Leaf Protein Concentrate Prepared by Spray-Drying

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Leaf protein concentrates were prepared by spray-drying the juice expressed from fresh alfalfa and pea vines. The concentrate was a green powder of low fiber content which could be fed to nonruminant animals. A product which might be further processed for human consumption was prepared by extraction of the spray-dried preparation with ethanol. Proximate analyses, amino acid analyses, estimation of the biological value of the protein, and analyses for some vitamins or growth factors were made and compared with other high protein feedstuffs. There appeared to be little or no loss of nutrients or nutritive value in the spray-drying process. On the basis of amino acid analyses and the pepsin pancreatin digest indices, the protein could be potentially equal or superior to high protein feedstuffs now available. The vitamin content of the spray-dried preparation was higher than that found in commercial dehydrated alfalfa products.

Methods for the preparation of leaf protein concentrate (LPC) have been studied for more than 20 years by Pirie and associates (*11*, *30*, *33*, *34*) at the Rothamsted Experiment Station and later by Chayen *et al.* (*12*) of the British Glues and Chemicals Ltd. Their object was to develop methods for the extraction of leaf proteins from green plants and to prepare a protein food which would be consumed by nonruminant animals including man. Leaves and other green plant material could be used as a protein source for humans and nonruminants, if the protein could be separated from the fiber. The nonruminants are efficient protein converters (*25*).

Pirie and associates (*15*, *30*) and Chayen *et al.* (*12*) described methods for large scale production of protein from leaf extracts. The process devised by Pirie involved pulping the green plants in a modified hammer mill and expressing the juice in a press. The protein concentrate was separated from the juice by heating. When the precipitate was pressed, a cake was formed which had the consistency and keeping properties of cheese. The procedure described by Chayen *et al.* (*12*) was similar except that the plant material was pulped with a large volume of liquid (liquid to solid ratio ranged from 5–15:1) and the protein was precipitated by acid. Byers (*8*) and Byers and Sturrock (*9*) have reported that leaf protein can be extracted from a large number of species.

Amino acid analyses (*22*, *41*) have shown that leaf protein concentrates have an adequate amount of all essential amino acids to serve as a high quality protein with methionine as the limiting amino acid. Early feeding trials suggested a low protein nutritive value (*10*, *11*, *13*, *14*, *20*). This may have been the result of improper processing. More recent studies (*6*, *18*, *19*) have indicated that leaf protein concentrate when properly processed has a nutritive value higher than soybean meal and equal to white fish meal when fed to rats, chicks, or pigs.

Waterlow (*40*) reported that infants recovering from protein malnutrition tolerated leaf protein concentrates added to a formula in which milk protein provided 50 to 75% of the protein. The nitrogen retention from such formulas in short-term balance trials was almost as good as from whole milk formulas. The results of recent feeding trials with humans and animals were summarized in an excellent review article (*31*).

Akeson and Stahmann (*2*) estimated the biological values of 14 samples of leaf protein concentrate prepared from eight different species by the method of Morrision and Pirie (*30*) and four samples from three different species prepared by the procedure of Chayen *et al.* (*12*) using the pepsin pancreatin digest index (*3*). The estimated biological values of leaf proteins were in general lower than values for egg and egg white but higher than beef, casein, soybean, yeast, wheat flour, gluten, zein, and gelatin. The values for leaf proteins were about equal to those of milk and lactalbumin.

The procedures which have been described for preparation of leaf protein concentrates (*12*, *15*, *30*) have two disadvantages. First, water-soluble nutrients such as sugars and amino acids are lost. Second, many problems were encountered with drying the moist leaf protein concentrate cake. The nutritive value fell seriously when dried at high temperatures. According to Morrison and Pirie (*30*), this loss of nutritive value was avoided by drying in a current of air at low temperature; however, the protein became hard and gritty when treated in this manner. Freeze-drying gave an excellent product but was not practical on a large scale (*30*). Other procedures for drying which involved acetone extraction (*30*) gave a satisfactory product with less loss of protein quality; however, the solvent extraction removed the lipids, and therefore, the feeding value was decreased from the energy standpoint.

