MOULD AND YEAST FLORA IN FRUIT JUICES AT DIFFERENT PLACES IN NICOSIA

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By

EGEMEN İNCE

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

NICOSIA, 2019

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Approval of Director of Graduate School of Applied Sciences

Prof. Dr. Nadire Çavuş

We certify this thesis is satisfactory for the award of the degree of Masters of Science in Food Engineering

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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To my family and all Cypriots…

ABSTRACT

Citrus is a common fruit type seen in Cyprus because of the convenient climate of the island. Citrus juices are acidic beverages (calculated pH 3 to 4) that have high sugar content. Because of the acidity and high sugar levels, acidolactic bacteria, moulds, and yeasts compose the typical microbiota present in citrus juices. Lactic acid bacteria are the primary spoilage bacteria in fruit beverages; however, their numbers are greatly reduced after pasteurization, concentration, and refrigeration. Molds and yeasts tolerate high-osmotic and low-pH conditions and grow at refrigerator temperatures and can therefore cause spoilage in the processed product as well.

The microbial inhabitant found on citrus fruits and juices is significantly determined by how foods are processed and preserved. To be used in this study, 40 different samples are collected from 22 different cafés and restaurants, which are known to produce their own fresh juices for customers from north side of the Nicosia. Our samples mostly consist of fresh orange juices rather than other fruit juice but for comparison, this study also contains 10 other different pasteurized juices as well.

The study is done with three different media to observe the growth of yeasts and moulds for each individual sample. Pour plate method for Rose Bengal media, Orange Serum agar and also instill Plate method for M-Green Yeast and Mold media are used for evaluations.

In general for this study; 85% of the fresh press fruit juices were found to be contaminated by at least one of the pathogenic yeast or mould in Nicosia. On the other hand no any growth of yeast and mold detected in processed juices.

Keywords: Fruit juices; fresh pressed; spoilage; contamination; fungi; mould; Nicosia

ÖZET

Sıcak ikliminden dolayı Kıbrıs'ta genellikle narenciye ürünleri yetişmektedir. Narenciye asitlik ve şeker oranı yüksek pH değeri 3 ve 4 arasında olan bir üründür. Bu nedenden dolayı bu ürünlerde ancakta asidofilik bateriler, küfler ve mayalar bulunup gelişebilen mikroorganizmalardır. Meyve sularında birincil bozulmaya yol açan mikroorganizmalar laktik asit bakterilerdir. Meyve sularına pastörizasyon, konsantrasyon ve soğutma işlemleri uygulanarak bu tür mikroorganizmaların üründe gelişmesi önlenebilir. Ancak küf ve mayalar yüksek ozmofilik, düşük pH derecelerinde ve soğuk ortamlarda bile hayatta kalıp gelişebilen microorganizmalardır. Böylece düşük pH, yüksek su aktivitesi ve soğuk şartlarda bile ürünlerde bozulmaya yol açabilirler.

Meyvelerde ve meyve sularında bulunan mikrobiyal flora ürünlerin nasıl işlenip nasıl korunduğuna göre değişir. Örnek olarak narenciye konsantresi üretiminde ürün suyu yüksek sıcaklıkta uçurulmadan önce iki saat boyunca çelik tanklarda tutulabilir. Bu işlem kontaminasyonun çoğalması için bir etmendir.

Pastörize edilmemiş meyve sularında *Saccharomyces spp.,* laktik asit bakteriler, *Candida* ve *Torulopsis* türleri gibi asite dayanıklı mikroorganizmalar tarafından bozulmaya yol açabilir. Bu çalışmada Kuzey Lefkoşa'nın farklı bölgelerinde bulunan 22 cafe ve restaurantlardan 40 farklı taze sıkılmış meyve suyu örneği toplanmış ve küf maya yükü analiz edilmiştir. Toplanan taze sıkılmış meyve suyu ürünleri arasında Kıbrıs'ın ikliminden dolayı en çok bulunan meyve olan portakal suyu en yoğunlukta olup, onun dışında karışık örneklerde toplanmıştır. Bir yandan ise karşılaştırma amaçlı marketlerden 10 adet işlenmiş meyve suları alınıp incelenmiştir.

Analizler Rose Bengal Chloramphenicol, Orange Serum Agar ve M Green Yeast and Mold olmak üzere üç farklı besiyeri üzerinde yürütülüp her besiyerine ayrı ayrı aynı ürün ekimleri gerçekleştirilmiştir.

Keywords: Meyve Suyu; Taze Sıkılmış; Bozulma; Kontaminasyon; Küf; Maya; Lefkoşa

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PEF: Pulsed Electric Field

HHP: High Hydrostatic Pressure

HPH: High Pressure Homogenization

RBC: Rose Bengal Chlortetracycline Agar

OSA: Orange Serum Agar

CFU: Colony Forming Unit

GHP: Good Hygiene Practices

pH: Hydrogen Ion Concentration

WHO: World Health Organization

FAO: Food and Agricultural Organization

G: Gram

MG: Milligram

L: Liter

%: Percentage

CHAPTER 1
INTRODUCTION

Fruits are considered to be a suitable habitat for microbial growth as they contain high levels of sugar, nutrients and water activity. However, as their Ph values are mostly at lower ranges, they serve as a better media for growing yeasts and molds than other species such as bacteria. Unlike yeast and molds, bacterial growth is optimal in neutral pH ranges (Tambekar, et. al. 2009).

Fruit juices are beneficial for human health as they have a high nutritional value, containing countless mineral, vitamins and other elements that human body requires. In many countries, fruits and fresh pressed fruit juices are sold in public areas, kiosks or cafés. Fresh pressed fruit juices are requested from customers because of their "Fresh Flavor" (Tambekar, et. al. 2009).

In Northern Cyprus, freshly pressed juices can be found almost everywhere. Fresh pressed fruit juices are not processed for protection. They are not heat-processed and contain no preservatives; therefore, they can be quickly spoiled by microorganisms, especially by acid-loving or acid-tolerant bacteria, as well as yeasts and molds.

1.1 Background

Many microorganisms are found in fresh fruits and fruit juices as environmental or raw material contaminants, but actually, very few of them can survive the acidic nature of the juices. Low oxygen levels also play an inhibitory effect on contamination of packaged products. Thus, contamination risk of unpackaged products with pathogen microorganisms is more likely than the contamination of processed products. Yeasts are the most significant group of microorganisms associated with spoilage of fruits and fruit juices. Their metabolic by products, for example, CO2, acid, and tainting compounds can cause alterations in taste, smell and appearance of the products. Even though the most spoilage is caused by yeasts and mould species in fruit-related products, acid-tolerant bacteria can

also play a minor role in spoilage of such products. (Hocking & Jensen, 2001; Jay & Anderson, 2001).

1.2 General Knowledge about Fruits and Spoilage

Most of the fruits have high acidity. Their pH value varies from 5.0 to 2.5 and this pH range is the predictor for what kind of microorganism can spoil the fruits. Most of the bacteria cannot survive the high level of hydrogen ion concentrations whereas yeast and molds can either easily grow or at least tolerate the acidic media. While ripening, the fruits pH increases, their defense barriers get weaker, their soluble carbohydrates start to accumulate and the fruit surface softens (Bates et. al., 2001).

Contamination can occur through the damage to the surfaces of fruits, such as punctures, wounds, cuts and splits that can happen during their growth or harvesting process. Some toxic moulds can even produce mycotoxins at low temperatures while being stored. On the other hand, pathogenic fungi can cause infections or allergies on susceptible individuals (Tournas, et al. 2005). Fungal infestation of fruits can occur on cultivation, harvesting, handling, transport, post-harvest storage or on marketing stages of the fruit.

