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Effect of heat treatment and gamma irradiation on *In vitro* starch digestibility of selected millet grains

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Abstract
The present investigation was conducted to find out the effects of heat or irradiation combined with heat on *in vitro* starch digestibility (IVSD). Sorghum, pearl millet, foxtail millet were used in the study. Whole (WC) and dehulled (DC) grains were treated either with heat (170°C) or irradiation at 1.0kGy / 2.5kGy and stored for 90 days. There was a significant (p<0.05) effect of treatments, storage and grain and their interactions on all the nutritional quality parameters with a result in increase in IVSD. The mean IVSD was 70.83 percent. The mean IVSD in untreated grains was 66.99 and 70.6 percent in WC and DC, which were improved by 6 and 4 percent due to irradiation combination treatment. The percent increase from 1.0 kGy to 2.5kGy was 5.27 in DC and 3.23 percent in WC grains. Among the three grains, sorghum had maximum IVSD followed by pearl millet and foxtail millet. Storage of both untreated and treated grains affected the IVSD (p<0.05) with a significant reduction (1.54%). Sorghum had slightly higher reduction during storage.

Keywords: IVSD, irradiation, millet, heat, storage

Introduction
Food irradiation is the process of exposing food to carefully controlled amounts of ionizing energy. “Irradiation is recommended as a safe and effective food processing method that can reduce the risk of food poisoning and preserve foods without detriment to health and with minimum effect on nutritional quality” (Kouba, 2003) [6]. It can be used to reduce insect infestation of grain, dried spices, and dried or fresh fruits and vegetables; inhibit sprouting in tubers and bulbs; retard postharvest ripening of fruits; inactivate parasites in meats and fish; eliminate spoilage microbes from fresh fruits and vegetables; extend the shelf life of poultry, meats, fish, and shellfish; decontaminate poultry and beef; and sterilize foods and feeds (Shea, 2000) [11].

Gamma-irradiation has been used to extend the shelf-life of food products and is often applied for the modification of food materials to change their physical properties (Waje and Kwon, 2007) [20]. The irradiation may generate active radicals which readily react with food components to change their molecular structure (Yu and Wang, 2007) [21].

In radiation processing of foods, the doses are generally measured in kGy (1,000 Gy). There are different modes of food irradiation; the mode used depends on the purpose: Low doses below 1 kGy are used to control insects, trichinae (parasitic worms) in pork, and ripening or sprouting of fruits and vegetables. Medium doses (1-10 kGy) are used to control both spoilage-causing microorganisms (such as molds) and bacterial pathogens. Low to medium doses are effective against food borne protozoans. High doses (greater than 10 kGy) are used to control microbial contamination of spices and with only enough heating to deactivate enzymes, to make other foods storable at room temperature that is shelf stable. High doses also can significantly reduce food borne viruses (Anonymous, 2010) [1].

During storage there may be some nutritional changes to the cereals, although for dry grains these changes will be small even over a period of several months. If grains are stored with a higher than ideal moisture content, grain and microbial amylases may begin to breakdown the starch, leading to a deterioration of grain quality. Several methods of drying are employed to reduce the moisture content to desirable levels. The effect of heating before irradiation is additive or slightly more than additive, ionizing radiation applied before heating is strongly synergistic in the inactivation of bacterial spores (Gombas and Gomez., 1978) [4]. High temperatures applied before radiation sensitize insects to radiation and hence allow the use of low dose (Tilton and Browser 1987) [18].

The chemical structure of irradiated food is less modified than heat-treated one and this technique avoids the use of potentially harmful chemicals (Siddhuraju *et al*., 2002) [14].
Millets are rich in bioactive phytochemicals, particularly phenolic acids and Phenolic compounds in millets are found in the soluble as well as insoluble-bound forms. Both hydroxybenzoic and Hydroxycinnamic acids and their derivatives are notably present in different types of millet grains in varying proportion**. (**Chandrasekara and Shahidi**,** 2011) [3]. Several workers (Bao *et al*., 2001, Sirisoontaralak and Noomhorm, (2007) [2, 16], Shin and Godber, (1996) [16], Yu and Wang, (2007) [21] studied effects of irradiation at varied dosages on rice.
Compared with other major cereals, millets remain much under-studied and under-utilized, though there has been increased interest in their utilization in recent years. Understanding the properties, structure, and potential uses of millets nutritional quality greatly contributes to the further development of millets as alternative functional crops. Starch in whole grains are generally low compared to dehulled grains. There are some studies on the starch digestibility of millet grains and as effected by processing such as fermentation, germination on improvement of IVSD. However, there were no studies found in the literature on the irradiation effect on the IVSD on different millets. Therefore, the present investigation was undertaken to study the effect of irradiation on the whole and dehulled millet grains.

