Czech University of Life Sciences Prague

Institute of Tropics and Subtropics

Department of Sustainable Techologies

Diploma thesis

Influence of selected natural juices containing ascorbic acid on browning inhibition during fruit drying

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DECLARATION

I, Anna Hubáčková, declare that this thesis, submitted in partial fulfilment of requirements for the master degree, in Institute of Tropics and Subtropics of the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

April 4th 2012

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Anna Hubáčková

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ABSTRACT

Drying is one of the most common food conservation techniques. However, during the process of drying, the colour of the surface of dried foods undergoes often undesirable changes. These changes are caused by non-enzymatic and enzymatic browning. The non-enzymatic browning mostly results from reactions between saccharides and proteins (so called Maillard reaction). The enzymatic brownign is in apples caused by enzymes with polyphenoloxidase activity, that catalyse oxidation of phenolic compounds in apples, when the plant tissue is disturbed and molecular oxygen is present. The products of oxidation – *o*-quinones then rapidly give rise to coloured pigments through polymeration.

'Dipping' and 'blanching' are techniques that can be used to slow down the enzymatic browning. 'Dipping' involves soaking the foods (apples) 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. In this thesis, dipping in natural juices with a hight ascorbic acid content was used. The species, whose fruits were selected for providing the juice are: *Sorbus aucuparia*, *Diospyros kaki* L., *Hippophae rhamnoides* L., *Actinidia deliciosa* and *Rosa canina* L. The effect of these juices on colour change compared to a freshly cut apple was evaluated through colorimetric methods. The effect of juices was also evaluted via sensory analysis panel, that focused on colour change, degree of browning, change of taste and overall acceptability of resulting colour and taste.

It is obvious from the results of both analyses, that out of the fruits tested, enzymatic browning is best suppresed by a macerate from *Rosa canina* L.

Discovery of natural, yet effective supressant of enzymatic browning greatly widens the possibilities of non-invasive food preservation.

KEY WORDS:

Apple, drying, browning, inhibition of browning, ascorbic acid, colorimeter

ABSTRAKT

Sušení patří mezi jednu z bežných konzervačních technik. V průběhu procesu sušení a uchování potravin se ale mění barva povrchu. Změna barvy je způsobena neenzymatickým a enzymatickým hnědnutím. Neenzematické hnědnutí je zapříčeneno hlavně reakcemi sacharidů s bílkovinnými proteiny (tzv. Maillradova reakce). Enzymatické hnědnutí je způsobeno oxidací fenolových sloučenin při porušení pletiva fenoloxydásami za přítomnosti kyslíku. Vzniklé o-chinonové sloučeniny následně polymerují za vzniku barevných pigmentů. 'Máčení' a 'blanšírování' patří mezi techniky, které se mohou využít při zpomalené enzymatického hnědnutí. Máčením se rozumí máčení potravin (jablek) v kapalinách a cílem tohoto procesu je potlačení aktivity enzymů, buď fyzicky zabráněním přístupu kyslíku, nebo jinak inhibováním enzymové activity. V této práci jsou potraviny máčeny v přírodních šťávách, které obsahují zvýšené množství kyseliny askorbové.

Plody, které byly pro přírodní šťávy vybrány jsou: *Sorbus aucuparia*, *Diospyros kaki* L., *Hippophae rhamnoides* L, *Actinidia deliciosa* and *Rosa canina* L. Vliv těchto šťáv na změnu barvy usušených jablek v porovnání s jablky čestvými byl vyhodnocen kolorimetrickým měřením. Vliv šťáv byl vyhodnocen i senzorickým hodnotícím panelem, který se zaměřil na změnu barvy, míru hnědnutí, změnu chuti a celkovou přijatelnost barvy a chuti.

Z výsledků kolorimetrického a senzorického hodnocení je zřejmé, že z testovacích šťáv je enzymatické hnědnutí nejvíce potlačeno při použití výluhu z plodů *Rosa canina* L. Nalezení přírodní, nicméně efektivní metody potlačení enzymatického hnědnutí značně rozšiřuje možnosti šetrné konzervace jídla.

KLÍČOVÁ SLOVA:

Jablka, sušení, hnědnutí, zamezení hnědnutí, kyselina askorbová, kolorimetr

PREFACE

Fruit is an importnat part of human nourishment, as it contains a number of beneficial and essential compounds, including sacccharides, vitamins, minerals, fiber and natural pigments. To secure the supply of fruit throughout the whole year it is necessary to store the fruit properly and to preserve it. Some of the oldest types of preserving foods are change of temperature and removing water from the matrix. However, through the drying process, fruit may lose much of its natural texture and colour. The colour change is caused by both enzymatic and non-enzymatic browning. One of the methods to suppress enzymatic browning is through the use of ascorbic acid. Since ascorbic acid may be difficult to obtain, however, the author attempts to find natural juices whose ascorbic acid content is high enough to suppress enzymatic browning during the drying process and subsequent storage.

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LIST OF ABBREVIATIONS

EB	Enzymatic browning
NEB	Non-enzymatic browning
AA	Ascorbic acid
PV	Peroxide values
HPD	Heat pump dehumidifier
MAHPD	Modified atmosphere heat pump dehumidifier
PPO	Polyphenol oxidase
PVPP	Polyvinyl Polypyrrolidone
POP	Phenolic Compounds and Oxidases

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1 INTRODUCTION

1.1 DRYING

Food mostly consists of various and complex biological materials, that serve as vital sources of energy and nutrients for humans (CHEN, 2008).

Drying, or dehydration, of foods is one of the most common methods used for preserving food. Its discovery and spread allowed people to become les 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 (VEGA-GÁLVEZ, 2011). As a method of food preservation, drying is used mainly for protection against spoilage caused by microorganisms and against pathogenic bakteria (CHEN, 2008).

The crucal part of the drying process is the removal of excess water from the food matrix. Because most biochemical reactions in biological materials need a certain level of humidity to occur, this removal o water in effect helps to control the activity of bioactive compounds and microorganisms in foodstuffs. Achieving a 'safe' moisture level can significantly lengthen the shelf-life of foods and can help to maintain their 'acceptable-to-excellent' edible status. The miscellaneous components of foods, such as proteins, fats and saccharides, vitamins, probiotics and various enzymes are also affected (CHEN, 2008).

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 chemici 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 maximise enzyme and microbial inactivation. On the other hand, when drying bacterial cultures, enzymes or vitamins, we seek to achieve little to no inactiavion (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. For example, drying in hot air, is usually not suitable for drying fruits and vegetables, since their high water content requires long drying times, which in turn lead to significant ganges in structure and colour and loss of nutrients (VEGA-GÁLVEZ, 2011).

The conventional method for dehydrating foods is heating air using an electric heater or a flue gas. In the case of a flue gas method, the heat transfer occurs primarily through convection. In general, the selecton of heating method influences greatly both energy cost and quality of drying and as such is a significant aspect of the drying. Microwave, radio frequency waves, infrared, refractance window and dielectric heating are examples of rating methods that use the electromagnetic wavelength spectrum, as these waves are a form of energy that interacts directly with matter. This interaction generates heat and greatly increases the drying rate. Recent developments have seen electrotechnology's rise to prominence in the field of drying, as it is able to considearbly improve both quality and efficiency of drying. Most drying processess fall into one of three broad, but distinct categories, differing from one another in the method used for removing water:

- thermal drying
- osmotic dehydration
- mechanical dewatering

Osmotic dehydration and mechanical dewatering are beyond the scope of this thesis, hence their description will be brief. In osmotic dehydration, a solvent or solution is applied to remove water by the process of osmosis, while in mechanical dewatering physical force, mainly through press, is used (RAHMAN, 2007).

1.1.1 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

On the drying curve, the drying rate is commonly represented as a function of drying time or water content. The curve exhibits three main stages of drying:

- Transient early stage, during which the product is heating up (transient period)
- Constant or first period, in which moisture is comparatively easy to remove
- Falling or second period, in which moisture is bound or held within the solid matrix (RAHMAN, 2007)

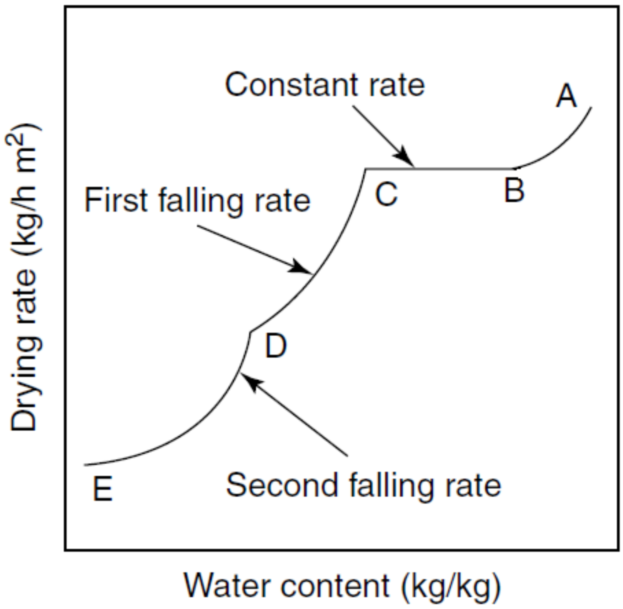

Figure 1.1 Typical drying rate curves: drying rate versus water content**Chyba! Nenalezen zdroj odkazů.**

Source: RAHMAN, 2007

1.1.2 AIR-DRYING METHODS

- **Convection Air drying**

Convection pneumatic dryers are utilized especially during industrial processing of cereals (wet milling of wheat and corn) as they are very useful and easily applicable for drying of fine-grained materials due to simple construction and low energy demand. The convection dryers are designed in a way to allow pneumatic transport of the wet material and its drying to occur concurrently. The particles of the processed material reach the size of (0.05-2.00) mm and its concentration is (0.05-2.00) kg of material per 1 kg of air. The material is dried by a heated agent (air or gas) which circulates at the speed (10-30) ms^{-1}. The water content of the material at the beginning of the drying process moisture can be w_1= (35-40) %. This moisture is reduced to w_2 = (10-15) %. The specific energy consumed to remove one kg of evaporable water is between (3900-5040) kJ kg^{-1}. The efficiency of convection pneumatic depends on whether the drying is direct or indirect. It is determined according to the thermal degree of utilization and reaches (66-75). The volume of water vapour in the dryer pneumatic pipe accounts for approximately (300-350) kg m-3h^{-1}. As the material is dried in matter of several seconds, this method is suitable for drying of foods sensitive to high temperatures (PRVULOVIC; TOLMAC, 2007).

- **Sun drying**

In earlier times, the only possibility to dry foods was by using direct solar energy. In this process, the material is placed on a flat surface or hanged in the air to be exposed to direct sunlight. Although sun drying is the cheapest method, it has a number of adverse effects on the final product, which include contamination from the environment or by insects and birds or bad odour. Other disadvantages of this type of drying could be floor space requirements, product losses and difficulty of controlling the process.

In case of climate unsuitable for sun drying or higher quality requirements, sun drying is most commonly replaced by mechanical air drying. Nowadays, both types enjoy a wide range of commercial uses (RAHMAN, 2007).

