Storage ability of Kulfi incorporated with Amaranthus (Rajgara)

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Abstract
The storage study of optimized Kulfi incorporated with Amaranthus was carried out at subzero temperature (-18 ± 2°C) up to acceptable level by the judges. After 60 days of storage at -18 ± 2°C, the average flavour, body and texture, colour and appearance and overall acceptability score of Kulfi were significantly decreased. During 60 days of storage, the average acidity value of Kulfi samples stored at -18 ± 2°C were significantly declined and the pH value of Kulfi non-significantly increased with the progress of storage. The melting rate of Kulfi samples were non significantly decreased with change in storage period. The SPC and YMC of the Kulfi samples stored at -18 ± 2°C, were increased non-significantly and significantly respectively, during 60 days of storage. The developed Kulfi had shelf-life of 60 days at -18 ± 2°C.

Keywords: Storage study, Kulfi, amaranthus, melting rate, standard plate count

1. Introduction
There has been a great increase in milk production in India during the post-independence era. It has increased from 55.6 MMT during 1991-92 to 187.7 MMT during 2018-19 at an average annual growth rate of 4.5%. Simultaneously the per capita availability of milk has also increased to 394 gms/ day in 2018-19. However due to cost constraints milk still remains a luxury item for a larger population and provide less than the minimum nutritional requirement. As a result, there is a wide spread protein malnutrition, especially in the weaker sections of society. Under these circumstances, utilization of food solids from the vegetable sources (like Amaranths, soybean, ground nut etc.) offers great promise to fight the malnutrition. Current advances in the food and nutrition sciences accentuate the substantial role of diet in modulation of various physiological functions and thus the health status of human body. As a result, new nutritional concepts, therapeutic nutrition in the form of dietetic foods have evolved, which focus on the diets specially designed to satisfy the specific nutritional requirements of different consumer group.

Kulfi is a 500 year old popular frozen dessert of Indian origin and it occupies a privileged position amongst the traditional Indian dairy products[1]. Kulfi is also known as Qulfi, Kulfa, Kulphy etc. [2]. The word Kulfi derives its origin from the Hindustani word Kulaf meaning a “lock” or a “container” that has to be unlocked. And, indeed the recess of the metal cone that encases the frozen delight has to be pried open to release the confection [1].

As per FSSR, Kulfi means the product obtained by freezing a pasteurized mix prepared from milk and/or other products derived from milk with or without the addition of nutritive sweetening agents, fruit and fruit products, eggs and egg products, coffee, cocoa, chocolate, condiments, spices, ginger and nuts. It may be frozen hard or frozen to a soft consistency. It shall have pleasant taste and smell free from off flavour and rancidity. It may contain permitted food additives. It shall contained minimum 36% TS, 10% milk fat and 3.5% protein [3].

Amaranth is a unique plant with a long and mysterious history. It is the object for science and also for business. The research on amaranth has increased the expectation of its utilization in different industry. Its characteristics viz. absence of gluten and special composition of oil led this plant to the position of very important plant for the future. And many value-added products can be made from it. Amaranths products are increasingly becoming popular especially amongst health conscious people.
Amaranth has the utmost amount of protein, two times the content of essential amino acid i.e. lysine, more dietary fiber, and 5–20 times calcium and iron compared to other grains [4]. High-protein amaranth flour containing 26–28% protein, 10–16% fat, and 40–52% starch has been produced through use of enzymes α-amylase and glucoamylase. Amaranth seeds (i.e. 13–21% protein) have been converted into value added protein concentrates (i.e. 52.5% protein). Amaranth protein isolates have protein content ranging from 79.4–85.4% [5]. Ingestion of gluten from rye, wheat, barley and other closely related cereal grains triggers the immune-mediated celiac disease in genetically susceptible individuals, and therefore such individuals need gluten-free diet [6]. Albumins and globulins which are easily digestible and are the chief parts of highly nutritive amaranth seed proteins, which have been considered as an important ingredient for such replacement. Substitution of 10-15% of wheat flour by amaranth flour enhanced the dough processing (increased binding of flour, production of CO₂), porosity of bread crumb (more regular with softer pores), and increased nutritive value of product; greater levels had some deleterious effect on bread making properties [7]. Amaranth flour contains remarkably high content of totalolate (53-73.0 μg/100 g dry wt.) than wheat flour (13.5 μg/100 g dry wt.) and hence used to enrich food like bread, noodles and cookies with this vitamin by replacing 40% of wheat by amaranth [8]. Invaluable advantages of Amaranth protein as a food supplement are gluten-free, high digestibility, favourable nutrition composition suggested for vegetarian and lysine content for children nervous system [9].

