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SPICE BASED TREATMENT TO INCREASE THE SHELF LIFE OF PANNER – CLOVE A PROMISING SPICE

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ABSTRACT

Panner is the important nutritious and whole some indigenous diary product which occupy a prominent place among traditional milk product and carry a lot of market potential. However, like any other milk products, panner too is perishable in nature having a very short shelf life. Little information is available on panner regarding the microbiology aspects during storage, which is the main cause of its spoilage. This study was carried out to identify the potential of spices as natural preservative agents to increase shelf life of panner. Of the 12 spices tested we found that clove (and clove oil) effective in enhancing the shelf life of panner. Further, the clove oil was found to be a better antagonistic agent compared to clove aqueous infusion. The *in vitro* antibacterial activity of clove oil was tested against the microorganism contaminating and spoiling panner by Agar-well diffusion assay. There was a direct correlation between the amounts of clove in the Agar well to that of zone of inhibition. The effective and lowest minimum inhibitory concentration of clove oil was found to be 0.01%. The clove oil treated panner could extend the shelf life to 40 days. However, except for hardening (observed after 20th day) the panner sample were free of contamination. Supported by the sensory valuation studies, we opine that clove oil could be an effective bio-preservative spice in increasing the shelf life of panner.

Keywords: Spices, clove oil, panner, shelf life

INTRODUCTION

Milk is considered as nature's most perfect food. Indigenous dairy products account for 15 – 20% of the annual production of milk in this country. Panner an analogue to cottage cheese is an indigenous coagulated milk product prepared by the addition of organic acid to milk at higher temperature **(Athar *et al.* 1989)**. Most panner is made from buffalo milk that contains 5% more fat than cow's milk. Despite its high moisture content, the texture and body of panner is firm and smooth. Panner enjoys the status of hante cuisine in Indian vegetarian cooking. It is widely used as a base material for the preparation of variety of culinary dishes. Panner contains approximately 53%-55% moisture, 23-25% fat, 17-18 % proteins, 2-2.5% lactose and 1.5-2 % minerals **(Kanawjia & Singh 1996)**. The major drawback is its short shelf life due to its high moisture content and low acidity **(Arora & Gupta 1980; Bhattacharya *et al.* 1971)**. To meet the increasing demand for the panner in the urban and the rural area, it is of utmost importance that one should ensure a long shelf life, particularly at ambient temperature.

Panner retains its freshness during storage for 3 days. Once the freshness of panner is lost after the 3rd day, undesirable changes commence. However, when the period of storage is extended to 6th day, the quality deteriorates to such extent as to making the product unmarketable **(Bhattacharya *et al.* 1971)**. It was observed that panner has a shelf life **(Bhattacharya *et al.* 1971)**, under refrigerate condition 18 days. Some studies have reported that adoption of more stringent measures during processing, packing and storage would help in producing a whole some product with longer shelf life. The preservative methods such as dehydration, freezing **(Arora & Gupta 1980)** or use of sorbic acid **(Singh *et al.* 1991)** are such attempts to increase shelf life. The ability of microbes to survive and grow at refrigeration temperature on milk product like panner is a public health concern. Once the packaging is disturbed, it is practically difficult to store panner (and avoid contamination) even at lower temperatures in refrigerators. The present investigation is directed to increase the shelf life of the panner at this junction.

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family Myrtaceae. It is native to Indonesia and used as a spice in cuisine all over the world. The name derives from French clou, a nail, as the buds vaguely resemble small irregular nails in shape. Clove is harvested primarily in Zanzibar, Indonesia and Madagascar; it is also grown in India and Sri Lanka. The compound responsible for the cloves aroma is eugenol. It is the main component in the essential oil extracted from cloves, comprising 72-90%.eugenol has pronounced antiseptic and anesthetic properties. Eugenol has been used in perfumeries, flavorings, essential oils and in medicine as a local antiseptic and anesthetic. Combining zinc oxide and eugenol forms zinc oxide eugenol which can be used as a filling or cement material used in dentistry. It is classified as an intermediate restorative material and has anesthetic and antibacterial properties. It is sometimes used in the management of dental caries as temporary fillings.