- **Solar drying**

Solar drying is a type of sun drying that makes use of radiation energy from the sun. The advantages of solar drying are that it does not produce any pollution and uses a renewable energy source, which is very ample and cannot be monopolized. The limitations of solar drying are similar to those of sun drying. They include large space and labour input requirements, no control of the drying rate and high risks of contamination by insects and microorganisms. These drawbacks prevent solar drying from being implemented in large-scale production.

According to their heating modes and the way of solar heat utilization, solar dryers can be divided into following two main groups:

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

Both groups can be further divided into following sub-classes: integral-type solar dryers, distributed-type solar dryers, mixed-mode solar dryers. These sub-classes differ primarily in the design arrangement of system components and the mode of utilisation of the solar heat (RAHMAN, 2007).

- **Passive solar dryers**

Passive solar dryers (also called natural circulation or natural convection systems) are operated utterly by solar energy. This energy is used to heat up air, which is then circulated through the material by either buoyancy forces, wind pressure or both forces combined. The size of the natural convection systems allows for them to be used on farm. They can be divided in direct (tent and box dryer) and indirect (cabinet dryer) systems (Weiss, 2003).

- **Active solar cabinet dryers**

Active solar dryers (also called forced convection or hybrid solar dryers) provide the possibility to modify the air flow and control temperature and moisture during the drying process, regardless of the climatic conditions. These properties, combined with the restriction of the bulk depth substantially improve the reliability of the dryers in comparison with the natural convection dryers, which offer much less possibility of control, resulting in lower effectiveness.

Implementing hybrid solar dryers can result in drying time reduction by three times and required space can decrease by 50 %. Therefore, forced convection dryers can reach a performance equal of a natural convection dryer with a collector six times as large. Fans are powered by electricity, which can be even produced using a photovoltaic panel, if utility electricity is unavailable. Almost all types of natural convection dryers can be operated by forced convection as well (Weiss, 2003).

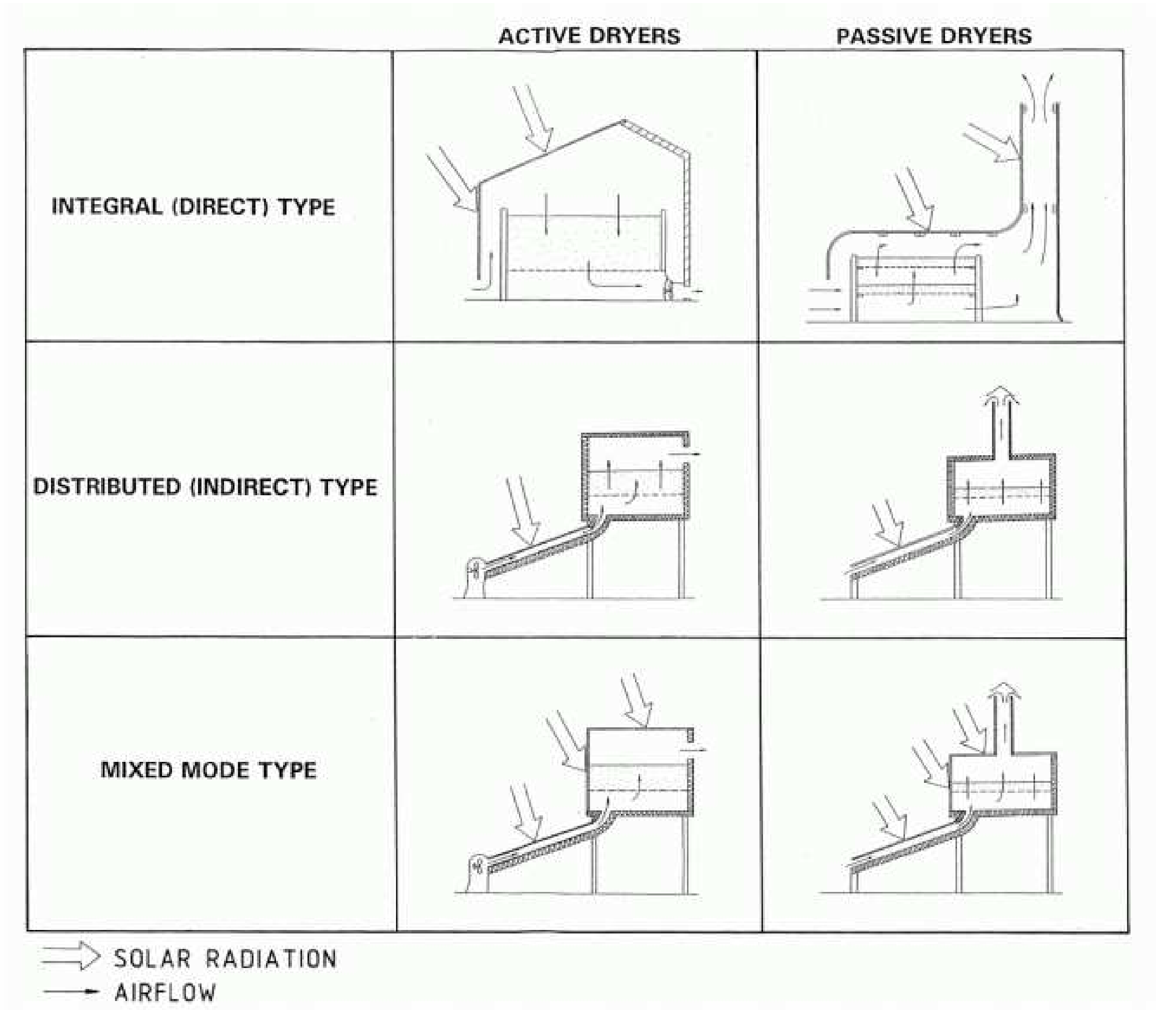

Figure 1. 2 Classification of solar dryers

Source: Weiss, 2003

1.1.2.1 LOW AIR ENVIRONMENT DRYING

- **Vacuum drying**

Although vacuum drying of lumber has been considered for many years, it has not been commercially used until recent years. The main advantage of vacuum drying is the lowering of the boiling point of water with decreasing air pressure, so that free water can evaporate at lower temperatures at nearly the same rate as it can with high-temperature drying above 100°C. Drying rate is therefore enhanced and the risk of damage of the material through high temperatures above 100°C is avoided. Vacuum drying is essentially high-temperature drying at low temperatures (SIMPSON, 1987).

- **Freeze drying**

Freeze-drying is alternatively called lyophilisation and is a crucial process for conservation of biological material that is susceptible to high temperatures. The spectrum of application of lyophilisation is very wide, from preserving food, over complex biotechnological or pharmaceutical products, to multiplying microorganisms. Beside food, it is useful in preserving other kinds of products, such as flowers, bacteria and fungi, medicinal drugs, medical devices, cosmetic goods, chemical materials (pigments, enzymes), etc.

In the process of lyophilisation, the solvent – which is usually water – and/or the suspension ‘medium’ are crystallized by freezing. The crystallization is followed by sublimation. There are three main steps of the drying process: freezing, the primary drying stage and the secondary drying stage. These basic stages consist of five operations, which are freezing, sublimation, desorption, vacuum pumping and vapour condensation.

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

- **Heat pump drying**

One of the simpler designs where the heat gained from the solar radiation is used to raise the drying air temperature is shown in figure. It represents a schematic layout of the various refrigeration components and a solar system integrating with the drying chamber. The inlet drying air passes through the drying chamber at point 1 and picks up moisture from the product. The moisture-laden air at point 2 is then directed to the evaporator coil. Typically, two types of evaporator systems exist. One is a direct expansion coil whereby the refrigerant undergoes a two-phase change from liquid to vapour to cool and dehumidify the air. The other is a chilled water system wherein the flow of chilled water to the coil is controlled for cooling and dehumidification. During the dehumidification process from point 2 to 3, the air is first cooled sensibly to its dew point. Further cooling results in water being condensed from the air. Both sensible and latent heats are then absorbed by the evaporator for boiling of the refrigerant. The recovered heat is "pumped" to the condenser. At the solar collector, the solar radiation from the sun is converted to sensible heat. Air passing through the number of pipes in the panel is then heated up. This heated air, passing through the heat exchanger at 4, is used to preheat the drying air before entering the condenser. The pre-heated and dehumidified air at 5 absorbs more heat from the condenser moving from point 5 to 6. The conditioned air at 6 is then one that is higher temperature and properly dehumidified which readily flows through the drying chamber at 1 for drying application (SOPIAN et. al., 2006).

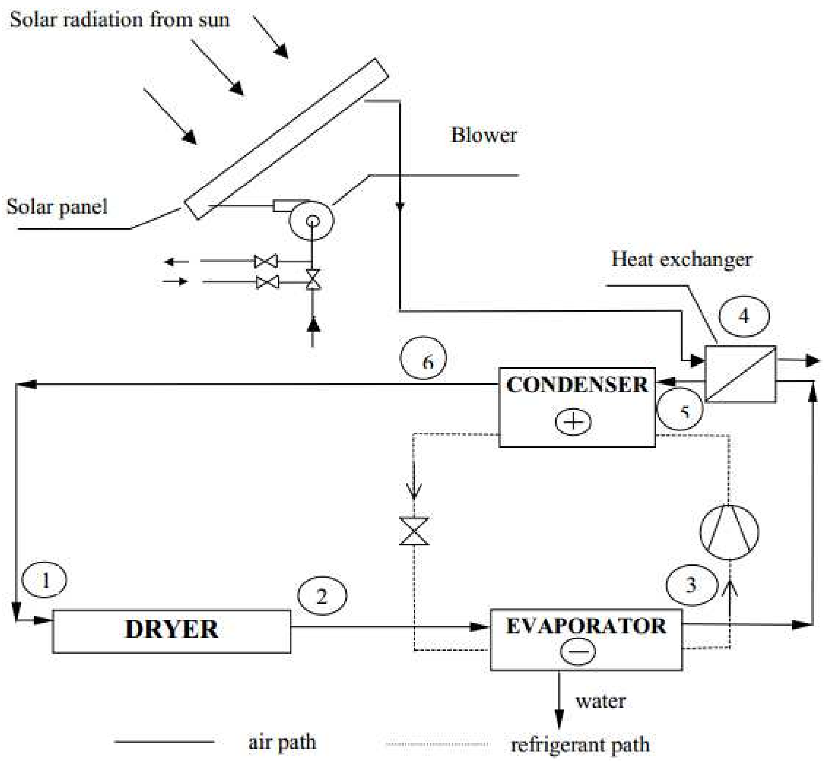

Figure 1. 3 Schematic representation of solar-assisted heat pump drying system
Source: SOPIAN *et. al.*, 2006

1.1.2.2 MODIFIED ATMOSPHERE DRYING

Modified atmosphere drying is a concept suitable for conserving quality of food susceptible to oxidation. It uses pump dryers with gaseous atmosphere of nitrogen or carbon dioxide.

The modified atmosphere drying has weaker adverse effects on the product, especially in terms of shrinking, colour change, types of moulds, and peroxide values (PV).

The fact that heat pump dehumidifier (HPD) drying is conducted in an enclosed, insulated chamber is made use of in the development of the Modified atmosphere heat pump dehumidifier (MAHPD) drying system. The air in the dehumidifier chamber is replaced with an inert atmosphere such as nitrogen, carbon dioxide, or their mixtures. Replacement of the air inside the chamber is easily carried out by exhausting the chamber using a vacuum pump and then breaking the vacuum using an inert gas. Vacuum exhaustion is a more cost-effective way to replace air than by direct purging with the specific inert gas. Replacement of air with carbon dioxide or nitrogen by purging requires over 50 volumes to achieve an oxygen level of less than 0.5 %.

