THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Nutritional Value and Quality of Processed Mango Fruits

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Front cover: Fresh and processed mango. *Photo by Margareth Rebelo*

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Abstract

Mango (*Mangifera indica* L.) is one of the most important tropical fruits commercialized and consumed worldwide, and an excellent source of fibre, bioactive compounds such as pro-vitamin A carotenoids, vitamin C (ascorbic acid) and phenolics. Mango was used in this thesis as a model fruit of fruits and vegetables that are rich sources of nutrients. They are highly perishable, prone to progressive undesired changes if stored untreated. As a result, large amounts of mango are lost annually in many areas of the world including Mozambique. To reduce postharvest losses of fruits, processing is recommended since it extends the shelf life and enable the availability off-season, and the promotion of consumption is a potential health enhancing strategy. However, processing of fruits and vegetables must be carefully designed to achieve a high sensory and nutritional quality of the final products. The aim of the present thesis was thus to evaluate the effect of conventional and novel processing techniques on the retention of vitamin C and β-carotene, two major bioactive compounds, in minimally processed or dried mango products.

The effect of osmotic dehydration (OD) with or without vitamin C or calcium prior to hot air-drying was evaluated in terms of the retention of vitamin C and β-carotene in dried mango. Fortification of OD solutions with either calcium or vitamin C was shown to be an efficient way to improve the retention of vitamin C and carotenoids in dried mango. OD pre-treatment reduced the hardness, drying time, and prevented colour changes.

The potential use of alternative blanching techniques to improve the retention of vitamin C and β-carotene in processed mango was explored using infrared (IR) and microwave (MW) dry blanching and blanching in a closed plastic bag immersed in water bath at (High-Temperature-Short-Time - HTST) or (Low-Temperature-Long-Time - LTLT), prior to hot air-drying. Complete inactivation of PPO was achieved, while a minor activity of AAO remained. An improved retention of vitamin C in dried mango was achieved using IR and MW dry blanching or water blanching in plastic bag in comparison to conventional water blanching. However, the retention of all-*trans*-β-carotene was higher in dried mango after water blanching than after dry blanching. In conclusion, a correct selection of suitable techniques and conditions for blanching can enhance the nutritional value of dried mango and inactivate enzymes that degrade vitamin C and β-carotene.

To address the problem of reduced sensory and nutritional quality during the processing of purées, the impact of acidification, addition of EDTA or water blanching at HTST, on the retention of vitamin C (L-AA and DHAA) and β-carotene was evaluated in mango purée. The results showed that the disruption of the cellular matrix during purée processing facilitates oxidative reactions of vitamin C and of all-*trans*-β-carotene unless protected by an initial blanching step, pH adjustments or addition of EDTA a chelating agent to inhibit AAO and PPO activities.

” I have fought a good fight, I have finished my course, I have kept the faith.”

(II Timothy 4:7)

LIST OF PUBLICATIONS

This doctoral thesis is based on the work contained in the following papers, referred to in the text by their Roman numerals.

I. Guiamba, I., Khan, M.A.M., Ahrné, L. and Svanberg, U. Retention of β-carotene and vitamin C in dried mango osmotically pretreated with osmotic solutions containing calcium and ascorbic acid.
Accepted in Food and Bioproducts Processing 2016.

II. Guiamba, I.R.F., Svanberg, U. and Ahrné, L. (2015). Effect of infrared blanching on the enzyme activity and retention of β-carotene and vitamin C in dried mango. *Journal of Food Science*. 80:E1235-E1242.

III. Guiamba, I. and Svanberg, U. Effects of blanching, acidification or addition of EDTA on vitamin C and β-carotene stability during mango purée preparation.
Accepted in Food Science & Nutrition 2016.

IV. Guiamba, I., Ahrné, L. and Svanberg, U. Enhancing the retention of β-carotene and vitamin C in dried mango using alternative blanching processes.
Submitted manuscript.

Related publications not included in the thesis:

Ráice, R., Guiamba, I., Arhné, L., Svanberg, U., and Bergenståhl, B. Effects of drying with or without blanching on composition of volatiles in dried mango fruit (*Mangifera indica L.*).
Submitted manuscript.

CONTRIBUTION REPORT

Paper I The author, Isabel Guiamba (IG), participated in the design of the study, planned the work and performed the experimental work. IG was responsible for interpreting the results together with the co-authors and wrote the first draft of the manuscript, which was finalized with contributions from the co-authors.

Paper II IG participated in designing the experiments and performed all the experimental work. IG was responsible for interpreting the data and writing the manuscript, with contributions from the co-authors.

Paper III IG was involved in designing the experiments, performed the experimental work and evaluated the results. IG wrote the first draft of the manuscript, which was finalized with contributions from the co-author.

Paper IV IG participated in the design of the study and conducted the laboratory work. IG was responsible for evaluating the data and writing the manuscript, with contributions from the co-authors.

ABBREVIATIONS

Abbreviation	Description
AA	Ascorbic acid
AAO	Ascorbic acid oxidase
DHAA	Dehydroascorbic acid
DW	Dry weight
EDTA	Ethylenediamine Tetraacetic Acid
FAO	Food and Agriculture Organization of the United Nations
HTST	High temperature and short time
IR	Infrared
IR65	Mango infrared blanched at 65 °C
IR90	Mango infrared blanched at 90 °C
L-AA	L-ascorbic acid
LTLT	Low temperature and long time
MW	Microwave
MW70	Mango microwave blanched at 70 °C
MW90	Mango microwave blanched at 90 °C
OD	Osmotic dehydration
ODAA50	Osmotic dehydrated mango in osmotic solutions 45 °Brix with 1% of vitamin C, hot air-dried at 50 °C
ODAA70	Osmotic dehydrated mango in osmotic solutions 45 °Brix with 1% of vitamin C, hot air-dried at 70 °C
ODCa50	Osmotic dehydrated mango in osmotic solutions 45 °Brix with 1% of calcium chloride, hot air-dried at 50 °C
ODCa70	Osmotic dehydrated mango in osmotic solutions 45 °Brix with 1% of calcium chloride, hot air-dried at 70 °C
OD50	Osmotic dehydrated mango in osmotic solutions 45 °Brix without additives, hot air-dried at 50 °C
OD70	Osmotic dehydrated mango in osmotic solutions 45 °Brix without additives, hot air-dried at 70 °C
PPO	Polyphenol oxidase
USDA	United States Department of Agriculture
U50	Untreated mango hot air-dried at 50 °C
U70	Untreated mango hot air-dried at 70 °C
VAD	Vitamin A deficiency
WHO	World health Organization
W65	Mango water blanched at 65 °C
W70	Mango water blanched at 70 °C
W90	Mango water blanched at 90 °C
W70P	Mango water blanched in plastic bag at 70 °C
W90P	Mango water blanched in plastic bag at 90 °C

1. INTRODUCTION

Mango *(Mangifera indica* L.*)* is one of the most important tropical fruits commercialized and consumed worldwide fresh or processed, having an attractive colour and distinct taste and aroma (Singh *et al*., 2000). Mango is an excellent source of fibre and bioactive compounds such as provitamin A carotenoids, vitamin C and phenolics that can easily be degraded during processing and storage. Fruits and vegetables, being rich sources of nutrients, have been of interest due to their potential health benefits in preventing several chronic diseases (Slavin and Lloyd, 2012).

Fruits are highly perishable due to enzymatic and non-enzymatic reactions that take place during maturation and storage, affecting nutritional, sensorial and physico-chemical properties. Although processing is considered an important tool to limit degradative reactions some vitamins and minerals can be lost during processing. Several processing methods have been explored with the aim to produce high quality products with extended shelf life, but there is a lack of information on the effect on bioactive compounds of exotic fruits such as mango and its products. The aim of this thesis was to apply conventional and novel processing techniques to study the effects on the retention of two major bioactive compounds in mango, vitamin C and β-carotene. In the addition, the effect of colour and texture was also evaluated.

Air-drying is a preservation method commonly used to extend the shelf life of fruits and reduce postharvest losses. However, high temperatures or long drying times negatively affects the quality of the dried products, particularly in terms of flavour, colour, texture, nutrients and rehydration capacity (Drouzas *et al*., 1999). Vitamin C and carotenoids are the most sensitive compounds when exposed to heat during the drying process (Kong and Singh, 2013). To improve the quality of dried products, pre-treatments such as osmotic dehydration and blanching are commonly used prior to air-drying particularly, for temperature sensitive products like fruits.

Osmotic dehydration (OD) involves the immersion of fruit in concentrated solutions of soluble solutes such as sugars or salts where both partial dehydration of the fruit and sugar uptake occur (Torres *et al*., 2006). However, during osmotic dehydration, leaching of water-soluble components from fruits such as minerals, vitamins and organic acids may be extensive (Chandra and Kumari, 2015; Yadav and Singh, 2014). Although a large number of studies on osmotic dehydration have been published in the last years, few studies have focused on changes on the nutritional compounds. Moreover, blanching is a frequently used method in fruit and vegetable processing to minimize undesired changes as a result of enzymatic reactions produced by enzymes such as polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) (Grandison, 2012). PPO affects the appearance and organoleptic properties of fruits and vegetables through browning reactions, and has been extensively studied in different fruits including mango. However, there is a lack of knowledge on the effect of AAO, an enzyme that catalysis the oxidation of L-ascorbic acid (L-AA) form to dehydroascorbic acid

(DHAA), which may lead to subsequent loss of vitamin C. Novel heating techniques that involve dry blanching by microwave and infrared electromagnetic radiation in combination with conventional drying processes of fruits and vegetables on the bioactive compounds are emerging. These methods have gained interest as alternatives to conventional water blanching, as they reduce the leaching of water-soluble compounds and thus are associated with improved nutritional value of the blanched products.

Addition of different enzyme inhibitors has been applied in the industry due to the increasing consumer demand for minimally processed food such as jams, juices and purées. This is to minimize the effect of enzyme activity that degrades nutrients and sensorial quality of processed products as a result of heat or oxidation. Although some studies of heat treatment are reported regarding vitamin C and β-carotene in mango purée (Lemmens *et al*., 2013; Vásquez-Caicedo *et al*., 2007), no reports were found on these bioactive compounds as affected either by acidifiers and chelating agents, or for the effect on the distribution of total vitamin C as L-AA and DHAA forms in mango products.

This thesis was therefore mainly focused on the investigation of different processing methods for preservation of fruits and vegetables with enhanced nutritional value using mango as a model fruit. Thus, studies were done on the effect of minimally processing of mango into purées and the impact of pre-treatments (osmotic dehydration with additives and blanching) prior to hot air-drying on the retention of vitamin C and β-carotene in dried mango.

Mango (*Mangifera indica* L.) was selected for this study due to its nutritional value and for being largely produced with significant postharvest losses in Mozambique. The agriculture census of 2009-2010 in Mozambique (INE 2011, Censo Agro-Pecuário 2009-2010) indicated that the mango tree is the most abundant fruit tree in the country, with more than fourteen million trees, followed by mandarin, orange and papaya. Thus, improved processing of fruits and vegetables, as well as promotion of consumption, is a promising strategy in improving the nutritional status of the population, besides being a possible source for exportation, and the results of this study may therefore be of considerable interest.

2. OBJECTIVES

The overall objective of this work was to evaluate the effect of different processing methods on the nutritional quality of minimally processed or dried mango to obtain products with high nutritional value and increased shelf life. To achieve this purpose, studies were initiated to investigate the retention of vitamin C and β-carotene in mango purée or in dried mango pre-treated by osmotic dehydration with additives or by alternative blanching techniques prior to hot air-drying.

The specific aims of the studies in this thesis were:

- To assess the variability of vitamin C and β-carotene content in mango cultivars used in different studies of this thesis.

- To evaluate the role of vitamin C or calcium as additives in osmotic dehydration solutions prior to hot air-drying in the nutritional value (vitamin C and β-carotene) of dried mango (Paper I);

- To compare the effect of conventional water heating and dry blanching methods, infrared and microwave performed at high temperature and short time (HTST) or low temperature and long time (LTLT) before hot air-drying on vitamin C and β-carotene retention in dried mango (Papers II and IV);

- To assess the influence of conventional water blanching in closed plastic bags on the retention of nutrients, especially the water-soluble nutrient vitamin C (Papers III and IV);

- To evaluate how HTST and LTLT blanching affect the enzymatic activity of polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) in mango (Papers II, III and IV);

- To investigate the impact of acidification, addition of Ethylenediamine Tetraacetic Acid (EDTA) (chelating agent) and HTST water blanching in closed plastic bags prior to matrix disruption on the retention of vitamin C (L-AA and DHAA) and β-carotene in mango purée (Paper III).

3. BACKGROUND

A review of the literature related to various aspects of the present thesis is presented in this chapter. This includes the importance of fruits and vegetables, and mango fruit characteristics. Issues associated to vitamin C and carotenoids bioactive compounds and to processing of fruits and vegetables are also discussed. Finally, the use of anti-browning and chelating agents in minimal processed products is as well explored.

3.1. Importance of fruits and vegetables

Fruits and vegetables are in general rich sources of vitamins, minerals and phytochemicals, as well as dietary fibre, providing compounds such as vitamin C and E, carotenoids and polyphenols (Khoo *et al.*, 2011; Masibo and He, 2009; Rodriguez-Casado, 2014; Slavin and Lloyd, 2012). Epidemiological studies have established a positive correlation between the intake of fruits and vegetables and prevention of cardiovascular disease (Dragsted *et al*., 2006; Ye and Song, 2008); neurogenerative diseases (Joseph *et al*., 1999), gastrointestinal and respiratory tract cancers, (Byers and Guerrero, 1995), and lung cancer (Yong *et al*., 1997). On the other hand, fibre is known to be of importance for normal gastrointestinal function and high intakes of dietary fibre are also suggested to lower the risk of cardiovascular disease and coronary heart disease (Slavin, 2013), diabetes (Hopping *et al*., 2010) and to maintain normal body weight (Slavin, 2008).

Thus, for a healthy diet, the World Health Organization (WHO) recommends a daily intake of at least 400 g (5 portions) of fruits and vegetables to provide a sufficient amount of nutrients and to lower the risk of chronic diseases (WHO, 2003). Many of the bioactive compounds also impart colour to plant foods (Bartley and Scolnik, 1995) and, therefore, colourful varieties of fruits and vegetables are especially recommended (Figure 3.1). As a result of this and other initiatives, people are becoming aware of the nutritional and health benefits of fresh fruits and vegetables.

Figure 3.1 Colourful varieties of fruits and vegetables

3.2. Mango fruit characteristics

Mango (*Mangifera indica* L.) is one of the most nutritious tropical fruits, native to southern Asia and especially Eastern India. The mango tree is from the anacardiaceae family and it was disseminated all over the world in the beginning of the sixteenth century, and there are currently around a thousand known varieties of mango (FAO, 2002). Cultivars such as Tommy Atkins (Figure 3.2), Haden, Kent and Keitt are the most produced and exported cultivars in many countries (Saúco, 2004). Mango is the dominant tropical fruit variety produced worldwide, followed by pineapples, papaya and avocado, all which are considered major tropical fruits. The global production of mango in 2010 was estimated to be about 35 million tons, accounting for nearly 50% of world tropical fruit production (FAOSTAT, 2012).

Figure 3.2 Mango fruit of the cv. 'Tommy Atkins'.

The mango fruit is fleshy, generally sweet in taste and varies considerably in size, shape, colour, flavour, and composition (FAO, 2002). Its yellow-orange characteristic colour is due to the presence of carotenoids. Like other tropical fruits, mango is seasonal, with relatively short postharvest shelf life due to its perishability. Mango has three parts of interest, the pulp, peel and seed, although mango pulp is the main part with utilization both domestically and industrially (Masibo and He, 2009). It is consumed fresh worldwide and used as an ingredient in processed products such as fruit juice blends, purées, jams, dairy products, desserts, fruit salads and snacks.

Mango is an excellent source of bioactive compounds such as provitamin A carotenoids, vitamin C and phenolics, as well as dietary fibre (Lemmens *et al*., 2013; Pott *et al*., 2003; Rincon and Kerr, 2010; Sogi *et al*., 2012), essential to human nutrition and health. Moreover, mango is known to contain other vitamins, carbohydrates and minerals such as calcium, iron and potassium, and to be low in calories and fat (Table 3.1).

Table 3.1 Average nutrient content per 100 g of raw mango pulp.

Component	Amount
Water, g	83.5
Energy, kcal	60
Protein	0.8
Total lipid (fat), g	0.4
Carbohydrates (by difference), g	15.0
Fibre (total dietary), g	1.6
Sugars (total), g	13.7
Calcium, mg	11
Phosphorus, mg	14
Iron, mg	0.16
Potassium, mg	168
Vitamin C (total ascorbic acid), mg	36.4
Vitamin A, RAE, µg	54
Vitamin A , IU	1082
Vitamin E (α-tocopherol), mg	0.90
Riboflavin, mg	0.04
Niacin, mg	0.67
Thiamin, mg	0.03
Vitamin B6, mg	0.12
Folate (DFE), µg	43

Source: taken in part from USDA (2011). RAE – retinol activity equivalent; IU – international unity; DFE – dietary folate equivalent.

The content of nutrients in mango is affected by factors such as cultivar, growing conditions, stage of maturity and storage (Lee and Kader, 2000; Mercadante and Rodriguez-Amaya, 1998). Processing of mango to obtain derivate products such as juices, purée, dried fruits and mango chutney is important because of its seasonality, since it prolong its shelf life. However, the full potential for processing has still not yet been explored in many areas of the world, including Mozambique. As a result, large amounts are lost annually

3.3. Vitamin C and carotenoids

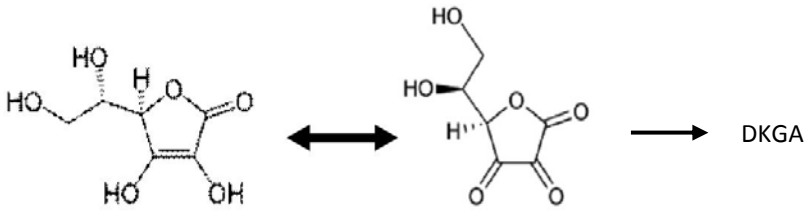
Several aspects related to vitamin C and carotenoids are presented in this section. Starting by the chemical structure and general properties, the role of these compounds in plant and its stability are discussed. In addition, sources and related factors, intakes, as well as its importance in the human nutrition and heath are also explored.

3.3.1. Vitamin C

3.3.1.1. Chemical structure and general properties

Vitamin C [L-ascorbic acid (L-AA)], an essential micronutrient for human nutrition, is a six-carbon lactone, which is synthesized from glucose by many animals (FAO, 2004).

Humans are unable to synthesize L-AA owing to their deficiency in L-gulonolactone oxidase, the enzyme that catalyzes the terminal step in L-AA (Nishikimi *et al*., 1994). Thus, humans must meet the required amount of vitamin C for normal metabolic functioning in the body through the diet, mainly fruits and vegetables that are plentiful sources of vitamin C. The principal biological active form of vitamin C in plant foods is L-ascorbic acid and, in minor part, its oxidized form, dehydroascorbic acid (DHAA) (see Figure 3.3), which also has vitamin C activity. Together they are defined to make up the total vitamin C in food (Santos and Silva, 2008; Wechtersbach and Cigić, 2007). For this reason, it is important to measure both L-AA and DHAA in fruits and vegetables to assess the total vitamin C content (Barrett and Lloyd, 2012; Oliveira *et al*., 2010).



L-Ascorbic acid Dehydroascorbic acid

Figure 3.3 Vitamin C structure and its conversion.

3.3.1.2 Stability of vitamin C

L-AA is known to be a very labile vitamin easily oxidised to DHAA during processing and storage, with subsequent irreversible hydrolysis to 2,3-diketogulonic acid (DKGA), leading to a loss of vitamin C activity (Barros *et al*., 2010; Lima *et al*., 2010; Santos and Silva, 2008). This has been the most common artefact in vitamin C analysis. The process of L-AA oxidation is a consequence of a damaging of plant tissue by cutting or crushing, which releases the endogenous plant enzyme ascorbic acid oxidase (AAO), the major degrading enzyme of L-AA in the presence of molecular oxygen (Saari *et al*., 1995). This enzyme has not yet been much investigated, and few studies on the effect of AAO during plant food processing are found in the literature (Leong and Oey 2012; Munyaka *et al*., 2010b; Yamaguchi *et al*., 2003). On the other hand, there are numerous studies on the effect of ripening, processing and storage on vitamin C content in fruits and vegetables (Azoubel *et al*., 2009; Sogi *et al*., 2012; Vasquez-Salinas and Lakshminarayana, 1985).

