Use of Recycled Dilute Alfalfa Solubles to Increase the Yield of Leaf Protein Concentrate from Alfalfa

Richard H. Edwards,* Donald de Fremery, and George O. Kohler

Controlled amounts of dilute alfalfa solubles were added to alfalfa feed material prior to juice expression in a twin-screw press. Solubles were added to chopped alfalfa, to ground alfalfa, and between extractions of doubly extracted chopped alfalfa. The effects of the solubles addition on the yield of the leaf protein concentrate (LPC) recovered and on the dewatering behavior of the feed material were determined. The dry matter content of the press cake was increased significantly by the double-extraction procedure but changed little when the solubles were added to chopped or to ground alfalfa prior to a single pressing. Reextraction of the press cake from chopped alfalfa increased the recovered yield of LPC by 55.6% when an equal weight of solubles was added to the press cake prior to the second pressing. The addition of the recycled dilute solubles to chopped alfalfa and to ground alfalfa increased the recovered yield of LPC by 14 and 23%, respectively. The increase in the recovered yield of LPC was proportional to the amount of solubles added, or to the decrease in the feed dry matter content resulting from the solubles addition. The highest LPC yields, which ranged from 14.9 to 21.4% of the alfalfa feed weight (dry basis), were obtained by adding solubles to ground alfalfa prior to a single pressing.

The process for making leaf protein concentrate (LPC) from alfalfa (*Medicago sativa* L.) or other green leafy crops involves the expression of green juice from the freshly harvested plant material, heat coagulation of the juice proteins, and separation of the resulting LPC curd from the residual deproteinized plant solubles. Dried alfalfa LPC contains 55–60% protein (N × 6.25), less than 3% fiber, and approximately 1000 ppm nonepoxide or pigmenting xanthophyll. LPC is an excellent ingredient in feeds for broilers, laying hens (Kuzmicky and Kohler, 1977; Kuzmicky et al., 1977), and swine (Cheeke et al., 1977).

The press cake and the alfalfa solubles are the other fractions from the process. The press cake, which is the major product, may be dehydrated and pelleted for use in cattle feeds. Following their separation from the LPC curd, the alfalfa solubles are a dilute solution of amino acids, sugars, minerals, vitamins, and other water soluble components. Generally, the solubles are remixed with the wet press cake prior to dehydration. Either the dilute solubles (4–7% dry matter), or solubles which have been concentrated to 50–70% dry matter by vacuum evaporation, may be blended with the press cake. Alternatively, the concentrated solubles may be used as an ingredient in liquid animal feed supplements. In either case, large amounts of water must be evaporated from the dilute solubles to obtain a useful end product.

We have recently published data on the yield of LPC obtained from field-chopped alfalfa and ground alfalfa feed material (Edwards et al., 1977a,b). The average yields were 8.53 and 15.2% of the alfalfa feed weight (dry basis) from the chopped feed and ground feed, respectively. While these yields are economically acceptable (Vosloh et al., 1976), the amount of protein extracted was lower than that obtained using more exhaustive extraction techniques (Arkcoll and Festenstein, 1971; Betschart and Kinsella, 1973; Chayen et al. 1961; Crook, 1946; Lexander et al., 1970; Pirie, 1971a; Poppe et al., 1970). These techniques have been used in laboratory experiments to determine the maximum amounts of protein which can be extracted from a given plant species. The procedures generally have involved the addition of large amounts of water, dilute alkali, or other solutions to the feedstock prior to juice expression and may have included multiple extractions of the plant material.

The adoption of such techniques for the commercial production of a feed-grade LPC appears economically impractical. Water streams or dilute alkali solutions added continuously to the feed material become part of the solubles flow stream. Each pound of water added to the feed material must eventually be evaporated in order to recover the solubles fraction in a usable form. In times of rapidly escalating energy prices, it is doubtful that the value of the additional LPC recovered due to the water addition is higher than the added evaporation costs.

The amount of juice produced per unit of wet or dry feed alfalfa is also an important economic consideration because it affects the capital and operating costs for presses, pumps, piping, heating steam, and curd separation equipment.

