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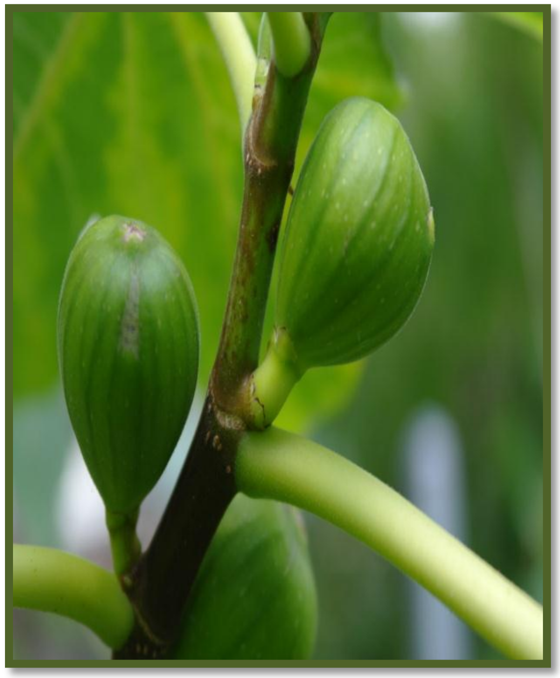
ISOLATION OF HEAT RESISTANT FUNGI FROM CANNED FRUITS



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ABSTRACT

Increase in the consumption of canned fruit juice rather than the fruit itself raises alarmonto the safety of these canned juices as they are stored for longer period of time unlike the fruit and dormant if spores present could germinate and cause spoilage. Heat-resistant molds are characterized by the production of ascospores or similar structures with heat resistance, in some instances comparable to bacterial spores. This enables them to survive even when they are thermally processed. So the presence of heat resistant molds in canned fruits is worthy of studying.In this research , heat resistant moulds exist incanned fruit juice and they can withstand the heat treatment/ pasteurization done at processing plants. Their presence might be due to contamination right from the farm fields or the processing company. Heat resistant moulds in canned fruit juice can pose a set or health hazard in the body which includes Diarrhea and Stomachache.

Keywords: Heat resistant molds,Contamination,antibiotic.etc

INTRODUCTION

Heat resistant molds can survive heat treatments used for fruit processing and can growand cause spoilage during storage. The heat resistance is attributed to the formation of sexual spores called ascospores and the heat resistance of the ascospores depends on the specie, strain, pH, heating medium and other growth conditions (Beuchat and Pitt, 2006). Spoilage fruits and fruit products by heat resistant molds have been recognized in several countries (Beuchat, 1978). *Byssochlamysfulva, B. nivea, Neosartoryafischeri, Talaromycesmacrosporus, T. bacillisporus,* and *Eupeniciltumbrefeldianum*have been most frequently encountered. These organisms are saprophytic rather than parasitic and usually contaminate fruits on or near the ground. *Byssochlamys*species have been recognized as spoilage molds in fruit since the early 1930s and have been extensively studied (Hull, 1979). Spoilage by other heat-resistant molds is a less serious problem recognized only in recent years. Consequently, less information is available concerning the behavior of these other genera in thermally processed fruit products (Beuchat and Pitt, 2006). Germination of ascospores may result in visible growth of mycelia on fruits and fruit products. Production of pectic enzymes by *Byssochlamys*can result in complete breakdown of texture in fruits and also can result in off-flavor development. Heat-resistant molds, therefore, may constitute a public health hazard as well as spoilage problems (Put and Kruiswijik, 1984).Some *Byssochlamys*species produce patulin, byssotoxin A, and byssochlamic acid, all having toxic effects on laboratory animals, *Neosartoryafischeri*is known to produce fumitremorgin A, B, and C, terrein, and verruculogen (Nelson, *et al.,* 1988).

OBJECTIVES

The aim of this research is to assess the presence or otherwise of heat resistant molds in canned fruit juice.

1. To isolate and identify the heat resistant molds in selected fruit juices.
2. To find the opinion of consumers regarding the contamination of the canned fruit juices.

HYPOTHESIS:

(i) Heat resistant molds are present in canned fruit juice.

MATERIALS AND METHOD

The method described by Murdock and Hatcher (1978) and modified by Beuchat and Pitt(2006) was used in this research.

SAMPLE COLLECTION

The samples of this research are canned Orange, Mango, Pineapple, Apple and Tomato juice. The Samples being already in liquid form hence no blending was required.