In comparison with common drying methods, fruit tissues dried by MAHPD showed improvement in colour, lower bulk density, retained porous structure, better rehydration properties and retention of nutritious values and flavour compounds. This shows that MAHPD is very suitable for preserving sensitive food and pharmaceuticals (RAHMAN, 2007).

1.2 COLOUR AND BROWNING REACTIONS

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 usually sets in in the middle of the drying period, probably as a result of the soluble components migrating towards the centre region. It is more intense towards the end of the drying period as well. Due to low moisture level in the sample, the occurrence of evaporative cooling is scarce and the temperature in the dried material increases.

Browning takes place at temperatures between 80 and 90°C with the rate growing with time and temperature. The colour change is caused by loss of sugars and amino acids (such as lysine, histidine, threonine, methionine and cysteine) from the tissue. Maillard browning advances most rapidly when moisture is reduced to 15-20 %, and then slows down again when the moisture content reaches 2 %. The colour does not change further by this moisture even during subsequent storage. The Maillard browning can be thus minimized by designing the drying systems or heating schedules to proceed quickly through the 20-15 % moisture range. Controlling of browning can differ according to the type of foodstuff dried. While in carbohydrate foods, it can be minimized by elimination of amines, in foods rich in proteins it is done by removal of reducing sugars. Near the end of the drying process, when the temperatures of the material and the drying 'medium' equalize and the moisture levels are low, the non-enzymatic browning reaches its highest rates. In heat pump driers, where the drying temperatures are lower, the extent of non-enzymatic browning is reduced (CHEN, 2008).

1.2.1 ENZYMATIC BROWNING

Enzymatic browning (EB) takes place in fruits after bruising, cutting or during storage. In fruit processing, especially in apples, pears, bananas, peaches, and grapes, it is crucial to be able to control this process.

However, browning to a certain extent is acceptable in some products. For example in apple processing, in certain products, like clarified apple juice, browning is even commercially desirable. On the other hand, in apple purée or cloudy juice browning must be prevented, as the colour should be yellowish or greenish to imply the freshness of the product.

Phenolic Compounds and Oxidases (PPO) is an example of an enzyme that can lower the quality of a food product by catalyzing the oxidation of phenolic compounds. The susceptibility to browning may depend on PPO activity and/or phenolic content. Polyphenol oxidase catalyzes the initial step in the polymerization of phenolics to produce quinones, which undergo further polymerization to insoluble dark brown polymers known as melanins. These melanins form barriers and have antimicrobial properties, which prevent the spread of infection or bruising in plant tissues. The formation of yellow and brown pigments in fruit products during EB reactions is controlled by the levels of phenols, the amount of PPO activity, and the presence of oxygen. During the browning of fruit tissue, the enzyme PPO, also called orthodiphenol oxidase or catecholase, catalyzes the oxidation of phenolic compounds related to catechol and containing two o-dihydroxy groups to the corresponding o-quinone. Most raw fruits contain polyphenols and PPOs, located in different compartments in the cell structure. When through damaging or processing (e.g. milling) enzyme, substrates and oxygen come into contact with each other, and a lot of reactions start that finally lead to the formation of insoluble brown pigments (melanins). The EB of fruit and vegetables is always considered as a quality loss of both fresh and processed food products (LOZANO, 2006).

1.2.2 NONENZYMATIC BROWNING

NEB via Maillard-type reactions is the most important route of colour deterioration in fruit juices as it causes unwanted alterations in colour and aroma.

Pyrolysis: results in a flavour change, which makes the product uneatable

Caramelization: when the simpler sugars lose water molecules from their structure, through a 1:2 and 2:3-enolization. This process is affected by pH. Through many intermediates, and in the pH 2–7 range, d-fructose for example can give rise to furans, isomaltol, and maltol, well-known bread crust flavor/aroma

Maillard-type reaction: of amino acids and proteins with carbohydrates

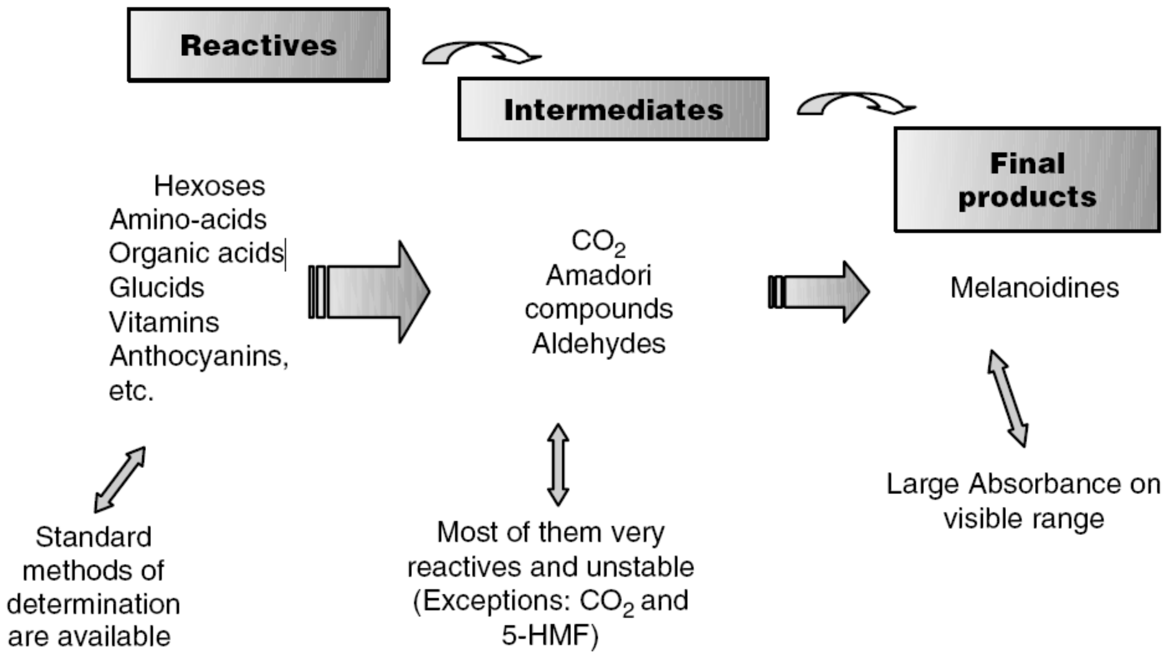

Fig. 1.4 Simplified scheme for NEB reactions in most fruit juices

Source: Lozano, 2006

The reaction begins to occur at lower temperatures and at higher dilutions than caramelization, as in fruit juices. The rate can increase by 2–3 times for each 108°C rise in temperature. Maillard reactions have three basic phases:

The initial reaction is the condensation of an amino acid with a simple sugar, which loses a molecule of water to form N-substituted aldosylamine. This is unstable and undergoes the Amadori rearrangement to form 1-amino- 1-deoxy-2-ketoses (or ketosamines), which can cause complex subsequent dehydration, fission, and polymerization reactions. One of the Maillard paths is a simple caramel reaction, catalyzed by amino acids.

The ketosamine products of the Amadori rearrangement can then react three ways in the second phase. One is simply further dehydration into reductones and dehydroreductones, which are essentially caramel products. Second is the production of shortchain hydrolytic fission products such as diacetyl, acetol, pyruvaldehyde, etc. These then undergo Strecker degradation with amino acids to aldehydes and by condensation to aldols. Negative aromas like 2 and 3-methyl-butanal are also formed.

Third path is the Schiff's base/furfural path. This involves the loss of 3 water molecules, then a reaction with amino acids and water. These also undergo aldol condensation and polymerize further into true melanoidins. These products react further with amino acids in the third phase to form the brown pigments and flavour active compounds collectively called melanoidins. These can be off-flavours. The outcome will depend not only on which amino acids and sugars are available, but also on pH, temperature, and concentration.

In general, high levels of amino acids favour both caramel and Maillard reactions, but dilution eliminates caramel reactions. At temperatures >1008°C pyrazines are produced. High levels of polyphenols favour Strecker degradation. Fruit juice concentrates containing more than 65 % total solids are normally stable from the standpoint of fermentation at any temperature, but when stored at relatively high temperatures, NEB reactions occur. NEB via Maillard-type reactions is the most important route of colour deterioration in apple juice. The reaction takes place between amino acids and reducing sugars present in the juice, decreasing the alpha-amino nitrogen content followed by undesirable colour, odour, and flavour ganges (LOZANO, 2006).

1.2.2.1 INHIBITION AND CONTROL OF ENZYMATIC BROWNING

Enzymatic browning cannot be fully avoided while pulping and juicing of fruit (unless access of oxygen is prevented), as it is a rapid reaction during which polyphenol oxidase catalyzes the oxidation of phenols to o-quinones, which are highly reactive compounds. O-Quinones originated in this way subsequently polymerize and form brown pigments (melanins). The brown colouring can be further enhanced when the melanins interact with other amino acids and proteins.

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.

Although avoiding the contact of the fruit surface with oxygen can be one way to slow down EB (as it is an oxidative reaction), after reintroduction of oxygen, the browning reaction is started once again. Oxygen can be eliminated by immersing the fruit in syrup or deoxygenated water or coating it with gas impermeable films.

Other way to control EB is to eliminate the enzymatic activity. Polyphenol oxidase can be denaturated by exposure to high temperatures, e.g. by steam blanching. This process is normally applied to fruits before canning or freezing.

Oxidation can be also prevented by means of chemical modification of phenolic substrates, such as chlorogenic acid, caffeic acid, and tyrosine. Certain chemical compounds react with the products of polyphenol oxidase activity and inhibit the formation of the coloured compounds produced in advanced, non-enzymatic reaction steps, which finally lead to the formation of brown compounds.

Chemical antibrowning agents have been ordinarily utilized for forestalling darkening of fruits and fruit products. They function in two modes to inhibit coloured product formation, either they act primarily on the enzyme or bind the substrate and/or products of the enzymatic reaction.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 preventing enzymatic browning in the fruit industry 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 triggering allergic reactions. Combinations of ascorbic acid (AA) with an acidic polyphosphate is highly effective with both juice and cut surfaces. On the contrary, combinations of betacyclodextrin with AA were effective in juice, but not on cut surfaces (LOZANO, 2006).

- **Preventive methods**

Temperature Control: NEB reaction is retarded by reducing the temperature

Process Optimization: concentration by evaporation is a very common practice in the fruit juice and pulp industry. Multiple-effect evaporators used in the fruit juice processing plants were designed to eliminate water under vacuum at relatively low temperatures. However, it is not unusual in practice to find very high temperatures in the first stage of processing. This can lead to changes both in colour and flavour of the juice, mainly due to NEB.

Ion Exchange Treatment: ion exchange resins have been used in the industry for discoloration of syrups, the hydrolysis of lactose, and the anthocyanin recovery from fruit bagasses among other applications (LOZANO, 2006).

