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Sensory evaluation of fruit bevarages treated with natural preservatives of plant origin

Diploma thesis

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DECLARATION

I hereby declare that my Diploma Thesis “Sensory Evaluation of fruit beverages treated with natural preservatives of plant origin” is my own work and all the sources I cited in the list in bibliography are true to the best of my knowledge.

Prague, 9.4.2015 Signature

AKNOWLEDGEMENT

I wish to express my sincere thanks to Supervisor Ing. Pavel Nový, Ph.D. Iam indebted to him for sharing his expertise, sincere and valuable guidance and encouragement extended to me throughout the thesis.

I take this opportunity to express gratitude to all the Department members for their support. Iam gratefull to the Dean of faculty and to the Head of Department, for providing me with all the necessary facilities for the research.

I am extremely thankful to lecturer Mrs.Kouřimská for her support and attention towards my work for allowing and conducting the experiment. Iam also thankfull to the staff memebers of the laboratory for their help in conducting experiments.

I am grateful to the God for the good health and well being that were necessary to complete this thesis.

I also thank my husband Ing. Pawan Kumar Pathak, for his encouragement, support and attention throughout my venture.

Last but not the least; I would like express my gratitude to all the evaluators who tasted the samples during their lecture.

Senzorické hodnoceni ovocných nápojů ošetřených přirodními konzervanty rostlinného původu

Souhrn

Ovocné džusy mají v současnosti velký význam. Kromě pasterizace bývají někdy obohacovány některými konzervanty jako např. oxid siřičitý nebo benzoáty. Syntetické konzervanty mohou způsobovat některé nežádoucí účinky jako je bolení hlavy, zvracení či alergické reakce. Jako alternativa konvenčních konzervačních technik by mohly být zavedeny jako částečná nebo plná náhrada antimikrobiální látky rostlinného původu. Nicméně vedle jejich konzervačního potenciálu je potřeba testovat také jejich možný vliv na změnu senzorických vlastností a tím také na spotřebitelskou akceptabilitu.

V této práci byl resveratrol, přidaný do vinného moštu, testovaný panelem hodnotitelů za účelem zjištění schopnosti hodnotitelů rozlišit obohacené vzorky od vzorků bez resveratrolu. Z výsledků trojúhelníkového testu bylo zjištěno, že hodnotitelé od sebe vzorky nerozliší. To naznačuje, že z hlediska chuti by jako konzervant byl resveratrol pro potencionální spotřebitele akceptovatelný.

Klíčová slova: aditiva, hroznový mošt, konzervační látky, Senzorická analýza, oxid siřičitý, resveratrol.

Sensory evaluation of fruit bevarages treated with natural preservatives of plant origin

Summary

Fruit juices are of great importace these days. Besides to pasteurisation, they are sometimes foritfied with preservatives like sulfur dioxide, benzoates, and other preservatives Synthetic preservatives can cause negative impacts on humans such as heachache, vomitting, allergic reactions etc. As an alternative to these, antimicrobial plant derived compoundscould be implemented as partial or complete alternative to conventional preservative techniques. However, next to their preservation potential, their possible impact on the sensory parameters and thus to consumers acceptability nedds to be evaluated.

In this study, resveratrol, was added to wine must and examined by evaluator panel using triangle test in order to determine the ability of judges to distinguish fortified samples from resveratrol free must. It was concluded that evaluators were not able to differentiate the two samples from each other which indicates that, regarding to the taste, resveratrol would be acceptable for potential consumers as a preservative

Key Words: food additives; grape must; preservatives; sensory analysis; sulphur dioxide; resveratrol

Contents

1. Introduction

Fruit juices have a balanced amount of nutrients, sugar, fats and salt. When consumed more, it is associated with decreased risk of heart coronary disease, Alzheimer's disease, prostate cancer etc. Today families are busy and mothers are mostly working, they don't have time to cook nutritious food or make homemade juices. Freshly squeezed juice is best for health. Pasteurisation is an alternative to preserve it for longer time and consume it later. However, these products don't meet all the needs of healthy consumer because of the loss of nutrients in their healthy diet (10).

Grapes have a close relationship with mankind since Bronze Age. Today grapes are also grown in temperate and tropical regions. They are mostly used in Europe (Hui Y.H. et al, 2006)

Antimicrobial plant phenolic extracts can be used as partial or complete alternative to sulphites. Wines when treated with antimicrobial phenolic extracts, remained microbiologically stable even during ageing in wooden barrels as worked by Rompinelli E.M.G., 2013. So, in this thesis we tried antimicrobials effects on fruit beverages i.e. on grape must expecting that it won't affect sensorial on taste, aroma to consumers. Volatile and phenolic profiles of Verdejo white wine remains significantly unmodified, so we chose white grape must for our experiment. Wines treated with these extracts till now didn't show any significant difference in sensory methods globally. Our trial is to encourage industries to produce such products more as this can open their gates for international market (Rompinelli E.M.G. et al, 2013).

This thesis discusses the shelf life of fruit juices. Factors effecting their extended shelf-life and they can't be consumed to few days or weeks once bottle is opened. Synthetic preservatives affect human health and people are addicted to their taste and sweetness. Now days, many people are adopting a healthier life and they need to be aware of these natural preservatives .this thesis proves that natural additives are healthier and not used yet in grape juices, musts or wines. Hereby we proved that it's good to use natural preservatives, like reseveratrol in grape products for extended shelf-life to lead a healthier life with less antioxidation activity and less microbial activity in grape must or other products.

2. Objective of work

Aim of this thesis is to deal with sensory evaluation of fruit beverages when treated with natural preservatives, in our case we used resveratrol. Aim is to see if it makes any difference for consumers in terms of appearance, consistency, taste, aroma, and colour. We tried to attempt this test by Discrimination method of sensory evaluation -Triangle test. In this test we chose untrained panels for better results expecting that samples won't have impact on health. Panellists were presented two sets of 3 samples, of which two were similar and one sample was different. Test was repeated in them twice so as to get more results to get better results statistically. Samples given to untrained panel was pure 100 % bio grape must and to other sample, same must was added with resveratrol of concentration 200 µg/ml prepared by a group of doctoral students at CZU, Prague.

The theoretical part of thesis deals with the composition of grapes, different preservatives used now days in market and how it affects human health. This work also proved that synthetic additives have some negative health impact whereas natural additives do not, until and unless not taken in overdoses or more than recommended. Literature part of this thesis explains some ISO norms used in Europe for sensory evaluation - Methodology for triangle test ISO 4120:2004; method of investigating sensitivity taste from ISO 3927: 2011 and ISO 3591: 1977 for apparatus in wine tasting glass.

Results got from untrained panellists were compared to our codes, i.e. code on the glass and order of their presentation. Number of correct and wrong answers were counted at a probability of 99% .Number of correct answers got from experiment were compared with our reference table in book- Senzoricka analyza potravin, Lab book .If the number of correct answers was more than the corresponded value, then our samples are statistically significant. Our reference for correct answers was n=17 for N=30 total number evaluators. And if get a correct answers less than n=17, then our samples are statistically insignificant. This may be because evaluators were not able to differentiate any difference between all three samples. This research can open new opportunities for industries to produce more juices with natural additives instead of using synthetic preservatives.

3. Literature Overview

3.1 Fruit beverages and shelf life

Fruits and vegetables have high concentrations of bioactive compounds including antioxidants which are beneficial to health. New trends have lead to a healthier and more convenient life for consumers. Food products claiming a functional capacity towards promoting health, extending beyond the general provision of essential nutrients, are accepted by consumers and likely to result in a decrease in mortality and a better quality of life within the population (Trujillo, 2011). In this way, new functional beverages based on fruit juices are more and more popular due to their health benefits. The market of functional food and beverages has experienced a rapid growth in recent years which represents an opportunity to produce new product and innovate for both food industry and scientific community. Here and now, less use of chemical preservatives is seen which had adverse effect on humans, now yeasts are practiced as they possess to extend shelf-life (Pimenta, 2009).

