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Aims and Scope

International Journal of Food Engineering Research (IJFER) is an international, peer-reviewed journal devoted to the publication of high quality original studies and reviews concerning a broad and comprehensive view of fundamental and applied research in food science&technology and their related subjects as nutrition, agriculture, food safety, food originated diseases and economic aspects.

IJFER is an international periodical published twice a year (April and October). The journal is published in both print and electronic format.

From The Editor

International Journal of Food Engineering Research (IJFER) has been publishing by Istanbul Aydin University Faculty of Engineering Department of Food Engineering since 2015. The journal covers wide ranges of area such as Food Processing, Food Preservation, Food Microbiology, Food Chemistry, Biotechnology, Nanotechnology, Novel Technologies, Food Safety, Food Security, Food Quality and their related subjects as nutrition, food and health, agriculture, economic aspects and sustainability in food production.

Food Engineering is getting more and more attention because it is directly related to human health. While the food and drinks we eat help to protect our health, on the other hand, improper conditions during the conversion of the raw material to the product, the use of poor quality raw materials, and the employees not working under hygienic conditions can cause the food harmful to health. Our aim in this journal is to include the recent research and reviews on food and beverages from field to fork. Articles submitted to the journal are accepted for publication after being reviewed by expert referees.

In the following years, the journal will include scientific activities such as symposiums, congresses, conferences and workshops held in the field of food science and technology, and information about the books published in this field. We hope that the journal will be a good resource for engineers, experts, researchers and students working in the food industry.

Prof. Dr. Z. Dilek Heperkan
Editor

International Journal of Food Engineering Research (IJFER)

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OCHRATOXIN A CONTAMINATION IN VINEGAR^{1*}

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin that is often produced by mold species such as *Aspergillus ochraceus*, *A. carbonarius*, *A. niger* and *Penicillium verrucosum*. Ochratoxin-producing molds widely grow up and form toxins in raisins, figs, coffee beans and grains. In this study, the presence of OTA in home-made vinegars was examined with high performance liquid chromatography (HPLC). 88% of vinegar samples were OTA negative. In one of the samples examined, the amount of OTA is above 2 µg/L, which is the limit value. OTA is a mycotoxin with nephrotoxic properties. OTA, which enters the body, is absorbed from the intestines and enters the blood and accumulates by transporting blood to tissues and organs. The organ most affected by OTA is the kidneys, which may lead to a kidney disease called nephropathy. The results obtained in this study showed that there was a low amount of OTA in vinegars made at home. Since the presence of OTA in vinegar may pose a health risk, the necessary precautions should be taken by examining the factors affecting the formation of OTA.

Keywords: *Ochratoxin, Vinegar, Health, Mycotoxin, Disease*

INTRODUCTION

Ochratoxin A (OTA) is a toxic compound produced by certain species of mold. In the genus *Aspergillus*, *A. ochraceus*, *A. carbonarius* and *A. niger* are the most well-known OTA producers. In the genus *Penicillium*, *P. verrucosum* and *P. nordicum* is the major species producing OTA [1]. OTA is an isocoumarin pentaacetate, chlorine-containing mycotoxin. Ochratoxin B (OTB), other derivatives of ochratoxin, does not contain chlorine, while ochratoxin C (OTC) is the ethyl ester of OTA [2]. Figure 1 shows the chemical structure of OTA. It is well soluble in polar organic solvents such as methanol, ethanol, chloroform, very slightly soluble in water however, insoluble in petroleum

and saturated hydrocarbons. It has a weak acidic property. In people who eat food and beverages contaminated with OTA, the kidneys are especially affected more than other organs. It can lead to kidney disease, which is known as lethal. Long time exposure to OTA with food and beverages can increase its nephrotoxic effect. This condition has been common, especially in Balkan countries, and this disease has been called Balkan Endemic Nephropathy (BEN) [1]. As a result of storing grain in improper conditions in villages, it was determined that the amount of OTA increased and led to disease [3]. But recent studies have shown that this condition encountered in the Balkans is related to exposure to toxins other than OTA

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[4]. Studies on the toxic properties of OTA have shown that OTA suppresses the immune system (immunotoxin), affects the liver (teratogenic) and is a potential carcinogen [5]. OTA can be found in body fluids such as blood, urine and breast milk. Because of this property, body fluids are used as biomarkers in people's exposure to OTA [6, 7].

OTA has been found to be positive in many foods, solid and liquid. It has been proven to exist in various cereals [8]. Moreover, the presence of OTA was determined in fruits such as dried figs, raisins; in beverages such as fruit juices, beer and wine; in products such as cocoa and coffee [9, 10]. The amount of OTA in raisins is more than 50% of the amount of OTA in fresh grapes. For example, the highest amount of OTA in fresh grapes is 1.5 µg/kg, while in raisins this amount is 98 µg/kg [11, 12]. In general, the amount of OTA in wine was also very low compared to raisins when the wines produced in various countries were examined. The highest OTA amounts found in wine were 0.815 µg/L in Turkey; 0.09 µg/L in Greece; and 0.144 µg/L in Spanish wines [13, 14, 15].

Vinegar is used to give flavor to food, salads and sauces and is not considered as basic foodstuff. However, it is known that making vinegar at home is common due to the use of excessive fruits on the one hand, and the health benefits of vinegar on the other. In addition, vinegar is used as a beverage by some consumers. In our country, there is no study on the presence of OTA in vinegar. The aim of this study was to examine the presence of ochratoxin A in home-made vinegar from different fruits.

MATERIAL

Vinegar samples were supplied from various provinces in their original containers and kept in the refrigerator till analyzed.

METHOD

Determination of OTA in Vinegar

OTA analysis in vinegar samples was determined by High Performance Liquid Chromatography (HPLC) system (Shimadzu serial no: L20225219693, model LC-20A) as specified in R-Biopharm application guide. HPLC requirements for OTA determination are given below. Mobile phase: acetonitrile:water:acetic acid (51: 47: 2 v/v/v); column: C-18 ODS-2 column (25cm x 4.6 mm x 5 µm); fluorescence detector: excitation: 333 nm, emission: 443 nm; flow rate: 1 mL/min; injection volume: 100 µL; pressure: minimum 0 bar, maximum 300 bar and column temperature: 40 °C.