The purpose of this investigation was to test the spray-drying of leaf protein concentrates and to compare the

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spray-dried products in composition and nutritive value with the more common high protein feedstuffs. Spray-drying the freshly extracted plant juice gave a green, water-soluble concentrate with no loss of nutrients or nutrient value. Tan leaf protein powders suitable for further processing for human consumption were obtained by extraction of the chlorophyll from the concentrates with 95% ethanol.

Methods and Materials

Plant Material. Alfalfa and pea vines were chosen for this study. Alfalfa produces the largest yields of protein per acre of the common crops grown in the United States (*1*), and may be one of the best plants to obtain high yields of leaf protein. Pea vines are a waste product and may be an inexpensive source of plant material for leaf protein concentrate production. First and second cuttings of alfalfa were obtained from the University of Wisconsin Experiment Station at Madison, Wis. Fresh pea vines were obtained from a commercial canning company near Madison.

Extraction. The juice was extracted from the plant material immediately after cutting with a machine, similar to those used at the Rothamsted Experiment Station (*15*). The plant material was pulped by a modified hammer mill and spread onto a continuous 12-inch wide belt. The juice was expressed from the pulped plant material by pressure exerted between the continuous belt and a perforated pulley. It was necessary to add a small amount of water during the extraction of the alfalfa to obtain a good extraction. The pea vines contained sufficient moisture to give a good extraction.

Spray-Drying. The expressed juice was spray-dried immediately after extraction and within 2 hours after cutting. There should be as short a time interval between cutting and processing as possible to avoid loss of nutrient value. The juice was spray-dried with a pilot Niro spray dryer which was built in Copenhagen. The juice was fed through a spinning disk atomizer at the rate of 2 liters per hour with an inlet air temperature of 170° to 200° C. and an outlet air temperature of 70° to 80° C. The dry material was collected in a glass jar at the bottom of a cyclone.

Little difficulty was encountered in spray-drying the plant juices. All of the spray-dried products were stored at −20° C. in 2 quart jars under an atmosphere of nitrogen until analyzed.

Removal of Chlorophyll. Chlorophyll was removed from the spray-dried plant juice by extraction with 95% ethanol at room temperature. One hundred grams of spray-dried plant juice was suspended in 400 ml. of 95% ethanol, stirred with a magnetic stirrer for 5 minutes, and then filtered through Whatman No. 40 filter paper. After washing with 50 ml. of 95% ethanol, the precipitate was resuspended in 400 ml. of ethanol, stirred, filtered, and washed as described above. Subsequent washing removed no further chlorophyll. The precipitate was dried under vacuum for 2 hours at 40° C. to remove the ethanol. Heating the ethanol filtrate to boiling precipitated no more protein. A light tan powder was produced which had a bland taste.

Analysis of Samples. Analysis of the samples consisted of a proximate analysis of the spray-dried juice, the spray-dried juice extracted with 95% ethanol, the residue remaining after expressing the juice, and the plant before expressing the juice. Complete amino acid determination and estimation of the biological value were made on the sprayed juice before and after extraction with ethanol. Vitamin analyses were made on the spray-dried juice from the second cutting of alfalfa.

Protein (N × 6.25), fat, and crude fiber analyses were made by the General Laboratory Division, Feed and Fertilizer Section, Wisconsin State Department of Agriculture. Moisture and ash were determined by standard AOAC methods (*5*).

Amino acid analysis was carried out on acid-hydrolyzed samples. Fifty milligrams of each sample was hydrolyzed with 25 ml. of 6*N* HCl under nitrogen atmosphere in a sealed 25 × 200 mm. borosilicate glass test tube for 22 hours at 110° C. The hydrolyzate was quantitatively filtered through acid-washed Whatman No. 42 paper and evaporated to dryness three times. The residue was dissolved in pH 2.2 citrate buffer and made to a volume of 25 ml. A 1-ml. aliquot of this solution was analyzed by a method slightly modified from that of Moore, Spackman, and Stein (*27, 28, 39*) on a Beckman-Spinco Model 120 amino acid analyzer. Acid and neutral amino acids were separated on a 60 × 0.9 cm. column of resin equivalent to Spinco 50A resin with 0.2*N*, pH 3.28 buffer at 50° C., which was changed to 0.2*N*, pH 4.25 buffer after 3 hours. Basic amino acids were separated on a 10 × 0.9 cm. column of resin equivalent to Spinco 15A resin with 0.35*N*, pH 5.28 buffer at 50° C.

