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Effect of Essential Oil of Tejpat, Black Pepper, and Cardamom as a Natural Food Preservatives for Sweet Orange (*Citrus sinensis*) Juice

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Abstract

Essential oil of tejpat (*Cinnamomum tamala*), black pepper (*Piper nigrum*) and cardamom (*Amomum subulatum* roxb.) collected by hydro distillation, and were tested as natural food preservative in orange juice (*Citrus sinensis).* The physiochemical changes such as pH, TSS, acidity, total microbial count, yeast and mold count were evaluated at fix time intervals of 4 hours. for 24 hours. It has been observed that the juice sample infused with black pepper essential oil demonstrated superior preservation of TSS, pH and acidity compared to the other variants. Orange juice supplemented with essential oils maintained the microbial count and yeast and mold count below 100 CFU/ml for up to 24 hours. After 24 hours of storage, all samples surpassed the total microbial count. Among the essential oils, black pepper essential oil demonstrated superior preservation compared to the others.

Keywords: Essential oil; *Cinnamomum tamala; Piper nigrum; Amomum* roxb; *Citrus sinensis*; shelf-life.

INTRODUCTION

Sweet orange, scientifically known as *Citrus aurantium*, is a member of the *Rutaceae* family and the Citrus genus, commonly referred to as sweet orange, and thrives in temperate and subtropical regions. The *C. aurantium* tree can reach a height of up to 6 meters, featuring leathery foliage and fragrant white blossoms (Azadi et al., 2012). Citrus ranks among the most significant fruit crops globally due to its substantial nutritional benefits, extensive fruit yield, and various processed products, as noted by Chaturvedi et al. (2001). Orange juice is both nutritious and revitalizing due to its vitamin C content, delightful tangy flavor, attractive hue, pleasant aroma, and health benefits (Kapoor et al., 2012). Nevertheless, after just a few hours of extraction, the juice begins to spoil, resulting in a loss of its color, flavor, and aroma. This spoilage is attributed to significant microbial presence and enzymatic activity, which degrade the sensory and nutritional properties of the juice, rendering it unsuitable for consumption. The primary factors contributing to this deterioration are likely the proliferation of microbes, chemical reactions, changes in structure, and the conditions under which it is stored (Kapoor et al., 2012). Additionally, potential health risks linked to the consumption of fruit juices containing synthetic preservatives have led to a notable shift in public perception regarding these drinks (Moses, 2018). Therefore, it is essential to explore additional antimicrobial substances derived from plant sources.

Lately, there has been a growing interest in utilizing plant-based antimicrobials in scientific studies and potentially for food preservation (Pandey et al., 2017). Essential oils (EOs) are typically intricate yet volatile substances that possess a strong aroma. These compounds are generated by fragrant plants as secondary metabolites in various parts of the plants (Bakkali et al., 2008). Essential oils, often referred to as volatile odoriferous oils, are fragrant oily liquids obtained from various parts of plants, such as leaves, peels, barks, flowers, buds, and seeds. These oils can be extracted from plant materials using several techniques, including steam distillation and expression. Among these extraction methods, steam distillation is particularly popular, especially for production on a commercial scale (Cassel and Vargas 2006). Essential oils are recognized for their antioxidant and antimicrobial properties, making them effective natural additives in food and food products. (Tongnuanchan and Benjakul, 2014). Because of the growing interest in natural additives, essential oils derived from various plants have gained more popularity, particularly when used alongside other preservation methods within the framework of “hurdle technology.” As a result, essential oils can act as alternative additives or processing aids in the realm of green technology.

MATERIALS AND METHOD

Extraction of essential oil

Cinnamomum tamala (*tejpat*), *Piper nigrum* (black pepper), and *Amomum subulatum roxb* (cardamom), plant spices were purchased from the local market of Pokhara, Nepal. The essential oil was obtained through the hydrodistillation method utilizing a Clevenger-type apparatus, following the procedures outlined in the European Pharmacopeia (1983).

Sample preparation

Ripe, mature, and uniform orange fruits were thoroughly washed with running tap water, manually peeled, deseeded, and the pulp blended using an electric juicer in a hygienic laboratory environment. The resulting orange juice was then strained through a clean muslin cloth and separated into four equal portions of 100 mL each. Essential oils (10 mL) and each oleoresin were individually dissolved in 90 mL of ethanol, then combined homogeneously with extracted juice (50 mL) in plastic cups. The control sample consisted solely of orange juice, while the control was made by mixing 100 mL of ethanol with orange juice (Kapoor et al., 2014).

