component in sterol glycosides, and hence the molecular species present in them are likely to be β-sitosterol or stigmasterol glucoside and sterol 6-acylglucoside, where the sterol could be β-sitosterol or stigmasterol and the acyl moiety palmitic, linoleic, and linolenic acid, respectively. On the basis of the earlier reports (Kiribuchi et al., 1966; Miyazawa et al., 1974; Mahadevappa and Raina, 1978b), the structure deduced for sterol glycoside would be β-D-glucopyranosyl-(1→3)-S, where S represents either β-sitosterol or stigmasterol and acyl would imply palmitic, linoleic, or linolenic acid.

The main sterol in plant sources reported earlier (Lepage, 1964; Kiribuchi et al., 1965; Sakata et al., 1973; Ito and Fujino, 1974; Miyazawa et al., 1974; Mahadevappa and Raina, 1978b; Fujino, 1978) is β-sitosterol with lesser proportion of stigmasterol. This is true of this legume also. Campesterol here constitutes about 3% which is in agreement with the earlier findings on pea and soybean (Miyazawa et al., 1974; Kiribuchi et al., 1966). It has earlier been established by using a cell-free particulate fraction of immature soybean seeds that sterol glycoside is biosynthesized from β-sitosterol and UDP-glucose. There is also a possibility that esterified sterol glycoside is formed likewise (Hou et al., 1967; Kates, 1970). A similar biosynthetic pathway for the formation of sterol glycoside and esterified sterol glycoside seems to be operating in this legume also. Stigmasterol could presumably substitute for β-sitosterol in the biosynthetic pathway to yield corresponding molecular species.

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Study of γ-Irradiated Starches Derived from Different Foodstuffs: A Way for Extrapolating Wholesomeness Data

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The foodstuff from which the investigated starches (maize, amylomaize, waxy maize, bread wheat, manioc, rice, potato, and haricot bean) derive only has a little influence upon their γ irradiation: the intermediate free radicals as the final radiolysis products are the same; the ratio between the maximal and minimal formation yields is always lower than 5; the different curves drawn (influence of irradiation dose, atmosphere, or water content) have similar shapes.

For reduction of food losses and for improvement of their nutritional quality, many approaches are used. Irradiation of food is among one of the recent ones. The appropriate studies required to ascertain the wholesomeness of irradiated food were discussed by different Joint FAO/IAEA/WHO Expert Committees (JECFI) in 1964, 1969, 1976, and 1980. In view of advances of knowledge, the 1976 Committee suggested (WHO, 1977) that "*Future evaluations of the wholesomeness of individual irradiated foods should take into consideration all relevant data obtained from tests on analogous irradiated foods and on repesentative food constituents*". Moreover, it is envisaged that radiation chemical investigations will eventually provide sufficient data to facilitate greatly the evaluations of irradiated foods. For instance, "*It was considered reasonable to take into account the radiation chemical studies on various starches and the absence of adverse effects in feeding studies with irradiated maize starch*" (WHO, 1977). Our laboratory, like others taking part in the CORC program (coordinated program in the field of radiochemistry of food and food components) of the IFIP (Inernational Food Irradiation Project), agreed that chemiclearance (Basson and Elias, 1978) was a rational approach to ascertain the wholesomeness of irradiated food. This work is designed to determine if there are variations in the amounts of radiolysis products from different starches. Our proposed method was not to undertake a chemical study as systematic as in the case of maize starch (Berger et al., 1977; Raffi et al., 1978); however, such a study would have been tedious and even unprofitable, our point being only to verify that the differences noticed after irradiation of these starches derive from various physical properties (degree of polymerization crystallinity, ...).

