



Innovative Co-fortification of Piperine and Curcumin in Paneer: Development, Quality Assessment and Shelf Life Analysis

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ABSTRACT

Background: The present investigation was aimed to standardize the level of co-fortification of piperine and curcumin in paneer and assess the quality and shelf life of the formulated paneer.

Methods: The research work is carried out in the laboratory of department of patronage of traditional and specialty foods of MIT school of food technology from 2023 to 2024. The three different proportions of piperine and curcumin extract powders were incorporated to identify the sensorially best co-fortification level in paneer. The quality parameters (Sensory, nutritional, chemical, microbial and antioxidants activity) were evaluated with shelf life study.

Result: The sensory evaluation of co-fortified paneer formulated with different concentration level of piperine and curcumin extract powder indicated acceptability for 0.30% level of fortification for both the ingredients with overall acceptability score of 8.6. Nutritional composition analysis showed enriched levels of protein and dietary fiber in fortified paneer. The co-fortification with piperine and curcumin enhanced the antioxidant activity of paneer without significantly affecting its sensory attributes. This research highlights the potential of co-fortifying paneer with piperine and curcumin to enhance its nutritional, functional value, thereby offering consumers a healthier and more sustainable dairy product option.

Key words: Curcumin, Fortification, Paneer, Piperine, Quality, Shelf life.

INTRODUCTION

India is regarded as an agrarian nation where most people practice vegetarianism. For these people, dairy products are a vital source of animal proteins in their diet. Paneer, a traditional Indian dairy product, has long been celebrated for its rich nutritional profile, including high protein and calcium content. However, in recent years, there has been a growing interest in fortifying paneer with bioactive compounds to enhance its health benefits and nutritional value. Among these bioactive compounds, curcumin and piperine have garnered considerable attention due to their numerous potential health-promoting properties.

Curcumin has strong anti-inflammatory effects, which can help reduce inflammation in the body. Chronic inflammation is linked to various diseases such as heart disease, cancer and Alzheimer's, so curcumin's anti-inflammatory properties are believed to be beneficial for overall health. It is a potent antioxidant, meaning it can neutralize harmful free radicals in the body. Free radicals are unstable molecules that can damage cells and contribute to aging and various diseases. By scavenging these free radicals, curcumin may help protect against oxidative damage. Some studies suggest that curcumin may have anti-cancer properties, including the ability to inhibit the growth of cancer cells and prevent the spread of tumours. Curcumin has been studied for its potential role in preserving cognitive function and protecting against neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Ghodke and Pawar, 2023). It may help reduce the buildup of amyloid plaques in the brain, which are characteristic of Alzheimer's disease. Curcumin may

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have several benefits for heart health, including improving endothelial function, lowering cholesterol levels and reducing the risk of blood clot formation. Curcumin reduces oxidative stress and interacts with numerous inflammatory molecular targets regulating inflammatory pathways related with most chronic diseases (Ghodke and Pawar, 2020). Though the bioavailability of curcumin is relatively low, black pepper extract (piperine) or fats may improve absorption. Piperine is a bioactive compound found in black pepper, responsible for its characteristic pungent flavor. It has been studied for its potential health benefits. Piperine is known to enhance the bioavailability of various nutrients and compounds, including curcumin, resveratrol, beta-carotene and vitamin B6. By inhibiting certain enzymes in the gut and liver that metabolize these compounds, piperine can increase their absorption into the bloodstream, thereby

maximizing their effectiveness (Ahmad *et al.*, 2012). Hence, co-fortification of these piperine and curcumin crude extract powders may provide an option to develop healthier traditional dairy products.

In the recent time, there has been an increasing trend of the consumers for foods fortified with natural bioactives such as piperine and curcumin, due to increased consumers' health awareness and reports regarding possible toxicological effect of chemical preservatives. Natural nutraceuticals and antioxidants have emerged in the food market as potential ingredients to prevent the auto-oxidation of fats, oils and fat containing food products. Thus, nutraceuticals like curcumin and piperine offer a promising natural alternative in fortification of food products (Devaki *et al.*, 2021). The combination of curcumin and piperine offers a synergistic approach to fortifying paneer, potentially providing enhanced health benefits beyond those of traditional paneer. By co-fortifying paneer with curcumin and piperine, it is possible to create a functional food product with improved nutritional value and additional bioactive properties. Hence in present investigation, the efforts are undertaken to explore the development and quality assessment of curcumin and piperine co-fortified paneer variants. The physicochemical characteristics, sensory attributes and shelf life stability of co fortified paneer were evaluated for their overall quality and potential health benefits.