Vinegar samples are filtered and 10 mL samples are taken. Samples are adjusted to 7,8 with 2M NaOH. It is centrifuged at 1600 rpm for 10 min by adding 10 mL phosphate buffered saline (PBS) on it. 10 mL of supernatant (upper phase) is taken and passed through the Ochraprerp immunoaffinite column. With 20 mL of PBS, the flow rate is 5 mL per minute so that the immunoaffinity column is washed. 1.5 mL of methanol: acetic acid (98:2 v/v) mixture is added to the column and OTA is recovered. 1.5 mL of water is added to it, vortexed, passed through a 0.45 µm filter, and 1.5 ml of mixture is taken to a glass vial. 100 µl is injected into the HPLC device. For OTA analysis, the retention time in the column is approximately 12 minutes. OTA recovery value in vinegar was determined as 86% (dilution factor=0.3).

RESULTS AND DISCUSSION

There is no legal regulation for OTA in vinegar in the Turkish Food Codex. Therefore, the results obtained from the study were evaluated by taking into account the limit value (2 µg/l) for vine and fruit

vines in Turkish Food Codex [16]. 88% of vinegar samples analyzed by HPLC were found to be OTA negative. In one of the OTA-positive samples, the amount of OTA is higher than 2 µg/L, which is the

limit value. Figure 1 shows the chromatogram (1 µg/L) and calibration curve of the OTA standard, and Figure 2 shows the chromatogram of the OTA positive vinegar sample.

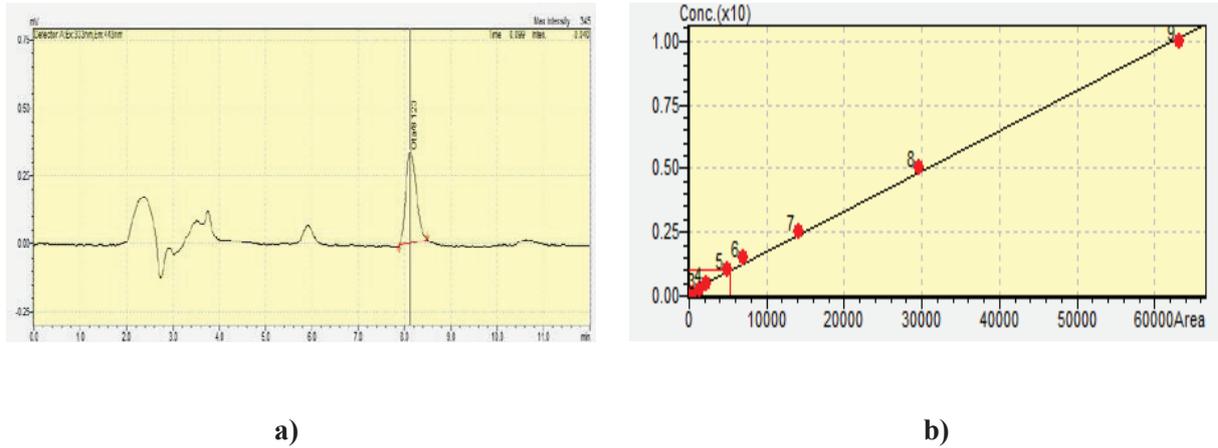


Figure 1. Chromatogram of OTA standard (a), Calibration curve of OTA standard (b)

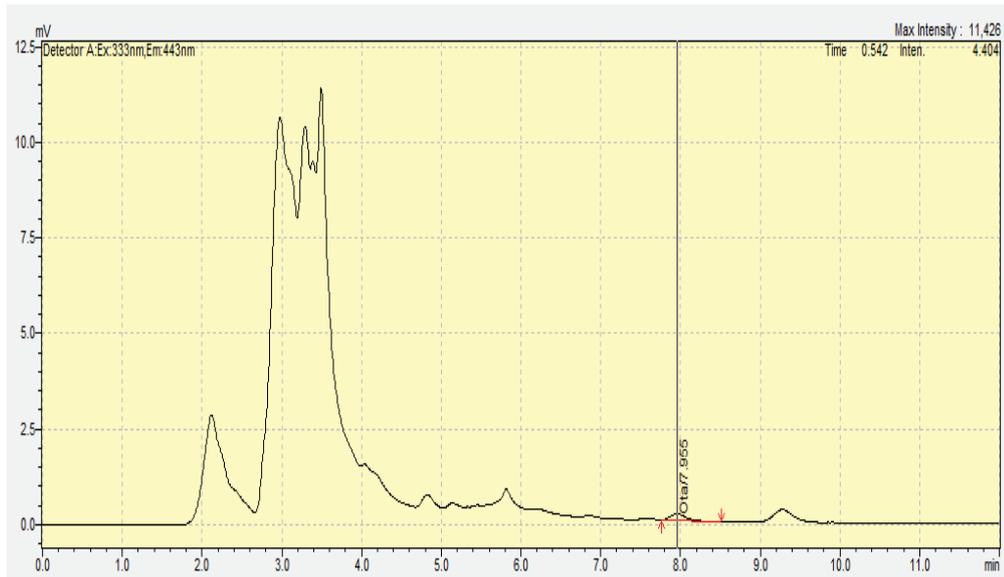


Figure 2. HPLC chromatogram of OTA positive vinegar sample

Ochratoxin A is a nephrotoxic, genotoxic, teratogenic, immunotoxic and possible carcinogenic mycotoxin. Although vinegar is not a basic foodstuff, it is often used to give flavor

to soups, salads, sauces and meals. In addition, it is also known that today, especially home-made vinegar is considered as a drink. In this study, a large proportion (88%) of home-made vinegars

were found to be OTA negative and only in one sample were found to be higher than the OTA limit value. However, since the presence of OTA in vinegar can pose a risk to public health, the factors affecting the formation of toxins should be examined. The raw materials and the equipment used during the production of vinegar at home, and environmental factors should be taken into account. In this study, people from whom vinegar samples were provided with positive results were contacted and it was determined that these people used fruits that were not suitable for table use, fell from trees and remained on moist soils in hot climate conditions. Moldy and damaged fruits should not be used in vinegar production. After the vinegar production is completed, the fruits should be separated and the vinegar should be kept in a cool environment in this way.