In addition to chemical studies, we also used electron spin resonance experiments in order to observe radioinduced radicals directly and to follow the influence of the

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Table I. Formation of Radiolytic Products in Starches Derived from Different Foodstuffs[h]

	product or function						
	carbonyl derivatives				acidities		
starch	total,[a] 10 kGy[e]	malonal.,[b] 10 kGy[e]	formal.,[b] 30 kGy[e]	acetal.,[b] 8 kGy[e]	formic,[c] 10 kGy[e]	total,[c] 10 kGy[e]	hydrogen peroxide,[d] 5 kGy[e]
MN	1.00	0.60	7.0	2.8	0.65	1.35	0.59
WM	1.20 +	0.60	8.2 +	5.1 +	0.85	2.65 +	0.38
AM	0.75	0.40	5.9	2.4	0.55	1.10	0.41
M	0.95	0.50	6.4	1.8	0.90	2.10	0.58
B	0.85	0.85 +	6.7	3.0	1.35 +	2.60	0.17 −
P	0.60 −	0.30	4.6	1.8	0.60	0.85 −	0.75 +
R	0.80	0.20 −	2.2 −	3.4	0.75	1.30	0.27
H	0.90	0.20	3.2	1.2	0.55	2.05	*f*
$\bar{R}$	0.9	0.5	5.5	2.7	0.77	1.75	0.45[g]
σ, %	21	50	37	45	35	40	45
r	2.1	4.6	3.8	4.1	2.5	3.2	4.4[g]
$\bar{G}$ (10 kGy)		1.2	4.8	8.7	2.0	4.6	2.4[g]

[a] Optical density. [b] Micrograms. [c] Microequivalents of H^+. [d] Micromolar. [e] Dose. [f] Value not determined; see Raffi et al. (1981b). [g] Averages on only seven starches. [h] The radioinduced quantities are per milliliter of aqueous extract (R) or per gram of starch (G). r is the ratio between the maximal (+) and the minimal (−) values. MN = maize; AM = amylomaize; WM = waxy maize; M = manioc; R = rice; B = bread wheat; P = potato; H = haricot bean.

Table II. Depolymerization and Kinetic Law for Radicals of Irradiated Starches Derived from Different Foodstuffs[h]

	product or function			
	radioinduced water-soluble products		macromolecule	
starches (equilibrium water content)	mass,[a] 40 kGy[e]	R.P.,[b] 60 kGy[e]	R.P.,[c] 10 kGy[e]	$10^3 \times k$,[d] 20 kGy[e]
MN (12.2)	4.5	7950	265	20.7 +
WM (12.5)	8.3 +	7700	330	11.5
AM (13.8)	3.4	7950	315	9.1
M (13.9)	7.5	7000 −	355 +	20.1
B (12.4)	4.0	8000 +	215	14.2
P (18.4)	2.7 −	7650	295	13.9
R (12.4)	5.7	*f*	180	14.1
H (12.2)	6.3	*f*	130 −	13.0
$\bar{R}$	5.3	7700[g]	260	14.6
σ, %	38	5	30	27
r	3.1	1.1[g]	2.7	2.3
$\bar{G}$ (10 kGy)	13.8			

[a] mg mL^{-1} (R) or mg g^{-1} ($\bar{G}$). [b] Limit reducing power (Raffi et al., 1981c). [c] Initial reducing power deducted. [d] k = kinetic constant, for "final radicals", in day^{-1}. [e] Dose. [f] Not determined. [g] Averages on only six starches. [h] r is the ratio between the maximal (+) and the minimal (−) values. MN = maize; AM amylomaize; WM = waxy maize; M = manioc; R = rice; B = bread wheat; P = potato; H = haricot bean.

starch variety and the radiolysis parameters upon them.

EXPERIMENTAL SECTION

Starches. Hereafter we shall consider the following types of starches: maize (MN), amylomaize or hylon (AM), waxy maize (WM), manioc (M), bread wheat (B), and potato (P) (supplied by Etablissements Roquette Frères, 62136 Lestrem, France), rice (R), and haricot bean (H).

These starches have a water content around 12–13% (18% for H); contents lying between 13 and 20% (18–25%) are obtained by mixing with the corresponding quantity of water; contents below 13% (18%) are obtained by low-temperature controlled dehydration under vacuum (freeze drier, Sogev, type Sublimac RP 12) or by drying on a fluidized bed (drier, Aromatic, type SRT 1).

