Extraction of Oleoresins from Cardamom as Natural Food Preservatives for *Citrus Sinensis* L. Juice

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Abstract

In this research**,** the spice seed *Amomum subulatum* Roxb. (Cardamom) was collected from Taung-Ngu Township. The extractions of oleoresins (methanol and acetone) from cardamom were carried out by Soxhlet extraction method. The oranges (*Citrus sinensis* L.) was collected from Ywe-Ngan Township in Shan State and prepared to obtain juice. The extracted oleoresins from cardamom had been used as natural food preservative for orange juice. During storage period, nutritional changes of prepared orange juice such as pH, titrable acidity, reducing sugar and vitamin C were tested. The change of pH was determined by digital pH electrode method. On the other hand, titrable acidity was measured by acid base titration. Moreover, reducing sugar was investigated by Lane and Eynon volumetric method. Finally, Vitamin C content was determined by redox titration method.

Keywords: Cardamom, Oleoresin, pH, Titrable acidity, Reducing sugar and Vitamin C

Introduction

In recent decades, the researcher has been emphasized for natural food preservatives have prompted research into their use in preserving easily spoilable foods. Natural food preservatives are synthesized from plant, animal, and microbial origins. The main application of natural preservatives in the food industry is to prevent the growth of undesirable bacteria. By adding the preservative materials from plant and fruit which are prevented the microbial growth into the packaging material which prolongs the shelf life of foods. (Bouarab Chibane L., 2019)

Amomum subulatum Roxb, (Cardamom) is a perennial plant. The constituent of the fruit is essential oil having typical characteristic flavor and have stimulant and stomachic, etc. Oleoresin exhibit antioxidant activity and have potential health benefits (Gupta PN., 1986). Nowadays, the consumers choose healthy and having no chemical preservatives which freshly juices are nutritious. Fresh fruit juices are abundant vitamins, minerals and protein. Fresh orange juice is a potential nutritious, aromatic and delicious fruit (Ahvenaine, R., 1996). Freshly extracted sweet orange juice was a healthful drink (Fraizer, W.C., 1967). By using cardamom oleoresin it has been made to prevent the growth of microorganism in the juice and enhance the shelf life of sweet orange juice.

The main points of this research work are to extract the oleoresin from *Amomum subulatum* Roxb. and treat for orange juice, to observe the nutritional changes such as pH, reducing sugar, titrable acidity and vitamin C on treated orange juice sample during storage period.

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Botanical Description of Cardamom

Figure 1. Cardamom

Scientific name - *Amomum subulatum* Roxb.
English name - Cardamom
Myanmar name - Phalar
Family - Zingiberaceae

Botanical Description of Sweet Orange

Figure 2. Sweet orange fruit

Scientific name - *Citrus sinensis* L.
English name - Orange
Myanmar name - Lain-maw-thi
Family - Rutaceae

Materials and Methods

Sample Collection

Cardamom was collected from Taung-Ngu Township. Sweet oranges were collected from Ywe-Ngan Township in Shan State.

Extraction of Acetone Oleoresins from Cardamom by Soxhlet Extraction Method

The cardamom sample about 50 g was weighed and placed in the thimble chamber of the Soxhlet apparatus. The acetone solvent (150 mL) was poured into the round bottle flask. Soxhlet extractor was placed onto a flask. The flask was heated at 56 ℃. During heating, the solvent was vaporized into the condenser and dripped into the thimble. This procedure was repeated about three days. After extraction, the oleoresin mixture solution was obtained. This mixture was dried at room temperature. Finally, the pure oleoresin mixture was yield.

Extraction of Methanol Oleoresins from Cardamom by Soxhlet Extraction Method

The cardamom sample about 50 g was weighed and placed in the thimble chamber of the Soxhlet apparatus. The methanol solvent (150 mL) was poured into the round bottle flask. Soxhlet extractor was placed onto a flask. The flask was heated at 56 ℃. During heating, the solvent was vaporized into the condenser and dripped into the thimble. This procedure was recycled and repeated about three days. After extraction, the oleoresin mixture solution was obtained. This mixture was dried at room temperature. Finally, the pure oleoresin mixture was received.