MATERIALS AND METHODS

Material

The present study was conducted at MIT School of food technology, Pune, Maharashtra, India during March 2023 to January 2024. The raw materials like buffalo milk, piperine and curcumin extract powders were purchased from the local market and online stores. Chemicals, reagents used for the preparation of different reagents were of analytical grade (AR) and were used from laboratories of MIT SoFT. The reagents required for analysis were freshly prepared according to standard procedures.

Processing technology of piperine and curcumin co-fortified paneer

The paneer was prepared as per the process given by Aneja *et al.* (2002) with slight modification and presented as flowchart in Fig 1. The buffalo milk containing 6 to 6.5% fat and 9% SNF was taken in stainless steel vessel and heated to 80 to 82°C followed by cooling to the 70°C temperature. Piperine and curcumin extract powder were incorporated in the milk at varying levels (0.30 to 0.50% each) and blended well. After cooling, citric acid was added in milk as a coagulant with continuous stirring. After complete coagulation, allow the curd was allowed to settle to sink to the bottom. The whey was then drained through a muslin clothe. The hot coagulum was pressed for 10-15 min. The pressed paneer blocks were removed from muslin cloth and dipped into the chilled water for absorption of moisture. The chilled paneer blocks were removed from

water for allowing them to drain, out and cut into desired pieces and packed into polythene bag. Finally, paneer was stored in refrigerator (4±1°C).

Sensory analysis

A group of judges evaluated the samples using a 9-point hedonic scale to assess characteristics such as colour, flavour, texture and overall acceptability. The product's appeal was gauged on a scale ranging from "like extremely" to "dislike extremely".

Proximate analysis

The moisture content and ash contents of both the control and spice-based paneer were determined following AOAC (2005) guidelines. Carbohydrate, protein and fat contents were analysed using the method outlined in AOAC (2005). Titratable acidity was measured according to AOCS standards, while pH was determined using a microprocessor-based digital pH meter. The DPPH technique was used to evaluate the antioxidant activity.

Microbiological analysis

Standard plate count, yeast and mold counts were determined using standard methods BIS(1983).

RESULTS AND DISCUSSION

Sensory profile of piperine and curcumin co fortified paneer

The sensory evaluation of fortified paneer at different concentrations of piperine and curcumin extract was carried out in comparison to control (Paneer without piperine and curcumin) sample. The rubric for sensory evaluation comprised of colour, appearance, flavour, texture, mouth feel and overall acceptability parameters. The semi trained panel set-up at MIT School of Food Technology assessed the quality parameters of developed test samples (S_0 , S_1 , S_2 and S_3) on hedonic scale of 9-points. The average scores for the samples are represented in graphical format in Fig 2.

For treatments S_0 , S_1 , S_2 and S_3 , the corresponding color and appearance scores were 8.40, 8.30, 7.90 and 7.00, respectively. S_1 had the highest score against other treated samples. The paneer sample's flavor score for treatments S_0 , S_1 , S_2 and S_3 were, in that order, 8.50, 8.70, 7.70 and 7.30. The paneer with 0.3% Piperine and 0.3% Curcumin received the highest score of 8.70 against rest of treated samples. The same trend recorded by Mishra *et al.* (2021) for the addition of black pepper extract. The highest scores were recorded by sample S_1 for the texture as compared to other piperine and curcumin treated sample.

Control S_0 and treatment S_1 received the highest overall acceptance scores, 8.70 and 8.40, respectively. (0.3% Piperine and 0.3% Curcumin) against other samples. Hence, 0.3% level of each piperine and curcumin was recorded to be best level for co fortification in paneer with highest overall acceptability.

Nutritional composition of fortified paneer with piperene and curcumin

Piperine and curcumin co fortified Paneer analyzed for selective nutritional proximate parameter and results depicted in Table 1 indicated nutritional upgradation of paneer.

It was discovered that the moisture level of paneer for treatments S_0 and S_1 was 51.59% and 52.27, respectively. With the addition of various combinations of piperine and powdered curcumin, the moisture content of paneer reduces. The treatment S_1 was discovered to be better than and comparable to S_0 (the control). The values are in good agreement with Eresam *et al.* (2015). The fat percentages of the treated paneer (S_1) and control (S_0) are,

respectively, 22.40 and 23.80. The decreasing trend was observed in fat in piperine curcumin treated sample. The lipid level of paneer dropped from 27.77 per cent according to Buch *et al.* (2012), who made the paneer by adding turmeric powder as a preservative.

