FUNCTION AND TOXICITY STUDY OF VEGETABLE PRESERVATIVE

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Abstract

Chemical preservatives are enormously used for fresh food preservation but have adverse effects on human health. Food additives are in high demand to inhibit the contamination of fresh and raw vegetables. The aim of the present study was to probe the effectiveness of food preservatives on microbial contaminants in fresh vegetables and to study the toxic effect of the same on mammalian cells using in-vitro cytotoxicity assays. In this study, 5 different vegetables were used for isolation of the contaminants against which citric acid and acetic acid were tested as the preservatives. The minimum inhibitory concentration (MIC) of them was found for different isolates taken from the vegetables. Two of them were identified to be *Salmonella enterica* and *Enterobacter tabaci*. The MIC of Citric acid on these isolates was found to be 1%, 1% and >15% respectively, and the MIC of acetic acid was found to be 0.6%, 0.6%, and 0.8% respectively whereas, the cumulative effect of both the additives showed the MIC of 0.6%. The cytotoxicity of these chemical preservatives was also guesstimated by employing two human cell lines that are INT 407 (human epithelial cells) and blood peripheral cells. The IC50 values were calculated employing GraphPad Prism software. The goodness of fit and ANOVA analysis revealed a significant correlation between the concentration of preservatives under study and cellular response. Moreover, non-significant changes in morphological patterns, cell growth patterns, and other cellular properties were scored among the cells under analysis. It can be said that the present study thus endeavors to lay a preliminary platform for understanding the spectrum of applications of food preservatives and their effect on the gut cells through in-vitro mode.

Keywords: citric acid, acetic acid, minimum inhibitory concentration, in-vitro toxicity

Background

Various physical and chemical methods have been used to preserve the fresh-cut vegetable quality. Chemical treatments include the application of chlorine, ascorbic acid, citrate and/or calcium salts for preservation. There are certain harmful effects of using chemicals for preservation. This study tries to evaluate the efficiency as well as toxic effects related to few of such commonly used chemical preservatives.

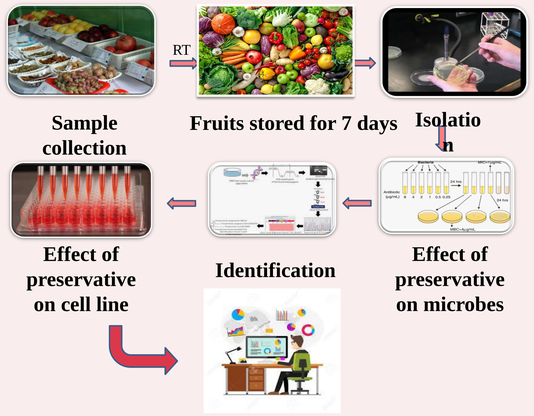
Hypothesis

“Topical application of citric acid and acetic acid reduces microbial contamination and its safe for consumption”

Objectives

- Isolation and Identification of microbial contaminants.
- Identifying the inhibitory concentration of different preservatives against contaminants.
- Determining the effect of preservative on human cell line.

Methodology



Results

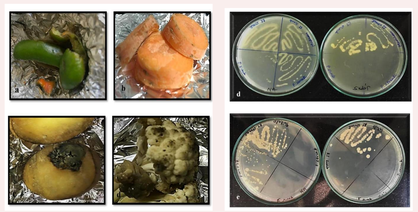


Figure 1 : Images of vegetables spoilage on day 7 stored in refrigerator

Figure 2: Micro-flora isolated at RT on Nutrient agar and Sabouraud’s agar for day 0 from flesh and surface a: Potato b: carrot

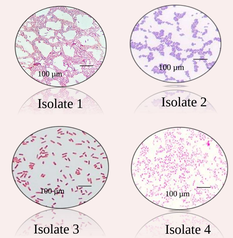


Figure 3 : Photo - micrographs of isolates

Table 1: Colony characteristics of isolates.