Besides being water soluble, which may lead to leaching, some reports indicate that the stability of vitamin C varies markedly as a function of conditions such as temperature and the presence of metal ions, oxygen and enzyme (AAO) activity, and light (Davey *et al*., 2000, Santos and Silva, 2008; Wechtersbach and Cigic, 2007). Thus, since

vitamin C is heat-sensitive, a long food processing time at elevated temperatures is detrimental. Owing to its instability, vitamin C is usually used as an indicator vitamin in processing.

3.3.1.3 Role of vitamin C in plants

As in humans, vitamin C is also an essential compound for plants. Recent studies indicate that vitamin C is a micromolecule substance widely existent in plants, where it fulfils essential roles in several physiological processes including plant growth and development, photosynthesis, plant antioxidant capacity, photoprotection, and prevention of oxidative stress, and it also acts as a co-factor for enzyme activity (Gallie, 2013; Smirnoff, 1996; Smirnoff and Wheeler, 2000; Zhang, 2013). However, very little is known about vitamin C metabolism in plants (Smirnoff, 2000). Vitamin C is synthesized in the inner membrane of the mitochondria (Siendones *et al.,* 1999) and is then distributed to all subcellular compartments, including the apoplast (cell wall), and is one of the most abundant metabolites in the chloroplasts (Rautenkranz *et al*., 1994; Smirnoff, 2000).

The action of vitamin C as an antioxidant is to protect plant cells by scavenging oxygen free radicals such as superoxide, hydroxyl radicals and singlet oxygen (Smirnoff and Wheeler, 2000). It is thus generally regarded that plant injury caused by stress comes from a depleted antioxidant system and membrane lipid peroxidation (Zhang, 2013). One of the key functions of vitamin C is therefore to protect the chloroplast from oxidative damage, and it can be assumed that other bioactive compounds stored in the chloroplast or chromoplast compartments, such as carotenoids, could also be protected from oxidative degradation.

3.3.1.4. Vitamin C and dietary intakes

Fruits and vegetables are reported to be the main contributors of vitamin C in the diet, with the percent contribution amounting to as high as 90% (Lee and Kader, 2000). Citrus fruits and juices are particularly rich sources of vitamin C, but other good sources are mango, papaya, strawberries, kiwi, tomato, green peppers, potatoes, broccoli, asparagus, spinach, melon, watermelon, cantaloupe and cranberries (FAO, 2001; Slavin and Lloyd, 2012). Reports of different values of vitamin C in mango as affected by several factors mentioned in section 3.1 are found in the literature. A range of 11 to 134 mg/100 g (FW) was reported by Manthey and Perkins-Veazie (2009) in five ripe mango cultivars (Ataulfo, Haden, Kent, Keitt and Tommy Atkins) from different locations, while a database of USDA (2011) reports an average vitamin C content in mango of 36.4 mg/100 g (Table 3.1). The vitamin C content in mango also decreases during ripening (Brecht and Yahia, 2009; Manthey and Perkins-Veazie, 2009); Vasquez-

Salinas and Lakshminarayana, 1985). The recommended nutrient intake (RNI) of vitamin C (Agriculture Organization of the United Nations (FAO)), taking into account the age and other factors, is shown in Table 3.2. In many developing countries, limitations in the supply of vitamin C are often determined by seasonality of the fruits and other social factors (FAO, 2001).

Table 3.2 Recommended nutrient intake (RNI) for vitamin C.

Group	RNI (mg/day)
Infants and children	
0-6 months	25
7-12 months	30
1-3 years	30
4-6 years	30
7-9 years	35
Adolescents, 10-18years	40
Adults	
19-65 years	45
65+ years	45
Pregnancy	55
Lactation	70

Source: FAO 2001

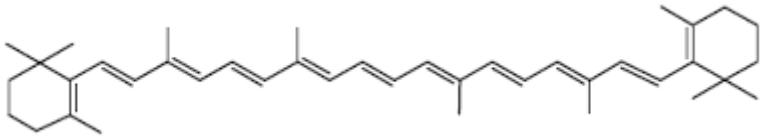
3.3.1.5. Role of vitamin C in human health

The attention to the role of vitamin C in nutrition and human health, especially as an antioxidant, has generated greater research. The biochemical properties of vitamin C are associated to its function as a reducing agent for many reactive species. Moreover, due to its solubility in water, vitamin C is considered the major antioxidant in plasma, playing a protective role (Frei *et al*., 1990). Vitamin C acts as an antioxidant to scavenge reactive oxygen species, keep membrane-bound antioxidant α-tocopherol in the reduced state, and is also a co-factor maintaining the activity of a number of enzymes. Furthermore, scurvy has been known for centuries to result from vitamin C deficiency. Vitamin C is also reported to enhance iron absorption and thus prevent anemia, improve collagen synthesis formation and the immune system, and prevent cardiovascular diseases (Block, 1993; Carr and Frei, 1999; Davey *et al*., 2000; Gibney *et al*., 2009; Kaur and Kapoor, 2001). On the other hand, the potential toxicity of excessive doses of supplemental vitamin C relates to intra-intestinal events and to the effects of metabolites in the urinary system (WHO, 2003). Some studies indicate that the protective action of vitamin C against cancers and other chronic disease (Byers and Guerrero, 1995; Yong *et al*., 1997) derive from the synergetic effect that exists between this nutrient and others found in the matrix provided by whole foods.

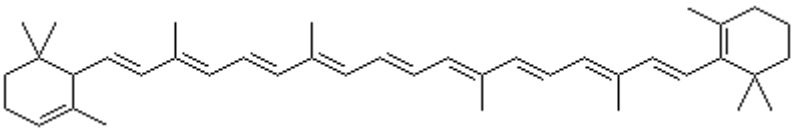
3.3.2. Carotenoids

3.3.2.1. Chemical structure and general properties

Carotenoids are widely distributed pigments synthesized by plants, microorganisms and animals, but not by humans (Briton, 1995; Furr and Clark, 1997; Rock, 1997; Young, 1991). Carotenoids are isoprenoid compounds, biosynthesized by tail-to-tail linkage of two C20 molecules, which produces the parent C40 carbon skeleton from which all the variations are derived (Britton, 1995). All carotenoids can be considered as lycopene, which has an acyclic structure with an inverted order at the molecule center (Delgado-Vargas *et al*., 2000), being as a whole a symmetrical molecule (Bartley and Scolnik, 1995). Cyclisation at one or both ends of the molecule will form one of the seven different typical end groups, with β-carotene being the only carotenoid containing two β-rings. Based on their composition, hydrocarbon carotenoids such as β-carotene, α-carotene and lycopene are called carotenes, or xanthophylls when oxygen is inserted in the structure, such as in β-cryptoxanthin (Figure 3.4).

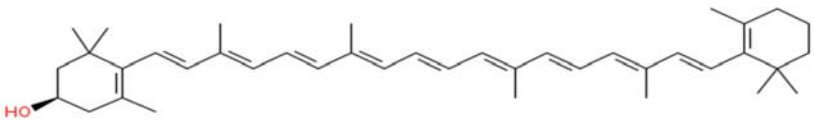


All-*trans*-β-carotene



All-*trans*-α-carotene

Lycopene



β-cryptoxanthin

Figure 3.4 Chemical structure of lycopene and provitamin A carotenoids.

The carotenoid structure is an extensive conjugated double-bonds system in which π-electrons are delocalized over the entire chain (Britton, 1995), an attribute that determines their molecular shape, chemical reactivity and light absorption in the visible region spectrum with a wavelength ranging from 400 to 500 nm (Britton, 1995; Rodriguez-Amaya, 2001). Moreover, carotenoids are found in nature primarily in the more stable *trans*-form (linear molecules), while *cis* isomers (bend molecules) are fewer due to their instability (Briton, 1995; Rock, 1997). Carotenoids are hydrophobic molecules with little or no solubility in water, but are soluble in organic solvents (Briton, 1995). Carotenoids such as β-carotene, α-carotene and β-cryptoxanthin, which have at least one unsubstituted β-ring (Figure 3.4), are vitamin A precursors that play an important role in human health (Rock, 1997), and β-carotene the most important provitamin A carotenoid.

3.3.2.2. Carotenoids in mango

Mango is considered a rich source of carotenoids. However, although a natural variation of carotenoids among mango samples is expected because of cultivar differences, climatic effects, stage of maturity at harvest and storage, some of the discrepancies in reported results are apparently due to the analytical procedures used (Mercadante *et al*., 1997; Rodriguez-Amaya *et al*., 2008). Thus, a large variation in carotenoids has been identified in different mango cultivars, with reports of increased content during ripening (Manthey and Perkins-Veazie, 2009; Mercadante and Rodriguez-Amaya, 1998; Vasquez-Salinas and Lakshminarayana, 1985). For instance, Cano and De Ancos (1994) identified thirty-three carotenoids in raw and frozen cv. 'Alphonso' mango fruit distributed among xanthophylls, carotenes and fatty acid esters. The major components identified were antheraxanthin, violaxanthin, *cis*-isomers and epoxy derivatives of lutein and β-carotene, while canned mango showed different patterns with β-carotene as the main compound (90%). However, most studies have shown that β-carotene is both the principal provitamin A carotenoid and the main pigment in mango (Chen *et al*., 2007; Masibo and He, 2009; Wilberg and Rodriguez-Amaya, 1995), which predominates in the form of all-*trans*-β-carotene. The presence of *cis*-β-carotene isomers in fresh mango has also been reported (Godoy and Rodriguez-Amaya, 1994; Pott *et al*., 2003; Vásquez-Caicedo *et al*., 2005).

Manthey and Perkins-Veazie (2009) evaluated β-carotene in five different mango varieties (see section 3.3.1.2) from different locations and multiple harvest stages. The average content of β-carotene was found to vary between 5 and 30 mg per kg of fresh mango, depending on the location and stage of maturity. The reports of different studies have shown that the carotenoid content in fresh mango varies considerably, and the results have either been reported on a fresh or dry basis, which limits data comparison. It is therefore recommended that nutrient data be expressed on a dry weight basis to account for changes in moisture (Barret and Lloyd, 2011). Table 3.3 shows carotenoids identified in fresh ripe mango from diverse studies, with evidence of a variation

between cultivars. Carotenoids in mango are also affected by the processing, which may result in a variation in the content and/or composition (Cano and De Ancos, 1994; Chen *et al*., 2007; Godoy and Rodriguez-Amaya, 1987; Lemmens *et al*., 2013; Mercadante and Rodriguez-Amaya, 1998; Pott *et al*., 2003; Vásquez-Caicedo *et al*., 2007).

Table 3.3 Carotenoids in fresh ripe mango (µg/g DW).

Carotenoid	Thaiwanese mango [1]	Thaiwanese mango [2]	Keitt [3][a]	Tommy Atkins [3][a]	Tommy Atkins [4]	Kent [4]	Thai Mango[b] [5]	Thai mango[c] [5]
Total β-carotene	39.2	18.6	39.4	34.1	45.9	57.0	82.4-121.9	18.0-26.0
All-*trans*-β-carotene	29.3	15.4	39.4	34.1	36.5	45.8	68.0-85.9	13.2-17.2
13-*cis*-β-carotene	1.5	1.4			9.4	11.2	7.9-18.0	2.5-4.7
9-*cis*-β-carotene							6.4-17.9	2.3-4.1
15-*cis*-β-carotene	7.2	1.3						
Neochrome	5.0	8.4						
Violaxanthin	4.6	5.3	105.9	131.8				
cis-violaxanthin	1.8	1.5	42.4	35.3				
Zexanthin	1.2	0.9	4.7	2.4				
Cis-Zexanthin		1.6						
Luteoxanthin	3.6	2.4	15.9	11.8				
*N*eoxanthin	1.4	1.4	11.2	28.8				
cis-*N*eoxanthin	0.5	0.2	1.8	5.9				
cis-Lutein	0.8	0.6						

[1] Chen *et al*. (2004); [2] Chen *et al*. (2007); [3] Mercadante and Rodriguez-Amaya, 1998; [4] Pott *et al*. (2003); [5] Vásquez-Caicedo *et al*. (2005). [a]Converted values with estimated dry matter content of 17%.
[b]Mango cultivars rich in β-carotene (*Kaew, Chok Ana, Maha Chanok, Nakmai #4)*.
[c]Mango cultivars poor in β-carotene (*Kiew Sawoei, Rad, Mon, Duen Gao, Okrong Thong)*.

3.3.2.3. Stability of β-carotene

The highly unsaturated structure that is characteristic of carotenoids creates an electron rich environment, which makes these compounds susceptible to various chemical reactions (Briton, 1995; Boon *et al*., 2010). β-carotene is the predominant carotenoid pigment found in mango. This pigment has been to a great extend investigated, and reports in the literature show that processing of fruits and vegetables, which includes preparation, treatments and storage may greatly affect its content (Chen and Huang, 1998; Lemmens *et al*., 2013; Rock, 1997; Vásquez-Caicedo *et al*., 2007), since β-carotene is prone to degradation mainly by oxidation and isomerization reactions, and oxidation is a major cause of carotenoid loss (Rodriguez-Amaya, 1999).

While carotenoids are naturally stabilized by the plant matrix, disintegration of the food cell matrix caused by cutting and/or crushing is known to induce oxidation and isomerization of β-carotene to a great extent by the increased exposition to oxygen, and the process is stimulated by the presence of endogenous oxidative enzymes, metals, light, low pH and co-oxidation with lipid hydroperoxides (Rodriguez-Amaya, 1999). Rodriguez and Rodriguez-Amaya (2007) showed that epoxycarotenoids and apocarotenals were formed during auto-oxidation of β-carotene with atmospheric oxygen through a free radical mechanism. These authors also reported that oxidation is accompanied by geometric isomerization, both E- and Z-isomers (*trans* and *cis* isomers) being subject to oxidation.

Thermal degradation of β-carotene is induced by a reversible *trans* to *cis* isomerization,

whose degree is directly correlated with the intensity and duration of heat processing conditions (Chen and Huang, 1998; Rock, 1997). The *cis* isomers may then subsequently undergo oxidation (Rodriguez and Rodriguez-Amaya, 2007). Increased *trans*-*cis* isomerization of β-carotene with a formation of 13-*cis* isomers in mango purée at pasteurisation temperatures of 85 to 93^{o} C, have been reported by Vásquez-Caicedo *et al*. (2007). Treatment at higher temperatures (> 100^{o} C) has also been shown to cause β-carotene isomerization in carrot purée (Lemmens *et al*., 2010) and in mango purée (Lemmens *et al*., 2013), with subsequent formation of 9-*cis*, 13-*cis* or 15-*cis*-β-carotene isomers. Lower temperatures used during conventional drying of mangoes (~75 ^{o}C) were characterized by elevated amounts of 13-*cis*-β-carotene, whereas sun-dried mango slices dried at ~60 ^{o}C contained increased amounts of 9-*cis*-β-carotene (Pott *et al*., 2003).

3.3.2.4. Role of carotenoids in plants

Carotenoids play a fundamental role in photosynthesis with double principal functions, as light-harvesting pigments and providing protection against photooxidation (Bartley and Solnik, 1995; Demmig-Adams *et al*., 1996; Young, 1991). In plants, carotenoids are located in subcellular organelles (plastids), called chloroplasts and chromoplasts (Bartley and Solnik, 1995).

In chloroplast thylakoid membrane, carotenoids are bound mostly to specific chlorophyll/carotenoid-binding protein complexes and serve as accessory pigments in photosynthesis, where they absorb light and transfer excitation energy to chlorophylls via singlet oxygen (Bartley and Solnik, 1995; Demmig-Adams *et al*., 1996). The protective role occurs by quenching triplet state chlorophyll molecules and scavenging singlet oxygen and other toxic oxygen species formed within the chloroplast (Demmig-Adams *et al*., 1996). A darker green colour is correlated with a high number of chloroplasts and therefore a high concentration of carotenoids. During the ripening of fruit and vegetables, chloroplasts differentiate into chromoplasts by disintegration of the thylakoid membranes (Delgado-Vargas *et al*., 2000). In chromoplasts, carotenoids are deposited in crystalline form or as oily droplets and contain plastids responsible for the yellow, orange and red colours of many fruits, vegetables and roots, and also in some birds and fishes (Bartley and Solnik, 1995). It is reported that carotenoids in mango occur in plastoglobular sub-structures as lipid droplets, while for instance carotenoids in crystal form predominate in carrot (Brackmann *et al*., 2011; Vásquez-Caicedo *et al*., 2006).

3.3.2.5. Carotenoids and human nutrition

3.3.2.5.1 Source and related factors

Vitamin A is an essential nutrient that is obtained by a dietary intake of preformed vitamin A (retinol and its esterified form, retinyl ester) or of provitamin A carotenoids. Preformed vitamin A is only found in food from animal sources including human milk, dairy products, egg yolk, meat, fish and liver (FAO, 2001). β-carotene is by far the most important provitamin A carotenoid, and other provitamin A pigments are α-carotene and β-cryptoxanthin. Carotenoids are found in several plant foods and generally yellow-orange vegetables and fruits provide most of the dietary β-carotene, α-carotene and β-cryptoxanthin, while dark green vegetables also provide significant amounts of β-carotene and non-provitamin A carotenoid lutein. Tomatoes (green and red) and tomato products are the major sources of lycopene in the diet (Rock, 1997). Thus, fruits and vegetables such as carrots, mango, papaya, apricots, pumpkins, squash, spinach, amaranth and young green leaves are good sources of provitamin A carotenoids (FAO, 2001; Rock, 1997). Animal food products such as milk and milk products, eggs, some fish and liver also have significant carotenoid content (Britton and Khachik, 2009; Rock, 1997). It is reported that foods containing provitamin A carotenoids tend to be less biologically available but more affordable than animal products (FAO, 2001). Thus, fruits and vegetables as a major source of provitamin A are of special importance for economically deprived populations. However, substantial losses of provitamin A may occur during storage and processing. Moreover, studies have shown that a low fat diet or absence of dietary fat significantly reduces absorption of carotenoids in the body (Dimitrov *et al*., 1988; Prince and Frisoli, 1993).

The seasonality of plant foods in some areas may lead to low intake of provitamin A carotenoids in periods off-seasons in populations that relay on indigenous fruits. In some cases, supplementation has been used as prophylactic against vitamin A deficiency (VAD). Single doses of 60 mg of retinol have been given to children in developing countries, an amount adequate to meet the child's needs for 4-6 months (Gibney *et al*., 2009). Food based approaches, such as food fortification and consumption of food rich in vitamin A and provitamin A have also been advocated as long-term approaches to controlling VAD (Haskell, 2013).

3.3.2.5.2. Conversions factors of vitamin A

Since dietary sources of vitamin A may be converted into retinol, FAO/WHO in 1967 introduced a concept of retinol equivalent (RE) that established the relationship among dietary sources of vitamin A (preformed and provitamin A carotenoids). One µg retinol was considered to be equal to 1 RE, and the conversion factor of dietary β-carotene to vitamin A was set to 6:1 by weight (i.e. 6 µg of β-carotene is converted to 1 RE) and 12:1 for all other provitamin A carotenoids (i.e., α-carotene and β-cryptoxanthin have

half of the provitamin activity of β-carotene). The assumptions behind these conversion factors were that the absorption of the β-carotene from a mixed diet was 1/3 of the absorption of vitamin A and that half of the absorbed β-carotene was converted to retinol in the intestinal mucosa. A new concept (retinol activity equivalent (RAE)) was introduced based on new research findings on carotenoid absorption with the following conversion factors: 12 µg of β-carotene to 1 µg RAE, and 24:1 for all other provitamin A carotenoids (IOM, 2001). Moreover, international units (IU) are often used in food composition tables, that is, 1 IU retinol = 0.3 µg retinol; 1 IU β-carotene = 0.6 µg β-carotene, and 1 IU retinol = 3 IU β-carotene.

3.3.2.5.3. Recommended intake of vitamin A

Recommended daily intake of vitamin A is defined based on the intakes required to maintain normal plasma concentrations of the vitamin, and prevent the appearance of deficiency-related syndromes, that is, night blindness and epithelial lesions of the conjunctiva and cornea of the eye, and for normal growth (FAO, 1988). The estimated requirements and safe levels of intakes according to FAO, taking into account age and gender differences in mean body weights are shown in Table 3.4.

