Extrinsic Tagging in Iron Bioavailability Research: A Critical Review

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Radiolabel techniques have gained acceptance as a powerful tool for measurement of iron bioavailability from foods. Intrinsic tagging, growing a food to incorporate a radioiron label, has become less common as extrinsic tagging increases in applications. The extrinsic tag technique, adding an exogenous radiolabeled iron spike to a test diet, assumes that a complete exchange of the extrinsic spike takes place with the iron naturally present in the food. This exchange has been verified under some conditions encountered in various foods and diets but has not been proven for all conditions encountered in foods. This review critically examines studies which have been conducted to validate the extrinsic tag in food systems. Problems that remain to be solved include conditions under which a complete exchange may fail and the effects of different foods and processes on the exchange.

Iron deficiency anemia is a widespread health problem throughout the world, despite the fact that the average daily diet contains iron far in excess of the amount needed for metabolic purposes. This is attributed to the low bioavailability of food iron. Bioavailability is a measure of that portion of iron in the diet which is capable of being absorbed by the gastrointestinal tract and subsequently stored or incorporated into heme. The Food and Nutrition Board, on compiling the RDA's, assume a 10% absorption, but this value can range from 0 to 50% (Lee and Clydesdale, 1979). Some of the dietary iron may be in a chemical form that is poorly absorbed. In addition, the diet may contain ligands or anions which can bind iron and inhibit its absorption. To ensure that a diet is adequate in iron, an accurate assessment of iron bioavailability is needed. This need has become even more apparent as rapid advancements in food technology and processing continue to provide an increasing number of new food products with varied composition (Smith and Rotruck, 1981).

Measurement of Iron Bioavailability. Various methods have been used to measure iron absorption from food. The chemical balance technique, i.e., difference between food and feces, is a method which directly measures iron absorption from the whole diet (Moore, 1968). But chemical balance may be too elaborate or costly to be used other than to study special problems or diets. It also gives little information about iron absorption from different meals within the same study.

Studies using intrinsically radiolabeled foods have provided useful information about the bioavailability of iron from specific foods (Moore and Dubach, 1951), but this kind of approach also has limitations (Bjorn-Rasmussen et al., 1974; Cook et al., 1972). It is not a valid measurement of iron absorption from the whole diet. This method is also expensive and requires the preparation of intrinsically labeled foods.

The extrinsic tag technique has recently become popular in iron bioavailability research. This technique has been used to measure iron absorption from both composite meals and from a whole diet. It has also been used to identify various factors which can influence the absorption of dietary iron and to evaluate the effectiveness of various intervention strategies.

The use of extrinsic tagging, however, has not been validated for all foods. There are indications that this technique may not work under all conditions encountered in complex food systems. The purpose of this review is to critically examine studies that have been conducted to validate the extrinsic tag technique and to encourage research on the different conditions present in various foods which may affect the reliability of this method. The extrinsic tag technique as described here is used only in the measurement of nonheme iron. The measurement of heme iron by the intrinsic tag technique has also been achieved (Bjorn-Rasmussen et al., 1974).

Extrinsic Tag Method. In the extrinsic tag method a trace amount of radiolabeled iron (extrinsic tag), usually $^{59}FeCl_3$ or $^{59}FeSO_4$, is added to a test diet. Bioavailability is measured by extrapolating the amount of radioiron absorbed to the quantity of iron dosed (Cook et al., 1972). The extrinsic tag method relies upon a complete isotopic exchange between the extrinsic tag and the endogeneous nonheme (intrinsic) iron in the diet (Hallberg and Bjorn-Rasmussen, 1972). It has been proposed that a common nonheme iron pool is formed by foods ingested in the same meal (Bjorn-Rasmussen et al., 1973; Cook et al., 1972). Isotopic exchange between native iron and the extrinsic tag is believed to occur within a common active segment of the pool called the "isotopic exchange pool". Thus, some researchers theorize that differences in the bioavailability of iron from single foods may be considered as differences in the relative size of the isotopic exchange pool (Hallberg, 1974).

