SHELF STABLE INTERMEDIATE MOISTURE PINEAPPLE (ANANAS COMOSUS) SLICES USING HURDLE TECHNOLOGY

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ABSTRACT

A process has been developed to prepare shelf stable Ready-To-Eat (RTE) intermediate moisture pineapple (Ananas comosus) slices, using hurdle technology. The combination of hurdles including osmotic dehydration, infrared drying and gamma radiation dose of 1 kGy, successfully reduced the microbial load to below detectable limit. The shelf life of the intermediate moisture pineapple slices was found to be 40 days at ambient temperature (26 ± 2 ºC). The untreated control samples spoiled within 6 days. The RTE intermediate moisture pineapple slices were found to have good texture, colour and sensory acceptability, during this 40 days storage.

Keywords: Pineapple; Hurdle technology; Osmotic dehydration; Infrared drying; Gamma radiation

Introduction

Pineapple (Ananas comosus) is one of the most popular tropical fruits. The fruit is known for its nutritive and health promoting properties. It is commonly used as table fruit or in desserts. The shelf life of ripe pineapple is short and limited to 4-6 days (Hajare et al, 2006). Fresh pineapple contains thick, thorny inedible peel and a large crown, which consumes storage space and also results in higher transportation costs (Fernandes et al, 2006). Therefore, value addition by processing to a RTE product, is an attractive alternative. Pineapple slices dipped in sugar syrup and canned are normally used around the world (Mortan, 1987). The canned pineapple is shelf stable but it is not liked by some consumers, due to its high level of sweetness.

The shelf life of peeled, sliced and polyethylene packed pineapple sold in market is 4-6 days, when stored at room temperature, whereas, pineapple slices when kept at 8-10 ºC, do not stay more than 8-10 days. Therefore, many alternate approaches were followed for pineapple preservation. The blanching treatment for microbial reduction and browning inhibition, affects freshness and taste. Cut pineapple undergoes enzymatic browning which can be inhibited by dipping in potassium metabisulfite solution (Adams & Moss, 2000). In osmotic dehydration, water is partially removed from the product by dipping in hypertonic sugar syrup (Pokharkar et al, 1997; Corzo & Gomes, 2004). The dipping in sugar solution reduces water
activity \( (a_\text{w}) \) to about 0.9 which is not enough for preventing bacterial, yeast and mold growth, these being involved in spoilage. Infrared drying offers a number of advantages including drying uniformity, reduced drying time, energy efficiency and high quality finished products (Afzal & Abe, 1998; Zhu et al, 2002). Due to limitations of individual preservation methods, a combination of these hurdles was used for maximizing pineapple shelf life. The intermediate moisture pineapple products are becoming popular, because these are shelf stable, retain nutritional value, are convenient to use and incur less transportation and storage costs (Leistner, 1992; Thakur & Singh, 1995). Radiation processing, a cold process, is being used as a preservation method for food commodities and is increasingly attaining new applications (Urbain, 1986; Diehl, 1990). This is one of the very effective alternatives for ensuring microbial quality and safety of minimally processed fruits and vegetables and is also recommended for processed pineapple (Shashidhar et al, 2007).

The objective of the present study was to prepare shelf-stable, ready-to-eat and safe intermediate moisture pineapple slices, using a combination of different hurdles.

Materials and Methods

Chemicals

Luria-Bertani agar (LBA), potato dextrose agar (PDA), Baird-Parker agar (BPA), violet red bile agar (VRBA), and egg-yolk tellurite emulsion were purchased from Himedia Laboratories, India. High density polyethylene packages (HDPE) of film thickness, 500 gauge and sucrose were obtained from a local market. Potassium metabisulfite solution and sodium chloride (NaCl) were purchased from S. D. Fine-Chem. Ltd., India.

