Exploring the potential of an Andean fruit:

An interdisciplinary study on the cape gooseberry (*Physalis peruviana L.*) value chain

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Mary Luz Olivares Tenorio

Thesis

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En memoria de Eliberto Olivares y Urias Tenorio

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Chapter 1.

General introduction

1.1. Background information

Fruit and vegetables are extremely important in human nutrition as sources of nutrients and non-nutritive food constituents and thereby for the reduction in disease risks. The intake of fruit and vegetables has found to be inversely associated to the risk of a number of relevant diseases such as cardiovascular disease and some types of cancer [1-7]. These health benefits are related to the presence of vitamins, minerals and dietary fibres but also to a broad range of phytochemicals and their subsequent radical scavenging activity to maintain an adequate level of non-enzymatic and enzymatic antioxidant defense [8]. These compounds are widely named as health-promoting compounds.

Cape gooseberry (*Physalis peruviana L)* is considered in Colombia as an exotic fruit with potential health-promoting compounds. This fruit has been part of Andean countries culture because usually the plants grow wild at high altitude. Since the 80´s, the production has been commercialized and at the moment the cape gooseberry is available in different countries of Europe, North America, Asia and Oceania. The cape gooseberry value chain faces certain constraints in its development. The fruit is highly perishable and there are several factors in cultivation and postharvest conditions that threaten the quality. Besides, there are governance issues such as low integration of actors and low knowledge about consumer preferences, that make the chain inefficient [9].

As a result of the diversification of the value chain, processed products have emerged such as jams, sauces, snacks, among others, giving alternatives to consumers for cape gooseberry consumption, also under the premise of the health-promoting compounds contents related to them.

1.2. Fruit and vegetables, phytochemicals and health properties

Consumption of fruit and vegetables and the relation with the reduction of certain diseases have been widely studied. A large number of epidemiological studies showed that the intake of fruit and vegetables is inversely associated to the risk of a number of cardiovascular (CVD), respiratory and digestive diseases and cancers [1-4, 10, 5, 11, 12, 6, 7, 13]. Other studies, however, did not find associations of fruit and vegetables consumption with incidences of breast cancer [14], stomach cancer [15], recurrence of colorectal adenomas [16] and lung cancer, CVD and total mortality [17, 18]. These controversies may be due to the complicating facts that fruit and vegetable consumption is not a single exposure, nor is cancer a single disease. Thus, the increase in fruit and vegetable consumption is highly encouraged [19].

Generally, those associations are related to the content of health promoting compounds such as phytochemicals and nutrients (vitamins, polyphenols, minerals, fibre) and their role to inhibit oxidative stress induced by free radicals, which are involved in the etiology of a wide range of chronic diseases [2, 12]. Nevertheless, it is not clear what the mechanisms responsible for association actually are. Dietary compounds are believed to work synergistically; therefore, a health benefit is not necessarily related to a single compound [20, 21, 10, 6].

Antiproliferative activities and cardio-protective effects are associated with the consumption of berry fruit such as strawberries, blueberries, blackberries, raspberries and cranberries [22]. Intake of flavonoids related to berries consumption was found to have an inverse relation with CVD (e.g., quercetin, kaempferol, myricetin, hesperitin, and naringenin [23]. In Finland, epidemiological associations related berries intake with a reduced risk of type 2 diabetes as found by a risk assessment, focused on flavonoids content [24]. In Norway, consumption of berries was found to be inversely related to all-cause mortality, CVD and cancer mortality in a risk assessment that evaluated data from Norwegian middle-age men [4]. Despite positive associations of berry consumption with disease prevention, these results cannot be extrapolated easily to other demographic conditions or type of berries.

A number of studies has been conducted to test health properties of *Physalis peruviana L* plant, cape gooseberry and calyx, concluding to a potential association of intakes with *in vitro* and *in vivo* anti-inflammatory activity [25, 26], inhibition of TPA-induced (12-O-tetradecanoylphorbol-13-acetate) oedema in mice [27], effectiveness in decreasing cadmium toxicity in rats [28, 8, 29], hepatoprotective, renoprotective and cholesterol suppression effects [8, 30-34], antidiabetic and antihypertension activity [35-39] and cytotoxic activity against *in vitro* non-specific cancer cells [40] and lung cancer cells [41]. These potential health benefits are associated with the presence of phytochemicals in the extracts such as withanolides, physalins, vitamin C, flavonoids and phenolics, among others. The main mechanism of these potential health benefits is claimed to be the radical scavenging activity of health promoting compounds that can maintain an adequate level of non-enzymatic and enzymatic antioxidant defense [28, 8, 36]. Nevertheless, mechanisms are not yet clear. So far, it is known that cape gooseberry contains vitamin C, β-carotene, certain phenolic compounds that consequently provide antioxidant activity. Besides, the fruit has high contents of sugars (fructose, glucose and sucrose) and relative good contents of fibers and other vitamins and minerals. These compounds can have different behaviours during thermal processing leading to release from the matrix, isomerization processes, degradation and also formation of compounds (e.g hydroxymethylfurfural HMF during Maillard reaction). More studies are required to get an insight on the presence and behaviour of health promoting compounds, mechanisms of potential health benefits and changes during processing of cape gooseberry.

1.3. *Physalis peruviana L*

Physalis peruviana L. is a plant that belongs to the *Solanaceae* family and genus *Physalis,* which has about 100 species and produces a fruit into a bladder-like calyx [42]. It is a perennial shrubby herb with heart-shaped leaves and usually reaches a height of 1-1.5 m, through a sympodial growth pattern [43, 44]. The plant can reach 2 m when pruned and with the use of rope and stick support [44, 45]. Yellow bell-shaped flowers are pollinated by insect or wind (National Research Council, 1989) but self-pollination is also common [46]. The calyx, a bladder-like organ, is small at the beginning of the fruit development, after the flower falls, it expands, ultimately forming a straw-coloured husk much larger (5 cm) and encloses the fruit [44]. The nearly round fruit are bright yellow berries with diameter of 1.25-2.5 cm and weight of 4-6 g and many flat seeds inside [44]. In Colombia, the best temperature to cultivate *Physalis peruviana L.* is between 13 and 16 °C [47] and at altitudes between 1800 and 2800 m.a.s.l. [48, 47]. Figure 1.1. shows images of the *Physalis peruviana L.* plant, flower, calyx and fruit.

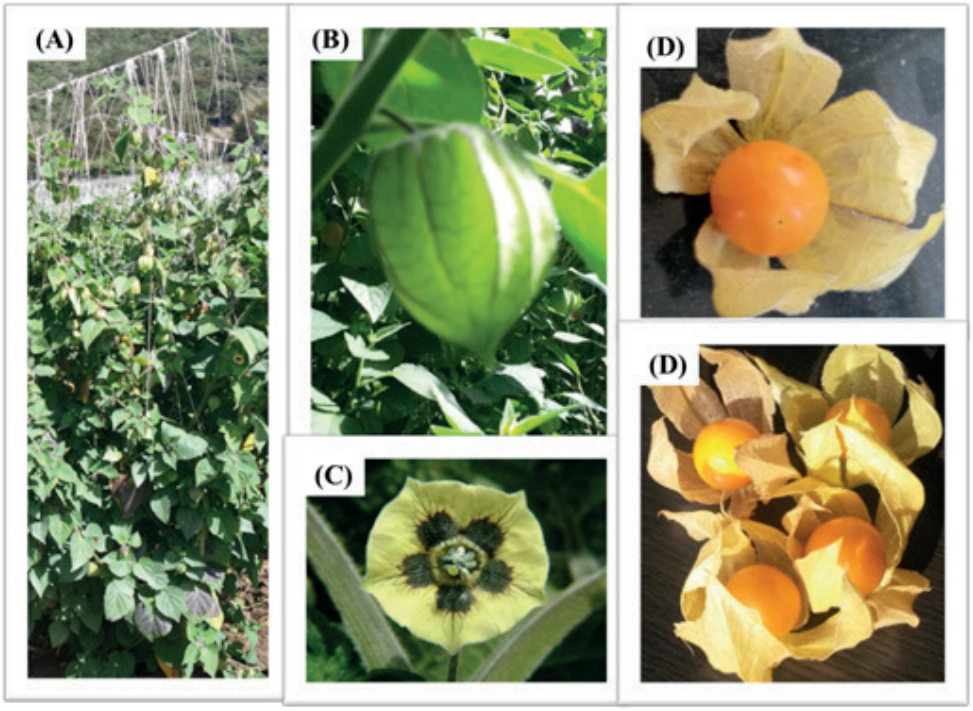

Figure 1.1. Images of *Physalis peruviana L.* plant (A), calyx (B), flower (C) and fruit (cape gooseberry) (D).

1.3.1. Ecology and distribution

Although *Physalis peruviana* L was already known by the Incas [49], the origin is not yet clear. So while Legge (1974) stated its origin in Peru, in the same areas as the tomato, Bartholomaus et al. (1990) claim that it comes from Ecuador and Peru. *Physalis Peruviana L* grows as a wild plant and semi-wild in higher areas and has expanded to almost all the highlands of the tropics and to various parts of the subtropics, including Malaysia and China, among others.

This species is found mainly in tropical America, the West Indies and Australia. Besides Colombia, there are also other producing countries, such as South Africa, New Zealand, Australia, Kenya and India. In Colombia it is very common to find wild populations in the Andean forests above 2,200 meters.

1.3.2. Common names

In literature, there is a vast number of names given to the fruit of the plant *Physalis peruviana L.* and a lot of confusion exists because of mixing up of fruit names with other species, such as *Physalis pubescens L., Physalis alkekengii L., Physalis angulata L., Physalis ixocarpa L., Physalis minima L.,* among others.

Several common English names are: 'Cape gooseberry', 'pichuberry', 'Peruvian ground-cherry', 'Peruvian-cherry', 'Inca berry', 'winter cherry', 'husk tomato', 'husk cherry', 'ground cherry', 'golden berry', 'gooseberry-tomato'. Frequently used names in Latin-America are: 'Aguaymanto', 'alquequenje', 'bolsa mullaca', 'camapu', 'capulí', 'cereza del Perú', 'motojobobo embolsado', 'mullaca', 'uchuva', 'uvilla'.

Other names: 'coqueret du Perou', 'groseiller du cap', 'mbotembote yandra', 'kospeli', 'maulanggua', 'tukiyadra', 'pohā'.

This thesis is about *Physalis peruviana L*. cultivated in Colombia where the fruit is locally called 'uchuva'. The English name used in this thesis is 'Cape gooseberry'.

1.4. Production of cape gooseberry in Colombia

Cape gooseberry grows wild in high-altitude between 1500 and 3000 m.a.s.l. [50] and in Colombia there are about 950 ha cultivated for commercial purposes [51]. The main cultivated areas are located in the departments of Antioquia, Boyacá, Cundinamarca and Nariño where the altitudes of Andean mountains favor the growth of the fruit (see figure 1.2). As illustrated in figure 1.3, the leading department is Boyacá, in the central part of the country which has the highest yield of production (18.9 ton/ha).

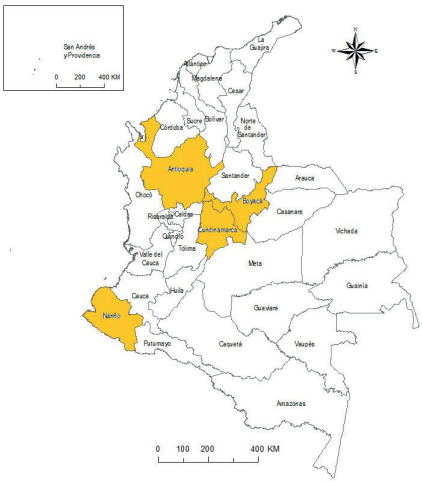

Figure 1.2. Location of production areas of cape gooseberry in Colombia in 2014. (Agronet, 2016)

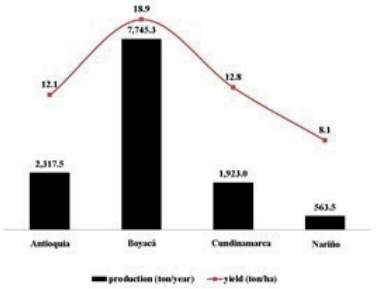

Figure 1.3. Production (tons) and yield (ton/ha) of the main production areas of cape gooseberry in 2014. (Agronet, 2016)

Production of cape gooseberry contributes to the rural economy of small and medium farmers and activities are conducted basically by family labor with a large involvement of women.

Optimal conditions for cultivation are 1800-2800 m.a.s.l., average temperature of 13 and 18 °C, a rainfall of 1000-2000 mm, relative humidity of 70-80% and fertile soil with a pH of 5.5-7.0 [52, 50].

The first harvest takes place 90-150 days after cultivation, depending on the altitude, being longer at higher altitude. Thereafter, the harvest is continuous and usually the picking of the fruit is done weekly. The plant can have a productive life up to two years [53].

Harvest activities are manual and because of the crucial effect of handling during harvest on the postharvest quality of the fruit, a careful and delicate picking up of the fruit is required.

The most serious problems facing producers are diseases due to fungal attack, bacteria, viruses and pest (such as *Epitrix cucumeris* and larvae of *Heliothis sp*), sometimes causing losses to the entire crop [54].

1.5. Colombian cape gooseberry value chain

Cape gooseberry is one of the most important fruit in Colombia in terms of international trade being the second most exported commodity after bananas [51]. Nowadays, Colombia is the biggest producer and exporter of cape gooseberry worldwide, as a result of a diversifying exports policy driven by public and private institutions since the 1980's. The competitors are in a minor proportion, other Latin-American countries [55-57]. Colombian cape gooseberry has a preferential position in international markets because of the fruit quality attributes (color and sugar content) and the constant supply [58-60, 56]. The main destination is the European Union, particularly countries such as The Netherlands, Germany and Belgium. For 2015, Colombia exported approximately US$ 25 million with a volume of 6,000 tons of fruit (Agronet, 2016). The current structure of the value chain is depicted in figure 1.4.

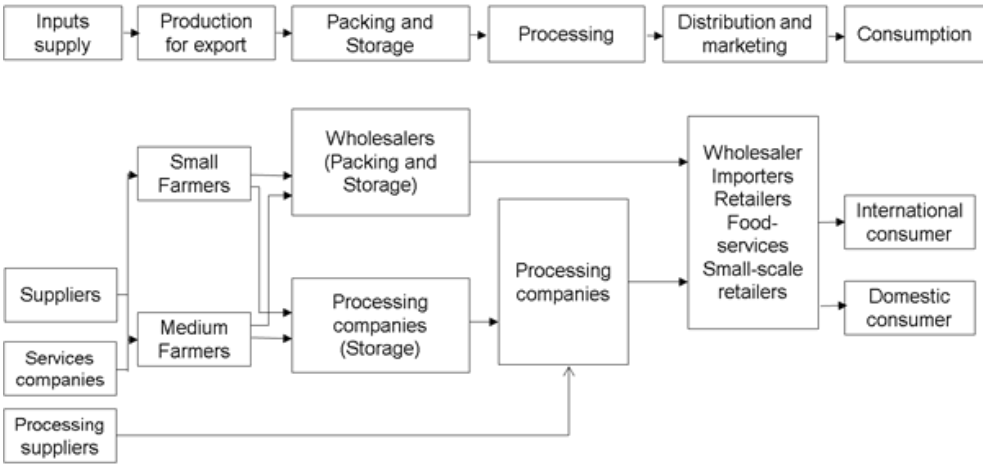

Figure 1.4. Colombian cape gooseberry value chain. Adapted from [61, 9]

The Cape gooseberry value chain comprises 6 main steps that involve inputs suppliers, small and medium farmers, local and international traders, food industry and consumers. The quality requirements imposed by the foreign market are related to appearance measured by colour and size, absence of pests and diseases and, of course, good taste (sweet and sour). These requirements are documented by a Colombian standard NTC 4580 which is based on the document CODEX STAN 226-2001[62, 63] (see appendix). The fruit fulfilling the high quality standards requirements goes to the international market, while the remaining

production goes either to processing or to the domestic market. Domestic consumption behaves depending on the international market; however the interest on the fruit from Colombian consumers has been gradually increased because of the belief of the high vitamins and minerals contents of the fruit. In figure 1.5 the consumption per capita of cape gooseberry is shown.

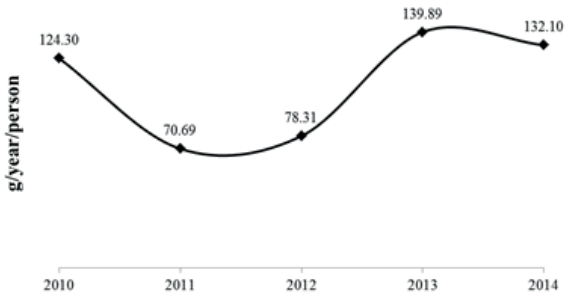

Figure 1.5. Consumption per capita of cape gooseberry (fresh and processed) in Colombia. Data: Calculations made based on data of AGRONET and DANE [51, 64]

The growth of the cape gooseberry market in the past ten years has been slow and the Colombian export of the fruit has been stable in contrast to what happened in the 90's, when a very significant increase (7-68% per year) took place [51]. Looking at the global market, the most traded fruits are apples, bananas, citrus and grapes [65, 55, 66] while cape gooseberry belongs to the category of other "fresh fruits" because of the small volume of traded fruit, meaning that cape gooseberry belongs to a niche market [65, 55].

The growth of the cape gooseberry value chain is believed to be restrained by several factors including economic crisis, country safety issues, infrastructure problems and the low level of farmers association [67, 68]. The following lists of critical factors has been identified [69, 9]:

- Unawareness of consumer preferences
- Lack of communication between actors of the chain
- Low efficiency in the cultivation and processing
- High production costs
- Lack of innovation, absence of formal organization of the value chain
- Low value added
- Low knowledge about the fruit among international consumers

The physical and chemical characteristics of the fruit make it a suitable raw material for

numerous products such as juices, jam, pulp, desserts, dehydrated fruit, oil, etc. [70-72] giving the opportunity for the cape gooseberry value chain to diversify and grow. Besides, the losses in preharvest and postharvest are about 45% of the production [73], usually not only because of diseases of pest of the plants, but also because the fruit does not fulfil the requirements of international markets. Thus, there is an opportunity to develop processed products. Currently, cape gooseberry is available in international markets in dried form; however, data about amount and characteristics of that trading is not available. The same situation is valid for other products such as jams and juices. In the domestic market in Colombia, jam, juices, preserves and comfitures are usually available, with an increasing consumption due to growing interest for the fruit and its presumed health properties by consumers (figure 1.5).

So, cape gooseberry seems to be a promising fruit to consume because of the contents of potential health-promoting compounds. This consumption would also promote the growth of the economy of small and medium farmers that currently are making a living out of the production of the fruit. Nevertheless, there are different knowledge gaps and barriers related to this fruit that restrain the growth of the production. First, the fruit has been widely claimed by marketers to have health properties, but reliable information confirming this statement is scarce and it is not clear which phytochemicals actually are present within the fruit, their contents and mechanisms of the potential health benefits. Second, nowadays cape gooseberry is offered in markets as processed food like jams, juices and snacks, and there is not enough evidence about the health promoting compounds in those processed products. Third, the cape gooseberry chain has issues of technological and organizational order that require investigation in order to get an understanding and consequently try to tackle problematics effectively. At the moment, the most important issues are the phytosanitary problems in production, the post-harvest losses that limit shelf life, the scattered information about health-promoting compounds, a weak integration of value chain actors and the low knowledge of the fruit in international markets. As the problems come from different fields of study, an interdisciplinary approach is proposed to investigate the cape gooseberry value chain and provide helpful information to reduce the gaps and barriers.

1.6. Considerations about the interdisciplinary study of the cape gooseberry value chain

In this thesis, we use terms from economic and management theories, such as the classification of the quality attributes of cape gooseberry: *search* are quality attributes that can be verified before purchase (e.g. color, size, amount); *experience* are assessed after the purchase has taken place (e.g. taste, texture); and *credence* cannot be evaluated, consequently are based on trust (e.g. health-promoting compounds contents) [74, 75]. These attributes can pertain to the product itself or to production and process methods. These methods may include aspects related to authenticity of origin, safety and environmental and socio-economic conditions of production (e.g. organic, fair trade). In many cases, the concept of the quality of a food is based on the process and production method rather than the product characteristics.

Moreover, several authors agree that a global value chain approach has a high impact in facilitating information transfer, enhancing learning, innovation and product quality by the good coordination of transactions [76-80]. Therefore the investigation on cape gooseberry value chain has been conducted with an interdisciplinary approach to achieve the objectives furthermore explained. In order to avoid misunderstanding in the interpretation of concepts and results we explain the definitions of chain we used in this document:

Supply chain: Organizations of activities required to move cape gooseberry from farm to customer (international or domestic). It also involves processing aspects.

Value chain: Organization of activities that the chain does to give valuable fruit or service for a specific market. It also involves transformation activities as well as strategic planning. [81].

1.7. Objectives and outline of the thesis

The objective of this thesis is to investigate the value chain of the cape gooseberry in an interdisciplinary perspective and contribute to the knowledge about health-promoting compounds contents and other quality attributes of cape gooseberry. Therefore this thesis aims:

1. To evaluate changes of health-promoting compounds and antioxidant activity in cape gooseberry in supply chain steps such as pre-harvest, post-harvest, processing (thermal and not thermal) and storage by literature analysis.

2. To investigate changes of quality attributes of cape gooseberry during post-harvest to make estimations about shelf-life of the fruit by evaluating the effect of temperature and the presence of the calyx and considering technological and consumer approaches.

3. To determine the effect of heat treatment on potential health affecting compounds such as ascorbic acid, β-carotene, phenolic compounds, hydroxymethylfurfural (HMF) contents and antioxidant activity of cape gooseberry.

4. To evaluate the degree of alignment among value chain actors of Colombian gooseberry quality attributes preferences and get and insight of the role of this alignment in the *scale up* process of the value chain.

In **Chapter 2,** a critical evaluation of current published data is presented on health-promoting compounds in cape gooseberry. This evaluation includes changes of various compounds in the supply chain steps (pre-harvest, postharvest, processing and storage) to give an insight of contents at consumption stage and to identify research gaps. **Chapter 3** gives a description of the postharvest changes of cape gooseberry with and without calyx, during low temperature storage, and shelf life studies were done based on quality attributes measurements and consumer study. A contribution towards preserving health-promoting compounds during cape gooseberry processing is presented in **Chapter 4**, in which the evaluation of thermal stability of such compounds and antioxidant activity is presented. **Chapter 5** studies managerial aspects of the cape gooseberry value chain by evaluating the degree of alignment among value chain actors regarding quality attributes in order to get insights about *scale up* barriers. In the final chapter (**Chapter 6**), a summary of the main findings of this thesis is given, followed by a critical discussion and implications for further research.

Chapter 2.

Health-Promoting Compounds in Cape gooseberry (*Physalis Peruviana L.*): Review from a supply chain perspective

Olivares-Tenorio, M.L., Dekker, M., Verkerk, R., van Boekel, M.A.J.S. (2016). Health-Promoting Compounds in Cape gooseberry (Physalis Peruviana L.): Review from a supply chain perspective. *Trends in Food Science and Technology.* 57(A):83-92

Abstract

Background

The fruit of *Physalis Peruviana L.*, known as cape gooseberry is a source of a variety of compounds with potential health benefits. Therefore, cape gooseberry has been subject of scientific and commercial interest.

Scope and Approach

This review paper evaluates changes of such health-promoting compounds and antioxidant activity in cape gooseberry, based on published literature and from a supply chain perspective, considering pre-harvest, post-harvest, processing (thermal and not thermal) and storage steps to give an insight of contents at consumption stage.

Key findings and Conclusions

Cape gooseberry has vitamin C (20 and 35 mg.100 g^{-1} FW), β-carotene (up to 2.0 mg.100g^{-1} FW), total phenolic compounds TPC (50-250 gallic acid equivalents.100 g^{-1} FW), phenolic acids (caffeic, gallic, chlorogenic, ferulic and p-cumaric acids), flavonoids (quercetin, rutin, myricetin, kaempferol, catechin and epicatechin) and antioxidant activity. There is not yet evidence of presence of physalins and withanolides in cape gooseberry as previous review papers have stated. The ripeness stage of cape gooseberry is a relevant factor affecting the content of many phytochemicals. Vitamin C and β-carotene contents are directly proportional to ripeness stage. The reported data in literature showed a large variation, likely caused by different raw material properties (origin, ripeness stage, growing conditions etc.) and differences in the employed analytical methods. Thermal and non-thermal processing have an effect on the extractability of the phytochemicals but also on the decrease of compounds and antioxidant activity. Relative stability to certain phytochemicals to processing suggest an opportunity to add value to supply chain with processed food containing health-promoting compounds.

Keywords: antioxidant activity, β-carotene, flavonoids, phenolic compounds, phytochemicals, supply chain, vitamin C.

2.1. Introduction

Cape gooseberry, also known as goldenberry, is the fruit of the plant *Physalis peruviana L*. that belongs to *Solanaceae* family and genus *Physalis*. This plant is native from the Andean Region and is cultivated currently in South American countries, especially Colombia, Peru and Ecuador. Cape gooseberry is a fruit with approximately 1.25 – 2.50 cm of diameter, 4 -10 g of weight, orange yellow skin and juicy pulp containing numerous small yellowish seeds [44].

Consumption of fruits and vegetables is inversely associated to the risk of cardiovascular (CVD), respiratory and digestive diseases and certain cancers [4, 5]. Although, other studies did not find these associations [17], the increase of fruit and vegetable consumption is advisable [19]. Anti-tumour, anti-diabetic, anti-inflammatory, anti-hypertension activities and cardio-protective effects have been associated with the consumption of berry fruits such as strawberries, blueberries, blackberries, raspberries and cranberries [4, 24] and have also been related to the plant *Physalis peruviana* (leaf and stems) and cape gooseberry [8, 82, 35, 32, 38]. The associations to health benefits are related to the content of phytochemicals such as vitamins, minerals, phenolic compounds, withanolides and physalins. Nevertheless, knowledge about health protective mechanisms is limited.

This review gives an overview of the state of art of changes of health-promoting compounds of cape gooseberry in a supply chain perspective, evaluating pre-harvest, post-harvest, processing and storage steps, giving an insight of compounds content at consumption stage (fresh and processed) and identifying knowledge gaps. The observed high variation in the reported data will be discussed, including the discrepancies and uncertainties in analytical methods that were used for the determination of the compounds.

2.2. Cape gooseberry supply chain

Cape gooseberry is the most exported tropical fruit in Colombia, after banana (Agronet, 2016), while the main cape gooseberry market is the European Union, especially the Netherlands, followed by Belgium and Germany [51]. Although most of the fruit is exported as fresh fruit, the physical and chemical characteristics of cape gooseberry make it a suitable raw material for numerous products such as juices, jam, pulp, desserts, dried fruit, etc. [9, 70, 83] which are currently available in the domestic and international markets. The Colombian cape gooseberry supply chain essentially involves 4 main stages: production, processing, distribution/marketing and consumption [84].

A supply chain approach in food quality is important to guarantee safety and quality from farm to table (consumption). Nowadays, consumers are increasingly interested about what they eat in terms of nutritional and health promoting properties. Actors in agri-food supply chains should not make unsubstantiated health claims that can mislead consumers, but provide accurate information about the nutritional and health-promoting compounds in products [74]. A report from the Colombian government has stated the need to have information about health-promoting compounds in cape gooseberry, not only in the fruit after harvesting, but also at consumption stage (fresh or processed) [9]. For the purpose of this review and what is reported in literature, the cape gooseberry supply chain evaluation has been focused on pre-harvest, post-harvest, processing and storage. In the cape gooseberry supply chain, like in any other agri-food supply chain, several factors affect the content of health-promoting compounds, such as the varieties of fruits, cultivation conditions, harvest time, storage, processing and consumer processing [85]. An analysis of these factors on compounds in cape gooseberry will be discussed based on published data.

2.3. Pre-harvest and post-harvest

2.3.1. Vitamin C

Vitamin C is the most abundant water-soluble antioxidant in the body. This nutrient is associated to the protection against cancer and CVC diseases, and to the beneficial effects on immune functions [86]. The theoretical mechanisms related to those benefits are essentially, the scavenging activity against free radicals and reactive oxygen species, the role in promoting collagen formation in the body, the inhibition of formation of N-nitroso compounds (carcinogenic nitrosamines), the participation as cosubstrate in catecholamine and carnitine biosynthesis and the protection of low density lipoprotein (LDL) cholesterol against oxidation [86].

Comparing various studies, a very high variation of data is observed, which is illustrated in figure 2.1 amounting up to 50-fold differences, ranging from of 18 to 929 mg.100 g^{-1} FW (fresh weight) of vitamin C.

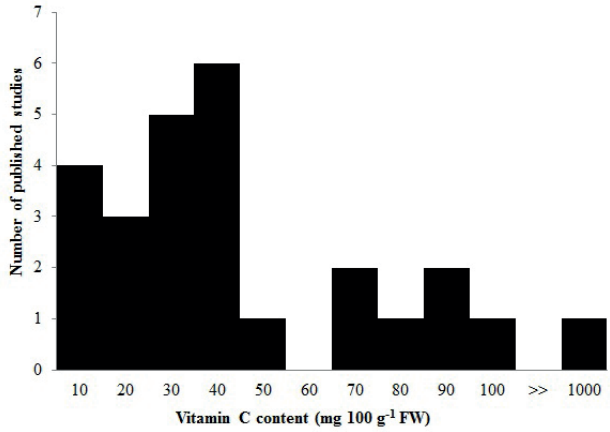

Figure 2.1. Histogram of Vitamin C values reported in literature for cape gooseberry (*Physalis peruviana L*)

The variation of vitamin C content can be caused by the used *analytical methods*. Titration method and HPLC are frequently used methods in reported studies. Titration is known to lack sensitivity and can lead to an overestimation of vitamin C content [87, 88]. Besides, differences in extraction procedures might cause variability in both, titration and HPLC methods [89]. Barcia et al. (2010) probably overestimated the content of vitamin C with an amount of 929 mg.100 g^{-1} FW [90]. Overestimation can be caused by a failure to separate interferences from the ascorbic acid peak in HPLC [91].

Another cause of variation of vitamin C content can be the different *varieties/cultivars/ ecotypes* used in cultivation. Due to the fact that information on cultivars is often missing in literature, the effect of them on vitamin C remains unclear. Differences in vitamin C content where found in cultivars from South Africa, *Giant, Inka* and *Golden Berry* [92], but for three ecotypes *Colombia*, *Kenya* and *South Africa* there were no significant differences [60]. Reported studies on vitamin C in cape gooseberry from Ecuador, Peru and Argentina are not comparable due to difference in experimental conditions or lack of reported information [93, 92, 94, 95]. *Location of cultivation* might me another cause of variation in results. Nevertheless, studies on cape gooseberry from different countries (South American countries, Egypt, Germany and Czech Republic) were not comparable because of the lack of detailed information about cultivation conditions. The altitude of cultivation did not have a significant effect on vitamin C content in cape gooseberry [60]. During *post-harvest,* presence of the calyx allowed the vitamin C stability [95].

An estimation of ripening stages was attempted to be able to compare results, using standard NTC 4580 [63] (see appendix). This standard classifies ripeness stage, according to colour, measured visually; °Brix, measured by refractometry and acidity, reported as % acid citric and measured by titration with a scale from 0 to 6, where 6 is the highest ripeness stage [63]. In figure 2.2, normalized data of vitamin C contents have been plotted as a function of ripeness stage. Only studies evaluating vitamin C in various ripeness stages were included.

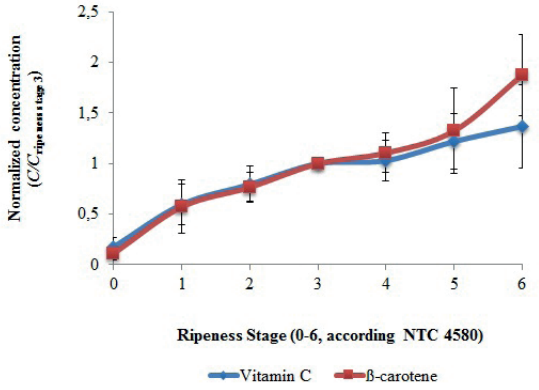

Figure 2.2. Normalized data of vitamin C and β-carotene contents in cape gooseberry (*Physalis peruviana L*) for ripeness stages (according to NTC 4580, 1999). Verticals bars represent standard deviations of estimated means (n=3-4). Data sources in references: [96-100, 95].

Some studies indicated a directly proportional association of vitamin C to ripeness stage [101, 99, 95]. Normalized data showed significant differences between means of vitamin C at the 6 stages ($P < 0.05$). Ascorbic acid can increase because of biochemical synthesis during the ripening process [99] as has been reported for tomatoes, peppers and guava [102, 103].

Cape gooseberry at the edible ripening stages (4-6) contains between 20 and 50 mg.100 g^{-1} FW of vitamin C. Therefore, cape gooseberry is a good source of vitamin C compared with other common sources, such as mango (15-36 mg.100 g^{-1} FW), comparable to orange (50 mg.100 g^{-1} FW), but less than guava (120-228 mg.100 g^{-1} FW) or marula (120 mg.100 g^{-1} FW) [102, 104-106]. Following international nutritional recommendations, the daily intake of vitamin C should be between 15 and 90 mg per day (depending on gender and age), thus, to comply this consumption with only cape gooseberry as vitamin C source, approximately 200 grams (approx. 20-50 berries) should be eaten [107-109].

2.3.2. Total Phenolic Compounds

Phenolic compounds form a group of secondary metabolites widely occurring in plants. These compounds may play a role in the inhibition carcinogenesis by affecting the molecular events in the initiation, promotion, and progression stages, especially, thanks to their antioxidant capacity. However, precision of the mechanism involved are still unclear [110]. There are four main groups of phenolic compounds: phenolic acids, flavonoids, stilbenes, and lignans [111].

Total Phenolic Compounds (TPC) contents in cape gooseberry and similar species have been reported showing a wide range of 2.5-934.9 mg.100 g^{-1} FW, usually expressed as gallic acid equivalents. This large variation is illustrated in figure 2.3.

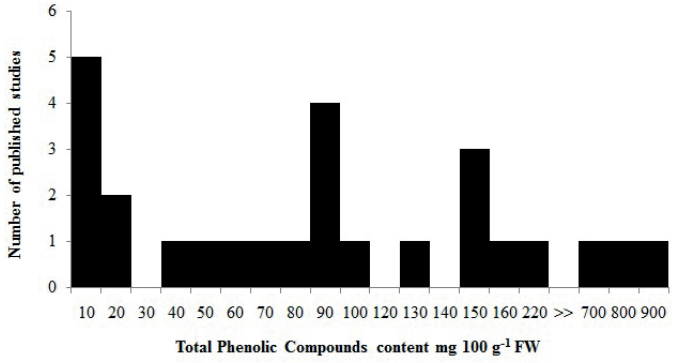

Figure 2.3. Histogram of Total Phenolic Compounds TPC values reported in literature for cape gooseberry (*Physalis peruviana L*)

The large variation of TPC contents might be caused by the different use of *varieties/cultivars/ ecotypes* [96, 92]. *Maturity stages* show higher phenolic compound contents when the fruit was still in the plant [96, 98, 112, 100, 95]. So far, there is not more published information about TPC content of cape gooseberry during pre-harvest and post-harvest. Variation of TPC content also can be due to *method of analysis*. Folin ciocalteau is the most widely used method. Nevertheless, there are variations in type of extraction solvent, extraction conditions, reaction time, standards used and wavelength. Studies reported low contents of TPC when no extraction was performed [83, 95]. TPC can also be underestimated when they are in bound forms because usually they are excluded from analysis [111]. From data reported for cape gooseberry, gallic acid is the most common standard used to quantify phenolic compounds and differences can arise when the standard used is caffeic acid (or other). Gallic acid can be less reactive to folin ciocalteau than caffeic acid, giving lower absorbance and affecting final results [113].

In figure 2.4, TPC contents have been plotted in relation to ripeness stages of the fruit according to NTC 4580 [63], using the same criteria as in section 3.1.