While these juice flows and the costs of evaporating the added water appear unacceptably high for commercial scale operation, the basic approaches are sound. It is generally understood that protein extraction is more efficient when a diluent is added to the feed material (Pirie, 1971b). Our own work has also shown that the yield of LPC from chopped alfalfa or ground alfalfa is inversely proportional to the dry matter content of the feed alfalfa (Edwards et al., 1977a,b). Accordingly, we have attempted to modify the extraction procedures to obtain the benefits of adding a feed diluent but in such a way that the overall evaporation load is not increased, and the juice flow streams are not excessively high.

The approach examined in this work was the use of the dilute alfalfa solubles as the feed diluent. In the preparation of feed-grade LPC, the dilute solubles stream is as acceptable as tap water for use as a diluent. Under steady-state conditions the solubles added to the feed are an internal recycle stream and only minor amounts of additional water are added to the overall process. The reason for the additional water is that the solubles must be cooled to 40–45 °C prior to their addition to the feed material, to prevent heat denaturation of the protein in the feed alfalfa. Therefore, the solubles must be reheated to 80–85 °C once they have become part of the green juice flow stream; condensate is formed then from the live steam added during the heating step. Basically, the same amount of water must be evaporated to obtain the final solubles fraction whether the recycle system is used or not.

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710.

Table I. Effect of Double Extraction and Solubles Addition on Press Cake Composition and Yields of Juice and LPC from Chopped Alfalfa[a]

	Feed material			Press cake			LPC[b]	
Extraction no.	Solubles added,[c] % press cake feed wt	Dry matter, %	Juice, yield,[c] % press feed wt	Dry matter, %	Protein, %	Fiber, %	Total yield,[d] % original alfalfa feed wt	Yield increase,[e] %
1	0	23.8	45.8	32.7	17.4	32.4	8.74	
2	0	32.7	14.9	38.9	15.9	33.6	10.0	14.4
2	42.1	24.9	45.5	37.9	15.4	34.5	12.5	43.0
2	91.3	20.1	58.8	38.4	14.4	37.1	13.6	55.6

[a] Protein, fiber, and LPC yield on dry basis; other analyses as is; original alfalfa 21.4% protein, 26.2% fiber. [b] LPC normalized to 40% dry matter before drying. [c] As is basis. [d] Dry basis; for one or both extractions, as applicable. [e] Compared to yield from first extraction.

Powling et al. (1954) first described a system in which dilute solubles were recycled to chopped alfalfa between extractions of the plant material to increase the overall yield of extracted protein. No yield data was given. Batley (1973) added dilute alfalfa solubles to chopped alfalfa prior to juice expression by sugar cane rolls during the commercial-scale preparation of LPC (reported by Bray, 1973). Bray (1973) reported an LPC yield of 5% with solubles addition, and Batley (1973) reported yields of 4.2 and 1.3%, with and without solubles addition, respectively. In neither case was the amount of solubles recycled nor the moisture content and quality of the raw material disclosed.

This paper reports the effects of adding controlled amounts of dilute solubles to the fresh alfalfa just prior to pressing on the yield of LPC recovered and on the dewatering behavior of the feed material. Three extraction procedures were used: (1) a single extraction of chopped alfalfa (2) a double extraction of chopped alfalfa, and (3) a single extraction of ground alfalfa. This paper is another in the Pro-Xan series, the name denoting the final LPC product and the procedures used by this laboratory, for the preparation of LPC from alfalfa.

