sponse is achieved. The effects of moisture on flavor release are especially critical in drying operations (Flink and Karel, 1972; Flink and Labuza, 1972; Rulkens and Thijssen, 1972a,b) and in the development of intermediate moisture foods. In addition, flavor microencapsulation techniques would benefit from a knowledge of these interactions.

The observed results are attributed to surface area and solubility effects, but they could be the result of molecular interactions between the flavor and the protein. Hence, flavor binding by protein in these model systems merits further study. Lipids and carbohydrates bind flavors (Maier, 1970; Nawar, 1966, 1971) which was demonstrated in this work by the direct correlation between the magnitudes of flavor binding by the various soy protein forms and their carbohydrate and lipid compositions. The headspace volatility of the flavors also increased upon the removal of the lipids from the LPC. The proteins did not bind the carbonyls in the corn oil solvent. This could be the result of the solubility of the lipophilic flavors in the oil so that they were less accessible to the protein whose solubility in oil was reduced compared to that in water.

Thus, in this study the effects of protein on the headspace volatilities of aldehydes and methyl ketones depended upon the amount, type, and composition of the protein, and the presence of solvents such as water and lipids. These results, however, should be more precisely quantified to aid in establishing the molecular mechanisms of flavor release or binding by proteins.

LITERATURE CITED

Betschart, A. A., Kinsella, J. E., *J. Agr. Food Chem.* **21**, 60 (1973).
Flink, J. M., Karel, M., *J. Food Technol.* **7**, 199 (1972).
Flink, J. M., Labuza, T. P., *J. Food Sci.* **37**, 617 (1972).
Jennings, W. G., *J. Food Sci.* **30**, 445 (1965).
Kinsella, J. E., *Chem. Ind. (London)*, 550 (1969).
Maier, H. G., *Angew. Chem., Int. Ed. Engl.* **9**, 917 (1970).
Nawar, W. W., *Food Technol.* **20**, 213 (1966).
Nawar, W. W., *J. Agr. Food Chem.* **19**, 1057 (1971).
Nelson, P. E., Hoff, J. E., *J. Food Sci.* **33**, 479 (1968).
Rulkens, W. H., Thijssen, M. A. C., *J. Food Technol.* **7**, 79 (1972a).
Rulkens, W. H., Thijssen, M. A. C., *J. Food Technol.* **7**, 95 (1972b).
Vananuvat, P., Kinsella, J. E., unpublished data, 1973.
Wientjes, A. G., *J. Food Sci.* **33**, 1 (1968).

Received for review June 13, 1973. Accepted March 4, 1974.

Composition and Nutritive Value of Cashew Nut to the Rat

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The proximate composition, amino acids, and protein quality of good grade and discarded cashew nut meals were studied. The crude protein in both sources was high and on oil extraction produced meals with comparable protein levels to those of peanut and soybean meals. The total amount of sulfur amino acids in the good grade meal was higher than in soybean, while these amino acids in both meals were higher than in peanut. The lysine and threonine levels were, however, lower than in soybean but comparable to those in peanut. The good grade meal was better digested and showed superior quality to the discarded kernel meal. Rats fed the good grade meal did not respond to methionine supplementation, but showed significant response to lysine, indicating an adequacy of the sulfur amino acids but a low available lysine level. Lysine but not methionine supplementation significantly improved the quality of the discarded kernel meal.

The desire for improved animal productivity, as well as better nutrition of the vast and fast-increasing population of the tropical and sub-tropical areas of the world, has given considerable impetus to evaluatory tests aimed at finding uses for some of the minor oilseeds and oilseed residues, quite distinct from the soybean, peanut, and cottonseed, which have long attained commercial importance, and for which nutritive values are fairly well documented. The cashew nut (*Anacardium occidentale*) is one oilseed with great potential and increasing commercial value, capable of joining the ranks of those mentioned above. The processing of the raw nut is now being carried out in many of the producing countries. In many of those only 60–65% are of commercial value while 35–40% of the nuts reaching the factory would be discarded either as broken kernels or as kernels scorched in the roasting process.

There has been limited work done on the nutritional qualities of cashew nuts. One study (Piva *et al.*, 1971) showed the extracted meal of some Tanzanian commercial grade kernel to be of high nutritive value and comparable to soybean meal. The work reported here was carried out to evaluate the discarded broken and scorched nuts in comparison with the good grade nuts, bearing in mind that while the good grade nuts and their extraction meal may find use in human nutrition, the discarded nuts could be a cheap source of protein in livestock feeding.