Juice from grapes is appealing for human palate because of its composition together holding sweetness, acidity and flavours. Juice can be consumed immediately or can be stored later after processing (e.g. pasteurisation).Grapes are also used to produce jellies and juice which is filled in bottles and used as natural sweetener. However, world production of grape juice is lesser as compared to wine or table grapes. Now grapes are in recent use due to its health benefits as they are natural source of extractable phenolics with antioxidative properties. Most of the times, phenolic compounds are extracted out of the material while processing grapes or musts. These are sod as nutritional supplement or used to support the antioxidant properties in food. Grape juice is now used as fruit ingredient because it has advantage of sweetness as

Figure 1 Grapes being pressed to make must

compared to other products in natural form. Generally, white grape are used as sweeteners and red grape to provide colour. Grape juice sweetened and diluted grape concentrates can be labelled as 100 % juice. Most of it is used as food additive. Juice colour and flavour is affected by high temperature .So it must be appropriate so that unwanted organisms are killed and juice quality is unaffected (Creasy G.L., Creasy L.L., 2009).

3.1.1 Food safety

Food borne diseases in processed fruit and vegetables has increased since eighties, which needs to be controlled now. Salmonella, Vertotoxigenic, E.coli and viruses like Hepatitis A are some common pathogens transmitted from food to humans. Some of them when treated by heat still remain pathogenic like Clostridium, Bacillus. They multiply in number and produce toxins and causediseases. By high hydrostatic pressure, E.coli and Salmonella can be killed (NguyenT.H., 2012).

Figure 2 Food safety

3.1.1.1 Aflatoxins

It is present in fruits but when used further for making juice or musts, is transferred to humans and makes food unhealthy. Aflatoxin is for humans carcinogenic. (Bates R.P. et al, 2001) It is produced by fungi Aspergillus flavis, a mould found in peanuts and peanut butter. Peanuts are great source of resveratrol too. Some sources of aflatoxin are-milk, cheese, corn, peanuts, and cottonseed, nuts, almonds, figs, spices, feed, and meat products. Animal products can be contaminated when they consume aflatoxin –contaminated feed. Highest risk of aflatoxin is from –corn, peanuts, and cottonseed. Its extent of contamination varies from geographic

location, agricultural practices of peanuts. It is susceptible to enter while being harvested, during storage, or processing. They have gained more attention than mycotoxins these days because of their potent carcinogenic effect and acute poisonous effects in humans. Due to aflatoxicosis in humans, many syndromes have been reported around the world. These syndromes are characterised by vomiting, abdominal pain, pulmonary edema, coma, convulsions, death with cerebral edema and fats involvement in liver, kidneys and heart. Due to its carcinogenic effects, IARC placed aflatoxin B1in the list of human carcinogens in 1988 (11).

3.1.1.2. Chemical contaminants

It can be from various things like- from pesticide residue is unsafe for human health. Some of the chemical contaminants according to FDA are acrylamide, benzene, dioxins, PCBs, ethyl carbamate, furan, melamine, perchlorate, radionuclide, toxic elements in food ware, pesticides, natural toxins, metals etc. FDA (Food and Drug Administration) in June 14, 2012 found Carbendazim in Orange juice products while doing there sampling update. It's a fungicide present in low levels in some orange juices imported from Brazil. Later several actions were taken due to addition of this unlawful pesticide residue and asked for action to remove from commercial domestic juices.

Existence of metals like Arsen, is present in environment naturally is a result of contamination for humans. It is found in water, air food and soil in organic and inorganic forms. FDA measures Arsen contents in juices since 1991.

Figure 3 Arsen in natural form

Lead is also a toxic element present in environment in small amounts and is exposed to humans from daily intake such as inhaling dust, eating food and drinking water. Other contaminant is lead, present in environment in soil. Lead in soil is absorbed by plants and

further enters human. It is advisable to wash food before consuming. It can affect various body systems like nervous system, kidneys, immune system. When children are exposed to even to small amount of lead, it leads to reduced IQ, impaired cognitive function , behaviour difficulties and other problems.1 ppm or 100ppb is the limit for lead consumption for children in candies. A maximum of 50 ppb of lead is allowed in fruit juices according to U.S. FDA.

Figure 4 Element coin, an example of Lead

Mercury is another chemical contaminant in FDA's list. It is mostly found in fish life-tilefish from Gulf of Mexico, Shark, Sword fish, and King mackerel. Fish absorbs methylmercury from their feed. Mercury content increases inside fish especially in lager fish with large life span. Methylmercury is present in fish tissues, so cleaning and cooking won't reduce its amount. But by removing its skin, belly fat, internal organs and later cooking can help reduced intake of methylmercury. Fish is great source of omega -3 fatty acids and vitamin D. Its high quality protein , vitamins , minerals are good for pregnant women for growth and development of kid and even in breastfeeding, in infants and childhood fish consumption is necessary. So it is recommended to eat fish 1-2 ounces per week for kids under six whereas2-3 ounces for kids between 6-12 years. (11)

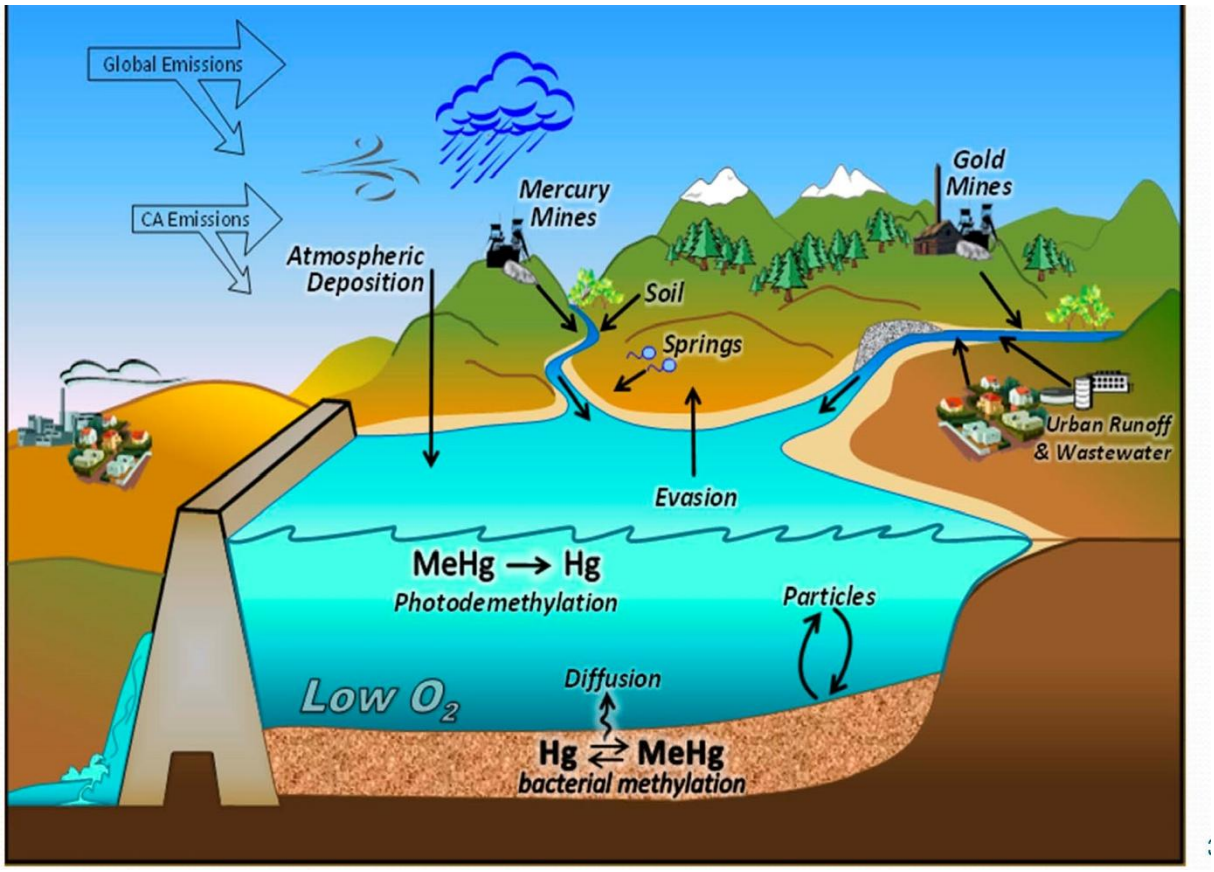


Figure 5 Sources of methylation of Mercury

3.1.2 Factors affecting shelf life of fruit beverages

In juice safety, its hazards and results are important to know so as to apply appropriate methods depending on juice concentrations, pH, sweetness, composition etc. The main factors affecting the shelf life are – Oxidation activity and microbial spoilage

