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PRESERVATION OF LEAF PROTEIN CONCENTRATE OBTAINED FROM *TRIFOLIUM RESUPINATUM* AND *PANICUM ANTIDOTALE*

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Microorganisms, Lipids, enzymes and moisture are the factors responsible for rapid deterioration of leaf protein concentrate at room temperature. Acetic acid (2%) successfully checked microbial and non-microbial deterioration of the leaf protein concentrate. The combined effect of emblic myrobalan leaf powder and acetic acid was more pronounced.

INTRODUCTION

The utilization of leaf protein for human consumption is one of the many possibilities for stamping out the problem of protein deficiency in the diet of underdeveloped countries. Much of the impetus for leaf protein research has come from the work of Pirie [1, 2].

The LPC obtained by the method of Morrison and Pirie [3] contained 66 - 75% moisture. It also contains a high proportion of unsaturated lipids (Lima *et al.* [4]), and a heavy load of microorganisms. The presence of all three substances adversely affect the life of the leaf protein product which shows signs of deterioration when stored at room temperature. This necessitates preservation studies of LPC. Lea and Par [5] tried various antioxidants and synergist mixtures but reported their failure to check the development of rancidity. Shah [6] used emblic myrobalan fruit and leaf powders for checking microbial and oxidative deterioration of LPC. He reported success in retarding the deterioration. Suba Rao *et al* [7] studied the effect of acetic acid on microbial induced deterioration of Lucerine LPC.

The present work was undertaken to determine whether shelf-life of LPC can be extended by checking the microbial activity and development of oxidative rancidity in the LPC of *Panicum antidotale* and of *Trifolium resupinatum.*

MATERIAL AND METHODS

Preparation of LPC. A sample weighing 2 - 5 kg of freshly picked green leaves was minced in an electric cryptomincer. The juice was then squeezed out of the pulp by hand in a cheese-cloth bag. The proteins in the collected juice were coagulated by gentle heating until the temperature of the juice reached 80 ± 2°C. Heating at this temperature lasted for 2 min. The coagulate was washed thoroughly with tap water and finally squeezed hard by hand, thus forming a hard cake. This final product was used for the experiments to be described and will be referred to here as LPC. Two types of leaves were used *Panicum antidotale* (Graminae) and *Trifolium resupinatum* (Leguminosae).

Checking of Deterioration. This was achieved by treating LPC with either acetic acid of dried emblic myrobalan or *amla* leaf powder. A sample of LPC was either homogenized with acetic acid (2% V/W) or *amla* leaf powder (1.2% W/w). The latter was prepared by drying the freshly picked leaves at 60°c for 24 hr. The dried leaves were then ground and used whenever required.

Chemical Analysis. The protein content of LPC was determined by the conventional kjeldahl method. Lipids were estimated according to Folch *et al* [8].

Determination of Rancidity. The development of rancidity and the effect of adding antioxidants to LPC were studied by manometric techniques. Conventional warburg apparatus was used and the reaction was carried out at 30 + 2°.

Microbial Growth. This was carried out on samples of LPC by the standard Koch's plate method. The plates were incubated at 28° up to 7 days.

RESULTS

Chemical Analysis of LPC. The results of the estimation of certain parameters of LPC of *P. antidotale* and *T. resupinatum* are shown in Table 1. This table shows that there is no appreciable difference in these concentrations between the two species. The high moisture content of LPC obviously promotes the growth of microorganisms

and the activity of hydrolytic and oxidative enzymes. In fact Shah [8] reported that the leaf protein cake does not keep well for more than 72 hr under room conditions. The fairly high level of lipids (11 - 16%, Table 1) renders LPC susceptible to oxidative rancidity. In this connection, it is relevant to mention that 80% of grass lipids are unsaturated [10] and spinach chloroplast lipids contain 70% unsaturated fatty acids (Mallick and Lea,) [11].

