Evaluation of the Effect of Plant Extracts (*Paederia foetida* and *Houttuynia cordata*) Incorporation on the Quality of Masala Paneer

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**Abstract**

Cow’s milk masala paneer was prepared by adding plant extracts of *Paederia foetida* and *Houttuynia cordata* along with crushed cumin, their levels were selected after conducting a pilot sensory analysis. Shelf-life study of the refrigerated sample was done at definite intervals based on its change in organoleptic, physico-chemical properties and microbiological qualities. Results showed no change in the organoleptic properties, physico-chemical properties revealed a significant difference in pH value, acidity, thiobarbituric acid number and free fatty acid between the control and the treatments. However, no significant difference in tyrosine value was found. Bacterial counts were within the FSSAI limit.

**Keywords** Masala paneer, *Paederia foetida*, *Houttuynia cordata*, Quality, Shelf-life

**Introduction**

Paneer is a non-renneted, unripened, non-melting and non-fermentative type of cheese. According to Food Safety and Standards Authority of India (2017), “chhana or paneer means the product obtained from any variant of milk, with or without added milk solids, by precipitation with permitted acidulants and heating”. It may contain permitted ingredients like acidulants (viz., lactic acid, citric acid, malic acid, vinegar, glucono- delta-lactone, sour whey), spices and condiments including salt for flavouring purpose only. The product shall conform to the compositional specification of maximum 65% and 60% moisture for chhana and paneer, respectively, and minimum milk fat of 50% on dry matter basis for both whereas, for low fat chhana and paneer a minimum fat of 15% is specified.

It is a highly perishable one and seeks for extension of its shelf-life. Considering this fact various studies have made viz., addition of sorbic acid (@0.15%) to milk and packaging it in waxed paper coated with sorbic acid (Aneja *et al.*, 2002); adoption of hurdle technology (Gokhale *et al.*, 2016) etc.
Wanjari (2016) has advocated the use of additives, modification in paneer manufacturing technology, surface treatments and use of various packaging materials for shelf-life extension of paneer.

However, one of the promising ways to extend shelf-life of milk products is the use of phyto-preservatives (Ismail, 2006). Phyto-ingredients (herbs and spices) are found to be more effective with lesser side effects. Many researchers have studied the effect of fruit extracts and peels as a natural antioxidant in paneer (Singh and Immanuel, 2014), betel nut extract in khoa (Sivakumar et al., 2014), ethanolic extract of arjuna bark and Vidarikand (Parmar et al., 2013; Gandhi et al., 2013) as well as juice of amla fruit (Ahmad et al., 1960) on shelf-life of ghee. Many of these medicinal plants also have been reported to possess antioxidant properties.

Both *H. cordata* and *P. foetida* are indigenous plants of Assam. Their proven antimicrobial and antioxidant properties offer a scope for their use to extend the shelf-life of paneer. In this study, an attempt was made to study the effect of the extracts of these two plants on the quality parameters and in extending the shelf-life of cow’s milk masala paneer.

**Materials and Methods**

**Procurement of milk**

Fresh cow’s milk for the study was procured from the Instructional Livestock Farm (Cattle Unit), College of Veterinary Science, Assam Agricultural University, Khanapara-22, Assam.

**Herbs and spices**

Fresh *P. foetida* and *H. cordata* leaves were procured from local vegetable market on regular basis. Authentication of the plants was done by Dr. P.J Handique, Professor and Head, Department of Biotechnology, Guwahati University –781014. Cumin seeds were procured from local market, washed with water, dried and then half ground. These half ground cumin seeds were stored in an air tight container at room temperature and used as and when required.

**Preparation of plant extracts**

Plant leaves (*P. foetida* and *H. cordata*) were thoroughly cleaned and washed, extracts were prepared separately in a mixture and grinder (Bajaj, CLASSIC 750) followed by filtration of the extracts.

**Masala paneer processing**

Masala paneer was prepared in the laboratory using standard method (Aneja et al., 2002) with slight modification. The milk used for
preparation of the masala paneer was heated to 86\(^\circ\)C (for 10 min) and subsequently cooled to 76\(^\circ\)C (within 10 min), crushed cumin and plant extracts were incorporated at this stage and allotted to the following groups: Control- T\(_1\) (Masala paneer with 0.25\% crushed cumin), Treatment- T\(_2\) (Masala paneer with 0.25\% crushed cumin and 4\% (v/v) \(P.\ foetida\) plant extract) and T\(_3\) (Masala paneer with 0.25\% crushed cumin and 4\% (v/v) \(H.\ cordata\) plant extract). Cumin was used only to add flavor to the product.