Storage

The control and treated samples having essential oils were stored at normal room temperature (25±5°C) for 24 h in laboratory condition. The effect of storage on physiochemical changes, and microbial count were observed after an interval of 4h.

Determination of moisture content

The moisture determination of the fresh orange was carried out by hot-air oven method (Ranganna, 2008)

Determination of titratable acidity and pH

Acidity was determined by titration with the standard sodium hydroxide (0.1N) solution and expressed as % malic acid (Ranganna, 2008). pH was determined using pH meter.

Determination of TSS

TSS was determined by using hand refractometer

Microbial analysis

In order to determine the total microbial count and yeast and mold counts, total plate count method as described by Aneja (2010) for the examination of juice samples using the plate count agar and potato–dextrose agar, respectively, were adopted

Total microbial count

The total microbial count was conducted following the method outlined by (Kapoor et al., 2014). Five g sample of juice was taken and diluted with 90 mL of Ringer's solution in a 1:10 ratio. After thorough mixing, 1 mL of the sample was added to 10 mL of dextrose-tryptone agar medium, which was then placed in a sterilized Petri dish at 45°C and mixed well. The mixture was subsequently incubated at 37°C to observe the colonies that developed in each Petri dish. The findings were recorded as the number of colonies present in 1 mL of the juice sample, expressed in cfu/mL.

Yeast and mold count

Yeasts and molds were quantified using acidified potato dextrose agar (PDA) following the Standard Methods for the Examination of Dairy Products (Kapoor et al., 2014). The procedures for the experiments remained identical to those used for the total microbial count, with the only variation being the substitution of dextrose-tryptone agar with PDA medium. All microbiological analyses were performed in triplicate, and the results are represented as colony-forming units per milliliter.

RESULTS AND DISCUSSION

Chemical composition of fresh sweet orange juice

The moisture content of citrus sinensis (Sweet orange) was measured at 85.23% as shown in **Table 1** below. Orange juice predominantly consists of water, typically containing between 85% and 90% water. This high-water content makes orange juice very perishable, necessitating specific preservation methods such as refrigeration, pasteurization, or freeze-drying to prolong its shelf life (Sharma et al., 2018). The acidity level and pH of citrus sinensis (Sweet orange) were found to be 2.83% and 5.02, respectively which is shown below **Table 1**. The acidity of both orange juice and the peel significantly affect their flavor, stability, and nutritional value. Typically, orange juice has a pH range of approximately 3.3 to 4.2, categorizing it as a moderately acidic drink. This acidity primarily results from organic acids, chiefly citric acid, which comprises more than 90% of the total acid content, alongside smaller quantities of ascorbic acid (vitamin C) and malic acid (Ladaniya et al., 2003). The low pH contributes to the tangy taste of orange juice and also serves as a natural preservative by inhibiting the growth of numerous bacteria, thereby extending the juice's shelf life (Boumediene et al., 2018). The total soluble solids (TSS) content of citrus sinensis (Sweet orange) was recorded at 10.3ºBx as shown in **Table 1** below. TSS, expressed in degrees Brix (°Brix), reflects the percentage of soluble solids in a liquid. In freshly squeezed orange juice, the TSS generally varies from 10 to 12 °Brix, influenced by factors such as the variety of orange, its ripeness, and the conditions under which it was grown (Kelebek & Selli, 2011).

Table 1 Chemical composition of fresh sweet orange juice.

Parameters	Percentage %
Moisture	85.23±1.25
Acidity	2.83±0.05
TSS	10.3±0.08
Ph	5.02±0.06

Values are means ± standard deviation of triplicate analysis

Effects of essential oils on the physiochemical properties of orange juice

TSS

The fresh juice extracted from citrus sinensis was subsequently blended with three different essential oils, and it was observed that the total soluble solids (TSS) of all samples decreased as storage days increased. The initial TSS of the control juice sample was 10.3°Bx, which diminished to 8.6°Bx over time. Likewise, the TSS values for S1 (black pepper), S2 (cardamom), and S3 (cinnamon) were recorded at 10°Bx, 9.7°Bx, and 9.6°Bx, respectively, after 24 hours of storage, as indicated in Table 2 below. The juice sample infused with black pepper essential oil demonstrated superior preservation of TSS compared to the other variants. The Total Soluble Solids (TSS) levels in orange juice generally decline over time while being stored; however, the degree of this reduction is influenced by variables like storage temperature, type of packaging, and microbial activity (Kapoor et al., 2009).

