Development of Gulabjamun by incorporating the Amaranthus hypochondriacus L. (Rajgara)

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Abstract
The present investigation was conducted to develop a value-added traditional Indian dairy products i.e. Gulabjamun by addition of Amaranthus (Rajgara) which made product more nutritious. The experiment was conducted to study the effect of admixing Amaranthus with Maida in five proportions (viz., 25:75 (T1), 50:50 (T2), 75:25 (T3), 100:00 (T4) and 00:100 (T5) w/w) on the product quality. The rate of addition of Amaranthus: Maida was kept at 20.0 % by weight of Khoa. It was found that among the four level studied Amaranthus: Maida @ 50:50 was most acceptable with respect to their flavor and overall acceptability scores and the values for rheological properties were nearer to control sample. The TS, fat, protein, total carbohydrate and ash content of Gulabjamun significantly (P<0.01) increased with increased in the proportion of Amaranthus. The acidity of Gulabjamun significantly varied in a narrow range. The value for pH, peroxide value and sugar syrup absorption of Gulabjamun was non-significantly varied. In conclusion, the study was successful in formulating an acceptable quality of Gulabjamun prepared by addition of Amaranthus: Maida in the ratio of 50:50, and the proportion was added @ 20 % by wt of Khoa as a binder which enhanced its overall acceptability.

Keywords: Gulabjamun, incorporating, Amaranthus hypochondriacus L. (Rajgara)

Introduction
Traditional dairy products and sweets are a significant part of Indian tradition. These products have immense cultural, religious, social, medicinal and economical importance and have been developed over a long period with the culinary skills of homemakers and halwais. The manufacture of traditional dairy products preserves milk solids for longer time at room temperature and adds value to milk, also provide substantial employment opportunity. It is projected that about 50 % of total milk produced in India is converted into traditional milk products. India has a conventional market for traditional dairy products but it also has prodigious export potential because of strong presence of Indian diaspora in many parts of the world [1].

Gulabjamun is a very prevalent Khoa based sweet. It is made by kneading Khoa and/or Maida (refined wheat flour), making balls, deep frying balls in oil/hydrogenated oil/Ghee till golden brown colour, soaking in sugar syrup and serving warm or cold. As it looks like monsoon fruit Jamun and is flavoured with rose water it got the name Gulabjamun.

Gulabjamun refers to the indigenous dairy product. And in India, it is considered by an unorganized nature of business. Almost all the parts of the nation exploit Gulabjamun as one of the indispensable and most commonly consumed sweet. Different states have different size and shapes of Gulabjamun viz; cylindrical, oval and spherical, but most usually found shape is spherical. Both round and cylindrical shaped Gulabjamun has golden to dark brown colour, soft to firm body and smooth texture and it is soaked in the sugar syrup. Variations include Pantua and Lalmohan (both Bengali sweets) with mixture of Chhana and Khoa.

As defined by Prevention of Food Adulteration Act, Khoa is an indigenous milk product obtained from cow or buffalo milk or a combination thereof by rapid drying. The moisture in Khoa shall not exceed 28 per cent and milk fat content shall not be less than 20 % of the product. Dhap Khoa containing 40-45 % moisture is normally used for its preparation. Like other sweets, the Gulabjamun has been mainly manufactured by halwais who implement small scale batch method.

Though there are huge differences in the sensory quality of Gulabjamun, the utmost preferred product should have smooth and round shape, brown colour, soft and a little spongy body, free from hard central core and lumps, uniform granular texture, mildly oily and cooked flavour,
free from doughy feel and fully juicy with sugar syrup. It shall have optimum sweetness.
The gross chemical composition of *Gulabjamun* varies extensively depending on several factors, such as composition and quality of *Khoa*, recipe of preparation, sugar syrup concentration etc. The composition of *Gulabjamun*, on the drained weight basis, varies from: moisture 25–35 %, fat 8.5–10.5 %, protein 6.0–7.6 %, ash 0.9–1.0 % and total carbohydrates 43–48 %.