Table 3.4 Estimated mean requirements and safe levels of intake for vitamin A.

Category and age	Mean requirement (µg RE/day)	Recommended safe intake (µg RE/day)
Infants and children		
0-6 months	180	375
7-12 months	190	400
1-3 years	200	400
4-6 years	200	400
7 years	200	450
7-9 years	250	500
Adolescents, 10-18 years	330-400	600
Adults		
Females 19-65 years	270	500
Males 19-65 years	300	600
65+ years	300	600
Pregnant women	370	800
Lactating women	450	850

Source: Adapted from FAO/WHO (2004)
RE (retinol equivalent) = 1µg retinol = 6 µg β-carotene = 12 µg other provitamin A carotenoids

3.3.2.6. Role of carotenoids in human health

Among the more than 700 carotenoids that are found in nature, only a few are predominantly observed in human plasma. The most common are β-carotene, lycopene, lutein, β-cryptoxanthin, and α-carotene, which are reported to constitute 90% or more of the circulating carotenoids in humans (Bieri *et al*., 1985; Rock, 1997). Carotenoid

pigments are expected to be found in hydrophobic areas of the cells, such as the inner core of membranes, except when in association with proteins allows them access to an aqueous environment (Britton, 1995).

A well-established function of carotenoids in human nutrition and health is vitamin A (retinol) activity. Vitamin A plays an important role in vision, and in cell growth and differentiation (FAO, 2001). In the visual system, vitamin A acts as a prosthetic group of light sensitive proteins in the retina, and a deficiency of vitamin A may result in an impairment of the ability to adapt to dim light or to night blindness. Prolonged or severe VAD leads to xerophthalmia, and the more advanced stages result in keratinization, that is, irreversible damage to the cornea (Gibney *et al*., 2009). VAD also affects growth and differentiation of epithelial cells, which consequently compromises immunity, resulting in major morbidity and mortality (Semba, 1998). Vitamin A deficiency is a major public health problem in large areas of the world, especially in young children (WHO, 2009).

Beyond their function as vitamin A precursors, carotenoids have been shown to exhibit additional biological activities, i.e. as natural antioxidants. However, there have been some concerns related to the antioxidant efficiency of carotenoids in the prevention of certain diseases. Results from randomized controlled trials, with supplementary pure β carotene in smokers showed no preventive effect on cardiovascular disease (Hennekens, *et al*., 1996), but increased risk for lung cancer development (Omenn *et al*., 1996). However, epidemiological studies show evidence of a positive link between higher dietary intake of food carotenoids and lower risk of chronic diseases (Donaldson, 2004; Ziegler, 1989). It has also been reported that carotenoids such β-carotene and lycopene act efficiently as scavenger of reactive oxygen and quenchers of singlet oxygen (Edge *et al*., 1997; Rao and Rao, 2007; Stahl and Sies, 2003). Lycopene has been associated with the highest antioxidant activity, followed by β-cryptoxanthin, β-carotene, lutein, zeaxanthin and α-carotene (Miller *et al*., 1996). On the other hand, at high concentrations of oxygen and carotenoids, autoxidation of carotenoids may occur, whereby carotenoid radical intermediates react with oxygen to form products that can initiate detrimental oxidation reactions, by acting as pro-oxidants (Briton, 1995; Young and Lowe, 2001).

3.4. Processing of fruits and vegetables

One of the main goals with food processing is preservation. Fruits are seasonal and highly perishable containing more than 80% water, which make them undergo progressive changes if stored untreated at ambient temperature due to the activity of naturally present enzymes such as AAO, PPO and peroxidase (POD). These enzymes are known to cause quality modifications in texture, flavour, colour and nutritive value (Yamaguchi *et al*., 2003; Leong and Oey, 2012). Therefore, application of processing methods that will reduce the water content, as well as inhibit enzyme activities, are

crucial to extending shelf life, avoiding wasting and increasing their availability off-season. Several studies have focused on processing methods that will minimize nutrient degradation in fruits and vegetables (Ali *et al*., 2012; Barrett and Lloyd, 2012; Chong *et al*., 2013; Wilberg and Rodriguez-Amaya, 1995), and some suggest that there are an increased bioavailability of nutrients as a result of processing (Lemmens *et al*., 2009; Rock *et al*., 1998).

3.4.1. Drying of fruits and vegetables

Dehydration is one of the oldest methods of food preservation. Its main objective is to extend the shelf life of the product by removing moisture and reducing the water activity, thus avoiding microbial growth and deteriorative chemical reactions (Drouzas and Schubert, 1996; Fellows, 1988). Drying is a complex process that is basically comprised of two fundamental and simultaneous processes, where heat is transferred by convection from the hot air to evaporate liquid from the product, and mass is transferred by convection as a liquid or vapor within the solid and as a vapor from the surface (Mujumdar and Menon, 1995). Simultaneous heating and moisture removal during drying affect both the physical and biochemical properties of food materials, such as nutrients (Kong and Singh, 2013; Pan *et al*., 2003), and the appearance of food materials such as colour. Consumer acceptability is affected as a result, and off-colour products are often unacceptable despite good taste and flavour (Piližota and Šubarić, 1998). The quality losses of the dried products are associated with the high temperatures or long drying times required for drying (Drouzas *et al*., 1999). Thus, to limit these problems, pre-treatments such as osmotic dehydration (Aktas *et al*., 2013; Azoubel *et al*., 2009; El-Aouar *et al*., 2003; Khan *et al*., 2008) or blanching (De Ancos *et al*., 1999; Galindo *et al*, 2005; Ruiz-Ojeda and Peñas, 2013) have been applied. However, more research on process-related retention of essential nutrients, such as vitamins C and β-carotene, in the final product, as brought about for instance by osmotic dehydration or dry blanching pre-treatments prior to drying, is required. In general, fruits and vegetables are dried to enhance their storage stability, minimize packaging requirements, and reduce transport weight and consequently transport costs (Sagar and Suresh, 2010). Dried fruit may be eaten as snacks or used in cookies or breakfast cereals, or used after rehydration in food processing that calls for fresh, canned or frozen fruit. It has the advantage of being available in off-season periods.

3.4.2. Pre-treaments and related quality effects

3.4.2.1. Osmotic dehydration

Osmotic dehydration (OD) is a process of partial removal of water by immersion of fruit in concentrated solutions of soluble solutes such as sugars or salts, where both

partial dehydration of the fruit and sugar uptake are obtained (Torres *et al*., 2006). OD has been widely used as a pre-treatment to improve quality, although it results in products with high water activity, requiring further drying or processing to enhance their shelf life. The process is characterized by three simultaneous countercurrent flows without involving phase change (Figure 3.5). A significant amount of water flows out of the food into the solution (water loss), a transfer of solutes from the solution into the food (soluble solids uptake) and a leakage of water-soluble solute molecules such as vitamins, minerals and organic acids, across the membrane into the solution.

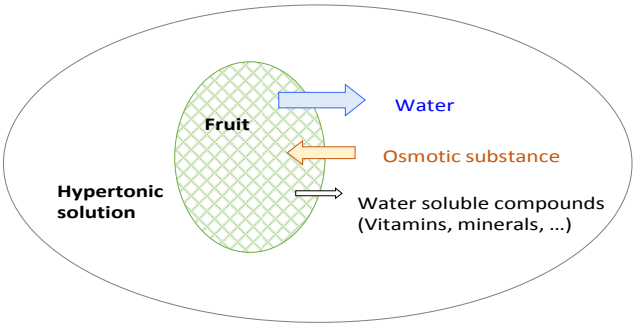


Figure 3.5 Schematic diagram of mass transfer during osmotic dehydration.

The driving force for the diffusion of water from the tissue into the solution is because of a difference in osmotic pressure between the food and its surrounding hypertonic solution (Sagar and Suresh, 2010), and the complex cellular structure of food acts as a semi-permeable surface (Chandra and Kumari, 2015).

Besides the advantage of osmotic dehydration in terms of lower energy consumption, reduced browning, improved texture, colour and appearance of fruits, osmotic dehydration can also be detrimental to the sensory and nutritional value of the dried product (Chavan and Amarowicz, 2012) due to the leakage of water-soluble compounds during OD. Such negative effects may be avoided by fortification of osmotic solutions with vitamins and minerals (Fito *et al*., 2001). This has been investigated for mango, apples and pineapple by an addition of vitamin C or calcium in the osmotic solution (Barrera *et al*., 2009; Nagai *et al*., 2014; Silva *et al*., 2014; Torres *et al*., 2006). However, the effect of subsequent drying was not investigated in these studies. Calcium fortification is expected to improve cell wall integrity as calcium interacts with the plant cellular matrix, forming bonds between pectins and other cellular wall components (Gras *et al*., 2003). It should be noted that, besides being a texture improver, calcium is an important mineral in human nutrition.

Although OD has been the focus of many studies, more research is needed to understand the impact on bioactive compounds such as vitamin C and carotenoids, particularly when OD is combined with other drying techniques. Degradation of vitamin C and carotenoids in dried fruits subjected to OD were reported in cashew apple (Azoubel *et*

al., 2009) and sea buckthom (Araya-Farias *et al*., 2014), while enhancing of vitamin C was found by An *et al*. (2013) in tomatoes.

3.4.2.2. Blanching

Blanching is frequently used prior to canning, freezing or drying of fruits and vegetables to minimize sensory and nutritional quality degradation caused by enzymes naturally present in plant food such as AAO (see Section 3.3.1.2), PPO and POD. These enzymes affect colour, produce off-flavours and reduce the nutritive value of most fruits and vegetables (Holzwarth, *et al*., 2013; Korbel *et al*., 2013). It is believed that these enzymes may affect vitamin C retention, although the activity for that purpose is often not evaluated (Barrett and Lloyd, 2012). Co-oxidation of carotenoids as a result of PPO activity has also been reported (Dorantes-Alvarez and Chiralt, 2000). Although it was not measured in the current study, POD is widely used as an indicator of blanching process adequacy (Brewer *et al*., 1994) and, contrary to AAO, several studies on the stability of PPO and POD in fruits and vegetables as affected by different thermal treatments have been reported in mango (Ndiaye *et al*., 2009; Vasquez-Caicedo *et al*., 2007; Wang *et al*., 2007) and in other fruits and vegetables.

Hot water and steam are the most commonly used heating techniques; however they are energy intensive processes, and the use of water results in the leaching of water-soluble nutrients (De Corcuera *et al*., 2004). Thus, alternative blanching techniques have been studied in order to improve the nutritional quality of processed fruits and vegetables and to reduce the environmental impact by reducing the amount of water consumption. Dry blanching techniques such as MW and IR radiation technologies have been studied to minimize losses of water-soluble compounds, inactivate enzymes with high-energy efficiency and improve the nutritional value of the blanched products (Ponne *et al*., 1994; Lin and Brewer, 2005; Vishwanathan *et al*., 2013). Although IR and MW heating are both transmitted as waves that are converted to heat, they differ in the heating form (see sections 3.5.2.2.1 and 3.5.2.2.2, respectively). Thus, although there are some reports in the literature of the application of microwave and infrared dry blanching, studies with subsequent drying are lacking. Vishwanathan *et al*. (2013) reported higher vitamin C retention in hot air-dried IR blanched carrot in comparison with dried water blanched samples.

The inactivation of enzymes during blanching is dependent upon both time and temperature, since under-blanching may enhance the activity of enzymes and may be more deleterious than no blanching, whereas over-blanching causes a loss of flavour, colour, vitamins and minerals (Fellows, 2009). Thus, pasteurization processes are designed to occur at specific parameters, usually at high temperature and short time (HTST) or at low temperature and long time (LTLT) (Singh and Heldman, 1984). However, processing by thermal treatment, which involves several heating steps in

order to inactivate oxidative enzymes and inhibit microbial growth, is known to degrade nutrients such as carotenoids and vitamin C by non-enzymatic oxidation reactions that increase with tissue disruption (Lemmens *et al*., 2013; Munyaka *et al*., 2010a; Vásquez-Caicedo, *et al*., 2007).

3.4.2.2.1. Infrared heating and its application for blanching fruits and vegetables

Infrared radiation (IR) is part of the electromagnetic spectrum, which lies between the visible and microwave portions at wavelengths of 0.78 to 1000 μm. Based on the wavelength, infrared radiation is divided into three categories: near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR). In general, FIR radiation is more advantageous for food processing because most food components absorb radiative energy in this region (Sandu, 1986). However, other reports state that FIR seems to be more efficient for drying thin layers, while drying thicker bodies should give better results with NIR (Nowak and Lewicki, 2004). IR radiation transfers thermal energy as an electromagnetic wave and becomes converted to heat when it impinges the food surface (Rastogi, 2012), by inducing changes in the electronic, vibrational and rotational states of atoms and molecules such as water (Krishnamurthy *et al*., 2008). The penetration depth of IR is very low, and this method of heating is thus especially suitable for thin layers of material with large surfaces exposed to radiation (Nowak and Lewicki, 2004). IR heating is generally applied in drying, baking, roasting, blanching, pasteurization and sterilization of food products. The application of IR for blanching showed that this technology preserved the cell integrity in carrot slices (Galindo *et al*., 2005), shortened drying times of apple slices (Nowak and Lewicki 2004), inhibited PPO enzyme activity in pears, sweet corn, carrots, French fries (Pan *et al*., 2005) and apples (Zhu and Pan 2009; Zhu *et al*., 2010). Sogi *et al*. (2012) assessed the efficacy of IR treatment for extending the shelf life of fresh-cut mangoes and found that the vitamin C content decreased in IR-treated fresh mango samples as compared with non-treated during 16 d of storage at 4 °C. In addition, a recent review of infrared blanching by Rastogi (2012) concluded that IR heating offers many advantages over conventional heating under similar conditions, having uniform heating, reduced quality losses and significant energy savings.

3.4.2.2.2. Microwave heating and its application for blanching fruits and vegetables

Microwave energy is an electromagnetic radiation in the frequency range between 300 MHz and 300 GHz, corresponding to wavelengths from 1000 μm to 1m. For microwave heating and drying of food, a frequency of 2.45 GHz is permitted, although a frequency of 915 MHz is also allowed for industrial purposes (Drouzas and Schubert, 1996). Microwave heating has been applied in a broad range of food processing such as drying,

tempering, blanching, cooking, pasteurization, sterilization and baking (Puligundla, *et al*., 2013). MW themselves do not produce heat, but heating is a consequence of the interactions between microwave energy and a dielectric food material such as water, which absorbs MW energy with a consequential rise in temperature (Singh and Heldman, 1984). Most foods contain a substantial amount of water and polar molecules, and, when these are subjected to a MW electric field, the molecules orient themselves according to the polarity of the field with constant rotation, and the molecular friction can then generate heat (Singh and Heldman, 1984). The heat is then directly transferred by means of conduction mechanisms throughout the entire mass of the radiated material from the inside out (Drouzas and Schubert, 1996). MW heating has advantages compared with conventional heat blanching such as in-depth heating (volumetric) in the absence of a temperature gradient and avoidance of the leaching of water-soluble components (Lin and Brewer, 2005). However, MW lack uniformity in heating and have a limited penetration range (Ramaswamy and Pillet-Will, 1992; Ramesh *et al*., 2002). The application of MW for blanching purposes was shown to inactivate oxidative enzymes in papaya, strawberry and kiwi purées, which resulted in minor losses of carotenoids (De Ancos *et al*., 1999). Ruiz-Ojeda and Peñas (2013) reported about a 50% higher retention of vitamin C in microwaved blanched green bean pods in comparison with conventional hot water treatment. Similar results were also reported by Muftugil (1986) and Brewer and Begum (2003) for microwaved green beans and by Ramesh *et al*. (2002) for spinach, carrot and bell peppers.

3.5 Minimal processing and the effect of antibrowning agents

In recent years, the demand of consumers has increased for minimally processed foods, such as juices and purées that have high sensory quality and nutritional value. Browning of fruits, vegetables and their products, as affected by enzymatic or non-enzymatic reactions, has been a major reason for quality loss during processing and/or storage (Manzocco *et al*., 2001; Pizzocaro *et al*., 1993). Enzymatic browning results from PPO catalyzed oxidation of phenolic compounds to quinones (Holzwarth *et al*., 2013; McEvily *et al.*, 1992; Park *et al*., 1980), and has been indicated as a main cause of colour change (Pizzocaro *et al*., 1993). Thermal treatment, on the other hand, is the main cause of non-enzymatic browning (McEvily *et al*., 1992; Pizzocaro *et al*., 1993). Enzymatic oxidation of ascorbic acid by AAO is the main cause of loss of the nutritional value of different fruit and vegetable products (section 3.3.1.2).

To overcome the problems induced by enzymatic browning, thermal treatment and anti-browning agents have been investigated and successfully applied in the industry. Consequently, it is known that processing by thermal treatment may degrade nutrients such as carotenoids and vitamin C by non-enzymatic oxidation reactions (Lemmens *et al*., 2013; Munyaka *et al*., 2010a; Vásquez-Caicedo, *et al*., 2007). However, little

information is found in the literature concerning the effect of anti-browning agents on nutrient retentions.

Inhibition of enzyme activity can be achieved using several anti-browning measures including reducing pH with carboxylic acids (Arpita *et al*., 2010; Son *et al*., 2001; Munyaka *et al*., 2010a) or by chelating Cu^{2+}, a co-factor for AAO and PPO activity with Ethylenediamine Tetraacetic Acid (EDTA) (Li, 2014; McEvily *et al*., 1992). EDTA, a complexing agent of metals such as iron and copper in the prosthetic group of the enzyme (Sapers *et al*., 1989), is a highly stable molecule. It has been proposed that, by combining different inhibitor agents, a synergistic effect may be achieved that will result in enhancement of the inhibition (Son *et al*., 2001). However, there are also reports of constraints on the use of anti-browning agents such as sulphites, due to their toxicity, although their efficiency in the inhibition of enzymatic and nonenzymatic browning has been emphasized (McEvily *et al*., 1992; Pizzocaro *et al*., 1993). Enzymatic browning in fruit systems has been investigated by several authors (El-Shimi, 1993; Özoğlu and Bayındırlı, 2002; Sapers *et al*., 1989), but not its effect on nutrient retentions. Munyaka *et al*. (2010a,b) reported both a higher retention of vitamin C and a higher ratio of L-AA in blanched broccoli and puréed broccoli acidified with acetate buffer to pH 4.3. More research on the effect of anti-browning or chelating agents on nutrients is thus needed.

4. MATERIALS AND METHODS

This chapter brings an overview of the studies included in this thesis and describes the materials and methods applied in the experimental work. The raw materials that were used, the way in which the samples were prepared, the pre-treatments and the hot air drying processes are described. Moreover, the procedures followed for the preparation of mango purée, enzyme activity measurements, vitamin C and carotenoids determination are presented. Finally, the analytical methods, as well as the statistical analysis are also described.

4.1. Study overview

A schematic outline of the studies is presented in Figure 4.1. In general, the impact of processing on the retention of bioactive compounds (vitamin C and β-carotene), using different methods was the focus. In paper I osmotic dehydration was combined with hot air-drying. In Papers II and IV the effect of blanching and drying was studied. Finally in Paper III, processing of mango fruits into purée was evaluated.