EXPERIMENTAL SECTION

Two grades of cashew nut meals were employed in these studies. A good grade kernel meal and a discarded kernel meal were both obtained as the unextracted kernels from the Western Nigeria Development Corporation (WNDC) processing factory in Ibadan, Western Nigeria. The processing treatment at the factory involved roasting of the whole nuts for 90 sec in cashew nutshell liquid (a caustic, vesicant liquid, primarily made up of mono- and dihydroxyalkylbenzenes and alkylphenolic acids (Kaufmann and Barve, 1967) obtained from the spongy mesocarp of the cashew nutshell) at 185°. The nuts were then cracked and the kernels dried at 105–110°. Manual peeling and final roasting in peanut oil for 5 min was carried out. Following roasting at 185°, in cashew nutshell liquid, the nuts that were scorched or heat damaged were separated from the undamaged nuts. The kernels from the scorched nuts together with broken pieces of the good grade kernel

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are referred to as the discards, while those that went through to the final grading are referred to as the good grade kernels.

Further treatment of the discard and good grade kernels in our laboratory involved the coarse milling of the samples and oil extraction in glass columns of 15 cm i.d. × 100 cm, using 5 l. of petroleum ether (bp 40–60°) for every 2 kg of sample. After oil extraction samples were further ground to pass through a 30-mm mesh sieve. They were then left in the open air to dry off the residual petroleum spirit, and later stored in screw-capped bottles at −5° until used.

Chemical Analysis. Proximate analyses were carried out on air-dried samples according to AOAC (1970) methods. Gross energy determinations were done with a Gallenkamp oxygen ballistic bomb calorimeter, while the mineral contents were determined by first wet-ashing the samples with a mixture of nitric, perchloric, and sulfuric acids, followed by flaming in a Perkin-Elmer atomic absorption spectrophotometer 290, using different lamps. The phosphovanadomolybdate method was used for the estimation of phosphorus (AOAC, 1970).

Amino Acid Analysis. The total amino acid composition of samples was determined using 100 mg of each sample hydrolyzed with 10 ml of 6 *N* hydrochloric acid in an atmosphere of nitrogen at 110° for 24 hr, using the Automated Hitachi–Perkin-Elmer amino acid analyzer, Model KLA-3B. Tryptophan was chemically determined using the method of Miller (1967).

Biological Evaluation. Biological studies were carried out with the cashew nut (good grade) kernel extracted meal and two samples of cashew nut (discard) extracted meal, with the purpose of comparing the nutritional values of the two products. Freeze-dried, ether-extracted, whole hen's egg was included as a standard reference protein for comparison. Parameters employed included protein efficiency ratio (PER), net protein retention (NPR), protein retention efficiency (PRE), biological value (BV), and net protein utilization (NPU). The apparent and true digestibility of these products were also assessed.

The basal diet contained corn starch (65.0%), glucose (5.0%), sucrose (10.0%), nonnutritive cellulose (5.0%), peanut oil (10.0%), mineral supplement (Miller, 1963) (4.0%), and a vitamin mixture (Miller, 1963) (1.0%). The protein sources were included in the basal diet at the expense of corn starch to the extent that they provided 10% crude protein in the final diet.

Animals and Management. In all a total of 40 male albino rats of the Wistar strain weaned at 21 days and weighing between 42 and 48 g were divided into five groups of eight rats each on the basis of weight and litter origin, such that the mean group initial weights were identical. Each group received either the egg, cashew nut (good grade kernel) extraction meal, or one of the cashew nut (discard) extraction meal samples; the fifth group was placed on the nitrogen-free diet of the same ingredient composition as the basal diet.

The rats were individually housed in perforated perspex cages with facilities for separate fecal and urinary collection. Dry feed and water were available *ad libitum*. Records were kept of weight gains and total feed consumption. The protein and nitrogen consumed were calculated from the total feed intake and protein or nitrogen content of the diet as determined by the Kjeldahl method (AOAC, 1970). The PER, NPR, and PRE were obtained from these data as defined by the National Academy of Sciences–National Research Council (NAS-NRC, 1963).

