ALFALFA FRACTIONATION

Effects of Various Procedures in Laboratory Processing of Fresh Alfalfa on Separation of Nitrogen and Solids from Fiber

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Experiments on the laboratory processing of fresh alfalfa to separate solids and nitrogen from fiber have been made. A small scale method, involving the use of added water, removed as much as 66% of the nitrogen and 45% of the solids from the fiber with a single extraction, when done under favorable conditions. Of the nitrogen removed about two thirds was heat-precipitable. Re-extraction of the fiber residue with additional water removed extra nitrogen and solids, the extent of removal apparently being dependent upon the proportion of water not removed from the fiber in the first extraction. In the use of a large scale method with added water and different equipment, the amount of nitrogen and solids separated from the fiber was much less than with the small scale method. The differences observed with the two processing methods were attributed to factors concerned with the extent of grinding, the temperature of grinding, the amount of water used in processing, and the manner of separation of the juice from residue. Other data are presented on the chemical compositions of some of the fractions prepared.

PROCESSING FRESH ALFALFA to separate nonfibrous from fibrous matter has been studied in a number of laboratories (*1*, *4*, *6*, *8*). These studies and some on other herbage (*4*, *6*) have shown that a material of high nitrogen and low fiber content can be separated by processing methods involving grinding or macerating and then squeezing or pressing the broken tissue to extrude the juice. Heat treatment of the juice has been reported to yield an insoluble product, high in nitrogen, which with some leaf crops accounts for as much as 50 to 60% of the protein (*7*). This report concerns the yield of solids and nitrogen from freshly harvested alfalfa by extractions with added water, and factors which may affect this yield.

Experimental

Most of the studies reported here were made using a small-scale process. The routine procedure used was as follows: Freshly harvested alfalfa in the early bloom stage was knifed into short lengths. Fifty grams of the chopped material and 115 ml. of distilled water, giving a water to solids ratio of about 10 to 1, were placed in the cup of a blender (Ivan Sorvall Omni-mixer) and ground for 5 minutes. During grinding, the cup was immersed in a cold water bath so that the temperature of the macerate after grinding was 15° to 18° C. The macerate was filtered by gravity through an 82-mesh silk bolting cloth and as much liquid as possible finally removed by hand squeezing operations. This process gave a liquid fraction referred to as the extract and a solid fraction referred to as the residue.

The extract was studied by measurements which included total volume and the water, solids, and nitrogen contents of 10-ml. aliquots. Water and solids were determined by drying at 100° C. for 16 hours, and nitrogen was determined by the Kjeldahl method (*2*). Dry matter (solids) and nitrogen were also determined on fresh alfalfa, and recovery of solids and nitrogen in the extract was calculated and expressed in terms of per cent of the total present in the 50 grams of alfalfa.

The measurements permitted the expression of the data as water recovered in the extract, as solids and nitrogen recovered in the extract, and as theoretically extractable solids and nitrogen. These values were the percentages of the total ingredient present in the alfalfa or, in the case of water, of the amount in the alfalfa plus the amount added at the grinding. The water-recovered values permitted a calculation of the theoretically extractable solids and nitrogen. This calculation was based upon the assumption that both the recovered and unrecovered water contained equivalent levels of extractable solids or nitrogen.

The routine procedure was varied in several studies to determine the effect of various factors on yield of extractables. To determine the effects of the ratio of water to solids in the grinding process, the procedure was altered only by the addition of water in various amounts to the alfalfa. The ratio of water to solids was calculated from the weight of water added and the weights of water and solids present in the alfalfa as determined by analysis.

To determine the effect of temperature of the grinding mixture on yields, the routine procedure was altered by keeping the cup of the blender immersed in a water bath maintained at about the temperature desired. The actual grinding temperature was considered to be that determined for the macerate after grinding.

The routine procedure was also compared with one where: (A) the sample was preground in a hand-operated meat grinder; (B) the macerated sample was filtered through the silk bolting cloth using a Büchner funnel covered with dental dam and suction to replace the hand squeezing operation; and (C) the residue was re-extracted twice with 115 ml. of water, repeating the grinding and squeezing process each time.

