Impact of Preharvest Relative Air Humidity and Postharvest Modified Atmosphere Packaging on Cucumber Fruit Quality

By

Haider Ali

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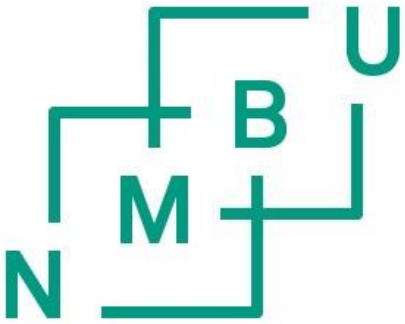

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Master Thesis

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Haider Ali

Table of Contents

Abstract

The effect of relative air humidity (RH) was tested on the cucumber (Cocktail, Quarto F1 cultivar) plant growth, morphology and fruit quality in controlled growth chambers. The plants were grown at moderate RH (60%) and high RH (90%) with the same temperature, CO_2 and irradiance. In addition, another experiment was conducted to test that effect of exogenous application of abscisic acid (ABA) and different packaging materials (modified atmosphere packaging bags and plastic folio) on the quality of commercial produced cucumber fruits stored at low temperature (TC) storage for 14 days and 21 days.

Cucumber plants and fruits responded strongly to the different RH conditions. The plant shoot length, number of leaves and fruit diameter was increased at high RH, while average leaf area, relative chlorophyll contents, number of side shoots were increased at moderate RH compared to high RH. Higher antioxidant capacity and total phenolics contents were observed in fruits from moderate RH. Through High Pressure Liquid Chromatography (HPLC) analysis 3 polyphenols (resveratrol, luteolin and apigenin) were identified in cucumber leaf samples and 6 polyphenols (apigenin, luteolin, quercetin 3 glycoside, quercetin, pinoresinol and resveratrol) were found in cucumber fruit samples. Moderate RH increased the resveratrol and luteolin concentration in cucumber leaves and increased the luteolin, quercetin 3 glycoside, quercetin and pinoresinol in cucumber fruit sample. Moderate RH not only affected the polyphenols contents, but it also influenced the sugars concentration in cucumber leaves and specifically in cucumber fruits. Significantly higher starch contents were found in cucumber leaves from high RH, while fructose, glucose and sucrose was not really effected by difference in RH. Stachyose and raffinose contents in leaves were significantly increased at moderate RH. On the other hand, fructose, glucose and starch was significantly higher in cucumber fruits from moderate RH. Furthermore, total nitrogen, potassium and boron contents were higher in cucumber leaves from moderate RH. Higher total nitrogen, total carbon, phosphorous and potassium contents were found in cucumber fruits from moderate RH, while calcium, magnesium, manganese and molybdenum contents in fruits were increased with increased RH. The sensory evaluation of cucumber fruits indicated that RH induced a more bitter taste perception and contained more water. On the other hand, cucumber fruits from moderate RH was perceived more sweet, better in

flavour and colour. Through correlation analysis, it was found that better flavour and good colour of cucumber fruits was due to starch contents and bitterness might be the taste of molybdenum.

In the second experiment, ABA application and MAP bags reduced the respiration of commercially produced cucumber fruits. Furthermore, the combined treatment of ABA with MAP bags effectively reduced physical weight loss, disease incidence and skin shrivelling of cucumber fruits. The ABA treated-MAP bagged and non ABA treated-MAP bagged fruits showed the lowest percentage of ion leakage, higher anti-oxidant capacity and total phenolics contents.

In summary, the cucumber plants grown at moderate RH performed better in terms of vegetative growth and produced good internal and external fruit quality. On the other hand, the cucumber fruits got extended storage life and maintained the better quality under MAP bagged condition.

1. Introduction

Cucumber (*Cucumis sativus.* L) belongs to family *Cucurbitaceae* and is native to the sub-tropical regions of South Asia (Miao et al., 2007). Cucumber is produced in open fields as well as in controlled conditions of greenhouses depending upon climate and geographical locations (Nonnecke, 1989). The plant of cucumber is a creeping vine and bears cylindrical, yellowish - dark green fruits which are not only used as culinary vegetable but are also used in medicine and cosmetics products (Sarhan and Ismael, 2004).

The world production of cucumber is 75 million tons, while Asia is the main cucumber producing continent with a share of 88% followed by Europe and America with 7.5% and 2.8% share respectively. In Asia, China is the major producer of cucumber with 54 million tons (FAOSTAT, 2014a). Mexico, Spain and Netherland are the major exporter of cucumber, while USA, Germany and Russia are the top three importers of cucumber (Anonymous, 2014). Due to unfavourable weather conditions in some countries such as Norway, China, Korea, Japan, Sweden, Netherland and Canada most of the cucumber are being produced under controlled conditions (Dorais et al., 2001)

Cucumber are consumed in different ways. It is consumed as fresh, raw, sauces, salad, pickle and as a component in many culinary dishes. Botanically cucumber is considered as fruit, while some consumers consider it as a vegetable (Malik and Bashir, 1994).

Cucumber contain 95.2% water. While, it is rich source of vitamin K (16%), antioxidants, phenols, potassium and some sugars (Bourn and Prescott, 2002).

It has been observed that the production of cucumber fruit at high RH in greenhouse reduces fruit quality in terms of nutritional value and shelf life, which is a serious problem (Beuchat, 1998). Cucumber fruits with a good quality at a moderate price is the demand of the market (Ahmed et al., 2004). Low shelf life due to loss of quality (membrane integrity, chilling injury, loss of chlorophyll, fruit weight loss and fruit softness) of many fruits in refrigeration is also a major concern of the consumers (Gine-Bordonaba et al., 2016). Cucumber is a tropical fruit and shows chilling injury (CI) symptoms at non-freezing temperature storage (Mao et al., 2007). Pre-harvest climatic conditions (light, relative air humidity and temperature) and postharvest storage temperature and packaging material are the key factors associated with cucumber fruit quality. Intensive production techniques and favourable environmental conditions

in the greenhouse, give high yield and excellent quality as compared to cultivation in open fields (Bot, 2003). However, the climate during cucumber development is important for the quality.

Mostly, in greenhouse conditions cucumber production takes place at high relative air humidity (85-90%). To ventilate warm humid air out of the greenhouse is expensive when the temperature outside is low and many growers keep the greenhouse more or less closed to save energy during autumn/winter. Under these conditions, although producers get higher yield, bigger fruits, higher plant, and high leaf area index and thick leaf (Jeon et al., 2006), these conditions are not the desirable traits for commercial cucumber production. Moreover, due to high RH stomata functionality may be reduced and do not close in response to closure signals like darkness but continue to transpire also during night (Fordham et al., 2001). After harvest, cucumber fruit may also transpire, due to open stomata and loose fresh weight, physical quality and shelf life.

After harvest the quality of fruit can be maintained by adopting some protocols such as postharvest packaging material and storage temperature. Packaging material not only enhance the aesthetic value, but also influence the fruit physiology. Packaging material slows down the respiration rate of fruit by creating a micro-climate of low oxygen and higher carbon dioxide concentration, which reduces water loss, increases the storage duration and sustain the quality of the fruit.

1.1. Objectives of the Study

This thesis consists of two parts with different experimental approach: (1) Growth experiment in controlled environment with RH as the main factor to study quality of cucumber fruits (2) Postharvest storage experiment with cucumbers fruits from a commercial grower. In both parts, the objective was to attain the goal of premium fruit quality. The aim in the two different experiments was

To study how different relative air humility conditions during plant production and fruit development influence on plant growth and fruit quality.

To study the impact of different packaging materials on the storability of commercial fruits and to test if the plant hormone abscisic acid is important for the fruits sensitivity to cold storage.

1.2. Literature review

1.2.1. Cucumber

Cucumber is a widely grown creeping vine in the gourd family, which bears cucumiform shaped fruits that are usually used as a vegetable (Garden, 1893; Reznicek et al., 2011). It is divided into three main varieties: slicing, pickling, and seedless. There are several cultivars which have been created by using these main varieties. The cucumber is being produced all over the world (Grubben, 2004; Reznicek et al., 2011).

Cucumber grows up trellises or other supporting frames. Cucumber plant have Viburnum leaves with large leaf area (10-16cm), dark green in colour, cordate, apically acute and rough surface (Zomlefer, 1994; Grubben, 2004 and Reznicek et al., 2011). The fruit of typical cultivars of cucumber is indehiscent cylindrical, but glabrous, elongated with tapered ends with number of seeds. Cucumbers have smooth or warty surface, green to yellow in colour, weigh 50g to 4kg. Each plant yields typically 25 fruits in a season (Grubben, 2004 and Lu et al., 2011).

1.2.2. Cucumber Industry

Cucumber is highly important commercial vegetable crop in the world. It ranks 4th following the potato, tomato and onion with an annual production of 75 million tonnes (FAOSTAT, 2014a). In Asia, China is major producer of cucumber with share of 57 million tonnes of total world's production in 2014 (FAOSTAT, 2014a). After Asia, Europe is second largest producer of cucumber (Figure 1.1).