Irradiations. They are carried out under air (dose studies) or nitrogen or oxygen (water content studies) at 25 °C with two ^{60}Co sources supplying dose rates of 0.60 and 6.2 kGy h^{-1}, respectively.

Measurements. The Brabender viscosity and the reducing power are directly studied on the macromolecule. All the radiolytic products are analyzed in the aqueous extract obtained as follows: X grams of irradiated starch are shaken for 1.5 h in $2X$ cm^3 of distilled water; then the suspension is filtered.

As all the methods have been detailed elsewhere (Raffi et al., 1981a–d), here we only give the principle of chemical titrations: colorimetry for the total of carbonyl derivatives (2,4-dinitrophenylhydrazine), malonaldehyde (thiobarbituric acid), formaldehyde (phenylhydrazine hydrochloride), and hydrogen peroxide [ammonium thiocyanide with iron(II) sulfate]; gas chromatography for formic acid (under methyl formate form) and acetaldehyde; pH-metry for total acidity; gravimetry for water-soluble dextrins; back-titration of iron(II) cyanide for reducing power.

Electron Spin Resonance. The spectra are recorded at room temperature on an ER 200 D 10 Bruker spectrometer combined with an Aspect 2000 computer.

RESULTS

Radiochemical Studies. The results summarized in Tables I and II correspond only to the irradiation of

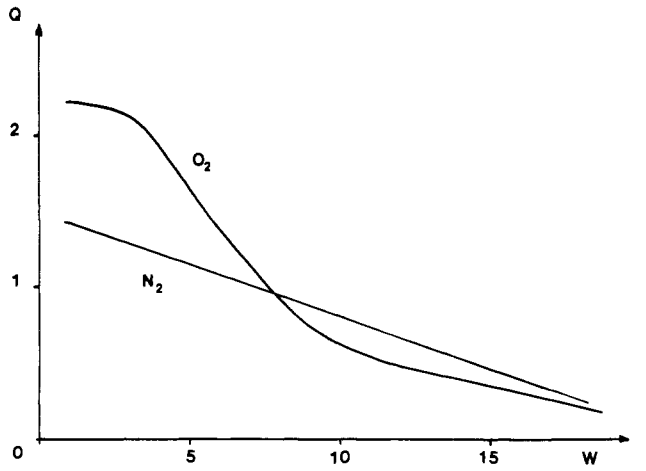

Figure 1. Quantity Q (optical density) of radioinduced malonaldehyde with regard to the water content, W (%), of maize starch. Irradiation dose of 20 kGy.

starches at equilibrium water content. The reason comes from the purpose of this study: applications to industrial radiopreservation of food and, more specifically here, radiodisinsectization of cereals. More details about the influence of irradiation parameters can be found elsewhere (Raffi et al., 1981a–d).

We give in Tables I and II the quantities R of radiolytic products found in the aqueous extract. The amounts G radioinduced by grams of starch are easily derived from R values, as the extraction efficiency does not vary significantly from one variety to another (Raffi et al., 1981a):

$$G\ (\mu g\ g^{-1}) \simeq 2.6R\ (\mu g\ mL^{-1})$$

In order to discuss the validity of the extrapolation of the data, we added in the tables the values $\bar{R}$ (average value of R) and r (ratio between maximal and minimal values or R).

Carbonyl Derivatives (Raffi et al., 1981a). At equilibrium water content, the quantities of carbonyl derivatives radioinduced in the different starches always have the same order of magnitude: r (Table I) varies from 2.1 (total carbonyl derivatives) to 4.6 (malonaldehyde).

Despite some small differences in the shape of the curves showing the influence of the irradiation dose (5 exceptions among the 32 curves), the above result remains valid.