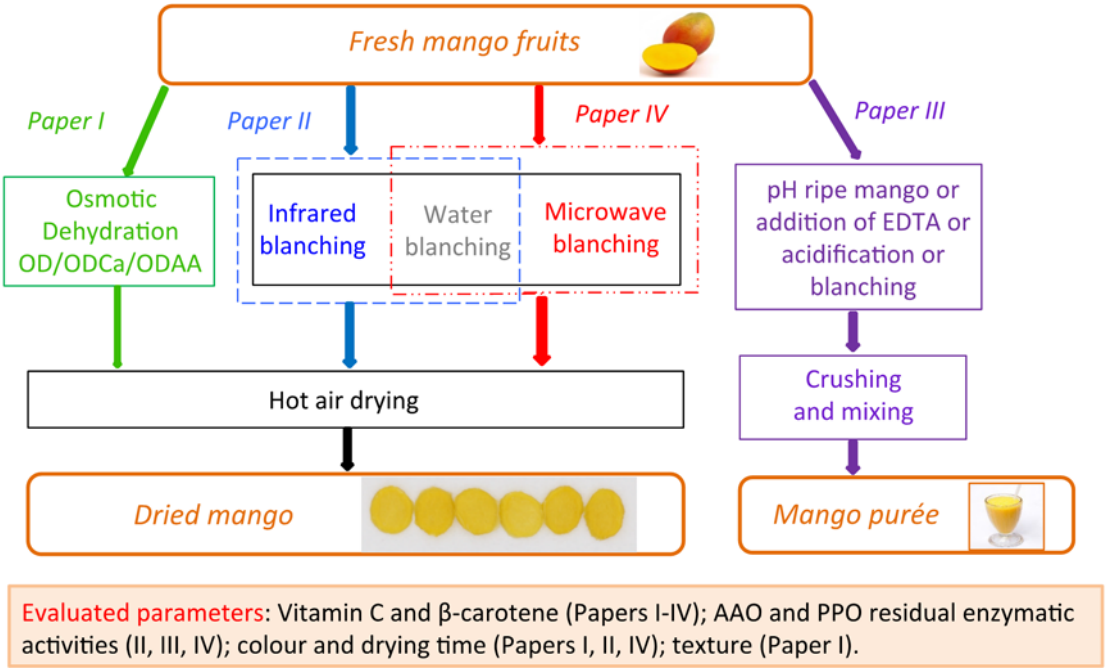


Figure 4.1 Schematic outline of the studies.

Paper I

The effect of osmotic dehydration (OD) with additives prior to hot air-drying on the retention of vitamin C and β-carotene was evaluated in medium ripe mango. Mango cylinders were immersed in osmotic sucrose solutions of 45° BRIX with or without 1% (w/w) calcium chloride or 1% ascorbic acid with a fruit/solution ratio of 1:10 (w/w), 15 h at 25 °C. Fresh untreated mango used as a control sample and OD-treated mango were dried in an air convection oven either at 50 °C or 70 °C. The effect of OD on vitamin C and β-carotene retention was determined in dried mango.

Paper II

In Paper II the influence of thermal pre-treatment using infrared (IR) blanching was investigated. IR dry blanching was applied and compared with conventional water blanching before hot air-drying of medium ripe mango. The purpose with the treatments was to inactivate polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) enzymes that affect the retention of vitamin C and β-carotene nutrients and may induce colour change. Processing was performed by blanching mango cylinders under similar temperature-time conditions either by IR heating or by immersion in a water bath for 2 min at 90° C (HTST) or for 10 min at 65 °C (LTLT). Untreated and blanched mango samples were hot air-dried at 70 °C. The effect of blanching was assessed by determination of enzyme residual activity and for vitamin C and β-carotene retention in dried mango.

Paper III

The impact of acidification with citric acid, addition of EDTA and water blanching at high temperature and short time (HTST), conducted at 90° C for 4 min, on the retention of vitamin C (L-AA and DHAA) and β-carotene was studied in mango purée 30 min after crushing. Anti-browning agents were used to minimize enzymatic oxidation, which affects quality during the processing of purées and juices. The aim of this study was to evaluate the stability of vitamin C (L-AA and DHAA) and β-carotene during processing of mango purée 30 min after crushing in the presence of different inhibitors, compared to the purée effect of blanched mango. The purées were produced crushing unblanched ripe mango flesh to achieve a pH of 5.0±0.1 with or without EDTA, representing the pH of ripe mango, and at pH 3.9±0.1 after acidification with citric acid. In addition, the purée of blanched mango was obtained after HTST water blanching fresh mango for a period of 4 min at 90 °C in closed plastic bags to inactivate PPO and AAO, prior to matrix disruption at pH 5.0.

Paper IV

As a follow-up of the dry-blanching process reported in Paper II, the effects of microwave (MW) dry blanching in comparison with water blanching, in a water bath (conventional) or in closed plastic bag, on inactivation of polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) enzymes, as well as retention of vitamin C (L-AA and DHAA) and β-carotene, were evaluated in medium ripe mango prior to hot air-drying (70 °C). Blanching conditions for MW and water blanching were 2 min at 90° C (HTST) or for 10 min at 70 °C (LTLT).

4.2. Processing methods

The experimental work of fresh sample preparation, pre-treatments and drying processes were performed at the SP Technical Research Institute of Sweden, while the analysis of vitamin C and β-carotene and enzyme determinations were done at the Division of Food and Nutrition Science, Chalmers University of Technology, in Sweden. A brief summary of practical processing steps applied in Papers I to IV is presented in Figure 4.2.

4.2.1. Raw material and sample preparation

Mango (*Mangifera indica* L.) samples used in all the studies were purchased at a local market and stored at 8-10 °C. The mango cultivars used were 'Tommy Atkins' (Papers I and II), 'Keitt' (Paper III) and 'Osteen' (Paper IV). Two or three mangoes were used for each experiment. They were taken from the store and kept at room temperature 24 hours before being used. All the experiments were performed in duplicate.

For Papers I, II and IV, a medium ripe mango was selected, washed with tap water, manually peeled, pitted and sliced in a dimmed room into cylinders of 2 cm in diameter and 0.5 cm thick just before the pre-treatments.

For Paper III, green-ripe mango was stored for three days at 8-10 °C and kept for 24 h at room temperature before use. They were then washed with tap water, manually peeled, pitted and cut into small pieces that were crushed unblanched or after blanching to produce mango purées.

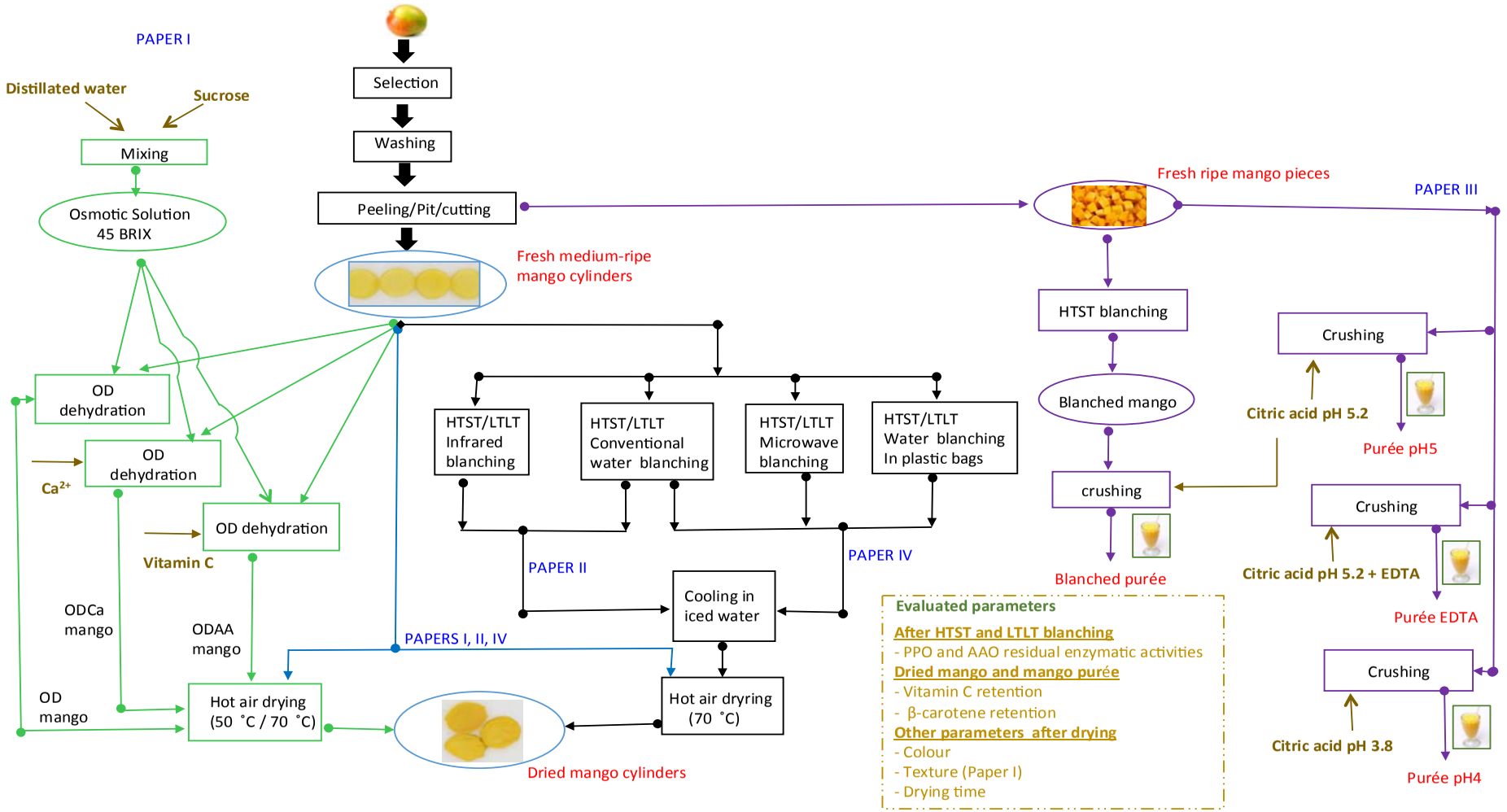


Figure 4.2 Schematic overview of experimental work in Papers I – IV.

4.2.2. Osmotic dehydration

Osmotic dehydration (OD) is a partial removal of water from plant tissue by immersion in hypertonic solutions. OD with and without additives was applied as a pre-treatment before hot air-drying in paper I. Osmotic solutions were prepared with commercial sucrose (refined sugar) purchased at a local market. Sucrose solution with 45 °Brix was prepared by dissolving the sugar in distilled water (Figure 4.2), and the refractive index of the fruit juice was measured using a refractometer (Digit = 32, Ceti Optical Instruments). The osmotic solutions with additives were obtained adding ascorbic acid or calcium chloride, corresponding to 1% of the mango samples pre-treated (~300 g), to an osmotic solution 45 °Brix with a fruit to solution ratio of 1:10.

Osmotic dehydration (OD) was carried out in 45 °Brix sucrose solutions without (OD) or with additives, 1% (w/w) calcium chloride (ODCa) or 1% ascorbic acid (vitamin C) (ODAA). The samples were osmotically dehydrated for a period of 15 hours at room temperature (~25 °C) and then hot air-dried at 50 °C or 70 °C. Samples of fresh, osmotically treated and dried mango were collected, frozen in a nitrogen environment and stored at -80 °C until determination of vitamin C and carotenoids.

4.2.3. Blanching

Blanching is a unit operation in which fruits or vegetables are heat treated to inactivate enzymes. There has recently been interest in finding new ways to improve the quality of blanched products, beyond conventional water blanching. In this work new blanching techniques based on infrared and microwave processing were compared with conventional blanching with water. The PPO and AAO enzyme residual activities were assessed to evaluate the effect of blanching treatments. Mango samples were blanched and cooled in iced water for 5 min to lower the residual enzymatic activity, prior to drying. Samples of fresh, blanched and dried mangoes were collected, frozen in a nitrogen environment and stored at -80 °C until determination of vitamin C, carotenoids and residual enzymatic activity. The details of the different blanching processes are described below.

4.2.3.1. Infrared blanching

Infrared blanching was performed in a near infrared (NIR) test oven in Paper II (Figure 4.2). This heating method is especially suitable for drying thin layers of material with a large surface exposed to radiation (Nowak and Lewicki, 2004). Preliminary tests were performed to identify the infrared conditions (power and time) required for the treatment of 300 g of sliced mango placed in an aluminum tray at the center of the oven.

The distance between the NIR emitters and the samples was always constant, 0.5 m above and below. A temperature profile similar to water blanching was achieved based on previous experience (Staack 2008; Eliasson *et al*., 2014) by systematically changing the IR power output between 100% and 8% during the blanching process. The temperature of the central point of mango cylinders was monitored using thermocouples previously calibrated and connected to a data log. The power sequences selected to maintain the required temperature profile for mango slices (65 °C for 10 min or 90 °C for 2 min) are presented in Table 4.1.

Table 4.1 Selected IR power output and time sequence of infrared blanching of mango cylinders at 65 °C and 90 °C to obtain a similar temperature history as water blanching.

		Blanching time (s)	
IR output power (%)	IR heat flux (kW/m^2)	IR65	IR90
100	14.3	25	72
40	5.8	55	-
30	4.4	-	48
10	1.5	280	-
8	1.2	240	-
Total blanching time (s)		600	120

4.2.3.2. Microwave blanching

Microwave blanching was studied in Paper IV (Figure 4.2) using a domestic MW oven with turntable plate. MW heating generates heat inside the food in the absence of a temperature gradient (De Ancos *et al*., 1999); however, in this method, the heating is not uniform (Ramesh *et al*., 2002; Ramaswamy and Pillet-Will 1992). Preliminary studies were carried out to find the MW conditions (power and time) required for the treatments of 200 g of mango cylinders. Thus, HTST at 90 °C required 1350 W/120 s, while LTLT at 70 °C required a set of power and time sequence of 1350 W/60 s + 420 W/540 s. The central point temperature of mango cylinders was recorded using an optic fibre connected to a data log.

4.2.3.3. Water blanching

Two types of water blanching were performed in this study (Figure 4.2). The immersion of mango samples in vacuum-packed plastic bag (Figure 4.3) (Papers III and IV) or directly in a plastic strainer (conventional water blanching) (Papers II and IV), in a thermostatic water bath at a pre-determined constant temperature for a corresponding blanching time. Water blanching in closed plastic bag avoids leaching of water-soluble compounds such as vitamin C and minerals, thus minimizing losses of nutritional quality. In the current work, the water blanching was carried out at the following

conditions: 300 g of mango cylinders blanched at HTST, 90 °C / 2 min or LTLT, 65 °C / 10 min (Paper II); 100 g of mango pieces blanched at HTST, 90 °C / 4 min (Paper III); or 200 g of mango cylinders blanched at HTST, 90 °C / 2 min or LTLT, 70 °C / 10 min (Paper IV). To monitor the temperature kinetics, the central point temperature of mango cylinders was measured using thin wire copper-constantan thermocouples previously calibrated and connected to a data log.

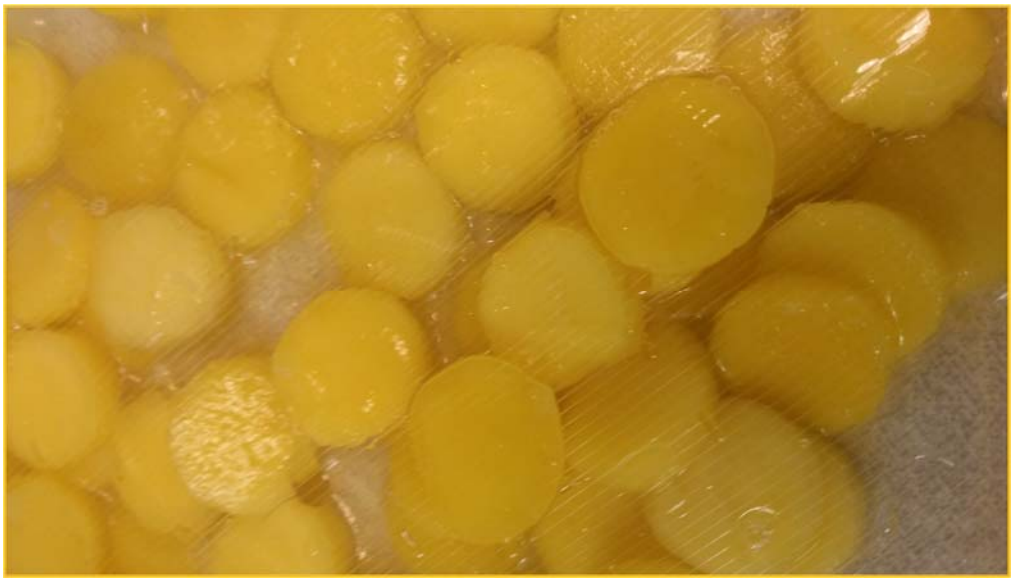


Figure 4.3 Blanched mango in plastic bag.

4.2.4. Preparation of mango purée

The use of enzyme inhibitors is possible means to extend the shelf life and food quality. In the present work, acidification with citric acid and an addition of a metal chelating agent, EDTA, to mango purée were evaluated and compared with blanched mango purée. Fresh mango (100 g) was homogenised for 30 s using a kitchen mixer (Braun MR 5550 MCA mixer, Germany) at two different pH levels of 0.2 M citric acid buffers (pH 3.8 or pH 5.2 with or without 2 mM EDTA), at a ratio of 1:1 (w/v). The final pHs of the mango purées were 3.9±0.1 or 5.0±0.1. The following purées were produced: purée of pH 3.9±0.1 that is within the recommended acidified pH for production of mango purée with improved microbial stability (FAO 2001); purée of pH of 5.0±0.1 representing the pH of ripe mango (Vazquez-Salinas and Lakshminarayana 1985), with or without EDTA. The purée of blanched mango pieces was obtained by crushing blanched mango in the presence of 0.2 M citric acid buffer (pH 5.2) after HTST blanching of mango pieces at 90 °C for 4 min in closed plastic bag (see section 4.2.3.3). The analyses of the nutrients were done in fresh mango and in purées 30 min after crushing. Samples for carotenoid analysis and enzyme determination were collected in

vacuum-packed plastic bags and stored within 30 min at -80 °C until analysis.

4.2.5. Drying processes

Drying is a water removal process that results in a solid product with a low moisture content. Hot air-drying is a commonly used method to preserve fruits and extend their shelf life. Untreated and pre-treated samples were dried either at 50 ºC or 70 ºC (OD dehydrated mango - Paper I) or at 70 °C (blanched mango - Papers II and IV) to reduce the moisture content to a level that ensures microbiological stability of the fruit given by the water activity of approximately 0.6. Drying was done using conventional hot air driers. In Paper I, a hot air-drier in a microwave convective drier previously described in detail by Pereira *et al*. (2007) (Figure 4.4A) was used, with an air velocity of 0.2 m/s, while an air circulation oven (Elektro Helios Garomat) (Figure 4.4B) with an air velocity of 1 m/s was used in studies presented in Papers II and IV.

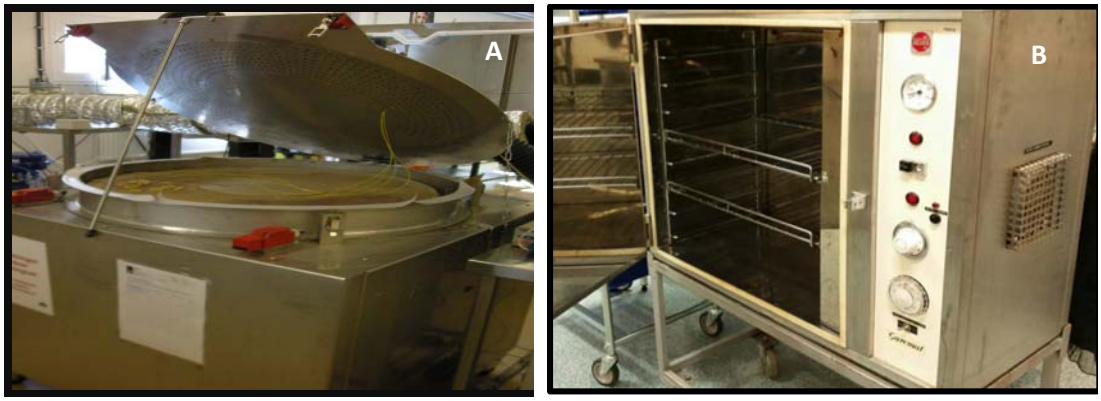


Figure 4.4 Driers at SP: A - Microwave-hot air-drier; B - air circulation oven.

4.3. Enzyme activity measurement

PPO and AAO enzymes were determined using extraction buffer solutions to prevent pH changes. The change in absorbance as a result of the reactions between the extract and the substrate was measured using a spectrophotometer and plotted against time. The enzyme activity was calculated from the slope of the curves, and the residual activity was expressed as the percentage ratio of the slopes between blanched and corresponding fresh mango samples. The extraction procedures and determination of PPO and AAO were done as follows:

4.3.1. Polyphenol oxidase (PPO)

Extraction and assay of PPO were carried out in Papers II, III and IV based on the procedure described by Palou *et al*. (1999) and Ndiaye *et al*. (2009) with some modifications. The enzyme was extracted in fresh (control) or blanched mango in McIlvaine citric-phosphate buffer (pH 6.5). The homogenates were centrifuged at 4 °C and the supernatant was collected and stored in ice prior to enzyme activity measurements. The reaction solution consisted of McIlvaine citric-phosphate buffer (pH 6.5), 0.175 M catechol substrate solution and PPO extract. The blank consisted of 2.5 mL McIlvaine buffer (pH 6.5) and 1 mL of 4-methylcatecol. To start the reaction between the enzyme and the substrate, the assay mixture was incubated at 40 °C for 20 s before measuring the absorbance at 420 nm for 3 min. The PPO activity (1 unit) was defined as an increase of absorbance of 0.001 at A_{420} /min/mL of extract.

