CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Department of sustainable technologies

Natural Compounds Used for Reduction of Enzymatic Browning in Food Processing

Bachelor thesis

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Supervisor:
doc. Ing. Jan Banout, Ph.D.

Author:
Jan Staš

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Department of Sustainable Technologies

Faculty of Tropical AgriSciences

BACHELOR THESIS ASSIGNMENT

Jan Staš

Sustainable Development in the Tropics and Subtropics

Thesis title

Natural compounds used for reduction of enzymatic browning in food processing

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Objectives of thesis

This study is focused on literature review and screening of potential natural preserva-tives, which inhibit enzymatic browning of food. The natural compounds are selected as potential anti-browning preservatives, which may be used as pretreatments during hot air and solar drying of food.

Methodology

Bachelor thesis is conceived as a literature research focused on the collection and comparison of scientific data. Collecting of these data was provided through internet sources to the extent that they have explanatory value about commonly used organic substances, which inhibit browning effect of food during storage and during drying. Sources of scientific literature were searched mainly through Web of Science, Scopus and Google Scholar. There were used 33 sources, with the majority of the scientific literature in the English language.Keywords for searching sources were: Enzymatic browning, anti-browning agents, prevent browning, drying, food storage

The proposed extent of the thesis

40

Recommended information sources

ABANO E. E., SAM-AMOAH L. K. Effects of different pretreatments on drying characteristics of banana slives. ARPN Journal of Engineering and Applied Sciences. 2011, Vol. 6, No. 3. ISSN 1819-6608

CHEN Xiao Dong. Drying Technologies in Food Processing. Singapore: John Wiley & Sons, 2008, 352. ISBN 978-1-4051-5763-6.

RAHMAN M. Shafiur. Handbook of Food Preservation. 2nd ed. US: CRC Press, 2007, 1088 . Food science and technology ; 167. ISBN 1574446061

Website: UNIDO; United Nation Industrial Development Organization, Preservation of fruits and vegetables by drying. 2012 <http://www.unido.org/fileadmin/import/32134_27PreservationFVone.9.pdf>

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Dean

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Statement

I declare that I have developed this thesis on the topic " Natural Compounds Used for Reduction of Enzymatic Browning in Food Processing" by myself and independently and I have quoted only from the sources listed in the bibliography. These data I have added by information which were the outcome of my own research.

In Prague 17th April 2015

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Jan Staš

Abstract

Drying or dehydration is one of the most popular methods for food preservation, despite it has some disadvantages. The main drawback is that dryed fruits or vegetables undergo browning reactions. Browning foods may be desirable, as in caramelisation, or undesirable, as in an apple turning brown after being cut. Foods, including beverages, can turn brown through either enzymatic or non-enzymatic processes. The prevalent use of sulfites as inhibitors of enzymatic browning in foods can cause allergic reactions sometimes exhibited by individuals with respiratory ailments. The main aim of this thesis is to present a comprehensive overview of organic substances, which inhibited enzymatic browning effect during storage and during solar drying. Potentially interesting organic inhibitors are summarized in the table as a result of this study. Alternatives to chemical substances include: ascorbic acid, citric acid, glutathione, bezoic acid, etc.

Key words: enzymatic browning, anti-browning agents, drying, prevent browning, food storage

Abstrakt

Sušení neboli dehydratace je jeden z nejpoužívanějších způsobů konzervace potravin, přestože má i některé záporné stránky. Hlavní nevýhodou je, že ovoce a zelenina při sušení podléhá procesu hnědnutí. Tato reakce může být žádoucí, jako tomu je u karamelizace, nebo nežádoucí, například jablečné hnědnutí po rozříznutí. Potraviny, včetně nápojů, mohou hnědnout kvůli enzymatickému nebo neenzymatickému procesu. Převládající používání siřičitanů, jakožto inhibitorů enzymatického hnědnutí potravin může způsobit alergickou reakci, zejména u jedinců s respiračním onemocněním. Hlavním cílem této práce je představit ucelený přehled látek přírodního původu, které potlačují efekt enzymatické hnědnutí při skladování a solárním sušení. Literární rešerše definuje sušení jako termín, klasifikuje typy sušení a rozděluje solární sušárny. Potencionálně zajímavé organické inhibitory jsou shrnuty v tabulce, jako výsledek této práce. Alternativami k chemickým látkám mohou být například: kyselina askorbová, kyselina citronová, glutathione, kyselina benzoická, apod.

Klíčová slova: enzymatické hnědnutí, agenti proti hnědnutí,sušení, předcházení hnědnutí, skladování potravin

Preface

This thesis focuses on the possibilities of reduction of enzymatic browning in food processing. Enzymatic browning is undesirable chemical process which degrades the product, which leads to large losses. Therefore it is very important to treated properly the food before processing. From this reason, there is a worldwide need to find alternative preservatives based on natural compounds, which could theoretically substitute traditionally used chemical substances as a pretreatment before food processing.

Each type of food needs a different kind of treatment to avoid the enzymatic browning as much as possible. For example in carbohydrate foods, it can be minimized by elimination of amines, in foods rich in proteins it is done by removal of reducing sugars. Also the concentration of selected treatment is very important and just small changes in concentration can cause different results.

There are various techniques for preventing of enzymatic browning, each of them trying to eliminate one of the key parts of the reaction, including oxygen, enzymes, copper or substrate. For example: dipping, blanching, spraying, coating, etc. 'Dipping' involves soaking the foods in liquids or solutions in order to suppress enzyme activity, either by physically preventing oxygen from interacting with the system, or otherwise inhibiting the enzymes.

The enzymatic inhibitors include acids, halides, phenolic acids, chelating agents, sulfites, and the substrate-binding compounds can be ascorbic acid, quinone couplers such as cysteine, and some other agents. The most commonly used method for ihnibiting enzymatic browning is the addition of sulphiting agents. PPO was successfully inhibited by application of sulphur dioxide (SO_2), but regulations were issued to restrict its utilization, as sulphites were responsible for 15 triggering allergic reactions. For this reaseon, organic compounds which have similar effect on PPO activity as chemical substances needs to be studied more intensively.

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1 Introduction

Solar radiation used for drying is one of the oldest applications of solar energy. It was used since the dawn of mankind mainly for food preservation but also for drying other useful materials as cloths, construction materials, etc. The first installation for drying by solar energy was found in South France and is dated at about 8000 BC. Solar heat was the only available energy source to mankind until the discovery and use of wood and biomass. Until today in remote small communities, not only in the so-called third world regions, but also in the western countries, people take advantage of solar radiation to dry and preserve small amounts of food.

Drying by solar energy is a rather economical procedure for agricultural products, especially for medium to small amounts of products, to preserve excess of production. It is friendly to the environment. It is still used for domestic up to small commercial size drying of crops, agricultural products and foodstuff, such as fruits, vegetables, aromatic herbs, wood, etc., contributing thus significantly to the economy of small agricultural communities and farms.

1.1 Drying

There are two main sources of food; plants and animals. Although various researchers have classified foods in different ways, generally they are categorized as perishable (which spoils very fast), non-perishable (relatively slower spoilage) (Rahman, 1999).

All variety of foods in our day to day life requires some form of preservation mainly to reduce or stop spoilage, to make it available throughout the year, to maintain desired levels of nutritional properties for the longest possible time span and to make value-added products. Amongst these, spoilage is the foremost reason for employing food preservation techniques. Spoilage or deterioration of food occurs during handling or due to mechanical, physical, chemical or microbial damage. Out of these, chemical and microbial damages are most frequent causes (Rahman, 1999; Mujumdar, 2007).

Microbial growth depends on the storage conditions and the moisture level in the product. Different micro-organisms have different growth rates depending on the conditions. There can also be several chemical and enzymatic changes during processing and storage of foods, e.g. browning.

Commonly employed methods for food preservation are: freezing, vacuum packing, canning, preserving in syrup, food irradiation, adding preservatives, and the most popular method, dehydration or drying. However, drying will never replace freezing and canning, because of their better ability to retaining the taste, appearance, and nutritive value of fresh food.