- **Restorative methods**

During fruit juice discoloration, chromophoric components are eliminated, without modifying if possible, the other components of the product. While in the case of apple juice polyphenols are to be removed, red coloration in orange juice is caused by anthocyanins. The adsorption capacity of certain substances eliminating colouring matter by adsorption is exploited for discoloration. Properties such as grain size, surface area, and porosity define adsorbers' capacity. This could be attributed to polyphenol adsorption. Adsorption forces are ruled by weak Van der Waals' forces, which are temperature dependent. Adsorption of colour is usually performed by adding activated carbon (AC) as slurry to the juice, since this gave better dispersion than the addition of dry carbon.

The synthetic polymer polyvinyl polypyrrolidone (PVPP), which has been used in the beverage industry since several years, is an absorbent, which selectively affine to polyphenols and tannins. PVPP is used not only for discoloration of fruit juices, but also to prevent haze formation after processing. The ability of PVPP to regenerate is the advantage of this product compared with the AC treatment (LOZANO, 2006).

- **Effect of the Ascorbic Acid (AA) content in colour change:** ascorbic acid (AA) does not inhibit polyphenol oxidase directly but acts as a reducing compound and reduces the orthoquinones to dehydroxyphenols. This action will continue as long as the concentration of ascorbic acid is sufficient to maintain a low concentration of quinones. As the concentration of AA is decreased, the quinone concentration increases and causes the formation of the brown pigments (LOZANO, 2006).

1.3 PRETREATMENTS IN FOOD DRYING

Pre-treatments are processes that diminish darkening of the dried fruits, especially the ones with light-coloured pulp, such as apples, which brown very quickly in contact with air and even continue darkening after having been dried (Websites UNIDO, 2012).

Drying of tropical fruits is connected with several drawbacks, such as loss of cellular structure, browning, loss of redrying ability, and case hardening. These changes negatively affect the market value and general 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. Sulphuring or using a sulphite dip is the most convenient pre-treatments for long-term storage of dried fruits. Unfortunately, sulphites may cause asthmatic reactions in a small portion of the asthmatic population (ABANO; SAM-AMOAH, 2011). Two basic types of pre-treatment are recognized: dipping and blanching (CHADWICK, 1995).

1.3.1 DIPPING

Dipping is the treatment used primarily to prevent fruits or vegetables from oxidizing.

- **Sulphuring, Sulphite dips:** sulphuring is an old method of fruit pre-treatment. It consists in burning sublimed sulphur in an enclosed container together with the fruit. The sulphur vapours permeate into the fruit and slow down spoilage and colour change of the fruit as well as prevents the loss of vitamins A and C. The same effect as sulphuring has sulphite dips in that it prevents the darkening in a long term. Moreover the process has the advantage of being quicker and easier. Either sodium bisulphite, which is a liquid form of sulphur, sodium sulphite or sodium metabisulphite that are USP (food grade) or Reagent grade (pure) can be used for dipping when mixed with water. It is the most effective and least expensive anti-oxidant. Only a food-safe sodium bisulphate designated for dehydration should be used (Websites UNIDO, 2012).

- **Ascorbic acid**: when diluted in water, it is a safe means of preventing fruit darkening, however without a long-term effect. Ascorbic acid reacts easily with oxygen so that it cannot be used by phenolase. Ascorbic acid also reduces the o-quinones formed by phenolase to the original o-dihydroxyphenolic compounds. It is however not effective enough in apples as the internal atmosphere of the slices contains oxygen (Websites UNIDO, 2012). Ascorbic acid (vitamin C) is present in all citrus fruits, from which dips are prepared in crystal, powder or in tablet form. The fruit is then soaked in an ascorbic acid solution for 10 minutes (CHADWICK, 1995).

- **Honey dip:** honey dips are applier quite commonly of dried fruits. Many dried fruits on the market have been treated this way. Dipping fruit in honey has the consequences of adding sweetness as well as energetic value to the final product (CHADWICK, 1995).

1.3.2 BLANCHING

Blanching, as means of pre-treatment, can be done either in boiling water or by steaming the foods. This pre-treatment is sometimes referred to as "checking" or "crazing". 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.

- **Water blanches:** fill a large pan over half full with water. Bring the water to a boil and stir the food directly into the boiling water. Cover and blanch for approximately 3 minutes. Remove food from the boiling water and place it into ice water to cool. Blot with a towel to remove excess water before drying (CHADWICK, 1995).

- **Steam blanching:** Steam blanching also helps retain colour and slow oxidation. However, the flavour and texture of the fruit is changed. Directions: place several inches of water in a large saucepot with a tight fitting lid. Heat to boiling. Place fruit not more than 2 inches deep, in a steamer pan or wire basket over boiling water. Cover tightly with lid and begin timing immediately. See below for blanching times. When done, remove excess moisture using paper towels and place on dryer trays (HARRISON; ANDRESS, 2010).

- **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 heightens the natural flavour of the product (CHADWICK, 1995).

2 AIM OF THE THESIS

The objectives are to evaluate the influence of fruit juices (*Sorbus aucuparia, Diospyros kaki* L., *Hippophae rhamnoides* L., *Actinidia deliciosa*, *Rosa canina* L.) containing ascorbic acid on degree of browning of dried fruit and to compare the influence of fruit juices and pure vitamin C.

3 MATERIALS AND METHODS

Natural juices from fruits of five species containing high amounts of vitamin C (*Sorbus aucuparia*, *Diospyros kaki* L. , *Hippophae rhamnoides* L. , *Actinidia deliciosa*, *Rosa canina* L.) and 1 % solution of ascorbic acid were prepared. Vitamin C content in the juices was measured by means of high-performance liquid chromatography. A single juice or ascorbic aqcid solution was applied always to five slices of apples (Idared), by soaking the slices for ten minutes.

The treated apple slices as well as an untreated control of 10 pieces were dried in a home fruit dryer. After drying, the degree of browning was measured on a colorimeter. The organoleptic properties of apples were also evaluated by fourteen trained panelists. The degree of browning of untreated apples, apples treated with fruit juices and apples treated with pure ascorbic acid were compared.

3.1 PLANT MATERIAL

Although up to 84 % of its mass consists of water, there are numerous organic and inorganic compouds in apples, which make its chemical composition quite complex. The most abundant components are soluble solids which make up 13.2 % of apple's mass. Sspecifically there is 2.3 % of pectin 1.3 % of glucose, 5.9 % of fructose, 2.1 % of sucrose, 0.37 % of total are acids, vitamins, and minerals and other biologically valuable substances. (PAUNOVIĆ, 2010).

These other micro biogenic and macro biogenic compounds found in apples include tannins, starch, cellulose, enzymes and phytohormones. Most represented chemical elements are carbon, nitrogen, oxygen, phosphorus, potassium, calcium, sulphur, iron and magnesium. Organic compounds' content in fruit depends on fruit cultivar, ripeness, physiological condition of the tree as well as soil and weather conditions. The average pH value is 3.57. (NOUR *et al*., 2010).

The apples used for the measurement of browning were of the Idared variety, which is of Czech origin. The apples were bought at a grocery store, washed in distilled water, sliced into slices 7 mm in width and immediately let to soak in of the juices for ten minutes. One group of apples was soaked in freshly prepared 1 % solution of pure ascorbic acid.

3.1.1 JUICE PREPARATION

Fruits of five species, chosen for their high vitamin C content were used for preparation of the juices, namely: European Mountain-Ash (*Sorbus aucuparia*), Dog Rose (*Rosa canina* L.), Seabuckthorn (*Hippophae rhamnoides* L.), Japanese Persimmon (*Diospyros kaki* L.) and Chinese Gooseberry (*Actinidia deliciosa*).

- ***Sorbus aucuparia*** belongs to the family Rosaceae Juss. Fruits contain 4-9.5 % sugar, 2.7 % organic acids, pectins, tannins, vitamin C (200 mg per 100 g), carotene (20 mg per 100 g), and vitamin P. Dry fruits contain plenty of vitamins and so are, together with blossoms, often used as a remedy in folk medicine (N.V. TEREKHINA, 2005).

- ***Rosa canina* L.** belongs to the family Rosaceae Juss. The fruits of the rose species, especially rose hip (dog rose), are rich in minerals, carotenoids, tocopherol, bioflavonoids, vitamins, sugars, phenolic compounds, tannins, organic acids, fruit acids, aminoacids, volatile oils, and pectin. Unsaturated and polyunsaturated fatty acids are also found in the seeds of rose hips. Fruit - hip consists from numerous (about 16) nutlets enclosing in gipantii locking after blossom. Furthermore, rose fruits also possess extraordinarily strong antioxidant and microbial capacity (KAZAZ *et. al*., 2009). Fruits are usually between 1.5-2.6 cm in length, naked, ovoid, rarely spherical, smooth. When mature the fruits are of bright orange-red colour. Average weight of a fruit is 1.7-3.2 g, amount of the flesh - up to 70.8 %. Fruits accumulate great amounts of vitamin C, carotenes and organic acids (DORONINA; TEREKHINA, 2005).

- ***Hippophae rhamnoides* L** belongs to the family Elaegnaceae Juss. The fruit of seabuchthars is quite rich in vitamins and other bioactive substances. It has high content of vitamin C, flavonoids, tannins, oils and oil soluble bioactive compounds as well as minerals (SAVA *et al*., 2001). Fruits are up to 1cm in length and resemble berries. They are orange to reddish in colour and contain only one seed. The seed itself contains vitamins, sugars (up to 6.6 %), organic acids (up to 2.5 %), and fatty oils (up to 9 % in pulp and 12 % in seeds).

- ***Diospyros kaki* L.** belongs to the family Ebenaceae. Fruits are round, conical, often oblate, or nearly square, enclosed in persistent calyx, their skin is thin, smooth and glossy. The colour varies - yellow, orange, red or even brownish-red kaki fruits can be found. The flesh similarily varies - it can be yellow, orange, or dark-brown. It is always juicy and gelatinous, but it may or may not contain seeds - dependenig on the variety (ORWA *et al*., 2009).

- ***Actinidia deliciosa,*** otherwise known as 'kiwi', belongs to the family Apterygidae. It is a good source of potassium and has a high potassium to sodium ratio. The fruits are a useful source of magnesium as well, but other minerals are not found in significant quantities. They also contain 2-3 % of pectin, and so are valued as a source of dietary fibre. Kiwi fruits are also high in vitamin C content and what is more, very little of the 85 mg ascorbate/100 g of fresh weight gets lost on storage or ripening (JANICK, 2008).

- **Origin and pre-processing of fruits used in the experiments**

The fruits of *Rosa canina* L. come from the region of Střední posázaví in the Czech Republic. The fruits of *Sorbus aucuparia* also come from Střední posázaví in the Czech Republic. The fruits of *Hippophae rhamnoides* L. were harvested in the Suchdol locality in Prague. The fruits of Chinese goosbery and *Diospyros kaki* L. come from Greece and Spain, respectively and both were purchased at a Prague grocery store.

All the fruits were obtained in October 2011. The juice from the fruits of *Rosa canina* L. was obtained by soaking the fruits in dark for 24 hours at the constant temperature of 18°C. The juices from the fruits of *Hippophae rhamnoides* L., *Sorbus aucuparia*, Chinese gooseberry, *Diospyros kaki* L. were prepared using a juicer. All juices were consequently frozen and kept at -15°C. until the soaking of apple slices took place.

