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Physico-chemical and Microbiological Study of Curd(yoghurt) Fortified With Spices

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ABSTRACT

Dairy products such as yogurt are widely used, so fortifying this food with spices is helpful to increase its nutritional value of it. Spices contain antioxidant activity, proteolytic activity, and bioactive compounds which are very helpful for human health. The main focus is to examine the technical element and microbiology involved in the manufacturing of various varieties of yogurt. Various types of yogurt were prepared by adding Black pepper, Cinnamon, Clove, and dry ginger. The yogurt formed was subjected to physicochemical and microbiological analysis. The time required for yogurt formation was found to be 10 h. The yogurt samples fortified with spices were found to be of smooth texture, white color having a pleasant smell and sour taste.

***Key words**: Yoghurt, Fortification, Spices, Physicochemical analysis, Microbiological analysis.*

Introduction

Curd, a fermented dairy product, is a staple in many households and is known for its various health benefits. India is the world's largest producer of milk, a significant amount of liquid milk is utilized for traditional dairy products. Incorporating herbal bioactives into conventional Indian dairy products facilitates to compete in the global functional food market, which is growing at a rapid pace (Sawale *et al.*, 2013).

The indigenous knowledge of traditional medicinal plants is becoming more widely recognized around the world. Since it is the world's largest producer of traditional medicinal herbs, India is considered the "Botanical Garden of the World" and "Land of Spices" (Modak *et al*., 2007). Herbs and their extracts have been used to fortify foods as preservatives, flavorings, medicinal agents, and food additives throughout history (Samah and Ahmed, 2019). Consumer knowledge and interest in adding herbs to dairy and food products to enhance dietary strategy and balanced nutrition for achieving health benefits from foods beyond providing basic nutrition have increased worldwide as a result of the advent of functional foods and consequently, the demand for value-added functional foods has grown worldwide (Kumar *et al.*, 2016). Scientific literature has established a clear correlation between food habits and disease prevention as evidenced by the impact of various diseases such as diabetes, obesity, osteoporosis, hypertension, and cardiovascular diseases on individuals' dietary choices (WHO, 2003).

Natural bioactive compounds, such as flavonoids, polyphenols, phenolic acid, terpenoids, sulfides, carotenoids, coumarins, lignans, saponins, curcumins, phthalides, and plant sterols, have been found in herbs and their extracts exhibiting several biological effects such as antimicrobial, anti-inflammatory, antioxidant, antiallergic and antihyperten-

sive effects. Herbs are also high in natural antioxidants which inhibit or prevent the oxidation process. Synthetic antioxidants used in the food industry may pose serious health risks due to their volatility and easy decomposition at high temperatures. Hence, herbal phytochemical-based natural antioxidants are in high demand among consumers. Phenolic compounds present in herbs demonstrate antioxidant properties and have been shown to prevent or reduce the risk of degenerative diseases such as diabetes, cancer, obesity, and cardiovascular disease(Poonia, 2020). Herbs' antimicrobial properties can effectively regulate the growth of spoilage and pathogenic bacteria in dairy products. Phenolic compounds are the primary antimicrobial compounds found in herbs and can be used as effective replacements for artificial antimicrobial agents used in food production. Phenolic compounds, such as tea catechins, coumaric acid, ellagic acid, oleuropein, and ferulic acid have been found to inhibit some pathogenic bacteria and fungi. Furthermore, the essential oils present in various herbs contain bioactive compounds that exhibit antimicrobial properties in addition to anti-inflammatory, anticarcinogenic, and other beneficial health-promoting activities. The use of synthetic chemical additives in food processing poses several health risks and essential oils present in herbs can be used as substitutes (Magdi, 2015; Shan *et al.,* 2011 and Bakkali *et al.,* 2005).

In recent years, there has been a growing interest in fortifying curd (Yoghurt) with different spices due to their potential health-promoting properties. The addition of spices to curd not only enhances its nutritional value but also imparts unique flavors and textures. However, the physicochemical and microbiological changes that occur during the fermentation process when spices are added to curd are not well understood. This has led to a surge in research investigating the effects of spices fortification on the curd. This study aimed to study the physicochemical and microbiological analysis of curd fortified with spices (Yoghurt) (Poonia, 2020; Burt, 2004).

Material and Methods

(Lab manual, 2015; Ranganadham, 2016; Jayaraman and Jayaraman, 1981; Harrigan and MacCane, 1976; Whitman, 2004; Chellapandi, 2007)

a) **Sample Collection and Preparation**: Samples of Black pepper (*Piper nigrum*), Cinnamon (*Cinnamomum venum*), Clove (*Synzigium aromaticum*), and ginger (*Zingiber officinale*) were collected from the local market of Karad, Maharashtra, India. The powders of these ingredients were chosen for the study. Pasteurized milk and curd were also procured from the same market.

