Table IV. Calculated Arginase Activity in Ice-Stored Shrimp

Postmortem age, days	$w^{1/2}$	Ornithine C,[b] mmol 100 g^{-1}	Arginase act., mmol 100 g^{-1} day^{-1}
2	2.130	0.0543	0.027[c]
5	2.287	0.108	0.025
8	2.332	0.178	0.028
9	2.362	0.203	0.030
12	2.467	0.257	0.031

[a] Weight (grams). [b] $C_0 = 0.004$ mmol 100 g^{-1}. [c] Calculated from the relationship $A = 2\{C + C_0[10 \exp(-k_L(w)^{1/2}t)]\}/t[1 + 10 \exp(-k_L(w)^{1/2}t)]$.

The high level of glycine suggested a physiological function. The lower levels in areas of suspected low salinities suggested that the physiological function might be osmotic pressure regulation.

The rate of loss of amino acids from shrimp tails during ice storage is affected by the rate of ice melt and by the size of the shrimp. Other factors such as position of the shrimp and ice particle size could affect the flow of melt water and, hence, rate of loss of amino acids. The increase in rate of loss with the square root of weight is the opposite direction expected if only diffusion were involved and the entire shrimp tail the site of the leaching action. This is further evidence that amino acid loss is *via* the anterior end of the shrimp tail.

The "natural flora" of shrimp is usually dominated by coryneform bacteria (Vanderzant *et al.*, 1970, 1971) which have little effect upon the free amino acid content of shrimp (Cobb and Vanderzant, 1971). Spoilage odors for all but one sample were not the typical putrid odors evident when shrimp were contaminated with proteolytic bacteria such as *Pseudomonas* species. Shrimp used in this study were checked for bacterial invasion by washing the shrimp tail for 5 min by shaking in water and comparing the bacterial counts with those determined on homogenates. There was no significant difference between the counts determined by the two methods suggesting that most bacteria were located on the surface of the shrimp. These observations probably explain why bacteria appeared to have little effect upon the free amino acid content of most samples.

The equations developed in this study provide methods of estimating some enzymic activities in shrimp tails during ice storage. For the limited size range of shrimp employed, prediction of the half-life of the glycine content and possibly other low molecular weight compounds can be made.

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Decaffeination. A Process to Detoxify Coffee Pulp

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The effect of a decaffeination process on the toxicity and nutritive value of coffee pulp was evaluated in rats. The decaffeination of the material was performed by extraction at 25° and by percolation at 94°. At 20 and 30% of the diet, the pulp subjected to either treatment showed a significantly ($P < 0.01$) lower feed efficiency ratio than the dehydrated coffee pulp. At the 50% level, the percolated pulp caused a significant decrease in the mortality index, in comparison to that observed with the dehydrated coffee pulp and or the decaffeinated pulp at 25°. A significant reduction in mortality was achieved with the latter by applying a complementary alcohol-extraction treatment. A high correlation was found between mortality and tannins, chlorogenic, and total caffeic acids ($r = 0.92$, 0.94, and 0.97, respectively). Decaffeination of the material could be effected both in the fresh or dehydrated states under equal processing conditions. It is concluded that this process offers an opportunity for the industrial use of coffee pulp.

During coffee processing the coffee pulp (term that includes the cherry peels and the pulp itself), the mucilaginous layer of the seed, and the seed husk are removed. The dry green coffee seed which represents around 20% (w/w) of the whole coffee cherry is the only part of the fruit utilized commercially at present (Bressani *et al.*, 1972).

Coffee pulp represents approximately 42% (w/w) of the whole coffee cherry (Bressani *et al.*, 1972). As an example we can mention that in 1971 approximately 2.7 million

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Table I. Per Cent Composition and Nitrogen Content of the Diets Tested[a]

Ingredients	Diet no.											
	1[b]	2	3	4	5	6	7	8	9	10	11	12
Casein	22.4	22.4	22.4	22.4	22.4	22.4	22.4	22.4	22.4	22.4	22.4	22.4
Starch	67.6	47.6	37.6	17.6	47.6	37.6	17.6	47.6	37.6	17.6	17.6	17.6
Dehydrated coffee pulp		20.0	30.0	50.0								
DCP[c] at 25°					20.0	30.0	50.0					
DCP at 94°								20.0	30.0	50.0		
DCP at 25° + alcohol treatment											50.0	
Dehydrated coffee pulp + alcohol treatment												50.0
Mineral mixture[d]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Soybean oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cod liver oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Nitrogen content	3.55	3.95	4.18	4.41	3.87	4.07	4.21	3.89	4.01	4.20	4.24	4.23