4.3.2. Ascorbic acid oxidase (AAO)

The activity of AAO was determined using the method described by Oberbacher and Vines (1963) with some modifications (Papers II, III and IV). AAO was extracted by mixing fresh (control) or blanched mango with phosphate buffer (0.1 M, pH 5.6, 0.5 mM EDTA). EDTA is an inhibitor of AAO activity. The homogenates were centrifuged at 4 °C and the resulting supernatant was collected and stored in ice prior to enzyme activity measurements. AAO activity was determined by measuring the decrease in substrate concentration (0.5 mM L-AA) for 3 min using a spectrophotometer at 25° C and 265 nm. The assay mixture consisted of 0.1 M phosphate buffer (pH 5.6, 0.5 mM EDTA), L-AA substrate and enzyme extract. The blank consisted of 2.9 mL of 0.1 M phosphate buffer (pH 5.6, 0.5 mM EDATA) and 100 µL of substrate solution. One unit of AAO activity was defined as a decrease in absorbance of 0.01 at A_{265}/min/mL of extract.

4.4. Determination of vitamin C (L-AA and total ascorbic acid)

Due to its susceptibility to oxidation, vitamin C content has been used as a quality indicator for food processes. To avoid losses that could lead to an underestimation of this nutrient by oxidation of vitamin C from L-AA to DHAA, which may be irreversibly hydrolysed to 2,3-diketogulonic acid, which has no vitamin C activity (Lima *et al*., 2010), during extraction and analysis precautions needs to be taken.

The extraction procedure and experimental considerations were largely based on the Davey *et al*. (2000). Vitamin C was extracted from fresh and processed samples by dissolving the samples in a meta-phosphoric acid extraction buffer (5% MPA, 1 mM EDTA) in Papers I and II, or sodium dihydrogen phosphate buffer (20 mM NaH_2PO_4,

pH 2.1, 1 mM EDTA) in Papers III and IV. Tubes were sonicated for 10 min and shaken well after 5 and 10 min and then centrifuged. The aliquots of the supernatants were diluted in McIlvaine buffer containing Tris-[2-carboxyethyl] phosphine hydrochloride (TCEP) in Papers I to IV and without TCEP (Papers III and IV), thereby enabling the determination of total ascorbic acid and L-AA, respectively. TCEP offers an efficient reduction of DHAA at an acidic pH (Wechtersbach and Cigić, 2007). The analyses were performed using a HPLC system consisting of two pumps, a cooled auto sampler at 8° C and an electrochemical detector. Separations were performed on a Thermo Aquasil C_{18} column (150 mm x4.6 mm, particle size 3µm). The mobile phase was phosphate buffer (50 mM, pH 2.8) and L-ascorbic acid was quantified by electrochemical detection in a glassy carbon flow cell against freshly made reference solutions containing known concentrations of L-ascorbic acid. The retention time for vitamin C was approximately 6.6 min. Results of L-AA and total vitamin C were expressed as milligrams per 100 g dry weight. The percentage retention of vitamin C was calculated as the ratio of vitamin C in the treated sample to that in the fresh sample x 100. All the extractions were made in triplicate.

4.5. Determination of β-carotene (*trans*/*cis* isomers)

The main problem in carotenoid analysis is its instability, and precautionary measures to avoid formation of artefacts and quantitative losses should thus be standard practice (Rodrigues-Amaya, 2001). The stages to be considered for carotenoid analysis are sampling and sample preparation, extraction, separation, identification and quantitation. In general, extraction must be carried out very quickly, with oxygen exclusion, protection from light, avoiding high temperature, and contact with acid, and using high purity solvents. Carotenoids are soluble in lipids or in nonpolar solvents, except when they form complexes with proteins and sugars Delgado-Vargas *et al*. (2000). Thus, they are insoluble in water and soluble in organic solvents, such as acetone and alcohol, and are readily soluble in petroleum ether, hexane and toluene (Machmudah and Goto, 2013), and since various carotenoids have different structure and characteristics no solvent is optimal for the extraction of all carotenoids (Delgado-Vargas *et al*., 2000).

High-performance liquid chromatography (HPLC) has been shown to be the most efficient technique for qualitative and quantitative analyses of carotenoids. This technique allows the easy monitoring of carotenoids with a UV-visible detector, and it has a diode array detector (DAD), which permits detection at several wavelengths and simultaneous tentative identification by UV spectral analyses (Delgado-Vargas *et al*., 2000). The HPLC method uses a polymeric C_{30} column aid separation, and identification and quantitation of various carotenoid isomers with better sensitivity and selectivity (Gupta *et al*., 2015). An HPLC reversed phase C_{30} column, which has the ability to resolve carotenoids and their isomers, was applied in this research.

4.5.1. Carotenoid extraction

During the extraction of carotenoids a different behavior was observed between mango matrixes of different cultivars used in this work. There was in some cases, a formation of precipitates, which may be complexes of carotenoids with proteins and sugars (Delgado-Vargas *et al*., 2000). This hindered the extraction of carotenoids with some solvents, and thus different organic solvent extractors had to be used. Thus acetone was applied in Papers I and II for cv. 'Tommy Atkins' mango, ethanol:hexane mixture (4:3 by volume) in Paper III for cv. 'Keitt' mango and hexane in Paper IV for cv. 'Osteen' mango. All the solvents contained 0.1% (w/v) butylated hydroxytoluene (BHT) antioxidant. The carotenoid analysis was performed according to Bengtsson *et al*. (2008) with slight modifications. Samples were freeze-dried; however, rehydration in water was required in mango samples before extraction with acetone (Papers I and II). Extractions were done in duplicate for each two sets of samples. The mango sample was added to a test tube and extracted with an adequate solvent. The test tube was vortexed and then centrifuged at 4750 g for 5 min. The resulting supernatant was saved in a new test tube. This was repeated up to four times until the residue was colourless. To the resulting extract, petroleum ether was added together with ultra-pure water to aid in the separation of two phases. The organic and water phases were separated by centrifugation at 4750 g for 5 min, and the organic phase was transferred to a new tube. This step was also repeated once. The pooled organic phase was evaporated in a heating block at 35° C under a stream of nitrogen. The residue was dissolved in 5 mL mobile phase (methanol:methyl tert-butyl ether (7:3, v/v)).

4.5.2. HPLC analysis

Carotenoids were analysed by reversed phase HPLC using a Waters 600 system equipped with UV–visible photodiode array detector, recording absorption spectra between 250 and 500nm. The separations of all-*trans*-β-carotene and 13-*cis*-β-carotene (an example is given in Figure 4.5) were carried out in a C_{30} carotenoid column. The analytes were eluted from the column with different proportions of methanol (MeOH) and methyl tert-butyl ether (MTBE), with a flow rate of 1 ml/min. The gradient initial conditions were 75% (v/v) MeOH and 25% (v/v) MTBE. At 17 min the ratio varied to 30% MeOH and 70% MTBE and at 26 min to 75% and 25% MeOH and MTBE, respectively, up to a finish at 30 min, with a flow rate of 1 ml/min. The gradient allowed separations of all-*trans*-β-carotene and its geometrical isomers (9, 13 and 15-*cis*-carotene). The retention times for all-*trans*-β-carotene and 13-*cis*-β-carotene were approximately 21.20 min and 17.00 min, respectively (Figure 4.5). Other isomers were not detected. Quantification of all-*trans*-β-carotene was done based on linear calibration curves of eight standard solutions of all-*trans*-β-carotene and using response factors for quantification of the *cis*-isomers according to Bengtsson *et al*. (2008). The injection volume was 20 μL. Carotenoids were identified using retention times and UV/vis absorption spectra. The concentration of all-*trans*-β-carotene was expressed as

micrograms per g dry matter, given as the mean of triplicate extractions. The percentage retention of all-*trans*-β-carotene was calculated as the ratio of all-*trans*-β-carotene in the treated sample to fresh sample x 100.

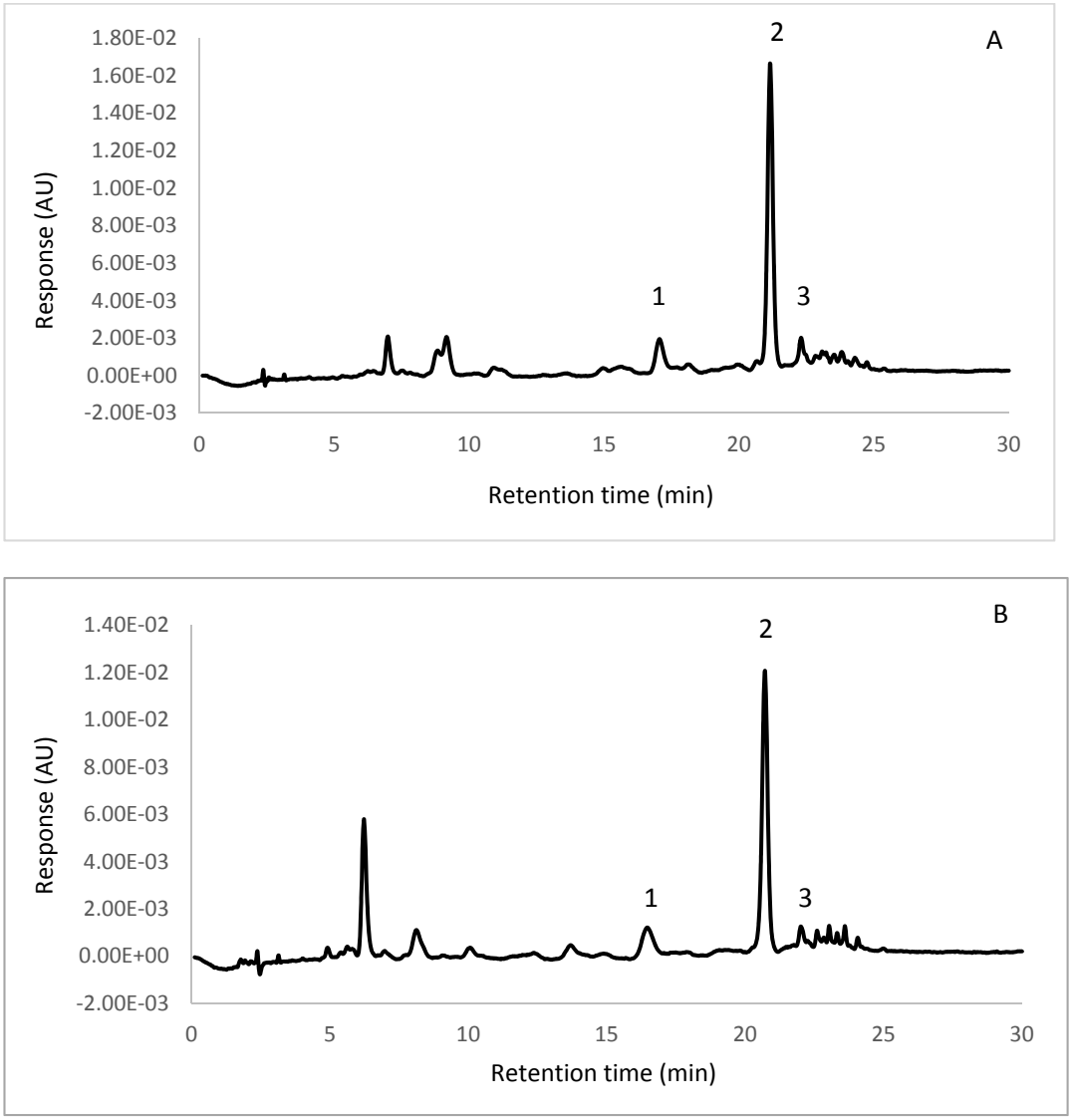


Figure 4.5 Representative HPLC profile of carotenoids from (A) fresh and (B) mango purée. 1, 13-*cis*-β-carotene; 2, all-*trans*-β-carotene; 3, probably 15-*cis*-β-carotene.

4.6. Analytical methods

Each of the following analyses were made in triplicates.

4.6.1. Moisture content

Moisture content expresses the amount of water present in the sample. The moisture content of fresh, pre-treated and dried mango was measured by drying samples in a vacuum oven at 80 °C and 900 mmHg until a constant weight was achieved (AOAC method 934.06, 2000) (Papers I - IV).

4.6.2. Water activity

Water activity is a tool used for evaluation of food stability. The water activity in fresh and hot air-dried mango (Papers I, II and IV) was measured using an AquaLab Series 3 - Decagon.

4.6.3. Colour measurements

Colour is an important parameter that may affect consumer acceptance of the product. To evaluate the effect of processing the colour of the samples was measured using a digital imaging system (Digieye) that measures the appearance of the product using a non-contact and non-destructive method. The measurement was based on the CIELAB parameters **L*** (lightness) and **a*** (redness), and the colour difference parameter (ΔE^*) was selected and calculated using the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where $\Delta x^* = x^* - x_0^*$(blank) and x^* represents L^*, a^* and b^*. The results were represented in the normalized form as ΔE^*dried (or blanched)/ΔE^*fresh, to compensate for variations in colour between mango from different batches (Papers I, II and IV).

4.6.4. Texture

The hardness of dried mango fruit was analysed using an Instron 5542 single-column universal testing machine (Instron, Norwood, MA, USA), and the cylindrical probe was 1.6 mm in diameter. The sample was placed over a 2.5 mm diameter hole, and the probe was allowed to completely penetrate the sample. The hardness of the texture was given

by the maximum force of compression, and the mean of ten independent measurements from each treatment is reported.

4.7. Statistical analysis

All the analyses were performed in triplicate of duplicate samples and the results are presented as mean ± standard deviation. Differences between variables were tested for significance by one-way analysis of variance (ANOVA) and Tukey´s HSD post hoc multiple range test. Differences were considered to be significant at $P < 0.05$ (or at a level of $\alpha = 0.05$).

5. RESULTS AND DISCUSSION

This chapter presents and discusses results of the work reported in Papers I to IV. The focus was improvement of the nutritional value of dried mango and purées. In the case of dried mango, the effect of pre-treatments such as osmotic dehydration with additives (vitamin C or calcium mineral) and dry blanching techniques such as infrared and microwave radiation as an alternative to conventional water blanching, were investigated. Blanching was done at conditions of high temperature and short time (HTST) or at low temperature and long time (LTLT) using AAO and PPO as indicator enzymes. The minimal processing of mango into purée was performed using different inhibitors of enzymatic activity. Some details related these processing methods are presented below:

- Osmotic dehydration without additives (OD) or with 1% calcium (ODCa) or 1% ascorbic acid (ODAA), prior to hot air-drying at 50 °C or 70 °C. Samples OD50 ODAA50, ODCa50, OD70, ODAA70 and ODCa70 represent the pre-treated mango dried either at 50 °C or 70 °C, while U50 and U70 are untreated mango samples dried at 50 °C or 70 °C, respectively – Paper I.

- Blanching at high temperature and short time (HTST) or low temperature and long time (LTLT) using infrared (IR), microwave (MW) or water (W) blanching, followed by hot air-drying at 70 °C. Samples are referred as IR65, W65, MW70, W70, W70P, IR90, MW90, W90 or W90P, where 65 and 70 indicate the blanching temperature at LTLT for 10 min, and 90 is the blanching temperature at HTST for 2 min. Samples with index P are blanched in a closed plastic bag – Papers II and IV.

- Processing of mango purée subjected to acidification, addition of EDTA and water blanching at HTST for 4 min in a plastic bag prior to homogenization at different pH, that is, pH 5.0±0.1 representing the pH of ripe mango and pH 3.9±0.1 the acidified condition (see section 4.2.4). The nutrients were analysed in all puréed samples 30 min after crushing, which are referred to as crushed unblanched at pH 5.0 (pH5), crushed unblanched at pH 5.0 with EDTA (EDTA), crushed at low pH 3.9 (pH4) and HTST blanched crushed at pH 5.0 (blanched) – Paper III.

The main objective was to evaluate the effect of processing on the stability of vitamin C and β-carotene in minimally processed mango or dried mango to obtain products with improved nutritional value. In addition, the impact of blanching to inactivate polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) enzymes, drying, texture time and colour variation in processed mango were also evaluated.

5.1. Characterization of fresh mango fruits

Different mango cultivars were used in this work. These were medium ripe mango cylinders of cv. 'Tommy Atkins' (Papers I and II) and cv. 'Osteen ' (Paper IV), while in Paper III a ripe mango of cv. 'Keitt' was used to prepare mango purée. The average moisture content and water activity of fresh mango was 0.87 ± 0.03 and 0.985 ± 0.006, respectively.

The nutritional quality of fresh mango was estimated by measuring the amount of total vitamin C and total β-carotene; the results are shown in Table 5.1. The vitamin C was measured as total vitamin C in Papers I and II, while in Papers III and IV the total vitamin C was calculated as the sum of L-AA and DHAA, both having vitamin C activity (Wechtersbach and Cigić, 2007; Santos and Silva, 2008). Significant differences in total vitamin C content was observed between cultivars Tommy Atkins and Osteen, with higher values for Tommy Atkins. The distribution of L-AA and DHAA in both Keitt and Osteen mango was found to be approximately 90 % and 10 %, respectively. Similar findings with a DHAA content of about 10% of the total vitamin C in fresh tropical fruits and vegetables including mango have been reported (Hernández *et al*., 2006; Wills *et al*., 1984). Therefore it is important to measure both L-AA and DHAA in fruits and vegetables to find the real value of their vitamin C content (Barrett and Lloyd, 2012; Oliveira *et al*., 2010).

Table 5.1 Total vitamin C and total β-carotene (mg/100 g DW) in fresh mango.

	Tommy Atkins	**Keitt**	**Osteen**
Vitamin C	132. 3±11.2[a]	97.4 ±33.3[a,b]	82.2 ±27.3[b]
β-carotene	4.8±0.2[a]	9.4±1.3[b]	1.8±0.6[c]

Samples showing the same letter in the same row are not significantly different (p>0.05).

β-carotene was calculated as the sum of all-*trans*-β-carotene and 13-*cis*-β-carotene isomers. The presence of 13-*cis*-β-carotene isomers in fresh mango has been reported by several authors (Godoy and Rodriguez-Amaya, 1994; Pott *et al*., 2003; Chen *et al*., 2007). The cultivars investigated in this study varied in their total β-carotene contents. Significant differences ($p < 0.05$) were observed in Osteen and Keitt mango cultivars (Table 5.1). The higher amount of β-carotene in cv. 'Keitt' could be due to its mature stage. Unripe mangoes are rich in vitamin C, while accumulation of β-carotene occurs during ripening of mango (Vasquez-Caicedo *et al*., 2005). According to Mercadante *et al*. (1997), a natural variation among mango samples is expected because of cultivar differences, climatic effects, stage of maturity at harvest, and time after harvest. The β-carotene and vitamin C contents found in the current research are in the same range reported by other authors in fresh mango flesh of different cultivars (Manthey and Perkins-Veazie, 2009; Pott *et al*., 2003; Vasquez-Caicedo *et al*., 2005).

5.2. Impact of blanching on polyphenol oxidase and ascorbic acid oxidase enzyme activity

The effects of new dry blanching techniques were evaluated in mango cylinders. The dry-blanching infrared at HTST, 90 °C / 2 min or LTLT, 65 °C / 10 min (Paper II), or microwave at HTST, 90 °C / 2 min or LTLT, 70 °C / 10 min (Paper IV) were compared with water blanching, in a water bath (conventional) or in closed plastic bag under similar time-temperature conditions. The polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) enzyme activities were assessed to evaluate the effect of blanching treatments. An example of a temperature profile is shown in (Figure 5.1). The stability of PPO and AAO was also analysed in mango pieces blanched in a water bath in closed plastic bags at HTST, 90 °C for a longer time duration, 4 min (Paper III).

