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# Manufacture and Evaluation of Paneer Using Lemon Rinds as a Value Added Ingredient

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author RY carried out the analysis of the study performed the statistical analysis and managed literature searches. Author SP designed the study, wrote the protocol and helped in writing and editing of the first draft of the manuscript. Author DP wrote the first draft of the manuscript. Author PP managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The present investigation was planned and conducted to evaluate the efficacy of two debittering agents viz. zinc sulphate and β-cyclodextrin in reducing bitterness of lemon rinds and to optimize the level of addition of debittered lemon rinds in lemon flavoured paneer (LFP). Optimization of the manufacturing parameters was done by using Response Surface Methodology (RSM) with central composite rotatable design. Based on the results obtained in this study it was concluded that the optimum parameters for debittering of lemon rinds was by treating lemon rinds in a solution of 50 mg zinc sulphate/100 ml water and 200 mg β-cyclodextrin/100 ml of water for 12 h at 7±1°C. The optimum level of addition of debittered lemon rind shreds in paneer milk was found to be 8.0 g/kg of milk. Both the debittering agents when used individually did not have any effect on bitterness but when used in combination resulted in significant improvement in flavour scores and total score of LFP. Yield of control paneer prepared from standardized milk (4.5% fat/ 8.5% MSNF) and LFP was 17 and 18%, respectively. Vitamin-C, fiber and zinc content of LFP was 7 mg/100 g, 0.6% and 11.36 mg/ kg of paneer, respectively. One serving of LFP is expected to provide 11% RDA of important trace element zinc.

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*Keywords: Lemon flavoured paneer; lemon rind; zinc sulphate; β-cyclodextrin; response surface methodology.*

## 1. INTRODUCTION

Paneer represents a variety of Indian soft cheese, which is used as a base material for the preparation of a large number of culinary dishes and is highly nutritious and wholesome dairy product. Paneer consists of the protein and usually all the fat, insoluble salts and colloidal materials, together with part of the moisture serum of the original milk, which contained lactose, whey proteins, soluble salts, vitamins and other milk components [1]. Health conscious consumers are interested in foods that have added beneficial compounds such as antioxidants, fiber, phenolics, phytosterols etc. Therefore, manufacturers add such functional ingredients to appeal to such consumers.

Lemon is an important medicinal plant of the family *Rutaceae*. *Citrus limon* (L.), belongs to family Rutaceae. On the bases of fruits and tree characters, true lemons are divided into four groups i.e. Eureka, Lisbon, Anomalous and sweet lemon. Most common variety grown in India is Kagzi Kalan lemon, which is characterized by its spherical, yellow, with apex slightly nippled, base rounded; rind thin, smooth, flesh acidic, light yellow, juicy, seedy (8-13 seeds. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz., leaves, rind, stem, root and flower) of lemon against clinically significant bacterial strains has been reported [2]. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [3,4]. Lemon peels are generally considered as waste products. The peels are rich in many essential constituents like antioxidants, fibre, etc. and can be used as functional ingredients in foods as well as for pharmaceutical purposes [5]. The majority of fruit peels exhibit 2 to 27 fold higher antioxidant activity than the fruit pulp [6-8]. Gorinstein et al. [9] reported that the content of ascorbic acid is higher in peels than in peeled fruits. The essential oil of lemon peels contains D-limonene, which is known for many health improving properties [10]. It improves the immunity, counters occasional feelings of depression, promotes clarity of thought and purpose, energizes and stimulates the mind and body, opens and releases emotional blocks and supports skin health and reduces the appearance of wrinkles [11].

Incorporation of functional ingredients like lemon rind will help to improve the quality, flavour and shelf-life of paneer thus making it attractive to health-conscious people. Addition of lemon rind resulted in a product which was extremely bitter and unacceptable. It has been reported that low molecular weight compounds, such as zinc lactate and zinc sulfate, can significantly decrease the bitterness elicited by a range of bitterants including caffeine, quinine hydrochloride, tetralone, and denatonium benzoate [12-14]. β-cyclodextrin is a commonly used cyclodextrin for this purpose.

Zinc is one of the most important trace elements present in the body with a great nutritional importance and that has a recognized action on more than 300 enzymes, participating in either their structure or in their catalytic and regulatory action [15,10]. Through interaction with specific amino acids (serine, threonine) on the extracellular portion of some bitter taste receptors, zinc sulfate may alter the integrity of TAS2R receptors, preventing them from functioning normally [12]. Zinc fortification of foods is an attractive and active strategy for decreasing zinc deficiency in the population and may confer additional value to the use of zinc sulfate in functional foods [15,16].