Moreover, the shapes of the curves showing the influence of starch water content are very similar: inhibiting effect (total carbonyl and malonaldehyde) or maximal value (formaldehyde and acetaldehyde). If irradiation is performed under oxygen, in place of nitrogen, the final effect is the same: an activating effect with the exception of malonaldehyde. In the latter case oxygen has an activating effect in the dry state and an inhibiting effect in the moist state; the intersection point, between "oxygen and nitrogen curves", is always at 2–3% below the equilibrium water content value (Figure 1).

Radioinduced Acidities (Raffi et al., 1981d). In a former study we had to define the two following acidities: the free acidity value is the quantity of sodium hydroxide put into the aqueous extract to reach the neutral pH; the total acidity is measured in the same way, but after a basic treatment of the aqueous extract (heating at 50 °C, pH 11).

The concept of free acidity must be used with caution and only to get an evaluation of the aqueous extract pH. Most of this acidity is due to formic acid (70–100% at doses lower than 15 kGy). The shape of the curve showing the influence of irradiation does on formic acid is always

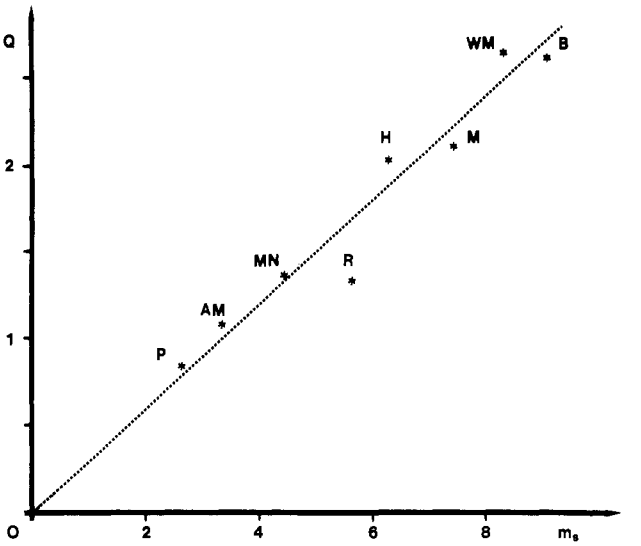

Figure 2. Relation between the total radioinduced acidity, Q (μequiv mL^{-1}), and the mass, m_S (mg mL^{-1}), of radiodextrins (irradiation: 10 kGy).

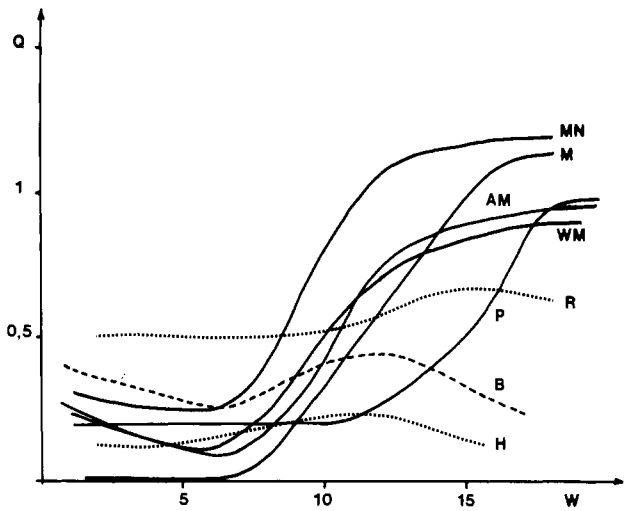

Figure 3. Quantity Q (optical density) of radioinduced hydrogen peroxide with regard to the water content, W (%). Irradiation dose of 5 kGy under oxygen.

the same and the values found are very similar (Table I): the ratio r is equal to 2.5.

In the case of total radioinduced acidity, r is equal to 3.2. Furthermore, there is a simple relation between this acidity and the mass of water-soluble dextrins (Figure 2).

The influences of the water content and of the storage time on these two acidities are not simple (the variations may be positive or negative depending on the starch) but quantitatively slight. Nevertheless, the oxygen always has an activating effect.