3.1.2 DETERMINIG THE VITAMIN C CONTENT IN JUICES

After unfrozing the juices, their ascorbic acid content was measured using High Performance Liquid Chromatography [HLPC]. Samples of juices were centrifuged in 1.5 ml Eppendorf test tubes at 14.500 rpm and then filtred through PTFE filter of 0.45 μm pore size. The samples were water diluted in proportion 20:1 and then analyzed at HLPC-DAD Dionex Summit (Dionex Inc, US). The chromatography conditions were as following: isocratic elution, mobile phase: 0.1 % TFA, flow rate: 0,8 ml/min, column: Gemini C-18, 250 x 4,6 mm, particle size: 5, μm (Phenomenex, Inc, USA) with a C-18 pre-column. Column temperature: 25°C, injection: 20 μl. The program duration was 7.5 minutes with elution time of the ascorbic acid at 6.2 minutes. Quantification 243 nm wavelength after external calibration by linear calibration for ascorbic acid of 0.004-1.000 mg/ml.

It was not possible to determin the ascorbic acid content in the fruits of *Diospyros kaki* L., most probably due to their high content of saccharides and resuling high viscosity of the measured sample. Available literature qutoes the average vitamin C contect at 0.62-0.70 mg per 100g.

3.1.3 DRYING OF THE TREATED AND UNTREATED APPLE SLICES

The drying process took place in a labortory with a constant temperature of 24.7°C and relative humidity of 24 %. Then both treated and untreated apple slices were laid on mesh screens and put into the fruit-drying oven. The temperature in the oven was set at 57°C and the constant air flow rate was 1,0 m/s. Before the drying process, the apple slices were weighed to determine their water content. Water content is defined as the loss of mass at experimental conditions. The samples are thermally dried at 80°C for 24 hours and then the difference in mass before and after the drying is measured. The water content is then calculated from the mass loss.

$$pH_2O = \frac{100\,(m - m_{dm})}{m}\ [\%]$$

m = mass of the sample before drying in grams

m_{dm} = mass of the sample after drying in grams

3.2 COLOUR MEASUREMENT

Colour, taste and appearance are all significant factors influencing people's decision process whenever food consumption is concerned. Colour and general appearance are important because mainly they create the first impressio a dish or a food product. On the other hand, texture together with flavour, as sub-categories of taste play a major part after the food is ingested. Vision psychology, science of colour, colour psychology, the technologies that measure colour and computer imaging, have all been developed in order to design foods, quantify colour and improve the quality of the foods (CHEN, 2008).

Colour is the result of an interaction of light with the observed object and consequently with the human eye. As light is the visible part of the electromagnetic spectrum, it comprises different wavelenghts. These can be either absorbed or reflected by the surface of the observed object. It is this interaction - combination of absorptions and reflexions of different wavelengths of light - and the light's subsequent interaction with the human eye that give rise to our perception of colour. Wavelengths are measured in nanometers (Color Guide and Glossary, 2004).

Nowadays, the colour measurement is usually performed using the CIELAB or the L^*–a^*–b^* system, established in 1976. The parameters L^*, a^* and b^* can be measured measured using a colorimeter such as the Hunter Lab instrument (CHEN, 2008).

The $L^*a^*b^*$ colour space makes use of the fact, that green and red are mutually exclusive colours (colour cannot be red and green at the same time), as are yellow and blue. In other words, the $L^*a^*b^*$ colour space is a 'colour-oponent'space. Thus, when a colour is expressed in the CIE L^* a^* b^* system, the a^* denotes the value on a scale from red to green, whereas b^* dentoes the value on a scale from blue to yellow. L^* then represents brightness (Color Guide and Glossary, 2004).

$L* = 0$ corresponds to Black; $L* = 100$ denotes White. There are hence 100 degrees between white and black, in other words, the shades of grey. As $a* \rightarrow +a$, the colour approaches pure Red; and as $a* \rightarrow -a$, the colour approaches pure Green. $-b$ towards pure Blue and $+b$ towards pure Yellow. The resulting colour is then a combination of $a*$ and $b*$ at the same level of brightness $L*$. Red, Blue and Yellow can act as primary colours to make up other colours by their combination (CHEN, 2008). The proper quantification and interpretation of such tristimulus colorimetry data is based upon trigonometric functions. A colour wheel subtends 360°, with red-purple traditionally placed at the far right (or at an angle of 0°); yellow, bluish-green, and blue follow counterclockwise at 90°, 180°, and 270°, respectively (RAYMOND G. MCGUIRE, 1992).

- **Colorimeter:** a colorimeter breaks the light down into its RGB components (in a manner similar to that of the human eye, a monitor, or a scanner) and then measures their respective intensity. The CIE $L^*a^*b^*$ system is them used to determine colour's numerical value and to plot a graph in a colour space.

- **Tolerance sphere:** In CIELAB, the control colour or original specification is precisely determined by measurement data in the $L^*a^*b^*$ colour space. Then a value of ΔE^* is calculated directly from the L^*a^* and b^* values. ΔE^* represents the total colour difference betweeen e.g. the control and the measured colour. ΔE^* can also be interpreted as a surface of a 'tolerance sphere' around the control colour. Samples that fall in the sphere are acceptable and those that fall beyond it are 'unacceptable'. The size of this sphere is chosen arbitrarily (RAYMOND G. MCGUIRE, 1992). ΔE^* is also very important for 'sensory' evaluation . The calculation of ΔE^*:

$$\Delta E * = \Delta L *2 + \Delta a *2 + \Delta b *2$$

Trace level difference	ΔE^* = 0–0.5
Slight difference	ΔE^* = 0.5–1.5
Noticeable difference	ΔE^* = 1.5–3.0
Appreciable difference	ΔE^* = 3.0–6.0
Large difference	ΔE^* = 6.0–12.0
Very obvious difference	ΔE^* > 12.0

(CHEN, 2008)

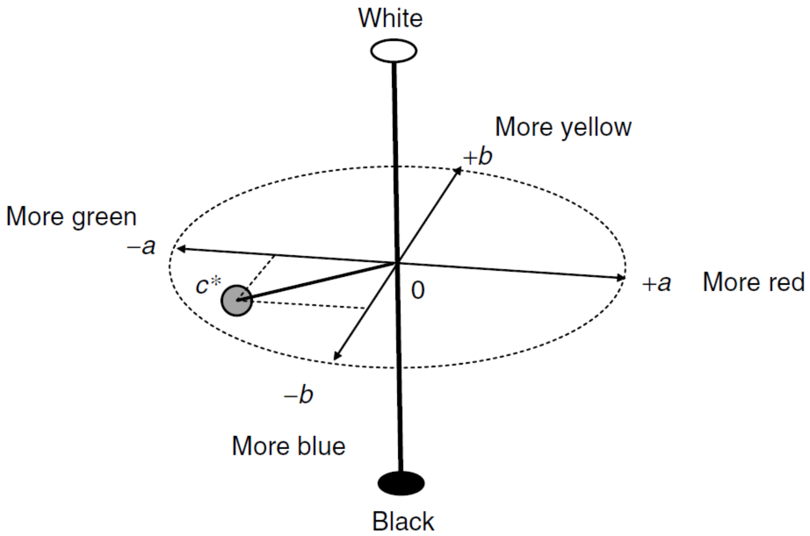

Figure 3.5 An illustration of the CIELAB colour expression systém

Source: CHEN, 2008

- **Browning index:** browning index *(BI)* represents the purity of brown colour and is generally accepted as a significant parameter (MOHAMMAD *et. al*, 2008). The formula for calculating the Browning Index is as follow (MOHAMMAD *et. al*, 2008):

$$BI = \frac{100\,(x - 0.31)}{0.17} \qquad x = \frac{a + 1.75L}{5.645 + a - 3.012b}$$

Browning index assumes values between 0 and 100, where 0 corresponds to black and 100 corresponds to white.

- **Metric chroma, Metric hue-angle *(h°)*:** both of these parameters can be calculated directly from the L^*a^* and b^* values. These parameters are for various reasons more appropriate for interpreting the colour analyzis then the L^*a^* and b^* values themselves. They serve purpose for example when we are interested in judging the efficacy of postharvest treatments. Chroma *C* is roughly analogous to colour saturation or intensity.

- **Chroma (Saturation index):** represents the hypotenuse of a right triangle created by joining points (0, 0), ($a*$, $b*$), and ($a*$, 0) (RAYMOND G. MCGUIRE, 1992). The greater the $C*$ the purer the colour appears to be (CHEN, 2008).

Hue angle: is defined as the angle between the hypotenuse and 0° on the $a*$ (bluish-green/red-purple) axis; $h°$ is calculated from the arctangent of $b*/a*$. The hue angle value corresponds to whether the object is red, orange, yellow, green, blue, or violet.

Since arctangent, assumes positive values in the first and third and negative values in the second and fourth quadrants we need the $h°$ to remain positive between 0° and 360° of the colour wheel for useful interpretation (RAYMOND G. MCGUIRE, 1992).

Chroma: $c *= \sqrt{(a *)^2 + (b *)^2}$

Hue angle: $hue = arctan\ (\frac{b*}{a*})$

3.2.1 COLOUR ESTIMATION

To determine the colour change properly, the apple colour characteristics were measured using MiniScan® XE Plus. sd The diameter of the test prod was 8mm. Controlization on a MiniScan® XE Plus model with 45°/0° geometry required reading of the black glass and white tile.

First freshly cut apples were measured, as a control. After that, apples treated with the fruit juices, 1 % ascorbic acid and the untreated apples were also measured. All these values were compared with the control.

- **The measurement of control:** Five halves of freshly cut apples of the Idared variety were measured six times each. All these values were averaged and the resulting value was taken as a control. That means, that the control is the average of thirty measurements of apple halves.

- **The measurement of colour change of dried apples:** the method was similar to the measurement of control - six values (three for each side) were taken for each of five slices and then the values were averaged. This way, the average colour for each kind of treated apples was obtained. The same procedure was applied for comparing freshly cut apples with apples that were treated with 1 % solution ascorbic acid and with apples that were not treated by any juice or solution.

3.3 SENSORY ANALYSIS OF DRIED APPLES

One part of sensory analysis was photodocumentation of apples. Pictures of all the apples were taken at identical conditions and the photographs may be used for later analysis.

Fourteen trained university students participated in the sensory analysis as panelists. In the course of the analysis, the participants first underwent a training session explaining the aim of the sensory analysis. During subsequent panel tasting, the participants filled out a questionnaire about the perceived colour change, degreee of browning, taste and overall attractivness of presented dried apples. The degree of browning and colour change were evaluated in comparison with freshly cut apples. Data gathared from the questionnaires was consequently converted into percentages. The questionnaire is in the sexction Annex A.

4 RESULTS AND DISCUSSION

4.1 ASCORBIC ACID CONTENT IN THE JUICES

Fruits that were used for making the juices had a certain amount of ascorbic acid. This amount was determined using HLPC, except in the case of *Diospyros kaki* L, where this method failed. The highest amount of ascorbic acid was found in the juice from *Sorbus aucuparia* and *Hippophae rhamnoides* L., About one third of that amount was found in the fruits of *Rosa canina* L. and *Actinidia deliciosa*. The average amount of ascorbic acid in the fruits of *Diospyros kaki* L. was taken from literature and is similar to that of *Rosa canina* L.