Acid hydrolysis destroyed tryptophan and cystine. Tryptophan was determined in a basic hydrolyzate by a method similar to that of Dreze (*17*). Fifty-milligram samples were hydrolyzed at 110° C. for 20 hours in 2 ml. of 5*N* sodium hydroxide under a nitrogen atmosphere in a sealed 18 × 150 mm. tube. After hydrolysis, the samples were neutralized with 6*N* HCl, and the precipitate was removed by centrifugation and washed three times with pH 2.2 citrate buffer. The supernatant and washings were combined and made to 25 ml. with pH 2.2 citrate buffer. A 2-ml. aliquot was separated on a 10 × 0.9 cm. column of resin equivalent to Spinco 15A resin with 0.38*N*, pH 4.26 citrate buffer at 30° C. Under these conditions, the tryptophan peak was eluted from the column in 4 hours and was completely separated from other amino acids. A regular 50-cm. column run required 12 hours for elution of tryptophan. Tryptophan was not determined with the 10-cm. column using the 0.35*N*, pH 5.28 buffer, since the tryptophan peak was eluted at the same time as ornithine which was produced from arginine during the basic hydrolysis.

Cystine was determined as cysteic acid with a performic acid oxidation method similar to that of Schram (*37*). Fifty-milligram samples were oxidized for 16 hours at 0° C. in 10 ml. of performic acid reagent (1 volume of 30% H_2O_2 to 9 volumes of 88% formic acid). After oxidation and addition of 4 ml. of 6*N* HBr, the reagent was removed by vacuum distillation. The sample was hydrolyzed and prepared for analysis in the manner described for the acid hydrolysis. The cysteic acid was determined on a 60 × 0.9 cm. column using 0.2*N*, pH 3.58 buffer at 30° C. The cysteic acid peak was eluted in about 50 minutes.

The biological values of the protein in the samples were estimated from the release of essential amino acids by pepsin digestion followed by pancreatin digestion as described by Akeson and Stahmann (*3*).

Vitamin analyses were carried out by the Wisconsin Alumni Research Foundation Laboratories, Madison, Wis., using the chemical methods cited. β-Carotene (*7*), thiamine (*5*), and riboflavin (*5*) were determined along with the xanthophyll (*7*), which has no vitamin activity but is an important pigment in alfalfa.

Results and Conclusions

Product Yield. The product of the spray-dried process was a green powder readily soluble or dispersible in water. An average yield of 27.2% of the total solids and 43% of the total nitrogen was obtained from the

alfalfa. A yield of 29.1% of the total solids and 43.9% of the nitrogen was obtained from the pea vine.

An average yield of 79.5 grams of chlorophyll-free protein concentrate was obtained from 100-gram samples of spray-dried alfalfa powder by extraction with 95% ethanol. Only 53.3 grams was obtained from a 100-gram sample of spray-dried pea juice. This indicated that the pea vine had a higher content of ethanol-soluble substances.

Proximate Composition. The proximate compositions of spray-dried juice, ethanol-extracted spray-dried juice, residue remaining after expressing the juice, and plant material before removal of juice are shown in Table I. For comparison, the proximate compositions of seven high protein supplements (*29*) were included. The compositions of the spray-dried juice from the two alfalfa cuttings were similar, except that the second cutting sample had a slightly lower protein content and slightly higher nitrogen-free extract content. The protein content of the pea vine preparation was about half that of alfalfa, while the nitrogen-free extract of the spray-dried alfalfa juice was correspondingly lower than that of pea vine. The ethanol-extracted preparations had compositions similar to the corresponding unextracted samples, except for lower fat content and higher protein content. The residue remaining after removal of the juice had a lower protein content and higher fiber content than the unextracted plant, but would still be an excellent feed for ruminant animals. This residue could be dehydrated to give a product only slightly inferior to 17% dehydrated alfalfa.