Effects of Adding Antioxidants to LPC. To test the effects of antioxidants for checking deterioration, the oxygen uptake of LPC, LPC + *amla* leaf Powder, LPC + acetic acid, was measured over period of up to 8 hr. The results are presented in Tables 2 and 3. At 30^{0}, the oxygen uptake was 4.2 ml 0_2/g protein/8 hr in the case of *P. antidotale* and 6 ml 0_2/g protein/8 hr. in the case of *T. resupinatum.* The addition of a natural antioxidant, *amla* leaf powder (with a concentration of 1.2% W/w) with or without autoclaving, reduced the uptake of oxygen by about 40% in the case of *P. antidotale* and 60% in the case of *T. resupinatum* (Tables 2 and 3). The effect of synthetic antioxidant on oxygen uptake are also shown in the Tables 2 and 3. The LPC containing 2% acetic acid shows that the oxygen uptake was almost doubled (Tables 2 and 3) in the samples which were squeezed after the addition of the acetic acid solution. However the samples, containing 2% acetic acid solution, which were not squeezed showed a reduced uptake of 0_2, i.e. 75% reduction in *P. antidotale* and 70% reduction in *T. resupinatum* (Tables 2 and 3). The reason for the enhancement of 0_2 uptake by squeezing LPC is not known, but might be possibly due to the leeching out of both the added and naturally occurring antioxidants and by further contamination with microorganisms.

The Combined Effects of Amla Leaf Powder and Acetic Acid. This set of experiments is similar to the experiments described in the previous sections except that acetic acid was also added to act as an antioxidant in conjunction with *amla* leaf powder. The results are also included in Tables 2 and 3. These tables show quite clearly that there was an even more pronounced reduction in the 0_2 uptake.

Effects of Amla Leaf Powder and Acetic Acid on Microbial Growth. The results of plating LPC (of *P. antidotale* and *T. resupinatum)* with or without antioxidants are shown in Tables 4 and 5. These results show very clearly the pronounced inhibition of microbial growth after the addition of acetic acid, whether squeezed or not. The inhibition of microbial growth was essentially to the same extent. This perhaps might explain at least in part the reduction of oxygen uptake after the addition of acetic acid (in the non-squeezed sample). It seems probable that the reduction of oxygen uptake is not only due to the arrest of oxidative processes but also due to the diminished 0_2 uptake by the diminished number of microorganisms. These results are in agreement with those obtained by Suba Rao *et al* [7] who used acetic acid to check the activity of microorganisms in Lucerene LPC. Arkcoll [12] used 1% acetic acid also which resulted in a complete arrest of microbial growth of moist leaf proteins.

The addition of natural antioxidant (*amla* leaf powder) slightly increased the number of organisms (i.e. from 436 to 523 colonies in *P. antidotal* and 402 to 480 colonies in *T. resupinatum*; Tables 4 and 5). This slight increase was probably due to the naturally occurring microorganism present in *amla* leaf powder which could be eliminated by autoclaving the *amla* leaf powder on the reduction of 0_2 uptake is due to the arrest of oxidative processes only (Tables 2 to 5).

DISCUSSION

The LPC obtained from *T. resupinatum* and *P. antidotale* contained high amounts of moisture (75-78%), lipids (11 - 15%). (Table 1) and microorganisms (Tables 4 and 5). These substances have been shown to affect adversely the life of the leaf protein product (Shah,) [6].

The present work shows quite clearly that the storage properties of LPC obtained from *T. resupinatum* and *P. antidotale* could be greatly improved by the arrest or impairment of oxidative reactions and of deterioration caused by microorganisms using various synthetic or natural agents. The arrest of deterioration is more pronounced when both types of antioxidants are used in conjunction with each other. An interesting point that emerges from this study is the increased oxygen uptake following squeezing by hand of LPC samples that had been treated with antioxidant (acetic acid 2% V/W).

This increase might be due to contamination by microorganisms, leeching out of antioxidants present in LPC and/or leaching out of the experimentally added antioxidant. The presence of flavenoids which act as naturally occurring antioxidants has been reported in the edible parts of a number of plants by Bate – Smith [13] and Swain [14]. It is also possible that some prooxidants get ridden by squeezing. Metal ions are reported to cata-

Table 1. Analysis of LPC obtained from *Panicum antidotale* and *Trifolium resupinatum*.