[a] All diets were supplemented with 5 ml of a vitamin solution (Manna and Hauge, 1953). [b] Diet 1 was prepared also at 4, 6, and 10% fiber levels using Alphacel commercial cellulose. [c] DCP = decaffeinated coffee pulp. [d] Hegsted *et al.* (1941).

metric tons of dry coffee pulp were produced in Latin America from a total dry coffee cherry production of 6.4 million metric tons (FAO, 1971). However, although isolated efforts have been made in different Latin American countries to utilize coffee pulp (Choussy, 1944; Jaffé and Ortiz, 1952; Squibb, 1945; Van Severen and Carbonell, 1947), this abundant natural resource is still regarded as a waste.

Since dry coffee pulp has been reported to contain from 8 to 11% protein with an amino acid profile on the same protein basis similar to that of soy, cotton, and fish flours, and a crude fiber content ranging from 16 to 28% (Bressani *et al.*, 1962, 1972; Choussy, 1944), most of the studies have been directed toward its use as animal feed. Nevertheless, other investigations have revealed a toxic effect when monogastric animals are fed rations containing from 15 to 30% coffee pulp (Bressani *et al.*, 1973; Jaffé and Ortiz, 1952). Similar effects have also been observed in ruminants (Braham *et al.*, 1973).

The toxic factors in coffee pulp are still unknown, although caffeine and tannins, which are present at relatively high levels in this by-product, have been among the most suspected substances (Bressani *et al.*, 1972). The toxic effects of caffeine in different animals are relatively well known (Cunningham, 1968; Hawkins and Davis, 1970), and their symptoms are similar to those observed in animals fed diets containing coffee pulp (Braham *et al.*, 1973; Bressani *et al.*, 1973).

Therefore, it was thought of interest to study the possibility of detoxifying the coffee pulp through a decaffeination process. Such a process would permit the use of coffee pulp as another source of caffeine, besides improving its quality as an animal feed.

The work reported herein describes the effect produced by decaffeination, under two temperature conditions, on the toxicity of coffee pulp using rats as experimental animals.

EXPERIMENTAL SECTION

The coffee pulp used in this study was obtained from a local coffee processing plant and corresponded to the 1973 crop.

Moisture, nitrogen, ash, crude fiber, ether extract, tannins, total solids, calcium, phosphorus, and iron determinations were carried out in duplicate according to the AOAC (1970). Protein was calculated by multiplying the nitrogen content by the customary conversion factor, 6.25. Sodium and potassium were determined by flame photometry (Coleman Model 6 C Junior) using the method described by the AOAC (1970).

Caffeine determinations were performed according to Ishler *et al.* (1948). Chlorogenic acid, caffeic acid, and total sugars were determined in triplicate following the methods described by Pomenta and Burns (1971). Glucose was used as a standard for the total sugar determinations. Total caffeic acid was calculated by adding to the caffeic acid figure the fraction contained in the chlorogenic acid.

Decaffeination was effected both in milled-fresh and milled-dehydrated coffee pulp, either at 25° or by percolation at 94° using potable water as solvent in both cases. At 25° decaffeination was carried out in two stages of 1-hr duration each, using a 1:330 dried coffee pulp:water ratio. The operation was carried out in a Lee 40-gal jacketted kettle with continuous agitation. The percolation at 94° (boiling water) was done in a home-type coffee percolator, in three stages of 1-hr duration each, using 300 g of dried coffee pulp and 8.5 l. of water per batch. The decaffeination conditions were based on preliminary experiments designed to determine the minimum time for maximum caffeine extraction yields under the cited conditions.

The alcohol treatment was carried out in a soxhlet apparatus for 6 hr using 95% ethanol as solvent, as described by Jaffé and Ortiz (1952).

A locally built tray-drier provided with a counterflow arrangement (similar to the Schilde Simplicitor drier, Model SG 5/XII) was used for drying both the fresh and treated coffee pulp with a hot end air temperature of 75°.