3.1.2.1 Oxidation activity

Oxidation occurs when cell walls break up and produces oxygen. Example when an apple is cut open, it turns brown, this is called oxidation. Generally oxidation does not affect juice quality but some nutrients in juice are affected .It is recommended to drink fresh juice as soon as they are made [3]. Polyphenols are usually oxidised by enzymes when plants are physically damaged. This process involves the reduction of oxygen molecules to generate superoxide anion and hydrogen peroxide which acts as defensive molecules. Carnosine, Quercetin are some antioxidants which protects discolouration and prevents formation of thiobarbituric acid. Resveratrol is also one of the antioxidant as it inhibits deoxygenase activity of lipoxygenase and acts by reducing lipoxygenase to its inactive form (Bekhit A.E.D., 2003).Oxidation can also happen when oxygen is dissolved in juices while packing or processing which causes browning of juice, reduces the quality and nutrients. Antioxidant rich

foods like polyphenols, are commonly form plant sources which make a positive addition to human diet (Beard P.C.W., 2011).

3.1.1.2 Microbial Spoilage

Microbial contamination results in survival or growth if pathogens. Later it leads to rapid spoilage of beverage. Juices contain water, sugars, organic acids, vitamins, and other trace elements thus providing an ideal environment for microbial spoilage. Yeasts are mainly found because of their high acid tolerance and the ability of many of them to grow anaerobically, resulting in CO_2 production, off-flavours and off-taste formation. *Pichia*, *Candida*, *Rhodotorula* and *Saccharomyces* are the genera of microorganisms mainly found in spoiled juices.

Zygosaccharomyces is the main spoilage yeast because it's resistant to stress environments and preservatives (Santos &Marquina, 2004; Santos et al.2009) .It is very tolerant to high sugar conditions and resistant to sulphite and sorbate levels inhibitory to other yeasts and bacteria (F.Esch, 1992). Its contamination results in re-fermentation, turbidity and CO_2 production in sweet wines or grape juice concentrates without producing off-characters. *Zygosaccharomyces* can be inactivated in wines, musts and grape juice by killer toxins and metabisulphite by reducing the levels of Potassium Metabisulphite. Killer toxins produced by *Pichia membranifaciens* are anti-spoilage agents in food and beverages when put in limitations to their stability to physical -chemical factors. Killer toxins control undesirable yeasts in winemaking, so they are valuable for controlling wine spoilage yeasts such as *Brettanomyces bruxellensis, Hanseniaspora uvarum and Saccharomycodes ludwigii.*

3.1.3 EU problems and remedies

Preservatives are required to ensure that food remain safe and unspoiled. The increasing number of food-poisoning worldwide has stimulated public awareness about food safety. The annual health care cost to trace foodborne pathogens like *Listeria monocytogenes, E.coli, Salmonella* was 5-6 billion euros per year. However, excessive use of preservatives suspects carcinogenic, tartogenic or residual toxicity, which is a problem for European consumer since long time. Pathogens are becoming resistant to food borne illness. Food Industry and European authorities have to alert consumers regarding food safety and quality (Anastasiadi M., 2009).

Consumers need to be educated that food with synthetic additives is not always safe. To get rid of such problems they need to switch to natural additives. There is also a need to develop

new types of preservation methods, use nontoxic harmless compounds and adopt natural alternatives/preservatives for consumer's safety and product's extended shelf- life (Anastasiadi M., 2009).

3.2 Preservation techniques

Preservatives are substances which keep beverages fresh looking, tasting longer and extend shelf-life by preventing them from rotting too quickly (Eunice B., 2015). Natural antimicrobials in combination with preservatives facilitate the replacement of chemicals in food preservation. Chemical preservatives should never be added in solid form to fruit juices. They should be first dissolved in small amount of juice/water and then incorporated with bulk o juice (Gupta, 1993).

Preservatives in EU labelled as E number. E numbers ranging from -

- E 100-199 are colorants,
- E 200-299 are preservatives,
- E 300-399 are antioxidants and acidity regulators,
- E 400- 499 are thickeners, stabilisers and emulsifiers,
- E 500-599 are pH regulators and anti-caking agents,
- E 600-699 are flavour enhancers,
- E 700-799 are antibiotics
- E 900-999 are miscellaneous and
- E 1100-1599 are additional chemicals

3.2.1. Pasteurisation

Liquid foods have been traditionally preserved by thermal treatments like pasteurisation, to prevent microorganism spoilage and contamination with pathogens. However, this treatment leads to the loss of healthy compounds and sensory properties of food (Roque et.al, 2015). Pasteurisation is done 2 times in juices. Primary pasteurisation is done after juice extraction at 95-98 ^{0} C for 10-30 sec. Main objective is to inactivate the enzymes. Second pasteurisation is done before filling the juice in container for juice with pH <4.2 at 95 ^{0} C for 15 sec. Main objective of second pasteurisation is to destroy contaminants reproduced in bulk storage before packing. After this method, juice can be consumed up to several weeks after opening the bottles. (7)

3.2.2. Use of biological preservative agent

Scapania. nemorea (a liverwort extract) has antimicrobial activity against food-borne pathogenic and spoilage microorganisms .When combined with heat, it controls spoilage yeast *Saccharomyces cerevisiae* in food like apple, orange-based beverages, (Bukvicki D., 2014). Scapania species can be used as natural preservatives. Plants from Scapaniaceae family have been chemically investigated (Guo et al., 2008) for its antifungal and antitumor properties. Yet it hasn't been reported to be used as a natural preservative in food industry.

3.2.3. Conventional preservatives

The use of conventional preservatives along with some formulation resists juice spoilage. Now world becoming eco-friendly and they are in favour of alternative and natural preservatives. Some of its examples -are citric acid, lactic acid, ascorbic acid, benzoic acid, essential oils, plant extracts including thyme, rosemary oils. Organic acids and essential oils are not currently suitable as substitute for conventional preservatives. A successful preservative is broadly effective against bacteria, fungus, yeasts, and moulds. Normally, food beverages have pH from 2-12 which are susceptible to fungi and bacteria whereas organic acids are more effective and is limited to pH from 2-6.If only organic acids are used as preservatives, and then there would be large difference in neutral and alkaline products.

Conventional preservatives are normally added <0.1 % to active ingredient, in contrast natural preservatives must be > 1% to achieve antimicrobial efficiency and for good performance to viscosity, odour, colour, pH, etc.

3.2.3.1. Colorants

From E100-109 are yellows, 110-119 are oranges, 120- 129 are reds, 130-139 are blues and violets, 140 -149 are greens, 150 -159 are browns and blacks, 160 -199 are gold and other colours. Commonly used colorants are Curcumin, Riboflain, Alkanin, Chlorophylls and Indigo carmine.

3.2.3.2. Antioxidants

Some examples of antioxidants are - ascorbic acid (Vitamin C), Sodium ascorbate, calcium ascorbate, extracts of rosemary, isopropyl citrate, etc. Vitamin C is an essential nutrient for the biosynthesis of collagen and certain hormones. Its intake can reduce the risk of cancer and cardiovascular diseases. On the other hand, it has been reported that diets rich in phenolic compounds correlate with the decrease of neurodegenerative disease and some cancer types.

In addition, both kinds of bioactive compounds are good contributors to the antioxidant activity of food (Roque et.al, 2015).

3.2.3.3. Preservatives

An environment friendly preservative is practically good only if its formulation doesn't fall apart in the product. The use natural alternatives for preservation is not simple as an ingredient substitution as there many problems to maintain safe, effective and stable products which are free from microbial contamination (12).Commonly used preservatives used in EU are as follows in table 1

Benzoic acid (benzoates) –Its amount depends on acidity of juice. It has been found that juice with pH 3.5-4.0; 0.06-0.1% of sodium benzoate (a salt of benzoic acid), is sufficient, but in less acidic juice like grape juice 0.3% is enough (Gupta, 1993).