Cyclodextrins have also been used as an additive to lemon and orange juice as they reduce the perceived bitterness of both naringin and limonin [17]. Cyclodextrins are cyclic oligosaccharides derived enzymatically from starch hydrolysates. Cyclodextrins usually bind bitter molecules at a fixed stoichiometry, usually 1:1 [18]. Cyclodextrins were capable of partially suppressing the bitterness of soy protein, soy protein hydrosylates [19] and olive oil [20]. In addition, β-cyclodextrin is nontoxic, edible, non-hygroscopic and chemically stable. Furthermore, in 1998 it was introduced into the Generally Recognized as Safe list of compounds (GRAS), because it is not absorbed in the upper gastrointestinal tract, and it is completely metabolized by the colon microflora [21]. Therefore, in order to reduce the bitterness of paneer samples incorporated with lemon rind, two bitter blockers viz. zinc sulphate and beta cyclodextrin were evaluated.

## 2. MATERIALS AND METHODS

Fresh, raw mixed (cow and buffalo) whole milk was procured from Anubhav Dairy, and standardized by mixing required quantity of skim milk and cream. The average composition of milk was 4.6±0.05% fat and 8.6±0.05% Milk Solids-Not-Fat (MSNF). Citric acid, β-cyclodextrin and zinc sulphate were procured from Loba-Chemie Pvt. Ltd., Mumbai, Maharashtra. Yellow coloured, Kagzi variety lemon were procured from local market in Anand, Gujarat, India.

### 2.1 Debittering and Processing of Lemon Rind Shreds

Good quality lemons of kagzi variety weighing about 60 to 75 g each were selected. After proper washing the lemons were soaked in 25 ppm chlorine solution for 10 min before peeling. Care was taken to exclude the albido, in order to obtain the rind or zest which is the thin coloured, outer layer. The lemon rinds were soaked in a 5% NaCl solution for 1 h at 7±1°C. Quantity of rinds obtained was 140 to 160 g per kg lemons. After draining, pasteurized chilled water (4X) by weight of rind and zinc sulphate (20 to 50 mg/ 100 ml of water) and β-cyclodextrin (80 to 200 mg/ 100 ml of water) were added and kept for 12 h at 7±1°C for debittering. The rinds were then drained to get processed lemon shreds (110-115 g). Debittered lemon rinds were shredded in a clean and sanitized grater attachment of Boss Food Processor, India. The shreds had an average length of 0.8 ± 0.1cm, average width and thickness of 0.3 ± 0.1 cm.

### 2.2 Preparation of Paneer

Three kg milk (4.5% fat/8.5% MSNF) was taken in a stainless steel vessel was heated to 90°C for 5 min and cooled to 80°C. Processed lemon rind shreds 8 to 15 g/ kg milk were added at this stage and stirred for one min. The milk was coagulated with citric acid (1.0% solution) at 75°C. The coagulant was added to the milk with slow stirring until a curd and clear whey separated out. The pH of whey at this stage was 5.4 to 5.6. The quantity of citric acid used ranged from 1.96 to 2.15 g/kg milk. The coagulum was allowed to settle for 5 min and the whey was drained through a clean, sterile muslin cloth. Care was taken so that the temperature of whey was maintained above 70°C. The curd was then collected and transferred to a rectangular shaped sterilized stainless steel hoop (15x10x9 $cm^3$) lined with a clean sterile muslin cloth. The coagulum was pressed for 15-20 min by applying a pressure of 2 to 3 $kg/cm^2$. The pressed block of paneer were removed from the hoop and immersed in pasteurized chilled water (3 to 5°C) for 2 h. The paneer blocks were removed from chilled water placed in a clean stainless steel dish for allowing the water to drain off for 10 min. On completion of draining, paneer blocks were weighed and their representative samples drawn as per method given in Indian Standards: IS 5162 [22] and subjected to compositional, sensory and rheological evaluation. After weighing and sampling, the chilled block of paneer was wrapped in 12 μ polyester + 50 μ LD/LLDPE laminated pouches and stored at refrigeration temperature (7±1°C) until further use.

LFP was prepared using different levels of level of processed lemon rind (g/kg of milk), zinc sulphate (mg/ 100 ml of water) and β-cyclodextrin (mg/ 100 ml of water) solution as suggested by Response Surface Methodology (RSM) to choose the best combination to get the most acceptable LFP. The level of processed lemon rind (A) (8 to 15 g/kg of milk), zinc sulphate (B) (20 to 50 mg/100 ml of water) and β-cyclodextrin (C) (80 to 200 mg/ 100 ml of water) were optimized by adopting a Central composite rotatable design (CCRD) consisting of total 20 experiments as shown in Table 1.