The addition of an extrinsic tag to a standard meal was first proposed by Pirzio-Biroli et al. (1958) as a practical approach to studying clinical abnormalities in the absorption of food iron, but they did not advocate that this method be used to measure iron absorption from the whole meal. A study by Schulz and Smith (1958) provided the first evidence that the absorption of an added radioiron tag might accurately reflect the absorption of food iron. In their study they compared the absorption of biosynthetically labeled milk iron (intrinsic) with that of an added radioiron spike (extrinsic tag). They found a ratio of extrinsic iron to intrinsic iron (E/I ratio) of near unity, indicating an almost identical absorption of the two radio isotopes. They also reported a similar finding using a test meal of eggs.

Finch reported at the 1969 WHO-IAEA meeting that when maize labeled biosynthetically with radioactive iron was given together with a drink containing a trace amount of a different iron label, about 40% more iron was absorbed from the extrinsic tracer than from the maize intrinsic tracer. Hallberg and colleagues (Hallberg, 1974) repeated Finch's study but instead of administering the extrinsic tag with a drink, they carefully mixed the extrinsic tracer in the dough before baking maize tortillas. They found identical absorption of the two tracers.

Other studies have reported similar findings, an extrinsic/intrinsic (E/I) ratio of near 1, for a number of different foods, as shown in Table I. The extrinsic and

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Table I. Comparative Studies between the Absorption of Intrinsic Tag (Biological Label) and Extrinsic Tag (Inorganic Radioiron Tracer Added to Food) from Different Foods

food prepared as	iron content of food, mg	extrinsic tag, mg	mean absorption, %[a] intr. tag	mean absorption, %[a] extr. tag	absorption ratio[b] (extr./intr.) mean values	absorption ratio[b] (extr./intr.) SEM[c]	species	literature reference
wheat								
roll	0.3	trace	29.3	27.2	0.93	0.02	human	Bjorn-Rasmussen et al. (1972)
roll	0.6	trace	2.9	3.0	1.01	0.04	human	Bjorn-Rasmussen et al. (1973)
white bread	0.3	0.7	12.8	12.6	0.98	0.03	human	Bjorn-Rasmussen (1973)
brown bread	0.6	0.7	3.7	3.8	1.13	0.08	human	Bjorn-Rasmussen (1973)
ground meal (cooked)	0.02	trace	20.8	23.4	1.16	0.14	rat	Monsen (1974)
ground meal (uncooked)	0.02	trace	25.8	27.9	1.10	0.07	rat	Monsen (1974)
bread	3.7	trace	7.9	9.0	1.14		human	Layrisse et al. (1973)
soybean								
meal (boiled, baked)	2.5	trace	2.6	2.7	1.04	0.02	human	Bjorn-Rasmussen et al. (1973)
meal (ground, cooked)	0.02	trace	56.1	58.4	1.06	0.07	rat	Monsen (1974)
biscuits	2.6	2.0	19.8	21.1	1.08		human	Sayers et al. (1973)
maize								
chapatis	4.6	trace	1.8	1.7	0.98	0.04	human	Bjorn-Rasmussen et al. (1972)
gruel (baked)	2.0	0.001	2.7	3.1	1.10	0.01	human	Cook et al. (1972)
gruel (baked)	2.0	0.5	3.9	3.8	0.98	0.02	human	Layrisse et al. (1973)
corn								
ground meal (cooked)	0.005	trace	24.7	28.4	1.15	0.07	rat	Monsen (1974)
ground meal (uncooked)	0.02	trace	12.0	13.3	1.15	0.21	rat	Monsen (1974)
ground meal (cooked)	0.02	trace	20.1	22.0	1.10	0.04	rat	Monsen (1974)
rice								
porridge (unmilled, unpolished grains)	1.0	trace	2.7[a]	4.6[b]	1.62	0.14	human	Bjorn-Rasmussen et al. (1973)
porridge (milled, polished grains)	1.0	trace	4.5[a]	5.2[b]	1.17	0.01	human	Bjorn-Rasmussen et al. (1973)
porridge (flour from milled, polished grain)	1.0	trace	7.0	7.0	1.01	0.02	human	Bjorn-Rasmussen et al. (1973)
black bean (boiled, mashed, cooked)	3.0	0.1	1.5	1.2	0.99	0.05	human	Cook et al. (1972)
egg								
omelet	4.0	trace	1.5	1.5	1.03	0.04	human	Bjorn-Rasmussen et al. (1972)

[a] Different letters in a row denote significant difference ($p < 0.05$) between absorption means. [b] Ratios reported are the slope of the extrinsic versus intrinsic regression line. [c] Standard error of the mean.

