carotenoid is difficult to separate chromatographically from the trollixanthin-like substance, which usually is found both as all-trans and as cis isomers, hence may sometimes be overlooked in the presence of somewhat greater quantities of the trollixanthinlike substances.

Nearly all subfractions obtained on chromatographing the diether diol peel fraction, three from the polyol peel fraction, and one from the monoether diol peel fraction, had very high single peaks on the spectrophotometer curves in the region of 300 to 325 mμ (in benzene). In the diether diol fractions, these peaks were successively at 314, 325, 314, and 303 mμ; in polyols at 300, 319, and 324 mμ; and in the monoether diol at 313 mμ. Similar peaks did not occur in the corresponding pulp fractions, but they had been found previously in fractions obtained from orange peel (*4*). These peaks may be caused by some of the less-volatile, peel-oil constituents, containing three or four conjugated double bonds.

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NUTRIENTS IN FROZEN FOODS

Amino Acids in Nine Frozen Vegetables

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Ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) were determined on composites of 102 sets of samples, representing 1953 and 1954 production seasons for nine frozen vegetables. Sample packages were taken from commercial processing lines of freezing plants in all United States production areas. Samplings were performed at statistically predetermined intervals to provide representative samples.

To supplement the limited data in the literature on nutrients in frozen foods, the National Association of Frozen Food Packers has undertaken the sponsorship of a comprehensive program in nutritional research. This report gives the results of the determination of ten amino acids in nine frozen vegetable products, which contain appreciable amounts of protein. Although arginine and histidine are not considered by all investigators to be essential for humans, they have been included in most previous work on fresh and prepared foods and, for this reason, were also included in this study. A previous paper from this laboratory by Burger and coworkers (*1*) reported the vitamin, mineral, and proximate composition of 51 frozen fruits, juices, and vegetable products, including the nine frozen vegetables used in this study.

Sampling and Sample Preparation

The statistical sampling plan employed has been described by Schmitt and Jessen (*6*). Packages were taken from commercial processing lines of freezing plants in all United States production areas. The samples were taken at statistically predetermined intervals to provide for representative sampling with regard to variables in weather, varieties, harvesting, processing, packaging, and grades. The coded sets were shipped under 0° F. refrigeration to a cold storage warehouse in Madison, Wis.

One hundred and two sets of samples were subjected to amino acid analysis. Each set was made up of an average of 38 packages of consumer-size frozen food packages. As described by Burger and associates (*1*), the sample sets were ground and thoroughly mixed, and assays on the frozen slurry were run as expeditiously as possible.

Assay Methods

Methods for proximate analysis have been described (*1*). The amino acids were determined by the microbiological method of Henderson and Snell (*2*). Tryptophan hydrolyzates were prepared by autoclaving the samples for 15 hours at 121° C., using 20 ml. of 5*N* sodium hydroxide per gram of protein. Com-

Table I. Proximate Composition of Frozen Vegetables

Frozen Food	No. of Sets	Solids, %	Ash, %	Ether Ext., %	Protein,[a] %	Carbohydrate, % Crude fiber	Carbohydrate, % Total (by difference)
Beans, baby lima	12	32.2	1.42	0.18	7.61	1.88	23.0
Beans, Fordhook lima	15	27.4	1.46	0.11	6.21	1.68	19.6
Broccoli spears	13	9.3	0.69	0.20	3.35	1.06	5.1
Brussels sprouts	11	11.5	0.84	0.15	3.28	1.19	7.2
Collard greens	3	11.0	1.04	0.38	3.27	1.01	6.3
Corn, cut	12	23.5	0.48	0.55	3.13	0.54	19.3
Peas, black-eyed	4	34.7	1.38	0.35	8.90	1.52	24.1
Peas, green sweet	26	19.0	0.72	0.31	5.30	1.83	12.7
Potatoes, French fried	6	36.8	1.06	6.08	2.63	0.56	27.0

[a] N × 6.25.