The levels of cumin and plant extracts were selected only after a pilot sensory analysis of the samples. Acidification of milk was done by using 2\% citric acid (v/v). Whey was drained through a sterilized muslin cloth and the curd mass thus collected was hooped and pressed for 15 min by applying a pressure of 2-3 kg/cm\(^2\), followed by immersion in chilled brine solution [\(\text{NaCl}\ 5\%\ (w/v)\)] for ½ h. Paneer samples were then removed from chilled water, kept in a cutting board with a gradient for 15 min to facilitate draining of excess brine followed by packaging. Masala paneer samples were then cut into pieces and vacuum packed in high density polyethylene (HDPE) films of 200 gauge (12x10") packaged in Sevana\textsuperscript{TM} (Electrical Appliances Pvt. Ltd., Kerala, India) at a vacuum pressure of 720 mg Hg and stored at refrigeration temperature for further analysis on days 0, 5, 8 and 10.

**Organoleptic properties analysis**

Sensory properties of the products were determined by using 7-membered semitrained panel through a 9-point hedonic scale with a maximum scores of 7 to 9 designated as ‘very good’ and the lowest of 1 to 3 marked as ‘unpleasant’. Eating quality attributes studied were change in the colour, appearance and odour of the product.

**Physicochemical analysis**

Physicochemical analysis of masala paneer samples for Titratable acidity was done by titration Method No. 920.124 of AOAC (1990), pH was determined as per BIS (1989) method, TBA number by Witte et al., (1970), Tyrosine Value by Strange et al., (1977), FFA by Koniecko (1979).

**Microbiological analyses**

Microbiological analysis of masala paneer sample were enumerated for Total Viable Count (TVC) in Plate Count Agar, coliforms and *Escherichia coli* in Endo agar, *Salmonella* and *Shigella* in Salmonella-Shigella Agar, *Staphylococcus aureus* in Mannitol Salt Agar and yeasts and moulds in Rose Bengal Chloramphenicol Agar procured from HiMedia, India (Harrigan and McCance, 1976). These results are reported as an average of two replicates.

**Statistical analysis**

Experimental data obtained from the experiment were expressed as the average of mean values ± standard error. Analysis of variance (ANOVA) with Honest Significant Difference (HSD) test for mean comparison was used to highlight significant differences among the masala paneer samples. Statistical tests were performed with a 5\% or 1\% significance level using the SPSS program version 20 (IBM Corp, 2011).

**Results and Discussion**

**Effect of addition of plant extract on organoleptic properties of masala paneer**

Over the storage period of the product, there was no change in the colour, appearance and odour attributes of the product. Odour was dominated by the smell of cumin over the storage period.
Effect of addition of plant extract on physico-chemical properties of masala paneer

pH

The pH of masala paneer during storage period exhibited highly significant (P<0.01) differences between the treatments. From the Table 1, it is evident that along with the increase in the storage period, a corresponding decrease in the pH values of the control as well as the treatment groups could be seen (Fig. 1). The pH value of T1, T2 and T3 samples varied between 5.504 ± 0.144 to 5.370 ± 0.116, 5.654 ± 0.113 to 5.544 ± 0.094 and 5.842 ± 0.059 to 5.702±0.050, respectively on 0 to 10d. Maximum value for pH was recorded for T3 at the end of storage period (10d) at refrigeration temperature. This might be related to the formation of lactic acid by lactose fermenters during storage (Yadav and Wadehra, 2016). However, higher levels of pH in T3 and T2 group of masala paneer might be due to the antibacterial action of fresh plant extracts (H. cordata and P. foetida, respectively) on the microbial population (Fu et al., 2013) of masala paneer resulting in slower production of lactic acid and corresponding higher pH values in the products. Similar findings were also reported by Yadav and Wadehra (2016) who found decline in pH levels of paneer due to incorporation of herbs and condiments in the range of 5.91 to 5.11. Wanjari (2016) reported reduction in pH values from 5.75 to 5.63 in garlic extract treated paneer over a storage period of 15d at 4±1°C.

Table.1 Effect of plant extract on pH and acidity of masala paneer during refrigerated storage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>0d</th>
<th>5d</th>
<th>10d</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>T1</td>
<td>5.504±0.144^a</td>
<td>5.420±0.110^b</td>
<td>5.388±0.118^b</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>5.654±0.113^a</td>
<td>5.586±0.092^ab</td>
<td>5.564±0.095^ab</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>5.842±0.059^a</td>
<td>5.758±0.049^a</td>
<td>5.734±0.051^a</td>
</tr>
<tr>
<td>Acidity</td>
<td>T1</td>
<td>0.286± 0.017^a</td>
<td>ab0.322±0.017^a</td>
<td>0.352±0.017^a</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>0.268±0.018^ab</td>
<td>ab0.298±0.021^a</td>
<td>0.324±0.024^a</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.218± 0.015^b</td>
<td>bc0.268±0.021^a</td>
<td>0.294±0.015^a</td>
</tr>
</tbody>
</table>