### 2.3 Physico-Chemical Analysis

Fat content in milk was estimated by Gerber's method (IS: 1479, Part I) [23]. The total solids of milk was determined by the standard procedure (IS: 12333) [24]. The titratable acidity of milk was determined by the method described in the IS: 1479 (Part II) [25]. The Moisture content in paneer was determined by according to IS: 10484 [26]. The fat content of paneer samples was determined by the Mojonnier method as described in IS: 2785 [27]. Protein content in paneer was determined by Kjeldahl method as per AOAC [28], using Kjel-plus digestion system (Model-KPS 006L) and Kjel-plus semi-automatic distillation system (Model-Distil M) of M/s. Pelican Instruments, Chennai. Ash content of all the samples was determined by procedure described in BIS [29]. Titratable acidity of paneer was determined by the procedure as described by Boghara and Rajorhia [30]. The pH of paneer was determined as described by O'Keefe et al. [31] using a Handheld digital pH meter (Mettler Toledo, Fivego). Fiber content was estimated by method reported by Madhu et al. [32] with slight modifications. Vitamin-C was estimated by method reported by Osborne and Voogt [33].

Zinc content was estimated by method as per FSSAI [34,35].

## 2.4 Sensory Evaluation of Paneer

Each block of paneer was cut into approximately 25 g rectangular pieces and served in petri dishes which were labelled with three digit codes in randomized order. The paneer samples were tempered to 10±2°C before judging. Sensory analysis of paneer samples was performed in a sensory evaluation laboratory. The sensory panel (n=10) was composed of staff members and post graduate students working in the department. Panellists were selected based on their consistent performance in triangle test and paired comparison test. The samples were evaluated using 100point score card as described in Indian Standards (IS: 15346) [36].

## 2.5 Textural Analysis

Compression testing of paneer samples was done with Lloyd Instrument, Hampshire, UK (Model No. 01/2962) using 5 KN load-cells which moved at a speed of 20 mm/min. All the textural measurements were conducted in a room maintained at 23±1°C temperature and 65±1% RH. The paneer samples were taken for texture measurement after tempering the same at 10±1°C for h. Cubic samples of the experimental paneer, with edges of 2.00±0.06 cm, were placed in the compression support plate in such a manner that fibers were oriented perpendicular to the cylindrical compression anvil. The cubic samples were compressed up to 70% of their initial size. Five cubic samples were used for each experimental paneer under study and the average value of these readings was reported.

## 2.6 Statistical Analysis

The data was analysed using Response Surface Methodology (RSM). To carry out their optimization in the final product formulation, an advanced statistical software program named Design Expert (Version 8.0.3) was employed.

# 3. RESULTS AND DISCUSSION

Lemon flavoured paneer (LFP) was prepared with varying level of processed lemon rind (g/kg of milk) using lemon rinds treated with varying levels of zinc sulphate (20 to 50 mg/ 100 ml of water) and β-cyclodextrin (80 to 200 mg/ 100 ml of water) as suggested by Response Surface Methodology to choose the best combination for manufacture of an acceptable product. The results of the study obtained for sensory scores, textural properties and selected compositional attributes of LFP are presented in Table 1. The regression analysis of suggested models for sensory scores, textural properties and compositional attributes of LFP is presented in Table 2.

## 3.1 Effect of Level of Processed Lemon Rind, Zinc Sulphate and β-cyclodextrin on Sensory Score of LFP

As seen in Table 2 that the calculated F values are more than the Table F values at 5% level of significance which indicate the significance of the model terms. It can also be seen that, the coefficient of determination ($R^2$) which reflects the proportion of variability in data explained or accounted by the model for flavour, body and texture, colour and appearance and total score were 0.9441, 0.8794, 0.8833 and 0.9326 respectively. A larger $R^2$ values suggest a better fit of the quadratic model. The Adequate Precision Value (APV) measure the signal to noise ratio. As seen in Table 2, the APV for sensory characteristics were greater than 4, which support the suitability of the model to navigate the design.

### 3.1.1 Flavour score

As seen in Table 1, the flavour score of LFP ranged from 37.75 to 44.67 out of 50. The values presented in Table 2 revealed, that the interaction of level of zinc sulphate and level of β-cyclodextrin (BC) showed significant ($P<0.01$) positive effect on flavour score. Level of processed lemon rind ($A^2$) and level of zinc sulphate ($B^2$) showed significant ($P<0.05$) positive effect on flavour score.