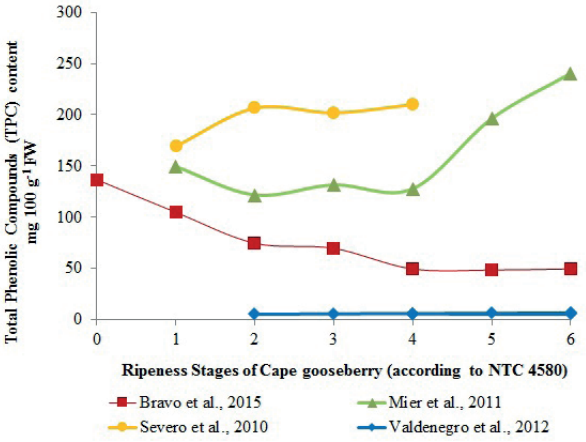

Figure 2.4. Total phenolic compounds TPC in cape gooseberry (Physalis peruviana L) for ripeness stages (according to NTC 4580, 1999). Data sources references : [96, 98, 100, 95].

In figure 2.4, there are discrepancies in the trend of TPC during ripening and changes of normalized data are not significant ($P > 0.05$). Contradictory results in figures 2.3 and 2.4, do not allow identifying the trend of TPC during maturity and ripening in cape gooseberry.

TPC contents of cape gooseberry at edible ripening stage (50-250 gallic acid equivalents.100 g^{-1} FW) are higher than what is reported for mango (56-193 gallic acid equivalents.100 g^{-1} FW) pineapple (94.3 gallic acid equivalents.100 g^{-1} FW) and banana (11.8 – 90.4 gallic acid equivalents.100 g^{-1} FW); similar to other fruits such as strawberry (160 gallic acid equivalents.100 g^{-1} FW), raspberry (114-178 gallic acid equivalents.100 g^{-1} FW), plums (174-375 gallic acid equivalents.100 g^{-1} FW) and cherry (105.4 ± 27.0 gallic acid equivalents.100 g^{-1} FW) [111]; but lower than what is reported in blackberry (417-555 gallic acid equivalents.100 g^{-1} FW) [111, 114].

2.3.3. Phenolic Acids and Flavonoids

Phenolic acids are believed to participate in the inhibition of tumour promotion and progression, therefore, they might be efficient preventing cancer in humans rather than helping in carcinogen treatment. However, mechanisms are still unclear.

Flavonoids are well-known to be able to scavenge a wide range of reactive oxygen, nitrogen, and chlorine species, such as superoxide, hydroxyl radical, peroxyl radicals, hypochlorous acid, and peroxynitrous acid. They can also chelate metal ions, often decreasing metal ion

prooxidant activity, contributing with the prevention of major age-related diseases [115].

Main phenolic acids identified in cape gooseberry are caffeic, chlorogenic, ferulic, p-coumaric and gallic acids [116-120]. However, there is not agreement in contents, probably due to differences in *method of analysis*. Main flavonoids identified and quantified in cape gooseberry are quercetin (0.1-10.9 $mg.Kg^{-1}$), rutin (1.7-6.7 $mg.Kg^{-1}$), myricetin (1.1-1.3 $mg.Kg^{-1}$), epicatechin (0.2-0.6 $mg.Kg^{-1}$), and catechin (3.8-6.7 $mg.Kg^{-1}$). Morine was not detected [121, 122, 116-118]. In hydrolysed cape gooseberry extracts, rutin (78.64 $mg.L^{-1}$), myricetin (4.67 $mg.L^{-1}$) and kaempferol (2.38 $mg.L^{-1}$) were found [122]. Rutin is unexpected because this is a glycoside of quercetin, thus it should be gone after hydrolysis. Contents of quercetin hereby reported are lower than in other berries and myricetin and kaempferol are present in cape gooseberry, while they were not detected in other berries [123]. The total flavonoid content assessed with a colorimetric assay was of 487 mg catechin equivalent.100 g^{-1} DW (dried weight) of extract [82] and 241 $mg.g^{-1}$ DW of fruit [124]. Differences in *analytical procedures* and *samples conditions* give variation in reported results. Thus, there is a remaining knowledge gap about what are the most important phenolic acids and flavonoids in cape gooseberry. Several studies reported flavonoids in *Physalis peruviana L*. leaves; however, they do not belong to the scope of this review.

2.3.4. Carotenoids

Carotenoids are convertible to vitamin A (approx. 10%) by enzymatic cleavage in the body. Thus carotenoids are believed to participate in cancer prevention because of their provitamin A activity, since this is essential for the normal maintenance epithelial cellular differentiation [125]. β-carotene is a quencher of singlet oxygen and also can inhibit lipid peroxidation. However, β-carotene is a relatively weak antioxidant [125].

The most important carotenoid in cape gooseberry is β-carotene being responsible of the yellow orange colour [60]. Large variation is observed in reported data ranging from 0.2 to 1074.7 mg β-carotene 100 g^{-1} FW, which is illustrated in figure 2.5.

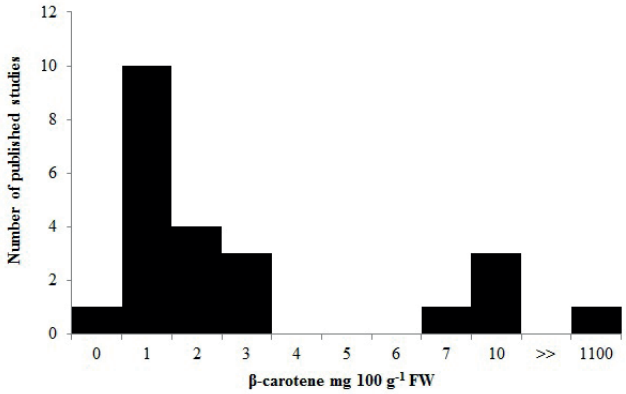

Figure 2.5. Histogram of β-carotene values reported in literature for cape gooseberry (*Physalis peruviana L*)

The variation causes are different *varieties/cultivars/ecotypes*, *cultivation conditions*, and *ripeness stage* as explained for previous compounds. β-carotene content can vary depending on chosen *methods for extraction and analysis* [126]. Unfortunately, sometimes authors use incorrect extinction coefficients in their calculation of carotenoids contents. Besides, carotenoids are sensitive to light, heat, air (oxygen), and active surfaces (with biological activity) (Scott, 2001), thus apart of taking precautions during extraction and analysis; the *standard used* needs to be checked to evaluate actual purity and avoid miscalculation of contents. Nevertheless, references evaluated in this review do not provide information about this evaluation. Figure 2.2 represents the normalize data of contents of carotenoids or β-carotene in different ripeness stages according to NTC 4580, as explained in section 3.1.

There is an increase of β-carotene content with ripeness in some studies [96, 98, 100]. Normalized data showed significant differences of β-carotene between ripeness stages ($P < 0.05$). The increase is expected in accordance with the development of the colour with ripening (from green to orange), which is found with NTC 4580 and in other studies [44, 97, 63]. Methods of analyses did not have significant differences ($P > 0.05$).

From the results evaluated in this review, the concentration of β-carotene in fresh and ripen (stage 5-6) cape gooseberry is ≤ 2.0 mg.100 g^{-1} FW, despite the substantial variation of data previously mentioned. USDA database reports as the most important sources of β-carotene, the sweet potato and carrot with 8.3-8.5 mg.100 g^{-1} FW. Within fruits group, the highest contents of β-carotene are in raw melon with 2.0 mg.100 g^{-1} FW and apricot with 1.1 mg.100 g^{-1} FW [127]. cape gooseberry is not included in the USDA database, however, Briones-Labarca et al. (2013) reported a content of 1075 mg.100 g^{-1} FW β-carotene, thus this value

could be an overestimation or misreport [128]. There is no specific recommended intake for β-carotene but there is for the REA (retinol activity equivalents) which is 400-950 μg/day [109]. The ratio of conversion of the β-carotene obtained from a food matrix to vitamin A in oil is 12:1 [129]. Therefore, a diet with 5 to 10 mg (depending on age and gender) of β-carotene would be recommended per day. That means that a portion of 50 g of cape gooseberry (10-15 berries) could provide 10-20% of the recommended daily intake of vitamin A.

2.3.5. Vitamins E and B_3 and B_6

As carotenoids, vitamin E (tocopherol) is a fat-soluble compound. The mechanisms involved in the health promotion are, as vitamin C, the inhibition of the formation of N-nitroso compounds in the stomach, the protection of selenium against reduction and polyunsaturated fatty acids in lipid membranes from oxidative damage. Vitamin E is thought to be the most important antioxidant found within lipid membranes in the body [130].

In cape gooseberry, there is not consensus about tocopherol content. Total tocopherol of 1.5 $\mu g.g^{-1}$ of fruit was reported [90] and considering that 2% of the fruit is the lipid part [131]. This amount is equivalent to 7.5 mg 100 g^{-1} lipid part. However, total tocopherols (including α+β+γ+δ) was reported to be 32.7 g Kg^{-1} lipid part, corresponding to 3,270 mg 100 g^{-1} lipid part, being extremely higher [131]. Restrepo et al., (2009) considered the content of vitamin E in cape gooseberry as negligible, while Vega-Galvez et al., (2016) reported an amount of 10.70 ± 0.28 g kg^{-1} (1,070 mg 100 g^{-1}) of α-tocopherol in the lipid portion of cape gooseberry fruit. δ-tocopherol was not detected in cape gooseberry [132]. β-tocopherol and γ-tocopherol were the major components in whole berry and seed oils, whereas δ–tocopherol and α-tocopherol were the main constituents in pulp and skin oil. The amounts of tocopherols in pulp oil found were (g kg^{-1}): α-tocopherol 28.3 ± 0.45, β-tocopherol 15.2 ± 0.85, γ -tocopherol 45.5 ± 2.35, δ -tocopherol 1.50 ± 0.05[83], corresponding to a total tocopherol of 90.5 g kg-1 (9,050 mg.100 g^{-1}). These values are so high in comparison to what is reported by USDA database, where the source with highest amount of vitamin E (α-tocopherol) is wheat germ oil with 149.4 mg 100 g^{-1}[127]. These discrepancies do not allow to draw conclusions about vitamin E in cape gooseberry.

Vitamin B6 (pyridoxine) and B3 (niacin) help the body convert carbohydrates to produce energy, help in the metabolism of fats and proteins and also help to regulate the nervous system. Contents of vitamins B_3 and B_6 reported for cape gooseberry pulp are 26.6 ± 0.9 mg.100 g^{-1} DW and 24.8 ± 0.2 mg.100 g^{-1} DW, respectively [132].

2.3.6. Minerals

Reported results on the mineral content of cape gooseberry are shown in table 2.1. cape gooseberry has high content of potassium and phosphorus compared to other fruits [53, 38, 133]. K is predominantly an intracellular cation, participating in the cellular uptake of molecules against electrochemical and concentration gradients, to the electro-physiology of nerves and muscle, and to acid-base regulation. P is used in a large variety of phosphorylated compounds which are needed for metabolic energy transfer and storage processes, enzyme activation and control [134].

Table 2.1. Content of minerals in cape gooseberry *(Physalis Peruviana L.)*

Mineral	**Content (mg 100 g^{-1} FW)**
Iron (Fe)	0.1 - 3.9
Magnesium (Mg)	34.7 - 120.1
Calcium (Ca)	7.0 - 37.7
Potassium (K)	55.3 - 501.9
Phosphorous (P)	34.0 - 54.9
Sodium (Na)	52.7
Zinc (Zn)	1.5
Copper (Cu)	0.7
Manganese (Mn)	0.7

Data sources references: [135-137, 83, 99, 138, 139]

Contents of Ca, Fe, Mn, Mg and Zn are low, in contrast with what has been claimed by some authors (Restrepo, Cortés, & Márquez, 2009). Contents of Mn, Zn, Cu, Se, Co, Ni, Cr, Na, Al, Ba, Sr, Rb were very low in the fruit [136, 138, 139], and Se was not detected [136].

2.3.7. Withanolides

Withanolides form a group of naturally steroids usually found in plants of the *Solanaceae* family and have received increasing attention from researchers because of its complex structural characteristics, potential bioactivity and large variety of health properties [140, 141, 41]. Research on the isolation and identification of these compounds is still ongoing and a number of withanolides have been obtained and characterized from *Physalis peruviana L*

plant parts [142, 143]. Nevertheless, there is no evidence of withanolides presence in cape gooseberry fruit [143], therefore, presumptive health benefits from these compounds cannot be claimed from cape gooseberry consumption as suggested in literature [144].

2.3.8. Physalins

Physalins are pseudo-steroids that have been isolated from plant of the genus *Physalis sp.* [145]. These compounds have been associated to anti-tumour, antibacterial and immunosuppressive activities [146-148, 145, 149]. The studies, however, as with withanolides, have not been conducted on the fruit but on the plant *Physalis.* So, there is no information on physalin content in cape gooseberry, nor evidence of mechanisms of beneficial activities of oral consumption of physalin compounds.

2.3.9. Antioxidant activity

Antioxidant activity is a property discussed widely in literature because oxidation has been related to several diseases like cancer, among others [150, 5]. cape gooseberry was reported to have less antioxidant activity than blueberries and cranberries [151]. Antioxidant activities determined by four methods, being DPPH assay the most used method. Reported data for antioxidant activity also have large variation in numbers, unit expressions and assays. These variation come from previous aspects discussed for antioxidant compounds. In figure 2.6, antioxidant activity has been plotted according to ripeness stage of the cape gooseberry, as explained in section 3.1.

However, this information is not enough evidence to make associations of specific health-promoting compound with antioxidant activity.

2.4. Processing and storage

Nowadays, cape gooseberry is available as dried fruit and in juices and jam. In this section, storage effect on health-promoting compounds is assessed as well as the effect of processing such as drying, pasteurization, enzymatic treatment and high pressure, according to availability of data..

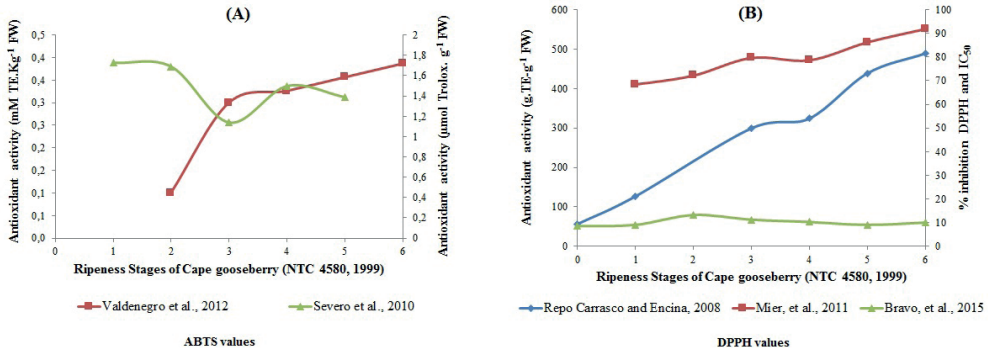

Figure 2.6. Antioxidant activity of cape gooseberry (*Physalis peruviana L*) assayed with ABTS method (2.6A) and DPPH method (2.66B) for ripeness stages (according NTC 4580, 1999) and reported data. In 2.6A, units are mM Trolox Equivalents. Kg^{-1} FW [95] and µmol Trolox Equivalent. g^{-1} FW [100]. In 6B, units are g Trolox Equivalents. g^{-1} FW [99]; % inhibition DPPH [98] and IC50 $g.L^{-1}$ [96].

2.4.1. Storage

In cape gooseberry packed in expanded polystyrene covered to vinyl film during 8 days of storage at 20 °C, carotenoids increased from 124 to 170 µg g^{-1}, and at 4 °C, the increase was from 124 to 142 µg g^{-1}. Similar effect was observed for phenolic compounds contents which at 20 °C increased from 210 to 360 mg GAE 100 g^{-1}, while at 4 °C, the increase reached an amount of 300 mg GAE 100 g^{-1}[100]. Antioxidant activity, however, remained stable at 20 °C and decreased at 4 °C from 1.55 to 1.3 µmol TE g^{-1}, assessed by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS assay [100]. These results suggested that cooling temperatures do not favour health-promoting compounds contents. Unfortunately, it is not mentioned whether the fruits were contained in the calyx or not. Vitamin C (from 28.58 – 31.65 to 13.05 – 13.24 mg.100 g^{-1}), phenolic compounds (from 6.12 to 6.02 mg GAE 100 g^{-1}) and antioxidant activity decreased (0.31 - 0.26 mM $TE.Kg^{-1}$) during 14 days of storage at 20°C without calyx [95]. These results, in addition to studies on ripening, previously discussed, suggest that calyx plays an important role in protecting the health-promoting compounds of the fruit. Preservation of antioxidant activity (0.31 - 0.32 mM $TE.Kg^{-1}$) assessed by ABTS assay was obtained with the use of 1-methylcyclopropene as inhibitor of ethylene biosynthesis on the fruit during post-harvest [95].

Storage of pasteurized cape gooseberry juice led to a reduction of carotenoids (from 70.1 to 68.66 $mg.ml^{-1}$), antioxidant activity (from 416.9 to 298 µM TE.100 g^{-1}) assessed by DPPH

assay, TPC (from 0.65 to 0.66 mg GAE.100 g^{-1}) and vitamin C (from 38.9 to 30.2 mg.100 g^{-1}) after 21 days of storage at 4 °C [70]. However, presented data are not consistent with carotenoids, TPC and antioxidant activity (DPPH) values reported in other researches as shown in sections 3.2, 3.3 and 3.8. Vitamin C content in the cape gooseberry powder obtained by spray drying was affected by storage temperature and vacuum conditions. At 30°C, vitamin C reduced (from 36.68 to 10.34 mg.100 g^{-1}) after six months under non-vacuum conditions. This reduction was lower reaching values of 25.64 and 21.78 mg.100 g^{-1} at 4 and 20 °C, respectively. The used of vacuum, under the same conditions, reduced the losses of vitamin C from 0.7 to 12 %, having more effect at 30 °C rather than at 4°C [152].

Total carotenoids (up to 6 mg.100 g^{-1}) and total phenolic compound (up to 90 mg GAE.100 g^{-1}) were stable in alginate coated fruits after 21 days of storage at 2°C. Antioxidant activity (assay with ABTS), however decreased (from 120 to 80 mg TE 100 g^{-1}) after one week. Authors did not find significant differences with control samples [153].

Effect of storage after high hydrostatic pressure processing will be discussed further on, at evaluation of non-thermal processing in cape gooseberry.

Scattered data on storage of fresh and processed cape gooseberry does not allow having a clear insight of health-promoting compounds during this stage.

2.4.2. Thermal processing

Thermal processing is the most common method to process cape gooseberry. Literature is mainly focused on drying (convection, drum, and freeze drying). Traditional pasteurization (low temperature, long time) and jam production has been studied as well [71].

TPC of dried cape gooseberry processed with microwave and convective methods showed a drastic decrease of these compounds (from 863 to 237 mg GAE.100 g^{-1} DW). There were not significant differences in the degradation of TPC, probably because polyphenol oxidases and peroxidases are not immediately inactivated during microwave drying, therefore they can speed up the degradation of TPC in the same way heat treatment does [154]. Antioxidant activity evaluated by DPPH assay also decreased (from 47.2 to 11.5 μmol TE g^{-1} DW), probably as a consequence of TPC decrease.

β-carotene content increased in convection drying when increasing temperature [155], probably for a better extractability caused by heat treatment [156]. In contrast, studies of convection drying reported degradations of 30-55% [112]. Carotenoids increased in a combined osmodrying–heat treatment processing, because of the temperature rather than because of concentration of osmotic solutions [157]. Discrepancies in results of β-carotene

do not allow understanding the effect of heat treatment on β-carotene nor the content of this compound in thermal processed food.

Retention of antioxidant activity (DPPH method) for jam and juice was higher at 90°C after approx. 30 min (about 9.7 μmol TE.g^{-1}) [70, 83, 71]. For drying processes, lowest retention of antioxidant activity (approx. 25%) was reported for microwave, convection and combined drying method (not plotted) [154]. Highest retention was reported for pasteurization, which has to do with shorter times of heat exposure [70, 83, 71]. Participation of β-carotene in the antioxidant activity is apparently low because even with the increase of β-carotene content, antioxidant activity is stable. The gathered information does not allow associating antioxidant activity and health-promoting compounds

In addition to thermal processing, the use of enzymes such as pectinase-arabanase, polygalacturonase, pectinase, hemicellulase and cellulose, has been tested to improve the yield in juice extraction. No significant decrease of TPC, ascorbic acid and antioxidant activity resulted of enzymes addition, but related to pasteurization treatment (80 °C and 10 min) [83].

In summary, there is dispersed information on the effect of thermal processing on the health-promoting compounds in cape gooseberry with high discrepancies in results, thus behaviour of compounds during thermal process are still unclear.

2.4.3. Non-thermal processing

While research on thermal processing of cape gooseberry is still in development, research about non-thermal processing is just emerging. The effect high hydrostatic pressure (HHP) for three pressures (control, 300Mpa, 400 Mpa and 500Mpa) and three processing times (1 min, 3 min and 5 min) on total phenolic compounds TPC, phenolic acids, antioxidant activity (ORAC, FRAP, DPPH), tocopherols, fibres, and vitamin B_3 and B_6 and minerals; and three pressures (control, 300, 400 and 500 Mpa) and 5 minutes of processing time on vitamin C, β-carotene and antioxidant activity (ORAC and DPPH) have been reported for cape gooseberry pulp [139, 132, 120]. In summary, after HHP treatment, the pulps had contents of about 17.5 mg of vitamin C 100 g-1 FW, 194.92 – 232.5 of β-carotene μg.g^{-1} FW, antioxidant activity values of 116.7 – 210.2 μg TE g^{-1} and TPC of 164.68 – 268.7 mg GAE 100 g^{-1}. Comparing to initial values, vitamin C and β-carotene were stable to HHP treatment which is different from what is reported for tomato (400 Mpa/15 min/25°C) where β-carotene increased remarkably (80%) given improvement of extractability and bioavailability [158]. Antioxidant activity increased and TPC decreased.

Effect on storage after HHP process was also evaluated at 4°C for 30 and 60 days. Content of

TPC in cape gooseberry pulp had contradictory results. After HHP treatment, an experiment showed an increase of TPC and a decrease after 60 day of storage at 4°C [132]. However, same authors reported another experiment where is not clear behaviour of TPC after HHP and storage [120]. Neither way no significant differences were found in TPC contents between pressures and between process times after HHP processing and storage ($P > 0.05$).

Antioxidant activity decreased during 30 and 60 days of storage at 4°C (with some samples exceptions) [139, 132, 120]. No significant differences were found in changes of antioxidant activity between pressures and times of processing, after processing and during storage ($P > 0.05$).

Vitamin C decreased approx. 81% after 30 days of storage at 4°C [139]. No significant differences were found in changes of vitamin C between pressures, after processing and during storage ($P > 0.05$).

β-carotene decreased after 30 days of storage [139]. HHP treatment has an effect on the content of α-tocopherol but not on (β+γ)-tocopherol. Higher pressure used, higher reduction of α-tocopherol during storage time [132]. There were significant differences of α-tocopherol at day 30 between pressures ($P < 0.05$), but not between processing times ($P > 0.05$).

B3 and B6 were reported to increase after HHP treatment [132]. Significant differences between pressures after HHP treatment were found only for B6, and after 30 days of storage at 4 °C for B3 and B6 ($P < 0.05$). No significant differences with processing time were found ($P > 0.05$).

There was no clear pattern for insoluble dietary fibre, soluble dietary fibre and total dietary fibre after HHP [132]. Therefore, effect of HHP on fibres is unknown.

In summary, HHP processing has shown to improve extractability of health-promoting compounds in cape gooseberry [128], especially, vitamins B3 and B6 and antioxidant activity [132, 120]. HHP might disrupt cell walls, increasing permeability and allowing solvent penetration and therefore, improve extractability and bioavailability of some compounds[159]. This effect could be positive when it relates to the bioavailability of the compounds at consumption. However, more information is required to make a proper assessment about behaviour of health-promoting compounds of cape gooseberry during HHP processing.

2.4. Conclusions

Health-promoting compounds of cape gooseberry have been reviewed from a supply-chain perspective, involving pre-harvest, post-harvest, processing and storage, in order to get an understanding of the presence of compounds at consumption stage. According to reported data, cape gooseberry is a source of vitamin C (20 and 35 mg.100 g^{-1} FW), β-carotene (up to 2.0 mg.100g^{-1} FW), total phenolic compounds TPC (50-250 gallic acid equivalents.100 g^{-1} FW), phenolic acids (caffeic, gallic, chlorogenic, ferulic and p-cumaric acids), flavonoids (quercetin, rutin, myricetin, kaempferol, catechin and epicatechin) and antioxidant activity. Presence of tocopherols, vitamins B3 and B6, minerals and fibre have been also reported. Contents of health-promoting compounds in cape gooseberry are relevant compared to other fruit sources for every type of compound. Consumption of fresh cape gooseberry provides a diversity of compounds, especially when the fruit fully ripened, as reported in this review. Contents of withanolides and physalins in cape gooseberry are not well studied yet, therefore it is not possible to make assumptions about the presence of those compounds in cape gooseberry, let alone whether there are health benefits.

Large variability of data has been found especially due to differences in methods of extraction and analysis, making a comparison of reported data an uneasy task. Moreover, varieties/cultivars/ecotypes and location of cultivation can have an effect of health-promoting compounds. In post-harvest, there are discrepancies in reported data. The effect of storage and calyx presence on health-promoting compounds and antioxidant activity remains unclear. Therefore, the contents of those compounds of fresh and processed cape gooseberry after storage are unknown.

Vitamin C is reported to be the most sensitive compound to thermal and non-thermal treatment. β-carotene did not show a clear pattern in processing, decreasing or increasing, probably because enhance of extractability or oxidations reactions. TPC and antioxidant activity were stable in thermal processing (90°C) and antioxidant activity increased in non-thermal treatment. HHP seems to improve the extractability of compounds. However, there is no systematic information yet to infer the effect of these processing on health-promoting compounds; therefore, more research into the content and bioavailability of these compounds at the consumption stage of processed cape gooseberry is needed.

2.5. Suggestion for future research

Because of the variety of health-promoting compounds in cape gooseberry and the large list of factors affecting their contents from cultivation to consumption, more research on this fruit is relevant. It is important to identify the effects of varieties/cultivars/ecotypes, cultivation and harvest conditions on phytochemicals and nutrients of cape gooseberry. The understanding of post-harvest physiology of the cape gooseberry is needed to know the role of calyx in health-promoting compounds preservation or degradation. Because processing is part of the supply chain, there is a big field of work for scientific research. Behaviour of phytochemicals and nutrients in cape gooseberry during storage, thermal and non-thermal processes requires more research. Last but not least, bioavailability of health-promoting compounds and the relation with antioxidant activity need to be researched in order to evaluate potential health benefits when cape gooseberry is consumed, so consumers can have the right information.

Acknowledgements

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Chapter 3.

Evaluating the effect of storage conditions on the shelf life of cape gooseberry

(*Physalis peruviana L.)*

Olivares-Tenorio, M.L., Dekker, M., van Boekel, M.A.J.S., Verkerk, R. (2016). Evaluating the effect of storage conditions on the shelf life of cape gooseberry (Physalis peruviana L.) *Submitted for publication.*

Abstract

Cape gooseberry is the fruit of the plant *Physalis peruviana L.* and has gained commercial and scientific interest for its potential contents of health-promoting compounds. An integral approach to estimate shelf life of cape gooseberry was conducted taking into account physicochemical, microbiological and nutritional changes and consumer acceptance. The experiments were performed for 5 independent harvest times during two years (2014-2015). The conditions of storage were temperatures of 4, 8 and 12 °C and a relative humidity of 80 %. Fruit with (Y) and without calyx (N) were packed into polyethylene terephthalate (PET) trays and polypropylene (PP) baskets, respectively. The experiment was conducted for a total of 76 d or shorter when the fruit was spoilt earlier. Fruit with the calyx showed a longer shelf life, while 8 °C was the temperature that gave longer shelf lives irrespective of the calyx presence. The critical quality attribute of shelf life without calyx was fungal growth, which determined consumer acceptance; and weight loss was the most critical quality attribute for the fruit with calyx. Studying various quality attributes in an integral way appeared to give a better understanding of the shelf life.

Keywords: ascorbic acid; β-carotene; fungal growth; modelling; survival analysis

3.1. Introduction

Shelf life is time that a food remains on an acceptable quality to the consumer. Shelf life estimation of a fresh food can be evaluated from a product and/or a consumer perspective; whereby a product view is related to changes occurring in the fruit, such as microbiological, physical, chemical, biochemical changes, and the consumer point of view is based on sensory evaluation [160]. Between the available techniques for consumer acceptance, survival analysis has emerged as a relative simple methodology to determine shelf life of foods [161, 162]. Survival analysis has been used before in fresh foods like lettuce and broccoli, using the current data methodology, where a consumer evaluates a single sample [163-165]. The combination of both perspectives (product and consumer) avoids the bias caused by the 'arbitrary' choices when deciding about shelf life based only on the product point of view; ultimately, it is the consumer who decides what is tolerated for consumption or not [166, 161]. An integral approach to study shelf life of foods based on combination of perspectives complies better with the quality definition of satisfying consumer needs [167].

Cape gooseberry is the fruit of the plant *Physalis peruviana L*. that belongs to the *Solanaceae* plant family and the genus *Physalis*. This fruit has a diameter of approximately 1.25 – 2.50 cm, 4 -10 g of weight, orange yellow skin and juicy pulp containing numerous small seeds; it is contained in a bladder-like calyx [44]. Cape gooseberry contains health-promoting compounds, especially ascorbic acid and β-carotene [168]. Few studies on postharvest behaviour of cape gooseberry have been reported [169, 170]. Nevertheless, shelf life estimations have not been conducted so far.

The present study aims to evaluate effect of storage conditions such as temperature (4, 8 and 12 °C) and presence (Y) or absence (N) of calyx on the shelf life of cape gooseberry under 80 % RH. This study brings an integrated approach that involves not only physicochemical and microbiological changes of the fruit as has been worked traditionally, but also incorporates the evaluation of health-promoting compounds (ascorbic acid and β-carotene) and consumer acceptance assessed by a survival analysis. This approach gives a more holistic view of the quality attributes changes that affect the shelf life of foods and allow making more accurate estimations based on product and consumer perspectives.

3.2. Materials and methods

3.2.1. Fruit material

Cape gooseberry (*Physalis peruviana L.* Ecotype Colombia) fruit grown in Pasca, Cundinamarca, Colombia (2,180 m.a.s.l.) were harvested from February to March 2014 and from March to April 2015 according to table 3.1. After harvesting, fruit were selected, choosing category I and extra with ripeness state No. 4 (for all experiments) according to the Colombian standard for cape gooseberry NTC 4580 [63] (see appendix). Fruit subjected to be studied with calyx were immediately placed into a dry chamber to reduce the humidity of the calix (36 h at 18 °C) and stored according to the experimental conditions described below. Fruit subjected to be studied without calyx were pealed after selection and packed in trays with approximately 300 g of fruit each and immediately stored according to the experimental conditions described below.

3.2.2. Experimental design and storage conditions

A full 2 x 3 factorial design was used. Factors: Calyx (presence: Y and absence: N) and Temperatures (4, 8, and 12 °C). Relative humidity (RH) was set at 80 %. Two different package conditions were used. Fruit without calyx (approx. 300 g) was contained in food grade perforated polyethylene terephthalate (PET) colourless squared trays with lid, 15.5 x 12.6 x 6 cm of dimensions and 8 orifices of 5 mm diameter, 4 in the lid and 4 in the bottom. Fruit with dried calyx (approx. 200 g) was put into food grade polypropylene (PP) round colourless baskets with cap and dimensions of 12 cm of diameter and 10 cm of height. The storage temperatures for harvests of 2014 were 8 and 12 °C, and for harvests of 2015 were 4, 8 and 12 °C. Samples were taken for physicochemical, phytochemical and fungal growth analyses every 12 d until the fruit was visually spoilt (35-44 d for fruit without the calyx and 76 d for fruit with calyx). For 2014, three independent experiments were conducted with independent fruit batches. For 2015, one experiment was conducted for storage at 8 and 12 °C and two independent experiments with independent fruit batches for 4 °C. A description of experiments conducted is given in table 3.1. For consumer evaluation, samples were taken weekly until reaching approximately 95 % of consumer rejection. One survival study was done for each year (table 3.1), plus preliminary pilot experiment and focus group.

Table 3.1. Set of experiments conducted for shelf life evaluation of cape gooseberry

Year	Harvest time	°C	Physicochemical	Phytochemical	Microbiological	Consumer Study
2014	March	8,12	x	x	x	One survival analysis study for 2014 (8 and 12 °C)
2014	April	8,12	x	x	---	
2014	May	8,12	x	x	---	
2015	March	4,8,12	x	x	x	One survival analysis for 2015 (4, 8 and 12 °C)
2015	April	4	x	x	x	

3.2.3. Freeze drying

Samples to be subjected to HPLC analyses were freeze dried in a FreeZone 2.5 L Benchtop Freeze Dry System Labconco. After dryness was achieved, samples were packed in aluminium foil bags, sealed under vacuum conditions and stored at -20 °C until analyses.

3.2.4. Physicochemical analyses

3.2.4.1. Titratable acidity

Five–six fruit (approximately 30 g) were blended and filtrated. The titration was done with 0.1 mol L^{-1} NaOH to an endpoint of pH 8.2 using a pH meter (HANNA instruments, inc, USA). Measurements were performed in triplicate of the same filtered sample, for each batch of experiment. The acidity was expressed as % citric acid.

3.2.4.2. pH

The pH of blended and filtered fruit was measured using a pH meter (HANNA instruments, inc, USA). Measurements were performed in triplicate of the same filtered sample.

3.2.4.3. Organic acids content

Approximately 0.25 g of freeze dried fruit was homogenized in KH_2PO_4 0.1 mol L^{-1} (pH 2.5). The solution was sonicated for 10 min. and centrifuged at 1,790 g for 10 min. The supernatant was centrifuged at 19,000 g for 10 min diluted and filtered (CA 0.2 µm) before HPLC analysis. Standard solutions of citric, oxalic, D-(+)-malic and L-(+)-tartaric acid (SIGMA-ALDRICH, USA) were prepared. Analyses were conducted in Dionex Ultimate 3000 RS diode array detector with a Prevail™ organic acids 250 x 4.6 mm 5µm Grace Alltech 88645 column. The mobile phase was KH_2PO_4 0.1 mol L^{-1} (pH 2.5) with a flow rate of 1 mL/min. Readings were made at wavelength of 210 nm. Measurements were performed in duplicate of fruit and extracts and results were expressed in g kg^{-1} of organic acid on fresh

weight basis.

3.2.4.4. Total soluble solids TSS

Total soluble solids TSS were determined with a refractometer (Brixco, 0-32) at 20 °C. Measurements were performed in triplicate on three different fruit juices obtaining by manual squeezing of a berry. Results are expressed in % of TSS.

3.2.4.5. Sugars content

Freeze-dried cape gooseberry (0.25 g) was homogenized in 25 mL 1:1 (v/v) Milli-Q water:ethanol (pure 100 %) solution. The samples were incubated at 50 °C for 60 min in a water bath and were mixed every 20 minutes with a vortex for 10 seconds. Subsequently, they were centrifuged at 3,200 g for 10 min. Part of the supernatant was diluted in the same ethanolic solution and filtered (RC 0.2 µm) before HPLC analysis. Standard solutions of D-sucrose (SIGMA), D-fructose and D-glucose (MERCK) were prepared. Analyses were conducted with HPLC with a Dionex Ultimate 3000 RS diode array detector equipped with a Polymer Laboratories PL-ELS 2100 evaporative light scatter detector (ELSD) (evaporator 120 °C, nebulizer 90 °C and carrier flow 1.0 mL/min) and an Alltech Prevail Carbohydrate ES Column 250 x 4.6 mm. The mobile phase was acetonitrile (Biosolve) and Milli-Q water 75:25 with a flow rate of 1 mL/min. Measurements were performed in duplicate of fruit and extracts and results were expressed in g kg^{-1} of sugar in fresh weight basis.

3.2.4.6. Maturity index

Maturity index was calculated based on NTC 4580 (ICONTEC, 1999), taking the ratio TSS/ titratable acidity, according NTC 4580 (ICONTEC, 1999).