Drying, or dehydration, of foods is one of the most common methods used for preserving food. Its discovery and spread allowed people to become less dependent on daily food supply, even despite the often difficult environmental conditions (Rahman, 2007).

The dehydrated foods industry has a prominent place among today's food industries all over the Word especially in developing countries (Vega-Gálvez, 2011).

The main effect of drying is reduced water activity in the dried foods. Reduced water activity preserves foods by preventing microbial growth and some undesirable chemical reactions. However, enzyme and bacterial inactivation that accompanies heat-based drying methods has both beneficial and adverse effects, depending on what biological material is processed. In the case of foods, the aim usually is to maximize enzyme and microbial inactivation. On the other hand, when drying bacterial cultures, enzymes or vitamins, we seek to achieve little to no inactivation (Rahman, 2007).

Different types of drying and their various operating variables affect food microstructures that are formed during the drying process, thus influencing the structure of the final product (Vega-Gálvez, 2011).

Selection of dryers for particular food product is itself a complex step as there are hundreds of dryers available and more than one dryer can suit a particular application. In developing countries food products are dried traditionally by open sun drying. Although this method is still common at several places for non-commercial use, there have been numerous efforts to develop advanced drying methods for food products on commercial scale (Kudra and Mujumdar, 2009).

1.1.1 Classification of dryers

There are many schemes used to classify dryers (Mujumdar, 2007; Van't Land, 1991). Classification of dryers on the method used for removing water is perhaps the most useful since it allows one to identify some key features of each class of dryers:

- thermal drying
- osmotic dehydration
- mechanical dewatering

Osmotic dehydration and mechanical dewatering are quite out of scope of presented thesis. Thus they were excluded from further classifications of drying systems.

1.1.2 Thermal drying

Thermal drying, which is one of the most widely used methods of drying foods, is a process where high temperatures are used to eliminate moisture from the material. The type or physicochemical state of the dried material and the drying process determine the mechanisms of moisture transfer. Thermal drying takes place in gaseous or void environment.

Thermal drying can be divided further into three types:

- 1. Air drying
- 2. Low air environment drying
- 3. Modified atmosphere drying

1.1.2.1 Air drying methods

Convection pneumatic drying

Application of the convection pneumatic dryers is represented especially in food industry in plants for industrial processing of grains (wet milling processing of wheat and corn). Generally, such dryers can be used for drying of meal-like and fine-kernel materials. Simple construction and a relatively low consumption of energy have enabled successful application of such dryers in the above stated industrial branches. The construction of the convection dryer enables simultaneous pneumatic transport of wet material and its drying. The material is are dried in matter of several seconds, therefore method is suitable for drying of foods sensitive to high temperatures (Prvulovic and Tolmac, 2007).

Sun Drying

The most basic method of drying is to spread the crop on a surface exposed to the sun (Heindl, 2000).

From an ancient times, solar drying was the only way, how to dry foods. In this process, drying material is laid on the ground or hanged in the air to be exposed to direct sun light. The advantage of solar drying is that it uses renewable energy source. Although sun drying is the cheapest and also least demanding way, how to dry foods, it has many disadvantages. For example the temperature rises without regulation. Drying is therefore uneven, and caramelized and crusted pieces result. The direct exposure to the sun also destroys color, vitamins and flavor, and there is chance of contamination with dust, dirt, insect infestation, and contact with other pests. They may also be drenched by rain or dew and, may need further drying if mold growth is to be avoided during storage. Sun-drying may take 2-14 days (De Guzman and Siemonsma, 1999).

In-store drying

In-store drying is also called low temperature drying or in-bin drying. Commonly it is used when grains or another crops are stored for longer time or until sold. Tropical climate weather conditions are less favorable for this type of drying, because of high ambient temperatures and relative humidity values (Rahman, 2007).

Spray Drying

Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas. This is the preferred method of drying of many thermally-sensitive materials such as foods and pharmaceuticals. A consistent particle size distribution is a reason for spray drying some industrial products such as catalysts. Air is the heated drying medium; however, if the liquid is a flammable solvent such as ethanol or the product is oxygen-sensitive then nitrogen is used (Mujumdar, 2007).

1.1.2.2 Low air environment drying

Vacuum Drying

Vacuum drying of lumber has been considered for many years, but until recently has not been in commercial use. The main attraction of vacuum drying is that by lowering the boiling temperature of water in vacuum, free water can be vaporized and removed at temperatures considerably below 100°C almost as rapidly as it can with high-temperature drying above 100°C. Vacuum drying is essentially high-temperature drying at low temperatures (Simpson, 1987).

Freeze drying

Freeze-drying, lyophilization or cryodesiccation is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze-drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase (Garrett, 2012)

Despite being used in a wide variety of products (such as pharmaceuticals and foodstuff), lyophilization presents a great challenge for scientists in the field, especially its usage for long-term conservation of living systems (Ciurzinska and Lenart, 2011).

Heat pump drying

The heat pump dryer is a further extension of the conventional convection air dryer with an inbuilt refrigeration system (Figure 1). Dry heated air is supplied continuously to the product to pick up moisture. This humid air passes trough the evaporator of the heat pump where it condenses, giving up its latent heat of vaporization to the refrigerant in the evaporator. This heat is used to reheat the dry air passing over the hot condenser of the refrigeration circuit and used to reheat the air within the dryer. The use of the heat pump dryer offers several advantages over conventional hot air dryers for drying food products, including higher energy efficiency, better product quality, the ability to operate independent of outside ambient weather conditions and zero environmental impact(Rahman,2007).

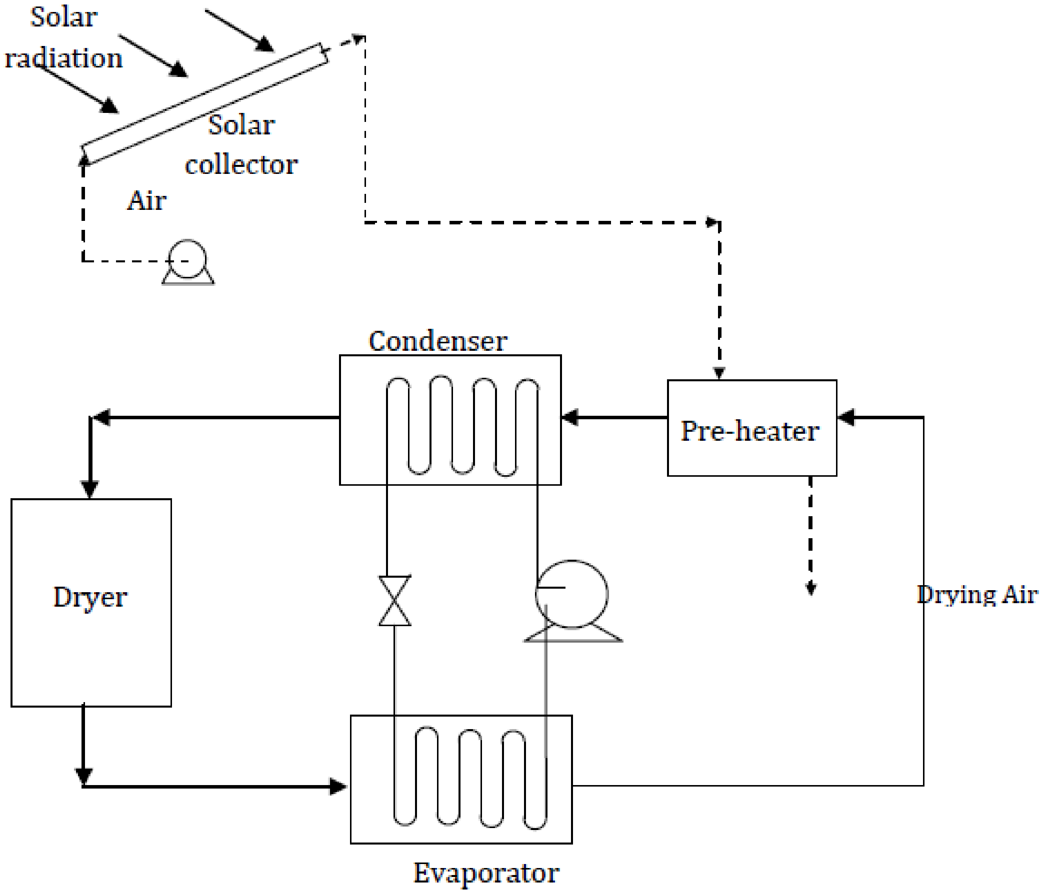

Figure 1: Solar assisted heat pump drying system

Source: (Jangam, 2011)

1.1.2.3 Modified atmosphere drying

This is practically a new concept of drying foods using heat pump dryers, which uses modified atmospheres such as nitrogen and carbon dioxide. These atmospheres help maintain better quality and preservation of constituents of foods, prone to oxidation. Technologies to create the modified atmosphere drying are now evolving. The modified atmosphere drying has weaker adverse effects on the product, especially in terms of shrinking, color change, types of molds, and peroxide values (Rahman 2007).