Table II. Grams of Amino Acids per Gram of Total Nitrogen

Frozen Food			Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Beans, baby lima	(12)[a]	Av.	0.41	0.22	0.41	0.47	0.42	0.06	0.31	0.27	0.07	0.40
		Max.	0.48	0.30	0.46	0.59	0.52	0.07	0.39	0.32	0.08	0.46
		Min.	0.32	0.17	0.35	0.38	0.35	0.05	0.25	0.24	0.06	0.36
Beans, Fordhook lima	(15)	Av.	0.43	0.21	0.42	0.49	0.39	0.06	0.29	0.25	0.08	0.39
		Max.	0.56	0.26	0.52	0.54	0.46	0.08	0.42	0.30	0.09	0.46
		Min.	0.30	0.12	0.36	0.42	0.32	0.05	0.18	0.21	0.07	0.31
Broccoli spears	(13)	Av.	0.32	0.11	0.25	0.27	0.35	0.07	0.17	0.19	0.07	0.27
		Max.	0.42	0.14	0.31	0.37	0.41	0.10	0.21	0.30	0.09	0.32
		Min.	0.20	0.08	0.19	0.19	0.23	0.06	0.13	0.10	0.05	0.17
Brussels sprouts	(11)	Av.	0.37	0.14	0.23	0.27	0.27	0.06	0.17	0.22	0.07	0.28
		Max.	0.42	0.17	0.29	0.35	0.31	0.07	0.21	0.37	0.08	0.46
		Min.	0.30	0.11	0.16	0.22	0.24	0.05	0.12	0.16	0.06	0.15
Collard greens	(3)	Av.	0.37	0.15	0.39	0.46	0.35	0.08	0.26	0.28	0.11	0.39
		Max.	0.41	0.16	0.57	0.48	0.41	0.08	0.34	0.31	0.12	0.43
		Min.	0.32	0.13	0.27	0.43	0.29	0.07	0.20	0.24	0.10	0.33
Corn, cut	(12)	Av.	0.24	0.17	0.27	0.67	0.29	0.13	0.26	0.26	0.05	0.35
		Max.	0.32	0.21	0.31	0.83	0.32	0.17	0.37	0.40	0.07	0.46
		Min.	0.10	0.09	0.20	0.46	0.26	0.09	0.18	0.19	0.04	0.28
Peas, black-eyed	(4)	Av.	0.48	0.20	0.37	0.48	0.43	0.09	0.35	0.23	0.08	0.40
		Max.	0.53	0.22	0.47	0.49	0.51	0.11	0.38	0.27	0.09	0.49
		Min.	0.45	0.18	0.29	0.45	0.35	0.07	0.32	0.21	0.07	0.35
Peas, green sweet	(26)	Av.	0.77	0.12	0.29	0.34	0.40	0.05	0.22	0.28	0.05	0.30
		Max.	0.96	0.17	0.45	0.40	0.58	0.07	0.31	0.49	0.09	0.35
		Min.	0.62	0.09	0.15	0.26	0.29	0.04	0.16	0.20	0.04	0.24
Potatoes, French fried	(6)	Av.	0.35	0.12	0.33	0.37	0.37	0.08	0.25	0.33	0.09	0.36
		Max.	0.45	0.15	0.56	0.44	0.42	0.10	0.36	0.44	0.11	0.44
		Min.	0.25	0.11	0.26	0.27	0.32	0.05	0.19	0.20	0.06	0.28

[a] Number of sample sets included in average.

Table III. Milligrams of Amino Acids per 100 Grams of Product

Frozen Food			Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Beans, baby lima	(12)[a]	Av.	492	263	492	577	500	70	386	333	89	490
		Max.	570	347	580	720	576	79	502	435	110	624
		Min.	330	220	440	384	418	59	280	300	77	436
Beans, Fordhook lima	(15)	Av.	430	211	413	486	391	59	283	245	76	381
		Max.	548	262	480	610	480	75	400	300	90	479
		Min.	300	120	350	400	320	50	175	214	62	320
Broccoli spears	(13)	Av.	171	61	134	144	185	38	93	99	37	147
		Max.	225	78	158	200	232	46	116	150	44	176
		Min.	100	50	101	120	160	30	73	60	30	92
Brussels sprouts	(11)	Av.	196	73	120	139	143	31	90	114	37	145
		Max.	220	86	150	180	160	39	137	192	42	230
		Min.	159	62	83	120	126	25	65	83	30	80
Collard greens	(3)	Av.	191	76	217	242	184	40	132	143	54	204
		Max.	238	95	360	300	264	46	150	150	60	275
		Min.	167	65	112	176	138	32	105	128	49	166
Corn, cut	(12)	Av.	118	83	135	336	142	66	133	128	24	174
		Max.	150	115	190	520	168	89	200	196	30	223
		Min.	54	50	108	240	124	50	80	90	16	142
Peas, black-eyed	(4)	Av.	703	288	516	685	614	123	495	329	109	573
		Max.	800	345	600	750	768	144	500	350	120	753
		Min.	570	240	450	580	544	104	490	306	88	460
Peas, green sweet	(26)	Av.	647	104	243	286	341	42	182	232	44	251
		Max.	769	145	350	380	456	60	250	375	66	321
		Min.	500	75	112	200	240	33	135	175	30	200
Potatoes, French fried	(6)	Av.	146	51	139	154	153	32	108	136	37	153
		Max.	200	62	250	182	176	37	160	163	40	200
		Min.	126	42	103	108	141	27	72	90	29	108