The NPU_{10} (carcass) values were calculated according to the revised equation of Bender and Doell (1957), using carcass nitrogen values obtained at the end of 10 days, from four rats each from the five groups of eight rats on the different proteins and the nitrogen-free group. For the determination of carcass nitrogen 20 of the 40 rats (4 per treatment) were sacrificed at the end of the 10th day and incisions made into the skull, thoracic, and body cavities. The rats were then dried in a hot air circulation oven at 85° to constant weight. The dissolution of the carcass for nitrogen determination was done by a modified method of Rippon (1958), the only modification being that it was found unnecessary to autoclave the dissolved carcass, as complete disintegration occurred on the addition of distilled water to a slurry of the dried carcass in concentrated sulfuric acid. The resultant dark red freely flowing solution was transferred to a 500-ml volumetric flask and made up to the mark. Duplicate samples were drawn for digestion and nitrogen determination (AOAC, 1970). The remaining groups of four rats per protein source were used to determine the apparent and true digestibility and biological value (BV) of the test protein. The metabolic fecal nitrogen was determined on the remaining four rats on the nitrogen-free diet. The balance studies involved a preliminary period of 7 days on the test diets, followed by a 7-day collection period. The feces and urine were collected in 1% (w/v) sulfuric and acetic acids, respectively, to prevent nitrogen loss. Nitrogen determinations were carried out on the bulked dried feces and urinary samples for each rat. A record of feed intake for the 7-day collection period was also kept and nitrogen or protein intake calculated on the basis of this and the determined nitrogen content of the test diets. The true digestibility, apparent digestibility, and BV were calculated from the relevant data as defined by NAS–NRC (1963).

In a second trial, 64 weanling male albino rats were used to study the effect on the protein quality of adding either 0.15% lysine-HCl and/or 0.20% DL-methionine to either of the two grades of cashew nut meal. Similar techniques and parameters as already described were employed.

All data relating to the protein quality indices and digestibility were subjected to analysis of variance (Steel and Torrie, 1960). Treatment means were compared using the Duncan's multiple range test (Duncan, 1955).

Table I. Comparative Chemical Composition of the Cashew Nut (Good Grade) Kernel Meal and the Cashew Nut Discard Kernel Meal

Item	% good grade kernel		% discarded kernel	
	Undefatted	Defatted	Undefatted	Defatted
Residual moisture	5.5	7.4	4.4	6.5
Crude protein	21.2	40.9	21.6	42.8
Ether extract	48.1	1.3	45.5	1.3
Crude fiber	0.8	1.5	2.3	4.1
Silica-free ash	3.3	5.3	3.8	6.8
Nitrogen-free extract	22.1	44.4	23.4	38.5
Calcium	0.04	0.06	0.03	0.06
Phosphorus	0.88	1.72	0.84	1.64
Sodium	0.005	0.02	0.016	0.03
Potassium	0.57	1.42	0.52	0.98
Magnesium	0.28	0.54	0.24	0.48
Iron	0.008	0.01	0.006	0.009
Copper	0.002	0.006	0.002	0.007
Zinc	0.004	0.009	0.003	0.007
Manganese	0.002	0.004	0.001	0.003
Gross energy, kcal/g	7.76	4.28	7.32	4.14

RESULTS AND DISCUSSION

Table I shows the proximate nutrient composition as well as the minerals in both grades of cashew nut meal.

Both of these sources had crude protein contents of 21.18 and 21.62%, respectively, in the unextracted kernel meal, and when the oil was removed yielded meals with protein contents between 40 and 43% depending on the

Table II. Total Amino Acid Composition of Cashew Nut (Good Grade) Kernel Meal and Cashew Nut Discard Kernel Meal, Compared to Those of Two Commonly Used Oilseed Meals, Peanut and Soybean Meals, and Whole Hen's Egg (Values in g/16 g of N)

