CZECH UNIVERSITY OF LIFE SCIENCES

PRAGUE

Faculty of Tropical AgriSciences

Plant-derived food preservatives as alternatives to sulfites

Bachelor thesis

Prague 2016

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DECLARATION

I hereby declare that I have written presented bachelor thesis "Plant-derived food preservatives as alternatives to sulfites" by myself with help of the literature listed in references.

Prague, 2016

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ACKNOWLEDGEMENT

I would like to express my thanks to my thesis supervisor Ing. Olga Leuner Ph.D. (Department of Tropical Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague) for the professional guidance and for giving me valuable recommendations. I would like to thank also to my family for the help they gave me.

ABSTRACT:

In present, fresh-cut foods, dried fruits and processed vegetables are often treated with sulfites against the spoilage and enzymatic browning. However, these additives cause undesirable health risks at a certain portion of the population. In addition, customers demand food without added chemicals. This Bachelor thesis is a literature review of substances with similar properties such as sulfites. These substances should have the ability to inhibit browning and undesirable microorganisms and act as antioxidants; substances of natural origin without health risks unchanging organoleptic properties of food and at the same time available for price allowing them to participate in tests on food models. As a result, sixteen compounds which exhibit very similar properties as sulfites were suggested for further research.

Key words: sulfites, additives, natural alternatives, sulfur dioxide, preservatives

ABSTRAKT:

V současné době se čerstvé krájené (tzv. "fresh cut") potraviny, sušené ovoce a zpracovaná zelenina nejčastěji ošetřuje proti kažení a hnědnutí siřičitany, které ovšem u jisté části populace způsobují nežádoucí zdravotní rizika. Kromě toho si zákazníci žádají potraviny bez přidání chemických látek. Bakalářská práce má za cíl formou literární rešerše zmapovat látky s vlastnostmi podobnými jako siřičitany - tj. schopností inhibovat hnědnutí a nežádoucí mikroorganismy a působit jako antioxidanty; látky rostlinného původu bez zdravotních rizik neměnící organoleptické vlastnosti potravin, zároveň dostupných na trhu za cenu, za kterou by se vyplatilo testovat je pro případné použití v testech na potravních modelech. Výsledkem této práce je seznam šestnácti látek, které vykazují velmi podobné vlastnosti jako siřičitany a které by se díky těmto vlastnostem daly teoreticky použít pro další testování.

Klíčová slova: siřičitany, přísady, přírodní alternativy, oxid siřičitý, konzervanty

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1 PREFACE

Food is transported across the world and if we demand fresh and healthy-looking food we must ensure their durability. Durability is currently ensured by the preservatives and antioxidants which are an effective protect before a bacterial growth. They reduce spoilage, stop fresh food from spotting and turning brown and help preserve medication and increase shelf life. The appearance of products is probably one of the most deciding factors for costumers. It is an indicator of food quality for fresh-cut fruits or vegetables. The bright and intensive color has a positive impact on the overall impression and the attractive appearance of food. One of commonly using protective food additives are sulfites collectively known as sulfiting agents. They are very good preservatives but can cause serious allergic reactions. It is the main reason why producers should reduce or even replace these substances with the alternatives without potential risk. Safer alternatives to sulfites seem to be plant-derived preservatives.

2 AIM OF THE THESIS

Aim of this study is to identify the natural substances which may be potential safe and effective equivalents to sulfites, having similar antimicrobial and antioxidant activity and ability to inhibit the Maillard reaction products and tyrosinase as sulfites and at the same time their price and other features (toxicity, organoleptic properties) allow them to be used in foods.

3 INTRODUCTION

3.1 Characteristic of sulfites

Sulfites are synthetic or natural preservatives with very good properties in liquid or gaseous form. They are in a group labelled as E220 - E228. The most important and using food additive is sulfur dioxide, a colourless, poisonous gas with a suffocating odour. It is heavier than air but cylinders of compressed liquid can explode in the heat of a fire. SO_2 reacts explosively in contact with sodium hydride. It ignites when it is mixed with lithium-acetylene carbide diamino or lithium acetylide ammonia. It easily dissolves in water and the liquid is heavier than water. At room temperature is a non-flammable [1].

Table 1:Sulfur dioxide and its derivatives - sulfite inorganic salts [9].

Label	Full designation	Chemical formula
E220	sulfur dioxide	SO_2
E221	sodium sulfite	Na_2SO_3
E222	sodium bisulfite	$NaHSO_3$
E223	sodium metabisulfite	$Na_2S_2O_5$
E224	potassium metabisulfite	$K_2S_2O_5$
E225	potassium sulfite	K_2SO_3
E226	calcium sulfite	$CaSO_3$
E227	calcium bisulfite	$Ca(HSO_3)_2$
E228	potassium bisulfite	$KHSO_3$

According to current legislation, Federation of the Food and Drink Industries of the Czech Republic (2012) is permitted to use: sulfur dioxide - E 220, sodium sulfite - E 221, sodium bisulfite - E 222, sodium metabisulfite - E 223, potassium metabisulfite - E 224, calcium sulfite - E 226, calcium bisulfite - E 227 and potassium metabisulfite - E 228 [3].

3.2 Sources and forms of sulfites

Sulfur compounds can be found in many forms as like sulfur amino acids, sulfates, sulfides and sulfites. SO_2 is released primarily from the combustion of fossil fuels (75-85 %). It is present in the atmosphere because of emissions from industry and volcanic activity. Commercially SO_2 is prepared by heating sulfidic ores or in a genuine form by elemental sulfur burning. The cheapest of all sources of SO_2 and also effective method of disinfection is direct burning. [1]

Sulfites can be common in a gas form from cylinders or in aqueous solution, available as pressurized liquid, which can be prepared by bubbling SO_2 into water or as dissolved metabisulfite salts. Storage and transportation of sulfur dioxide demands special steel containers and it makes a source of SO_2 costly [16].

Sulfites are also naturally present in many beverages and food. Especially yeasts naturally produce some amount of sulfites during a fermentation of wines and beers [19]. In winemaking is commonly used liquid SO_2 which is free from impurities. Grapes and fresh-cut fruits are exposed to fumes of burning SO_2 before dehydration or transporting [16].

Sulfur compounds can be found also like a free or bound SO_2. The term "free SO_2" includes gaseous SO_2, H_2SO_3, HSO_3^- and SO_3^{2-}. The term "bound SO_2" includes sulfur dioxide bound to compounds such as aldehydes, ketones and sugars. The term "total SO_2" means the sum of bound and free SO_2 [27].

3.3 Uses of sulfites

Sulfur dioxide is mainly applied for production of dried fruits, jams, vegetables and products made from fish or sea-food but also for alcoholic beverages like wine or beer to protect their aroma [13]. They are also included in bakery and meat products [3].

Sulfites are used like an antimicrobial or antioxidant agents and have many another uses such as bleaching or reducing agents, pH control agent or stabilizing agent [19]. As a preservatives are especially effective in acidic foods [2]. Furthermore, sulfur dioxide and sulfites are used to inhibit the growth of acetic and milk bacteria and yeast in wine production and storage. Although the wine makers try to limit the use of sulfites

and sulfur dioxide to a minimum their complete elimination is not currently possible. Their versatile usability is hard to replace [3].

3.3.1 Antioxidant and Antimicrobial inhibition

Antioxidant properties

Sulfites have considerable antioxidant properties. Antioxidants are substances which prolong the shelf life of food and protect it against deterioration caused oxidation by atmospheric oxygen. Oxidation may occur in particular rancid fats or changes in colour foods. This category also includes substances labelled E300 - E321. Sulfites are synergistic substances in category of the antioxidants named an “oxygen traps” [2].

Antimicrobial/Antiseptic properties

Sulfites inhibit spoilage caused by the pathogenic microorganisms (bacteria, yeasts or moulds). It is generally known that the surfaces of untreated foods, fruits, vegetables, meat, etc. contain wide range of microorganisms which cause food spoilage, respectively whole range of undesirable changes. Most often it is a change in smell, colour, taste and shape. Under certain conditions, however, may produce hazardous substances for human health. Under suitable conditions for the growth of microorganisms can be considered: sufficient moisture foods, the availability of nutritional substances, suitable pH, a suitable environment, such as temperature, quantity of oxygen, osmotic pressure etc. [2].

3.3.2 Anti-enzymatic properties

Because of these properties, fruits, vegetables or non/alcoholic beverages have longer durability and preserved colour, stabilized aroma and flavour. Sulfites can also prevent the enzymatic tyrosinase oxidation leading to black spot formation. They can inhibit an enzymatic or non-enzymatic browning [13]. Durability of fresh-cut products is disrupted by physiological processes in the plant (maturation and aging), tissue damage (compression, cuts, tears) and pathogenic infections [9,26]. These processes cause the undesirable browning.

Enzymatic browning

Enzymatic browning occurs as a result of the main oxidative reaction involving two oxidoreductases enzymes named polyphenol oxidase and peroxidase.

Polyphenol oxidase is an enzyme naturally found in the plant vacuoles or in chloroplasts and in them bound to the thylakoid membrane. Polyphenol oxidase catalyses two different but successive oxidation reactions. It is the hydroxylation of monophenols to o-diphenols, relatively slow reaction, give colourless products [17] and the oxidation biphenyls to o-quinones, a rapid reaction, give coloured products [12,15,22]. Oxidation of present phenols to quinones results in gradual colour changes (brown pigments) resulting spontaneous polymerization formed quinones [8]. Peroxidase is an enzyme which influences oxidative deterioration. Also has an important function in enzymatic browning and could promote darkening in fruits or vegetables during processing and preservation [6].

These reactions of oxidoreductases generally induce losses or changes of door, flavour or nutritional value [23].

