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Research Article

Safety evaluation of honey incorporated orange squash by microbial challenge studies

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Abstract

Orange squash was developed with part substitution of sugar with honey. Based on organoleptic and antimicrobial properties the amount of honey that can replace sugar in orange squash was decided. The modified orange squash was then challenged with the common spoilage causing bacteria in squash and selected food borne pathogens. Challenge Study was carried out on the honey incorporated orange squash by growth inhibition method which showed that the orange squash with 50% honey passed the challenge studies.

Introduction

Food safety is the most important aspect in food industry which takes into account the food borne illnesses caused by microorganisms (Prescott et al 2008).Fruit juices due to low pH are commonly contaminated by *Yeasts*, *Molds*, and *Lactic acid bacteria*. Unpasteurized fruit juice can be contaminated with pathogens like *Salmonella spp*. and *E.coli O157:H7* (López-Gómez et al 2012). Spoilage of fruit juice caused by the *Alicyclobacillus* species is a major threat to the beverage and fruit juice industry (Zeki et al, 2010). Pathogenic strains of *Escherichia coli, Salmonella, Staphylococcus,* and *Listeria monocytogenes* continue to cause serious outbreaks of foodborne illness and frequently occur in unpasteurized orange juices (Bucknow 2013). Thus, fruit juices are at a threat due to food borne pathogens and spoilage causing microorganisms.

Microbiological challenge testing is used to determine the ability of a food product to support the growth of spoilage organisms and/or pathogens (www.fda.gov).Survival of the pathogens prove the unsafety of the product for consumption. Cultures of single organism or a mixture of organisms can be inoculated in the food product and their presence or absence of growth can be attributed to the safety of the food product. Challenge studies give information about Food safety ,Quality and Stability of any new or reformulated food product (US FDA 2009). In addition, if Microbial challenge testing is carried out properly, information is obtained on the types of microorganisms capable of growing in the product, so that risks of food borne illnesses and poisoning can be assessed. It also helps to determine whether a food has the ability to "kill-off" any pathogens or toxins that may accidentally enter the product (Curale et al 2001).

Krushna et al (2007) had shown that honey could be used as a natural preservative for milk owing to its hydrogen peroxide activity and combination of various sugars. In addition honey possesses several health benefits such as hypoglycemic, immunomodulatory, etc. (Erejuwa et al 2012; Cortés et al 2011)

Our previous study showed that honey exhibits antimicrobial property in orange squash (Sharma et al, 2014)(Pimentel 2013). The present study carried forward the previous research towards testing of the ability of honey incorporated orange squash to support or inhibit selected spoilage and pathogenic microorganisms through challenge testing protocol.

Materials and Methods

Product Development:

Orange squash was prepared using the standard recipe as given by Thangam (1965) and the most inhibitory variety of honey was incorporated in the squash at different levels as partial replacement of sugar (Fig. 1) (Sharma et al 2013).

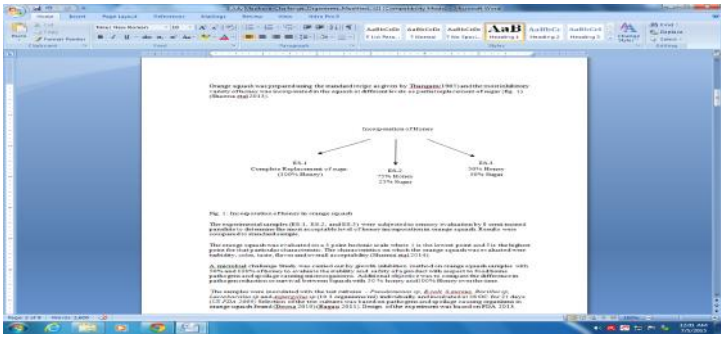

Fig. 1: Incorporation of honey in orange squash

The experimental samples (ES-1, ES-2, and ES-3) were subjected to sensory evaluation by 8 semi-trained panelists to determine the most acceptable level of honey incorporation in orange squash. Results were compared to standard sample.

The orange squash was evaluated on a 5 point hedonic scale where 1 is the lowest point and 5 is the highest point for that particular characteristic. The characteristics on which the orange squash was evaluated were turbidity, color, taste, flavor and overall acceptability (Sharma et al 2014).