These results are in accordance with those reported by Keast et al. [37] who investigated the taste and somatosensory effect of zinc salts (chloride, iodide, sulphate, bromide, acetate). He reported that zinc was a potent inhibitor of bitterness (>70% reduction in bitter taste), but did not affect salt, savory or sour taste. It has been reported that low molecular weight compounds, such as zinc lactate and zinc sulphate, can significantly decrease the bitterness elicited by a range of bitterants including caffeine, quinine hydrochloride, tetralone, and denatonium benzoate [12-14]. β-cyclodextrin is also commonly used cyclodextrin for this purpose. Cyclodextrins usually bind bitter molecules at a fixed stoichiometry, usually 1:1 [18]. Cyclodextrins were capable of partially suppressing the bitterness of soy protein, soy protein hydrosylates [19] and olive oil [20].

**Table 1. Experimental design matrix and sensory characteristics, textural properties and compositional attributes of LFP**

| Run | (A) | (B) | (C) | Flavour Score (Out of 50) | B & T Score* (Out of 35) | C & A Score@ (Out of 10) | Total Score[1] (Out of 100) | Hardness (N) | Chewiness (Nmm) | Cohesiveness | Fat (%) | Moisture (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 11.50 | 35.00 | 240.91 | 39.00 | 29.00 | 8.00 | 81.00 | 11.46 | 0.41 | 26.48 | 24.56 | 53.65 |
| 2 | 11.50 | 35.00 | 140.00 | 39.00 | 29.00 | 8.00 | 81.00 | 11.77 | 0.38 | 23.62 | 24.44 | 51.09 |
| 3 | 08.00 | 20.00 | 200.00 | 40.00 | 27.50 | 8.00 | 80.50 | 11.85 | 0.40 | 25.67 | 25.15 | 51.47 |
| 4 | 11.50 | 35.00 | 140.00 | 37.75 | 29.00 | 8.00 | 79.75 | 14.40 | 0.40 | 30.66 | 25.68 | 50.96 |
| 5 | 11.50 | 35.00 | 039.09 | 40.25 | 27.50 | 7.75 | 80.50 | 10.51 | 0.45 | 27.83 | 25.51 | 50.11 |
| 6 | 15.00 | 50.00 | 200.00 | 43.00 | 29.67 | 8.34 | 86.01 | 13.50 | 0.42 | 32.74 | 19.80 | 52.46 |
| 7 | 05.61 | 35.00 | 140.00 | 43.00 | 30.67 | 8.34 | 87.01 | 12.76 | 0.42 | 30.23 | 24.36 | 48.58 |
| 8 | 11.50 | 60.23 | 140.00 | 42.67 | 32.00 | 8.34 | 88.01 | 13.19 | 0.42 | 31.63 | 21.06 | 50.69 |
| 9 | 15.00 | 20.00 | 080.00 | 44.34 | 31.00 | 8.00 | 88.34 | 12.26 | 0.42 | 27.74 | 20.62 | 52.95 |
| 10 | 08.00 | 50.00 | 200.00 | 44.67 | 32.00 | 8.67 | 90.34 | 14.35 | 0.43 | 35.58 | 22.12 | 49.05 |
| 11 | 11.50 | 35.00 | 140.00 | 39.00 | 29.00 | 8.00 | 81.00 | 16.16 | 0.45 | 43.40 | 19.80 | 50.03 |
| 12 | 11.50 | 09.77 | 140.00 | 41.50 | 30.00 | 8.00 | 84.50 | 15.61 | 0.45 | 42.18 | 19.10 | 51.03 |
| 13 | 08.00 | 50.00 | 080.00 | 40.00 | 29.33 | 9.00 | 83.33 | 18.69 | 0.48 | 54.49 | 21.30 | 51.29 |
| 14 | 11.50 | 35.00 | 140.00 | 39.00 | 29.00 | 8.00 | 81.00 | 15.63 | 0.44 | 40.37 | 20.70 | 51.76 |
| 15 | 11.50 | 35.00 | 140.00 | 39.00 | 29.00 | 8.00 | 81.00 | 17.64 | 0.46 | 51.70 | 20.70 | 49.73 |
| 16 | 15.00 | 20.00 | 200.00 | 40.00 | 29.50 | 8.00 | 82.50 | 14.97 | 0.40 | 33.34 | 21.07 | 51.56 |
| 17 | 08.00 | 20.00 | 080.00 | 44.33 | 30.67 | 8.67 | 88.67 | 11.16 | 0.43 | 27.50 | 24.85 | 51.69 |
| 18 | 11.50 | 35.00 | 140.00 | 39.50 | 29.33 | 8.00 | 81.83 | 11.45 | 0.42 | 25.37 | 20.72 | 51.19 |
| 19 | 15.00 | 50.00 | 80.00 | 41.67 | 28.00 | 7.34 | 82.01 | 17.83 | 0.44 | 47.23 | 20.11 | 52.29 |
| 20 | 17.39 | 35.00 | 140.00 | 42.33 | 28.34 | 7.34 | 83.01 | 18.81 | 0.46 | 50.39 | 18.00 | 50.26 |