1.2 Solar-energy Drying

Solar drying has not yet widely commercialized. Solar dryers are equipment, generally of small capacity and based rather on empirical and semi-empirical data than in theoretical designs. The majority of the numerous solar dryer designs, which are available, are used mainly for drying of various crops either for family use or for small-scale industrial production. Solar radiation, in the form of solar thermal energy, is an alternative source of energy for drying especially to dry fruits, vegetables, agricultural grains and other kinds of material, as wood, etc. This procedure is especially applicable in the so-called "sunny belt" world wide, i.e. in regions where the intensity of solar radiation is high and sunshine duration long. It is estimated that in developing countries there exist significant post harvest losses of agricultural products, due to lack of other preservation means, that can be saved by using solar dryers.

Solar drying is an extension of sun drying that uses radiation energy from the sun. Solar drying is a non-polluting process and uses renewable energy. Moreover, solar energy is an abundant source, that cannon be monopolized. However, solar drying has some disadvantages that limit its in large-scale production. It also needs large areas of space and high labor inputs and there is again possibility of insect infestation and microbial contamination. (Rahman, 2007)

1.2.1 The principle of solar drying

Solar drying technique uses the energy from the sun to heat a stream of air, which in turn flows by natural or forced convection through a bed of the commodity to be dried.

Scientists opined that in addition to the quality improvement, heat energy from collectors could also be stored, if needed, which permits drying to continue during the night or cloudy periods (Jose, 2004).

1.2.2 Importance of solar dryers in developing countries

In comparison with developed countries has agriculture in developing countries an irreplaceable role in terms of the number of economically active population, the agricultural sector employs an average of 50% of the economically active population in developing countries (FAO, 2015). Moreover, agriculture in developing countries constituted in 2013 average 12% of GDP (World Bank, 2015).

Agricultural production in developing countries faces a number of problems that cannot be discussed here due to their extent. In general we can say that most of them are economic, technological and political character. One of the main problem is the lack of adequate technology for the processing of food, whether plant or animal origin, inappropriate cultivation methods and fertilization, poor marketing, poor logistics and high post-harvest losses. It is estimated that the above problems in developing countries have resulted in 10-40% losses in food production (Murthy, 2009).

A partial solution of this situation may be the introduction of appropriate technologies for processing of agricultural products. Here, offers primarily the drying as one of the oldest methods of post-harvest processing that is used in agriculture. Even in developing countries, it is now possible to observe an increase of small and medium-sized enterprises oriented on drying as agricultural products processing. Drying of agricultural products in developing countries mainly provides these positives (Jon and Kiang, 2008):

• increases agricultural production through the introduction of alternative products on the market,

• generates job opportunities in urban and rural areas,

• reduces the losses of harvest fresh produce (fruits, vegetables, and others)

• improves nutrition of the producers themselves (farmers) due to possible reserves of dried products outside their harvest season,

• generates a new source of income for farmers,

• creates new value-added products.

Despite the technological level of the food industry, the vast majority of agricultural products in developing countries is processed by drying directly on the sun (Imre, 2007). The technique of drying of agricultural products on the sun has a disproportionately large losses (up to 30% of total production) and it is mainly due to the factors as damage of products by domestic animals, rodents and birds during drying, exposing to the direct impact of climate (rainfall, dew, wind, etc.), contamination of products by insects, bacteria or mold.

For many reasons, suitable alternative to traditional drying on the sun in developing countries could be solar dryers. Above all, we must realize, that more than 80% of all food in developing countries is produced by small producers or small-farmers (Murthy, 2009), who do not have needful money capital, to invest it in relatively expensive sophisticated technologies for food processing. Solar dryers could be a possible options for farmers in developing countries, mainly, because of their advantages as low input and operating costs, simple construction, easy service without complicated electronic and mechanical features, more efficient compared with traditional drying methods and easy maintenance and replacement of spare parts.

Another and equal important fact, which contributes to the usage of solar dryers for agriculture products processing is changing human view in the field of usage energy in developing countries. In the last two decades, we can observe some effort on energy independence, environmental protection and interest in the usage of renewable energy sources at the expense of fossil fuels (Imre, 2007).

1.2.3 Classification of solar-energy drying systems

All drying systems can be classified primarily according to their operating temperature ranges into two main groups of high temperature dryers and low temperature dryers. However, dryers are more commonly classified broadly according to their heating sources into fossil fuel dryers (more commonly known as conventional dryers) and solar-energy dryers. Strictly, all practically-realized design of high temperature dryers are fossil fuel powered, while the low temperature dryers are either fossil fuel or solar-energy based systems.

To classify the various types of solar dryers, it is necessary to simplify the complex construction and various modes of operation to the basic principles. Solar dryers can be classified based on the following criteria:

- Mode of air movement
- Exposure to insulation
- Direction of air flow
- Arrangement of the dryer
- Status of solar contribution

Another useful classification is based on (1) the method of transferring heat to the wet solids or (2) the handling characteristics and physical properties of the wet material. The first method of classification reveals differences in dryer design and operation, while the second method is most useful in the selection of a group of dryers for preliminary consideration in a given drying problem.

A classification chart of drying equipment on the basis of heat transfer is shown in Figure 3 (Ekechukwu, 1999; Sharma et al., 2009).

This chart classifies dryers as direct or indirect, with subclasses of continuous or batch wise operation. Solar-energy drying systems are classified primarily according to their heating modes and the manner in which the solar heat is utilized (Belessiotis, 2011). In broad terms, they can be classified into two major groups, namely:

- passive solar-energy drying systems (conventionally termed natural-circulation solar drying systems) and,
- active solar-energy drying systems (most types of which are often termed hybrid solar dryers).