Amino acids	Cashew nut meals		Peanut meal[a]	Soybean meal[a]	Whole hen's egg[a]
	Good grade	Discards			
Arginine	10.70	9.87	12.30	7.57	6.10
Histidine	2.06	1.96	3.04	2.68	2.43
Isoleucine	3.86	3.79	3.58	4.58	6.29
Leucine	6.51	6.63	7.09	7.94	8.82
Lysine	4.04	3.86	3.90	6.12	6.98
Methionine	1.40	1.38	0.91	1.25	3.36
Cystine	1.78	1.68	1.14	1.64	2.43
Methionine + cystine	3.18	3.06	2.05	2.89	5.79
Phenylalanine	3.89	3.74	5.60	5.68	5.63
Tyrosine	2.37	2.68	4.34	4.32	4.16
Phenylalanine + tyrosine	6.26	6.42	9.94	10.00	9.79
Threonine	3.10	3.09	3.04	3.82	5.12
Tryptophan	1.37	1.34	1.24	1.26	1.62
Valine	5.80	5.23	4.27	5.51	6.85
Alanine	3.70	3.77	4.19	4.83	5.92
Aspartic acid	9.20	9.13	11.82	11.46	9.02
Glutamic acid	18.74	19.42	21.12	17.70	12.74
Proline	3.72	3.46	5.06	5.10	4.16
Serine	4.76	4.35	5.33	5.32	7.65
Glycine	4.60	4.16	6.30	4.63	3.31

[a] Values reported for these meals were also obtained in our laboratory using similar techniques as described for the cashew nut meals.

efficiency of oil extraction. This level of protein in the residual meal is comparable to that found in other commonly used oilseed meals, like soybean (Wolf, 1970) and peanut meal (Woodroof, 1969).

The percentages of lipid in the undefatted samples of both grades were high and also comparable to those normally encountered in peanut, sunflower seed, and soybean seed.

The meals from the good grade kernel had a low crude fiber content. The level of crude fiber in the discard meal was, however, higher than in the good grade meal, due perhaps to incidental contamination of the discards by pieces of the cashew nutshell but this level was not high enough to be considered detrimental to the digestibility of its protein.

Of all the mineral constituents, phosphorus was the most abundant and its level is even higher than the value for this element in peanut meal (Woodroof, 1969). This was closely followed by the values for potassium, which were identical in both the good grade and discard meals, and higher also than peanut meal. There was, however, a low content of calcium compared to soybean and peanut meal in both the discard and good grade meals. The magnesium contents in these two meals were just about as high as the values recorded for soybean (Markley, 1950) and peanut (Woodroof, 1969). The levels of iron in the two grades of cashew nut meal were considerably higher than the levels encountered in many oilseeds, particularly those of peanut meal, soybean meal, and cottonseed meal. Zinc was, however, low in the cashew nut samples compared to soybean and peanuts (Roach *et al.*, 1968).

The undefatted samples of both sources showed high gross energy values, undoubtedly due to their high oil content. On fat extraction, these values were considerably reduced, but were still comparable to the values for residual meals from the oil extraction of other oilseeds.

Table II shows the total amino acid composition of the cashew nut meals compared to peanut and soybean meals, as well as whole hen's egg. A comparison of the amino acid patterns of the good grade and the discard meal shows very little difference. The lysine and threonine contents of both the discard and the good grade meals in common with peanut were lower than those reported for egg and soybean meal. The good grade meal has a total sulfur amino acid content (methionine + cystine) higher than in soybean meal. Both grades have total sulfur amino acid contents higher than in peanut but lower than in whole egg. They also contain marginally higher quantities of tryptophan than in either peanut meal or soybean but lower than in hen's egg. The other essential amino acids appear to be well represented in the cashew nut meals.

Table III. Comparative Protein Quality of Cashew Nut (Good Grade) Kernel Meal, Cashew Nut Discard Meal, and Freeze-Dried Ether-Extracted Whole Hen's Egg

	Cashew nut good grade	Cashew nut discard		Whole hen's egg
		I	II	
Wt gain at 10 days, g	14.8b[a]	7.1c	3.9d	26.8a
Feed intake, g	67.6	66.0	51.4	27.7
Protein intake, g	7.3	6.36	5.24	6.78
PER	2.01b	1.12c	0.76d	3.94a
NPR	4.01b	3.13c	2.86c	6.04a
PRE	64.2b	50.1c	45.7d	96.6a
NPU	63.0b	46.7c	41.3d	94.0a
BV	68.6b	55.3c	48.9d	98.4a
Apparent digestibiity, %	83.8b	77.9c	77.4c	93.7a
True digestibility, %	91.8b	84.6	84.3	98.4a

[a] Means in the same row not marked by the same letter are significantly different from each other ($P < 0.05$).

