Int.J.Curr.Microbiol.App.Sci (2020) *9*(12): 240-249

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 12 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.912.032

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

Rashmi Rekha Saikia[1*], Trishna Borpuzari[1], Mineswar Hazarika[1], Rita Nath[2] and Arup Kumar Sharma[1]

[1]Department of Livestock Products Technology, [2]Department of Veterinary Biochemistry, College of Veterinary Science, AAU, Khanapara, Ghy, Assam, India

**Corresponding author*

Keywords

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

Article Info

Accepted:
04 November 2020
Available Online:
10 December 2020

A B S T R A C T

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 it's 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.

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.

Paederia foetida and *Houttuynia cordata* are two locally grown plants which are used in traditional medicine of Assam since long. *P. foetida* (Bhedailata in Assamese) is a well-known medicinal plant used extensively in traditional medicine for its therapeutic value (Chanda *et al.,* 2013) and antioxidant property (Chanda *et al.,* 2013; Kumar *et al.,* 2014). *P. foetida* plant can be used as an antibacterial agent (Uddin *et al.,* 2007). It also has DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (Soni *et al.,* 2013).

H. cordata is another medicinal plant and is used in the indigenous medicinal system of Southeast Asia. It is commonly known as Musundori (Assam), Dokudame (Japan), E-Sung-Cho (Korea) (Gogoi and Kalita, 2014). Its extract has antimicrobial (Chikane *et al.,* 2003; Kim *et al.,* 2008; Sekita *et al.,* 2016; Li *et al.,* 2017) as well as antioxidant properties (Choi *et al.,* 2002; Chen *et al.,* 2003; Nuengchamnong *et al.,* 2009).

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 86^0C (for 10 min) and subsequently cooled to 76^0C (within 10min), 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 15min by applying a pressure of 2-3kg/cm^2, followed by immersion in chilled brine solution [NaCl 5% (w/v)] for ½ h. Paneer samples were then removed from chilled water, kept in a cutting board with a gradient for 15min 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 200gauge (12x10″) packaged in Sevana^{TM} (Electrical Appliances Pvt. Ltd., Kerala, India) at a vacuum pressure of 720mg 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 semi-trained 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 T_1, T_2 and T_3 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 T_3 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 T_3 and T_2 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^{0}C.

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

Parameters	Treatments	0d	5d	8d	10d
P^H	T_1	5.504 ± 0.144^{a}	5.420 ± 0.110^{b}	5.388 ± 0.118^{b}	5.370 ± 0.116^{b}
	T_2	5.654 ± 0.113^{a}	5.586 ± 0.092^{ab}	5.564 ± 0.095^{ab}	5.544 ± 0.094^{ab}
	T_3	5.842 ± 0.059^{a}	5.758 ± 0.049^{a}	5.734 ± 0.051^{a}	5.702 ± 0.050^{a}
Acidity	T_1	$_{b}0.286\pm 0.017^{a}$	$_{ab}0.322\pm0.017^{a}$	$_{a}0.352\pm 0.017^{a}$	$_{a}0.372\pm0.015^{a}$
	T_2	$_{b}0.268\pm0.018^{ab}$	$_{ab}0.298\pm0.021^{a}$	$_{ab}0.324\pm0.024^{a}$	$_{a}0.352\pm0.025^{a}$
	T_3	$_{c}0.218\pm 0.015^{b}$	$_{bc}0.268\pm0.021^{a}$	$_{ab}0.294\pm0.015^{a}$	$_{a}0.336\pm0.029^{a}$

Means with common superscripts column wise and subscripts row wise does not differ significantly. Data are expressd 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

Parameters	Treatments	0d	5d	10d
TBA	T_1	$_{c}0.136\pm0.004^{a}$	$_{b}0.237\pm 0.013^{a}$	$_{a}0.285\pm 0.022^{a}$
	T_2	$_{c}0.120\pm0.005^{b}$	$_{b}0.211\pm0.013^{ab}$	$_{a}0.243\pm0.010^{ab}$
	T_3	$_{c}0.120\pm0.003^{b}$	$_{b}0.191\pm0.007^{b}$	$_{a}0.230\pm0.009^{b}$
Tyrosine value	T_1	12.95±0.45	13.53± 0.45	14.20± 0.48
	T_2	12.66± 0.52	13.29± 0.57	13.61± 0.55
	T_3	12.60± 0.66	13.15± 0.72	13.50± 0.71
FFA value	T_1	$_{c}0.15\pm0.01^{a}$	$_{b}0.21\pm0.01^{a}$	$_{a}0.27\pm0.01^{a}$
	T_2	$_{b}0.14\pm0.01^{a}$	$_{a}0.18\pm0.01^{ab}$	$_{a}0.21\pm0.01^{b}$
	T_3	$_{b}0.13\pm0.01^{a}$	$_{ab}0.16\pm0.02^{b}$	$_{a}0.18\pm0.01^{c}$