2. Materials and Methods
The experiment studies were conducted in the department laboratory, Department of Dairy Technology, G.N. Patel College of Dairy Technology, S.D. Agricultural University, (Gujarat) India. The objective of the present study is to increase the functional value of Kulfi by incorporation of one of the most underutilized pseudocereal i.e. Amaranthus that make it more useful and acceptable to undertake its commercial production.

2.1 Ingredients/ Materials
Raw materials such as milk, Amaranthus flour, sugar, stabilizer & emulsifier, spices & condiments, and Artificial/Natural flavours and colourants were used in the experiment. Milk / Skim milk / Cream were obtained from the fresh, raw mixed (cow and buffalo) milk received at Mini Dairy Plant of GN Patel College of Dairy Technology. Good quality Amaranthus free from stones, dust, insects and other impurities was procured from local market for preparation of Kulfi. Good quality commercial grade cane sugar, procured from the local market was used for preparing sugar syrup. Stabilizer i.e. sodium alginate was used for manufacturing of Kulfi, was obtained from the local market of Palanpur. Artificial/Natural flavours i.e. Mawa flavour used for manufacturing of Kulfi, was obtained from the local market of Palanpur.

All glassware such as conical flask, beakers, volumetric flasks, measuring jars were cleaned using detergents and sterilized using hot air oven set at 160-180 °C temperature for 2 hours before use. High purity commercially available chemicals/ media were used in the investigation for different analyses. All the chemicals, used for this study was of AR grade. The equipment used in the investigation was as follows: cream separator, Kulfi mould, candy making machines, hardening tunnels and storage unit.

2.2 Preparation of Kulfi
2.2.1 Amaranthus based Kulfi
Kulfi mix was prepared using milk, cream, skim milk powder, Amaranthus flour, sugar, stabilizer i.e. sodium alginate @ 0.15% and artificial/natural flavours i.e. Mawa flavour. It was standardized to 10% fat, 10% SNF and 15% sugar. Part of SNF was adjusted by adding the Amaranthus flour. Amaranthus: SMP in Kulfi mix was added in a 25:75 (T1), 50:50 (T2), 75:25 (T3), 100:0.0 (T4) and 100:0.0 (T5) ratios. Formulated Kulfi mix was pasteurized at 80 °C for 25 sec. followed by cooling at 4 °C. Artificial/Natural flavours i.e. Mawa flavor @ 0.3% was added to the mix. Then the Kulfi mix was filled in to the Kulfi mould covered with the lid. Moulds were transferred to candy making machine, set at -20 °C for freezing. After complete freezing, Kulfi was transferred to deep freezer maintained at -18 ± 2 °C (for overnight) for hardening. The Kulfi was kept in deep freezer until further used. Four different experimental Kulfi i.e. T1 (25% replacement of SMP), T2 (50% replacement of SMP), T3 (75% replacement of SMP) and T4 (100% replacement of SMP), were prepared. Different proportions of Amaranthus flour in Kulfi as indicated in Table 1.

2.2.2 Control Kulfi
The control Kulfi sample (i.e. T5) was prepared without addition of Amaranthus flour in Kulfi mix, which was standardized to 10% fat and 10% MSNF and 15% sugar. SNF was adjusted with the skim milk powder only.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proportions of Amaranthus: SMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>(25: 75)</td>
</tr>
<tr>
<td>T2</td>
<td>(50: 50)</td>
</tr>
<tr>
<td>T3</td>
<td>(75: 25)</td>
</tr>
<tr>
<td>T4</td>
<td>(100: 00)</td>
</tr>
<tr>
<td>T5 (control)</td>
<td>(00: 100)</td>
</tr>
</tbody>
</table>

Optimization was done on the basis of sensory score and Kulfi with Amaranthus: SMP @ 25: 75was found most suitable on the basis of sensory score. Based on the sensory acceptability of the product, it was decided to prepare Kulfi incorporated with Amaranthus: SMP @ 25: 75. And control product without incorporation of Amaranthus was also prepared in identical manner. Both the products were stored in deep freezer maintained at –18 ± 2 °C for 60 days and were analyzed periodically for physico-chemical properties (i.e. pH, acidity and melting rate), sensory (i.e. flavor, colour and appearance, body and texture and overall acceptability) and microbiological (i.e. standard plate count, yeast and mold count and coiform count) parameters. The analysis of products stopped when it was found to be unacceptable by the sensory panel.