Plant species	No. of samples	Moisture %	Lipids	Protein %	Ash %
Panicum antidotale	6	78.2 ± 1.5	11.0 ± 2.3	59.9 ± 4.1	22.2 ± 1.5
Trifolium resupinatum	6	75.1 ± 2.2	15.6 ± 1.2	54.8 ± 5.2	13.7 ± 2.2

On dry matter basis; ± Standard deviation

Table 2 Effect of acetic acid and/or amla leaf powder on the absorption of oxygen by *Panicum antidotale* LPC at 30 ± 1°C, pH 7.

Treatment	No. of samples	Time in hr.	ml. of oxygen/g of LPC
LPC (without treatment)		2	0.10 ± 0.01
		4	0.60 ± 0.03
	4	6	2.40 ± 0.03
		8	4.20 ± 0.05
LPC + Acetic acid (2% V/W)		2	0.00 ± 0.00
(Final concentrate, non-squeezed)		4	0.02 ± 0.01
	4	6	0.80 ± 0.04
		8	1.02 ± 0.04
LPC + Acetic acid (2% V/W)		2	0.34 ± 0.10
(final concentrate, squeezed)		4	2.1 ± 0.08
	4	6	4.00 ± 0.04
		8	7.10 ± 0.05
LPC + non autoclaved *amla* leaf powder		2	0.04 ± 0.01
(1.2% (W/W).		4	0.6 ± 0.03
	4	6	1.8 ± 0.03
		8	3.2 ± 0.02
LPC + autoclaved *amla* leaf powder		2	0.00 ± 0.00
(1.2% W/W)		4	0.30 ± 0.03
	4	6	0.80 ± 0.03
		8	2.60 ± 0.06
LPC ± autoclave *amla* leaf powder		2	0.04 ± 0.01
(1.2% W/W) + Acetic acid (2% V/W)		4	0.08 ± 0.02
(Final concentrate, non-squeezed)	4	6	0.11 ± 0.02
		8	0.14 ± 0.03

Table 3. Effects of acetic acid and/or amla leaf powder on the absorption of oxygen by *Trifolium resupinatum* LPC at 30 ± 1°C and pH 7.0

Treatment	No. of samples	Time in hr.	Ml. of oxygen/g of LPC
LPC (without treatment)		2	0.80 ± 0.03
		4	2.10 ± 0.02
	4	6	4.30 ± 0.03
		8	6.00 ± 0.11
LPC + acetic acid (2% V/W)		2	0.10 ± 0.01
(Final concentrate, non-squeezed)		4	0.40 ± 0.03
	4	6	1.20 ± 0.01
		8	2.30 ± 0.02
LPC + Acetic acid (2% V/W)		2	1.20 ± 0.03
(Final concentrate, squeezed)		4	3.00 ± 0.02
	4	6	6.20 ± 0.05
		8	10.50 ± 0.05
LPC + non autoclaved *amla* leaf powder		2	0.40 ± 0.05
(1.2 W/W)		4	1.00 ± 0.03
	4	6	2.10 ± 0.04
		8	3.80 ± 0.04
LPC + autoclaved *amla* leaf powder		2	0.08 ± 0.03
(1.2% W/W)		4	0.80 ± 0.03
	4	6	1.50 ± 0.03
		8	2.80 ± 0.05
LPC + autoclaved *amla* leaf powder		2	0.03 ± 0.03
(1.2% W/W) + acetic acid 2% V/W		4	0.80 ± 0.02
(Final concentrate, non-squeezed)	4	6	1.10 ± 0.03
		8	2.00 ± 0.03

Table 4. Effects of acetic acid and/or amla leaf powder on microbial growth in *Panicum antidotale* leaf protein concentrate

Treatment	No. of samples	No. of colonies/g of LPC	Microorganims Observed
LPC	3	436 ± 20	Cocci Bacilli
LPC + AA^1	3	20 ± 4	Bacilli
LPC + AA^2	3	16 ± 4	Bacilli
LPC + ALP^1	3	523 ± 15	Cocci Bacilli *Rhizopus spp.* *Penicillium* spp. *Aspergillus niger*
LPC + ALP^2	3	442 ± 20	Cocci Bacilli *Penicillium* spp
LPC + AA^1 + ALP^2	3	24 ± 5	Bacilli Penicillium spp.

