(1967) with the important ions being m/e 30, 42, a relatively large parent ion and peak due to α -cleavage of the alkyl side chain, with subsequent loss of NOH, as well as a peak at P-17 due to the loss of the hydroxyl radical. An important pathway can be described in the following scheme for the m/e 42, except in the case of dimethylnitrosamine, which bypasses the α -cleavage step.



The phenyl-substituted nitrosamines all have an appreciable parent peak and a strong P-30 peak. The benzyl-substituted nitrosamines have a strong parent peak and a strong m/e 91 peak, which in some cases is the base peak of the spectrum. The m/e 91 is analogous to the splitting of the bond β to the phenyl ring, as described by Grubb and Meyerson (1963).

Currently, a more specific and detailed study is in progress utilizing both high-resolution mass spectrum and the metastable spectrum of each compound. This will allow us to more fully explain the fragmentations that occur.

The determination of N-nitrosamines in food products and other natural substances is complicated by the presence of large numbers of interfering compounds. Therefore, adequate isolation and cleanup procedures are necessary to permit sampling of the nitrosamines so that the spectra described in this paper can be utilized.

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Processing of Cauliflower Leaf Waste for Poultry and Animal Feed

A. Lyle Livingston,* Richard E. Knowles, Jon Page, Donald D. Kuzmicky, and George O. Kohler

Leaf waste from commercially grown cauliflower was dehydrated in a pilot scale alfalfa dehydrator to give dried meals suitable for poultry and cattle feeds. Separation of the poultry and cattle meal fractions was accomplished via air classification of the dried plant material. The poultry meal fraction contained 375 to 620 mg/kg of xanthophyll and 26 to 31%protein, while the cattle meal contained 17 to 21% protein. The xanthophylls in the poultry meal were

ommercial production of vegetables in the United States results in more than 4 million tons of fresh vegetable wastes annually (Willaman and Eskew, 1948; U.S. Dept. Agr. Stat., 1969). A large portion of these

as effective in pigmenting broiler skin as the xanthophylls in dehydrated alfalfa meal. No undesirable flavor was imparted to poultry meat by cauliflower meal. Pressing of cauliflower leaf prior to dehydration increased its solids content and lessened the quantity of water to be evaporated per pound of dried product by nearly 30%. The pressed and dehydrated meals were almost equal in quality to the unpressed dehydrated meals.

wastes is green leaf plant materials that are removed at the packing shed. An estimated 60,000 tons of cauliflower leaf wastes are produced annually in the California Salinas Valley. Presently, following removal of the flowers for the fresh and frozen vegetable markets, leaf and stem portions are chopped and returned to the fields where they constitute an odor problem as they decompose to form a green manure.

Prior studies at this laboratory have been concerned with

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the dehydration of alfalfa (Livingston *et al.*, 1966, 1968, 1970), including the separation of the dehydrated meal into leaf and stem fractions (Chrisman and Kohler, 1969). By careful dehydration procedures, meals rich in nutrients and vitamins for poultry or cattle feeds have been prepared. More recently, a process has been developed (Kohler *et al.*, 1969) for the dejuicing of fresh alfalfa by pressing between sugar cane rolls and the preparation of a protein xanthophyll concentrate from the expressed juice. Oelshlegel *et al.* (1969) prepared similar leaf protein concentrates from a variety of green plant wastes and found the amino acid composition (except methionine) to be adequate for most human food needs. The pressed bagasse was utilized as silage for cattle feed.

Due to increasing pressure to reduce pollution and to develop an additional source of income from the cauliflower crop, the following study was undertaken to determine if leaf wastes could be dried in a pilot alfalfa dehydrator to yield quality meals for poultry and cattle feeds. To reduce the evaporation load in the dehydrator, trials were also made in which leaf wastes were partially dejuiced by pressing before drying.

MATERIALS AND METHODS

Collection of Whole Plants. Prior to the dehydration trials, 12 whole cauliflower plants of two varieties (Pearl and Snowball) from two different fields were selected at random, the flowers removed, and the leafy portions of each plant placed in a plastic bag, frozen between layers of dry ice, and freeze-dried. A leaf-stem separation was manually made and the two fractions were analyzed for carotene, total xanthophyll, individual xanthophylls, as well as protein, fat, fiber, ash, phosphorus, and calcium.

