Wholesomeness of Irradiated Cocoa Beans. The Effect of γ Irradiation on the Chemical Constituents of Cocoa Beans

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Analysis of cocoa beans after irradiation at doses of 0, 0.10, 0.20, 0.50, 2.00, and 5.00 kGy showed no significant differences in respect of reducing sugar, total amino acids, total fat (and its nature, as determined by iodine value, free fatty acid value, saponification value, refractive index, slip point, and specific gravity), alkaloid, and protein (total and soluble) contents. Results from thin-layer chromatography and gas–liquid chromatography of the fats and methyl esters, respectively, further suggest that the nature of the fat is unaffected by irradiation up to a dose of 5 kGy. The significance of these observations is discussed in relation to the possible acceptance of radiosterilization of cocoa beans commercially.

Moldiness and insect infestation are the two major factors which limit the storage life of prepared cocoa beans in Ghana. While an acceptable solution in the form of chemical control appears to have been found for the insect problem, the problem of moldiness remains largely unresolved.

It is, therefore, not surprising that one of the most frequent defects of cocoa beans exported from cocoa-producing countries, like Ghana, is the presence of a number of molds on the embryo of prepared beans. More than 11 distinct genera of molds have been identified on cocoa beans by Bunting (1928). These include species of *Aspergillus*, *Circinella*, *Mucor*, *Penicillium*, and *Absidia*.

Invariably, the presence of these molds deteriorates the value of cocoa intended for human consumption, also cocoa and chocolate manufacturers are unable to utilize such beans in appreciable quantities on account of the flavor the molds impart to finished products. Furthermore, since the toxins (e.g. aflatoxin) and other metabolic products produced by the molds are hazardous to health, there is a need to find an effective means of preventing moldiness of cocoa beans.

The initial (i.e., primary) infection is believed to occur at the time of preparation of the beans for the market, and it is estimated that the embryo of every single bean is already infected by the time the cocoa beans leave the farmer's hand for the market. As long as the moisture content of the beans remains low, the fungal spores remain dormant, but as soon as the moisture content rises above 8%, molds begin to grow profusely (i.e., secondary moldiness). Since cocoa beans are highly hygroscopic, 8% moisture content is easily attained under (a) storage conditions in ships during export and/or (b) under relative humidities above 80% which are a common feature of most cocoa-producing countries.

In Ghana, efforts have mainly been concentrated on keeping the moisture content of the beans low in order to suppress secondary moldiness; but this is difficult to maintain and requires either special storage conditions, or repeated drying and rebagging. Furthermore, the maintenance of a very low moisture content of the beans is not in the best interest of the producer since cocoa is marketed on weight basis.

Amuh (unpublished data) has shown that secondary moldiness of fresh beans (i.e., beans less than 2 months old) can be prevented for at least a year (at 27 °C; 80% RH, with a corresponding moisture content of 8–10%) when the beans are irradiated with a dose of 0.5 kGy from a ^{60}Co source, while those older require 5 kGy. However, for irradiated food to be wholesome for human consumption, it must satisfy a number of conditions. These include (a) there should be no harmful products formed and (b) the food value should not be lowered, i.e., there should be no undesirable changes in the chemical constituents. The present study is, however, concerned with investigating whether the irradiated beans meet the second requirement. The aim of this work, therefore, is to determine whether or not at the effective doses (0.5 kGy for new beans; 5 kGy for old beans) and other doses, the chemical constituents of cocoa beans are affected by γ irradiation.

MATERIALS

Nonfumigated cocoa beans (500 lb) were obtained from the Cocoa Research Institute, Tafo. Moisture content was 3.70%. γ irradiation source: a 220 γ cell no. 129 with a dose rate of 6.22×10^5 rad/h.

EXPERIMENTAL SECTION

Uniform cocoa beans (500 g) were sealed into small polythene bags and irradiated in the γ cell with different doses. The shells of the beans were then removed by hand and the cotyledons ground to fine powder in a coffee grinder and used for subsequent analysis for the following constituents: (a) Fat content (including fatty acid analysis and characterization of the extracted fat using the iodine value, saponification value, slip point, refractive index, specific gravity, and free fatty acid values). (b) Protein content (total and soluble) and constituent amino acids. (c) Sugar content (reducing, and total sugars). (d) Alkaloids: theobromine and caffeine. All the constituents were analyzed within 48 h after irradiation. Samples were kept at room temperature (27 °C) prior to analyses.

Fat Analysis. Total fat content was obtained by extraction of 5 g of finely ground beans with petroleum spirit (40–60 °C) in a Soxhlet extractor for 18 h. Fat for the determination of other parameters was obtained by extraction of about 50-g portions of ground beans for 4 h.