All these changes aids in making the fruits more prone to fungal infections. Spoilage with fungi can cause unpleasant taste and odors, discoloration and more importantly, it can lead to serious poisoning. On the other hand, toxic fungi can also produce mycotoxin which is additionally hazardous for human health (Tournas, et. al. 2006).

Mycotoxins are produced by filamentous fungi and they can have serious adverse effects on animals and susceptible humans. Mycotoxins are a variety of substances that can be found in nearly all major taxonomic groups. Their toxic effects can occur with acute, subacute or chronic onset in both animals and humans and some of them can be mutagenic or carcinogenic; or teratogenic (Drusch, et. al. 2003).

However, not all the fungal manifestations result in mycotoxin formation. The strain of the fungus is the most important indicator for whether there will be mycotoxin formation or

not. In addition to the species and strain of the fungus, the physical, chemical and environmental factors also play critical roles in mycotoxin formation (Drusch, et. al. 2003).

On the other hand, the presence of fungi does not necessarily mean mycotoxin formation, and vice versa, because of the stability of mycotoxins, they can still be present in the fruit even though there is no fungi infestation anymore. Moreover, a single strand of fungi can produce different types of mycotoxins, and a specific type of mycotoxin can be produced by several different fungi (Drusch, et. al. 2003).

Furthermore, mycotoxins can lower the quality of plant seeds, decrease their lifespan and cause a reduction in the quality and qualifications of all types of products. They can especially cause a decline in nutritional quality of foods. Because of that, mycotoxins are counted as one of the serious issues concerning global public health, agriculture and economics (Arias et. al. 2002).

When it comes to fruit juices, the type of the fruit used for making juice is the main indicator for spoilage with mycotoxins. The contamination of juice usually occurs due to the usage of lower quality fruits (Drusch, et. al. 2003).

Some fungi are plant pathogens and they can interfere with natural growth pattern of the plants and fruits that they infest. Because of that, they can cause early deterioration and can halt harvesting which in return leads to an economical loss in long run (Tournas, et. al. 2006).

The mycotoxin contamination has not only caused health issues but also results in economic losses, especially on trading area. Although huge numbers and variety of mycotoxins exist, only a few of them are observed to be found in foods. The mycotoxins that are most commonly found in foods are toxins, ochratoxin A, patulin, and mycotoxins which are produced by *Fusarium* species (Drusch, et. al. 2003).

In addition to these, controlling the microbial spoilage of fruits before harvesting, controlling the harvesting conditions, storage of the fruits at low temperatures or under the controlled environments after the harvest, and application of fungicides are important factors of spoilage. The use of synthetic fungicides is shown to be effective on prevention of the spoilage but some fungi can still become resistant to the commonly used pesticides.

Storage of the fruits at lower temperatures slows down fungal growth significantly and extends the shelf life of the fruit, but still, many fungi can grow at low temperatures and cause substantial damage, especially if the fruits are stored for extended periods of time (Arias et. al. 2002).

When fresh fruit juices are pasteurized, these microorganisms are denatured and the shelf-life of the products are extended, assuming the microbial load is lowered. Pasteurization inhibits the organisms which are contaminated after harvest, pathogens and other heat sensitive microbes. Most research shows that yeasts are the dominant fungi in fruit-based beverages, fruit juices and fruit concentrates. Yeast spoilages can change color, increase the turbidity of fruit juices and produce CO_2. Freezing or refrigerating techniques are used for controlling yeast spoilage in fruit concentrates. Freezing or refrigerating does not inhibit most yeasts but it may slow down their growth. However, in room temperatures, rates of surviving cells increase, increasing the risk of spoilage (Tournas, et. al. 2006).

1.3 Aim of the study

The aim of this study is to compare the fruit juice samples that are collected from different producers that are located in Nicosia (commercially packed and freshly pressed juices) and to examine the microbial quality they own.

The following steps are applied when growth of different microorganisms is detected in the samples:

• Isolation of the microorganisms such as yeasts and moulds from juice samples collected from the available café's in Nicosia

• Identification of isolates with the help of miscellaneous biochemical tests

CHAPTER 2
THEROTICAL FRAMEWORK

2.1 Fruit juice

The fruit and vegetable juice vintage is as old as agriculture. Juice is a drink made from the removal or squeezing of the natural liquid found in fruit and vegetables. It is intended for ready to eat, collected via a mechanical system from intact, fully developed fruits, preserved particularly by physical means. In simpler words, juice includes tissues and cells as the extractable fluid content of fruits. Sugar or acid addition can be permitted but it must be adequate with the individual standards. Fruits and vegetables assemble diverse and complex category of substances. The minerals, acids, carbohydrates, anthocyanin, carotenoids, vitamins, fibers, amino acids, compounds of flavor, and bioactive substances are relevant with fruits and vegetables. During proces, these compounds are necessarily to transmitted to the beverage or concentrate (Aneja, 2005).

2.2 Health benefits of fruit juices

Eating of fruit and vegetables helps to block of many diseases that deteriorate, essentially cancer and cardiovascular diseases. Many researches has concluded that fruit and vegetables are having major act in reducing the risk of chronic diseases that have been shown to be causing ichor in the long term. Published many reports are admitted that fruit based beverages are plays a major role in diminishing the formation of cancer and illness of Alzheimer for humans (Delichatsios and Welty, 2005).

Fruit juices sodium level is low and potassium level is high, which promotes maintaining the normal blood pressure. Fruit juices do not contain fat, this is also favorable for the cardiovascular system of humans (Delichatsios and Welty, 2005).

2.3 Spoilage

Aneja et al. (2008) Defines the food spoilage as “a change in the smell, taste or appearance of food that makes it unacceptable to the consumer”. Spoilage of fruit and vegetable juices

is mainly determine from of their naturally bearing osmofilic and acid tolerant microorganism. Their high sugar levels and own water activity is ideal media for for microbial growth. Their high acidity makes them harder than bacterial growth to yeast and fungal spoilage. Great parts of contaminated bacteria are wiped out because they cannot survive at low pH levels (Aneja et. al., 2008; Kalia and Gupta, 2012).

2.4 Sources of contamination

Vegetables and fruits used in juice processing are defined as different possible spoilage microorganisms at the time being cultivation, collecting and shipment, classification of fruit or the extraction of juice processes. Basically observed microorganisms on fruit surfaces are mostly soil inhabitants. Tracks are also another source for advertise the microbes. They can transmit soil grains, aerial spores and flooding water. Fruit and fruit based products are can be poison with molds and yeasts which are transmitted by insects. Another potential sources of microbial contaminations are flavorings which are added into, using water, machines, filling lines and additives that are using in the process of fruit juice (Wareing and Davenport, 2005).

2.5 Spoilage microorganisms

Unprocessed fruit juices like fresh juices are more sensitive for contamination than processed fruit juices as their essence are in contact with air and microorganisms that at the atmosphere contains at the time of collection. Yeasts, molds and lactic-acid bacteria are all indicators for the raw product quality. The target of pasteurization of fruit juices are killing the heat resistant fungi and *Bacillus coagulans* and *Clostridium pasteurianum (C. pasteurianum)* which are makes spores (Aneja et. al., 2008, Wareing and Davenport, 2005). The other groups of fruit and fruit juice spoiling organisms are described below.

2.5.1 Sources

Fruit and fruit juices are commonly contaminated with yeasts and moulds, often from insect damage or poor hygiene practices. Fallen fruit should thus be avoided where possible, for all of the risks outlined below. Sugars and sugar concentrates are commonly

contaminated with osmophilic yeasts, for example *Zygosaccharomyces rouxii (Z. rouxii)*. Growth is slow in concentrated solutions, but one cell per container of diluted stock is enough to cause spoilage. Flavorings, water and other chemicals are all potential sources of microbial contamination. Process machinery and filling lines are particularly problematic and strict hygiene is essential for processed products (Wareing and Davenport, 2005).