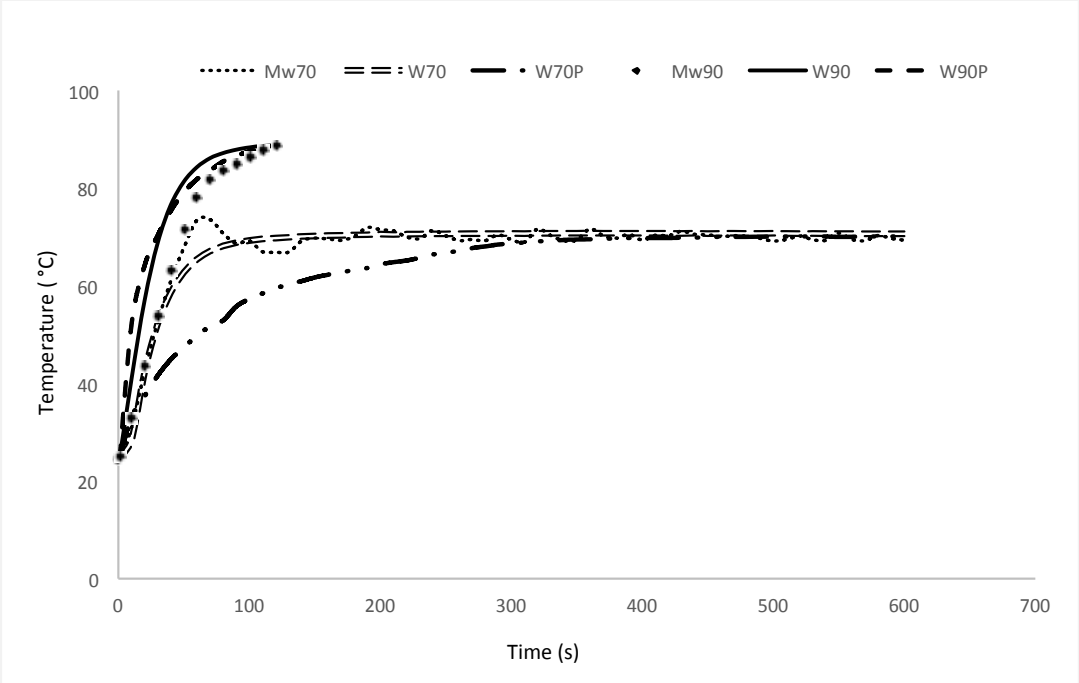


Figure 5.1 Temperature profiles during LTLT and HTST water and microwave dry blanching.

A complete inactivation of polyphenol oxidase (PPO) enzyme was achieved with all thermal processing, i.e. in water, IR and MW blanching. That was evidenced by the curve of absorbance vs time, during the reaction between the PPO enzyme extract and catechol substrate (not shown), where no changes in absorbance and colour (Figure 5.2A) were observed in the reaction mixture buffer. In contrast, changes in absorbance and browning (Figure 5.2B) were observed in reaction mixture buffer of fresh mango, indicating the existence of PPO that catalyzed the oxidation of catechol (colourless) to enzyme-substrate complex (brown colour). In the presence of the enzyme in the extract,

adequate substrate binds to enzymes forming the enzyme-substrate complex, as illustrated in Figure 5.2C.

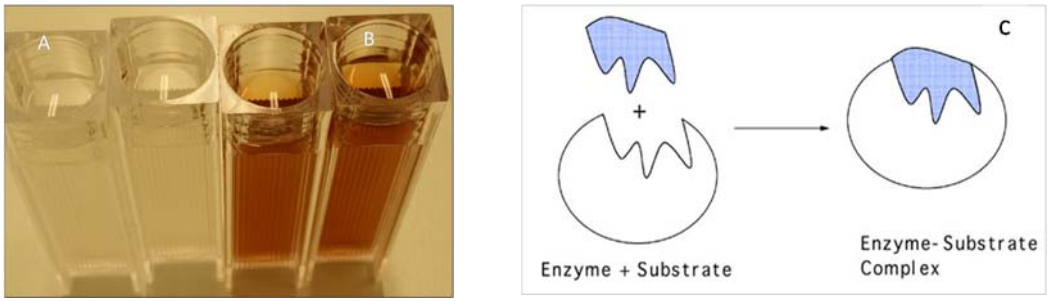


Figure 5.2 Colour variation in mixture buffer after PPO extract and catechol substrate reaction A-blanched mango and B-fresh mango (duplicate samples). C-illustration of enzyme-substrate complex binding (Source: Casiday and Frey, 1988).

On the other hand, some residual enzymatic activity of AAO was detected in mango samples after the treatments, as shown in Table 5.2. These results indicate that AAO in mango seems to be more resistant than PPO. The results on PPO are consistent with earlier reports in the literature for mango (Wang *et al*., 2007; Ndiaye *et al*., 2009; Vásquez-Caicedo *et al*., 2007); however, the stability of this and other enzymes appears to depend on cultivars, processing methods and conditions. For instance, the PPO and AAO activities in fresh mango were 121.6±26.4 and 5.3±0.25 units, respectively, for cv. 'Keitt', while the activities for cv. 'Osteen' were 68.0±24.4 and 1.7±0.2 units, respectively. Information on AAO in mango is scarce. Cardello *et al*. (1993/1994) showed that AAO in the cellular wall of mango of the Haden cultivar was thermolabile above 80 °C.

Table 5.2 Residual AAO activity (%) after infrared, microwave and water blanching.

Osteen mango (Paper IV)	Untreated	MW70	W70	W70P	MW90	W90	W90P
Residual AAO activity	NA	18.2±2.5^{a}	6.6±1.8^{b}	14.3±4.5^{a}	11.9±3.4^{a}	6.2±2.0^{b}	17.2±5.0^{a}
Tommy Atkins mango (Paper II)	Untreated	IR65	W65		IR90	W90	
Residual AAO activity	NA	31.1±6.1^{a}	29.4±7.8^{a}		8.7±1.8^{b}	14.8±3.4^{c}	

NA – not applicable. Samples showing the same letter in the same row are not significantly different ($p>0.05$).

5.3. Effect of processing on the stability of vitamin C in mango products

The evaluation of vitamin C in mango was performed in fresh, blanched, dried and puréed samples. The vitamin C results in processed samples were expressed as the retained percentage of the original amount present in the fresh mango.

5.3.1 Effect of osmotic dehydration prior to hot air-drying on the retention of vitamin C in dried mango

The vitamin C retained in untreated and osmotically pre-treated mango with and without calcium and dried at 50 °C or 70 °C is shown in Figure 5.3. A high retention of vitamin C ($p < 0.05$) was observed in untreated mango dried at 50 ºC (76.4%) as compared with mango dried at 70 ºC (57.1%). As vitamin C is heat sensitive, a higher drying temperature was expected to cause a higher degradation, as well as a higher enzymatic degradation, due to a higher activity of ascorbic acid oxidase. Osmotic pre-treatment without additives resulted in significantly lower vitamin C retention in mango dried at the lower temperature (50 ºC) compared to untreated mango, although no such difference was observed between mango samples dried at 70 ºC. Similarly, Azoubel *et al*. (2009) and Araya-Farias *et al*. (2014) observed a lower retention of vitamin C in samples exposed to osmotic dehydration. This finding was attributed to the leaching of vitamin C from the product to the osmotic solution (Yadav and Singh, 2014) and to chemical degradation during subsequent drying (Azoubel *et al*., 2009). The addition of Ca to the osmotic solution significantly improved vitamin C retention, when compared to OD samples without additives, i.e. 53.3% (ODCa50) and 60.1% (ODCa70) in relation to 37.2% (OD50) and 51.3% (OD70), respectively, while the addition of ascorbic acid in osmotic solutions increased the vitamin C content to several times higher than in the fresh mango (Paper I). The higher retention of vitamin C in dried pre-treated mango in osmotic solutions fortified with calcium may be associated with the decrease in cell wall permeability since calcium can interact with the plant cellular matrix, forming bonds between pectins and other cellular wall components, modifying its structural response (Gras *et al*., 2003). The findings from this study shows that the fortification of OD solutions with vitamin C may be strategically used to improve the nutritional quality of dried fruits.

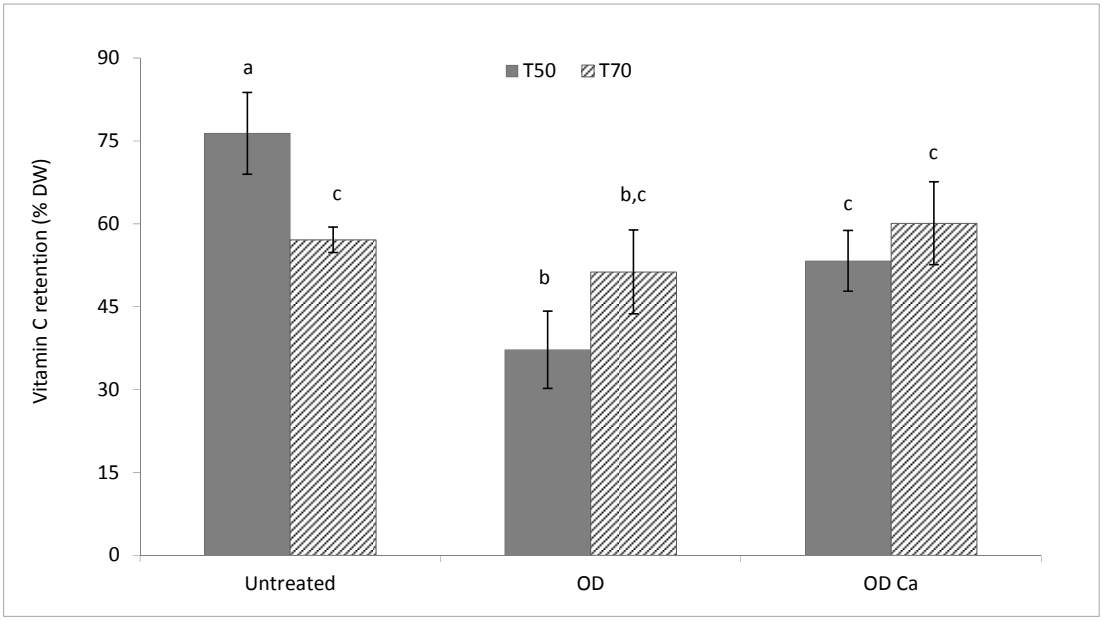


Figure 5.3 Vitamin C retention in dried untreated and OD treated mango. Samples showing the same letter are not significantly different (p>0.05).

5.3.2. Effect of blanching prior to air-drying on the retention of vitamin C in dried mango

Table 5.3 shows the retention of vitamin C in mango affected by blanching and drying processes. MW blanching (MW70, MW90) and water blanching in closed plastic bag (W70P, W90P) either at low or high temperatures had no effect on the vitamin C content, and the retention was ~100% (Table 5.3A). The retention after conventional water blanching was however significantly lower, 86.2% (W90) and 81.5% (W70). The drying process, except for MW70 and untreated samples where the retention was 81.8% and 82.9%, respectively, caused no additional degradation of this nutrient. Blanching mango in closed plastic bag resulted in comparable retention values of vitamin C as in MW heating. This may be associated with limited leaching of water-soluble nutrients in both processes.

Similar to the results in the MW blanching study, a high degradation of vitamin C was observed using conventional water blanching in comparison with infrared blanching (Table 5.3B). The retention of vitamin C in dried LTLT treated mango was negatively affected by both the blanching and drying processes, while no further degradation of vitamin C was caused by drying in the final HTST blanched products. Moreover, a significantly higher retention of this nutrient ($P < 0.05$) was obtained in dried untreated mango (93.0%), followed by IR and water HTST treatment with 78.8% and 54.8%, respectively. The lowest vitamin C retention was found in dried IR and water LTLT blanched samples, 43.3% and 30.1% ($P < 0.05$), respectively. The degradation of vitamin C in the mango during the drying stage could be associated with thermal

breakdown of the matrix structure (Ponne *et al*., 1994), which may have caused oxidation of vitamin C in the presence of oxygen.

Table 5.3 Vitamin C retention (% DW) in blanched and dried mango subjected to infrared (Paper II), microwave (Paper IV) and water (Paper II, IV) blanching.

A – (Paper IV)	**Untreated**	**MW70**	**W70**	**W70P**	**MW90**	**W90**	**W90P**
Blanched	NA	99.7±6.4	81.5±6.4	100.4±3.1	101.6±2.7	86.2±3.4	102.2±5.3
Dried	82.9±2.0	81.8±4.5	78.6±2.5	99.0±4.8	97.1±7.3	86.7±2.6	101.0±4.4

B – (Paper II)	**Untreated**	**IR65**	**W65**	**IR90**	**W90**
Blanched	NA	69.2±2.9	50.7±9.6	88.3±1.0	61.4±5.3
Dried	93.0±7.3	43.4±3.9	30.1±6.7	78.8±10.9	54.8±5.2

The relation between vitamin C retention and the residual AAO enzymatic activity observed after blanching water and IR blanching (Paper II) is shown in Figure 5.4. A direct relation between vitamin C retention in dried mango and the residual AAO enzymatic activity was observed. A higher degradation of vitamin C occurred after LTLT treatments that could be attributed to a higher level of AAO residual activity in that samples, while lower reduction of vitamin C at HTST was associated with a lower remaining enzymatic activity (see section 5.2). A negative effect of AAO activity on vitamin C retention during fruit and vegetable processing has been reported (Munhyaka *et al*., 2010a).

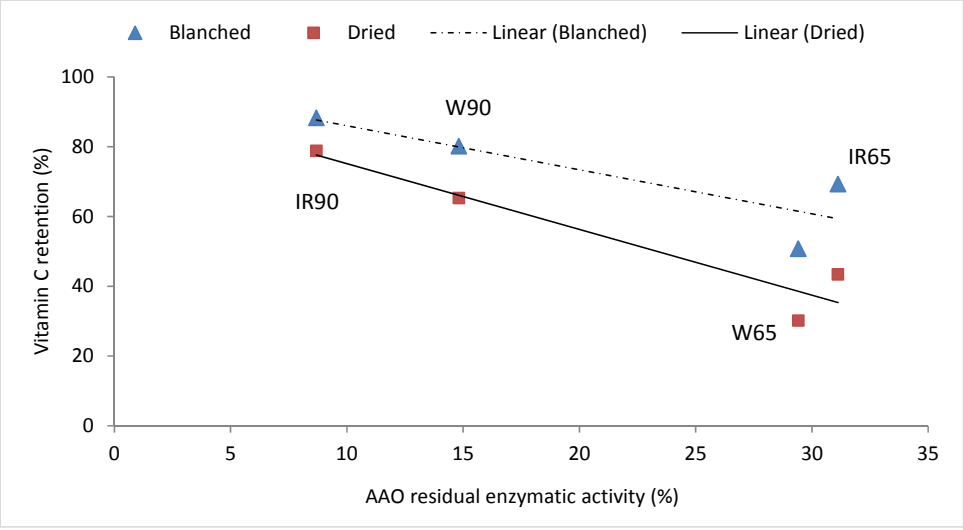


Figure 5.4. Vitamin C retention in dried infrared and water blanched mango versus residual ascorbic acid oxidase enzymatic activity.

Moreover, in order to gain a better understanding of the contribution of L-AA and DHAA to the total amount of vitamin C in mango, these components were measured in the MW blanching study (Paper IV), and the distribution in the fresh and dried mango

is shown in Figure 5.5. The retention of vitamin C was mainly as L-AA, with significantly higher levels in fresh and dried untreated mango. Higher levels of DHAA were found in the blanched samples, and there was a trend toward a higher percentage in the LTLT treated samples compared with the HTST treated samples. The effect of both long blanching time and comparatively higher remaining AAO residual activity was associated with a higher oxidation of L-AA in MW70 mango samples (Table 5.2) (see details in Paper IV).

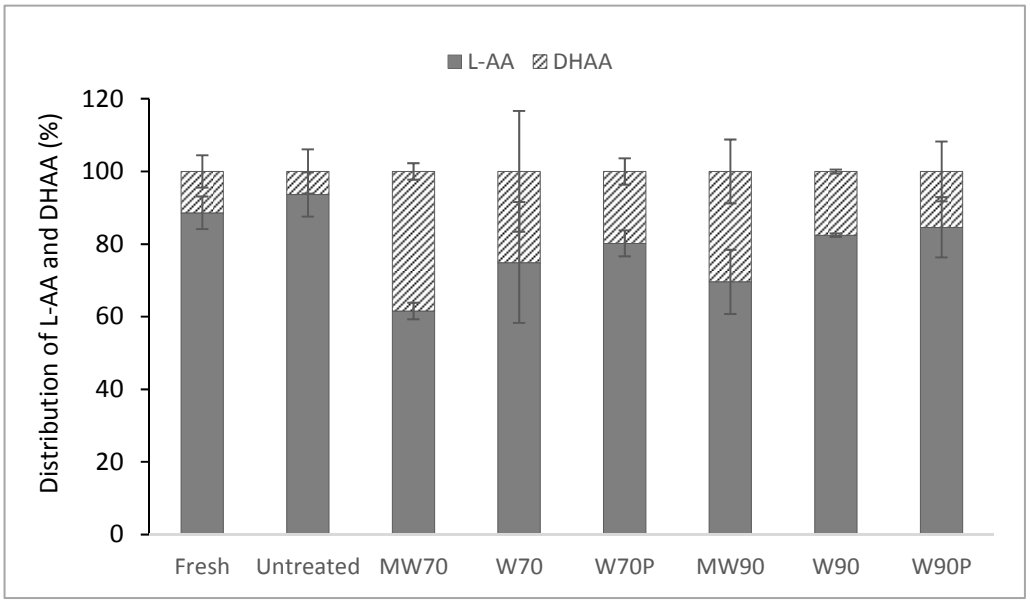


Figure 5.5 Total vitamin C distribution as L-AA and DHAA in fresh and dried mango.

In summary, compared with conventional treatment, infrared and microwave blanching resulted in a better retention of vitamin C, which may be explained by lesser leaching losses during processing. Similar results were presented by Ramesh *et al*. (2002) and Lin and Brewer (2005). It would thus be convenient from a nutritional point of view to reduce vitamin C losses by using IR or MW blanching instead of water blanching at similar temperature. On the other hand, the retention of vitamin C was significantly higher for MW blanched than for IR blanched mango, and LTLT blanching resulted in a higher degradation of vitamin C compared with HTST blanching. The higher retention of vitamin C in mango subjected to microwave blanching agrees with the results in microwaved blanched vegetables (Ponne *et al*., 1994; Muftugil, 1986; Ruiz-Ojeda and Peñas 2013; Ramesh *et al*., 2002; Jeevitha *et al*., 2013, 2014), and infrared blanched (Vishwanathan *et al*., 2013).

5.3.3. Effect of blanching, acidification and addition of EDTA on the retention of vitamin C in mango purée

The results in vitamin C retention in processed mango purées are shown in Figure 5.6. Compared to the total content of vitamin C (L-AA + DHAA) in fresh samples, the retention of total vitamin C was about 90% in purées at pH 4 and 5, and approximately 100% in purées of blanched mango and in purées with added EDTA. No significant differences were observed between fresh and processed samples, meaning that there were low losses of vitamin C as a result of irreversible DHAA degradation to 2,3-diketogulonic acid (DKGA). HTST heating of mango in water using closed plastic bags that could avoid leaching of soluble solids was found to positively influence the total retention of vitamin C (L-AA + DHAA). Significantly lower vitamin C retention (~60%) was shown in HTST treated mango without plastic bag protection in Paper II.