Materials and Methods

Raw materials such as milk, *Khoa*, refined wheat flour, *Amaranthus*, sugar, *ghee*, natural and permitted flavours, colours and condiments were used in the experiment. Fresh, raw mixed (cow and buffalo) milk received at Mini Dairy Plant of G N Patel College of Dairy Technology was used as the base material for *Khoa* manufacture. *Ghee* was procured from Mini Dairy Plant of G N Patel College of Dairy Technology, SDAU, SK Nagar or from the local market. Good quality *Amaranthus* free from stones, dust, insects and other impurities was procured from local market. Good quality commercial grade cane sugar, procured from the local market was used for preparing sugar syrup. Cardamom used for flavouring of *Gulabjamun* was obtained from the local market of Palanpur. The *Amaranthus* flour was free from any objectionable flavor and had an average composition of 09.84% moisture, 05.90 % fat, 14.34 % protein.

**Preparation of Khoa**
The *Khoa* was prepared from fresh standardized milk. It was employing heating in a jacketed kettle with constant manual stirring-cum-scraping, keeping steam pressure 0.5-1.0 kg / sq. cm. till it reaches to a semi solid consistency. *Khoa* was removed from the kettle, allowed to cool to room temperature and was used within 24 h for preparation of *Gulabjamun*.

**Preparation of Gulabjamun**

*Gulabjamun* was prepared as per the method described by Ghosh *et al* (1986) [2]. It involves proper blending of *Khoa*, *Amaranthus/Maida* at different rate, baking powder and water (optional) to make homogenous and smooth dough. Small amount of water was added in case of dough is hard and do not roll into small balls. The mix was made fresh every time. The small balls formed from the dough were deep fried in ghee to golden brown colour and subsequently transferred to 60% sugar syrup maintained at about 60°C. It takes about 2 hours for the balls to completely absorb the sugar syrup.

**Physico-chemical Analysis**

*Gulabjamun* soaked in sugar syrup were tempered at 40 °C for 20 min. They were then kept on a sieve of about one square centimetre mesh to allow the sugar syrup to drain for 10 min. The *Gulabjamun* were then cut into small pieces and mixed thoroughly to form a paste, which was then tested separately for different chemical constituents. Total solids of gulabjamuns were determined as per the standard procedure using Mojonnier Milk Tester Model-D [3]. Fat content was determined by Gerber method as per the procedure described by Ladhani and Mulay (1974) [4]. The total nitrogen / protein content were determined by semi-micro Kjeldahl method AOAC (2002) [5]. Ash content was determined by procedure described in ISI: 1479 (1961) [6]. Total carbohydrate was derived by difference of sum of the major constituents like moisture, protein, fat and ash from 100. The acidity of *Gulabjamun* was determined by method described in IS: 1166 (1973) [7] for condensed milk. The pH of *Gulabjamun* was measured using digital pH meter. The method described by Franklin and Sharpe (1963) [8] for cheese was used and Peroxide value was determined by the method as described in AOAC (1970) [9]. Compression testing of *gulabjamun* were subjected to uniaxial compression to 50% of the initial sample height, using a Food Texture Analyser.

**Sensory evaluation**

Controls as well as experimental samples of *Gulabjamun*, were subjected to sensory evaluation by an expert trained panel of judges were selected. The *Gulabjamun* samples were evaluated using a 9 - point hedonic scale. The judges were also requested to give criticisms for each attributes of the samples.
Statistical analysis
Data obtained from various experiments during optimization, was statistically analyzed by using statistical software SPSS with completely randomized design (CRD).

Results and Discussion
Optimization of rate of Amaranthus for the acceptable Gulabjamun
During preliminary trials, Amaranthus: Maida in the ratio of 25:75, 50:50, 75:25, and 100:00 was tried for Gulabjamun preparation as a binders. The carbohydrate content of Gulabjamun prepared from less than 20 per cent binder was disintegrated during the frying. Based on the preliminary trials different proportion of Amaranthus: Maida, at the rate of 20 % of Khoa was selected for further study. The Gulabjamun was prepared with four different levels of Amaranthus: Maida i.e. 25:75 (T1), 50:50 (T2), 75:25 (T3), 100:00 (T4) and 00:100 (T5) Control. The all lots of Gulabjamun were prepared using the methods, delineated in Figure 1 and studied for their effect on compositional, physico-chemical, sensory and rheological characteristic.