2.5.2 Yeasts

There are over 800 species of yeasts currently described (Barnett *et al.*, 2000), but only about 10 species are commonly associated with spoilage of foods prepared in factories operating good standards of hygiene and using correctly applied chemical preservatives (Pitt & Hocking, 1997). Others are found if something goes wrong during manufacture; for example, incorrect preservative level, poor hygiene or poor-quality raw ingredients.

One group of yeasts are well adapted to growth in processed or unprocessed fruit juices and can able to cause spoilage in fruit juices from very low cell numbers. The characteristics of these yeasts are osmotolerance, aggressive fermentation, resistance to preservatives (particularly weak organic acids) and a requirement for vitamins. *Zygosaccharomyces bailii (Z. baili)* is a typical example of this group, and this group corresponds closely with 10 key spoilage yeasts (Lawlor et. al., 2009).

Another group is spoilage/hygiene types, able to cause spoilage of fruit juices, but only if something goes wrong during manufacturing, for example, unsterilized instrument which using for pressing fruits for fresh juices or too low a level of preservative, ingress of oxygen, defective of pasteurization or poor standards of hygiene for processed products. These yeasts will not grow in fruit juices, even if present in high numbers, and are typical of the yeasts found in many factories; the higher the count, the worse the hygienic state of production area (Lawlor et. al., 2009).

2.5.2.1 Yeasts resistant to preservatives

Resistance to preservatives is a serious threat to the stability of fruit juices. Some of the yeasts that are resistant to preservatives are *Z. bailli, Candida krusei, Saccharomyces bisporus, Schizosaccharomyces pombe* and *Pichia membranifaciens*. Resistance to preservatives has been associated with the ability of cells to tolerate constant intracellular pH declines caused by phosphofructokinase enzyme. *P. membranifaciens* is regarded as the target microorganism for optimization of thermal pasteurization of fruit juices because of its resistance to heat, moderate amount of salt, SO_2, sorbic, benzoic and acetic acids (Lima et al. 2009; Stratford, 2006).

2.5.3 Moulds

Overgrowth of a different array of moulds can occur because of the poor hygiene standards of the instruments used for pressing fruits. Contamination can cause spoilage, discoloration and other general problems associated with evident mould growth. Xerophilic fungi are example of this group which causes spoilage about poor hygiene standard. In addition to this, growth of heat-resistant moulds can also cause serious contamination of the heat-processed juices. Heat-resistant moulds can result in steady growth of the mould within the processed juices (Tournas et. al. 2006).

Heat-resistant moulds that can cause contamination of processed fruit juices are *Aspergillus ochraceus (A. ochraceus), Aspergillus tamarii, Aspergillus flavus (A. flavus), Byssochlamys nivea, Byssochlamys fulva Eupenicillium brefeldianum Neosartorya fischeri, Paecilomyces variotii, , Phialophora mustea, Talaromyces flavus, Talaromyces trachyspermus* and *Thermoascus aurantiacum*. Some of the other moulds are also *Penicillium notatum, Penicillium roquefortii* and *Cladosporum* spp. Mould contamination of the factory is an indicator for poor hygiene conditions. Various types of heat-resistant spores can be observed: ascospores, chlamydospores and sclerotia. Many of the above moulds can be found on fruits during the pre- and post-harvest periods. Most of the mould species are oxygen-dependent. Growth can be displayed on surface mats when there is abundant spore production. Some mould species can produce extracellular degradative

enzymes, such as pectinases. Detection of heat-tolerant moulds takes place by plating out a sample from heat-shocked juices (Lawlar et. al., 2009).

2.5.4. Mycotoxins

Fungi that are found within or on the surface of foods can produce toxic secondary metabolites which are known as mycotoxins. They can be hazardous for human and animal health. Patulin is the most commonly observed mycotoxin within fruit juices, particularly apple juices. It commonly occurs due to production of juices from the reserved apples. As mould growth advances with time in infected apples, so does the levels of patulin. The use of windfall apples for juice production is also another factor on spoilage. Prevention of usage of windfall apples, proper filtration of juice materials and pressing the fruits quickly after the harvest are some methods that can be applied to reduce the incidence of patulin formation in juices. Patulin can be eliminated by fermentation of fruits to cider or by the extra addition of ascorbic acid to the juice extract. Within Europe, the European Union has set a limit of 50 g/kg of patulin in both apple juice and cider. A recent survey which was held in Chile showed that 28% of samples of apple products (juice and concentrates) were patulin, exceeding the maximum acceptable level (Wareing and Davenport, 2005).

2.5.5 Heat resistant fungi

Pasteurized fruit juices can be spoiled by heat resistant fungi. Primary heat resistant moulds are *Byssochlamys nivea*, *Byssochlamys fulva, Neosartorya fischeri, Eupenicillium brefeldianum* and *Talaromyces macrospores*. These moulds survive commercial heat pasteurization process which is applied to fruits and fruit products, due to the presence of heat resistant ascospores they contain. *Byssochlamys spp.* are most widely detected moulds that cause the spoilage of heat processed fruits (ICMSF, 2005; Salomao *et al*., 2007;Lawlor *et al*., 2009). Kutama *et al*. (2009) reported that heat-resistant moulds such as *Byssochlamys*, *Neosartorya* and *Talaromyces* were observed in orange, mango, tomato and pineapple juices. The presence of heat-resistant fungi such as *Paecilomyces variotii, Aspergillus tamari, A. flavus* and *A. ochraceous* has been reported in sixty packaged Nigerian fruit juices consisting of mango, pineapple, orange and tomato (Obeta and

Ugwuyani, 1995). Chlamydospores, sclerotia and aleuriospores are the resistant structures/spores produced by implied moulds (Voldrich et. al., 2004).

2.5.6 Bacteria

Bacteria naturally occur in low numbers on fresh fruits and vegetables. Still, some bacteria, such as heterofermentative lactic acid bacteria (LAB), acetic acid bacteria, *Erwinia spp., Enterobacter spp*., *Clostridium spp.*, *Alicyclobacillus acidoterristeris*, *Propionibactreium cyclohexanicum*, *Pseudomonas spp*. and *Bacillus spp.* have been reported to be degenerative in cut fruits and juices (Pitt & Hocking, 1997).

2.6 Pathogenic microorganisms

In tropical countries, consumers choose fresh-cut fruits and juices rather than their processed counterparts. Because of their fresh look and original nutritional and sensory attributes, fruit juices sold by the street merchants are consumed regularly by the local people. In India, regardless of their differences in income and age, a large group of the people consume freshly squeezed fruit and vegetable juices, but the presence of pathogenic microorganisms in street-sold fruit juices have been reported in various parts of India such as Vishakhapatnam, Amravati, Nagpur, Kolkata, Mysore and Tirumula. Other researchers from other parts of the world have also carried out various studies on the microbiological condition of street vended fruit juices. It is reported that there have been some *Salmonella spp*. and *Staphylococcus aureus (S. aureus)* in orange juice extracts which were produced by squeezing machine that are used in restaurants. The foodborne outbreaks associated with consumption of fruit juices have also increased due to the existence of pathogens in fruit juices (Parish, M., 2000).

2.7 Factors affecting shelf life of juices

The shelf life of a food defines the time interval within the food is accepted to be safe to be consumed and/or has an adequate quality to the consumers. The shelf-life is also defined as the time elapsed to reach a microbial load of 6 log cfu/mL which is determined experimentally. The shelf life can be affected by both the intrinsic and extrinsic factors.