Gamma radiation treatment

Gamma radiation treatment was carried out in a cobalt-60 Gamma Chamber-5000 (GC-5000, BRIT; dose rate 7.65 kGy/h) at Food Technology Division, BARC. The packed samples were treated at different doses of gamma radiation (250 Gy, 500 Gy and 1 kGy) at ambient temperature. Non-irradiated samples were used as control. After radiation treatment, the samples were stored at ambient temperature (26 ± 2°C) and relative humidity (56 ± 3 % RH). The radiation dosimetry was carried out by placing Fricke dosimeters.

Pineapple processing

Pineapples (six) procured from a local market were cleaned with water, crowns removed and peeled manually. The fruits were then transversely cut into 8 slices, each approximately of 1 cm thickness. The slices were dipped in potassium metabisulfite solution (0.25%) for 2 h, followed by immersion in sugar (sucrose) solution (70%, 16 h). The slices were taken out of the sugar solution, drained on a two layered muslin cloth and dried in infrared (IR) dryer (Sakav, Shirsat Electronics, Mumbai) at 80 °C/1 h to bring the \( a_\text{w} \) to 0.82. The slices were then packed in high density polyethylene bags, sealed, radiation-treated and stored at ambient temperature (26 ± 2°C). Such processed pineapple slices named in this paper as Intermediate Moisture (IM) pineapple slices were periodically examined and subjected to the following analyses, up to a period of 40 days of storage.

Microbiological analysis

Total Bacterial Count (TBC) and Yeast and Mold Count (YMC) were determined as described by ICMSF (2002). Individual pineapple slices (25g) were aseptically homogenized for 2 min in stomacher bags with 75 ml sterile saline water solution (0.85% NaCl) using Stomacher Blender (Stomacher Lab Blender, model 400, Seward, U.K.). Serial dilutions were made in sterile saline and spread plated in duplicate. Media employed were plate count agar, potato dextrose agar for determination of TBC and YMC, respectively. The microbial analyses of pineapple slices were undertaken periodically during storage.
**Water activity (a_w) measurement**

A small piece of pineapple slice weighing approximately 2 g was used, for determination of water activity using a water activity meter (AqualabCX2T, Decagon Devices, USA). The measurement was taken with samples drawn from four slices and the average value was expressed as water activity of the product.

**Moisture content**

To determine the moisture content, weighed pineapple slices were kept in a hot air oven (Metlab Scientific Instruments, India) at 100°C, till the weight remained constant. The percentage decrease in weight was expressed as moisture content (AOAC, 1995). The measurement was replicated with the samples drawn from four different slices and the average was taken as moisture content of the product.

**Colour measurement**

Colour of the central region of pineapple samples was measured by reflectance measurement, using a Minolta CM-3600D Spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan). The reflectance of whole visible spectrum (360 to 780 nm) was recorded at wave length intervals of 10 nm. D65 lamp was used as reference light source and the detector was fixed at an angle of 10° with respect to the light source (Hajare et al 2006). The colour parameters L* (Lightness), a* (Redness), b* (Yellowness) were analyzed with using JAYPAK 4808 software (Quality Control System, Version1.2).

**Texture analysis**

Texture was analyzed using a Texture Analyser (TA.HD plus, Stable MicroSystems, Godalming, Surrey, UK) with a P/2N needle probe. The probe speed and penetration depth were 0.5 mm/s and 5 mm, respectively. The hold time was 0.01 s and the trigger force was set at 10 g force. The probe travel distance was optimized for 5 mm. Texture was expressed in the unit of gram resistance force (g) measuring resistance offered by the sample to the penetrating needle probe (Hajare, Saroj, Dhokane, Shashidhar & Bandekar, 2007). The instrument was calibrated before each use. In sliced pineapple, tissue firmness varies from centre to periphery and hence, texture was measured at various points starting from the central pith region to the peripheral region of the edible portion.