[a] Number of sample sets included in average.

plete racemization was assumed and all tryptophan values in the tables are doubled to correct for this (4). For the other amino acids, samples were hydrolyzed by the same autoclaving procedure with 40 ml. of 3N hydrochloric acid used per gram of protein. Hydrolyzates were neutralized, filtered to remove the humin (3), and stored under toluene at 5° C.

Lactobacillus delbrueckii 3 was used in the assay of arginine, *Streptococcus faecalis* R for threonine, and *Lactobacillus arabinosus* 17-5 for tryptophan. *Leuconostoc mesenteroides* P-60 was used for histidine, isoleucine, leucine, lysine, methionine, phenylalanine, and valine. United States Pharmacopeia reference standards were used in all assays except for arginine and histidine, for which commercial products were used.

Results and Discussion

Table I gives the proximate composition of the frozen vegetables used in this study. The amino acid assay results are presented in Tables II and III. In Table II the results are expressed as grams of amino acid per gram of total nitrogen in the frozen food. Table III gives the results in milligrams of amino acid per 100 grams of product. The figures in Table III represent 1.06 times more than the amounts present in a serving consisting of one third of a 10-ounce package of the vegetable.

The tables give average, maximum, and minimum values for each group of vegetables. Values which deviated markedly from the average were checked by repeat assay.

The legumes are highest in protein content and, on an absolute basis, tend to be higher in the amino acids studied (Table III). However, on the basis of grams of amino acid per gram of nitrogen the amino acid pattern in the various products is similar.

The biological protein value of diets is determined by the combination of available amino acids in each meal. It is important to provide, in a proper balance, those amino acids which the body cannot synthesize in adequate amounts. Other amino acids may have a sparing action on these "essential" amino acids. Qualitative studies have established the fact that valine, leucine, isoleucine, methionine, threonine, phenylalanine, tryptophan, and lysine are required in the diet for maintenance of nitrogen equilibrium and prevention of subjective symptoms in normal adult man; a beginning has been made toward quantitative measurement of minimal requirements to provide a basis for estimation of a "safe" intake (5). The vegetable products included in this study are ordinarily consumed in a mixed diet with other protein foods. The amino acid data reported herein will facilitate the estimation of the supplementary protein value of these products.

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MOLASSES FERMENTATION

Continuous Fermentation

Alcoholic Fermentation of Blackstrap Molasses

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Continuous alcoholic fermentation has been studied intensively and various patents have been granted for alcohol production (8, 13, 15, 16, 19-21, 23, 27).

Alzola (2) described a continuous fermentation process in which the sterilized mash goes through successive fermentors connected in a series. The tanks were agitated by the carbon dioxide produced during the process. In 1945, he studied a new continuous process (3), using a column divided into six parts. The mash was introduced into the bottom of the column and also agitated by the carbon dioxide produced.

The possibility of performing the continuous fermentation in a single flask was first investigated by Bilford and coworkers (6) in 1942, who carried out the experiments on a laboratory scale, thus reducing equipment requirements. Owen (26), in 1948, used a glass column divided in six parts; the mash was fed continuously into the top of the column. The continuous fermentation of beet juice was described and discussed by Mariller and coworkers (22). The study of continuous alcoholic fermentation using two connected fermentors was started by Asai and coworkers (4, 5). Borzani (9) examined the economical aspects of continuous alcoholic fermentation of molasses using a single fermentor and mechanical agitation. The work was carried out on a pilot-plant scale with the same equipment used in the batch process.

The striking advantages that a continuous fermentation has over the corresponding batch process (24) justified a systematic study of the factors involved. The following factors, that influence the continuous alcoholic fermentation of blackstrap molasses in a single vessel, are considered in this paper: sugar concentration of feed mash, feed rate, agitator speed, and fermentor capacity.

Apparatus

The equipment shown in Figure 1 consisted of two steel fermentors: 100-liter capacity (45 cm. in diameter by 80 cm. in height) and 1800-liter capacity (120 cm. in diameter by 188 cm. in height). The agitators in Figures 2