Effect of γ Irradiation and Storage Conditions on Amino Acid Composition of Some Iraqi Dates

Hamid Auda* and Hussain AL-Wandawi

The influence of γ radiation in the range of 30–1000 krad and storage at zero and room temperature (25–30 °C) on the total amino acid composition of fully mature dates of four varieties (Sayer, Zahdi, Hillawi, and Khastawi) was investigated. Variation of different individual amino acids was found to be varied with regard to radiation dose, storage temperature, and varietal differences.

Dates represent an abundant source of carbohydrates; however, large quantities of stored dates are annually lost due to insect attack.

Although dates are usually disinfested by fumigation with methyl bromide, reinfestation does occur, thus causing a high percent of deterioration. Low doses of γ radiation were used successfully for disinfestation of dates (Ahmed, 1976). Wholesomeness studies on irradiated dates revealed no genetic or adverse effects due to the irradiation of date when mammalian cells and laboratory animals were used as indicators (IFIP Report, 1974–1978).

The effect of γ irradiation on the amino acid composition (free and total) of several varieties at different stages of fruit maturation was previously reported (Auda et al., 1973, 1976).

The present work is part of a series of investigations concerning the chemical changes induced in dates upon γ irradiation, with special attention being paid to their amino acid constituents as an attempt to support the wholesomeness studies on irradiated dates. Other studies on chemical changes induced by radiation in some date components such as carotenoids, protein, and carbohydrates were also carried out (Auda et al., 1973, 1978).

MATERIALS AND METHODS

Date Samples. Four local varieties of dates, namely Sayer, Zahdi, Hillawi, and Khastawi, were obtained from Za'afarania Horticultural Experimental Station and visually sorted for uniform size and maturity. The samples were either used immediately or stored in a deep freeze.

Irradiation and Storage. Samples (fruits) of each variety were irradiated (in the range of 30–1000 krad) in a ^{60}Co γ cell 220 (Atomic Energy of Canada Ltd.). For storage studies two varieties (Zahdi and Sayer) were irradiated with 100 and 270 krad and stored for 3 weeks at room temperature (25–30 °C), while the other two varieties (Hillawi and Khastawi) were irradiated with 30, 100, 270, and 1000 krad and stored for 2 months at 0 °C. Nonirradiated samples were also stored at the above two storage temperatures.

Total Amino Acid Analysis. Total amino acids of all treatments were analyzed on a Beckman Model 120C amino acid analyzer and according to our standard method (Auda et al., 1976).

RESULTS AND DISCUSSION

Values for total amino acids of irradiated and nonirradiated dates for varieties Sayer and Zahdi are presented in Table I. The data revealed that the concentration of various amino acids was varied, and it can be noticed, however, that a dose of 270 krad caused an increase in some amino acids. With the exception of cystine, the amino acid composition of the varieties Khastawi and Hillawi irradiated with 30, 270, and 1000 krad (Table II) is in agreement with the above observation. There was, however, a slight increase in values with 270 krad, and it is worthy to mention that most of them showed some variation in concentration. The results of total amino acids of dates irradiated and stored at room and zero temperature are shown in Tables III and IV, respectively. With few exceptions the amino acid composition is found to be basically unaffected by postirradiation storage.

Table I. Amino Acid Content (mg/10 g Dry Weight)[a] of Irradiated and Nonirradiated Date[b] (Varieties Saver and Zahdi)

amino acid	Sayer			Zahdi		
	krad			krad		
	0	100	270	0	100	270
Lys	4.11	4.88	4.67	3.18	3.81	2.94
His	1.76	1.92	1.90	1.49	1.44	1.79
Arg	4.63	5.03	4.93	4.85	4.81	4.48
Asp	8.29	9.48	9.18	16.63	16.25	19.02
Thr	2.57	2.80	2.73	2.92	3.62	3.55
Ser	3.39	3.59	3.69	3.91	4.27	4.88
Glu	16.04	15.57	15.81	20.34	18.19	20.55
Pro	9.34	8.99	9.55	4.83	4.58	5.29
Gly	5.99	6.24	6.36	7.36	7.74	8.12
Ala	5.71	6.26	6.26	8.09	7.99	8.20
Cys	2.55	2.32	2.02	2.11	1.40	1.82
Val	3.77	3.95	4.32	5.03	5.00	4.88
Met	0.73	0.41	0.96	1.38	1.09	1.25
Ile	2.72	2.74	2.05	2.75	2.90	3.03
Leu	5.12	5.30	5.66	5.35	5.69	5.48
Tyr	1.41	1.71	1.74	2.06	2.59	2.50
Phe	2.96	3.22	3.37	2.67	3.04	3.00

[a] Average of duplicate analysis. [b] Forty fruits per sample.