Tab. 4.1 The amount of ascorbic acid in juices

Natural juices	Ascorb acid [mg/ml]
Sorbus aucuparia	1,79
Rosa canina L.	0,57
Hippophae rhamnoides L.	1,65
Diospyros kaki L.	Not detected
Actinidia deliciosa	0,50

4.2 THE WATER CONTENT IN APPLES

The average water content in apples is 80-90 %. Apples treated with *Diospyros kaki* L or *Rosa canina* L. juice and those that were left untreated contained the most water. Less water was found in apples treated with *Hippophae rhamnoides* L., *Sorbus aucupari*a and *Actinidia deliciosa*. The least water was present in apples treated with 1 % solution of ascorbic acid.

Tab. 4.2 Water content in apples

Dried apples	m	m_{dm}	MC
Sorbus aucuparia	0.014	0.0066	88,2
Rosa canina L.	0.013	0.0013	90,1
Hippophae rhamnoides L.	0.011	0.0011	89,5
Diospyros kaki L.	0.017	0.0011	93,4
Actinidia deliciosa	0.012	0.0016	86,2
1 % solution AA	0.011	0.0014	87
Untreated apples	0.012	0.0009	92,6

*m = mass of sample before drying in kilograms, m_{dm} = mass of sample after drying in kilograms , MC = moisture content [%]

4.3 COLORIMETRIC MEASUREMENTS

- **Colour measurement of the control**

The control freshly cut apples show a light shade of green. Yellow and slighthly green shade is only very light.

Tab.4.3 Colour of the Control

	L*	**a***	**b***
Control	86,78	-0,29	25,71

- **Colour measurement of apple slices treated with natural juices, 1 % ascorbic acid solution and of untreated samples**

+Δ*L* = sample is lighter than the control: apples treated before drying are lighter than freshly cut. No apples achieved this value.

-Δ*L* = sample is darker than the control: all the apple samples are darker than the control The closest equivalent to the control were *Rosa canina* L., *Sorbus aucuparia*, *Diospyros kaki* L., *Actinidia deliciosa*, untreated apples, *Hippophae rhamnoides* L. And the farthest value from the control was 1 % solution of AA.

+Δ*a* = sample is redder than control: the closest equivalents to the control were *Rosa canina* L., *Diospyros kaki* L., *Actinidia deliciosa*, *Sorbus aucuparia*, *Hippophae rhamnoides* L., untreated apples and the farthest values from the control was 1 % solution of AA.

-Δ*a* = sample is greener than control: appleas treated before drying are not greener on the surface that freshly cut.

+Δ*b* = sample is yellower than control: samples closest to the control were *Hippophae rhamnoides* L., *Actinidia deliciosa*, *Diospyros kaki* L, untreated apples. The farthest values from the control was *Sorbus aucuparia*.

-Δ*b** = sample is bluer than control: the closest equivalent to the control were 1 % solution of AA and *Rosa canina* L.

Δ*E** = the closest equivalent to the control were the samples treated with *Rosa canina* L. The second smallest difference was with the apples treated with *Diospyros kaki* L. and *Actinidia deliciosa juices*. The colour difference was more pronounced with the samples treated with *Hippophae rhamnoides* L. juice and with the apples that were not treated at all. Samples treated with the 1 % ascorbic acid solution and *Sorbus aucuparia* juice showed the greatest difference in colour.

Tab. 4.4 Colorimetric raw data and derived values for sample treated with natural juices, 1 % ascorbic acid solution and untreated apples

Dried apples	***L****	***a****	***b****	**Δ*L****	**Δ*a****	**Δ*b****	**Δ*E****
Sorbus aucuparia	82.076	7.272	40.979	-4.704	7.562	15.269	18.113
Rosa canina L.	84.291	4.405	23.180	-2.052	4.695	-2.530	8.509
Hippophae rhamnoides L.	77.481	7.821	27.153	-9.299	8.111	1.443	13.145
Diospyros kaki L.	80.462	5.557	31.442	-6.318	5.847	5.732	10.516
Actinidia deliciosa	79.342	6.208	27.464	-7.438	6.498	1.754	10.815
1 % solution of AA	74.088	11.565	25.285	-12.692	11.855	-0.425	17.764
Untreated apples	77.783	8.185	31.892	-8.997	8.475	6.182	13.976

- **Calculation of Hue angle, Saturation index, Browning index**

Data used for the calculation of Hue angle, Saturation and Browning index was obtained from the measurements taken at the Miniscan XE Plus. Parameters *L*, a*, b** were used. Apples treated with the juice from Actinidia deliciosa, Hippophae rhamnoides and 1 % ascorbic acid solution showed the least browning. Still quite low degree of browning was found with the samples treated with *Diospyros kaki* L. juice and with the untreated apples. The browning index was highest with the samples treated with *Sorbus aucuparia* and *Rosa canina* L.

Tab. 4.5 Calculation Hue angle, Saturation index, Browning index

Dried apples	***h°***	***C****	***BI***
Sorbus aucuparia	79,939	41,619	73,529
Rosa canina L.	79,24	23,595	73,53
Hippophae rhamnoides L.	73,933	28,257	50
Diospyros kaki L.	79,977	31,93	52,941
Actinidia deliciosa	77,262	28,156	47,059
1 % solution AA	65,418	27,804	52,941
Untreated apples	75,604	32,925	58,823

4.3.1 COLORIMETRIC MEASUREMENTS

Apple samples treated with either natural juices, 1 % ascorbic acid solution or not treated at all before drying did not achieve a lighter shade than a freshly cut apple. The comparatively lowest degree of browning was found in apples, treated with juices from Rosa canina, whereas higher degree of browning was observed with apples treated with 1 % ascorbic acid solution. Also, none of the apples achieved a greener shade than the control. All the dried apples showed an increase in the yellow colour component with the most prominent difference measured on the *Sorbus aucuparia* samples. The smallest difference in the yellow component was found with the *Hippophae rhamnoides* L. and *Actinidia deliciosa* samples A certain amount of blue colour component was found with the ascorbic acid and *Rosa canina* L. samples.The results show, that according to the ΔE^* values (total colour difference), the samples treated with *Rosa canina* L. samples were in colour the closest to the control. On the other hand, the *Sorbus aucuparia* samples were in total, the farthest. Collected data is in the section Annex B.

4.4 SENSORY ANALYSYS RESULTS

- **The colour change in comparison with fresh apple**

The results of sensory analysis revealed, that perceived colour change was rated as ‘moderate’ in samples treated with *Rosa canina* L.and ascorbic acid (AA) solution. In the *Actinidia deliciosa*, *Hippophae rhamnoides* L., *Diospyros kaki* L. and untreated samples, the colour change was rated as ‘medium’ and the e samples treated with *Sorbus aucuparia* achieved the level ‘significant’.

- **Browning as compared to fresh apple**

The results of sensory analysis showed, that samples treated with the juice from Rosa *canina* L. displayed the lowest degree of browning. There was a ‘medium’ degree of browning in apple slices treated with the AA solution, with juice from *Actinidia deliciosa*, *Sorbus aucuparia* and *Diospyros kaki L*. and those that were left untreated. The strongest browning effect was observed in the *Hippophae rhamnoides* L. samples.

- **Overal appearance**

Another part of the sensory analysis dealt with samples’ appearance. The appearance was not compared with the control; rather it focused on the overall acceptability by the consumer.

The most positive grades were given to apples treated with the 1 % AA solution, *Rosa canina* L. juice and to the untreated samples, while samples treated with *Actinidia deliciosa*, *Diospyros kaki* L., *Sorbus aucuparia* mostly obtained the ‘satisfactory’ grade. On the other hand, the grade for apples treated with juice from the fruits of *Hippophae rhamnoides* L. averaged between ‘satisfactory’ and ‘unacceptable’

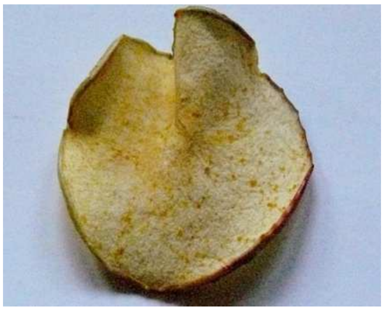

Figure 4.6 *Sorbus aucuparia*

Source: Hubáčková, 2011

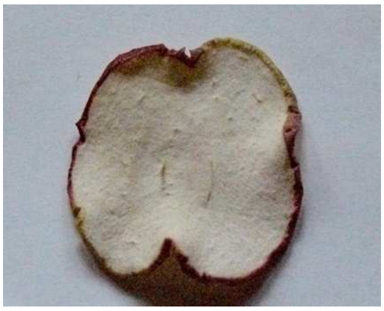

Figure 4.7 *Rosa canina* L.

Source: Hubáčková, 2011

Figure 4.8 *Hippophae rhamnoides* L

Source: Hubáčková, 2011

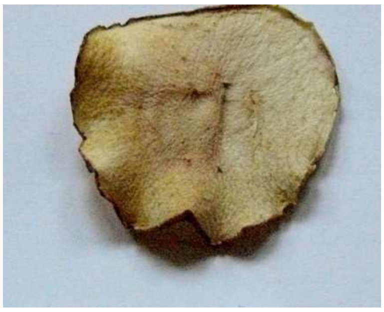

Figure 4.9 *Diospyros kaki* L.

Source: Hubáčková, 2011

Figure 4.10 *Actinidia deliciosa*

Source: Hubáčková, 2011

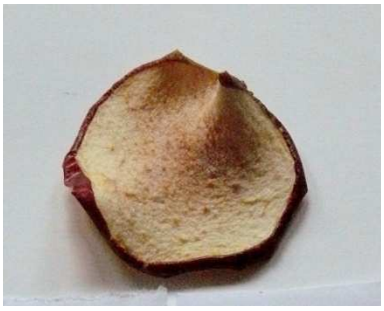

Figure 4.11 1 % solution AA

Source: Hubáčková, 2011

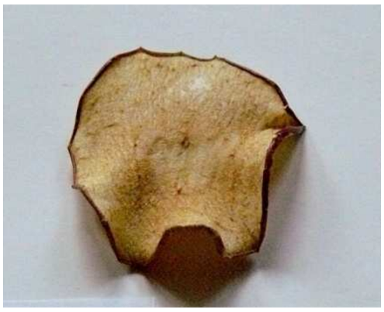

Figure 4.12 Untreated apples

Source: Hubáčková, 2011

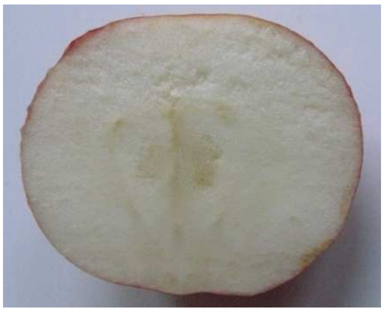

Figure 4.13 Freshly cut appple

Source: Hubáčková, 2011

- **Sweetness, bitterness and sourness**

The fourth aspect evaluated via sensory analysis was taste, specifically sweetness, bitterness and sourness of the dried apples. The least sweeet of dried apples were the *Rosa canina* samples. Sweet taste was more pronounced in the apples treated with juices from *Hippophae rhamnoides* L., *Diospyros kaki* L., *Actinidia deliciosa* and AA solution. The sweetest were notreated apples.

Most apples showed only a 'mild' degree of bitterness, only *Sorbus aucuparia* caused the samples to be rated as 'medium' with regard to bitterness.