EQUIPMENT AND PROCEDURE

The equipment used and the procedure for determining the experimental yield of LPC have been discussed previously (Edwards et al., 1977a,b). Basically, field-chopped alfalfa or ground alfalfa was fed to a twin-screw press (CE-Bauer, No. 585 Helipress) for dewatering. Alfalfa grinding was done in a 40 in. diameter vertical hammer mill (Owens Mfg. Co., Verdon, Neb., Master Crusher). The yield of juice (100 × mass wet juice/mass wet press feed) and the processing rate were determined by collecting the total juice and press cake exiting from the press for a timed interval. A screened aliquot (1500 g) of juice at pH 8.0–8.5 was heated to 85 °C to precipitate the LPC. The LPC curd was drained in a nylon cloth bag and pressed to approximately 40% dry matter in a hydraulic press. The yield of wet pressed coagulum at exactly 40% dry matter was determined by calculation using wet and dry basis mass balances. The yield of LPC (100 × mass dry LPC/mass dry alfalfa) was then calculated from measurements of the juice yield, the dry matter content of the fresh alfalfa, and the amount of dry LPC curd obtained after normalization to 40% dry matter. By normalizing the wet curd to 40% dry matter, the soluble solids content is limited to 8–10%, and much of the LPC yield variations due to soluble solids content alone are eliminated (Edwards et al., 1975; Kohler et al., 1977).

Dilute solubles containing 5 to 6% dry matter were obtained by diluting vacuum concentrated alfalfa solubles with tap water. Dilute solubles at several known rates were sprayed onto the alfalfa as it excited the feeder by means of a positive displacement pump (Moyno, 1L6) with a variable speed drive. The solubles were added prior to any grinding step. The pH of the whole green juice from the chopped alfalfa feed material was raised to 8–8.5 by adding ammonium hydroxide or anhydrous ammonia to the fresh alfalfa and solubles prior to pressing.

Ground alfalfa was dewatered without pH adjustment and the resulting juice was adjusted to pH 8–8.5 with ammonium hydroxide. Chopped alfalfa was double extracted by first pressing the alfalfa without solubles addition and then repressing the press cake to which controlled amounts of solubles had been added. In all experiments, the control (fresh alfalfa with no solubles added) was run first.

ANALYTICAL METHODS

Wet samples were freeze-dried and ground prior to analytical determinations. Crude fiber and Kjeldahl nitrogen were determined by standard AOAC methods (AOAC, 1970) and reported on a dry basis. Crude protein was calculated as Kjeldahl nitrogen × 6.25. Dry matter content was determined by drying samples for 2 to 3 h at 110 °C in a forced draft oven.

RESULTS AND DISCUSSION

Results from the double extraction of chopped unground alfalfa are shown in Table I. Repressing of the cake from the first extraction without the addition of solubles resulted in an increase in the dry matter content of the final press cake from 32.7 to 38.9% and improved the total yield of LPC by 14%. When solubles were added before reextraction, larger LPC yield increases were obtained. The dry matter content of the final press cake remained constant, with or without the addition of solubles. Press cake protein content decreased and fiber content increased, with increasing application of solubles. When approximately an equal weight of solubles was added to the press cake, and the mixture was reextracted, the total LPC yield increase (55.6% relative) and the press cake dry matter content (38.4%) approximated that found for a single pressing of ground alfalfa of the same composition (Edwards et al., 1977b).

Addition of solubles to chopped alfalfa, prior to a single pressing, increased the LPC yield less dramatically than press cake reextraction, but would be a simpler and less expensive procedure to implement. The results are shown in Table II. The dry matter content of the press cake remained constant, indicating that all the free water applied to the feed was being removed during the operation. For this alfalfa, containing 20% dry matter, the relative yield increase was proportional to the amount of solubles added to the chopped feed. The relative percent yield increase (Y) can be expressed as $Y = 0.280X + 0.475$ where X is the amount of solubles added, expressed as a percentage of the wet alfalfa feed weight. The correlation

Table II. Effect of Solubles Addition on Press Cake Composition and the Yields of Juice and LPC from Chopped Alfalfa[a,b]

	Feed material			Press cake			LPC	
Experiment no.	Solubles added,[c] % alfalfa feed wt	Dry matter, %	Juice, yield,[c] % press feed wt	Dry matter, %	Protein, %	Fiber, %	Yield,[d] % alfalfa feed wt	Yield increase,[e] %
1	0	20.1	49.0	29.5	19.8	31.8	8.93	
	11.1	18.6	57.2	29.1	19.5	32.4	9.14	2.4
	23.2	17.4	63.0	30.8	19.3	32.4	9.75	9.2
	41.0	15.9	67.0	30.7	19.4	32.3	9.97	11.6
2	0	20.1	50.1	32.4	14.9	38.7	8.88	
	14.9	18.3	60.8	31.9	16.0	37.6	9.18	3.4
	34.1	16.6	64.5	33.0	16.0	38.5	9.90	11.5
	59.0	15.0	69.7	34.5	14.8	38.2	10.3	16.0