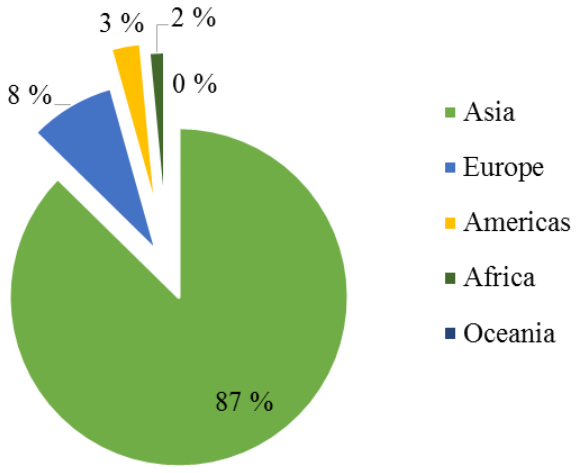


Figure 1.1: Worldwide Cucumber production by continents (FAOSTAT, 2014a). Source; http://www.fao.org/faostat/en/#data/QC/visualize

1.2.3. Cucumber Industry in Norway

The agriculture industry in Norway shares 1.6% of total GDP (Gross domestic Product) (SSB, 2015). Cucumbers dominates other greenhouse crops and accounted for 51% of the total production. (SSB, 2015). While in overall vegetables commodities, cucumber ranks 3rd with respect to production (Figure 1.2).

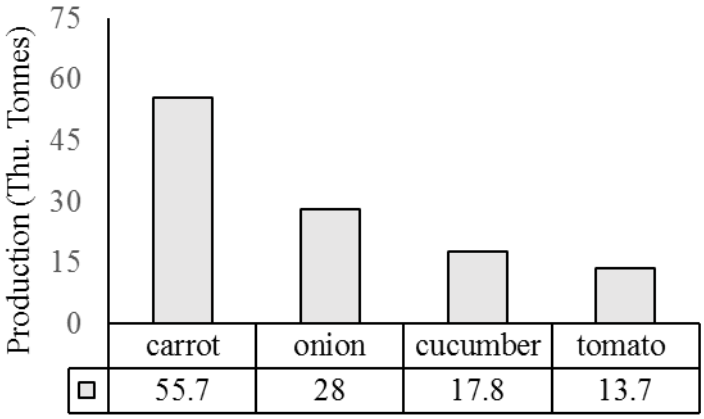


Figure 1.2: Top four vegetable crops of Norway by annual production (FAOSTAT, 2014b).
Source; http://www.fao.org/faostat/en/#data/QC

1.2.4. Quality

In reported literature, there are a number of definitions of term "Quality" exist. But, according to Shewfelt (1999) "Quality is a term frequently used but rarely defined." It means the definition of quality depends on stakeholders of supply chain groups i.e., producers, wholesalers, retailers and consumers. Usually, it is described in terms of physical appearance, biochemical compounds and sensory attributes (Cuartero and Fern´andez-Munoz, 1999). The general quality parameters of cucumber fruits are colour, size, surface smoothness, disease, fruit softness, skin bruises, shrivelling, physical injury, shelf life, taste, flavour and water contents (Ennis and O'sullivan, 1979). The cucumber fruit with more green in colour, big in size, have smooth surface, blemishes and disease free, good in taste and flavour are considered high in quality.

1.2.5. Nutritional Importance

Cucumbers are consumed fresh (Slicing cucumbers) and pickled. The juice from the cucumber leaves aid digestion and induce vomiting (Fern, 1997; Grubben, 2004). Most of the cucumber fruit biomass consist of 90-95% water. 100g serving of cucumber contains 3.63g of

carbohydrates, 0.65g protein, 5% Pantothenic acid, 3% of Pyridoxine and Riboflavin, 147mg Potassium, 0.28mg Iron, 24mg Phosphorus and 13mg Magnesium. Surprisingly, they are a rich source of vitamin K and vitamin C with a share of 13.6% and 4.5% of dry matter respectively (Lixandru, 2014).

1.2.6. Therapeutic importance

In past few decades, the concept of using natural foods has changed and offered an advance practical approach through which consumers can attain optimal health by reducing the risk of chronic diseases (Bordbar et al., 2011). Cucumbers are rich source of vitamins (Vitamin K and C), phytochemicals (apigenin, quercetin and luteolin) and pinoresinol. Phytochemicals and pinoresinol have a strong antioxidant and anti-inflammatory effects (Anonymous, 2017). Cucumbers also contains cucurbitacin compound, which is a phytonutrient and belongs to a large family of triterpenes. Cucurbitacin and pinoresinol has been reported to have an anti-cancer benefits (Lixandru, 2014).

Apart from antioxidant and anti-inflammatory activities of phenols in cucumber, the potassium in cucumber is also an important intracellular electrolyte. This ability of potassium intake reduces the blood pressure, control heart rate and minimize the chances of heart attack (Lixandru, 2014). The vitamin K in cucumbers plays a key role in promoting blood coagulation, osteoblastic (bone formation) activity and bone strengthening (Anonymous, 2017).

1.2.7. Climate and plant growth

Climatic factors are referred to abiotic factors and include water, rainfall, light, temperature, relative air humidity, CO_2 and air movements. They influence plant growth and development directly and indirectly. A lot of research work has been reported on the effects of rainfall and water (Edmond et al., 1975; Eagleman, 1985; Miller, 2001), light (Devlin, 1975; Edmond et al., 1975; Manaker, 1981; Abellanosa and Pava, 1987), temperature (Devlin, 1975; Poincelot, 1980) and wind on plant growth and development. But, very little consideration have been given to influence of air movement and relative air humidity (Miller, 2001). All climatic factors are associated with photosynthesis, transpiration, transportation of water, plant growth and development and other physiological processes in plants.

The fundamental process for carbon (C) accumulation, growth, and biomass production in plants is photosynthesis. All climatic factors such as quality and intensity of light, temperature and relative air humidity influence the photosynthesis. These factors also indirectly affect biomass production and plant growth (Bakker, 1995). Phytochrome, light receptor respond to light quality and triggers multi-component signals to induce fundamental cellular processes and controls the plant height (Reed et al., 1993). Low temperature reduces the water absorption and slows down the physiological process, which reduce the plant growth (Skálová et al., 2015). These factors not only affect the plant growth, but also control external quality, internal quality and organoleptic attributes of vegetable products (Gruda, 2005).

1.2.8. Relative Air Humidity and Plant Morphology

High relative air humidity (RH) severely affects the plant production in greenhouses. Most specifically, winter climate of Northern countries, is not quite friendly for ventilation and energy saving (prone to heat loss) (Mortensen, 2000). It has been reported that high RH have strong effects on plant morphology such as increase in plant height (Hoffman and Rawlins, 1971; Mortensen, 1986; Mortensen and Gislerød, 1990; Mortensen and Fjeld, 1998; Mortensen, 2000; Leuschner, 2002; Jeon et al., 2006), biomass and length of shoots (Hoffman et al., 1971; Mortensen, 1986; Mortensen and Gislerød, 1990; Mortensen and Fjeld, 1998; Mortensen and Gislerød, 1999; Mortensen, 2000; Jeon et al., 2006), more leaf area (Mortensen, 2000; Leuschner, 2002; Jeon et al., 2006; Hovenden et al., 2012), less leaf thickness (Leuschner, 2002; Torre et al., 2003) and chlorophyll contents (Mortensen and Gislerød, 1990; Jeon et al., 2006).

The increase in leaf area index (LAI) has been linked to photosynthesis, carbon assimilation and carbon metabolism (Jeon et al., 2006). Torre et al. (2003) reported reduction in leaf thickness, which was attributed to a decrease in size of spongy and mesophyll cells under the epidermis of leaves. It was also observed that the size of intercellular air-spaces increased under high RH condition. Same kind of findings were showed by Leuscher (2002). Most of previous studies reported the increase in leaf area at high RH (Van de Sanden & Veen, 1992; Roriz et al., 2014), which is due to cell expansion in epidermal cells (Carins-Murphy et al., 2014). But Innes, (2015) stated that the leaf area and leaf length not really get influenced by RH level. Van de Sanden (1985) and a recent study by Jakobsen (2016) reported opposite findings, they found the increase in leaf area of cucumber plant at moderate RH.

There are very few studies have reported the effects of RH on chlorophyll contents. Only one study stated the increase of relative chlorophyll contents in response to increase in RH (Jeon et al., 2006). On the other hand, Innes, (2015) and Jakobsen, (2016) found less chlorophyll contents in plants under high RH.

1.2.9. Carbohydrates and polyphenols in cucumber

Stachyose, raffinose, sucrose, glucose, fructose and starch were found in cucumber plant leaves, as mentioned before (Alam, 2016). While fructose, glucose, stachyose and starch are primary sugars of cucumber fruits. Most of previous studies reported effect of temperature and light on photosynthesis and carbohydrates production in plants (Taji et al., 2002). Riesmeier et al. (1994) reported that these assimilates translocate in the form of sucrose, while in cucumber they also found as stachyose. That sugar is considered as predominant form of transport sugar in cucumber family (Hendrix, 1982; Webb and Gorham, 1964), but in fruits there was not stachyose found. On transportation to fruits, the stachyose metabolized into sucrose and later converted into glucose and fructose (Gross and Pharr, 1982). More photosynthesis take place at high RH, because stomata remain open and excessive CO_2 remain available to the plant (Grange and Hand, 1987). Previously very little study have been done on photosynthesis activity and RH.