Table 1 Preservatives E numbers ranging from E 200- E 299

Code	Names	Code	Names	Code	Names	Code	Names
E200	Sorbic acid	E220	Sulphur dioxide	E236	Formic acid	E265	Dehydroacetic acid
E201	Sodium sorbate	E221	Sodium sulphite	E237	Sodium formate	E266	Sodium dehydroacetate
E202	Potassium sorbate	E222	Sodium bisulphite	E238	Calcium formate	E270	Lactic acid
E203	Calcium sorbate	E223	Sodium metabisulphite	E239	Hexamine	E280	Propionic acid
E209	Heptyl p-hydroxybenzoate	E224	Potassium metabisulphite	E240	Formaldehyde	E281	Sodium propionate
E210	Benzoic acid	E225	Potassium sulphite	E242	Dimethyl dicarbonate	E282	Calcium propionate
E211	Sodium benzoate	E226	Calcium sulphite	E249	Potassium nitrite	E283	Potassium propionate
E212	Potassium benzoate	E227	Calcium hydrogen sulphite	E250	Sodium nitrite	E284	Boric acid
E213	Calcium benzoate	E228	Potassium hydrogen sulphite	E251	Sodium nitrate	E285	Sodium tetraborate
E214	Ethylparaben	E230	Biphenyl, diphenyl	E252	Potassium nitrate	E290	Carbon dioxide
E215	Sodium ethyl para-hydroxybenzoate	E231	Orthophenyl phenol	E260	Acetic acid	E296	Malic acid
E216	Propylparaben	E232	Sodium orthophenyl phenol	E261	Potassium acetate	E297	Fumaric acid
E217	Sodium propyl para-hydroxybenzoate	E233	Thiabendazole	E262	Sodium acetates		
E218	Methylparaben	E234	Nisin	E263	Calcium acetate		
E219	Sodium methyl para-hydroxybenzoate	E235	Natamycin, Pimaracin	E264	Ammonium acetate		

3.2.3.4. Sulphur dioxide SO2 (sulphites)

SO_2, a food additive in Europe is marked by "E" number .It ranges from E 220–E 228 such as sulphur dioxide (E 220), sodium sulphite (E 221), sodium hydrogen sulphite (E 222), sodium metabisulphite (E 223), potassium metabisulphite (E 224), calcium sulphite (E 226), calcium hydrogen sulphite (E 227) and potassium hydrogen sulphite (E 228).[4]

Figure 6 -Wine containing sulphites

Fruit juices can be preserved for up to one year with pottasium metabisulphite, a source of sulphur dioxide by addition of 0.1% sulphurous acid and store containers at 15^0C. It retards oxidation, prevents against discolouration and loss of flavour (Gupta, 1993). It is useful for mechanically harvested grapes which decay quickly (Creasy G.L., Creasy L.L., 2009). SO_2 is good preservative to protect wines from alterations by virtue of its anti-oxidative, antimicrobial effects against lactic acid bacteria. But its high concentrations result in human health risks, like headache, allergic reactions, fatigue, asthma, itching (Roque, 2015).

- Negative impact of SO2 – Allergic reactions has been reported in some sulphite-sensitive people. Quantity ranging from 10-50 mg shows sulphite ingestion and has symptoms like dermatitis, urticaria, angioedema, abdominal pain and anaphylaxis. Its adverse reactions are rare. Asthmatics have greater risk of experiencing reaction to sulphite containing food. It also causes inactivation of proto-oncogenes, inactivation of tumour suppressor genes, and even lung cancer. Excess SO_2 has toxic effects on human health, resulting in headaches, nausea and asthmatic reactions. To reduce the growing microbial population in musts, 30–50 mg/L SO_2 is preferred value to be added. At this point, it is ineffective against aerobic yeast and lactic acetic acid bacteria (Guerreo F.R., 2015). A maximum of SO_2 limit up to 200 mg/L is allowed.

Food with more than 10 mg/kg or 10 mg/L is not considered to be sold (Guerreo F.R., 2015).

3.2.4. Alternatives to conventional preservatives

To successfully commercialize market, juice is stabilized by various methods-

- HIPEF- High Intensity Pulsed Electric Fields, a non-thermal technology used as an alternative to extend the shelf life with high added value. It inactivates the spoilage or pathogenic microorganisms and enzymes (Trujillo, 2011).
- HPP - High-pressure Processing, inactivates microorganism and enzymes without compromising the nutritional and sensory quality of food (Roque et.al, 2015).
- UV radiation, ultrasound and bacteriocins, dimethyl dicarbonate and use of lyzozyme - are other alternative methods.
- Natural preservatives - Antimicrobial plant extracts rich in polyphenols can be availed as total/partial alternative to sulphites during winemaking. Consumers don't recognise any difference between wines treated and untreated with antimicrobial phenolic extracts in winemaking (Eva M. et al, 2013).

3.2.5 Plant derived antimicrobials

Some of the plant derived antimicrobials disscussd in this thesis are quercetin, epigallocatechin gallate, luteolin, rutin and resveratrol.

- Quercetin – It is a flavonoid found in many fruits and vegetables, leaves and grains. It is used in supplements, in food and beverages as antimicrobials. It is present in human's daily diet and helps in biological activities such as antioxidant, antiviral, anticancer, antimicrobial and anti-inflammatory. Its derived forms are used into drugs for treatment of diseases caused by oxidative stress and lethal viruses (Maalik A., et al, 2014).

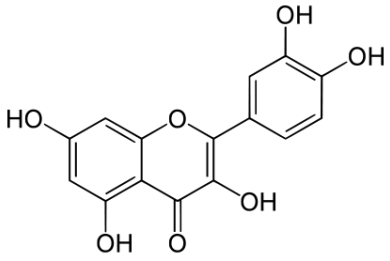


Figure 7 Chemical structure of Quercetin

- Rutin - An article by Sanches M.C.et al, 1999, found that wines have higher antioxidant activity than grape juices and red wines have highest among all wines. This antioxidative activity was due to free radical scavenging capacity. He measured major polyphenolic constituents in standards like-rutin, ferulic acid, tannic acid, Gallic acid, resveratrol, BHA, quecetin, caffeic acid. He found that free radical scavenging activity of Gallic acid was highest, whereas for rutin, tannic acid, caffiec acid, activities were intermediate and for resveratrol was lowest.

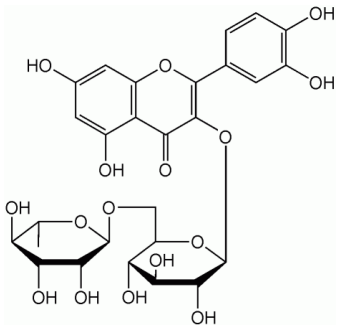


Figure 8 Chemical structure of rutin

- Epigallocatechin gallate - Bub A. Et al, 2003 concluded that juices have physiological effects in vitro including antioxidative, immunomodulatory and antigenotoxic effects. With his study he showed that plasmamalondialdehyde decreases with juice consumption. Intake of fruit juice reduces the reduction activity if oxidative DNA damage.
- Luteolin - Some potentially anticarcinogenic flavanoids like quercetin, kaempferol, myricetin, apigenin, luteolin are commonly used in fruit beverages. They can be detected by RP-HPLC, UV detection. Flavonoid levels in white wine are below 1 mg/l. In red wines and grape juice, quercetin levels below 5 mg/l. Lemon juice has 7mg/l and tomato juice 13mg/l. No luteolin was found in his analysis in wines, grape juice, grapefruit juice and tomoto juice. In black tea infusions, quercetin was found ranging from 10-25 mg/l, kaempferol from 7-17 mg/l and myricetin from 2-5mg/l as said by Hertog M.G.L. et al, 1993. They found that flavonoid level in tea bags was higher than loose tea leaves.