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BIOPLASTICS USED IN RENEWABLE PACKAGING IN THE FOOD INDUSTRY^{1*}

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ABSTRACT

Food waste from different sources is an environmental burden. In food technology, plastics and polymers are an alternative option for food packaging, food preservation and preservation, and recycling of food waste. Today, almost all plastics are produced synthetically and have much better properties than naturally occurring plastics. The raw materials of all modern plastics are petroleum and natural gas. Due to the non-degradable properties of these raw materials, it is supported to reduce the cost of production in plastics by offering an environmentalist approach option. In this review, for polymers such as polyhydroxy alkanoates (PHA) Poly (3-hydroxybutyrate) (PHB), Polylactic acid, Polylactide aliphatic copolymer (CPLA), Polycaprolactone (PCL), polyhydroxy-co-3-butyrate-co-3-valerate (PHBV) focuses on available technologies for polymers. Fermentation technologies based on pure and mixed cultures are of particular importance in the preparation of raw materials (prepared from food waste) for true bioplastic production. In this study, alternative methods are provided for the evaluation of food wastes, their economical/technical approaches meeting the expectations and applicability, and the reduction of waste by solving food wastes (FW) with environmentally friendly renewable polymer packages.

Keywords: *Bioplastics, Polymers, Polyhydroxy Alkanoate (PHA), Food Waste (FW)*

INTRODUCTION

Bioplastics are perishable materials whose raw materials are composed of renewable raw materials. Bioplastics are alternatives compatible with sustainable nature [1]. Plastics are biodegradable; It is sustainable as raw material and can be obtained from oil [2]. The United Nations Food and Agriculture Organization (FAO) has

defined food waste as “food losses in quality and quantity throughout the supply chain process that takes place during the production, post-harvest and processing stages”. Food losses that occur at the end of this chain become defined as “food waste (FW)”, which is dependent on consumer behavior, purchasing intent and retailer marketing strategy [3]. Due to the use of plastics in a wide variety

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of applications, plastic waste production has increased 200 times in the last 60 years worldwide. Bioplastics are preferred because they reuse less energy and less waste for plastic waste reuse [4, 5].

Poly-hydroxy alkanooates (PHA) as active ingredients for bioplastics can be produced to prevent the use of excess carbon-nitrogen, oxygen or phosphorus [5]. PHA are biodegradable polyesters of various hydroxy-alkanoates [6]. Economic, ethical, environmental and engineering studies can be supported with sustainable PHA production. Instead of fossil sources, biomass such as starch, cellulose, wood and sugar can be used for plastic production. Their use is also among common methods for a sustainable environment [7]. The use of cost-effective biodegradable natural biopolymers such as rice husk, wheat straw and corn ground can be expanded [8]. Synthetic polymer production is an environmental burden [9]. The conventional petro-polymer average energy requirement of the bioplastics to be produced is lower in terms of global warming rates (47 MJ kg^{-1} compared to 67 MJ kg^{-1}) [10, 11]. In many ways, bioplastics are a good alternative to replace petroleum-based plastics [12, 13].

Recycling methods are preferred for reuse of plastic waste. Manual or automatic devices should be used before recycling plastics. Subsequently, large amounts of used plastic are needed to use other regimes such as combustion, pyrolysis, hydrogenation, gasification and thermal cracking [13].

In the use of recycled materials or using renewable resources, two strategic methods are used, namely directives and dependence on fossil resources, to reduce CO_2 [14]. The role of bio-based sugar and lipids are very important in the food packaging industry to increase sustainability [15, 16, 17]. However, large-scale use of PHBV in packaging may result in fragility and thermal instability [18, 19, 20].

BIOPLASTICS THAT CAN BE USED IN FOOD PACKAGING

Food waste occurs at all stages including post-production sustainable supply chain, shopping and consumption [21]. According to the researches, an average of 90 million tons of food waste is produced by the European Union in a year. Some of this data (38%) originates from the food production sectors [22]. The shelf life of the products is related to the durability of the packaging. The mechanical and/or barrier properties of the packaging material must remain stable and can be operated without any problem until destroyed during storage. The material should then be efficiently biodegradable. The most important variables to control the stability of the biologically based packaging material are proper water activity, pH, oxygen, nutrients, temperature and storage time. The disadvantage of biodegradable starch-based films is their hydrophilic character, which leads to low stability when exposed to different environmental conditions. Therefore, moist foods must have limited storage times [23]. Polyalkanoate use in the food industry should be widely preferred in beverage bottles, coated cardboard milk cartons, glasses, fast food packages [24]. In some bioplastic grades, in terms of cost and performance in their production, PHA and polylactides are usually easily processed into films, with greater efficiency than standard plastics, but are more costly than synthetic analogues [25].

Films made from proteins and carbohydrates are oxygen-tolerant due to tightly packed, regular hydrogen bonded network structures [26]. The results of a PE film containing a normal polyethylene (PE) film and 6% corn starch were evaluated in the packaging of broccoli, bread

and minced meat stored under normal time and temperature conditions. They discovered that the addition of starch ($0\pm 28\%$) in polyethylene films does not impair the thermal permeability and does not accelerate microbial growth in minced meat. Another study found that when fresh mushrooms were covered with gluten film and stored at 10°C for 5-6 days, a modified atmosphere with 2-3% CO_2 and 2-3% O_2 developed during the storage period [27]. In addition, chitosan (14.5% by weight) cellulose (48.3%) and polycaprolactone [glycerol (36.2%) and protein (1.0%)] were synthesized as packaging materials for the storage of fresh vegetables [28]. It showed that minced meat improved reddish surface color by matching with samples.

Polyhydroxy alkanooates (PHA)

Poly-hydroxy alkanooates (PHA) are polyesters of various hydroxy-alkanoates. Over 100 are defined

as units of different monomers. PHA has the lowest molecular weight and is one of the most common polymers in nature. Its biodegradability has been demonstrated compared to conventional plastics [29, 30]. Unique features of PHA are considered to be good oxygen barrier, water vapor barrier, oil/odor barrier. Such superior physico-chemical properties of PHA promote its use in a variety of fields, including food packaging. In addition, medical applications are used in different fields, including energy and fine chemicals [31]. It is determined that global PHA production from commercial producers reached 2.05 million tons in 2017. Food products preferred in the PHA industry are sugarcane and vegetable oils. The current industrial costs of PHA production are 5-10 times higher than that of petroleum polymer derivatives [29]. PHA production is one of the most preferred methods among alternative raw materials for the search for large-scale (compared to traditional raw materials) cost-effective raw materials [32, 33]. Figure 1 shows the PHA biosynthesis scheme.