A lot of studies have been conducted regarding influence of RH on plant growth, transpiration, stomatal conductance, photosynthesis, transport of mineral and water (Hoffman and Rawlins, 1971; Ford and Thorne. 1974; Tibbitts and Bottenberg, 1976; Tibbitts, 1979; Gislerod, et al., 1987; Gislerod and Nelson, 1989; Gislerod and Mortensen. 1990; Bakker, 1991; Torre. et al., 2001; Carins- Murphy, et al., 2014). But very few studies have focused on the effect of RH on polyphenols in leaves or fruits. Cucumber plant contain a lot of polyphenols and some of them are identified as apigenin, quercetin, luteolin (Hertog, et al., 1992; Chu, et al., 2000; Lugast and Hovari, 2000) and pinoresinol (Peñalvo, et al., 2005; Milder, et al., 2005; Peñalvo, 2007). But little researched base information is available about RH effect on phenols concentration in plants.

1.2.10. Greenhouse relative humidity and Fruit quality

Preharvest relative air humidity in greenhouse not only influence the plant growth and physiology, but also effect the fruit quality and shelf life. High and low vapour pressure deficit (VPD) have various impacts on postharvest fruit quality of cucumber and tomatoes. Fruits, cut

flowers, and ornamentals grown at high RH showed poor postharvest keeping quality, due to water loss, chilling injury at low temperature and less tolerance to stress (Mortensen and Fjeld, 1998; Mortensen, 2000; Torre et al., 2003). High RH (Low VPD) caused a decrease in the fresh weight of marketable tomatoes (Holder and Cockshull, 1990). Tomatoes grown at high RH face physiological and ripening disorders after harvest (Mulholland et al., 2001). Fricke and Krug (1997) reported a finding that the cucumber fruit lost the quality under various humidified treatments. Bakker et al. (1987) recommended that variation in day and night-time humidity provided excellent cucumber fruit quality and better storage life. Cucumber fruits contain stomata on peel and stomata of cucumber fruits grown at high RH behave same as stomata of plant leaves (Mortensen, 2000).

1.2.11. MAP bags and fruit physiology

The respiration is a metabolic process, which play major role in deterioration of the fresh produce, and it aims at the oxidative breakdown of complex organic substrates into simple molecules such as CO_2 and H_2O with the production of energy (Fonseca et al., 2002). Respiration rate of fresh produce depends on the storage conditions, particularly temperature, RH and gaseous composition. Respiration rate can be slow down by decreasing the O_2 concentration as well as increasing the CO_2 concentration in the environment (Saltveit, 2002; Rocculi et al., 2006). Modified atmosphere packaging (MAP) is one of the most important food preservation methods. By using MAP bags, the respiration rate slows down through creating the microclimate in the bags, which helps to extend the storage life and maintain the natural quality of fresh produce (Martínez-Ferrer et al., 2002).

1.2.12. MAP bags and chilling injury

Chilling injury (CI) is a common physiological disorder of many tropical and subtropical fruits, vegetables and ornamental crops which arises during the low temperature storage (Cabrera and Saltveit, 1990). Exposure of chilling-sensitive crops to cold temperature (<12 °C) caused variable symptoms that included uneven ripening or discoloration, higher water loss, increased surface pitting, wilting, fruit softening upon warming and increased permeability of the cellular membranes (Cabrera and Saltveit, 1990; Wang, 1993; Lelièvre et al., 1995). CI becomes more

severe with longer storage times and/or at lower temperatures (Zagory and Kader, 1988). Differences in chilling sensitivity have been reported in tomatoes (Autio and Bramlage, 1986).

Normally due to aminocyclopropane-1-carboxylic acid (ACC) synthase activity, the ethylene production is a common symptom of chilling injury (Wang, 1987). The production of polyamine titres increased in plants with CI and other stresses such as osmotic shock, variation in UV radiation, oxygen deficiency stress, low pH, as well as K^+ and Mg^{2+} deficiency (Serrano, et al., 1997; Wang, 1987). Apart from ethylene production, the electrolyte or ion leakage is also associated with CI, fruits or some plant cells lost their membrane permeability at chilling temperature (McCollum and McDonald, 1991). In tomato fruits the ion leakage due to chilling injury did not show immediate increase on exposure to chilling temperature (Saltveit, 2002). Treating the lemon and avocado fruits with CO_2 (10−40%) before low temperature storage reduced CI symptoms, while avocado tolerated low temperature with treatment of low concentrations of O_2 (Wang, 1987; Pesis, et al., 2000). On the other hand, modified atmospheres packaging diminish the chilling injury symptoms in many fruits (Cabrera and Saltveit, 1990). It has been reported that the Xtend® film (XF) was more effective to reduce the symptoms of CI in mango fruits as compared to micro-perforated polyethylene (PE) film (Pesis, et al., 2000).

1.2.13. Exogenous ABA application and fruit quality

Although plants produce ABA endogenously, but the exogenous application of ABA also influence the plant physiology and has been implicated as a regulatory factor (Heino, et al., 1999). Spomer (1979) reported that the exogenous application of ABA on cucumber seedlings increased the membrane integrity and reduced the chilling injury and ion leakage, while Rinkin et al. (1976) noticed that exogenous application of ABA increased the level of endogenous ABA and also increased the tolerance of plant tissues to chilling. Phenol concentration and phenylalanine ammonia-lyase (PAL) activity increased rapidly with exogenous application of ABA in strawberries fruits (Jiang and Joyce, 2003). Previously it has been reported that the opening of stomata regulated the elevation of Ca^{2+} in guard cells and down-regulated the K^+ ions (Schroeder and Hagiwara, 1989) and H^+-ATPases (Kinoshita et al., 1995), which provided basic and mechanized approach for ABA and influence of Ca^{2+} to inhibit K^+ uptake.

2. Materials and Methods

The present study was conducted at the Centre of climate regulated Plant Research (SKP), Norwegian University of life Sciences, Norway during May, 2016 to October, 2017. The experiments were conducted in 2 phases.

2.1. Experiment 1: effect of relative air humidity on plant growth and fruit quality

2.1.1. Seedling Production

The seeds of cucumber (Cocktail, Quarto F1 cultivar, L.O.G. As) were sown directly into 30 (12 cm) pots in a greenhouse compartment (20 – 25 °C, RH 70%, ambient CO_2 and 100µmole.m^{-2}.s^{-1} PAR from high pressure Sodium (HPS) lamps) on 9th May, 2016. The Sphagnum peat (pH 5.0 – 6.0 and salinity ca. 1.5 – 2.5) produced by Degernes Torvstrøfabbrikk AS, (Degernes, Norway) was used as growing media.

The climate in greenhouse was controlled by a PRIVA system (Priva, De Lier, The Netherlands). HPS lamps (Osram NAV T-400W, Munich, Germany) were used to meet the daily light requirement of plants and light intensity was measured with Li-Cor Model L1 250 Quantum Sensor (Li-Cor Inc., Lincoln, NE, USA). To attain the uniform germination, pots were covered with polyethylene sheet. On emergence of the seedling the sheet was removed. Seedlings were irrigated daily. On second leaves stage (27th May, 2016), the plants were moved to controlled environment growth chambers (Figure 2.1).

Figure 2.1: Cucumber Plants seedlings, before transfer to growth chambers

2.1.2. Experiment set-up

The 22 healthy plants were subjected to controlled relative air humidity growth chambers. 11 plants were placed in each chamber of moderate 60% and high 90 % RH (Figure 2.2). Other climatic conditions (23 °C temperature, ambient (400ppm) CO_2 and 200µmol.m^{-2}.s^{-1} PAR HPS lights with 20 light and 4 dark interval) were common in both chambers.

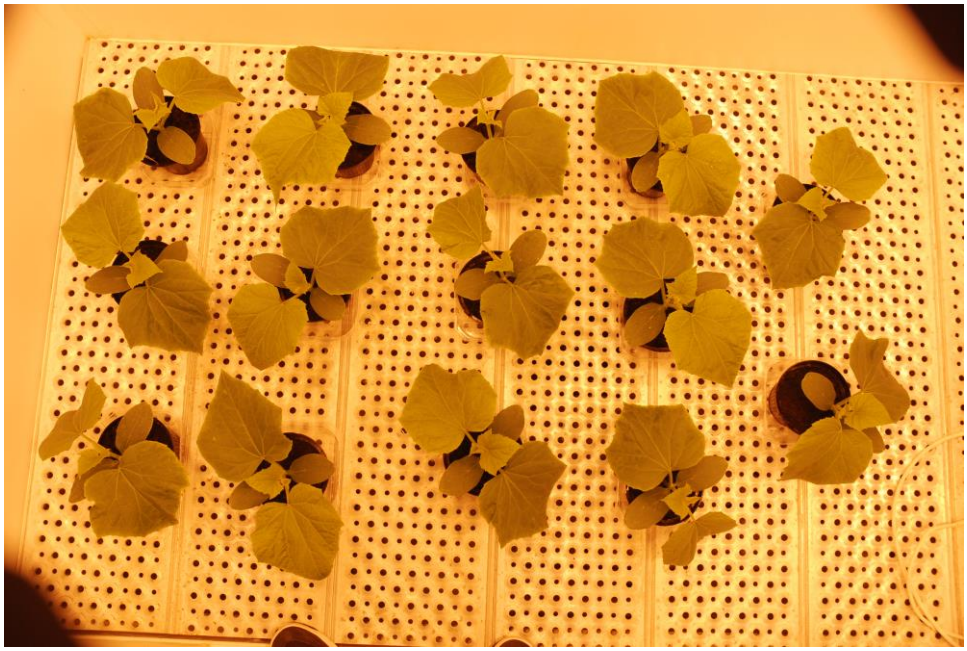


Figure 2.2: cucumber plants in growth chambers

2.1.3 Irrigation and plant maintenance

The plants were irrigated thrice a week with 50/50 mixture of Kristalon™ Indigo (7.5% NO_3, 1% NH_4, 4.9% P, 24.7% K, 5.7% S, 4.2% Mg, 0.027% B, 0.2% Fe, 0.06% Mn, 0.027% Zn, 0.004% Cu and 0.004% Mo, Yara Norge AS, Oslo, Norway) and YaraLiva® Calcinit™ Calcium nitrate solution (14.4% NO_3, 1.1% NH_4, 19.0% Ca, Yara Norge AS, Oslo, Norway) and 4 time a week with normal tap water.