2.3 Physico-chemical analysis
The pH of Kulfi was measured using digital pH meter. The method described by Franklin and Sharpe [10] for cheese was used. About 20 ml of sample was taken for measuring the pH directly. Titratable acidity of the product was determined by titrimetric method as described in IS:1166 [11] specifications for condensed milk. Melting rate was determined by emptying the Kulfi samples from the moulds on an iron mesh (9 squares per linear inch) placed over a glass funnel having 10 cm
outside diameter as suggested by Ashokraju et al. [12]. The whole assembly was kept over a preweighed glass measuring cylinder (capacity 100 ml) and placed in an oven maintained at 30 °C without air circulation. The weight of the cylinder was taken after 30 minutes and difference in weight is expressed as melting rate in grams per 30 minutes.

2.4 Microbiological analysis
Standard Plate Count, Yeast and Mold count and Coliform count for Kulfi sample were determined by method as per IS: 5550 [13] with slight modifications. For determining SPC, Eleven grams of sample were aseptically be weighed and transferred into 99 ml sterile tri-sodium citrate buffer flask. Further dilution was prepared using 9 ml sterile tri-sodium citrate buffer blanks. Suitable dilutions (selected based on preliminary study conducted) of each sample were transferred (1.0 ml) aseptically into sterile petri plates and thereafter 10 to 15 ml of molten SPCA was added. The plates were incubated in an incubator maintained at 37±1 ºC for 48 h, and the number of colony forming units (CFU/g) were noted and expressed in log10 CFU/g of sample. All the plates were prepared in duplicate. The Yeast and Mold count of all the samples were determined by the same method as described for SPC except that the medium used was potato dextrose agar (PDA) along with added tartaric acid to set pH 3.5 of agar and the plates were incubated 25±1 ºC for 3 to 5 days. The coliform count of all the samples were determined by the method as described for SPC except that the medium used was violet red bile agar (VRBA), and the plates were incubated at 37±1 ºC for 24 to 48 h.

2.5 Sensory evaluation
The product was subjected to the sensory evaluation by judges for colour and appearance, flavour, body and texture, and overall acceptability criteria. Fresh product at 0 days and the stored product (10, 20, 30, 40, 50 & 60 days, stored at – 18 ± 2 ºC) were served to judges for sensory evaluations. The score given by them on 9 point hedonic scale was taken to determine the acceptability level of product.

3. Results and Discussion
3.1 Changes in physico-chemical attributes of Kulfi during storage
Amaranthus added Kulfi (experimental) and control Kulfi samples stored at -18 ± 2 ºC, were analyzed for changes in titratable acidity, pH and Melting rate value at interval of 10 days. The physico-chemical attributes of Amaranthus added Kulfi and control Kulfi during storage are presented in Table 2.