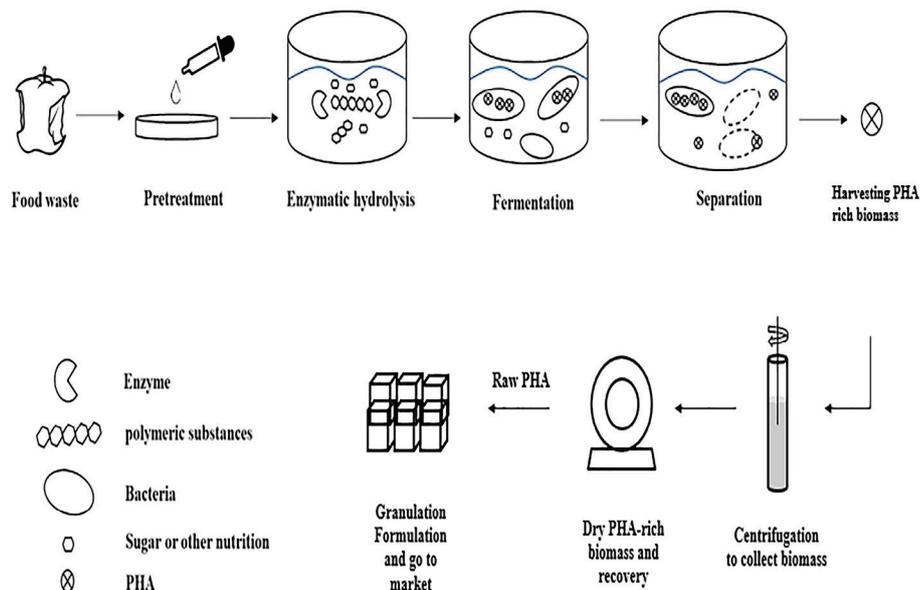


Figure 1. PHA biosynthesis scheme [4].

Table 1. Characteristics of food wastes with high usability in PHA production.

Type of food waste	Potential materials	Properties	References
Consumed and used cooking oils	tail oil, margarine oil, extra virgin olive oil seed	High lipid (oil) content can be converted to biodiesel (fatty acid methyl esters: FAMES)	[34]
Animal wastes	Blood, fats, large intestine rumen residues	High nitrogen content or high levels of BOD and COD	[35]
Organic plant waste	Fruits, vegetables, herbs, plants, greens prina	These fractions consist of important sources of sugar, lipids, carbohydrates, and mineral acids. Provided water, soluble sugar and celluloseSuccinic acid production	[36, 37]
Domestic waste	waste meal, peanut and walnut shell	High protein, starch, fat and fatty acids content	[38]

Table 1 describes some of the old methods for producing PHA, as well as their errors and benefits. In particular, as a result of 145 article reviews published after 2010, current information on valuation techniques for FW has been summarized, focusing on PHA production.

Table 2. Advantages and disadvantages of some commonly used PHA production methods [36]

Production method	Advantages	Disadvantages
Production with pure bacterial cultures	These single isolates may have the ability to produce and biologically improve PHA.	Sterilization is needed to ensure efficient production
	Great potential to reduce PHA production cost	Sometimes the production process may require substrates such as pretreatment or physicochemical or biological hydrolysis.
Production by substrate hydrolysis	Many substrates can be suitable for production	Usually several steps and / or solvents are required to prepare the hydrolysates of the substrate.
	It can be an effective method for the use of various substrates that are normally difficult to use	Since it requires a separate hydrolysis step before production, it can be a time consuming method

PHB (poly-hydroxy butyrate)

The polymeric material properties of PHAs are considered a good alternative for petroleum-derived synthetic plastics. Another emerging application of PHA is enantiomeric pure 3-hydroxybutyric acid (3-HB), which acts as an intermediate for the synthesis of many chiral drugs [39]. High production costs are biodegradable of PHAs. This is one of the main factors that limit its broader use. Improvement in PHA production strategies may result in lower costs, which enables wider

use of PHA in daily life [40]. This has created a worldwide interest in the efficient production of PHB at low cost by new microorganisms. PHB can also be synthesized from sugars and fatty acids by de novo fatty acid biosynthesis and oxidation pathways [41, 42]. The three most unique features of PHB are (I) 100% water resistance, (II) 100% biodegradability, (III) thermoplastic processing ability. It can be easily processed in standard industrial plastic plants, and it is highly fragmented with its water resistance and soil contact.

PLA (polylactic acid)

Polylactic acid (PLA) is the polymer with the highest potential for commercial large-scale production of renewable packaging materials. PLA materials have a good water vapor barrier and also have relatively low gas permeability. There may be agricultural resources such as raw materials, corn or wheat, and alternatively agricultural waste products such as whey or green juice can be used [43]. They are the most promising and versatile biopolymers. Sugar, which is a renewable resource that is biodegradable easily, is its raw material. PLA is the controlled depolymerization of lactic acid monomer from sugar fermentation [44]. It can be recycled in terms of transparency, molecular weight, processability, high resolution. PLA is water absorbent, thereby providing hydrolysis and splitting of ester linkages that are automatically catalyzed by carboxylic acid end groups. It is easily processed with thermoform, which is the real technology in the packaging of foods. This material is currently used for short shelf life products in food packaging application.

The PLA components were examined by extraction tests under which the polymer samples were exposed to food simulating solvents under the temperature/time conditions that the foods would be exposed to while in contact with the PLA. In the samples examined, acid and oil formation was observed in foods. It has been concluded that lactic acid (dimers, trimers, etc.) represents very small and safe amounts [15].

Aliphatic polyesters

Biodegradable aliphatic polyesters are similar to PE and PP polymers in terms of their other properties except mechanical properties. These polymers are formed by the polycondensation reaction of glycol and aliphatic dicarboxylic acid obtained from renewable raw materials. They are odorless and can be used for

beverage bottles. They can biodegrade in 2 months, giving carbon dioxide and water in soil and water [15].