The change in amino acid values was not high for most amino acids when dates were irradiated and stored at 0 °C (Table IV). In the Hillawi variety, arginine and tyrosine, however, showed a reduction at 30 krad, while serine and glutamic acid were increased for the same dose. On the other hand, histidine, arginine, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, and phenylalanine were all reduced at the 30-krad dose for the variety Khastawi. At 270 krad only proline was significantly reduced in variety Hillawi and was slightly increased in variety Khastawi. At 1000 krad a varietal difference was observed; thus most amino acids were reduced in value for variety Khastawi while the majority of them increased in value for the variety Hillawi.

It may be concluded, however, that radiation treatment up to 1 mrad of four varieties of Iraqi dates induced a slight variation in amino acid composition when irradiated at

Iraqi Atomic Energy Commission, Nuclear Research Centre, Department of Biology and Agriculture, Tuwaitha, Baghdad, Iraq.

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Table II. Amino Acid Content (mg/10 g Dry Weight)[a] of Irradiated and Nonirradiated Date[b] (Varieties Hillawi and Khastawi)

amino acid	Hillawi krad 0	Hillawi krad 30	Hillawi krad 270	Hillawi krad 1000	Khastawi krad 0	Khastawi krad 30	Khastawi krad 270	Khastawi krad 1000
Lys	8.70	7.71	8.82	8.78	10.02	9.45	10.48	9.02
His	3.67	3.27	3.66	3.64	4.12	3.88	4.47	3.82
Arg	8.15	7.37	8.09	8.73	8.50	8.36	8.80	8.01
Asp	17.91	16.53	20.61	18.23	16.72	16.69	18.57	16.53
Thr	6.82	6.35	7.02	6.70	6.35	6.29	6.82	6.31
Ser	7.99	7.84	8.26	7.77	7.82	7.33	8.10	8.03
Glu	24.56	22.50	25.29	26.11	23.08	22.25	24.81	23.04
Pro	17.45	15.35	20.92	16.94	15.12	14.59	16.31	15.35
Gly	12.63	11.51	13.07	12.72	12.57	11.92	13.21	12.59
Ala	13.17	12.54	14.03	13.93	9.42	9.08	10.05	9.77
Cys	5.31	5.42	5.74	5.62	5.53	5.96	6.31	6.24
Val	9.15	7.77	8.67	9.11	9.37	8.85	9.28	8.70
Met	2.68	1.73	2.49	2.34	2.67	2.73	3.04	2.77
Ile	6.93	5.90	6.60	6.99	6.36	6.64	6.40	6.70
Leu	12.79	11.61	12.95	12.69	11.65	12.02	11.94	11.86
Tyr	3.41	2.69	3.20	3.63	3.31	3.98	4.00	3.94
Phe	7.79	6.78	7.60	7.84	7.32	7.00	7.74	6.97

[a] Average of duplicate analysis. [b] Forty fruits per sample.

Table III. Amino Acid Content (mg/10 g Dry Weight)[a] of Irradiated Dates[b] after 3 Weeks Postirradiation Storage at Room Temperature (20–25 °C)