The biological trials were run using weanling rats of the Wistar strain from INCAP's animal colony. The rats were distributed in groups of three males and three females each, placed in individual all-wire-screen cages with raised bottoms. The basic diet administered in all experiments contained casein, corn starch, minerals, and vitamins; the corn starch was then replaced by different levels

Table II. Per Cent Chemical Composition of Fresh and Dehydrated (75°) Coffee Pulp

Ingredients	Fresh pulp	Dehydrated pulp at 75°
Water	84.66	5.02
Ether extract	0.64	4.00
Crude fiber	2.62	16.40
Protein (N × 6.25)	1.90	11.90
Ash	1.39	8.71
Caffeine	0.33	1.27
Tannins		2.40
Chlorogenic acid		2.60
Caffeic acid		0.24
Total caffeic acid		1.56
Total soluble sugars		8.83

of the untreated dehydrated coffee pulp, and of the pulp subjected to the different treatments evaluated in the study. Standard diets were also prepared adjusting the fiber level, at the expense of starch of the basic diet, to a content similar to that calculated for the different rations containing coffee pulp. The per cent composition and nitrogen content of all diets are shown in Table I. All feeding trials lasted 28 days; food was administered *ad libitum*, and drinking water was available at all times. Weights and feed consumption were recorded weekly. The feed efficiency ratio was determined as the ratio of grams of feed intake/grams of weight gain in 28 days.

RESULTS AND DISCUSSION

The per cent composition of the fresh and dehydrated coffee pulp (on an "as is" basis) is presented in Table II. It is of interest to note that the caffeine content of coffee pulp is similar to that reported in the literature for green coffee from which such alkaloid is commercially extracted (Sivetz, 1963). Caffeine values obtained for the green coffee grains corresponding to the coffee pulp used in this study also indicate that both materials contain similar amounts of caffeine.

Likewise, it is of interest to note the high moisture content of the fresh coffee pulp. The former would indicate that dehydration of the material could be necessary, especially if the process proposed to utilize the pulp would imply transportation of raw material and/or if the process itself could not be applied to the moist raw material.

Table III presents the chemical composition (on an "as is" basis) of the samples subjected to the different extraction treatments studied. As the data show, coffee pulp treated by percolation with boiling water gives the lower residual levels of caffeine, tannins, total sugars, and caffeic and chlorogenic acids. These values are followed by those of the pulp treated with water at 25° plus a complementary alcohol extraction, and correlate very well with the caffeine and total solids extraction yields obtained through the different treatments (Table IV). Very similar composition values and extraction yields were obtained utilizing either milled-fresh or milled-dehydrated coffee pulp. This indicates that the extraction process was unaffected by the direct use of fresh or moist coffee pulp.

The crude fiber level appears to increase proportionally to the degree of extraction obtained for total sugars; in every case the fiber values for the treated samples were higher than those obtained for the dehydrated coffee pulp. Apparently, the percentage of protein content was not affected by any of the treatments.

The mineral compositions of the dehydrated coffee pulp and of those samples subjected to the different extraction treatments studied are given in Table V. As the data reveal, sodium and potassium tend to decrease with any of the extraction treatments applied. Iron tends to remain at the same level, while calcium and phosphorus increase through the extraction process. The high potassium:sodium ratio determined for the dehydrated coffee pulp is in accordance with the findings reported by Bressani *et al.* (1972). This high ratio does not seem to be affected by any of the processes studied.

The weight gain, feed consumption, feed efficiency ratio, and mortality data obtained with the different diets are shown in Table VI. When coffee pulp was included at the 20 and 30% levels in the diet, the weight gain and feed efficiency ratio were significantly ($P < 0.01$) improved by the decaffeination treatment, either at 25 or at 94°. A better weight gain and feed efficiency ratio was always obtained with the standard diets, presenting a similar fiber content (4 and 6%) to that calculated for the coffee pulp diets at the 20 and 30% levels, thus indicating that fiber content is not the only factor affecting such indices. Furthermore, the standard diet, to which 10% fiber was added to simulate the fiber content of those diets with 50% coffee pulp, caused no mortality and induced a much better feed efficiency ratio than those rations containing 50% of either untreated or treated coffee pulp.