3.2.4.7. Weight loss

The weight of cape gooseberry contained in each package was measured at day 0 and at the sampling day using a precision balance of accuracy of 0.01 g (DENVER, USA). Measurements were performed in duplicate and results are reported as percent of weight loss per initial fruit weight.

3.2.4.8. Colour

Colour was measured using the CIELAB-system. L*, a* and b* values of Cape gooseberries were measured with a Konica Minolta Chroma Meter Chroma Meters CR-400. Before measurement, white calibration was conducted with a white calibration plate. Measurements were performed in triplicate on three different fruit.

3.2.4.9. Firmness

Firmness was determined using a texture analyser TA-T-Pro (Brookfield Engineering Laboratories, Inc, USA). Speed of 0.5 mm/s, compression distance of 5 mm and a trigger of 4 g with a TA9 stainless steel needle probe with 1.0 mm of diameter were used. Measurements were performed in triplicate on three different fruit and recorded in g of force (maximum penetration force).

3.2.5. Fungal growth

Two methods were used to assess fungal growth. Mould count was obtained by the pour plate method using agar base oxytetracycline glucose yeast extract OGYE (DIFCO, USA) as growth medium. 1 mL of the sample dilution (10^{-2}, 10^{-3}, 10^{-4}) was poured into sterile Petri dishes and about 10 mL of agar was dispensed on it. After swirling and solidifying, the plates were inverted and incubated at 25 °C for 5 d. The colonies were counted and the number of colonies per plate was multiplied by the dilution factor to obtain the total viable mould counts per g of the original sample. A second method consisted in a visual estimation of presence of mould in the fruit within the PET tray because this was conducted only for fruit without calyx. Individual fruit showing mycelial development were counted and were expressed as % of fruit infected with respect the total number of fruit units within the tray. A preliminary identification was conducted by isolating the two most prominent moulds in the samples. A sterile needle was used to extract spores of the moulds and subsequently placed on petri dishes with the same agar used for mould count. Plates were incubated at 25 °C for 5 d. Sterile tape was used to extract a part of the mycelium of the mounds and place on sterile microscope slides. Two drops of blue lactophenol were put on the sample to be able to make the observation in a microscope. Visual preliminary identification was conducted by examination of the morphology of the mycelium and comparing to literature.

3.2.6. Nutritional contents

3.2.6.1. Ascorbic acid

Ascorbic acid content was determined according to the procedure described by Hernandez et al., (2006) with modifications, using RP-HPLC with a Dionex Ultimate 3000 RS diode array detector and a Polaris 5 C18 A column 150 x 4.6 mm, 5µm. The mobile phase was prepared by adding 0.2 % orthophosphoric acid (Merck) into Milli-Q Water (v/v). Ascorbic acid eluted after 5.5 min at a flow rate of 1.0 mL/min with a UV- detector at a wavelength of 245 nm. For the sample preparation, 3.5 mL of 3 % metaphosphoric MPA and 0.1 % tert-Butylhydroquinone THBQ solution was mixed with 0.25 g of freeze dried fruit. The

suspension was homogenized with an Ultra Turrax (IKA-Werke, T-25 Basic) at high speed for 1 min. Thereafter, the extract was centrifuged for 5 min at 1,790 g and 4 °C. The supernatant was collected and the pellet was treated twice more with the same procedure. The combined supernatants obtained from the three extraction procedures were again centrifuged for 10 min at 11,700 g and 4 °C. The extract was filtered using 0.2 µm CA filters (Minisart) and put into amber vials prior to HPLC analysis. To quantify the vitamin, a standard calibration was prepared using ascorbic acid (Sigma-Aldrich, ≥ 99 %) dissolved in 3 % MPA-0.1 % THBQ solution. Measurements were performed in duplicate of fruit and extracts and results were expressed in g kg^{-1} of ascorbic acid on fresh weight basis.

3.2.6.2. β-carotene

The β-carotene content in cape gooseberry fruit was determined following procedure described by Bushway (1986) using RP-HPLC with a Dionex Ultimate 3000 RS diode array detector. The eluent was 92.5 % pure methanol (BioSolve), 7.5 % pure tetrahydrofuran THF (BioSolve) and 0.1 % trimethylamine TEA (Sigma). 20 µL of the extract was injected; the column used was Vydac C18 218TP54 4.6 x 250 mm, 5 µm with a pre-column 30-40 µm, 3.9 mm x 40 mm. β-carotene eluted after 25 minutes with flow rate of 1.0 mL/min. Compounds were scanned and recorded at a wavelength range of 220-550 nm. 0.25 g of freeze dried fruit was added to 4 mL Milli-Q water, 10 mL pure hexane (HPLC, Biosolve) and 0.4 mL, 0.1 % butylated hydroxytoluene (BHT, Sigma). The suspension was homogenised with an Ultra turrax (IKA-Werke, T-25 Basic) for 2 min, then flushed with nitrogen and centrifuged for 10 min at 1,560 g and 4 °C. The supernatant was carefully collected and the pellet was mixed with 10 mL of pure tetrahydrofuran (THF, BioSolve), homogenised with Ultra turrax for 1 min, flushed with nitrogen and centrifuged for 10 min at 1560 g and 4 °C. The combined supernatants were evaporated and dried at 40 °C and 270 mbar. The extracted β-carotene was dissolved with 5 mL THF and 5 mL eluent solution. The extract was filtered with 0.2 µm RC filter (Minisart RC 15) and put into amber vials (Grace) before HPLC analysis. A calibration curve of β-carotene (Sigma) ranging from 0.4 to 200 µg/mL was used as standard. The purity of the β-carotene standard was checked according to the method reported in literature (Scott, 2001). Measurements were performed in duplicate of fruit and extracts and results were expressed in g kg^{-1} of β-carotene ascorbic acid on fresh weight basis.

3.2.7. Consumer acceptance evaluation: Survival Analysis

Prior to survival analysis data gathering, a focus group and pilot experiment were performed in order to identify the critical quality attributes of the cape gooseberry [171] and also to validate data gathering questionnaires. The focus groups consisted of 8 consumers from Bogota, Colombia, whom informed us about the most important quality attributes. They

manifested dislike for fruit with calyx because they were not able to visualize fruit quality when buying. In the pilot experiment, 38 consumers of cape gooseberry from 20 to 60 years old (mean age 38 years ± 12), employees and students of Fundación Universitaria de Colombia were recruited. Trays of cape gooseberry without calyx were stored at 8 °C and 12 °C and every week one different tray was taken to evaluation. The same consumers were asked to evaluate colour, flavour, taste, texture and general appearance according to their preference, during 8 weeks (56 d). Results of the pilot experiment pointed general appearance as the most important quality attribute for consumers when buying and/or eating cape gooseberry. Because general appearance can be evaluated visually without sample destruction and during preliminary pilot experiment it was difficult to reach the same people every week for the study, the chosen methodology was current-status survival analysis based on the evaluation of a single sample [163, 165].

The consumer study based on survival analysis was conducted for samples of cape gooseberry without calyx placed in PET trays. Every week the same trays were evaluated only visually by different consumers. The consumers recruited were located in different places of the northern part of Bogotá, Colombia. For 2014, fruit stored at 8 and 12 °C were evaluated by 798 consumers (from 20 to 60 years old with gender equality 50/50) over 8 weeks (56 d). The whole consumer study was repeated in 2015. In this year, PET Trays were stored at 4, 8 and 12 °C and samples from two independent fruit batches were evaluated at 4 °C (See table 3.1). The same locations were chosen and 914 consumers participated in the study (from 20 to 60 years old with gender equality 52/48 male/female) over 9 weeks (63 d). In the two studies, consumers were asked the question 'are you willing to consume this fruit?' for every sample. Each consumer had to evaluate two (in 2014) and four samples (in 2015), only one time, and simply respond 'yes' or 'no' for every sample, according to their willingness to consume, based on general appearance only [165]. The number of weekly respondents was changed deliberately in order to increase the number of responses when the rejection rate was getting higher, close to final shelf life [162]. The studies were conducted until more than 90 % of the consumers rejected the fruit.

3.2.8. Statistical analysis

Analyses of variance ANOVA between years, between harvests, between temperatures and between presence of calyx (Y, N) during the studied period were evaluated with the statistical package SPSS statistics 22 [172] and using a confidence coefficient of 95 % (α=0.05). Data related to organic acids content, TSS, sugars, ascorbic acid and β-carotene contents were corrected based on weight loss to avoid bias in results interpretation. Parameter estimation and simulations of rejection rate of samples by consumers were conducted to find a mathematical expression with the best data fitting in a survival analysis model (Weibull,

exponential, logarithmic transformation) [173, 161] with the software package Athena Visual Workbench®, Version 14.2, (http://athenavisual.com) using a confidence level of 95 %.

3.3. Results and discussion

3.3.1. Physicochemical changes

Physicochemical results of the five experiments conducted in 2014 and 2015 (table 3.1) did not vary significantly ($P > 0.05$), therefore data were combined. Titratable acidity, organic acids content, pH, TSS, sugars content, and colour did not change significantly when the fruit contained the calyx or not (Y, N) ($P > 0.05$).

3.3.1.1. Titratable acidity, organic acids and pH

A reduction in titratable acidity was found over storage time for all three temperatures ($P < 0.05$) from 2.1 to 0.8 % citric acid equivalent. Storage temperature had an effect of this reduction ($P < 0.05$). At higher temperature, higher reduction of titratable acidity was seen. The main organic acid identified in the fruit was citric acid with 16.8 ± 1.6 g kg^{-1} at day 0 which also decreased over storage time 9.7 ± 2.0 g kg^{-1} ($P < 0.05$), affected equally by storage temperature. Malic acid and tartaric acid were also found but in lower concentrations,1.8 ± 0.3 g kg^{-1} and 1.7 ± 0.3 g. kg^{-1}, respectively, at day 0. Malic acid and tartaric acid acids remained stable during storage time at the three studied temperatures ($P > 0.05$). Oxalic acid was not detected within our samples.

In line with the decreased acidity, the pH of cape gooseberries showed a gradual increase in storage time from 3.8 (day 0) up to 4.7 after 76 d at 12° C. The decrease of H^+ ions is a consequence of the reduction of organic acids: citric acid represented 84 % of the total of organic acids analysed (including ascorbic acid), therefore it has the highest influence on the pH value. Our results agree with previously reported data for changes in organic acids during storage of cape gooseberry, mango, husk tomato, berries and grapes [174-176]. The diminishing acidity during ripening can be explained by increased respiration where transformation of acids to other compounds could take place and a reduced ability of the fruit to synthesize acids with ripening [177, 178].

3.3.1.2. TSS and sugars content

TSS decreased for all three storage temperatures (from 13.4 % ± 0.3 to 9.6 % ± 0.9) and after day 60 showed a slight increase (until 11.3 % ± 1.0). The sugars identified in cape gooseberry fruit were sucrose, glucose and fructose, in accordance with a previous report [52, 179]. Results of sucrose, glucose and fructose contents in g kg^{-1} for the three temperatures during

storage are shown in table 3.2. For all data combined (2014 and 2015; with calyx Y and without calyx N), glucose and fructose increased slightly during the first 20 d of storage and then remained stable, while sucrose decreased ($P < 0.05$).

Table 3.2. Sugars content (g kg^{-1}) of cape gooseberry during storage time at three temperatures and 80 % RH. SD standard deviation (n=4-16)

Storage temperature	Storage time (days)	Sucrose	SD	Glucose	SD	Fructose	SD
4 °C	0	26.6	4.1	16.3	2.7	8.9	1.0
	24	17.4	8.3	20.8	5.5	15.7	5.1
	35	16.2	4.5	21.4	8.5	14.0	4.4
	44	14.3	5.9	19.7	2.9	14.6	4.1
	55	14.2	4.2	21.0	3.9	14.9	3.5
	76	18.6	6.7	19.7	2.2	15.9	3.6
8 °C	0	52.5	2.2	25.9	0.7	23.7	1.2
	24	36.4	4.7	31.0	1.1	28.9	0.9
	35	31.9	2.2	28.4	3.1	28.1	2.5
	44	23.0	3.5	32.9	0.9	31.3	1.5
	55	30.5	2.9	25.7	1.7	26.9	1.2
	76	24.7	1.9	24.6	2.2	28.0	2.7
12 °C	0	47.7	7.3	24.7	4.5	23.0	3.6
	12	33.6	7.6	26.7	1.3	26.1	1.9
	24	24.0	0.6	26.7	1.6	26.6	1.6
	35	24.2	4.7	28.5	2.4	29.8	1.8
	44	24.2	1.3	32.5	0.8	35.8	1.9
	55	23.4	1.6	22.0	3.2	24.3	3.6
	76	21.5	2.1	18.6	2.3	21.7	1.3

Since sucrose was the major sugar (about 50 %), its decrease over time explains the initial decrease of TSS. Production of fructose and glucose was not so significant to make an effect on TSS. Slight increase of TSS at the end of storage could be the result of interference of other compounds because TSS is affected in different manners by organic acids and salts presence [180], however, during ripening, fruit are also subjected to changes in pectin solubility (explained in section 3.1.6.) [181], having a potential effect on TSS increase. In section 3.1.6. and 3.3.1., the transformation of pectin from insoluble to soluble is discussed as well as the increase of vitamin C during post-harvest. Because sugars are used as source of energy, reduction of TSS and sucrose could be related to the maintenance of cellular metabolism including the synthesis of vitamin C (explained in section 3.3.1.) [182, 183]. Our results

differ from previous research where TSS increased slightly during storage of cape gooseberry [93]. Sugar content changes in this study were in line with previous studies on other berries, husk tomato and grapes [174-176].

3.3.1.3. Maturity index

The calculated maturity index (Ratio TSS/titratable acidity) (figure 3.1) for experiments combined at three temperatures (all times), showed that this ratio was approx. 7.1 for experiments conducted at 8 and 12 °C, corresponding to a maturity index 4 (According to NTC 4580) [63]. Fruit of experiments of 4 °C showed a ratio value of 6.5 which is between maturity index 3 and 4. Figure 3.1 depicts how fruit continued to ripen during post-harvest storage. Storage at 12 °C showed the most rapid ripening of the fruit, followed by 8 and 4 °C. This trend follows the climacteric behaviour as reported for cape gooseberry by other authors [184, 170, 93, 185]. At lower temperature, the ripening process was obviously slower.

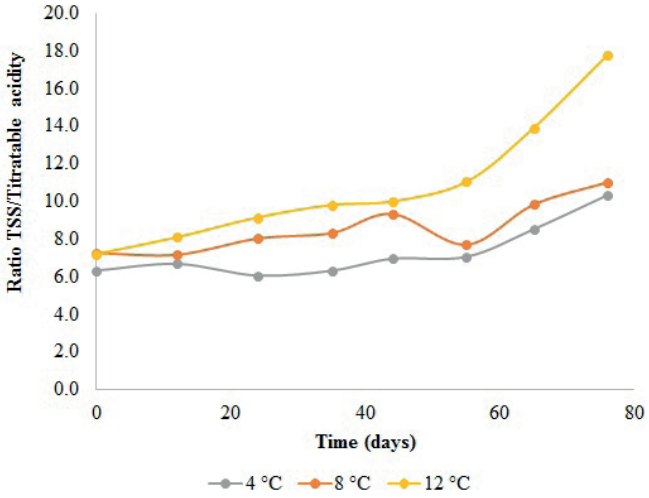

Figure 3.1. Effect of temperature on maturity index (Ratio TSS/Titratable acidity) of cape gooseberry fruit stored at 80 % RH.

3.3.1.4. Weight loss

Weight loss was affected by temperature during storage time ($P < 0.05$) as shown in figure 3.2. After 76 d of storage at 12 °C, fruit lost about 17 % of weight. Results in figure 3.2 are presented combined for fruit with and without calyx because no significant differences were found ($P > 0.05$).

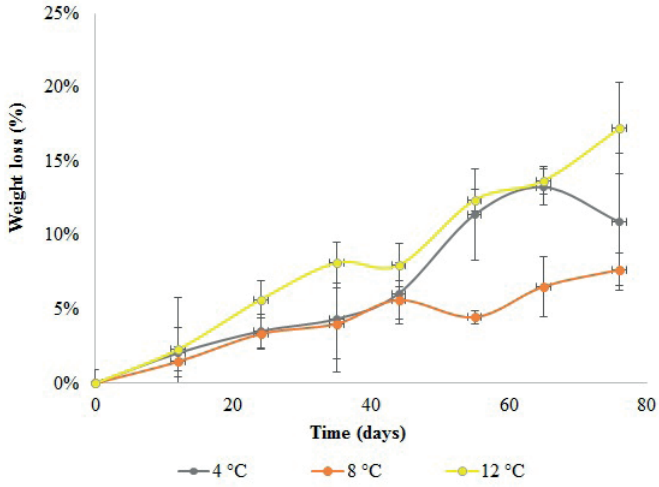

Figure 3.2. Effect of temperature and time on weight loss of cape gooseberry fruit at 80 % RH ($P < 0.05$). Vertical bars represent standard deviation Weight loss vs time (n=4-16); Weight loss vs Temperature (n=36-52).

Our results were higher compared to data of weight loss reported previously for *Physalis ixocarpa* without calyx, where fruit were stored at 25 °C and weight loss was monitored for 5 weeks, losing 5 to 12 % of weight [174]. Calyx removal was found to promote the loss of weight in *Physalis peruviana L.* fruit at first d of storage at 16 °C and after 15 d of storage. Thereafter, the weight loss was the same for the fruit with and without the calyx, reaching 15 % of weight loss after 22 d at 80 % of RH. [186]. Tomatoes (*Solanum lycopersicum*, also from *Solanaceae* family) lost 6-7 % of weight when stored at 21 °C for 16 d [187]. The rate of weight loss at 12 °C was 0.49 % per day which is higher than for cape gooseberry (0.22 % per day) and for 5 °C was 0.15 % per day in tomatoes during 7 d of storage [188] comparable to cape gooseberry with a rate loss of 0.14 % per day at 4 °C during first 12 d of storage. Therefore, at higher storage temperature, higher weight loss. For the fruit with calyx (Y), weight loss was one the most important quality attributes that denoted loss of quality, at least, the attribute that changed more rapid. Considering 6 % as a commercial standard limit for weight loss, fruit stored at 4 °C reached that level at approx. day 38, while at 8 and 12 °C, times were approx. 62 and 24 d respectively. Weight loss in cape gooseberry corresponds to water loss caused by the difference of relative humidity between the environment and the fruit [189, 69, 170].

3.3.1.5. Colour

The colour expressed as CIELAB values did not change significantly with temperature nor

with presence or absence of calyx (Y, N) ($P > 0.05$). Differences in colour were noticed over storage time ($P < 0.05$). L* decreased (from 57.1 to 50.7), a* remained stable (≈ 10.0) and b* decreased (from 47.3 to 35.4). This behaviour indicates a change from yellow to orange colour which is related to β-carotene synthesis (see 3.3.2). Colour values were similar to earlier reported data of colour in cape gooseberry [155].

3.3.1.6. Firmness

Firmness was affected by temperature ($P < 0.05$) and the presence or absence of calyx (Y, N) ($P < 0.05$) as shown in figure 3.3. Firmness decreased over time, faster at higher storage temperature. As expected, the fruit without the calyx (N) was less firm because of the lack of protection from environmental conditions calyx gives to the berry.

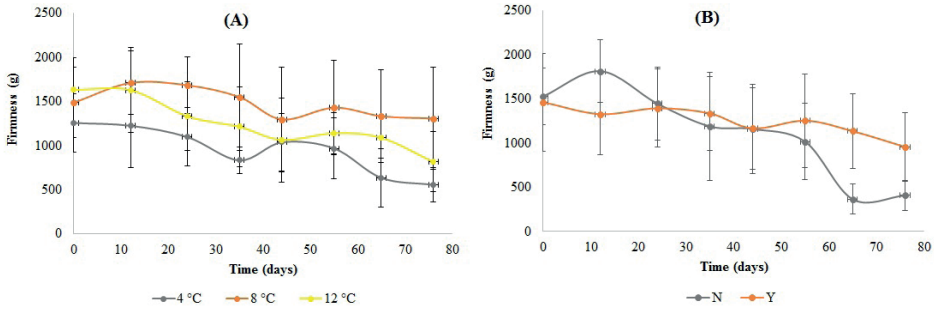

Figure 3.3. Effect of time and temperature (A) and presence 'Y' or absence 'N' of calyx (B) on Firmness (g) of cape gooseberry fruit at 80 % RH (data of three temperatures combined). Vertical bars represent standard deviation SD (n=6-15).

Decrease in firmness in cape gooseberry was previously reported to be inversely related with the ripening stage [190], similar to our results where firmness decrease is related to the maturity index with Pearson's coefficients of -0.795, -0.735 and -0.859 for storage temperatures of 4, 8 and 12 °C, respectively. The mechanism behind firmness decrease of cape gooseberry fruit is a coordinated series of enzymatic activities, where after the start of ethylene synthesis, respiratory rate increases synchronized with increased level of polygalacturonase activity resulting in pectin transformation from insoluble to soluble, leading to gradual softening of cape gooseberry fruit [191, 181]. Variation in absolute values of firmness could be explained by natural variation of the fruit specially coming from different environmental temperatures and dry or rain seasons.

3.3.2. Fungal growth

Fungal growth results are presented in figure 3.4. Fungus development varied drastically with the presence (Y) or absence (N) of the calyx and the harvest times (year) ($P < 0.05$). Figure 3.4 depicts the growth of fungi evaluated with mould count (CFU g^{-1}) and level of mycelium growth (%), visually assessed, in experiments with fruit without calyx (N). As statistical differences were detected at 8 and 12 °C between harvest times ($P < 0.05$), all harvest times were plotted. Fungal growth of samples with calyx was negligible during the time studied. Mould count is not the most suitable method to analyse fungal growth as it is for bacterial growth, given the high complexity of fungi quantification [192]. Therefore, percentage of growth mycelium has been used to evaluate the quality of a fruit [193] and for our results, it gave more conclusive data.

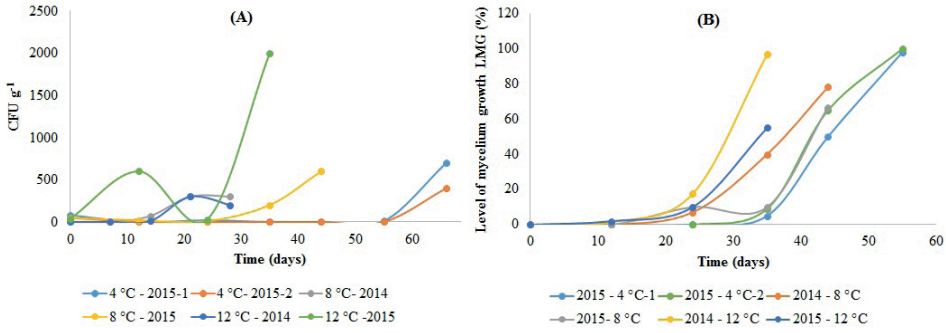

Figure 3.4. Effect of temperature and time on fungal growth in cape gooseberry without calyx 'N' at 80 % RH. CFU *colony forming-unit* g^{-1} (A) and Level of mycelium growth (LMG) (%) (B).

Fungal growth appeared to be the most critical attribute for the shelf-life of cape gooseberry without calyx (N). Higher temperature resulted in higher counts at shorter time in samples stored at 12 °C, followed by stored samples at 8 °C. Mould count in samples stored at 4 °C remained stable. Mycelium growth showed a different pattern for 4 and 8 °C. At 4 °C, in contrast with what is reported in mould count, the mycelium of fungus was visible earlier. At 8 °C, there was a large difference between years. The harvest of 2014 presented visible mycelium and growth sooner than harvest 2015 (Figure 3.4B). In comparison with storage at 4 °C, 8 °C of harvest 2015 did not show difference in the level of mycelium growth over storage time. The fruit at 4 °C, for the two experiments conducted, presented a very wet surface, which could be a result of chilling injury of the fruit that promoted humidity, thus, the growth of fungi. Chilling sensitivity was reported previously in husk tomatoes (*Physalis ixocarpa*) stored at 2.5 and 5 °C [194]. Since the fruit of our experiments were planted in

tropical conditions (average temperature 20 °C), low temperatures during post-harvest might predispose it to pathogenic attacks, like in other tropical fruit cases (Paull, 1990, 1999). The cape gooseberry usually has a waxy surface due to a terpene resin that is developed in the calyx and could protect fruit against pests during development of the fruit [195, 196]. Previous studies reported that acylsucroses are the main component of the resins covering fruit of *Physalis sp.* [197]. We noticed visually when preparing samples for analyses, that after calyx removal, these covering substance on the fruit increased significantly the moisture, making a good substrate for microbial growth in post-harvest. However, the measurement of this substance was not subject of study in our experiments.

Two types of moulds were observed, a very white cotton-like mould with a high-spread capacity; and a black one that usually occurred as big spots on the fruit. Sometimes, the fungus mycelium presented a grey appearance.

Conducted preliminary identification of the most common two moulds in samples suggested the presence of *Sclerotinia sclerotiorum* and *Phoma sp*. This agrees with studies conducted with cape gooseberry [54, 198]. Nevertheless, other fungi have been found in cape gooseberry such as *Alternaria sp., Colletotrichum gloeosporioides* and *Botrytis cinerea* [54]. Besides, common fungi in cape gooseberry are related to medium acidification [199] and in our experiments, the titratable acidity decreased. Moreover, *Sclerotinia sclerotiorum* growth is related to production of oxalic acid [200] and we did not detect oxalic acid in our samples. Thus, there are no conclusive results about the species of fungi found in samples and a detailed taxonomic study is required to determine them. The presence of fungi is related to cultivation conditions rather than post-harvest contamination because they are associated to diseases, pest and weeds of the plant *Physalis peruviana L* [54]. Nevertheless, enzymatic changes of pectin in plant cell walls, makes fruit more vulnerable to fungal infection [199]. In section 3.1.6., we related firmness loss to a possible enzymatic action during ripening. Cape gooseberry pH (up 4.7) also gives favourable conditions for fungal growth (shown in 3.1.1.). When comparing microbiological results with physicochemical changes, fungal growth is correlated to the ripening process. Since the fruit with calyx (Y) gave negligible results in fungal growth, the exposure of the fruit to environmental conditions and extra moisture produced by exudation after calyx removal are the most important aspects to consider. In post-harvest storage, the most usual control measure to retard respiration, thus ripening, is the use of ethylene antagonists but such control does not inhibit directly fungal growth [201]. Previous studies using cleaning and disinfection in cape gooseberry without calyx increased more respiration, promoting even more fungal growth [169]. Therefore, further investigation regarding inhibition of fungal growth in cape gooseberry without calyx is recommended.

3.3.3. Ascorbic acid and β-carotene

3.3.3.1. Ascorbic acid

Because the presence (Y) or absence (N) of calyx and harvest times and years (2014 and 2015) on ascorbic acid content changes were not significant ($P > 0.05$), the results are shown combined. Figure 3.5 shows the trend of ascorbic acid content during storage time at the three temperatures studied.

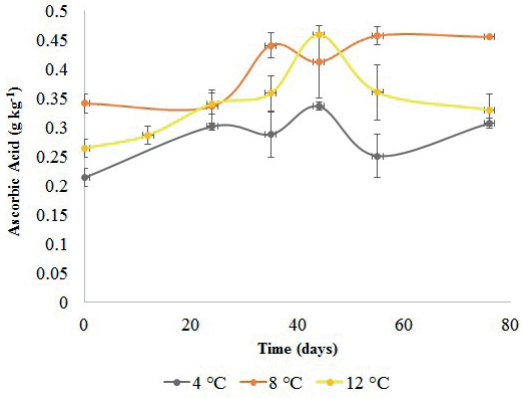

Figure 3.5. Effect of time and temperature on ascorbic acid content (g kg^{-1}) of cape gooseberry fruit at 80 % RH. Vertical bars represent standard deviation SD (n=8-24).

Ascorbic acid increased at the three temperatures until a certain period of post-harvest time, thereafter degradation took place at 12 °C. At 4 and 8 °C, after reaching a peak, the ascorbic acid remained stable during the studied time. Posterior decrease of ascorbic acid at 12 °C probably can be due to oxidation processes. Oxidation can occur in the presence of catalysts, oxidase enzymes promoting ascorbic acid losses [183]. Similar results were reported for *Physalis ixocarpa* (husk tomato) with respect to the role of the calyx and post-harvest storage temperature (4 °C and 20 °C) with the increase of ascorbic acid [189]. Our study presented a similar trend of ascorbic acid variation like what has been reported for tomato (*Solanum lycopersicum*, also from *Solanaceae* family), where ascorbic acid increased during post-harvest time at 20 °C and then decreased [202]. This is an uncommon pattern since most of the fruit show a decrease of ascorbic acid in post-harvest. Losses (20.4 %) in ascorbic acid were reported in guava at 11 °C, after 12 d [203]. In strawberries, the effect of temperature and storage time was significant on the total ascorbic acid concentrations of the fruit; for the first 2 d of storage, ascorbic acid remained stable irrespective of storage temperature (0.5 °C, 10 °C and 20 °C). Until day 4, in strawberries stored at 0.5 °C and 20 °C ascorbic acid declined (approx. 13.5 % and 14.8 %, respectively) while at 10 °C it was stable [204]. On the

contrary, in cape gooseberry, ascorbic acid decreased rapidly during storage at 20 °C after the calyx was removed [95].The difference between our results and those from Valdenegro et al., (2012) can be explained by the difference in temperature, because their experiments were conducted at 20 °C, probably promoting degradation of ascorbic acid. Experiments evaluating ascorbic acid at different stages of ripening of cape gooseberry showed an increase in the ripened fruit [101, 99, 95]. Because cape gooseberry is a climacteric fruit, the ripening continues during post-harvest, which can explain the increase in ascorbic acid at first 35-50 d at the three evaluated temperatures.

3.3.3.2. β-carotene

Our analyses on carotenoids showed that β-carotene was the prominent carotenoid in cape gooseberry since we did not identify any other carotenoid. Although another study reported α-carotene and β-cryptoxhantin in Colombian variety of cape gooseberry, the reported amounts were not high and β-carotene accounted for 95 % of the carotenoids [60]. β-carotene did not change significantly between harvests times ($P > 0.05$), therefore data are presented combined (figure 3.6).

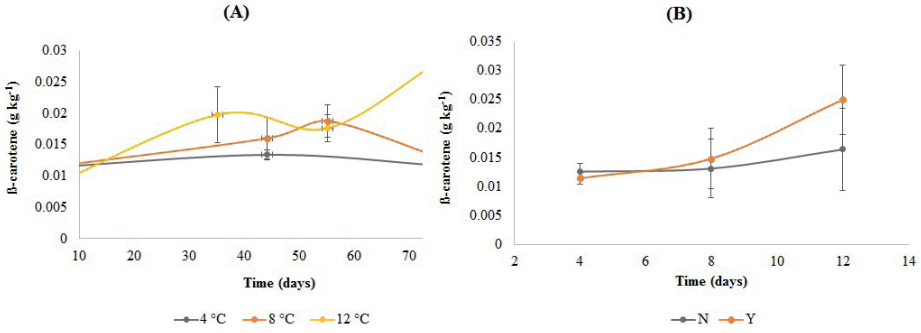

Figure 3.6. Effect of time and temperature (A) and presence 'Y' or absence 'N' of calyx (B) on β-carotene content (g kg^{-1}) of cape gooseberry fruit at 80 % RH. Vertical bars represent standard deviation SD β-carotene vs time (n=8-24); β-carotene vs temperature (n=12-36).

Effects of temperature and presence (Y) or absence (N) of the calyx were found ($P < 0.05$). β-carotene increased at 12 °C, at 8 °C first increased and then decreased; and at 4 °C, it remained stable. The presence of the calyx played a role in β-carotene development. When the fruit was removed from the calyx, β-carotene increased at 8 and 12 °C, at 4 °C there was no significant change. Experiments on cape gooseberry have shown an increase of β-carotene related to ripening stages [96, 98]. This explains the colour development of the fruit turning from green to yellow and then to orange [96, 63, 98, 100]. Increase in carotenoids was also

observed in tomatoes (*Solanum lycopersicum)* [202] and in husk tomato (*Physalis ixocarpa*), where chlorophyll values decreased with time due to the synthesis or expression of various pigments such as carotenoids [205, 189, 206].

3.3.4. Consumer acceptance evaluation: Survival Analysis

Survival analysis was made by the evaluation of consumer rejection probabilities calculated from the data obtained for the fruit without calyx (N) at different storage times, evaluated temperatures and harvest years, according to table 3.1 (figure 3.7). The Weibull, log-normal and normal distributions were tested. Weibull distribution model (equation 1) was selected because it had the lowest Akaike criterium [207].

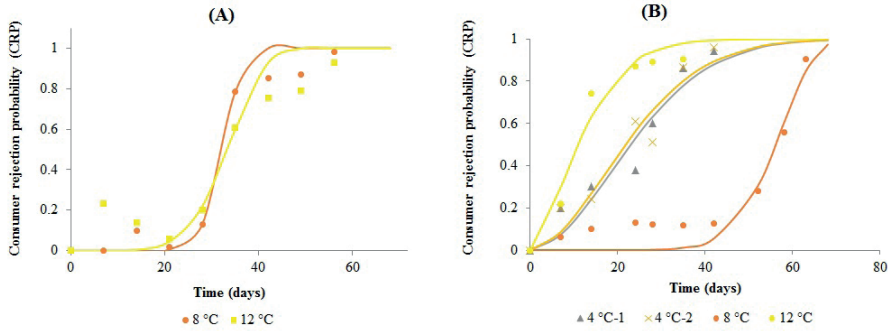

Figure 3.7. Consumer rejection probability CRP as a function of storage time for studies 2014 (A) and 2015 (B). Lines are Weibull models and points are experimental data.

$$F(t) = F_{sev}\left(\frac{\ln(t)-\mu}{\sigma}\right) \qquad \text{(equation 1)}$$

F_{sev} represents the survival function of the smallest extreme value distribution and and are the model's parameters shown in table 3.3 [163, 173]. Figure 3.7 shows the data and the model. As mentioned, surveys were conducted for fruit stored at three temperatures (4, 8 and 12 °C) and 80 % HR without calyx only.

Table 3.3. The parameters μ and σ of the Weibull model for the experimental data and Confidence intervals CI (95 % confidence) and shelf-life values estimated of cape gooseberry with 50 % probability of rejection by consumer's data and Confidence intervals CI (95 % confidence)

Temperature/Harvest time	μ± CI	**σ± CI**	**Shelf-life values (days) ± CI (95 %)**
8 °C – 2014	3.51 ± 0.02	0.09 ± 0.03	32.5 ± 2.7
12 °C – 2014	3.57 ± 0.04	0.17 ± 0.05	33.4 ± 2.7
4 °C - 2015-1	3.33 ± 0.08	0.54 ± 0.13	23.0 ± 2.7
4 °C - 2015-2	3.29 ± 0.09	0.56 ± 0.16	21.8 ± 2.7
8 °C – 2015	4.07 ± 0.02	0.12 ± 0.03	56.1 ± 2.7
12 °C – 2015	2.64 ± 0.10	0.67 ± 0.14	10.9 ± 2.7

To estimate shelf life, authors usually choose rejection probability of 50 % (= 0.5) [163, 208, 209]. From this, shelf lives of cape gooseberry thus calculated based on the value and the estimations are shown in table 3.3.

In 2014, no differences between the rejection rate of fruit stored at 8 °C and 12 °C were found. For 2015, shorter shelf life was obtained at 12 °C with 10.9 ± 2.7 d. At 4 °C, the shelf-life was 23.0 ± 2.7 and 21.8 ± 2.7 d and at 8 °C, 56.1 ± 2.7 d. These variations between rejection rate and harvests time confirm findings of the pilot experiment, where the critical quality attribute of cape gooseberry shelf-life was general appearance, which is highly affected by fungal growth, one of the most rapid noticeable change in the fruit (by consumers). Shelf life predictions by the survival analysis are in concordance with fungal growth that varies depending on harvests time (years). As explained in section 3.2., cultivation and harvesting conditions might have an effect in fungal growth. However, high moisture on fruit surface after calyx removal and chilling injury reported previously at 4 °C in the fruit, also affected the general appearance of the fruit, thus, leading to consumer rejection of the cape gooseberry without calyx (N). Although the difference between harvest times is evident, the temperature that showed better results from the consumer point of view was 8 °C.