The tendrils of plants were removed twice a week and on fruiting stage the pinching of diseased and small alternate fruits was also practiced twice a week. After one and half week (6th June, 2016) in growth chambers, 6 plants from each chamber were removed and used for initial data collection of growth parameters. Remaining 5 plants were left for fruiting. On fruit setting, 1, 2, 4 and 6 fruits were marked for fruit harvesting. The size of marked fruits were analyzed on alternate day to see maturation stage. On consistent reading, the fruits were considered ready to

harvest. The fruits harvesting was done on 29th June, 2016. 1 fruit from each plant was sampled for phytochemicals and sugars analysis. 1 fruit from each plant was used for dry matter percentage, minerals analysis, total phenols and antioxidants analysis, while remaining 2 fruits were used for sensory evaluation.

The data collection included physical parameter of plant and fruit growth, biochemical analysis and organoleptic evaluation. Furthermore fruit weight loss was also recorded. Analysis of dried fruits and leaves was also carried out to determine mineral concentration.

2.1.4 Data collection

2.1.5 Growth data and Physical Analysis

Following parameters were included in physical analysis.

- Plant Height (measuring tape)
- Number of Leaves (count)
- Number of fruits (count)
- Number of side shoots (count)
- Average Leaf Area Index
- Chlorophyll contents
- Fruits Length (Measuring tape)
- Fruit Diameter (Vernier Calliper)
- Dry matter percentage

Every leaf from each plant was removed, counted, weighed (fresh sample weight) and used for measuring the leaf area (LA). The LA was measured by using LI-3100 Area meter (Li-Cor, Inc., Lincoln, Nebraska, USA). The leaf area was divided by counted leaves and average leaf area was calculated.

Chlorophyll contents were measured using CL-01 Chlorophyll content meter (Hansatech Instruments Ltd, Narborough Road, Pentney, King's Lynn, Norfolk, UK). This field-portable, hand-held device determined relative chlorophyll content using dual wavelength optical absorbance (620 and 940nm) measurements from leaf samples. Relative chlorophyll content was displayed in the range 0 – 2000 units. The CL-01 features simple 2 button operation device. The reading was taken by placing the plant leaf between optics. It is auto-calibrating device.

The fruit length was measured from stylar end to blossom end. While fruit diameter was measured from both sides and centre of fruit. Dry matter was calculated on the basis of initial weight (before storage) and final weight (at the end of storage period) according to following formula;

$$\text{Dry matter (\%)} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

The leaves and fruits were divided into 2 parts. Half of leaves and fruits were used for dry matter contents (later on dried samples were used for minerals analysis) and remaining half were freeze dried and used for biochemical analysis.

2.1.6 Biochemical analysis

Biochemical analysis was done for the following compounds

1. Total phenolic and Anti-oxidants capacity (FRAP)
2. Phenolic compounds
3. Photo assimilates (Sugars)

2.1.6.1 Total Phenolic and Anti-oxidants capacity (Fruits)

Sample collection and preparation

For measuring the anti-oxidant activity and total phenolic concentration, a KONE-lab was used. The fresh cucumber fruits (10 fruits) from each treatment (2 treatments) were stored at -20 ºC after harvest. The frozen samples were placed at room temperature for melting. After melting the sample was homogenized using hand blender. 3g of homogenized sample was taken in 50 ml centrifuge tube. The 30 ml of acidified (10 mM HCl) methanol was added in tube. The sample was vortexed for 30s. After vortex, the samples were sonicated in water bath at 0 ºC for 15 minutes followed by centrifugation for 10 minutes at 4 ºC and 4000 rpm. Supernatant was poured into Eppendorf-tube and centrifuged again for 3 minutes at 4 ºC and 132000 rpm.

2.1.6.1.1Anti-Oxidant activity (FRAP- assay)

The Ferric reducing ability of plasma (FRAP) assay was used to measure the concentration of total anti-oxidants. The method was based on the colour changes appeared when the TPTZ-Fe^{3+} (2,4,6-tri-pyridyl-s-trizine) complex was reduced to the TPTZ-Fe^{2+} form in the

process of antioxidants. An intense blue colour with the absorption maximum at 593 nm developed. The samples were measured at 600nm. An aqueous solution of 500 µM $FeSO_4 \times 7.H_2O$ was used for calibration of the instrument.

The calculation of standard was done by using following equation

$$\text{FRAP value of sample (µM)} = \text{Abs (sample)} \times \frac{\text{FRAP value of Std}}{\text{Abs (Std)}}$$

2.1.6.1.2 Total phenolic contents (TPC) determination

The TPC of cucumber was determined using the Folin–Ciocalteu (FC) method as outlined by Ainsworth and Gillespie (2007) with some modifications. The extracted samples (100 µL) were mixed with FC reagent (200 µL) in a fresh eppendrof tube and vortexed with the help of vortex mixer (SLV-6, MyLabTM, Seoulin BioScience, Korea) thoroughly for a few seconds. After adding 800 µL of 700 mM sodium carbonate (Na_2CO_3) were again vortexed for few seconds and incubated at room temperature for 2 h. TPC were determined at 765 nm. The TPC were expressed as mg GAE 100g-1 against the standard curve of gallic acid (Figure 2.3).

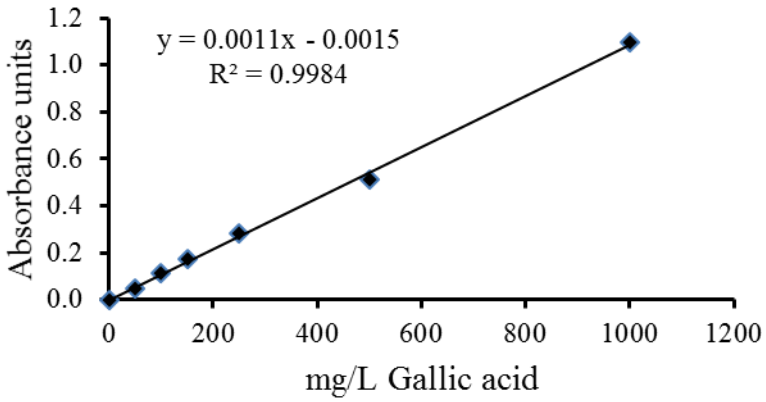


Figure 2.3 Standard curve of gallic acid for determination of TPC

2.1.6.2 Polyphenols compounds

Sample preparation

After harvest, the leaves, fruit were peeled with common home use potato peeler. The peel and pulp samples were placed into test tubes (50 mL) and immediately frozen using liquid

nitrogen. The samples were placed into -80 ºC freezer. Before extraction the samples were freeze dried and grinded.

Procedure

The polyphenols were analyzed by high-performance liquid chromatography (HPLC) (Agilent, Series 1100, Germany), consisting of a binary pump (G1312A), a thermostated auto-sampler (G1329A), a thermostated column oven (G1316A) and a diode array detector (G1315B). The phenolic metabolites were separated using a Zorbax SB-C18 (4.6 × 60 mm) HPLC column (Agilent Technologies, USA). The samples were re-dissolved in 600 µL methanol: water (1:1) and eluted (flow rate 2 ml min^{-1}) using the methanol: water gradient (Julkunen-tiitto et al., 1996). The auto injection volume was 20 µL, and all runs were performed at 30 ºC. The phenolic metabolites were identified by comparing their retention times and UV spectrum with those of standards.

Extraction of phenolic compounds

20 mg of plant material (grinded) was taken into an Eppendorf vial and 600 µL MeOH (methanol) was added into the vial and homogenized for 30 s. The vial was left in ice bath for 15 minutes followed by centrifugation for 3 minutes on high speed (18000 rpm). The supernatant was poured into marked reagent vial (6 – 10 ml). 600 µL MeOH was added to residue (the rest of plant material) and homogenized again for 30 s. after that material was centrifuged for 3 minutes on high speed and supernatant was poured into same reagent vial. The same procedure was repeated 3 times more and supernatant was collected into reagent vial. MeOH was evaporated from the collected extract using vacuum concentrator. The extract was stored in 4°C until analysis.