3.2.5.1. Resveratrol

Grape (*Vitis vinifera*) is considered as major source of phenolic compounds among different fruits, associated with health benefits as natural antioxidants, including resveratrol, anthocyanins, etc. Research has shown that polyphenols from grapes give health benefits,

such as reducing the incidence of cardiovascular diseases, improvement of neuronal function with aging and neurodegenerative diseases, antitumor, anti-inflammation, and antimicrobial. Phenolic compounds are absorbed by the human digestive system, and enter into the blood successfully with no potential toxicity. High levels of phenolic compounds are found in wine, seeds and skins of grapes, and wine would be considered as a good source of grape phenolic compounds due to containing high levels of potentially bioactive polyphenols (Enqin X. et al, 2014).

It is a member of natural polyphenols it has antimicrobial effects found in the skin of grapes, peanuts and berries. It is used as conservative. It can also affect sensory testing. Its amount in bevarages depends on various factors such as grape variety, environmental factors, and extraction parameters. There has been an increasing trend towards increasing the concentrations of resveratrol and total phenolics in thermovinification by increasing fermentation temperature, must freezing, and extended maceration during the winemaking process. Yet no data has been found for reseveratrol on factors like yeast selection, juice run-off, or cold-soak treatments (Cvejic J., 2015).

Figure 9 some good sources of Resveratrol - red grapes, eucalyptus, blueberries, peanuts, peanut butter etc.

3.2.5.1.1. Benefits of resveratrol

Since resveratrol has antioxidative properties, it protects the human body against damage linked to increased risk of cancer and heart disease. That's the reason why many traders are selling resveratrol supplements thses days (capsules exracted from plant *Polygonum caspidatum*). Others are made from red wine or grape extracts. Resveratrol has gained attention for its disease combating benefits such as –

Figure 10 Night serum containing resveratrol with anti-aging properties

- Anti-aging – Reduces inflammation.
- Heart disease – by preventing the oxidation of LDL (Low Density Lipoprotein) or bad cholesterol which makes difficulty for platelets to stick together and form clots which lead to heart stroke.
- Cancer- by limiting the growth of cancer cells
- Alzheimer's disease - by protecting nerve cells from damage and built up of plaque which leads to Alzheimer's disease (Pasinetti, 2014).
- Diabetes - by preventing insulin resistance. (1)

3.2.5.1.2 Side effects of resveratrol supplements

So far, studies have not discovered any side-effects if resveratrol taken in higher doses. But its supplements may interact with blood thinners and medications such as aspirin, ibuprofen, increasing risk of bleeding. There isn't any dosage recommendation in studies because it varies from supplement to supplement. In normal supplements 250-500 mg of resveratrol is present. People may consume 2 g or more per day. (2)

Figure 11 Resveratrol supplement capsules

3.3 Effects on sensory analysis

Juices containing preservatives have sensory effects such as on taste, aroma, texture, shelf-life etc. Natural preservatives when added in appropriate amount are sensorial good and also beneficial for health. Here are list of some compounds which effects makes sensory changes in food beverage.

- Polyphenols - relate to sensory qualities such as colour, bitterness, and astringency, which are relevant in wine, tea, grape juice, and other products. These occur naturally varying from simple phenolic acids to complex polymerized tannins. The use of polyphenols against carcinogenesis proved to be suitable alternative for prevention and/or therapeutic purposes (Gollucke A.P. et al, 2013).
- Methoxypyrozins (MPs) –MPs are group of compounds found in grapes, wines which are responsible for "green or vegetative" aromas in wines like Cabernet Sauvigon, Sauvigon Blanc, Bodeuax. They contribute to sensory characteristics in wines. Some common MPs are: 3-isobutyl-2-methoxypyrozine, 3- isopropyl-2methoxypyrazine and 3- sec-butyl-2-methoxypyrozine.Its contents may vary due to viticulture practices, light exposure, maturation, climate and soil (Sidhu D., 2013).
- Linalool: 3-mercaptohexyl acetate - Aroma difference in sensory analysis in wine and grapes is due to the ratio of linalool: 3-mercaptohexyl acetate. It contributes to floral and sweet notes in wine. (Campo E., 2005).
- Terpenes – Are flavouring agents. Its content depends on grape varieties ranging from 500-1700 µg/l. Some common terpenes are alcohols, aldehydes, hydrocarbons, oxides of mono terpenes, sesquiterpenes. They are bounded with oligosaccharides in odourless glycoside form and require glycosidase enzymes to liberate terpenes (Hui Y.H. et al, 2006)

3.3.1 Sensory evaluation techniques

There are several types of sensory evaluation methods done on various food products.

3.3.1.1 Discrimination/Difference Test

These are the simplest and most sensitive form of testing. These are used to determine-whether a difference in some specific attribute exists or not between two samples. One sample can be preferred to another sample too. Unfortunately this method cannot determine how large the difference between samples is. Some commonly used Difference Tests are -

- Paired comparison - Two samples are compared, one has to be selected (1-tailed) or, where preference is asked either sample can be correct (2-tailed)
- Triangle test - only one response can be correct
- To control the difference- duo-trio; multiple comparison

3.3.1.2 Ranking Procedures

In this method three or more samples are presented at the same time, arranged in order of intensity or degree of some attribute e.g."Rank A, B and C in order of increasing sweetness". This method is not affected by serving procedure i.e. coded samples, interval between tasting, orders of presentation etc. Number of samples is not more than 4-6.Unfortunately, this method gives no information on the size of the differences between samples. (6)

3.3.1.3 Scaling procedures- Panelists define by using a scale to explicit their perception by measuring the size of sensory attribute. Types of scales can be as follows:

- Verbal – words used to divide the scale
- Numerical – uses numbers
- Line / unstructured
- Arrangement of scale can be:
    - ✓ unipolar – scale 0-9 ; none- extreme
    - ✓ biploar –extremely soft - extremely hard
- It can be objective or subjective

Unipolar verbal scale (9 point)-e.g. "Bitterness"- none- extreme

□	□	□	□	□	□	□	□	□
none		slight		moderate		very		extreme

Unipolar numerical scale (9 point) – "Rancidity" 0-9

0	1	2	3	4	5	6	7	8	9
Absent		slightly			moderately		very		extremely

Unipolar line scales

not sweet very sweet

Bipolar line scale

___	_____	____
extremely smooth	extremely rough

Bipolar verbal comparative scale (5 point) –"Sweetness"

□	□	□	□	□
much less than standard	slightly less than standard	the same as standard	slightly more than standard	much more than standard

In this thesis we used triangle test because it is extensively used in food analysis to determine difference between two products by sensory detection. The assessors must select the different sample from a group of three, two identical and one different .This method helped us to see if consumers can percept any difference between normal must and must with natural additive which was our aim.

- ISO 4120:2004 - According to ISO 4120:2004, Sensory analysis, Methodology for Triangle test, explains the procedure to determine perceptible sensory differences or similarity between two products (if exists). It's a forced-choice method. It applies to whether difference can exist in a single sensory attribute or in several attributes. This method is meant to be statistically more efficient than other tests like duo-trio test, but it has limited use with products that exhibit strong carryover flavours. This method is used where difference in products is unknown i.e. there is no difference between samples and there is no indication of attributes responsible for difference. It is good for homogenous products. Changes in a product are made by ingredients, processing, packaging, handling, storage which we can test by sensory analysis methods (L.L. Rogers, 2010) [5]
- ISO 3972:2011 -Sensory Analysis –Methodology -Method of investigating sensitivity of taste. Aim of this standard is to teach assessors to recognise tastes and find difference between them. It teaches assessors to know and familiarise with different types of threshold tests (like- stimulus threshold, satiation threshold, recognition

threshold, difference threshold).It also aware the assessors of their own sensitivity of taste. It enables supervisors to carry out a preliminary categorisation of assessors. (8)

- ISO 3591:1977-Sensory analysis-Apparatus-Wine tasting glass- This International standard specifies the characteristics of a wine-tasting glass to be used for sensory analysis of wines. This glass may be used for examining all types of tests like simple testing, profile analysis etc. for all organoleptic characteristics of wine samples such as colour, clarity, solubility, bouquet, flavour etc. (9)

The statistical interpretation of this test is based on the binomial law with p = 1/3 (null hypothesis, no differences between the two tested products). When the number of correct answers of a triangle test equals or exceeds the critical value compatible at a given probability level with a binomial distribution with parameter 1/3, the null hypothesis is rejected and significant differences between samples are concluded. The hypothetical value of 1/3 of the binomial parameter is rejected, not the binomial nature of the observed results. This implies to consider that the assessors are still exchangeable since all of them have a common probability p (greater than 1/3) of giving a correct answer (Carbonell et.al, 2007).

