INFLUENCE OF IONIZING RADIATION ON PROTEIN DEGRADATION BY ENDOGENOUS PROTEASES IN POULTRY VISCERA

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Abstract

In poultry processing industry, viscera accounts for nearly 30% of the byproducts. It contains 10-13% proteins, 24-27% lipids and possesses 32–64% of the total proteolytic enzymes. A rapid method based on the degradation of tissue proteins by endogenous enzymes has been developed in our laboratory to retrieve 80-90% of the tissue proteins in the form of protein hydrolysates from poultry viscera (Bioresource Technol. 96 [1276-1284], 2005). The efficacy of this method is now evaluated in tissues hygienised by gamma radiation. Exposure to 5 to 10 kGy reduced the microbial load of poultry viscera by 4 to 6-log cycles while 20 kGy extended the shelf life to more than 60 days. Irradiation did not affect the activities of aminopeptidases, dipeptidyl peptidases and alkaline proteases while it inactivated aspartic protease only marginally. Consequently, the degradation of proteins by tissue autolysis was also unaffected by radiation processing. Thus, ionizing radiation could be used to extend the shelf life of poultry viscera, which in turn, could significantly reduce the cost of protein hydrolysate production.

Introduction

Poultry industry is flourishing globally with nearly 6.8 x 10^7 tonnes being processed every year (FAO statistics, 2005). It produces large quantities of organic by-products including the viscera, blood, feet, head and feathers (Ockeraman and Hansen, 2000); the utilization and disposal of which pose increasing challenges. The viscera, which constitutes nearly 30% of the total wastes, is rich in proteins and proteolytic enzymes (Jamdar et al 2005). It is estimated that in India, more than 1,44,000 tonnes of poultry viscera are produced annually in organized sector alone. In the absence of suitable methods for the preservation and retrieval of nutrients, major portion of this by-product is being discarded resulting in environmental pollution (Fransen et al 1996) and loss of valuable nutrients (Rao et al 1996). We have developed a cost effective process based on tissue autolysis for the retrieval of intestinal proteins in the form of high quality protein hydrolyzates. One of the major constraints in this process is the high microbial load in viscera, which rapidly putrefy the tissue during handling and storage. The widely used methods such
as heat sterilization (Giri et al 2000, Nengas et al, 1999), acid stabilization (Cai et al 1995) and fermentation (Kherrati et al, 1998; Rao et al, 1996) are not suited to our scheme of protein retrieval, because of inactivation of tissue proteases (Jamadar et al 2004) and inadequate decontamination (Haapapuro et al, 1997). Thus, the need for a suitable method, which ensures decontamination of poultry viscera without the inactivation of proteases, has been felt.

In this context, application of gamma irradiation, which is recognized as an environment friendly cold sterilization process with least physical and chemical changes to food constituents, (Molins, 2001; Durante, 2002) holds promise. Tissue enzymes are relatively resistant to gamma radiation while microorganisms are highly susceptible. In the present paper, we report the status of protein degradation by endogenous proteases in radiation hygienized poultry viscera.

Methods

Poultry viscera, brought from local market were washed in tap water, blotted, weighed, sealed in polyethylene bags (15 cm x 20 cm) and exposed to different doses of gamma radiation (5kGy, 10 kGy and 20 kGy) in a package 60Co γ-irradiator (dose rate 46 Gy/min) at ambient (26°C) temperature. The samples were stored at 4°C or 26°C.

Total aerobic bacteria, coliform bacteria and yeast and moulds were determined (BAM Online). The tissue samples were analyzed for protein, fat, moisture and ash according to AOAC method (1984). Lipid peroxidation was monitored in terms of thiobarbituric acid (TBA) values. (Alur et al 1995). The tissue autolysis was carried out at pH 2.8 and 60°C and the levels of TCA (10%) soluble peptides at different time intervals were estimated by Miller’s method. Total Volatile Basic Nitrogen (TVBN) was determined according to Farber and Ferro (1956).

Activities of aspartic protease, aminopeptidases were determined according to Jamdar and Harikumar (2005). Activity of alkaline proteases was determined as per method described by Thakore and Harikumar (1995).