Radioinduced Hydrogen Peroxide (Raffi et al., 1981b). Hydrogen peroxide is found only if the irradiation is carried out under oxygen. As can be seen on Table I and Figure 3, the curves are very similar with an inflection point at 2 or 3% below the equilibrium water content. The special shape of curves obtained for B, R, and H may derive from the fact that B, R, and H are the raw materials where the radioinduced quantities of acid have extreme values; indeed, Berger and Saint-Lèbe (1971) already noticed the great influence of "starch pH" on the formation of hydrogen peroxide.

Radiodepolymerization (Raffi et al., 1981c). The radiodepolymerization of maize starch was already studied

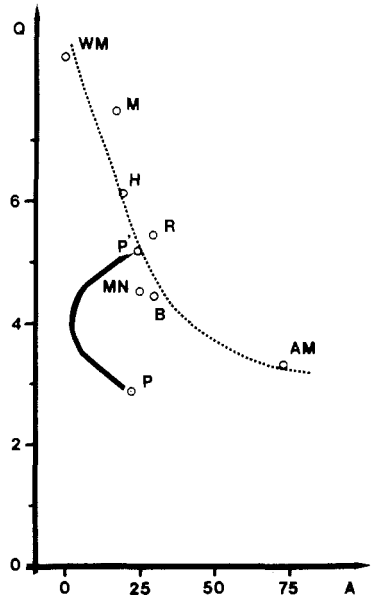

Figure 4. Relation between the mass, Q (mg mL^{-1}), of water-soluble products and the percentage, A (%), of amylose of starches (see the text for signification of P').

under its theoretical (Raffi et al., 1980) and experimental aspects (Michel et al., 1980). It is worth noticing that all the varieties studied here behave similarly under irradiation. If the masses of water-soluble radiodextrins are different (Table II), they are, as previously predicted (Raffi et al., 1980), related to the percentage of amylose (Figure 4). The only apparent exception, potato starch (point P of the graph), is due to the fact that the equilibrium water content is 18.4% instead of 12–14% for the other starches; after corrections at 13%, the point P' obtained falls on the curve.

For these masses, the ratio r is 3.1, but it is only 1.1 for the reducing power of the radiodextrins and 2.7 for the reducing power of the macromolecule.

Partial Conclusion. Indeed, there is no marked difference between all the starches studied: if the values R vary from one variety to another, r is always lower than 5.

But the experiments must be carried out the same week, as the equilibrium water content, and consequently R, is very sensitive to the atmosphere temperature and moisture. Thus, important variations may be found from one experiment to another in some particular cases; for instance, we found for water-soluble radiodextrins of B masses equal to 9.2 and 4 mg mL^{-1} in two series of experiments; so the respective points on Figures 2 (relation to total acidity) and 4 (relation to the amylose percentage) are not comparable and must not be related to each other.

Electron Spin Resonance Studies. Most of the numerous ESR experiments which were undertaken on irradiated polysaccharides are 10 years old or more: 60% before 1969; only 15% after 1975. A comprehensive discussion and extensive literature on this subject can be found in the report by Kochetkov et al. (1979), which points out the difficulty of such experiments. These early results contain some contradictions: irradiated starch and cellulose display identical or distinct spectra; irradiated starch (or cellulose) from various raw materials exhibit identical or different ESR signals; oxygen (as well as water) has no effect upon the spectrum, decreases the spin concentration, or alters the spectrum shape.

While the spectrometers were greatly improved, the recent investigations are oriented toward low-temperature experiments (Abaghian and Apresyan, 1979; Plonka et al., 1979), action of hydrogen atoms (Abaghian, 1979), and use of sensitizers (Merlin and Fouassier, 1980). Our purpose being chemiclearance, here we carried out a systematic study on dry starches, generally irradiated under nitrogen and at room temperature.

