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Dual Role of Raspberry Ketone as Antioxidant and Flavoring Agent in Citrus Flavors

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Abstract

Objectives: To improve the shelf life and protect the citrus oils degradation. Citrus oils have been used since long in flavor and fragrance industry and degrade periodically when exposed to oxygen, high temperatures and low pH. Strategic studies employing an alternative antioxidant may find a solution. **Method:** Antioxidant study was performed by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), pH assessment, and sensory analysis, to test the response of raspberry ketone on citrus oils -orange and lemon oil to improve the shelf life. **Findings:** Raspberry ketone exhibits 70% antioxidant activity in citrus oils (lemon and orange oil) when compared to the antioxidants BHA (76.5%) and tocopherol (76.7%). Antioxidants stabilize the pH of citrus oils. The pH drop of citrus oils is approximately from 4.58 to 3.77, and in the presence of antioxidants, it ranges from 4.52 to 4.07. The sensory scale reports that the likeability of citrus oils with raspberry ketone is good when evaluated in black tea and hardboiled candy, without any off-taste, compared to BHA and tocopherol at a 0.15% dosage. Thus, the addition of raspberry ketone as a flavor and fragrance agent ($\leq$ 0.15%) can also serve as an antioxidant to combat the self-degradation process of citrus oils. **Novelty:** This study reveals a solution to protect citrus oil degradation by using Raspberry ketone, a flavoring agent as well as antioxidant to avoid the usage of BHA and tocopherol.

Keywords: Citrus oils; BHA; Tocopherol; Raspberry ketone; Sensory; DPPH agent

1 Introduction

Citrus essential oils are present in the peel of the citrus fruits. They are aromatic, refreshing and embedded in the flavedo part of the peel. Extraction of citrus oils are primarily done from the peel of the fruit, and they consist of both volatile (85-99%) and non-volatile compounds (1-15%) [1]. There are different extraction methods other than steam distillation, such as cold pressed method, solvent extraction, and carbon dioxide extraction. Citrus oil undergoes self-degradation if it is not stored under cold temperature conditions [2]. Citrus fruits are rich in ascorbic acid, and essential oils, and the peel of citrus fruit is rich in polyphenols and flavonoids, which act as an antioxidant for the short term, depending on storage conditions. Consumption of citrus fruits provides excellent health benefits and acts as preservatives for edible oils [3]. Abundant research has been known since the archaic era, but one of the most challenging points to avoid degradation of citrus oils is to sustain stability under different storage conditions.

There are various intrinsic and extrinsic factors that impact the stability or shelf life of citrus oils, especially storage conditions, which influence the process of degradation. The chemical composition of citrus oils reveals they are sensitive and decompose, releasing off notes because of various factors like light, oxygen, and temperature [4]. Citrus oils undergo self-degradation process due to autooxidation under accelerated conditions when stored in UV light and visible light [5]. During the degradation process aldehydes present in citrus oil break down periodically during storage and release acids, which indicates there is a decline in pH value after degradation [6]. Antioxidants play a vital role in preserving food and its derivatives.

Natural components of green tea, rosemary, spice extracts and tocopherols were tremendously used as antioxidants in flavor; however the challenging aspect is that natural extracts are expensive with less yield. Chemical antioxidants like Ascorbic acid, Sodium ascorbate, and Calcium ascorbate used in soft drinks, jams, and BHT (Butyl hydroxy anisol) and BHA (Butyl hydroxy toluene) are used in sweets, butter, and flavoring substances, but they are prohibited in many countries as they are considered as carcinogens. Antioxidant activity can be measured by DPPH assay (2,2-Diphenyl-1-picrylhydrazyl. DPPH in coordination with antioxidants undergoes reduction to form DPPHH and changes the color from purple to yellow [7]. Research studies were done to find an alternative antioxidant to overcome the degradation process of citrus oils. The challenge of maintaining the stability of citrus oils is continual, as oxidized components are released when not properly stored, which releases off-notes that are not acceptable in the flavor industry, where aroma and taste are key parameters to be measured.

Stability studies can be reconfirmed by performing sensory analysis. Sensory analysis will give us a better understanding of the extent of degradation and the use of antioxidants to prevent or slow down the degradation process. Though there are many analytical instruments, human sensors cannot be completely replaced or compared, and hence the organoleptic study of individuals derives the sensory attributes of the food or flavor [8]. Sensory attributes of citrus oils can be characterized based on the odor and taste. The fruity and sourness of citrus oils are the main key descriptors due to the high content of limonene and aldehydes. To understand the sensory profile of essential inputs, evaluations were recorded from both trained and untrained panel members [9]. This research aims to investigate and conduct a new research using a flavoring agent, raspberry ketone, on citrus oils as an antioxidant, with a comparative study using BHA and tocopherol as standard antioxidants.