Non-enzymatic browning

Non-enzymatic browning is one of the most important reactions occurring during storage and food processing. It is the reaction reducing sugars (fructose, glucose, maltose, xylose) with amino compounds (amino acids and proteins) in food. During the reaction a series of highly reactive carbonyl compounds react with each other and also with amino compounds. A complex of these reactions is called as Maillard reaction and causes brown pigments (melanoidins) in food [25].

Also occurs to a formation of important organoleptic properties and because of these products have a characteristic colour, door and flavour. Reaction is typical for bakery products, especially bread, when the temperature changes during the cooking. It causes the characteristic brown colour, flavour and aroma of bread crust. In wines, Maillard reaction supports nutty, coffee, caramel or spicy tones of colour. It is more intensive with a higher temperature during maturation or a longer-term maturation of wines. Also due to the Maillard reaction beers are golden or dark. These properties are mostly assessed positively by the tasters.

On the other hand occurs to the formation of some compounds with mutagenic and carcinogenic properties. These mutagens don’t occur during a cooking in water.

These reactions are generally monitored because of toxic substances such as a nitrosamine [14].

3.4 Negative effects

3.4.1 Effects on asthmatics

Sulfites are listed in the Code of Federal Regulations (CFR) as GRAS (Generally Recognized As Safe). However, sulfites additives have some side effects and can cause extremely health problems for sensitive people specifically asthmatics. Sensitivity to sulfites can develop at any age. It poses a risk during inhalation or drinking beverages containing sulfites. These substances can cause allergic reactions such as a headache, skin problems - hives and itchiness, digestive problems - diarrhoea, gastric irritation, nausea, flushing, dizziness or respiratory problems - trouble swallowing, wheezing, trouble breathing, so called sulfite-asthma and in extreme situations anaphylactic shock which can results in death, because the person is not able to breath and experiences drop in blood pressure [5]. For very sensitive patients, an intake of less than 10 mg sulfite might be enough to provoke an asthma attack. The prevalence of sensitivity to sulfites in adult asthmatics was determined to be approximately 5 % [7].

3.4.2 Effects on sensitive people

Sulfur dioxide is acidic irritant gas and attacks olfactory receptors. The highest maximum incidence in the air should not exceed 350 $\mu g/m^3$ for 1 hour and within 24 hours 125 $\mu g/m^3$. Sulfur dioxide is toxic even at low concentrations. Sudden escape SO_2 from pressure cylinders can lead to blindness and death. Symptoms of acute intoxication are whooping cough, eye irritation, and chest pain. The symptoms of chronic poisoning include cracking a tooth enamel, also eye irritation and respiratory or olfactory disorders. Thus, asthmatics and individuals with respiratory problems should avoid sulfites [1].

Sulfites are potent inhibitors of certain enzymes, and thus may have different effects on different biochemical processes in the body. Some studies point to the inhibition of enzymes associated with the synthesis or activation of neurotransmitter active in the nucleus accumbens which can lead to tinnitus (ringing in the ears),

hyperacusis or other diseases associated with decreased dopaminergic or serotonergic activity [24]. Sulfites are also undesirable for children because of hyperactivity [18].

3.4.3 Effect on vitamins

Sulfites react with numerous vitamins and cause their loss (B1, B2, C, and K) or even can cause a destruction of these vitamins in a higher concentration. It is ensured that they are not used in meat and other foods recognized as a source of vitamin B_1 (thiamine) [21].

3.5 Dosage of sulfur dioxide

The quantity of sulfites is regulated by norm so it is needed to control their content in foods. Maximum permitted quantity of these substances in food is expressed in total sulfur dioxide regardless its origin and function in food [3]. Delivery of sulfur dioxide is usually expressed in anhydrous form, in units of mg/l (milligram per litter) or g/hl (gram per hectolitre). The sulfur dioxide in the wine is supplied in a gaseous form, as a solution SO_2 or in solid form such as sodium hydrogencarbonate, potassium sulfite and potassium metabisulfite. The effect of adding to the wine is the same irrespective of the form [28].

The legislated maximum concentration of sulfur dioxide allowed in wines has been gradually reduced or even has been eliminated and replaced by other effective preservatives. According to Life Sciences Research Office (1985) the total intake of sulfur dioxide is about 10 mg/day for 1 person [19].

The most common method for determination of sulfites in wine is iodometric titration which can determines both - free and bound sulfites. To determine the sulfites are also used faster instrumental methods, e.g. the molecular/atomic spectrometry, electroanalytical methods - e.g. coulometry, amperometry or separation, e.g. capillary electrophoresis or isotachophoresis chip [3].

Sulfites in the combination with alcohol can increase the symptoms of a nausea and headache. The International Organization of Vine and Wine (OIV) has been reducing the maximum concentration allowed in wines, which is nowadays 150 mg/l for red wines and 200 mg/l for white wines [8]. Different limits apply to special wines and wines with high residual sugar. For example in the wine-growing countries outside the

European Union; in the States of America, Canada, Japan and Australia are the same limits for all wines 350 mg/l of total sulfur dioxide.

The pathogenesis of reactions to sulfites are considered the three mechanisms. The most important mechanism appears to be the inhalation of sulfur dioxide released from sulfites. It is believed that sulfur dioxide causes bronchial spasm via cholinergic reflex mechanism. At doses of 100 to 200 mg of sulfites in capsule or 10 to 100 mg of sulfites/ml solution usually for each asthmatic appears irritation within the bronchial hyper-reactivity that is common among asthmatics. Asthmatics are allergic to sulfites react to much smaller doses. Response threshold is individual from 0.6 mg SO_2 (equivalent to 1 mg $K_2S_2O_5$) to more than 100 mg (corresponding to 200 mg $K_2S_2O_5$). The second mechanism is the reaction mediated by IgE antibodies which are responsible for the anaphylactic reactions after administration of very low doses. Finally, it was found asthmatics sensitive to sulfites have reduced an activity of sulfite oxidase in dermal fibroblasts. Further it was found that the probability of reaction is dependent on the ratio between free and bound sulfites. Some foods bind to its constituents sulfites tighter and therefore is less likely to react as opposed to foods that have more free sulfites. The probability of reaction can be higher there where are sulfites in a fluid with a low pH. Both promote the release of SO_2 [7].

Acute toxicity is set at 1500 mg/kg; this value is approximately similar to the value of common salt and sodium bicarbonate, which is commonly used in the food industry. Chronic toxicity is set at 0.35 mg/kg. The lethal dose of sulfites for 50 % of the population is in the range of 0.7 to 2.5 g per 1 kg of body weight [28].

3.5.1 Permitted dosage of sulfur dioxide

There are dozens of preservatives with antimicrobial effects, however, are not always harmless. On the basis of expert knowledge was processed a list of preservatives which comply with the health requirement in relation to the maximum authorized quantity of food; the maximum level (ML) [2].

Table 2: Terms of use of sulfur dioxide and compounds which may be used in the manufacture of foods or food groups [2].

Food or food group	ML SO_2 $mg \cdot l^{-1}$ /$mg \cdot kg^{-1}$
crustacean and cephalopods fresh and (deep) frozen (in the edible part)	150
crustacean from family *panaeidae solenceride, aristeidae* (the edible part):	
- to 80 units	150
- 80 - 120 units	300
- over 120 units	300
crustacean and cephalopods cooked from family *panaeidae solenceride, aristeidae* (the edible part)	
- to 80 units	135
- 80 - 120 units	180
- over 120 units	270
dried salted cod (*Gadidae*)	200
hamburger meat with a minimal contain of vegetable or cereals 4 %	450
analogues of meat, fish and crustaceans based on protein	200
biscuits, crackers	50
nonextruded snacks based on potatoes and cereals	50
puffs	30
starches (except starches in infant formulas, follow-on formulas and processed cereal-based foods and baby foods)	50
sago	30
dehydrated potatoes	400
peeled (raw) potatoes	50
raw potato products (including frozen potatoes)	100
potato dough	100
dried white vegetables	400
ground horseradish and products thereof	800
vegetables and fruits in vinegar, oil or brine (except olives)	100
processed white vegetables (including frozen)	50
dried ginger	150
dried tomatoes	200
onions, garlic and shallots (processed)	300
yellow peppers in salt brine	500
vacuum packed sweetcorn	100
processed mushrooms (including frozen)	50
dried mushrooms	100
dried apricots, peaches, grape berries, plums, figs	2000
dried bananas	1000
dried apples and pears	600
other dried fruits, nuts in shell	500
fresh lychees (the edible part)	10
jams, jellies, marmalades (except extra jam and extra jelly) and similar processed fruit products including energy-reduced	50
jams, jellies, marmalades made from sulfited fruit	100
candied, crystallized or glacé fruit and vegetables or candied dried fruit and peel of angelica or citrus according to the decree. no. 157/2003 Coll.	100
fruit pie fillings	800
gelling fruit extract	100
liquid pectin for sale to the final consumer	250
canned cherries, rehydrated dried fruit and lychees	50
pickled lemon slices packaged	50
desiccated coconut	200

Food or food group	ML SO_2 $mg \cdot l^{-1}$ /$mg \cdot kg^{-1}$
pickled walnuts	10
condiments based citrus juice	40
carbohydrates	20
glucose	50
other sugars	40
starch syrup	50
confections based on glucose and glucose or starch syrup	350
sweet dessert sauce (topping)	250
orange, grapefruit, apple and pineapple juice for catering	50
lemon juice, lime juice	350
other concentrates based on fruit juice	250
non-alcoholic wine	200
table wine	10
cider, fruit wine and sparkling fruit wine	200
mead	200
wine and cider vinegar	170

Food or food group	ML SO_2 $mg \cdot l^{-1}$ /$mg \cdot kg^{-1}$
concentrated grape juice for home wine-making	2000
molasses, syrup to salads (liquid sugar)	70
concentrates based on fruit juice containing less than 2.5 % barely (barely water)	350
non-fermented grape juice for sacramental purposes	70
distilled alcoholic beverages containing whole pears	50
alcohol and non-alcoholic beer	20
yeast beer from barrel	50
French mustard (Dijon)	500
other mustard	250
gelatine	50
breakfast sausages	450
Longaniza and Butifarra fresca	450
Mostarda di frutta	100
made wine	260
salsicha fresca	450

3.6 Methods for reducing concentration of sulfites

Food spoilage caused by improper growth of microorganisms that have resulted change organoleptic properties of food or even that the food can become physically harmful. People are trying to avoid these undesirable reactions since time immemorial. They use a variety of prevention which we generally call the preservation of food. One of these methods is the use of preservatives [2].

