Nutrient Changes during Alfalfa Wilting and Dehydration

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Four wilting and dehydration trials were conducted on a commercial scale in a major alfalfa producing area. Alfalfa was wilted in windrows from 1 to 27 h, then dehydrated. Moisture decrease during wilting varied from 10 to 26%, 25 to 48%, and 36 to 47% in 4, 10, and 23 h, depending upon the weather conditions. Carotene retention during wilting 1 to 10 h plus dehydration varied from 41 to 100%, while xanthophyll retention varied from 61 to 97%. Carotenoid retention was inversely correlated with moisture decrease during wilting and directly correlated with dehydrated alfalfa meal moisture ($P > 0.001$). Starch decreased during wilting while the total and reducing sugars either remained unchanged or increased. Protein decreased up to 18% in 23 h of wilting while fiber increased 23%. Lysine increased slightly during 10 h of wilting.

The great need for conservation of fuel during forage drying has prompted alfalfa dehydrators to consider partial sun-curing or wilting as an integral part of their operations. The alfalfa dehydration industry came into being largely as a means of eliminating the large field losses that may occur during sun-drying and to provide a forage product with a guaranteed content of nutrients for use in high-quality poultry and animal feeds. Dehydrated alfalfa has generally been found to be an excellent source of carotene, xanthophyll, and other nutrients for poultry rations (Livingston et al., 1969) and animal feeds (Bechdel et al., 1940). These differences in nutritive quality of dehydrated alfalfa compared to sun-cured hay make it important for the alfalfa dehydrator as well as the feed formulator to know what nutrient changes occur during field wilting and what fuel savings may be achieved without significantly lowering the quality of dehydrated alfalfa meal.

The study presented here is a continuation of a preliminary report presented at the American Dehydrators Association convention in Orlando, Fla., Feb 2, 1976 and described in a recent trade journal article (Livingston et al., 1976).

EXPERIMENTAL SECTION

Four trials were conducted during June and July, 1975 at a commercial alfalfa dehydration plant near Maize, Kans. These trials are designated Kan-1 (June 18, field I), Kan-2 (June 20, field II), Kan-3 (July 22, field I), and Kan-4 (July 23, field II). Two rectangular alfalfa fields in close proximity were selected for this study. The alfalfa in field I was Lahonton variety and for the June study was second-cut, $^1/_4$ bloom, 30–35 cm high and for the July study, third-cut, $^1/_2$ bloom, 26–30 cm high. The alfalfa in field II was Northrup King variety and for the June study was second-cut, $^1/_4$ bloom, 32–36 cm high and for the July study third-cut, $^3/_4$ bloom, 26–30 cm in height. The alfalfa around the outer perimeter of each field was harvested separately to square-off the field and assure a uniform stand and was not used in this study. Field I contained approximately 12 ha and was 250 m in width while field II contained nearly 20 ha and was 570 m in width. Direct cut alfalfa was harvested from both sides of the fields (employing a Field Queen harvester with a 4.3-m cutter bar) to serve as the unwilted controls. The times of windrowing and picking-up were chosen so as to include the midday wilting hours in each wilted harvest. A Hesston windrower with a 4.3-m cutter bar was used to cut the alfalfa and deliver it into windrows approximately $^3/_4$ m wide by 10–15 cm. deep. Following wilting the windrows were picked up by a Field Queen harvester employing a 2-m windrow pick-up head. The wilted alfalfa was chopped as it was picked-up and quickly transported (about 1.5 km) to the dehydrator, promptly transferred to the feeder, and dehydrated in a Guaranty Performance 12-40 triple-pass drum with a rated capacity of 15 700 kg of 70% moisture raw material per hour. The dehydrator inlet and outlet temperatures were automatically controlled by means of an Anacon infrared reflectance meal moisture analyzer.

Duplicate samples of fresh green chops were collected at the feeder elevator by means of a long handled pan designed to obtain a representative sample of the alfalfa for use as freeze-dried standards, and corresponding samples of dehydrated meal and pellets were collected for each condition.