CPLA (Polylactide aliphatic copolymer)

Poly lactide aliphatic copolymer (CPLA) is a mixture between aliphatic polyesters, such as dicarboxylic acid orglycol, with hard (like PS) and soft flexible (such as PP) properties, depending on the amount of aliphatic polyester in the mixture with renewable sources. It can be easily processed up to 200 °C. The amount of carbon dioxide produced during heating combustion does not produce toxic substances, nearly half of that produced from commercial polymers such as PE and PP. It dissolves completely after 12 months in the natural environment and starts to deteriorate in 5 to 6 months. Food waste begins to decompose after 2 weeks [15].

PCLA (Polycaprolactone)

Polycaprolactone (PCL) is a completely biodegradable polymer resulting from the polymerization of non-renewable raw materials such as crude oil. Chemical resistance against liquids such as water, oil and chlorine is strong. Due to its low melting point, it is a thermoplastic polymer that is easy to process and has a very short degradation time. It is not possible to apply it in food applications. However, by combining with materials such as starch, biodegradable material can be obtained which can be broken down at low cost [15].

Starch-based polymers

Depending on the percentage of starch and other ingredients such as additives (coloring additives, flame retardant additives), the properties of these materials are variable. Starch, consumed by the microbial effect, speeds up the breakdown of the polymer chain by producing pores in weakening materials. This processing time is quite long, but if

the mixture is blended with starch, the processing time can be accelerated by 60%. Starch can also be transformed into foamy material using water vapor by replacing the polystyrene foam as packaging material. It can be pressed on trays or disposable dishes, consumed by the microbial medium in about 10 days, giving only water and carbon dioxide as a by-product [15].

PHBV

Polyhydroxy-co-3-butyrate-co-3-valerate (PHBV)

is an aliphatic polyester produced by bacterial fermentation of sugars and lipids. Biodegradability can be thermally processed through the ease of processing, injection molding, extrusion, low moisture permeability, and acceptable mechanical properties. However, large-scale use of PHBV in packaging is limited by its fragility, thermal instability, and narrow processing window. Table 3 shows comparisons between some common plastics and bioplastics [45]. Figure 2 shows ways to convert energy from biomass [4].

Table 3. Comparison between some common plastics and bioplastics [45].

Polymer	Moisture permeability	Oxygen permeability	Mechanical properties
Cellulose	High- medium	high	high
Cellulose acetate	medium	high	medium
Starch	high	Low	high
polylactate	medium	high	high
Low density polyethylene	Low	high	medium
Polystyrene	high	high	Low-medium

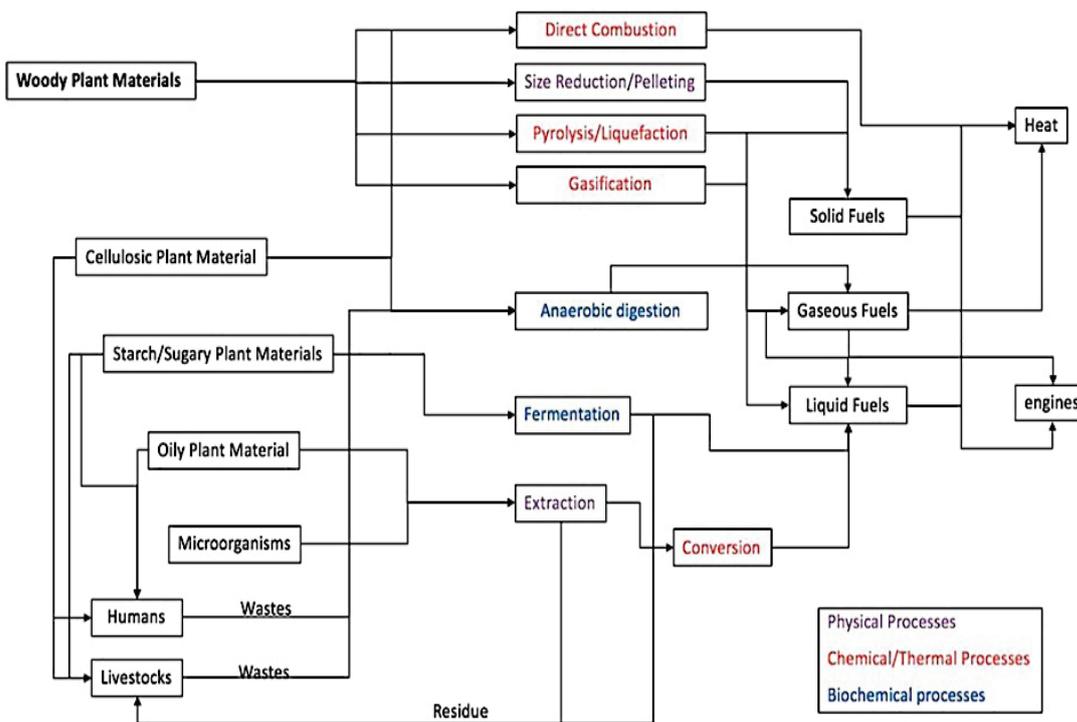


Figure 2. Ways to convert energy from biomass [4].

CONCLUSION

Bioplastics, which are natural polymeric materials that have been widely developed in the last two decades, have become one of the most active areas of research due to their biocompatibility and biodegradability. Generally, it can be applied as a solvent in bioplastics, packaging industries, spray, device materials, electronics, agricultural products and various chemical environments. Bioplastic production is an essential strategy in terms of economic and environmental burden aimed at linking biotechnology processes, maximizing the use of food waste and increasing the potential income of the entire bioprocess chain. The production of consumed food wastes, environmental problems caused by wastes (eg air pollution and CO₂ gas emissions) need to be reduced. It should also be used as a commercial packaging for the applicability of long shelf life in foods. For all these reasons, this review showed that FW has the potential for important environmental problems for bioplastic production. In addition, based on additive/mixed culture and fermentation technologies, PHA, Poly (3-hydroxybutyrate) (PHB), Polylactic acid (PLA), Polylactide aliphatic copolymer (CPLA), Polycaprolactone (PCL), polyhydroxy-co-3-butyrate-co It focuses on the use of -3-valerate (PHBV) polymers. As a result of all these data, it was concluded that PHA production may be a more suitable technology item for FW production.