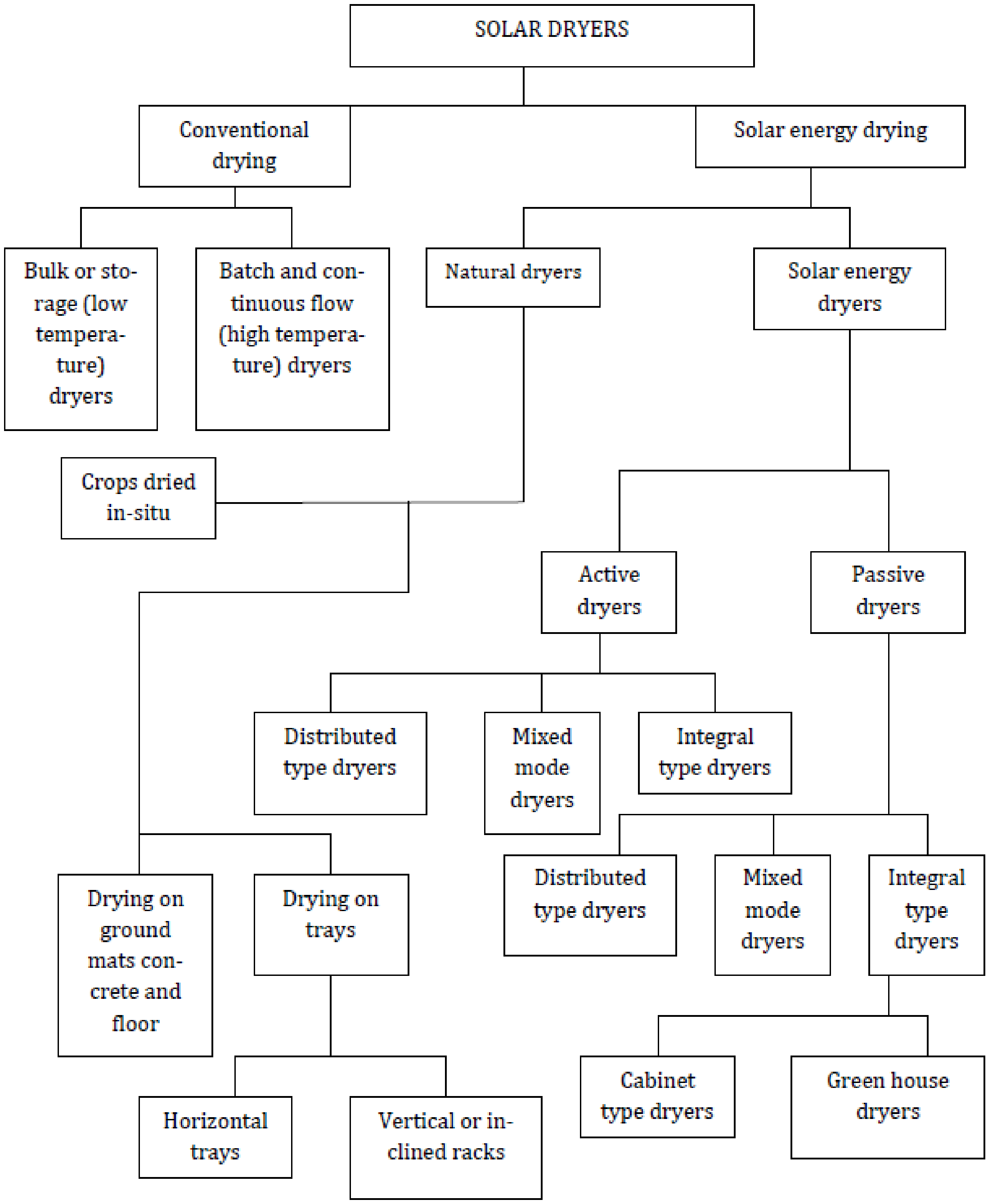

Figure 2: Classifications of dryers and drying models

Source: (Ekechukwu, 1999)

Three distinct sub-classes of either the active or passive solar drying systems can be identified namely:

- integral-type solar dryers;
- distributed-type solar dryers; and
- mixed-mode solar dryers.

The main features of typical designs of the various classes of solar-energy dryers are illustrated in Figure 3, showing three main groups for solar dryers on the basis of the energy sources used (Leon et al., 2002). The design of solar dryers is adjusted to the quantity, character, and designation of the material to be dried as well as to the energy sources used and accordingly, various types of solar dryers have been developed and are in use to date.

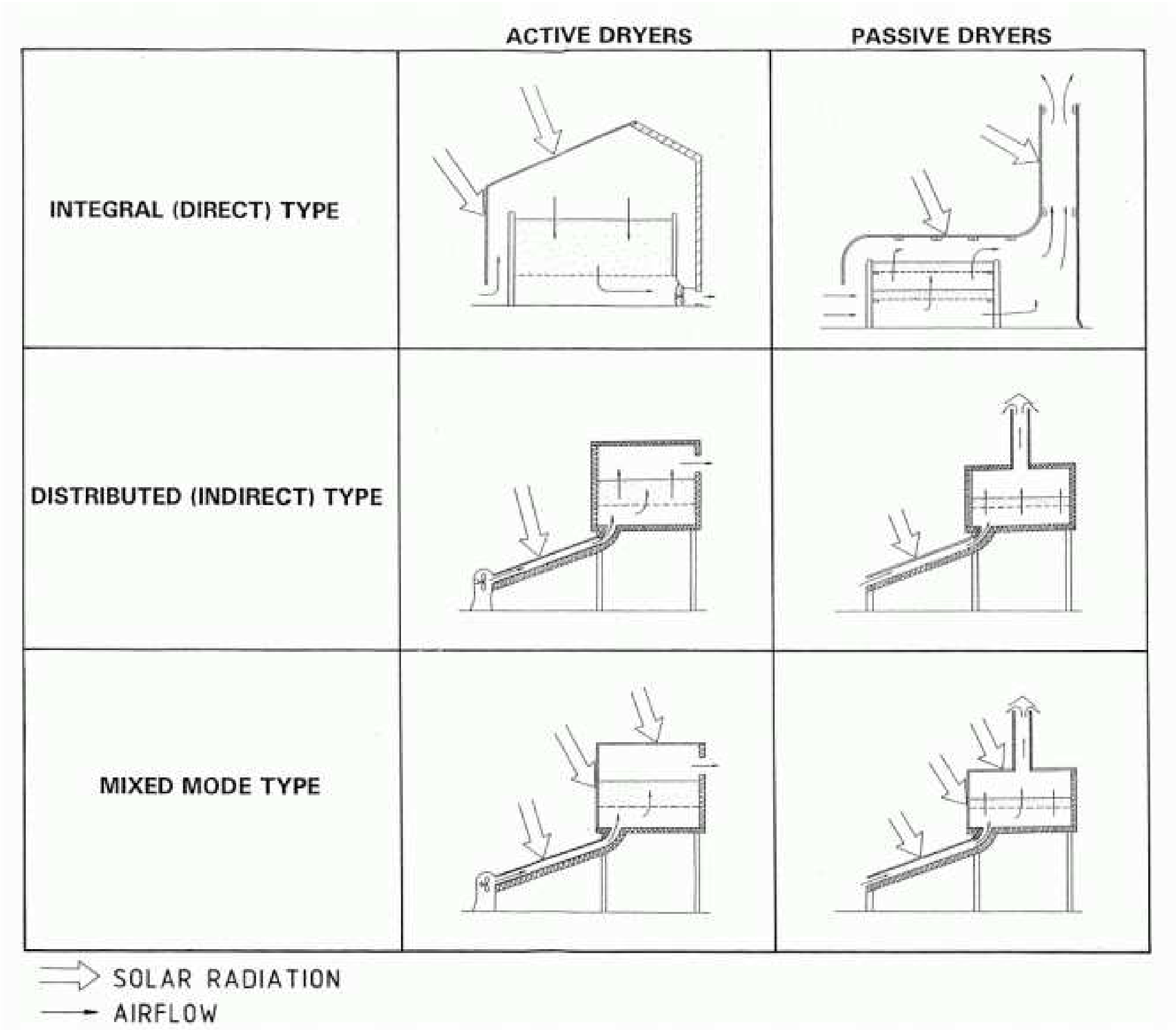

Figure 3: Typical solar energy dryer designs

Source: (Ekechukwu and Norton, 1999)

The mode of drying can be differentiated into direct and indirect, depending whether the product is directly exposed to solar radiation or dried in the shade. In direct mode, the product itself serves as absorber, i.e. the heat transfer is affected not only by convection but also by radiation according to the albedo of the product surface. Therefore, the surface area of the product being dried has to be maximized by spreading the crop in thin layers. To obtain uniform final moisture content, the crop has to be turned frequently.

It should be noted, that sunlight may affect certain essential components in the product e.g. chlorophyll is quickly decomposed. Due to the limitation of the bulk depth, such dryers need large ground surface areas. If grounds are scarce, indirect mode type of dryers are preferred for drying large quantities (Weiss, 2003).