Effect of rate of addition of Amaranthus on composition and physico-chemical properties of Gulabjamun.

Table 1: Physico-chemical properties of Gulabjamun made using varying level of Amaranthus

<table>
<thead>
<tr>
<th>Constituents*</th>
<th>25:75 (T1)</th>
<th>50:50 (T2)</th>
<th>75:25 (T3)</th>
<th>100:0 (T4)</th>
<th>Control (T5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>0.18±0.05</td>
<td>0.24±0.06</td>
<td>0.34±0.06</td>
<td>0.43±0.06</td>
<td>0.08±0.06</td>
</tr>
<tr>
<td>TS</td>
<td>67.25±0.38</td>
<td>68.54±0.39</td>
<td>70.23±0.36</td>
<td>71.29±0.35</td>
<td>66.15±0.28</td>
</tr>
<tr>
<td>Protein</td>
<td>0.66±0.03</td>
<td>0.83±0.03</td>
<td>0.91±0.05</td>
<td>0.74±0.04</td>
<td>0.60±0.02</td>
</tr>
<tr>
<td>Ash</td>
<td>0.21±0.02</td>
<td>0.20±0.01</td>
<td>0.21±0.02</td>
<td>0.33±0.01</td>
<td>0.01±0.04</td>
</tr>
<tr>
<td>Acidity</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>pH</td>
<td>0.64±0.04</td>
<td>0.63±0.05</td>
<td>0.60±0.01</td>
<td>0.65±0.02</td>
<td>0.67±0.05</td>
</tr>
<tr>
<td>Peroxide</td>
<td>0.00±0.00</td>
<td>0.03±0.00</td>
<td>0.02±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>Sugar Syrup Absorption</td>
<td>110.15±0.50</td>
<td>110.64±0.50</td>
<td>110.71±0.54</td>
<td>111.51±0.60</td>
<td>109.38±0.66</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. Total carbohydrate was obtained by difference, * all the values are in per cent except for pH, peroxide value expressed as milli-equiv. O2/kg, Acidity expressed as lactic acid.

This part of the study was carried out the influence of rate of addition of Amaranthus on compositional and physico-chemical attributes of experimental samples of Gulabjamun. Five batches of Gulabjamun viz. T1, T2, T3, T4 and T5 were prepared. Effect of Amaranthus on composition of Gulabjamun are presented in table 1. The total solid content of Gulabjamun added with five different proportion of Amaranthus: Maida i.e. T1, T2, T3, T4 and T5 were found 67.25, 68.54, 70.23, 71.29 and 66.15 % respectively. The total solid content was higher in T4 (i.e. 71.29) as compared to other treatments. Average total solid content of experimental Gulabjamun were ranged from 66.15 to 71.29 %. It can be seen from the table 1 that total solid content of experimental Gulabjamun increased significantly with increased in rate of addition of Amaranthus. The fat content of experimental Gulabjamun was found in range from 2.01 to 2.33 per cent respectively. Ash content of Gulabjamun was increased with increased in the rate of addition of Amaranthus. It can be seen from the table 1 that addition of Amaranthus at different rate had significant effect (P<0.01) on ash content of Gulabjamun. The values of ash for T1, T2 and T3 were statistically at par with each other. Acidity values varied in a very narrow range of 0.32 (T3, T4) to 0.33 (T1, T2 and T5) per cent. The tabulated values and relevant statistical analysis showed that there was statistical significant (P<0.05) difference found in the acidity of experimental Gulabjamun. The pH values varied in a narrow range of 6.60 (T3) to 6.70 (T5). T5 (i.e. control) sample had higher pH value i.e. 6.70 than all other sample. The tabulated values and relevant statistical analysis showed that there was not any statistical significant (P<0.05) difference found in the pH of experimental Gulabjamun. The values were ranging between 109.38 and 111.51 g/100 g. At higher level of Amaranthus addition, the sugar syrup absorption to some extent increased but the values were statistically not significant. The rate of addition of Amaranthus had no significant effect (P<0.05) on sugar syrup absorption. The data obtained for peroxide values were ranging between 0.31 and 0.32. The rate of addition of Amaranthus had no significant effect (P<0.05) on peroxide value.

