLE 2. EFFECT OF EXTRACTION TECHNIQUES ON PN AND NPN OF JUICE OF *Trifolium alexandrinum*.

Pulping machines	Percentage of Protenous Nitrogen (PN) and Non-Protenous Nitrogen (NPN)											
	January						March					
	Belt pressing		Hand pressing		IBP pressing		Belt pressing		Hand pressing		IBP pressing	
	PN	NPN	PN	NPN	PN	NPN	PN	NPN	NP	NPN	PN	NPN
IBP pulper	90·00	10·0	89·0	11·0	87·1	12·9	89·0	11·0	87·0	12·0	85·1	12·9
Crypto meat mincer	87·0	13·0	86·1	13·9	85·0	15·0	84·8	15·0	84·5	15·5	83·3	16·7
Power operated sugarcane crusher	—	—	—	—	—	—	83·8	16·2	81·0	79·0	79·0	21.0
Hand operated sugarcane crusher	—	—	—	—	—	—	80·7	19·3	71·6	28·4	70·0	30·0

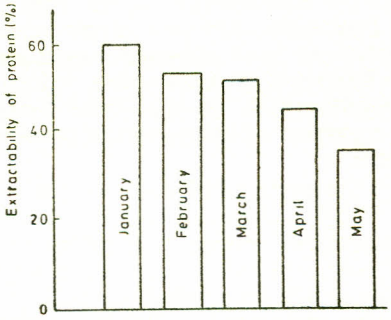

Fig 1. Variation in the exrtactabilily of protein (using IBP pulper and hand pressing)

tents of the plant which was 91.6% in January, 90.6% in February, 90% in March, 89.7% in April and 83.6% in May. Similar observations have been made by Shah *et al.*[19] during studies on the extractability of proteins from various grasses.

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