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INVESTIGATION OF THE EFFECT OF BULGUR ADDITION IN FERMENTED POMEGRANATE VINEGAR PRODUCTION*

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ABSTRACT

In this study, microbiological, physicochemical and sensory properties of pomegranate vinegar produced from pomegranate grains were examined in the laboratory. Pomegranate vinegars produced in the study were made in two ways: bulgur added and bulgur free. Total acidity, pH, color, Brix values were measured in pomegranate vinegars produced. Coliform, yeast-mold, and lactic acid bacteria were studied in chromogenic coliform agar (CCA), Potato dextrose agar (PDA), and Man Rogosa Sharp (MRS) media, respectively. It has been observed that the pH of bulgur added vinegars varies between 3.10-3.47 during fermentation and between 2.90-3.32 for bulgur free vinegars. Dry matter values were determined as 2.2-4.0 Brix in bulgur added samples and 2.1-4.2 Brix in bulgur free samples. The total acidity of bulgur added samples ranged from 8.10-22.05 % during the fermentation period and from 6.30-13.5 0% for bulgur free samples. It has been observed that bulgur free pomegranate vinegars obtained as a result of fermentation are clearer, deteriorate later and receive more sensory acclaim. It has been determined that the addition of bulgur during vinegar production does not provide an advantage.

Keywords: *Pomegranate vinegar, Bulgur added vinegar, Fermentation, Sensory properties*

INTRODUCTION

Pomegranate (*Punica granatum* L.) fruit, one of the oldest known edible fruits is also widely consumed both fresh and in processed forms such as juice, wine, jam, vinegar [1, 2, 3, 4]. Pomegranate fruits are known to contain phenolic ingredients known to be natural antioxidants such as gallotannins, ellagic acid and anthocyanins, as well as active ingredients

such as carbohydrates, minerals and vitamin C [5, 6, 7, 8]. Some studies have shown that natural antioxidants found in pomegranate fruit can reduce the risk of chronic disease and prevent disease progression by protecting people against oxidative stress [9, 10]. Recently, pomegranate vinegar has been produced by applying various technologies, with the preservation of the functional properties

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of pomegranate fruit and improving the sensory properties with new aroma components [11]. Vinegar is a special product produced from various foods since very old years, used as a flavoring agent and preservative. Various vinegars are produced by using different raw materials and production methods all over the world, especially in the Far East and European countries [12].

According to TSE 1880 EN 13188 vinegar standard, vinegar is defined as “a unique product produced biologically from agricultural liquids or other substances by two-stage fermentation of alcohol and acetic acid”. In this standard vinegar varieties, according to the raw materials used in the production of vinegars is classified as: wine vinegar, fruit vinegar, fruit wine vinegar, cider vinegar, alcohol vinegar, cereal vinegar, malt vinegar, flavored vinegar and others. Of these, wine (grape) vinegar has been defined as” vinegar obtained only from wine (only from fresh grapes) by fermentation of acetic acid by biological pathways” [13]. Vinegar production is a batch process consisting of two stages as ethyl alcohol and acetic acid fermentation. The first stage is ethyl alcohol fermentation, and yeasts break down sugar into ethyl alcohol by anaerobic pathway. In acetic acid fermentation, which is the second stage, ethyl alcohol is oxidized to acetic acid under aerobic conditions by vinegar (acetic acid) bacteria such as *Acetobacter* and *Gluconobacter* [14]. Vinegar production can be produced in different ways, such as slow (traditional) method, orleans (French) method, pastor method, generator method and immersion methods. In these methods, alcohol fermentation is carried out in the raw material first in the slow method. When the alcohol level rises to 13%, acetic acid bacteria increase on the liquid

surface, forming a membrane (mother of vinegar). The mother of vinegar formed on the surface allows ethyl alcohol to be converted into acetic acid. This method produces vinegar quite slowly. Some of the vinegars produced in our country are also produced by generator method. In order to provide a wide surface for vinegar bacteria, wood chipper, grain-removed corn cob parts and similar materials are used in the fermenter. In submerge method, acetic acid bacteria multiply inside the substrate without fillers. Because the filling material is not used, there are no problems caused by the filling material. In this method, vinegar production is 30 times faster than generator method. Fermentation is carried out at 24-29 °C with *Acetobacter* culture by continuous mixing in an environment containing 8-12% alcohol. Fermentation occurs on the inside of the liquid, not on the surface. During fermentation, oxygen is delivered to the environment in a controlled manner. With this method, very high proportions of vinegar can be produced in a short time. 5-10 tons of vinegar with an acidity of 4-6% in 24 hours can be produced by this method [15].

Vinegar quality depends primarily on raw material. The chemical composition of vinegar is directly related to the raw material. The second factor affecting quality is production technique. The production technique is determined after many years of research. Other parameters that affect quality are microorganisms, added ethanol concentration and vinegar concentration (in commercial production), O₂ amount, fermentation temperature and duration (aging), storage, bottling and pasteurization. 80% of vinegar is water, 20% is organic acids, alcohols, polyphenols, amino acids [14].

Vinegar can be produced naturally and artificially.

Natural vinegars are obtained by fermentation, artificial vinegars are obtained by the addition of acetic acid. Artificial vinegar is largely colorless because it contains no substance other than water and acetic acid, and artificial vinegar and natural vinegar can be easily distinguished as it does not contain vitamins and other fermentation byproducts formed during acetic acid fermentation. Artificial vinegar is not allowed in many countries.

The beneficial effects of vinegar on health are caused by bioactive compounds such as organic acids, amino acids, phenolic compounds and melanoidins. These compounds found in vinegar have been reported to have antimicrobial, antioxidant, antidiabetic, anticarcinogenic, antitumor, antiinfection effects [12]. For instance, black rice vinegar (kurosu), traditionally produced in Japan, was found to be richer in phenolic substances than wine and apple vinegar, and its antioxidant activity was also higher compared to other vinegars. Vinegar is a fermented product produced by obtaining raw materials from a variety of different ways. In this study, positive effects on health are scientifically proven pomegranate fruit is used. Various characteristics of vinegars were studied which were produced in 2 different types as bulgur added and bulgur free.