Materials and Methods

Procurement of raw Materials

Sorghum and foxtail millet grains were collected from RARS, Nandyal, ANGRAU and pearl millet from RARS, Palem, PJTSAU. All the grains were cleaned to remove foreign material and stored in polythene bags until used under dry and cool conditions away from insects and pests.

Dehulling

The grains were dehulled in an abrasive dehuller (Gurunanak Engineering Co, Hyderabad) up to 17 percent removal of bran. The dehulled grain collected through bottom opening along with hull. The bran was separated from the dehulled grain through the sieve attached to the dehuller. The separated grain was further winnowed to ensure grain without any adhering bran.

Heat treatment

High moisture content of the grains is one of the factors for grain spoilage. The moisture content can be reduced by giving heat treatment. Many types of dryers can be used; however, using rotary drier is more effective as there will be continuous agitation during drying providing uniform heat to all the grains. In the present experiment electric rotary dryer (S k Engineering, New Delhi) was used which can be operated continuously for large quantity of grain. It has provision for adjusting the temperature and the rpm. For the present study 10 kg of whole and dehulled grains of all three millets were exposed to heat treatment at a temperature of 150-170°C for 1.5 min at 300 rpm. The treated grains were then packed in polythene pouches in desired quantity of 500 g quantity for further use.

Gamma irradiation

Gamma Chamber 5000 was used for giving radiation treatments. It is a compact shelf shielded Cobalt 60 gamma irradiator providing an irradiation volume of approximately 5000cc. Irradiation is one of the processing technologies currently available for the inactivation of microorganisms, and it has proven successful in ensuring the safety and extending the shelf life of foods (Mahapatra *et al.*, 2005) [8]. The millet grains were irradiated using cobalt – 60 gamma sources. Two different dosages *viz*., 1.0kGy and 2.5 kGy were used based on the previous studies. Grains of 500 g were packed in polythene pouches and exposed to the irradiation as guided by technical staff of the university.

Estimation of *in vitro* starch digestibility

Starch digestibility (*in vitro*) was estimated by using pancreatic amylase and the liberated maltose was measured by using dinitro salicylic acid reagent (Singh and Jambunathan, 1982) [15]. 25 mg of sample was dispersed in 1.0 ml of 2.0 M phosphate buffer. pH 6.9. 0.5 ml of enzyme buffer was added to the sample suspension and incubated at 37*C for 2 hrs. After the incubation period, 2 ml of 3-5 DNSA reagent was quickly added and the mixture was heated for 5 min in a boiling water bath, and after cooling the solution was made up to 25 ml with distilled water and filtered. The absorbance was measured at 550 nm. A blank was run simultaneously. 4ml of maltose standard was taken and the reaction from the addition of 3-5 DNSA standard was followed. The values were expressed as mg of maltose released /g sample.

Storage studies

All the treated grains and control samples of 500g were stored for 30, 60, 90 days in HDPE pouches at 34°C to 36°C of temperature and 23% of humidity. The estimations were done at the end of 30th day, 60th day and, 90th day.

Study design

The study was conducted using 3x8x4 factorial design, i.e 3 types of grains, 8 types of treatments and 4 levels of storage were used for the study. The effects of these factors were studied on *in vitro* starch digestibility.

Table 1: The details of treatments used for the study

Treatments	Grains	Storage period (days)
Control –Whole grain	Sorghum	0
Control- Dehulled grain	Pearl Millet	30
Heat treated –Whole grain	Foxtail Millet	60
Heat treated –Dehulled grain		90
Heat and 1.0kGy Irradiated -Whole grain		
Heat and 2.5kGy Irradiated - Whole grain		
Heat and 1.0kGy Irradiated -Dehulled grain		
Heat and 2.5kGy Irradiated - Dehulled grain		

Results and Discussion

Effect of heat and irradiation: Irrespective of the grain and storage, it can be observed that there was a marginal yet statistically significant (p<0.05) improvement in the IVSD when heat treated and further significant improvement was observed when heat was combined with irradiation i.e 4.63 and 4.06 percent in WC and DC grains respectively(Table 1)The raw maize flour and raw bean flour starch digestibility after 120 min increased from 0 kGy to a maximum value at 2.5 kGy then declined between 5 and 10 kGy to values lower than that of the control. Low-dose irradiation may improve the starch digestibility of the grains by opening up starch molecules through depolymerization. (Urbain, 1986) [19] However, at higher doses, starch digestibility *in vitro* may be reduced owing to the formation of inhibitors of amylolytic enzymes as in Maillard reactions or owing to the formation of resistant starch made up of short amylose chains (Colonna *et al*., 1992).