4. Materials and methods

4.1 Chemicals

Resveratrol was obtained from BDL (Turnov, CZ). The following chemicals were used as solvents: ethanol (Penta, Prague, CZ), dimethylsulfoxid (DMSO) (Lach-Ner, Neratovice, CZ), Tween 80%, Polyethylenglykol (PEG) a Cyklodextrin (všechny Sigma-Aldrich, Prague, CZ). Acetonitrile and acetone (Merc, Prague, CZ), orthophosphoric acid (Lach-Ner, Neratovice, CZ) and deionized water were used for HPLC analysis.

4.2 Resveratrol solubility

The resveratrol alone showed very low solubility in must. Therefore, several solvents (ethanol, DMSO, Tween 80% and PEG) were used in order to try to increase the resveratrol solubility. As the last step, the attempt to increase the resveratrol solubility using an inclusion complex with cyclodextrin was made.

4.2.1 Resveratrol complex with cyclodextrin

β-cyclodextrin-resveratrol inclusion complex was prepared at the Department of Crop Production using a co- precipitation method (Bhandari et al. 1998) . 500 mg of β-cyclodextrin was dissolved in distilled water (15 ml) at 60 ºC. Then the solution was cooled to 40°C and resveratrol in ethanol was slowly added during continuous agitation. In total, 100 mg of resveratrol was loaded into the complex. The vessel was then sealed and stirred for 3 h and refrigerated overnight at 4 ºC. The cold precipitate was recovered using vacuum-filtration and washed with 1.5 mL of distilled water, then dried at 75 ºC in a vacuum oven for 24 h. The final dry complex powder was stored in airtight glass desiccators at room temperature.

4.2.2 HPLC analysis

To examine the effectiveness of the cyclodextrine complex formation, the content of resveratrol in the complex was tested by high-performance liquid chromatography using the liquid chromatography LC500 (INGOS, Prague, CZ) equipped with UV-vis detector and RP-18e (5 µg) column. The column temperature was set to 24°C, sample volume of 20 µL was injected and 25% acetonitrile in water (adjusted to pH 3 using orthophosphoric acid) was used as a mobile phase using constant flow of 1.5 mL/min and isocratic elution. The measurement was carried out at the wavelength of 306 nm. The analysis was performed at the Department

of Quality of Agriculture Products. Quantification was evaluated using linear calibration curve.

4.3 Prepare Samples

One beaker filled with Clear Juice was Malverina, a bio grape must bought from supermarket in Czech republic. Second beaker contained grape must with resveratrol at the concentration of 200 mg/L. The resveratrol was dissolved in PEG before addition to the must, whereas the final concentration of PEG in the must was 0.5%.The must sample was 100% Bio product and non-proliferated, Produced in Czech Republic. Compositions written on bottle were as follows –Non alcoholic drink. Fruit juice made from organic farming, pasteurised. With maturation of grape must, it can produce grape tartar on bottle which is not a racing product. Average nutritional values in 100 ml: Energy value 303 kJ/ 73 kcal, protein < 0.13 g, saccharides 17.1 g, from which sugars 17.1g, fat <0.1 g, from this saturated fatty acid <0.001%, fibres <0.01%, sodium 0.00243g. Date of manufacture 20.9.2013.Minimal durability till 31.12.2016. Producer: Vinselekt Michelovský a.s.-Luční 858, Rakvice, CZ 69103.

Figure 12: Malverina 100 % bio product grape must for sensory evaluation

Second beaker contained resveratrol + grape must of 200 µg/ml concentration. 45 Glasses was prepared for 15 consumers, 3 glasses each. Glasses were covered with aluminium foil so that consumers don't recognise colour difference and solubility difference by sight. If Clear juice is A, and Resveratrol mixed with juice is B, there were 6 possibilities of serving samples (AAB, ABA, BAA, BBA, BAB, and ABB). So, groups A and B were randomly put in serving trays keeping in mind to serve in above 6 possible orders.

4.4 Serving samples

Samples of volume 30 ml were filled in wine tasting glasses OIV (KOBERN, Dubnany, CZ) covered with aluminium foil to avoid the evaluators decision process influence the optical changes caused by resveratrol addition. The sets of samples were served at room temperature 20 ± 2 ^{0}C. 30 set of samples were presented to testers. 2 groups- Resveratrol (200 µg/ml) and Clear juice (100 % clear). Juices were filled 30 ml in each glass. Total number of 15 consumers with two repetitions per person, we got total results for 30 assessors between age group of 20-25 years. Of which 6 were male and 9 were females. Out of 15 consumers 13 were healthy and 2 were having cold.

Figure 13- Wine glasses covered with aluminium foil with codes at CŽU laboratory

4.5 Evaluators panel

The panel of evaluators for the triangle test consisted of 15 untrained judges, of which 9 were female and 6 male with age range from 20 to 25 years. Each judge evaluated two sets of samples with about one hour break between the sets. From the total of 15 assessors, 14 were students and 1 was female staff member. Time of analysis was 14:30- 15:30.

4.6 Instructions to evaluators

Oral instructions given to consumers included the following points.

1. 3 glasses with grape must

2. Taste all 3

3. 2 are similar and 1 is different - Triangle test

4. Observe for different sample.

5. Re-tasting allowed for one sample only and only once

6. Fill the Evaluation form (Table 2) for triangle test

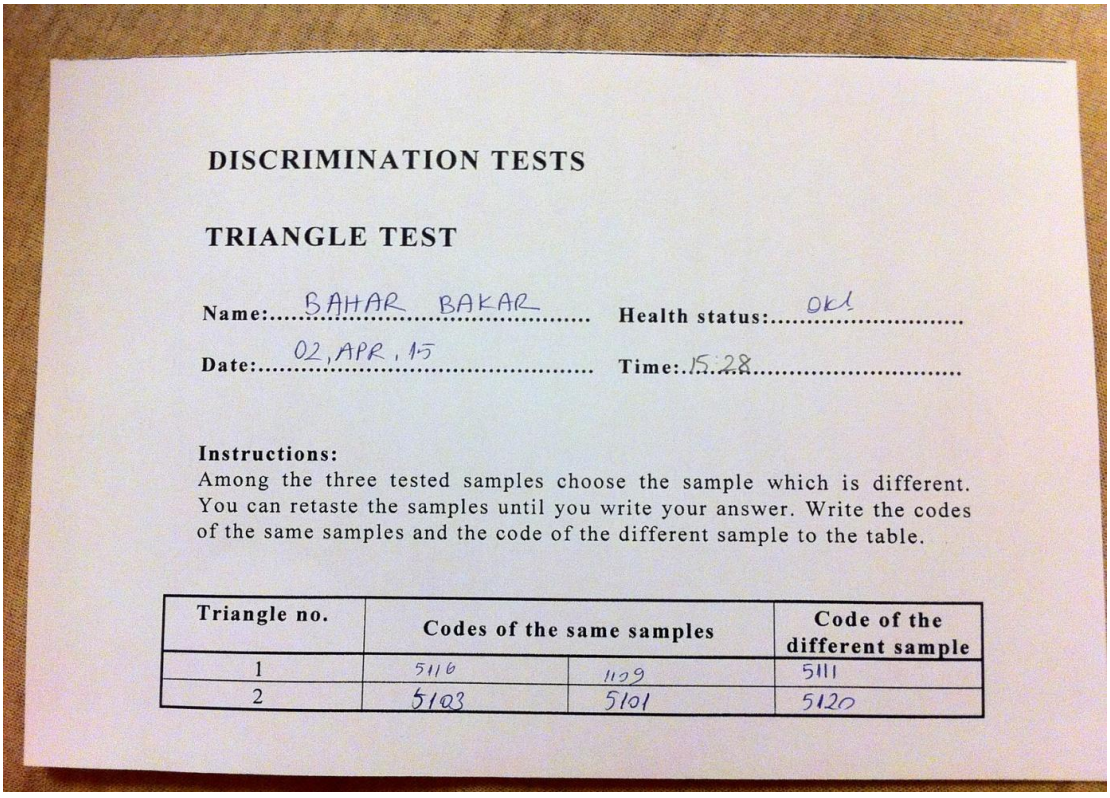
7. Repeat steps 1-6 in second round after 30 min with new samples

4.7 Collecting data

Written instructions were given to consumers given in Table 3. Consumers were supposed to fill codes in the table in the appropriate columns- 2 similar and 1 different taste. After first analysis a break of 30 min was given and again new samples were served.