All samples were ground through a no. 40 screen and analyzed in duplicate for carotene and xanthophyll by the procedure of Livingston et al. (1971b). Total and reducing sugars were determined by the method of Potter et al. (1968) and starch was determined by the method of McCready et al. (1950). Amino acids were determined by the method of Kohler and Palter (1967). Nonprotein nitrogen was determined by extraction of meals with 10% trichloroacetic acid solution, followed by Kjeldahl determination of N on extract aliquots (Oser, 1965). Meal moisture was determined by drying at 105 °C for 20 h in a forced draft oven.

RESULTS AND DISCUSSION

Wilting Conditions and Moisture Decrease. During wilting the farmer and dehydrator operator are dependent upon the weather. The Kan-1 study began with cloudy skies and threatening rain. The air temperature was high (26–33 °C) with relatively high humidity (50–77%). However, in spite of these apparently adverse weather conditions for field-drying, relatively rapid moisture reduction was achieved (Figure 1), due to a great extent to strong, steady winds (17–27 knots).

The Kan-2 study was begun two days after Kan-1, employing an adjacent alfalfa field. Although the moisture content of the fresh alfalfa was slightly lower than that of Kan-1, the rate of moisture reduction was essentially the same. Rapid wilting was again achieved primarily due to the wind velocity (13–18 knots), in spite of the limited sunshine (40–70%) and high humidity (52–82%). Rain on June 21 forced cancellation of an intended overnight study begun on June 20.

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Table I. Carotenoid Retention during Alfalfa Wilting and Dehydration

Field treatment	Moisture, %		Drier outlet temp, °C	Carotene, mg/kg[a]			Xanthophyll, mg/kg[a]		
	Green[b] chops	Dehy meal		Green[b] chops	Dehy meal	Reten, % (wilt + dehy)	Green[b] chops	Dehy meal	Reten, % (wilt + dehy)
Kan-1									
Direct cut	77.3	3.4	138	229	197	86	281	256	91
Direct cut	76.9	6.0	122	218	190	87	293	258	88
Windrow 4 h	64.2	4.6	114	185	165	74	265	259	90
6 h	57.7	5.8	106	164	164	74	234	217	76
10 h	50.3	6.1	97	155	142	64	231	187	65
10 h	48.0	2.2	127	137	133	59	216	184	64
23 h	41.3	5.4	93	118	104	47	193	149	52
Kan-2									
Direct cut	73.8	6.8	121	212	198	93	271	239	88
Direct cut	75.5	6.1	131	200	182	93	255	220	86
Windrow 4 h	64.9	6.3	110	184	173	84	263	225	86
7 h	54.2	6.0	110	152	134	65	212	208	79
10 h	48.9	5.7	96	137	134	65	211	206	78
Kan-3									
Direct cut	70.3	3.03	149	260	240	92	326	299	98
Direct cut	70.6	7.64	118	260	226	87	325	282	87
Windrow 4 h	44.1	7.0	75	153	138	53	232	219	67
8 h	26.0	7.1	71	136	127	49	220	211	65
10 h	22.7	7.4	64	134	106	41	216	199	61
23 h	23.8	7.3	75	126	99	38	196	174	54
Kan-4									
Direct cut	72.6	1.7	149	264	234	89	300	216	72
Direct cut	73.4	6.6	114	243	242	100	325	285	88
Windrow 1 h	68.5	6.6	114	242	241	100	302	304	97
2 h	65.8	6.0	113	225	194	76	284	260	84
3 h	60.6	6.6	112	221	174	69	275	237	76
4 h	54.5	6.5	92	183	170	67	242	201	65

[a] Moisture free basis. [b] "Green chops" constitutes the fresh alfalfa at the dehydrator site.