1.2.3.1 Passive solar dryers

In a passive solar dryer, air is heated and circulated naturally by buoyancy force or as a result of wind pressure or in combination of both. Normal and reverse absorber cabinet dryer and greenhouse dryer operates in passive mode. Passive drying of crops is still in common practice in many Mediterranean, tropical and subtropical regions especially in Africa and Asia or in small agricultural communities. These are primitive, inexpensive in construction with locally available materials, easy to install and to operate especially at sites far off from electrical grid. The passive dryers are best suited for drying small batches of fruits and vegetables such as banana, pineapple, mango, potato, carrots etc. (Hughes et al., 2011).

They can be either direct (e.g. tent and box dryer) or indirect (e.g. cabinet dryer). Natural-circulation solar dryers depend for their operation entirely on solar-energy (Weiss, 2003)

Tent solar dryers are consist of a frame of wood poles covered with plastic sheet. This is the main reason, why are these dryers cheap and simple to construct. Important is, that black plastic should be used on the wall facing away from the sun. They are usually used for fruit, fish, coffee or other products for which wastage is otherwise high. Even so, they have disadvantage of being easily damaged by strong wind.

The aim of solar cabinet dryers is mainly to improve product quality by reducing contamination by dust, insect infestation, and animal or human interference. It consists of a hot box with a transparent top and blackened interior surfaces. Ventilation holes in the base and upper parts of slide walls maintained a natural air circulation (Szulmayer, 1991).

A number of other designs of passive solar cabinet dryer in configuration to that developed by Brace Research Institute have been built and tested for a variety of crops and locations. Ezekwe (1981) reported a modification of the typical design shown in Figure 4 equipped with a wooden plenum guiding the air inlet and a long plywood chimney to enhance natural circulation, accelerating the drying rate by about 5 times over open-sun drying.

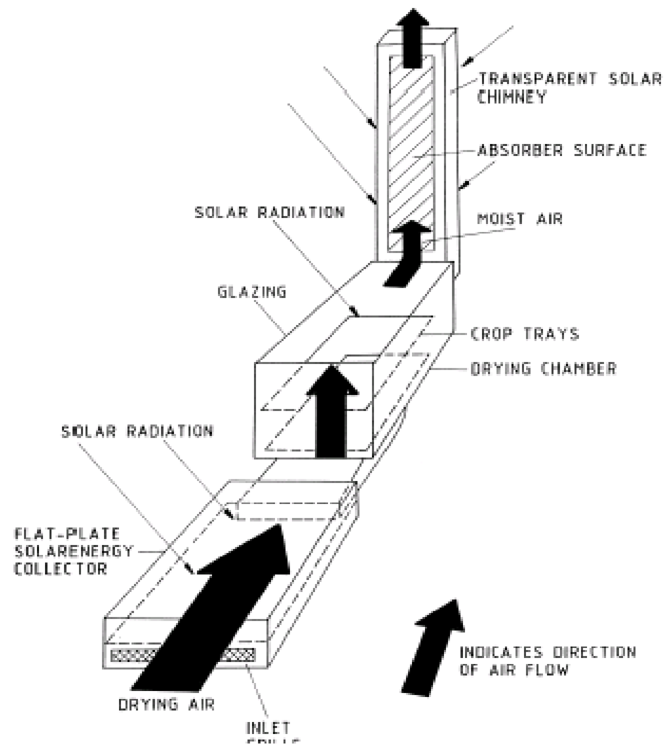

Figure 4 Features of a typical mixedmode natural-circulation solar-energy dryer

Source: Ekechukwu and Norton, 1999

A hybrid type passive solar-energy dryer would have the same typical structural features as the indirect-type and direct-type (i.e. a solar air heater, a separate drying chamber and a chimney), and in addition has glazed walls inside the drying chamber so that the solar radiation impinges directly on the product as in the direct-type dryers.

1.2.3.2 Active solar dryers

Active solar dryers system are constructed including external means, like fans, ventilators or pumps, for moving the heated air, as a solar energy, from the collector area to the drying beds. By their application, all active solar dryers are forced convection dryers. A classical active solar dryer depends on solar energy only for the heat source, while for air circulation uses motorized fans or ventilators. These dryers are commonly used in large-scale commercial drying operations in combination with convectional fossil-fuel to have a better control over drying by inhibiting the effect of fluctuations of solar insolation on the drying air temperature. Active solar dryers are known to be suitable for drying higher moisture content foodstuffs such as papaya, kiwi fruits, brinjal, cabbage and cauliflower slices. A variety of active solar-energy dryers could be divided into either direct-type, indirect-type or hybrid dryers. (Visavale, 2012)

Indirect-type active solar drying systems

These active dryers have a separate collector and drying unit. They are usually composed of four basic components viz., a solar air heater, drying chamber, a fan for air circulation and ductings (Visavale, 2012). Figure 5 shows a typical in-direct active solar dryer.

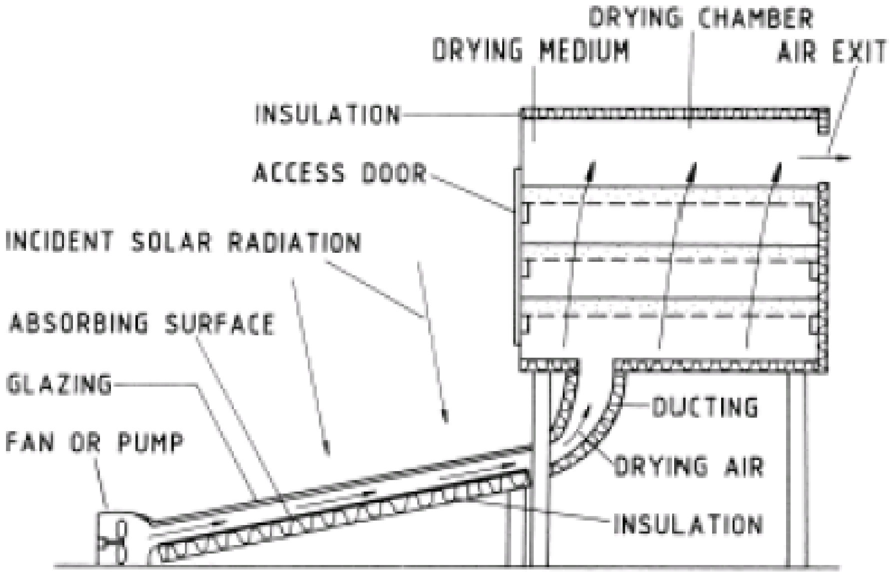

Figure 5 Features of a typical distributed-type active solar energy dryer

Source: Ekechukwu and Norton, 1999

The direct-type active solar drying systems are designed with integral solar energy collection unit. Generally, three distinct designs of direct-type active solar dryers can be classified viz., the absorption type, storage type and greenhouse dryers (Visavale, 2012).

The hybrid solar dryers combine the features of a solar energy with a conventional or some auxiliary source of energy and can be operated either in combination or in single mode with either source of energy. These dryers generally are medium to large installations operating in the range of 50-60%, and compensate the temperature fluctuations induced by the climatic uncertainties (Visavale, 2012).

1.2.4 Enzymatic browning during solar drying

Many crops, fruits and vegetables are immediately after harvest treated and dried. Pretreatment helps to slow down the activity of enzymes.

Drying is applied to prolong the fruit shelf life. However, the appearances of dehydrated fruit may change significantly owing to the Maillard reaction, pigment degradation, enzymatic browning and ascorbic acid oxidation during drying (Manzocco 2001).

Solar drying conditions play an important role in determining the quality of the final product, especially in terms of its antioxidant activity, colour and pro-healthy properties. Therefore it is very important to choose optimal drying method for food. The reduced levels of the polyphenolic compounds or other active compounds found in dried food were related in correlation to the antioxidant activity, and indicated that the decrease of antioxidant activity resulted from the degradation of biologically active compounds at high temperatures, due to chemical, enzymatic or thermal decomposition (Nicoli, 1999).