Sourness was least pronounced in apples treated with *Diospyros kaki* L. and in untreated samples. Samples treated with *Actinidia deliciosa*, *Hippophae rhamnoides* L., *Sorbus aucuparia* and *Rosa canina* L. showed a 'medium' degree of sourness, whereas those treated with AA solution were rated as the most sour.

- **Overal flavour**

Apples treated with the juice from *Hippophae rhamnoides* achieved the highest rating of overall flavour. The *Rosa canina* L., *Diospyros kaki* L., *Actinidia deliciosa*, *Sorbus aucuparia*, 1 % solution of AA and untreated samples received mostly a 'satisfactory' rating.

Tab. 4.6. Evaluation of the questionnaire

Sample		Color		
No.	Name	The color change in comparison with fresh apple	Browning compared with fresh apple	Overal appearance
1	*Sorbus aucuparia*	2,6	2,1	2,1
2	*Rosa canina* L.	1,0	1,0	1,8
3	*Hippophae rhamnoides* L.	2,1	3,0	2,6
4	*Diospyros kaki* L.	2,3	2,3	2,0
5	*Actinidia deliciosa*	1,9	2,0	1,9
6	1 % solution of AA	1,3	1,6	1,9
7	Untreated apples	2,3	1,8	1,7

Sample		Flavour				Overal preference
No.	Name	Sweetness	Bitterness	Sourness	Overal flavour	
1	*Sorbus aucuparia*	1,8	1,5	2,1	2,3	4,6
2	*Rosa canina* L.	1,5	1,3	2,1	1,8	4,1
3	*Hippophae rhamnoides* L.	1,9	1,4	1,8	1,5	2,9
4	*Diospyros kaki* L.	2,0	1,3	1,4	2,0	3,5
5	*Actinidia deliciosa*	2,0	1,3	1,6	2,1	4,1
6	1 % solution of AA	1,6	1,4	2,6	2,3	4,6
7	Untreated apples	2,1	1,1	1,5	2,0	4,1

4.5 EVALUATION OF THE RESULTS OF MEASUREMENTS AND SENSORY ANALYSIS

Untreated apples: the untreated apple samples show ‘medium’ total colour difference *(ΔE)* compared to the samples treated with natural juices and 1 % AA solution. The *Browning Index* value was also in the ‘medium’ range. Based on the results of sensory analysis, the “colour change in comparison with the fresh apple” (further “colour change”) was very pronounced. Only the colour change of *Sorbus aucuparia* samples was percieved as stronger. “Browning compared with freshly cut apple” (further only “browning”) was also pronounced, however it did not score the highest among the other juices. According to the panelists, these apple were rated the sweetest, least sour and least bitter of all the samples. Most of the untreated samples achived ‘satisfactory’ grade in the Overall flavour category.

1 % solution of Ascorbic acid: The colorimetric results show a significant total colour difference compared to the other samples. Browning index is in the ‘medium’ range. The sensory analysis in the visual categores - colour change and browning was graded as ’slight’. Sweetness and sourness ranked as ‘medium’ and bitterness ranked only ‘mild’. As well as the untreated aples, these samples achieved mostly ‘satisfactory’ score in the Overall flavour category. Compared to the other samples, these apple slices were more sour and less sweet. It should be noted, however, that the samples treated with the AA solution displayed very different degrees of browning in different parts of the cut. It seems (as can be seen from the photographs ANNEX B), that browning was much more marked in the centre of an apple slice, than on the sides, where the colour was significantly paler. The reason for this phenomenon is unknown and it was reproduced.

***Sorbus aucuparia*:** These samples have the highest value of total colour difference, but very high Browning index, which indicates low degree of browning. Their total colour difference was much higher than that of untreated apples. Sensory analysis supports the colorimetric data in both colour change and browning categories. ‘Significant’ colour change and a ‘medium’ degree of browning was reported. Overall appearance ranked mostly ‘satisfactory’. In all three of these categories the *Sorbus aucuparia* samples scored worse than untreated apples and apples treated with 1 % AA. Sweetness, bitterness and sourness were graded ‘mild’ and overall flavour was found to be most often ‘satisfactory’. However, even here the apples treated with 1 % AA solution achived better results.

***Rosa canina* L.:** the value of the total colour difference was the lowest out of all samples. Also, the Browning Index was among the highest which indicates a very light shade. The sensory analyisis of *Rosa canina* L. samples is one of the best rated. Colour change and browning both ranked as ‘slight’ and the values are on average lower than those of untreated apples and apples treated with 1 % AA solution. Overall appearance ranks as ‘satisfactory’ and almost achieves the grade of untreated samples. Sweetness and sourness of the samples both achieve the ‘mild’ grade, while bitterness was graded on averag ‘medium’. Overall flavour ranked ‘satisfactory’. *Rosa canina* L. samples achieve on average better results than both the untreated and the AA samples.

***Hippophae rhamnoides* L.:** the value of the total colour difference was average compared to other samples and it is on a level similar to the untreated apples. Also, the Browning Index was comparatively low - even lower than BI of untreated apples and the AA solution treated ones. The sensory analysis of *Hippophae rhamnoides* L. samples shows, that colour change and browning both ranked as ‘medium’. Overall appearance ranked “unacceptable. Sweetness, sourness and bitterness all ranked ‘medium’ and overall flavour ranked ‘satisfactory’. In the categorz overall flavor the *Hippophae rhamnoides* L. samples achieved on average better results than both the untreated and the AA samples

***Diospyros kaki* L.**: The value of the total colour difference was quite low compared to other samples and it’s on a level similar to untreated apples. The browning index was also low. Untreated apples achieved worse results, although the difference is not very pronounced. In sensory analisis of *Diospyros kaki* L samples colour change and browning both ranked as ‘slight’. Overall appearance ranked as ‘satisfactory’. Sweetness was perceived to be significant, sourness and bitterness both ranked ‘mild’ and overall flavour ranked ‘satisfactory’. *Diospyros kaki* L. achieved similar results to those of 1 % AA solution samples.

***Actinidia deliciosa*:** in samples treated with *Actinidia deliciosa*, the total colour difference was the lowest from all the tested samples. Also Browning index was the lowest from all the tested samples. According to the colorimetric measurements, the *Actinidia deliciosa* samples achieved better results than both untreated apples and those treated with 1 % AA solution. According to the sensory evaluation, both “colour change” and browning ranked close to ‘medium’, while overall appearance was found to be ‘satisfactory’. Sweetness and sourness were both graded as ‘medium’ whereas bitterness was ‘mild’. Overall flavour was ‘satisfactory’. *Actinidia deliciosa* achieved the same sensoric results as both untreated apples and the samples treated with 1 % solution of ascorbic acid.

5 CONCLUSION

The aim of this thesis was to experimentally evaluate the influence of fruit juices with high content of ascorbic acid on enzymatic browning in dried apple slices. The degree of browning, overall colour change and taste of the dried apple samples were measured via sensory analysis and colorimetric techniques. Colorimetric measurements were carried out on a colorimeter MiniScan® XE Plus. Fourteen panelists participated in the sensory analysis. The fresh apple slices were used as control for comparison of browning and colour difference (*ΔE*).

According to the colorimetric measurements, the apple samples treated with juice from the fruits of *Rosa canina* L. showed the smallest total colour diference, the lowest degreee of browning after drying. Sensory analysis of samples treated with *Rosa canina* L. supports these findings as *Rosa canina* L. samples achieve the best score in categories ‘degree of browning in comparison with a freshly cut apple’ and ‘colour change in comparison with a freshly cut apple’ and the second best score in the ‘overall appearance’ category. *Rosa canina* L. samples also scored second best in the ‘overall flavour’ category of sensory testing. It may be concluded that from all the five natural juices tested, the *Rosa canina* L. juice is clearly the best substitute for the 1 % ascorbic acid solution, which is commonly used in the foodstuff drying industry.

The rest of the juices did not provide equally good improvements over the untreated samples or the 1 % ascorbic acid solution. Although, *Sorbus aucuparia* scores well on the Browning Index, it has the highest Total Colour Difference of all tested juices. It also scores second worst in the sensory analysis, where in categories ’browning compared with fresh apple‘ and ‘browning in comparison with fresh apple‘ it rated between ,’medium’and ‘significant‘. Most importantly, the *Sorbus aucuparia* samples scored poorly in the ‘overall flavour’ category of sensory analysis, probably of the bitter and sour taste they acquired from the rowan juice.

The unknown affect of many bioactive chemical compounds in the fruits of selected species makes it difficult to identify precise reasons for the overall results. However, given that the main reason for browning of dried apples is assumed to be the phenoloxidase activity, further research might focus on the PPO inhitors that may be present in the *Rosa canina* L. and *Sorbus aucuparia*, apart from ascorbic acid.

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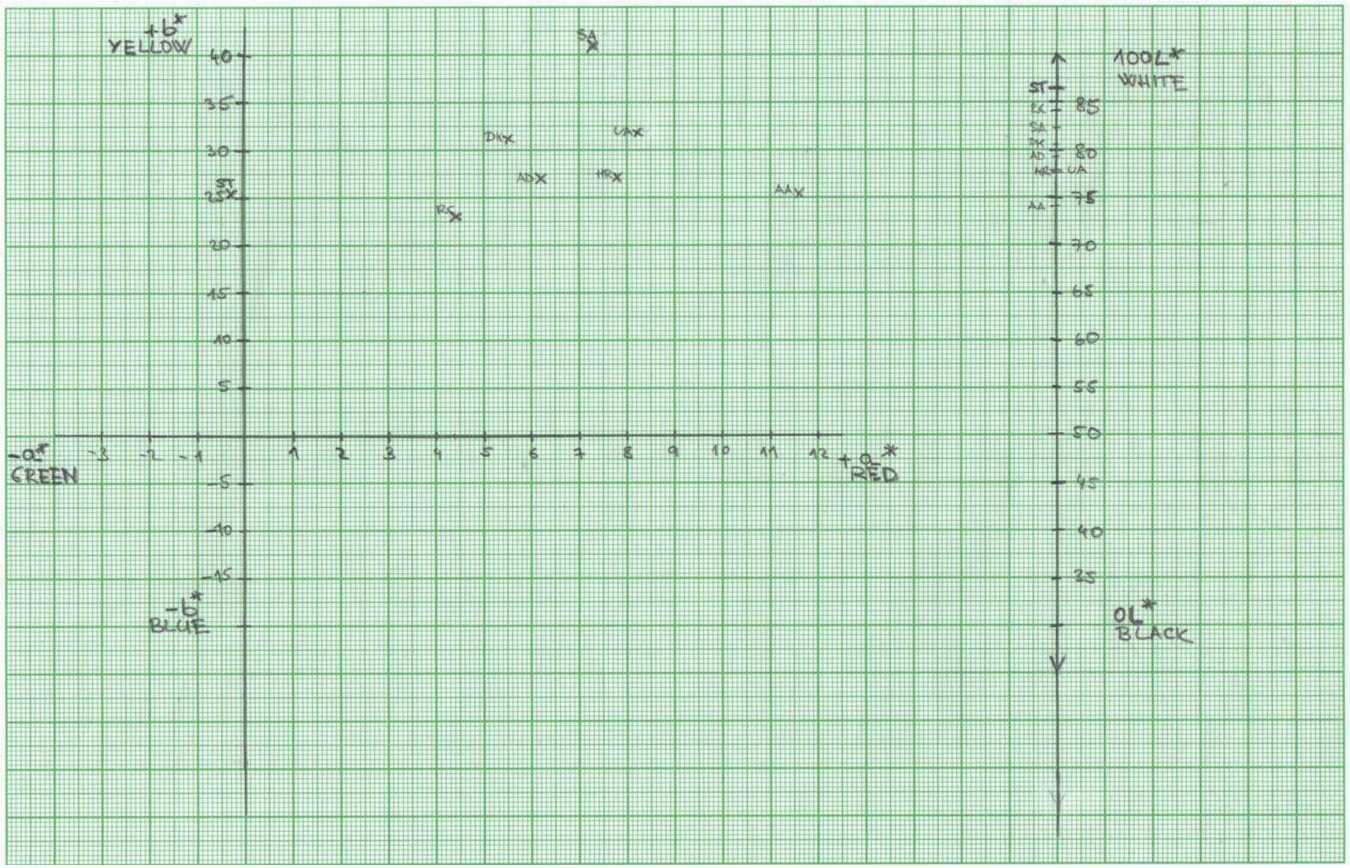
7 ANNEX