**Body & Texture @ Colour and Appearance [1]Full marks 5 out of 5 were allotted to packaging; A, B and C refer to the processed lemon rind (g/kg milk), zinc sulphate (mg/100 ml water) and β-cyclodextrin (mg/100 ml water) respectively*

**Table 2. Regression analysis of suggested models for sensory characteristics, textural and compositional properties of LFP**

| | | Sensory characteristics | | | | Textural properties | | | Composition | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | | **Flavour Score (Out of 50)** | **Body & Texture Score (Out of 35)** | **Colour & Appearance Score (Out of 10)** | **Total Score (Out of 100)** | **Hardness (N)** | **Chewiness (Nmm)** | **Cohesiveness** | **Fat (%)** | **Moisture (%)** |
| Intercept | | 38.85 | 29.05 | 7.99 | 80.90 | 14.95 | 35.39 | 0.43 | 21.37 | 50.95 |
| Linear | A | -0.0817 | -0.3843** | -0.3179* | -0.7840** | 1.2077* | 2.3222 | 0.0013 | -1.6487* | 0.5363* |
| Level | B | 0.1931 | 0.2704 | 0.0916 | 0.5552 | 0.8045** | 5.2390* | 0.0082* | -0.7660* | -0.1385 |
| | C | -0.3494 | 0.1605 | 0.0307 | -0.1581 | -0.2486 | -2.3358 | -0.0106* | -0.0247 | 0.0760 |
| Interactive | AB | -0.0012 | -0.7487* | -0.1650** | -0.9150 | -0.2651 | -2.2512 | -0.0044 | 0.600** | 0.6275* |
| Effect | AC | -0.4187 | 0.0837 | 0.2500 | -0.0850 | 0.2191 | 1.4812 | 0.0040 | -0.1225 | 0.0875 |
| | BC | 1.8337* | 1.1262* | 0.1675** | 3.1275* | -1.4741* | -4.6462** | -0.0046 | -0.030 | 0.0100 |
| Quadratic | $A^2$ | 1.4742* | 0.1784 | 0.0052 | 1.6579* | 0.2345 | 1.8277 | -0.0002 | -0.1101 | -0.4470* |
| Level | $B^2$ | 1.2691* | 0.7069* | 0.1219** | 2.0981* | 0.2426 | 0.7865 | 0.0014 | -0.5468** | 0.0620 |
| | $C^2$ | 0.3994 | -0.2652 | 0.0176 | 0.1518 | -1.4625* | -2.8232 | 0.0003 | 1.2527* | 0.5976* |
| R2 | | 0.9441 | 0.8794 | 0.8833 | 0.9326 | 0.8513 | 0.8750 | 0.8746 | 0.9324 | 0.8707 |
| Model F-value | | 18.78 | 8.10 | 8.41 | 15.37 | 6.36 | 3.83 | 7.75 | 15.32 | 7.48 |
| APV | | 11.20 | 11.21 | 11.04 | 11.19 | 8.53 | 8.84 | 11.82 | 12.84 | 11.42 |
| Suggested Model | | Quadratic | Quadratic | Quadratic | Quadratic | Quadratic | Quadratic | Quadratic | Quadratic | Quadratic |

**Significant at 1% level (P<0.01); **Significant at 5% level (P<0.05); R2 coefficient of determination; APV Adequate Precision Value; FDM Fat on Dry Matter; A, B and C refer to the processed lemon rind (g/kg milk), zinc sulphate (mg/100 ml water) and β-cyclodextrin (mg/100 ml water) respectively*

### 3.1.2 Body and texture score

The body & texture scores of LFP depicted in Table 1 indicated variation from 27.50 to 32.00 (out of 35). The values presented in Table 2 revealed, the level of processed lemon rind showed significant (P<0.05) negative effect on body and texture score. The interaction effect between level of processed lemon rind and level of zinc sulphate (AB) showed significant (P>0.05) negative effect on body and texture score. Interaction effect between of level of zinc sulphate and level of β-cyclodextrin (BC) showed a significant (P<0.05) positive effect on body and texture score.

The results obtained in this study are similar to those reported by Kahraman and Ustunol [38], who reported that although zinc-fortified cheese was harder as determined by the texture analyzer, the untrained consumer panel did not detect differences in the sensory attributes and overall quality of the cheeses.