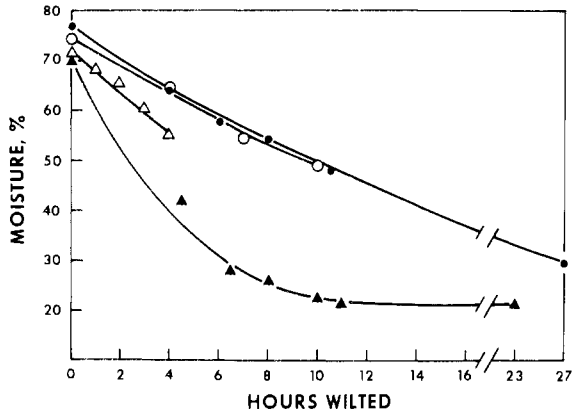

Figure 1. Moisture decrease during alfalfa wilting: (●—●) Kan-1, June 18; (○—○) Kan-2, June 20; (▲—▲) Kan-3, July 22; (△—△) Kan-4, July 23.

In contrast to the Kan-1 and Kan-2 studies, Kan-3 and Kan-4 were conducted under ideal wilting conditions with clear skies, a slight wind (7–13 knots), low humidity (23–48%), and high temperature (26–38 °C). This led to very rapid moisture reduction, particularly during Kan-3 in which during 4 h of wilting the alfalfa moisture content was reduced from 70.3 to 44.1% (5.22 to 1.74 kg of water per kg of dry matter). The following day, Kan-4 trial, the ideal wilting weather changed somewhat with an increase in humidity (43–59%), while both the temperature (26–33 °C) and wind velocity decreased slightly (3–6 knots). In 4 h of wilting the moisture of the alfalfa decreased from 73.4 to 54.5% (6.07 to 2.64 kg of water per kg of dry matter). Field losses, particularly those due to leaf-shatter, were very severe during the picking-up and loading of the alfalfa in the Kan-3 study even after only 4 h of wilting. Only moderate field losses were observed after 4 h of wilting in the other three studies. However, these losses became visually evident upon wilting to a moisture content of less than 50%, and more so when the alfalfa was wilted overnight to a moisture content of less than 30%. It has previously been estimated by Hoglund (1964) that as much as 25% of sun-cured alfalfa hay may be lost in the field when the hay is picked up.

Reduction in the moisture of the wilted alfalfa enabled both the dehydrator inlet and outlet temperatures to be greatly decreased while still producing dehydrated meal of 6–7% moisture. This was particularly the case following 10 h of wilting in Kan-1 and Kan-2 or 4 h of wilting in Kan-3 and Kan-4, in which the inlet temperature was reduced to less than 500 °C and the outlet temperature to under 130 °C. It was also possible to produce a high-moisture dehydrated meal following overnight wilting to under 30% moisture.

Retention of Carotene and Xanthophyll. Previous studies, Livingston et al. (1968, 1970) of this laboratory have shown that xanthophyll and carotene retention during alfalfa dehydration was maximized by producing a high-moisture meal. Therefore, during these trials it was intended to produce at least a 6–8% moisture dehydrated alfalfa meal. In order to demonstrate the effect of meal moisture upon carotenoid retention, lower moisture meals were also produced. As shown in Table I, xanthophyll was more stable than carotene during wilting in all of the trials. However, during dehydration, except during production of the lowest moisture meal (Kan-4, direct cut), carotene and xanthophyll were about equally stable. Considering both wilting and dehydration, xanthophyll was substantially better retained than carotene. This was particularly the case during wilting to low moisture levels. In the Kan-3 study, only 59% of the initial carotene was retained during wilting for 4 h compared to 71% of the xanthophyll, while additional quantities of both were lost during dehydration even to relatively high meal moisture levels. During Kan-3 and Kan-4, there was a much more rapid loss of moisture during wilting than in Kan-1 and Kan-2. During these

Table II. Changes in Carbohydrates during Alfalfa Wilting[a]