3.3.5. Shelf life estimations

As our study integrates evaluations of quality from different perspectives, figures 3.8 and 3.9 represent shelf lives estimation based on the measured parameters of cape gooseberry quality and/or consumer acceptance. Figure 3.8 depicts quality attributes that denoted loss of quality more rapid for fruit with calyx (Y). All data from 2014 and 2015 are presented combined for fruit with calyx (Y).

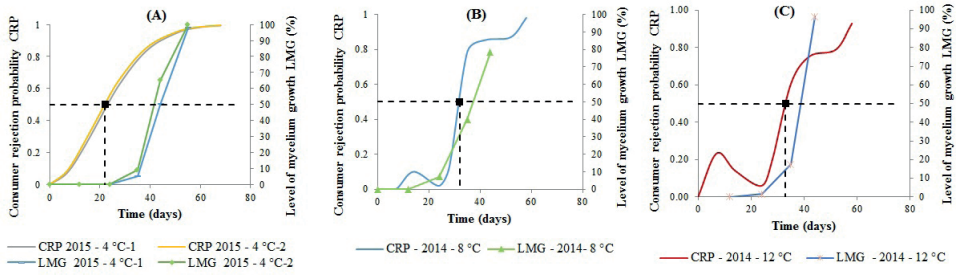

Figure 3.8. Shelf lives of cape gooseberry with calyx 'Y' at 4 (A), 8 (B) and 12 °C (C). Horizontal dashed lines represent the cut-off point (■) of 6 % as the acceptance limit of weight loss and relate cut-off point with shelf life. Vertical dashed lines related cut-off point con firmness values.

Weight loss and firmness were the most important changes in the fruit with the calyx (Y). Considering a 6 % of weight loss (commercially used) a limit for delivering fruit quality, 8 °C gives longest shelf life values (approx. 62 d), followed by 4 °C (approx. 38 d) and 12 °C (approx. 24 d). Cape gooseberry with calyx is sensitive to storage temperature, when temperature is low (4 °C) it might suffer chilling injury, increasing weight losses and loss of firmness. At high temperature (12 °C), transpiration processes take places more rapid, stimulating these changes as well. Cut-off points of shelf life for the three temperatures are related to firmness values from 800 and 1400 g, which corresponds to a loss of firmness of about 14 % for samples stored at 8 and 12 °C and 24 % at 4 °C.

Figure 3.9 shows the level of mycelium growth (LMG) and the consumer rejection probability (CRP), which were the critical factors in shelf life of fruit without the calyx (N).

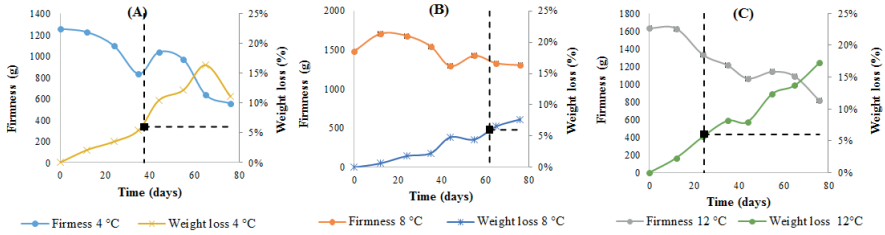

Figure 3.9. Shelf lives of cape gooseberry without calyx 'N' at 4 (A), 8 (B) and 12 °C (C). Horizontal dashed lines represent the cut-off point (■) of 0.5 as the acceptance limit Consumer Rejection Probability (CRP) and relate cut-off point with Level of Mycelium growth (LMG). Vertical dashed lines relate cut-off point with shelf life.

In figure 3.9, shelf life estimations for fruit without the calyx (N) are depicted (4 °C -2015 and 8 and 12 °C of 2014 as examples). We have already mentioned that consumer rejection was the parameter determining shelf life in this presentation. Since fungal growth had close relation with consumer rejection, they are both plotted. For fruit at 4 °C, the rejection of consumers starts before the fungal growth is visible because of the chilling injury of the fruit that made the surface of the fruit wet, affecting the general appearance and promoting rejection from consumers. Shelf lives values were already given in table 3.3. Our results are similar to a previous study on cape gooseberry store at 7 °C, where shelf life was about 40 d (García, 2014).

Because our approach involves also health-promoting compounds, we show the amount of ascorbic acid and β-carotene present at the end of each estimated shelf life. Results are in table 3.4 based on data presented in section 3.3.

Table 3.4. Estimation of ascorbic acid and β-carotene contents in cape gooseberry with and without calyx, stored at 4, 8 and 12 °C, at the end of shelf life

Temperature Harvest time	Presentation	Estimated Shelf-life values (days)	Ascorbic acid* g kg^{-1}	β-carotene* g kg^{-1}
4 °C - 2015- 1-2	Calyx (Y)	38.0	0.33 - 0.34	0.013 – 0.014
8 °C - 2014-2015	Calyx (Y)	62.0	0.44 – 0.47	0.016 – 0.021
12 °C - 2014-2015	Calyx (Y)	24.0	0.32 – 0.36	0.015 – 0.024
8 °C - 2014	No calyx (N)	32.5	0.42 – 0.46	0.013 – 0.019
12 °C - 2014	No calyx (N)	33.4	0.33 – 0.39	0.013 – 0.019
4 °C - 2015-1	No calyx (N)	23.0	0.29 – 0.31	0.010 – 0.012
4 °C - 2015-2	No calyx (N)	21.8	0.29 – 0.31	0.010 – 0.012
8 °C - 2015	No calyx (N)	56.1	0.44 – 0.47	0.016 – 0.021
12 °C - 2015	No calyx (N)	10.9	0.27 – 0.30	0.006 – 0.007

*Values taken from the closest data point in time

Ascorbic acid and β-carotene are present in high contents in comparison with other fruit sources [106, 127], during post-harvest storage, at the three evaluated temperatures and with and without calyx, therefore, health-promoting compounds from cape gooseberries are present at any stage of post-harvest time.

3.4. Conclusions

Cape gooseberry (*Physalis peruviana L.*) is a climacteric fruit with difficulties to retain an acceptable level of quality attributes. An integrated approach to shelf life estimation showed that storage temperature has a large effect on the shelf life of the fruit. No differences between the two years, harvest times and presence (Y) or absence (N) of calyx for physicochemical, ascorbic acid and β-carotene changes were found, which means that in our experiment, the calyx did not play a big role in the ripening process in storage time. Differences in experiments of cape gooseberry without calyx (N) came from fungal growth and subsequently, from consumer acceptance, measured by a survival analysis study. The removal of calyx is the crucial factor in fungal growth because increased the moisture of the fruit surface, promoting the growth of fungi. Consumer rejection due to signs of fungal growth determined shelf life of cape gooseberry without the calyx. Shelf life of cape gooseberry without calyx (N) was found to be about 10 d at 12 °C, 20 d at 4 °C and about 50 d at 8 °C in harvest 2015. For harvest 2014, shelf life was about 33 d for both 8 °C and 12 °C. Main quality attributes to be noticed are weight loss and firmness. Taking into account a commercially accepted weight

loss of 6 %, the shelf life of fruit with calyx (Y) is about 62 d at 8 °C with 80 % of HR. At 4 °C 38 d and 24 d at 12 °C showing a strong effect of temperature for fruit with calyx (Y). This is relevant for exports of fruit, where transportation times can be longer than 22 d.

In summary, the temperature of 8 °C is better suitable to store cape gooseberry in comparison with 4 and 12 °C, either with calyx (Y) or without (N) under the studied conditions (80 % RH). The shelf life is longer when the calyx is not removed. Contents of ascorbic acid and β-carotene are still relevant at the end of the shelf life of cape gooseberry.

The use of an integral approach to estimate the shelf life of fresh foods is a suitable methodology to understand the changes of the product form different perspectives because it permits to correlate quality attributes changes with consumer acceptance. The use of survival analysis allows to have more insights about consumer acceptance since the number of consumers participating in the study is higher than in trained panels. Besides, the fact that they are actual consumers gives more reliability to shelf life estimations.

Acknowledgments

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Chapter 4.

Thermal stability of phytochemicals, HMF and antioxidant activity in cape gooseberry *(Physalis peruviana L)*

Olivares-Tenorio, M.L., Verkerk, R., van Boekel, M. A.J.S., Dekker, M., (2016). Thermal stability of phytochemicals, HMF and antioxidant activity in cape gooseberry (*Physalis peruviana L*). Submitted for publication.

Abstract

Changes in the content of phytochemicals (ascorbic acid, β-carotene, catechin and epicatechin), hydroxymethylfurfural (HMF) and antioxidant activity of the fruit cape gooseberry were studied at 40, 60, 80, 100 and 120 °C for various times. These compounds were measured with HPLC and antioxidant activity with offline and HPLC-online DPPH assays. Various kinetic models were evaluated as well as the temperature dependence using the Arrhenius model. Ascorbic acid degradation was described by a first order reaction (reference rate constant $k_{80\,°C} = 3.5 \times 10^{-3}$ min^{-1} and activation energy $E_a = 44.8$ kJ mol^{-1}). β-carotene was not degraded at all temperatures studied and followed an isomerization reaction from trans- to cis-β-carotene isomers from 80°C onwards, however, modelling of this phenomenon was not possible with the obtained data because of the apparent increase in concentration upon heating. Formation of HMF was described with a consecutive zero and first order reaction model because it involved the production of a precursor of the compound with $k_{1\text{-}80\,°C} = 9.4 \times 10^{-3}$ mol (dm^3)$^{-1}$ min^{-1}; $E_{a\text{-}1} = 112.3$ kJ mol^{-1}. The second step in the formation reaction of HMF was characterized by $k_{2\text{-}80\,°C} = 1.9 \times 10^{-3}$ min^{-1}; $E_{a\text{-}2} = 103.7$ kJ mol^{-1}. The content of catechin and epicatechin increased at 40 and at 60 °C decreased. More than three competing reactions were identified, which made kinetic modelling not well possible. Antioxidant activity with offline DPPH ($k_{80\,°C} = 1.0 \times 10^{-3}$ min^{-1} and apparent $E_a = 38.4$ kJ mol^{-1}) and HPLC-online DPPH ($k_{80\,°C} = 4.3 \times 10^{-3}$ min^{-1} and apparent $E_a = 49.5$ kJ mol^{-1}) assays were characterized by a fractional first order conversion model. Comparison with kinetics found in other fruits showed that cape gooseberry is a source of various health-promoting compounds that appeared to be relatively more stable to heat treatment. This makes cape gooseberry suitable for the preparation of processed food, such as jam, juices and dehydrated fruit with health-promoting compounds contents.

Keywords: ascorbic acid; β-carotene; DPPH assay; flavonoids; health-promoting compounds; heat treatment; modeling.

4.1. Introduction

Cape gooseberry is the fruit of the plant *Physalis peruviana L.*, original from Andean countries and currently available in international markets [210, 211]. The most commercialized cultivar is the ecotype Colombia. Cape gooseberry contains health-promoting compounds such as vitamin C [96, 168, 95], β-carotene[60], flavonoids [121, 122] and shows antioxidant activity [96, 121, 100, 95]. This fruit is currently commercialized as fresh and dried fruit, and it is often used as raw material to produce juices, jam, etc. Although, some studies have attempted to evaluate the losses of some health-promoting compounds and antioxidant activity after heat treatment of cape gooseberry [70, 71], a systematic study aimed to evaluate the kinetic of those stabilities in this fruit has not been reported [168]. Previous researches had reported the stability of ascorbic acid and flavonoids during heat treatment on other food products [104, 212, 213] as well as the changes in carotenoids contents [156], concluding differences in stability based on food matrix characteristics. Thus, there is still knowledge gap about the thermal stability of phytochemicals and antioxidant activity in cape gooseberry.

The present study evaluates the changes in the content of ascorbic acid, catechin, epicatechin, β-carotene, HMF and antioxidant activity assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assays (offline and online-HPLC). Kinetic modelling has been conducted, when possible, to describe the changes as affected by time and temperature to make a contribution for optimising process design towards preserving health-promoting compounds of cape gooseberry.

4.2. Materials and methods

4.2.1. Fruit material

Cape gooseberry (*Physalis peruviana L.*, ecotype Colombia) fruits grown in Pasca, Cundinamarca, Colombia (2.180 m.a.s.l.) were harvested, selected and immediately sent to The Netherlands by ship (three weeks of transportation under 9 °C ± 2 and 75-85% of relative humidity). Based on preliminary tests, we considered that changes in phytochemicals contents during those transportation times were negligible. Once the fruit was received in the laboratory of Wageningen University & Research, approximately 1 kg per experiment was peeled (detached from calyx), cut in halves, frozen and freeze dried. Fruit material was kept at -20 °C in aluminium foil trays with cap until experiments were conducted.

4.2.2. Heat treatment

As the physical characteristics of fresh cape gooseberry did not allow to work properly the

samples in the lab, freeze dried fruit was pulverised (Retsch, MM 400, Germany) and 1.2 g was introduced in glass tubes with caps (10 cm of length, 1.0 cm of diameter). The fruit powder was dissolved in 4.8 mL of water to re-establish the original moisture content of the fruit; a headspace of 4 cm remained. Samples were flushed with nitrogen before heating. A probe going through the cap was used to monitor the temperature of the samples in the tubes. Heat treatments of samples were conducted in a heating block (Liebisch 33649, Bielefeld, Germany). After a predetermined heating time, tubes were cooled down in cold water and samples were analysed immediately. Time taken to reach the required heating temperature (5–10 min, depending on the temperature) was excluded from the kinetic parameter analysis. The reported heating times start from the moment that the desired temperature was reached. The scheme of temperatures and times used for this study is shown in table 4.1. Because the rate of degradation varies depending on the temperature, to have a more accurate estimation of the rate constants it is necessary to use different incubation times for each temperature. Heat treatments were conducted in duplicate (or quadruplicate) with different batches as indicated below for each compound/antioxidant activity evaluated.

Table 4.1. Heating times and temperatures of cape gooseberry samples

Temperature (˚C)	Time (min)					
40	0	360	1020	2400	4140	5580
60	0	80	315	960	2460	4140
80	0	40	160	300	990	1200
100	0	5	40	120	240	350
120	0	2.5	5	10	40	120

4.2.3. Ascorbic acid analysis

Ascorbic acid content was determined according to the procedure described by Hernandez et al., (2006) with modifications (especially for the extraction method for cape gooseberry), using RP-HPLC with a Dionex Ultimate 3000 RS diode array detector and a Polaris 5 C18 A column 150 x 4.6 mm, 5µm [89]. The mobile phase was prepared by adding 0.2% orthophosphoric acid (Merck®) into Milli-Q Water (v/v). Ascorbic acid eluted after 5.5 min at a flow rate of 1.0 mL min^{-1}, detection was with a UV- detector at a wavelength of 245 nm. For the sample preparation, 3.5 mL of 3% metaphosphoric acid (MPA) and 0.1% tert-Butylhydroquinone (THBQ) solution was mixed with approximately 1.25 g of heated cape gooseberry sample. The suspension was homogenized with an Ultra Turrax (IKA-Werke, T-25 Basic) at high speed for 1 min. Thereafter, the extract was centrifuged for 5 min at 1,790 g and 4 °C. The supernatant was collected and the pellet was treated twice more with the same procedure. The combined supernatants obtained from the three extraction procedures

were again centrifuged for 10 min at 11,700 g and 4 °C. The extract was filtered using 0.2 µm CA (cellulose acetate) filters (Minisart®) and put into amber vials prior to HPLC analysis. To quantify the vitamin, a standard calibration was prepared using ascorbic acid (Sigma-Aldrich®, ≥ 99%) dissolved in 3% MPA-0.1% THBQ solution. Heat treatments were conducted two times for independent batches. Measurements were performed in duplicate of heated samples and extracts and then averaged to get one data set per batch (2). Results were expressed in mg ascorbic acid 100 g^{-1} fresh weight (FW).

4.2.4. β-carotene analysis

The β-carotene content in cape gooseberry fruit was determined following the procedure described by Bushway (1986) with modifications (especially for the extraction method for cape gooseberry), using RP-HPLC with a Dionex Ultimate 3000 RS diode array detector [214]. The eluent was 92.5% pure methanol (BioSolve®), 7.5% pure tetrahydrofuran THF (BioSolve®) and 0.1% trimethylamine TEA (Sigma-aldrich®). 20 µL of the extract was injected; the column used was a Vydac C18 218TP54 4.6 x 250 mm, 5 µm with a pre-column 30-40 µm, 3.9 mm x 40 mm. β-carotene eluted after 25 minutes with a flow rate of 1.0 mL min^{-1}. Compounds were scanned and recorded at a wavelength range of 220-550 nm. Approximately 1.0 g of heated cape gooseberry sample was added to 3 mL Milli-Q water, 10 mL pure hexane (HPLC, Biosolve®) and 0.4 mL of 0.1% butylated hydroxytoluene (BHT, Sigma-aldrich®). The suspension was homogenised with an Ultra turrax (IKA-Werke, T-25 Basic) for 2 min, then flushed with nitrogen and centrifuged for 10 min at 1,560 g and 4 °C. The supernatant was carefully collected and the pellet was mixed with 10 mL of pure tetrahydrofuran (THF, BioSolve®), homogenised with Ultra turrax for 1 min, flushed with nitrogen and centrifuged for 10 min at 1,560 g and 4 °C. The combined supernatants were evaporated and dried at 40 °C and 270 mbar. The extracted β-carotene was dissolved with 5 mL THF and 5 mL eluent solution. The extract was filtered with 0.2 µm RC (regenerated cellulose) filter (Minisart®) and put into amber vials (Grace, Alltech) before HPLC analysis. A calibration curve of β-carotene (Sigma-aldrich®) ranging from 0.4 to 200 µg/mL was used as standard. The purity of the β-carotene standard was checked according to the method reported in literature (Scott, 2001). Heat treatments were conducted twice for independent batches. Measurements were performed in duplicate of heated samples and extracts and then averaged to get one data set per batch (2). Results were expressed in mg 100 g^{-1} FW of trans-β-carotene and cis-β-carotene. trans-β-carotene and cis-β-carotene were identified by a shift in retention time after heat treatment and by a visual spectrum analysis.

4.2.5. Flavonoids and hydroxymethylfurfural (HMF) analysis

Flavonoids in heat treated cape gooseberry fruit were identified and quantified with RP-

HPLC following the procedure described by Bandoniene & Murkovic (2002) with some modifications (especially for the extraction method for cape gooseberry) [215]. Approximately 1.5 g of heated cape gooseberry sample was dissolved in 70% methanol (BioSolve®). The suspension was mixed with vortex (VWR vv3) every 10 min for 30 min at room temperature. Thereafter, the suspension was centrifuged for 10 min at 1,790 g and 4 °C. The supernatant was collected and homogenised with pH 2.5 solution of water/trifluoroacetic acid (TFA, Merck®) TFA with a ratio of 1:1 (v/v). The sample was filtered through 0.20 µm CA filter (Minisart®) and placed into HPLC vials (Grace, 1.5 mL) before HPLC analysis. Series of calibration curves of quercetin di-hydrate, kaempferol, myricetin from Fluka® and naringenin, (+-)-catechin, (-)- epicatechin, rutin hydrate, Kaempferol from Sigma-aldrich® were prepared in methanolic solutions from 0.4 to 200 µg mL^{-1}.

The flavonoids extract was analysed by Dionex Ultimate 3000 equipment with a Polaris 5 C18 A column 150 x 4.6 mm, 5µm. The mobile phase consisted of a solution of pH 2.5 Milli-Q/ TFA and pure acetonitrile (Biosolve®). The flow rate was 1.0 mL min^{-1} and compounds in the sample were scanned and recorded at a wavelength range of 220-370 nm.

Hydroxymethylfurfural (HMF) was analysed in the same extract as used for flavonoids analysis; the results were read at 280 nm in the same chromatogram. A calibration curve was prepared with HMF (Sigma-aldrich®) from 0.4 to 200 µg mL^{-1} for quantification. Heat treatments were conducted twice for independent batches for flavonoids and four times for HMF. Measurements were performed in duplicate of heated samples and extracts and then averaged to get one data set per batch (2 for flavonoids and HMF). Results were expressed in mg flavonoid or HMF 100 g^{-1} FW.

4.2.6. Antioxidant activity

DPPH assay (Offline)

The 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazinyl (DPPH) assay was conducted according to Brand-Williams (1995) [216]. A working solution was prepared by dissolving DPPH ($6*10^{-5}$ M) (Sigma-aldrich®) in 100% methanol. 3.9 mL of this solution was mixed with 0.1 mL of the same flavonoid extract prepared according to method 2.5. The mixture was incubated in a water bath of 25 °C during 30 min in dark conditions and continuous shaking. The absorbance of the solution was measured at a wavelength of 515 nm in a spectrophotometer (Cary 50 UV-Vis; cuvettes of 1 cm^2). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox (Sigma-aldrich®) was used as standard with a calibration curve from 18.9 to 750 µg.g^{-1}. Heat treatments were conducted four times for independent batches. Measurements were performed in duplicate of heated samples and extracts and then averaged to get one data

set per batch (4). Results were expressed as μg trolox equivalent g^{-1} FW.

DPPH –HPLC Online assay

This online-HPLC method was used to determine the radical scavenging ability of individual compounds and standards after HPLC separation with Dionex Ultimate 3000, based on the method described by Koleva et al., (2000) [217] and Boldoniene et al., (2002). The antioxidant activity determination of this assay is based on the screening of the induced bleaching of DPPH solution which is detected as negative peaks photometrically at 515 in a post-column. The flavonoid extract was prepared as mentioned in Section 2.5. The working solution of DPPH had the same composition of the common DPPH assay (Offline) described previously.

The system of the online HPLC-DPPH assay had the following elements: an eluent pump (HPG – 3400 Binary High Pressure Gradient pump); a photodiode array detector (DAD-3000 RS) equipped with deuterium and tungsten lamp; an analytical flow cell 13 μL, 10 mm, SST, DAD-3000; a pump for delivery of DPPH solution (3100A isocratic pump); a UV-vis absorbance detector (VWD-3400 RS) equipped with deuterium and tungsten lamp and an analytical flow cell 11 μL, 10 mm, SST, VWD-3400RS. Separations were performed on reversed phase analytical column Polaris 5 C18-A 150 x 4.6mm HPLC column. Two reaction coils used were: the reaction knit with its volume of 375 μL and the blue coil made of PEEK tubing with its dimension: 0.010" (inch) × 50 foot (equivalent to a volume of 748 μL). These two coils were connected with each other and interfaced between the UV detector and DPPH reagent pump via a T-junction. The mobile phase consisted of a solution of pH 2.5 Milli-Q/ TFA and pure acetonitrile (Biosolve®). The flow rate was 0.7 mL min^{-1}. Compounds in the sample were scanned and recorded at a wavelength range of 260-380 nm. In the post-column, the mobile phase was the DPPH solution with a flow of 0.5 mL min^{-1} and negative peaks of DPPH reaction with compounds in the sample were scanned and recorded at a wavelength of 515 nm. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox (Sigma-Aldrich) was used as standard with a calibration curve from 18.9 to 750 μg g^{-1}. Heat treatments were conducted twice for independent batches. Measurements were performed in duplicate of heated samples and extracts and then averaged to get one data set per batch (2). Results were expressed as μg trolox equivalent g^{-1} FW.

4.2.7. Kinetic modelling

Where appropriate, kinetic models were applied to the data obtained with the goal to extract kinetic parameters such as rate constants (k) and activation energies (E_a), which can then be used to make predictions about stability. The general approach was to first find a suitable model describing the behaviour at each temperature studied and then to check whether the

most suitable model could be applied to all data at once (global modelling). Most temperature dependent reactions in foods can be modelled via the Arrhenius equation (1), describing the relation between a rate constant *k* and absolute temperature *T*:

$$k = k_0 exp\left(-\frac{E_a}{RT}\right) \quad (1)$$

in which k_0 is the so-called pre-exponential factor and E_a the activation energy; R is the general gas constant. For statistical reasons (to avoid strong correlation between k_0 and E_a), it is better to reparametrize this equation (van Boekel, 2009):

$$k = k_{T_{av}} exp\left(-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{av}}\right)\right) \quad (2)$$

In which T_{av} is the average value of the temperatures used and k_{Tav} the rate constant at this average temperature. For global modelling, this expression for k can be substituted in a kinetic model, for instance for a n^{th}-order model describing degradation of a compound with concentration c:

$$\frac{dc}{dt} = -kc^n \quad (3)$$

it becomes:

$$\frac{dc}{dt} = -k_{T_{av}} exp\left(-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{av}}\right)\right) c^n \quad (4)$$

if the Arrhenius model holds for all data studied, then this equation can be applied to all data simultaneously to extract the parameters k_{Tav} and E_a as parameter estimates [160]. Further details will be given in the Results and Discussion section for each compound studied.

The software used for numerical integration of equations like the one depicted in equation (4) and parameter estimation via nonlinear least squares was Athena Visual Studio v. 14.2 (www.athenavisual.com).

4.3. Results and Discussion

4.3.1. Ascorbic acid

Ascorbic acid was not stable; it decreased at each temperature studied. The problem we were faced with was that batches were variable, i.e., each batch had a different initial concentration

and this induced a problem when the heat treatment was replicated: the variation induced by the treatment (which can be measured via replication) comes on top of the variation present in the batches and it is not possible to separate the two effects. There are two possibilities to deal with this:

- Analyse each batch individually; this has the advantage that the data are used as they are, the disadvantage is that the initial concentrations are variable
- Normalize the concentrations as C/C_0. This has the advantage that the initial concentrations are the same (namely normalized to 1) but it introduces extra errors in the transformed data because of the experimental uncertainty in the initial concentration

We decided to try both options. We first applied the n^{th}-order model (equation 4) to each batch with its own initial concentration, assuming for the moment that the Arrhenius equation applies (this will be validated afterwards). The parameter estimation with the 95% of certainty were:

$k_{80°C} = 1.8 \times 10^{-3} \pm 2.1 \times 10^{-3}$ $(dm^3\ mol^{-1})^{0.2}\ s^{-1}$; $E_a = 46.5 \pm 2.5$ kJ mol^{-1}; $n = 1.2 \pm 0.3$

Although the order appears to differ slightly from one, we nevertheless assumed first order kinetics because it is within the confidence interval and it allows us to compare with literature results on vitamin C degradation in which first-order kinetics is usually assumed [218, 219]. We then determined first-order rate constants at each temperature to check the validity of the Arrhenius equation; this is shown in figure 4.1.

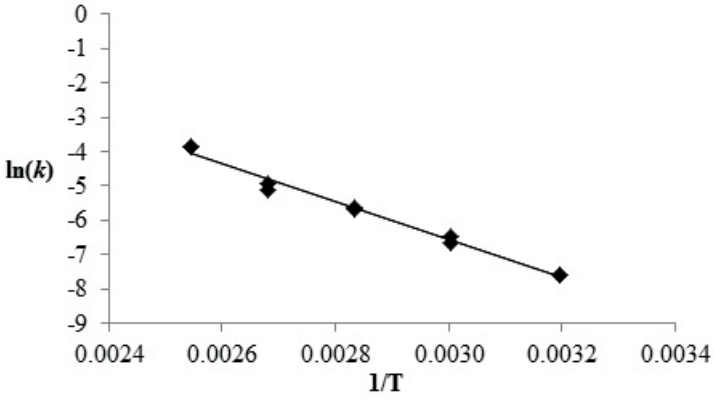

Figure 4.1. Arrhenius plot for first-order rate constants obtained from individual batches

This plot shows that the data set fulfils the Arrhenius law, which allows us to globally model all data at once via equation (4) with n=1.

The parameter estimation results for the case of modelling each batch were:

$k_{80°} = 3.5 \times 10^{-3} \pm 2.3 \times 10^{-3}$ min^{-1}; $E_a = 44.8 \pm 2.3$ kJ.mol^{-1}

The resulting fits for the two batches are shown in Figure 4.2.

Taking into account that the model with the two parameters is applied to all data at once, the fits are reasonable; the precision of the estimate of activation energy (E_a) is quite good, while the imprecision for the rate constant ($k_{80\,°C}$) at average temperature is rather high. When modelling the normalized concentrations, the results were (plots not shown):

$k_{80\,°C} = 3.5 \times 10^{-3} \pm 2.5 \times 10^{-4}$ min^{-1}; $E_a = 45.4 \pm 2.5$ kJ mol^{-1}; $C_i/C_0 = 0.98 \pm 0.03$

The values of the parameter estimates are about the same as with modelling the batches separately but remarkably the precision of the rate constant at 80 °C is one order of magnitude better, with a very close estimation of C_i/C_0 to 1, what is expected. The lag plot and the normal probability plot show the normal distribution of the residuals and also their randomness which indicated the modelling is suitable for the data set (See figure 4.3).

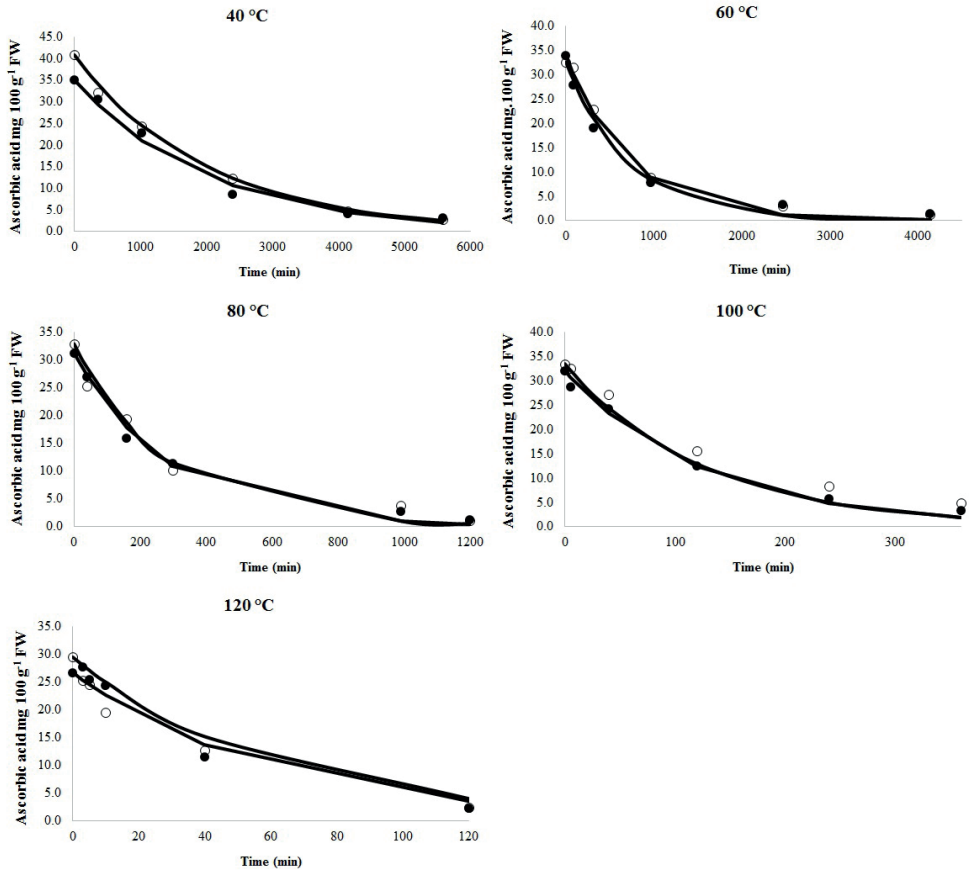

Figure 4.2. Thermal degradation of ascorbic acid in cape gooseberry (Physalis peruviana L.) at 40, 60, 80, 100 and 120 °C for Batch 1 (●) and Batch 2 (○). Symbols represent the experimental data and lines represent the global first-order model fit with the parameters $k_{80°}$= 3.5 x 10^{-3} min^{-1}; E_a = 44.8 kJ.mol^{-1}.

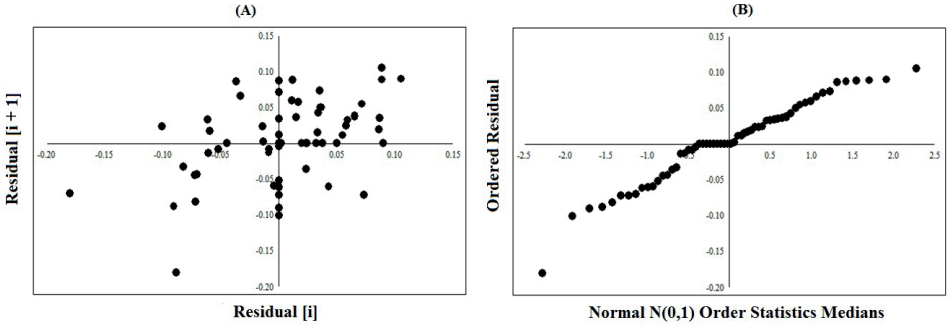

Figure 4.3. Lag plot (A) and Normal probability plot (B) of normalized modelled data of ascorbic acid.

Ascorbic acid in Cape gooseberry showed a lower rate constant than what is reported for cupuaçu nectar (*Theobroma grandiflorum*) ($k_{80\,°C}$= 32 x 10^{-3} min^{-1}) [220]. Using the estimated parameters, the calculated $k_{100\,°C}$= 8.0 x 10^{-3} min^{-1} for ascorbic acid in cape gooseberry is lower than what has been reported for mango ($k_{100\,°C}$= 1.3 x 10^{-1} min^{-1}), guava ($k_{100\,°C}$= 1.2 x 10^{-1} min^{-1}) and similar to marula ($k_{100\,°C}$= 7.2 x 10^{-3} min^{-1}) [104]. A calculated $k_{90\,°C}$= 5.3 x 10^{-3} min^{-1} is lower than what is reported for orange juice ($k_{90\,°C}$= 1.8 x 10^{-1} min^{-1}) [221]. A calculated $k_{120\,°C}$= 16.6 x 10^{-3} min^{-1} is more than double the degradation rate constant k of squeezed tomatoes ($k_{120\,°C}$ = 7.1 x 10^{-3} min^{-1}) and squeezed orange ($k_{120\,°C}$ = 7.6 x 10^{-3} min^{-1}) [219]. Activation energy E_a of degradation of ascorbic acid of cape gooseberry was higher than in mango (E_a =39 kJ mol^{-1} in the most stable fraction) and marula (E_a =29 kJ mol^{-1} in the most stable fraction) [104]; lower than for guava (E_a =58 kJ mol^{-1} in the most stable fraction), for squeezed tomato (E_a =106.7 kJ mol^{-1}) and for squeezed orange (E_a =117.1 kJ mol^{-1}) [219]. Ascorbic acid degradation in cape gooseberry seems to be less temperature dependent and showed a lower degradation rate constant than other common sources of vitamin C.