HPLC analysis

The dried extract was removed from freezer. 200 µl MeOH and 200 µl distilled water was added into dried extract. The ultrasound bath was used to dissolve the material. The material was poured into Eppendorf vial and centrifuged. The supernatant was poured into a HPLC vial and a lid was put on. The sample was ready for analysis. The standard curve for some compounds was obtained from reported study (Figure 2.4a and 2.4b) (da Graça Campos & Markham, 2007).

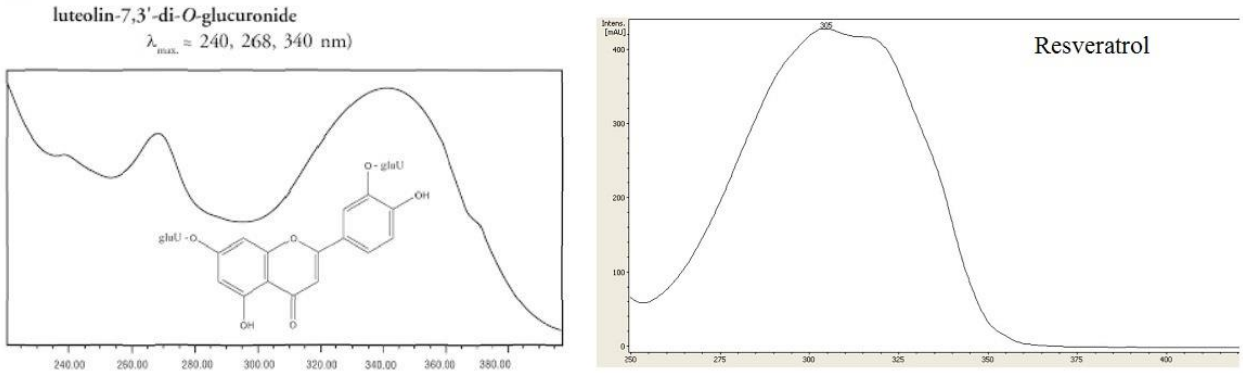


Figure 2.4a: The HPLC Chromatograph showing Standard curve for luteolin-7, 3'-di-*O*-glucuronide and resveratrol.

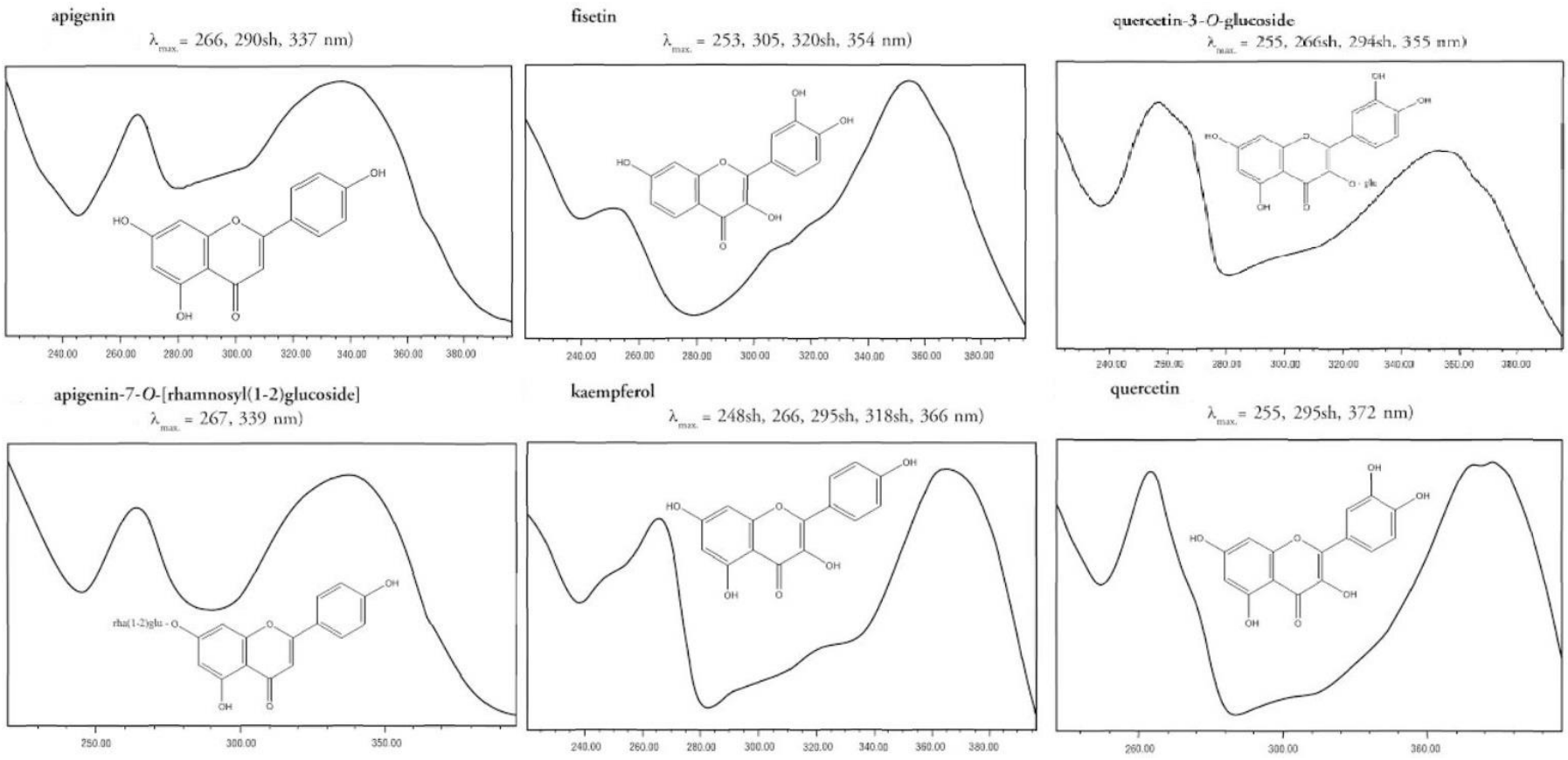


Figure 2.4b: The HPLC Chromatograph showing Standard curve for apigenin, Fisetin, quercetin-2-*O*-glucoside, apigenin-7-*O*-[rhamnosyl(1-2) glucoside], Kaempferol and quercetin.

2.1.6.3 Carbohydrates

The amount of carbohydrates (stachyose, raffinose, glucose, sucrose and fructose) in leaves and fruits of cucumber were analyzed by following Gross and Pharr, (1982). From leaves and fruits 250 mg of freeze dried samples (2 treatments and 5 replications) were taken in each test tube. The carbohydrates were extracted through heating the samples in 1.5 ml of 80% ethanol at 70 ºC for 30 minutes using ultrasonic bath. After heating, the samples were centrifuged at 15000 rpm for 3 minutes. Supernatant from each tube was collected in separate tube. The ethanol was removed from the supernatant at 60 ºC by using the vacuum desiccator (Eppendorf AG 22331, 8 Hamburg, Germany). After that, 1 ml water was added into dried extract and heated at 60 ºC for 30 minutes followed by centrifugation at 15000 rpm for 3 minutes. The supernatant was collected separately and filtered through a 0.45 µm GHP membrane filter (Millipore) before HPLC.

Separation of Carbohydrates

After extraction, the samples were analyzed through HPLC. Carbohydrates were separated on the basis of their adsorption characteristics and it was analyzed by passing the solution through a column (Agilent Hi-Plex Ca USP L19, 4.0 * 250 mm). The separation was achieved through refractive index detector. For the mobile phase, water was used as solvent and flow rate was 0.3 ml/min and the temperature of column was 80 ºC. 10 µl of extracted sample was injected by the HPLC. Eluted carbohydrates were identified and quantified on the basis of their retention time and area of standard sugars (Figure 2.5).