### 3.1.3 Colour and appearance score

The colour & appearance scores of LFP depicted in Table 1 indicated variation from 7.34 to 9 (out of 10). The values presented in Table 2 revealed, the level of processed lemon rind showed significant (P<0.05) negative effect on colour and appearance score. The interaction of level of processed lemon rind and level of zinc sulphate (AB) and interaction effect between level of zinc sulphate and β-cyclodextrin (BC) showed significant (P<0.05) negative and positive effect on colour and appearance score respectively. The level of zinc sulphate ($B^2$) showed significant (P<0.05) positive effect on colour and appearance score.

This study indicated a definite influence of level of processed lemon rind (g/kg milk), level of zinc sulphate (mg/ 100 ml of water) and level of β-cyclodextrin (mg/ 100 ml of water) on the colour and appearance score. Addition of lemon rind in any form of paste and powder resulted in a yellowish tinge to the product. Whereas addition of lemon shreds resulted in uneven appearance which was not liked by panellists.

### 3.1.4 Total score

The total score of LFP depicted in Table 1 indicated variation from 79.75 to 90.4 (out of 100). The values presented in Table 2 revealed, the level of processed lemon rind showed significant (P<0.05) negative effect on total score. The interaction of level of zinc sulphate and level of β-cyclodextrin (BC) showed significant (P<0.01) positive effect on total score of LFP. Also at higher levels of processed lemon rind ($A^2$) and zinc sulphate showed significant (P<0.05) positive effect on total score at quadratic level.

Addition of lemon rind resulted in a product which was extremely bitter and was found to be unacceptable to panellists which resulted in significant reduction total score of paneer containing lemon rind**.** This study indicated a definite influence of level of processed lemon rind (g/kg milk), level of zinc sulphate (mg/ 100 ml of water) and level of β-cyclodextrin (mg/ 100 ml of water) on the on the total score. Both the debittering agents when used individually did not have any effect on bitterness but when used in combination resulted in significant improvement in flavour scores and hence, total score.

The results are also in agreement with those reported by Abd-Rabou et al. [39]. In their studies Edam cheese was fortified by different sources of dietary zinc such as zinc acetate, zinc chloride and zinc sulphate at a level of 150 mg zinc / kg cheese curd. The authors reported that fortified cheeses with zinc salts were scored higher with respect to all sensory attributes, samples were more tasty and had better flavour intensity. Moreover, Edam cheese fortified with zinc acetate had better properties along the ripening period and reached the highest scores earlier than the other treated cheeses or control, except for body and texture properties which were decreased in the last week.

## 3.2 Effect of Level of Processed Lemon Rind, Zinc Sulphate and β-cyclodextrin on Textural Properties of LFP

Textural properties chosen as responses for LFP to monitor its quality are Chewiness (Nmm), Cohesiveness and Hardness (N). The values of these responses along with their factors as per run order are given in Table 1. The coefficient of determination ($R^2$) for chewiness, cohesiveness and hardness were 0.8750, 0.8746 and 0.8513 respectively (Table 2).

### 3.2.1 Chewiness

The values of chewiness (Nmm) of LFP shown in Table 1 ranged from 23.62 to 54.49 Nmm. The values presented in Table 2 revealed with

increase in level of zinc sulphate increases the chewiness of paneer significantly (P<0.01).

#### 3.2.2 Cohesiveness

The values of cohesiveness of paneer shown in Table 1 ranged from 0.38 to 0.48. The level of zinc sulphate showed significant (P>0.01) positive effect on cohesiveness of LFP. A significant (P<0.01) negative effect showed by level of β-cyclodextrin cohesiveness. This indicates that the increase in the level of β-cyclodextrin decreased cohesiveness of LFP.

#### 3.2.3 Hardness

The values of hardness of LFP shown in Table 1 ranged from 10.51 to 18.81 N. The level of processed lemon rind showed significant (P<0.01) positive effect on hardness of LFP. This indicates increase in level of processed lemon rind increased hardness. The level of zinc sulphate showed significant (P<0.05) positive effect on hardness of LFP.

Thus, this study indicated a definite influence of level of processed lemon rind (g/kg milk), level of zinc sulphate (mg/ 100 ml of water) and level of β-cyclodextrin (mg/ 100 ml of water) on the hardness of paneer. Addition of lemon rind shreds in paneer led to a product with hard body, which was less cohesive and had uneven coarse texture due to the presence of lemon rind paste which did not allow the proper aggregation of casein micelles. The samples were criticized by panellists for hard body, this could be attributed to presence of fiber in lemon rind and cations such as $Zn^{++}$ because of treated with zinc sulphate, which could have resulted in more closer linkages of protein resulting in increased hardness.