Due to the side effects dangerous to health are used alternative methods which can reduce concentration of sulfites in food. These methods are technological, physical or chemical.

3.6.1 Technological methods

Malo-lactic fermentation is a biological process which leads to conversion of malic acid (malate) to lactic acid (lactate) and carbon dioxide. Malic acid is a bitter tasting dicarboxylic acid which occurs in many bitter or sour food. It is naturally found in apples and wine grapes. During this process frequently occur to degrade citric acid Malo-lactic fermentation has many functions in wine production. It includes reducing acidity, bacterial stability of the final product, influencing flavour of wine and reducing the consumption of sulfur dioxide. Reduction of sulfur dioxide consists primarily in a bacterial stabilization of wine and the abolition of binding sites, where the free SO_2 could bind. However, there are too few studies and is necessary further research [28].

Sur-lie technology was founded in a French region Burgundy and the meaning is: "on the yeast lees". After alcoholic fermentation and gross sludging wine is left on the fine yeast lees and it gradually leads to the yeast autolysis – dissolution of the cell wall - and thus the transition of various substances contained within the yeast in wine. Saving sulfites consist in the power of reducing yeasts. Wines of sur-lie types are characterized by longer shelf-life [30].

Tangential filtration also called cross-flow filtration. Tangential filters are able to filter wine that the filtrate is sterile without the presence of yeast and bacteria. This method can be used for termination of alcoholic fermentation and microbial stabilization before bottling [29].

3.6.2 Physical methods

Pasteurization is a widely used method of food preservation which is based on a heat treatment. Pasteurization means a liquidation of pathogenic organisms and ensures a harmless product. Pasteurization can be practiced at an elevated temperature for a short period or at a low temperature applied for a longer time. This method is most useful in the pasteurization of wines with an average quality which have a problem with stabilization [32].

The wine yeasts are more sensitive to heat, so warming up to 45 or 48 ° C sterilizes the wine and the bottle [28]. However, this method is not used much in oenology because it has an impact on a sensory characteristic of wine.

Ultraviolet radiation is an electromagnetic radiation which is tested to reduce the concentration of SO_2. It is a process with a short wavelength shorter than the visible spectrum which is below 400 nm [32]. This method has a similar inhibitory activity against pathogenic microorganisms as SO_2 without undue influence of the sensory characteristics of wine and other quality parameters (pH, tartaric acid, alcohol content). Microbial inhibition caused by ultraviolet radiation is based on the rearrangement of nucleic acids which directly affects the ability to reproduce of microorganisms. The final efficacy depends on the appearance and characteristics of the product (colour, absorbance, density, dissolved and suspended solids) which can prevent the penetration of ultraviolet radiation to microorganisms [32]. Ultraviolet radiation has not any negative effect to wine from the organoleptic standpoint but very limiting factor in use of this technology are the financial costs [33].

Ultrasound is an acoustic wave with a frequency higher than the human audible threshold which corresponds to approximately 20 kHz [32]. It is known that ultrasound has a lethal effect on the yeast cells, but not their spores [33]. The positive effects of ultrasound are tested for treating the wine and reducing the addition of sulfur dioxide. The killing of microorganisms is mainly due to thinning of cell membranes, a local temperature increase and the formation of free radicals [34].

Pulsed electric field (PEF) is a technology that represents a quick, without-heating, a highly effective technique for inhibiting pathogenic organisms in foods and the organoleptic properties are not changed [32].

The method is based on the action of electric field pulses in the order of microseconds about high intensity in the values of 15 to 50 kV/cm. The intensive electric field generates the changes of potential on cell membranes which are irreversible and lead to collapse the membrane structure [32]. The technology can be used in the winery as an alternative method to kill up even to 99.9 % microflora which causes devaluation musts and wines. For white grapes was even found a significant

increasing their pomace (about 20 to 50 %). The method is more effective when is used before pressing than in during the process. Furthermore musts treated PEF technologies have proven to be less susceptible to browning. Positive effects were also found in the production of red wines where the PEF has contributed to a better extraction of polyphenols and anthocyanins from the skin of grapes [32].

High hydrostatic pressure (HHP) has a huge potential in the context of reducing the dose SO_2. This method is new for antimicrobial stabilization of wine and beer. It was found that HPP destructs the pathogenic microorganisms, and also improves certain organoleptic characteristics of the beer or wine, without adversely influence of the important quality parameters (colour, pH and turbidity) [32]. In this method is used a pressure in the range from 400 to 600 MPa. These pressure values have proved a high effectivity in the destruction of microorganisms present in wine [35]. Use of HHP method for two minutes with a pressure of 400 MPa and an initial temperature about 20°C is able to kill all microorganisms in the wine. It was demonstrated that inhibition of microorganisms increases with increasing pressure and the length of its effect [32].

Light pulses

The antimicrobial properties of light pulses are studied in food such as honey and milk, however, could be prospective for the treatment of wine. The technology of light pulses is based on action of electromagnetic waves in a wavelength range from ultraviolet an infrared spectrum in a few pulses per second. Each pulse acts for a very short period of time in the range of 100 nanoseconds to 2 milliseconds. A disadvantage of light pulses is that depend on the optical properties of the medium because the transport of electromagnetic waves to the treatment liquid takes place only near the surface [32].

Ohmic heating

Use of ohmic heating for the purpose of microbial stabilization of liquid foods is the subject of research. It could be useful in oenology. This thermal method is based on the electrical resistance of the liquid attached to the electrodes. Usually is applied electric field intensity exceeding 1 kV/cm. Ohmic heating appears to be an effective and quick method without heat shock, ensuring pasteurization, fading and dehydration. The primary effect is to increasing the temperature of the liquid, the second is the direct

effect of the electric field [32]. Effects on the organoleptic changes are questionable and further studies are needed.

3.6.3 Chemical methods

Ascorbic acid (also called a vitamin C) is the best-known preservative to sulfites. (Smith, 2008) It is present in the wine grapes in a small amount of about 50 mg/l must. However, content of the ascorbic acid rapidly decreases during the fermentation and therefore it is not in the wine basically. In the oenology ascorbic acid is used as a reducing agent, an antioxidant. Its maximum concentration is 150 mg/l but the recommended dosage is between 50 mg/l and 100 mg/l. The higher concentrations could influence the taste of wine. Ascorbic acid is widely used in combination with sulfur dioxide [28]. Sulfur dioxide and ascorbic acid have different antioxidant properties. SO_2 is an antioxidant with a weak effect which persists even in the presence of an oxygenation. SO_2 can prevent ferric turbidity which occurs rapidly after aeration. The reaction of oxygen with ascorbic acid is almost 1700x faster than the reaction of oxygen with SO_2. Therefore ascorbic acid has immediate effect which can eliminate the damage caused by sudden and intense aeration but it works only as long as the wine is in permanent contact with the air [32].

Colloidal Silver Complex (CSC) or colloidal silver complex is used for its antimicrobial properties. It has been found that colloidal silver is antimicrobial to a wide range of Gram negative and Gram positive bacteria. CSC also has some antifungal and anti-virus-inflammatory properties which are now also used in medicine [32]. It has been shown that the application of 1 g per 1 kg CSC grapes acts as an effective antiseptic which is able to control the evolution of acetic and lactic acid bacteria while the growth of Saccharomyces cerevisiae is not disturbed. Using of CSC occurs also to changes the substances. Wines have a lower content of alcohol and acetaldehyde compared with wines which are treated only with SO_2. The results indicate that the presence of CSC modifies the metabolism of yeast which leads to reduced production of ethanol compared to wines treated with SO_2. Wines treated with CSC additionally showed not many changes in the chemical and sensory characteristics except for a susceptibility to oxidation of white wines. CSC therefore appears as a promising

antiseptic for the wine industry. But the disadvantage is the lack of antioxidant activity. This shortcoming could be offset by a combination with another antioxidant for example ascorbic acid [32].

Dimethyl Dicarbonate (DMDC) is a food additive. The European Commission in 1995 authorized the use of DMDC as preservatives non-alcoholic flavoured drinks, alcohol-free wine and liquid tea concentrates at a maximum concentration of 250 mg/l [7]. DMDC was very effective against yeasts and fungi and at doses of hundreds of milligrams per litter during bottling. But during storage its effectiveness decreased [28]. The application of this oenological product can reduce the required dose of SO_2, but the low sulfidation dose is needed in terms of protection against oxidation. DMDC decomposes to methanol and carbon dioxide. That toxic methanol is in dispute whether it is possible to use the additive. DMDC is currently permitted in Europe with the maximum dose of 200 mg/l in the bottling of wines with higher residual sugar than 5 g/l as is apparent from the European Commission Decree 2006. DMDC is a strong antimicrobial agent whose maximum inhibitory effect occurs soon after its addition. The advantage of the preparation can be a low dependence on the effectiveness of pH. Very effective use of DMDC has been shown to treat botrytised wines because there was a rapid reduce adding of SO_2 during storage. However, DMDC effect is only temporary and bacteria are more resistant than yeast [31].