Moreover, in forced convection drying, phytochemical compounds can oxidise because the plant material has greater contact with oxygen. Direct exposure to the hot air also destroys the colour, vitamins, and flavour of fruits, therefore new and innovative techniques that increase the drying rate and enhance product quality have achieved considerable attention in the recent past (Chong, 2013)

1.3 Enzymatic browning and pre-treatment of food before drying

1.3.1 Browning reaction

Browning reactions induce changes in the dried material such as colour and texture change, nutritional value decline and formation of off-flavours. Two types of browning reactions are recognized: enzymatic and non-enzymatic. Concerning the drying process, the non-enzymatic reactions are more significant. They include two basic types of reaction: caramelization and Maillard browning. The rate of non-enzymatic reactions is influenced by moisture level, temperature, pH and the composition, whereby the highest browning rate is reached in the intermediate moisture range and declines with increasing moisture.

Browning reaction requires oxygen, phenolic compound and polyphenol oxidizes and is usually initiated by the enzymatic oxidation of monophenol into O-diphenol and O-diphenol into quinines, which undergo further non-enzymatic polymerization lead to the formation of pigments (He et al. 2008). Enzymatic browning is a result of the action of endogenous polyphenol oxides (enzymatic browning) followed by the spontaneous polymerization of quinonoid compounds with other food components (Iyengar R. 1992).

Enzymatic browning may occur in many fruits and vegetables. When the tissue of fruits is cut or pealed, it rapidly darkens on expose to air. As a result, conversion of phenolic compounds to brown melanin occurs. The browning phenomenon usually impairs the sensory properties of products because of the associated changes in color, flavor and softening. The enzymes involving the browning reaction are monophenol monoxyganese or tyrosinase, diphenol oxidase or catachol oxidase polyphenol oxides. For mushroom, browning occurs as a result of two distinct mechanisms of phenol oxidation which is activation of tyrosinase. Tyrosinase is an enzyme belonging to the polyphenoloxidase family or spontaneous oxidation (Martinez and Whitaker 1995; O. Nerya et al 2006).

1.3.2 Polyphenoloxidase

Polyphenol oxides catalyses two basic reactions (shown in Figue 5) which is hydroxylation to the o-position adjacent to an existing hydroxyl group of the phenolic substrate and oxidation of diphenol to o-benzoquinones. Both reactions utilize molecular oxygen as a co-substrate. Whether a single enzyme system exhibits both monoand di- phenol oxides activities is still unclear. However, when both monophenol- and diphenol oxidizes are present in plants, the ratio of monophenol to diphenol oxides activity is usually 1:10 or as low as 1:40 (Marshall et al., 2000).

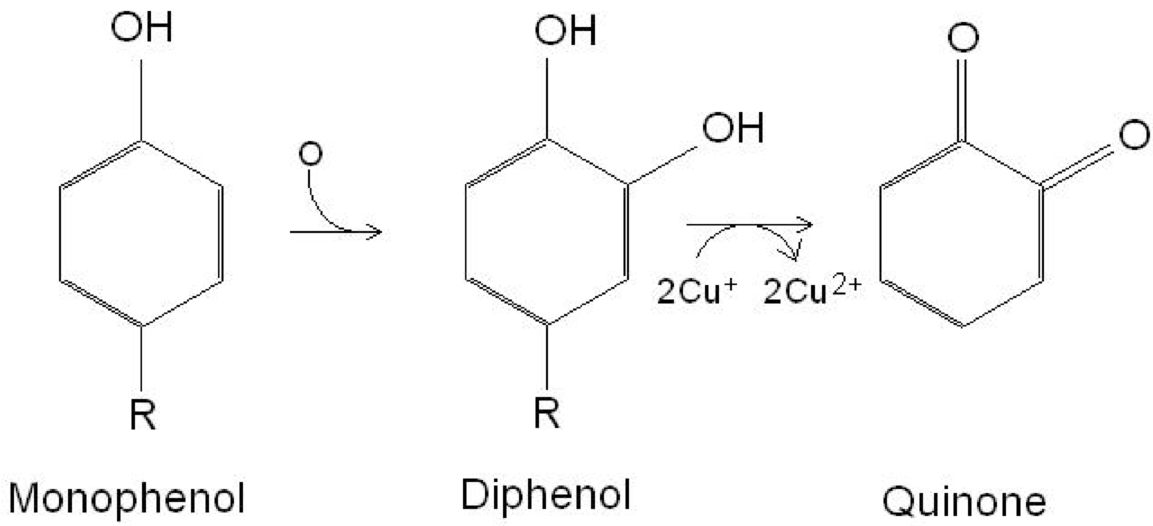

Figure 5: The reactions catalyzed by polyphenol oxidase in plants. First the hydroxylation of monophenol to a diphenol. Then the oxidation of diphenol to a quinone

Source: Queiroz, 2008

1.3.3 Postharvest technologies and enzymatic browning

Postharvest technologies have allowed food industries to meet the global demands of local and large-scale production and intercontinental distribution of fresh produce that have high nutritional and sensory quality. Harvested products are metabolically active, undergoing ripening and senescence processes that must be controlled to prolong postharvest quality. Inadequate management of these processes can result in major losses in nutritional and quality attributes, outbreaks of foodborne pathogens and financial loss for all players along the supply chain, from growers to consumers.

Optimal postharvest treatments for fresh produce seek to slow down physiological aging of senescence and maturation, reduce/inhibit development of physiological disorders and minimize the risk of microbial growth and contamination. In addition to basic postharvest technologies of temperature management, an array of others have been developed including various physical (heat, irradiation and edible coatings), chemical (antimicrobials, antioxidants and anti-browning) and gaseous treatments. (Mahajan, 2014)

1.3.4 Pretreatments in food drying

Pretreatments are processes that reduce darkering of dryed fruits, especially the ones with ligh-coloured pulp, such as apples, which brown very rapidly in contact with air and even continue darkering after having been dried (Unido, 2015).

Drying of food is connected with many disadvantages, such as browning, loss of cellular structure, loss of redrying ability and case hardening. These changes negatively change the market value and also acceptability of the products. Pre-treating will help maintain colour (reduces oxidation of foods such as fruits and vegetable keeping them from turning a rust-like colour), nutrients (helps retain the minimal loss which tends to occur during the drying process) and texture (the change for fibre deterioration in the produce is lessened. There are various methods of pre-treatment applied in fruit drying, e.g. lemon juice, slat solution, honey dip, ascorbic acid, sulphuring, osmotic pre-treatment, and blanching.

For long-term storage of dried fruit, sulphuring or using a sulphite dip are the best pretreatment. However, sulphites found in the food after either of these treatments have been found to cause asthmatic reactions in a small portion of the asthmatic population. Thus, some people may want to use the alternative shorter-term pre-treatment (Abano and Sam-Amoah, 2011). There are two basic types of pre-treatments: dipping and blanching (Chadwick, 1995)

1.3.4.1 Dipping

Dipping is the treatment, which is usually used to prevent fruits and vegetables from oxidation.

- **Sulphuring, sulphite dip**

Sulphuring is an old method of retreating fruits. Sublimed sulphur is ignited and burned in an enclosed box with the fruit. The sulphur fumes penetrate the fruit and act as a pretreatment by retarding spoilage and darkening of the fruit. The sulphur fumes also reduce the loss of vitamins A and C. The same effect as sulphuring has sulphite dips in thatr it prevents the darkering in lorg term. Moreover, this procces is quicker and easier. Either sodium bisulphite, which is a liquid form of sulphur, sodium sulphite or sodium metabisulphite that are food grade or Reagent grade (pure) can be used for dipping when mixed with water. It is the cheapest, but most effective antioxidant. Only a food-safe sodium bisulphate designated for dehydration should be used (Unido, 2012).

- **Ascorbic Acid**

Ascorbic acid (vitamin C) mixed with water is a safe way to prevent fruit browning. However, its protection does not last as long as sulphuring of sulphating. Ascorbic acid reacts easily with oxygen and diminish oxygen that will be used by phenolase. Ascorbic acid also reduces the o-quinones formed by phenolase to the original o-dihydroxyphenolic compounds. Protection aga inst browning last as long as any ascorbic acid remains. Ascorbic acid (vitamin C) is not satisfactory for apples since internal atmosphere of the slices contains oxygen (Unido, 2012). Ascorbic acid (vitamin C) is present in all citrus fruits, from which dips are prepared in different formy (in crystal, powder or in tablet). The fruit is then soaked in an ascorbic acid solution for 10 minutes (Chadwick, 1995).