	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Size	1mm (watery)	1mm	Pinpoint	1mm-2mm
Shape	Circular	Circular	Circular	Circular
Colour	Pale white	Pale transparent	White	Whitish
Margin	Entire	Entire	Entire	Entire
Elevation	Raised	Raised	Raised	Raised
Opacity	Opaque	Translucent	Opaque	Opaque
Consistency	Mucoid	Mucoid	Powdery	Mucoid
Gram nature	Gram negative	Gram positive	Gram negative	Gram negative

Table 2: MIC of acetic acid on isolates

Concentration of citric acid	Concentration of acetic acid	Colony 1 (0 hr)	(24 hr)	*Enterobacter tabaci* (0 hr)	(24 hr)
0.2	0.2	+ + +	+ +	+ + +	+ +
0.2	0.4	+ + +	+	+ + +	+
0.2	0.6	+ +	-	+ +	-
0.2	0.8	+ +	-	+ +	-
0.2	1	+	-	+	-
0.4	0.2	+ + +	+ +	+ + +	+ +
0.4	0.4	+ +	+	+ +	+
0.4	0.6	+ +	-	+ +	-
0.4	0.8	+	-	+	-
0.4	1	+	-	+	-
0.6	0.2	+ +	+	+ +	+
0.6	0.4	+ +	-	+ +	-
0.6	0.6	+	-	+	-
0.6	0.8	+	-	+	-
0.6	1	-	-	-	-
0.8	0.2	+	-	+	-
0.8	0.4	+	-	+	-
0.8	0.6	-	-	-	-
0.8	0.8	-	-	-	-
0.8	1	-	-	-	-
1	0.2	-	-	-	-
1	0.4	-	-	-	-
1	0.6	-	-	-	-
1	0.8	-	-	-	-
1	1	-	-	-	-

Table 3: MIC of citric acid on isolates

Concentration of citric acid	Isolate 1 (0hr)	(24hr)	*Salmonella enterica* (0hr)	(24hr)	*Enterobacter tabaci* (0hr)	(24hr)
0.2	+ + +	+ +	+ + +	+ +	+ + +	+ + +
0.4	+ + +	+ +	+ + +	+ +	+ + +	+ + +
0.6	+ +	+	+ + +	+	+ + +	+ + +
0.8	+	+	+ + +	-	+ + +	+ + +
1	+	-	+ +	-	+ + +	+ + +
2	-	-	-	-	+ + +	+ + +
3	-	-	-	-	+ + +	+ + +
4	-	-	-	-	+ + +	+ + +
5	-	-	-	-	+ + +	+ + +
6	-	-	-	-	+ +	+ +
7	-	-	-	-	+ +	+ +
8	-	-	-	-	+ +	+ +
9	-	-	-	-	+ +	+ +
10	-	-	-	-	+ +	+ +
11	-	-	-	-	+	+
12	-	-	-	-	+	+
13	-	-	-	-	+	+
14	-	-	-	-	+	+
15	-	-	-	-	+	+

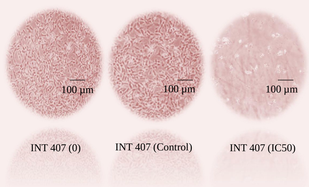


Figure 4 : Photomicrographs of INT 407

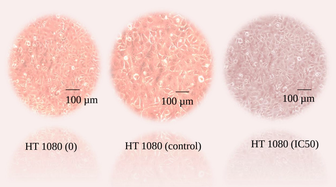


Figure 5 : Photomicrographs of HT 1080

Conclusion

- Acetic acid is more effective as a preservative compared to citric acid.
- In-vitro cytotoxicity studies showed that citric acid has negligible effect on both normal gut intestinal cell lines (INT 407) and Human lymphocytes.
- Acetic acid exhibited cell death at higher concentration on INT 407 as well as human lymphocytes.
- Citric acid can be generally regarded as safe for human consumption while acetic acid should be preferred at lower concentration.
- The cumulative effect of citric acid and acetic acid exhibited almost a similar response as used individually.
- The application of citric acid and acetic acid as an anti-cancer molecules was evaluated on HT 1080 (human fibrosarcoma cells) wherein a decrease in cell density was recorded in inverse proportion to the concentration of citric acid, acetic acid or combined.

References

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