[a] Protein, fiber, and LPC yield on dry basis; other analyses as is. [b] Original alfalfa no. 1: 21.2% protein, 26.6% fiber; no. 2: 20.9% protein, 28.7% fiber. [c] As is basis. [d] Dry basis; LPC normalized to 40% dry matter before drying. [e] Relative increase compared to yield without solubles addition.

Table III. Effect of Solubles Addition on Press Cake Composition and the Yields of Juice and LPC from Ground Alfalfa[a]

	Feed alfalfa		Feed to press			Press cake			LPC	
Experiment no.	Protein, %	Fiber, %	Solubles added,[b] % alfalfa feed wt	Dry matter, %	Juice, yield,[b] % press feed wt	Dry matter, %	Protein, %	Fiber, %	Yield,[c] % alfalfa feed wt	Yield increase,[d] %
3	23.5	25.5	0	18.6	69.6	36.4	14.6	38.4	18.5	
			20.8	16.5	74.8	36.6	14.9	38.9	18.6	0.5
			40.8	15.1	78.2	37.5	14.4	38.5	20.3	9.7
4	20.7	26.1	0	20.5	62.3	38.8	13.6	38.4	13.8	
			25.7	17.6	71.5	40.0	13.9	36.5	15.1	9.4
			42.6	16.3	74.8	39.5	12.5	39.2	16.0	15.9
			57.2	15.4	77.6	39.7	12.8	38.2	16.5	19.6
5	20.5	25.6	0	21.4	64.6	38.8	12.1	36.8	15.8	
			29.4	18.0	72.7	39.4	12.8	35.3	18.3	15.8
			47.0	16.6	76.0	38.4	11.9	38.4	18.8	19.0
			66.4	15.4	79.8	40.4	11.5	37.8	19.5	23.4
6	17.7	30.6	0	18.5	67.2	35.7	12.3	43.0	12.1	
			57.1	13.8	79.4	38.1	10.9	44.9	14.9	23.1
7	25.0	19.3	0	19.3	59.9	28.6	17.6	27.4	20.1	
			15.5	17.4	65.6	31.0	16.7	27.7	21.4	6.5

[a] Protein, fiber, and LPC are dry basis; other analyses as is. [b] As is basis. [c] Dry basis; LPC normalized to 40% dry matter before drying. [d] Increase compared to yield without solubles addition.

coefficient, r, was 0.958. Thus, the addition of solubles equal to 50% of the wet alfalfa feed weight would result in a yield increase of 14.5%. This addition of solubles would approximately double the juice flow stream (mass wet juice/mass dry alfalfa) compared to the control treatment.

The results of the present work are difficult to compare with those of Batley (1973) since no experimental conditions were given. He reported that the addition of solubles increased the LPC yield by 218%, from 1.3 to 4.2%. This yield increase is 10 to 15 times greater than those found in the present study. However, the yield of 1.3% obtained by Batley (1973) from chopped alfalfa using sugar cane rolls compares with average yields of 8.9% obtained in the present work and 8.5% obtained in previous work with the same press (Edwards et al., 1977a). This difference in yield reflects both the inefficiency of the former operation and improvements in LPC technology in the intervening years. The inefficiency of the Batley pressing operation must have been largely responsible for the 218% increase in yield obtained by the solubles addition.

The results of those experiments in which the solubles were added to ground alfalfa are shown in Table III. As more solubles were added, the LPC yield increased. The LPC yield increases, for a given amount of added solubles, were larger with drier alfalfa feed material. In general, the dry matter content of the press cake remained constant but the protein decreased and fiber increased, as more solubles were added to the feed. Once again, at the maximum levels of solubles addition, the juice flow streams were approximately twice that of the control treatment.