Published data on proximate composition of gulabjamun affected by addition of Amaranthus are not available for comparison, however the values observed in the present study are very close to those reported by Sharma and Zariwala (1978) [10], Minhas et al (1985) [11], Prajapati et al (1991) [12], (1992) [13], Deshmukh et al (1993) [14], Adhikari et al 1994 [15], Thakar et al (1994) [16], Chaudhari (2016) [17].
Effect of different rate of Amaranthus on sensory attributes of Gulabjamun

In product development process, sensory quality plays a vital role in deciding the acceptability of the product. The sensory parameter chosen to assess the quality of gulabjamun was flavour, body and texture, colour and appearance and total score. The result are presented in table 2. The flavor scores of experimental gulabjamun are depicted in table 2. The mean values for flavour of experimental Gulabjamun ranged from 6.96 to 7.81. The highest score for flavour i.e. 7.81 was observed in sample T2. Addition of different rate of Amaranthus had significant effect (P<0.05) on flavour score of Gulabjamun. However, the flavour score of sample T2, T3, T5 and T1, T4, T5 were statistically at par with each other. The data obtained for body and texture score of Gulabjamun was presented in table 2. Body and texture scores of experimental Gulabjamun was ranging from T4 (6.69) to T2 (7.85). The highest mean value for body & texture associated with T2, followed by T5, T3, T1 and T4. The body and texture score for different sample were statistically significant (P<0.05). It can be seen from tabulated values that the sensory scores for colour and appearance varied from 7.27 (T4) to 8.14 (T2). A statistically significant difference (P<0.01) was observed for colour and appearance of experimental Gulabjamun. The average sensory scores for overall acceptability of Gulabjamun are depicted in table 2. The addition of different level of Amaranthus i.e. T1, T2, T3, T4, and T5 had overall acceptability scores of 7.28, 7.88, 7.32, 6.67 and 7.24 respectively. It can be observed that sample containing Amaranthus: Maida in the ratio of 50: 50 (T2), was preferred with highest score i.e. 7.88 compared to other treatments. It is evident from table 2 that overall acceptability of Gulabjamun was significantly affected (P<0.01) by level of Amaranthus used in present study. The overall acceptability of different Gulabjamun samples were ranked in order of T2>T3>T1>T5>T4. The result obtained for overall acceptability in present study was comparable with the findings of Dewani and Jayprakashas (2002) [18] who reported overall acceptability score for Gulabjamun ranged from 6.50 to 8.16. Thaware (2011) [19] mentioned the score for overall acceptability that ranged as 7.58 to 8.24 for prepared Gulabjamun blended with potato powder as substitute of Maida. Patil (2002) [20] conveyed overall acceptability score ranged from 7.96 to 8.20 for formulation of Gulabjamun prepared from goat milk. The Gulabjamun made using Amaranthus: Maida in the ratio of 50: 50 (T2), had significantly (P<0.01) superior scores for flavour, body & texture and colour & appearance (table 2). Hence, it obviously led to such Gulabjamun scoring significantly (P<0.01) higher overall acceptability score compared to other experimental Gulabjamun. This shows that addition of Amaranthus: Maida in the ratio of 50:50, in the formulation of Gulabjamun enhances its overall acceptability.