The results obtained in this study are somewhat similar to those reported by Kahraman and Ustunol [38], who reported that although zinc-fortified cheese was harder as determined by the texture analyzer. In our studies also panellist reported that samples treated with zinc sulfate were harder than control. These authors hypothesized that the addition of zinc sulphate to cheese milk before clotting contributes to the divalent cation equilibria similarly to the addition of calcium. Divalent cation zinc provides for more bridging between caseins and for more crosslinking similar to calcium. The more rigid protein network that is produced then allows for better fat entrapment into the curd consistent with higher fat content of zinc-fortified cheeses in this study [38].

### 3.3 Effect of Level of Processed Lemon Rind, Zinc Sulphate and β-cyclodextrin on Compositional Attributes of LFP

Compositional properties chosen as responses for LFP to monitor its quality are fat and moisture content of paneer. The values of these responses along with their factors as per run order are given in Table 1 and the coefficient of determination ($R^2$) for fat and moisture 0.9324 and 0.8707 respectively (Table 2).

#### 3.3.1 Fat

The values of fat shown in Table 1 ranged from 18 to 25.68%. The level of processed lemon rind and level of zinc sulphate showed significant (P>0.01) negative effect on LFP. This indicates that the increases the level of processed lemon rind and level of zinc sulphate decreases the fat content at linear level. A significant (P<0.01) positive effect on fat content was found with higher level of β-cyclodextrin ($C^2$).

Thus, this study indicated a definite influence of processed lemon rind (g/kg milk), level of zinc sulphate (mg/ 100 ml of water) and level of β-cyclodextrin (mg/ 100 ml of water) on fat content of paneer. The results are in agreement with those reported for Zinc-fortified Cheddar cheese containing 228 mg of zinc/kg of cheese was manufactured from milk that had 16 mg/kg food-grade zinc sulphate added. Zinc-fortified cheese had slightly higher fat content [38]. However, results Rathod [40] reported that addition of different levels of lemon pulp extract did not significantly affect the fat in chicken nuggets. Aleson-Carbonell et al. [41] also observed that the presence of lemon albedo did not modify (P>0.05) the fat content in the beef burger.

Moreover, in studies conduted by Kahraman and Ustunol [38], the authors postulated that addition of zinc sulphate to cheese milk before clotting contributes to the divalent cation equilibria similarly to the addition of calcium. Divalent cation zinc provides for more bridging between caseins and for more crosslinking similar to calcium. The more rigid protein network that is produced then allows for better fat entrapment into the curd consistent with higher fat content of zinc-fortified cheeses in this study.

### 3.3.2 Moisture

The values of moisture shown in Table 1 ranged from 48.58 to 53.65%. The level of processed lemon rind showed significant (P>0.01) positive effect on LFP. A significant (P<0.01) negative effect on moisture content was found with the higher level of processed lemon rind ($A^2$) and significant (P<0.01) positive effect with level of β-cyclodextrin ($C^2$) at quadratic level.

In our study it was observed that with increase in level of lemon rind there was a progressive decrease in moisture content of paneer. The decrease in moisture content of samples containing lemon rind could be attributed to the higher acidity of samples containing lemon rind. In this study it was found that all the samples containing lemon rinds treated with debittering agents (zinc sulphate) had lower moisture content. Similar results are reported by Chauhan and Chandra [42] prepared paneer enriched with fiber from milk standardized to 5% fat and 8.5% MSNF. Coconut powder was incorporated at four different levels from 1.0 to 2.5% (w/w of milk). The moisture % of fibre enriched paneer ranged from 54.06 to 50.82%. With increase in level of coconut powder there was a decrease in moisture content compared to a moisture content of 54.78%.

However, the results with respect to moisture are not in accordance with Fernande-Gines et al. [43] who studied the effect of addition of lemon albedo in bologna sausages and observed that moisture content was affected (P<0.05) by albedo type and concentrations. The different levels of lemon pulp extract used during preparation of chicken nuggets were found to be significantly affect the moisture content. The difference in the results could be attributed to water released from the meat matrix during the cooking process being retained by albedo, which has a high water holding capacity, due to their soluble compounds, mainly pectin, which may constitute up to 25% of the Lemon albedo [44-46].

The predicted formulation for optimized LFP from RSM analysis consisted of addition of processed lemon rind @ 8.0 g/kg of milk. Treating lemon rinds with zinc sulphate 50 mg/100ml water and β-cyclodextrin 200 mg/100 ml of water. The predicted sensory scores, textural properties and compositional attributes of LFP are depicted in Table 3. The final product was manufactured employing the suggested formulation and the actual results obtained were compared with these predicted values of the criteria/responses selected for process optimization. The calculated t-values of for all the parameters are reported in Table 3. The calculated t-values being less than the table values, it is inferred that there was non-significant (P<0.05) difference between the predicted and actual values of responses.

