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Biological and Biochemical Changes in Two Nigerian Species of Sorghum (SK.5912 and HP3) following Premalting-γ-Irradiation Treatment

A. O. Uwaifo

Two Nigerian species of sorghum—*Sorghum acaudatum* (SK.5912) and *Sorghum guineense* (HP3)—were γ-irradiated prior to malting on a Cobalt irradiator. The species were exposed to the following doses—0.22, 0.44, 1.76, and 4.95 krd—in the assays for diastatic power, β-amylase, and α-amylases, while in the assays for germinative energy and lengths of rootlets and acrospire they were exposed to a dose range of 0–5 krd. A dose of 1.76 krd raised the diastatic power, β-amylase, α-amylase, germinative energy, and lengths of rootlets and acrospire maximally relative to those of the unirradiated sorghum in the species studied. This effect of 1.76 krd was, however, higher in SK.5912 species than in HP3 species. This dose also raised DNA levels in the two species relative to that of the unirradiated species but had the reverse effect on protein levels in the two species.

γ-Irradiation has been reported to have predilection for proteins, enzymes, DNA, RNA, amino acids, and nucleoproteins in its effects on biological materials (EL-Meteiny et al., 1973).

The nature and degree of these effects are dose dependent (Holms, 1957). γ-Irradiation effects on barley is one of those most extensively studied (Tipples and Norris, 1963; Adams and Nilan, 1958) to date, this being so because of its utilization in the manufacture of beer.

In this study the results obtained when two sorghum species are exposed to different doses of γ-irradiation prior to malting are presented. The biological and biochemical parameters monitored after irradiation and subsequent malting include (i) DNA levels, (ii) diastatic power, (iii) β-amylase and (iv) α-amylase activities, (v) protein levels, (vi) germinative energy, (vii) length of rootlets, and (viii) length of acrospire.

MATERIALS AND METHODS

γ-Irradiation. A total of 75 g of sorghum devoid of extraneous particles (stones and broken seeds) was placed in a glass receptacle provided along with the irradiator (^{60}Co γ irradiator, Model 3500, Noratom-Norcontrol A/S, Holmenveien 20, Oslo 3, Norway) and irradiated at a dose rate of 400 ± 40 rd/min at room temperature (25 ± 2 °C). The effective doses to which the sorghum species were exposed in the different assays are shown in the figures for these assays.

Malting. The γ-irradiated and unirradiated sorghum were malted along the lines outlined in the work of Dyer and Novellie (1966).

Determination of the Activities of the Amylases and Diastatic Power. β-Amylases was determined in the malted γ-irradiated and unirradiated sorghum species by the method outlined in the work of Kneen and Sandstedt (1941) while α-amylase in the two species of sorghum was determined by the method of Meredith (1976). The diastatic power of irradiated and unirradiated sorghum species was determined by the method of Novellie (1962).

Determination of DNA and Protein. DNA was assayed by the diphenylamine method of Burton (1956) and protein by the method of Lowry et al. (1951). Bovine serum albumin BSA (SIGMA) and crystalline DNA (Sigma) were used as standards in the assays.

RESULTS

As depicted in Figure 1, 2.0 krd seems to have elevated maximally the germinative energy and growth of rootlets in sorghum species SK.5912 and HP3. Growth in the two

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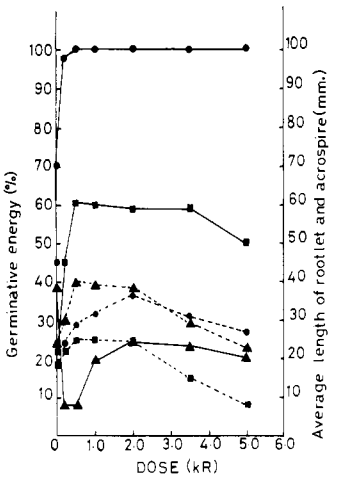