pH

The fresh juice of citrus sinensis was further combined with three different essential oils, and it was observed that the pH of all samples decreased with the increase in storage days. The control sample of juice had an initial pH of 5.02, which subsequently decreased to reach 4.0. Likewise, the pH values for S1 (black pepper), S2 (cardamom), and S3 (cinnamon) were recorded as 4.7, 4.5, and 4.2 after 24 days of storage, as detailed in Table 2 below. Among the juice samples, the one with black pepper essential oil exhibited superior pH preservation compared to the other samples. The pH tends to decline progressively as titrable acidity rises. The drop in pH observed during storage could be attributed to acids produced from fructose, glucose, and various carbohydrates (Kapoor et al., 2007). This reduction in pH might be linked to the elevated concentration of essential oil, which leads to a relatively higher acidity and other biochemical alterations that contribute to the decrease in pH (Kapoor et al., 2009).

Acidity

The fresh juice from citrus sinensis was combined with three different essential oils, and it was observed that the acidity of all samples increased as the number of storage days progressed. The acidity of the control juice sample started at 2.83 and rose to 3.2% after storage. Likewise, the acidity levels of S1 (black pepper), S2 (Cardamom), and S3 (Cinnamon) were recorded at 2.86%, 2.92%, and 2.93% after 24 days of storage, as indicated in Table 2. Among the juice samples, the one with black pepper essential oil demonstrated superior preservation of acidity compared to the other samples. The rise in acidity during storage can be attributed to the breakdown of lactose (Grewal and Jain 1982) resulting in the conversion of sugars into acids (Kapoor et al., 2007) and microbial contamination, which produces lactic acid from hexose sugars. A comparable increase in acidity percentage was noted for kinnow by (Panesar et al., 2000) and for orange juice by (Kapoor et al., 2012).

Effect of essential oils on total microbial count of orange juice during storage

In all the samples depicted in the **Figure 1**, microbial loading increased with prolonged storage time. Orange juice supplemented with essential oils maintained the microbial count below 100 CFU/ml for up to 24 hours. After 24 hours of storage, all samples surpassed the total microbial count. Among the essential oils, black pepper essential oil demonstrated superior preservation compared to the others. The effectiveness of essential oil is attributed to the higher percentage of monoterpene hydrocarbons, which may be key in inhibiting microbial growth, as noted by (Kapoor et al., 2014), who described their synergistic effects. Kapoor et al. (2014) also highlighted the combined action of different phenolic compounds, piperine, flavonoids, and various other components that suppress the growth of these microbes. The antifungal properties of the essential oils evaluated may stem from their phenolic, alcoholic, and aldehydic components, which have been identified as antimicrobial agents (Kapoor et al., 2014). Generally, cinnamon essential oil is recognized as a strong antimicrobial substance (Burt, 2004).

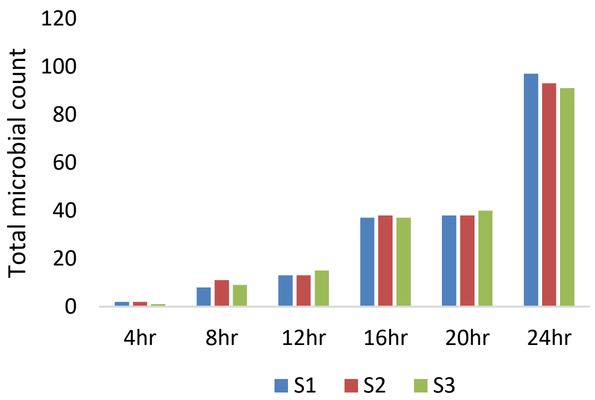

Figure 1. Effect of essential oils on total microbial count of orange juice during storage.
Here, microbial count of control sample 1CFU/ml after 4 h exceed more than 100 CFU/ml. S1 (black pepper), S2 (Cardamom) and S3 (Cinnamon).