2 Methodology

- Citrus oils extracted by steam distillation from citrus peel-Lemon and orange [10]
- pH meter
- Stability chamber, Refrigerator
- DPPH agent-(2,2-Diphenyl-1-picrylhydrazyl) from Sigma Aldrich -2.4mg DPPH dissolved in 100ml of Methanol solution was taken for this experiment
- BHA, tocopherol, and Raspberry ketone of 100% purity from Mane were taken as antioxidants

2.1 DPPH assay of citrus oil and pH Assessment

Selected 25μl of citrus oils (Lemon and orange-1% in Methanol) with 0.1% of antioxidants BHA/tocopherol/raspberry ketone were taken in separate test tubes and mixed with 975μl of DPPH solution (2.4mg in 100ml Methanol). All the samples were kept for incubation, with positive controls having BHA, tocopherol, and flavoring agent raspberry ketone. Raspberry ketone antioxidant activity against citrus oils was compared with positive controls, and a blank was maintained without any antioxidant. After incubation, spectrophotometric readings were recorded at 525-528nm and the values are incorporated into the formula below [11]. Color difference was also recorded from purple or deep blue to yellow.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{(OD\ blank\ -\ OD\ sample)}{OD\ blank} \times 100$$

The pH of lemon and orange oils was analysed after one month for samples stored at different temperatures (4°-8°C in a VC cooler and 42°C in a stability chamber) using a pH meter to check the efficiency of raspberry ketone as an antioxidant, compared with BHA/tocopherols.

2.2 Preparation of Hard-boiled candy and black tea

After one month, all the citrus oils with Raspberry ketone (0.15%, 0.25% and 0.5%.) stored at different temperatures were evaluated in hard boiled candy and black tea. For hard boiled candy, ingredients were taken as per Table 1 in a small vessel and heated up to 140°C and citrus oils with selected antioxidants, citric acid, and sunset yellow colour were added and mixed well. The contents were poured into the mold and allowed to cool.

Table 1. Formulation for hard boiled candy

S.No	Ingredients	Quantity (g)
1	Citrus oil with Raspberry ketone	0.35%
2	Water	14.2
3	Sugar	47.2
4	Glucose syrup	38.5

Citrus oils with antioxidants (0.15%, 0.25% and 0.5%.) were dosed at 1% in Tata Gold tea base and mixed uniformly. The samples were allowed to stand overnight at room temperature. For brewing, 2g of sample tea base was taken in a beaker, and 80 ml of boiling water was added. A blank, without any oils or antioxidants, was used for comparison. The contents were brewed for 5 minutes (without stirring while brewing). After 5 minutes, the contents were mixed once (single stir) and filtered using a tea strainer, then proceeded for tasting.

2.3 Sensory analysis of Citrus oils

A total of 10 trained panellists were selected for evaluation to assess the attributes of citrus oils. Six attributes were considered for the orange and lemon oils: juiciness, peeliness, pithiness, zestiness, sweetness, and sourness. For all the attributes, hedonic scaling was recorded on a scale from 1 to 9 (1 = dislike to 9 = likeability). The intensity of each sample at different dosages of antioxidants (0.1%–0.5%) was recorded to understand the interference of raspberry ketone (berry profile) in citrus oils, and comparative studies were performed with BHA and tocopherol.

Sensory analysis to determine the attributes and hedonic scaling profiling of orange oil and lemon oil in hard-boiled candy and tea were performed by maintaining triplicates for each test sample. The obtained data were represented as the mean of triplicates ± standard deviations (SD). The results were interpreted using a two-way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT). The major differences between each sample were correlated at the ratio of ($p \leq 0.01$) [12].

3 Result and discussion

Literature review discloses citrus oil has effective antioxidant activity, but it is unstable over a period and to overcome this, antioxidant (natural/synthetic) is used predominantly [13].

3.1 Antioxidant activity & pH assessment

In the present study, the antioxidant activity results reveal that after one month of shelf-life, the selected lemon oil exhibited 2.2%, orange oil 24%, and the test antioxidant raspberry ketone 70%, with positive controls such as BHA (76.5%) and tocopherol (76.7%). Orange oil with raspberry ketone exhibited ±68%, with BHA ±81%, and with tocopherol ±82%. Lemon oil with raspberry ketone displayed ±62%, with BHA ±83%, and with tocopherol ±83%. The antioxidant activity of citrus oils increased upon the addition of BHA, tocopherol, and raspberry ketone, separately (Figure 1).