The main differences in composition of the spray-dried juice and the unextracted plant were the protein and fiber contents. The unextracted plant had greater than 20% fiber while the spray-dried juice had only about 1%. The low fiber content of the spray-dried juice would permit one to feed larger proportions to nonruminant animals than is possible with dehydrated alfalfa. However, the consumption of high concentrations of alfalfa by certain nonruminant animals such as poultry may be limited by the presence of toxic saponins or phenolic compounds which inhibit growth and cause diarrhea (*32*). Since not all such compounds are soluble, it is likely that the amount in the juice will be less than that in the alfalfa. The protein content of the spray-dried juice was 1.5 to 2 times greater than that of the unextracted plant. The spray-dried preparations were lower in protein than the other commercial high protein feeds. Leaf protein concentrates may be prepared which contain 60% protein (*22*) by the procedure of Morrison and Pirie (*30*), but the water-soluble nutrients would be wasted. The fiber contents of the spray-dried preparations were as low as or lower than all the protein concentrates listed. The nitrogen-free

Table I. Composition of Spray-Dried Juice, Spray-Dried Juice Extracted with 95% Ethanol, Residue after Expressing Juice, and Plant Material before Expressing Juice

Sample	Moisture	Protein	Fat	Fiber	N-Free Extract	Ash
Spray-dried juice						
Alfalfa (first cut)	4.9	34.9	6.6	0.7	40.2	12.7
Alfalfa (second cut)	4.2	31.3	5.8	0.9	45.7	12.1
Pea vine	4.8	18.5	2.5	1.4	60.0	12.8
Spray-dried juice extracted with 95% ethanol						
Alfalfa (first cut)	2.6	42.8	0.6	0.8	38.8	14.4
Alfalfa (second cut)	2.3	37.0	0.6	1.2	44.8	14.1
Pea vine	3.1	26.2	0.5	1.6	47.0	21.6
Residue after expressing juice[a]						
Alfalfa (first cut)	7.4	15.9	3.1	32.6	34.6	6.4
Alfalfa (second cut)	5.8	15.5	2.8	31.0	38.2	6.7
Pea vine	6.3	9.7	3.3	28.5	41.2	11.0
Plant material before expressing juice[a]						
Alfalfa (first cut)	5.5	21.7	4.5	23.0	37.5	7.8
Alfalfa (second cut)	5.1	19.5	3.6	24.1	40.6	7.1
Pea vine	5.5	12.2	3.4	21.4	44.6	12.9
Other high protein feedstuffs[b]						
Fishmeal[c]	8.0	60.9	6.9	0.9	5.0	18.3
Tankage[d]	7.2	59.4	7.5	1.9	2.6	21.4
Soybean meal[e]	8.3	50.4	1.0	3.2	31.0	5.6
Cottonseed meal[f]	9.0	41.6	2.0	10.7	31.1	6.8
Corn gluten meal[g]	8.4	43.2	2.2	3.8	38.9	3.5
Linseed meal[h]	8.5	35.2	4.6	8.9	36.7	5.7
Alfalfa meal[i]	7.3	17.7	2.5	24.0	38.4	10.1

[a] Air dried for 48 hours at 140° F. [b] Morrison (*29*). [c] All analysis. [d] Digester process, 60% protein. [e] Dehulled, solvent extracted. [f] Solvent extracted. [g] All analysis. [h] Expeller, all analysis. [i] Dehydrated, all analysis.

extract was higher in the spray-dried preparations than in the protein feedstuffs listed.

Amino Acid Composition and Protein Quality. When comparing protein feeds, the quality is important. An important factor determining protein quality is amino acid composition (Table II). In general, all spray-dried preparations had similar amino acid compositions when expressed as per cent of total amino acids recovered. The compositions were similar to those reported in the literature for leaf protein concentrate. Gerloff, Lima, and Stahmann (*22*) reported that methionine was the limiting amino acid and that the other essential amino acids were present in adequate amounts for a high quality protein. A comparison of the methionine content of the spray-dried preparation with that of the commercial protein supplements showed that the methionine content of the leaf protein was as high as or higher than that of all the protein supplements except fish meal and possibly corn gluten meal. The lysine content of the spray-dried preparations was lower than that of fish meal, about the same as that of tankage and soybean meal, and higher than that of cottonseed meal, corn gluten meal, and linseed meal. Lysine is the limiting amino acid for these last three feeds. The tryptophan content of leaf proteins was higher than that of all the proteins listed. In comparing the essential amino acid composition of leaf proteins with the high protein feeds, the authors concluded that the leaf proteins could have a biological value equal to or greater than the high protein feeds listed with possible exception of fish meal.