The mortality rate (6/6) of the animals fed the diet prepared with dehydrated coffee pulp at the 50% level was significantly decreased (1/6) when coffee pulp was treated by percolation at 94°. This did not occur (5/6), however, when the decaffeination was effected at 25°, a fact indicating that temperature had an effect in solubilizing the toxic substances present in the coffee pulp. Nevertheless, when the coffee pulp decaffeinated at 25° was subjected to a complementary alcohol extraction treatment, mortality at the 50% level was significantly reduced reaching the

Table III. Chemical Analysis of Coffee Pulp Treated by Different Procedures (%)

	Percolation	Extracted at 25°	Extracted at 25° + alcohol	Alcohol extraction
Water	6.21	7.02	8.43	8.63
Ether extract	4.50	5.03	2.02	2.43
Crude fiber	28.11	24.83	23.84	17.62
Protein (N × 6.25)	11.19	11.25	9.75	11.19
Ash	2.71	3.43	3.81	7.60
Caffeine	0.02	0.31	0.29	0.76
Tannins	0.53	1.81	0.93	1.00
Chlorogenic acid	0.35	1.42	0.35	0.90
Caffeic acid	0.00	0.35	0.00	0.10
Total caffeic acid	0.18	1.06	0.18	0.56
Total soluble sugars	1.54	3.70	3.21	6.04

Table IV. Extraction Efficiency of Coffee Pulp by Different Procedures

Extraction procedures	Caffeine extd, %	Total solids extd, %
Alcohol	69.53	19.10
Water (25°)	78.11	28.33
Water (25°) + alcohol	84.65	35.50
Percolation	99.06	29.01

same values obtained when providing rations prepared with the percolated coffee pulp. The feed efficiency ratio, obtained with the coffee pulp decaffeinated at 25° plus a complementary alcohol treatment, was significantly ($P < 0.01$) lower than that determined for the percolated coffee pulp; this finding indicates the beneficial effects derived from the alcohol treatment of the pulp, already reported by Jaffé and Ortiz (1952). However, when coffee pulp subjected only to the alcohol treatment was administered to the rats at the 50% level, the feed efficiency ratio was significantly ($P < 0.05$) higher than that found for coffee pulp decaffeinated at 25° and subjected to a complementary alcohol treatment. Likewise, mortality with the former diet (3/6) was greater than that induced by the latter (1/6), a finding that once more demonstrates the beneficial effects of the water extraction treatment.

Analysis of the data did not reveal any correlation between the mortality caused by the diets containing 50% of the untreated or treated coffee pulps and their caffeine content. Nevertheless, a high correlation was found between mortality and the tannin, chlorogenic, and total caffeic acids content (r = 0.92, 0.94, and 0.97, respectively), demonstrating the possible use of such substances as indicators of the degree of detoxification of coffee pulp.

It is of interest to note that one of the symptoms of toxicity of coffee pulp, which consists in the loss of hair by the animals, tended to disappear as the tannins, chlorogenic, and total caffeic acid levels were reduced. This finding is particularly interesting in view of the fact that caffeic acid has been reported to produce low systemic toxicity and a sensitization type of dermatitis in humans (Stecher, 1968).

Based on the above statements, and considering that decaffeination of coffee pulp by percolation at 94° can produce higher extraction yields in a much shorter time than those reported for green coffee grains (Sivetz, 1963), we consider that the decaffeination process offers good possibilities both to industrialize and to detoxify coffee pulp. The fact that this process can be applied to milled-fresh as well as to the milled-dehydrated material, producing equal extraction yields, further supports this possibility.

In addition, preliminary results indicate that coffee pulp is a relatively easy to dry material, a quality that offers the alternative of dehydrating it prior to its processing, when such step is considered necessary due to needs of transportation, storage, or the like.

Table V. Mineral Content of Dehydrated Coffee Pulp and Treated by Different Procedures (mg %)

Minerals	Dehydrated at 75°	Percolated	Extd at 25°	Extd at 25° + alcohol	Alcohol extd
Calcium	329	350	369	517	332
Phosphorus	65	152	130	130	161
Iron	26	33	25	26	19
Sodium	53	32	29	10	29
Potassium	2903	950	1051	1051	2102

Table VI. Biological Characteristics of Dehydrated Coffee Pulp and Subjected to Different Extraction Treatments, Compared to Those of the Standard Diet at Different Fiber Levels