Fatty acids

Some higher fatty acids, with the number of carbons in the chain 16 and 18, are important for activation of fermentation, while acid containing 8 to 10 carbons in the chain have fungicidal activity. Studies have shown a positive effect on the stabilization of sweet wines. And the applied dose should not exceed 10 mg/l [28]. It has been found that the combination of 150 mg SO_2 and 9 mg of fatty acids per litter has the same effects as 250 mg of sulfur dioxide. This method allows the preservation sweet wines at only 40 mg/l of free SO_2. But due to the intense aroma of these acids and their esters was observed a slight influence on the change of flavour of the treated wines [28]. Sensory reliably detectable dose of fatty acids, however, pointed to the application of 30 mg/l [32]. Fungicidal mechanism is not very known. Studies have shown that octanoic acid and decanoic acid are toxic to yeast, especially at low pH. Further it was found that

the toxicity is increased by the application of the two acids than in the treatment by various acids. The treatment of wine may also be used by combinations six-, eight- and ten-carbon acids in dose of 3 mg/l [33].

Inert gases are recommended in storing wine as a protection against the adverse effects of chemical and microbial oxidation. The oxidation can be limited by the number of ways. One of the options is storing wine in tanks. This storage method is inappropriate if the wine is continuously taken away. Another option is to use tanks with so-called floating lid which reduces the contact surface with the air, but their efficiency is questionable. Satisfactory results provide storage of wine in partially filled tanks under inert gas atmosphere without contact with oxygen. Permissible inert gases are nitrogen, carbon dioxide and argon. Argon gas is seldom used because it is less soluble than the other (4 l/hl) and is more expensive. Carbon dioxide is very soluble in wine (107.2 l/hl), and therefore cannot be used alone. Sometimes is used in a mixture with nitrogen. Nitrogen is the most common inert gas. It is used with a low content of oxygen which has no influence on wine. Nitrogen is less soluble than oxygen in the wine but unlike the oxygen does not react with the components of the wine. In the method of storing under an inert atmosphere is important airtightness of tanks and overpressure of inert gas. It is checked by pressure gauge because of possible leaks. Storage tanks are also equipped with a pressure relief valve which eliminates the potential consequences of excessive pressurization [28].

Lysozyme is a crystalline protein substance that occurs naturally in the saliva, egg albumen and breast milk. The lysozyme is for example used in the food industry for the preservation of hard cheeses. Lysozyme is an enzyme capable of destruction Gram positive bacteria such as lactic acid bacteria. Treatment with lysozyme leads to lysis of the cell walls and subsequently death of the cell. But it was found also to have antimicrobial properties against Gram-negative bacteria such as acetic bacteria [28] or against yeasts [33]. One of the uses, is in the wine making, where is an assumption of slower fermentation. Lysozyme in this regard prevents eventual dominance of lactic bacteria or yeast and thus reduces the risk of premature termination of alcoholic fermentation. Undesirable growth of bacteria can be controlled by 200 to 300 mg/l lysozyme. Delivery of lysozyme, however, is not possible to ensure protection against

re-fermentation wines with a high residual sugar or antibacterial stability against acetic bacteria that cause volatile acidity [28].

Natamycin also known as Pimaricin is a substance with fungicidal properties permit to preservation of hard cheeses. Its activity consists in changing the cell membrane permeability which could stop the alcoholic fermentation and begin control the antimicrobial stability of the wine. As an antibiotic is allowed locally in South African wines but cannot be exported. In Australia, the US or the European Union this substance is not approved as an oenological additive [33].

Nissin is a substance with antimicrobial effects. Nissin was first isolated from the bacterium Streptococcus lactis. Its antimicrobial properties particularly against Gram positive bacteria are higher at lower pH [33]. Some strains of bacteria have a higher resistance so the effects continue to be scrutinized [31].

Sorbic acid is a white crystalline substance soluble in ethanol and has low water solubility. For this reason, in oenology is used as the sodium and potassium salts which are soluble. Fungicidal properties have been studied in many experiments. In laboratory experiments was shown that a dose of 150 mg/l sorbic acid at pH 3.1 the wine was sufficient to suppress secondary fermenting whereas at pH 3.5 it was necessary to apply 300 mg/l. Besides the effect on normal fermentative yeasts was observed fungicidal effect on the wild strain of Candida organisms occurring in the surface of the wine [28].

Sorbic acid has a low antibacterial effect against acetic and lactic bacteria. In case of increase growth of lactic acid bacteria decomposition of the sorbic acid can occur resulting in a defect wine - smell of geranium [31]. The dose required to inhibit the development of bacteria would be ranged between 500 to 1000 mg/l. The described properties show selectivity against microorganisms contained in the wine which unlike from sulfur dioxide suppress growth of yeasts and not bacteria. In this context the sorbic acid have to be unconditionally used in the combination with sulfur dioxide. But its addition can be reduced high concentrations of SO_2. Suitable application is in case of preservation of wines with higher residual sugar and low pH as a protection against re-fermentation [28].

Currently, the list of additives recorded over 2500 compounds. In food production is used about 350 substances [2].

Table 3: Table of authorized food additives in the Czech Republic (updated November 2012) in accordance with Commission Regulation (EU) no. 231/2012 [2].

Label	Name of compound	Use
E 200	Acid sorbic	Preservative
E 202	Potassium sorbate	Preservative
E 203	Calcium sorbate	Preservative
E 210	Benzoic acid	Preservative
E 211	Sodium benzoate	Preservative
E 212	Potassium benzoate	Preservative
E 213	Calcium benzoate	Preservative
E 214	Ethyl- *p*-hydroxybenzoane	Preservative
E 215	Ethyl- *p*-hydroxybenzoane sodium	Preservative
E 218	Methyl- *p*-hydroxybenzoane	Preservative
E 219	Methyl- *p*-hydroxybenzoane sodium	Preservative
E 220	Sulfur dioxide	Preservative, antioxidant
E 221	Sodium sulfite	Preservative, antioxidant
E 222	Sodium bisulfite	Preservative, antioxidant
E 223	Sodium metabisulfite	Preservative antioxidant
E 224	Potassium metabisulfite	Preservative, antioxidant
E 226	Calcium sulfite	Preservative, antioxidant
E 227	Calcium hydrogen sulfite	Preservative, antioxidant
E 228	Potassium hydrogen sulfite	Preservative, antioxidant
E 234	Nisin	Preservative
E 235	Natamycin	Preservative
E 239	Hexamethylentetramine	Preservative
E 242	Dimethyl dicarbonate	Preservative
E 249	Potassium nitrite	Preservative, stabilizer
E 250	Sodium nitrite	Preservative, stabilizer
E 251	Sodium nitrate	Preservative, stabilizer
E 252	Potassium nitrate	Preservative, stabilizer
E 260	Acetic acid	Preservative, acidity regulator
E 261	Potassium acetate	Preservative, acidity regulator
E 262	Sodium acetate	Preservative, acidity regulator, sequestrant
E 263	Calcium acetate	Preservative, modified starch, thickener, acidity regulator, stabilizer

Label	Name of compound	Use
E 270	Lactic acid	Preservative
E 280	Propionic acid	Preservative
E 281	Sodium propionate	Preservative
E 282	Calcium propionate	Preservative
E 283	Potassium propionate	Preservative
E 284	Boric acid	Preservative
E 285	Sodium tetraborate (borax)	Preservative
E 290	Carbon dioxide	Packing gas, preservative, carrier, solvent
E 296	Malic acid	Preservative, acidity regulator
E 297	Fumaric acid	Preservative, acidity regulator
E 385	Disodium calcium salt diamintetraacetate	Antioxidant, preservative, sequestrant
E 1105	Lysozyme	Preservative

3.7 Natural alternatives to sulfites

The use of additives in food is not any breakthrough technology. The natural additives have been commonly used for centuries without evidence of any effects on human health so therefore can be considered as candidates for human health completely harmless or safe. For example Vitamin C, lactic acid and the like. Some of them act directly as the source of vitamins or minerals. Additives, which are essential for the present used technological methods of the food production, are a part of the commonly available foods. These substances on the one hand enhance their attractiveness, taste, texture, etc. but on the other hand one of these substances are problematic. It is need to be remind and emphasized that modern food technology cannot work without certain additives. If the additives fell into disuse it would mean produce unsaleable foods. The result would be reduced shelf-life, freshness, appearance, taste and in many cases also digestibility. A return to the old technologies is almost impossible in relation to the availability of necessary raw materials [2].

One of the problematic substances may be preservatives, specifically sulfites. As a result of negative effects of sulfiting agents, there has been a considerable focus on identifying suitable alternatives to use in foods. In nature, there are many materials having components with antimicrobial effects. These materials are therefore for their self-preservation capabilities used in the food industry. Many other substances are produced synthetically and in foods is added intentionally [2, 4]. It should be noted that for a food production can be used only those additives which undergone a thorough toxicological tests and showed no health problems. The Czech Republic belongs to the countries, where are the strictest conditions for use of additives [2].

A few of currently used natural alternatives to sulfites are described in the chapter “Methods for reducing concentration of sulfites” above. The natural alternatives to the sulfite agents are not easy to identify. Possible alternatives usually provide a narrower range of benefits, are often less effective and are almost always more expensive.

4 RESULTS AND DISCUSSION

In this study were described 82 natural substances as the possible alternatives to sulfites (Tab. 4). These substances were divided to three tables by different properties with an antioxidant and antimicrobial properties (Tab. 5), with ability to inhibit the tyrosinase enzyme (Tab. 6) or products of Maillard reaction (Tab. 7) as described by Čamková in 2013 [4].

For each compound were determined toxicity, price, extraction method or availability, appearance or organoleptic properties, common uses and state of matter or solubility. The degree of toxicity was described as a non-toxic, low or high. The price of compounds was calculated to currency 1$ for 1 gram in the time period from January to March 2016. N/A means that the information was not available. Especially toxicity is needed for further studies. The results are summarized in the Tables 4-7.