Protein Digestibility. Both the apparent and true digestibility values for the two grades of cashew nut are shown in Table III, in comparison with whole hen's egg, a protein source which is nearly always completely digested. The apparent and true digestibility values of egg protein were significantly higher than the values for the good grade kernel meal which were in turn significantly higher than those for the discard kernel meal ($P < 0.05$). The discarded kernels were essentially heat-scorched kernels. It is quite possible that the reduced digestibility of this protein source is associated with heat damage. Ford (1965) and Neishem and Carpenter (1967) have all demonstrated reduced proteolysis of heat-treated cod fillets, *in vitro* and *in vivo*. The above cited literature tends to suggest that the reduced digestibility observed for the cashew nut discard kernel meal may be due to a more severe heat damage of this product when compared to the good grade kernel.

Protein Quality Indices. The different protein quality indices for the different foodstuffs are presented in Table III.

In terms of body weight gains rats receiving the egg diet gained significantly more total weight than those on the cashew nut (good grade) kernel meal which in turn gained significantly ($P < 0.05$) more weight than rats receiving either cashew nut discard meals I or II. The sample of cashew nut discard meal designated II was significantly ($P < 0.05$) poorer than the sample designated I , in terms of the live-weight gain.

The PER, NPR, PRE, NPU, and BV for these protein sources are also shown in Table III. All these parameters for the whole egg were significantly ($P < 0.05$) superior to those for the other protein sources. The two samples of

Table IV. Amino Acid Supplementations of Cashew Nut Scrap Kernel and Good Grade Kernel Extraction Meal; Protein Quality Indices

	Wt gain at 10 days, g	Protein intake, g	PER	NPR	PRE	NPU	BV	AD, %	TD, %
Cashew nut scrap kernel meal + no amino acid	7.2a[a]	6.36	1.12a	3.13a	50.9a	46.0a	54.5a	78.2a	83.9a
Cashew nut scrap kernel meal + 0.15% lysine-HCl	21.3d	8.14	2.51ef	4.10b	65.9b	65.6bc	74.0c	76.4a	84.4a
Cashew nut scrap kernel meal + 0.15% lysine-HCl + 0.20% DL-methionine	17.3bc	7.30	2.39de	4.15b	66.4b	65.0b	77.4c	76.1a	84.9a
Cashew nut scrap kernel meal + 0.20% DL-methionine	8.3a	6.39	1.31b	3.30a	52.9a	50.0a	57.9a	76.9a	86.2a
Cashew nut good grade extraction meal + no amino acids	15.3b	7.30	2.11c	4.06b	64.8b	65.9bc	79.8cd	83.8b	91.4b
Cashew nut good grade extraction meal + 0.15% lysine-HCl	20.6cd	7.60	2.71g	4.42cd	71.8cd	68.4bc	83.2de	82.7b	90.8b
Cashew nut good grade extraction meal + 0.15% lysine-HCl + 0.20% DL-methionine	18.3bcd	6.80	2.67fg	4.57d	73.1d	70.4c	84.8e	84.6b	92.1b
Cashew nut good grade extraction meal + 0.20% DL-methionine	15.1b	6.70	2.23cd	4.25bc	68.1bc	66.4bc	81.6de	84.9b	91.9b

[a] All means in the same column with the same letter are not significantly different ($P < 0.05$).