Effect of essential oils on yeast and mold count of orange juice during storage

The yeast and mold counts increase with prolonged storage period as shown in **Fig 2**. Initially The rise in yeast and mold, along with their microbial contamination, may also result from the proliferation of acid-producing bacteria (Azhdarzadeh & Hojjati, 2016). According to Tserennadmid et al. (2011), lemon essential oil was effective in suppressing the growth of S. cerevisiae in clear apple juice. This finding is reinforced by Ndagijimana et al.'s (2004) study, which demonstrated similar effects when orange juice was treated with the same essential oils. (Eissa et al., 2008) found that apple juices infused with lemon grass, clove, and rosemary extracts exhibited the strongest suppression of yeast and molds (Y and M) as well as bacteria (B), while those treated with basil and sage extracts showed slightly lower levels of inhibition after 4 weeks at 4°C.

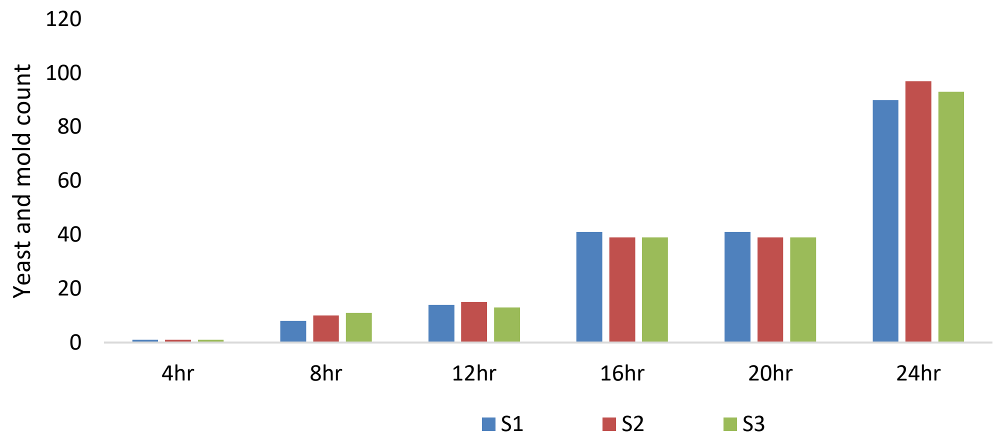

Figure 2. Effect of essential oils on yeast and mold count of orange juice during storage
Here, microbial count of control sample 1CFU/ml after 4 h exceed more than 100 CFU/ml. S1 (black pepper), S2 (Cardamom) and S3 (Cinnamon)

Sensory evaluation

The mean value for color was highest in sample S1 and lowest in sample S3. The essential oil does not affect the color of the samples, but panelists favored the black pepper sample over the others. The control sample had the lowest color value due to the rapid change in color of the untreated juice, which led to noticeable browning. The highest mean value for flavor was found in sample S1, while the lowest was in the control sample S. Many panelists preferred the taste of the essential oil juice in comparison to the control sample. The mean values for aroma were quite similar across all samples, but the black pepper essential oil juice received the highest ratings, as most panelists preferred its aroma over the others. The overall acceptability was highest for sample S1 and lowest for the control sample S3.

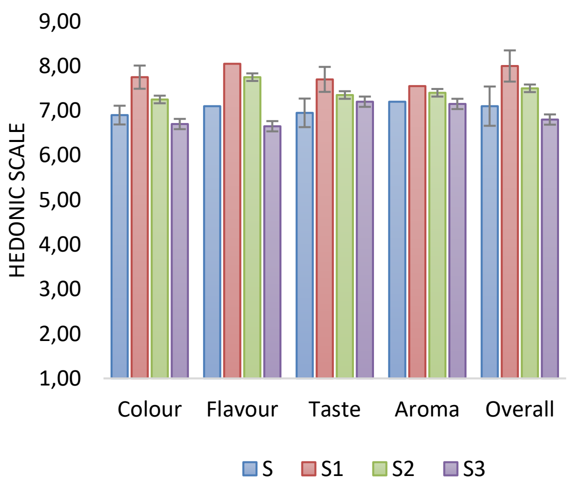

Figure 3. Sensory evaluation
S (control), S1 (black pepper), S2 (Cardamom) and S3 (Cinnamon)

CONCLUSION

The current research indicated that essential oils from black pepper, tejpat, and cinnamon can effectively inhibit microbial growth, which poses a risk to human health. Therefore, it can be inferred that incorporating black pepper, *tejpat*, and cinnamon essential oils into orange juice could aid in extending its shelf life by slowing down the proliferation of spoilage microorganisms. The longevity of orange juice has been shown to improve. These findings could serve as a significant basis for creating efficient natural preservatives.

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Conflicts of interest: The authors declare no conflict of interest

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