Prior experiments show that the radicals follow a complicated kinetics. During the first 1 or 2 h, the spectra are severely modified, the kinetics over the main lines being of second order. Then, after an intervening time (several days or weeks according to the foodstuff), the spectra undergo slight variations and decrease with a first-order rate, the final spectrum being common to all the investigated starches. However, as can be seen on Figure 5, the "initial" spectra, recorded 2–3 h after irradiation, on starches derived from several raw materials, are not identical. Moreover, the water content affects the initial spectra, particularly if present during the irradiation, but

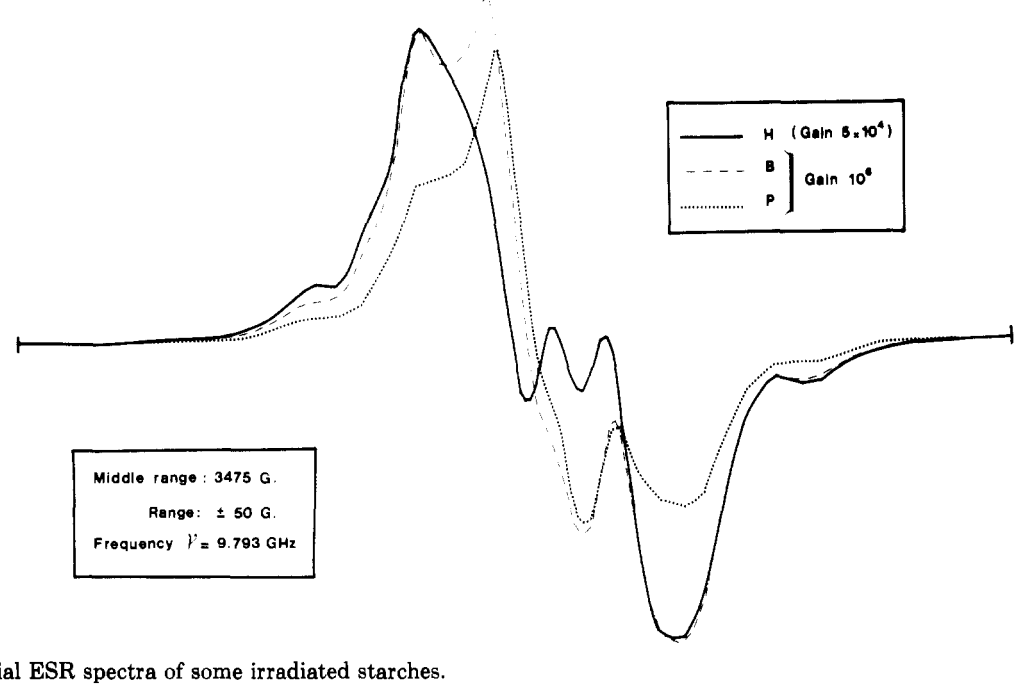

Figure 5. Initial ESR spectra of some irradiated starches.