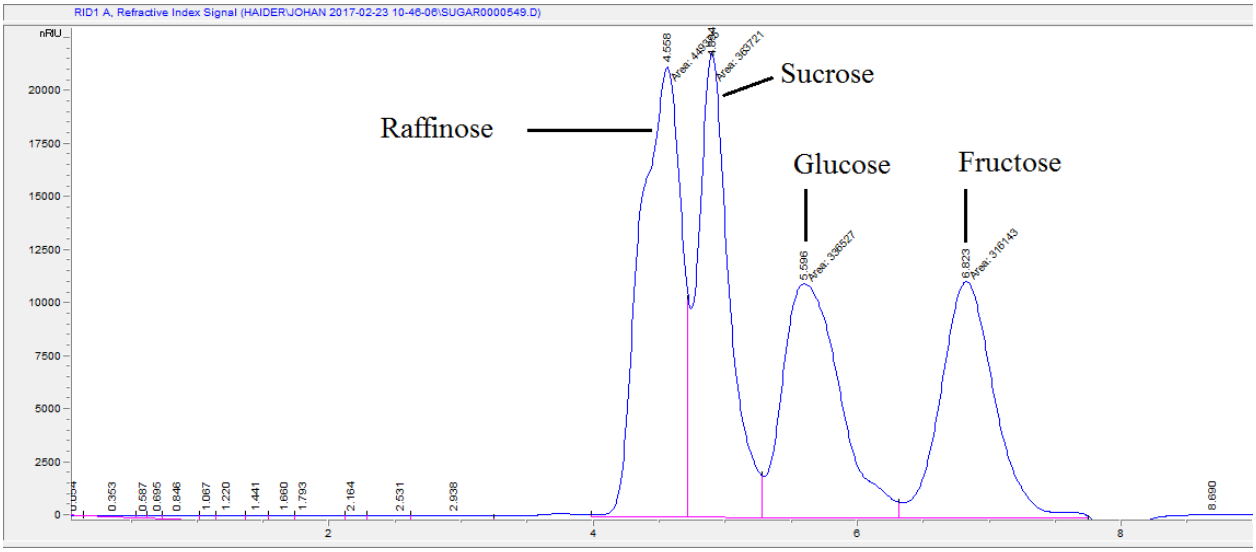


Figure 2.5: The HPLC Chromatograph showing Standard curve for sugars (0.1% of each Stachyose, Raffinose, Sucrose, Glucose and Fructose) according to their retention time

2.1.6.4 Mineral analysis

Element analysis (Mg, Ca, S, P, Fe, K, B, Mo, Mn and Na) were performed on leaves and fruits samples taken at the time of harvest by using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Total nitrogen content was measured by the use of the Dumas method (Bremmer and Mulvaney, 1982). Five replications were taken of each treatment. Following process was adopted to accomplish the analysis.

Sample collection

(i) leaves sample

(ii) fruit sample

Leaves sample preparation

Steps involved are as follows

(i) Leaves collection

(ii) Drying

(iii) Crushing/grinding

After harvesting mature leaves were selected. Samples were kept in brown envelops with tiny holes made with punch machine. Leaves was further dried in oven at 60 ºC for 48 hours. After that, grinding was carried out with electric grinder until powder form. Samples were stored in labelled plastic vials.

Fruits sample preparation (for dry matter contents)

(i) Weighing

(ii) Chopping

(iii) Hot air drying

(iv) Grinding/Crushing

100 gm of fruit was weighed with the help of weighing balance then chopping was carried out on a chopping mate with a sharp knife to divide the cucumber into minute pieces. After that drying was done using a hot air oven at 60ºC, and samples were weighed periodically after 24 hours until the weight turn out to be constant after (48 to 50 hours). The final weight was noted to calculate dry matter using the following formula.

$$\text{Dry matter \%} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

Process was terminated on grinding. All samples were leaded towards minerals analysis.

2.1.7 Organoleptic Evaluation

The fruits were evaluated at ripening for organoleptic acceptability on the basis of taste, pulp colour, and texture and over all liking using the 9 point hedonic scale described by Peryam and Pilgrim (1957). Ten judges were called in the panel for organoleptic evaluation of treatments.

Hedonic scale (Peryam and Pilgrim, 1957)

Product: _____ Date: _____

Name of Judge: _____ Signature: _____

Instructions: (*Please read the instructions carefully before filling the blanks*)

1. This is an organoleptic evaluation form for different cucumber samples.
2. Please follow the numerical system for scoring the samples.

Dislike extremely……..1 Like slightly……...6

Dislike very much…….2 Like moderately……7

Dislike moderately……3 Like very much……..8

Dislike slightly……...4 Like extremely……...9

Neither like nor dislike……5

- Please do not disturb the sequence of the samples provided.
- Please wash the tongue before testing next sample, with the water provided.

Sr.No.	Taste	Pulp Colour	Texture	Flavour	bitterness	Water cont.	Over Liking
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

2.2. Experiment 2: storability of commercial fruits

This experiment was conducted to analyse the impact of MAP bag, exogenous application of ABA and different temperature conditions influenced the cucumber fruit quality, storage duration and chilling injury sensitivity.

2.2.1. Experimental Layout

Plant material

The plant material was comprised of physiologically mature green cucumber fruits with equal size and weight of 150 to 200 grams. The fruits were sourced from commercial green house (Espedal Hansdelsgarteri AS, Lier, Buskerud, Norway) on 3rd March, 2017. The average greenhouse conditions during production were as follow (Table 2.1).

Table 2.1: experiments layout

Temperature (°C)	RH (%)	CO_2 (ppm)
20-22	80-85	1100-1150

Fruit were harvested manually along with the pedicel to avoid sap injury. After harvesting fruit were packed in corrugated boxes and transported to cold storage facility near Centre of climate regulated Plant Research (SKP), Norwegian University of Plant Sciences, Norway, using private vehicle at 20°C. Upon arrival at SKP, the fruit were graded, grouped, sprayed (half of fruits) with ABA (500µM), dried, packed in Xtend® MA/MH (modified atmosphere/ modified humidity) bags (StePac L.A. Ltd. Tefen, Israel) and plastic folio wrapping, placed in boxes, taken fresh weight, and subjected to low temperature storage conditions (11°C ±1; 85% RH) for 14 and 21 days (Table 2.2).

After each removal from storage room, the fruits were used for data regarding weight loss, physical appearance, chilling injury or fungal rot and ion leakage. While, bagged fruits were analysed for gases. After taking data, the fruits were wrapped again in plastic folio, weighed and placed at ambient temperature of 22-25 °C for 2 days (concept of retail market). After 2 days, the data about weight loss and physical appearance was noted. After taking data the fruits were wrapped and placed in normal fridge at 5-6 °C: 70-80% RH for 2 days (concept of consumers). Later on final data was noted and fruits were sampled for ion leakage, total anti-oxidant and total phenolic compounds.

Table 2.2: experiments layout

Main Factor	Sub-factor	No. of Removals	Replications
Map Bag	ABA Treated	2	2
	Non ABA	2	2
Plastic Folio Film	ABA Treated	2	2
	Non ABA	2	2

2.2.2. Data Collection

2.2.2.1. Gases concentration

The respiration of bagged fruits was analysed by using CO_2 analyser (Anagas CO_2 Analyser). The concentration of CO_2 was measured in percentage.

2.2.2.2. Physical data

2.2.2.2.1. Weight Loss

Fresh weight loss was calculated on the basis of initial weight (before storage) and final weight (at the end of storage period) according to following formula;

$$\text{Weight Loss (\%)} = \frac{\text{Initial weight - Final weight} \times 100}{\text{Initial weight}}$$

2.2.2.2.2. Skin shrivelling

Skin shrivelling of cucumber fruits was assessed by using scales used by Malik and Singh (2005). Skin shrivelling was recorded by using the scale as follows; 1: nill; 2: <10% affected area; 3: 10-25% affected area; 4: 25-50% affected area; 5: >50% affected area.

2.2.2.3. Ion Leakage

Electrolyte leakage was determined on eight disks (4 mm × 1 cm) taken with a cork borer from skin tissue from the surface. Disks were immersed in 20 mL of 0.3 M mannitol in glass vials, which were agitated at 20 °C for 120 min. Ion leakage was measured as the amount of increased conductivity ($\mu S\ cm^{-1}$) of the solution. After that, disks were boiled for 30 min and cooled to room temperature and the total conductivity was measured. Chilling-induced ion leakage was expressed as the percent of the total conductivity leaked per hour (Gonzalez-Aguilar et al., 2000).

2.2.2.4. Total Phenolic and Anti-oxidants (Fruits)

For determination of total phenolic and anti-oxidants compounds the same procedure was adopted (as mentioned in experiment 1).

3. Results

3.1. Experiment 1

3.1.1. Comparison of growth and morphology of cucumber plants and fruits produced in different RH conditions

3.1.1.1 Growth and Morphological parameters

3.1.1.1.1 15 days under controlled conditions

After 15 days under controlled conditions, the plant growth and morphology of 'Quarto F1' cocktail cucumber were slightly affected by RH (RH). No significant effects of RH on average leaf area, relative chlorophyll contents and number of fruits were found. However, the average leaf area (5.4%) and total chlorophyll contents (7.7%) were higher in plants grown at moderate RH compared to high RH. While the number of leaves and shoot length were significantly higher in plants grown at high RH with 20.5% and 35.4% difference respectively compared to moderate RH. Additionally the plants grown at moderate RH had 31.25% more side shoots compared to plants produced in high RH (Table 3.1).

Table 3.1: Growth and morphological Parameters of 'Quarto F1' cocktail cucumber plants after 15 days under different RH conditions (60% RH and 90% RH)

Parameters	60% RH	90% RH		
	Mean	Mean	SE	LSD
Avg. leaf area per leaf (cm^2)	139.35^a	132.15^a	5.6397	NS
Relative Chlorophyll content	19.53^a	18.12^a	1.0356	NS
No. of Leaves	7.8^b	9.4^a	0.3162	S
No. of Side Shoots	4.2^a	3.2^b	0.4243	S
Shoot length (cm)	42.140^b	57.060^a	1.9499	S
No. of Fruits	8.8^a	10.0^a	1.1489	NS

Means in rows not sharing similar letters differ significantly at $P \leq 0.05$; NS = Non-significant; S= Significant

3.1.1.1.2 On Harvesting

At the stage of harvest (33 days after start of treatments) the vegetative growth and fruit morphology were significantly affected by the different RH conditions (Table 3.2). Average Leaf Area, relative chlorophyll contents, percentage dry matter (biomass) of leaves and fruits, number of side shoots and fruit length were significantly higher in plants grown at moderate RH (60%) compared to high RH (90%) (Table 3.2). An increase in average leaf area

(7.5%), relative chlorophyll contents (17.2%), percentage dry matter (2.7%), number of side shoots (17.1%) and a higher number of fruits (18.9%) were observed in plants grown at moderate RH compared to high RH. On the other hand, a higher number of leaves (9.6%), longer shoots (9.0%) and an increase in fruit diameter (41.7%) were noticed in plant grown at high RH than moderate RH (Figure 3). While number of fruits were non significantly different, but a slightly higher number of fruits were found in plants grown in high RH conditions (Table 3.2).