Effect of addition of different rate of Amaranthus on rheological properties of Gulabjamun

The textural characteristics of Gulabjamun were greatly influenced by its composition, type of raw material used, and manufacturing practices / parameters followed. The properties studied were hardness, cohesiveness, chewiness, gumminess, and springiness. The results obtained on rheological / textural profile of Gulabjamun are presented in table 3. It can be seen from table 3 that the average values of hardness of Gulabjamun made from different proportion of Amaranthus: Maida was ranging from 3.33 N (T4) to 16.51 N (T2) respectively. The rate of addition of Amaranthus had significant effect (P<0.01) on hardness of Gulabjamun. The hardness of Gulabjamun for T1 (11.20 N) was statistically at par with that of T5 (11.34 N) and was statistically different (P<0.01) than T2 (16.51 N), T3 (9.05 N) and T4 (3.33 N). Our finding is in accordance with Vasava et al (2018) [21] who manufactured a farali Gulabjamun having hardness of 3.90 N. Chaudhari (2016) [17] reported that the average of hardness of experimental Gulabjamun prepared using Moraiyo were ranged from 5.10 to 8.16 N and increased significantly with increased in rate of addition of Moraiyo. Yawale and Rao (2012) [22] investigated textural profile analysis of effect of Maida level in Khoa powder Gulabjamun mix and reported the hardness, which ranged from 2.65 to 4.90 N. The values presented in table 3 indicate that cohesiveness of Gulabjamun was ranging between 0.17 (T4) to 0.40 (T5). T5 (control) sample had significantly highest value of cohesiveness i.e. 0.40. The addition of Amaranthus had a significant effect (P<0.01) on cohesiveness of Gulabjamun.

Table 2: Influence of varying level of Amaranthus on the sensory score of Gulabjamun

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Gulabjamun made using different level of Amaranthus (w/w) (Amaranthus: Maida)</th>
<th>SEM</th>
<th>CD (0.05)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25:75 (T1)</td>
<td>50:50 (T2)</td>
<td>75:25 (T3)</td>
<td>100:0 (T4)</td>
</tr>
<tr>
<td>Flavour (10)</td>
<td>7.27±0.10</td>
<td>7.81±0.06</td>
<td>7.34±0.18</td>
<td>6.96±0.24</td>
</tr>
<tr>
<td>Body &amp; Texture (10)</td>
<td>7.11±0.14</td>
<td>7.85±0.06</td>
<td>7.29±0.27</td>
<td>6.69±0.39</td>
</tr>
<tr>
<td>Colour &amp; Appearance(10)</td>
<td>7.58±0.10</td>
<td>8.14±0.10</td>
<td>7.71±0.13</td>
<td>7.27±0.22</td>
</tr>
<tr>
<td>Overall Acceptability(10)</td>
<td>7.28±0.12</td>
<td>7.88±0.13</td>
<td>7.32±0.24</td>
<td>6.67±0.24</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, Figures in parentheses indicates maximum score.

Table 3: Effect of addition of different rate of Amaranthus on rheological properties of Gulabjamun

<table>
<thead>
<tr>
<th>Treatment (Amaranthus: Maida)</th>
<th>Hardness (N)</th>
<th>Cohesiveness</th>
<th>Gumminess (N)</th>
<th>Springiness (mm)</th>
<th>Chewiness (Nmm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (25:75)</td>
<td>11.20±0.12</td>
<td>0.32±0.00</td>
<td>0.68±0.02</td>
<td>0.89±0.08</td>
<td>32.21±0.45</td>
</tr>
<tr>
<td>T2 (50:50)</td>
<td>16.51±0.10</td>
<td>0.33±0.01</td>
<td>0.89±0.24</td>
<td>0.99±0.07</td>
<td>50.42±1.62</td>
</tr>
<tr>
<td>T3 (75:25)</td>
<td>16.51±0.10</td>
<td>0.26±0.00</td>
<td>0.89±0.24</td>
<td>0.99±0.07</td>
<td>50.42±1.62</td>
</tr>
<tr>
<td>T4 (100:00)</td>
<td>11.34±0.11</td>
<td>0.17±0.00</td>
<td>0.89±0.24</td>
<td>0.99±0.07</td>
<td>50.42±1.62</td>
</tr>
<tr>
<td>T5 (00:100)</td>
<td>11.34±0.46</td>
<td>0.40±0.01</td>
<td>0.89±0.24</td>
<td>0.99±0.07</td>
<td>50.42±1.62</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.
The data revealed that T1 (0.32) and T2 (0.33) were statistically at par with each other and were significantly ($P<0.01$) differing from T3 (0.26), T4 (0.17) and T5 (0.40). Vasava et al. (2018) [21] who manufactured a farali Gulab jamun having cohesiveness of 0.25. Chaudhari (2016) [17] reported that the average of cohesiveness of experimental Gulab jamun prepared using Moraiyo were ranged from 0.18 to 0.25 and she reported that the cohesiveness of Gulab jamun increased significantly with increase in rate of addition of Moraiyo. Singh et al. (2009) [23] examined the texture profile of Gulab jamun made with the soy flour and reported that cohesiveness increased with increase in the level of soy flour. Adhikari (1993) [24] described the textural characteristic of Khoa and Gulab jamun made from cow milk and reported that cohesiveness of laboratory and market sample Gulab jamun was 0.35 and 0.39 respectively. Yawale and Rao (2012) [22] studied textural profile analysis of effect of Maida level in Khoa powder Gulab jamun mix, and reported the cohesiveness ranged from 0.25 to 0.30.