4.3.2. β-carotene

The data obtained on β-carotene revealed the presence of both the trans- and cis- form (figure 4.4). At 40 °C, not much seemed to happen. At 60 and 80 °C, a slight increase in carotene was apparent with also a hint of isomerization of trans- into the cis- form. Clear signs of isomerization can be seen at 100 and 120 °C. Trans-cis isomerization of β-carotene has been described before, eventually leading to an equilibrium between the two forms (Colle, 2013). The apparent increase in carotene is most likely attributable to release from the food matrix upon heat treatment [222]. This phenomenon of apparent increase in carotene content upon moderate heating makes it not well possible to apply a kinetic

model; one cannot distinguish between the increase due to extractability, changes due to isomerization and possibly also degradation. At 100 and 120 °C, where isomerization is most apparent, we tried to model the data according to the following scheme:

$$\textit{trans-}\beta\textit{-carotene} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \textit{cis-}\beta\textit{-carotene} \qquad (5)$$

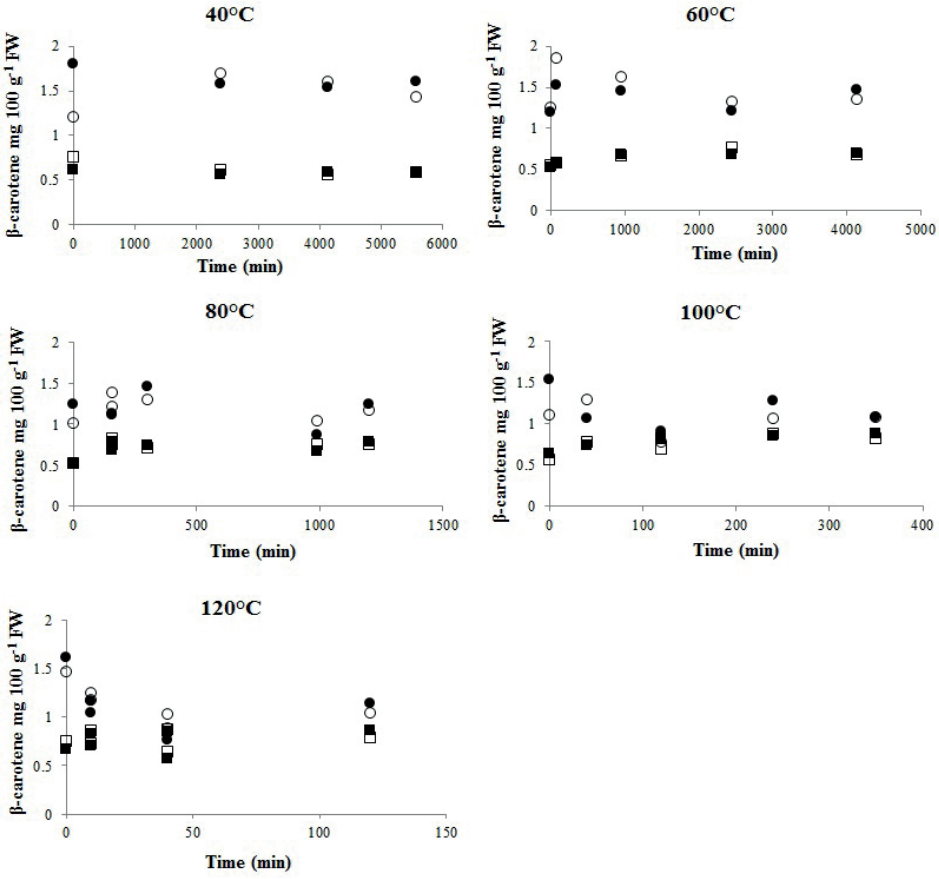

Figure 4.5. Thermal formation and degradation of catechin (batch 1 ● and batch 2 ○) and epicatechin (batch 1 ■ and batch 2 □) in cape gooseberry (*Physalis peruviana L.*) at 40, 60, 80, 100 and 120 °C.

However, the dataset did not contain enough information to extract parameters k_1 and k_2 and their temperature dependence; the imprecision in the parameters was too high. When comparing the total content of carotene (the sum of cis- and trans-) it became apparent that

there was a slight increase at all temperatures except at 120 °C where the content was, within experimental error, constant (data not shown). In conclusion, it can be stated that the total content of β-carotene is more or less stable during heating, but there is some isomerization from trans- to cis-carotene.

This result differs from β-carotene in hexane solution, where all-trans-β-carotene was converted to isomers at 100 °C in 11 min [223]. In carrot juice, however, at 100 °C a minor isomerization of β-carotene took place starting only at 120 °C [224, 225]. The stability of β-carotene is probably related to the food matrix and heat processing increases the amount of cis-β-carotene, explaining the high amount of these isomers in processed food [222].

4.3.3. Flavonoids

In our experiments we searched for flavonoids such as quercetin di-hydrate, kaempferol, myricetin, naringenin, catechin, epicatechin and rutin. Despite several attempts and the use of HPLC assisted by MS (mass spectrum), from the large amount of peaks we found in the methanolic extract, we only succeeded to identify catechin, epicatechin, rutin, quercetin di-hydrate, myricetin and kaempferol in fresh fruit samples. However, from samples after heat treatment , we could only quantify catechin and epicatechin. Previous research identified quercetin, rutin, myricetin, epicatechin, catechin and kaempferol in cape gooseberry [121, 122, 116-118, 124] and although we replicated the procedures of extraction, hydrolysis (if required) and analysis, we could not come up with the same results. We therefore limit the discussion to catechin and epicatechin; the fate of these compounds is presented in figure 4.5.

Catechin and epicatechin at 40 °C showed a strong increase followed by some degradation at 60 °C (however, only after a substantial initial increase of catechin) while at 80, 100 and 120 °C only degradation, and perhaps some epimerization, was observed. It is known from literature that epimerization already occurs at the lower temperatures: it can start at 25 °C [212, 213]. However, in our results the total amount of catechin and epicatechin increased quite strongly at 40 and 60 °C, which cannot be attributed to epimerization because then the total amount should remain the same. It seems that especially catechin is formed from another flavonoid compound. As with carotenoids, part of the increase could perhaps be explained by better extractability but catechins are much less hydrophobic than carotenoids and the effect of a better extractability after a modest heat treatment will be limited.

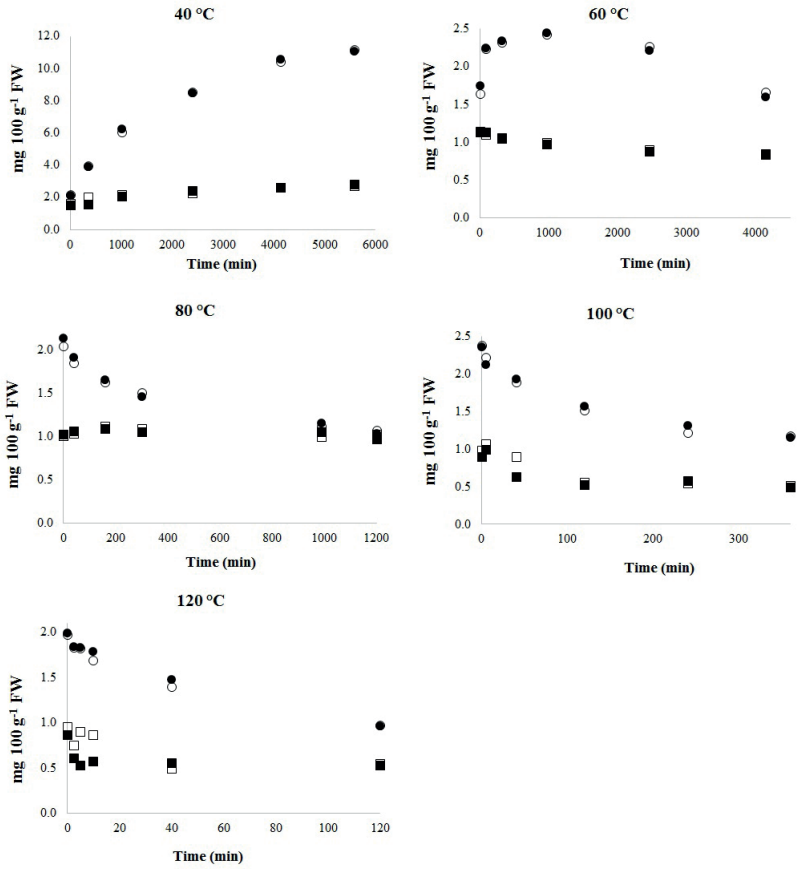

Figure 4.5. Thermal formation and degradation of catechin (batch 1 ● and batch 2 ○) and epicatechin (batch 1 ■ and batch 2 □) in cape gooseberry (*Physalis peruviana L.*) at 40, 60, 80, 100 and 120 °C.

If we consider again the possibility to apply a kinetic model, we are now faced with three phenomena that interfere with each other: 1) better extractability leading to higher apparent concentration, 2) formation of catechin from another compound, 3) epimerization, 4) degradation of catechin and epicatechin [226, 212]. The data obtained do not allow separating these four effects and so it makes no sense to apply a kinetic model.

Epimerization in tea drinks and aqueous solutions has been shown to be affected not only by temperature, but also by the presence of metal ions and pH [227, 226, 213]. Thus, all these

factors need to be considered before being able to apply kinetic modelling of degradation or epimerization. In cape gooseberry, there are metals that might affect the behaviour of catechin compounds [228]. The low pH (around 4.0) can also facilitate an epimerization reaction next to degradation [226]. From the plots in figure 4.6, catechin degrades faster than epicatechin which is different from what is reported for aqueous solutions [212], thus, the food matrix might play a role in this different behaviour and that requires more study.

4.3.4. HMF

HMF formation showed a clear lag phase at 60 °C followed by an exponential increase at all temperatures, thus, in contrast to many reports in literature on HMF formation, it cannot be described by a zero order reaction in cape gooseberry; also a first order formation reaction did not perform well (not shown). When considering the formation route of HMF, a lag phase can be expected: first, other compounds need to be formed before HMF can be formed Therefore, we applied a consecutive model of zero order reaction for the formation of HMF intermediates and a first order reaction model for the formation of HMF from these intermediates. This consecutive model thus includes the conversion of a compound (A) into a precursor compound (B) of HMF followed by 1st order formation of HMF (B→HMF). Compound A and B could be sucrose and fructose, respectively, since sucrose hydrolysis can occur at these temperatures and fructose is a reactive precursor of HMF; however, also other intermediates are formed from fructose before HMF can be formed, so the nature of A and B remains unclear for the moment [229, 230].

$$A \xrightarrow{k_1} B \xrightarrow{k_2} HMF \qquad (6)$$

A = initial compound, B= precursor compound of HMF and HMF is the concentration at certain time, and k_1 and k_2 are the respective rate constants. First, we determined consecutive zero and first order rate constants at each temperature to check the validity of the Arrhenius equation, using two batches as shown in Figure 4.6.

This plot shows that the data set fulfils Arrhenius law, which allows us to globally model all data at once via equation (4) with n=0 and n=1, respectively.

The parameter estimation results were (± 95% confidence intervals):

$k_{1\text{-}80\ °C} = 9.4 \times 10^{-3} \pm 7.8 \times 10^{-3}$ mol $(dm^3)^{-1}$ min^{-1}; $E_{a\text{-}1} = 112.3 \pm 23.3$ kJ mol^{-1}

$k_{2\text{-}80\ °C} = 1.9 \times 10^{-3} \pm 3.6 \times 10^{-3}$ min^{-1}; $E_{a\text{-}2} = 103.7 \pm 57.3$ kJ mol^{-1}

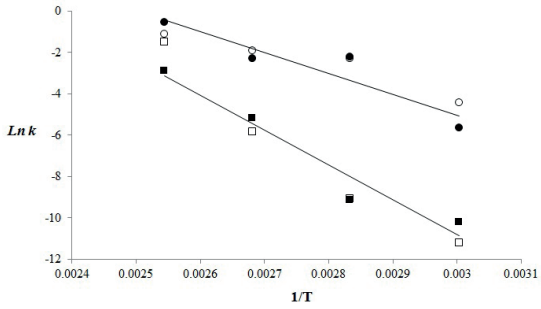

Figure 4.6. Arrhenius plot for consecutive zero (Batch 1 k_1, ●; Batch 2 k_1, ○) and first order rate (Batch 1 k_2, ■; Batch 2 k_2, □) constants obtained from cape gooseberry.

The results of the global modelling are shown in figure 4.7. In figure 4.8 are depicted the lag plot and the normal probability plot. Lag plot shows randomness with a small indication of correlation between the data. Normal probability plot shows a deviation from a linear line, thus the residuals are not normally distributed. The statistical analysis of the regression casts some doubt on the validity of the regression. Therefore, the results of the regression should be interpreted with caution. In other words, the trend could reasonable but the extractions of values out of this model might be slightly different from the ones reported in this study.

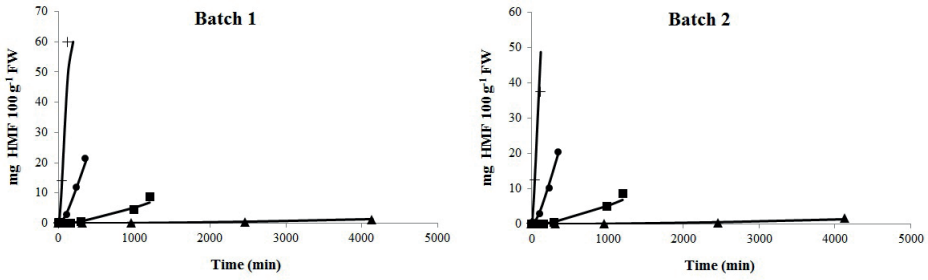

Figure 4.7. Formation of hydroxymethylfurfural HMF in cape gooseberry (Physalis peruviana L.) at 60°C (▲), 80 °C (■), 100 °C (●) and 120 (+) for the two batches evaluated. Symbols represent the experimental data and lines the global model fits according to consecutive zero-first-order model. $k_{1\text{-}80°C}$ = 9.3 x 10^{-3} mol $(dm^3)^{-1}min^{-1}$; $E_{a\text{-}1}$ = 112.3 kJ mol^{-1}, $k_{2\text{-}80°C}$ = 1.9 x 10^{-3} min^{-1}; $E_{a\text{-}2}$ = 103.7 kJ mol^{-1} as parameters

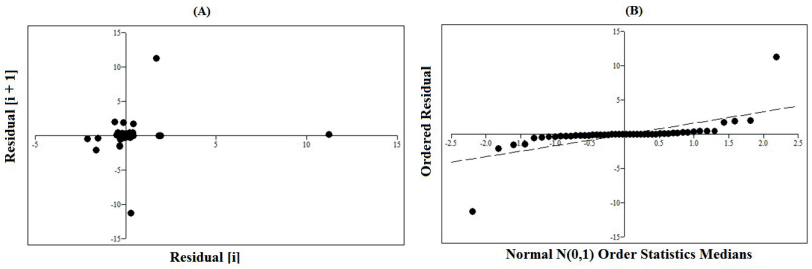

Figure 4.8. Lag plot (A) and Normal probability plot (B) of modelled data of HMF.

HMF formation has been described as a zero-order reaction for milk and apple cider [229, 231] and as a first-order reaction for honey and apple juice [232, 230], possibly due differences in carbohydrate profiles of the various food systems. HMF formation in milk is linked to the Maillard reaction and its temperature dependence reflects that with an E_a of 118.6 ± 8.2 kJ mol^{-1} [231]. In honey, the temperature dependence with an E_a of 226 kJ mol^{-1} [230] is much higher, possibly reflecting sugar degradation rather than Maillard reaction. HMF formation in apple juice with E_a = 126.2 kJ mol^{-1} [232] resembles Maillard reaction again in terms of magnitude of activation energy. In cape gooseberry, the lag phase has a $k_{80\,°C}$ = 1.9 x 10^{-2} mol $(dm^3)^{-1}$ min^{-1} with a moderate temperature dependence and in the formation phase $k_{80\,°C}$ = 1.2 x 10^{-3} min^{-1} with a E_a = 103.7 kJ mol^{-1}. Reaction rates in apple cider have been reported to be $k_{80\,°C}$ = 1.4 x 10^{-2} ppm s^{-1}, $k_{120\,°C}$ = 4.4 x 10^{-2} in milk, $k_{100\,°C}$ = 1.9 x 10^{2} in honey and $k_{80\,°C}$ = 1.0 x 10^{3} min^{-1} in apple juice [232], however, the kinetics was based on zero order formation of HMF, therefore direct comparison is not possible. Explanation of the lag phase presented before formation of HMF are the type and content of compounds involved [233] and the content of solutes in products, that could make HMF formation reactions to be slower in cape gooseberry than in other products [232]. For instance, fructose has shown to be five times more reactive than glucose; fructose is not the most abundant sugar (1.2 - 1.3 $g.100^{-1}$ FW) in cape gooseberry (ecotype Colombia) in comparison to sucrose (2.5 – 3.5 $g.100^{-1}$ FW) [52, 179] but its concentration is one order of magnitude higher than what is formed in amounts of HMF, so it will not be limiting. Thus, the lag phase before the HMF formation is probably caused by the formation of intermediate compounds [233]. Besides, the presence of other compounds such as catechin can react with HMF, forming oligomeric compounds, thus, degrading and inhibiting HMF formation [234, 235].

4.3.5. Antioxidant activity

The DPPH assay was conducted to assess antioxidant activity both with offline (traditional assay) and with online HPLC-DPPH methods; similar DPPH values were obtained. For

kinetic modelling, the simple n[th] order model did not give acceptable results. Therefore, a fractional conversion model was used:

$$\frac{dc}{dt} = -k_d(c - c_f) \qquad (7)$$

c_f refers to the final concentration at infinite time.

Arrhenius law was tested first for the individual batches and the results are shown in figure 4.9.

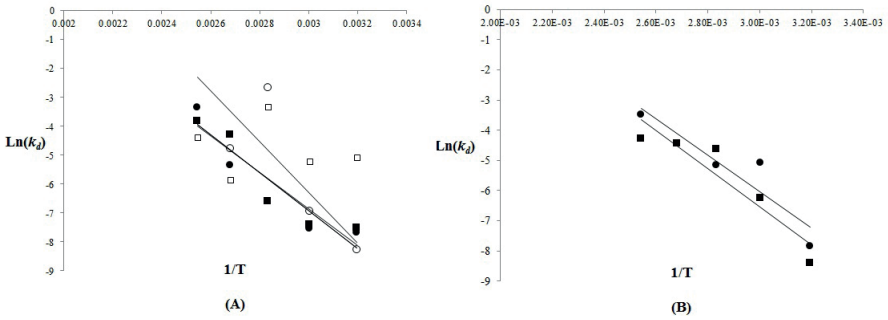

Figure 4.9. Arrhenius plots for first-order rate constants obtained with the fractional conversion model from batches 1 (●), 2(○) 3 (■) and 4 (□) in Offline DPPH assay (A) and online HPLC assay for batches 1 (●), 2 (■) (B).

As the data set is not contradictory to the Arrhenius law, the entire data sets of all batches (all times and temperatures) were modelled simultaneously (global fitting) to obtain the rate constants at reference temperature (80 °C) and the apparent activation energies, as follows (± 95% confidence intervals):

Offline DPPH antioxidant activity

$k_{80\,°C}$ = 1.0 x 10^{-3} ± 0.3 x 10^{-3} min^{-1}; E_a = 38.4* ± 7.3 kJ mol^{-1}; c_f = 246.6 ± 57.5 µg Trolox Equivalent 100 g^{-1} FW.

Online HPLC-DPPH antioxidant activity

$k_{80\,°C}$ = 4.3 x 10^{-3} ± 1.8 x 10^{-3} min^{-1}; E_a = 49.5* ± 10.3 kJ mol^{-1}; c_f = 244.8 ± 20.7 µg Trolox Equivalent 100 g^{-1} FW.

* These values represent apparent activation energies, since they are not describing the temperature effect of a single chemical reaction

Data of offline DPPH are shown in figure 4.10 and online HPLC-DPPH are in figure 4.11.

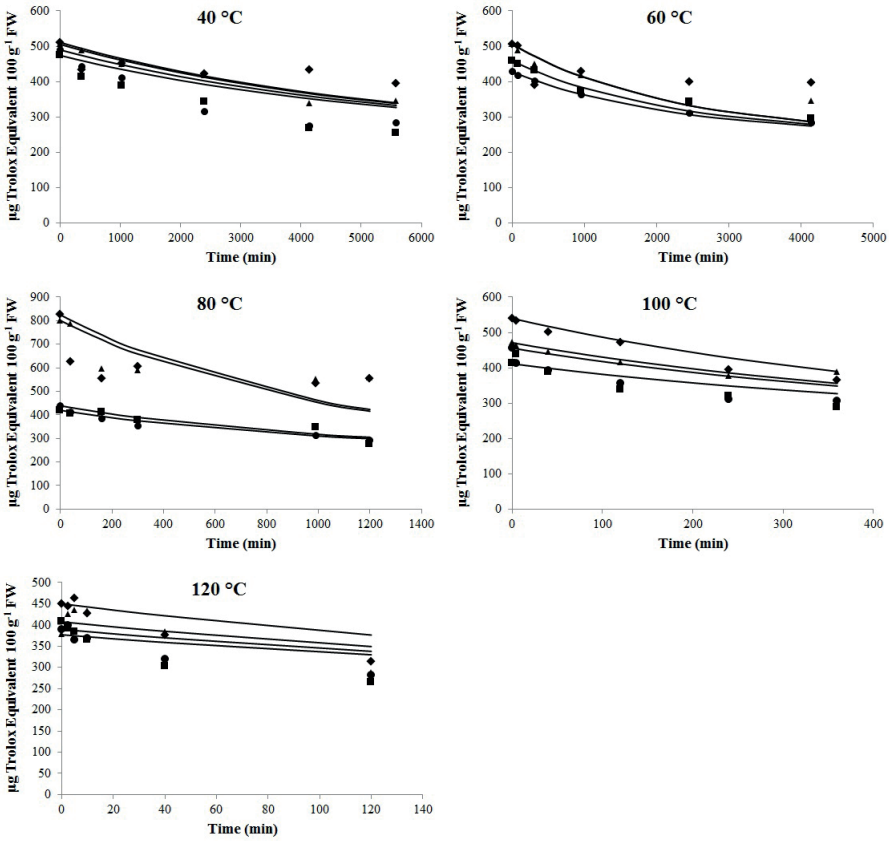

Figure 4.10. Thermal reduction of total offline DPPH values (antioxidant activity) in cape gooseberry (Physalis peruviana L.) at different temperatures for the four evaluated batches: Batch 1 (♦), Batch 2 (▲), Batch 3(●) and Batch 4 (■). Symbols represent the experimental data and lines the global model fits according to the fractional conversion model. $k_{80°C}$ = 1.0 x 10^{-3} min^{-1}; E_a = 38.4* kJ mol^{-1}; c_f = 246.6 ± 57.5 µg Trolox Equivalent 100 g^{-1} FW.

* These values represent apparent activation energies, since they are not describing the temperature effect of a single chemical reaction

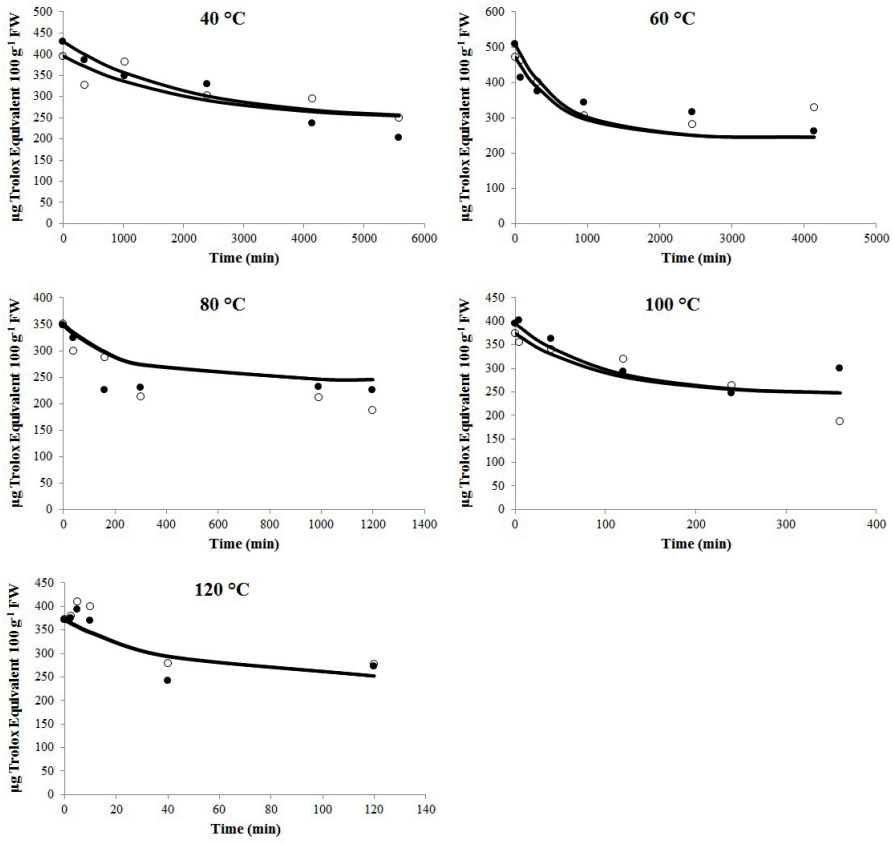

Figure 4.11. Thermal reduction of total online-HPLC DPPH values (Antioxidant activity) in cape gooseberry (Physalis peruviana L.) at different temperatures for the two evaluated batches: Batch 1 (●) and Batch 2 (○). Symbols represent the experimental data and lines the global model fits according to the fractional conversion model. $k_{80°C}$ = 4.3 x 10^{-3} min^{-1}; E_a = 49.5* kJ mol^{-1}; c_f = 244.8 µg Trolox Equivalent 100 g^{-1} FW.

* These values represent apparent activation energies, since they are not describing the temperature effect of a single chemical reaction

We also did the excersice with normalized data of both DPPH assays getting the following results:

Offline DPPH antioxidant activity: $k_{80°C}$ = 3.6 x 10^{-3} ± 1.2 x 10^{-3} min^{-1}; E_a = 39.8* ± 6.7 $kJ.mol^{-1}$ (plots not shown) and c_f/c_0= 0.7 ± 0.03.

Online HPLC-DPPH antioxidant activity: $k_{80°C}$ = 4.5 x 10^{-3} x 10^{-3} min^{-1}; E_a = 42.8* kJ mol^{-1} (plots not shown) and c_f/c_0= 0.6 ± 0.05.

DPPH values were reduced after heat treatment in both DPPH methods, giving comparable kinetic parameters (k_{80C}, E_a and c_f). Appendices 4 and 6 are depicting results combined for the two negative peaks obtained at 550 nm of online HPLC-DPPH assay, giving total DPPH antioxidant activity. These two negative peaks were compared with peaks obtained at 280 nm to determine the compounds that contributed to antioxidant activity. From spiking and comparing retention times, ascorbic acid and catechin and epicatechin were the compounds that gave a negative signal at 550 nm after reacting with DPPH reagent. The time required from the separation of the compound in the HPLC column to the signal in the post-column of DPPH analysis was approximately 2 min. Non-polar solvent extract was also tested with DPPH offline method to assess the antioxidant activity of β-carotene, however, due to the low antioxidant activity of β-carotene no clear results were obtained. Ascorbic acid had the major role in DPPH value, while catechin and epicatechin had only a small contribution. Figure 4.12 shows the plot of the correlation between offline and online HPLC- DPPH assays.

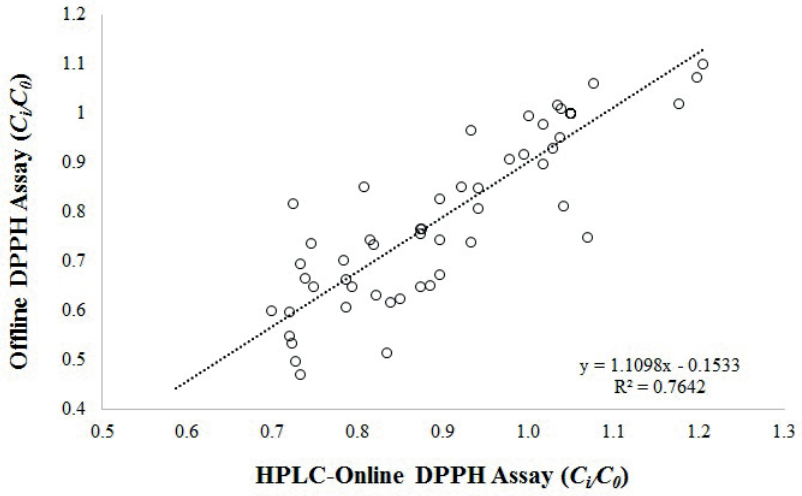

Figure 4.12. Correlation of antioxidant activity (offline DPPH with HPLC-online DPPH values). Pearson's coefficient is 0.89 and R^2=0.76. Symbols represent the experimental data and line the linear trendline.

Pearson's coefficients of the correlation between ascorbic acid concentration and offline and online HPLC-DPPH are 0.86 and 0.82, respectively. Epicatechin and catechin concentration had low correlation with antioxidant activity (Pearson's coefficients > 0.25) because of the high concentrations of ascorbic acid in comparison with flavonoids. From these results, it is concluded that the antioxidant activity of cape gooseberry, as assessed with DPPH assays, was mainly due to ascorbic acid rather than flavonoids; also the activation energy corresponds to the one found for this compound. In summary, antioxidant activity showed degradation during heat treatment with similar pattern as shown by ascorbic acid.

4.4. Conclusions

Thermal stability of different phytochemicals and antioxidant activity of cape gooseberry were assessed. Ascorbic acid degradation was described with first order reaction kinetics, and appeared to be until 15 times more stable to heat than other common good sources of vitamin C. β-carotene was also stable to heat and some isomerization from *trans*- to *cis*- was observed. Kinetic modelling of this lipophilic compound was not well possible because of the apparent increase of the compound caused by heat treatment, interfering with the isomerization reaction of β-carotene. Several peaks were detected in the attempt to identify flavonoids from methanolic extract of cape gooseberry. Only catechin and epicatechin could be identified after heat treatment. These compounds increased at 40 °C and showed degradation above 60 °C. The obtained data, however, did not allow kinetic modelling because catechin followed patterns of formation, degradation and epimerization, experiments need to be devised to unravel these various reactions. The best fit of HMF formation was obtained with a two-step consecutive zero- and first order reaction, respectively. The formation of this compound in heated cape gooseberry was slower compared to other products, probably due to less reactivity of the sugars of the cape gooseberry or the presence of compounds that inhibit HMF formation (catechins). Antioxidant activity was assessed by two DPPH assays (offline and online HPLC). Reduction of antioxidant activity could be described by a fractional conversion model and had comparable reaction rates and apparent E_a as ascorbic acid, which is concluded to be the compound that has the major role in the antioxidant activity measured with DPPH assay for cape gooseberry. In conclusion, this study showed that various health promoting compounds in cape gooseberry are subject to thermal degradation or formation but not to the extent that they are no longer present after heating.

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Chapter 5.

The quality-attributes-alignment perspective and its implications for the agri-food value chains *scale up*

Olivares-Tenorio, M.L., Dekker, M., Verkerk, R., van Boekel, M.A.J.S., Pascucci, S. (2016). The quality-attributes-alignment perspective and its implications for the agri-food value chains *scale up*. *Submitted for publication.*

Abstract

There are several aspects hindering the agri-food value chains *scale up*. Globalising agri-food value chains implies consumer orientation approaches that increase complexity and require high degree of coordination. We open the debate on implications for *scale up* process by studying the potential role of alignment issues, particularly focusing on preferences and perceptions of value chain actors in relation to quality attributes. We argue that when agri-food value chain has high degree of complexity, there is an increase of the likelihood of misalignment among actors, subsequently restraining a *scale up* process and affecting the performance of the value chain. From that premise, we suggest different pathways to *scale up* agri-food value chains. A case study based on cape gooseberry CG value chain has been embedded into the investigation to illustrate the theoretical models developed. CG value chain is an example of a fruit currently being in niche markets and facing difficulties to *scale up* to global. The CG value chain case study had three parts in which we involved quantitative and qualitative research methods and non-parametric statistics to support our theory building. Case study findings show a high degree of misalignment among actors that combined to the current low degree of complexity, characteristic of the *scale up* pattern where the chain is situated, might require a progressive *scale up* process, mitigating the potential risk-taking that going global brings. This paper contributes to theory and managerial practices on globalization of agri-food value chains by giving a consumer approach to tackle management and governance issues and facilitates the organization of activities to improve performance of agri-food value chain. Moreover, it is also a contribution to consumer-driven concepts to envision agri-food products as a subject that require to be studied from a value chain perspective in order to achieve consumer satisfaction.

Keywords: Quality; Customer Satisfaction; Organization; Strategy Development; Case Studies; Cluster Analysis; Conceptual Theory Development; Nonparametric Statistics; Qualitative Data Analysis

5.1. Introduction

Globalisation of agri-food value chains take along a set of challenges given by the continuous changing context the agri-food products, i.e. increased consumer awareness of food safety and healthiness and demand of diversity of food products [236, 74]. From an organizational design and value chain governance perspective, the *scale up* to global markets could be seen as performance indication that requires a consumer orientation approach which subsequently increases the overall complexity of the agri-food value chains, given the rise of the number of involved actors, type of interactions, standards, information and quality uncertainty increase [81, 74, 237]. Besides, the number and type of quality attributes also contribute in increasing the complexity [74]. This high degree of complexity of globalized agri-food value chains should be face with a suitable degree of coordination which is achieved by an efficient vertical integration [81]. The vertical integration is often related to alignment among actors [238-240]. Alignment is defined a dynamic capability in which firms of a supply chain engage to understand consumer requirements, codify and communicate them effectively along the chain [241-244]. This alignment can have different natures, therefore, given the consumer oriented approached required in agri-food value chains, we have focused this study of alignment on the preferences and perception related to quality attributes of vale chain actors.

This is a grounded theory investigation integrated to a case study. Herewith, we make contributions to both research and managerial practice by developing a theoretical model to contribute to the understanding of the role of complexity of agri-food value chains on alignment and *scale up* processes. For that purpose we set models of agri-food value chain *scale up* patterns and pathways to *scale up*. The case study is based on the Colombian Cape Gooseberry (CG) which is an example of local value chain currently experiencing challenges in the attempt to *scale up* at a global level [65, 55, 9]. CG value chain illustrates how the theoretical model can be interpreted and used to configure strategies to conduct a suitable *scale up* process, based on the current degree of alignment situation and attempting to mitigate the risk-taking of the agri-food value chain. The aim of this study is to inform scholars and practitioners focused on other agri-food value chains which are dealing with issues in moving from niche to a global scale, on how contextual and products aspects can have implication for complexity and subsequently for alignment in the value chain, affecting the *scale up* as one potential performance indicator of the value chains.

In the case study, the degree of alignment related to quality preferences and perceptions among actors on the *scale up* process of the CG value chain was empirically evaluated with a three-part research. Data from actors (farmers, traders, consumers) along the Colombian CG value chain have been gathered. These data were used to make an approachment in

answering the following research questions:

RQ1: How does the alignment of actors regarding quality attributes preferences and perceptions affect the *scale up* of the agri-food value chain?

RQ2: What strategy can be taken to *scale up* CG value chain based on the degree of alignment among actors of the agri-food value chain regarding quality attributes preferences and perceptions?

5.2. Conceptual background

5.2.1. From niche to global agri-food value chains

The concept of value chain goes beyond the consideration of physical activities required to bring a product to end users as it is described for supply chain, involving as well marketing and distribution aspects to respond to consumer needs in an efficient way [245, 76]. Several authors agree that value chain approach has a high impact in facilitating information transfer, enhancing learning, innovation and product quality by the good coordination of transactions [76-80].

Agri-food value chains have been facing several changes in the past years such as globalisation, liberalisation of market, increased awareness of food safety, healthiness of food and new products demands [236, 77, 246, 80, 74, 75, 247]. Thus, the need to introduce new technologies, the leadership in decision making on only one or few actors of the value chain [246], the high load of information to be transferred [236], the failure to meet consumer needs and the need to increase vertical integration [248, 249] have become crucial factors to succeed in global markets.

Bringing the kiwifruit (Chinese gooseberry) case as a reference, the key factors that turned this value chain from niche to global had both marketing and management basis. The presence of a leading value chain actor (e.g. Zespri co.) as a result of a vertical integration, which organised activities to invest in marketing research and promotion and the new organisation of the value chain allowed the understanding of the key drivers behind international consumer preferences enabling value chain actors to push the product acceptance globally [66]. The development of different varieties of fruit in order to attract more consumers has been also crucial, and the role of consumer preferences has been widely taken into account to redesign the value chain relationships [250-252, 249, 253, 66]. That important development could not be possible without the collaboration among stakeholders to conduct research and development activities. Besides the specific case of the kiwifruit value chain, the process of globalisation of agri-food value chains has been associated to the presence of strong leadership in the

decision making [246, 80, 254, 247], the use of new technologies to deal with the high load of information related to the increased number of transactions [236], the capacity to quickly manage changing consumer preferences, and the increase of vertical integration [248, 249]. Those activities underlie in the field of supply chain management and subsequently affect the performance of value chains. Therefore, the *scale up* of local agri-food value chains to a global scale could be seen as a performance indicator of a supply chain and requires moving from a supply-oriented to a so called market-oriented or consumer-driven governance model [81, 246, 74]. This governance model should contribute in advocating the improvement of performance in value chain by minimizing the production and transaction costs [255].