Table 2- Evaluation Form given to untrained paneslists

DISCRIMINATION TESTS- Triangle test Name- Health- Date- Time-			
Instructions- Among the 3 tested samples, choose the sample which is different. You can re-taste the samples until you write your answer. Write the codes if the same samples and the code of different samples to the table			
Triangle set number	Code of similar samples		Code of different sample
01			
02			



DISCRIMINATION TESTS

TRIANGLE TEST

Name: BAHAR BAKAR **Health status:** OK!

Date: 02, APR, 15 **Time:** 15.28

Instructions:
Among the three tested samples choose the sample which is different. You can retaste the samples until you write your answer. Write the codes of the same samples and the code of the different sample to the table.

Triangle no.	Codes of the same samples		Code of the different sample
1	5116	1129	5111
2	5103	5101	5120

Figure 14 - An example of evaluation form collected from evaluators after tasting

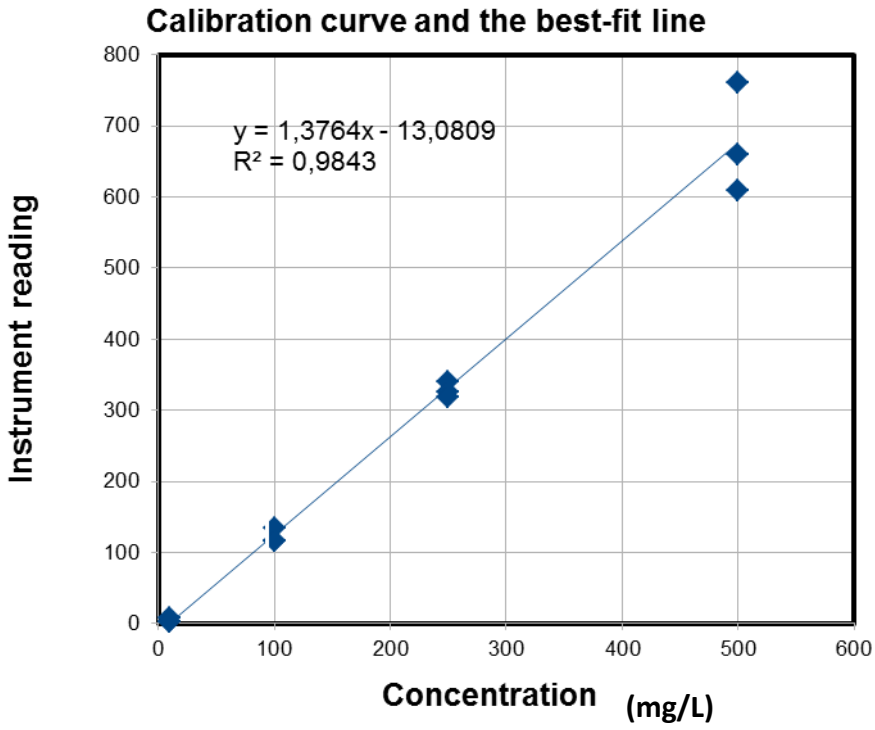
5. Results

5.1 Solving the resveratrol solubility

Based on previously reported growth inhibitory activity of resveratrol against wine spoilage microorganisms, the concentration of 200 mg/mL was selected for sensory evaluation tests. Unfortunately, resveratrol showed very low solubility in must. Therefore, several solvents were tested in order to increase the resveratrol solubility. However, when the dissolved resveratrol was added to the must, it started to precipitate and to settle to the bottom of the test tube rapidly. The attempt to solve the solubility problem using inclusion complex with cyclodextrin (4.2.1) failed as well. The solubility was not improved significantly, although the concentration of resveratrol in the complex measured by HPLC and quantified using linear calibration curve (Figure xx), was determined to be 35.12 µg/mL of complex.

Finally, dispersion of resveratrol in must at the concentration of 200 mg/L was prepared for the sensory evaluation. The dispersion was improved by 0.5% of PEG so that he resveratrol remained evenly dispersed for approximately 5 min before the beginning of its slow settlement. This time interval was considered to be long enough for the distribution and evaluation of one sample set. However, for this reason, the samples had to be served immediately after filling the tasting glasses with samples.

Figure 15 - Calibration curve for resveratrol quantification



5.2 Sensory analysis

According to our reference table for Triangle test in Laboratory Book , Senzorická Analýza Potravin; Pokorný J.,Valentová H., Pudil F.,1999; (Table3.) the minimum number of expected positive answers for 30 people was 17 positive answers with a level of probability 99 % so as to have statistically significant samples. Values lower than 17, samples are insignificant and values more than 17 were significant.

Table 3- Reference table from Senzoricka analyza potravin (Pokorny J.et al, 1999). Here n= minimum number of positive answers for N=total number of evaluators.

Celkový počet posudků *N*	Minimální počet kladných odpovědí *n*	Celkový počet posudků *N*	Minimální počet kladných odpovědí *n*	Celkový počet posudků *N*	Minimální počet kladných odpovědí *n*
5	5	21	13	37	20
6	6	22	14	38	21
7	6	23	14	39	21
8	7	24	14	40	22
9	7	25	15	41	22
10	8	26	15	42	22
11	8	27	16	43	23
12	9	28	16	44	23
13	9	29	17	45	24
14	10	30	17	46	24
15	10	31	18	47	25
16	11	32	18	48	25
17	11	33	19	49	25
18	12	34	19	50	26
19	12	35	19		
20	13	36	20		

For calculating values of conclusiveness at the level of probability at P=99%.

Out of 15 evaluators 9 were women, 6 were men. Our test was repeated twice with different samples .So total number of females now becomes 9*2 = 18 and total number of males become 6*2= 12 males. Out of 18 females 11 answered correctly and 7 wrong. Whereas in males, memory to remember samples was low. Out of total 12 males, only 5 answered right and 7 answered wrong.

On the basis of our Discrimination test (Table 2), we compared the results with Table 3 and created Table 4.

Table 4- Triangle test-Acceptability of number of correct answers

Total no. Of samples (N)	No. Of correct answers (n)	No. Of wrong answers	Probability of right answers	Test statistically conclusive or not At P =99 %
30	16	14	17	Not

For 30 evaluators, according to our reference table 2, n=17 was minimum number of positive answers. Here in our case n=16, which is lower than the reference value shows that our samples were statistically insignificant.

To 15 assessors we presented 2 sets of triangles. Here N= 30.From which we got n=16 right answers, which is value lower than corresponding tabular value n= 17. So, we can conclude that, for N= 30 at level P=99%, difference between resveratrol with juice and clear juice is statistically inconclusive.

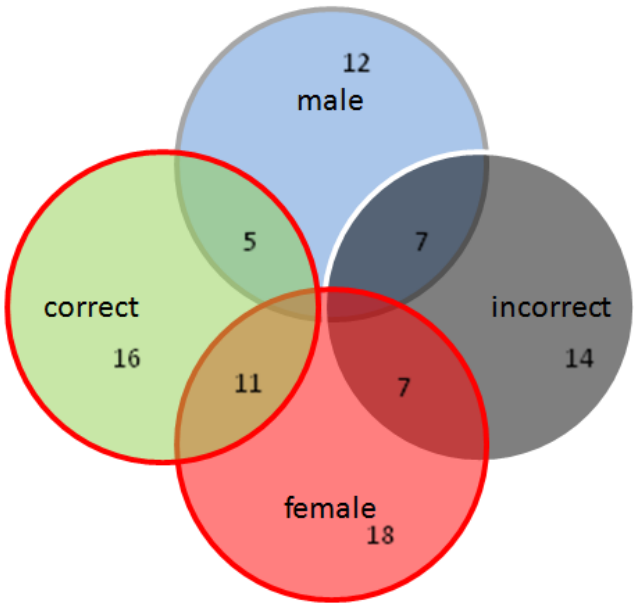


Figure 16 Venn diagram based on gender evaluation and correct / incorrect answers. Here total number of evaluators is 30 of which 12 were male and 18 women. 5 Males answered correctly and 7 incorrectly. On the other hand, out of 18 females, 11 answered correctly and 7 incorrect.