As piperine and curcumin powder were combined at varying levels, the resulting paneer's protein content increased. For treatments S_0 and S_1 , respectively, the generated product's protein content varied from 15.30 to 16.10. S_0 (15.30) has the lowest protein content while S_1 (16.10) has the highest. A higher proportion of varying quantities of piperine and curcumin powder resulted in a lower final product's carbohydrate content. Regarding carbohydrate, the average values for treatments S_0 and S_1

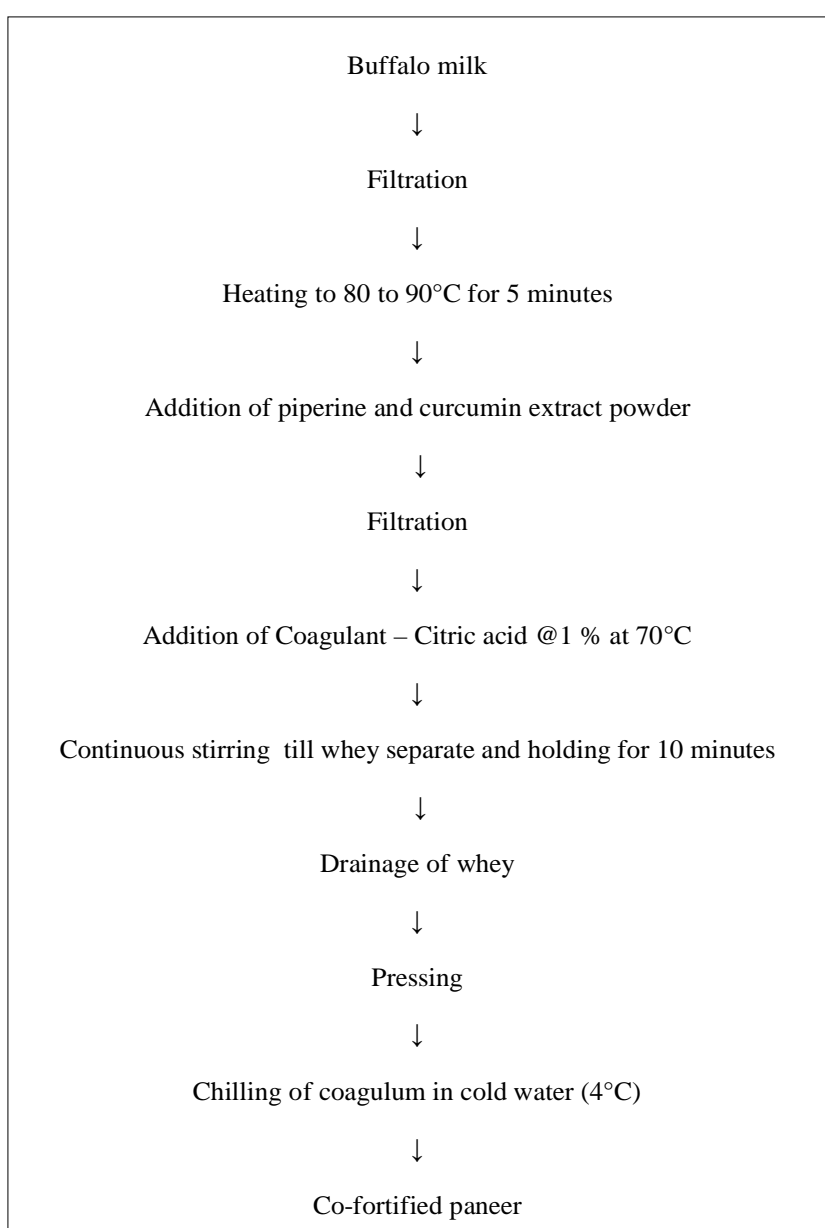


Fig 1: Method of preparation of co-fortified paneer (Aneja *et al.*, 2002).

were 4.48 and 5.73 correspondingly. The more or less similar values were recorded by Singh *et al.* (2018) and Mhatre (2018) for paneer with different spices.