cashew nut discard kernel meal showed significantly poorer protein quanity indices compared to the good grade kernel meal. These lower values for the discard meal might be attributable to heat damage and reduced digestibility (84.6%) for the discard meal as against the higher value (91.8%) for the good grade kernel meal and hence a reduced availability of the essential amino acids, as explained by Melnick and Oser (1949) who stated that heat treatments reduce the nutritive value of proteins for animals through their effect of reducing the rate of proteolytic attack on the protein *in vivo*. The differences between the discard samples designated I and II in protein quality could also be explained in terms of the differences in the degree of heat scorching and therefore in the extents to which some of the constituent amino acids are rendered unavailable. It was noticed that the drop in protein values reported for the cashew nut discard meals was much more than could be accounted for by the drop in digestibility of the protein, *i.e.*, over a 50% drop in weight gain in one sample and over 74% in another sample. There were more than 40% drops in NPU and other protein quality indices as against a drop of 6% in digestibility units, when comparing the discard kernel meals with the good grade kernel meal. Miller *et al.* (1965) had noticed a similar trend in that the nutritional value of heat-damaged proteins fell considerably more than digestibility. *In vitro* experiments of Ford (1965) indicated some differential release of amino acids from heat-damaged cod fillet, with the residual peptides from the digestion with pepsin and papain showing a particularly high lysine content, thus suggesting that in the intact animal a lot of the lysine in heat-damaged proteins may be lost to the animal in uncleaved peptide residues. Neishem and Carpenter (1967) in *in vivo* studies also with cod fillet showed that the undigested protein and peptides leaving the small intestine are fermented in such a way that nitrogen is absorbed in a form such as ammonia with little nutritional value. Thus, unavailable amino acids in the protein were probably not absorbed although some of their nitrogen might have been released by microorganisms in the lower intestinal tract and thus apparently digested. The above discussion shows that several factors are operative in heat-damaged proteins that are not encountered in nondamaged proteins, and such factors may explain the differentials in quality between the good grade cashew nut kernel meal and the discard meal, which showed identical chemical and amino acid compositions.

Amino Acid Supplementation of the Cashew Nut Meals. Table IV summarizes the results of the protein quality parameters for amino acid supplementation of 10% protein, cashew nut good grade kernel meal, and cashew nut discard kernel meal diets.

Addition of methionine alone to the scrap kernel meal resulted in a slight but nonsignificant increase in the total gains as well as nonsignificant improvements in NPR, PRE, NPU, and BV. The good grade kernel responded even less to additional DL-methionine. Piva *et al.* (1971) had also reported the nonresponse of rats to methionine supplementation of Tanzanian cashew nut meals. It is unlikely that methionine is a limiting amino acid in cashew nut. Moreover, its cystine content (1.68 g/16 g of N) is high compared to most other oilseeds and this, along with the methionine present in the intact protein, might be sufficient to meet the requirements of the rats for the sulfur-bearing amino acids.

Addition of lysine alone and lysine and methionine to the cashew nut discard kernel meal resulted in a 200–300% increase in the total gains at 10 days which was significantly ($P < 0.05$) greater than for the unsupplemented form. Addition of lysine alone and lysine and methionine to the cashew nut good grade kernel meal resulted also in a significant increase in total gain over and above those of rats on the unsupplemented meal. Rats receiving diets of the discard meal with added methionine and lysine gained significantly less weight than those on the discarded meal with added lysine and this appeared to be associated with the lower feed intake of this group. There was a remarkably significant improvement in all of the protein quality indices in the discard meal, while the good grade meal showed smaller but also significant ($P < 0.05$) improvement in the protein quality indices. It would appear that responses to amino acid supplementation were only achieved to any significant extent in those diets to which lysine was added. This seems to suggest that available lysine is a limiting factor in the cashew nut meals studied for rat growth. Much of the lysine may have been bound

in complex forms to the associated carbohydrate, carboxylic groups of glutamic or aspartic acids, amide groups, or oxidation products of the associated lipids. The more severe heat treatment and damage to the cashew nut discard kernel might thus explain the differences between the discard and the good grade kernel, which had received a milder heat treatment, in terms of overall protein quality and the different extent to which they responded to amino acid supplementation.

It appears that cashew nut discard meal could be of considerable value in diets, provided its production is carefully controlled to minimize heat damage. Its value in practical-type diets therefore needs to be more carefully studied.

ACKNOWLEDGMENT

The guidance and help of J. K. Loosli in the preparation of this manuscript is gratefully acknowledged.