**Sensory evaluation**

A preliminary sensory testing was performed by an experienced panel familiar with pineapple fruit characteristics. This panel comprised of 15-20 researchers from different sections of Food Technology Division, BARC. This testing was done in a Taste Panel Laboratory in individually partitioned compartments, under a controlled-environment (Meilgaard, Civille & Carr, 1999). Sensory analysis was carried out on radiation- treated and non-treated processed samples along storage. Panelists were previously briefed and trained to distinguish different pineapple samples and score their respective quality attributes including appearance, colour, texture, odour, taste and overall acceptability. A 7-point hedonic scale (1-very poor, 2-poor, 3-fair, 4-satisfactory, 5-good, 6-very good, and 7-excellent) was used, for grading the samples during evaluation.

**Statistical analysis**

All the above detailed experiments were repeated in three different batches. In each batch, 3-4 replicates were taken for every tested parameter. The data obtained was expressed in terms of Mean and Standard Deviations (SD). The mean values were compared using one-way ANOVA (ANalysis Of VAriance) test for significance of their difference (P < 0.05). The data was analyzed using the Origin 6.1 software version v6.1052 (B232) (OriginLab Corp., Northhampton, Mass., USA).
Results and Discussion

Effect of pineapple processing

The potassium metabisulfite dip, minimized pineapple slices browning which was visible. Browning occurs in cut pineapple due to polyphenol oxidase (PPO) action. This enzyme is located in plastids and cytoplasm inside the cell and phenolic substrates are located in vacuole. Due to cutting, the subcellular compartmentation in pineapple slice is lost. The enzyme and substrate come in contact with each other and in the presence of oxygen, the reaction results in the formation of quinones, which auto polymerize to form brown melanin-like pigments (Vamos-Vigyazo, 1981). Potassium metabisulfite water solution releases SO$_2$ which inactivates the PPO enzyme (Madero & Finne, 1982). The osmotic dehydration alone reduced $a_w$ of pineapple slices from 0.97 to 0.9. The $a_w$ below 0.82 was achieved only after infrared drying.

The $a_w$ and moisture content of the IM pineapple slices were about 0.82 and 37%, respectively and the change was found to be insignificant ($P < 0.05$) during 40 days of storage. The HDPE packaging retained the product moisture content. Polyethylene is known to have good moisture barrier properties (Basantia, Arora, Seth & Singh, 2000). It has been observed that the thickness of the pineapple slices is crucial for uniform drying by infrared. The slices with thickness greater than 1 cm showed uneven drying and took longer time to dry to the desired level. In such slices, $a_w$ could not be maintained below 0.82 during storage, due to improper drying.

Microbiological analysis

The mean values of total bacterial count and yeast and mold count in fresh pineapple slices, were found to be in the range of $10^2$ to $10^3$ cfu/g. There was an increase in TBC, YMC of freshly cut pineapple slices after 6 days at ambient storage temperature. The difference in microbial load between potassium metabisulfite dipped and freshly cut slices on day 1 of storage was found to be insignificant ($P < 0.05$), whereas, osmotic dehydration was found to decrease the bacterial load. Moreover, the increase in bacterial load and YMC of osmotically dehydrated slices during 6 days of storage was insignificant ($P < 0.05$). The combination of osmotic dehydration with infrared drying, decreased TBC and YMC which remained constant up to 6 days of storage (Table 1). Most bacteria fail to grow at an $a_w$ of less than 0.82 (Ledward, 1981). Intermediate moisture products in which $a_w$ is reduced to inhibit microbial growth, can be stored for extended periods under ambient conditions (Ledward, 1981). However, reduction of $a_w$ alone could not bring counts below the detection limit and so, mold growth was observed in samples during storage.