Cauliflower Leaf Dehydration. Fresh cauliflower trim wastes from a commercial packing plant were chopped in a conventional field silage chopper (International Harvester Model No. 350). The whole chops were either directly dehydrated in a pilot scale Arnold dehydrator (Model SD45-12) or, in some trials, first dejuiced by passing between sugar cane rolls prior to dehydration. Operation of these rolls for the pressing of fresh alfalfa prior to dehydration has been described by Spencer *et al.* (1970).

The dehydration throughput time of the chops was regulated by means of a variable speed fan on the outlet side of the dehydrator. Although the outlet temperature of the dehydrator was kept constant for a particular study, the inlet temperature varied automatically, depending on the quantity of fresh cauliflower entering the unit. Following dehydration the chops were conveyed by an elevator into an air-classifier where a leaf-stem separation was made in the same manner as that employed by Chrisman and Kohler (1968) to fractionate dehydrated alfalfa.

Analysis. Fresh cauliflower samples were collected in

plastic bags at the dehydrator elevator, frozen with dry ice, and later freeze-dried. These samples and dehydrated cauliflower samples were ground through a No. 40 screen and analyzed for total xanthophyll and carotene by the procedure of Livingston *et al.* (1971b), for nonepoxide xanthophyll (Livingston *et al.*, 1969), and for individual xanthophylls (Nelson and Livingston, 1967).

Amino acids were determined by the modified ion exchange procedure of Kohler and Palter (1967), employing a modified Phoenix amino acid analyzer.

Moisture determinations were made by drying the meals in a forced draft oven at $105 \,^{\circ}$ C for 24 hr.

Broiler Pigmentation. Dehydrated cauliflower leaf meal, two lots of alfalfa meal (pelleted and reground), corn gluten meal, and two levels of pure lutein were fed at levels to give comparable pigmentation responses based on chemical analyses. A low pigment white milo diet was also included as a control diet. Day-old White Rock cockerels were fed the respective rations for 28 days. Two replicates of 12 chicks per replicate were used for each ration. Composition of the ration, analysis of toe web punches, and visual scoring of shank color employing the Roche color fan have been previously described (Kuzmicky *et al.*, 1968).

Evaluation of Flavor of Cauliflower-Fed Chickens. Additional day-old broiler chicks were depleted of pigment for 10 days and then fed similar diets for 8 weeks. They were commercially dressed and flavor tests were conducted on the roasted meat. The birds were halved, washed, dried, weighed, and placed on racks in open shallow aluminum pans. They were roasted at 325°F in a revolving oven to an internal thigh temperature of 185°F. Cooking time was between 1 hr, 10 min and 1 hr, 25 min, depending on size of bird.

One taste panel was held each day for 6 days. Four birds were cooked each day, one from each diet group.

Four portions of breast meat, four of dark meat (thigh and drumstick), and four of skin (no seasoning added to any) were served to each of five panelists at each of the six sessions. Sodium lights were used in the panel room to mask color differences. Judges were asked to rate the flavor in each group. There were 30 judgments in each category (five panel members, six replications).

RESULTS AND DISCUSSION

A preliminary study of the two varieties of freeze-dried cauliflower plants indicated that a very high carotene and xanthophyll feed material might be prepared by leaf-stem separation of the dried cauliflower plants (Table I). The high level of stem compared to leaf, particularly in the Pearl variety, made a leaf-stem separation of the dried cauliflower plant essential if a rich poultry pigment source were to be prepared. The ratios of individual xanthophylls were very similar to that found in freeze-dried alfalfa meals (Livingston *et al.*, 1968).