Preparation of the Orange Juice Samples using Methanol and Acetone Oleoresins

Two kinds of oleoresins (methanol and acetone) extracted from cardamom were used in the preservation of orange juice. The extracted orange juices were divided into five equal batches of 150 mL, each kept in clean and dry plastic bottles. Following treatments were done:

T_0 (Control I) - 150 mL of orange juice

T_1 (Control II) - 250 μg of ethanol and 150 mL of orange juice

T_2 (methanol oleoresin) - 225 μg of ethanol dissolved in 25 μg of methanol oleoresin and 150 mL of orange juice

T_3 (acetone oleoresin) - 25 μg of acetone oleoresin dissolved in 225 μg of ethanol and 150 ml of orange juice

(i) T_0 Control I (ii) T_1 Control II (iii) T_2 Methanol Oleoresin (iv) T_3 Acetone Oleoresin

Figure (3) Four types of orange juice sample bottles

Storage Time

The control and treated juice samples were stored at refrigerated temperature (4°C) for 35 days under laboratory conditions. The effect of storage on nutritional changes such as pH, titrable acidity, reducing sugar and vitamin C were determined weekly. Each analysis was carried out in triplicate on initial days, 7 days, 14 days, 21days, 28 days and 35 days.

Determination of pH

Each pH values of orange juice sample were measured using a digital pH meter. It was calibrated with buffer solutions of pH-(4 to 9).

Determination of Titrable acidity (Ranganna, S. 1986)

The mainly acid in orange is citric acid. Titrable acidity of juice samples was determined by titrating with against sodium hydroxide (NaOH). 10 g juice sample was dissolved in boiled distilled water and cooled It was diluted and filtered. 25 mL

of this filtrate was titrated against 0.1N sodium hydroxide (NaOH) using phenolphthalein as an indicator.

Determination of Reducing Sugar (Lane, J.H, 1983)

Sugar contents in orange juices were determined by Lane and Eynon volumetric method.

Standardization of Fehling's solution

Standard dextrose solution was placed into a 50 mL burette. 10 mL of Fehling's solution was placed into a 100 mL conical flask and titrated with standard dextrose solution to affect the reduction of all the copper. The flask containing the mixture was heated and gently boiled for 2 min. After two minutes of boiling, 1mL of methylene blue indicator solution was added while it was boiling to complete the titration. While the contents of the flask begin to boil, standard dextrose solution (1 or 2 drops at a time) from the burette was added until blue color of indicator disappeared within one min.

Procedure of Reducing Sugar

10 mL of juice samples containing about 2-2.5 g sugar was transferred to 100 mL volumetric flask, it was diluted to reach graduated mark and 2 mL of 45 % lead acetate was added to precipitate the protein present in the sample. 2 mL of 22 % potassium oxalate was added along the side of the flask to precipitate excess lead used in clarification and filtered. The clarified filtrate was placed in the burette instead of dextrose solution. It was titrated with mixed Fehling A and B (1:1) using Lane and Eynon volumetric method.

Determination of Vitamin C (Ranganna, S. 1986)

The ascorbic acid content in orange juice can be determined with stabilizing agent such as 20 % metaphosphoric acid. Ascorbic acid of juice samples were determined by using 2, 6-dichlorophenol indophenol visual titration method.

Standardization of Dye

10 mL of standard ascorbic acid was placed in a conical flask and it was titrated with indophenol solution until faint pink color persists for 15 seconds.

Procedure of Vitamin C

50 mL of juice sample was dissolved in 25 mL of 20 % metaphosphoric acid as stabilizing agent and volume was made up to 100 mL and filtered. 10 mL of above solution was taken and titrated with dye solution until a faint pink color persists for 15 seconds.

Results and Discussion

Yield Percent of Oleoresins from Cardamom

In all the cases, the determinations were repeated three times and the mean value was used. According to the results, the yield percent were also compared between oleoresins extracted from methanol and acetone. It was found that the yield of methanol extract was slightly higher than acetone extract in Table 1. Therefore, the yield may be depended on the solvent used.