Previous reports state that synthetic antioxidants like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were used as positive standard controls by Umit Erdogan to test the efficiency of essential oils' antioxidant activity [14]. The above results were compared, and the percentage of DPPH activity for orange (24-25%) and lemon (33-34%) fruits was noted, with Vitamin C showing 160-170% for orange and 217-220% for lemon [15]. Radical scavenging, determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity, revealed that the purple color of DPPH changed to yellow by forming diphenylpicrylhydrazine. The antioxidant activity of the citrus essential oils was shown to be 65% for *C. limonum*, 72% for *C. paradisi*, and 76.02% for *C. reticulata*, which was lower when compared to the BHT control [16].

pH assessment was done after one month, as the weekly data difference was very minimal (±0.01 to 0.02) for the samples stored at refrigerated temperatures (4-8°C, VC cooler) and in the stability chamber at 42°C. The pH of orange oil and lemon oil dropped in both temperature settings, especially at the accelerated temperature. When these oils were stored along with antioxidants (BHA/tocopherol) and the test antioxidant or flavoring agent raspberry ketone, there was a ±0.1-0.3 less pH drop compared to pure oils without antioxidants (Figure 2). There is limited research available on the effect of pH, although reports suggest that added antioxidants help stabilize citrus oils by inhibiting the oxidation process [17]. Oxidation can be prevented by storing the essential oils and fats at lower temperatures, or alternatively, by using proven antioxidants like BHA and BHT [18].

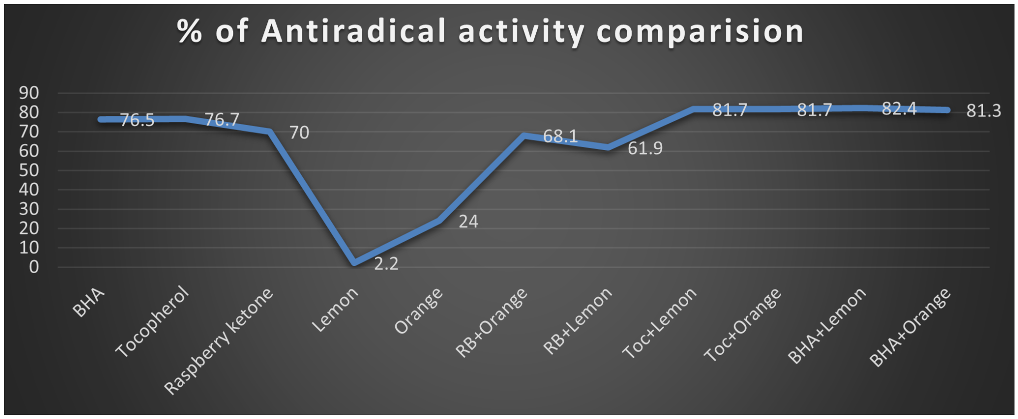

Fig 1. Comparative study of Raspberry ketone as antioxidant on orange and lemon oils

Lemon oil has a major central component that deteriorates, and its pH was found to be 3.5 $\pm$ 0.5[18], compared to 5.3 $\pm$ 0.27 in an emulsion format[19].