 Table I.
 Carotenoid Analysis of Two Varieties of Cauliflower Leaves

								% Individual xanthophylls in leaf fraction					
	Dry	weights, g ^a	Caro mg	Carotene, mg/kg ^a		Xanthophyll, mg/kg ^a		Deoxy-			Neox-	Violox-	
Variety	Leaf	Stem	Leaf	Stem	Leaf	Stem	lutein	lutein	\mathbf{X}_2	\mathbf{X}_1	anthin	anthin	
Pearl ^b	40.3	106.2	527.0	24.0	998.2	44.9	68.4	1.2	2.1	3.8	11.2	13.3	
Sx	6.2	8.6	26.2	3.1	63.0	5.7	1.9	0.1	0.2	0.4	0.6	1.8	
Snowball ^b	38.4	52.3	562.1	31.0	1156.0	63.2	69.1	1.2	2.7	3.3	8.3	15.4	
Sx	8.0	6.6	13.9	2.9	25.8	7.5	1.5	0.2	0.3	0.3	0.4	1.0	

^a Moisture-free basis. ^b Avg of duplicate analysis of six separate plants. $S\bar{x} = standard error of the mean$.

	Protein		Fat		Fiber		Ash		Calcium		Phosphorus	
Variety	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Pearl ^b	36.6	17.8	5.3	2.2	8.1	13.3	14,2	11.4	2.6	1.0	0.68	0.50
Sx	1.3	1.1	0.2	0.1	0.2	0.5	0.6	0.4	0.2	0.1	0.01	0.01
Snowball ^b	32.4	18.9	6.2	2.7	9.5	14.7	16.1	14.9	3.8	1.9	0.68	0.40
Sx	0.6	0.6	0.2	0.1	0.4	0.7	0.1	0.3	0.1	0.1	0.02	0.01

Table III. Dehydration of Waste Cauliflower Leaf

Meal sample and dryer temperature at outlet	Weight,	Moisture of meal, %	Carotene, mg/kg ^a	Xantho- phyll, mg/kg ^a	Protein, [%] a	Fat, $\%^a$	Fiber, $\%^a$	Ash, $\%^a$	Calcium, % ^a	Phos- phorus, $\%^a$
Trial 1, 275°F										
Freeze-dried whole plant		2.9	190.0	398.0	24.6	4.06	13.5	10.72	1,74	0.69
Dehydrated whole plant	100.0	6.1	176.8	280.1	24.1	3.06	13.7	11.13	2.09	0.67
Dehydrated leaf fraction	53.8	8.1	256.2	420.3	28.1	3,64	12.5	11.60	2.07	0.75
Dehydrated stem fraction	46.2	17.0	65.6	119.4	21.3	2.43	14.5	10.65	1.48	0.54
Pressed freeze-dried whole plant		2.9	184.7	373,5	23.0	3.48	14.6	9.55	1.66	0.69
Pressed dehydrated whole plant	100.0	8.8	185.4	298.5	24.1	3.11	14.8	10.55	1.77	0.70
Pressed dehydrated leaf fraction	58.2	4.9	238.7	375.0	26.0	2.87	12.5	10.67	1.70	0.76
Pressed stem fraction	41.8	16.2	67.9	119.3	20.7	2.25	15.9	10.65	0.93	0.54
Trial 2, 250°F										
Freeze-dried whole plant		3.4	245.0	441.0	23.3	4.98	13.8	13.75	2.34	0.55
Dehydrated whole plant	100.0	10.3	239.0	338.0	25.1	3.61	13.5	13.37	2.26	0.54
Dehydrated leaf fraction	51.4	7.2	303.1	419.0	26.7	3.72	12.7	13.55	2.61	0.66
Dehydrated stem fraction	48.6	23.0	31.2	55.4	17.2	1.88	15.3	11.85	1.60	0.42
Pressed freeze-dried whole plant		9.2	210.0	441.0	22.6	2.79	13.13	13.10	2.31	0.55
Pressed dehydrated whole plant	100.0	12.1	228.7	389.0	21.5	2.85	14.7	12.38	2.24	0.53
Pressed dehydrated leaf fraction	58.0	7.3	281.8	460.0	25.6	3.36	15.2	12.40	2.43	0.62
Pressed dehydrated stem fraction	42.0	21.7	33.2	60.0	17.7	1.78	16.5	11.74	1.93	0.48
Trial 3, 250°F										
Freeze-dried whole plant		3.1	265.3	648.2	26.2	4.52	11.8	15.37	2.52	0.61
Dehydrated whole plant	100.0	15.3	244.1	413.4	26.7	4.10	13.2	17.70	2.26	0.64
Dehydrated leaf fraction	55.5	7.2	383.7	599.0	31.0	4.34	11.1	18.01	2.49	0.71
Dehydrated stem fraction	44.5	24.3	19.8	42.2	19.2	2.58	14.8	15.16	1.94	0.44
Pressed freeze-dried whole plant		3.7	235.3	506.0	23.5	4.18	14.5	14.52	2.64	0.61
Pressed dehydrated whole plant	100.0	12.1	230.8	420.0	24.2	3.47	15.1	15.50	2.08	0.59
Pressed dehydrated leaf	64.4	8.0	334.0	620.0	26.9	4.16	14,1	15.81	2.68	0.68
Pressed dehydrated stem	35.6	21.9	84.0	176.0	19.3	2.42	16.0	14.53	1.80	0.53
^a Moisture-free basis.										