Table 4: Sulfiting agents

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Sulfur dioxide	GRAS	[100]	0,28	[65] 3.1.2016	volcanoes, industrial processes, acid rain, atmosphere, coal and petroleum	[38]	colourless gas or white liquid with suffocating door	[38]	preservative, reducing, purifying, oxidizing, a germicide and bleaching agent, pH controller, refrigerant, heat transfer fluid and selective solvent, in winemaking	[38]	liquid/gaseous, easily soluble in water	[38]
Sodium sulfite	GRAS	[100]	0,14	[65] 3.1.2016	salt of sulfurous acid	[38]	white or yellow odourless crystalline powder with salty taste	[38]	antimicrobial, antioxidant preservative, dechlorinating, bleaching, stabilizer, developer agent	[38]	solid, soluble in water and glycerol	[38]
Sodium bisulfite	GRAS	[100]	4,6	[65] 3.1.2016	combination of sodium, hydrogen, sulfur and oxygen ions	[38]	white or yellow crystalline powder with sulfurous door	[38]	preservative, antiseptic, antioxidant, bleaching and reducing agent	[38]	solid, easily soluble in water, slightly soluble in ethanol	[38]
Sodium metabisulfite (SMBS)	GRAS	[100]	0,06	[65] 3.1.2016	Campden tablets	[38]	white or yellow crystalline powder with sulfurous door	[38]	disinfectant, reducing, antiseptic, freshen and bleaching agent	[38]	solid, easily soluble in water (with acidic aqueous solution) and glycerine, slightly soluble in ethanol	[38]
Potassium metabisulfite	GRAS	[100]	0,16	[65] 3.1.2016	Campden tablets	[38]	white or slightly yellow crystalline powder with sulfurous door	[38]	preservative, antioxidant, antibacterial, antiseptic, bleaching, dechlorinating agent	[38]	solid, soluble in water with acidic aqueous solution, insoluble in ethanol	[38]
Potassium sulfite	forbidden in CZ	[3]	0,09	[65] 3.1.2016	by-product of coal combustion	[101]	white crystal powder, odourless, tasteless	[38]	preservative, antioxidant, bleaching agent	[38]	liquid/solid, soluble in water, slightly soluble in alcohol	[38]

Table 4: Sulfiting agents (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Calcium sulfite	permitted only in a limited range of food in CZ	[3]	N/A	-	by-product of coal combustion	[101]	white crystal powder, odourless, tasteless	[38]	preservative, antibacterial	[101]	liquid/solid	[101]
Calcium bisulfite	N/A	-	N/A	-	by-product of coal combustion	[101]	yellow liquid with a strong sulfur dioxide door	[2]	preservative, in making wood pulp and as a disinfectant and antichlor	[2]	liquid/solid	[101]
Potassium bisulfite	N/A	-	N/A	-	by-product of coal combustion	[101]	white crystalline powder	[38]	preservative, disintegrating agent, for preparation of potassium salt	[38]	gas/solid, soluble in water, insoluble in ethanol	[38]