Diet	Av wt gain,[a] g/rat per 28 days	Av feed consump., g/rat per 28 days	Feed efficiency ratio[b]	Mortality
Basic diet	176	370	2.13	0/6
Basic diet + 4% fiber	156	348	2.27	0/6
Basic diet + 6% fiber	157	361	2.34	0/6
Basic diet + 10% fiber	145	370	2.47	0/6
Dehydrated coffee pulp (20%)	92	358	4.08	0/6
Dehydrated coffee pulp (30%)	48	273	5.61	2/6
Dehydrated coffee pulp (50%)				6/6
Pulp decaffeinated at 25° (20%)	144	350	2.47	0/6
Pulp decaffeinated at 25° (30%)	96	271	2.95	0/6
Pulp decaffeinated at 25° (50%)				5/6
Pulp decaffeinated at 94° (20%)	141	412	2.91	0/6
Pulp decaffeinated at 94° (30%)	120	395	3.12	0/6
Pulp decaffeinated at 94° (50%)	28	253	8.94	1/6
Pulp decaffeinated at 25° + alcohol extraction (50%)	73	334	4.71	1/6
Alcohol-extracted pulp (50%)	58	337	5.97	3/6

[a] Average initial weight, 58 g. [b] Feed efficiency: feed intake/weight gain.

The economic feasibility of the whole process is presently under study. The possibility of using the final extraction residue as a substrate for single-cell protein production is also under active investigation. If this proves to be the case, a final product with a lower fiber and higher protein content could be obtained, with the desirable characteristics in a material intended for the production of concentrates for monogastric animals.

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The Use of Activated Charcoal to Remove or Inactivate Mouse Growth Inhibitors Present in Soybean Whey

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The whey solution (pH 4.4 supernatant) from raw soybean meal was subjected to charcoal filtration procedures as a means of finding a practical method of removing or inactivating the animal growth inhibitors present in this soybean fraction which is currently discarded as a polluting waste product. Filtering the pH 4.4 supernatant through activated charcoal removed or inactivated more than 98% of the trypsin inhibitors, while about half of the carbohydrates and one-quarter of the starting protein remained in the filtrate. When fed in diets to mice, this filtrate slightly reduced their weight gains indicating most of the growth inhibitor activity in the pH 4.4 supernatant was also removed or destroyed. Washing the charcoal successively with 0.3 *N* NaOH, 0.15 *N* NaOH, and 0.2 *N* HCl resulted in the recovery of additional quantities of protein and carbohydrates. The growth inhibitor activity of these fractions was low and diluted enough so as not to cause any marked reductions in growth rates of mice. The protein elution patterns on Sephadex G-25 of the various charcoal filtrated fractions indicated that the charcoal filtrate contained mostly large molecular weight proteins (>than 5000 molecular weight), whereas the 0.30 and 0.15 *N* NaOH filtrates contained primarily peptide and amino-sized compounds, and the 0.2 *N* HCl filtrate contained a small amount of various sized protein and peptides. This treatment scheme of the soybean whey fraction appears to be an effective practical method for recovering a substantial portion of its nutrients for human or animal food use, and at the same time removing or destroying virtually all of the trypsin inhibitors and most of the animal growth inhibitors present in this soybean fraction.

The poor nutritive value of raw (unheated) soybean meal has been attributed to several heat labile components which reduce animal growth (Liener and Kakade, 1969; Mickelsen and Yang, 1966). Most of these compounds are contained in the soybean whey solution, which is the water and acid (pH 4.0–4.5) soluble fraction remaining after acid precipitating most of the soy proteins (Rackis *et al.*, 1963; Schingoethe *et al.*, 1970). The soybean whey contains 20–33% of the total solids and 5–8% of the total protein present in the original soybean meal (Rackis *et al.*, 1963; Schingoethe *et al.*, 1974), that is presently discarded as a waste product of soy protein processing because of these growth inhibitor problems. The objectives of this research were (1) to devise a practical method of separating the proteins and peptides in soybean whey from the carbohydrates, and (2) to devise a practical method of removing or inactivating the growth inhibitors present in the soybean whey solution. The ultimate results could make possible the utilization of at least part of the currently wasted soy by-product for animal or human foods.

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MATERIALS AND METHODS

Preparation of Soybean Fractions. Soybean whey solution (pH 4.4-S) was prepared from Corsoy variety soybeans by previously described methods (Schingoethe *et al.*, 1970). Fractionation using activated charcoal proceeded as outlined in Figure 1. One liter of the pH 4.4-S was mixed thoroughly with approximately 30 g of activated charcoal (U.S.P., powder, Mallinckrodt Chemical Works, St. Louis, Mo.) before filtering and the resulting filtrate (charcoal filtrate) saved for analysis and growth assays. The charcoal was then washed in order with equal vol-