Although the stemmy fraction seemed too low in either carotene or xanthophyll to be of value as a poultry feed, the proximate analyses indicated that an excellent cattle feed might be prepared from this fraction (Table II), while the high protein and mineral content and the low fiber of the leaf fraction further enhanced its value as poultry feed.

These preliminary studies provided reason to undertake a pilot-scale dehydration study of cauliflower plant waste. Dehydrator conditions were similar to those used to dehydrate alfalfa and grasses. Results demonstrated that while the dried leaf contained 6 to 10% moisture the stem was still very wet at nearly 20% moisture. This necessitated a leaf-stem separation. The dehydrated product was processed through the air-classifier and separated into leaf and stem fractions. An excellent separation was achieved by this means as indicated by the high xanthophyll of the leaf fraction and low xanthophyll of the stem (119 mg/kg) (Table III, trial 1). The yield of leaf and stem was 53.8 and 46.2\%, respectively.

A limiting economic factor in the dehydration of cauliflower waste is its high moisture content (90 to 93%). This of course means a great deal more water has to be evaporated to obtain 1 lb of dehydrated cauliflower meal as compared to a material such as alfalfa, which may have an initial moisture content of only 76 to 84%. The freshly chopped cauliflower waste was therefore pressed to reduce the moisture load by means of a three-roll mill developed at this laboratory for use in pressing of alfalfa (Spencer *et al.*, 1970). About 35% of the total water was removed and the solids content of the bagasse was increased to 12.0%. Although there was a loss of soluble solids in the pressed juice, the carotene, xanthophyll, and protein content of the pressed and dehydrated leaf and stem fractions was nearly equal to that of the unpressed dehydrated products. The protein and other nutrients in the pressed juice might be recovered by a process similar to that developed at this laboratory for the pressed stem fraction proved more difficult to separate from the leaf, as evidenced visually and by a higher proportion of leaf to stem (58.2 to 41.8%).

A second dehydration study was made (Table III, trial 2), in which the dehydrator was operated at 250° F compared with 275° F. The freshly chopped cauliflower wastes were dehydrated with/without pressing as in trial 1. The pressed and dehydrated leaf fraction actually contained more xanthophyll than the unpressed. This might be attributed to the lower moisture and consequent shorter drying time of the pressed leaf fraction, resulting in less loss of xanthophyll. The high

	Initia	l, mg/kg	8 weeks,	% retained	12 weeks, $\%$ retained		
Meal sample	Carotene	Xanthophyll	Carotene	Xanthophyll	Carotene	Xanthophyll	
Whole dehydrated	209.6	296.3	27.0	45.8	19.8	35.9	
Whole dehydrated ^b	209.6	296.3	78.9	78.5	78.5	73.4	
Whole pressed dehydrated	213.8	342.0	28.9	46.0	20.1	36.3	
Whole pressed dehydrated ^b	213.8	342.0	85.0	74.5	81.5	70.5	
Leaf fraction dehydrated	251.0	353.0	36.3	57.3	26.0	39,6	
Leaf fraction dehydrated ^b	251.0	353.0	90.2	89.2	85.4	83.6	
Leaf fraction pressed dehydrated	261.7	426.2	32.8	48.6	22.2	36.5	
Leaf fraction pressed dehydrated ^b	261.7	426.2	78.5	71.9	71.0	68.4	
Whole freeze-dried	206.4	443.2	55.4	61.2	43.8	56.2	
Whole freeze-dried ^b	206.4	443.2	68.7	70.5	65.4	68.0	