LFP prepared using the optimized solution of debittering shreds with zinc sulphate @ 50 mg/100 ml water and β-cyclodextrin @ 200 mg/100 ml of water and addition of debittered lemon rind shreds @8.0 g/kg of milk. The compositional, textural properties and sensory characteristics of the lemon flavoured paneer (LFP) was compared with a paneer (CP) made from standardized milk (4.5% fat/ 8.5 % MSNF). The average values of the chemical composition of the developed lemon flavoured paneer i.e. LFP and CP are showed in Table 4.

**Table 3. Comparison of predicted v/s actual values of responses selected**

| Response | P Value | Predicted Value * | Actual Value @ | Cal. t-Value# | Significance |
|---|---|---|---|---|---|
| Flavour[1] | 0.1147 | 44.18 | 44.89 | 1.844 | NS |
| Body & Texture[1] | 0.6935 | 32.27 | 32.143 | 0.4136 | NS |
| Colour & Appearance[1] | 0.0584 | 8.65 | 8.414 | 2.3335 | NS |
| Total Score[1] | 0.5953 | 90.11 | 90.429 | 0.5608 | NS |
| Chewiness | 0.2471 | 31.9903 | 35.25 | 1.2823 | NS |
| Cohesiveness | 0.1608 | 0.4211 | 0.4113 | 1.5996 | NS |
| Hardness | 0.179 | 11.88 | 12.4239 | 1.5215 | NS |
| Fat | 0.3145 | 22.31 | 21.996 | 1.0974 | NS |
| Moisture | 0.4095 | 49.85 | 50.34 | 0.8864 | NS |

*1Sensory score on 100 point score card; * Predicted values of Design Expert 8.0.3 package; @ Actual values are average of seven trials of optimized product; # t-values found non-significant at 5% level of significance; NS = Non-significant; Tabulated t-value = 2.447 (Calculated t-value less than tabulated value)*

**Table 4. Comparison of proximate composition and sensory attributes of LFP with regular paneer**

| Components | Lemon flavoured paneer (LFP) | Regular paneer (CP) |
|---|---|---|
| Moisture (%) | 51.55 | 53.68 |
| Fat (%) | 25.60 | 25.20 |
| Protein (%) | 17.13 | 17.05 |
| Ash (%) | 1.28 | 1.86 |
| Carbohydrate (%) | 4.44 | 2.21 |
| Acidity (%LA) | 0.51 | 0.46 |
| Vitamin C (mg /100g) | 7 | <1 |
| Total Dietary Fiber (%) | 0.6 | - |
| Zinc (mg / kg paneer) | 11.36 | <1 |
| Flavour Score (Out of 50) | 44.17 | 45.20 |
| Body & Texture Score (Out of 35) | 32.27 | 32.55 |
| Colour and Appearance Score (Out of 10) | 8.65 | 8.69 |
| Total Score (Out of 100) | 90.11 | 91.44 |
| Yield (kg paneer /100 kg milk) | 17 | 18 |

It can be seen from Table 4 that the vitamin-C, Fiber and Zinc content of LFP was 7 mg/100 g, 0.6% and 11.36 mg/ kg of paneer respectively. While in control samples, Vitamin-C, fiber and Zinc was <1 mg/100g, 0 % and <1 mg/kg paneer respectively. It was found the zinc content in LFP was 11.36 mg/ kg paneer. Considering a serving size of 100 g, one serving of paneer would provide 1.136 mg zinc which represents about 11% RDA. According to Dietary guidelines for Indians [47], Recommended daily allowance for zinc for Indians is 10 to 12 mg/day. One serving of LFP is expected to provide 11% RDA with respect to zinc.

According to guidelines provided by FDA [48] on food labelling, the product label must include % daily value (% DV), to designate both the daily reference value (DRV) and recommended daily intake (RDI). In order to make a "good source of micronutrient", the finished product must ideally contain 10 to 19% of DV per serving, since the zinc content was in this range, the product can be labelled as "good source of zinc".

## 4. CONCLUSION

Based on the results obtained in this study it can be concluded that the optimum parameters for manufacture of lemon flavoured paneer using: processed lemon rind 8.0 g/kg of milk, zinc sulphate 50 mg/100 ml water and β-cyclodextrin 200 mg/100 ml of water. Both the debittering agents when used individually did not have any effect on bitterness but when used in combination resulted in significant improvement in flavour scores and total score of LFP. Yield of control and LFP was 17% and 18% respectively. Vitamin-C, Fiber and Zinc content of LFP was 7 mg/100 g, 0.6% and 11.36 mg/ kg of paneer respectively. One serving of LFP is expected to provide 11% RDA with respect to zinc.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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