Chemical analysis of piperine and curcumin co-fortified paneer

Chemical parameters of piperine and curcumin fortified samples are presented in Table 2.

The percentage of acidity in paneer made using varying combinations of piperine and curcumin powder treatment was found to be 0.46, 0.38, 0.31 and 0.29 for S_0 , S_1 , S_2 and S_3 accordingly. S_0 had the greatest acidity percentage (0.46%), while S_3 had the lowest (0.29%). Each treatment was very different from the others. The decreasing trend of acidity was observed with increasing level of piperine and curcumin level. The similar trend was recorded by Buch *et al.* (2012). The rise in values of pH from 4.80 to 5.59 was recorded with increase in co fortification level of piperine

and curcumin in paneer. The similar result was recorded by Khobragade *et al.* (2020a).

Effect of piperine and curcumin co-fortification on antioxidant activity of paneer

Antioxidant activity as one of the functional attributes of paneer was assessed and the effect of incorporation of bioactive compounds (Piperine and curcumin) on paneer is presented graphically in Fig 3.

Curcumin and piperine are well recognized as an antioxidants. The value of S_0 9.59% was found to be the lowest antioxidant percentage and S_3 45.58% to be the highest. As piperine and curcumin extract powder were fortified from 0% to 0.50%, rising trends were seen. This trend suggests that higher levels of fortification with piperine and curcumin extract powder result in significantly enhanced antioxidant activity in paneer. Antioxidants like piperine and curcumin are known for their ability to

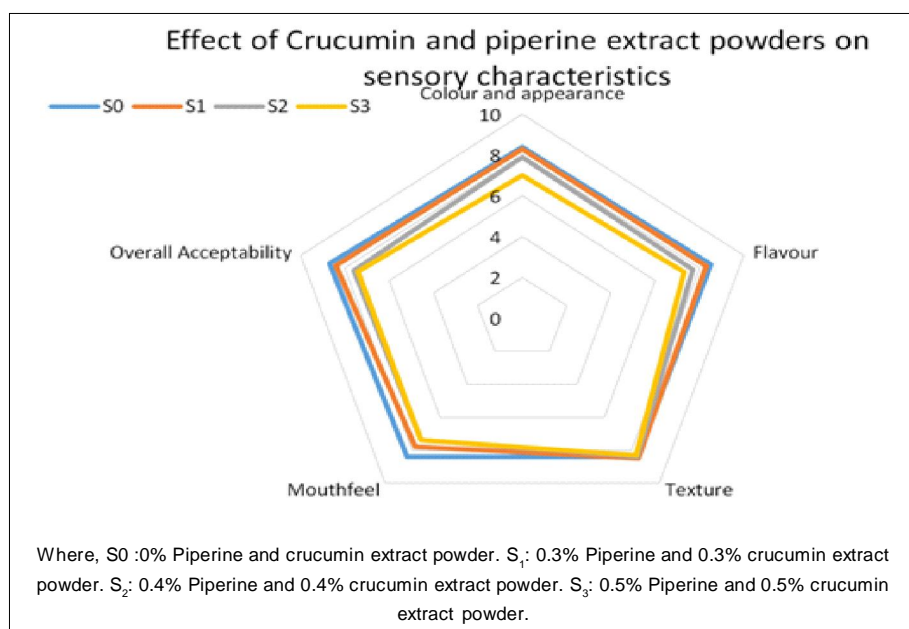


Fig 2: Sensory profile of co-fortified paneer.

Table 1: Proximate analysis of co-fortified paneer.

| Sample code | Moisture (%) | Crude fat (%) | Protein (%) | Crude ash (%) | Crude fibre (%) | Carbohydrate (%) |
|-------------|--------------|---------------|-------------|---------------|-----------------|------------------|
| S_0 | 52.27±0.45 | 23.80±0.12 | 15.30±0.36 | 1.55±0.31 | 0.60±0.64 | 4.48±0.26 |
| S_1 | 53.59±0.35 | 22.40±0.31 | 16.10±0.21 | 1.80±0.74 | 0.90±0.88 | 5.21±0.78 |
| S_2 | 50.20±0.25 | 21.03±0.11 | 16.20±0.47 | 2.03±0.28 | 0.88±0.22 | 5.66±0.32 |
| S_3 | 52.60±0.19 | 23.60±0.02 | 18.52±0.57 | 2.12±0.32 | 1.03±0.46 | 5.73±0.18 |

*Average of three reading and±standard deviation.