Table IV. Carotene and Xanthophyll Storage Stability in Cauliflower Leaf Meals^a

moisture content of the stem fractions after dehydration and separation (17.0 to 24.3%) may require an additional stem drying operation if this fraction is to be stored.

In the first two trials, cauliflower plants were processed in February and March. In April a third trial was made, which included dehydration of a leafy cauliflower plant of the Pearl variety. Again the freshly chopped plant material was dehydrated with/without pressing (Table III, trial 3). In this study an exceptionally high xanthophyll leaf fraction was prepared by air-classification of the dehydrated whole meal. This fractionation procedure gave 55.5% leaf to 44.5% stem for the unpressed material, and 64.4% leaf to 35.6% stem for the pressed material. The xanthophyll was found to be 621.0 mg/kg in the pressed and dehydrated leaf fraction, compared to 600.0 mg/kg in the unpressed dehydrated leaf. Although there was loss of protein, carotene, and xanthophyll to the press juice, as evidenced by comparison of the whole freezedried plant analyses before and after pressing, the lower throughput time in the dehydrator for pressed cauliflower again resulted in a lower loss of the labile xanthophylls during drying.

Cauliflower is grown over a 10-month period in the state of California. However, in midwestern and eastern parts of the United States, it is grown over a shorter season; therefore, it

Table V.Amino Acid Analysisa of Dehydrated CauliflowerMeals (g of amino acid/16 g of nitrogen)

Amino acid	Leaf fraction	Stem fraction
Lysine	4.53	3.51
Histidine	1.81	1.34
Ammonia	3.35	4.97
Arginine	4.16	3,11
Aspartic acid	9.08	7.74
Threonine	4.26	2.95
Serine	5,38	3.73
Glutamic acid	16.86	24.83
Proline	5.00	4.61
Glycine	4.32	3.00
Alanine	5.12	4.32
Valine	5.22	3.83
Isoleucine	3.80	2.56
Leucine	6.66	4.30
Tyrosine	2.78	1.75
Phenylalanine	4.40	2.67
Methionine	1.67	1.10
Cystine	1.23	1.02
% N recovered	79.53	78.68

^a Average of duplicate analysis, mfb.

would be necessary to store cauliflower leaf meal produced in these areas for a period of several months in order to provide a continuous supply for the feed industry. Previous studies at this laboratory (Knowles *et al.*, 1968; Thompson, 1950) and others (Silker *et al.*, 1944) have demonstrated that considerable losses of carotene and xanthophyll may occur unless protective measures such as inert gas storage (Graham, 1944) or the addition of the antioxidant ethoxyquin (Thompson, 1950) are carried out. To ascertain the stability of the carotene and xanthophyll in dehydrated cauliflower meals, samples were stored in open shell vials at 90°F and analyzed periodically. The results are presented in Table IV.

In the dehydrated untreated meals, 43 to 54% of the initial xanthophyll was lost after 8 weeks of storage. Addition of ethoxyquin reduced this loss to only 11 to 28% over the same storage period. Although the carotene was more rapidly lost in the untreated meals (64 to 73% after 8 weeks of storage), it became slightly more stable than the xanthophyll after addition of ethoxyquin. Both the carotene and xanthophyll were more stable in the freeze-dried meal than in any of the dehydrated meals. It has been previously observed that the carotenoids in freeze-dried alfalfa are more stable than in the corresponding dehydrated meal (Knowles *et al.*, 1968). This increased stability was attributed to the natural antioxidants present in the freeze-dried meal, which are partly destroyed during hot-air dehydration.