Table1. Percentage of oleoresins (Methanol and Acetone) in Cardamom

Extracted Sample	Yield (%)
Methanol oleoresin	6.26 %
Acetone oleoresin	5.33 %

Determination of pH

Table 2. pH Changes of Juice Samples with Storage Time

Days	pH			
	T_0	T_1	T_2	T_3
I_1 (Initial day)	4.0	4.0	4.0	4.70
7	4.42	4.39	4.40	4.48
14	4..59	4.40	4.45	4.49
21	4.70	4.52	4.50	4.40
28	4.95	4.69	4.87	4.57
35	5.00	4.71	4.95	4.62

T_0 = Control I
T_1 = Control II
T_2 = Methanol Oleoresin
T_3 = Acetone Oleoresin

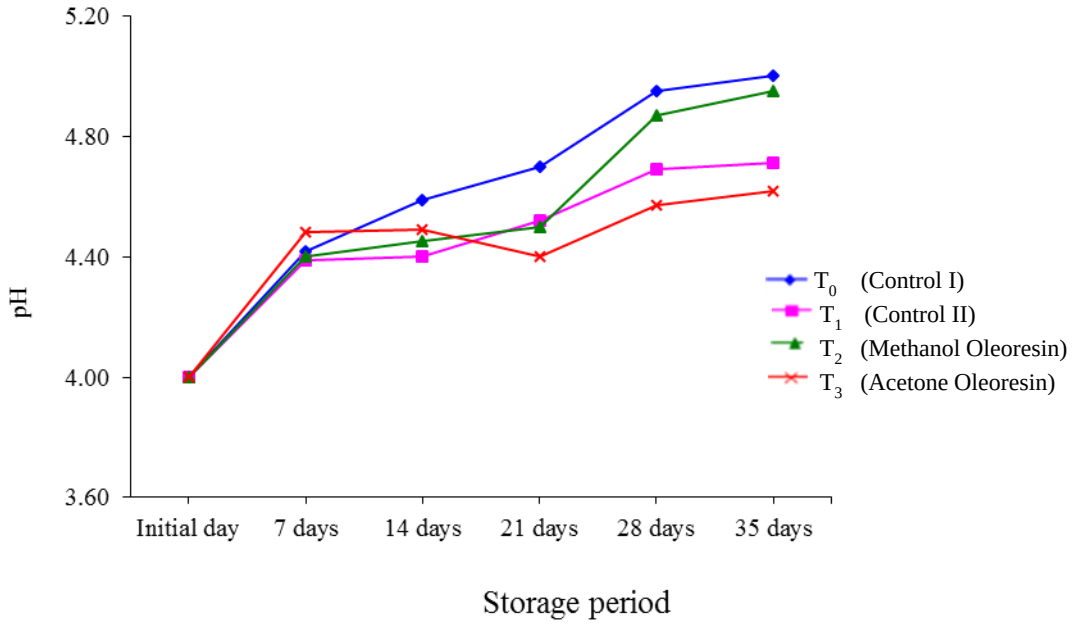

Figure 4. Effect of cardamom oleoresins on pH of sweet orange juice during storage

In this figure, an increase of pH in all treatments during storage was observed. Although maximum pH value was found in T_0 (Control I) but minimum pH values were noted in T_3 (Methanol Oleoresin). Furthermore, gradual increase was observed in pH during storage. The increase in pH was due to a decrease in acidity and increase in total sugar content.

Determination of Titrable acidity

Table 3. Percent Acidity of Juice Samples with Storage Time

Days	Titrable Acidity (%)			
	T_0	T_1	T_2	T_3
I_1 (Initial day)	0.625	0625	0.625	0.625
7	0.40	0.54	0.51	0.58
14	0.32	0.46	0.36	0.54
21	0.23	0.34	0.31	0.44
28	0.11	0.30	0.22	0.39
35	0.09	0.23	0.19	0.27

T_0 = Control I
T_1 = Control II
T_2 = Methanol Oleoresin
T_3 = Acetone Oleoresin