intrinsic tracer have also been shown to be absorbed to the same extent when the labeled food item was part of a composite meal. Table II is a list of meals and their composition. When eggs and wheat flour, two foods which differ markedly in iron bioavailability, were biosynthetically tagged with separate isotopic labels and served as an omelet, absorption of both radioiron tracers was equal (Bjorn-Rasmussen et al., 1973). Bjorn-Rasmussen et al. (1973) have also demonstrated identical plasma radioactive postabsorption curves of the intrinsic and extrinsic tracers. These findings suggest that a rapid, almost complete isotopic exchange was taking place between the endogenous nonheme food iron and extrinsic tracer in these meals, supporting the common nonheme iron pool theory. The only food which has been reported to show exception was unmilled unpolished rice (also shown in Table I). In this case the dense outer aleurone layer of the rice grain is believed to have impaired the diffusion of iron, preventing a complete exchange (Bjorn-Rasmussen et al., 1973).

Conditions Required for Successful Tagging. In order for a complete isotopic exchange to occur between the native nonheme food iron and the extrinsic tracer, two conditions need to be met. First, the extrinsic tag and the food must be well mixed before absorption occurs and, second, the added tracer and the nonheme iron compounds in the meal must both be in a suitable chemical form for isotopic exchange, i.e., sufficiently soluble and dissociated (Hallberg, 1980). The solubility characteristics of iron salts are in part determined by chemical characteristics of a particular food, such as pH and the presence of chelators (Lee and Clydesdale, 1979). Smith and Rotruck (1981) have shown that the absorption of the extrinsic tag, as $^{59}FeCl_3$, decreases as the pH of the food is increased from 4 to 7. Although they did not examine the effect of pH on iron exchange, a possible interpretation of their data is that the efficiency of the extrinsic tag exchange is a function of pH, among other variables. Food pH has not been reported in other studies which have used the extrinsic tag. Insoluble iron salts cannot be used as an extrinsic tag because of their poor absorption (Amine and Hegsted, 1974). The added tracer, however, does not have to be in the form of a simple fully dissociated inorganic iron salt. An E/I ratio of near unity has been reported when sodium ferric EDTA, a strong heptadentate iron chelate, was used as the extrinsic tag (Layrisse and Martinez-Torres, 1977).

Addition of soluble iron salts up to 60 mg does not affect the accuracy of the method as seen in Table III. The efficiency of the method also does not appear to be affected by large amounts of nonheme iron or by the size of the meal itself (Monsen, 1974). A list of meals varying in size and iron content is given in Table II.

The exchange process has been studied only indirectly by measuring the absorption of the extrinsic and intrinsic tag. Very little is known about how the extrinsic tag exchanges with the various forms of nonheme food iron, such as soluble, insoluble, ferrous, ferric, elemental, and chelated iron, and the factors that can influence the exchange. It is not known if the isotopic exchange is completed before consumption or if a complete exchange requires the mixing and acidification which takes place in the upper gastrointestinal tract. The exchange process does not appear to be affected by rapid gastric emptying, gastric acidity, or the iron status of the individual (Table IV).

Table II. Comparison of the Absorption of Intrinsic Tag vs. the Absorption of Extrinsic Tag When the Labeled Food Was Part of a Whole Meal

food with intrinsic label	meal composition (iron content, mg)	mean absorption, %		absorption ratio (extr./intr.)		species	literature reference
		intr. tag	extr. tag	mean values	SEM		
wheat							
dough (baked)	arepa dough, rice, black beans, ground meat (3.5 mg)	8.8	9.1	1.06	0.02	human	Layrisse et al. (1973)
dough (baked)	arepa dough, rice, black beans (4.5 mg)	4.5	4.5	1.01	0.01	human	Layrisse et al. (1973)
dough (baked)	black beans, rice, maize or wheat, meat (4.3 mg)	3.0	3.3	1.13	0.04	human	Layrisse et al. (1976a)
rice							
boiled, steamed	potato and onion soup (5.5 mg)	2.0	2.6	1.3		human	Sayers et al. (1974)
maize							
boiled, homogenized	corn, instant potatoes, lean beef, bread, margarine, peaches, ice milk (4 mg)[a]	3.4	4.0	1.06	0.02	human	Cook et al. (1972)
flour	black beans, maize-soybean flour, plantain, meat (7.7 mg)	3.4	4.1	1.21[b]		human	Layrisse and Martinez-Torres (1977)
flour	black beans, maize-soybean flour, plantain (5.9)	5.1	6.2	1.21[b]		human	Layrissee and Martinez-Torres (1977)
arepas (baked pancakes)	black beans, maize, rice, meat (4.5 mg)	0.9	1.1[c]	1.22[b]		human	Layrisse et al. (1976a)