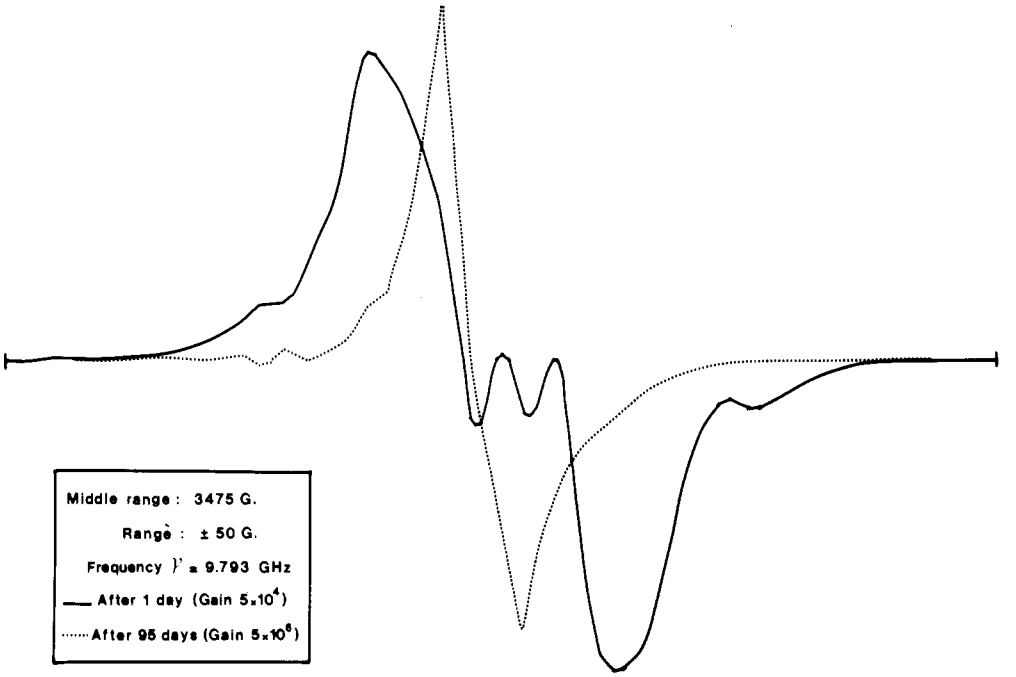

Figure 6. Final ESR spectrum, common to all the irradiated starches (dotted line), compared to the initial ESR spectrum of haricot bean starch (solid line).

not the "final" spectrum (Figure 6). An oxygen effect is also observed on the first step of the kinetics.

In fact, the final first-order rate appears to be similar for all the foodstuffs. So, the observed differences come from distinct initial kinetics. A detailed kinetic study would be fairly complex, the radicals being randomly oriented in the powder state, but might be done using models (maltotriose, ...) and spin-trapping experiments. Nevertheless, the final kinetic constants calculated from 20 to 80 days are indicated in Table II; in this experiment the ratio r between the extreme values is equal to 2.3 for "dry" starches (water content: 2–4%), but it is clear that the drier the starches, the more similar the kinetics.

How can these variations be explained? We have to consider that starch is not a simple macromolecule but contains crystalline as well as amorphous fractions. The various initial kinetics may reflect differences in molecular arrangements (Arthur et al., 1966). As already pointed out for acid hydrolysis (Robin et al., 1974), the penetration kinetics of protons, water, or oxygen molecules differ in crystalline and amorphous parts: crystalline regions "produce" free radicals with longer lifetimes than in less ordered fractions (Ahmed and Rapson, 1972). The structure and percentage of crystalline parts vary with the foodstuff, which may be sufficient to explain the observed kinetic differences.

Despite the fact that powder spectra are only poorly resolved, computer simulations seem to indicate that they are identical. Therefore, since the chemical nature of the glucosidic chains of the different starches is the same and since the kinetic decreases of spin concentrations are closely related, there is a new presumption to ascertain that the main radioinduced radicals are the same for any starch.

CONCLUSION

In summary, this work shows that γ irradiation leads to similar effects upon starches from various raw materials: the final radiolysis products are identical, and it can be expected that the main radicals, which exhibit similar ESR signals and kinetic behavior, are likely the same for the eight investigated starches and, extrapolating, for any one. The only variations found in the results are related to physical (crystallinity, ...) or chemical (amylose percentage, ...) differences. The case of starches must be compared to other foodstuffs (Taub et al., 1976; Basson and Elias, 1978) and leads once more to the conclusion (WHO, 1977, 1981) that radiation treatment must actually be considered as a process and not as a food additive. This conclusion should be helpful for health authorities in evaluating petitions based on wholesomeness data. As a matter of fact, it is mainly on the ground of such studies that the 1980 Joint WHO/FAO/IAEA Expert Committee (Genova, Oct 27–Nov 3) has just ascertained the wholesomeness of foods irradiated at doses below 10 kGy (WHO, 1981); indeed, with radiochemical conclusions, it is rational to extrapolate the results of toxicity assays, i.e., animal feeding studies of mutagenicity tests, from individual foods to other varieties of the same class of food without further biological testing.

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Studies on the Proteins of Poppy Seed (*Papaver somniferum* L.)