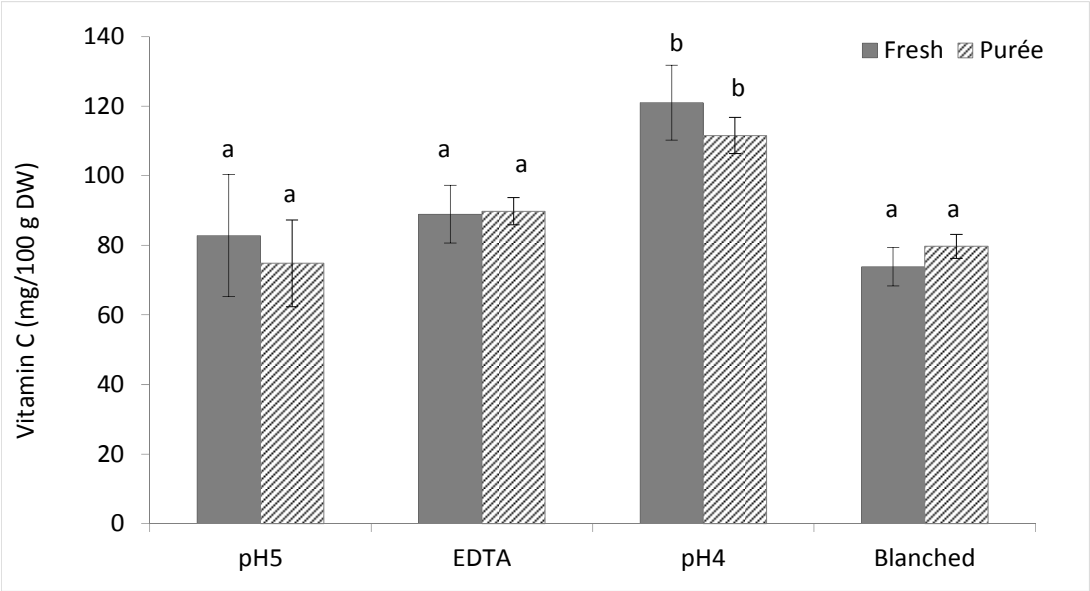


Figure 5.6 Total vitamin C (L-AA and DHAA) content in fresh mango and mango purée 30 min after different treatments. Samples showing the same letter are not significantly different ($p>0.05$).

Figure 5.7 shows the percentage distribution of L-AA and DHAA in fresh mango and in puréed samples 30 min after being processed. The percentage distribution in fresh mango, and purées of blanched and acidified (pH4) mango, was similar, and vitamin C occurred mainly as L-AA. However, the percentage distribution of L-AA in EDTA purée was significantly lower, while purée at pH5 had a complete conversion to DHAA. Blanching and lowering of pH were associated with inactivation of oxidative enzyme activity of AAO, preventing the oxidation of L-AA to DHAA. The results of this study show that the effect of enzymatic oxidation of vitamin C (pH5) in mango purée was larger than the oxidation caused by thermal treatment (blanched). Similar findings were reported in studies on broccoli (Munyaka *et al*., 2010a,b).

The addition of EDTA could also protect L-AA from oxidative degradation to DHAA, which may be attributed to the fact that EDTA chelates the copper in the prosthetic group of the enzyme and thereby inhibits its activity. The results also show that the combination of citric acid with EDTA at pH 5.0 was more efficient in inhibiting the AAO activity than citric acid alone at pH 5.0, since complete oxidation of L-AA to DHAA was observed with citric acid alone. Hernández *et al*. (2006) showed that adding EDTA diminished ascorbic acid (AA) loss during extraction of fruits for AA analysis. It has been reported in the literature that combinations of inhibiting agents may result in enhancement of the inhibition (Son *et al*., 2001; Özoğlu and Bayindirli, 2002).

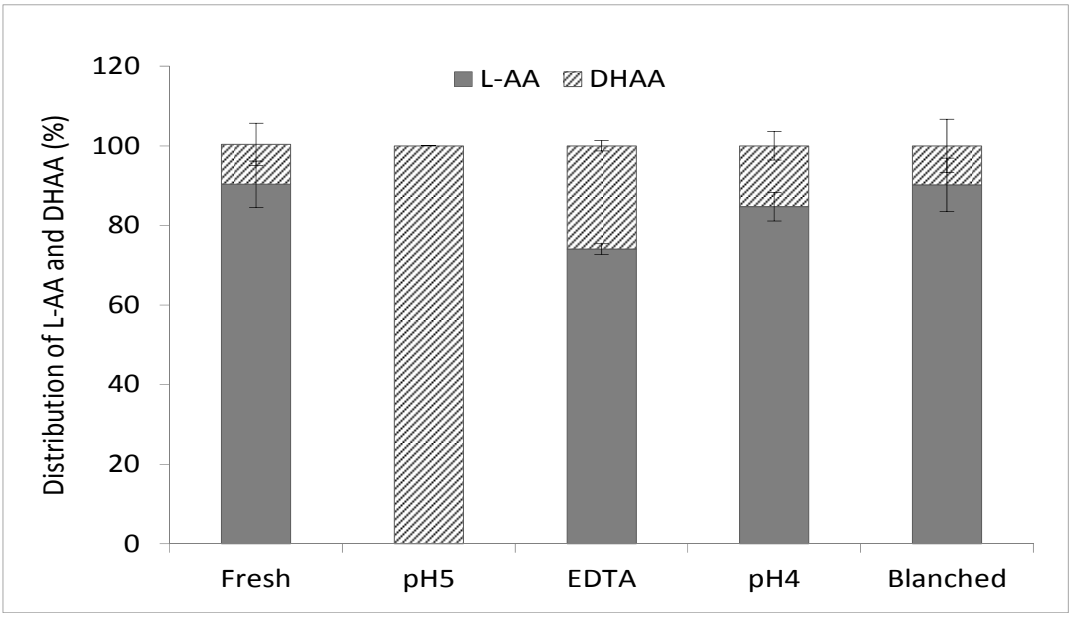


Figure 5.7 Percentage distribution of L-AA and DHAA in fresh and mango purée.

5.4. Effect of processing on the stability of β-carotene in mango

β-carotene was analysed in fresh or untreated and in processed mango. The results are presented as the retention of all-*trans*-β-carotene and the ratio of 13-*cis*-β-carotene to all-*trans*-β-carotene (% DW), as affected by the processing.

5.4.1. All-*trans*-β-carotene retention in mango products

5.4.1.1. Effect of osmotic dehydration prior to hot air-drying on the retention of all-*trans*-β-carotene in dried mango

Studies in the literature of β-carotene and its *trans*-*cis*-isomerization in food materials associated with pre-treatments with osmotic solutions and further drying are scarce.

The results of all-*trans*-β-carotene retention in dried untreated mango and osmotically pre-treated prior to hot air-drying are shown in Figure 5.8. The retention of all-*trans*-β-carotene in dried untreated mango at 50 °C (83.0%) was found to be significantly higher ($p<0.05$) as compared to untreated samples dried at 70 °C (75.0%). Similar results were reported by Pott *et al*. (2003), showing a retention of about 70% for untreated mangoes conventionally dried at 75 °C. The retention of this nutrient in dried untreated mango was not significantly different from dried mango osmotically pre-treated, fortified with calcium (ODCa) or ascorbic acid (ODAA), and dried at 70 °C (~78%). A lower retention of this nutrient was shown in OD pre-treated samples with additives dried at 50 °C or without additives (OD) dried at both temperatures, which retained ~60%. Araya-Farias *et al*. (2014) evaluated the effect of OD in combination with convective or vacuum drying on sea buckthorn fruits and found a substantial reduction of the vitamin C and the total carotenoid content, which was associated with high water leakage of the vitamin C and heat degradation of the carotenoids. According to the results obtained by Moore (2003), processed mango products fortified with antioxidants such as vitamins C and E had improved preservation of carotenoid structure and functions.

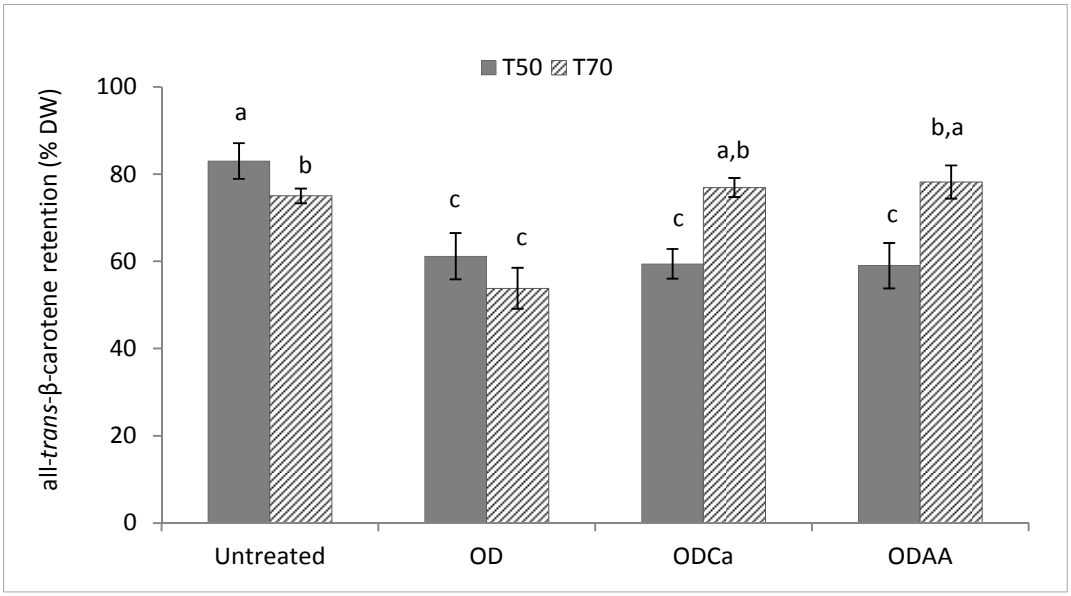


Figure 5.8 All-*trans*-β-carotene retention in dried untreated and OD treated mango. Samples showing the same letter are not significantly different ($p>0.05$).

5.4.1.2. Effect of blanching prior to hot air-drying on the retention of all-*trans*-β-carotene in dried mango

The retention of all-*trans*-β-carotene in dried mango subjected to IR, MW or water blanching, prior to hot air-drying, is presented in Table 5.4. In general, water blanching

retained more all-*trans*-β-carotene in blanched and dried mango than IR and MW heating. This may be associated to the hydrophobic characteristics of carotenoids, i.e., to little or no solubility in water (britton, 1995). Thus, water blanching either at LTLT or HTST (W65, W70, W70P, W90, W90P) resulted in high retention values (~100% or more) of all-*trans*-β-carotene in dried mango. Blanching mango in plastic bag did not make difference on the retention of all-*trans*-β-carotene in dried mango comparatively to conventional water blanching. There was a tendency to better retention of all-*trans*-β-carotene in MW blanched mango (~82%) compared to IR blanched samples (~75%). On the other hand, a different behaviour was observed in untreated dried mango samples, where cv. 'Tommy Atkins' mango (Paper II) retained 86% of this nutrient, while a severe degradation was noted in cv. 'Osteen' dried untreated mango, where the retention was around 57%. This could be due to the PPO activity, which might cause co-oxidation of the all-*trans*-β-carotene when exposed to high temperature and in the presence of oxygen (Dorantes-Alvarez and Chiralt, 2000; Xianquan *et al*., 2005), and may also be associated with differences in cultivar, stage of maturity and growing conditions (Mercadante and Rodriguez-Amaya, 1998). On the other hand, carotenoids have a highly unsaturated structure, and are prone to isomerization during processing and storage due to the effects of chemical, mechanical and thermal stresses (Boon *et al*., 2009; Mao *et al*., 2009; Qian *et al*., 2012). No studies of β-carotene stability at IR or MW blanching followed by hot air-drying were found in the literature. Our findings suggest that the retention of all-*trans*-β-carotene was more dependent on the type of the blanching process (water, IR or Mw) than on the blanching conditions time/temperature.

Table 5.4 All-*trans*-β-carotene retention (% DW) in dried mango subjected to infrared (Paper II), microwave (Paper IV) and water (Papers II, IV) blanching.

A – (Paper IV)	**Untreated**	**MW70**	**W70**	**W70P**	**MW90**	**W90**	**W90P**
Dried	56.9±4.9	82.7±8.1	103.7±0.9	100.8±8.5	81.1±2.1	87.8±1.1	90.1±2.0
B – (Paper II)	**Untreated**	**IR65**	**W65**		**IR90**	**W90**	
Dried	86.4±3.0	73.6±3.6	93.2±5.2		76.9±2.9	91.4±5.1	

5.4.1.3. Effect of blanching, acidification and addition of EDTA on the retention of all-*trans*-β-carotene in mango purée

In mango processed as purée as a result of different treatments, the average retention of all-*trans*-β-carotene after 30 min was found to vary between 64.8 – 72.2%, as shown in Figure 5.9. The purée prepared with added EDTA had a slightly higher retention (72.2%) than the purée of blanched samples (64.8%). The degradation of all-*trans*-β-carotene was attributed to disintegration of the food cell matrix caused by cutting and/or crushing, which highly induces oxidation and isomerization of β-carotene by the

increased exposition to oxygen, and the process is stimulated by the presence of endogenous oxidative enzymes metals, light and co-oxidation with lipid hydroperoxides (Boon *et al*., 2010; Rodriguez-Amaya, 1999). The higher amount of all-*trans*-β-carotene in EDTA samples in comparison with blanched samples suggests that there might be some protective effect from EDTA. Qian *et al*. (2012) reported that EDTA was highly effective in inhibiting colour loss (β-carotene encapsulated within oil-in-water nano-emulsions), which was attributed to its ability to strongly chelate and inactive the transition metals that normally promote carotenoid oxidation. Thus, EDTA can act as an inhibitor of PPO activity by chelating the copper in the prosthetic group of the enzyme (Sapers *et al*., 1989).

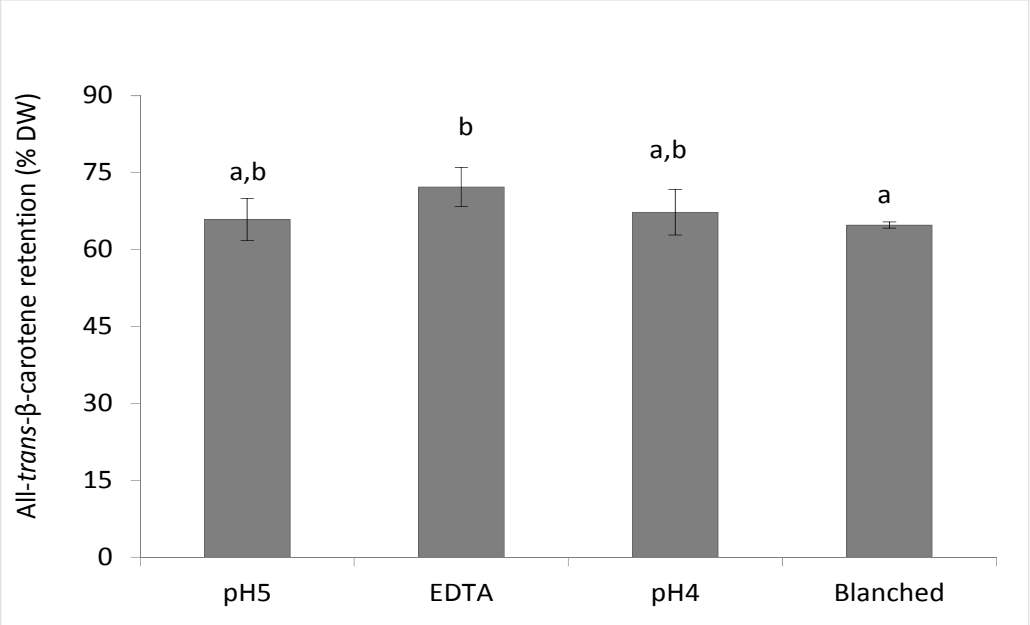


Figure 5.9 All-*trans*-β-carotene retention in mango purée. Samples showing the same letter are not significantly different (p>0.05).

5.4.2. Effect of processing on the ratio of 13-*cis*-β-carotene to all-*trans*-β-carotene in mango products

Cis-isomers may be found in fresh fruits and may also result from *trans*-*cis* isomerization of all-*trans*-β-carotene. *Cis*-isomers have lower biological potency than the corresponding *trans*- β-carotene, and quantification of the isomers of provitamin A individually is required to determine the vitamin A value of foods more accurately (Godoy and Rodriguez-Amaya,1994).

The influence of processing on 13-*cis*-β-carotene isomer in the mango products under consideration in this thesis (fresh, dried and purées) was evaluated as the ratio of 13-*cis*-β-carotene (%) to all-*trans*-β-carotene, and the results are shown in Figure 5.10. Generally, higher ratios of 13-*cis*-β-carotene to all-*trans*-β-carotene between 13 and 18

% were observed in fresh mango of the cv. 'Tommy Atkins' (Figure 5.10A and B), while Keitt and Osteen mango cultivars showed lower values (Figure 5.10, C and D, respectively). In general, the ratios of 13-*cis*-β-carotene to all-*trans*-β-carotene remained unchanged after hot air-drying untreated samples either at 50 or 70 °C compared to the ratio in fresh samples (Figure 5.10A, B and C). Increased ratios were observed in dried mango after IR (Figure 5.10B) and MW (Figure 5.10C) pre-treatments, and no specific trend was seen in conventional water blanching. Processing mango as a purée, in acidified samples (pH4) or EDTA, had no effect on *trans*-*cis* isomerization; however the ratios of 13-*cis*-β-carotene to all-*trans*-β-carotene increased with blanching and at pH5 (Figure 5.10D). Being highly unsaturated, carotenoids are prone to isomerization and oxidation during processing, as previously described. Reports in the literature show that heat treatment promotes isomerization of the carotenoids in foods, from *trans* to *cis* isomeric forms, and that the degree of isomerization is directly correlated with the intensity and duration of heat processing (Cheng and Huang 1998; Vásquez-Caicedo *et al*., 2007; Achir *et al*., 2011; Lemmens *et al*., 2013) and with the presence of oil in the process (Bengtsson *et al*., 2010).

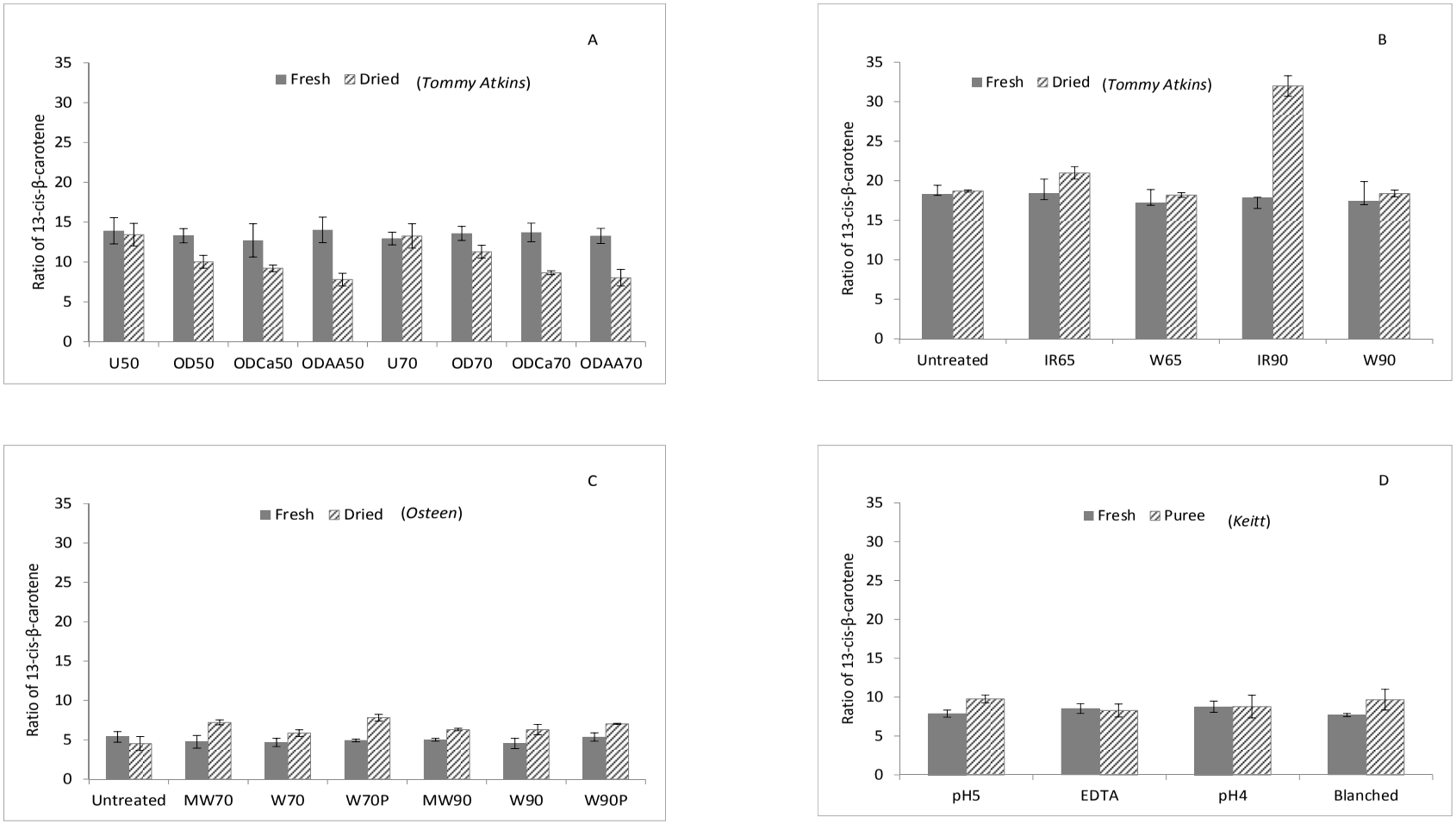


Figure 5.10 The ratio of 13-*cis*-β-carotene to all-*trans*-carotene in relation to fresh and dried mango subjected to: A - OD pre-treatment, B - IR blanching, C - MW blanching, D - mango purée processing.