Iodine value, free fatty acid value, saponification value, refractive index, slip point and specific gravity were all determined as recommended by Standard Methods of Chemical Analysis Vol. 2B.

TLC of the fats was carried out on silica gel G plates (0.005 mL of ether solution containing 250 mg of fat/mL of ether was spotted on plates coated with silica gel, 200

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[1]Deceased.

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μm thick). The lipid spots were located by either spraying with 0.1% Rhodamine B solution or exposure to iodine vapor.

Methyl esters were prepared using the method of Jamieson and Reed (1965) and gas chromatographed at 240 °C on a 10% SE 30 column; argon (17 lb/sq in.) was the carrier gas. The individual esters were identified using known references prepared in similar fashion and quantified by the method of triangulation (height × width at one-half height).

Proteins. Pearson's (1970) modification of the Kjeldahl method was used for determination of total proteins, while the methods of Rohan and Stewart (1966) and Pearson (1970) were used for analysis of soluble nitrogen.

Amino acid analyses: Qualitative data were obtained using a Locarte amino acid autoanalyzer after acid hydrolysis (6 M HCl at 110 °C for 24 h in vacuum). Quantitative results were obtained by the method of triangulation.

Sugar Analysis. *Extraction.* The procedures used was developed from the methods of Nelson (1944), Clegg (1956), Rohan and Stewart (1966), and Reineccius et al. (1972).

Two grams of finely ground cocoa beans was weighed directly into a 250-mL centrifuge tube. Thirty milliliters of hot 20% methanol was added and the mixture stirred with a glass rod and allowed to stand for 5 min with occasional stirring. The mixture was then centrifuged at 800*g* for 10 min and the supernatant fluid decanted. The extraction was repeated with 25 mL of hot 20% methanol. The two extracts were combined and pH adjusted to 10 with a 0.3 M solution of sodium hydroxide. Five milliliters of saturated lead acetate solution was added with stirring to precipitate the polyphenols. After waiting for 5 min, the mixture was centrifuged and the centrifugate decanted through a small plug of cotton wool in a funnel to remove fat globules. The light yellow extract was passed through a glass column (12 × 1 in.) containing a 2-in. layer each of Celite 545–zinc metal powder (50:50 w/w) and Celite 545. The eluate was collected and transferred into a 100-mL volumetric flask, and the volume made up with distilled water. It was found necessary to remove the fat globules before passing the extract through the column since the globules tended to plug the column spaces and so reduced the flow rate of the eluate.

Sugar Estimation. This was done on 1 mL of eluate from the zinc column using the spectrophotometric method of Nelson (1944) which measures reducing sugars. Total sugars were estimated as reducing sugars after acid hydrolysis (Rohn and Stewart, 1966).

Alkaloid Determination. Theobromine and caffeine were determined (using defatted samples) by the spectrophotometric method of Englis and Miles (1954).

RESULTS AND DISCUSSION

The effect of γ radiation on sugar (reducing and total) and amino acids is shown in Tables I and II.

A look at the results obtained from sugar analysis (Table I) shows that there is no significant difference between the control and irradiated samples. It might, therefore, be logical to assume that up to the dose of 5 kGy, γ radiation has no effect on the reducing sugars of cocoa beans. A similiar observation has been made by Kodenchery and Nair (1972) when working with potatoes. These findings, therefore, enhance the possibility of accepting irradiated beans for chocolate manufacture so far as the reducing sugar content is concerned, inasmuch as sugars are necessary for the development of the characteristic chocolate color and aroma through their reactions with amino acids

Table I. Effect of γ Radiation on Constituents of Cocoa Beans[a]

dose, kGy	sugar, mg/g sample: free reducing	sugar, mg/g sample: total	fats: % age fat	fats: iodine value	fats: FFA[b] (oleic)	fats: sap. value[b]	fats: RI[b] (40 °C)	fats: sp gr[b] (25 °C)	fats: slip point, °C	alkaloids, mg/g sample: theobromine	alkaloids, mg/g sample: caffeine	proteins, % age: total	proteins, % age: soluble
0	7.9 ± 1.4	22.1 ± 0.10	52 ± 1.40	34.0 ± 0	0.707	185 ± 3.0	1.456	0.8987	32–34	4.02	1.16	13.7 ± 0.05	5.22
0.10	6.5 ± 1.3	20.8 ± 0.9	53 ± 0.19	36.2 ± 0.3	0.736	182 ± 3.3	1.454	0.8985	32–34	3.56	0.88	14.3 ± 0.07	5.60
0.20	7.2 ± 1.3	22.1 ± 2.2	53 ± 0.68	36.2 ± 0.4	0.722	181 ± 0	1455	0.8970	30–31	4.16	1.18	13.6 ± 0.14	7.40
0.50	6.7 ± 1.3	19.5 ± 1.5	55 ± 0.44	34.5 ± 0.5	0.687	183 ± 0	1.456	0.9034	32–34	4.20	1.34	13.6 ± 0.12	9.16
2.00	7.5 ± 1.4	20.8 ± 1.5	53 ± 1.50	35.8 ± 0.2	0.724	185 ± 2.2	1.454	0.8991	32–34	4.40	0.76	15.4 ± 0	5.70
5.00	8.0 ± 1.4	22.1 ± 1.6	53 ± 1.50	34.7 ± 0	0.750	185 ± 2.6	1.455	0.9244	32–34	4.34	0.80	14.6 ± 0.04	5.40