Table 5: Antioxidant and Antimicrobial inhibition

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Amento-flavone	N/A	-	5	[38], 4.1. 2016	*Selaginella tamariscina*, *Cnestis ferruginea*	[36, 37]	brown-yellow color, characteristic door	[38]	antifungal agents; antioxidant	[36, 37]	solid	[38]
Baicalein	N/A	-	5	[38], 4.1. 2016	*Scutellaria baicalensis*, *Scutellaria rivularis*, *S.galericulata* , white wine	[38,39, 41,42]	light yellow color	[38]	inhibit the growth of cancer cells, inhibit fibrillation, antioxidant	[40]	solid, soluble in water	[43]
Butein	N/A	-	5,5	[38], 4.1. 2016	*Dalbergia odorifera*	[44]	white or yellow crystal powder	[38]	antioxidant	[44]	solid, soluble in water and ethanol	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Caffeic acid	N/A	-	0,25	[38], 4.1. 2016	wine	[45]	Yellow crystal powder, characteristic odour	[38]	antioxidant in vitro/in vivo, inhibit carcinogenesis	[38, 45]	solid, soluble in water	[38]
Catechin	N/A	-	0,15	[38], 4.1. 2016	*Camellia sinensis*, green tea, grape seeds	[38, 46]	yellow or brown powder with tea perfume odour	[38]	antioxidant	[46]	solid, soluble in water and ethanol	[38]
Cinnamic acid	low	[48]	0,05	[38], 4.1. 2016	*Cinnamomum cassia*	[38]	white-yellow or brownish red powder, characteristic odour	[38]	antibacterial, antiviral and antifungal properties	[48]	solid; with methyl, ethyl, and benzyl esters for the perfume industry	[38]
Daidzein	N/A	-	0,15	[38], 4.1. 2016	*Glycine max*, soybean isoflavone	[49, 50]	white-yellow-brown powder	[38]	anti-carcinogenic, antiviral and antioxidant properties	[49]	solid	[38]
Dihydro-5,6-dehydroka-wain (DDK)	N/A	-	N/A	-	*Alpinia zerumbet*, *Alpinia speciosa*, by chloroform, kava-kava	[51, 52]	white crystalline compound	[52]	inhibition to β-carotene oxidation, antifungal and antiseptic activity, neuroprotective	[51, 52]	solid, soluble in methanol	[47]
Epicatechin (EC)	low	[54]	1	[38], 4.1. 2016	Dark chocolate, cocoa beans, *Camellia sinensis* - green and black tea	[53]	off-white to pale pink powder, characteristic odour	[38]	antiviral, antimalarial and anti-carcinogenic properties, stronger antioxidant activity than vitamin C and E	[38, 53, 54]	liquid/solid; soluble in water, ethanol, methanol and acetone. A little impact on the taste of end product	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Epigallocate-chin-3-Gallate (EGCG)	N/A	-	0,25	[38,56], 8.1. 2016	*Camellia sinensis* - green tea	[55]	brown yellow powder, characteristic odour	[38]	strong antioxidant and anti-carcinogenic activity	[55]	liquid/ solid, soluble in water or ethanol	[38]
Ellagic acid	low	[59]	0,2	[38], 8.1. 2016	Fruits (berries, pomegranate) and nuts	[57]	reddish brown powder, characteristic odour	[38]	antioxidant, anti-fibrosis, anti-carcinogenic and whitening activity	[58]	solid, soluble in ethanol, acetone/water or methanol	[38,57]
Esculetin	N/A	-	0,5	[38], 8.1. 2016	horse chestnut tree (*Aesculus chinensis*), *Euphorbia lathyris*	[38, 60]	white or yellowish crystalline powder, characteristic odour	[38]	antibiotic and antimicrobial activity similar to vitamin P, treating dermal hyperpigmentation	[38, 60]	solid, fluorescent dye, slightly soluble in water	[38]
Esculin	low	[62]	1	[38], 8.1. 2016	horse chestnut tree (*Aesculus Hippocastanum*), *Fraxinus sieboldiana blume*	[38, 61]	white or yellow-brown crystalline powder, characteristic odour and bitter taste	[38]	antioxidant and anti-carcinogenic properties, for a treatment of neurodegenerative diseases	[61]	solid, fluorescent dye, soluble in ethanol and water	[38]
Ferulic acid	low	[63]	0,028	[65] 12.3. 2016	a complex of cereals, Chinese medicine herbs	[63, 64]	white/yellow crystalline powder, characteristic odour and taste	[38]	antioxidant, antimicrobial, anti-cancer activities, for production of vanillin, edible films	[63]	solid, soluble in hot water, ethanol and ethyl acetate, slightly soluble in ether	[38]
Gallic acid	low	[66]	0,068	[65] 12.3. 2016	*Rheum palmatum*, Chinese gallnut, sumac tree, hazel tree, tea leaves, oak bark	[38]	White/yellow or light brown needle crystal powder, characteristic odour and taste	[38]	Anti-inflammatory, anti-fungal, anti-viral, anti-cancer properties, antioxidant	[38, 67]	solid, soluble in hot water, ethyl ether, ethanol, acetone and glycerine, hardly soluble in cold water	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Genistein	low	[68]	0,047	[65] 12.3. 2016	soybeans, pea pods, and other legumes, *Flemingia vestita* and coffee	[38]	white/light yellow powder, characteristic odour and taste	[38]	carcinogenic, antioxidant	[38]	solid, soluble in dimethylsulfoxide, ethanol and the usual organic solvents, in dilalkalies with yellow color	[38]
Hesperetin	non-toxic	[70]	0,2	[38] 12.3. 2016	citrus fruits, *Citrus Aurantium, Citrus sinensis*	[38, 69]	yellow/brown powder, characteristic odour, high intense sweet taste	[38]	Anti-virus, anti-inflammatory, anti-allergic properties, antioxidant	[38, 69]	solid, good solubility in water and ether	[38]
Hesperidin	non-toxic	[70]	0,044	[65] 12.3. 2016	citrus fruits, *Citrus Aurantium, Citrus reticulata, Citrus sinensis*	[38]	yellow/light powder, characteristic odour and taste	[38]	antioxidant, anti-inflammatory, anti-carcinogenic and cholesterol lowering actions	[38]	solid, soluble in ethanol and water	[38]
Chlorogenic acid	low	[38]	0,054	[65] 12.3. 2016	Green coffee bean, *Coffea Arabica, Eucommia ulmoides* Oliver	[38]	Brown to White powder, characteristic odour and taste	[38]	anti-virus, anti-bacteria, anti-ages, reducing blood pressure effects	[38]	solid, good solubility in water and ethanol	[38]
Isovitexin	low	[71]	0,268	[65] 12.3. 2016	Green bean, *Ficus microcarpa, Oryza Sativa*	[38, 71]	yellow powder	[38]	pharmacology experiments	[38]	liquid/solid	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Kaempferol	low	[72]	0,117	[65] 13.3. 2016	Fruit of *Sophora japonica*, tea, broccoli, *Delphinium*, Witch-hazel, Grapefruit, **B**russels sprouts, Kaempferia galanga	[38]	Light yellow-green-brown powder, characteristic odour and taste	[38]	anti-cancer, inhibiting growth, epilepsy, anti-inflammatory, strong antioxidant, treating cough and bronchitis	[38]	solid, soluble in water and ethanol, soluble excellent in alcoholic drink	[38]
Kurarinone	N/A	-	0,1	[38] 13.3. 2016	*Sophora angustifolia* and *Sophora flavescens*	[38]	White crystalline powder, characteristic odour and taste	[38]	anti-tumour, a diuretic, anti-allergic, anti-bacterial, anti-pathogens, immune function	[38]	solid, soluble in water and ethanol	[38]
Kuwanon G	N/A	-	0,05	[38] 13.3. 2016	mulberry tree, *Morus alba*	[73]	white/brown powder, bitter-sweet taste	[38]	antibacterial, antioxidant properties; cosmetics, pharmaceutical industry	[38, 73]	liquid/solid, soluble in hot water	[38]
Luteolin	low	[74]	0,187	[65] 13.3. 2016	Legumes peanut shell, *Arachis hypogaea*	[38]	yellow green/reddish brown powder, characteristic odour and taste	[38]	antioxidant, anti-inflammatory properties	[38]	solid, soluble in water and ethanol	[38]
Morin hydrate	N/A	-	0,027	[65] 13.3. 2016	mulberry bark, Osage orange	[38]	yellow-white crystalline powder, characteristic odour and taste	[38]	antioxidant, anti-inflammatory, anti-cancer properties	[38]	solid, soluble in water and ethanol	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Myricetin	N/A	-	0,1	[38] 13.3. 2016	*Myrica rubra*, *Ampelopsis grossedentata*	[38]	brown-yellow-white-green powder, characteristic odour and taste	[38]	anti-aging, anti-inflammatory, anti-tumour, anti-mutation, prevention of dental caries	[38]	solid, soluble in water and ethanol	[38]
Naringenin	N/A	-	0,03	[65] 13.3. 2016	*Citrus Aurantium*	[38]	red brown/ light yellow-white powder, characteristic odour and bitter taste	[38]	Antisepsis, anti-inflammatory, anti-cancer, antioxidant, antispasmodic, choleric activity	[38]	solid, soluble in alcohol, ether and benzene. almost insoluble in water	[38]
Naringin	N/A	-	0,066	[65] 13.3. 2016	*Citrus Paradisi*, *Citrus grandis*, *Citrus Aurantium*	[38]	yellow-white powder, characteristic odour and bitter taste	[38]	Anti-inflammatory, anti-viral, anti-mutation, anti-carcinogen	[38]	solid, soluble in water	[38]
Neohesperi-din	N/A	-	0,1	[38] 13.3. 2016	*Citrus Aurantium*	[38]	white/yellow powder, characteristic odour and intense sweet taste	[38]	treat hypertension, reduce the brittleness of capillary and prevent microvascular bleeding, Infarction	[38]	solid, hardly soluble in cold water, alcohol, insoluble in ether and inorganic acids	[38]
Nobiletin	N/A	-	0,365	[65] 13.3. 2016	*Citrus Aurantium*	[38]	white/yellow/brown crystalline powder, characteristic odour and taste	[38]	Increasing capillary toughness, lowering cholesterol, Treating cardiovascular disease, Anti-virus and anti-inflammatory	[38]	solid, soluble in water and ethanol	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Oxyresve-ratrol	N/A	-	0,223	[65] 14.3. 2016	*Morus alba*, mulberry wood	[75]	yellow/brown-white powder, characteristic odour and taste	[38]	antioxidant, anti-inflammatory, anti-cancer, lowering blood lipid, antibiosis	[38]	solid, soluble in water	[38]
P-coumaric acid	N/A	-	0,018	[65] 14.3. 2016	*Fagopyrum Cymosum Meisn*	[38]	white powder	[38]	cytostatic activity, immune-active agent, inhibitor of stilbene oxidase	[38]	solid, insoluble in water, soluble in ethanol	[38]
Phloridzin	N/A	-	0,041	[65] 14.3. 2016	Apple tree bark, *Malus pumila*, bark of the apple, pear, cherry…	[38]	white/brown powder, characteristic odour and taste	[38]	antioxidant, anti-diabetics, anti-cancer, anti-tumour	[38]	solid, soluble in hot water and ethanol	[38]
Piceatannol	low	[76]	0,25	[65] 14.3. 2016	Giant knotweed	[38]	White crystalline powder, characteristic odour and taste	[38]	anti-aging, anti-cancer, anti-tumour, antibacterial, fungal inhibitor	[38]	liquid/solid, poorly soluble in water, soluble in methanol and chloroform	[38]
Protocate-chuic acid	N/A	-	0,133	[65] 14.3. 2016	*Stenoloma Chusanum Ching*, *Salvia miltiorrhiza Bunge*	[38]	White crystalline powder, characteristic odour and taste	[38]	antioxidant, anti-aging, antibacterial, relieving asthma effects, treatment of chronic bronchitis	[38]	liquid/solid, soluble in ethanol, methanol, water	[38]
Quercetin	low	[77]	5	[65] 14.3. 2016	*Sophora japonica*	[38]	yellow green powder	[38]	anti-asthmatic, anti-cancer, inhibit histamine	[38]	liquid/solid, soluble in aqueous alkaline solutions, insoluble in water	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Quercitrin	low	[77]	5 000	[65] 14.3 .2016	*Sophora japonica*	[38]	yellow crystalline powder, characteristic odour and taste	[38]	treating asthma, chronic bronchitis, antiviral agent	[38]	liquid/solid, soluble in aqueous alkaline solutions, insoluble in water	[38]
Resorcinol	N/A	-	0,135	[65] 14.3. 2016	rice *(Pyricularia oryzae)*	[102]	white/yellow/brown flakes, characteristic odour and taste	[38]	hair dye, pharmaceutical, pesticide, rubber adhesive, antifungal, for cosmetics	[38]	solid, easily soluble in water, alcohols and ethers	[38]
Resveratrol	non-toxic	[103]	1 508	[65] 14.3. 2016	*Polygonum cuspidatum*, Red grape skin, Giant Knotweed, peanuts, mulberry	[38]	light yellow/white/brown powder	[38]	antibiotic antivirus, anti-tumour, anti-aging, resists the hepatitis, anti-inflammatory	[38]	liquid/solid, soluble in water	[38]
Rutin	non-toxic	[78]	1 960	[65] 14.3. 2016	*Saphora japonica*, *Rheum* species	[38]	yellow powder without odour, characteristic odour and taste	[38]	anti-inflammatory, anti-viral, treat hemorrhoids, varicosis and microangiopathy	[38]	solid, soluble in methanol	[38]
Sanggenon D	N/A	-	10 000	[38] 14.3. 2016	*Morus Cathayana*, *Morus Alba*, mulberry	[38]	yellow crystalline powder	[38]	antioxidant, hypotensive activity	[38]	solid	[38]
Scopoletin	N/A	-	6 700	[65] 14.3. 2016	*Epimedium*, *Spolia Japonica*	[38]	white crystal powder	[38]	antioxidant, anti-cancer, antiseptic	[38]	liquid/solid, slightly soluble in water	[38]
Syringic acid	N/A	-	470	[65] 14.3. 2016	*Kalimeris indica*	[38]	white crystal powder	[38]	Antioxidant, anti-endotoxin	[38]	liquid/solid	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
Tannic acid	N/A	-	0,17	[65] 14.3. 2016	medical gallic acid, burnt gallic acid, sulfonamides	[38]	light yellow/brown powder	[38]	leather tanning agent, rubber peptidizer, protein medicinal preparation, dressing inhibitor, wines clarifying agent and blue-black ink	[38]	solid, soluble in ethanol, water, acetone	[38]
Umbellife-rone	N/A	-	1 340	[65] 14.3. 2016	*Rutaceae*	[38]	white crystal powder	[38]	antifungal agent	[38]	solid, soluble in ethanol, hot water, ether	[38]
Vanillic acid	N/A	-	1 360	[65] 14.3. 2016	*Vanilla planifolia*	[79]	white/yellow crystal powder	[38]	flavoring agent, Anti-mutagenic, anti-angiogenetic, anti-colitis, anti-sickling;	[38]	solid, soluble in ethanol, ether, slightly soluble in water	[38]
Vanillin	N/A	-	1 840	[65] 14.3. 2016	*Vanilla planifolia*, vanilla bean, roasted coffee, Chinese red pine	[38]	white to slightly yellow powder, sweet cream odour	[38]	flavoring agent, in sweet food, perfumes, pharmaceuticals	[38]	solid, soluble in ethanol	[38]
Vitexin	non-toxic	[85]	6 600	[65] 14.3. 2016	Hawthorn Berry extract, *Vitex agnus-castus*	[38]	brown/yellow/red powder, characteristic odour and taste	[38]	antibacterial, anti-cancer	[38]	solid, soluble in water and ethanol	[38]
3,5-Dicaffeoyl-quinic acid (Isochloroge-nic acid A)	N/A	-	200 000	[65] 14.3. 2016	Honeysuckle, globe artichoke, *Caucasian whortleberry*, *Aster scaber*, *Ipomoea batatas*	[38]	white powder	[38]	neuroprotective effects, antiviral and anti-hepatotoxic activities	[38]	solid, soluble in ethanol and acetone, very slightly soluble in ethyl acetate	[38]