MATERIALS AND METHODS

Material

Pomegranate that purchased from a local market as raw material for the production of pomegranate vinegar used in research. In this study 2 different vinegars were produced, one of them was produced as bulgur free and the other one was produced with bulgur.

Method

Pomegranate Vinegar Made on a Laboratory Scale

The traditional method was used in the production of pomegranate vinegar. 780 grams of pomegranate seeds separated from their shells were added into 3-liter glass jars, after crushing the grains, water was added until 400 mL of space remained on it. After adding 11 grams of sugar to both jars, bulgur (1 tablespoon) wrapped in muslin was added to a jar. All vinegars made are kept in the study at 22 °C. Mixing was carried out every day until the pomegranate seeds collapsed to the bottom. When pomegranate seeds collapsed to the bottom and mother of vinegar formed on it, fermentation was stopped (fermentation lasted 28 days) and then filtered. After the preparation of pomegranate vinegar, samples were taken and analyzed in 1, 7, 14, 21 and 28 days.

Determination of Total Acidity

Determination of total acidity in vinegar samples was made by titrimetric method. After 20 mL vinegar sample was completed to 100 mL with distilled water, 20 mL of mixture was taken to erlenmeyer, 1-2 drops of phenolphthalein were dripped on it and titrated with 0.1 N NaOH solution until pH 8.1. The total acidity was calculated in % acetic acid (g/ 100 mL) according to the amount which used in titration [16].

pH Measurement

A sample of vinegar was taken into the beaker and pH was measured at room temperature using a pH meter (Mettler Toledo S220) probe.

Color Measurement

Color measurement of vinegars was performed with Tindometer (Lovibond PFX880) device. Before reading the device, the device is calibrated. Vinegar samples were placed on the lovibond tintometer and CIE L*a*b* values were read. L* value indicating lightness (L*: 0 black, L*: 100 white), a* value indicating redness and greenness (+a: red, 0: gray, -a: green), and b* values indicating yellowness and blueness (+b: yellow, 0: gray, -b: blue) degree of pomegranate vinegar. Measurements were made in 2 parallel and averages were taken.

Determination of Water-Soluble Dry Matter (Brix)

The determination of Brix was made using the Abbe refractometer (Leica Reichert Abbe AO Mark II). 2-3 drops of vinegar were dripped on the refractometer. Results are expressed in ° Bx.

Microbiological analysis

10 mL of bulgur added and bulgur free vinegars were added to sterile stomacher bags separately, homogenized by mixing with peptone water in a ratio of 1:10. Samples for microbiological analysis were taken in 7., 14., 21. and 28. days. Potato dextrose agar (PDA) for yeast and mold, Man Rogosa Sharp (MRS) for lactic acid bacteria and Chromogenic coliform agar (CCA) for coliform were used as media. The petries were left incubation for 48 hours at 22°C, 37°C and 37°C respectively. Colonies formed at the end of 48 hours were counted and the number of microorganisms in 1 mL was determined.

RESULTS AND DISCUSSION

Determination of Total Acidity

Change in total acidity values (acetic acid %) in samples taken in 1, 7, 14, 21 and 28 days in bulgur

added and bulgur free pomegranate vinegars produced is shown in Figure 1. The total acidity of bulgur added and bulgur free pomegranate vinegar was found to be between 8.1%-22.05% and 6.3% and 13.5%, respectively (Figure 1). In a study, it was observed that the acidity value of pomegranate vinegar is between 9% and 10% [17]. In another study, the acidity of pomegranate vinegar was found to be 4.63 % [18]. It is observed that the acidity level changes according to the production method of pomegranate vinegar. It has been observed that the addition of bulgur to pomegranate vinegar increases the acidity level of vinegar and negatively affects its sensory properties.

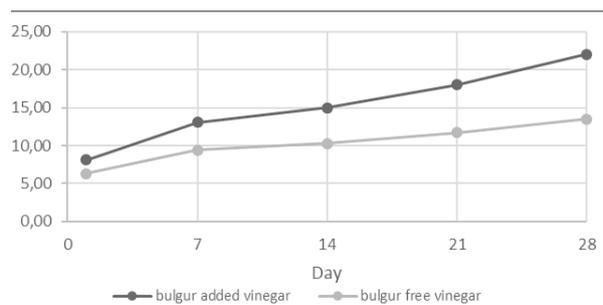


Figure 1. Change in total acidity values of pomegranate vinegars in fermentation process (acetic acid %)

pH Measurement

The change in pH during the fermentation period in bulgur added and bulgur free pomegranate vinegars were examined in samples taken at 1, 7, 14, 21 and 28 days and given in Figure 2. In bulgur added pomegranate vinegar, the pH value was 3.47 at the beginning of fermentation and 3.10 at the end of the fermentation. The change in pH in bulgur free pomegranate vinegar was found to be 3.32-2.90. In a study, it was observed that the pH of pomegranate vinegar during the 5-day fermentation

period ranged from 3.41-3.24 [19]. In another study, it was observed that the pH of pomegranate vinegar during the fermentation period was 2.98 on average [18]. It was determined that the pH values measured in the study were compatible with the literature.

A decrease in pH was observed due to an increase in acidity in both vinegar. It was determined that the acidity in bulgur vinegar was very high in addition to this the decrease in pH was also excessive. In vinegar samples, mother of vinegar formation began to be observed after the 14th day (Figure 2). It is thought that the sudden increase in pH is associated with the formation of mother of vinegar (Figure 3).