Figure 1. Germinative energy (percent) and average lengths of rootlets and acrospire (millimeters) of sorghum species (SK.5912 (—) and HP3 (---) following γ-irradiation and subsequent malting. (●) Germinative energy (percent). $-V_2/V_3$ (variance ratio) = 1.71 × 10^3 (ϕ_2 = 1, ϕ_3 = 16). Variance difference is significant at P = 0.05. V_2 = variance between irradiation doses; V_3 = variance within doses in three experiments of three samples per experiment. (■) Length of rootlets (Lr); (▲) length of acrospire (La). Standard deviations of Lr and La at a dose of 1.0 krd: Lr = 60 ± 3.24 (SK.5912) and 20 ± 1.89 (HP3); La = 20 ± 1.05 (SK.5912) and 35 ± 1.15 (HP3). Coefficient of variation: Lr = 5.4% (SK.5912) and 9.5% (HP3); La = 5.3% (SK.5912) and 3.3% (HP3). Lr and La were determined in 100 viable seeds in triplicate in three experiments.

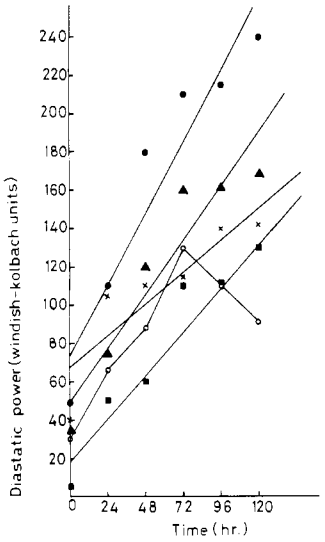

Figure 2. Diastatic power of SK.5912 following γ-irradiation and subsequent malting. (×) Unirradiated (control), r = 0.89; (●) 1.76 krd, r = 0.95; (▲) 0.44 krd, r = 0.94; (○) 4.95 krd, r = 0.74; (■) 0.22 krd, r = 0.97. Variance ratio (V_2/V_3) of variance between doses (V_2, ϕ_2 = 4) to variance within doses (V_2, ϕ_3 = 40) = 0.544 × 10^3. Variance difference is significant at P' = 0.05. Total of nine determinations in three experiments.

species, as indicated in all the measured growth parameters except germinative energy, after reaching optima as depicted in Figure 1 fell gradually, reaching their minima at 5.0 krd. A total of 1.76 krd raised the diastatic power optimally in the two species of sorghum over a malting time period of 120 h. The diastatic power was, however, relatively higher in SK.5912 than in HP3 (Figures 2 and 3). This dose (1.76 krd) also raised α- and β-amylase activities in the irradiated cereals relative to those in the unirradiated control in both species (Figures 4 and 5). The concentration of DNA in irradiated sorghum relative to that in unirradiated sorghum in the two species was also

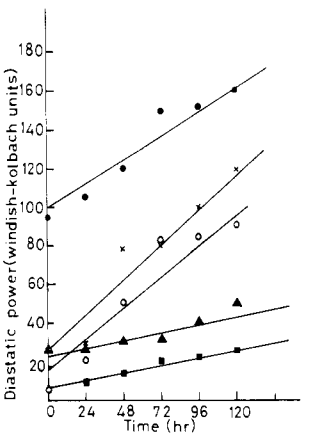

Figure 3. Diastatic power of HP3 following γ-irradiation and subsequent malting. (×) Unirradiated (control), r = 0.97; (●) 1.76 krd, r = 0.97; (▲) 0.44 krd, r = 0.92; (○) 4.95 krd, r = 0.96; (■) 0.22 krd, r = 0.99. Variance ratio (V_2/V_3) of variance between doses (V_2, ϕ_2 = 4) to variance within doses (V_2, ϕ_3 = 40) = 1.90 × 10^3. Variance difference is significant at P' = 0.05. Total of nine determinations in three experiments.

elevated by this dose (1.76 krd), but here again the DNA level was raised to a higher degree in SK.5912 than in HP3 (Figure 6). Also, whereas in SK.5912 the optimum level of DNA observed was on the fourth day of malting, for HP3 it was on the third day. Protein concentration, however, fell in the two species to a minimum on the first complete day of malting, rose to a maximum on the second day, and then fell gradually to a minimum on the sixth day (Figure 7).