MYCOLOGICAL ANALYSIS

Malt extract agar (MEA) was used as the culture media, and it was prepared according to the manufacturing guidelines/instructions. Growth of bacterial spores that may survive the heat shock has been prevented by the inclusion of an antibiotic (erythromycin) as described by Pitt and Hocking, (1997). Finally it was sterilized by autoclaving at 121°C for 15minutes.

Pour plate method was used in preparation of plates for incubation. Five (5) plates were prepared for each fruit; 2ml of each sample was carried by using a sterilized 5ml syringe and transferred into a petridish. Malt extract media (prepared) was then poured into each plate; the content is then mixed gently by shaking the plates clockwise then counter-clockwise. It was allowed to solidify on a labeled surface to obtained even distribution, the plates then were inverted.

The plates were incubated at 30°C in an incubator machine for seven (7) days, the control samples were like-wise incubated. After the incubation period (7 days), each colony was then morphologically studied and recorded.

IDENTIFICATION

The identification of heat resistant molds was done according to the procedure described byBeuchat and Pitt (2006). And it was achieved by morphological examination of colonies formed, and by microscopic examination. The size of the colonies were measured by using ruler and recorded. Hand magnifier was used to aid view. The Isolates were Identified by making reference to the taxonomic key described by Beuchat and Pitt (2006).

MICROSCOPIC EXAMINATION

A wet mounts was prepared by putting a small piece of fungal filament using sterilizedinoculating needle in a clean free-grease glass slide. A drop of lactophenol was added for clear view. This was then covered by cover slip and examine under x40 by xlOO objective lens. The mycelium was observed i. e whether the hyphae are coenocytic or septate, branched or unbranched. The arrangement of spores were observed and also recorded. Also the diversity was recorded by grouping the species into different taxonomic genera.

RESULTS

A total of 31 fungal isolates were obtained from the five canned fruit samples screened.The isolate were classified by grouping into three taxonomic genera namely; *Byssochlamys, Talaromyces*and *Neosartorya.*Table A shows the average diameter covered by the isolates on the plates with the highest mean recorded in Orange and least in Apple. Table B showed the isolates and their respective frequency on the plates, the highest number of isolates was also recorded in Orange juice. Table C shows the percentage of respondents that encounter changes in their fruit juices and those that still consume the fruit juices when changes are noticed. Table D shows the percentages of the respondent's perception on the cause and source of contamination. Table E shows the percentages of the respondent's fruit juice which contamination occur most and the effect on health when consumed. All results are presented below:

Table A

SHOWING AVERAGE DIAMETERS COVERED BY THE ISOLATES ON THE PLATES

PLATE NUMBER	TOMATO	ORANGE	MANGO	PINEAPPLE	APLLE
1	2.1	2.5	3.0	2.9	0.0
2	2.4	3.3	2.8	4.1	0.0
3	2.0	4.6	4.4	3.6	2.0
4	3.0	2.4	1.9	4.3	2.2

5	**3.0**	**2.9**	**0.0**	**0.0**	**2.5**
TOTAL	**12.5**	**17.7**	**12.1**	**14.9**	**6.7**
MEAN	**2.5**	**3.5**	**2.4**	**3.0**	**1.3**

TABLE B
SHOWING THE ISOLATES AND THEIR FREQUENCY ON THE PLATES

Fruits	Isolates	Frequency	Percentage
Orange	*Byssochlamysnivea*	3	33.3%
	Byssochlamysfulva	3	33.3%
	Neosartoryafisherii	3	33.3%
Control	-	-	-
Mango	*Neosartoryafisherii*	2	25%
	Byssochlamysfulva	5	62.5%
	Byssochlamysnivea	1	12.5%
Control	-	-	-
Apple	*Talaromycesmacrosporuos*	3	100%
Control	-	-	-
Tomato	*Talaromycesmacrosporuos*	2	33.3%
	Neosartoryafisherii	4	66.7%
Control	-	-	-
Pineapple	*Byssochlamysfulva*	2	40%
	Byssochlamysnivea	3	60%

Control	-	-	-

TABLE C
SHOWING THE PERCENTAGES OF RESPONDENTS THAT ENCOUNTER CHANGES IN FRUIT JUICE AND THOSE THAT STILL CONSUME THE JUICE AFTER THE CHANGES ARE NOTICED.

CHANGES ENCOUNTERED	PERCENTAGE	CONSUMPTION AFTER POILAGE	PERCENTAGE
Yes	66.25%	Yes	19.72%
No	33.75%	No	80.28%

TABLE D
SHOWING THE PERCENTAGES OF THE RESPONDENTS PERCEPTION ON THE CAUSE AND SOURCE OF CONTAMINATION RESPECTIVELY.