One study of a different process on a larger scale was also made. Here, 80 pounds of freshly cut alfalfa in an early bloom stage, to which 60 pounds of flaked ice were added, were ground with a power-driven meat grinder (Enterprise, 2-ton-per-hour capacity) having $^3/_8$-inch openings in the extrusion plate. As much liquid as possible was separated from the ground mass by means of a screw-type lard press. The liquid obtained by this means was measured and further fractionated by heating to 95° C. by steam intrusion. This yielded a heat-precipitable matter. After the

Table I. Effect of Water to Solids Ratio and of Temperature during Grinding on Recovery of Solids and Nitrogen in Extracts

Variable in Grinding	Water Recovered in Extract, %[a]	Recovery in Extract, %[b]		Theoretically Extractable, %[c] (Calculated)	
		Solids	Nitrogen	Solids	Nitrogen
Water-Solids Ratio[d]					
7.2	77.7 ± 0.3	45.9 ± 0.9	67.1 ± 1.0	59.1	86.3
8.6	83.4 ± 0.6	48.1 ± 0.8	70.1 ± 0.7	57.7	84.0
11.6	86.9 ± 0.4	48.9 ± 0.9	70.6 ± 1.5	56.3	81.2
17.4	90.8 ± 0.5	50.9 ± 0.8	74.2 ± 1.0	56.1	81.7
Temperature, ° C.					
15	84.6	46.9	70.9	55.5	83.8
18	84.1	45.1	66.0	53.6	78.5
30	83.1	41.5	56.3	50.0	67.7
38	83.7	41.9	55.9	50.1	66.8
48	84.5	40.3	51.0	47.7	60.3
58	82.6	35.7	38.5	43.2	46.6
68	81.2	35.1	33.6	43.2	41.4
79	75.2	32.1	26.3	42.8	34.9
90	76.8	33.4	24.4	43.5	31.8

[a] $\frac{\text{(Wt. water in extract)}}{\text{(Wt. water in alfalfa) + (wt. water added)}} \times 100$

[b] $\frac{\text{(Wt. solids or N in extract)}}{\text{Wt. solids or N in alfalfa}} \times 100$

[c] $\frac{\text{(\% Solids or N recovered in extract)}}{\text{(\% Water recovered in extract)}} \times 100$

[d] Data of water-solids experiment are mean values of duplicate samples with the same batch of alfalfa. The percentage deviation of the duplicate values from the means is given.

Table II. Effects of Other Variations in Procedure on Recovery of Solids and Nitrogen in Extracts

Procedure	Water Recovered in Extract, %	Recovery in Extract, %		Theoretically Extractable, % (Calculated)	
		Solids	Nitrogen	Solids	Nitrogen
(A) Effect of Method of Preparation for Grinding					
Routine procedure[a]	83.7	47.1	66.7	56.3	79.7
Preground with meat grinder[b]	83.6	44.7	61.7	53.4	73.8
(B) Effect of Method of Extruding Juice from Macerate					
Routine procedure	83.6	52.1	72.5	62.2	86.7
Pressure filtration[b]	79.1	45.4	59.6	57.3	75.3
(C) Effect of Re-extracting Residue					
Routine procedure[a]	83.7	47.1	66.7	56.3	79.7
Re-extracted twice	99.7	57.5	79.4	...	...

[a] Data are for the same run.
[b] Pregrinding and pressure filtration treatments made under room temperature conditions.

heated extract had been kept for 24 hours in the cold, the precipitate had settled, and the supernatant liquid was removed slowly by siphoning. The remainder of the liquid was removed by vacuum filtration through filter paper. The precipitate obtained was dried at 70° C. in a forced-draft oven, weighed, and finely ground.