Table 3.2: Morphological Parameters of 'Quarto F1' cocktail cucumber plants and fruit at the time of harvest under different RH conditions (60% RH and 90% RH), n = 9

Parameters	60% RH	90% RH		
	Mean	Mean	SE	LSD
Average Leaf Area per Leaf (cm^2)	143.63^a	133.60^b	2.0324	S
Relative Chlorophyll Contents	20.530^a	17.520^b	0.7490	S
Dry Matter Leaf (%)	15.274^a	14.872^b	0.0592	S
No. of Leaves	33.2^b	36.4^a	0.4472	S
No. of Side Shoots	16.4^a	14.0^b	0.4000	S
Shoot length (cm)	177.12^b	193.08^a	5.0930	S
No. of Fruits	29.6^a	31.4^a	1.1489	NS
Fruits diameter (mm)	23.396^b	33.152^a	1.6574	S
Fruit length (cm)	8.42^a	7.08^b	0.4391	S

Means in same row not sharing similar letters differ significantly at $P \leq 0.05$; NS = Non-significant; S= Significant

Figure 3: Pictorial view of cucumber fruits from different RH conditions.

3.1.2 Biochemical analysis of cucumber leaves and fruits under different relative air humidity conditions

3.1.2.1 Biochemical analysis

3.1.2.1.1 Total phenolics and Anti-oxidant capacity (FRAP)

Although total phenolic contents and anti-oxidant capacity in fruits were significantly affected by RH (appendix 1 and 2), and the anti-oxidant capacity was almost double in cucumber fruits produced in moderate RH as compared to high RH. Furthermore, the total phenolic contents were also higher (24.8%) in fruits sample of moderate RH (Figure 3.1).

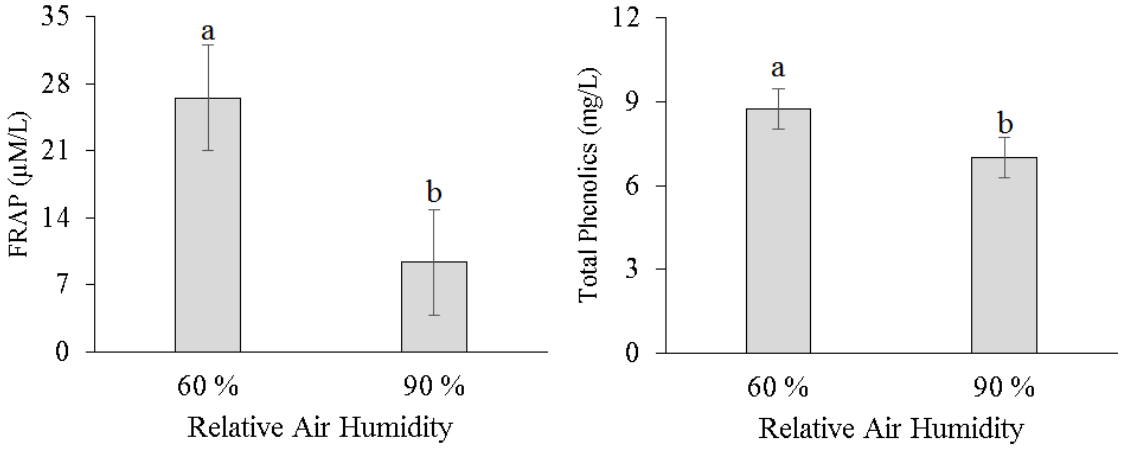


Figure 3.1: FRAP (µM/L) and Total phenolics contents (mg/L) in 'Quarto F1' cocktail cucumber fruits from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 5.5138 and 0.7215, the different letters on the bars express significant difference, n=9

3.1.2.1.2 Polyphenols in cucumber plant leaves, fruit pulp and peel

The High Pressure Liquid Chromatography (HPLC) chromatograms of the phenolic fractions in cucumber leaves, fruit peel and fruit pulp were analysed. Some of the peaks were identified according to saved standards of polyphenols in the system library. According to the retention time, almost 18 peaks were common in each leaf sample (Table 3.3) and 3 of them were identified as resveratrol, luteolin and apigenin by comparing their spectrums with standard spectrum (Figure 3.2, 3.3 and 3.4). Unidentified polyphenols in the different treatments were compared on the basis of peak area. While identified polyphenols were compared on the basis of their concentration, which was measured by comparing the standard compound against samples measurements.

In leaves, resveratrol and luteolin and 3 unidentified peaks were significantly different between the RH treatments. While apigenin was non significantly affected by RH (Appendix

3). Higher concentration of resveratrol, luteolin and apigenin was observed in leaf samples from moderate RH (60% RH) as compared to leaf sampled from higher RH (90% RH) (Figure 3.7) and 2 unidentified polyphenols were also significantly higher in leaves samples from moderate RH. The remaining 12 peaks were non significantly different for both treatments (Table 3.3). Peak with retention time 21.8 showed higher value for polyphenols concentration in leaves samples from higher RH (90% RH) (Table 3.3).

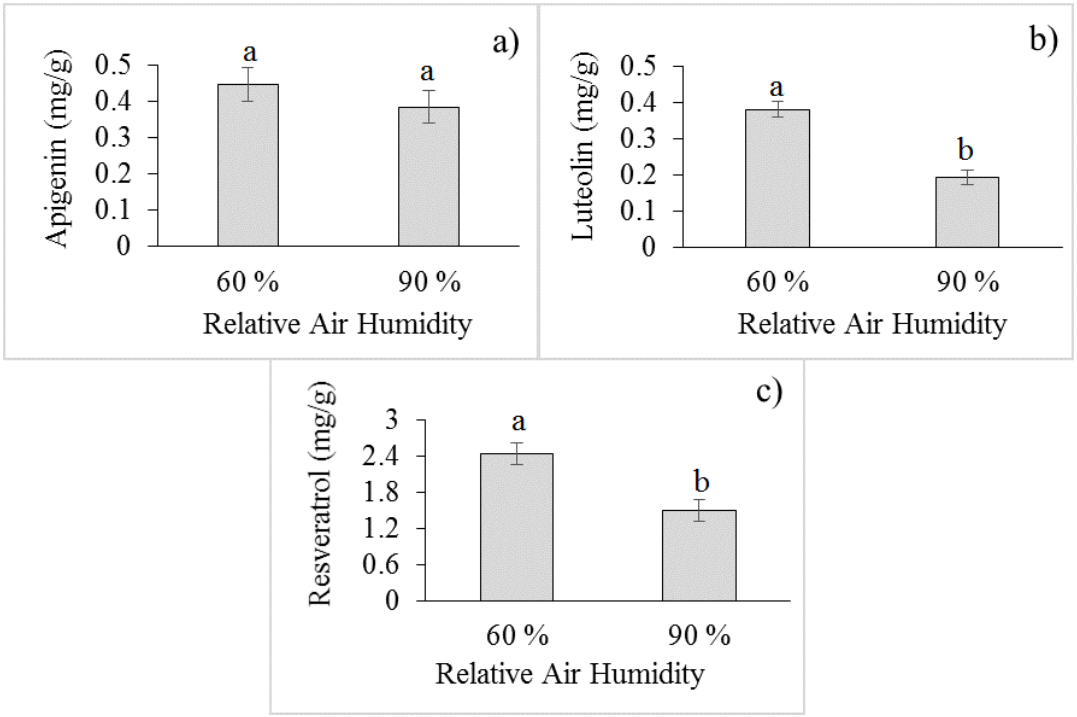


Figure 3.7: a) Apigenin (mg/g), (b) luteolin (mg/g) and (c) resveratrol (mg/g) in 'Quarto F1' cocktail cucumber leaves from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 0.0454, 0.0206 and 0.178, the different letters on the bars express significant difference n=9

On the other hand, almost 8 peaks of different polyphenols was observed in fruits samples from both experimental treatments. 6 peaks were identified polyphenols (apigenin, luteolin, quercetin 3 glycoside, quercetin, pinoresinol and resveratrol) on the basis of their spectrum match (Figure 3.2, 3.3, 3.4, 3.5, and 3.6) and 2 peaks were found as unknown compounds