Table 5: Antioxidant and Antimicrobial inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Appearance / Organoleptic properties	Ref.	Common use(s)	Ref.	State of matter/ Solubility	Ref.
4,5-Dicaffeoyl-quinic acid	N/A	-	10 000	[38] 14.3. 2016	Honeysuckle	[38]	white crystalline powder	[38]	detoxification anti-pyretic, antibacterial anti-inflammatory	[38]	liquid/solid	[38]
4-Hydroxy-cinnamic acid	non-toxic	[38]	1 870	[65] 14.3. 2016	Propolis, *Brassica juncea*, Rice Hull	[81, 82,83]	white/yellow crystalline powder, characteristic odour and taste	[38]	antioxidant, skin whitening agent, anti-cancer	[38]	solid, soluble in water	[38]
4-Chloro-salicylic acid	N/A	-	8	[65] 14.3. 2016	derivative of salicylic acid	[105]	white crystalline powder	[38]	antimicrobial and antifungal properties	[104]	solid, slightly soluble in water	[38]

Table 6: Tyrosinase inhibition

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Organoleptic properties	Ref.	Most significant usability	Ref.	State of matter/ Solubility	Ref.
Aloesin	N/A	-	0,023	[38] 15.3.2016	*Aloe vera*, *Aloe barbadensis Miller*, bitter sap	[38]	white/brown powder, light odour	[38]	anti-inflammatory, anti-cancer, cure acne, inhibit the growth of bacteria	[38]	liquid/solid, soluble in water and alcohols	[38]
Anacardic acid	low	[84]	15 300	[65] 15.3.2016	Shell of cashew nuts	[38]	yellow/white powder	[38]	anti-cancer	[38]	solid	[38]
Andalasin A	N/A	-	N/A	-	*Morus macroura*	[85]	N/A	-	antifungal properties	[85]	N/A	-
Anemonin	high	[86]	10 000	[38] 15.3.2016	*Pulsatilla chinensis (Rununculaceae)*, Anemone chinensis	[38]	yellow/brown powder, characteristic odour	[38]	antibacterial, treating noxious heat and blood dysentery, genital problems, anti-amoeba	[38]	liquid/solid, soluble in water	[38]

Table 6: Tyrosinase inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Organoleptic properties	Ref.	Most significant usability	Ref.	State of matter/ Solubility	Ref.
Arbutin	N/A	-	1 560	[65] 15.3.2016	Bearberry	[38]	white crystal powder, characteristic odour and taste	[38]	antibacterial, skin lightening, anti-inflammatory effect	[38]	solid, soluble in water	[38]
Delphinidin	non-toxic	[87]	47 700	[65] 15.3.2016	Bilberry, *Vaccinium Uliginosum*	[38]	Dark-violet/blue powder, characteristic odour and taste	[38]	anti-inflammatory, anti-aging, cure eyes diseases	[38]	solid, soluble in water and ethanol	[38]
Dieckol	non-toxic	[88]	N/A	-	*Ecklonia cava*, marine brown algae	[88]	white powder	[89]	Antioxidant, anti-inflammatory, anti-fungal properties	[88]	solid	[89]
Dihydromorin	N/A	-	10	[38] 15.3.2016	*Moraceae (Morus, Artocarpus, Chloro phora)*	[38]	white powder	[38]	anti-tumour activity	[38]	N/A	-
Eckol	N/A	-	N/A	-	*Wakame algae*	[38]	N/A	-	improvement and the prevention of Alzheimer's disease and dementia	[38]	N/A	-
Fisetin	non-toxic	[90]	640	[65] 15.3.2016	Smoketree, Boxwood	[38]	Yellow to brown crystalline powder	[38]	Antioxidant, anti-inflammatory	[38]	liquid/solid, soluble in ethanol, acetone and acetic acid	[38]
Hinokitiol	N/A	-	0,099	[38] 15.3.2016	*Cupressaceae* trees, Western red cedar	[38]	Yellow crystalline powder, characteristic odour	[38]	Antimicrobial, antiseptic, anti-allergic, in cosmetics	[38]	solid, soluble in water	[38]
Chalcomoracin	N/A	-	N/A	-	Mulberry, *Morus Alba*	[91,92]	N/A	-	Antifungal properties	[91]	N/A	-
Isoliquiritigenin	N/A	-	32 800	[65] 15.3.2016	Licorice, *Glycyrrhiza glabra*	[38]	Light yellow powder, light odour, characteristic taste	[38]	antibacterial, anti-inflammatory, anti-viral, detoxifying, AIDS, and epigastric abdominal	[38]	Liquid/solid, soluble in water	[38]

Table 6: Tyrosinase inhibition (continued)

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Organoleptic properties	Ref.	Most significant usability	Ref.	State of matter/ Solubility	Ref.
Isoliquiritin	N/A	-	10 000	[38] 15.3.2016	*Allium chinense, Glycyrrhiza glabra*(licorice), *Trifolium subterraneum*	[38]	Light yellow/white crystal powder, characteristic odour	[38]	Anti-inflammatory, anti-tumour, anti-viral, promote immunity, anti-cancer	[38]	solid	[38]
Norartocarpetin	N/A	-	N/A	-	*Artocarpus communis, Moraceae*	[93,94]	N/A	-	Antioxidant, anti-tyrosinase activity, potential for use as a whitening agent	57	Poor solubility in aqueous systems and oils	58
Norkurarinol	N/A	-	N/A	-	*Sophora flavescens*	[95]	N/A	-	Anti-inflammatory properties	[95]	N/A	-
Panduratin A	N/A	-	N/A	-	*Kaempferia pandurata*	[96]	N/A	-	potential for use as an anti-inflammatory agent	[96]	N/A	-
Papyriflavonol A	N/A	-	N/A	-	*Broussonetia papyriferra*	30	N/A	-	Antimicrobial, antifungal	30	N/A	
Pelargonidin	N/A	-	20 400	[65] 16.3.2016	*Geranium, Pelargonium hortorum*	[38]	Brown powder, characteristic odour and taste	[38]	anti-depressant, astringent, antiseptic, deodorant, and to treat diarrhea and irritable bowel syndrome	[38]	solid	[38]
Tectorigenin	N/A	-	22 160	[65] 16.3.2016	*Belamcanda chinensis, Baptisia, Centrosema, Dalbergia, Ononis spinosa, Iris germanica*	[38]	White/yellow crystal powder	[38]	Anti-inflammatory, hypoglycemic, cytotoxic activity	[38]	solid, soluble in ethyl acetate	[38]
Veratric acid	N/A	-	0,61	[65] 15.3.2016	*Phlebia radiata*	[97]	White/light yellow/brown crystalline powder	[38]	Antimicrobial agent, antiviral	[38]	solid, slightly soluble in water	[38]

Table 7: Maillard reaction products inhibition

Name of compound	Toxicity	Ref.	Price ($ for 1 g)	Ref. Date	Extraction method/ availability	Ref.	Organoleptic properties	Ref.	Most significant usability	Ref.	State of matter/ Solubility	Ref.
Apigenin	low	[38]	19 700	[65] 15.3.2016	*Apium graveolens*	[38]	Light yellow/brown powder, characteristic odour and taste	[38]	Antioxidant, anti-cancer, anti-inflammatory, antiviral	[38]	solid, soluble in hot ethanol and in dilute potassium hydroxide	[38]
Cirsilineol	N/A	-	146 200	[65] 15.3.2016	*Artemisia vestita Wall*	[98]	N/A	-	Anti-cancer	[98]	N/A	-
Cirsimaritin	N/A	-	302 000	[65] 16.3.2016	*Rosmarinus officinalis*	[30]	powder	[38]	Antibacterial, cytotoxic activity, anti-cancer, antidepressant	[38]	solid	[38]
Coumestrol	N/A	-	5 240	[65] 16.3.2016	Alfalfa (*Medicago sativa*), soybeans, brussels sprouts, spinach	[38]	Brown/yellow/green powder, characteristic odour and taste	[38]	antibacterial	[38]	solid, good solubility in water	[38]
Daphnetin	N/A	-	15 200	[65] 16.3.2016	*Daphne korean nakai, Daphne odora*	[38]	White or grey crystal powder	[38]	Anti-cancer	[38]	liquid/solid, soluble in water	[38]
Mangiferin	N/A	-	0,55	[65] 16.3.2016	*Musa paradisiaca*	[38]	Light yellow powder, natural banana odour and characteristic taste	[38]	treating inflammatory bowel disease, anti-bacterial, fungicidal, calming, and curing sugar diabetes	[38]	solid, insoluble in water	[38]
Myricitrin	N/A	-	33 300	[65] 16.3.2016	China Baybarry (*Myrica rubra*), walnuts	[38]	Yellow/red/brown crystalline powder, characteristic odour and taste	[38]	Antioxidant, anti-tumour, anti-inflammatory	[38]	solid	[38]
Plantagoside	N/A	-	0,098	[38] 16.3.2016	*Plantago Asiatica*	[38]	Light brown/yellow powder, characteristic odour	[38]	Antibacterial, anti-inflammatory, antiviral	[38]	solid	[38]
Puerarin	low	[99]	610	[65] 16.3.2016	*Pueraria Mirifica, Pueraria Lobata*, Kudzu root	[38]	White/yellow-brown powder, characteristic odour and taste	[38]	Anti-cancer, enhancing immunity, protecting myocardial cell	[38]	liquid/solid, soluble in water and ethanol	[38]

4.1 Classification of compounds

The natural compounds were classified into several groups according to their suitability for further in vivo testing depending on their properties and price:

- not suitable (high toxicity and/or price)
- suitable if the toxicity is low (unknown at the moment)
- suitable for further testing

Not suitable

In this study, a compound which presumably cannot be a possible alternative to sulfites, despite its ability to inhibit tyrosinase, is Anemonin due to its high toxicity. Ingestion of this substance causes inflammation in the mouth, causing abdominal pain. Mouth ulcers and damage to the digestive tract may occur. Poisoning is accompanied by diarrhea and can detect blood in the urine. If the poisoning is fatal, convulsions can occur [106].