<table>
<thead>
<tr>
<th>Storage Period (Days)</th>
<th>Acidity</th>
<th>pH</th>
<th>Melting rate</th>
<th>Acidity</th>
<th>pH</th>
<th>Melting rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.16 ± 0.00</td>
<td>6.72 ± 0.02</td>
<td>38.56 ± 0.99</td>
<td>0.16 ± 0.00</td>
<td>6.72 ± 0.02</td>
<td>35.38 ± 0.87</td>
</tr>
<tr>
<td>10</td>
<td>0.16 ± 0.00</td>
<td>6.72 ± 0.02</td>
<td>38.49 ± 0.95</td>
<td>0.17 ± 0.00</td>
<td>6.72 ± 0.02</td>
<td>35.31 ± 0.85</td>
</tr>
<tr>
<td>20</td>
<td>0.17 ± 0.00</td>
<td>6.72 ± 0.02</td>
<td>38.38 ± 0.94</td>
<td>0.17 ± 0.00</td>
<td>6.71 ± 0.02</td>
<td>35.24 ± 0.82</td>
</tr>
<tr>
<td>30</td>
<td>0.17 ± 0.00</td>
<td>6.72 ± 0.02</td>
<td>38.27 ± 0.91</td>
<td>0.18 ± 0.00</td>
<td>6.71 ± 0.02</td>
<td>35.19 ± 0.82</td>
</tr>
<tr>
<td>40</td>
<td>0.18 ± 0.00</td>
<td>6.71 ± 0.02</td>
<td>38.20 ± 0.86</td>
<td>0.18 ± 0.00</td>
<td>6.70 ± 0.02</td>
<td>35.13 ± 0.78</td>
</tr>
<tr>
<td>50</td>
<td>0.19 ± 0.00</td>
<td>6.71 ± 0.02</td>
<td>38.12 ± 0.82</td>
<td>0.19 ± 0.00</td>
<td>6.70 ± 0.02</td>
<td>35.04 ± 0.74</td>
</tr>
<tr>
<td>60</td>
<td>0.19 ± 0.00</td>
<td>6.70 ± 0.02</td>
<td>38.03 ± 0.78</td>
<td>0.19 ± 0.00</td>
<td>6.70 ± 0.02</td>
<td>34.95 ± 0.70</td>
</tr>
</tbody>
</table>

Means with at least one letter common are not statistically significant using Fisher's Least Significant Difference. Figures placed after ± indicates Standard Error of Mean. Acidity and Melting rate expressed as% lactic acid and g/30 min. respectively.

3.1.1 Titratable acidity
It can be seen from the Table 2 that the changes in acidity of experimental and control Kulfi shows the same trend during the entire storage period i.e. up to 60th days of storage. Statistical analysis revealed that significantly marginal increased in acidity from 0.16 to 0.19% LA was found in both Kulfi samples (i.e. control & experimental) stored at -18 ± 2 °C during 60 days of storage. This increase in acidity was found to be significant (P<0.01) with change in storage period for Amaranthus added Kulfi and for control Kulfi as well. Nigam [14] reported that the slight increase in acidity from 0.270 to 0.306% LA was found in Chhana based Kulfi and control samples stored at -18 ± 2 °C during 60 days of storage. This increase in acidity was found to be highly significant (P<0.01) with change in storage period for Chhana based Kulfi and for control Kulfi as well.

3.1.2 pH value
The average pH value for control and experimental Kulfi are presented in Table 2. It can be seen from the table that, during 60 days of storage average pH value of Kulfi samples, stored at -18 ± 2 ºC declined a bit from 6.72 to 6.70 for both the sample i.e. experimental and control Kulfi samples. It was observed form the Table 3 that both Kulfi samples showed non-significant (P>0.05) decrease in pH value with change in storage. The same trend was observed by Nigam [14] who noticed that the average pH value of Chhana based Kulfi and control Kulfi samples stored at -18 ± 2 ºC declined significantly (P<0.01) from 5.965 to 5.948 and 6.430 to 6.414 respectively during 60 days of storage.

3.1.3 Melting rate
The effect of storage on melting rate of Kulfi is depicted in Table 2. As it could be seen from the table that the storage days increased, the melting rate non significantly (P>0.05) decreased in both control and experimental Kulfi during storage. Statistical analysis revealed that the melting rate does not show any significant change all over the entire storage period. The melting rate of control Kulfi and experimental Kulfi decreased from 38.56 to 38.03 and 34.95 g/30 min, respectively. The results are in agreement with the
findings of Thomas et al. [15] who reported that with the development of storage periods, the melting rate of Kulfi was decreased in both control and experimental Kulfi. The melting rate in case of control decreased from 17.76 to 17.10 whereas in case of experimental Kulfi the melting rate was decreased from 17.48 to 17.05 ml/15min as the period of storage increased from 0 to 50. Jha [16] who conducted shelf life studies of low fat and low calorie Kulfi at -15 °C for 60 days. He found that the meltdown rate in ml/min increased from 14 to 19 in experimental sample compared to 14 to 16 in case of control.