Where,

S_0 : 0.0% Piperine and 0.0% curcumin extract powder.

S_1 : 0.3% Piperine and 0.3% curcumin extract powder.

S_2 : 0.4% Piperine and 0.4% curcumin extract powder.

S_3 : 0.5% Piperine and 0.5% curcumin extract powder.

neutralize free radicals, which can contribute to various health benefits, including reducing oxidative stress and inflammation. Therefore, increasing the fortification level appears to be an effective strategy to enhance the antioxidant properties of paneer. The similar rise in antioxidant activity in cheese with addition of spice powder was recorded by Arkan *et al.* (2024).

Shelf life assessment piperine and curcumin co fortified paneer

The shelf life of a product denotes its capacity to remain viable for a specific duration without compromising its quality. This storage duration also reflects the product's market viability. Presently, consumer preferences lean towards foods devoid of chemical preservatives due to concerns over potential toxicity. There's a growing insistence on food items with extended shelf lives and minimal risk of causing foodborne illnesses. Additionally, there's a rising demand for foods enriched with natural ingredients. Responding to these consumer demands, the food industry is increasingly exploring natural alternatives to synthetic antimicrobial compounds. Spices present a compelling option for enhancing food safety. Renowned for their medicinal, preservative and antioxidant attributes, spices not only add flavor but also extend the shelf life of food. Some spices exhibit bacteriostatic or bactericidal properties, effectively inhibiting bacterial growth, while others prevent food from turning rancid through their antioxidant effects.

The standardized and overall acceptable level of piperine and curcumin co fortification was recorded as 0.3% each. Hence, standardized finished paneer along with control sample was evaluated for shelf life at refrigerated temperature ($4\pm1^{\circ}\text{C}$) using Polyvinylidene dichloride as packaging material. The co fortified paneer was analyzed for Physico-chemical (Titrable acidity, pH and antioxidant) and microbial yeast-mold, Colliform count and total plate count.

Effect of refrigerated storage on titrable acidity and pH of co-fortified paneer

The standardized piperine and curcumin cofortified paneer was subjected to shelf life study. The acidity values were determined during storage study and graphically represented in Fig 4. Both the co-fortified and control paneer's acidity rose when they were kept in a refrigerator. For control paneer, acidity increased from 0.46 to 1.9% lactic acid in 15 days, while for co-fortified paneer, 0.38 to 1.09% lactic acid in 15 days. An increase in paneer's titrable acidity was a sign that lactose-fermenting bacteria had caused bacterial deterioration. Compared to control paneer, co fortified paneer developed its acidity at a slower rate. The similar increasing trend was recorded by Sachdeva and Singh (1990).

As shown in Fig 5, the pH values of the piperine and curcumin-fortified paneer (S_1) and the control paneer (S_0) were 4.80 and 6.20, respectively. The graph showed that after the paneer was refrigerated, the pH of both the control and fortified paneer dropped. In 15 days, the pH of the control paneer (S_0) dropped from 4.80 to 3.89, while for the co-fortified paneer, it went from 6.20 to 5.80. The pH of

Table 2: Acidity and pH of piperine and curcumin co-fortified paneer.

| Samples | Acidity % | pH % |
|---------|--------------|--------------|
| S_0 | 0.46 ± 0.5 | 4.80 ± 0.6 |
| S_1 | 0.38 ± 0.5 | 5.02 ± 0.3 |
| S_2 | 0.31 ± 0.2 | 5.30 ± 0.4 |
| S_3 | 0.29 ± 0.4 | 5.59 ± 0.5 |

*Average of three reading and \pm standard deviation.

S_0 : 0% Piperine and curcumin extract powder.

S_1 : 0.3% Piperine and 0.3% curcumin extract powder.

S_2 : 0.4% Piperine and 0.4% curcumin extract powder.

S_3 : 0.5% Piperine and 0.5% curcumin extract powder.