Field treatment	Green chop[b] moisture, %	Total sugar, %	Reducing sugar, %	Starch, %
Kan-1 June 18 (time cut)				
Direct cut (7:30 a.m.)	76.9	2.59	1.67	3.54
10 h wilt (8:00 a.m.)	50.3	3.87	1.63	2.22
Direct cut (2:00 p.m.)	70.3	4.33	2.89	4.74
27 hour wilt (1:00 p.m.)	29.5	4.74	1.57	2.78
Kan-2 June 20 (time cut)				
Direct cut (6:45 a.m.)	75.5	2.63	1.42	2.64
10 h wilt (8:00 a.m.)	48.9	3.59	1.84	2.05
Kan-3 July 22 (time cut)				
Direct cut (8:00 a.m.)	70.0	2.83	1.45	2.69
10 h wilt (8:30 a.m.)	22.7	3.68	1.69	2.03
Direct cut (2:00 p.m.)	70.3	4.96	3.21	3.32
23 h wilt (1:00 p.m.)	23.8	5.18	1.47	2.48

[a] Freeze-dried for analyses. [b] "Green chop" constitutes the fresh alfalfa at the dehydrator site.

Table III. Effect of Wilting and Dehydration upon Proximate Analyses[a]

Field treatment	Moisture, % Green[b] chops	Moisture, % Dehy meal	Protein, %	Fiber, %	Fat, %	Ca, %	P, %	Ash, %
Kan-1								
Direct cut	77.3	3.4	19.8	29.6	3.0	1.4	0.27	8.6
Direct cut	76.9	6.0	19.5	30.2	3.0	1.2	0.25	8.0
Windrow 6 h	57.7	5.8	18.8	30.9	2.8	1.0	0.25	7.6
Windrow 10 h	50.3	6.1	19.0	32.8	2.7	1.0	0.26	7.6
Windrow 10 h	48.0	2.2	19.1	32.6	2.7	1.1	0.22	7.5
Windrow 23 h	41.3	5.4	18.2	31.2	2.6	1.1	0.24	7.8
Kan-2								
Direct cut	73.8	6.3	18.9	33.2	2.5	1.1	0.25	8.4
Windrow 4 h	64.9	6.3	19.2	32.6	2.4	1.0	0.26	8.2
Windrow 10 h	48.9	5.7	18.1	33.5	2.7	1.1	0.25	8.9
Kan-3								
Direct cut	70.3	3.0	23.4	24.0	3.4	1.5	0.35	8.8
Windrow 4 h	44.1	7.0	20.3	28.9	2.8	1.3	0.27	7.6
Windrow 7 h	28.0	6.7	20.2	28.1	2.8	1.4	0.34	8.5
Windrow 10 h	22.7	7.4	19.5	28.5	2.8	1.4	0.33	7.7
Windrow 23 h	27.7	7.8	18.9	30.8	2.8	1.3	0.33	7.8
Kan-4								
Direct cut	72.6	1.7	21.5	25.4	3.4	1.4	0.34	8.3
Direct cut	73.4	6.6	20.7	25.7	3.1	1.3	0.26	7.9
Windrow 1 h	68.5	6.6	21.8	27.4	3.2	1.3	0.32	8.0
Windrow 3 h	60.6	6.6	20.7	30.1	2.8	1.3	0.31	6.8
Windrow 4 h	54.5	6.5	19.8	26.6	3.2	1.3	0.32	8.3

[a] Moisture free basis. [b] "Green chops" constitute the fresh alfalfa at the dehydrator site.

times of rapid moisture decrease, the carotene and xanthophyll were also very unstable and poorly retained. Since during wilting for more than 4 h and during periods of rapid moisture decrease large losses of both carotene and xanthophyll occurred, it would seem desirable not to wilt alfalfa below 60% moisture if the meal product is to be used primarily as a source of carotene and xanthophyll.

Carbohydrate Changes During Wilting. Table II shows the changes which were found to occur in the composition of the carbohydrates before and during wilting. In Kan-1 and Kan-3, the initial (direct cut) total sugars, reducing sugars and starch were higher in the afternoon cut alfalfa, due to photosynthesis during the day. However, following windrowing, starch decreased while the total sugars increased and reducing sugars increased somewhat less or remained the same during the 10 h wilting; during overnight wilting, total sugars increased only slightly while reducing sugars decreased. These changes were apparently due to the actions of plant enzymes following cutting and during wilting, so long as sufficient moisture remained. Starch is hydrolyzed into simple sugars which are in turn metabolized to other compounds.