- **Ethyl oleate dip**

Ethyl oleate increase the evaporation rate of water in the initial stages of drying, acts as surfactant by increasing the spreading of free water within the sample and remove the skin wax / cell wall of certain products such as grapes, maize grains, starch pastes (Unido, 2012).

- **Honey dip**

Many store-bought dried fruits have been dipped in a honey solution. A similar dip can be made at home. Honey dipped fruit is much higher in calories. Honey dips are applier quite commonly of dried fruits.. Dipping fruit in honey has the consequences of adding sweetness as well as energetic value to the final product (Chadwick, 1995).

1.3.4.2 Blanching

Blanching, as means of pre-treatment is sometimes referred to as "checking" or "crazing". It can be done either in boiling water or by steaming the foods. This method is most effective on tough-skinned fruits such as grapes, plums, cranberries, etc. By boiling fruits in water for 1 to 2 minutes, the skin "cracks" and allows the moisture to escape more quickly, thus reducing the drying time.

Blanching can achieve a renge of objectives, depending on the aplication. For many fruits and vegetable as well as seafoods that are frozen, canned, or dehydrated, blanching inactives enzymes and reduces microbial loads to improve food safety and increase shelf life. For potatoes that become Franch fries, blanching also leaches sugars to improve product texture and color. For some meat and poultry products as well as bagels and potato products that are ready-to-eat, blanching cooks the products. For nuts, blanching can facilitate peeling or kill microbes such as salmonella, depending on the temperature and duration of the blanching operation. Within each of these applications, a food processor has a variety of water blanching and steam blanching technologies to choosen from (Johnson, 2011).

- **Steam blanching**

With steam blanching, product is exposed directly to food-grade steam that is typically 212^{o}F (100^{o}C) as it is conveyed within a chamber. Some steam blanchers use convection technology that forces the steam through the bed of product in a single layer to achieve quick blanching. To minimize the product’s exposure to heat, some steam blanchers follow the heat penetration stage with a holding stage that allows the core temperature of the product to rise without the addition of more stream (Johnson, 2011).

Steam blanching also helps retain colour and slow oxidation. However, the flavour and texture of the fruit is changed.

- **Water blanching**

Both immersion and deluge water blanching, whether achieved with a rotary, auger, double draper or belt-conveying system, have one thing in common: product is exposed directly to food-grade water, that is typically ranges in temperature from 158^{o}F to 212^{o}F (70^{o}C to 100^{o}C) (Johnson, 2011).

- **Electric steamers**

Electric steaming is probably the best way of blanching. The electric steamer preserves the nutrients as well as the colour of the food and eightens the natural flavour of the product (Chadwick, 1995).

2 Objectives

This study is focused on literature review and screening of potential natural preservatives, which inhibit enzymatic browning of food. The natural compounds are selected as potential anti-browning preservatives, which may be used as pretreatments during hot air and solar drying of food.

3 Methodology

Bachelor thesis is conceived as a literature review focused on the collection and comparison of scientific data. Collecting of these data was provided through internet sources to the extent that they have explanatory value about commonly used organic substances, which inhibit browning effect of food during storage and during drying. Sources of scientific literature were searched mainly through Web of Science, Scopus and Google Scholar. The majority of the scientific literature was in English.

4 Results and discussion

We have analyzed 33 natural compounds, which are mostly used for their inhibitory effect on polyphenoloxidase activity, which causes enzymatic browning in foods during processing. All of these compounds have got at least some inhibitory effect on polyphenoloxidase activity. It cannot be clearly presented, which of these substances is the most effective, because it depends on more conditions, than those evaluated in this study such as kind of food, different methods of application of the treatment substance and its concentration

However it was found, that the most commonly used and also the most effective natural compounds, which inhibited the polyphenoloxidase activity include Ascorbic acid, which demonstrated 100% inhibitory effect in the combination with *Brassicaceae* extract (Altunkaya and Gökmen, 2009). Ascorbic acid is also very effective in combination witch *Rosa canina* extract, inhibitory effect in this case was 95% (Federico Zocca et al., 2011). On the other hand, Altunkaya (2011) shown, that effect of Ascorbic acid acts more as an antioxidant than as an enzyme inhibitor because it reduces the initial o-quinone formed by the enzyme to the original diphenol before it undergoes secondary reaction which leads to browning.

Another effective acids are: Citric acid, Kojic acid, Cinamic acid, Benzoic acid, Phytic acid, which also shown 100% inhibitory effect on PPO activity in apple juice (Cheema and Sommerhalter, 2015).

Another organic compound, which proved to be a good inhibitor, which act as antioxidant is 4-hexylresorcinol that inhibited polyphenoloxidase activity, for example, in red delicious apple to 12.4% (Hesham A. Eissa, 2006).

Martı´nez-Alvarez (2007) shown, that spraying of 4-hexylresorcinol based formulation to prevent enzymatic browning in Norway lobster during chilled storage, inhibit polyphenoloxidase aktivity to 9%.

Yaguang Luo et al.(2011) described anti browning effect of calcium ascorbate, which completely inhibited PPO activity in apple slices, after dipping in treatment solution for one minute.

All investigated substances are summarized in table 1, where are shown different effects depended on used foodstuff, method of application and on the most effective concentration.

According to the theoretical knowledge about natural compounds, which have got inhibitory effect on enzymatic browning we conclude, that some of founded compounds could be potential alternatives to the commercially used treatments such as sulfites, whose sides effects on human health were many time discussed in the literature. For example they can cause breathing difficulty within minutes after eating a food containing it, due to allergic reaction (California Department of Public Health, 2015). Byatt (2011) stated that sulfites and related compounds are powerful inhibitors of enzymatic browning, but their use is limited since they can produce adverse reactions in a significant proportion of the population. Safe and effective alternatives to sulfites could be very useful to the food processing industry.

Table 1
Comprehensive overview of organic substances inhibiting enzymatic browning

Name of active substance	Used foodstuff	Method of application	Most effective concentration	Effect	Reference
Ascorbic Acid	Fresh lettuce	homogenized in 9 ml of distilled water	0.50%	Temporary inhibition of browning	1
	Red delicious apples	dipped in the treatment solution for 5 min	-	inhibit PPO activity to 11.4%	6
	Red delicious apples	dipped in the treatment solution for 5 min	0.5mg/100ml water	inhibit PPO activity to 63.5%	17
	Rojo Brilante	dipped in the antioxidant solution for 3 min	1.12%	effective protection against PPO	2
	Potato	combined with reaction medium	100 µl of 0.5 g/l AA	inhibit PPO activity to 15.6%	7
	Apple jucie	mixed with crude PPO extract	0.05mM	inhibit PPO activity to 14%	14
+ Dog rose extract	Artichokes	combined with reaction medium	100 µl DRE and 100 µl of 0.5 g/l AA	inhibit PPO activity to 5.04%	7
+ Pomegranate	Artichokes	combined with reaction medium	100 µl PGE and 100 µl of 0.5 g/l AA	inhibit PPO activity to 50.13%	7
+ BPW	Grape	combined with reaction medium	10 mg LPW /1.0 ml of DW and 100 µl of 0.5 g/l AA	100% inhibition of PPO	26
Citric Acid	Fresh lettuce	homogenized in 9 ml of distilled water	0.50%	inhibitory effect on PPO activity	1
	Rojo Brilante	dipped in the antioxidant solution for 3 min	0.21%	inhibitory effect on PPO activity	2
	Red delicious apples	dipped in the treatment solution for 5 min	-	inhibit PPO activity to 31.2%	6
	Apple jucie	mixed with crude PPO extract	10mM	inhibit PPO activity to 59%	14

Table 1
Comprehensive overview of organic substances inhibiting enzymatic browning (Table continuing)