Leonhardt, *et al.* (1983) [7] showed that the digestibility of wheat, oat, barley and rye straws can be increased by up to 80% by treatment with γ-rays or accelerated electrons, as both types of radiation depolymerize cellulose and hemicellulose. Many authors (Hennig *et al.*, 1982 and Pritchard *et al.*, 1962) [5, 9] have reported that the size of the irradiation dose greatly affects fibre digestibility. Sandev and Karaivanov, (1979) [10] reported that decreases in the crude content of fibre in alfalfa hay, grain straw, corn cobs and wheat bran were directly proportional to the increasing dose of radiation. In rice varieties with different resistant starches, the *in vitro* starch digestibility decreased with low resistant starch rice and increased with high resistant starch rice. It was explained that irradiation decreases apparent amylose content and gelatinization temperature and changed the starch granule structure, while increasing V-type crystalline. Starch enzymatic hydrolysis rate was reduced following irradiation, and the effect of irradiation on reducing starch digestibility was negatively correlated with resistant starch content Shu *et al.* (2013) [13].

Effect of grains: Among the three millets foxtail millet exhibited lowest IVSD followed by pearl millet and sorghum.

Effect of storage: There was a gradual significant decrease (p<0.05) in the IVSD from 71.90 to 71.2, 70.47 and 69.75 per cent at 30, 60 and 90 days respectively. The increase in starch digestibility during storage may be due to hydrolysis of starch into simpler products that are easily digested. Storage of wheat, maize, sorghum for 2 and 4 months exhibited increased IVSD, a higher IVSD in sorghum, where values of starch digestibility were significantly higher after 2 and 4 months of storage with fresh grains (Sudesh and Kapoor, 1992) [17]. Further, it can be observed that the highest protein containing grains exhibited lowest IVSD and vice versa.

Interaction effects: The interaction of treatment and grain (Fig.1) indicated that compared to untreated, treated grains had higher IVSD. Among the higher dose irradiated grains DHEI 2.5 sorghum grains had highest IVSD (77.89%), followed by DHEI 2.5 (77.89%) and WHEI 2.5 of pearl millet. The per cent increase of IVSD from control to treatments was highest in WHEI 2.5 (10.50%) in pearl millet and lowest in DHE in foxtail millet (Fig.4). All the three grains during storage had a significant reduction in IVSD (Fig.2). Foxtail millet and pearl millet had a similar reduction (1.57 %), whilst sorghum had a slightly higher reduction (1.74%). Even after 90 days, only sorghum had above the mean IVSD. There was a significant effect of treatment and storage interaction on IVSD. Among all the treatments including DC and WC, maximum IVSD was exhibited by DHEI 2.5 followed by DHEI 1.0 and WHE I 2.5 treatments throughout the storage period (Fig.3). Least IVSD was found in WC and WHE grains. Generally dehulling improves the IVSD and the percentage depends on the degree of the dehulling. In the present study, it was observed that the improvement in IVSD due to irradiation at 2.5 kGy was significantly higher than that occurred due to dehulling. (Fig. 3). This suggests that irradiation up to 2.5kGy can be used as a technique for improving starch digestibility.

Table 2: Effect of Treatment, storage and grain type on *in vitro* digestibility of starch

Main Effects	IVSD (g Maltose) % *
Treatment	
DC	70.60±0.01
DEHE	71.10±0.02
DEHEI1.0	73.13±0.03
DEHEI2.5	74.85±0.02
WC	66.99±0.01
WHE	67.895±0.02
WHEI1.0	69.91±0.01
WHEI2.5	72.17±0.03
Storage (days)	
0	71.90±0.01
30	71.2015±0.02
60	70.4752±0.02
90	69.7508±0.01
Grains	
Foxtail Millet	66.6398±0.03
Sorghum	74.1566±0.01
Pearl Millet	71.7048±0.01

*Statistically significant (p, 0.05)