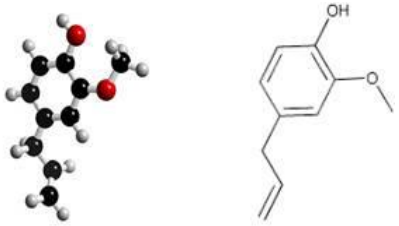

Figure 1 a) 3D structure of Eugenol, b) Chemical structure of Eugenol

A renewed interest in "natural preservation" is stimulated by present food safety concerns, growing problems with microbial resistance and rise in the production of minimal processed food joined with "green" image policies of food industries. In recent times, there has been renewed interest on spices as sources of antimicrobial agent for a new, effective and affordable spice with antimicrobial activity. However, there is no information on systematic work with special reference to extend shelf life of panner using spice. A feasibility study was therefore under taken to test the efficacy of a range of spices (viz., clove, cinnamon, cumin, vanilla, pepper, turmeric, star anise, bay leaf, nutmeg, cardamom & clove oil) in extending the shelf life of panner. Our results suggest that the 5% clove oil treatment can extend the shelf life of panner appreciably.

MATERIAL AND METHODS

Panner as cubes was purchased from the market cold shelf and was left in refrigerator. Luria Broth, Nutrient Agar was procured from HIMEDIA. Clove oil

(Technical grade from NIRMAL CHEMICALS, Bangalore) was purchased from a local market at Davangere, Karnataka, India.

Isolation of contaminating culture from spoilt panner

Panner from the market shelf was purchased and the packaging was unsealed. Panner was then transferred to a petri plate and incubated at refrigerator temperature (4^{o}C) for 48 hours. After 48 hours, a loop of panner contaminating culture from the surface of panner was smeared and streaked onto nutrient agar plate and incubated overnight at 37 ^{0}C. The following day the isolates were transferred to slants and maintained at 4 ^{0}C till use and stored.

Preparation of spice aqueous infusion

The standard method for preparation of spice aqueous infusion was followed **(Gupta *et al.* 2009)**. Individual spices were dried and grounded in a mixer grinder to obtain the fine dry powder. The obtained spice was weighed using a single pan electronic weighing balancer (Sartorius) The spice aqueous infusion was obtained by maceration process. Individual spice powder was soaked independently in distilled water (10mg of powder in 9.09 ml distilled water) in a 100 ml Erlenmeyer flask for 48 hours at room temperature with intermittent shaking. The flasks were closed with cotton plug and aluminum foil. The soaked spice were then crushed to fine paste in mortar and piston. The whole sample was transferred to centrifuge tubes and centrifuged at 5000 rpm for 10 min at room temperature. The clear supernatant was collected and used as aqueous infusions for further studies.

Study of growth of PCO-A (as OD at 600nm) on different spice aqueous infusion

The effect of different spices on the growth of PCO-A was studied. To the flasks containing Nutrient broth (50ml) 1000ppm of the spices (Cardamom, cinnamon, clove, cumin, nutmeg, pepper, salt, star anise, bay leaf, turmeric and vanilla) were spiked in individual flasks along
with 1.5% inoculum. NaCl (salt) was used as negative control for growth. Later for every 2hrs the turbidity was read at 600nm

a. Effect of different spice on the contaminating organism – By Agar well diffusion method: Study of zone of inhibition: PCO-A were swabbed on the agar plates to study the zone of inhibition by cinnamon, clove by the standard procedure given by **Prabuseenivasan *et al.* (2006)**. The spices of different concentrations were dispensed (viz., 1000ppm, 1% and 10%) into the individual bore. The plates were incubated for 24 hrs at 35 ^{0}C to check the zone of inhibition.

b. Effect of different concentration of clove on contaminating organism – By Agar well diffusion method: study of zone of inhibition: 5% cove oil was dispensed into well of agar plates at different concentrations viz., 1µl, 2µl, 3µl, 4µl, 5µl, 10µl, 20µl, 40µl. The plates were incubated for 24 hrs at 30 ^{0}C. The zone of inhibition was measured.

Effect of clove oil on PCO-A – Media spiked with different concentrations of clove oil

The growth of PCO-A was studied in liquid media (LB). To each flask of Luria broth 1% (from overnight grown LB) of PCO-A was transferred and media was fortified with clove oil viz., 100ppm, 500ppm, 1000ppm, 1%, 2% and 5%. At 24 hrs regular interval the growth was read turbid metrically (OD 600nm).