Previous work with both chopped and ground feed material showed the LPC yield to be linearly related to the dry matter content of the feed material and other variables (Edwards et al., 1977a,b). At constant alfalfa protein content, fiber content, and temperature, a 1% (absolute) decrease in the dry matter content of the feed alfalfa resulted in LPC yield increases of 0.197 and 0.151% (absolute) for chopped (unground) and ground feed, respectively. The relationships were valid over the range of 15.2 to 24.2% dry matter for the chopped alfalfa, and 16.9 to 27.0% for the ground material. A similar relationship exists when solubles are added to the feed material to reduce its dry matter content. A graph of the increase in LPC yield (%, absolute) vs. the decrease in feed dry matter content (%, absolute) due to the addition of solubles is shown in Figure 1. The correlation coefficient and the 95% confidence interval for the intercept were 0.974, −0.57 to +0.14 and 0.914, −1.66 to +0.44 for the chopped feed and ground feed, respectively. The intercepts are not significantly different from zero since, in either case, zero is included within the 95% confidence intervals. If the equations are recalculated so that they pass through the origin, they may represent a better approximation of the effect of the solubles addition. The recalculated equations are $Y = 0.27X$ and $Y = 0.57X$ for the chopped feed and ground feed, respectively.

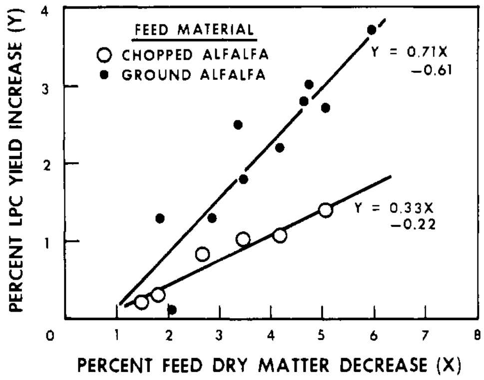

Figure 1. The effect of decreasing the feed dry matter content (percent, absolute) by the addition of dilute solubles on the yield of LPC (percent, absolute) from alfalfa.

The increase in LPC yield was greater when the change in feed dry matter content was due to solubles addition than when the change occurred in the natural feed material from the field. This may be due to the fact that feed adjusted to a given moisture content by the addition of dilute solubles contains more free water than the natural feed material at the same moisture. This extra free water is able to flush out more chloroplasts and soluble protein from cells broken before or during the pressing operation. The response of solubles addition to the ground alfalfa was greater than that of the chopped alfalfa, presumably because of the higher initial concentration of soluble protein and chloroplast fragments in the feed to the press as a result of the cell rupture obtained during the grinding operation.

The maximum LPC yields obtained by the addition of solubles to ground feed material ranged from 14.9 to 21.4% of the alfalfa feed weight (dry basis). These yields are higher than those obtained from alfalfa in our previous pilot-scale work (Edwards et al., 1977a,b; Kohler and Bickoff, 1971), or other pilot-scale experiments with alfalfa. Koegel et al. (1974), McDonald et al. (1954), and Raymond and Tilley (1957) have reported LPC yields of 8.4, 9.2, and 13.2%, respectively.

Addition of recycled alfalfa solubles to ground alfalfa feed material would appear to be highly desirable for the commercial production of LPC. An additional 10–20% increase in LPC yield may have a significant positive effect on the economics of the process. It is also noteworthy that the yield increases from the addition of solubles are greater for feed alfalfa with higher dry matter contents, and that "free water" addition produces a greater effect than when the water is present in the plant cells. Finally, the large increases in LPC yield obtained by the addition of solubles between extractions of chopped alfalfa suggests that further experimentation should be done using the same system with ground alfalfa feed material.

ACKNOWLEDGMENT

The authors wish to thank K. V. Smith and associates for help in operating the equipment, L. V. Richards and associates for the sample analyses, and R. E. Miller and B. E. Knuckles for helpful discussions. The authors also express their appreciation to the Processing Division, Newhall Land and Farming Company, for supplying the fresh alfalfa.