[a] Meal was homogenized. [b] Absorption ratio was not calculated in the study; thus, an arithmetic ratio is listed. [c] Extrinsic tag was added in a drink in the middle of the meal.

Table III. Effects of Iron Fortification on the Absorption of Intrinsic Tag and Extrinsic Tag from Food

food labeled with intr. tag	meal composition (Fe content, mg)	added Fe, mg	mean absorption, %		absorption ratio (extr./intr.)		species	literature reference
			intr. tag	extr. tag	mean values	SEM		
maize								
gruel (baked)	single item (2.0)	$FeCl_3$, 0.5	3.85	3.81	0.98	0.02	human	Layrisse et al. (1973)
gruel (baked)	single item (2.0)	$FeCl_3$, 5.0	1.66	1.56	0.93	0.02	human	
gruel (baked)	single item (2.0)	$FeCl_3$, 10.0	1.22	1.16	1.00	0.02	human	
gruel (baked)	single item (2.0)	$FeCl_3$, 20.0	0.5	0.5	1.10	0.02	human	
gruel (baked)	single item (2.0)	$FeCl_3$, 60.0	0.37	0.44	0.99	0.06	human	
maize								
gruel (baked)	gruel, meat (3.0)	$FeCl_3$, 1.0	5.8	6.4	1.03	0.04	human	Layrisse et al. (1973)
gruel (baked)	gruel, meat (3.0)	$FeCl_3$, 5.0	8.6	9.4	1.07	0.01	human	
gruel (baked)	gruel, meat (3.0)	$FeCl_3$, 20.0	3.87	5.1	1.04	0.02	human	
gruel (baked)	gruel, meat (3.0)	$FeCl_3$, 60.0	2.3	2.4	1.04	0.01	human	
flour	black beans, maize-soy flour, rice, plantain, meat (7.7)	Fe(III)EDTA, 5	3.4	4.1	1.20[a]		human	Layrisse and Martinez-Torres (1977)
		Fe(III)EDTA, 25	6.0	6.8	1.13[a]			
		Fe(III)EDTA, 50	4.9	5.3	1.08[a]			
maize								
flour	black beans, maize-soy flour, rice (5.9)	Fe(III)EDTA, 5	5.1	6.2	1.21[a]		human	Layrisse and Martinez-Torres (1977)
	same as above	Fe(III)EDTA, 25	7.0	7.0	1.00[a]			
	same as above	Fe(III)EDTA, 50	4.3	4.2	0.97[a]			

[a] Ratio was not calculated in the study; thus, an arithmetic ratio is listed.

In original studies on food iron absorption from whole meals, the extrinsic tag was uniformly distributed throughout all food items in the meal to ensure adequate mixing. However, a later study reported an E/I ratio close to 1 when an extrinsic tag was mixed with only one food item in the meal (Bjorn-Rasmussen et al., 1976), and this procedure has gained general acceptance.

Factors Which May Cause Incomplete Extrinsic Tagging. Little is known about the effects of processing on the validity of the extrinsic tag method. There is evidence to suggest that the processing history of a food may influence the iron bioavailability measured by this method (Smith and Rotruck, 1981). Various forms of cooking, e.g., boiling and baking, appear to have no effect on the exchange efficiency of the extrinsic method (Bjorn-Rasmussen et al., 1973; Bjorn-Rasmussen, 1973;Cook et al., 1972).