b) Preparation of Curd by Adding Species: Curd was prepared in the laboratory using pasteurized milk. Thirty milliliters of milk were collected in sterile beakers, boiled, and cooled to 45°C. In each of the five beakers, 30 ml of milk was added and 1 ml of curd sample was inoculated into each beaker. The flasks were labeled F1, F2, F3, and F4 and inoculated separately with 5mg each of black pepper (*Piper nigrum*), cinnamon (*Cinnamomum verum*), clove (*Synzigium aromaticum*), ginger (*Zingiber officinale*), respectively. All beakers were kept for incubation at 30 ^{0}C and observed after 4-6 h for curdling. After the formation of curd, samples were analyzed for physicochemical and microbiological properties after 12, 24, 36, and 48 h.

c) Preparation of Curd by Traditional Method (Control set): Curd was prepared in the laboratory using traditional ingredients. Pasteurized milk was used and 30 ml of it was collected in the sterile beaker, boiled, and cooled to 45°C. One milliliter of homemade curd was added to the milk and the beaker was kept covered for incubation at 30 ^{0}C. The curd was observed after 6, 12,24, 36, and 48h and analyzed for its physicochemical and microbiological properties for comparison with fortified curds (Yoghurts).

d) Physico-chemical Analysis of Fermented Milk and Curds Fortified with Spices

Following tests were carried out to determine the physicochemical properties of the fermented milk (curd) and curds fortified with the spices:

The organoleptic tests

- Smell determination: Curd was picked with a spoon and its aroma and smell were noted.
- Taste determination: Curd was picked with a spoon and its taste was noted.
- Color determination: Curd was picked with a spoon and its color was noted as white /off-white color.
- Texture and Consistency determination: Curd was picked with a spoon and its texture was noted as loose or close to close texture by cutting with the knife.

The titratable acidity and pH: It was determined by titrating a 10 g curd sample with 0.1 N NaOH using 0.5% phenolphthalein as an indicator. The titratable acidity was calculated using the formula: 0.1 X Burette reading X100/Sample taken in g. The pH of the curd sample was measured at different stages of sample preparation including during fermentation, in the finished product, and during storage using a pH meter (Chellapandi, 2007)

The total solid content was determined using the gravimetric method. (Chellapandi, 2007)

Specific gravity was determined using a specific gravity bottle method.

The fat content was determined using Gerber's butyrometer method.

The protein content of fermented milk was determined using the Biuret method (Lowry *et al*., 1951).

e) Microbiological analysis of fermented milk: (Cruickshank, 1985; Sivakumar and Kalaiarasu, 2010):

Determination of Total Viable Count for bacteria

The Total Viable Count (TVC) of the curd samples was determined using the standard plate count method by inoculating 1 ml of diluted sample on MRS medium and nutrient agar plates by the spread plate method and incubated at 30 °C for 48 h.

Determination of coliform count: Coliform count was determined by the MPN method.

Microscopic examination of curd sample:

The curd sample was subjected to Gram staining and the slide was observed under a microscope. The results were noted down.

Plate count for yeast and molds was taken using the standard plate count method on Sabouraud's agar.

Results and Discussion

The time required for curd formation was found to be an average of 10 h (Photoplate 1).

The study examined the quality and properties of curd fortified with black pepper over a period of 48 h. The curd exhibited a pleasant smell, sour taste, firm body, soft consistency, white color, and smooth and thin texture for up to 24 h. However, after 24 h, the curd had a rancid smell, sour taste, slightly yellow color, and ropy texture.

Photoplate 1. Showing curd samples fortified with different spices and control set

The pH of the curd decreased from 4.8 at 12 h to 4.4 at 24 hours and then remained constant at 4.5-4.4 at 36-48 h. The titratable acidity increased from 2.2% at 12 h to 4.0% at 48 h. The fat content remained constant at 4.3% for up to 24 h. but then decreased (lipolysis-rancidity) to 3.5% at 36-48 h. The sugar content decreased from 3.5g at 12 h to 2.5g at 48 h, while the protein content decreased from 6.5g at 12 h to 4.0g at 48 h. (Table 1).

The study investigated the quality and properties of curd fortified with cinnamon over a period of 48 h. The curd had a pleasant smell, sour taste, soft consistency, white color, and smooth texture for up to 24 h. However, after 24 hours, the curd had a rancid smell, sour taste, slightly yellow color, and ropy texture.

The pH of the curd decreased from 5.1 at 12 hours to 4.0 at 48 h. The titratable acidity increased from 2.5% at 12 h to 3.9% at 48 h. The fat content decreased from 4.2% at 12-24 h to 2.8% at 36-48 h. The sugar content remained constant at 3.5-3.7g up to 36 h and then decreased to 3.1g at 48 h. The protein content decreased from 6.5g at 12 h to 5.0g at 48 h. (Table 1).