Proximate Composition. During the pick-up and transfer of the wilted alfalfa considerable loss of dry matter occurred, particularly during the handling of the lower moisture wilted alfalfa such as in the Kan-3 study. Much of this loss was leaf tissue, and the data from Kan-3 in Table III indeed shows a loss of protein (from leaves) and increased fiber (from stems). In Kan-1 after 10 or more h of wilting and in Kan-3 after 4 or more h of wilting there was a decrease in crude fat during wilting.

Amino Acids. Table IV presents the effects of wilting and dehydration upon the amino acids of alfalfa. Prior dehydration studies (Livingston et al., 1971a) have shown that certain of the essential amino acids, particularly lysine and methionine, are very susceptible to loss during dehydration to low meal moisture levels. Therefore, to evaluate meal moisture as well as wilting effects the direct cut alfalfa and 10 h wilted alfalfa were dehydrated to both low and to medium moisture levels. In general, amino acid levels have been found to vary directly with meal moisture levels. Differences in amino acid content of the meals dehydrated to similar moisture levels are therefore due to the effects of wilting. In this present study, although cystine and methionine were relatively unaffected during wilting, there was a significant increase in lysine after 10 h of wilting. This was an unexpected benefit of wilting and it will require further investigation to determine the source of the additional lysine.

Table IV. Retention of Amino Acids during Alfalfa Wilting and Dehydration (g of amino acid/16 g nitrogen (dehydrated meal))

Hours wilted	Direct cut	Direct cut	4	10	10	23
Moisture of wilted alfalfa	76.9	77.3	64.2	50.3	48.0	41.3
Dehy meal moisture, %	6.0	3.4	4.6	6.1	2.2	5.4
Amino acid						
Lysine	5.61	4.13	5.10	6.16	4.20	5.21
Histidine	2.43	2.06	2.25	2.43	2.08	2.04
Ammonia	2.23	1.94	2.00	2.25	1.96	1.98
Arginine	4.88	4.13	4.45	4.77	4.00	4.05
Aspartic acid	11.88	9.90	11.17	12.87	10.14	11.49
Threonine	5.06	4.16	4.47	4.95	4.04	4.29
Serine	5.32	4.17	4.71	5.56	4.47	4.74
Glutamic acid	11.04	9.49	9.75	10.32	8.87	9.14
Proline	4.60	3.77	4.19	5.48	4.16	5.23
Glycine	4.65	4.66	4.69	5.19	4.47	4.49
Alanine	6.17	4.98	5.15	5.77	4.88	5.01
Valine	6.00	5.46	5.80	6.11	5.53	5.69
Isoleucine	5.31	4.44	4.61	5.08	4.29	4.38
Leucine	8.47	7.25	7.41	8.05	6.93	6.98
Tyrosine	3.58	2.84	2.91	3.28	2.70	2.81
Phenylalanine	5.48	4.58	4.72	5.24	4.56	4.58
Methionine	1.90	1.42	1.56	1.93	1.43	1.56
Cystine	1.15	0.96	1.15	1.14	1.10	1.18
% nitrogen recovery	90.1	75.9	80.1	90.0	75.4	78.6