Table 1: Microbiological analysis of pineapple slices during storage at ambient temperature ($26 ± 2°C$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Bacterial Count</th>
<th>Yeast and Mold Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Day 6</td>
<td>Day 1</td>
</tr>
<tr>
<td>Freshly cut</td>
<td>3.0 b</td>
<td>5.5 c</td>
</tr>
<tr>
<td>Potassium metabisulfite dip</td>
<td>2.9 b</td>
<td>6.2 c</td>
</tr>
<tr>
<td>Osmotic dehydration</td>
<td>2.1 d</td>
<td>2.4 d</td>
</tr>
<tr>
<td>Osmotic dehydration and infrared drying</td>
<td>2.0 e</td>
<td>2.1 e</td>
</tr>
<tr>
<td>Potassium metabisulfite dip, osmotic dehydration and infrared drying</td>
<td>2.0 e</td>
<td>2.1 e</td>
</tr>
</tbody>
</table>

*The data was expressed as logarithm of colony forming unit (cfu/g) and represents the mean of at least 12 slices per treatment. The mean values with different superscript (b, c, d, e) are significantly different ($P < 0.05$) as analyzed by one way ANAlysis Of VAriance (ANOVA)
There was a minor increase in b* value, which were insignificant (p < 0.05), at the end of storage time in every sample (data not shown).

**Texture analysis**

Pineapple slices texture decreased in every sample during storage, many times significantly. The cutting process resulted in tissue disruption and membrane breakdown leading to the release of hydrolytic enzymes. Enzymatic degradation of cellulose, hemicellulose and pectin led to texture losses during storage (Myung 2006). This effect, being pronounced in freshly cut samples in the present study, was not reduced by the potassium metabisulfite dip or its combination with osmotic dehydration. Similar effects of such pre-treatments have also been reported by Karim et al (2008) for air dehydrated pineapple slices. Infrared drying was found to restore texture which remained stable up to 6 days of storage (Table 4). Drying by infrared radiation has also been reported earlier, to stabilize the texture of sugar-infused IM blueberries during storage (Shi et al, 2008). This texture retention could be due to sugar infusion into the intercellular spaces (Stojanovic & Silva, 2006). The combination of potassium metabisulfite dip, osmotic dehydration and infrared drying treatments, were fairly successful in maintaining texture values up to 6 days of storage.

The change in texture due to radiation treatment was found to be insignificant (P < 0.05) till 40 days of storage (data not shown). As earlier reported, a radiation dose of 2 kGy did not affect the texture of minimally processed pineapple slices when stored at 10°C for 12 days (Hajare et al 2006). López-Malo and Palou (2008) also reported the insignificant effect of combined preservation methods such as blanching and osmotic dehydration, on texture of pineapple slices.
Table 3: Colour analysis of pineapple slices during storage at ambient temperature (26 ± 2 ºC)\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L* (Lightness)</th>
<th>a* (Redness)</th>
<th>b* (Yellowness)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 \textsuperscript{b}</td>
<td>Day 5 \textsuperscript{b}</td>
<td>Day 1</td>
</tr>
<tr>
<td>Freshly cut</td>
<td>70 ± 2</td>
<td>58 ± 3</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Potassium metabisulfite fitedip</td>
<td>72 ± 2</td>
<td>59 ± 3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Potassium metabisulfite dip and osmotic dehydration</td>
<td>72 ± 2</td>
<td>64 ± 2</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Osmotic dehydration Potassium metabisulfite and infrared drying dip, osmotic dehydration, and infrared drying</td>
<td>70 ± 2</td>
<td>54 ± 5</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>74 ± 3</td>
<td>68 ± 1</td>
<td>3.7 ± 1.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The data represents mean ± SD of at least 9 slices per treatment. The corresponding mean values for day 1 and day 5 across columns superscripted as \textit{b} are significantly different (P < 0.05). The mean \textit{a*} values for day 1 and day 5 along the rows are significantly different (P < 0.05) as analyzed by one way ANalysis Of VAriance (ANOVA).

**Sensory evaluation**

Panelists gave overall scores ranging between 4 (satisfactory) and 5 (good) for the IM pineapple slices, with no statistical significant differences due to storage days or radiation doses. The scores for colour were better than texture, aroma, taste and overall acceptability. For many of these parameters a minor, though not significant, decrease in average scores was observed during storage (Table 5). These findings are in agreement with those of Hajare et al (2006), who also reported no significant effect of 2 kGy radiation dose, on sensory parameters of minimally processed pineapple slices, when stored at 10 ºC for 12 days.