A microbial challenge Study was carried out by growth inhibition method on orange squash samples with 50% and 100% of honey to evaluate the stability and safety of a product with respect to food borne pathogens and spoilage causing microorganisms. Additional objective was to compare the difference in pathogen reduction or survival between Squash with 50 % honey and100% Honey over the time.

The samples were inoculated with the test cultures - *Pseudomonas sp, E.coli, S.aureus, Bacillus sp, Lactobacillus sp* and *Aspergillus* sp (10 3 organisms/ml) individually and incubated at 28 OC for 21 days (*US FDA 2009)* Selection of the test cultures was based on pathogens and spoilage causing organisms in orange squash found (Doona 2010) (Bagasi 2011). Design of the experiment was based on FDA 2013.

To determine product stability, the inoculum was diluted so that a final concentration of approximately 102-103 cfu /g of product was attained (Scott 2005). The challenge tests of this study were designed to simulate as closely as possible the natural contamination level in foods (low-level inoculum) (Eija et al 2000).

At different time intervals aliquots were plated on media from these samples and plates were checked after incubation for survival of respective cultures. Nutrient agar was used to detect the presence of following organisms *Pseudomonas ,E.coli ,S.aureus and Bacillus* sp where as *Lactobacillus* sp was detected using Rogosa agar and for *Aspergillus* sp Sabouraud agar was used (Difco manual 2009).

Viable counts were determined at zero time, after 2 days, 8 days ,15 days and 21 days of storage of the samples. Count of the squash samples with 50% honey and 100% honey without adding challenge organisms were also determined and treated as controls. Uninoculated controls were maintained and total bacterial and mould counts were determined.

Physical appearance of both types of orange squash samples was noted after 21 days of storage of the samples.

Results and Discussion

Product Development:

Honey was incorporated in orange squash at various levels, i.e., 100%, 75% and 50% of original sugar content in the recipe, as shown in table no. 1.

Table-1: Incorporation of Honey in Orange Squash.

Samples	**Standard Recipe**	**Experimental Recipe (Honey incorporation)**
	Sugar/Liter	Honey/Liter
Standard recipe-A	450 gms	0 gms (0 %)
Experimental recipe-B	0 gms	450 gms (100%)
Experimental recipe-C	112.5 gms	337.5 gms (75%)
Experimental recipe-D	225 gms	225 gms (50%)

As per our previous study, squash samples with 50 % and 100 % honey that showed better antimicrobial and organoleptic properties were selected for the present study (Sharma etal 2013).

Challenge Study:

Growth inhibition studies were carried out to demonstrate safety of the formulation at ambient temperature.100% substitution with honey sample of squash was next on organoleptic scale hence used with the most suitable sample (50%) for comparison.The purpose of selecting 50% and 100% honey incorporated orange squash samples for the challenge study was to ascertain the level of honey incorporation that would inhibit the challenge organisms while not hampering the organoleptic qualities of the orange squash.

(www.fda.gov/Food/FoodScienceResearch/.../ucm094154.htm)

Before inoculating the challenge organisms it was determined whether the honey incorporated orange squashes exhibited growth of any organism on the agar plate after incubating the squashes for 24 hours at 28 0C.Baseline level of microorganisms present inhoney incorporated orange squash were determined as a control.

Controls without any inoculation of the challenge organisms showed product to be safe and of good quality microbiologically.

Table. 2: Microbial growth in Uninoculated Orange Squash.(Control)

Dilution	**50% Honey Incorporated Orange Squash**	**100% Honey Incorporated Orange Squash**
	Unchallenged	
Neat	No growth	Fungal Growth
10-1	No growth	No growth

The control plates showed that 50% honey incorporated orange squash did not have any microorganisms and although the 10-1 dilution of 100 % honey incorporated orange squash did not show any growth; its neat variant showed fungal growth which could be due to high sugar content in the squash (Wareing and Davenport, 2008). In previous study based on organoleptic quality and antimicrobial effect of both the samples, it was proved that 50 % honey incorporated orange squash was a better product (Sharma etal 2013).

Table. 3: Appearance of squash after 21 days of challenge test.