Table V. Changes in Nonprotein Nitrogen during Wilting and Dehydration

Field treatment	Green chop moisture, %	Dehy meal moisture, %	Nonprotein nitrogen/total nitrogen[a]	
			Freeze-dried meal, %	Dehy meal, %
Kan-1				
Direct cut (6:30 a.m.)	77.3	3.4	18.7	18.3
Direct cut (7:00 a.m.)	76.9	6.0	17.3	18.2
Windrow 4 h	64.2	4.6	21.0	21.5
Windrow 6 h	57.7	5.8	22.6	21.9
Windrow 10 h	50.3	6.1	21.9	22.4
Windrow 23 h	41.3	5.4	21.9	22.6
Windrow 27 h	28.5	6.4	24.5	21.0
Direct cut (2:00 p.m.)	75.6	3.7	17.6	18.9
Direct cut (2:30 p.m.)	75.4	6.0	17.9	19.0
Kan-3				
Direct cut (7:30 a.m.)	70.6	2.5	18.6	17.3
Direct cut (8:00 a.m.)	70.0	6.4	18.3	18.2
Windrow 4 h	44.1	7.0	25.4	22.8
Windrow 6 h	28.0	6.7	25.2	23.8
Windrow 10 h	22.7	7.4	24.9	21.4
Windrow 20 h	27.7	7.8	23.8	22.4
Direct cut (2:00 p.m.)	70.3	3.0	18.4	17.9

[a] Nonprotein nitrogen is expressed as percent of total nitrogen.

Nonprotein Nitrogen. The principle source of nonprotein nitrogen in alfalfa would be free amino acids, soluble peptides, stachydrin, and nucleic acids. As shown in Table V, there was an increase in the level of nonprotein nitrogen relative to total nitrogen in Kan-1 and Kan-3 meals after 4 h of wilting. Since there was no additional increase in nonprotein nitrogen upon further wilting, it appears that the plant enzymes which convert protein into nonprotein nitrogen are inactivated as the moisture in the wilting alfalfa is reduced below a certain level. Nonprotein nitrogen may be more available than protein nitrogen for certain poultry such as broilers or turkey poults. (However, for many ruminant animals intact or protected proteins may be better utilized (McGilliard, 1972)). Thus, wilting alfalfa for short periods can result in an increase in the level of nonprotein nitrogen which may be advantageous for certain feeds purposes.

Wilting for periods longer than 10 h or to moisture levels below 50% may lead to serious losses of dry matter in the field, but many of the nutrients essential for animal feeds do not seem adversely affected by field wilting for short periods to medium moisture levels. Dorn et al. (1976) found essentially no difference in the feed quality of wilted and direct-cut dehydrated alfalfa when included in the rations of sheep. However, carotene and xanthophyll were rapidly lost under certain field wilting conditions in the present study. These losses plus possible additional losses during dehydration make it important to carefully analyze for carotenoids those meals prepared from alfalfa that has been field-wilted prior to dehydration and are intended for use in hen or broiler rations. In this way the well-known quality standards of dehydrated alfalfa for feed purposes can be maintained and valuable fuel may be conserved during the drying process by field-wilting prior to dehydration.

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LITERATURE CITED

Bechdel, S. E., Clyde, A. W., Cromer, C. O., Williams, P. S., Pennsylvania State College, *Agric. Exp. Sta. Bull.* **396**, 12 (1940).
Dorn, C., Klopfenstein, T., Ogden, R. L., Kehr, W. R., *Feedstuffs*, **48**, **A4**, Feb 9 (1976).
Hoglund, C. R., *Mich. Agric. Exp. Econ-Rep.*, 947 (1964).
Kohler, G. O., Palter, R., *Cereal Chem.* **44**, 512 (1967).
Livingston, A. L., Allis, M. E., Kohler, G. O., *J. Agric. Food Chem.* **19**, 947 (1971a).
Livingston, A. L., Knowles, R. E., Amella, A., Kohler, G. O., Arnold, W. N., Smith, K. V., *Feedstuffs*, **48**, **A8** Feb 9 (1976).
Livingston, A. L., Knowles, R. E., Kohler, G. O., *J. Assoc. Off. Anal. Chem.* **54**, 981 (1971b).
Livingston, A. L., Knowles, R. E., Kohler, G. O., *ARS Tech. Bull.*, 1414 (1976).
Livingston, A. L., Knowles, R. E., Nelson, J. W., Kohler, G. O., *J. Agric. Food Chem.* **16**, 84 (1968).
Livingston, A. L., Nelson, J. W., Kohler, G. O., *J. Assoc. Off. Anal. Chem.* **52**, 617 (1969).
McCready, R. M., Guggolz, J., Silviera, V., Owens, H. S., *Anal. Chem.* **22**, 1156 (1950).
McGilliard, A. D., *J. Am. Oil Chem. Soc.* **49**, 57 (1972).
Oser, B. L., "Hawk's Physiological Chemistry", 14th ed, The Blakiston Co., Toronto, 1965, p 1029.
Potter, A. L., Ducay, E. D., McCready, R. M., *J. Assoc. Off. Anal. Chem.* **51**, 748 (1968).