Intrinsic factors include pH of the product, oxidation–reduction (redox) potential, water activity, availability of nutrients, the presence of antimicrobial compounds and competing microflora. Extrinsic factors contain storage temperatures and storage period, relative humidity conditions during storage and the packaging material characteristics. Among intrinsic factors, pH and water activity are the most prominent factors affecting the spoilage rates. Ideal pH for bacterial growth is around 6.5-7.5 but they can tolerate a pH range from 4 to 9 in most cases. Yeasts are more tolerant to low pH values than the bacteria. However, when compared, moulds can grow in the broadest pH value range. Therefore, microbial growth in foods can be controlled by increasing the acidity of the products (Davidson, 2001; Mosqueda-Melgar *et. al*., 2008; Lawlor *et. al*., 2009).

In previous times, fruit juices were considered to be harmless foods because of their low pH levels caused by naturally occurring organic acids they contain. Organic acids alter several systems of the targeted organisms. Organic acids has direct effect on pH of the substrate and growth medium as they cause an increase in proton concentrations, reduce the internal cellular pH by causing the ionization of undissociated acid molecules, and cause the disruption of the substrate transport by altering the cell membrane permeability. In addition to disrupting the substrate transport, organic acids can also inhibit NADH oxidation, which results in the elimination of the supplies of reducing agents to the electron transport systems Water activity of juices is linked with Brix. Brix is used to specify the percentage of soluble solids found in juices and is one of the most important factors used for evaluation of the quality of the juices. Microorganisms cause fruit juice spoilage as they cause the fermentation of sugars. This results in Brix value increase due to the conversion of complex sugars into monosaccharides. Extrinsic factors such as temperature also has an impact on the shelf life of juices. The shelf life of freshly squeezed, un-pasteurized orange juice is lower than 20 days at 1°C. Low temperature is essential during manufacturing and storage of the juices. The main aim of the low temperature storage is to increase the shelf life by slowing down the deteriorating chemical reactions which in return inhibit the microbial growth. Therefore, the combination of reduction in chemical, biochemical and microbial kinetics can increase the shelf life of fresh and processed foods (Aneja *et. al*., 2008, Wareing and Davenport, 2005).

2.8 Preservation of fruit juices

Food preservation is defined as to control the microorganisms which can cause the spoilage in products and pathogenic organisms which are can make disease for human. Preservation of fruit juice depends on the refrigeration standard, pasteurization level, pH level, and chemical preservatives which are added to products. For fruit juices pasteurization process often done by increasing the temperature of product to 85-95°C up to 2 minutes. Pasteurization process is effective about inhibition of pathogens such as e-*Escherichia coli* and *Salmonella spp.* if it is done right. But if product was contaminated with heat resistant bacteria and heat resistant fungi before pasteurization these bacteria are can survive despite of high temperature effect. Also this thermal treatment affects the sensory and nutritional quality of product (Aneja *et. al*., 2008).

Against disadvantage of the increasing the temperature of products new techniques are developed. Example of these non-thermal techniques are pulsed electric field (PEF), high hydrostatic pressure (HHP), high pressure homogenization (HPH), ultrasound techniques and irradiations for inactivate microorganisms and increase the shelf life of foods (Normey et. al., 2007, Rupasinghe and Yu, 2012).

The best application for fruit juice treatment is the High Hydrostatic Pressure (HHP). In HHP process they do not increase the temperature of product up to 20°C, they expose the product to 400MPa pressure for a few minutes. The effect of this pressure of applied is squeeze the cell membrane of microorganisms and inactivates some key enzymes which are used in DNA replication and transcription processes (Kuldiloke and Eshtiaghi, 2008).

CHAPTER 3

RELATED RESEARCH

In 2018 B. Alemayehu was study about microbial quality of fresh fruit juices prepared in juice houses of Dangila town, Awi zone, Amhara regional state, Ethiopia in Haramaya University. In that study they determine the microbial quality by quantifying the total aerobic viable bacterial count, Aerobic spore forming bacterial count, Staphylococcal count, Yeast and Mould count, using spread plate count method with appropriate media and Entrobactriaceae count, Total coliform count and Faecal coliform count, were determined by using the most probable number method. They used XLD agar for detecting of *Salmonella spp.*, Selenite Cystine broth and *Salmonella Shigella* agar for detecting *Shigella spp.,* Mannitol Salt Agar for *S. aureus* and potato dextrose agar for Yeast and Mold count.

In another comprehensive study at 2015 S. A. M. Mahgoub and G. A. El-Shourbagy are study with 360 fruit juice samples for determine the Microbiological and physicochemical quality of fruit juices sold in Egypt. They study with 6 different juices and analyzed total bacterial count, total spore-forming bacteria count, coliforms, *E. coli*, yeasts & molds, and *Staphylococcus spp*. according to standard methods of APAH. Also they use Rose Bengal Chloramphenicol agar for detecting yeasts and molds as like us and then they incubate the plates at 25°C for 5 days.

At the other study Tournas at. al. (2006) analyzed yeasts and molds in fruit salads and fruit juices. In their study they distillates the juice samples 10 times and plates 0.1ml sample to Rose Bengal Chloramphenicol for see the growth of yeasts and molds. Also they incubates plates 5 days at 25°C. They found much higher contamination from minimally processed juices and they attribute this situation for low-quality or the lack of adherence to good manufacturing practices.

CHAPTER 4

MATERIAL & METHODS

4.1. Sampling

To be used in this study, 40 different samples are collected from 22 different cafés and restaurants, which are known to produce their own fresh juices for customers from north side of the Nicosia. Citrus is a common fruit type seen in Cyprus because of the convenient climate of the island. For this reason, our samples mostly consist of fresh orange juices rather than other kind of fruit juices. In addition to all these, this study also contains 10 other different pasteurized juices for comparison.

Figure 4.1: Map showing the amount of samples taken from selected regions of Northern Nicosia

Table 4.1: List of the fruit juices which were collected by region

Sampling Sites	Fruit Juices	Total Sample
Köşklüçiftlik	Orange Juice	3
	Pineapple Juice	1
	Pomegranate Juice	1
	Carrot Juice	2
	Apple Juice	3
Metehan	Orange Juice	1
	Carrot Juice	1
	Apple Juice	1
Ortaköy	Orange Juice	4
	Apple - Carrot Juice	1
Kaymaklı	Orange Juice	5
	Pomegranate Juice	2
	Apple - Carrot Juice	1
Surlariçi	Orange Juice	4
	Pomegranate Juice	2
Taşkınköy	Orange Juice	2
	Lemonade	2
Hamitköy	Orange Juice	2
	Lemonade	2
Supermarkets (Processed Fruit juices)	Orange Juice	4
	Mandarin Juice	1
	Mix Fruit Juice	2
	Pineapple Juice	1
	Apple Juice	2

4.2. Reagents

4.2.1 Used Media

The study is done with 3 different media to observe the growth of yeasts and molds for each individual sample. We used pour plate method for Rose Bengal media and Orange Serum agar and also Spread Plate method for M-Green Yeast and Mold media.