The amino acid composition alone does not determine the nutritive value of a protein. The rate and degree of release of amino acids during the digestion process are also important determinants of the protein quality. Processing can alter the nutritive value of a protein. Since nutritive values of leaf proteins are drastically reduced by excessive heat, it is necessary to check new processes like the one described to see whether nutritive value has been lost. Because relatively small amounts of leaf protein concentrate were prepared, the biological value was estimated by the pepsin pancreatin digest index (*3*). These results are summarized in Table III along with values for other foods computed by the same methods. There was little difference between estimated biological values of the spray-dried alfalfa samples, either before or after extraction with ethanol. These preparations had values very similar to those reported in the literature for leaf protein concentrates prepared according to the method of Morrision and Pirie (*30*) and Chayen *et al.* (*12*). These preparations were about equal in biological value to lactalbumin and milk and superior to casein, beef, yeast, soybean, cottonseed meal, wheat flour, gluten, zein, and gelatin. The estimated values for the pea vine preparations were somewhat lower than for the alfalfa but would still be as good as or better than soybean and cottonseed protein.

The authors concluded from this study that the spray-drying and ethanol-extraction procedures did not have a deleterious effect on the nutritive value of the leaf protein concentrates. Before final evaluation can be

Table II. Amino Acid Composition of Spray-Dried Leaf Protein Preparations and Other High Protein Foodstuffs

	Amino Acids[a]																	
	Essential[b]								Nonessential									
Protein Source	Lys.	Phe.	Met.	Thr.	Leu.	Ileu.	Val.	Try.	Arg.	His.	Tyr.	Cys.	Asp.	Ser.	Glu.	Pro.	Gly.	Ala.
Spray-dried juice																		
Alfalfa (first cut)	5.5	6.4	1.7	5.6	8.0	4.6	5.6	1.7	5.9	2.5	4.7	1.0	13.8	5.2	12.0	4.9	5.1	5.7
Alfalfa (second cut)	6.4	6.9	1.4	5.3	8.6	4.9	5.9	1.8	6.7	2.4	4.7	1.3	11.3	4.9	11.4	4.7	5.3	6.0
Pea vine	5.6	5.2	1.6	6.3	7.1	4.4	5.9	1.5	5.7	2.3	4.3	1.1	14.7	5.7	12.8	4.8	4.6	6.4
Spray-dried juice extracted with 95% ethanol																		
Alfalfa (first cut)	6.0	6.5	1.4	5.2	8.5	4.8	5.7	1.6	6.3	2.5	5.0	1.1	12.1	4.9	11.5	5.2	5.5	6.1
Alfalfa (second cut)	5.9	6.6	1.7	5.3	8.6	4.8	5.8	1.6	6.1	2.4	5.2	1.2	11.6	4.8	11.7	5.4	5.4	6.0
Pea vine	5.5	5.5	1.6	5.4	7.2	4.0	5.2	1.4	5.9	2.4	4.6	1.1	15.9	5.8	13.3	4.6	4.9	5.7
Other high protein feedstuffs[c]																		
Leaf protein concentrate[d]	6.3	6.0	2.1	5.2	9.8	5.3	6.3	1.6	6.5	2.2	4.2	0.7	9.9	4.8	11.7	5.1	5.7	6.6
Fishmeal	10.4	4.2	3.0	4.6	8.4	6.0	5.7	1.1	6.5	2.5	3.0	1.3	...	...	13.8	...	7.2	...
Tankage	6.7	4.5	1.3	4.0	8.6	3.2	7.1	1.2	6.1	3.2	...	...	...	...	...	...	...	...
Soybean meal	6.2	4.8	1.7	3.9	8.2	6.4	5.0	1.4	6.0	2.5	3.2	1.4	...	...	17.0	...	5.7	...
Cottonseed meal	3.9	4.6	1.2	2.6	5.3	3.7	4.3	1.2	8.0	2.2	2.5	2.4	...	...	15.1	...	5.8	...
Corn gluten meal	1.9	6.7	2.3	3.2	17.5	5.3	5.1	0.5	3.2	2.3	2.3	1.4	...	...	19.2	...	3.5	...
Linseed meal	3.7	4.2	1.3	3.4	5.7	5.4	4.8	1.4	8.1	2.0	2.8	1.7	...	...	...	...	...	...
Alfalfa (dehydrated)	6.3	4.5	0.6	4.0	7.4	5.1	4.5	1.7	4.5	1.7	3.4	...	...	...	...	...	...	...