Results and Discussion

Data presented in Table 1 shows the influence of gamma radiation on microbial population of chicken intestine during storage. A dose of 5 kGy extended the shelf life for 5 and 10 days at 26°C and 4°C respectively. The corresponding values for 10 kGy were 10 and 20 days. A dose of 5-10 kGy, which reduced the total viable count of bacteria, yeast and moulds by 4 to 5 log_{10} units was sufficient to eliminate coliforms completely (<1 CFU/g tissue), while 20 kGy rendered the samples sterile. Similar degree of kill by ionizing radiation has been observed by Borrely et al. (1998) who showed that ionizing radiation at 3kGy could reduce the count in raw sewage by 5 log_{10} units. The efficacy of gamma radiation to eliminate both vegetative as well as spore forms of microorganisms from flesh foods have been demonstrated by many investigators (Molins, 2001; Farkas, 1998). Significantly, total count in irradiated samples (5 kGy and 10 kGy) even after storage for 10-20 days at 4°C (Table 1) remained 3-4 log cycle lower than that in unirradiated control (1.094 x 10^{7} CFU/g). No significant organoleptic degeneration was also discernible for these samples. This, along with the observation that low dose of ionizing radiation do not inactivate tissue proteases (Jamdar and Harikumar,
suggest that irradiation (5 kGy and 10 kGy) combined with storage at 4°C could be effectively employed for extending the shelf life of poultry viscera. The biochemical changes induced by microbial proliferation as well as gamma radiation are monitored in terms of known parameters such as TVBN and TBA values (Table 2) (Alur et al. 1995). The levels of TVBN increased eight fold within 18h in unirradiated samples at ambient temperature while irradiated sample showed similar increase only after 62 days. The increase in TVBN value in unirradiated sample stored at 26°C indicates microbial spoilage (Alur et al. 1995), while that in irradiated samples (20 kGy) could be attributed to production of non-protein nitrogen by autolytic degradation of proteins followed by decarboxylation and deamination. As expected, the TVBN values were lower in samples stored at 4°C, which could be ascribed to inhibition of bacterial proliferation and retarded activity of hydrolytic enzymes. One of the important parameters that has to be reckoned with in radiation hygienization of flesh foods, is lipid peroxidation. TBA values in unirradiated samples were 2.0 to 2.5 times lower than the
corresponding irradiated samples throughout the storage period. The tissue showed significant increase in TBA levels immediately after irradiation as well as during storage (2.2 and 2.5 fold during 20 days at 4°C and 26°C). Increase in lipid peroxidation, though to a limited extent (1.3 fold) was also observed in unirradiated samples during storage which could be predominantly due to atmospheric oxygen, while an additional participation of radiation induced free radicals could explain the enhanced peroxidation in irradiated samples (Al-kahtani et al. 1996; Hampson et al. 1996). Our observation that TBA values diminished by 45% on prolonged storage (62 days), is in conformity with earlier reports of Auburg et al (1993), showing decreased levels of TBA.
during post irradiation storage. Data on the levels of aspartic protease, cathepsin B, alkaline proteases, and aminopeptidases are presented in Table 3. Exposure to a dose of 20 kGy resulted in 3%, 20%, 12%, 8% and 14.1% loss in activity of aspartic protease, alkaline protease, arg-aminopeptidase, phe-aminopeptidase and ala-aminopeptidase respectively. The levels of TCA soluble peptides released during autolytic degradation of irradiated (20 kGy) chicken intestine at pH 2.8 and 60°C are presented in Table 4. No significant difference was found in protein degradation after radiation treatment. The observation that irradiation did not cause any significant change in proteases activity as well as tissue autolysis is important while considering radiation hygienized poultry viscera for the preparation of protein hydrolysates or as a source of proteolytic enzymes for commercial applications. Moreover, the process, assures decontamination with little change in proximate composition, which is in agreement with the observations of Farag et al. (1999). Thus, irradiation could be used to hygienize poultry viscera before utilizing it for the preparation of value added products such as protein hydrolysates and proteolytic enzymes with minimum risk of spreading pathogenic organisms.

### Table 4: Effect of irradiation on tissue autolysis

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<th>Time (h)</th>
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### References


Dr Sahayog N. Jamdar joined BARC in 1996 through BARC Training School (39th batch) and is working in the flesh food Biochemistry section, Food Technology Division. His major contribution is in understanding structure function relationships of proteases and preparation of value added products from poultry and fish processing wastes.

Dr P. Harikumar is presently the Head, Flesh Food Biochemistry Section, Food Technology Division. He joined BARC in 1967 after graduating from the university of Kerala, obtained M.Sc. and Ph.D. degrees from the University of Mumbai. He has carried out postdoctoral research at Roche Institute of Molecular Biology, Nutley, New Jersey, USA (1982-1984) and at University of Medicine and Dentistry, New Jersey, USA, (1998-1999) on “The Role of lysosomal proton ATPase and Ca\(^{2+}\) in the regulation of intracellular protein catabolism.” His major contribution is in understanding the structure-function relationships of lysosomal proteases and their role in regulating the quality attributes of irradiated flesh foods and in the preparation of value added products from poultry and fish processing wastes.