Table 4: Texture analysis of pineapple slices during storage at ambient temperature (26 ± 2 ºC)\textsuperscript{a, b, c, d}.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly cut</td>
<td>185\textsuperscript{a} ± 26</td>
<td>111\textsuperscript{b} ± 30</td>
</tr>
<tr>
<td>Potassium metabisulfite dip</td>
<td>175\textsuperscript{a} ± 22</td>
<td>113\textsuperscript{b} ± 34</td>
</tr>
<tr>
<td>Potassium metabisulfite dip and osmotic dehydration</td>
<td>125\textsuperscript{a} ± 17</td>
<td>106\textsuperscript{a} ± 17</td>
</tr>
<tr>
<td>Osmotic dehydration and infrared drying</td>
<td>138 ± 23</td>
<td>137 ± 26</td>
</tr>
<tr>
<td>Potassium metabisulfite dip, osmotic dehydration, and infrared drying</td>
<td>125 ± 36</td>
<td>113 ± 37</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The data represents mean ± SD of measurements of at least 12 slices.  
\textsuperscript{b} The corresponding mean values for day 1 and day 6 across columns in each zone are significantly different (P < 0.05) as analyzed by one way ANalysis Of VAriance (ANOVA).  
\textsuperscript{c} The texture was expressed in the unit of gram resistance force (g) measuring resistance offered by the sample to the penetrating needle probe.

**Conclusion**

The combination of hurdles including potassium metabisulfite dip, osmotic dehydration, infrared drying, HDPE packaging and a radiation dose of 1 kGy was found to be effective in extending the shelf life of pineapple slices up to 40 days under ambient storage temperature, whereas the control non-treated slices spoiled within 6 days. The potassium metabisulfite dip was indispensable for browning inhibition. Osmotic dehydration and infrared drying reduced \textit{a*}, which prevented microbial growth. A radiation dose of 1 kGy was found to be effective in eliminating the residual microbial load on these pineapple samples, thus ensuring microbial safety of the product sensorily accepted throughout storage.
Table 5: Effect of radiation on sensory characteristics of intermediate moisture pineapple slices during storage at ambient temperature (26 ± 2 ºC)*b.

<table>
<thead>
<tr>
<th>Radiation Dose (Gy)</th>
<th>Days of Storage</th>
<th>Colour</th>
<th>Texture</th>
<th>Aroma</th>
<th>Taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>5.0±0.9</td>
<td>4.8±0.7</td>
<td>4.7±0.6</td>
<td>5.1±0.7</td>
<td>5.0±0.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.9±0.9</td>
<td>4.6±0.8</td>
<td>4.5±0.7</td>
<td>3.8±1.0</td>
<td>4.5±0.8</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>4.6±0.9</td>
<td>4.4±0.8</td>
<td>4.1±0.7</td>
<td>3.9±0.8</td>
<td>4.3±0.9</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>5.3±0.7</td>
<td>4.6±0.7</td>
<td>4.6±0.6</td>
<td>4.5±0.7</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5.0±0.9</td>
<td>4.3±0.5</td>
<td>4.3±0.6</td>
<td>4.6±0.6</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>4.8±0.9</td>
<td>4.2±1.2</td>
<td>4.0±0.8</td>
<td>4.6±0.9</td>
<td>4.5±0.9</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
<td>5.2±1.2</td>
<td>4.0±1.2</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>4.1±1.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.6±0.5</td>
<td>4.4±0.8</td>
<td>4.5±1.0</td>
<td>4.9±0.7</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>4.6±0.7</td>
<td>3.9±1.0</td>
<td>4.0±0.7</td>
<td>4.4±0.9</td>
<td>4.1±1.0</td>
</tr>
</tbody>
</table>

*a The data represents mean ± SD of sensory scores of 12 slices. The mean values in a column are not significantly different (P < 0.05) as analyzed by one way ANalysis Of Variance (ANOVA). b Data in the table denote the scoring in a 7-point hedonic scale (1-very poor, 2-poor, 3-fair, 4-satisfactory, 5-good, 6-very good, and 7-excellent.