amino acid	Sayer krad 0	Sayer krad 100	Sayer krad 270	Zahdi krad 0	Zahdi krad 100	Zahdi krad 270
Lys	4.30	4.31	4.45	3.83	3.41	3.68
His	1.82	1.70	1.67	1.82	1.41	1.64
Arg	4.78	4.70	3.91	5.54	4.81	6.15
Asp	8.95	8.87	7.29	17.64	16.84	20.75
Thr	2.85	2.62	2.61	4.30	4.06	3.70
Ser	3.60	3.45	3.40	5.49	5.17	5.51
Glu	16.02	16.83	14.34	21.91	20.40	24.94
Pro	9.88	9.54	8.27	5.51	5.00	5.88
Gly	6.18	5.89	5.92	9.65	8.51	9.17
Ala	5.93	5.64	5.83	10.11	9.06	9.57
Cys	3.40	3.13	2.99	2.16	2.94	3.90
Val	4.35	4.11	3.63	5.99	5.23	5.41
Met	0.87	0.75	0.74	0.83	0.80	0.67
Ile	2.81	3.10	2.70	3.67	2.71	3.69
Leu	5.34	5.01	4.94	6.57	6.34	6.91
Tyr	1.40	1.45	1.59	3.04	3.16	2.86
Phe	3.75	3.63	3.00	4.00	4.10	4.24

[a] Average of duplicate analysis. [b] Forty fruits per sample.

room temperature and that the total constituents remained nearly unchanged. Variation in radiation response of various individual amino acids was observed among the different varieties investigated. These results are in partial agreement with those of Srinivas et al. (1972, 1974) on wheat and shrimp, respectively. Radiation treatment of dates showed some change in total amino acids after storage at zero and room temperatures. Changes in amino acid constituents were observed for most amino acids for the storage at room temperature when the 100-krad dose was applied (Table III). Storage at zero temperature also showed reduction in the concentration of some amino acids for all doses of γ radiation (Table IV). The radiation response of amino acids for the two storage temperatures was varied with different doses and varieties.

In general, most amino acids decreased in value upon storage. With only a few exceptions, such loss was significant. The dose of radiation also seemed to vary in its effect; 30- and 1000-krad doses showed higher changes in value for most amino acids than 270 krad for samples stored at 0 °C temperature. This variation in radiation response was also true for the storage experiment at room temperature. Variation in amino acid composition could be due to the wide variability among the individual sam-

Table IV. Amino Acid Content (mg/10 g Dry Weight)[a] of Nonirradiated and Irradiated Date[b] after 2 Months Postirradiation Storage at Zero Temperature

amino acid	Hillawi krad 0	Hillawi krad 30	Hillawi krad 270	Hillawi krad 1000	Khastawi krad 0	Khastawi krad 30	Khastawi krad 270	Khastawi krad 1000
Lys	8.04	8.05	8.19	8.40	8.86	8.22	8.00	8.76
His	3.60	3.62	3.58	3.49	4.17	3.90	4.05	3.66
Arg	8.20	7.79	8.84	8.35	8.80	8.27	8.43	7.52
Asp	18.36	18.34	17.68	20.45	16.35	15.84	16.76	14.59
Thr	6.24	6.61	6.21	6.67	6.71	6.36	6.74	5.81
Ser	7.37	7.82	7.26	8.18	7.67	7.38	7.60	6.96
Glu	22.93	24.72	22.36	25.55	23.50	22.21	23.20	20.75
Pro	18.35	18.52	14.83	17.50	14.98	15.29	15.39	13.75
Gly	12.00	15.36	11.73	13.15	12.68	11.77	12.82	11.51
Ala	12.05	13.05	11.51	14.15	9.70	8.86	9.70	8.66
Cys	6.15	5.69	7.30	6.87	7.14	6.73	6.74	6.34
Val	8.46	9.35	7.27	8.75	9.48	8.38	9.54	8.58
Met	2.03	1.71	2.12	1.93	2.87	2.57	2.79	2.20
Ile	5.94	6.59	5.86	6.77	6.77	5.98	6.81	5.88
Leu	11.25	12.22	11.25	12.72	12.20	11.29	12.22	10.61
Tyr	3.84	3.01	4.80	4.02	3.29	3.40	3.32	3.19
Phe	6.79	7.39	6.89	7.67	7.52	6.85	7.47	6.49

[a] Average of duplicate analysis. [b] Forty fruits per sample.

ples. This in fact exists even among fruits carefully sorted according to their initial color and texture. These factors may contribute to the variation in the experimental results.