Analyses were made for solids and nitrogen on the fresh alfalfa, on aliquots of the unheated extract, and on the dried precipitate. In addition, a sample of the fresh alfalfa was processed by the small-scale method previously described to obtain data for purposes of comparison. Representative samples of the alfalfa, the small-scale process extract and residue fractions, and the heat-precipitated fractions from the large-scale process were analyzed by the AOAC methods for proximate analysis, calcium, phosphorus, and carotene (*2*). The method of Norman and Jenkins as modified by Common (*9*) was used for lignin and that of Crampton and Maynard (*5*) for cellulose.

Results

The data in Table I illustrate that varying the water to solids ratio over a rather wide range had only a small effect on solids and nitrogen recovery in the extract. Both increased somewhat as the water was increased. Since the fibrous residue should retain about the same amount of water, regardless of the amount added to the mixture, the data for water recovered in the extract are what might be expected, and to some extent they explain the results for recovery of solids and nitrogen in the extract. There is no apparent explanation, however, for the decrease in values for theoretically extractable solids and nitrogen with increasing water to solids ratio.

The temperature of grinding appears, from the data in Table I, to have had a great effect on recovery of solids and especially of nitrogen in the extract. This effect was that the recoveries of these constituents were lowered when the temperature of grinding was increased. The extract volumes—i.e., water recovery—were also lowered when the temperature of grinding was high. This response may be attributed to the tendency of the denatured proteins to increase the bulk of the residue and thereby cause a lowered recovery of water. The data on the effect of grinding temperature were taken from a single experiment, but they are representative of the results obtained in several trials.

A further study of the temperature effect is illustrated in Figure 1. The results in this figure were obtained in the same experiment as that reported in Table I. In addition to measuring the solids and nitrogen theoretically extractable, the amount of each that could be removed from the liquid phase by centrifugation at 8000 × G for 30 minutes was determined. The amount of extractable solids that were soluble (not removed by centrifugation) remained quite constant over the entire temperature range, but the extractable insoluble solids were highest at 15° C. and decreased up to temperatures of about 50° C. Results of a similar nature were obtained for nitrogen except that the extractable, soluble nitrogen decreased at the higher temperatures. At the 90° C. blending temperature, the nitrogen in soluble form represented 21.5% of the total present in the alfalfa. Since the extractable portion may con-

tain as much as 23% of the alfalfa nitrogen as a nonprotein form (as measured by trichloroacetic acid removal of the protein from a different alfalfa extract), it appears that processing at high temperatures serves to remove soluble nitrogen largely in nonprotein form. The data of the figure indicate that deviations occurred in the extraction patterns with the sample ground at 58° C.; however, these variations are attributed to experimental error.

In Table II, the data show that pregrinding does not increase the efficiency of removal of the extractables. In fact, it decreases the efficiency to some extent, possibly because of the temperature effect already discussed. Pressure filtration also decreased the efficiency of extraction, and here the decrease may have been the result of a lesser removal of particulate matter caused by a greater retention of the particles by the residue with this type of treatment.

Re-extraction (Table II) removed essentially the same amount of solids and nitrogen that the data for the routine procedure indicated were theoretically extractable. Further observations, for which data are not shown, were made. Considerable variation in the speed at which the blender was run had no effect on efficiency of extraction as long as visible plant structure was broken up. Furthermore, silk bolting cloth with meshes ranging between 82 and 150 were all equally suitable for use.

Using the large-scale process, the yield of extractable solids was considerably less than it was for the small-scale process (Table III). In part, this decreased yield probably was the result of the removal of a smaller percentage of the liquid from the ground mixture. This might, to some degree, be improved by using more ice in the grinding, giving a higher water to solids ratio. Since, however, the values for theoretically extractable solids and nitrogen were lower for the large-scale process, the efficiency of grinding is no doubt also involved. Finally, the temperature effect discussed previously may be important, since it is doubtful that the added ice can maintain a low temperature throughout the mass during grinding.

The proportion of heat-precipitable material in the liquid phase was quite constant (34 to 36% of the solids and 61 to 66% of the nitrogen in the liquid portion), tending to be only slightly lower in the material from the large-scale process.