5.2.2. Agri-food value chains and alignment challenges

The literature on supply chain emphasizes the crucial role of integration, thus, the required process of market orientation to entry to global markets must involve every actor of the chain [256]. This premise has increased the interest among scholars and practitioners to look integration from a value chain perspective [257-259]. Market orientation of value chains is often associated with consumer-driven product development, in which consumers preferences for quality attributes become crucial matter [260]. Therefore, in agri-food value chains issues such as food safety and healthiness, as well as the diversity of food products, have become relevant [236, 74]. Consequently, elements related to consumer preferences about quality attributes need to be communicated and transferred to all other actors along the chain (upstream and downstream), therefore creating a great deal of coordination and integration among them [261, 256, 74] improving performances of value chains in global scenarios [241]. Recently, supply chain literature has recognised the importance of alignment among actors as a dynamic capability in which firms of a supply chain engage to understand consumer requirements, codify and communicate them effectively along the chain [241-244]. However, the achievement of an appropriate degree of alignment can be hinder by an ineffective knowledge transfer cause by differences in expectations, preferences, abilities, motivations and priorities among actors [261, 262]. This aspect increase the uncertainty in the value chain because of the lack of knowledge of actors about crucial factors related to the consumer which consequently negatively affect the capability of the value chain to achieve performance goals efficiently [263]. Besides, engaging in a process of internationalization of a product which has been previously marketed only in a given regional context, forces value chains to be able to quickly respond and adapt to preferences and needs coming from new typologies of customers (or consumers) (Jaeger et al., 2003; Lee et al., 2012) and increase the volume of products to be traded, and/or change/improve their quality attributes and standards, in order to meet an increased demand with differentiated preferences (Menard, 2005; Lee et al., 2012; Bonany et al., 2014). These changes have a direct effect on the degree of complexity which requires a suitable organization and integration in order to maintain the

value chain functioning efficiently. The increased complexity within the chain can hamper on knowledge transfer which is required to improve performance of value chain, causing misalignment among actors. [264, 265, 249].

5.2.3. The cape gooseberry value chain case

Cape gooseberry (CG) is the fruit of the plant *Physalis peruviana L.*, native from the Andean region. The fruit is available in domestic market in Colombia and international markets as fresh and processed food. The appearance of fresh CG makes it to be currently used extensively for dessert decoration in several European countries [73, 9]. The fruit contains relevant contents of certain vitamins and antioxidants [168] making it an interesting fruit to diversify the consumption of healthy foods.

In Colombia, CG is one of the most important fruit in terms of international trade, being the second most exported tropical fruit after banana [51]. The main destination is the European Union, particularly countries such as The Netherlands, Germany and Belgium. In 2015, these three countries traded 93% of the CG exported from Colombia with a market value worth of approximately US$ 25 million and a volume of 6,000 tons of fruit [51]. At the moment, Colombia is among the main producing countries worldwide. [59, 56]. Moreover, Colombian CG has a preferential price position in international markets because of its high quality properties (colour and sugar content) and a constant all year round supply [58-60, 56]. However, the value chain is still too fragmented in comparison with other fresh fruit value chains operating at international level [266, 260, 66]. The chain displays a very traditional structure that comprises 6 main stages, involving several inputs suppliers, small and medium farmers, local and international traders, food industry, and domestic and overseas consumers. CG production with high quality (usually determined by size and colour) is exported, while the remaining production goes either to processing or to domestic fresh fruit market.

The Colombian CG case can be considered as an example of a local value chain currently experiencing challenges in *scaling up* and go global to give more profit to actors of the chain. CG has been traded in European markets for about two decades but is still positioned in a niche segment of the market. The increase of exports from Colombia has been 2% from 2010 to 2015 [51], experiencing peaks in 2011 and 2012 but the fruit is not yet visible in the international market trade statistics because of low trade volumes [65, 55]. Statistics about production and trade of the last 5 years show that the attempt to *scale up* has been rather unsuccessful [51]. Moreover, from a preliminary exploration in countries to which CG has been exported, people revealed not to know the fruit well and are not aware of relevant quality attributes (data not shown). Previous reports highlighted the lack of integration of the CG value chain and the limited knowledge about preferences of consumers about quality attributes as the main possible barriers for the *scale up* process of the Colombian CG value

chain [9, 210, 68]. Nevertheless, barriers to *scale up* of CG value chain are not limited to the lack of knowledge of value chain actors about the preferences of international consumers of the product attributes. They include a wider set of factors, such as lack of communication between actors of the chain, a low efficiency in the cultivation and processing of the fruit and lack of innovation and research [9]. All these factors have to be considered in the *scale up* process, and particularly when trying to understand how to move CG value chain from a niche to a global scale, in case actors of the chain are interested in globalisation of the berry.

5.3. Theoretical model

5.3.1. Agri-food value chain *scale up* patterns

The proposed model has theoretical roots in the concepts of agri-food global value chain governance, economics, quality attributes, consumer-driven approach, food quality complexity and supply chain management. Value chain governance is the coordination of exchanges of information between firms [78] and has important impact in the chain efficiency [81, 74, 240]. Features of governance of the value chain are the degree of complexity of the information required to be transferred along the chain, the ability of that information to be codified and the capability of suppliers to fulfil those requirements [81]. As a value chain go from niche to global, the number of actors, requirements and transactions increase [237], the degree of complexity rises, therefore, this *scale up* requires a suitable governance model [267]. The degree of complexity from a food quality point of view can be defined in terms of product and process complexity [268], then in a value chain this degree is related to the number of quality attributes, the number of interactions within the chain and the degree of product novelty [269]. Agri-food products can have a variety of quality attributes. According economics concept, quality attributes types are *search*, *experience* and *credence* [74, 75]. *Search* refers to quality attributes that can be verified during the purchase process (e.g. colour, size, amount); *experience* attributes are assessed after the transaction (purchase) has taken place (e.g. taste, texture); and *credence* attributes cannot be evaluated, they are consequently based on trust (e.g. organic production, health-promoting compounds contents) [74, 75]. In general, in globalised food products there is a shift of quality attributes preferences of products from *search* to *experience* and *credence* attributes [270]. This further increases complexity of the value chain through a potential *scale up* process because aside of an increased number of interactions (number of transactions and amount of product to trade, the number and nature of quality attributes also may rise, increasing the uncertainty in the value chain [269, 268] represented by the likelihood of lack of knowledge along the chain [263, 74]. We relate this potential uncertainty with the likelihood of misalignment in the agri-food value chain. This is how quality attributes, complexity and alignment within the value chain shape the process of *scaling up*, and ultimately the globalisation of food products (Bonany et al., 2014). As we

already discussed, alignment in the value chain is a capability difficult to reach because of different preferences and perception of actors. In a consumer-driven value chain preferences should be address towards to meet preferences of consumers. These preferences could be measured in terms of quality attributes. Therefore alignment of value chain actors regarding quality attributes preferences of the consumers is required to guarantee food quality and consumer satisfaction and thus, contribute to the enhancement of degree of capability of the value chains to enter more global scenarios, improving their performance [236, 271, 265].

According to explained concepts, we have developed a model of "*Agri-food value chain scale up patterns*" connected to the likelihood of misalignment. These patterns are depicted in figure 5.1 and they are associated to different well-known niche and globalised value agri-food chains in order to make instructive representations. In this model the degree of complexity is determined by the type of quality attributes (X-axis), and the number of transactions (Y-axis) of an agri-food the value chain, typically increased when going from a nice to a global market.

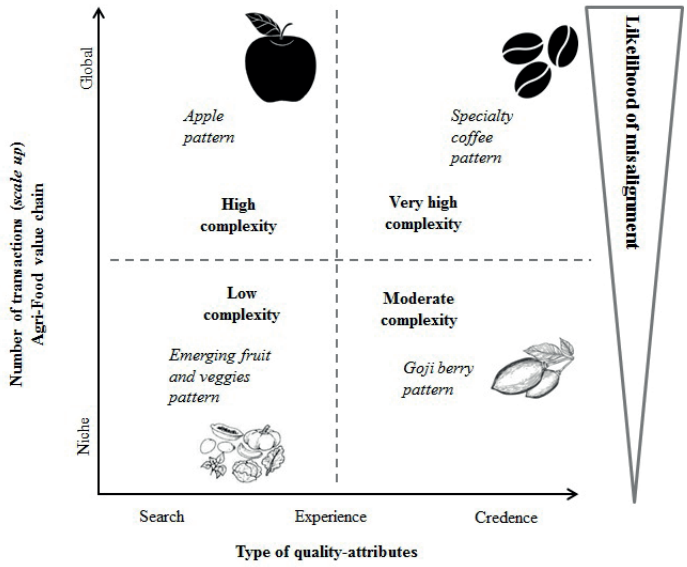

Figure 5.1. Theoretical model. Agri-food value chain scale up patterns

We have defined the number of transactions as the number of actors of the value chain, of trading processes and/or requirements to comply from customers, consumers and legalization.

The first pattern refers to chains that resemble the case of "*specialty coffee products".* In this case we observe a chain which has *scaled up* in terms of both number of transactions and types of quality attributes considered, which combine *search*, *experience* and *credence*

attributes (e.g. fair trade, organic, certifications). These products are also regularly and widely consumed. These characteristics make this typology of value chain to have a high degree of complexity and be more likely to have issues of misalignment among actors operating in the chain and final consumers, particularly in terms of product quality-preferences [237]. Agri-food value chains that follow this pattern to *scale up* manage to combine a high degree of product diversification, diffuse quality certification and standardization processes along the chain, as well as coordination imposed by leading value chain actors such as international traders and/or retailers. Within this pattern we can find value chains of agri-food products such as tea, cocoa, organic and fair-trade certified food products [272].

The second *scale up* pattern (*apple)* refers to what has been experienced by several fresh agri-food products such as apple, pear and orange. In this case the process of diversification of the product has been limited mainly to *search* and *experience* quality attributes, while *credence* aspects are to some extent less relevant. This pattern is also characterized by a high degree of complexity because it involves a large number of transactions [81]. Although, according to our view a high complexity of the value chain makes it prone to misalignment, the alignment between value chain actors and final consumer preferences has been successfully achieved through an intense process of standardization of the production, combined with a limited diversification and use of certification and brands [74] as a consequence of an effective integration of the chain. In other words, only few varieties have been selected and *scale up,* while quality standards, certification and branding are mainly controlled or managed by the retailers rather than by producers or traders.

The third pattern, which we defined "*goji berry"*, is obviously inspired by the goji berry value chain, in which product diversification includes *credence* components mainly associated to the promise of health-promoting compounds contents. Value chains which follow this *scale up* pattern are typically dominated by a strong player which is also managing the production stage. As in the case of goji berry, kiwifruit or tropical fruit such as pineapple or banana, branding and quality standards are controlled and managed by few international traders/ corporations while the role of retailers is somewhat limited in the process of alignment with consumer preferences [273]. This *scale up* pattern is characterized by a moderate degree of complexity because it involves a lesser number of actors involved (who are part of the number of transactions). In this case, the likelihood of misalignment could be mitigated by the control and strong integration provided by the value chain leader. The forth *scale up* pattern refers to so-called "*emerging fruit and veggies"*. These are value chains of products with potentials to globalise, since they are appreciated in regional and/or niche international markets. Within this pattern we can find emerging fruit and veggies, such as kale and sweet potato. This pattern is characterized by a low degree of complexity because it involves a short number of transactions (actors, legislations) because the product is present locally or at

niche level and involves mainly *search* and *experience* types of quality attributes. However these value chains are still too fragmented while lacking the presence of actors with a strong leadership.

Regardless the specific pattern followed, several studies have emphasized that *scale up* processes are associated to high likelihood of misalignment among actors, and particularly with final consumer preferences, demanding high degree of coordination, information and control costs [238-240]. As seen in the *apple* and *goji berry* patterns, likelihood of misalignment is reduced by more integration among actors, often lead by a dominant/ powerful company either at processing, trading or retailing level [274, 81, 78]. However integration may be difficult to realize, or too costly, particularly in the early stage of the *scale up* and globalisation process, thus leaving the misalignment problem unsolved. This may constitute a bottleneck for the *scale up* and globalisation process, disrupting the opportunities that several local producers may gain when their products are sold from niches into global chains. Although we propose there is a relation between complexity and alignment in the value chain, it is still unknown at what stage of the value chain the misalignment is more likely to occur, namely whether between consumer, trader or farmers. Furthermore, it is still unknown whether specific issues of misalignment are due to quality attributes preferences and perceptions *per se* rather than for other buyer-seller issues (e.g. lack of communication or infrastructure). These issues have implications in terms of how to tackle the misalignment problem. Understanding the nature and degree of misalignment is relevant to design effective value chain configurations, particularly in a process of *scale up*. For that reason, given out consumer-driven approach, we have set alignment as the capability of the agri-food value chain to have a common goal towards to meet consumer preferences. Hence, the measure of degree of alignment/misalignment would be subjected to the matching of preferences and perception of value chain actors comparing with consumer preferences.

5.3.2. Pathways to *scale up*

From above discussion we stated that to *scale up* from niche to global markets an agri-food value chain required a governance model with market oriented value chain, consequently dealing with increased complexity and quality uncertainty [81, 74, 267, 237]. This high complexity and uncertainty might require a vertical integration to allow a better communication and information transfer [76-78, 261, 80]. This implementation of integration might require an important investment that can enhance a financial risk [275], thus this vertical integration must make a careful management of resources (Ralston et al, 2011). The risk in supply networks is positively related to the degree of complexity which is risen in globalisation processes [276]. From the model developed and depicted in figure 5.1, we have set a number of pathways that agri-food value chains can take to *scale up* (figure 5.2A). The

chosen pathway should be subjected to the degree of complexity of the value chain, such that the complexity is positively related to the risk-taking level of the agri-food vale chain (figure 5.2B) [276].

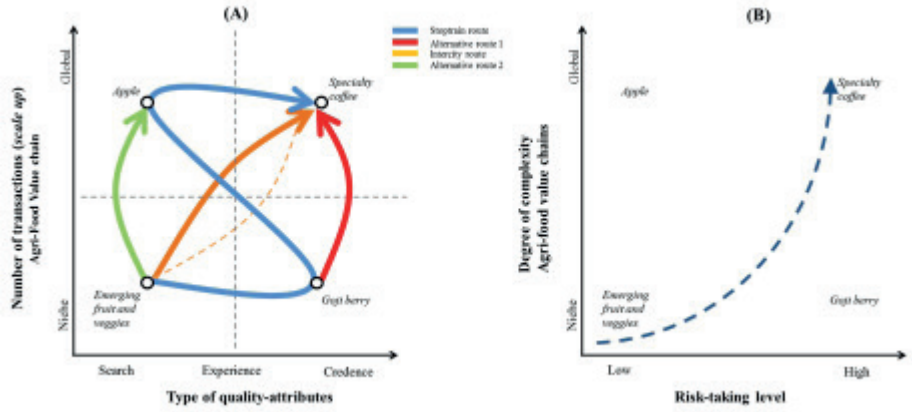

Figure 5.2. *Scale up* pathways and risk-taking level

The model has 4 alternative pathways for *scaling up* which define the movement within the four agri-food value chain *scale up* patterns described in figure 5.1. These pathways have been graphically inspired by a train routing map. The first pathway can be defined as a "*stop-train route*". This means that the agri-food value chain could *scale up* progressively starting by exploring and investing in offering more varieties of fruit, giving added value such as special certifications, investigating and promoting health properties, or entering in the organic or fair trade market. Taking this direction, the first step would be to move to the *scale up* pattern of "*goji berry*" and then continue with the other two quadrants of figure 5.1. This pathway might require less risk and investment from actors of the value chain, at least straightaway, in comparison to the other directions. It also allows progressively improving the coordination and degree of alignment of the value chain. When being in the quadrant of "*goji berry*" *scale up* pattern, the agri-food value chain faces a trade-off, continuing in the "*stop train*" to go to the "*apple*" pattern or taking the "*alternative route 1*" to go to "*specialty coffee*" pattern. Once more, it depends on the capabilities of the value chain at that point and the situation at the moment with respect consumers preferences. If consumer preferences increase especially in *credence* quality attribute, the only option to *scale up* for the value chain would be to move "*specialty coffee*" *scale up* pattern. In this pattern, the degree of complexity is the highest; therefore value chain will need to learn from the gained experience in offering *credence* quality attribute and to generate investment in providing high volumes of production. Here again, the issue of misalignment required to be solved by a proper coordination model.

Going to "*apple¨* pattern will require stronger vertical integration to reduce likelihood of misalignment, thus, the investment could be focused on communication and alliances. "*Alternative route 2*" moves the value chain from *¨emerging fruit and veggies¨* pattern to *¨apple¨* pattern. This pathway would be followed in case the agri-food value chain wants to continue offering the same type of quality attributes but in a global level. This means that consumers are still looking for physical properties of the fruit and/or price. In this pathway, the investment in founding a strong integration is needed as well as in improving quality of the fruit and production in order to get the capabilities to attend the large volumes of demand of this *scale up* pattern.

The most ambitious and risk-taking pathway the "*intercity*" because the agri-food value chain would move from the most basic *scale up* pattern to the most complex. The reason an agri-food value chain might chose this option is when consumer preferences are addressed to *search*, **e***xperience* and *credence* quality attributes.

5.4. Methodology

5.4.1. Research approach

The case study of CG value chain has been designed to be qualitative, quantitative and exploratory, thus involving multiple data gathering methods and tools. The three-phase research framework is depicted in figure 5.3.

5.4.2. Phase I: Empirical analysis

The empirical analysis has been structured in a *consumer study,* in which 3 focus groups (25 consumers), 105 face-to-face surveys and 304 online surveys with people in Europe (The Netherlands) have been conducted, and a *value chain actor's study*, in which 66 face-to-face interviews with value chain operators were performed. All in all, the empirical investigation has involved a total of 500 participants. Table 5.1 shows the descriptive statistics of participants in the empirical investigation.

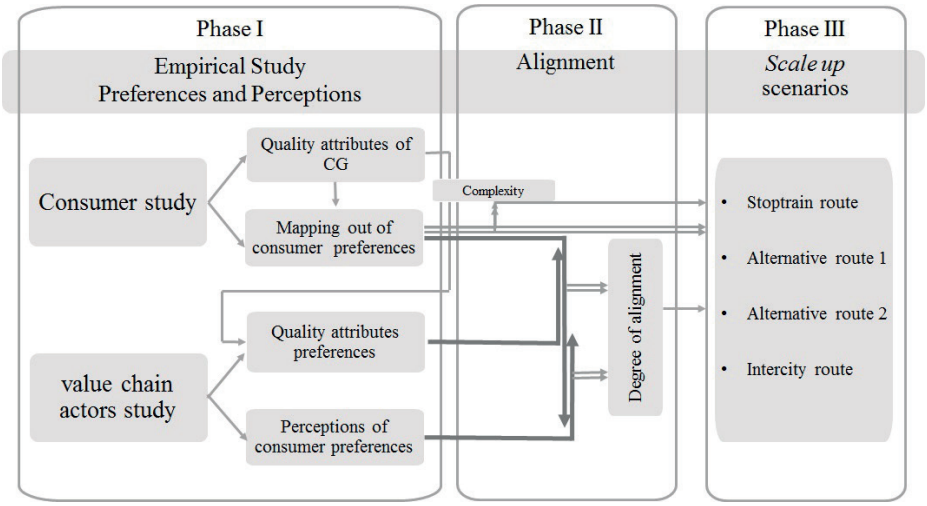

Figure 5.3. Research framework

Consumer Study

The consumer study involved three elements: consumer focus group, face-to-face survey and online survey. The aims of this study were to define the main quality attributes of the CG and their descriptors as well as to map out consumer preferences of the Colombian CG.

Quality attributes of CG: The first stage of the research has been to define the main quality attributes of the CG and their descriptors. For that purpose, a convenience sampling was conducted and 3 experienced-consumer focus groups were performed in Colombia in April-May 2013, with groups of 7-10 people (total of 25 people) (table 5.1). The selection of Colombian consumers was motivated by the need to investigate quality attributes specified by experienced consumers who are familiar with the fruit. During the sessions, consumers were asked questions about what they value when buying CG, frequency of consumption, ways of consumption, reasons of consumption, descriptors of quality attributes, preferences in quality attributes and the most common quality issues they usually face with the fruit. Sessions were moderated by two researchers and involved samples tasting. Although price was a quality attribute considered of high importance for Colombian consumers, they did not know international prices, therefore the descriptors for this attribute were obtained by asking export companies.

Table 5.1. Descriptive statistics of participants

Consumers (Colombia)	*n* = 25		
Age (years old)	*n*	*Gender*	*n*
Under 18	0	Female	17
18 -25	12	Male	8
26- 35	9	*Country of residence*	*n*
36-50	4	Colombia	25
Over 51	0	*Country of nationality*	*n*
		Colombia	25
Participants (Europe)	*n* = 409		
Age (years old)	*n*	*Country of residence*	*n*
Under 18	3	The Netherlands	400
18 -25	75	Other EU*	6
26- 35	114	Non-EU**	3
36-50	85	*Country of nationality*	*n*
Over 51	132	The Netherlands	305
Gender	*n*	Other EU*	51
Female	159	Non-EU**	53
Male	250		
Traders	*n* = 22***		
Local retailer (Europe)	3	Exporter (Colombia to Europe)	9
Distributor (Europe)	10	Importer (Europe from Colombia)	12
Processors	*n* = 11***		
Dried fruit	11	Juice	2
		Jam	2
Farmers	*n* = 33		
Cultivation region	*n*		
Nariño	1		
Cundinamarca	19	Small farmer <4999 plants	28
Boyacá	13	Medium farmer >5000 plants	5

*Other European Country; **Non-European Country: ***number of companies (usually with more than one role or product)

Mapping out of consumer preferences: Convenience sampling with face-to-face survey and online survey were used to collect information about CG consumption. Because this is research about barriers of the *scale up* of CG value chain and The Netherlands is the biggest import country of the Colombian CG, empirical consumer research was done in that country. The aim of this mapping was to identify groups of people in order to illustrate the current situation of the CG value chain in terms of consumption *versus* quality attributes. Participants have been clustered according to their actual frequency of consumption or willingness to consume CG, and their preferences regarding *search*, *experience* and *credence* quality attributes. This information was used as base to support our understanding of the complexity of consumer-driven elements and the type of quality attributes to be considered in the *scale up* process of the CG value chain based on figure 5.1. The mapping has been conducted using a two-step cluster analysis with the statistical package for social sciences SPSS[172].

Face-to-face survey: Using the insights gathered in the Colombian consumer focus groups, face-to-face surveys have been designed to be used in the analysis of Dutch consumption of CG. To implement the field surveys, the retailing company Albert Heijn supported the project by allowing making the surveys in some of their supermarkets. Four stores were chosen to conduct the study based on the top 10 stores selling CG and also on geographic location. The purpose of this selection was to increase the probability to recruit into the study a higher number of actual consumers of CG.

Questionnaires were developed and validated to collect information from consumers and non-consumers. These questionnaires had English and Dutch versions and were filled out *in situ* digitally with the use of Qualtrics tool (https://www.qualtrics.com/). Interviewees were asked about their preferences in quality attributes of CG and their willingness to become a habitual consumer. For that purpose, digital questionnaires included a set of pictures from where they could select their choices of quality attributes. Quality attributes of CG were asked based on NTC 4580 (see appendix 1) [63]. Samples of the product were also available *in situ* to further inform interviewees about the fruit. A total of 105 valid face-to-face surveys were conducted in August and September 2015 by two researchers in Albert Heijn stores in Amsterdam, The Hague, Roermond and Maastricht.

Online survey: Same questionnaires adapted and sent to a database of the Food Quality and Design group (Wageningen University and Research), containing people from The Netherlands. The survey was also sent to employees and students of Agro-technology and Food Science department of Wageningen University and Research, The Netherlands. Online questionnaires links were sent out in August 2015 and were kept available for one month. A total of 304 valid responses were obtained.

Value chain actors study: Among the value chain actors, three specific groups have been

targeted (farmers, processors and traders). Questionnaires were developed and validated to collect the information about quality attributes preferences of value chain actors and their perceptions of consumer preferences. Questionnaires were accompanied with a set of pictures to facilitate their choices. Quality attributes of CG were asked based on NTC 4580 (see appendix 1) [63]. The selection of data gathering methods were made based on efficiency criteria (time, resources and willingness to participate) as described in table 5.2.

Table 5.2. Data gathering methods

Category of value chain actor	Gathering method	Number of participants
Farmers	Face-to-face interview	33
Traders	Face-to-face interview	22
Processors	Face-to-face interview	8
	Online-survey	3
Total		**66**

Farmers were approached by face-to-face interviews (with the use of the validated questionnaire) within a convenience sampling. Interviews were conducted by two researchers in May 2015, reaching out 32 farmers located in Boyacá (Ventaquemada) and Cundinamarca (Gamma), which are relevant CG production regions in Colombia [51]. One farmer was interviewed in Germany along with other value chain actors.

Traders such as exporters, importers and distributors have been approached. Purposive sampling and face-to-face interview (with the use of the validated questionnaire) were conducted. 22 face-to-face interviews were eventually performed in two main professional fairs in Germany, namely FRUIT LOGISTICA 2016 in Berlin and BIOFACH 2016 in Nurnberg.

At the same venues we also interviewed CG *processors*. The current number of processors of CG operating in Colombia is not really high (about 20). Therefore purposive sampling was used, approaching 8 CG processing companies with a face-to-face interview and 3 with an online survey.

Preferences and perceptions: in this study, preferences refer to a greater liking of certain alternatives. Preferences of consumers, farmers, traders and processors were assessed with data obtained from the consumer and value chain actors' studies (farmers, traders, processors). Perceptions denote the ability to be aware of something. In this research, perceptions of actors (farmers and traders) in relation to the preferences of consumers about quality attributes were assessed based on the questioning of actors about what they believe what consumers prefer with respect to the presence of calyx and the most valuable quality attributes consumer ranked. In the perception assessment, processors were not included because they do not trade

fresh fruit.

Preferences and perceptions of quality attributes were assessed by frequentist inferences with SPSS. Chi squared test was used to estimate the significant differences in preferences and perceptions between groups (consumers, processors, traders and farmers) [277].

5.4.3. Phase II. Alignment

The degree of alignment of the CG value chain actors was assessed from two points of view: a) the extent of alignment or similarity between actors (consumer, traders, processors and farmers) regarding their quality attributes preferences; and b) the extent of alignment or matching of value chain actors (traders and farmers) with respect to perceived quality attributes preferences of consumers. For the first case, we are referring to preferences of quality attributes of value chain actors and for the second, to the perception of actors of the value chain in relation to consumer preferences. Alignment was evaluated based on chi squared test [277] with the use of SPSS.

5.4.4. Phase III. *Scale up* pathways

The study of a suitable *scale up* pathway to globalise CG value chain has been conducted using the model of figure 5.2 and taking into account results on degree of alignment and *scale up* pattern of the agri-food value chain.

5.5. Results

5.5.1. Quality attributes of CG

Table 5.3 shows the list of quality attributes of CG and their descriptors as defined by the experienced consumer focus groups in Colombia. We have subsequently classified these attributes in terms of *search*, *experience* and *credence* features.

Table 5.3. Quality attributes of CG and descriptors

No./Quality attributes*	Descriptors	Type of quality attribute
1.Presence of calyx	With calyx/Without calyx/Indifferent/both	*Search*
2.Colour of berry	0;1;2;3;4;5;6 according to NTC 4580**	*Search*
3.Colour of calyx	0;1;2;3;4;5;6 according to NTC 4580**	*Search*
4.Size of berry	2.5 cm; 2.1 cm; 2.0 cm; 1.8 cm; 1.6 cm; 1.4 cm	*Search*
5.Taste	Sour/sweet	*Experience*
6.Texture	Hard/soft	*Experience*
7.Price (fruit with calyx, 100 g)	< €1.25; €1.25-2.00; > €2.00	*Search*
8.Price (fruit without calyx, 250 g)	< €3.50; €3.50-5.00; > €5.00	*Search*
9.Origin	Country specific	*Credence*
10.Type of production	Organic/traditional	*Credence*
11.Certification	Having/Not having GPA certification	*Credence*
12.Spoilage	Presence/absence of moulds	*Experience*
13.Shelf-life	Short (1-5 days)/long < 5 days	*Experience*
14.Nutritional aspects	vitamins and antioxidants	*Credence*

*There is not a specific order in the list. ** Standard NTC 4580 in Appendix 1.

5.5.2. Mapping out CG of type of quality attributes consumer preferences

Based on the outcome of the focus group analysis we used the selected and above presented quality attributes to figure out the preferences of surveyed people and to group them accordingly. The result of the two-step cluster analysis indicated a number of 5 clusters with a silhouette measure of cohesion and separation close to 1.0 (good). Figure 5.4 shows the 5 clusters obtained from responses of participants of the face-to face and online surveys.

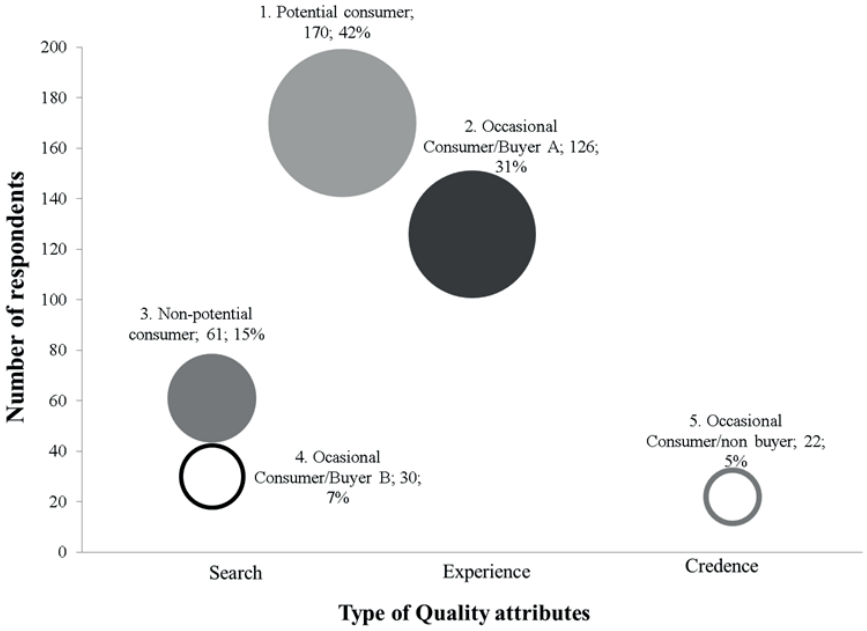

Figure 5.4. Mapping out of consumer preferences

"Potential consumers" (1) was the biggest cluster with 170 participants (42%), this group of people are generally non-current consumers that might or might not know the fruit and express a willingness to consume it and their interest is related to *search* and *experience* quality attributes, especially taste and price. Second biggest cluster (2) has 126 people (31%) that are occasional consumers and buyer consumers (A). These people are interested in *search* and *experience* quality attributes such as taste and permanent availability. Third cluster has been named non-potential consumers (3) who are people that did not show interest in CG but have stated to be interested in *search* quality attributes (15%). Fourth group (4) are again occasional consumers/buyers (B), people that consume and participate in purchase and look for *search* quality attributes like size and price (7%). The last cluster has 22 occasional consumers/non-buyers (5) who are into *credence* quality attributes, especially, health properties contents and organic or local production (5%). This type of people does not participate in the purchase, but consume the product bought by someone else, or in restaurants and hotels. Overall, the majority of participants showed to look more for *search* quality attributes such as price, colour of the berry, *experience* quality attributes like taste and shelf life and to a lesser extent for *credence* quality attributes such as health properties and organic production

5.5.3. Alignment among value chain actors related to preferences of quality attributes

Evaluated preferences of value chain actors (consumers and other actors) about quality attributes are depicted in figures 5.5, 5.6 and 5.7. In this evaluation, responses from all participants of the consumer study were taken as consumer responses. The number of responses *N* is higher than the total number of participants, because the frequentist analysis takes into account the total of responses, and people could make more than one choice in their selections for most of the evaluated quality attributes.

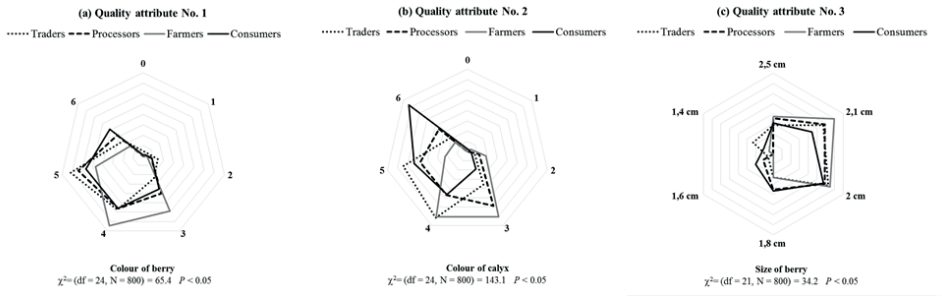

Figure 5.5. Preferences of quality attributes of actors of CG value chain

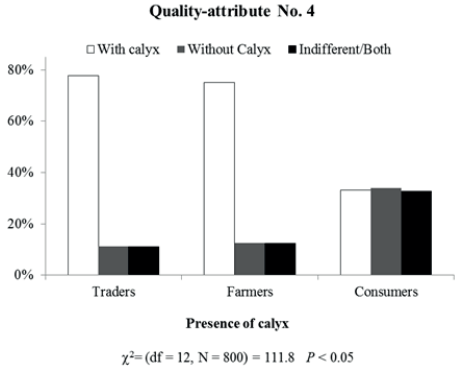

Figure 5.6. Preferences of actors of CG value chain respecting the presence of calyx

Figure 5.7. Most valuable quality attributes for CG value chain actors

Quality attributes such as colour of berry (figure 5.5a), colour of calyx (figure 5.5b), size of berry (figure 5.5c) and presence of calyx had significant differences between actors ($P < 0.05$). At a glance one can see there is misalignment in these aspects. However, in some cases preferences of quality attributes between actors of the chain could differ because of the different circumstances required to meet costumer and consumer requirements. For instance, the fact that agri-food products continue changing during the process from cultivation to consumption, actors (traders, farmers) of the value chain take measures to provide fruit with the characteristics required by consumers. This might be the case of colour of the berry (figure 5.5a) in CG case, where farmers move down preferring yellow colours (3-4) in contrast to consumers that prefer orange colours (5-6). In case of preferences for the colour of calyx had higher differences because consumers prefer brown instead of green (figure 5.5b). Farmers, however, prefer green, what could also be related to the anticipation of farmers to deliver suitable fruit to consumers.

Preferences in size of berry, however, showed misalignment among actors (figure 5.5c). While farmers and traders seem to have preference for big sized fruit, consumers have also chosen small berries in their preferences, arguing that they like the appearance of small berries and also they could get more units per package. Since size of the berry is not changing along the processes of the chain, this cannot be named anticipation of upstream value chain actors.

Preferences related to calyx presence are also misaligned (figure 5.6). Farmers and traders prefer the fruit with the calyx, probably to facilitate the logistic process and maintain the shelf life of the fruit. Nevertheless, participants of the consumer study have shown a different preference being interested in the fruit without the calyx and sometimes just being indifferent with this quality attribute. This result clearly shows how the preferences of actors hinder the achievement of consumer wants (olive), showing misalignment and indicating the need for more vertical integration and knowledge transfer.

Rankings of the most valuable quality attributes also show differences (figure 5.7). As

expected, actors expressed their preferences based on their own interests. That is how for farmers, the most valuable quality attributes are colour of the calyx, ripeness stage and size of the berry because they get paid with fixed prices according to these attributes. Traders, however, expressed their preference in shelf life, price and food safety. For consumers, the most valuable quality attribute is price, followed by colour of the berry and ripeness stage. In summary, farmers and traders did not match the most valuable quality attributes for consumers. However, traders once again are closer to know when stating price and shelf life as consumer most valuable quality attributes.

5.5.4.Alignment among value chain actors related to perceptions of consumer quality attributes preferences

Differences in perception of farmers and traders and consumer preferences were found ($P < 0.05$) (figure 5.8). In short, farmers and traders have erroneous notion of what consumers prefer in terms of presence of calyx and most valuable quality attributes.