SENSORY ANALYSIS - QUESTIONNAIRE

Sample		Color		
No.	Name	The color change in comparison with fresh apple	Browning compared with fresh apple	Overal appearance
1	*Sorbus aucuparia*			
2	*Rosa canina* L.		1 = slight browning on the cut surface	
3	*Hippophae rhamnoides* L.	1 = slight color change		1 = very good
4	*Diospyros kaki* L.	2 = medium color change	2 = medium brown	2 = satisfactory
5	*Actinidia deliciosa*	3 = significant color change	3 = significant	3 = unacceptable
6	1% solution of AA			
7	Untreated apples			

Sample		Flavour				Overal preference						
No.	Name	Sweetness	Bitterness	Sourness	Overal flavour	Order by preference: 1 = most preferred, 7 = least preferred						
1	*Sorbus aucuparia*											
2	*Rosa canina* L.											
3	*Hippophae rhamnoides* L.	1 = mild	1 = mild	1 = mild	1 = very good							
4	*Diospyros kaki* L.	2 = medium	2 = medium	2 = medium	2 = satisfactory							
5	*Actinidia deliciosa*	3 = significant	3 = significant	3 = significant	3 = unacceptable							
6	1% solution of AA											
7	Untreated apples											

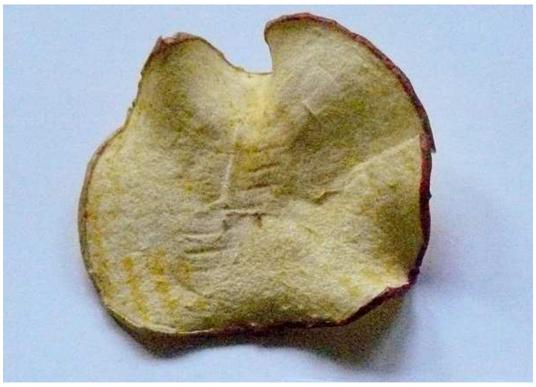
COLORIMETRIC MEASUREMENT

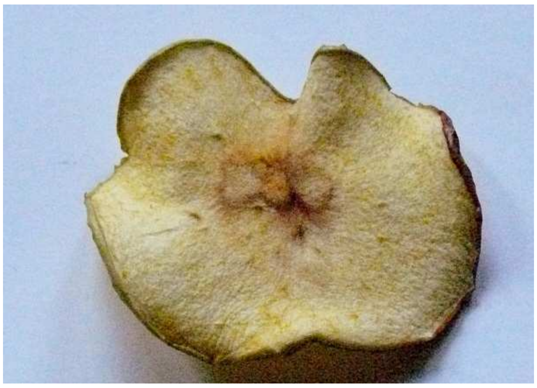


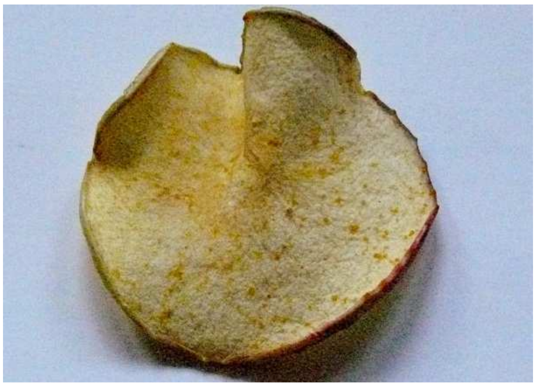
*SA = *Sorbus aucuparia*, RC = *Rosa canina* L.,HR = *Hippophae rhamnoides* L, DK = *Diospyros kaki* L., AD = *Actinidia deliciosa*, 1% solution of AA, UA = Untreated apple

PHOTOGRAPHS OF DRIED APPLE TREATED WITH DIFFERENT NATURAL JUICES

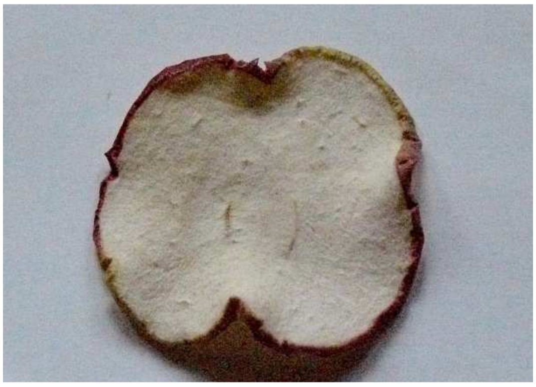
- ***Sorbus aucuparia***

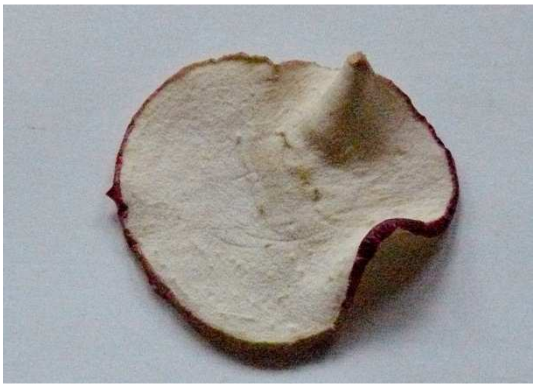
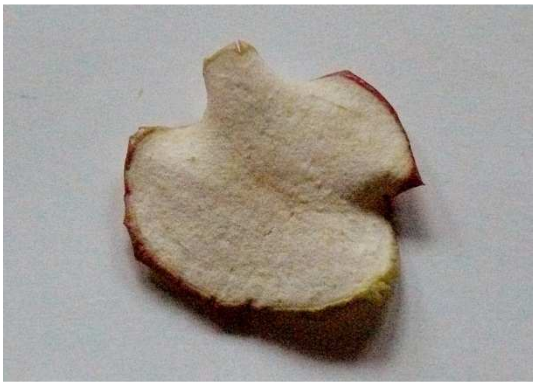






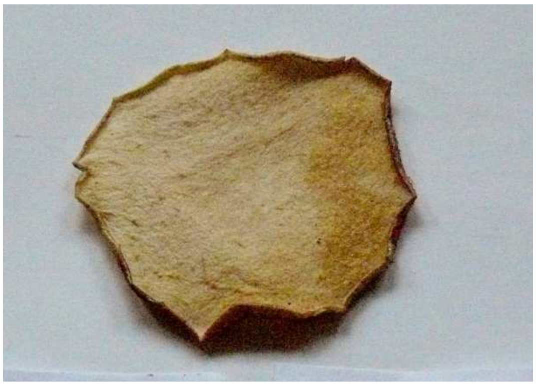
- ***Rosa canina* L.**

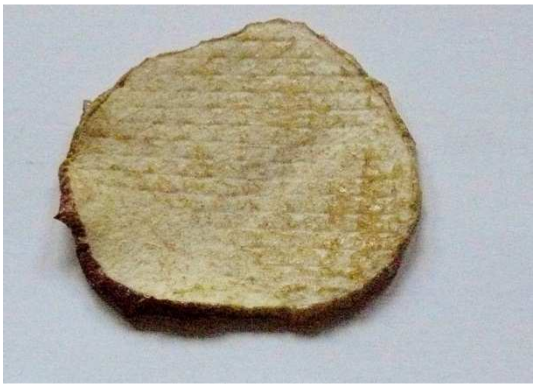


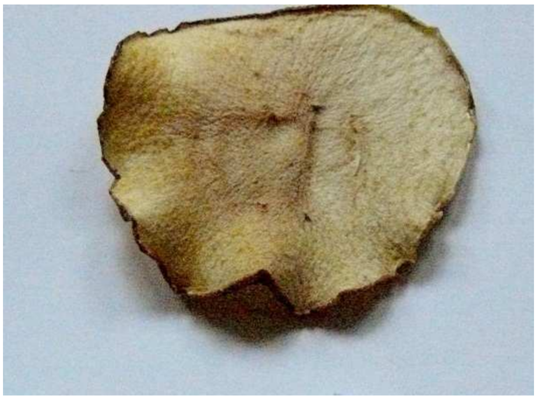




- ***Hippophae rhamnoides* L.**

- ***Diospyros kaki* L.**





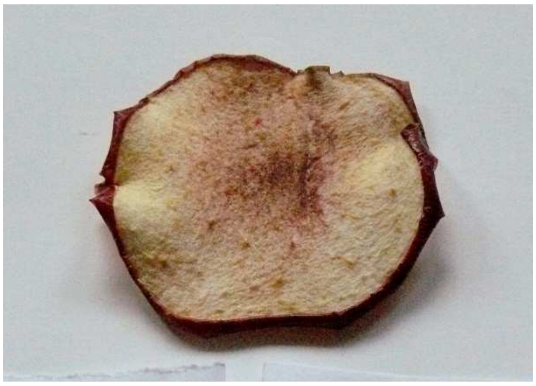
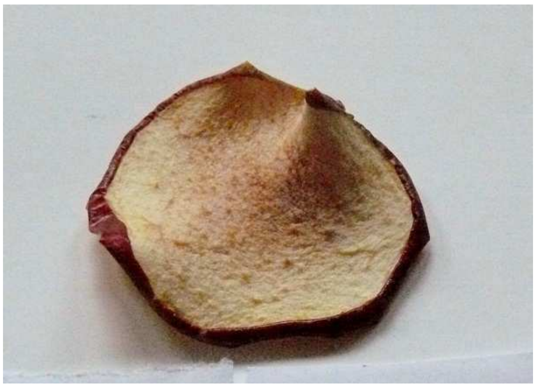


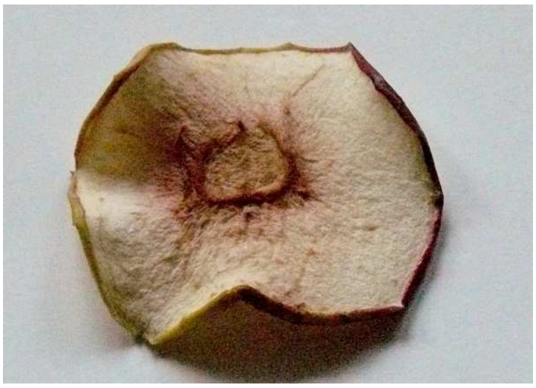
- ***Actinidia deliciosa***





- **1 % solution AA**




- **Untreated apples**