[a] Amino acids expressed as per cent of total amino acids recovered. [b] Required by adult man (*35, 36*). [c] Data taken from Morrison except for leaf protein concentrates (*29*). [d] Average of values reported by Gerloff, Lima, and Stahmann (*22*) for 25 samples prepared according to method of Morrison and Pirie (*30*) and four samples prepared according to method of Chayen *et al.* (*12*).

Table III. Estimated Biological Values of Spray-Dried Leaf Protein Preparations[a]

Sample	Estimated Biological Value[a]
Spray-dried juice	
Alfalfa (first cut)	80
Alfalfa (second cut)	81
Pea vine	68
Spray-dried juice extracted with 95% ethanol	
Alfalfa (first cut)	80
Alfalfa (second cut)	83
Pea vine	70
Other foodstuffs determined by same method[b]	
Whole egg	97
Egg white	87
Lactalbumin	84
Milk	83
Leaf protein concentrate[c]	83
Casein	76
Beef	75
Yeast	71
Soybean[d]	65
Cottonseed meal[e]	64
Wheat flour	50
Gluten	45
Zein	26
Gelatin	17

[a] Biological values estimated by pepsin pancreatin digest index (*3*). [b] Akeson and Stahmann (*2*). [c] Average of 14 samples prepared according to Morrison and Pirie (*30*). [d] The biological values range from 57 to 75 depending on preparation (*26*). [e] Biological value from rat-feeding trials (*26*).

Table IV. Partial Vitamin Analysis of Spray-Dried Alfalfa Juice and Other High Protein Feedstuffs

Sample	β-Carotene, Mg./100 G.	Thiamine, Mg./100 G.	Riboflavin, Mg./100 G.	Xanthophyll, Mg./100 G.
Alfalfa, spray-dried[a]	53.3	0.64	2.22	91.6
Alfalfa, dehydrated[b]	16.1	0.04	1.23	25.7
Fishmeal tankage[c]	...	0.13	0.68	...
Soybean meal[c]	0.02	0.38	0.35	...
Cottonseed meal[c]	0.02	0.64	0.60	...
Corn gluten meal[c]	1.63	0.02	0.15	...
Linseed meal[c]	0.02	0.57	0.33	...

[a] Values from WARF. [b] American Dehydrators Association (*4*). [c] Morrison (*29*).

made, feeding trials should be carried out, for toxic factors such as saponins may lower the feed value.

Vitamins. Vitamin analyses are shown in Table IV. The spray-dried alfalfa juice had a β-carotene content which was higher than the other protein supplements. The β-carotene content of the spray-dried preparation was about three times the average value found for dehydrated alfalfa (*4*). The thiamine content of spray-dried alfalfa juice was higher than all the other supplements listed except for cottonseed meal which was equal to that of spray-dried alfalfa juice. The riboflavin content was higher than that for the other high protein feedstuffs. From these data, the authors concluded that spray-drying is an excellent means of preservation of the vitamins tested. This product was a rich source of xanthophylls, which have no vitamin activity but are important in pigmentation.

In addition to the vitamins which have been identified and characterized, certain unidentified water-soluble factors are present in the juice of grass and alfalfa which are required for optimal growth of chicks (*24*) and other animals (*21*, *23*). Analyses (*16*) indicate that this spray-dried product is a very rich source of the grass juice factor.

Acknowledgment

The authors express their appreciation to Daniel O. Noorlander and the Dairy Equipment Co. for the machine used in the plant juice extraction; to the Department of Dairy and Food Industries of the University of Wisconsin for the use of the Niro spray-drier; to Joseph Schroeder for his technical assistance in extracting the juice and for the amino acid analyses; to P. H. Derse for vitamin analyses; and to W. B. Griem for the proximate analyses.

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Received for review January 31, 1966. Accepted October 10, 1966. Work supported by grants from the Herman Frasch Foundation for Agricultural Research. Published with the approval of the Director of the Wisconsin Agricultural Research Experiment Station.