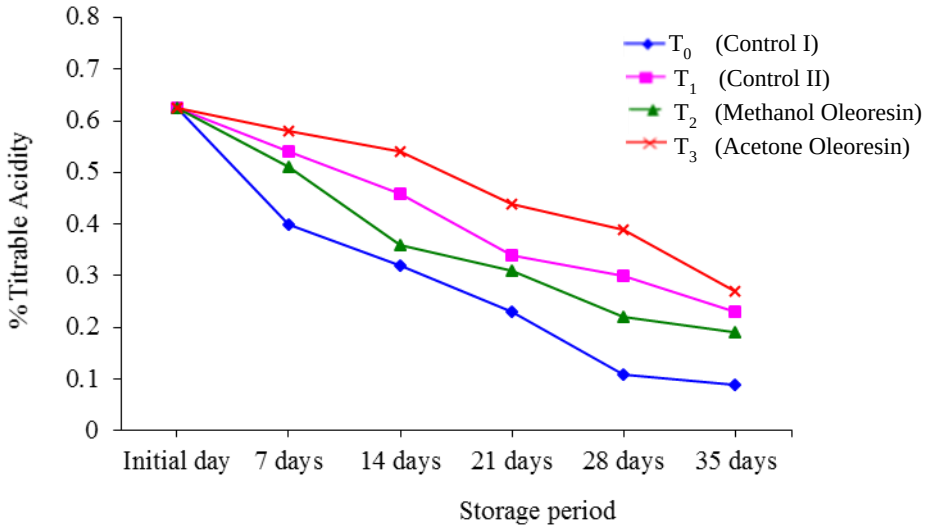

Figure 5. Effect of cardamom oleoresins on titrable acidity of sweet orange juice during storage

This figure showed change in titrable acidity of sweet orange juice upon storage at 4±1°C with various treatments weekly. The decrease in acidity because of it was conversion of acid to sugar. T_3 (Methanol Oleoresin) has highest value, whereas lowest acidity value was observed in T_0 (Control I). During 35 days of storage, the percentage of titrable acidity was decreased.

Determination of Reducing Sugar

Table 4. Reducing Sugar Contents of Juice Samples with Storage Time

Days	Reducing Sugar (%)			
	T_0	T_1	T_2	T_3
I_1 (Initial day)	5.9	5.9	5.9	5.9
7	10.2	7.7	6.1	6.1
14	11.5	10.8	10	8.7
21	13	10.4	11.7	10.1
28	14.7	13.5	13	11.2
35	15.3	14.6	14.1	12.8

T_0 = Control I
T_1 = Control II
T_2 = Methanol Oleoresin
T_3 = Acetone Oleoresin

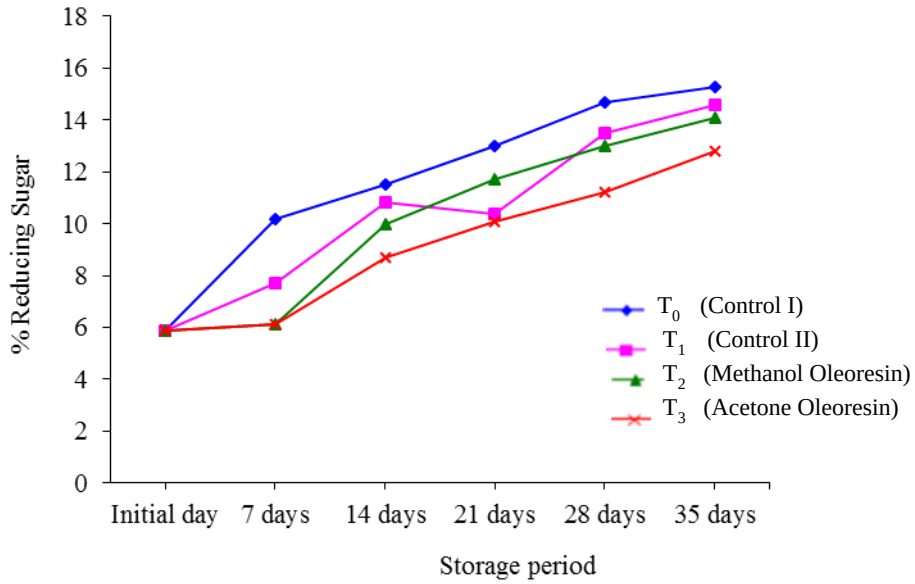

Figure 6. Effect of cardamom oleoresins on reducing sugars of sweet orange juice during storage

According to the results, the reducing sugar contents increased with storage period in Table 4 and Figure 6. It was the dehydration as a result of moisture loss and decrease in acidity of fruit juice by physiological changes during storage. Comparison of treatments showed that the highest value for reducing sugar is T_0 (Control I) whereas the lowest value was found in T_3 (Methanol Oleoresin).