### 3.2 Changes in sensory attributes of Kulfi during storage

Sensory evaluation plays a vital role in product development and determining the shelf life of a product as well. All deteriorative changes i.e. oxidative, lipolytic, proteolysis, browning, acidity development, microbial and textural changes are collectively correlated with sensory quality and thus lead eventually to rejection of the stored product. From the consumers point of view, it is one of the primary characteristics based on which the quality of product is decided. The effect of storage on sensory qualities of Kulfi i.e. flavour, body and texture, colour and appearance and overall acceptability are shown in Table 3.

#### 3.2.1 Flavour

It is evident from the Table 2 that the flavour scores of control sample remained same without any significant difference up to 10th day of storage and subsequently decreased significantly along with the progress of storage period, while in case of experimental Kulfi the flavour score significantly decreased throughout the storage periods. The general mean for flavour score of experimental Kulfi (i.e. 7.23) was higher as compared to control Kulfi (i.e. 7.19). The comparison of the present findings with those of other scientists for similar frozen products has been made under section 3.2.4.

#### 3.2.2 Body and texture

It is apparent from the Table 2 that the changes in body and texture of both the sample showed the same trend. The body & texture score for control and experimental Kulfi decreased significantly throughout the entire storage periods. At the end of the storage period i.e. 60th days, the scores for body and texture attribute of control and experimental Kulfi were 6.43 and 6.27 respectively. The general mean for body & texture score of control Kulfi (i.e. 7.34) was slightly higher as compared to experimental Kulfi (i.e. 7.21). The comparison of the present findings with those of other scientists for similar frozen products has been made under section 3.2.4.

#### 3.2.3 Colour and appearance

After 60 days of storage, average colour & appearance score of control and experimental Kulfi samples declined from 7.30 to 6.01 and from 7.49 to 6.08, respectively. It is evident from the Table 3 that the colour & appearance scores of control sample remained same without any statistically significant difference up to 10th day of storage and subsequently decreased significantly with the advancement of storage period up to 60th days, while in case of experimental Kulfi the colour and appearance score significantly decreases throughout the storage periods. The general mean for colour & appearance score of experimental Kulfi (i.e. 6.75) was slightly higher as compared to control Kulfi (i.e. 6.71). The comparison of the present findings with those of other scientists for similar frozen products has been made under section 3.2.4.

#### 3.2.4 Overall acceptability

It can be seen from the Table 3 that the overall acceptability scores of control and experimental Kulfi showed significant change all over the storage period. The overall acceptability score for experimental Kulfi was higher compared to the control Kulfi up to the 20th days of storage. After 20th days onwards, the control Kulfi fetched the higher score compare to experimental Kulfi. At the end of the storage period i.e. 60th days, the scores for overall acceptability of control and experimental Kulfi were 7.14 and 7.11 respectively. Thomas et al. [15] concluded that score for all the sensory attributes of control as well as experimental sample of Kulfi i.e. lactose hydrolyzed functional Kulfi prepared using 2% oat flour, 1% flaxseed oil and 4% Whey Protein Concentrate were decreased slightly at each subsequent analysis interval. At the end of storage i.e. 50 days the product was found acceptable on the basis of overall acceptability score in both the cases i.e. control as well as experimental. He concluded from the results that lactose hydrolyzed functional Kulfi can be stored up to 50 days without affecting its sensory attributes. At the end of storage the experimental sample was scored 7.20, 7.95, 7.76.
and 7.27 for flavour, body and texture, colour and appearance and overall acceptability, respectively while the control Kulfi scored 7.64, 7.36, 7.22 and 7.40 respectively. Nigam reported that the flavour, body, texture, colour and appearance and melting quality scores of Chhana based Kulfi and control Kulfi decreased marginally throughout the storage period up to 60 days. Schaller-Povolny reported highly significant (P≤0.01) effect of storage period on iciness of inulin containing reduced fat ice cream, which ultimately affected the other sensory characteristics of frozen products. Jha conducted shelf life studies of low fat and low calorie Kulfi at -15 °C for 60 days. Sensory scores for all the attributes of control as well as experimental sample of Kulfi were decreased slightly at each subsequent analysis interval. At the end of storage the experimental sample was scored 7.50, 7.40, 7.21 and 7.20 for colour and appearance, body and texture, flavour and overall acceptability, respectively while the control Kulfi scored 7.68, 7.88, 7.70 and 7.60 respectively.