In irradiated HP3 the concentration of protein hardly fell at all after the rise on the second day while in the unirradiated the fall was sharp on the third day (Figure 7). In both species, however, the concentration of protein was consistently higher in the unirradiated than in the irradiated sorghum, except in HP3 in which the reverse position was the case after the second day of malting (Figure 7).

DISCUSSION

The effects of γ-irradiation on the growth and development of cereals are widely reported in the literature (Adams and Nilan, 1958; Kamazir, 1969; Saric, 1958; Ehrenberg, 1955). In general, results obtained in this study on the effect of γ-irradiation on germinative energy and growth of rootlets and acrospire are very similar to those reported in the above studies (see Figure 1).

On the contrary, whereas the effect of γ-irradiation on α- and β-amylases in barley malt at doses up to 100 krd is not significant (Tipples and Norries, 1963), in our studies significant stimulatory effects were observed at 0.22–1.76 krd. The optimal stimulatory effect observed at 1.7 krd prompted the study on the effect of this dose on biochemical parameters such as α- and β-amylase activities and protein and DNA concentrations. Also, EL-Meteiny et al. (1973) had reported the predilection of γ-irradiation for biomolecules such as proteins, RNA, DNA, amino acids, and nucleoproteins. In our studies, the stimulation on DNA observed may be a manifestation of increased DNA synthesis that is a necessary corollary of increased mitotic activity that also is a reflection of increased growth activity attending malting processes. Again, the initial sharp rise of DNA concentration that was more pronounced in the irradiated than in the unirradiated species of sorghum (Figure 6) may be due to the dissociation of nucleoproteins from DNA following radiation (Kamazir, 1969).

It is also possible that the increased in DNA concentration in the γ-irradiated sorghum species relative to that

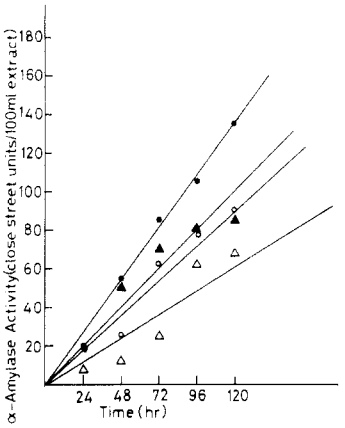

Figure 4. α-Amylase activities in sorghum species following γ-irradiation and subsequent malting. γ-Irradiated (1.76 krd): (●) SK.5912, $r = 0.99$; (▲) HP3, $r = 0.97$. Unirradiated (control): (○) SK.5912, $r = 0.98$; (△) HP3, $r = 0.95$. Variance ratio (V_2/V_3) of variance between treatments (V_2, $\phi_2 = 1$) to variance within treatments (V_3, $\phi_3 = 16$) = 0.35×10^3 for SK.5912 and 0.26×10^3 for HP3. Variance difference is significant at $P' = 0.05$. Total of nine determinations in three experiments.

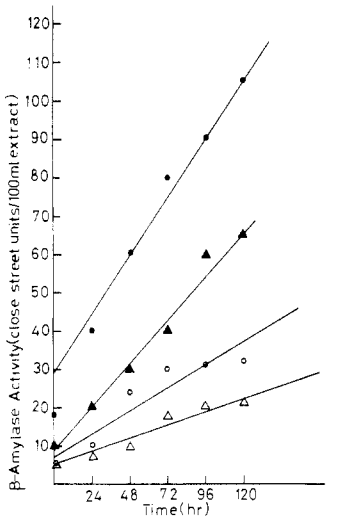

Figure 5. β-Amylase activities in sorghum species following γ-irradiation and subsequent malting. γ-Irradiated (1.76 krd): (●) SK.5912, $r = 0.99$; (▲) HP3, $r = 0.99$. Unirradiated (control): (○) SK.5912, $r = 0.93$; (△) HP3, $r = 0.97$. Variance ratio (V_2/V_3) of variance between treatments (V_2, $\phi_2 = 1$) to variance within treatments (V_3, $\phi_3 = 16$) = 1.15×10^3 for SK.5912 and 0.85×10^3 for HP3. Variance difference is significant at $P' = 0.05$. Total of nine determinations in three experiments.