The data obtained for gumminess content of Gulab jamun along with their statistical analysis are presented in table 3. The mean values of gumminess of Gulab jamun were ranging from 0.60 N (T4) to 5.80 N (T2) as the minimum and maximum values respectively. Addition of Amaranthus had a significant effect ($P<0.01$) on gumminess of Gulab jamun. All the treatments studied, were statistically differed with each other. Amongst the different treatments studied, gumminess value of Gulab jamun made from Amaranthus only (T4 i.e. Amaranthus: Maida @ 00:100), was statistically lower. Vasava et al. (2018) [21] who manufactured a farali Gulab jamun having gumminess of 1.41 N. Chaudhari (2016) [17] reported that the average gumminess of experimental Gulab jamun prepared from Moraiyo as a binder were ranged from 0.93 to 2.11 N. Gumminess of experimental Gulab jamun increased significantly with increase in rate of addition of Moraiyo. Singh et al. (2009) [23] studied the texture profile of Gulab jamun made with the soy flour, and reported that gumminess increased with increase in the level of soy flour.

The average values of springiness of Gulab jamun were ranging from 6.21 (T4) to 8.99 (T2, T5) mm as indicated in table 3. Data shows that use of Amaranthus had a significant effect ($P<0.01$) on springiness of Gulab jamun. Amongst different treatments studied, T5 (Control) and T2 (50:50) had significantly higher springiness ($P<0.01$) than other treatments, while T4 (Amaranthus: Maida @ 100:00) was having significantly higher value of gumminess. It was revealed from the table 3 that the gumminess of Gulab jamun prepared by adding Amaranthus: Maida @ 00:100 was statistically lower than any other sample. The value for gumminess of this study is higher than the values of Chaudhari (2016) [17] who reported that the average gumminess of experimental Gulab jamun prepared from Moraiyo as a binder were significantly ranged from 6.18 to 16.07 Nmm. Vasava et al. (2018) [21] who manufactured a farali Gulab jamun having cohesiveness of 7.73 mm. Adhikari (1993) [24] examined the textural characteristic of Khoa and Gulab jamun made from cow milk and reported that the cohesiveness of laboratory and market sample of Gulab jamun was 3.60 and 3.40 respectively. Yawale and Rao (2012) [22] studied textural profile analysis of effect of Maida level in Khoa powder Gulab jamun mix and reported that the increase in the level of Maida increased the cohesiveness of Gulab jamun.

Conclusion
Thus, it can be concluded on the basis of this study that a proportion of Amaranthus: Maida @ 50:50, added at the rate of 20% by wt of Khoa, was most suitable for manufacture of gulabjamun. Addition of Amaranthus along with Maida resulted in the improvement in sensory and rheological properties of resultant gulabjamun. And this developed Gulabjamun contained Amaranthus (Rajgara) which provides better nutrition and this pseudocereal is the gluten free and with an exceptionally high protein content as compared to the true cereals. It is a reasonably well-balanced food with functional properties that have been shown to provide medicinal benefits.

Reference
5. AOAC. Method for micro kjeldahl method. Introduction to the chemical analysis of foods, Association of official analytical chemists, USA, 2002, 210-211.