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Chickpea Seed Proteins: Conformational Changes in 10.3S Protein during Germination

K. Ganesh Kumar,*[1] Lalgudi V. Venkataraman, and A. G. Appu Rao

The changes that occur in the major 10.3S storage protein of chickpea during germination have been monitored by viscosity, heat coagulation, α-chymotryptic digestion, circular dichroism, and fluorescence spectral measurements. The hydrodynamic method suggested an alteration in the asymmetry, whereas from the CD spectral studies, it was inferred that the limited ordered structure of the 10.3S protein was considerably reduced during germination. The heat coagulation and free -SH group measurements indicated that changes in the quaternary structure occurred possibly through the scission of one of the three disulfide bonds present in 10.3S protein. Evidence of conformational changes in 10.3S protein during germination is also provided by the increased susceptibility of 6-day germination-modified (GM) protein to α-chymotryptic digestion as compared to 10.3S protein.

In our earlier study on chickpea seed germination (Ganesh Kumar and Venkataraman, 1978), we reported on the gross modification occurring in the storage proteins. Among the two major storage proteins, it was observed that 6.9S protein degraded faster in the initial stages, whereas 10.3S protein was found to do so in the later stages of germination. There are many structural constraints on the proteins which are operative and become important in the selective degradation of a protein fraction. In this work we have attempted to study the structural and conformational changes occurring in the major 10.3S storage protein of chickpea seed during germination. We have usually chosen a 6-day germination period since this represents the period of maximum degradation (Ganesh Kumar and Venkataraman, 1978). The germination-modified form of 10.3S has been isolated from the germinated cotyledon, which will be referred to as germination-modified (GM) protein. The structural and conformational characteristics of 10.3S and GM proteins were determined using the techniques of viscosity, heat coagulation, circular dichroism, in vitro enzymic hydrolysis, and fluorescence spectral measurements.

EXPERIMENTAL SECTION

Seeds and Germination. Seeds of chickpea (*Cicer Arietinum*) were obtained locally. They were surface sterilized with $HgCl_2$ (0.1% w/v) and thoroughly washed with distilled water. Germination was carried out in autoclaved moist vermiculite and grown at room temperature (28 ± 2 °C) in darkness. Only distilled water was provided during the germination period. Cotyledons of 3-, 6-, and 9-day germinated seeds alone were taken up for protein studies. The cotyledons were powdered in a hand mill passed through a 100-mesh sieve and were defatted with hexane. Finally they were dried at 40 °C and used as such for further study.

Preparation of 10.3S Protein. The defatted chickpea flour was extracted with 10% (w/v) NaCl in solute-to-solvent ratio of 1:10 for 2 h on a mechanical shaker. The slurry was centrifuged at 5000 rpm for 30 min. The supernatant was diluted with 10 volumes of distilled water. The resultant precipitate was collected by centrifugation dissolved in 10% (w/v) NaCl solution. Another dilution was performed with 20 volumes of distilled water. The precipitate obtained in this step was further purified by DEAE-cellulose chromatography.

DEAE-Cellulose Chromatography. The DEAE-cellulose column (2 × 20 cm) was equilibrated with 0.01 M borate buffer of pH 7.8 containing 0.25 M NaCl (buffer I). The protein solution (~30 mg/2.0 mL) in buffer I was applied on the column and eluted with the buffer containing 0.25 M NaCl (100 mL). The fractions collected were discarded. Then a continuous linear gradient was set up in buffer I from 0.25 to 0.40 M NaCl (500 mL). The fractions (4.0 mL each) were monitored at 280 nm and the protein eluting in the range of 0.30 to 0.33 M were collected, dialyzed against distilled water, and lyophilized.

Preparation of Germination-Modified (GM) Protein from Germinated Chickpea Cotyledons. The isolation of germination-modified proteins was similar to the preparation of 10.3S with the following modification in the DEAE-cellulose chromatographic step. The column was equilibrated with 0.01 M borate buffer of pH 7.8 containing 0.29 M NaCl; the protein also in the same buffer was applied on the column and a continuous linear gradient

Protein Technology Discipline, Central Food Technological Research Institute, Mysore-570013, India (K.G.K., L.V.V.), and the Department of Biophysics and Genetics, University of Colorado Medical Center, Denver, Colorado 80262.

[1]Present address: Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

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