The data of Table IV illustrate differences in chemical composition of the dry matter in several preparations as compared to that of the alfalfa used in preparing each. Yield data for these preparations were presented in Table III. Of the constituents measured, ether extract and carotene are the ones for which quantitative recovery was low. For ether extract, only about half of that present in the fresh alfalfa can be accounted for in the extract and residue, while in the case of carotene only about one fourth can be accounted for. Although the low recoveries of these ingredients from the fractions could have been caused by an unfavorable drying condition, both the fractions and the fresh alfalfa were dried at a similar oven temperature—i.e., 70° C.—in preparation for analysis.

The data in Table IV indicate that the extract yields a dried solid high in nitrogenous constituents, fairly high in minerals and nitrogen-free extract, and low in fiber. The residue, high in fiber, has a composition indicative of a reasonably good roughage feed. The precipitate formed on heating was especially high in protein, having a good concentration of carotene and very little fiber.

Table III. Comparison of Small and Large Scale Processing Methods

Processing Method Used	Water-Solids Ratio in Grinding Mixture	Water Recovered in Extract, %	Recovery in Extract, %			Theoretically Extractable, % (Calculated)	
				Solids	Nitrogen	Solids	Nitrogen
Small scale	10.4:1	81	Total	35	65	43	81
			Heat-precipitable	12	43	15	53
Large scale	5.8:1	63	Total	22	39	35	63
			Heat-precipitable	8	24	12	38

Table IV. Comparison of Chemical Composition of Alfalfa Dry Matter with that of Dry Matter of Some Fractions (Dry Basis)

Material Analyzed[a]	Ether Extract, %	Crude Fiber, %	(N × 6.25), %	Ash, %	NFE, %	Calcium, %	Phosphorus, %	Lignin, %	Cellulose, %	Carotene, μg./Gram
Fresh alfalfa	2.6	32.2	17.2	8.1	39.9	1.7	0.2	8.4	22.7	89
Extract from small scale process	1.4	0.5	32.7	14.9	50.5	2.8	0.5	...[b]	...[b]	62
Residue from small scale process	1.3	45.2	10.4	5.0	38.1	1.3	0.1	11.3	34.0	3
Heat-precipitable matter in extract from large scale process	3.3	1.0	54.6	8.4	32.7	2.0	0.6	...[b]	...[b]	240

[a] Materials were dried from 24 to 48 hours in a forced draft oven at 70° C. and then ground in a Wiley mill prior to the analyses.
[b] Not determined.

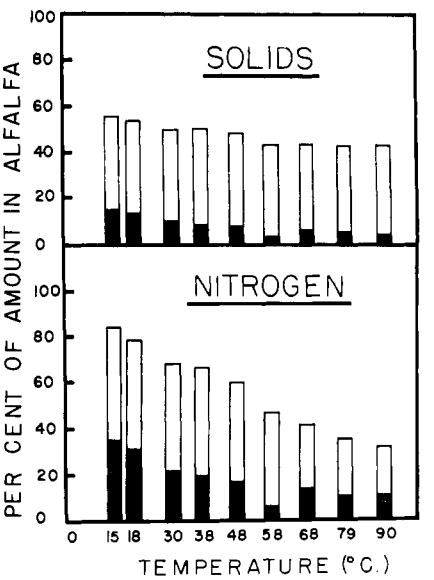

Figure 1. Effect of temperature of grinding on extractability of solids and nitrogen from alfalfa

□ Soluble extracted matter
■ Insoluble extracted matter

Discussion

Early researchers had demonstrated that 80 to 90% of the nitrogen of leaves could be removed by repeated water extraction (*3*). The studies reported here indicate that about 70% of the nitrogen is removed in small-scale processing, using a single extraction, where water is added during the grinding process and the temperature is kept at about 15° to 18° C. during grinding. This is a somewhat better yield than that reported by Pirie (*7*), who employed a single extraction method without the addition of water.

The addition of water to fresh alfalfa at an approximate rate of 2 to 1 appears to be about as effective as the use of larger amounts, either as concerns the

extraction of solids or the extraction of nitrogen. A grinding process which gives a high degree of rupture of cells appears to be important in obtaining good yields. Maintaining a low temperature is especially important in obtaining efficient extraction of the nitrogenous constituents.