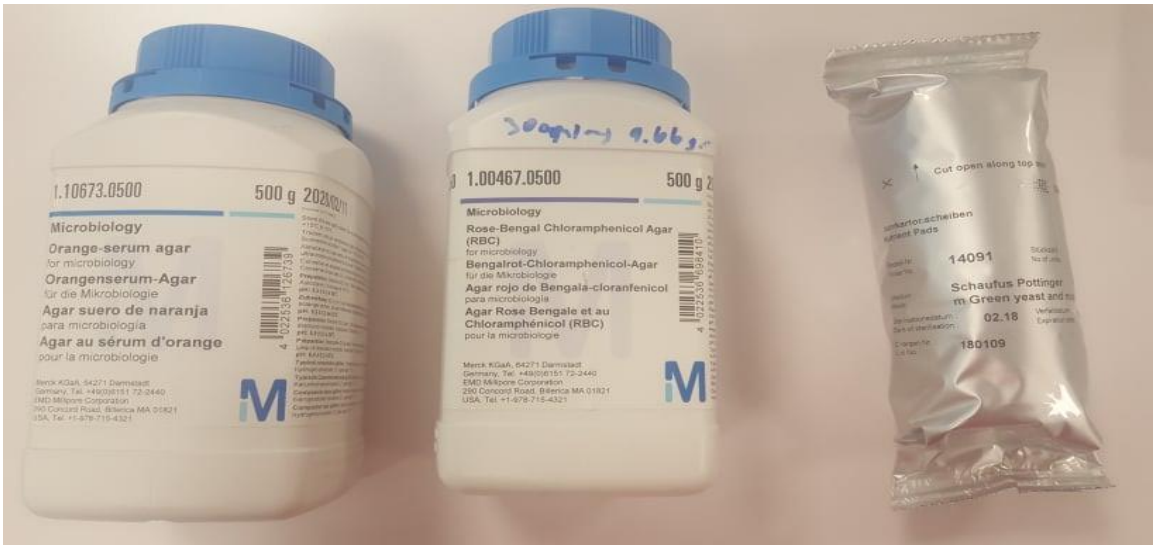


Figure 4.2: Media which was used for analyzes

Rose Bengal Chlortetracycline Agar (RBC):

A specific type of medium developed by Jarvis in 1973 that contains chlortetracycline and is used for counting of moulds and yeasts in food samples. Nowadays, instead of chlortetracycline, it is more commonly used with chloramphenicol. Rose Bengal makes it possible to keep the size and height of the mould colonies under control and assists the counting process by giving fungi coloured dyes. Chloramphenicol aids in inhibiting the growth of most of the bacteria in the media, thus aids in keeping the media uncontaminated (King et. al, 1979).

Composition of Media

Table 4.2: İngredients of Rose Bengal Chloramphenicol (RBC)
(gr/L of deionized water)

Mycological peptone	5.0
Glucose	10.0
Di-potassium hydrogen orthophosphate	1.0
Magnesium sulphate.7H_2O	0.5
Rose Bengal	0.1
Chloramphenicol	0.1
Agar	15.0

In earlier times, the selective medias used were added acids to aid in inhibition of bacterial growth, but later on, instead of acidic additions, some specific antibiotics were added to media, which did not interfere with PH much and made it possible to grow a vast range of fungi in the given media. By doing so, it was observed that some damaged fungal spores recovered in the given conditions as well (Koburger et. al. , 1985).

Another refinement was made possible by the addition of rose bengal to prevent the rapidly spreading moulds reproduction. Rose Bengal chlortetracycline agar (RBC) is the most successful fungal enumeration medium for producing a desired result in current usage nowadays (Koburger et. al., 1985).

Miscellaneous fungicidal compounds are often added to the media to prevent the growth of unwanted fungi species and aiding in the growth of the desired ones instead (King et al, 1979).

Rose Bengal chlortetracycline (RBC) is a neutrally-selective medium, which is recommended for the exact counting of molds and yeasts in foods, water and other environmental materials. Bacteriological peptone is the main provider for the nitrogen, vitamins, minerals and also amino acids. Dextrose is the fermentable simple carbohydrate

which is used as an energy source. Potassium phosphate is used as the buffer. Magnesium sulfate provides sulfur and other trace elements to aid in survival. Rose bengal is a selective agent that prevents the growth of bacteria and limits the size and height of faster-growing molds that are unwanted in the media, and also aids in the development and detection of other (desired) slower-growing yeasts – molds, which in the end appears pink-colored. Chloramphenicol is a selective agent, which is used for inhibition of bacterial growth. It is a suitable antibiotic for neutral media as it is heat-resistant and has a wide spectrum effect on bacterial killing. Bacteriological agar is the stiffing agent (Beuchat, 1992).

Orange Serum Agar (OSA)

The microbial inhabitant found on citrus fruits and juices is significantly determined by how foods are processed and preserved. Even a minor percentage of contamination of fruits can fill the operating equipment with spoilage organisms. Equipment's used for fruit juice preparations are often found to be the weighty source of contaminations. There are many specific processes, or areas, where microbial overgrowth can occur such as pipelines, conveyors, extractors, finishers, mills, and presses. Aerobic plate counts can be used as a guide for evaluating the sanitation of citrus fruit and juice processing equipment (Kator, and Rhodes, 2003).

In the preparation of citrus concentrates, juice can be kept in stainless steel tanks for up to 2 hours before high-temperature evaporation process. Throughout this time the product is most vulnerable to microbial contamination.(Vanderzart and Splittstoesser, 1992).

Convenience citrus fruit juices and products have a pH value range of 2.9 to 4.0. For example, the pH of orange juice is usually 3.0 to 4.0 and other acidic foods like tomato juice have pH that ranges from of 3.9 to 4.4. Because of the low pH of fruits and fruit juices, aciduric molds and yeast are the microbes that are most often came across with in contaminated citrus products. (Kator, and Rhodes, 2003).

Food-related yeasts and molds contain several hundred species. Such organisms, as being tolerant for wide range of pH and temperature changes, as well as their variety of hydrolytic enzymes, has the ability to grow in most of the low pH food. Spoilage of foods by yeast and molds result in significant losses to the producer, processor and also the consumer. Several of the foodborne molds, and very likely yeast, may be dangerous to human and animal health due to their ability to produce mycotoxins. Yeast and molds may also induce allergic reactions or infections in susceptible individuals such as elderly people or people having a compromised immune system due to chemotherapy or other drug usages (Vanderzart and Splittstoesser, 1992).

Orange Serum Agar is specially formulated for the isolation, cultivation and enumeration of the mold and yeasts seen in citrus foods, juices and other foods with low pH values. Orange Serum Agar contains: casein peptone as a nitrogen source, yeast extract to provide B-complex vitamins for growth stimulation, and dextrose as a carbohydrate source. orange powder is added to the media to establish an acidic media that benefits the recovery of aciduric organisms and potassium phosphate, which serves as a buffer (Vanderzart and Splittstoesser, 1992).

Composition of Media

Table 4.3: İngredients of Orange Serum Agar (OSA)
(Grams per liter of deionized water)

Peptone from casein	10.0
Yeast Extract	3.0
Orange Extract	5.0
D(+) glucose	4.0
Di-Potassium hydrogen phosphate	3.0
Agar – Agar	17.0

M Green Yeast And Mold

This classical composition is used by the food industry for the detection and enumeration of yeast and moulds by the membrane filter method which was adopted by ISO to be applied in cork stoppers for alcoholic and non-alcoholic beverages in the 10718:2002 Standard. The composition of the culture broth includes Bromocresol Green indicator that aids in the visualization and counting of fungal colonies found in the beverages. The fungi appear to be green due to the diffusion of the dye into the colonies (due to an alkaline reaction). The end products of the microbial growth diffuse into the medium, reduce the pH and turn the indicator into yellow (due to an acidic reaction). Bacterial growth is inhibited by the acidic pH of the media. The membrane filter is rolled onto the surface of the medium to prevent the formation of air bubbles. Plates are incubated at 30 ± 2ºC for 3 days. Colonies are observed and counted on each plate at least every 24 hours. After incubation, colonies appearing on the filter surface can be counted. Mould colonies generally appear green and filamentous whereas yeast colonies are green and opaque (Mac Faddin, 1985).

Composition of Media

Table 4.4: İngredients of M Green Yeast and Mold

(Grams per liter of deionized water)

Dextrose	50.0
Peptone	10.0
Yeast extract	9.0
Magnesium sulfate	2.1
Potassium phosphate	2.0
Diastase	0.05
Thiamine	0.05
Bromocresol green	0.026
Agar agar	15.0

4.3 Preparation media for plating of samples

4.3.1 Rose Bengal Chlortetracycline (RBC): 32.2 g RBC media putted in to 1 liter of purified water and mixed. This mixture is heated in boiling water and agitated frequently until completely dissolved. When all particles are dissolved the mixture was autoclaved at 121°C for 15 minutes. After autoclave cooled to 50-45°C and media used for plating.