5.5. The influence of pre-treatment on weight change and drying time

Pre-treatments, either osmotic dehydration or blanching, resulted in weight losses in mango cylinders with the exception of blanched samples in closed plastic bag. The weight change was significantly higher during infrared or microwave dry blanching than in water blanching conditions. This could be associated with rapid heating of the material and the drying action of IR (Paper II) and MW (Paper IV), which caused moisture vaporization (Hebbar *et al*., 2004; Muftugil 1986; Ramesh *et al*., 2002). The loss of fruit material and water-soluble solids by leaching was thought to be the probable cause of the weight loss in water blanching and in osmotic dehydration (Paper I).

Untreated mango cylinders, and osmotically pre-treated or pre-treated by blanching in water, IR or MW, were hot air-dried at 50 °C and/or 70 °C (Paper I, II, IV) in order to reduce the water activity to a level that ensures microbiological stability of the fruit (~0.6). It was observed that comparatively higher drying times were required to dry untreated and LTLT water blanched samples. Infrared and microwave blanching significantly reduced the drying time of mango as compared to water blanching. The same behaviour of a shorter drying time was shown in dried OD treated mango in comparison with dried untreated, and a higher drying temperature shortened the drying time more. The reduction in drying time was attributed to weight loss during the pre-treatment processes. This finding is in agreement with reports in the literature (Azoubel *et al*., 2009; El-Aouar *et al*., 2003; Mastrantonio *et al*., 2005; Wishwanathan *et al*., 2013).

5.6. The influence of the pre-treatment prior to hot air-drying on the colour and appearance of dried mango

Colour is usually the most influential factor in evaluations of food by consumers, and the off-colour products are often unacceptable in spite of their good taste and flavour (Piližota and Šubarić 1998). The effect on colour of osmotic dehydration, water, IR and MW blanching prior to hot air-drying, as well as in drying untreated mango, was evaluated in Papers I, II and IV. Figure 5.11 shows the colour variation of dried mango, as affected by the temperature and the treatments. The total colour difference parameter, ΔE^*, was selected to represent the colour of mango, and the results were normalized with the colour of a fresh sample ($\Delta E^*/\Delta E^*$fresh) to compensate for variations in colour between mango from different batches and varieties. The results show that the drying temperature did not influence the colour of dried mango subjected to the same treatment (Figure 5.11A). In general, the colour was not affected by blanching or osmotic dehydration pre-treatments prior to hot air-drying in comparison with the colour of fresh mango ($\Delta E^*/\Delta E^*$fresh = 1). The absence of a colour change could be associated with osmotic dehydration in preventing enzymatic browning by inhibition of polyphenol oxidase (PPO) activity in osmosed samples (Chavan and Amarowicz, 2012; Yadav and Singh, 2014), or inactivation of the enzyme during the blanching processes (see section 5.2), since PPO is the

enzyme responsible for browning (Palma-Orozco *et al*., 2012; Ioannou and Ghoul 2013). However, an increase in the ($\Delta E^*/\Delta E^*$fresh) ratio was shown in dried OD treated mango without additives, dried at 70 °C (Figure 5.11A), and in dried HTST IR blanched mango (Figure 5.11B), which indicates that there was a slight darkening in those samples. ODCa mango dried at 50 °C (Figure 5.11A) resulted in a slight reduction of the ($\Delta E^*/\Delta E^*$fresh) ratio, that is, it tended to be lighter than the fresh ones. On the other hand, untreated and water blanched mango did not show a specific trend in colour variation, being lighter, darker or with preserved colour. Changes in colour could be related to the thermal treatments, which induce several reactions such as pigment degradation, especially carotenoids, Maillard reactions and oxidation of ascorbic acid (Barreiro *et al*., 1997; Korbel *et al*., 2013). The Maillard reaction and ascorbic acid oxidation have been shown to produce a yellow brown colour in mango dried at high temperature (70^o C) (Chong *et al*., 2013).

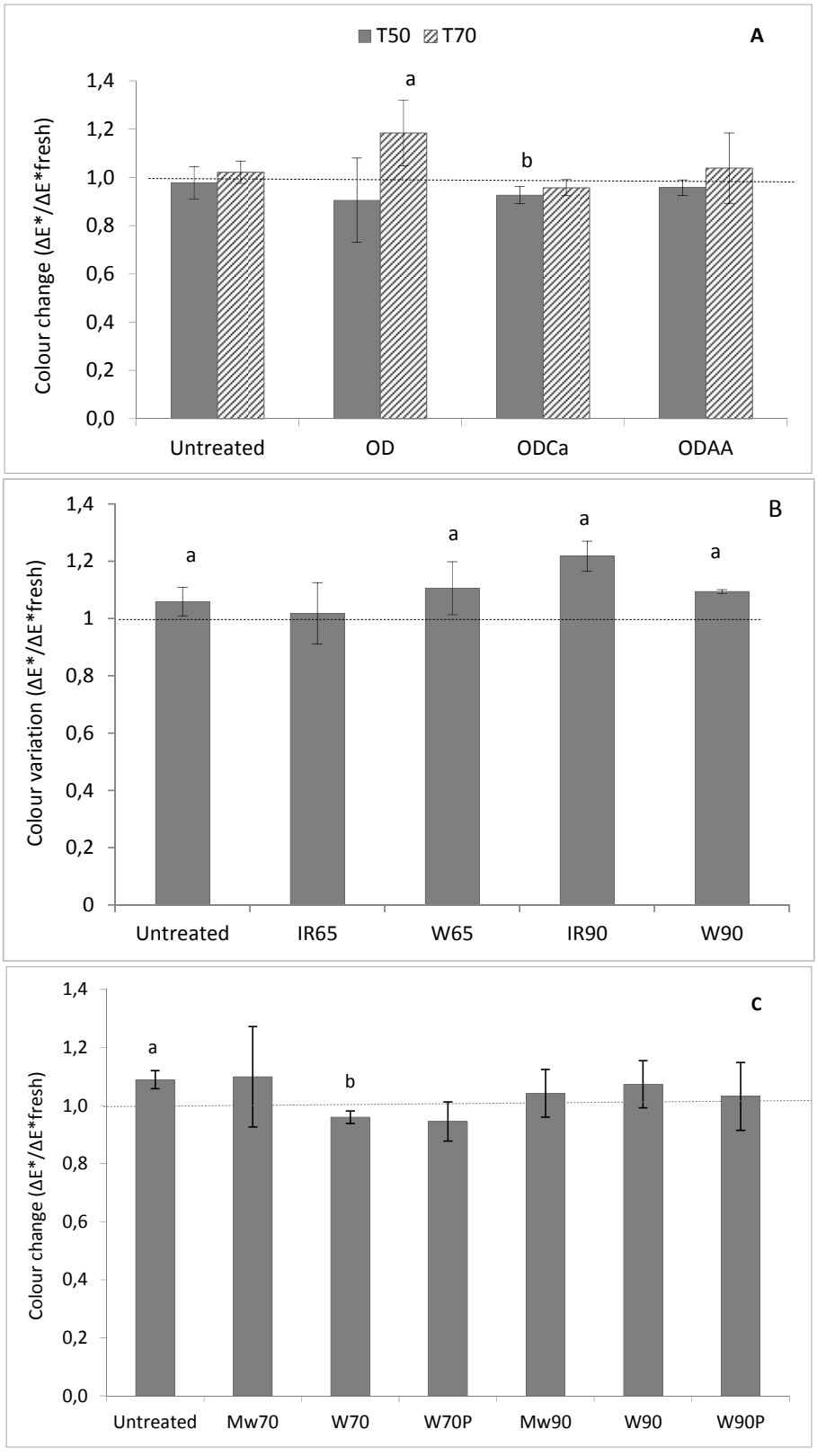


Figure 5.11 Colour variation in dried untreated mango subjected to: A - OD pre-treatment, B - IR blanching and C - MW blanching, prior to air-drying. With the letters **a** and **b** are samples with the ratio ($\Delta E^*/\Delta E^*$fresh) significantly different ($p<0.05$) from the corresponding fresh mango. Samples **a** are darker, while **b** are lighter.

Furthermore, the pre-treatments improved the appearance of the final mango dried products. In general, the appearance of pre-treated mango did not significantly differ from fresh mango (Figure 5.12A), as shown in Figure 5.12B for osmotic dehydrated mango without additives dried at 70 °C in comparison with dried untreated (Figure 5.12C) at the same temperature.

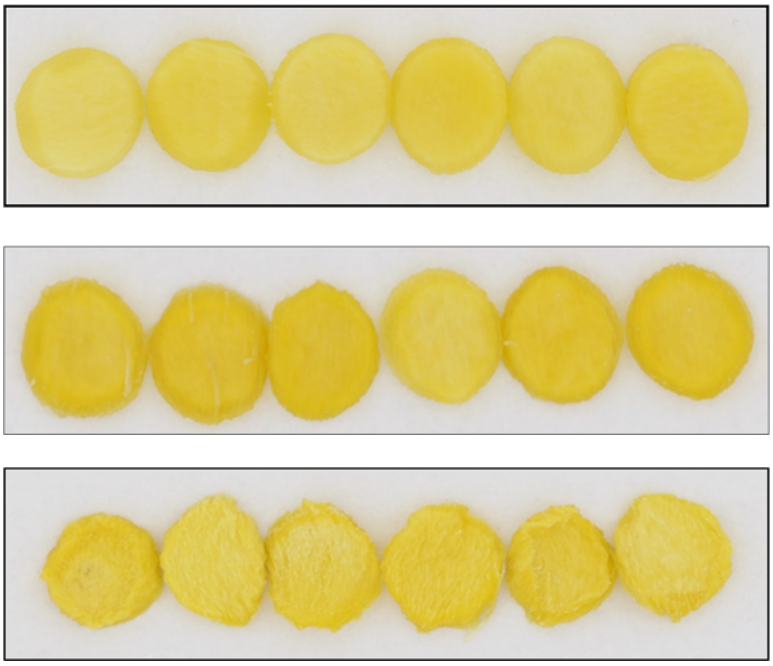


Figure 5.12 Representative mango samples, A - fresh mango cylinders, B - OD treated mango dried at 70 °C and C - untreated mango dried at 70 °C.

5.7. **The effect of osmotic dehydration prior to hot air-drying on the texture of dried mango**

The influence of the OD on the texture of the dried mango was measured as the maximum force required to perforate a mango cylinder using a puncture test, and the results of hardness of dried OD pre-treated or untreated mango are shown in Figure 5.13. OD pre-treated mango cylinders showed a significantly lower perforation force, that is, a softer texture, compared with dried untreated samples. Similar results of decreased hardness and increased elasticity in the texture of dried OD pre-treated apples were reported by Atkas *et al.* (2013). According to Nimmanpipug and Therdthai (2013), the reduction of hardness in osmotic treated samples could be associated to water migration from the cell, and the water loss causes a decrease in the tension that the liquid exerted against the cell wall. No clear effect of calcium on improving the texture of dried samples was observed, which may be attributed to the lower concentration used

in this study. A pronounced effect of calcium in mango texture was shown by Torres *et al*. (2006) with a concentration of 2%.

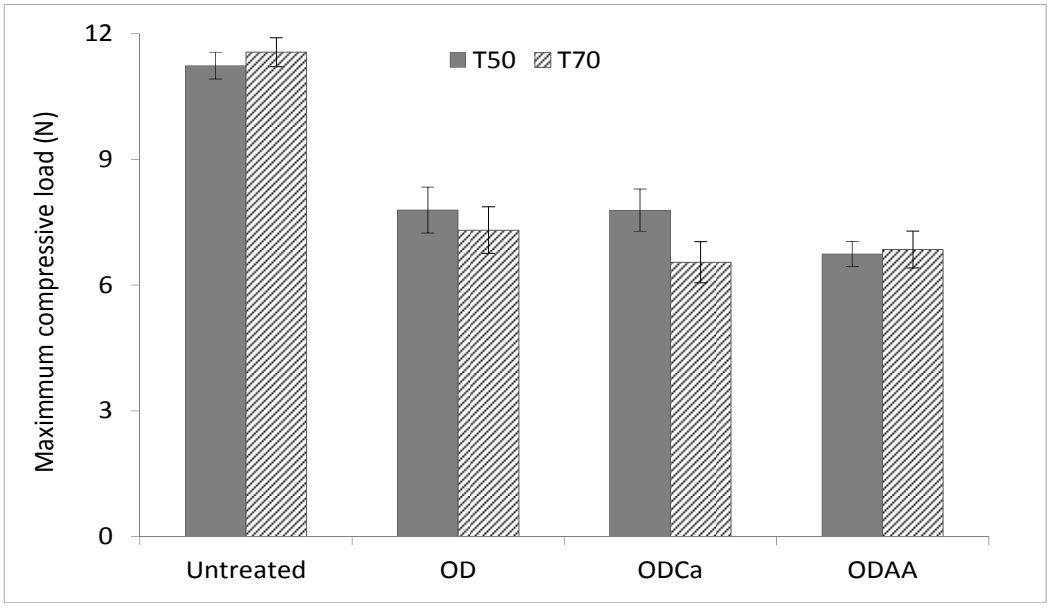


Figure 5.13 Maximum compressive load.

6. CONCLUSIONS

A diversity of processed products derived from fruits such as mango, with enhanced sensory quality and nutritional value, may be obtained with a selection of appropriate technologies and processing conditions, taking into account the matrix structure of the products. In the present thesis, studies were carried out to evaluate the effect of conventional and novel processing techniques on the retention of two major bioactive compounds in mango, vitamin C and β-carotene in minimally processed or dried products. The novel processing techniques investigated may be applied to improve the retention of nutrients in fruit products. However the nutrient nature, i.e., water or lipid soluble, also play an important role. The main conclusions from these studies are:

- The nutrient content of total vitamin C and β-carotene differed among the mango cultivars used in this thesis, i.e., cv. 'Tommy Atkins', cv. 'Keitt' and cv. 'Osteen'. Significant differences in the amount of total vitamin C was observed between cultivars Tommy Atkins and Osteen, with higher content in mango of cv. 'Tommy Atkins'. On the other hand, the β-carotene content was significantly different between all cultivars. Mango of cv. 'Keitt' and cv. 'Osteen' showed the higher and lower values, respectively.

- The ascorbic acid oxidase (AAO) was more heat stable than polyphenol oxidase (PPO), which was completely inactivated in all blanching processes applied, while AAO showed a low remaining activity for the conditions tested in this work. This enzyme has an impact on vitamin C degradation.

- Osmotic dehydration prior to hot air-drying of mango was detrimental to vitamin C due to leaching into the OD solution of water-soluble nutrients. However, fortification of osmotic solutions with calcium or vitamin C was seen to be an efficient way to improve the retention of vitamin C. A similar trend of lower retention of all-*trans*-β-carotene was found in OD treated mango and retentions comparable to untreated dried mango were only obtained in OD treated mango with additives at 70° C. OD with and without additives reduced the ratio of 13-*cis*-β-carotene to all-*trans*-β-carotene. OD softened the texture of dried mango, and no specific effect of calcium was observed at concentrations considered in this work.

- Alternative blanching techniques, infrared (IR) and microwave (MW) dry blanching and blanching in closed plastic bag prior to hot air-drying, are potential methods for inactivating relevant enzymes such as AAO and PPO and improving vitamin C (water-soluble and heat sensitive) retention in dried mango. Comparable results concerning high retentions of vitamin C like those obtained at LTLT and HTST MW dry blanching (~100%) can be achieved with water blanching in a closed plastic bag prior to hot air-drying, with subsequent high contents in dried mango in both processing conditions. The distribution of vitamin C as L-AA and DHAA in fresh and dried mango was mainly as L-AA, but with relatively higher amounts of DHAA in blanched samples, thus showing an influence of blanching on the oxidation of L-AA to DHAA. In contrast,

LTLT or HTST water blanching before hot air-drying are effective ways to improve the retention of carotenoids (water insoluble, prone to isomerization) in dried mango. However, no changes were introduced by blanching in plastic bag on the retention of all-*trans*-β-carotene, compared to conventional water blanching. The pre-treatment with MW heating showed better retention of vitamin C in dried fruits as compared to IR heating, with a similar trend for the all-*trans*-β-carotene, while increased ratios of 13-cis-β-carotene to all-*trans*-β-carotene were observed in dried mango exposed to both pre-treatments.

- The disruption of the cellular matrix during purée processing facilitates oxidative reactions of vitamin C and of all-*trans*-β-carotene unless protected by an initial blanching step, pH adjustments or the addition of a chelating agent, EDTA, to inhibit oxidative enzymes such as AAO and PPO. The total retention of vitamin C was close to 100% in mango purée pre-treated with a blanching or puréed with addition of EDTA at a pH of 5.0, and about 90% in purées with added citric acid at pH 3.9 and 5.0, respectively. However, a complete conversion of L-AA to DHAA was obtained in mango purée at pH 5.0, while vitamin C occurred mainly as L-AA (~90%) in fresh mango and in purées of blanched and acidified (pH 3.9) mango. A significantly lower value of L-AA (74%) was found in purée at pH 5.0 with EDTA.

- The retention of all-*trans*-β-carotene was significantly reduced in all mango purées as a result of isomerization or co-oxidation of carotenoids. The retention in mango purée with EDTA was significantly higher than in the purée of blanched mango, explained by the inhibitive effect of EDTA on PPO activity. The ratios of 13-*cis*-β-carotene to all-*trans*-β-carotene were not changed in purées with EDTA and at pH 3.9, while the ratios increased in purées at pH 5.0 and in purées with an initial blanching step.

- Pre-treatments, either osmotic dehydration or blanching, may result in weight losses with the exception of blanching samples in closed plastic bags. The weight change was significantly higher after infrared and microwave dry blanching compared with water blanching, which led to a comparatively reduced hot air-drying time for IR and MW treated mango. The drying time was also reduced in OD treated mango. Longer drying times are required to dry untreated and LTLT water blanched samples.

- The pre-treatments carried out prior to hot air-drying prevented, in general colour change in dried mango, which could be attributed to PPO inactivation during thermal treatments or inhibition by sugar solutions.

7. FUTURE OUTLOOK

The effect of conventional and novel processing techniques on the retention of vitamin C and β-carotene has been compared in thesis and has provided new knowledge, in minimally processed or dried mango products. Some suggestions for continued work in this issue are the following:

- The enzyme ascorbic acid oxidase (AAO) is the major degrading enzyme of L-AA during processing and storage of fruits and vegetables in the presence of oxygen. However, studies on AAO in fruits are limited. Thus a better understanding of the effect of this enzyme during the processing of different fruits is required.

- The results reported in this thesis show that the use of alternative thermal processing, such as MW, IR dry blanching and blanching in plastic bag prior to hot air-drying, improves the retention of vitamin C, a water-soluble and heat sensitive nutrient. The potential of using these pre-treatments in a diversity of fruits and vegetables, combined with other drying techniques and different processing conditions, should be explored for the evaluation of both vitamin C and carotenoids.

- On the other hand, in-depth studies of the efficiency of osmotic dehydration pre-treatment of fruits, either with or without additives, including different concentrations of additives and/or osmotic solutions prior to further drying, in the nutritional value of different food matrices is also recommended in order to find specific beneficial conditions for improvement of the retention of the nutrients.

- The sensorial and nutritional qualities of minimally processed foods, such as juices and purées, are usually negatively affected by endogenous enzymes (for instance PPO, AAO, POD), and an anti-browning agent has been used to minimize this effect. However, information is lacking concerning the effect of anti-browning on nutrient retention. Thus, it would be of great value to have more studies on this issue.

- The effect of processing fruits on the availability of nutrients such as carotenoids must also be investigated.

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