Grinding methods which do not give a high degree of rupture of cells while maintaining a low temperature, such as the large-scale laboratory process studied here, apparently cannot be expected to give a good yield of extractable material. The studies reported here on methods of expressing the juice from the ground mass are too limited to allow for concise conclusions, but it appears that the small samples and the manner of the squeezing were both compatible to good yields.

The processing of freshly harvested alfalfa for a good yield of material, high in protein, would appear possible through the use of grinding, pressing, and heat treatment similar to what is described here. A residue having some apparent value as a roughage feed is also obtained. However, a third fraction in liquid form, which contains much of the nutritive value of the alfalfa, results from the processing. While the heat-precipitated material might be easily dried and the residue might be stored and fed as silage, a concentration of the liquid fraction would be costly and difficult. Nevertheless, when processing grasses for their protein is considered, the methods discussed here appear more suitable than alkali extraction methods or methods not involving the addition of water. Feeding studies on the various fractions are essential, however, to a more thorough evaluation of the methods.

Because the data obtained from extracting the alfalfa could be closely duplicated with similar or different samples, little attention was given to factors such as moisture content of the alfalfa, during the work. Samples which contained between 63 and 72% of moisture showed no relationship between moisture content and extractability of solids or nitrogen when the collections were made between late August and the middle of October. Further, no changes attributable to advancement of the season were evident during the same period. Data recorded with a sample collected in June (Table III) showed some divergence from those obtained with samples collected in September (Table I) in that a lower percentage of extractable solids was obtained with the sample collected at the earlier time. No differences of nitrogen extractability were apparent between the samples collected in June and September, however. Thus, it seems evident that variables such as the season of harvest and the stage of maturity should receive additional attention in alfalfa extraction work.

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ALFALFA CONCENTRATES IN NUTRITION

Protein Quality of an Alfalfa Concentrate

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THE USE OF FRESH HERBAGE as a raw material for the preparation of protein concentrates has received attention in recent years (*12*). Some investigators have processed plants such as alfalfa and Italian ryegrass to obtain the juice, a liquid suspension containing soluble protein, nonprotein matter and chloroplasts. The juice upon treatment with steam yielded a heat-precipitable material of high nitrogen and low fiber content. This material was suitable for use in high energy diets in either wet or dry form (*3, 9, 13*).

Feeding studies with the heat-precipitable material have indicated that it is a fair to moderately good source of protein in practical diets. Work with chicks showed that diets containing the material in dry form supported rather good growth although the rate of growth was somewhat inferior to that obtained with a diet supplemented with casein (*3*). The diets containing the alfalfa preparations were improved by cholesterol supplementation and those containing either alfalfa or other herbage preparations were improved by lysine supplementation. Experiments using a wet preparation from alfalfa for chicks suggested that the material was equal to or slightly inferior to fish meal (*9*). In work using a dried concentrate from alfalfa for laying hens, egg production was similar for diets that contained either an alfalfa concentrate or fish meal (*9*).

The work reported here involved rat feeding experiments with an alfalfa concentrate, an alfalfa meal, and some supplements as dietary nitrogen sources. Cholesterol, a compound known to counteract largely the growth inhibitory property of alfalfa meal (*11*), was also used as a supplement.

Experimental

The alfalfa concentrate was prepared by processing several varieties of fresh alfalfa at the prebloom and early bloom stages. Following the preparation of the dry concentrate by a large scale process as described in a previous report (*6*), the material was stored in glass bottles at room temperature prior to feeding, the storage intervals being about 2 months for Experiment 1 and 18 months for Experiments 2 and 3. The material, ground through a 1-mm. sieve with the Wiley mill, was analyzed (*1*) and found to contain about 50% protein (N × 6.25) and 1% crude fiber.

The protein sources used in the diets had the following nitrogen contents as determined by the Kjeldahl method (*1*):

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