Effect of clove oil (infusion) on panner

Panner were cut into 1/1/1 cm cubes, weighing around 25 gm each and were packed into 400 gauge high density polyethylene pouches for use. The cubes were further transferred to a 250 ml Erlenmeyer flask containing 100ml of distilled water. 5% of clove oil aqueous infusion was spiked, since the clove oil is hydro soluble. The flask were closed with cotton plug and aluminum foil. To ensure proper mixing, the flask were incubated on a rotary shaker set to 150 rpm at room temperature. After 24 hours the panner were then shifted to refrigerator and stored for 1-5 days. Every 24 hours the surface of the panner was swabbed and inoculated on a fresh agar plate to check for contamination.

Sensory evaluation

Paneer were examined for sensory properties during the entire experimental duration. On each day cubes were randomly sampled for the studies. Clove oil treated panner and untreated panner (as control) were examined for changes in odor, color, sliminess and overall appearance by five trained and five semi trained panelists. The panelists were asked to record their preference on a hedonic scale ranging from 1 to 5, where 1 represented extreme disliking as described by **Kumar and Srinivasan (1982)** for Khoa.

RESULTS

Isolation of microorganism from contaminated and spoiled panner

Two colonies very distinct from each other were observed (Plate I), Panner contaminating organism A (PCO-A) and panner contaminating organism B (PCO-B). One colorless colony (PCO-A) appeared within 24 hrs and the other distinct color colony (PCO-B) appeared on 2nd day. For study convince we concentrated on one of the colony, PCO-A which was colorless.

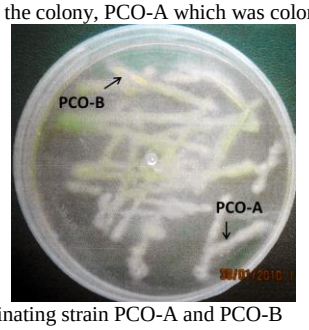

Plate I Panner contaminating strain PCO-A and PCO-B

Growth kinetics of PCO-A on spice infusion

The growth PCO-A was observed on0 day to 4th day and the results are recorded on Table I from the result it is evident that of the 10 tested spices, 8 of them encourage growth (The growth as OD 600λ at 16 hrs were appreciable). Cinnamon, clove supported least growth as compared to all the spices tested and clove seems to relatively more antibacterial. On the other hand, Cardamom, pepper, nutmeg, star anise, bay leaf, turmeric and vanilla failed to inhibit growth of the contaminating organism PCO-A. Star anise encouraged the maximum growth which means it had the least antibacterial activity. Cinnamon, clove inhibited the growth which means it has antibacterial activity. Thus, our further study thus focused only on these two spices Cinnamon and clove.

Table I OD (600nm) growth of PCO-Ain different spices (Growth at OD 600λ).

Sl. No.	Spice sample	0hr	16hrs	22hrs	38hrs
	Salt (Negative control)	0.00	**0.06**	0.12	0.21
1	**Clove**	**0.00**	**0.15**	**0.24**	**0.27**
2	**Cinnamon**	**0.00**	**0.24**	**0.27**	**0.27**
3	Nutmeg	0.00	**0.33**	0.33	0.18
4	Pepper	0.00	**0.33**	0.30	0.36
5	Turmeric	0.00	**0.33**	0.21	0.33
6	Cardamom	0.00	**0.36**	0.33	0.24
7	Vanilla	0.00	**0.42**	0.36	0.39
8	Bay leaf	0.00	**0.42**	0.36	0.24
9	Cumin	0.00	**0.54**	0.15	0.21
10	Star anise	0.00	**0.54**	0.36	0.42

Effect of different spice on PCO-A – By Agar well diffusion method: Study of zone of inhibition

The spices diffused radially into the agar medium layer and exert its toxic effect on the isolate (panner contaminating organism). Subsequently a zone of inhibition developed around the bore which showed that spices which we used were having antimicrobial properties. The results are given as photographic plates. After 24hrs of incubation of plates, cinnamon did not show an inhibitory effect and thus had no zone of inhibition, the clove showed the clear zone of inhibition (Plate II). Thus clove was used for further studies.