Name of active substance	Used foodstuff	Method of application	Most effective concentration	Effect	Reference
	Ataulfo mango extract	combined with reaction medium	20mM	100% inhibition of PPO	21
Kojic Acid	Amasya apple juice	combined with 50 ml of jucie	4mM	inhibit PPO activity and bleaches melanin	11
	Ataulfo mango extract	combined with reaction medium	20mM	100% inhibition of PPO	21
Cinamic Acid	Golden delicious apples	added to apple jucie	1mM	prevent browning	22
Cinamic Acid+XG	Pear slices	coated with solution	XG powder: 2.5 g/L+CA: 1g/L	inhibited PPO activity approx. by three times	19
Oxalic Acid	Fresh lettuce	homogenized in 9 ml of distilled water	0.50%	inhibit PPO activity by chelating copper	1
Benzoic Acid	Ataulfo mango extract	combined with reaction medium	20mM	inhibit PPO activity to 44%	21
Salicylic Acid	Chinese chestnut	mixed with enzyme extract solution	0.5g/L	inhibit PPO activity to 46.3%	4
Phytic Acid	Apple jucie	mixed with crude PPO extract	0.1mM	100% inhibition of PPO	14
EDTA	Ataulfo mango extract	combined with reaction medium	20mM	inhibit PPO activity to 23%	21
4-hexyl resorcinol	Red delicious apples	dipped in the treatment solution for 5 min	0.1 mg/100ml water	inhibit PPO activity to 12.4%	17
	Norway Lobster	spraying of 4-hexyl resorcinol based formulation	0.05%	inhibit PPO activity to 9%	20

Table 1
Comprehensive overview of organic substances inhibiting enzymatic browning (Table continuing)

Name of active substance	Used foodstuff	Method of application	Most effective concentration	Effect	Reference
	Apple jucie	combined with apple juice	0.35g/L	100% inhibition of PPO	25
Acidified Sodium Chlorite	Apple slices	dipped in the solution for 1 min	300 mg L^{-1}	100% inhibition of PPO	15
Calcium Chlorite	Apple slices	dipped in the solution for 1 min	20g L^{-1}	inhibit PPO activity to 21%	15
N-acetyl cysteine	Loquat juice	mixed with ChA (1.0mM of final concentration)	0.3mM	100% inhibition of PPO	16
Cysteine L-glucose	Red delicious apples	dipped in the treatment solution for 5 min	500 mL/100ml Water	inhibit PPO activity to 15.5%	17
L-cysteine	Loquat juice	mixed with ChA (1.0mM of final concentration)	0.3 mM	inhibit PPO activity to 20%	16
	Red delicious apples	dipped in the treatment solution for 5 min	0.5 mg/100ml Water	inhibit PPO activity to 29.4%	17
	Golden delicious apples	added to apple jucie	1-1.8 mM	inhibitor of PPO, but this concentration produced undesirable odour and a bleaching effect	22
Reduced Glutathione	Red delicious apples	dipped in the treatment solution for 5 min	0.5mg/100ml Water	inhibit PPO activity to 18.2%	17
Glutathione	Loquat juice	mixed with ChA (1.0mM of final concentration)	0.2mM	inhibit PPO activity to 25%	16

Table 1

Comprehensive overview of organic substances inhibiting enzymatic browning (Table continuing)

Name of active substance	**Used foodstuff**	**Method of application**	**Most effective concentration**	**Effect**	**Reference**
	Ataulfo mango extract	combined with reaction medium	2mM	100% inhibition of PPO	21
	Grape juice	combined with 0.04% glutathione solution	100 mL of 0.04% GTS and 100 mL PPO extract	100% inhibition of PPO	24
Calcium Ascorbate	Apple slices	dipped in solution for 1 min	20g L^{-1}	100% inhibition of PPO	15
Sodium Ascorbate	Ataulfo mango extract	combined with reaction medium	2mM	100% inhibition of PPO	21
Allium, Brassica extracts	Avocado pulp	lyophilized vegetable extract were added to final concentration	10g/100g	inhibited PPO activity up to 15 days of refrigeration	5
Grape seeds extract	Fresh lettuce	combined with reaction medium	0.75mg/L	inhibit PPO activity to 50%	8
Grape leaves extract	Fresh lettuce	heated GLE combined with reaction medium	90mg/mL	inhibit PPO activity to 45%	9
Onion extract	Taro	dipped in heated onion extract	3.1mg/L	inhibit PPO activity to 46.2%	12
Honey	White cabbage	added to 2g of homogenate and 3 ml of distilled water	60g/L	reduced browning activity to 31%	10
PFH	Apple jucie	combined with juice	3.5g/L	inhibit PPO activity to 14.5%	25
Oil from pine leaves	Fresh lettuce	dipped in 1% phytoncide essential oil diluted with ethanol	-	inhibiting effect against PPO and POD activity	13
Whey protein concentrate	Fresh lettuce	added to 3g of homogenate and 9 ml of distilled water	28mg/L	inhibit PPO activity to 40%	18

Table 1

Comprehensive overview of organic substances inhibiting enzymatic browning (Table continuing)

Name of active substance	Used foodstuff	Method of application	Most effective concentration	Effect	Reference
Arginine+GLC	Apple pureé	added to 10g of homogenate	1 mL	inhibit PPO activity to 25%	23
	Potato cubes	dipped in solution	1 mL	inhibit PPO activity to 49%	23
Histidine+GLC	Apple pureé	added to 10g of homogenate	1 mL	inhibit PPO activity to 48%	23
	Potato cubes	dipped in solution	1 mL	inhibit PPO activity to 47%	23

Note: EO: electrolyzed, DW: Distilled water, PPO: popyphenol oxidase, GLE: grape leaves extract, DRE: Dog rose extract, PGE: Pomegranate extract, ChA: Chlorogenic Acid, XG: Xanthan gum, CA: Cinamic Acid, EDTA: Ethylenediaminetetraacetic acid, GLC:Glucose, PFH: Palo fierro honey extract, (L)BPW: (lyophilized) Brassicacaea processing water, mM: millimolar (10^{-1} mol/dm3), L^{-1} = L

(1) Altunkaya and Gökmen, 2009; (2) Ghidelli et al., 2013; (3) Rojas et al., 2008; (4) Zhou et al., 2015; (5) Bustos et al.,2005; (6) Kim and Hung, 2014; (7) Zocca et al., 2011; (8) Altunkaya and Gökmen, 2012; (9) Altunkaya, 2014; (10) Nyawali et al., 2015; (11) Iyidogan and Bayındırlı, 2004; (12) Lee, 2007; (13) Kim et al., 2014; (14) Du et al., (2012); (15) Luo et al., 2011; (16) Ding et al., 2002; (17) Eissa et al., 2006; (18) Altunkaya, 2011; (19) Sharma and Rao, 2014; (20) Alvarez et al., 2007; (21) Cheema and Sommerhalter, 2015; (22) Özoglu and Bayındırlı, 2002; (23) Mogol et al., 2010; (24) Wu, 2014; (25) Rosa et al., 2011; (26) Zocca et al., 2010.

5 Conclusion

Enzymatic browning is a significant problem during food processing. Many scientists deals with finding the most suitable natural substance that can inhibit popyphenoloxidase activity, which cause enzymatic browning.

The aim of this thesis was to present a comprehensive overview of natural preservatives, which inhibit enzymatic browning effect during storage. Thirty-three compounds have been found and summarized in this thesis.

According to comparison of all founded natural compounds, we are in agreement with the majority of published studies, that ascorbic acid is the most effective antibrowning agent, especially in combination with some other natural compounds. Also citric and kojic acid showed very good results in inhibition of enzymatic browning.

We suppose, that natural compounds, whose effect against enzymatic browning were proved during storage and which are summarized in the Table 1, have got some potential to be used in drying industry, where they can be used as pre-treatments of dryed products during hot-air drying and solar drying.

Due to our literature research, we can state that there is no similar summary of antibrowning agents as in this thesis. That is why more studies focused on this issue are needed.

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