LPC = Leaf protein concentrate
AA^1 = Acetic acid 2% V/W (final concentrate, non squeezed)
AA^2 = Acetic acid 2% V/W (final concentrate, squeezed).
ALP^1 = Amla leaf powder 1.2% W/W (not autoclaved)
ALP^2 = Amla leaf powder 1.2% W/W (autoclaved).

Table 5. Effect on *Amla* leaf powder and/or acetic acid on microbial growth in *Trifolium resupinatum* leaf protein concentrate

Treatment	No. of samples	No. of colonies/g of LPC	Microorganisms Observed
LPC	3	402 ± 20	Cocci Bacilli *Penicillium* spp. *Rhizopus* spp. *Aspergillus niger*
LPC + AA^1	3	24 ± 5	Bacilli
LPC + AA2 LPC + AA^2	3	32 ± 6	Bacilli
LPC + ALP^1	3	480 ± 15	Cocci Bacilli *Penicillium* spp. *Rhizopus* spp. *Aspergillus niger*
LPC + ALP^2	3	412 ± 15	Cocci Bacilli *Penicillium* spp.
LPC + AA^1 + ALP^2	3	32 ± 4	Bacilli *Penicillium* spp.

LPC = Leaf Protein Concentrate
AA^1 = Acetic acid 2% V/W (final concentrate, non-squeezed)
AA^2 = Acetic acid 2% V/W (final concentrate, squeezed)
ALP^1 = Amla leaf powder 1.2% W/W (not autoclaved)
ALP^2 = Amla leaf powder 1.2% W/W (autoclaved).

lize oxidation of lipids [1.5]. High concentration of minerals catalysts are present in *P. antidotal* (22.2%) and *T. resupinatum* (13.7%) (Table 1). It is possible that a combination of these mechanisms underlies the increased 0_2 uptake caused by squeezing.

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REFERENCES

1. N.W. Pirie, Science, **152**, 1701 (1966).
2. N.W. Pirie, *Leaf Protein, its Agronomy, Preparation, Quality and Use*, ed. N.W. Pirie, (IBP Handbook 20, Blackwell, Oxford 1971).
3. J.E. Morrison, and N.W. Pirie, J. Sci. Food. Agr., **12**, 1 (1961).
4. I.H. Lima, T. Bichardson, and M.A. Stahmann, J. Agr. Food Chem., **13**, 143 (1965).
5. C.H. Lea, and L.J. Par, J. Sci. Food Agr., **12**, 785 (1961).
6. F.H. Shah, J. Sci. Food Agr., **19**, 199 (1963).
7. M.S. Suba Rao, N. Singh and G.J. Parsanappa, J. Sci. Food Agr., **18**, 295 (1967).
8. J. Folch, M. Lees and G.H. Sloane-Stanley, J. Biol, Chem., **226**, 497 (1957).
9. F.H. Shah, Ph.D. Thesis, University of London (1963).
10. Gorton, G.A., Nature, **187**, 511 (1960).
11. L.R. Mallick, and F.A. Lea, J. Food Sci., **26**, 356 (1961).
12. D.B. Arkcoll, J. Sci. Food Agr., **24**, 437 - 445 (1973).
13. E.C. Bate - Smith, *Pharmacology of Plant Phenols* (ed. J. W. Fairbrain, Academic Press, New York, 1959).
14. T. Swain, *Economic Importance of Flavenoid Compounds*, Chap. 16, ed. T.A. Geisman, (Macmillan Co., 1962).
15. N. Uri, Chem. Res., **50**, 375 (1952).