H. Srinivas and M. S. Narasinga Rao*

The proximate composition of three varieties of poppy seed, namely, "Dhawla Bada", "Dhawla Chotta", and commercial, showed that the commercial variety had a slightly lower content of protein and oil. Poppy seed meal proteins showed minimum solubility in water around pH 6.6 and a maximum at pH 9.2; in 1 M NaCl and 2% sodium hexametaphosphate solution, maximum solubility was at pH 6.6. Gel filtration on Sepharose 6B gave three peaks, and in polyacrylamide gel electrophoresis one major and several minor bands were observed. In ultracentrifugation four peaks having $s_{20,w}$ values ranging between 0.8 and 1.3, 6.1 and 6.6, 9.1 and 10.3, 14.1 and 15.1 were obtained. The major fraction had an $s_{20,w}$ value of 9.1–10.3. No trypsin inhibitor or hemagglutinin activity was detected in the meal. The proteins of poppy seed were rich in aspartic and glutamic acids and arginine. The amino acid composition did not reveal major differences among the three varieties.

Poppy seeds, from the fruits of *Papaver somniferum* L. (Papaveraceae), are grown in the temperate and subtropical regions of the world. In India, the seeds are mainly grown in the states of Madhya Pradesh, Uttar Pradesh, and Rajasthan. The seed is a good source of protein and oil. Traditionally the seeds are used in food preparations like curries, breads, sweets, and confectionary. Some work is reported on the composition of the oil (Cosovic and Prosteric, 1973; Yarosh and Megorskaya, 1975; Beare-Rogers et al., 1979) and the nutritive value of the proteins (Satyanarayana et al., 1956; Eklund and Agren, 1975). Practically no data are available, however, on the composition and physicochemical characteristics of the proteins of poppy seed. The present investigation deals with the characterization of the proteins from three varieties of poppy seed.

MATERIALS AND METHODS

Materials. Poppy seed varieties "Dhawla Bada" and "Dhawla Chotta" were obtained from the Government Opium Factory, Neemuch, Madhya Pradesh, India, and the commercial variety was obtained through M/s Organon, Calcutta, India.

Preparation of Defatted Poppy Seed Meal. Poppy seed was thoroughly cleaned to remove the impurities and then flaked. The flakes were solvent-extracted 6 times with hexane and air-dried to remove the solvent. The defatted meal was ground to a fine powder and passed through a 85-mesh sieve (BSS).

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Proximate Composition. Moisture, protein ($N \times 6.25$), ether extractives, and ash were determined by AOAC (1975) methods. Fiber was estimated by the neutral-detergent fiber method of analysis (Goering and Vansoest, 1970).

Nitrogen Solubility. Two grams of poppy seed meal was mixed with 20 mL of solvent and the pH of the slurry was adjusted to the desired pH by the addition of 2 N HCl or 2 N NaOH. The solvents used were water, 1 M NaCl, and 2% sodium hexametaphosphate (SHMP) in water. The suspension was mechanically shaken for 1 h at room temperature (~28 °C) and centrifuged at 6000 rpm for 30 min, and the pH of the clear supernatant was noted. Aliquots (10 mL) were used for nitrogen determination by the Kjeldahl method. The solubilized nitrogen was expressed as percentage of the total meal nitrogen.

Gel Filtration. Sepharose 6B-100 (Pharmacia Fine Chemicals) which had been equilibrated with 1 M NaCl was packed into a column (1.5 × 100 cm). About 70 mg of protein in 1 M NaCl was applied to the column. The column was eluted with 1 M NaCl solution at a flow rate of 25 mL/h. Fractions (3 mL) were collected in an automatic fraction collector and the absorbance was monitored at 280 nm.

Polyacrylamide Gel Electrophoresis. Electrophoresis was performed according to the method of Davis (1964) using 7.5% gels. In the anionic system, electrophoresis was done in a 0.025 M Tris–glycine buffer of pH 8.3; for the cationic system a 0.05 M β-alanine–acetic acid buffer of pH 4.5 was used. Approximately 200 μg of protein was applied to the gel. Electrophoresis was done for 60 min at 3 mA/tube. The gels were stained with 0.05% Coo-

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