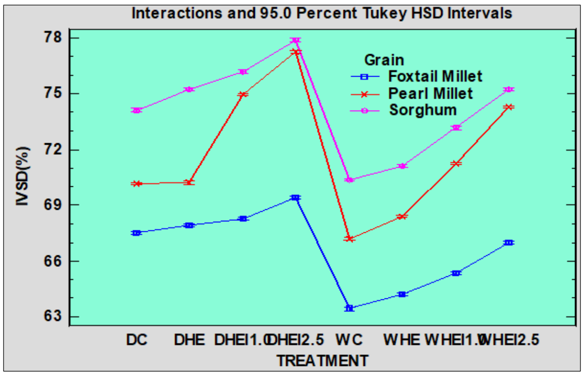

Fig 1: Interaction plots of grain and treatement on IVSD

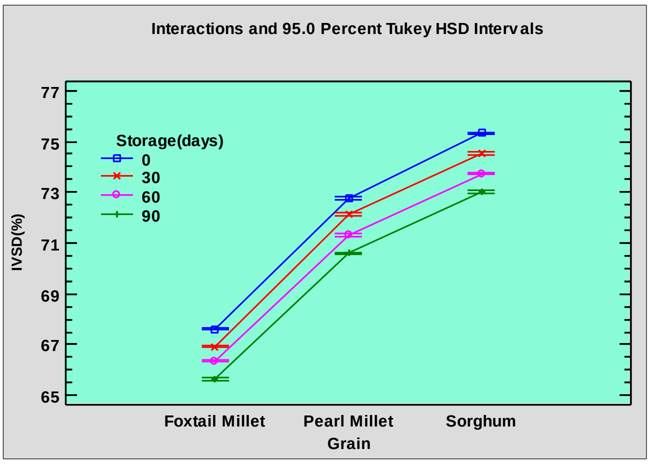

Fig 2: Interaction plots of grain and storage on IVSD

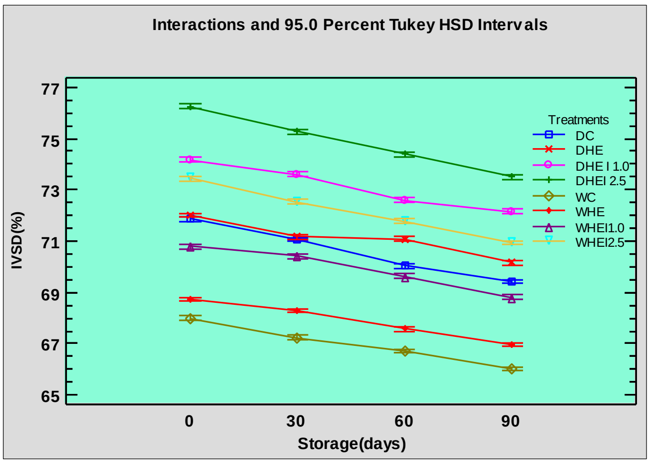

Fig 3: Interaction plots of treatment and storage on IVSD

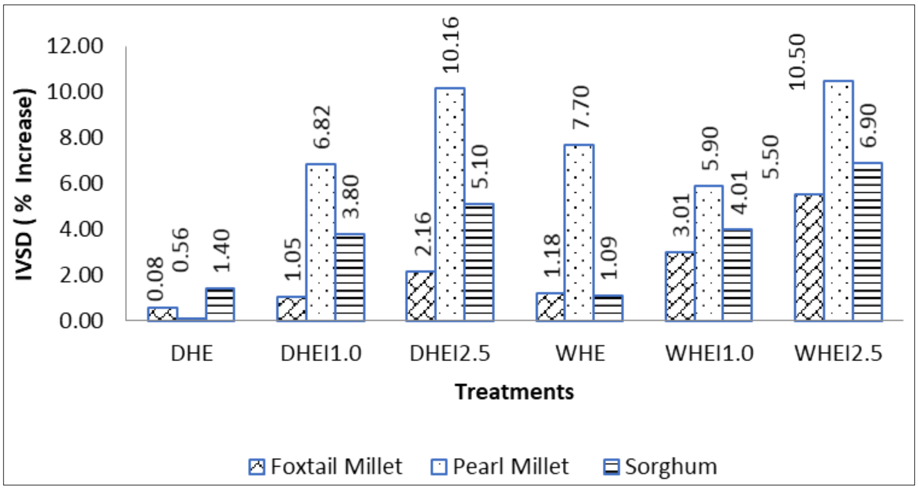

Fig 4: Percent increase of *in vitro* starch digestibility from control to treatments

DHE - Dehulled heat treated, DHE1.0- Dehulled heat and irradiated (1kGy)
DHE2.5- Dehulled heat and irradiated (2.5kGy), WHE - Whole heat treated
WHE1.0 Whole heat and irradiated (1.0kGy), WHE2.5 – Whole heat and irradiated (2.5kGy)

Conclusion

Maximum IVSD was found in sorghum followed by pearl millet and foxtail millet. The mean IVSD of the grains was 70.83 percent. The effect was more pronounced in whole grains (4.63%) than in dehulled grains (4.06%). Storage of both untreated and treated grains affected the IVSD ($p<0.05$) with a significant reduction (1.54%). The interaction studies of grain and storage revealed that foxtail millet had the lowest IVSD during the storage. Sorghum had highest IVSD compared to pearl millet but lower than foxtail millet. The storage losses can be minimized with the use of 2.5kGy irradiation. The interaction of grain and treatment revealed that highest IVSD in sorghum were found in heat combined with irradiation at 2.5 kGy treated grains.

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