CAUSE OF SPOILAGE	PERCENTAGE	SOURCE OF CONTAMINATION	PERCENTAGE
Virus	0%	Farm fields	30.26%
Bacteria	35.21%	Processing company	43.42%
Fungi	64.79%	Retail shops	26.37%

TABLE E
SHOWING THE PERCENTAGES OF THE RESPONDENT'S FRUIT JUICE WHERE CONTAMINATION OCCUR MOST AND THE EFFECT ON HEALTH WHEN CONSUMED

MOST ENCOUNTERED FRUIT JUICE	PERCENTAGE	EFFECT ON HEALTH	PERCENTAGE
Orange	25%	Stomachache	36.21%
Mango	25%	Diarrhea	15.52%
Pineapple	12.5%	Both stomachache and diarrhea	12.07%
Tomato	35.94%	None	29.31%

Apple	1.56%	Other effects	6.90%

DISCUSSION

The result of this experiment showed that, three genera of heat resistant molds wereisolated and identified namely; *Byssochlamys, Talaromyces, Neosartorya,* out of the five plates of orange cultured. There was growth on arwo of the tested samples. This showed the occurrence or contamination of heat resistant molds in orange and tomato samples, translating to a total of 17.7cm and 12.5cm total diameter and 3.5cm and 2.5cm mean diameter for orange and tomato juice respectively. Apple juice sample has the least with only three positive plates and a total diameter of 6.7cm and 1.3cm mean diameter covered on the plates.

Stolk and Samson (1981) observed the presence of heat resistant mould in canned fruit juice samples, including all the five used in this study and confirmed the presence of different species including *Byssochlamyssp, Talaromycessp, Neosartorya sp.* Contamination of fruit during harvest as well as growth of fungi on lesions and wounds have been described by Moyer *et aL,* (1969) as a major cause of contamination in canned fruit juice. Presence of heat resistant moulds in pipes of processing companies is also a source of contamination according to Murdock and Brokaw (1958).

*Byssochlamyssp*was described by Beuchatt and Pitt (2006) as the most economic important heat resistant mould in canned fruits and this may be due to the production of byssochlamic acid which breaks down the fruit structure. They also have toxic effect. According to Beuchat and Pitt (2006), *Byssochlamysfulva*produce colonies that cover the whole Petridish and relatively sparse, with low or somewhat floccose, with conidia production heavy, uniform and colored olive brown, while *B. nivea*produce white to slightly gray colonies on malt extract agar. Therefore the colonies formed can be confirmed to be *B. fulva*and *B. nivea.* This is in agreement with the results of this research.

Byssochalmys spp. was first reorganized as a spoilage agent in Great Britain by Oliver and Rendle (1934) and for a time it appeared to be a localized problem. However in recent years, spoilage outbreaks due to this mould has been recognized in almost all the continents of the world (Maunder, 1989) The occurrence of *Neosartoryaspp*on orange sample only was due to the facts that, this species grow only on food with low pH. Orange has a pH of 1.8-2.9 while that of tomato, pineapple and apple have a range of 2.5-3.7, 3.2-4.4 and 3.2-4.5 respectively, *Talaromycesspp*usually grows in pH range of 2.9-3.3 and therefore are more likely to be present in high rate in tomato and apple than the other two samples (Maunder, 1989).

Due to high concentration of sugar (carbohydrate) in orange, *Byssochlamysspp*are found to be more abundant. *Byssochlamysspp*grow best at high concentration of sugars (Norman, 1980). The occurrence of these organism may be attributed to their ability to produce resistant spores, *Byssochlamysspp*are more resistant to high temperature. In conjunction to this, *Byssochlamysspp*has been implicated in the spoilage of fruits and vegetables in Nigeria (Uzuegbu and Emiforiye, 1984). All the samples showed contamination by these heat resistant molds. This may be due to improper handling right from field or during transportation or in during processing. (Pitt and Hocking, 1985).

RECOMMENDATIONS

1. It is also recommended by virtue of this research that canned fruit juices should be stored at low temperatures as high temperature could activate the ascospores of the molds and cause spoilage.
2. Contaminated juices should not be consumed as they may pose health hazard.
3. Processing companies should ensure that all their fruits have bearable or minimum concentration of contaminants when relieved from the farm and they should employ better processing techniques such as flash pasteurization which ensures better safety.

CONCLUSION

It can be concluded from the results of this research that heat resistant moulds exist incanned fruit juice and they can withstand the heat treatment/ pasteurization done at processing plants. Their presence might be due to contamination right from the farm fields or the processing company. Heat resistant moulds in canned fruit juice can pose a set or health hazard in the body which includes Diarrhea and Stomachache.

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