[a] Each result is a mean of three separate analyses ±SEM. [b] FFA, free fatty acid value; sap., saponification; RI, refractive index; sp gr, specific gravity.

Table II. Effect of 5 kGy of γ Radiation on Constituent Amino Acids of Cocoa Bean Protein

amino acid	concn, nmol mg^{-1} sample	
	control	exptl
aspartic acid	63	56
threonine	26	24
serine	37	34
glutamic acid	84	83
proline	35	33
glycine	48	49
alanine	43	39
cysteine	Tr[b]	Tr
valine	39	36
methionine	8	7
isoleucine	23	21
leucine	40	36
tyrosine	17	14
phenylalanine	26	24
histidine	11	10
lysine	35	32
arginine	30	28
total	565[a]	526[a]

[a] $P > 0.05$, test of significance was done using variance ratio test. [b] Trace.

Table III. Effect of γ Radiation on Fat and Constituent Fatty Acids of Cocoa Beans

dose, kGy	R_f of fat[a]	percentage in fat		
		palmitic acid	stearic acid	linoleic and oleic acid
0	0.59	21	44	35
0.10	0.60	26	45	29
0.20	0.60	20	45	35
0.50	0.60	22	48	30
2.00	0.60	25	49	26
5.00	0.60	27	47	26

[a] Solvent system, chloroform/methanol (98:2, v/v).

(Maillard reaction) in nonenzymatic browning (Rohan, 1963, 1964). Rohan and Stewart (1966) have shown that a deficiency of reducing sugars is an essentially important factor limiting the development of optimum chocolate flavor during roasting.

Beans irradiated with a dose of 5 KGy were analyzed quantitatively for the total amino acid content. Results (Table II) indicate that irradiation at this dose has no significant effect ($P > 0.05$) on the amino acid content although there is a general tendency of the amino acids to decrease; the total amino acid content of the control and irradiated beans are 565 and 526 nmol mg^{-1} sample, respectively. Thus, so far as amino acid content is also concerned, irradiated beans can be used for chocolate manufacture. There is a clear evidence from this work that cocoa indeed contains all of the essential amino acids; therefore cocoa protein is of high quality.

Effect on Cocoa Fat. As for the simple sugars and amino acids, there is no significant difference in the total fat content of control and irradiated beans (Table I). Analysis of the fat by TLC on silica gel G plates show only one spot in each of the irradiated and control beans; the R_f of the control was 0.59, while that of the irradiated beans was 0.60 (Table III). These results suggest that the nature of the fat has not been affected by irradiation. However, it appears that above 0.20 kGy, the amount of saturated fatty acids (palmitic and stearic) tend to increase at the expense of unsaturated ones (linoleic and oleic, Table III). This should not present any health hazard since these changes are not significant when compared to the control values.

Table IV. Comparism of the Organoleptic Properties of Control and Irradiated Cocoa Beans[a]

organoleptic charact.	control beans	irrad. beans
texture[b]	brittle	more brittle
odor[c]	cocoa odor	sharp odor
color[c]	dark	lighter in color
taste[c]	cocoa taste	slightly bitter

[a] Beans were roasted at 165 °C for 1 h, deshelled and used for testing. Six people constituted the panel. [b] Evaluation was done on whole beans. [c] Evaluations were carried out on ground (powdered) beans.

The only marked difference (visual observation) noted in the fats extracted is that of color. There appears to be a gradual decrease in the intensity of the cocoa fat (yellow from irradiated beans) as the dose increases above 0.50 KGy. This may be due to the gradual destruction and/or alteration of the flavonoid compounds. Similar changes have been reported to occur in wheat (Deschreider, 1966) where it has been shown that although the carotenoids disappeared completely at the dose of 40 kGy, there was no change in the chemical composition of the lipids. In addition, Van Kooij (1978) has just reported that even though fat from cocoa beans irradiated at 0.46 and 3.67 kGy have an o.d. of 5.3 and 5.1, respectively, as compared to a control value of 7.2, there was no sifnificant difference in the chemical composition of the fats. Although these insignificant changes in color and reduction in unsaturated fatty acids may imply a marginal free radical damage to the lipids which may subsequently affect storage stability of the irradiated cocoa beans, the free radicals would be expected to decrease rather than increase with time since they are extremely short-lived. Inasmuch as these changes are not significant (when compared with control values) within 48 h postirradiation, it is unlikely that they would be significant when the beans are stored for longer periods.