4.3.2 Orange Serum Agar (OSA): 42.0 gr of medium dissolved in 1 liter purified water and mixed with heating as like RBC until all particles are dissolved. After the autoclaving at 121°C for 15 minutes the media used for plating.

4.3.3 M Green Yeast And Mold: This media is ready to use media for membrane technique. All ingredients are stored in small pasteboard. Sample is instilled in to this pasteboard medium.

4.4 Equipment

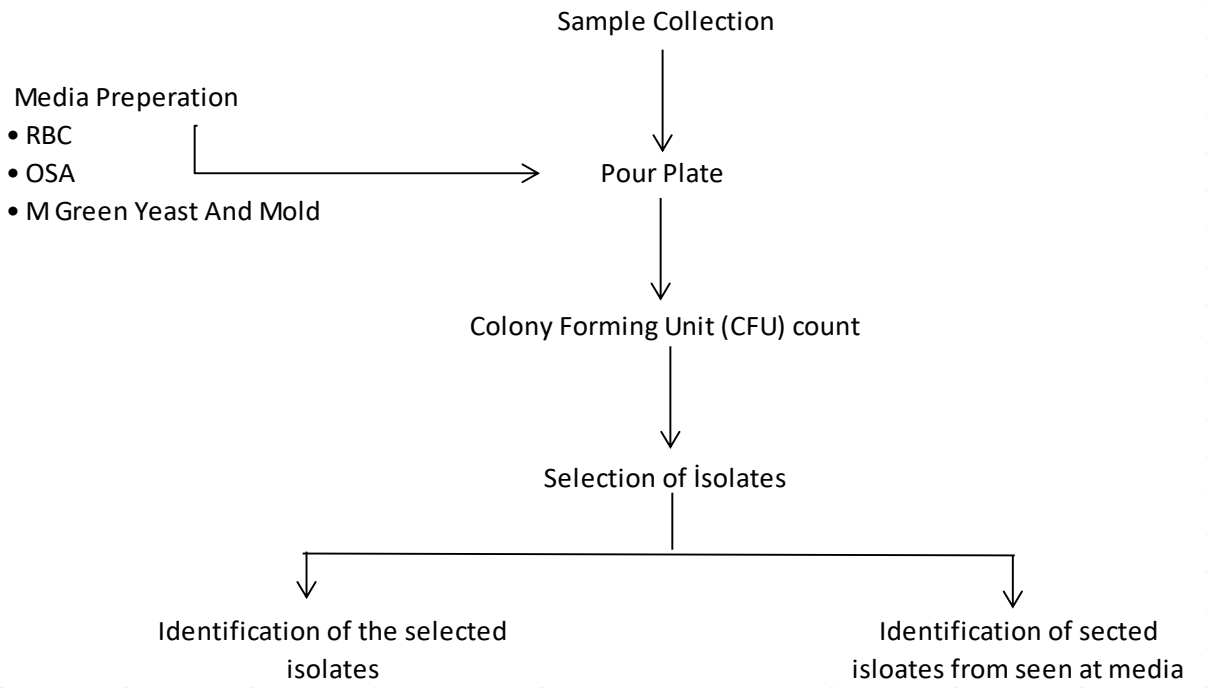
- Laminar airflow cabinet (Model-BLB Biological Sterile cabin)
- Incubator (Poleko, 1LW-53STD, Turkey)
- Autoclave machine (Model: Wise WAC 80 Liter Daihan Scientific Co. ltd, Korea)
- Glass wares, laboratory distillation apparatus- fractional distillatory set up, microscope, petri-dishes, slants, micro-pipettes, Bunsen burner, hot plate, clamp stands, electric balance, etc.

4.5 Methods

4.5.1 Sample processing:

After collecting samples these methods are applied to the samples for see the growth of yeast and mold flora.1ml of fruit juice samples are plated with pour plate method to RBC and OSA. On the other hand 1ml of sample instill to the M Green Yeast And Mold media. Then same identification techniques are done for selected isolates.

4.5.2 Flowchart of the study design:



4.5.3 Pour Plate:

For both RBC and OSA media 1ml of sample was dropped in to the center of sterile petri dishes with using sterile pipette. Then poured approximately 15 ml of agar which prepared before.

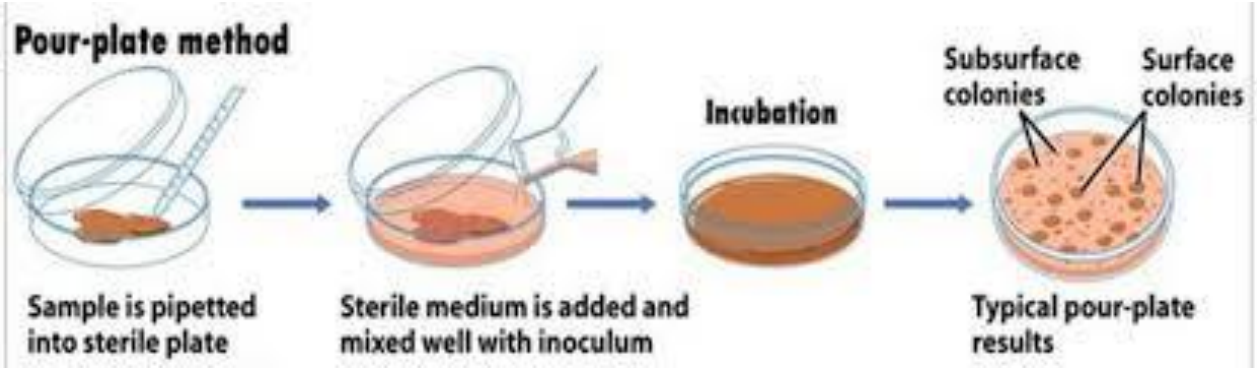


Figure 4.3: Describing the poor plate method (intranet.tdmu.edu)

4.5.4 Identification Techniques

For Yeasts: After incubation of OSA, RBC and M Green Yeasts and mold media yeast colonies are selected and isolation techniques are applied. These yeast colonies are inoculated to corn meal agar with streak plate technique and after than petri dishes are incubated in 24-25°C for 24-48 hour. After incubation detection of arthrospore and chlamydospore are examined.

For Moulds: After incubation of OSA, RBC and M Green Yeasts and mold media mould colonies are selected and isolation techniques are applied. These mould colonies are identificated with typical of mycelium or dust structure of them. More over identification of spaces cellophane band technics are used for identification macro and micro colony structures.

CHAPTER 5

RESULTS AND DISCUSSION

In developing countries, millions of people ingest drinks, meals, and snacks sold by street food vendors. These street products provide a fair source of nutrients to many sectors of the community.

Street-vended fruit juices are well enjoyed by consumers, because of their taste, cheap price, and availability at right time (FAO, 1988; Ohiokpehai, 2003). However, street foods frequently cause gastrointestinal problems (diarrhea and vomiting) due to their inappropriate handling and serving methods. (WHO, 2002). Street Juice trade vendors are mostly unaware of good hygiene practices (GHP) and causes of diarrheal diseases, which can raise the risk of street food contamination (Bhaskar et. al., 2004).

Therefore, the conditions of street food preparation and vending raise many concerns about consumers' health. In most cases, running water is not accessible at vending sites; hands and utensils cleaning are usually done in one or more buckets, and sometimes without any cleanser. Wastewaters and garbage are discarded nearby, serving as a nutrient source for insects and rodents.