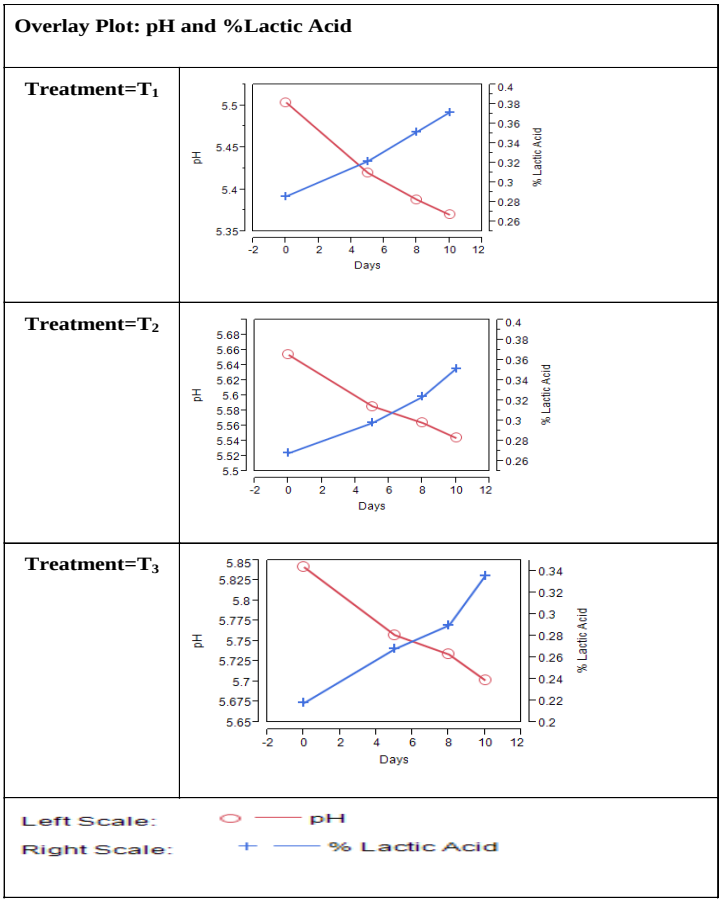
Means with common superscripts column wise and subscripts row wise does not differ significantly. Data are expressd as mean± SE (n=5)

Table.3 Effect of plant extract on total viable count (log_{10}cfu/g) of masala paneer during refrigerated storage

Treatments	0d	5d	8d	10d
T_1	ND	3.76 ± 0.072^{ac}	3.86 ± 0.014^{ac}	3.93 ± 0.022^{ad}
T_2	ND	3.27 ± 0.135^{ab}	3.32 ± 0.150^{b}	3.52 ± 0.208^{ab}
T_3	ND	3.16 ± 0.198^{b}	3.21 ± 0.208^{b}	3.29 ± 0.231^{bc}

ND-Not detected, means with common superscripts column wise does not differ significantly. Data are expressd 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 T_3 (0.336±0.029%) and the highest was recorded in T_1 (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 T_2 and T_3 groups of masala paneer as compared to that of the control group (T_1) 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 T_2 and T_3 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* (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 T_2 and T_3 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 T_3 group followed by12.66± 0.52 to 13.61±0.55 mg/100g in T_2 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 (T_1) group. Lower levels of proteolysis indicated in the treatment groups (T_2 and T_3) 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 T_3 group, followed by T_2 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. T_3 group (with *H. cordata* extract) exhibited minimum count on both 5 and 10d of storage. Treatment T_2 with *P. foetida* extract showed statistically non-significant apparently higher values than T_3 during the entire storage period. Maximum TVC was noticed in (T_1) 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); Eresam *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 T_2 and T_3 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 (T_2 and T_3) with plant extracts may be recommended for its health beneficial effects like antioxidants and antimicrobial agents.

Acknowledgments

The authors express sincere gratitude and thankfulness to the Dean, Faculty of Veterinary Science, AAU, Khanapara, Guwahati – 22, Dr B.N. Saikia for providing the necessary facilities and financial aid to carry out the research programme successfully, Dr R.N. Borpuzari, professor and Dr. M. Raquib, Asst. Professor, Dept. of Livestock Products Technology and Dr. J. Hussain, Professor, Dept. of Livestock Production and Management, College of Veterinary science, AAU, Khanapara, Ghy-22 for extending help in writing of this manuscript.

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How to cite this article:

Rashmi Rekha Saikia, Trishna Borpuzari, Mineswar Hazarika, Rita Nath and Arup Kumar Sharma. 2020. Evaluation of the Effect of Plant Extracts (*Paederia foetida and Houttuynia cordata*) Incorporation on the Quality of Masala Paneer. *Int.J.Curr.Microbiol.App.Sci.* 9(12): 240-249. doi: https://doi.org/10.20546/ijcmas.2020.912.032