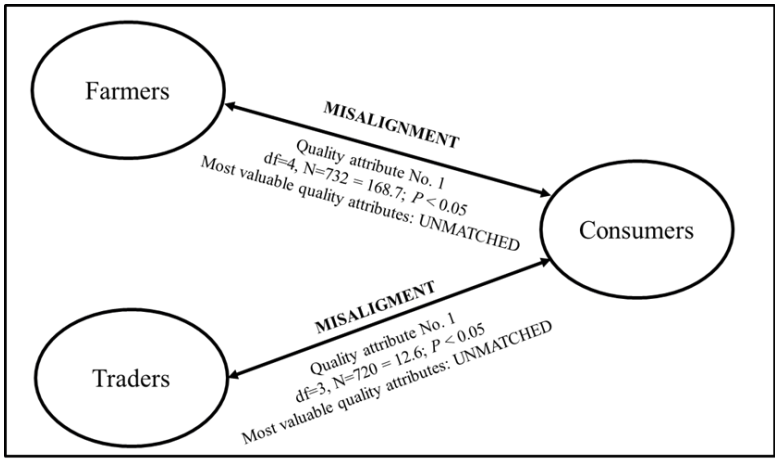

Figure 5.8. Misalignment among farmers and traders in relation with consumer preferences

Figure 5.8 represents the misalignment among farmers and traders in relation with consumer preferences. When farmers and traders were asked about what they believed consumer preferred in relation to presence of calyx and most quality attributes, they did not match consumer preferences. However, traders were closer to know when stating price and shelf life as consumer most valuable quality attributes. Aside of preferring the presence of the calyx, farmers and traders actually believe consumers also have those preferences, which is far from the complete truth. The fact that the fruit is commonly used as decoration in European countries, probably make farmers and traders assume that consumers prefer the fruit with the calyx. However, we already gave evidence that consumers are willing to buy the fruit without

the calyx. A third part of consumers, did not mind at all about the presence of absence of the calyx, especially for those who were not familiar with the fruit. About the most valuable quality attributes, consumers stated that price is very important when deciding to buy the fruit, so are the colour of the berry and ripeness stage (specially assessed by colour of the berry). Nevertheless, traders and farmers think that consumers are looking for food safety, colour of calyx and size of the berry.

Overall, results on preferences and perceptions highlight the presence of issues of misalignment in CG value chain. Farmers are more misaligned with consumer preferences. Traders and processors have a similar degree of alignment but still do not match all consumer preferences. This low degree of alignment highlights that the CG value chain has a low vertical integration, thus a low degree of coordination.

5.5.5. Pathways to *scale up* CG value chain

In this study we have detected that CG value chain have misalignment issues that might be restraining a *scale up* process, keeping the chain within a "*emerging fruit and veggies*" agri-food value chain *scale up* pattern for almost two decades. The question remains about how to envision a *scale up* strategy at value chain level, taking into account the misalignment issues. Using the pathways to *scale up* model (figure 5.2); we attempt to figure out how Colombian CG value chain can go global, if so desired. The results on CG value chain also showed that current and potential consumers are looking for *search* and *experience* quality attributes. However, there is an emerging trend of looking for *credence* quality attributes in this fruit, namely organic production and health-promoting compounds. This trend can be developed by transferring information along the value chain about these needs in order to coordinate a change to offer consumers what they want and *scale up* the value chain. Based on the degree of complexity characteristic of the *scale up* pattern where CG value chain belongs and the misalignment among actors found, the "*stop train*" pathway could be a convenient route to follow as depicted in figure 5.9.

The current misalignment of the value chain can be solved progressively while extending the offer of products with *credence* quality attributes. The reason we think CG value chain should move to increase the offer in terms of quality attributes rather than in terms of volumes is also because of the large number of competing fruits that have gained a big share of the market and are very known by consumers, leaving CG value chain in a weak position.

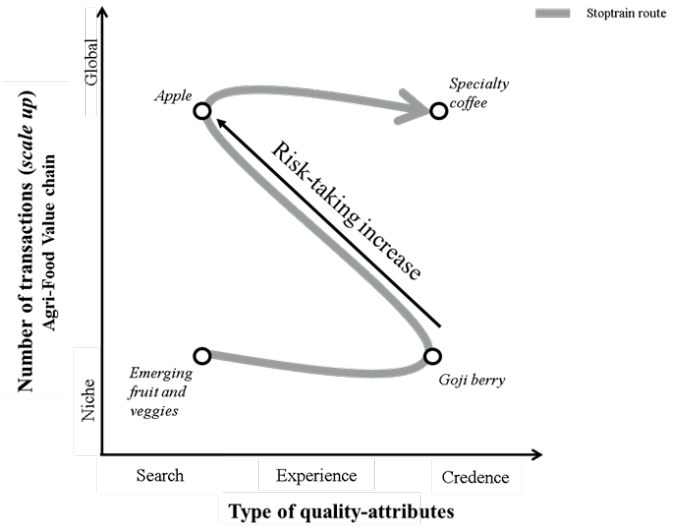

Figure 5.9. Stoptrain route to *scale up* CG value chain

This pathway might require less risk and investment from actors of the value chain, at least straightaway, in comparison to the other pathways. It also allows having a progressive improvement of coordination and degree of alignment. According to the chosen pattern, the most risk-taking movement would be when going global as depicted in figure 5.9, where the CG value chain might need to invest considerably in marketing and coordination strategies and probably in infrastructure to provide high volumes of fruit. Therefore, it is suggested to reinforce vertical integration and mitigate misalignment before going to global markets in order to moderate the risk-taking when going global.

5.6. Conclusions

This study advances in agri-food value chains literature by developing a theoretical model to identify agri-food value chains patterns of *scale up* in terms of the complexity of the value chain, based on the number of transactions involved and the number and type of quality attributes, in a frame of consumer-driven and vertical integrated value chain structure. The *scale up* patterns were related to the likelihood of misalignment of the value chains. Moreover, the model was complemented by pathways of *scale up* model. The case of Colombian CG value chain was integrated to the theoretical development with a three-part empirical research in order to illustrate the theory building. This empirical part involved consumer and value chain actors' studies with the participation of 500 actors of the value chain and allowed us to set the quality attributes of the CG, the consumers preferences, the preferences and perceptions of value chain actors in relation to CG quality attributes and subsequently, evaluate alignment among actors in relation to preferences and perception of quality attribute

in order to be able to provide pathways to *scale up* the CG value chain.

Based on results, the CG value chain is closer to the *emerging and fruit veggies* value chain of *scale up* pattern where the complexity is low. Nevertheless, farmers, processors and traders are misaligned regarding the preferences of consumers. In general traders are the actors that have more knowledge about consumer preferences probably because they are closer to consumers in the value chain structure.

In order to tackle misalignment issues, we proposed the *"stop train"* as the most suitable pathway to *scale up*, because it allows the CG value chain to progressively *scale up* by promoting the interest of *credence* quality attributes while working on solving its misalignment troubles by building a proper coordination structure of the chain, and meanwhile mitigating risk-taking before going to global markets. This paper contributes to literature on globalization of agri-food value chains by giving a consumer approach in order to tackle governance issues and facilitates the organization of activities when trying to *scale up.* Moreover, it is also a contribution to consumer-driven concepts to envision agri-food products as a subject that require to be studied from a value chain point of view because there are several factors along the chain that need attention in order to achieve consumer satisfaction.

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Chapter 6.

General discussion

6.1. Introduction

Cape gooseberry is a fruit cultivated in Andean countries. Currently it is available in different places worldwide belonging to niche markets, namely a small number of people demanding for it. In Colombia, the production of this berry grew considerably in the 90´s until the middle of 2000´s, since then the production and trade have been stable. There are several aspects that restrain the success of the cape gooseberry to *scale up* to bigger markets. On the one hand, there are governance issues of the value chain that make it inefficient. And on the other hand, the fruit is highly perishable, thus there are issues along the chain that affect the quality of the fruit. Cape gooseberry has been associated with potential health benefits and specially with the contents of certain phytochemicals and antioxidant activity. Nowadays, this perceived quality attribute is used as a marketing driver to promote the consumption of cape gooseberry as a health or so-called "superfood". However, there was only scattered reliable information about the presence and content of health-promoting compounds in cape gooseberry. This research investigated the cape gooseberry chain with an interdisciplinary approach of food quality and managerial and economical sciences. This approach evaluated quality attributes of the cape gooseberry during the supply chain, including the changes in the contents of health-promoting compounds; and assessed the degree of alignment of the actors in the chain regarding their preferences of the quality attributes, and potential barriers for the *scale up* of the cape gooseberry value chain were investigated.

6.2. Main findings

In **chapter 2**, a critical literature review showed scientific information on the content of various health-promoting compounds in cape gooseberry, such as vitamin B, C, and E, β-carotene, phenolic compounds, minerals and fibre. Although there was a large variation in published values, a comparison between results of different papers was conducted, finding a trend of increasing contents of vitamin C and β-carotene during post-harvest, related to ripeness stages. However, in general not much information was found on the behaviour of health promoting compounds and antioxidant activity during pre-harvest, post-harvest, storage and processing. Thus, further investigation on this aspect was suggested. In **Chapter 3** cape gooseberry was found to be a climacteric fruit with difficulties to retain an acceptable level of quality attributes. The experiments conducted aimed to estimate shelf life of cape gooseberry. Temperature has an effect on the shelf-life of the fruit. A temperature of 8 °C gave a longer shelf life for fruit with and without calyx, compared to 4 and 12 °C. The removal of calyx is a crucial factor in fungal growth because of the increased moisture on the surface of the fruit, promoting the growth of fungi in the fruit without calyx. Consumer

rejection, mainly based on visible fungal growth, determined the shelf life of cape gooseberry without the calyx. Shelf life of cape gooseberry without calyx was found to be about 33-50 days at 8 °C and 80% relative humidity. The shelf-life was longer when the calyx was not removed (up to 62 days). Contents of ascorbic acid and β-carotene were not reduced at the end of the shelf life of cape gooseberry. In **chapter 4**, the kinetics of changes in the contents of the phytochemicals is described. Ascorbic acid degradation after heat treatment (40-120 °C) was described with first order reaction kinetics, and it appeared that ascorbic acid was up to 15 times more stable to heat than other common sources of vitamin C. β-Carotene was stable to heat and some isomerization from *trans*- to *cis*- was observed. Catechin and epicatechin were identified in the samples but kinetic modelling of the available data was not possible because these compounds follow simultaneous patterns of formation, degradation and epimerization. Formation of HMF was modelled by a zero order formation of a precursor compound followed by a first order HMF formation reaction. Antioxidant activity (DPPH) was strongly correlated to the content of ascorbic acid and this compound was found to have the major contribution of the antioxidant activity measured by DPPH. This study showed that various health-promoting compounds in cape gooseberry are subject to thermal degradation or formation but not to the extent that they are no longer present after heating. The main result described in **Chapter 5** is a conceptual model to typify value chain *scaling up* patterns and relate them to the likelihood of misalignment of quality preferences and perceptions of actors. Quality attributes and product descriptors were defined and the preferences of consumers and upstream actors of the value chain were assessed. Although there is a low complexity in the current cape gooseberry value chain, there is a low degree of alignment which is a potential barrier for the *scaling up* process. A conceptual model of scenarios was provided in order to give orientation to the value chain about directions to take for *scaling up*, according to the alignment of the actors.

6.3. Methodological considerations

As explained in chapter 1, an interdisciplinary approach has been used to investigate the cape gooseberry value chain. This section discusses the chosen methodologies of the different studies presented in this thesis.

6.3.1. Fruit material

Cape gooseberry (*Physalis peruviana* L., ecotype Colombia) used in all the experiments of this thesis was grown in Pasca, Cundinamarca, Colombia (2.180 m.a.s.l.). Although this guarantees the absence of variation due to regional differences in the experiments, it was not possible to draw conclusions about the possible effect of cultivation processes or soil characteristics. The use of fruit from different farms, in a systematic way, could have given

hints about the effect of cultivation conditions on health-promoting compounds contents or shelf life estimations.

6.3.2. Literature review

Since cape gooseberry is quite popular in Andean countries, most of the research about the fruit has been conducted in countries like Brazil, Colombia, Ecuador and Peru. Authors from Chile and Argentina have also published papers. Although there are quite some English reports, most of the literature was in Spanish and Portuguese, most of them in peer-reviewed journals. There were also already published reviews on this fruit, but they were limited to a small number of publications probably because of language limitations or interest of the authors. Moreover, those reviews combined cape gooseberry data with reports on the plant *Physalis peruviana L.*, which is widely used in folk medicine, thus, there was no certainty about the actual phytochemicals in the fruit, different from what is in the plant. Thus, a new review paper was written, taken into account all the scientific research reported worldwide. The first decision made about this review was to focus on potential health-promoting compounds in cape gooseberry because that was the quality attribute that was most claimed by actors in the chain and also because of the high interest of consumers in this matter. Besides, there was no good conclusive review on the occurrence of such compounds in this fruit. During the compilation of results, different reported units were found, therefore they were converted to one type of unit.

Using a food chain approach, the literature review was divided in different supply chain steps when possible with the available information. The study of cape gooseberry revealed several gaps that unfortunately could not be closed with this review.

6.3.3. Shelf life study

One of the technological issues of the cape gooseberry chain is the waste of fruit during post-harvest storage and transport [69]. Shelf life is usually short (30 -40 days) and the shipping process is long (20 days), shortening the time for distribution and consumption steps. This study on the shelf life of cape gooseberry was conducted based on an integral approach proposed by Van Boekel (2009), where physicochemical, microbiological, nutritional properties and consumer acceptance are evaluated.

The chosen variables for the experiment were temperature of storage and presence/absence of the calyx. Studies on cape gooseberry were reported for storage temperatures of 4, 12 and 18°C [278-280, 93], however, the focus was not shelf-life, therefore only some analyses were conducted and for different periods of time, remaining estimations of the fruit shelf life as a gap to investigate. Data were collected from two years of harvest, for the first year

(2014), 8 and 12°C were evaluated. These temperatures were chosen because they are in the range of the current temperatures used in the containers when shipping (9 ± 2 °C). The close results between these two temperatures, especially in the consumer study, resulted in the addition of another temperature (4 °C) for experiments of 2015.

The evaluation of different cultivation conditions can give more clues about causes of fungi growth by assessing the efficiency of phytosanitary measures. Besides, complete identification of the fungi could be done and also a comparison of identification of fungi from cultivation field. A more detailed knowledge of the fungi could have supported to draw recommendations to prevent the growth. This type of study could help to make estimations of the cultivation conditions that promote the growth of fungi during post-harvest.

An integrative approach to measure shelf life is very helpful to understand what happens with the product from different perspectives. Nevertheless, it is a long and costly process. The best way to improve such studies is by getting a good understanding of the most important changes that limit the shelf-life, and focus on that. This can be done by preliminary tests, observation of the changes of the fruits during storage and also consumer focus groups. Consumers are the most experienced people in the food they consume. Indeed, when one does not have a hint about the issues that limit shelf-life, it is required to try to understand the problem from different perspectives.

The most relevant insights came from the consumer study. This was because trained panels were not used but actual consumers and they gave a lot of information, not just about the quality issues of the fruit, but also about their preferences. At the end of the day, this is what companies are looking for, the satisfaction of consumer. Thanks to the methodology developed by previous research [163] the use of survival analysis was possible, which to some extent gave more conclusive results than the other measurements, because in the end it was the consumer who judged the end of the shelf-life. The other measurements such as physicochemical and microbiological are very important to get insights about how to tackle the issues with the food under study, but the consumer indeed has a big impact in deciding when the food is not suitable for consumption. At least, this statement applies for the cape gooseberry. Probably, for other products, there are safety issues that cannot be noticed by consumer, therefore the limitation of shelf-life requires other aspects to be studied.

6.3.4. Kinetic modelling study

Processed food such as jam, juices, comfitures among others are one alternative for the cape gooseberry chain to diversify. Because the interest of consumer is to have health diets and get intake of health-promoting compounds, this study aimed to evaluate the kinetic behaviour of

such compounds during heat treatment.

The effect of heat treatment on ascorbic acid, β-carotene, flavonoids, HMF content and antioxidant activity was evaluated at 5 different temperatures and 6 heating times. The same conditions of heat treatment were conducted for all compounds and for the antioxidant activity. Probably for β-carotene more extreme conditions could have been used, given the stability of the compound. However, since such extreme conditions are unlikely to be used during food processing, the experimental design was kept the same.

The most important methodological improvements in this respect are to gain more knowledge about all the flavonoids and phenolic compounds in cape gooseberry because a large number of peaks in chromatograms was observed, but it was not possible to identify them all. Thus, potential more health-promoting compounds can be present in the fruit and that is still unknown. Catechin and epicatechin were the only quantified flavonoids in this thesis.

6.3.5. Alignment of value chain actors

Using an own-designed conceptual model, research was conducted in three parts (chapter 5). Part I was an empirical analysis with consumer and value chain actors involving the participation of 500 people in The Netherlands and Colombia. The most important methodological consideration was about the selection of people, which was based on practical considerations. Colombian consumers participated in describing quality attributes because they are more familiar with the cape gooseberry. However, for the consumer study, only people living in the Netherlands were considered, since the interest was to get insight about international consumers (especially Europe). The Netherlands is probably not the most important European consumer country because from the surveyed people 42% did not know the fruit (chapter 5). However, statistical trade data shows it to be the country with highest imports of cape gooseberry from Colombia, probably intended to be re-exported. Anyhow, considering the fact that the research itself was conducted in The Netherlands, it was more practical to approach contacts within this country and to be able to conduct the high number of surveys.

Unfortunately, it is uncertain who the actual consumers of cape gooseberry are. From the experience gained during the data gathering, the participation in conferences and unformal conversations with people in some countries, apparently in Germany the fruit seems to be more popular, however, there is no documented data on that consumption. This research relied basically on trade data, but countries such as The Netherlands, United kingdom and Germany might act as intermediaries and the cape gooseberry could end in another different country, even outside Europe.

6.4. Discussion and interpretation of results

6.4.1. Health-promoting compounds of cape gooseberry

Cape gooseberry is a source of a variety of compounds with potential health benefits. The review paper (chapter 2) evaluated changes of such health-promoting compounds and antioxidant activity from a supply chain perspective based on literature. In this section a complementary analysis of health promoting compounds in cape gooseberry has been conducted considering information from published literature and data obtained in this thesis.

The reason to give special attention to health-promoting compounds in cape gooseberry is the increasing interest in the fruit related to its health properties. People are constantly seeking for food that helps them to have a healthy diet. Thus, consumers are interested to know what is in the product they consume and have reliable information about the potential health-benefits they could get out of the cape gooseberry consumption.

This section provides discussion of three main factors:

- Factors that are considered to have an effect on health-promoting compounds contents along the chain, and the gaps of knowledge that are remaining for the specific cape gooseberry case.
- Contents of health-promoting compounds along the cape gooseberry supply chain.
- An intake assessment.

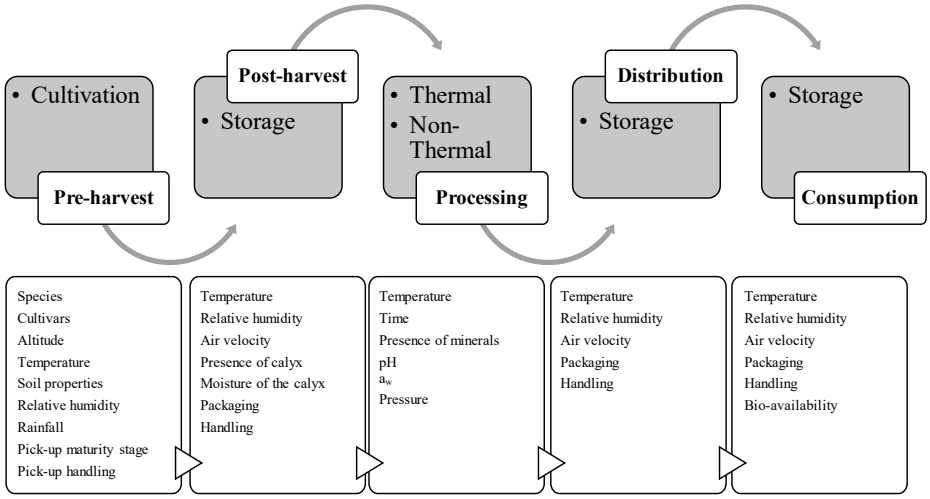


Figure 6.1. Potential factors affecting health-promoting compounds contents in cape gooseberry

6.4.2. Factors affecting health-promoting compounds in the cape gooseberry chain

A summary of all factors considered to have an effect on the health promoting compounds contents in agri-food products, focusing on cape gooseberry is depicted in figure 6.1.

Pre-harvest: Cultivation factors

Genus, Species and cultivars play an important role in the content of health-promoting compounds as reported by several studies on other products [111, 281, 113, 282]. For this thesis, the focus was on *genus Physalis* and *Species Physalis Peruviana L.* This species has Taxonomic Serial No.: 30606 and there is no documented information regarding types of varieties or cultivars (ITIS, 2014). In practice, few varieties/cultivars/ecotypes are used for production and a seedbank has been created from some institutions in Colombia. It is known that there are differences among varieties/cultivars/ecotypes regarding compounds such as vitamin C, β-carotene and phenolic compounds [96, 60, 92, 95]. However, the selection of seeds for production is usually based on obtaining quality attributes such as high sugar content, high sized fruits or resistance to pests. Thus, there is no information about specific varieties/cultivars/ecotypes that could give higher contents of health-promoting compounds. The selection of varieties/cultivars/ecotypes should aim to get fruit with all quality attributes

according consumer expectations, consequently involving health-promoting compounds contents.

Environmental conditions such as *altitude, temperature, soil properties, relative humidity, UV-B radiation and rainfall* might have a specific influence on the health-promoting compounds depending on the compound. For instance, no significant effect of environmental conditions on the content of vitamin C was found in cape gooseberry cultivated at an altitude of 2300-2690 m.a.s.l., mean air temperature of 17ºC-12.5ºC, mean soil temperature of 19.5ºC-19.8ºC, relative humidity of 66.6%/79%, daily sunshine hours of 5.3-5.4 and UV-B radiation of 148 mW-160mW. Nevertheless, these same conditions had a significant effect on β-carotene, at higher altitude the contents of this compound where higher [60]. The effect of environmental conditions in cultivation on health-promoting compounds has been documented for other fruits to some extent [111, 283]. Thus, information about how to enhance the contents of these compounds, based on factors such altitude, temperature, soil properties, relative humidity and rainfall is limited.

Harvesting conditions: This is a relevant factor for almost all type of fruits [111, 283]. In the case of cape gooseberry, it is known how the maturity stage of the fruit at the harvesting moment causes big differences in health-promoting compounds contents [96, 98, 112, 99]. Since cape gooseberry is a climacteric fruit (chapter 3), the ripeness process continues after being separated from the plant. In case of vitamin C, β-carotene, phenolic compounds and antioxidant activity, documented studies give information about the increase of those compounds/activity after harvesting [96, 98, 99]. Usually, harvest is performed when the fruit has green-yellow to yellow colour in order to allow more time for post-harvest, processing and distribution activities.

Post-harvest: Storage

Post-harvest storage is definitely a fact to consider regarding health-promoting compounds. In the case of cape gooseberry, the presence of calyx is believed to play a crucial role in maintaining the quality of the fruit [179]. Chapter 3 showed that this is indeed the case. However, the presence of calyx did not play a role in ascorbic acid and β-carotene changes. The *handling* then might have a role because rough removal from the plant gives potential cracking of the fruit, making it more vulnerable to oxidation processes [111, 283]. In cape gooseberry, this phenomenon has not been studied, thus the effect remains unclear.

The use of post-harvest ethylene treatment induced losses of phenolic compounds, antioxidant activity and ascorbic acid in cape gooseberry, while 1-methylcyclopropene treatment hardly affected these compounds after 14 days of storage at 20 °C [95].

Processing: Thermal and non-thermal

Thermal and non-thermal processing is expected to have an effect on health-promoting compounds. In cape gooseberry, thermal processing leads to degradation of ascorbic acid, catechin, epicatechin and antioxidant activity. β-Carotene was stable and there were signs of isomerization. Formation of HMF was shown. The pH, a_w and the presence of metals can also influence degradation, formation, epimerization and isomerization of compounds under heat treatment, but these effects have not yet been studied for cape gooseberry.

Non-thermal treatment of cape gooseberry has not been studied much, except for some research on High Hydrostatic Pressure processing (HHP). The effect of different temperatures, times and pressures used in HHP is still unclear. However, depending on the food matrix, HHP could improve extractability of health-promoting compounds, or cause degradations [139, 132, 120, 72]. Information about isomerization and epimerization processes is not available for HHP of cape gooseberry.

Distribution and consumption: storage

Storage of fresh fruit or processed fruit has different requirements. For fresh cape gooseberry, it is important to consider the temperature (chapter 3), the relative humidity, the ventilation (air velocity) that allows maintaining the proper temperature and humidity. The presence of calyx definitely gives protection of the fruit against different forms of damage, but health-promoting compounds did not seem to be affected by that (chapter 3). Packaging might have an effect on health-promoting compounds of processed fruit, e.g., by the amount of oxygen in the headspace and the permeability rate of oxygen through the packaging material. However, this field has not been studied yet for cape gooseberry products. Not only the content of health promoting compounds is important, but also their bioavailability. So far, there is no literature on this aspect related to cape gooseberry.

6.4.3. Intake assessment: a part of a health-benefit assessment

In previous sections the importance of the intake of food with health-promoting compounds is important for the potential prevention of diseases. Thus, it is important to estimate the intake of compounds based on the food that people consume and also the frequency in which those food are consumed.

In chapter 3 and 4, findings related to vitamin C, ß-carotene, total phenolic compounds (TPC), flavonoids (quercetin, rutin, myricetin, kaempferol, catechin and epicatechin) and antioxidant activity were provided. It was concluded that there is no evidence of the presence of withanolides and physalins in the fruit as has been suggested by previous papers on

Physalis peruviana L. This plant has shown health-promoting compounds in leaves, root and calyx, but the studies on withanolides and physalins were not focused on the fruit.

The estimation of the content of health-promoting compounds at the consumption stage has been performed based on literature data as well as on experimental data obtained in this thesis project.

A risk assessment methodology was used to make calculations of the intake of the chosen health-promoting compounds. Risk assessment is a scientific evaluation of known or potential adverse health effects which result from human exposure to a foodborne hazard. The purpose of risk assessment is to provide a scientifically based estimation of risk of that hazard to a population [167]. For this attempt, the inverse concept of risk assessment has been considered, since the presence of health-promoting compounds is highly desired for potential diseases prevention, instead of posing a health risk. Therefore, the modified risk assessment scheme has been named "health-benefits assessment" and the framework is depicted in figure 6.2.

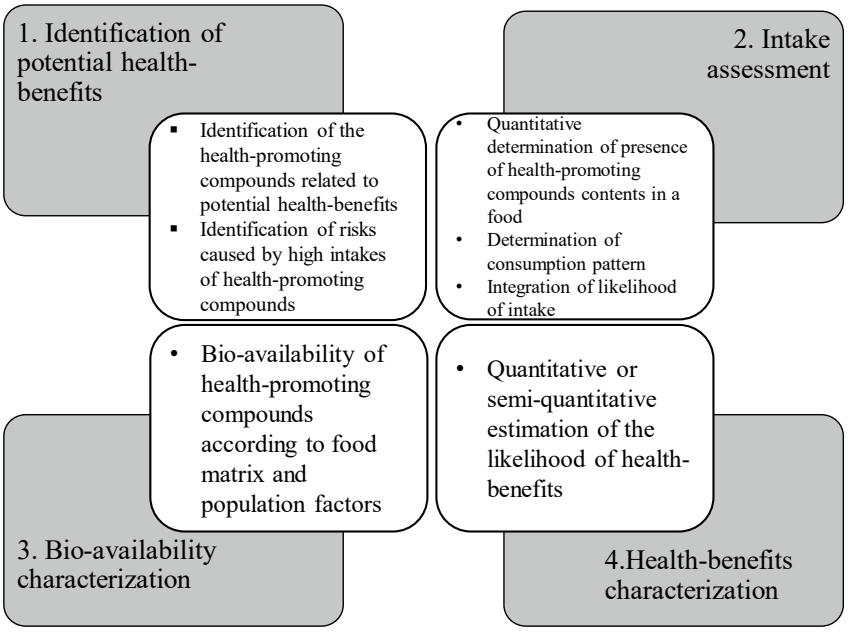


Figure 6.2. Framework of health-benefits assessment in foods

Following figure 6.2, to conduct a health-benefits assessment, the least information required is:

6

- Information on potential health-promoting compounds related to potential benefits.

- Information on potential risks related to the high consumption of the health-promoting compounds.

- Quantitative information of health-promoting compounds in different stages along the food chain.

- Consumption data on the studied food in a specific geographical region.

- Bio-availability of the health-promoting compounds under study according to the food matrix and specific characteristics of the population studied.

For cape gooseberry it is not possible to make a complete health-benefits assessment with the available data but it does allow conduction of an intake assessment.

6.4.4. Approximation of health-promoting compounds contents of cape gooseberry at main stages of the supply chain

The required information discussed in this section is about data on the presence of health-promoting compounds in cape gooseberry. These data have been estimated for each stage of the supply chain in order to approximate the contents of health-promoting compounds at the consumption stage. The following assumptions were made:

Cultivation:

Contents of TPC, ascorbic acid and β-carotene are presented as ranges involving data for ripeness stages 4, 5 and 6, which are the most common edible stages. Data sources: Ascorbic acid [99]; β-carotene [96, 98-100]; TPC [96, 98, 100] and antioxidant activity (DPPH) [99].

Storage:

Post-harvest storage effects on ascorbic acid and β-carotene are presented for a storage time up to 76 days according to data obtained in chapter 3. Temperatures have been averaged (8 ± 4 °C). These temperatures represent the actual ones currently used post-harvest in the international chain. Relative humidity was 80 %. Since results of this thesis did not show significant difference between fruit with and without the calyx ($P > 0.005$), these results were also averaged.

Processing and storage of processed food:

The effect of thermal and non-thermal processing was evaluated, although nowadays in the market, cape gooseberry foods based on non-thermal processing are not available. Non-thermal processing data were taken from published literature on cape gooseberry pulp. The initial values were kept the same as the cultivation stage. The effect of HHP processing was taken from reported data assuming a similar behaviour with fresh fruit as reported in cultivation [139, 120, 72]. The effect of storage after 30 days of HHP were taken from the same literature.

Data of thermal process effects were taken from the results of chapter 4. Initial values were not changed since they were consistent with the values of cultivation. There were no available data to plot TPC. For the storage after thermal treatment, the initial values were taken from the experiments of chapter 4, temperature of 80 ℃ and 40 minutes as a simulation of a standard pasteurization process. This selection was made based on the available data from the kinetic study since it had the closer conditions to a standard heat treatment. The behaviour during storage after heat treatment was taken from a study on cape gooseberry juice [70].

There was no information on β-carotene changes during storage at these conditions.

Distribution:

Distribution of fresh fruit and processed product was studied using the data evaluated for storage for the two scenarios, taking into according thermal and non-thermal processing.

Consumption:

From the integration of all calculations made in every stage of the supply chain, the content of health-promoting compounds at consumption stage was approximated.

The total quantitative estimation of health-promoting compounds in cape gooseberry is depicted in figure 6.3.

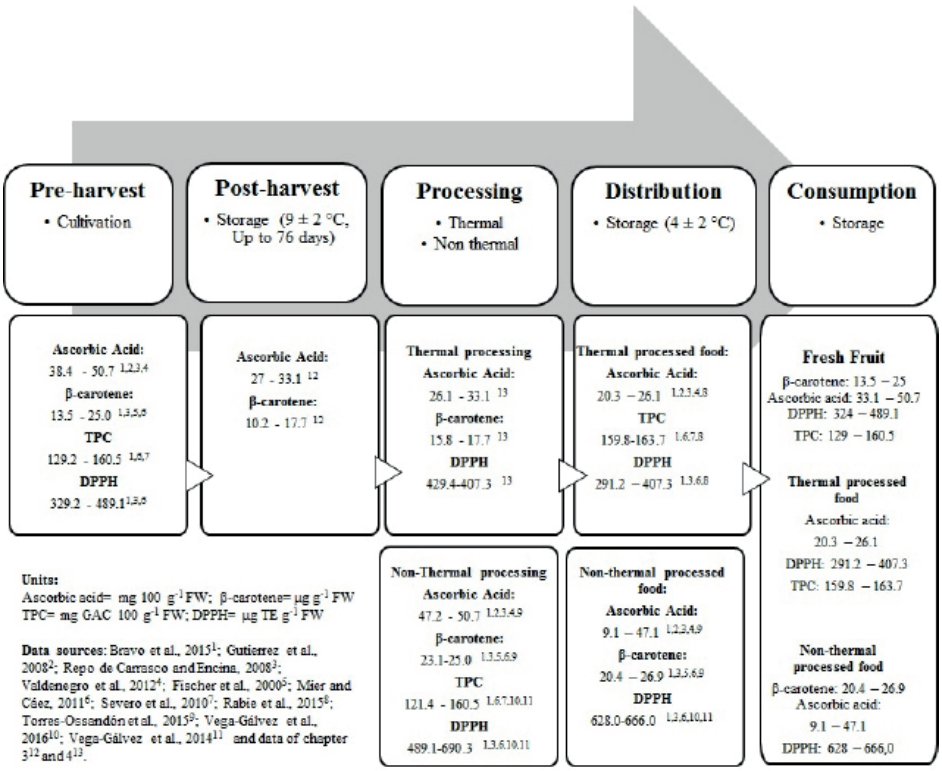
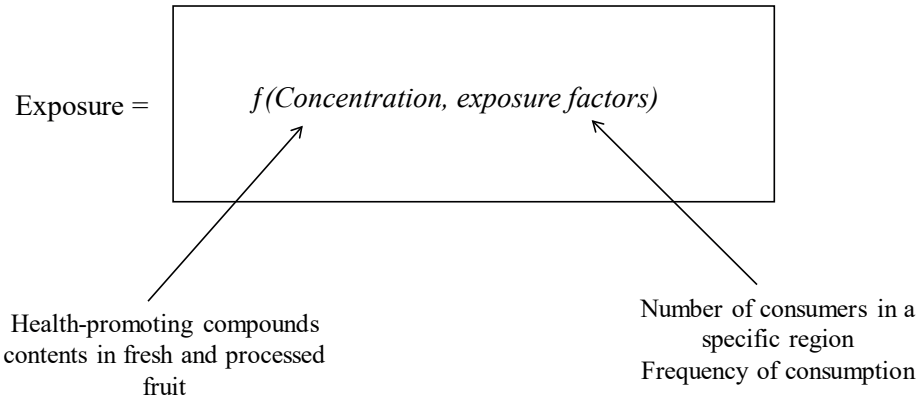


Figure 6.3. Approximation of health-promoting compounds contents in cape gooseberry in different stages of supply chain

6.4.5. Intake assessment of health-promoting compounds from cape gooseberry in Colombia and The Netherlands

As previously mentioned, the data obtained in this thesis, unfortunately, only allowed doing the health-benefit assessment until intake because there is no information about bio-availability of the compounds from cape gooseberry and quantitative information on their health beneficial effects. The exercise to illustrate how to conduct an intake assessment based on health-promoting compounds is done with vitamin C (ascorbic acid) and β-carotene, according to the following expression:



Concentrations of vitamin C and β-carotene have been estimated for fresh fruit and processed food in the previous section. *Intake factors* are related to the number of people that consume the fruit and their patterns of consumption. By integrating these two items one can get the amount of vitamin C and β-carotene people get from the consumption of cape gooseberry. As data about consumption related to Colombia and The Netherlands are available, those calculations allow an estimate of the intake.

Colombia case

In the introduction chapter 1, it was shown that the consumption of cape gooseberry (both fresh and processed) was estimated to be 132.10 g/year/person for 2014. However, for the calculation of the intake assessment, this value could lead to bias of intake of cape gooseberry because it takes the whole population and in this case it is important to be specific on the population that are actual consumers. A preliminary survey was conducted with the participation of 128 people from Colombia. These data are not shown in the previous chapters but the descriptive statistics of the survey is depicted in table 6.1.