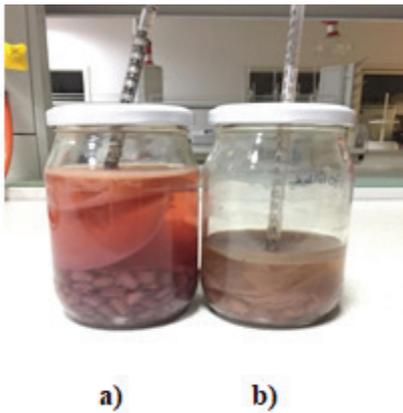


Figure 2. Mother of vinegar formed in pomegranate vinegars; a) bulgur free vinegar, b) bulgur added vinegar

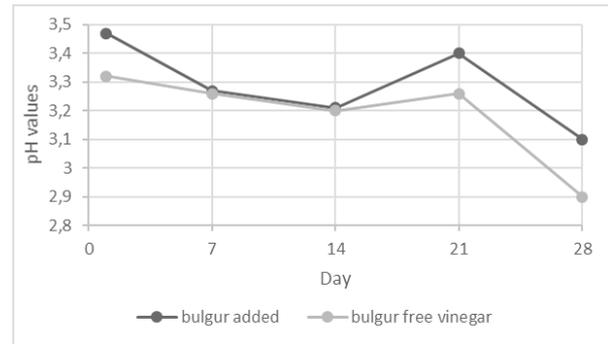


Figure 3. pH values of pomegranate vinegars

Determination of Water-Soluble Dry Matter (Brix)

Water-soluble dry matter values (Brix, °Bx) in bulgur added and bulgur free pomegranate vinegars are given in Figure 4. During the fermentation, the values of water-soluble dry matter in bulgur added and bulgur free pomegranate vinegar were measured as 4-2.2 °Bx, 4.2-2.1 °Bx, respectively. It was thought that the decrease in Brix value was due to the decrease in the rate of sugar by microorganisms using the sugar found in the environment. It was found that the values of water-soluble dry matter determined in vinegars are similar to the literature [20].

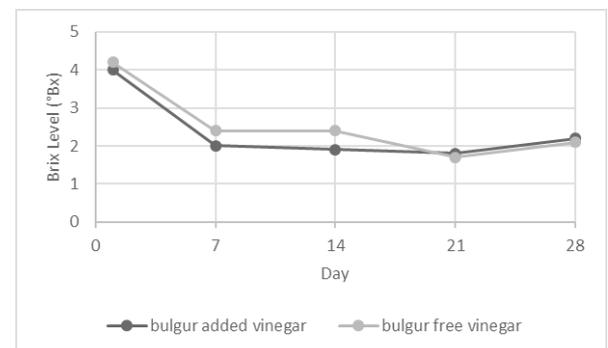


Figure 4. Water-soluble dry matter values of vinegar samples (°Bx)

Color Measurement

The values of color measurements made with lovibond tintometer in bulgur added and bulgur free pomegranate vinegars are given in Table 1.

L* value indicating lightness, a* value indicating red and green, and b* values indicating yellow and blue in bulgur added vinegar compared to a sample of bulgur free vinegar it was found higher up to the 21st day. But in bulgur free vinegar a sudden increase in values measured on the 28th day has been observed. Color is one of the important

parameters that affect the consumer’s purchase of vinegar [21]. In a study, it was determined that L* values ranged from 0.28 to 20.15, a* values ranged from 0.09 to 14.88 and b* values ranged from 0.43 to 14.11 in grape and apple vinegars produced by traditional methods [22]. The data in the literature coincides with the data in our study.

Table 1. L*a*b* values in bulgur added and bulgur free pomegranate vinegars

Day	Color Parameter	Bulgur Added	Bulgur Free
1	L*	9.87	7.025
	a*	16.70	12.98
	b*	12.73	7.84
7	L*	17.49	2.19
	a*	11.18	3.76
	b*	12.62	2.29
14	L*	15.8	3.42
	a*	7.36	4.65
	b*	8.27	3.15
21	L*	1.86	1.60
	a*	1.26	2.00
	b*	1.46	1.34
28	L*	3.13	15.77
	a*	1.96	9.93
	b*	2.23	11.25

Microbiological Analysis

E. coli coliform was not found in the samples during the 4-week fermentation period in bulgur

added and bulgur free vinegar. The changes in the number of lactic acid bacteria and yeast in vinegars are given in Table 2.

Table 2. The number of lactic acid bacteria and yeast in the fermentation process in bulgur added and bulgur free vinegar

Days	Number of lactic acid bacteria Log ₁₀ CFU/mL		Number of yeasts Log ₁₀ CFU/mL	
	Bulgur added pomegranate vinegar	Bulgur free pomegranate vinegar	Bulgur added pomegranate vinegar	Bulgur free pomegranate vinegar
7	6,209	6,149	6,110	6,041
14	5,257	5,875	6,354	6,320
21	-nd	-	-	-
28	-	5,531	5,447	5,875

nd: not determined

Both the number of lactic acid bacteria and the number of yeast were found to be similar at the beginning of fermentation in bulgur added and bulgur free vinegars. But after the 2nd week, the analysis was not carried out, because bulgur added vinegar was deteriorated. In bulgur free vinegar, the number of LABs decreased by about 1 log at the end of the 4-week fermentation period. Similarly, the number of yeast has decreased.

At the beginning of fermentation in pomegranate vinegar, the pH of vinegar was also found to be low (approximately pH: 3.5). A further decrease in pH during the fermentation period was expected to prevent the development of lactic acid bacteria. But bulgur added vinegars were removed from the trial because they deteriorated at the end of 2 weeks. In bulgur free vinegar, it has been observed that they maintain LAB vitality, although the pH drops to 2.9 at the end of fermentation. In fact, they are generally undesirable to be present in vinegar, as LAB and other rod-shaped bacteria cause the formation of sensory undesirable compounds [23]. But in our study, it was determined that lactic acid bacteria maintain their viability despite the decrease in pH. It is believed that this condition does not lead to an undesirable development in bulgur free vinegar, but

lactic acid bacteria also play a role in the disfavor and degradation of bulgur added vinegar.

CONCLUSION

Vinegar is a special product that has been produced all over the world using different methods with different fruits and vegetables since very old years. The vinegar microbiota consists mainly of acetic acid bacteria, lactic acid bacteria and yeasts. Acetic acid bacteria produce acetic acid, on the one hand, improving the flavoring property of vinegar, and on the other hand, allowing it to be used as a natural antimicrobial. The bioactive components in its composition contain many different functional properties. Pomegranate fruit is rich in phenolic compounds such as flavonoids (anthocyanin, catechins and other complex flavonoids), polyphenols, fatty acids, aromatic compounds, amino acids, tocopherols, sterols, phenolic alkaloids. Due to these phytochemicals in its content, the medicinal and economic importance of pomegranate vinegar is quite high. In our study, it was determined that bulgur used in the production of many vinegars is not compatible with pomegranate vinegar, which leads to the deterioration of the vinegar in a short time, so the addition of bulgur in the production of pomegranate vinegar is not recommended.

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