References


ABOUT THE AUTHORS

Mr. Sudhanshu Saxena did his M.Sc. in Biotechnology from Madurai Kamaraj University, Madurai in the year 2003. After graduating from the 47th batch of Training School, he joined the Food Technology Division. His research interest includes radiation preservation of food products and development of shelf stable fruits.

Mr. B. B. Mishra did his M.Sc. in Botany from Ravenshaw College, Utkal Univesity, Orissa in the year 1999. After graduating from the 44th batch of Training School, he joined the Food Technology Division. His research interest includes radiation preservation of foods of plant origin, development of shelf stable fruit products and inhibition of polyphenol oxidase mediated enzymatic browning, in fruits and vegetables.
Dr. Ramesh Chander joined the Food Technology Division in 1975 after graduating from BARC Training School. He received his PhD from the University of Mumbai and MSc from H.A.U. both in Microbiology. He was a Post Doctoral fellow at the University of Florida, USA from 1988-1990. He is a fellow of the “Maharashtra Academy of Sciences”. He is the recipient of the Outstanding Paper Award in the pharmaceutical field, conferred by the Controlled Release Society at Orlando, Florida (USA) on July 28, 1992. He is the author of more than 40 publications. His work pertains to the development of safe, shelf-stable meat products using hurdle technology. He has invented Vibro-thermal disinfester for disinfection of food grains. He has also transferred the technology for foldable solar dryer. He has investigated several natural antioxidants for minimizing lipid peroxidation in processed food. His major contribution is development of “Artificial Viral Envelopes” for targeted intracellular delivery of peptides, enzymes, toxins and gene constructs, for which he holds a US and a European patent. He was actively involved in the upgradation of KRUSHAK for meeting the compliance requirements of USDA-APHIS for radiation processing of mangoes for export to USA. As a result of his efforts, Indian mangoes could be exported to USA after a gap of 18 years. He retired from BARC in April, 2009 as Head, Meat & Meat Products Section, Food Technology Division, BARC.

Dr. A.K. Sharma joined the Training School of BARC, Mumbai, in 1975, after finishing Masters degree in Microbiology from National Dairy Research Institute, Karnal. His early work related to aflatoxin biosynthesis and control in food commodities, for which he was awarded a Ph.D. degree from the University of Mumbai in 1984. Dr. Sharma was a DBT Post-Doctoral Associate at the Department of Plant Pathology, University of California, Riverside, USA during 1990-1991, where he worked on the molecular mechanism of host-pathogen interactions in plants. He studied food irradiation at the Royal Institute of Quality Control of Agricultural Products (RIKILT), Wageningen, The Netherlands. Dr. Sharma has made substantial contributions to the field of food irradiation technology with special reference to microbiological safety of foods, radiation processing of food commodities including spices and mango. He was the project manager of KRUSHAK irradiator project at Lasalgaon, set up for demonstrating low dose applications of radiation, for preservation of agricultural commodities. As a result of his efforts, Indian mangoes could be exported to USA after a gap of 18 years. For his outstanding achievements in the field of radiation and radioisotope applications in food technology Dr. Sharma has been awarded INS Award-2006 by the Indian Nuclear Society, and Group Achievement Award-2007 of the Department of Atomic Energy. He has more than 300 publications in the national and international journals, including chapters in books and encyclopedias, and articles in international and national conferences. Dr. Sharma has been the apex coordinator for three plan projects in the field of applications of radiation technology for food preservation and hygienization. He has represented India in the co-coordinated research programmes as well as an expert of the International Atomic Energy Agency. He has been the secretary of the Environmental Mutagen Society of India and Vice-President of the Indian Society for Environmental Science & Technology. Dr. Sharma is currently Outstanding Scientist & Head, Food Technology Division, BARC, and also holds adjunct position of Senior Professor, Homi Bhabha National Institute, Mumbai.