3.3 Microbiological changes in Kulfi during storage
The microbiological analysis as could be observed in Table 4 indicates that the Standard Plate count, Coliform count and Yeast and Mold count of both control and experimental Kulfi was within the maximum limit as prescribed in FSSAI standards for frozen dessert.

### 3.3.1 Standard Plate Count (SPC)
The SPC count of experimental and control Kulfi are presented in Table 3. It can be seen from the table that the Standard Plate Count of the Kulfi prepared by using Amaranthus and control Kulfi samples stored at -18 ± 2 °C increased non-significantly (P < 0.05) from 4.67 to 5.14 log CFU/g and 4.60 to 5.11 log CFU/g respectively after 60 days of storage. The storage period did not have any significant (P < 0.05) effect over the change in SPC for both of the samples. This result is in consonance with Thomas et al. who observed that the SPC of lactose hydrolyzed Kulfi supplemented with oat flour, flaxseed oil and WPC and control Kulfi increased from 4.7 to 5.00 and 4.52 to 4.87 log CFU/g respectively during storage period. Nigam found that the Standard Plate Count of the Chhana based Kulfi and control Kulfi samples stored at -18 ± 2 °C, significantly (P ≤ 0.01) decreased from 8.12 to 7.72 and 7.26 to 6.87 log CFU/g after 60 days of storage.

### 3.3.2 Yeast and Mold count
Yeast and Mold growth is one of the major factors in spoilage of the milk based sweets and desserts as Yeast and Mold can grow at lower moisture content as well as in sugar containing products and it affects appearance and renders the product unsafe for consumption. Yeast and Mold count was absent up to the 50th days and 40th days of storage at -18 ± 2 °C for control and experimental Kulfi respectively. From the 50th days onwards the Yeast and Mold count of control Kulfi significantly increased to 1.00 log CFU/g, while in case of experimental Kulfi the Yeast and Mold count significantly increased to 1.32 log CFU/g after the 60th days of storage. Statistical analysis revealed that the changes in Yeast and Mold count were highly significant (P < 0.05) after the 50th days and 40th days of storage for control and experimental Kulfi respectively. Nigam reported the Yeast and Mold count of Chhana based Kulfi samples and control Kulfi samples, stored at -18 ± 2 °C decreased from 3.05 to 2.40 log CFU/g and 2.79 to 2.39 log CFU/g, respectively. Statistical analysis revealed that the changes in Yeast and Mold count was highly significant (P ≤ 0.01) with change in storage period for both type of Kulfi samples.

### 3.3.3 Coliform count
Coliforms which are indicator of hygienic conditions maintained during production and packaging of products, were absent in all fresh and stored products irrespective of type of samples and storage intervals. This indicates that hygienic conditions were appropriate during the entire product preparation and packaging of Kulfi prepared by using Amaranthus and control Kulfi as well. This result is supported by Nigam who reported the coliform was absent in experimental and control sample throughout the entire storage period. Thomas et al. reported that the coliforms was absent in experimental Kulfi on 40th day of storage. In experimental Kulfi the coliform count was observed from 40th day of storage. The coliform count in experimental Kulfi on 40th and 50th days of storage was 1.0 log CFU/g and 1.3 log CFU/g, respectively.

### 4. Conclusion
From the present storage study, it can be concluded that Kulfi prepared by using Amaranthus: SMP @ 25:75 had 60 days shelf life when stored at - 18 ± 2 °C. Storage had led to deterioration in sensory quality and physico-chemical parameters. Increased in acidity and decreased in pH and melting rate was observed in storage period. All the sensory attributes i.e. flavor, colour and appearance, body and texture and overall acceptability score decreased significantly during storage.
storage which led to its rejection by sensory panelists. The total bacterial count and yeast and mold count of experimental Kulfi also notably affected with the progress of storage. Owing to its unacceptable sensory quality, Kulfi was rejected after 60 days of refrigerated storage.

5. References
3. FSSR. Food Safety and Standards Act, Rules and Regulations, Professional Book Publishers, New Delhi, India. 2011; 150:156.