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Solubility Properties of Fraction I Proteins of Maize, Cotton, Spinach, and Tobacco

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The solubility characteristics of the major leaf protein, fraction I protein, of four plant species (tobacco, spinach, cotton, maize) have been determined and compared. The solubility in ammonium sulfate solutions was identical for all four proteins. Furthermore, all four proteins underwent isoelectric precipitation at about pH 4.5. The proteins of tobacco, spinach, and cotton (C_3 species) were precipitated only above 60 °C, but that of maize (a C_4 species) was partially precipitated at temperatures as low as 43 °C. Tobacco fraction I protein crystallized in low-salt media only when treated with $MgCl_2$ and $NaHCO_3$ and dialyzed at pH values around 7.5. At higher pH the protein remained soluble; at lower pH noncrystalline precipitates were obtained. Temperature had little effect on the crystallization. Crystallization could be obtained by (1) treatment with $MgCl_2$ and $NaHCO_3$ in the presence of salt, followed by dialysis in salt-free buffers, or by (2) direct addition of $MgCl_2$ and $NaHCO_3$ to protein in salt-free buffer. $CaCl_2$ and $MnCl_2$ replaced $MgCl_2$ effectively. The fraction I proteins of spinach, cotton, and maize did not precipitate or crystallize in low-salt media under a wide range of conditions of temperature and pH.

Interest in leaf protein concentrates for animal and human consumption has increased in recent years as a result of an anticipated world-wide need for alternate protein sources and a desire to derive maximum usefulness from agricultural crops. With leaf protein concentrates intended for human use, it is desirable to separate the soluble protein fraction from the pigmented chloroplast membranes as well as from other flavor-producing components to give a protein concentrate of neutral flavor. One successful purification procedure has been developed which utilizes selective heat precipitation of the pigmented membrane and soluble protein fractions of alfalfa (Edwards et al., 1975).

Fraction I protein is the predominant soluble component of leaf homogenates and thus is a major fraction of most colorless leaf protein concentrates (Kawashima and Wildman, 1970; Sarkar et al., 1975). The name "fraction I" arises from the observation that soluble leaf proteins are separated by ultracentrifugation or gel chromatography into two molecular weight classes, fractions I and II (Wildman and Bonner, 1947; Sarkar et al., 1975). The higher molecular weight class, about 500 000 to 600 000, is fraction I protein. In tobacco leaves, up to 50% of the soluble protein is fraction I protein (Kawashima and Wildman, 1970).

In those plants whose primary photosynthetic product is 3-phosphoglyceric acid, the C_3 plants, fraction I protein consists almost entirely of a single protein, ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) (Kawashima and Wildman, 1970; Siegel et al., 1972; Jensen and Bahr, 1977), which is responsible for the incorporation of CO_2 during photosynthesis. The ribulose-1,5-bisphosphate carboxylases of spinach and tobacco have been extensively studied. The molecular weight of ribulose-1,5-bisphosphate carboxylase is 560 000 (Paulsen and Lane, 1966; Trown, 1965; Pon, 1967; Ridley et al., 1967). SDS–polyacrylamide gel electrophoresis reveals two sizes of subunits: 55 000 and 12 000 daltons, respectively. The native protein probably consists of eight subunits of each size (Rutner and Lane, 1967; Baker et al., 1975).

The fraction I protein of tobacco and several related species can be crystallized by dialysis of crude leaf extracts against low ionic strength buffers. Following a centrifugation step to remove particulate matter and a chromatography step to remove phenolic compounds, fraction

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