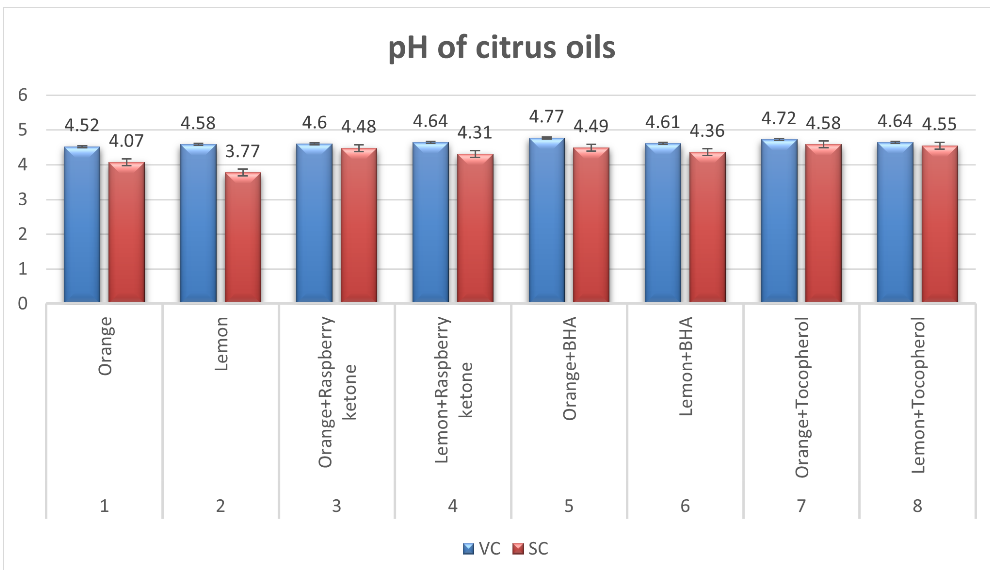

Fig 2. pH assessment of citrus oils after 1 month with and without antioxidants in cold storage temperature 4-8°C (VC-cooler) and accelerated temperature - 42°C (SC-stability chamber)

3.2 Sensory analysis of Citrus oils

Sensory analysis was performed for both orange and lemon oils in hard-boiled candy and black tea. The attribute rankings were recorded based on a nine-point hedonic scale, ranging from 1 = Dislike extremely to 9 = Like extremely, for the orange and lemon descriptors: juicy, peel, pithy, sourness, zest, and sweetness. Sweetness is less perceivable in lemon oil compared to orange oil. Orange oil sensory analysis indicates it is juicier, less peely, sweeter, less sour, slightly pithy, and zesty, whereas lemon oil is zestier, more peely, and has pleasant sour, juicy notes (Table 2). In the current research, Table 3 describes the citrus oils after one month without antioxidants as more peely, pithy, and less juicy. The citrus oils with positive controls BHA/tocopherol are better in juiciness, and the test antioxidant raspberry ketone samples are fresh, juicy, less pithy, less peely, and juicy without any change in profile. Blank samples of tea without antioxidants and citrus oils are not astringent in taste, whereas the samples with antioxidants are less astringent. In the case of hard-boiled candy, the blank sample is sweet in taste without any aroma, as the samples were not incorporated.

Table 2. Sensory descriptors of orange and lemon oil using hedonic scale-9 = like extremely to 1 = dislike

S. No	Attributes	Orange oil	Lemon oil
1	Juicy	9	9
2	Sour	8	8
3	Sweet	9	0
4	Fresh	9	9
5	Peely	5	5
6	Pithy	6	5

Table 3. Performance of antioxidants with and without antioxidants in citrus oils and Average of Panel score by hedonic scale-9 = like extremely to 1 = dislike

S. No	Attributes	BHA	Tocopherol	Raspberry ketone	Without antioxidants
1	Juicy	7	6	9	3
2	Sour	5	6	7	4
3	Sweet	7	7	9	2
4	Fresh	7	7	8	3
5	Peely	6	5	7	4
6	Pithy	6	5	7	5

No study has been recorded on the active flavor and fragrance ingredient as an antioxidant for citrus oils stability; thus, the current study is unique. The trained panel can easily perceive the taste and smell based on their experience with evaluation [(20)]. Major citrus oils are extracted from the peel, and the flavors developed in aroma and taste can be tested using a hedonic scale. The nine-point hedonic scale is used to understand the preference for any flavor [(21)].

In the current research, the taste and aroma of lemon and orange oil were assessed by sensory panel members. Techniques like flavor profile attribute analysis for essential oils, with qualitative assessment using a 1-9 rating scale, were preferred to describe the oils or any food flavors. Descriptor analysis was helpful to describe and derive the attributes and understand consumer requirements. Trained panelists, such as flavorists and quality control teams, play a key role in assessing or describing the attributes of aroma components. The essential oils' descriptive profiling was performed on a spider map considering the descriptors and intensity. A 9-point hedonic scale, with 9 = like extremely and 1 = dislike extremely, was used to assess mixed citrus and lemon oils, setting attributes like color, taste, aroma, and overall preference [(22)].

4 Conclusion

The increase in demand for food and the usage of citrus juices in various areas of RTD beverages is not sufficient to maintain the longevity of the taste. This study reports a novel approach by using raspberry ketone in citrus oils to improve stability. Raspberry ketone enhances the sweetness of the sample without releasing off-notes, compared to BHA and tocopherol. The results are promising, as raspberry ketone alone exhibits 70% antioxidant activity, which increases to approximately 82% when applied to citrus oils. Sensory panel members preferred the samples with raspberry ketone compared to BHA/tocopherol in terms of taste and aroma. Further research is in progress to confirm the changes through NMR and IR. This research suggests that raspberry ketone can be used as an antioxidant in the flavor and fragrance industry, as BHA is no longer considered viable under future regulatory standards, and tocopherol is more expensive compared to other antioxidants.

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