From total 30 evaluators 53 % of answers were correct and 46 % were answered wrong. Since there was visible difference between the samples with reseveratrol and without it, evaluators were not able to recognise it. It may be because of glasses covered with aluminium foil. There was a clear difference in solubility. Juice with reseveratrol was more transparent as compared to the pure juice.

6. Discussion

In our test, we selected 30 untrained students could be better if this test was done on both trained and untrained panellists and for other characteristics. This test could be later done on different age groups and compare if consumers were able to differentiate natural additives than normal juice or not. It can be also practiced to other juices like orange juice, grape juice and even strawberry must, orange must and so on.

Results can be also affected by time of analysis because it was after lunch time from 14:30 - 15:30.This test can be later done with more assessors for e.g. 50 to get more results and get statistically sufficient data. In our case, we got 53 % correct and 46 % incorrect answers, so if this test was attempted on more people, we can get more values and can compare with different age groups and according to gender. When we describe this test on basis of gender, we found that out of 15 evaluators 9 were women, 6 were men. From 9 female students, only 4 answered correctly all samples. On the other hand, males were not able to recognise much .Only 1 male student was able to recognise all samples correctly. There was clear optical difference in samples with resveratrol and clear must. Clear juice was clear and when resveratrol was added juice's density changed and became denser.

Pasteurisation and convectional preservatives significantly lowers the nutritive value of juices that's why people are accepting natural preservatives. This work can be further extended by working on different samples with different natural preservatives and account consumer's acceptability. In this thesis we proved that resevratrol can be used as natural preservative in food industry because it acceptable from sensory point of view. Its percentage depends on other juice concentrations, pH and other factors. It is important in man's daily life and known for various biological activities such as antioxidant, antiviral, anticancer, antimicrobial and anti- inflammatory. Its various forms can be used to cure diseases caused by oxidative stress and lethal viruses.

Plant phenolic compounds like epigallocatechin gallate presents effects in vitro changes with antioxidative, immunomodulatory and antigenotoxic effects.It can help reduce the DNA damage. Juices when preserved with cyanidin polyphenols and epigallocatechin gallate have no accumulation of plasma polyphenols. Juice consumption with such preservatives increases lymphocyte proliferative responsiveness. Juice consumption enhances antioxidative effect and reduces oxidative DNA damage with stimulated immune cell functions

We also found that wines have higher antioxidant activity than grape juices and red wines have highest among all wines. This antioxidative activity is due to free radical scavenging capacity. Some major polyphenolic constituents in standards like-rutin, ferulic acid, tannic acid, Gallic acid, resveratrol, BHA, quecetin, caffeic acid, we can state that free radical scavenging activity of Gallic acid is highest, than rutin, tannic acid, caffeic acid. Whereas, for resveratrol is lowest. Aromatic constituents can be characterised by using headspace solid phase micro extraction (HS-SPME). The use of preservatives has an impact on cooked fruits as well.

An environment friendly preservative is practically good when formulation doesn't fall apart in the product. Conventional preservatives can be normally used <0.1 % to active ingredient, whereas natural preservatives can be > 1% to achieve antimicrobial efficiency and for good performance to viscosity, odour, colour, pH, of product.

Essential oils extracts from Thymus kotschyanus and Carum copticum can be evaluated for efficacy in control of postharvest fungal decay as didn't show any negative impact on sensory quality of grapes. These compounds can be used for preservation to reduce the postharvest losses (Marandi R.J., 2010)

Antimicrobial plant extracts can be used as an alternative to conventional preservative techniques. We searched for natural polyphneols, antioxidants existing already existing in nature. They are natural so won't have any side efffetcs until and unless taken in recommended dosage. Few of them are Quercetin, Resveratrol, Rosemary extracts, Luteolin, Epigallocatechin gallate and Rutin. Some added preservatives used and approved in EU ranges from E 200-299 (Espina L., 2014).

Red wine and grape juice consumption effects in vitro effects of resveratrol , caffeic acid, gallic acid , quercetin and rutin on NTPDase –nucleoside triphosphate diphosphate , adenosine deaminase activities (ADA) in patelets and platelet aggregation from streptozotochin induced diabetic rats. In vitro tests with resvertrol , rutin, quercetin, gallic acid, caffeic caid decreases the hydrolysis of platelets in diabetic rats in presence of all polyphenols. It is stated that grape juice and red wine and all polyphenols are able to modulate the ectoenzyme actitvity. It can be used as prevention of platelet aggregation abnormality and vascular complications in diabetic state (Schmatz R., in 2013)

ISO norms helped us a lot to carry out our experiment for. Triangle test by explaining the procedure to determine perceptible sensory differences or similarity between two products (if exists). This method was more statistically efficient than other tests like duo-trio test, because it has limited use with products that exhibit strong carryover flavours. This method is used where difference in products is unknown i.e. there is no difference between samples and there is no indication of attributes responsible for difference. It is good for homogenous products. Changes in a product are made by ingredients, processing, packaging, handling, storage which we can test by sensory analysis methods. ISO standard helped assessors to recognise tastes and find difference between them. It teaches assessors to know and familiarise with different types of threshold tests .It also stated about apparatus of wine in wine glasses for all organoleptic characteristics of wine samples such as colour, clarity, solubility, bouquet, flavour etc. [9]

Anticarcinogenic flavanoids like quercetin, kaempferol, myricetin, apigenin, luteolin are commonly used in fruit beverages. They can be detected by RP-HPLC, UV detection. Flavonoid levels in white wine found in literature used were below 1 mg/l. In red wines and grape juice, quercetin levels below 5 mg/l. (Hertog M.G.L., 1993)

It can be also also used in thermovinification to make wines by using appropriate processing methods and appropriate amounts within EU limits and according to consumer's acceptability.

7. Conclusion

We tested Resveratrol by sensory analysis by adding in 100 % bio grape must. Triangle test was selected as sensory evaluation method. ISO norms and Laboratory books were our guidance to perform tests. astly, we compared the results of all evaluators and accounted their acceptibility to sensory analysis. Results were compared with the reference Laboratory Book. So that it can be used further in juce industries and preserve with this natural polyphenol already existing in wines. Our results collected from evaluators were statistically insignificant, concluding that evaluators were not able to differentiate it with normal juice in triangle sensory test. This indicates that resveratrol if used as andditive it will be acceptable to consumers by taste. Since it was not refused by our evaluators, it can be used in upcoming days in food industry. We can conclude that this Triangle test was statistically insignificant. Consumers couldn't recognise much difference between the samples added with resveratrol and without resveratrol. In our test, we found that out of 18 women 11 answered correctly and out of 12 men, only 5 answered correctly.

This data opens new possibilities to add natural additives in fruit juices, musts and even wine, as in our case, grapes are used in all three products. This thesis can help juice producing food chains to see consumer's acceptance to natural additives, proving that they don't need synthetic preservatives any more. And, for them natural preservatives are better than synthetic ones for their better health and extended shelf- life of juice/ musts.

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24. Figure 11- Reseveratrol supplement capsules- http://www.bestdietpillreviewsforwomen.com/resveratrol-customer-reviews-side-effects-report/
25. Figure 12 – Malverina 100 % bio product grape must for sensory evaluation
26. Figure 13 - Wine glasses covered with aluminium foil with codes at CŽU laboratory
27. Figure 14 - An example of evaluation form collected from evaluators after tasting
28. Figure 15 - Calibration curve for resveratrol quantification
29. Figure 16 Venn diagram based on gender evaluation and correct / incorrect answers
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