Means with common superscripts column wise and subscripts row wise does not differ significantly. Data are expressed as mean± SE (n=5)

Table.2 Effect of plant extraction TBA number (mg malonaldehyde/kg), tyrosine (mg/100g) and FFA value (% oleic acid) of masala paneer during refrigerated storage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>0d</th>
<th>5d</th>
<th>10d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA</td>
<td>T1</td>
<td>0.136±0.004^a</td>
<td>0.237± 0.013^a</td>
<td>0.285± 0.022^a</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>0.120±0.005^b</td>
<td>0.211±0.013^ab</td>
<td>0.243±0.010^ab</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.120±0.003^b</td>
<td>0.191±0.007^b</td>
<td>0.230±0.009^b</td>
</tr>
<tr>
<td>Tyrosine value</td>
<td>T1</td>
<td>12.95±0.45</td>
<td>13.53±0.45</td>
<td>14.20±0.48</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>12.66±0.52</td>
<td>13.29±0.57</td>
<td>13.61±0.55</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>12.60±0.66</td>
<td>13.15±0.72</td>
<td>13.50±0.71</td>
</tr>
<tr>
<td>FFA value</td>
<td>T1</td>
<td>0.15±0.01^a</td>
<td>0.21±0.01^a</td>
<td>0.27±0.01^a</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>0.14±0.01^a</td>
<td>0.18±0.01^ab</td>
<td>0.21±0.01^b</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.13±0.01^a</td>
<td>ab0.16±0.02^b</td>
<td>0.18±0.01^c</td>
</tr>
</tbody>
</table>

Means with common superscripts column wise and subscripts row wise does not differ significantly. Data are expressed as mean± SE (n=5)
Table.3 Effect of plant extract on total viable count (log_{10}cfu/g) of masala paneer during refrigerated storage

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0d</th>
<th>5d</th>
<th>8d</th>
<th>10d</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>ND</td>
<td>3.76±0.072&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.86±0.014&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.93±0.022&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₂</td>
<td>ND</td>
<td>3.27±0.135&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.32±0.150&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.52±0.208&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₃</td>
<td>ND</td>
<td>3.16±0.198&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.21±0.208&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.29±0.231&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ND-Not detected, means with common superscripts column wise does not differ significantly. Data are expressed as mean± SE (n=5)

Fig.1 Effect of plant extract on pH and titratable acidity (% l.a) of masala paneer during refrigerated storage

Titratable acidity

Effect of *P. foetida* and *H. cordata* on per cent lactic acid content of masala paneer during storage exhibited significant differences (P<0.05) between treatments and a highly significant (P<0.01) variation was observed between days of storage. From the Table 1 it could be seen that there was a gradual increase in the per cent lactic acid content of all the treatment groups from 0 to 10d of storage at refrigeration temperature. A fall in the pH value of all the groups along with a concomitant increase in the per cent
lactic acid content at 0, 5, 8 and 10d could be noted (Fig. 1). Lowest per cent lactic acid content was noted in T₃ (0.336±0.029%) and the highest was recorded in T₁ (0.372 ± 0.015%). The findings of the present study are in accordance to the findings of Sachdeva and Singh (1990), Eresam et al., (2015). The increase in acidity might be due to production and subsequent utilization of some of the acidic and/or basic compounds formed as a result of microbial activity (Sachdeva and Singh, 1990). The pH of the paneer might also be affected by the coagulant (citric acid) used in acidification of paneer (Gokhale et al., 2016; Shanaziya et al., 2018). However, lower lactic acid content of T₂ and T₃ groups of masala paneer as compared to that of the control group (T₁) were might be due to the antimicrobial activity of the added P. foetida and H. cordata fresh plant extracts which might have slowed down the lactic acid production by inhibiting microbial growth (Fu et al., 2013; Soni et al., 2013).

Thiobarbituric Acid Number (TBA)

Gradual increases in TBA number were noticed in all the groups from 0 to 10d of storage. Results showed significant differences (P<0.05) between the groups and highly significant variations (P<0.01) between days of storage in Table 2. TBA numbers in T₂ and T₃ treatment groups were found to be much lower i.e., 0.243±0.01 and 0.230±0.01 mg MDA/kg, respectively than the control group with 0.285±0.02 mg MDA/kg on 10d of storage at refrigeration temperature. This might be due to the potential antioxidant activity of the phenolic substances present in the plant extracts of P. foetida and H. cordata (Osman et al., 2009; Chanda et al., 2013) and H. cordata (Nuengchamnong et al., 2009, Fu et al., 2013) added to masala paneer which in turn prevented lipid oxidation. Results of the present study are supported by the findings of Wanjari (2016).