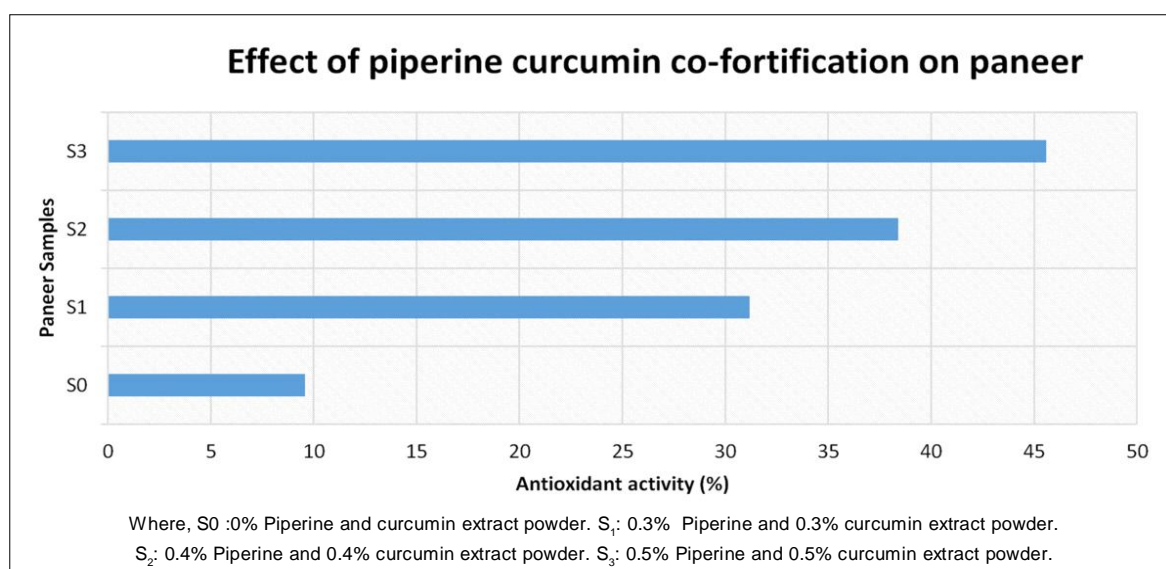


Fig 3: Antioxidant activity profile of co-fortified paneer.

paneer samples was also found to drop after storage, according to Arora and Gupta (1980).

Effect of refrigerated temperature on antioxidant activity of co-fortified paneer during storage

The study evaluated the antioxidant activity of control paneer (S_0) and co-fortified paneer (S_1) with piperine and curcumin extract powder over 15 days presented graphically in Fig 6, revealing significant differences. Initially, the control paneer exhibited an antioxidant activity of $9.59 \pm 0.5\%$, which gradually declined to $7.26 \pm 0.08\%$, while the co-fortified paneer started at $31.19 \pm 0.45\%$ and decreased to $28.03 \pm 0.51\%$. Despite the decrease, the antioxidant activity in the co-fortified paneer remained substantially higher than in the control

throughout the study period. This suggests that fortification not only boosts initial antioxidant levels but also provides a more sustained antioxidant effect over time, potentially enhancing both the nutritional quality and shelf life of paneer.

Microbial parameter assessment of co-fortified paneer during storage

The microbial parameters (Standard plate count, yeast and mould and coliform count) were assessed during storage study to establish shelf life of standardized piperine curcumin co-fortified paneer and presented in Table 3. The increase in standard count, yeast and mould count was recorded during 15 days duration at refrigerated temperature in both control and standardized samples.

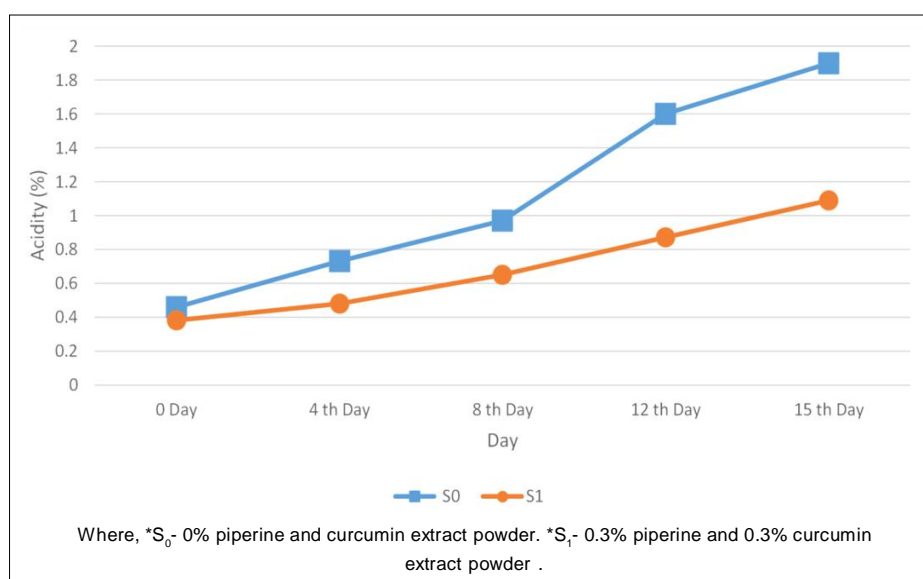


Fig 4: Effect of co-fortification on acidity of paneer during storage at $4 \pm 1^\circ\text{C}$.