Some of the juices are not accurately protected against flies which may be a food borne pathogen transmitter. Street vended juice are rarely preserved at safe food storage temperatures. Moreover, there is another potential health risk associated with primary contamination of foods by pathogenic bacteria as well as subsequent contamination by vendors during preparation, handling, and cross-contamination (Mosupye and van Holy, 2000).

This study intends to establish the hygienic status of street vended juices and their impact on street food contaminations. The analyses revealed presence of microbial contaminations of varying degrees in both fresh press juices and processed juice beverages.

A total of 50 samples from different areas on Nicosia were analyzed (Table 5.2). 40 of them were fresh press fruit juice and the remaining 10 were processed fruit juice. The fresh press fruit juices were obtained from different cafes located in different regions of Nicosia and the other 10 were obtained from supermarkets. 34 of 40 fresh press fruit juice samples were found to be contaminated by yeast and moulds or both. From 40 fresh pressed juice samples, 4 different types of pathogenic yeast and 2 different types of pathogenic mould were isolated. In general, 85% of the fresh press fruit juices were found to be contaminated by at least one of the pathogenic yeast or mould.

Citrus is the most consumed and preferred type of juice in Cyprus, therefore, most of the samples were chosen to be from citrus fruit family, orange juice being the most and it was studied in both fresh press group and processed juice group. 90.48% of the analyzed fresh press orange juices were found to be contaminated by yeast and mould. 7 out of 21 fresh press orange juice samples were contaminated by moulds which were detected as *Aspergillus spp.* 5 out of 21 fresh press orange juice samples were contaminated by black yeasts and 7 out 21 fresh press orange juice samples were contaminated another pathogenic yeasts and molds as *Penicillum, Candida* and *Trichosporon.* There was no pathogenic yeast or mould detected in the remaining 2 samples of fresh press orange juice. On the other hand, 4 processed orange juices were included in our study and there was no contamination with any pathogenic organism (Table 5.1).

In addition to orange juices, fresh press pomegranate, pineapple, apple-carrot, carrot and apple juices were also included in our study. 5 fresh press pomegranate juices were analyzed and 2 of them were found to be contaminated from *Aspergillus spp.* and 1 of them contaminated with yeast *Rhodoturla spp.* 4 samples were obtained from fresh press lemonade, 2 of them were observed to be contaminated by *Rhodotorula spp.* The remaining 2 were observed to be uncontaminated with pathogenic yeast or moulds. 4 fresh

press apple juice were collected from points and all samples were to be contaminated with any pathogenic yeast or mold. 2 samples of fresh press apple juices are contaminated with *Aspergillus spp*. and another 2 samples were contaminated with *Trichosporon spp.* 3 fresh press carrot juice was included in the study and all of them were contaminated with different yeasts or moulds. 1 of them contaminated with *Aspergillus spp.* and other ones were contaminated with yeasts namely *Rhodotorula spp.* and *Trichosporon spp.* When it comes to fresh press apple-carrot juice, 2 samples were evaluated and 1 of them was found to be contaminated with pathogenic mold; *Aspergillus spp*. whereas the other one was also contaminated by *Penicillium spp* (Table 5.1).

1 fresh press pineapple juice was also examined in this study and contamination with *Aspergillus spp.* was seen (Table 5.1).

When it comes to the processed fruit juices, 4 orange juices, 2 mixed fruit juices, 2 apple juices, 1 mandarin juice and 1 pineapple juice was evaluated in our study. There was no contamination and in addition, no growth of the naturally occurring yeast or mold was observed on processed fruit juices (Table 5.1).

Table 5.1: Totally collected samples and isolated pathogens

	Types of Fruit Juices	Total Sample	Growth Yeast And Molds	*Aspergillus spp*	*Peniciilium spp*	*Rhodotorula spp*	*Candida albicans*	*Trichosporon spp*	*Black yeast*	Percentage of pathogen detected juices
Fresh Pressed Juices	Orange Juice	21	19	7	2	-	2	3	5	90
	Pineapple Juice	1	1	1	-	-	-	-	-	100
	Pomegranate Juice	5	3	2	-	1	-	-	-	60
	Apple - Carrot Juice	2	2	1	1	-	-	-	-	100
	Lemonade	4	2	-	-	2	-	-	-	50
	Carrot Juice	3	3	1	-	1	-	1	-	100
	Apple Juice	4	4	2	-	-	-	2	-	100
Processed Fruit Juices	Orange Juice	4	Not seen	-	-	-	-	-	-	0
	Mandarin Juice	1	Not seen	-	-	-	-	-	-	0
	Mix Fruit Juice	2	Not seen	-	-	-	-	-	-	0
	Pineapple Juice	1	Not seen	-	-	-	-	-	-	0
	Apple Juice	2	Not seen	-	-	-	-	-	-	0

For this study 10 samples were obtained from Köşklüçiftlik region. 1 pathogenic yeast and 1 pathogenic mold were isolated from 3 fresh press orange juice samples of this region. 1 pathogenic mold were found in 1 pineapple juice sample and also 1 pathogenic mold and 2 pathogenic yeast among 3 fresh press apple juices. Also 1 pathogenic mold and 1 pathogenic yeast were detected from carrot juice samples in this region. Totally 8 pathogenic yeasts and molds were detected in 10 samples from this region (Table 5.2).

None of the pathogenic yeast or mold was detected in 3 samples that were collected from Metehan region (Table 5.2).

For Ortaköy region, total number of 5 samples were collected and 2 pathogenic yeast and 2 pathogenic molds were isolated from these samples. Among all the regions, with the percentage of 80, it was the area where the most pathogenic microorganisms were detected (Table 5.2).

8 fresh press juice samples were collected from Küçük kaymaklı region, 5 of the samples were fresh press orange juice. 3 pathogenic molds and 1 pathogenic yeast were detected from those samples. 2 fresh press pomegranate juice samples collected from this region and they are also contaminated with one pathogenic yeast or mould. Only 1 sample of apple-carrot juice mixture was obtained from this area and it was observed to be contaminated with pathogenic yeast (Table 5.2).

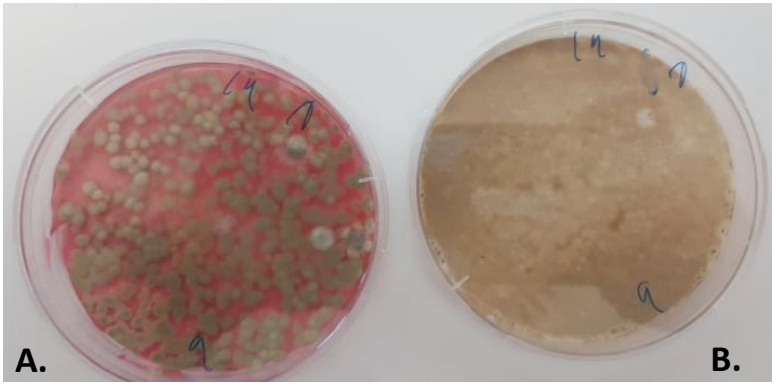


Figure 5.1: A: *Aspergillus spp.* Isolated with Rose Bengal Chlortetracycline Agar from orange juice in Küçük Kaymaklı region. **B:** Aspergillus spp. Isolated with Orange Serum Agar from orange juice in Küçük Kaymaklı region.

When we come to the Surlariçi region, 4 fresh press orange juice samples were obtained and 1 of them were found to be contaminated with pathogenic moulds and another 3 samples were contaminated with pathogenic yeasts. 2 fresh press pomegranate juices which were also obtained from this area were evaluated and there were no contamination with any kind of mould or yeast (Table 5.2).

Figure 5.2: *Aspergillus spp.* Isolated with Orange Serum Agar from orange juice in Surlariçi region.