Quercitrin, Anacardic acid, Apigenin, Delphinidin, Hydroxycinnamic acid, Fisetin, Resveratrol, Rutin, Puerarin and Vitexin are not suitable because of their very high price.

Suitable if the toxicity is low

Toxicity wasn't detected in all substances so their research is needed for more results. Thus these substances could be used as alternatives to sulfites but they are not tested enough.

Compounds with antioxidant and antimicrobial properties: P-coumaric acid, Morin hydrate, Naringenin, Phloridzin, Kuwanon G, Naringin, Kurarinone, Myricetin, Neohesperidin, Protocatechuic acid, Resorcinol, Catechin, Daidzein, Tannic acid, Oxyresveratrol, caffeic acid, Epigallocatechin-3-Gallate (EGCG), Nobiletin, Esculetin, Amentoflavone, Baicalein, Butein, 4-Chlorosalicylic, Syringic acid, Umbelliferone, Vanillic acid, Vanillin, Sanggenon D, 4,5-Dicaffeoylquinic acid, 3,5-Dicaffeolquinic acid (Isochlorogenic acid A), Scopoletin, Dihydro-5,6-dehydrokawain (DDK).

Compound with tyrosinase inhibition: Aloesin, Hinokitiol, Veratric acid, Dihydromorin, Arbutin, Isoliquiritin, Pelargonidin, Tectorigenin, Isoliquiritigenin,

Andalasin A, Eckol, Chalcomoracin, Norartocarpetin, Norkurarinol, Panduratin A, Papyriflavonol A.

Compounds with ability to inhibit Maillard reaction products: Plantagoside, Mangiferin, Cirsilineol, Daphnetin, Cirsimaritin, Myricitrin, Coumestrol.

Suitable for further tests

Some substances showed the best properties with no or low toxicity. These substances could be used as alternatives to sulfites.

Compounds with antioxidant and antimicrobial properties: Non-toxic: Hesperidin, Hesperetin. Low toxicity: Ferulic acid, Genistein, Cinnamic acid, Chlorogenic acid, Gallic acid, Kaempferol, Luteolin, Ellagic acid, Piceatannol, Isovitexin, Epicatechin (EC), Esculin, Quercetin.

Compound with tyrosinase inhibition: Non-toxic: Dieckol.

4.2 Research of suitable compounds

The following list describes the substances which exhibit the best properties very similar to sulfites, having low or no toxicity and are the most affordable.

Furthermore, these natural alternatives to sulfites are described in terms of research. Results showed substances with many uses in cosmetics, pharmaceutical and food industry. It is not excluded that the compounds have been tested also in another research than it is shown below. It means these substances can have another beneficial properties.

Hesperidin

Hesperidin is a compound found primarily in unripe citrus fruit used as an antioxidant. Food sources include orange, grapefruit, lemon, and tangerine juice.

Benefits of hesperidin:

Chronic Venous Insufficiency - According to a study published in International Angiology [108], supplements containing a combination of hesperidin, extract of the herb Butcher's broom, and ascorbic acid may help treat chronic venous insufficiency.

Hemorrhoids - In a study in Angiology [120], a supplement containing a combination of hesperidin and diosmin (another antioxidant compound available in citrus fruits) was found to aid in the treatment of hemorrhoids.

Osteoporosis - A study from the Journal of Nutrition [121] suggests hesperidin shows promise for the prevention for postmenopausal osteoporosis.

Cholesterol - In a study on rats [122], scientists found that supplementing the animals' high-cholesterol diets with hesperidin and vitamin E helped reduce cholesterol levels in their blood.

Cancer - According to a preliminary study published in Phytotherapy Research [123], scientists found that hesperidin inhibited the spread and growth of cancer cells.

Negative effects:

Hesperidin may trigger a number of side effects, including abdominal pain, diarrhea, contact dermatitis, and nausea [124].

Hesperetin

Hesperetin is a compound found primarily in citrus fruit. Food sources include orange and grapefruit fruits – especially in the peel. Hesperetin exhibits estrogenic, anticarcinogenic and antioxidative properties. Hesperetin reduces cholesteryl ester mass and have antioxidant, anti-inflammatory, anti-allergic, hypolipidemic, vasoprotective and anticarcinogenic actions [125].

Ferulic acid

Ferulic acid is a phenolic compound found in fruits and vegetables. Recently, several studies have shown that ferulic acid acts as a potent antioxidant. Furthermore, it is able to inhibit the expression and/or activity of cytotoxic enzymes. Based on this evidence, ferulic acid has been proposed as a potential treatment for many disorders including cancer, cardiovascular diseases, Alzheimer's disease, skin disease and diabetes mellitus. However, new efforts and resources are needed in clinical research for the complete evaluation of Ferulic acid therapeutic potential in chronic diseases [109]. Ferulic acid has already been successfully used in food industry for other purposes [139].

Genistein

Genistein is an isoflavone isolated from a soy bean. It has many biological activities including anti-inflammatory, antioxidant and antiangiogenic properties. It has been shown that genistein inhibits many type of cancers including prostate cancer [110]. Genistein could be also a prospective potent agent for treating diabetes mellitus [120].

Cinnamic acid

Cinnamic acid is a phenylalanine naturally found in plant tissue. Cinnamic acid and its derivatives have shown a variety of pharmacologic properties. However, little is known about the antiglycation properties of cinnamic acid and its derivatives. They also act as an antioxidant and have antimicrobial properties [111].

Cinnamic acid is a hormone-like substance which regulates cell growth and differentiation in plants. Efficient agents with low toxicity are important in cancer treatment. Few studies have reported on the effects of cinnamic acid on tumour cells especially in treatment of leukaemia [112].

Chlorogenic acid

Chlorogenic acid is a polyphenol compound and is used as an antioxidant and anti-obesity agent. Recently study has shown that chlorogenic acid may be beneficial for the prevention and treatment of anti-inflammatory diseases [113].

Gallic acid

Gallic acid is a phenolic compound present in various plants such as blackberry, blueberry, strawberry, grapes, hazelnut, walnut, cashew nut, plums, mango, tea, wine and so on. The studies have shown gallic acid has neuroprotective actions in different models of neurodegeneration, neurotoxicity and oxidative stress [114].

Kaempferol

Kaempferol is a polyphenol found in various fruits and vegetables. Many studies have described the beneficial effects of dietary kaempferol in reducing the risk of chronic diseases, specifically cancer. Kaempferol acts like an antioxidant defence against free radicals which promote the development of cancer. Inhibits cancer cell growth and angiogenesis and induces cancer cell apoptosis [115].

Luteolin

Luteolin is a flavonoid compound often found in leaves of plants. Recent study has shown luteolin has valuable therapeutic properties against P-dependent human breast cancer. Luteolin disrupts tumour progression by blocking several processes that are vital to P-dependent tumour development [116]. It has been also found luteolin plays major role in the antioxidant activity of the various plants [126,127].

Ellagic acid

Ellagic acid is a polyphenol compound found in fruits such as pomegranates, walnuts or strawberries [128]. Recent study has shown that ellagic acid is able to prevent or treat ocular complications of diabetes such as retinopathy and cataract [129].

Piceatannol

Piceatannol is a natural stilbene occurring in various plants. Recent study has shown that grapes (*Vitis vinifera* L. cv. Cabernet Sauvignon) have significant amounts of piceatannol [130]. Piceatannol has an anti-cancer, anti-oxidative, anti-microbial, anti-inflammatory activities. It suggests that piceatannol might be a potentially useful nutritional and pharmacological biomolecule [131].

Isovitexin

Isovitexin is a flavonoid and exhibits a potential antioxidant activity. It is isolated from mungo beans and it has an anti-inflammatory and anti-diabetic effects [132].

Epicatechin (EC)

Epicatechin is a flavonoid isolated from a tea or cocoa [107]. It is present in many foods that affects vascular function. Recent study has shown that epicatechin may reduce cardiovascular disease risk [133]. Another study has shown anti-cancer effects of epicatechin [134].

Esculin

Esculin is a coumarin with an antioxidant property. Recent study has shown that esculin could prevent intestinal inflammatory disease. [135]. It has also an anti-cancer activity including growth inhibition of human leukaemia cells [136].

Quercetin

Quercetin is a flavonoid naturally found in many fruits and vegetables. It has an anti-cancer, vasorelaxant and antioxidant effects in cardiovascular diseases. In study where was investigated improve of some risk factors of cardiovascular disease, quercetin yet exerted slightly pro-inflammatory effects [137]. Another study has shown quercetin can reduce oxidative stress and atherosclerosis in animal models [138].

Dieckol

Dieckol is a phlorotannin derivative isolated from brown seaweed, *Ecklonia cava*. It has been studied for a treatment of diabetes. This compound shows antithrombotic and profibrinolytic activities and also an effect on hair growth [118]. According to the findings of these studies dieckol can be developed as a therapeutic agent for type II diabetes [117]. Another study had shown that dieckol can be developed as a therapeutic agent for liver disease by oxidative stress [119].

5 CONCLUSION

To serve as natural alternative to sulfites, a compound needs to meet several prerequisites: possess antioxidant and antimicrobial activity, be able to inhibit tyrosinase and the products of Maillard reaction, have low toxicity and have low price. The results of this thesis show that there is no a single compound that would unequivocally meet all the needs. There are several that may be suggested for further *in vitro* or *in vivo* tests, such as: Hesperidin, Hesperetin, Ferulic acid, Genistein, Cinnamic acid, Chlorogenic acid, Gallic acid, Kaempferol, Luteolin, Ellagic acid, Piceatannol, Isovitexin, Epicatechin (EC), Esculin, Quercetin and Dieckol.

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