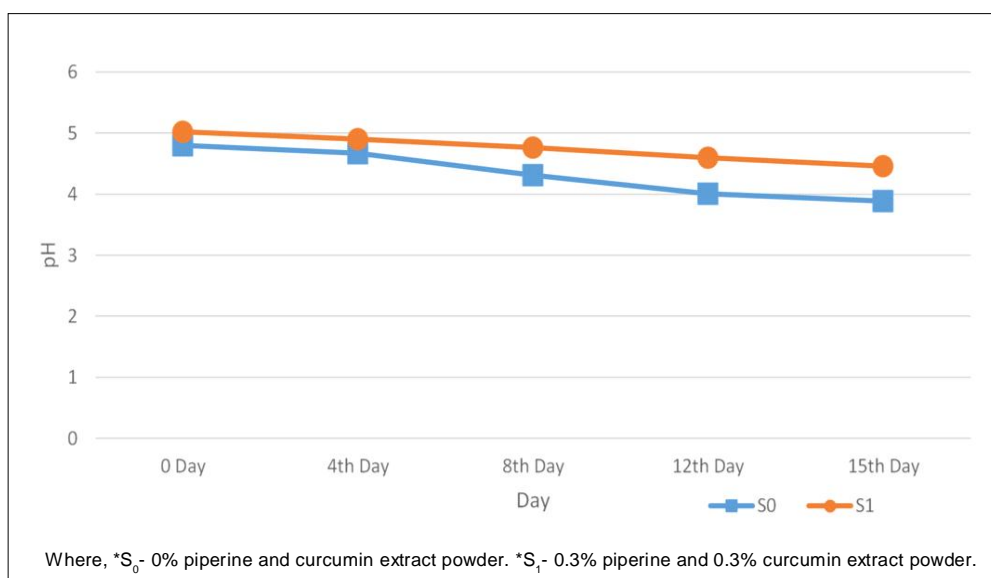


Fig 5: Effect of co-fortification on pH of paneer during storage at $4 \pm 1^\circ\text{C}$.

The all counts were found to be within prescribed limits of FSSAI (Standard Plate count- 35×10^4 , Yeast and mold count- 1×10^2 CFU/g, Coliform count- 1×10^2 CFU per g). It is observed that piperine curcumin co-fortified standardized paneer exhibited slower rate of growth of microorganism

compared to control sample. This may be attributed to antimicrobial activities of both bioactive compounds in paneer. The similar trend was recorded by Jyoti *et al.* (2022) for paneer spread incorporated with ginger and garlic.

Table 3: Microbial parameters of control and co fortified paneer during refrigerated storage at $4 \pm 1^\circ\text{C}$.

| Storage period (days) | Standard plate count (CFU/gm) | | Yeast and moulds (CFU/ gm) | | Coliforms (CFU/gm) | |
|-----------------------|-------------------------------|------------------|----------------------------|------------------|--------------------|----------------|
| | S ₀ | S ₁ | S ₀ | S ₁ | S ₀ | S ₁ |
| 0 Day | 16×10^4 | 15×10^4 | 6×10^1 | 4×10^1 | Nil | Nil |
| 4 Day | 21×10^4 | 18×10^4 | 9×10^1 | 7×10^1 | Nil | Nil |
| 8 Day | 25×10^4 | 21×10^4 | 11×10^1 | 11×10^1 | Nil | Nil |
| 12 Day | 27×10^4 | 24×10^4 | 13×10^1 | 14×10^1 | Nil | Nil |
| 15 Day | 31×10^4 | 29×10^4 | 21×10^1 | 18×10^1 | Nil | Nil |

Where,

*S₀- 0% piperine and curcumin extract powder.

*S₁- 0.3% piperine and 0.3% curcumin extract powder.