in the unirradiated ones may be a manifestation of a rise in replication of DNA, one of the molecular repair processes, following damage inflicted by γ-irradiation.

The protein concentration, which seems to have fallen sharply within the first 24 h of malting in the irradiated and unirradiated in the two species of sorghum, was higher in the unirradiated than in the irradiated for the first 48 h of malting in HP3 and for 120 h in SK.5912. This result is compatible with the observation of higher growth in the irradiated than in the unirradiated on the grounds that the hydrolysis of proteins to amino acid is concomitant of inhibition of water by cereals—a normal prelude to germination (Goodwin and Mercer, 1974).

On the whole, 1.76 krd seems to have had a benign effect on the two sorghum species studied, particularly with regard to their potential utilization in the brewing industry. In this regard, sorghum species SK.5912 seems to be a

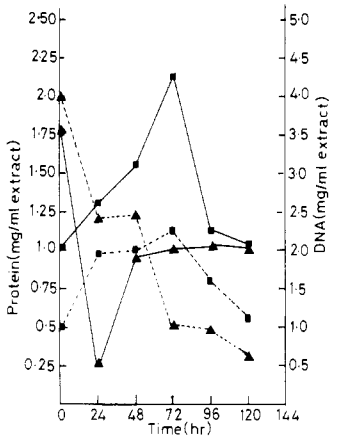

Figure 6. DNA and protein concentrations in HP3 after γ-irradiation (1.76 krd) and subsequent malting. (---) Unirradiated (control); (—) irradiated (1.76 krd). (■) DNA; (▲) protein. Variance ratio (V_2/V_3) of variance between treatments (V_2, $\phi_2 = 1$) to variance within treatments (V_3, $\phi_3 = 16$) = 63.8 (protein) and 47.4 (DNA). Variance difference is significant at $P' = 0.05$. Total of nine determinations in three experiments.

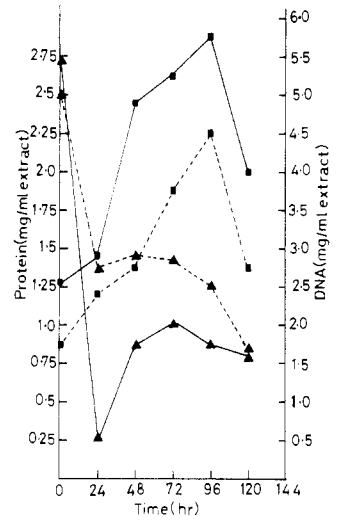

Figure 7. DNA and protein concentrations in SK.5912 after γ-irradiation (1.76 krd) and subsequent malting. (---) Unirradiated (control); (—) irradiated (1.76 krd). (■) DNA; (▲) protein. Variance ratio (V_2/V_3) of variance between treatments (V_2, $\phi_2 = 1$) to variance within treatments (V_3, $\phi_3 = 16$) = 45.83 (protein) and 310.80 (DNA). Variance difference is significant at $P' = 0.05$. Total of nine determinations in three experiments.

better prospect.

Registry No. β-Amylase, 9000-91-3; α-amylase, 9000-90-2.