Other tests carried out in the present work to characterize the fats from control and irradiated samples, namely, iodine value, free fatty acid value, saponification value, refractive index, specific gravity, and slip point (Table I), also confirm that irradiation up to a dose of 5 kGy has no appreciable effects on fats of cocoa beans; thus fat from irradiated beans can be used for chocolate manufacture or for other purposes so far as the lipid content and composition are concerned.

Effect on Alkaloids. As in the case of the other constituents, results from analysis of theobromine and caffeine content (Table I) of control and irradiated beans indicated that these alkaloids are not affected by γ irradiation when up to a dose of 5 kGy is used. The values for theobromine and caffeine in each sample are around 4.0 mg and 1.0 mg/g sample, respectively.

Organoleptic Tests. Since the sum total of the effects (if any) of irradiation on the chemical constituents would be to affect the flavor of the beans, it was felt that a more direct approach was to evaluate the beans organoleptically; therefore, control and beans irradiated with 5 kGy were roasted and then evaluated.

The results, shown in Table IV, indicate some slight differences in organoleptic properties. Even though the significance of these differences is difficult to evaluate quantitatively, it is noteworthy that these changes (brought about by irradiation) have not affected the fat which is the constituent of greatest importance in chocolate manfacture. Loaharanu (1975) has carried out quantitative analysis of color and odor of cocoa beans irradiated up to 5 kGy with

γ rays and has reported that there was no significant difference between control and irradiated beans.

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Amino Acid Composition of Whole Cells of Different Yeasts

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The amino acid composition of the intact cells of eight yeast species (*Saccharomyces cerevisiae*, *Candida utilis*, *Kluyveromyces fragilis*, *Saccharomyces uvarum*, *Schwanniomyces castellii*, *Saccharomycodes ludwigii*, *Pichia membranaefaciens*, *Lipomyces starkeyi*) was determined. High contents of threonine and lysine and a deficiency in methionine and cystine were apparently a characteristic of all the species considered. *Saccharomycodes ludwigii*, *Pichia membranaefaciens*, and *Lipomyces starkeyi* showed a very low protein content. Amino acid profiles and protein contents of *Saccharomyces uvarum* and *Schwanniomyces castellii* were comparable to those of *Candida utilis*.

Among the novel food sources presently being developed and studied, single-cell protein (SCP) from yeast holds a prominent role (Chen and Peppler, 1977). In fact, yeasts are highly efficient producers of protein from different carbon sources, show an elevated protein content ranging from 38.8 to 70.7% of dry weight (FAO, 1970), and have a reasonably high lysine content as well as sufficient amounts of threonine and tryptophan.

Though many studies have been done on the subject of food yeasts, these normally involve but a few species such as *Candida utilis*, *Saccharomyces cerevisiae*, and *Kluyveromyces fragilis*. The selection of these yeasts was not necessarily due to careful screening surveys. *Candida utilis*, for example, was isolated by chance as a contaminant in a German yeast factory and then used for its exceedingly rapid growth and ability to use a wide range of carbon sources. On the other hand, *Kluyveromyces fragilis* was chosen primarily because of its ability to utilize lactose from whey while *Saccharomyces cerevisiae* does not represent an actual choice, being itself an imposed, abundant byproduct of the alcoholic beverage industry.

In this work five additional yeasts were compared to the three above-mentioned species in relation to their protein contents and amino acid patterns.

MATERIALS AND METHODS

Organisms and Growth Conditions. The species used in this study are listed in Table I. Basal medium was yeast nitrogen base (Difco) supplemented with 2% glucose. Cells were grown on a rotary shaker (150 rpm) at 28 °C to the end of the exponential phase of growth (usually 15–18 h of culture), collected by centrifugation, washed three times with distilled water, and freeze-dried.

Cell Analysis. Total nitrogen content was determined in a Merz apparatus, Model C (Heraeus Co., Hanau, West Germany). Total protein (true protein) was estimated by the method of Lowry et al. (1951) with bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard. Acid hydrolysis of freeze-dried cells was performed according to Puerser and Beuchler (1966). Amino acid profiles were determined by chromatography on an Optica S.A.S. Aminolyser (Milan, Italy) by the procedure of Mondino (1967). Tryptophan was estimated by the procedure of Opienska-Blauth et al. (1963) after hot alkali hydrolysis (Brown and Rose, 1969). Sulfur-containing amino acids were oxidized with performic acid to cysteic

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