The bioavailability of nonheme iron is influenced by a number of dietary factors (Hallberg, 1981). Certain substances in the diet, e.g., ascorbic acid, meat, and fish, enhance the absorption of iron, while other substances such as phytates, certain fibers, and teas can render the iron less available for absorption by forming insoluble or undissociated iron complexes with iron. Changes in iron

Table IV. Effect of Clinical Disorders on Absorption of Intrinsic Tag and Extrinsic Tag

food with intrinsic label and clinical disorder	mean absorption, %		absorption ratio (extr./intr.)		species	literature reference
	intr. tag	extr. tag	mean values	SEM		
soybean						
subjects with achlorhydria	0.7	0.7	1.00	0.01	human	Hallberg (1974)
subjects with rapid gastric emptying	0.6	0.6	0.97		human	Hallberg (1974)
subjects with severe Fe deficiency	5.7	5.8	1.02	0.02	human	Hallberg (1974)
subjects with Fe deficiency	67.2	79.2	1.18	0.50	rat	Monsen (1974)
wheat						
subjects with Fe deficiency	66.1	76.4	1.18	0.17	rat	Monsen (1974)
corn						
subjects with Fe deficiency	62.4	72.3	1.18	0.04	rat	Monsen (1974)

Table V. Effects of Ascorbic Acid on the Absorption of Intrinsic Tag and Extrinsic Tag from Different Foods

food labeled with intr. tag	meal composition (iron content, mg)	ascorbic acid concn, mg	mean absorption, %		absorption ratio (extr./intr.)		species	literature reference
			intr. tag	extr. tag	mean values	SEM		
wheat								
dough (baked)	black beans, rice, wheat, meat (4.3)	100	5.4	5.1	0.9	0.03	human	Layrisse et al. (1976b)
bread	single item (2.2)	50	5.3	5.9	1.15		human	Sayers et al. (1973)
rice								
boiled, steamed	onion and potato soup (2.6)	100	11.8	12.3	1.3		human	Sayers et al. (1974)
maize								
baked gruel	single item (2.0)	500	22.0	22.5	1.01	0.01	human	Cook et al. (1972)
porridge	single item (0.5)	200	16.0	17.0	1.06	0.01	human	Bjorn-Rasmussen et al. (1973)
porridge	single item (4.9)	50	12.1	13.1	1.15		human	Sayers et al. (1973)
porridge	single item (3.0)	100	22.6	25.3	1.13		human	Sayers et al. (1973)
porridge	porridge, egg (3.6)	250	24.8	25.2	1.03		human	Sayers et al. (1973)
porridge	single item (4.5)	100	15.5	17.4	1.12		human	Sayers et al. (1973)
soybean								
biscuit	single item (2.6)	100	14.6	15.1	1.11		human	Sayers et al. (1973)
corn								
ground (cooked)	single item (0.02)	5	39.5	45.1	1.16	0.04	rat	Monsen (1974)

Table VI. Effects of Desferrioxamine on the Absorption of Intrinsic Tag and Extrinsic Tag from Different Foods

food labeled with intr. tag	iron concn of food, mg	desferrioxamine concn, mg	mean absorption, %		absorption ratio (extr./intr.)		species	literature reference
			intr. tag	extr. tag	mean values	SEM		
maize, gruel (baked)	2.0	500	0.6	0.7	1.14	0.04	human	Cook et al. (1972)
wheat, bread	0.6	500	1.11	0.3	0.40[a]	0.08	human	Bjorn-Rasmussen (1973)
corn, ground (cooked)	0.02	5	6.1	7.5	1.24	0.08	rat	Monsen (1974)

[a] Usually the extrinsic tag is taken with the meal. In this study, the tag was consumed following the meal.

bioavailability are believed to be the result of changes in the relative size of the isotopic exchange pool (Hallberg, 1974). What determines iron bioavailability is thought by some to be the net effect of these enhancing and inhibiting factors on the size of the isotopic exchange pool.

Several studies have been conducted to determine if factors that influence the bioavailability of dietary iron could also affect the exchange efficiency of the extrinsic tag. The addition of ascorbic acid to selected foods and composite meals has been shown to equally affect the absorption of both the extrinsic and intrinsic tracers (Table V). In other studies desferrioxamine has been added to a food to simulate the effect of a chelating substance which might theoretically be present in a diet. Desferrioxamine forms a stable complex with iron which is poorly absorbed. The absorption of the two tracers were affected equally by the addition of desferrioxamine to the food. However, when desferrioxamine was consumed immediately after ingestion of the food, a significantly lower absorption of the extrinsic tag was observed when compared to the intrinsic tracer (Table VI).