Determination of Vitamin C

Table 5. Vitamin C Contents of Juice Samples with Storage Time

Days	Vitamin C or Ascorbic acid (%)			
	T_0	T_1	T_2	T_3
I_1 (Initial day)	37.8	37.8	37.8	37.8
7	36.3	37.1	36.8	37.3
14	35.9	36.7	36.5	37
21	34.9	36.6	35.5	36.8
28	33.7	34.1	35.3	35.2
35	32.5	33.6	34.0	34.4

T_0 = Control I
T_1 = Control II
T_2 = Methanol Oleoresin
T_3 = Acetone Oleoresin

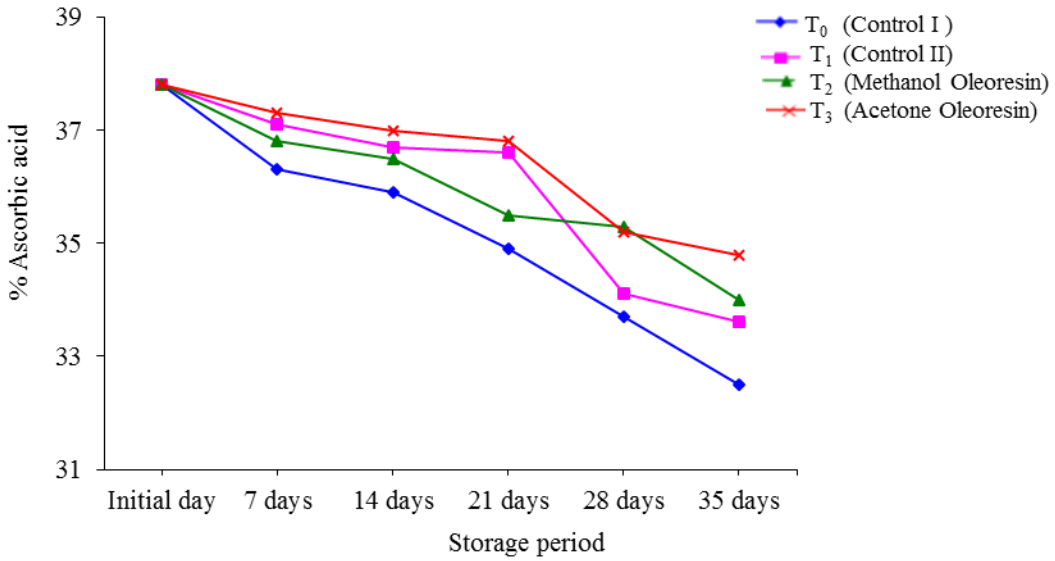

Figure 7. Effects of cardamom oleoresins on ascorbic acid of sweet orange juice during storage

In figure 7, the highest value for ascorbic acid content was found in T_3 (Acetone Oleoresin) whereas the lowest for T_0 (Control I). It is regarded that the oxidation of ascorbic acid to dehydroascorbic acid by enzyme ascorbicnase. Data regarding storage intervals showed that in all the treatments, ascorbic acid contents were decreased as the storage period was prolonged.

Conclusion

In conclusion, the oleoresins (methanol and acetone) from cardamom were extracted by Soxhlet extraction method. These results were 6.26 % and 5.33 %. In this research, T_0 (Control I), T_1 (Control II), T_2 (Methanol Oleoresin) and T_3 (Acetone Oleoresin) samples were treated in the orange juice samples. Among them, the nutritional values of acetone oleoresin juice samples, pH values were found to be 4.62, titrable acidity were 0.27 %, reducing sugar content was 12.8 % and vitamin C content was 34.4 %. Acetone oleoresin (T_3) was more maintained and improved the quality of the juice than the control sample orange juices (T_0 and T_1) and methanol oleoresin (T_2). The acetone oleoresin orange juice sample was controlled the growth of microorganisms in the juice and inhibited the fermentation. Therefore, acetone oleoresin can be applied as natural preservative which effectively extends the shelf life of the orange juice.

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