LITERATURE CITED

Association of Official Analytical Chemists, Official Methods of Analysis, 11th ed, Washington, D. C., 1970.
Bender, A. E., Doell, B. H., *Brit. J. Nutr.* **11**, 140 (1957).
Duncan, D. B., *Biometrics* **11**, 1 (1955).
Ford, J. E., *Brit. J. Nutr.* **19**, 277 (1965).
Kaufmann, H. P., Barve, J., *Fette Seifen Anstrichm.* **69**, 437 (1967).
Markley, K., "Soybean and Soybean Products," Interscience, New York, N. Y., 1950.
Melnick, D., Oser, B. L., *Food Technol.* **3**, 57 (1949).
Miller, D. S., *Nat. Acad. Sci.-Nat. Res. Counc. Publ.* **No. 1100**, 34 (1963).
Miller, E. L., *J. Sci. Food Agr.* **18**, 381 (1967).
Miller, E. L., Carpenter, K. J., Milner, C. K., *Brit. J. Nutr.* **19**, 547 (1965).
Neishem, M. C., Carpenter, K. J., *Brit. J. Nutr.* **21**, 399 (1967).
Nat. Acad. Sci.-Nat. Res. Counc. Publ. **No. 1100** (1963).
Piva, G., Santi, E., Ekpenyong, T. E., *J. Sci. Food Agr.* **22**, 22 (1971).
Rippon, W. P., *Brit. J. Nutr.* **13**, 243 (1958).
Roach, A. G., Sanderson, P., Williams, D. R., *Analyst* **93**, 42 (1968).
Steel, G. D., Torrie, J. G., "Principles and Procedures of Statistics," McGraw-Hill, New York, N. Y., 1960.
Wolf, W. J., *J. Agr. Food Chem.* **18**, 969 (1970).
Woodroof, J. G., *World Rev. Nutr. Diet.* **11**, 142 (1969).

Received for review July 22, 1973. Accepted February 5, 1974.

Loss of Free-Radical Signal during Induction Period of Unsaturated Lipids Containing Nitroxide Antioxidants

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Stable free-radical nitroxides have marked antioxidant activity in unsaturated lipids. The amount of residual nitroxide can be estimated from the electron paramagnetic resonance (epr) signal. With three different nitroxides in squalene at 37° the signal gradually decreased during the induction period. Only when it was no longer or barely detectable, did active uptake of oxygen begin. At 50° with squalene and with menhaden oil, active oxidation began while the nitroxide signal was still measurable. In squalene at 37°, the three nitroxides studied, Tempol (2,2,6,6-tetramethyl-4-piperidinol-*N*-oxyl), Synvar 611 (4′,4′-dimethylspiro[5α-cholestane-3,2′-oxazolidin]-3′-yloxyl), and Synvar 614 (2-[10-carboxydecyl]-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl), had relative antioxidant activities at equivalent molarities of approximately 2:1.2:1. A simplified method for following weight gain and epr signal without transfer of sample is described.

Free radicals are involved in the autoxidation of lipids, but their half-lives are usually so short that they cannot easily be detected by electron paramagnetic resonance (epr) spectroscopy. We have taken advantage of the fact that stable free-radical nitroxides have antioxidant activity (Weil *et al.*, 1968) to study their fate during the induction period. In general the oxidation of the lipid was inhibited until the signal was no longer detectable. Our observations thus parallel those quoted by Rozantsev (1970) concerning the thermal oxidation of certain polymers: "... radical inhibitors completely block the oxidation of polyamide until they are exhausted, and, after the end of the induction period, the rate of oxidation approximates the rate of the uninhibited oxidation of the polyamide (Neiman *et al.*, 1965)."

MATERIALS AND METHODS

The nitroxides used were Synvar 611 (4′,4′-dimethylspiro[5α-cholestane-3,2′-oxazolidin]-3′-yloxyl), Synvar 614 (2-[10-carboxydecyl]-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl), and Tempol (2,2,6,6-tetramethyl-4-piperidinol-*N*-oxyl). The first two were from Synvar Associates and the third was a gift of A. Keith. Some observations on Tempol as an antioxidant appeared in a previous publication (Weil *et al.*, 1968). The substrates for antioxidant assays were squalene (Eastman) and a sample of menhaden oil that had been molecularly distilled (National Marine Fisheries Service, Seattle, Wash.). Each was further purified before use by silicic acid chromatography (Olcott and Van der Veen, 1968).

The methods used for evaluating antioxidant activity and measuring epr spectra including the following.

Method 1. Watch-glass covered 10-ml beakers containing 200 mg of substrate with and without additive were held in constant temperature draft ovens. Once or twice daily they were removed from the oven, tested for rancidity by odor, cooled to room temperature, and weighed (Olcott and Einset, 1958). At intervals an approximately 50-μl sample was transferred to a quartz tube, the epr spectra were measured with a Varian Model E-3 X-band spectrometer, and the sample was returned to the beaker. Signal intensities were determined from the height of the center line of the three-line nitroxide spectra.

Method 2. Halves of filter papers (Whatman No. 1, 4.25 cm) were rolled into small cylinders and inserted into

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