In a more recent study Bjorn-Rasmussen and Hallberg (1979) have reported that the absorption of ferric and ferrous iron is affected differently by iron absorption inhibitors such as phytate. Similarly, Martinez-Torres et al. (1981) have found that the absorption of ferric iron was greater than ferrous iron in the presence of the amino acid cysteine. These data suggest that the absorption of the extrinsic tag and the various forms of nonheme food iron may be affected differently by enhancers and inhibitors present in the diet. This would be most possible if the extrinsically added iron has a different valence than the endogenous food iron.

Contaminant iron may also be responsible for an incomplete exchange with the added tag. Foods can become contaminated with iron from a variety of sources, e.g., dust,

Table VII. Organ Doses following Oral Ingestion of 1 μCi of ^{59}Fe and 5 μCi of ^{55}Fe for Dual Radioiron Absorption Measurement[a]

	dose, mrd		
	^{55}Fe	^{59}Fe	total
whole body	3	2	5
blood	24	7	31
bone marrow	8	7	13
spleen	13	10	25
liver	7	9	16
lower large intestine	4	33	37

[a] A 10% absorption of both isotopes is assumed. The doses of 1 μCi of ^{59}Fe and 5 μCi of ^{55}Fe are those usually used (Bothwell et al., 1979).

handling, processing, and residual soil on vegetables and cereals. Water used in cooking, drinking, and processing may also be contamined with iron.

Hallberg et al. (1983) have shown that in diets contaminated with iron, up to 50% of the nonheme iron does not exchange with the extrinsic tag. Thus, it is extremely important to consider the presence of contaminating iron when using this method. Very little is also known about the bioavailability of the various forms of contaminating iron. However, there have been recent attempts to quantitate this problem (Hallberg and Bjorn-Rassmusen, 1981; Hallberg et al., 1983; Rosanoff and Kennedy, 1982).

Amine et al. (1972) have also shown that the extrinsic tag technique will not give an accurate measurement of iron absorption from a diet which contains insoluble iron. Therefore, this method cannot be used to monitor iron absorption from foods fortified with insoluble iron salts. Monsen (1974) has noted that in both human and animal studies the extrinsic iron label has consistently been absorbed at a slightly higher rate than the intrinsic label. A greater absorption of the extrinsic iron label would be indicative of a partial or incomplete isotopic exchange.

Safety and Ethical Considerations. Although the extrinsic tag technique can be a powerful tool for iron bioavailability research, it is not always considered appropriate for use in human studies. Safety and ethical considerations impose limitations on the use of ^{55}Fe and ^{59}Fe with children and adults of reproductive age (Carni et al., 1980). On the other hand, Bothwell et al. (1979), provide substantial evidence to support the case that the radioiron extrinsic tag method is safe and acceptable to use in human subjects. These authors duly note that "such studies must be justified on the basis of the scientific information which they might be expected to provide".

The most commonly used isotopes in extrinsic tagging are ^{55}Fe and ^{59}Fe. Table VII gives the expected organ dose following a typical oral ingestion of 1 μCi of ^{59}Fe and 5 μCi of ^{55}Fe, for dual iron absorption measurements. It should be noted that these radiation dosages are relatively crude and are intended only to represent an order of magnitude. Bothwell et al. (1979) have suggested that the dose of radiation received in clinical investigations with ^{55}Fe and ^{59}Fe is best put into perspective by comparison with other types of radiation exposure. Individuals are exposed to approximately 100 mrd per year from background sources of radiation (Marx, 1978). Another 100 mrd per year per person would be contributed by radiological examinations. A double isotope study of iron absorption using the cumulative data in Table VII is estimated to be equivalent in whole body irradiation to the amount received by individuals from natural radiation and from radiological examinations during a period of 1–2 weeks (Bothwell et al., 1979). Other types of radiation exposures are far above the levels that result from investigations with radioiron. For example, a complete gastrointestinal series (barium and enema) results in a radiation exposure of 300–600 mrd.

The controversy over the ethics of using radioiron techniques in human subjects will likely continue. All agree that volunteers for these types of studies be fully informed of the objectives of the study and its possible risks. In the future the use of enriched stable isotopes of iron may provide a more acceptable alternative to radioactive isotopes (Miller and Van Campen, 1979). At present, the cost of stable isotopes and their analysis are greater than radioisotopes, and successful applications of stable isotopes are very limited (Janghorbani and Young, 1982).