Challenge Organisms	**50% Honey Incorporated Orange Squash**	**100% Honey Incorporated Orange Squash**
S. aureus	OK	OK
E. coli	OK	OK
Pseudomonas sp	OK	OK
Bacillussp	OK	OK
Lactobacillus sp	OK	Hardened
Aspergillus sp	OK	Growth
Uninoculated Squash	OK	*Aspergillus* growth seen

All the squash samples were checked visually for appearance after three weeks of storage. It was seen that 50 % honey incorporated squash appeared norma where as appearance of 100 % honey incorporated squash was found to be unacceptable with samples inoculated with *Aspergilluss p*and *Lactobacillus sp* as shown in table 3. It would be unsafe to consume such orange squash.

Table 4 . Results of growth inhibition study at different time intervals and types of orange squash.

Squash with 50 % honey						**Squash with 100% Honey**				
Storage Time in Days	**0**	**2**	**7**	**14**	**21**	**0**	**2**	**7**	**14**	**21**
Test cultures										
S. aureus	1X103	1X103	0	0	0	3X103	1X10	0	0	0
E. coli	1X103	0	0	0	0	1X103	0	0	0	0
Pseudomonas sp	1X103	0	0	0	0	2X103	10X101	0	0	0
Bacillussp	25X103	0	0	0	0	43X104	1X103	5	0	0
Lactobacillus sp	6X103	0	0	0	0	4X103	0	0	0	0
Aspergillus sp	Growth	Growth	0	0	0	Growth	Growth	0	0	Growth
Appearance of squash after 21 days	Normal					Growth of *Aspergillus* was seen				

Table -5: Percentage reduction in the number of organisms observed over the period of time during growth inhibition study

	Squash with 50 % Honey				Squash with 100% Honey			
StorageTime in Days	**2**	**7**	**14**	**21**	**2**	**7**	**14**	**21**
Test cultures								
S. aureus	0	100	100	100	99.666	100	100	100
E. coli	100	100	100	100	100	100	100	100
Pseudomonas	100	100	100	100	95	100	100	100
Bacillus	100	100	100	100	99.76	99.99	100	100
Lactobacillus	100	100	100	100	100	100	100	100

Table -6:Log reduction in the number of test organisms over 21 days

	Squash with 50 % Honey				Squash with 100% Honey			
Storage Time in Days	**2**	**7**	**14**	**21**	**2**	**7**	**14**	**21**
Test cultures								
S. aureus	0	3	3	3	2	3	3	3
E. coli	3	3	3	3	3	3	3	3
Pseudomonas	3	3	3	3	2	3	3	3
Bacillus	3	3	3	3	1	2	4	4
Lactobacillus	3	3	3	3	3	3	3	3

After 2nd day of inoculation with the cultures of *E.coli*, *Pesudomonas sp,Bacillus sp, Lactobacillu sp* in orange squash in 50% honey, the samples showed three log reduction in number i.e 99.9 % killing . S.aureus survived on day 2 but showed 3 log reduction in number after one week (Table 6) (Tanner B) . From these results it can be concluded that the product with 50% honey is safe for human consumption, as challenge organisms did not survive beyond seven days of storage. The developed product is of good quality and honey can aid in the preservative action shown by citric acid. Inactivation study proved orange squash with 50 % honey as stable and safe product for consumption. Squash with 100 % honey also showed three fold reduction of *Lactobacillus* sp and *E.coli*, but *S.aureus* and *Pseudomons sp* showed only two fold reduction on day 2 and 3 fold reduction from 7 th day onwards. *Bacillus sp* showed more resistance as it showed one fold reduction on day 2 and two fold reduction on seventh day.(Table 6)(https://www.biofilm.montana.edu/files/CBE/documents/KSA-SM-07.pdf).

The orange squash preparation has 1% citric acid as a preservative .In the formulation sugar was partially replaced with honey as honey has better functional properties like anti-bacterial property, low pH, high viscosity, hygroscopicity and peroxide activity. All these factors added to the role of honey as a preservative as shown by this challenge studies (Table 4 and 5).

Thus, the microbiological quality of the product with 50% honey studied remained unchanged during the storage period at 28°C as microbial number declined to zero after two days of storage. (www.fda.gov/ Food/FoodScienceResearch/.../ucm094154.htm).

The developed product was found to be safe against microbial contamination with good keeping quality and hence suitable for human consumption.

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