Table 4: Sensory evaluation of control and co-fortified paneer during storage at ($4 \pm 1^\circ\text{C}$).

| Storage period (day) | 0 Day | | 4 Day | | 8 Day | | 12 Day | | 15 Day | |
|-----------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | S ₀ | S ₁ | S ₀ | S ₁ | S ₀ | S ₁ | S ₀ | S ₁ | S ₀ | S ₁ |
| Colour | 8.40 | 8.30 | 7.80 | 7.50 | 7.40 | 7.30 | 7.30 | 7.20 | 7.10 | 7.00 |
| Appearance | 8.50 | 8.70 | 8.20 | 7.30 | 7.20 | 7.10 | 6.90 | 6.90 | 6.70 | 6.00 |
| Taste | 8.50 | 8.40 | 7.50 | 7.40 | 7.30 | 7.20 | 6.80 | 6.50 | 6.50 | 6.40 |
| Texture | 8.60 | 8.50 | 7.60 | 7.60 | 7.30 | 7.40 | 7.20 | 7.30 | 7.00 | 7.00 |
| Mouth feel | 8.40 | 8.10 | 7.40 | 7.40 | 7.10 | 7.20 | 6.80 | 6.50 | 6.50 | 6.00 |
| Overall acceptability | 8.47 | 8.40 | 7.60 | 7.45 | 7.25 | 7.25 | 7.05 | 6.97 | 6.82 | 6.50 |

Where,

*S₀- 0% piperine and curcumin extract powder.

*S₁- 0.3% piperine and 0.3% curcumin extract powder.

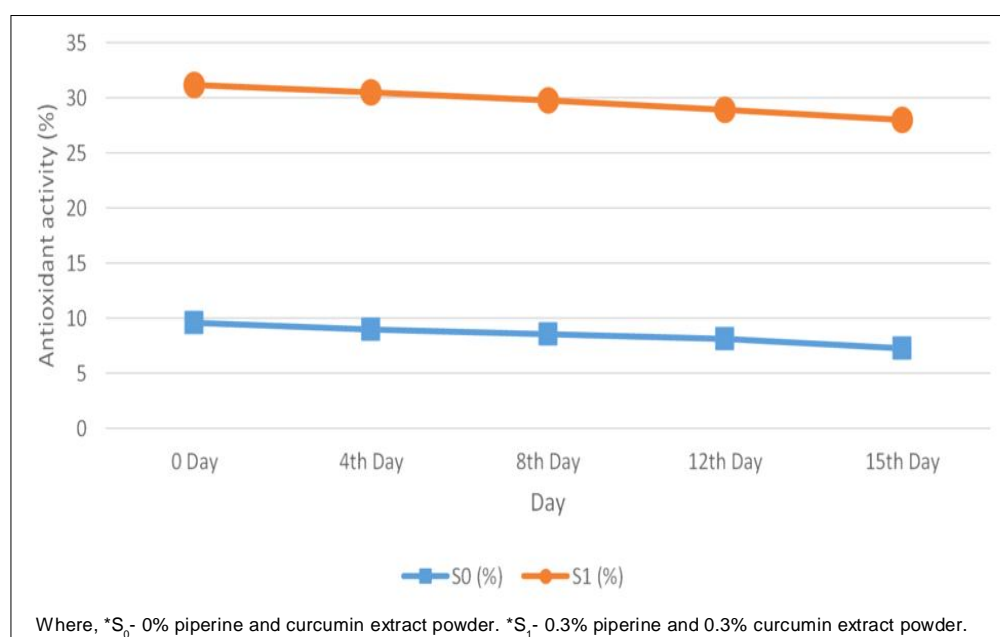


Fig 6: Effect of co-fortification on antioxidant activity of paneer during storage at $4 \pm 1^\circ\text{C}$.

Sensory evaluation of co-fortified paneer during storage

The sensory evaluation of the piperine and curcumin co-fortified paneer during storage from 0 to 15th Days is presented in Table 4. The decline in the sensory score was recorded as days advanced but found to be acceptable at the end of 15th day.

CONCLUSION

The fortification of piperin and curcumin as novel ingredient synergistically can offer novel opportunities to enhance functionality of dairy industry. The co-fortification at 0.3% of piperine and curcumin in paneer lead to nutritional upgradation, enhanced antioxidant activity with safe storage tenure of 15 days without affecting on sensorial attributes. Thus, utilization of these nutraceuticals provides a viable ingredient option for dairy product development.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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