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Polyphenol Changes in Sorghum Grain during Malting

C. William Glennie

Bird-resistant and non-brid-resistant sorghum cultivars were malted to study the effects of tannin on malting as well as the change in polyphenols during malting. No difference could be found in the percent germination nor in the root and shoot production of the malts of the two cultivars. There was an increase in the anthocyanidin content of the roots and shoots during malting. Since there was also an increase in the anthocyanidin content of the non-bird-resistant kernels, it would appear that there is no migration of anthocyanidins from the kernels to the roots and shoots. A transmission electron micrograph of the testa after malting showed a testa very similar to that before malting. The tannin in the bird-resistant sorghum picked up extra protein or peptides during malting. Although it was difficult to establish the source of this extra material, amino acid analysis suggested that it was not from the prolamin storage protein in the endosperm.

Although sorghum beer has been traditionally brewed only in Africa, studies involving the malting of sorghum grain have been carried out in several countries having hot climates. In Sri Lanka it was hoped to replace imported barley malt with locally produced sorghum malt (Jayatissa et al., 1980). In Nigeria, studies involved the brewing of the lager beer (Okafor and Aniche, 1980), as well as the local Otika (Ogundiwin and Tehinse, 1981), from malted sorghum grains. In South Africa, sorghum is still malted for the brewing of traditional sorghum beer although some of the beer is now brewed by modern industrial methods (Novellie, 1968).

Besides geographical diversity, these sorghum malting studies covered a wide variety of modification changes. Unlike barley, gibberellic acid has little effect on amylase production in sorghum and it is probable that this production is a function of the scutellum rather than the aleurone layer (Daiber and Novellie, 1968; Aisien, 1980). The carbohydrate products of enzyme modification were established as glucose, maltose, and maltotriose (Aisien, 1982). Since malted sorghum is now used for brewing other types of beer as well as the traditional one, enzyme composition and activity are of considerable importance. The development of enzyme activity in germinating sorghums of different varieties has been reported by Nout and Davies (1982). They concluded that most sorghum malts were not desirable for conventional lager but some varieties were acceptable for production of a "tropical lager".

Because of their high diastatic power and good agronomic properties, bird-resistant sorghums are being produced extensively in South Africa. The large amounts of tannin found in the testae of these bird-resistant grains can inhibit enzymes essential to the brewing process (Daiber, 1975). This inhibition is caused by the tannins reacting with the enzymes to form insoluble complexes. Besides reducing enzyme activities, these tannins can also reduce the nutritional quality of the grain. Hence, there are numerous reports in the literature regarding the problems of tannin in sorghum grain. Such aspects as tannin structure, biosynthesis, location in the kernel, biochemical effects, and bird resistance are ably discussed by experts in proceedings edited by Hulse (1979).

The research literature on malting is very extensive and much has been reported on tannin, but very little has been reported on the effect of malting on tannin or on the effect of tannin on malt quality. Chavan et al. (1981) reported that approximately 73% of the tannins of a high-tannin sorghum were lost during germination. Also, they found that seedling growth was suppressed in these high-tannin grains and that the rate of starch and protein degradation was reduced. In another study it was found that after 6 days of malting some of the sorghum tannins required more solvent to elute them from a Sepharose CL-6B column than was necessary before malting (McGrath et al., 1982). The fate of the tannins during malting have now been more extensively studied, especially in respect to their location in the testa. Besides the tannins, the fate of other phenolic compounds such as anthocyanidins, phenolic acids and the cyanogenic compound, dhurrin, have been studied during malting and these studies are now reported.

MATERIALS AND METHODS

Malting. Three cultivars of sorghum grain, Barnard Red, NK283 and SSK30, were harvested from the 1981/1982 crop. The first two are not bird resistant while the third one is. The grain was surface sterilized with 0.2% Adcodyne (an idophore) and steeped for 16 h (water changed after every 3 h with a 1-h air rest). Malting was carried out in nylon mesh bags loaded so that the layer of grain was three to five kernels thick. A Forma Scientific incubator was used to keep the temperature at 28 °C and the relative humidity at 99%. The grain was watered twice a day by steeping (1 × 5 min, 1 × 10 min) in water containing 0.05% hypochlorite. Excess moisture was removed

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