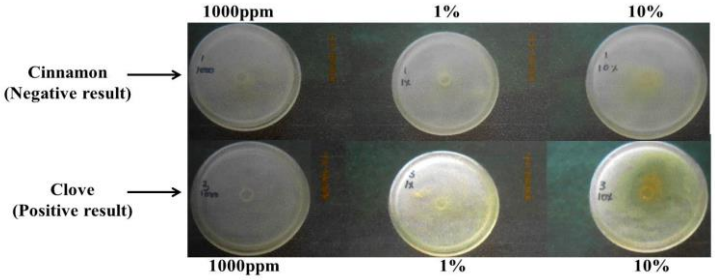

Plate II Clear zone of inhibition of clove

Effect of different concentration of clove oil on contaminating organism – By Agar well diffusion method: Study of zone of inhibition

Since clove proved as a suitable spice, a range of concentrations (1µl-40µl) were studied and the results are documented as Table II and Plate III. It is evident from

Table 2 that diameter of zone of inhibition varied from 1cm to 2.5cm. However there was no difference in diameter of zone of inhibition at 2µl to 5µl concentration.

Table II Zone of inhibition of different concentration of clove oil

Sl.No.	Concentration (µl)	Diameter of zone of inhibition (cm)
1	1	1
2	2	1.5
3	3	1.5
4	4	1.4
5	5	1.5
6	10	1.8
7	20	2
8	40	2.5

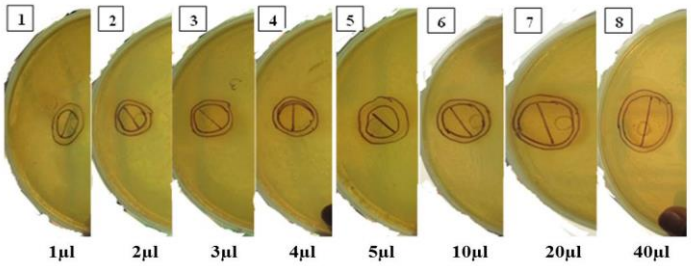

Plate III Zone of inhibition of different concentration of clove oil on PCO-A

Effect of clove oil on growth on PCO-A

Antiseptic properties of plant volatile oils have been recognized since antiquity **(Doaman & Deans 2000).** Cloves are used in Ayurveda, Chinese medicine and western herbalism. Clove oil found to be more effective as compared to clove extract **(Gupta 2009)**. Indeed clove oil is commonly used as anesthetic in relieve of toothache in dentistry. General studies have demonstrated potent antifungal **(Park *et al.* 2007)**, antiviral **(Chaieb *et al.* 2007a)** and anti-bacterial effects of clove **(Lopez *et al.* 2007)**. The antimicrobial sensitivity of clove oil against gram negative and gram positive and fungus shows a broad spectrum of activity **(Ayoola *et al.* 2008)**.

The Figure II shows growth of PCO-A in liquid media with different concentrations of clove oil (100ppm to 5%). From the graph it is obvious that spiked clove oil seems to be toxic to the organism. There was a direct relation between the growth inhibition and amount of clove oil. As the clove oil increased the growth was retarded. 100, 500 & 1000 ppm had least effect. Beyond 1000 ppm clove oil inhibited growth drastically. Hardly any growth was observed at 5%.

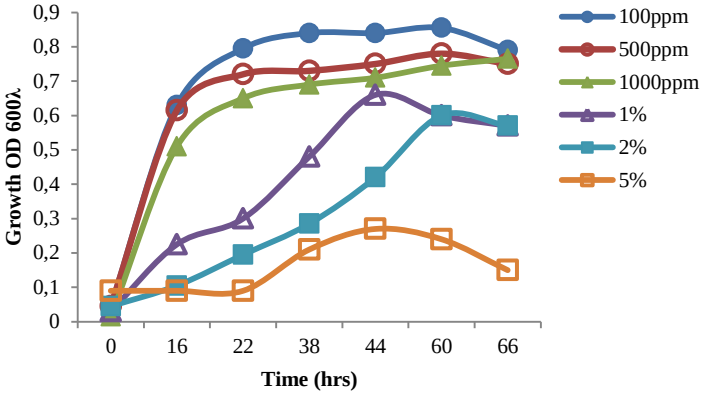

Figure II Growth of PCO-A in liquid media with different concentration of clove oil

Study of shelf life of panner treated with clove oil (5%)

From the Table 3 it is clear that clove oil treatment has a remarkable role in increasing the shelf life. The panner not treated with clove oil got contaminated on the 1st day. Panner treated with clove oil (duration of a panner 1 to 5 day) was never contaminated till 30th day but got harden after 20th day.