A further study by this laboratory showed that several of the essential amino acids in alfalfa may undergo significant losses during dehydration, unless dehydrator outlet tempera-

Table VI. Broiler Fed to C	Pigmentation hicks from 0	from -4 Weel	Xanthophyll ks of Age	Sources
Supplement	% of ration	mg of xantho- phyll per kg ration	Pigment toe web discs, $(\mu g/100$ cm ²)	Visual score ^a
Control diet			10. 9	0
Lutein		5.5	41.5	1.4
Lutein		11.0	80.0	2.7
Dehydrated alfalfa 1 ^b	3.97	11.0	61,6	
Dehydrated alfalfa 2 ^b	3.55	11.9	51.8	1.6
Dehydrated cauliflowe	r ^b 2.16	11.2	49.4	1.6
Corn gluten meal ^e	3.60	11.0	49.3	2.0

^a Employed 15-blade Roche color fan for scoring. ^b Analyzed by the procedure of Livingston *et al.* (1971b); dehydrated alfalfa meals 1, 2, and the dehydrated cauliflower contained 298.5, 308.8, and 561.2 mg/kg of xanthophyll, respectively. ^c Analyzed by the procedure of Quackenbush *et al.* (1970); contained 296.0 mg/kg of xanthophyll.

Table VII. Evaluation of Flavor of Cauliflower-Fed Chickens F Panel Ratin

Summary	of	Panel	Ratings
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None										
110116			Slight			Moderate	;		Strong	
Light Dark Supplement meat meat	Skin	Light meat	Dark meat	Skin	Light meat	Dark meat	Skin	Light meat	Dark meat	Skin
Basal control 24 18	25	4	12	5	1			1		
5.85% alfalfa 17 26	22	10	3	7	3	1	1			
6.49% cauliflower 22 27	27	6	3	3	2					
10% cauliflower 20 23	24	7	7	5	3		1			

ture and meal moisture are carefully controlled (Livingston et al., 1971a). Analyses of typical samples of cauliflower leaf and stem meal are presented in Table V and indicate that both fractions are good sources of the essential amino acids and would provide a sufficient level of these nutrients for poultry and animal feeds.

Broiler feeding trials at this laboratory showed that the birds readily consumed the cauliflower leaf meal when included in the rations at a level of 2 to 10%. Shank pigmentation was found to be comparable to that provided by the xanthophylls of either corn gluten or dehydrated alfalfa meal when fed at similar xanthophyll levels (Table VI).

The pelleted and reground alfalfa meal 1 was deposited at a slightly more efficient rate than any of the other three supplements. Kohler et al. (1968) previously presented evidence that pelleting and regrinding increases xanthophyll availability. This same effect might be expected if dehydrated cauliflower meal were pelleted and reground prior to feeding to broilers.

Although the corn gluten meal xanthophylls were deposited in the shank to the same extent as the cauliflower or alfalfa meal xanthophylls, the visual score for corn was slightly higher due to the presence of zeaxanthin, which imparts a redder hue to the skin compared to the golden color imparted by lutein.

Evaluation of cauliflower fed broilers was carried out by comparing cooked meat flavor with that of control and alfalfafed birds. A trained panel detected no off-flavor in birds fed either 6.5 or 10% dehydrated cauliflower leaf meal in the diet, as shown in Table VII. All birds were adequately tender. Therefore, there should be no objection to feeding such levels as far as palatability is concerned.

Cauliflower is a member of the crucifier plant family, which may contain glucosinolates. These compounds have been shown (Van Etten et al., 1969) to hydrolyze enzymically to form isothiocyanates, thiocyanates, and nitriles which may be harmful to poultry. To ascertain if these might be of sufficient level in cauliflower meals as to be deleterious, freezedried and corresponding dehydrated meals were analyzed for these compounds. None were detected. It appears, therefore, that dehydration of cauliflower plant waste can yield products which are palatable and wholesome, containing adequate protein and amino acids; leaf-stem separation yields a product rich in carotenoid pigments, a valuable constituent of poultry rations, while the balance of the material should be useful for cattle feed.

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Received for review July 14, 1971. Accepted October 8, 1971. Agricultural by-products for animal feed must conform with government regulations relating to pesticide residues. Reference to a com-pany 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.