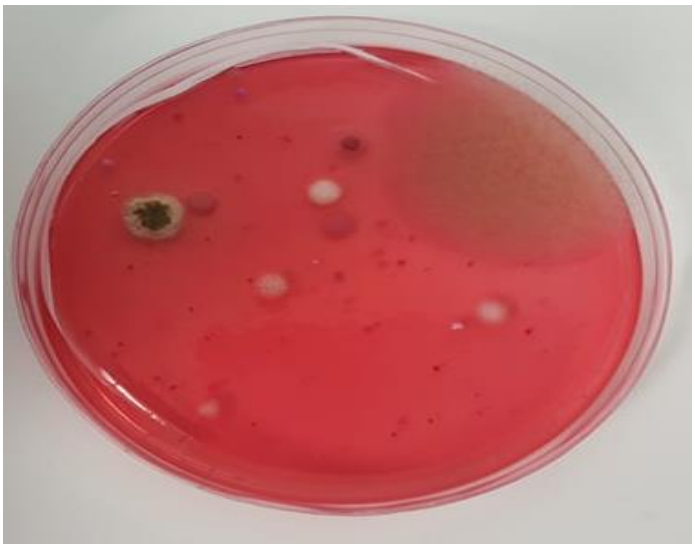


Figure 5.3: *Aspergillus spp.* Isolated with Rose Bengal Chlortetracycline from orange juice in Surlariçi region.

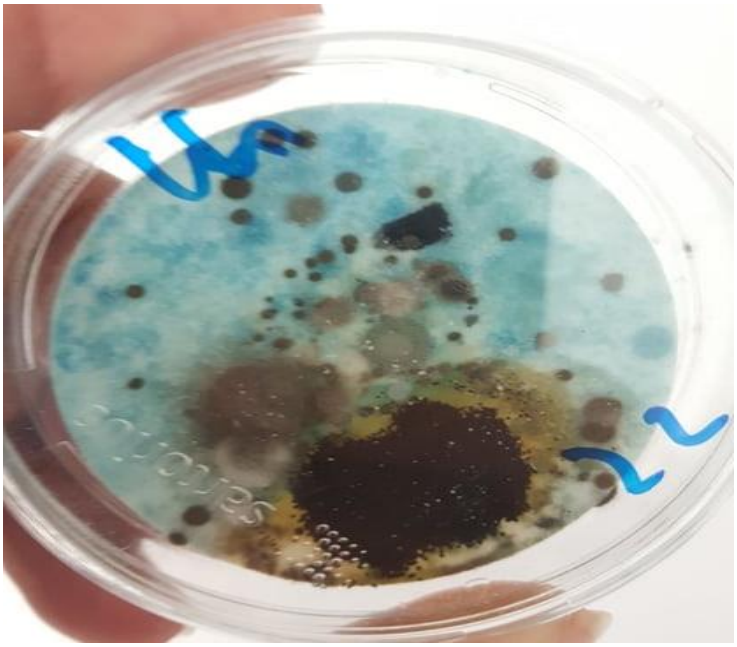


Figure 5.4: *Aspergillus spp.* Isolated with M Green Yeast and Mold media from orange juice in Surlariçi region.

For Taşkınköy region, 2 fresh press orange juices and 2 fresh press lemonade were analyzed. Only 1 of the samples of lemonade and 1 of the samples of orange juice was observed to be contaminated with pathogenic yeast or mold (Table 5.2).

In Hamitköy region, 2 fresh press orange juices and 2 fresh press lemonade were obtained. In both groups, 1 of each sample were found to be contaminated by a pathogenic yeast (Table 5.2).

On the other hand, 10 different samples of processed juices were obtained from different supermarkets. Out of 4 orange juice, 2 mix fruit juice, 2 apple juice, 1 mandarin juice and 1 pineapple juice, none of them were found to be contaminated by any kind of pathogenic yeast or moulds (Table 5.2).

Table 5.2: Totally collected samples and number of pathogens detected by region

Sampling Region	Type of Fruit Juice	Total Samples	Pathogenic Molds	Pathogenic Yeasts
Köşklüçiftlik	Orange Juice	3	1	1
	Pineapple Juice	1	1	-
	Pomegranate Juice	1	-	-
	Carrot Juice	2	1	1
	Apple Juice	3	1	2
Metehan	Orange Juice	1	-	-
	Carrot Juice	1	-	-
	Apple Juice	1	-	-
Ortaköy	Orange Juice	4	1	2
	Apple - Carrot Juice	1	1	-
Kaymaklı	Orange Juice	5	3	1
	Pomegranate Juice	2	1	1
	Apple - Carrot Juice	1	1	-
Surlariçi	Orange Juice	4	1	3
	Pomegranate Juice	2	-	-
Taşkınköy	Orange Juice	2	1	-
	Lemonade	2	-	1
Hamitköy	Orange Juice	2	-	1
	Lemonade	2	-	1
Supermarkets (Processed Fruit juices)	Orange Juice	4	-	-
	Mandarin Juice	1	-	-
	Mix Fruit Juice	2	-	-
	Pineapple Juice	1	-	-
	Apple Juice	2	-	-

Tournas et al. (2006) were isolated *Rhodotorula rubra* from apple cider, *Candida sake* from apple juice, *Candida lambica, Kloeckera apis, Fusarium spp., Geotrichum spp,*

Penicillium spp., from citrus juice (Grapefruit juice) in the Washington, DC. They used Dihloran Rose Bengal Chlortetracycline and Potato Dextrose agar for plate. Their result is 22 percentage of fruit juice samples are contaminated with fungi.

In another study made from Durush and Ragap (2003) for check the mycotoxin level of fruit juices. They reported that the founding in Grapefruit juice, *A. flavus, Aspergillus parasiticus,* in Orange juice, *A. flavus,* for fruit juices. Also in North Nicosia *Aspergillus spp.* is the most isolated mold from fresh press orange juice in our study.

On the other hand Deak and Beuchat (1993a). are isolated 21 species yeasts from fruit juice concentrates. Their most frequently isolated species were *Saccharomyces cerevisiae* (24.7%), *Candida stellata* (22.1%), and *Z. rouxii* (14.3%). Also they isolated *Rhodotorula mucilaginosa* from 24% of apple juice concentrate.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

Fresh fruits are playing important role in human diets. Fruits and their juices are having important contributions of health and nutritional benefits. Nutritionist are always advises to have rich in fruits and vegetables in our diet for well balanced. Including vitamin C and vitamin A makes them important about protection of body to several dieses. This study aimed to determining the microbial quality of fresh pressed fruit juices in north side of Nicosia and comparison with processed juices. Most of restaurants, cafes or bars don't know what is the contamination, importance of contamination and good hygiene practices, also they don't know how to do well sanitizing. In general 85% pathogens are detected from analyzed fresh pressed fruit juices samples totally collected from north side of Nicosia. *Aspergillus spp*. is the most founded pathogen in the samples. The importance of this mold is it can produce mycotoxins such as patulin and ochratoxin which are having cancerogenic affect for human. Most of countries are having set limits and they are controlling the products which consumption. On the other hand 7 pathogenic molds are detected from 40 fresh pressed samples. These pathogens are can also cause important disease for human.

Recommendation:

This case can be preventing with giving training to all restaurants and cafes in Cyprus by municipal or health ministry. In Cyprus about sunny and hot climate people always wants to drink something because they lost liquids from their body. On the other hand doctors and nutritionists are always advising the fruit and fruit juices for health. The important point is here that training is necessary about contamination and sanitation for food preparation places. Because human eats what they prepare, and health is close with what they eats.

- Presence of Rhodotorulla, Black yests, Trichosoporon is also should be investigated in other studies.
- Attraction of Aspergillus spp. and Penicillum spp. shows that examination of mycotoxins are should be done in Cyprus.
- Personal hygiene and plant sanitation should be applied for producers.
- Training and audits are should be done properly.

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