This study investigated the effect of Clove fortification on the quality attributes of curd over time. The curd fortified with Clove was formed within 10 hours and exhibited a pleasant smell, sour taste, firm body, soft consistency, white color, and smooth and thin texture up to 24 h. However, after 24 h, it developed a rancid smell, sour taste, firm body, soft consistency, slightly yellow color, and ropy texture. The pH decreased from 5.5 to 4.0 over 48 h, while the titratable acidity decreased from 3.5% to 2.5%. The fat content remained constant at around 4.3%, while the sugar content decreased from 3.5 g to 2.65 g. The protein content also decreased from 5.6 g to 3.3 g over the 48-h period. These results suggest that Clove fortification has a significant effect on the quality attributes of curd over time (Table 2).

curd fortified with Dry Ginger formed within 10

Table 1. Study of Physicochemical properties of curd(Yoghurt) fortified with Black pepper and Cinnamon

Sr. No.	Test	Curd fortified with Black pepper.				Curd fortified with Cinnamon.			
		After 12h	After 24h.	After 36h.	After 48h.	After 12h.	After 24h.	After 36h.	After 48h.
1	Smell	Pleasant	Pleasant	Rancid	Rancid	Pleasant	Pleasant	Rancid	Rancid
2	Taste	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour
3	Body	Firm	Firm	Firm	Firm	Firm	Firm	Firm	Firm
4	Consistency	Soft	Soft	Soft	Sticky	Soft	Soft	Soft	Soft
5	Color	White	White	Slightly yellow	Slightly yellow	White	White	Slightly yellow	Slightly Yellow
6	Texture	Smooth	Smooth	Smooth	Ropy	Smooth	Smooth	Smooth	Ropy
7	pH	4.8	4.4	4.5	4.4	5.1	5	4.5	4
8	Titratable acidity	2.20%	2.25%	3.70%	4.00%	2.50%	2.60%	2.90%	3.90%
9	Fat %	4.3	4.3	3.5	3.5	4.20%	4.20%	2.80%	2.80%
10	Sugar	3.5g	3.5g	2.7g	2.5g	3.7g	3.5g	3.5g	3.1g
11	Protein	6.5g	6.3g	5.03g	4.0g	6.5g	5.7g	5.5g	5g

Table 2. Study of Physicochemical properties of curd (Yoghurt) fortified Clove and Dry Ginger

Sr. No.	Test	Curd fortified with Clove.				Curd fortified with Dry Ginger.			
		After 12 hrs.	After 24 hrs.	After 36 hrs.	After 48 hrs.	After 12 hrs.	After 24 hrs.	After 36 hrs.	After 48 hrs.
1	Smell	Pleasant	Pleasant	Rancid	Rancid	Pleasant	Pleasant	Rancid	Rancid
2	Taste	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour
3	Body	Firm	Firm	Firm	Firm	Firm	Firm	Firm	Firm
4	Consistency	Soft	Soft	Soft	Soft	Soft	Soft	Soft	Soft
5	Color	White	Slightly yellow	Slightly yellow	Slightly Yellow	White	Slightly yellow	Slightly yellow	Slightly Yellow
6	Texture	Smooth	Smooth	Smooth	Ropy	Smooth	Smooth	Watery	Ropy
7	pH	5.5	5.1	4	4	5.8	5.6	4.9	4.2
8	Titratable acidity	3.50%	2.80%	2.80%	2.50%	2.50%	3.50%	4.20%	4.50%
9	Fat %	4.50%	4.30%	4.30%	4.30%	3.80%	3.80%	3.80%	2.80%
10	Sugar	3.5g	3.5g	2.7g	2.65g	4.5g	4.2g	4.2g	3.8g
11	Protein	5.6g	5.0g	4.7g	3.3g	5.7g	5.2g	5.2g	4.0g

hours and had a pleasant smell, sour taste, soft consistency, white color, and smooth texture up to 24 h. After 24 h, it had a rancid smell, sour taste, firm body, soft consistency, slightly yellow color, and ropy texture. The pH of curd fortified with Dry Ginger decreased from 5.8 to 4.2 over 48 h, while the titratable acidity increased from 2.5% to 4.5%. The fat content decreased from 3.8% to 2.8%, and the sugar content decreased from 4.5g to 3.8g (Table 2).

While the curd made without the addition of spices(Control) had an acidic smell, soft texture, and yellow color after 24 h.

Conclusion

- Curd fortified with Dry Ginger (Sunthi) showed stable pH, titratable acidity, taste, smell, and consistency even after 36 h at 30 ^{0}C.
- The addition of spices during curdling enhances the shelf life of curd.
- Dry Ginger (Sunthi) can be used as a natural preservative for curd at 30 ^{0}C.
- Further studies should be conducted to analyze the microbiological aspects of curd fortified with Dry Ginger (Sunthi), including the isolation and characterization of microorganisms.

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Table 3. Results of microbiological analysis of fermented milk(Yoghurt):

Name of test	Curd fortified By adding black paper herb powder	Curd fortified By adding cinnamon herb powder	Curd fortified By adding clove herb powder	Curd fortified By adding ginger herb powder
SPC for bacteria	3.25 $X10^5$	2.89 $X10^5$	3.45 $X10^5$	3.20 $X10^5$
SPC for Yeast and Molds	24	25	21	28
Coliform Count	Less than <2	Less than <2	Less than <2	Less than <2
TVC	2.6 $X10^6$	2.8 $X10^6$	3.0 $X10^6$	2.5 $X10^6$

the research facilities to conduct the work.

Conflicts of Interests

The authors declare that there are no conflicts of interests

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