Another factor for lesser TBA numbers in T₂ and T₃ groups may be attributed to high pH values recorded in these two treatment groups. Fox and McSweeney (1998) reported that low pH enhances oxidation of unsaturated fatty acids resulting in formation of hydroperoxides. These hydroperoxides further degrade to yield carbonyls and other compounds which in turn are related to increase in TBA number (O’Connor and O’Brien, 1994). Hence, in the present study lesser TBA number obtained might also be attributed to high pH in the products indicating antibacterial activity of these two plant extracts (Kim et al., 2008; Chanda et al., 2013; Fu et al., 2013).

Results of the study also correlates with that of the results of free fatty acid values presented in Table 2 which also have shown a similar trend. Free fatty acids themselves get oxidized more readily in their free forms than their combined form with glycerides of normal fat and also catalyze oxidation of fat. This in turn might be related to increase in TBA number (Deeth and Fitz-Gerald, 1976).

Tyrosine value

No significant difference in tyrosine value between treatments and days of storage were observed. From the Table 2, it is evident that along with the increase in the storage period, there was a minimal increase in the mean tyrosine values in all the treatment groups. Minimum mean tyrosine value ranging between 12.60±0.66 to 13.50±0.71mg/100g was recorded for T₃ group followed by 12.66±0.52 to 13.61±0.55 mg/100g in T₂ group on 0 to 10d of storage. While the maximum mean tyrosine value of 12.95±0.45 to 14.20±0.48 mg/100g was recorded in control (T₁) group. Lower levels of proteolysis indicated in the treatment groups (T₂ and T₃) of masala paneer incorporated with plant extracts (P. foetida and H. cordata, respectively) might be
attributed to the antimicrobial activity of the plant extracts on proteolytic microorganisms (Fu et al., 2013; Soni et al., 2013). These findings are in accordance to the findings of Kashyap, (2009); Yadav and Wadehra (2016); Wanjari, (2016).

Free Fatty Acid (FFA)

The effect of plant extract on the FFA content of masala paneer exhibited highly significant difference (P<0.01) between the treatments and days of storage with a gradual increase in the free fatty acid value (% oleic acid) of different treatment groups of masala paneer. From the result presented in Table 2, minimum (0.18±0.01%) FFA contents in masala paneer could be observed in T3 group, followed by T2 group indicating the antibacterial activity of plant extract on lipolytic organisms (Fu et al., 2013; Soni et al., 2013). It might also be due to potent antioxidant activity of the plant extracts (Chanda et al., 2013; Fu et al., 2013). Similar increasing trend in the content of FFA in paneer during storage are reported by Kashyap (2009); Yadav and Wadehra (2016) and Kamble and Seth, (2017).

Effect of plant extract on microbiological quality of masala paneer

Total viable count

Total viable count showed highly significant difference (P<0.01) between the treatments. From the Table 3, it is evident that on 0d, the samples of all the treatment groups had nil TVC. However, it increased gradually from 5 till 10d of refrigeration storage. T3 group (with H. cordata extract) exhibited minimum count on both 5 and 10d of storage. Treatment T2 with P. foetida extract showed statistically non-significant apparently higher values than T3 during the entire storage period. Maximum TVC was noticed in (T1) control group. These were attributed to the antibacterial activities of H. cordata (Kim et al., 2008; Fu et al., 2013) and P. foetida plant extracts on the masala paneer samples (Chanda et al., 2013; Soni et al., 2013), respectively. However, in the present study, all the counts were below the FSSAI (2015) limit (<35x10^4/g).

Coliform count and other organisms

None of the paneer samples of treated group showed presence of coliforms over the entire storage period of 10d. However, control samples showed coliform counts in between 8d to 10d but the counts were <25/plate. These findings are supported by the findings of Rani et al., (2014b); Eresa et al., (2015).

Results of the present study also showed nil growth of yeasts and moulds, E. coli, Staph. aureus, Salmonella and Shigella in all the paneer samples. This might be due to marked antibacterial activity of P. foetida (Uddin et al., 2007; Soni et al., 2013) and H. cordata (Lu et al., 2006; Ahmed et al., 2012) in T2 and T3 group of masala paneer samples, respectively and might be due to hygienic conditions of production of masala paneer.

In conclusion the incorporation of fresh plant extracts in masala paneer was effectively reached. Shelf-life of the product as anticipated from its change in organoleptic properties and physico-chemical properties like titratable acidity, pH, TBA and FFA showed superior results. Plant extract also have a significant effect on the total viable count (P<0.01) between the treatment groups. In comparison, the extract of H. cordata was found to be better than P. foetida in production of phyto-preserved masala paneer from cow’s milk. On the whole, the results of the present study showed that masala paneer (T2 and T3) with plant extracts may be recommended for its health beneficial effects like antioxidants and antimicrobial agents.
Acknowledgments

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