Table III Shelf life (in days) of clove oil (5%) treated panner

Duration of clove oil on Panner (days)	Sampling days to study contamination						
	0	**1**	**2**	**3**	**4**	**20**	**30**
0	No treatment	C	C	C	C	C	C
1	NC	NC	NC	NC	NC	NCH	NCH
2	NC	NC	NC	NC	NC	NCH	NCH
3	NC	NC	NC	NC	NC	NCH	NCH
4	NC	NC	NC	NC	NC	NCH	NCH
5	NC	NC	NC	NC	NC	NCH	NCH

Note: **C** – **C**ontamination, **NC** – **N**o **C**ontamination, **NCH** - **N**o **C**ontamination but panner has become **H**ard

Sensory valuation

Further, the changes in the organoleptic traits of panner treated with clove oil were studied. The results are recorded as Table 4. It is very clear from the tabulated result that the clove oil treated panner that the overall appearance even on the 30th day was acceptable.

Table 4 Changes in the organoleptic traits of panner with clove oil

	Control			Treated		
	Storage time (days)			Storage time (days)		
	0	**3**	**5**	**0**	**20**	**30**
Odor	4	3.5	3	4	4	4
Color	4	4.2	3	4	4	4
Sliminess	4	4.1	3.2	4	4	4
Overall appearance	4	3.4	3	4	4	4

Note: Scores on a 5 point hedonic scale (5-extreame liking, 1-extreame disliking)

DISCUSSION AND CONCLUSION

Panner is obtained by direct acidification at denoted temperature hence, its micro flora arises mainly from post processing contamination namely from air, utensils, workers, handling conditions, packaging materials and raw milk. The various microbial groups present in panner including *staphylococcus* species and from coliform. **Sachdeva & Singh (1990)** reported that fresh panner had a total plate count of 10^1 to 10^3 cfu gm^{-1} whereas for spoiled samples counts range from 158×10^4 cfu gm^{-1} to 45×10^6 cfu gm^{-1} panner. Psychotropic bacteria which are initially low in number increased by 2 log cycles on day 7 of storage.

Today the issue of food preservation has become more complex, with increasing concern over the presence of chemical residue in foods. Also the demand for non-toxic natural preservatives is increasing every day because of harmful effects of food preservatives **(Shelef 1984)**. From the result we conclude that clove oil is an excellent agent in inhibiting the growth of the panner contaminating organism, thus increasing the shelf life of panner. 5% of clove oil solution treated for 10min could increase the shelf life to greater extent. Except for the fact the panner had hardened. The modes of action by which microorganisms are inhibited by essential oils and their chemical compounds seem to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipids bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents **(Juven *et al.* 1994)** and impairment of bacterial enzymes systems **(Farag *et al.* 1989)**.

The inhibitory activity of clove is due to the presence of several constituents, such as eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone. Phenolic compounds are known to have anticidant and antimicrobial properties **(Pelezar *et al.* 1998)**. Clove oil is obtained from the flower buds of *Syzigium aromaticum syn, Eugenia aromaticum or Eugenia coryophyllata*, family Myrtaceae **(Chaieb *et al.* 2007a)**. Eugenol is a phenol. GC-MS analysis of the clove oil extract showed eugenol, eugenol acetate, caryo-phyllene as the major constituent. Clove oil has 79.2% eugenol **(Ranasinghe *et al.* 2002)**. Members of this class are

known to be either bactericidal or bacteriostatic agents, depending upon the concentration used **(Dorman *et al.* 2000)**. The phospholipid bilayer of the microbial cytoplasmic membrane are hypothesised to be senstivie to the phenolic compounds causing increased permeability leading to imparement of bacterial enzyme system. These compounds were strongly active despite their relatively low capacity to dissolve in water **(Hili *et al.* 1997)**.

Clove is traditionally used in the treatment of oral candidiasis and athlete's feet. The essential oil extracted from the dried flower buds of cloves is used for acne, warts, scars and parasites. Researcher has shown that clove oil is an effective mosquito repellent. However, clove oil is toxic to human cells. If ingested or injected in sufficient quantity, it has been shown to cause life threatening complications **(Prashar *et al.* 2006)**, including Acute Respiratory distress syndrome, Fulminant hepatic failure and central nervous system disorder. In addition clove are anti-mutagenic, anti-inflammatory and antioxidant **(Chaieb *et al.* 2007b)**. This study confirms that clove oil possess antibacterial activity. Hence it represents an alternative source of natural antibacteria substances for use in food system to prevent the growth of food-borne bacteria anbd extend the shelf life of the paneer. The authours belive pretreatment of panner with clove oil would not affect the nutritional quality of the panner, but a detail organoleptic study needs to be done.

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