Table 6.1. Descriptive statistics of participants in preliminary research on Colombian consumption (data not shown in this thesis)

Participants	*n* = 128		
Age (years old)	*n*	*City of residence*	*n*
Under 18	2	Bogotá	70
18 -25	14	Facatativá	58
26- 35	25		
36-50	24	*Consumption of*	
Over 51	6	*cape gooseberry*	*n*
Not reported	57	Consumers	89
		Non-consumers	39
Gender	*n*		
Female	58	*Consumption frequency*	*n*
Male	70	Twice a week	1
		Once a week	7
Country of residence	*n*	Every two weeks	9
Colombia	128	Once a month	72
Country of nationality	*n*		
Colombia	128		

From the surveyed population, 69 % were consumers of cape gooseberry. This survey was random, therefore the assumption about the consumption of the whole population could be made. The frequency that most of the participants had chosen was “once a month”, that frequency of consumption is taken as reference for the calculation. The estimation of consumption leads then to 5 berries every month per person. Therefore, the approximation of consumption of cape gooseberry of adults in Colombia is 300 grams/year.

Considering the Colombian population data as,

Total population 2015: 48,000,000: Age 15-59: 62.26 % and over 60: 11.1%: the target population is 48,000,000 x 0.73 = 35 million of people.

Taking the lowest and highest scenario of fresh and processed cape gooseberry for vitamin C and β-carotene (previous estimation), one person might consume per year 27.3- 152.1 mg of vitamin C and 4,050 – 8,070 µg of β-carotene from cape gooseberry. The standard recommendations of these two compounds are a daily consumption of vitamin C of 30 mg/day and of β-carotene, 4800-6000 µ/day for adults (adults are the population that consume the cape gooseberry). The consumption of cape gooseberry has then the following contribution to the health-benefit of health-promoting compounds intake in adults in Colombia:

Vitamin C = 0.2-1.4%

β-carotene = 0.02- 0.04%

These percentages mean that the fruit is currently not a relevant source of vitamin C and β-carotene of adults in Colombia. The intake of vitamin C from cape gooseberry is higher when the fresh fruit is consumed rather than the processed food. The opposite happens with β-carotene, where the highest intake is obtained by processed food.

The Netherlands case

The attempt to make the intake assessment for The Netherlands does not aim to give a substantial contribution because of the low intake of the fruit. However, because in chapter 5, a study was conducted with 409 people in The Netherlands who were asked about cape gooseberry consumption. This information allows making estimations of intake to make comparisons with the Colombian exercise. From the 409 people, 75% were Dutch and the rest European and non-European people currently living in The Netherlands. From this sample, the following results were obtained:

Consumers of fresh fruit: 36.4% of the population sample

Consumer of dried fruit: 6.8% of the population sample

For the purpose of this exercise, both fresh and processed food consumers were grouped in the same 36.4% because they can be assumed to be overlapping. The people were also asked about frequency of consumption giving the following results:

Weekly: 1%

Monthly: 5%

Occasionally: 94%

Because the frequency of consumption for the majority of people was occasional, that frequency was taken as reference. Most of the people stated that the consumption occurred in season time, in restaurants or bought once in a while. From the responses of these consumers, an estimation of a consumption of 2-3 berries every six months per person was made. The consumption of cape gooseberry of adult consumer population is then 30 grams/year.

Considering the population data as,

Total population 2015: 16,900,000: Age 15-64: 67.7% and over 65: 14.9%: the target population is 16,900,000 x 0.83 = 14 million people.

It is not correct to make inferences of the total population based on the surveyed population sample because the sampling was purposive, to get the highest possible number of current

consumers. Thus, by making estimations with import fruit data in The Netherlands, an assumption on actual consumer population was made. Thus, the 36.4% of the population being occasional consumer would be about 100 times less (this value is uncertain). The number of occasional consumers in The Netherlands is then 50,814, with a consumption of 30 g/year. The consumption per capita of the adult population of The Netherlands would be 0.11 g/year/person. The contribution of cape gooseberry in health-promoting compounds intake is then seen to be completely negligible.

6.4.6. Shelf life of cape gooseberry

Temperature

Temperature has an effect on the shelf-life of cape gooseberry. Usually, the lower temperature, the better preservation of the fruit, but this is not the case for the experiments described in this thesis. The temperature giving better shelf-life was 8°C, 4°C led to chilling injury of the fruit without the calyx, promoting excessive moisture that favoured the growth of fungi. Storage at 12 °C also showed growth of fungi faster than at 8°C. The fruit with calyx did not have development of fungi at the same speed, therefore the shelf-life was longer. Two aspects we like to address from these results. The first aspect is the importance of the chilling injury in cape gooseberry; one common problem for this fruit is the cracking of the fruit that has been previously investigated and is attributable to the low presence of calcium and boron in the fertilization of the soil in the cultivation stage [284]. Thus, cultivation conditions could have an effect of this chilling sensitivity. In all experiments presented in this thesis, fruits from the same cultivation farm were used, therefore we could not compare between different cultivation conditions. Besides, the cultivation conditions of the used fruit are not known in detail.

The second aspect is the similarity of results that were obtained in experiments at 8 and 12 °C, especially for harvest 2014. For instance, the results from the consumer study of 2014, were similar. See figure 6.4.

The differences presented in other experiments came from the growth of fungi. As described before, the presence of fungi is a problem of cultivation [54]. Therefore, the temperature during storage could be higher, saving energy during transportation of the fruit.

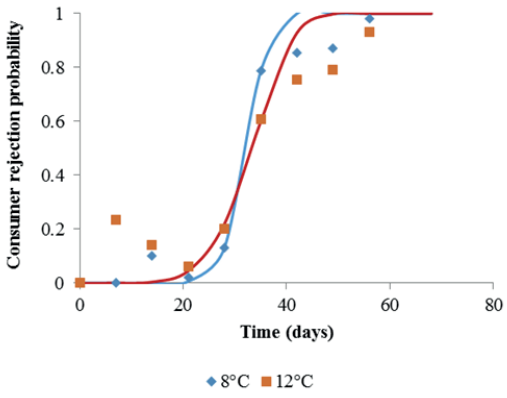


Figure 6.4. Survival analysis of cape gooseberry (without calix) stored at 8 and 12 °C.

Presence of calyx

The function of the calyx is believed to protect the fruit from bruises, physical damage, and is also a gas and moisture barrier. In addition, the calyx has an important role in the development of the fruit in the first 20 days of growth [179]. Once the calyx is removed, oxidation processes can start by the exposure to light and more available oxygen, causing decrease in the content of the antioxidant compounds [95]. Nevertheless, results presented in this thesis did not show that large differences. Indeed, the fruit lasted longer when it was within the calyx, but only because the growth of fungi was slower. As an illustration, figure 6.5 shows the behaviour of citric acid over time, and tartaric acid under the 3 studied temperatures, with and without calyx.

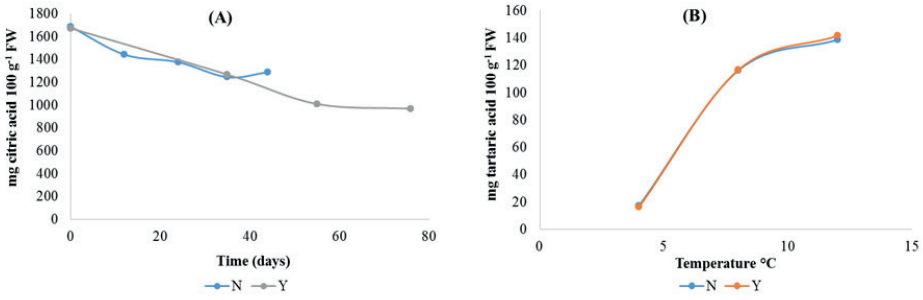


Figure 6.5. Results on cape gooseberry. A. Citric acid contents over a period up to 76 days with fruit with calyx (Y) and without calyx (N). B. Tartaric acid at three different temperatures (4, 8 and 12 °C) during storage time of fruit with calyx (Y) and without calyx (N)

The fact that most experiments did not show significant differences with or without calyx, highlight

fungi as the main shelf life problem of the fruit because the fruit is rejected by consumer.

6.4.7. Challenges in kinetic modelling

Kinetic modelling could be done for ascorbic acid degradation, the formation of HMF and the decrease of antioxidant activity during thermal treatments. However, complications were encountered when using different batches. Each batch had a different initial concentration and this induces a problem with replication of the heat treatment. First, the analysis of each batch individually but with global modelling of all data at once gave reasonable fits as well as reasonable k_d and E_a. Second, normalizing the concentrations as c/c_0 gave similar parameter values and better confidence intervals (95%). Although normalization is expected to introduce extra error of the experimental uncertainty, the results show that this is a potential way to conduct kinetic modelling.

Most of the literature uses a first order reaction model to describe ascorbic acid degradation. This study showed the reaction order to be around 1.2. This raises questions about model discrimination that is done in kinetics studies. Studies found in literature usually do not discuss whether or not a first order reaction actually applies, it is just assumed that this is the case. Thus, this is an important missing factor from current published papers. Moreover, it is usually not explained how replicates are used in kinetic modelling. When there are differences in the data between batches, it is best to model the batches separately, while still using global fitting. Another criticism on kinetic papers is the omission of precision of parameters. If given at all, usually the standard deviation is reported, however, confidence intervals give more information about the uncertainty of the parameters because confidence intervals take the number of data into account in contrast to the standard deviation.

The experiments of chapter 4 were conducted in real food (cape gooseberry), thus the modelling was more complicated as with simple model solutions. There are various factors that can interact with the reactions that are attempted to be modelled, such as the improved extractability of compounds by heat treatment and the competing other reactions happening simultaneously. Moreover, when studying thermal stability in real foods, there are other aspects that might have an effect on the studied reactions such as the pH or the presence of certain metals [226, 212], hampering the understanding of the chemical reaction produced by the exposure to heat.

The online-DPPH antioxidant activity assay suggested that the main compound contributing to the antioxidant activity was ascorbic acid, with a very small contribution from catechin and epicatechin. Nevertheless, the question remains on the contribution of flavonoids in the total antioxidant activity and what the unidentified compounds are in the chromatograms that did not match any known flavonoid or phenolic acid in the experiments.

By conducting another antioxidant analysis than DPPH, the results could have been different

because every antioxidant activity has different mechanisms [285], therefore, the antioxidant activity from other compounds could have been revealed when using another test.

6.4.8. Prediction of ascorbic acid and DPPH values

By using the Arrhenius equation introduced in chapter 4 and the obtained of the parameters $k_{80°C}$= 3.5 x 10^{-3} ± 2.3 x 10-3 min^{-1} and E_a = 44.8 ± 2.3 kJ.mol^{-1} for ascorbic acid, a prediction in Athena software of ascorbic acid concentration has been conducted. This prediction took into account reported processing in literature. Data on temperatures and times for the elaboration of juice, jam and dried fruit for cape gooseberry are available [155, 70, 71].

Pasteurized juice case

Rabie et al. (2015) reports data of cape gooseberry juice. The variables used are a temperature of 90 °C and 10 min of processing. Initial ascorbic acid is provided (40.30 mg 100 g^{-1}) as well as the concentration of ascorbic acid after heat treatment. Besides, the ascorbic acid was measured during storage time up to 21 days at 4 °C. Data of the predictions for ascorbic acid in cape gooseberry juice are shown in figure 6.6 for heat treatment and also for storage.

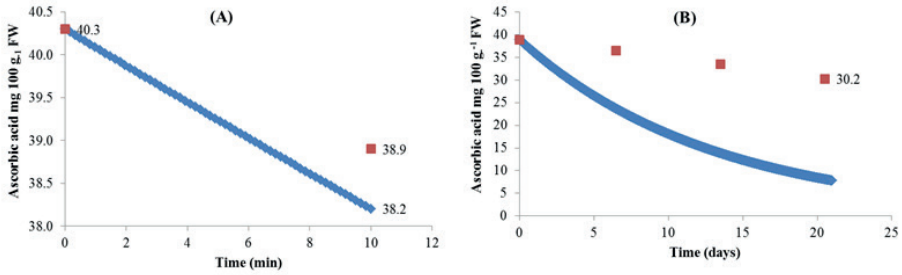


Figure 6.6 Predictions of ascorbic concentrations during pasteurization of cape gooseberry juice. Symbols represent the experimental data taken from [70]. The dotted lines represent the models based on the obtained parameters.

In figure 6.6A, the model predicts very well the final concentration of ascorbic acid, that might be because of the similar characteristics of the fruit used for the experiment and the juice did not have additions of other components. Nevertheless, the model did not perform well in predicting the storage time. This is definitively because of the different conditions respecting to the ones in experiments for parameter estimation. In the study of shelf life reported, the juice was in glass bottles, probably under dark conditions and little present of oxygen, changing the degradation pattern.

Jam case

Rutz et al (2012) reports the elaboration of jam under two different process. The processes vary because of the ingredients, therefore the variables temperature and time differed. The prediction modelling was conducted for DPPH values with the parameters obtained in chapter 4, $k_{80°C}$ = 1.0 x 10^{-3} ± 0.3 x 10^{-3} min^{-1}; E_a = 38.4* ± 7.3 kJ.mol^{-1}; cf = 246.6 ± 57.5 µg Trolox Equivalent 100 g^{-1} FW. The plot is depicted in figure 6.7.

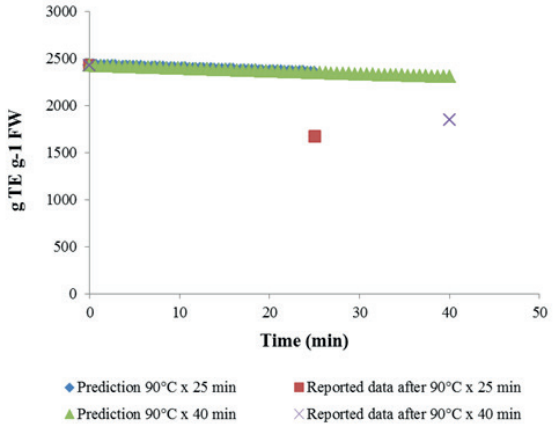


Figure 6.7. Predictions of antioxidant activity (DPPH) during preparation of cape gooseberry jam. Symbols represent the experimental data taken from [71]. The dotted lines represent the models based on the obtained parameters.

Model did not perform well in predicting the results obtained by the authors. However, there is an inconsistency in the results, because the degradation of DPPH values was higher after 25 min than after 40 min at the same temperature. The explanation for this might be the concentration process that takes place during jam preparation that could increase the apparent health-promoting compounds contents responsible of the antioxidant activity. Thus, the model must not necessarily be suitable for this type of product because the food matrix has been changed.

Dried cape gooseberry

The same attempt of prediction was made for dried cape gooseberry with data reported by Lopez et al., (2013). The predictions were made for ascorbic acid with the same parameters as the juice example. Plots are shown in figure 6.8.

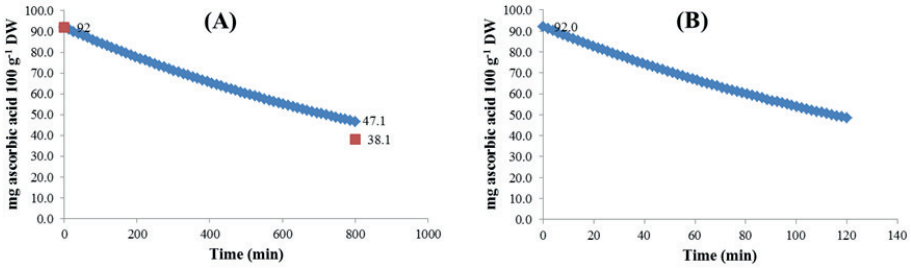


Figure 6.8. Predictions of ascorbic concentrations during drying process of cape gooseberry. Symbols represent the experimental data taken from [155]. The dotted lines represent the models based on the obtained parameters. Conditions are 50°C for 800 minutes (A) and 90 °C for 120 minutes (B).

The results of the prediction for 50 °C is reasonable for the time of 800 min. However, the authors claimed not to find ascorbic acid after the treatment under 90 °C for 120 min. These differences raises doubt about the data of the experiments presented by authors because of the difference in degradation patterns [155].

In general, the performance of the models obtained in chapter 4 are reasonable good, taking into account that the comparison are made with very different studies that have been conducted under completely different conditions.

6.4.9. The combination of food quality and value chain concepts to assess agri-food value chains

The definition of food quality is to meet or exceed consumer expectations [167]. Consumers are every time more aware of food safety and healthiness and are continually seeking diversity of foods [236, 74]. Consumer protection is not uniquely a matter of food or user safety, but also of supplying reliable information that facilitates consumer choices [286]. Several aspects affect food quality along the agri-food chain [85]. Thus, a chain approach is especially important to guarantee quality from farm to table (consumption).

Although food quality in the past has been a field of study of food technology or sciences related to food, the raised complexity of activities of agri-food chain has been also a subject of study of economics and management practitioners, especially in the area of value chain [74]. From a value chain point of view, final quality of products is the result of a coordination between chain actors [75, 240]; the consumer is an actor of the value chain that qualifies the product [287] and constitutes the starting point in buyer-driven value chains [249].

The figure 6.9 has been designed to illustrate the relation between quality attributes of the food chain from food quality literature and quality attributes from economic theories.

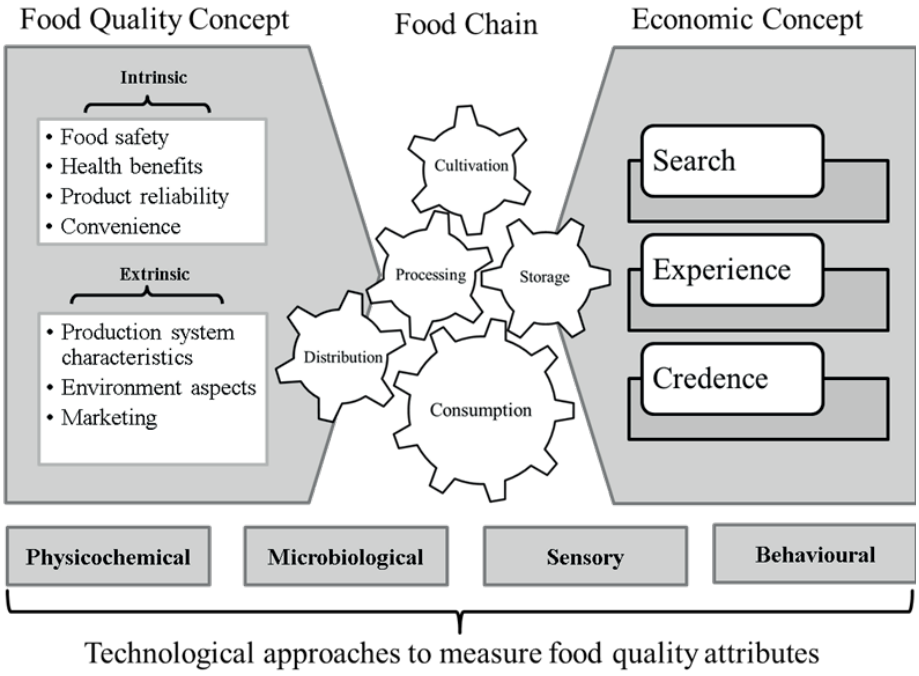


Figure 6.9 Food quality attributes according food sciences and economic concepts

Food quality practitioners usually describe quality attributes as intrinsic and extrinsic. Intrinsic attributes are directly related to the physical product properties, while extrinsic attributes refer to production system characteristics, environmental impact or marketing (See figure 6.9) [167]. Economic and management theories classify quality attributes in: search aspects as quality attributes that can be verified before purchase (e.g. color, size, amount); experience aspects as assessed after purchase has taken place (e.g. taste, texture); and credence aspects cannot be evaluated, consequently they are based on trust (e.g., health-promoting compounds contents) [74, 75].

Agri-food value chains have been facing several changes in the past years. Complex phenomena such as globalization, liberalization of markets, increased awareness of food safety, healthiness of food and new products demands [236, 77, 246, 80, 74, 75, 247] have brought some issues to deal with, such as the need to introduce new technologies, the leadership in decision making on only one or few actors of the value chain [246], the high load of information to be transferred [236], the failure to meet consumers' needs and the need

to increase vertical integration [248, 249].

The study of how to improve food chain efficiency, however, is in infant stage for food quality practitioners, compared to the degree of development of theories about value chain in other disciplines. Since agri-food products are prone to quality variation, uncertainty increases [246, 74]. This aspect requires an efficient governance structure, vertically integrated, buyer-driven, with clear, well defined and subsequently formalized and communicated quality expectations among all actors [81, 246, 74, 75]. Governance in global value chains is the process of organizing activities with the purpose of achieving the goals along the chain [81].

The concept of a global value chain has to deal with the mentioned issues in value chains with international scope [81]. Global value chain goes beyond the consideration of physical activities required to bring a product to end users, but also involves marketing and distribution aspects to respond to consumers' needs in an efficient way [245, 76]. Several authors agree that the global value chain approach has a high impact in facilitating information transfer, enhancing learning, innovation and product quality by the good coordination of transactions [76-80]. Features of the governance of the value chain are the level of complexity of the information required to be transferred for a transaction, the ability of that information to be codified and the capability of suppliers to fulfill those requirements [81].

From the results obtained on the research of the value chain, it was found that the international consumer still is unaware of the existence of the cape gooseberry, let alone the health-promoting compounds contents, with the consequence that the intake of such compounds from cape gooseberry is insignificant. This, with the fact of the low alignment among actors of the value chain, shows that the value chain is not really communicating effectively. Besides, the fruit is known with so many different names, restraining the positioning of it. Therefore, there must be an effort on promoting the fruit in the international markets, when wanting to go global. Particularly two aspects are worth mentioning: unify the name of the fruit and give information regarding health-promoting compounds. The intake could become more relevant with increased consumption. This definitely requires leadership organizing activities for promoting, investigation and innovation to really give value to consumers. The big portion of surveyed people in chapter 5 are potential consumers, thus there is a potential for entering in that market, making the demand for the fruit more stable. Whatever actor takes the leadership of the chain, it is required to tackle the problem, for which the aspects described in this thesis form a basis.

6.5. Implications and recommendations

This thesis has explored different aspects of the cape gooseberry value chain closing some knowledge gaps but revealing that there is still information required to understand the value chain better.

First of all, it is important to study food quality from an interdisciplinary perspective. This interdisciplinarity might come with managerial sciences or with other technological scientific fields, such plant, marketing, policy making science, among others.

For the improvement of cape gooseberry quality, it is relevant to study the effect of cultivation environment on the presence of fungi in post-harvest. Probably, the answer is as simple as the following of good manufacturing practices in production. The option to make improvements on seed brings another interdisciplinary collaboration: the development of cape gooseberry seed with the quality attributes demanded by markets and more resistance to pests and fungi.

Regarding health-promoting compounds, an introduction on the topic was given. There is still much unclear about the flavonoids present in the fruit. Claims of health-properties require also scientific evidence.

The study of withanolides and physalins is an interesting field of explore, given the potential presence of these compounds in the solanoceae family species and the ongoing research providing evidence on potential health-benefits of these two type of compounds.

The bio-availability of health-promoting compounds is also an important gap to close. It is unclear what the bioavailability of health-promoting compounds in cape gooseberry is.

In fact, the value chain requires a lot of collaborative work between actors to comply with international consumer demands. This collaboration should be done by an effective integration of the value chain. This can be conducted with the leadership of one actor category. Usually, this is conducted by large retailing companies. However, because the fruit is a niche market, there are no powerful actors in the value chain. Therefore, the association of farmers conjoint with traders can make this integration happen and try to tackle the problems presented in this thesis.

6.6. Main conclusions

Cape gooseberry is a source of health-promoting compounds, namely vitamin C, β-carotene, phenolic compounds and has antioxidant activity properties, but in terms of intake it does not contribute much with the present consumption.

Various health promoting compounds in cape gooseberry are subject to thermal degradation or formation but not to the extent that they are no longer present after heating.

The main issue for shelf-life of fresh cape gooseberry is the growth of fungi. The presence of calyx was shown to restrain the growth of fungi but did not show any other effect regarding the rest of evaluated quality attributes.

The temperature giving longer shelf life was 8 °C (up to 62 days for fruit with calyx and up to 56 days without calyx) under 80% of relative humidity.

Vitamin C and β-carotene were relatively stable after storage time during post-harvest, under the studied temperatures.

The estimation of health-promoting compounds through the supply chain gave contents of 13.0 – 26.9 μg β-carotene g^{-1} FW and 31.0 – 50.7 mg vitamin C $100g^{-1}$ FW for fresh or processed cape gooseberry at consumption stage.

The intake of vitamin C decreases with the intake of processed food because of thermal degradation during processing. On the contrary, the intake of β-carotene is potentially higher in these products than in the fresh fruit.

The intake assessment conducted based on the current consumption of cape gooseberry, concluded that the contribution of this fruit to the daily recommendation intake of vitamin C and β-carotene in Colombian and Dutch adult population is negligible.

Cape gooseberry is a very little consumed fruit because is not well-known in international markets. However, it has potential to improve by first facing alignment issues, by integrating the value chain and by developing strategies to effectively plan the route to follow in order to scale up.

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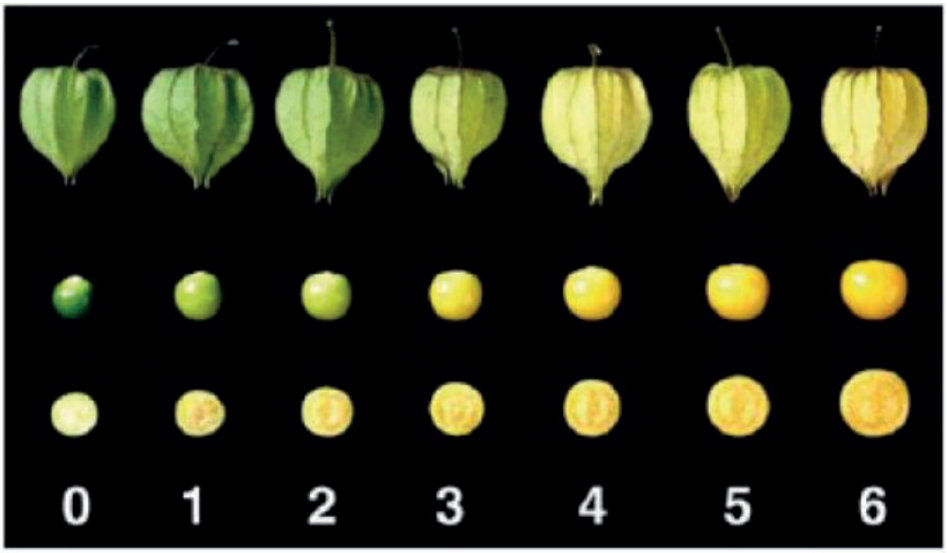
Appendix

Specifications of ripeness stages of cape gooseberry. Colombian Standard. Fresh fruits. CAPE GOOSEBERRY. NTC 4580 (ICONTEC, 1999)



Summary

Exploring the potential of an Andean fruit:

An interdisciplinary study on the cape gooseberry (*Physalis peruviana L.*) value chain

Cape gooseberry is a fruit cultivated in Andean countries. Currently it is available some international markets, besides the domestic Andean market. Colombia is the major producer and export country at the moment. The value chain of cape gooseberry faces several barriers of technological and governance nature. This research is an interdisciplinary study on the Colombian cape gooseberry value chain. It aimed to evaluate quality attributes of the fruit during the supply chain, including the changes in the contents of health-promoting compounds; and also assessed the current situation of the value chain regarding degree of alignment of the actors.

In **Chapter 2** a critical evaluation of current published data was presented regarding health-promoting compounds in cape gooseberry. There was found scientific evidence of the presence of vitamin C, β-carotene, phenolic compounds, vitamin E and B, minerals and fibre in cape gooseberry. It was also revealed that withanolides and physalins have not been studied in the fruit. Despite the fact of the large variation of data, methods and units, the analysis of health-promoting compounds during pre-harvest, post-harvest, storage and processing was attempted. An increasing trend of contents of vitamin C and β-carotene during post-harvest, related to ripeness stages was found. This chapter gives a contribution on the state of art on phytochemicals and antioxidant activity in cape gooseberry. Nevertheless, no strong evidence is available to a complete insight of the behaviour of health promoting compounds and antioxidant activity during pre-harvest, post-harvest, storage and processing. Thus, the continuation of the investigation on this aspect was suggested.

The postharvest changes of cape gooseberry with and without calyx were shown in **Chapter 3.** This study aimed to investigate the effect of storage temperature and the presence of the calyx of the fruit on the shelf-life of cape gooseberry. Experiments involved the measurement of different quality attributes and a survival analysis that complemented the research from a consumer perspective. Cape gooseberry was found to be a climacteric fruit with difficulties to retain an acceptable level of quality attributes. Temperature has an effect on the shelf-life of the fruit. A temperature of 8 °C gave a longer shelf life for fruit with and without calyx, compared to 4 and 12 °C. The removal of calyx is the crucial factor in fungal growth because of the moisture on the surface of the fruit, promoting the growth of fungi in the fruit without calyx. Consumer rejection, mainly based on visible fungal growth, determined the shelf life

of cape gooseberry without the calyx. Shelf life of cape gooseberry without calyx was found to be about 33-50 days at 8 °C and 80% of relative humidity. The shelf-life was longer when the calyx was not removed (up to 62 days). Contents of ascorbic acid and β-carotene were not reduced at the end of the shelf life of cape gooseberry. This chapter gives hints of the most important quality attributes that determine shelf-life, making an important input for further research. Indeed, the study aimed to decrease the fungal growth is highly required.

A contribution to the knowledge on health-promoting compounds of cape gooseberry after heat processing is presented in **Chapter 4**. The evaluation of thermal stability of such compounds and antioxidant activity was conducted. The kinetics of changes in the contents of the phytochemicals is described, when possible. Ascorbic acid degradation after heat treatment (40-120 °C) followed first order reaction kinetics, and was up to 15 times more stable to heat than other common good sources of vitamin C. β-Carotene was stable to heat and some isomerization from trans- to cis- was observed. Catechin and epicatechin were identified in the samples but kinetic modelling of the data was not possible because these compounds follow patterns of formation, degradation and epimerization at the same time. Formation of HMF was modelled by a zero order formation of a precursor compound followed by a first order formation reaction. Antioxidant activity (DPPH) was correlated to the content of ascorbic acid, therefore this compound has the major contribution of the antioxidant activity measured by DPPH in cape gooseberry. In conclusion, this study showed that various health promoting compounds in cape gooseberry are subject to thermal degradation or formation but not to the extent that they are no longer present after heating. Thus, this gives opportunity to food industry to develop product based on cape gooseberry that have potential to contain relevant amounts of health-promoting compounds.

Chapter 5 studies the value chain of the cape gooseberry by evaluating the degree of alignment among value chain actors regarding quality attributes preferences in order to get insights about *scale up* barriers. One of the main results described in this study is a conceptual model to typify value chain *scaling up* patterns, relating them to the likelihood of misalignment among of actors. The quality attributes and descriptors of cape gooseberry were defined thanks to the participation of Colombian consumers of the fruit. The empirical study that also involved actors of the cape gooseberry value chain showed a low degree of alignment which is a potential barrier for the *scaling up* process. A scenarios model was provided in order to give orientation to the value chain about directions to take for *scaling up*, according to the alignment of the actors. This chapter is considered a contribution to literature on globalization of agri-food value chains by giving a consumer approach in order to tackle governance issues and facilitates the organization of activities when trying to *scale up*. Moreover, it is also a contribution to consumer-driven concepts to envision agri-food products as a subject that require to be studied from a value chain point of view because

there are several factors along the chain that need attention in order to achieve consumer satisfaction

In **Chapter 6**, a summary of the main findings of this thesis was given, followed by discussion and implications for further research. In summary the most important conclusions of this thesis are:

Agri-food value chains can face different barriers restraining their efficient performance. Those barriers come from different aspects, not only technological. Thus, interdisciplinary studies are needed to make contribution on the state of art of those value chains.

Cape gooseberry is a source of health-promoting compounds and has antioxidant activity properties. Such health promoting compounds in cape gooseberry are subject to thermal degradation or formation but not to the extent that they are no longer present after heating. Vitamin C and β-carotene were relative stable after storage time during post-harvest.

The main issue for shelf-life of fresh cape gooseberry is the growth of fungi.

The intake assessment conducted based on the current consumption of cape gooseberry, concluded that the contribution of this fruit to the daily recommendation intake of vitamin C and β-carotene in Colombian and Dutch adult population is negligible.

Cape gooseberry is indeed a very low consumed fruit because is not well-known in international markets. However, it has potential to improve performance by first facing alignment issues, integrate the value chain and develop strategies to effectively plan the route to follow in order to *scale up.*

About the author

Mary Luz Olivares Tenorio born on 22nd November 1976, in Bogotá, Colombia. She followed Bachelor studies in Food engineering at Fundacion Universitaria Agraria de Colombia- Uniagraria in Bogotá. After graduation she worked in several food companies in the field of food quality. In 2004 she was granted a NUFFIC scholarship for an MSc in Food Quality Management at Wageningen University from which she graduated in 2006. In 2007 Mary Luz started working at UNIAGRARIA as a university lecturer and in 2009 she was appointed as Director of the Food Engineering program. In 2012 she started her PhD at Wageningen University thanks to a NUFFIC PhD fellowship. During the four years of the PhD program, Mary Luz combined the activities related to her academic program with the duties from the job position in UNIAGRARIA. In 2016 she was granted the scholarship WOMEN IN SCIENCE of L'Oréal-UNESCO to continue with a postdoctoral research related to the PhD topic.

List of manuscripts and publications

Olivares-Tenorio, M. L., Verkerk, R., van Boekel, M.A.J.S & Dekker, M. Thermal stability of phytochemicals, HMF and antioxidant activity in cape gooseberry (*Physalis peruviana L*) (submitted)

Olivares-Tenorio, M. L., Dekker, M., van Boekel, M.A.J.S & Verkerk, R. Evaluating the effect of storage conditions on the shelf life of cape gooseberry (*Physalis peruviana L.*) (submitted)

Olivares-Tenorio, M. L., Dekker, M, Verkerk, R., van Boekel, M.A.J.S &. Pascucci, S. The quality-attributes-alignment perspective and its implications for the agri-food value chains *scale up* (submitted)

Olivares-Tenorio, M. L., Dekker, M., Verkerk, R., & van Boekel, M. A.J.S. (2016). Health-Promoting Compounds in Cape gooseberry (*Physalis Peruviana L.*): Review from a supply chain perspective. Trends in Food Science & Technology (57A) 83-92.

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Overview of completed training activities

Discipline courses and international conferences (11 ECTS)

Reaction kinetics in food science (2012), Wageningen, The Netherlands

EFFOST annual meeting (2013), Bologna, Italy (poster presentation)

II conference of institutional research UNIAGRARIA (2013), Bogotá, Colombia (oral presentation)

International conference of food marketing research (2014), Arhus, Denmark (oral presentation)

I day of reflection in research UNIAGRARIA (2015), Facatativá, Colombia (oral presentation)

Innovations in Food Packaging, Shelf Life and Food Safety (2015), Erding, Germany (oral presentation)

International Conference on Nutrition and Food Sciences WASET (2015), Zurich, Switzerland (oral presentation)

General courses (9.4 ECTS)

VLAG PhD Week (2012), VLAG Graduate School, Baarlo, The Netherlands

Techniques for Writing and Presenting a Scientific Paper (2013), Wageningen Graduate Schools, Wageningen, The Netherlands

Scientific Publishing, Wageningen University Library (2012), Wageningen, The Netherlands

Scientific Writing (2013), Wageningen Graduate Schools, Wageningen, The Netherlands

Project and Time Management (P&TM) (2013), Wageningen Graduate Schools, Wageningen, The Netherlands

Data Management (DM) (2012), Wageningen Graduate Schools, Wageningen, The Netherlands

Competence Assessment (CA) (2012), Wageningen Graduate Schools, Wageningen, The Netherlands

Presentation skills (2012), Wageningen University Languages services, Wageningen, The Netherlands

Teaching and supervising Thesis students (2012), Wageningen University Educational Staff Development, Wageningen, The Netherlands

Entrepreneurship (2014), Tradeit, Tralee, Ireland

Optional courses and activities (9.8 ECTS)

VLAG Research proposal (2012)

International excursion (2014), Food Quality and Design, Singapore and Thailand

Preparation international excursion (2013-2014), Food Quality and Design, Singapore and Thailand

Scientific Meetings (2012-2016)

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