SUMMARY

The extrinsic tag technique has proven valid for several foods under certain experimental conditions. But, this method cannot yet be considered proven with regards to all types of foods. The extrinsic tag method is not appropriate for monitoring iron absorption from a diet that contains insoluble forms of iron. The validity of this technique relies upon the basic assumption that the extrinsic tag exchanges completely with all endogenous nonheme food iron. At present is is not known how completely the different forms of nonheme iron are labeled by an extrinsic tag. This is important in light of studies which have suggested that iron inhibitors may affect the extrinsic tag differently than some forms of nonheme iron in foods. Research on food factors which can impair a complete isotopic exchange is scant. Thus, interpretation of bioavailability data from extrinsic tag research requires consideration of inhibitors of exchange which may be present in the food or diet.

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ARTICLES

Insect Attractants: Volatiles of Hydrolyzed Protein Insect Baits

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The volatile components of a widely used commerical hydrolyzed protein insect bait have been isolated both by Tenax trapping and by vacuum steam distillation continuous extraction. The volatile concentrates obtained were analyzed by capillary gas–liquid chromatography–mass spectrometry and 43 components identified. Major components include phenylacetaldehyde, acetic acid, furfuryl alcohol, 2-acetylfuran, benzaldehyde, methional, 2-acetylpyrrole, and furfural. Unusual components include some aldol condensation products such as 5-methyl-2-phenyl-2-hexenal and 5-methyl-2-[(methylthio)methyl]-2-hexenal.

A number of investigators [e.g., Hagen et al., (1976), van Emden and Hagen (1976), and Miller and Haarer (1981)] have explored the use of hydrolyzed protein preparations as baits for certain insects such as the green lace wing (*Chrysopa carnea*) and the Mediteranean fruit fly (*Ceratitis capitata*, Wiedemann). These insects seem to be attracted to the hydrolyzed protein by the volatile compounds associated with it. The hydrolyzed protein mixtures are assumed to be related in composition to the "honeydew" extruded by aphids which, in nature, can apparently supply a suitable diet for both the adult and larva of certain insects (Hagen et al., 1976).

Some of these baits have been used in large-scale programs to combat insect pests such as in the 1981 program in California to eradicate the Mediteranean fruit fly. The main bait used in California for this purpose was the commercial hydrolyzed protein bait "Staley Protein Bait No. 7" (abbreviated PIB-7).

There seems to be very little published information on the identity of the volatile compounds associated with these baits which must attract the insects initially to the hydrolyzed protein. Some recent studies have been reported by Morton and Bateman (1981), who identified 39 compounds in two different hydrolyzed protein preparations, one of which was PIB-7.

The present study was aimed at further identification of the major volatiles associated with the commerical PIB-7 hydrolyzed protein bait.

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EXPERIMENTAL SECTION

Materials. The main sample of hydrolyzed protein was "Staley Protein Bait No. 7" (corn gluten hydrolyzed) manufactured by A. E. Staley Manufacturing Co., Protein Division, Decatur, IL (PIB-7). Two different lots of this material were examined. This material is an aqueous (44–50% solids) viscous liquid, pH 3.5–4.5, described as containing 18–25% protein (as amino acids) and 6–12% carbohydrate (mostly sugars) and 6–13% salt. An enzyme-hydrolyzed protein for comparison was "Ardemine Autolysate enzymatic protein hydrosylate of Brewers yeast" manufactured by Unilab Research Corp., Berkeley, CA.

Isolation Using Tenax Traps. The traps as described previously (Buttery et al., 1982) were made from Pyrex glass and contained a 1.3 cm diameter × 7 cm long (1.7 g) column of Tenax GC adsorbent. One liter of the hydrolyzed protein preparation was placed in a 12-L flask and stirred with a magnetic stirrer. Purified air (500 mL/min) was passed into the flask (via a Teflon tube) and out through the trap by applying suction to the outlet of the trap. The isolation was continued for 24 h at room temperature. The trap was then removed and the trapped material eluted with freshly distilled diethyl ether. The ether extract was concentrated by using a warm water bath and low hold up distillation columns.

Isolation by Vacuum Steam Distillation Continuous Extraction. This was carried out in essentially the same way as described previously by the authors for other materials [e.g., Buttery and Kamm (1980)] at 100 mmHg pressure. In this case methyl *tert*-butyl ether, purified by distillation through a 20-plate column, was used as the