LITERATURE CITED

Association of Analytical Chemists, "Official Methods of Analysis", 11th ed, Washington, D.C., 1970.
Arkcoll, D. B., Festenstein, G. N., *J. Sci. Food Agric.* **20**, 49 (1971).
Batley, W. R., Jr., U.S. Patent 3 775 133 (Nov 27, 1973).
Betschart, A. A., Kinsella, J. E., *J. Agric. Food Chem.* **21**, 60 (1973).
Bray, W. J., *Chem Eng.* **80**, 76 (1973).
Chayen, I. H., Smith, R. H., Tristram, C. R., Thirkell, G. R., Webb, T., *J. Sci. Food Agric.* **12**, 502 (1961).
Cheeke, P. R., Kinzell, J. H., de Fremery, D., Kohler, G. O., *J. Anim. Sci.* **44**, 772 (1977).
Crook, E. M., *Biochem. J.* **40**, 197 (1946).
Edwards, R. H., de Fremery, D., Kohler, G. O., Proceedings of the 12th Technical Alfalfa Conference, Overland Park, Kans., 1974, 1975, p 67.
Edwards, R. H., de Fremery, D., Mackey, B. E., Kohler, G. O., *Trans. ASAE* **20**, 423 (1977a).
Edwards, R. H., de Fremery, D., Mackey, B. E., Kohler, G. O., *Trans. ASAE*, in press (1977b).
Koegel, R. G., Barrington, G. P., Bruhn, H. D., Proceedings of the 4th Annual Alfalfa Symposium, Madison, Wis., 1974, 1974, p 13.
Kohler, G. O., Bickoff, E. M., in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", IBP Handbook No. 20, Pirie, N. W., Ed., Blackwell Scientific, Oxford, 1971, Chapter 7.
Kohler, G. O., Edwards, R. H., Knuckles, B. E., de Fremery, D., Miller, R. E., Currence, H. D., American Society of Agricultural Engineers, Raleigh, N.C., June 26–29, 1977, Paper No. 77-1058.
Kuzmicky, D. D., Kohler, G. O., *Poult. Sci.* **56**, 1510 (1977).
Kuzmicky, D. D., Livingston, A. L., Knowles, R. E., Kohler, G. O., Guenthner, E., Olson, O. E., Carlson, C. W., *Poult. Sci.* **56**, 1504 (1977).
Lexander, K., Carlsson, R., Schaler, V., Simonsson, A., Lundborg, T., *Ann. Appl. Biol.* **66**, 193 (1970).
McDonald, A. N. C., Hamblin, H. J., Cashmore, W. H., Report No. 43, National Institute of Agricultural Engineers, Bedfordshire, England, 1954.
Pirie, N. W., in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", IBP Handbook No. 20, Pirie, N. W., Ed., Blackwell Scientific, Oxford, 1971a, Chapter 3.
Pirie, N. W., in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", IBP Handbook No. 20, Pirie, N. W., Ed., Blackwell Scientific, Oxford, 1971b, Chapter 5.
Poppe, J., Tobback, P. P., Maes, E., *Lebensm.-Wiss. Technol.* **3**, 79 (1970).
Powling, W. T., Powling, W. T. L., Davis, P. S., British Patent 705 369 (March 10, 1954).
Raymond, W. F., Tilley, J. M. A., *Colon. Plant Anim. Prod.* **6**, 3 (1957).
Vosloh, C. J., Jr., Edwards, R. H., Enochian, R. V., Kuzmicky, D. D., Kohler, G. O., Agricultural Economic Report No. 346, Economic Research Service, U.S. Department of Agriculture, 1976.

Received for review August 25, 1977. Accepted December 8, 1977. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable. Presented in part at the 81st National Meeting, American Institute of Chemical Engineers, Kansas City, Mo., April 11–14, 1976 and the 1977 Summer Meeting, American Society of Agriculture Engineers, Raleigh, N.C., June 26–29, 1977.