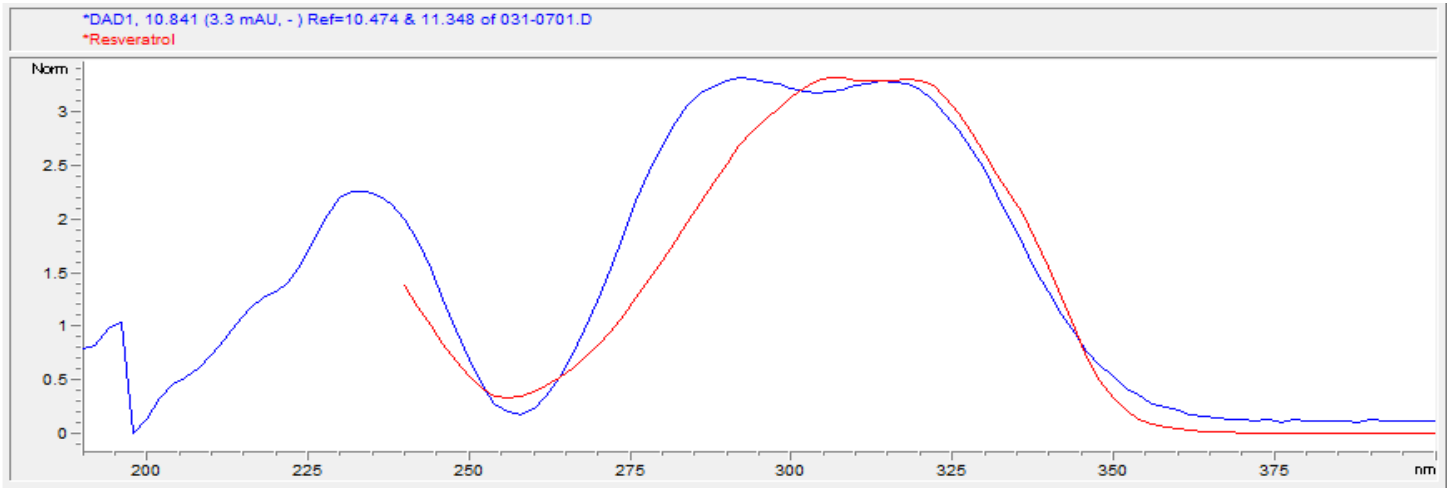


Figure 3.2: Resveratrol HPLC spectrum, red line presents the standard compound, blue line is spectrum of resveratrol in samples

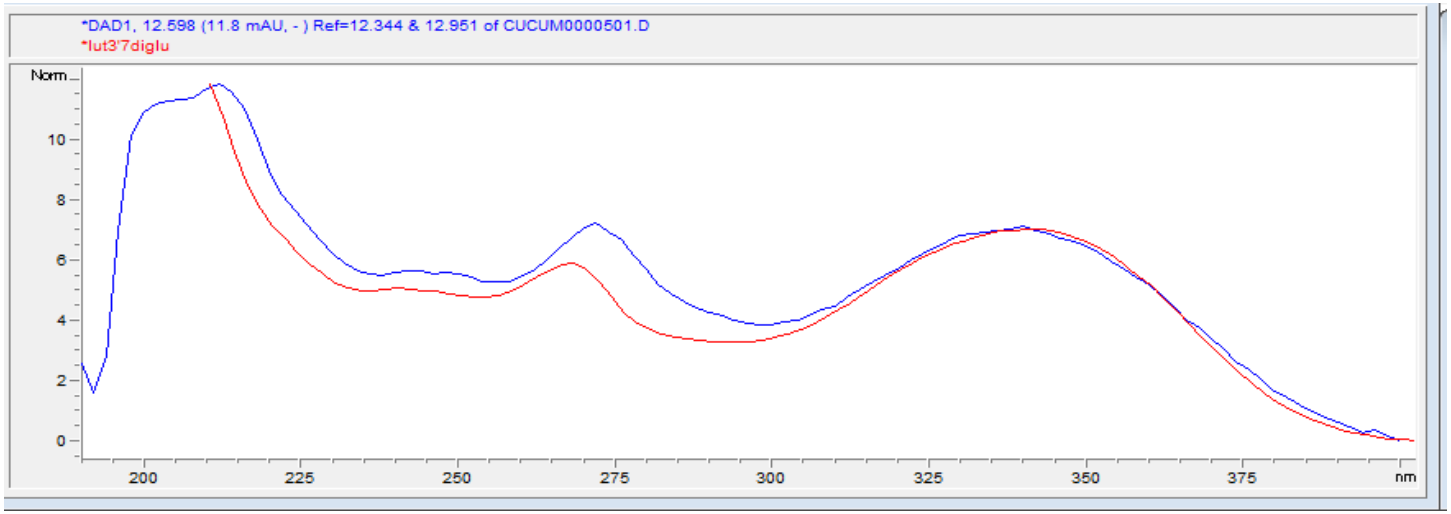


Figure 3.3: Luteolin HPLC spectrum, red line presents the standard compound, blue line is spectrum of luteolin in samples

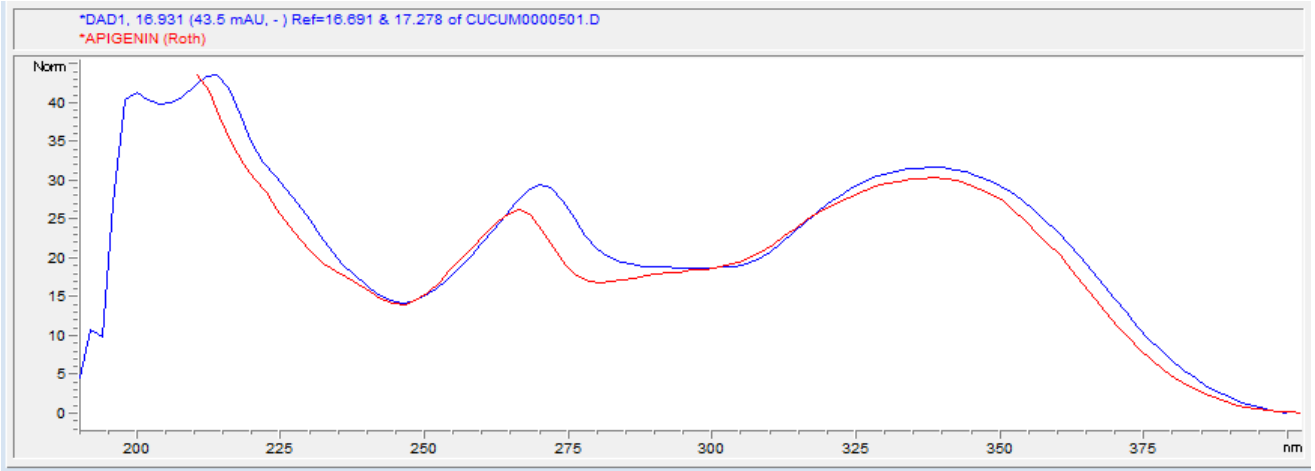


Figure 3.4: Apigenin HPLC spectrum, red line presents the standard compound, blue line is spectrum of apigenin in samples

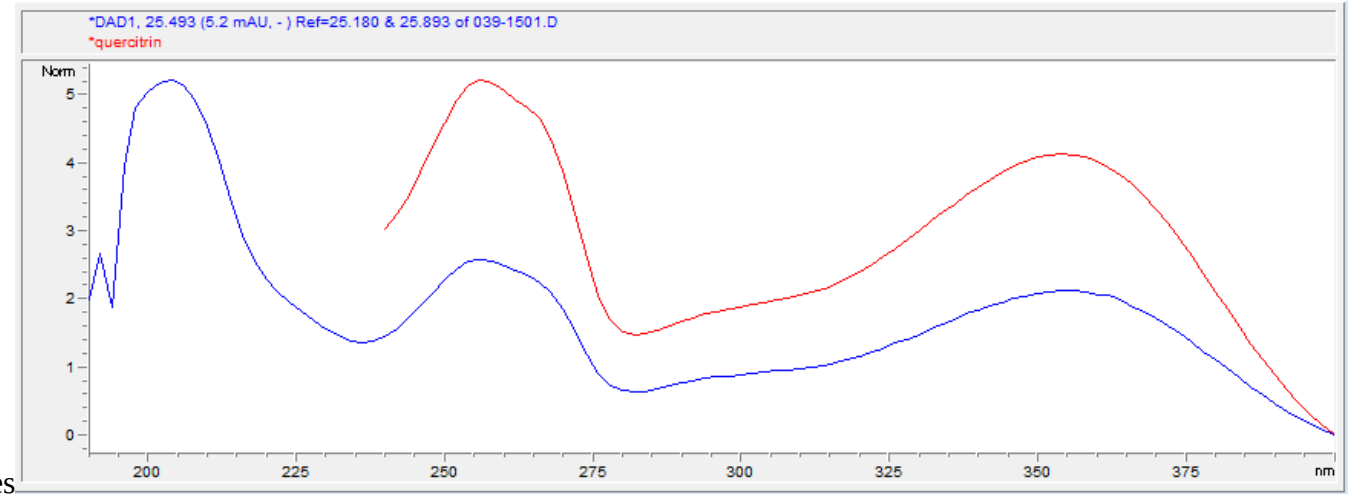


Figure 3.5: Quercetin HPLC spectrum, red line presents the standard compound, blue line is spectrum of quercetin in samples

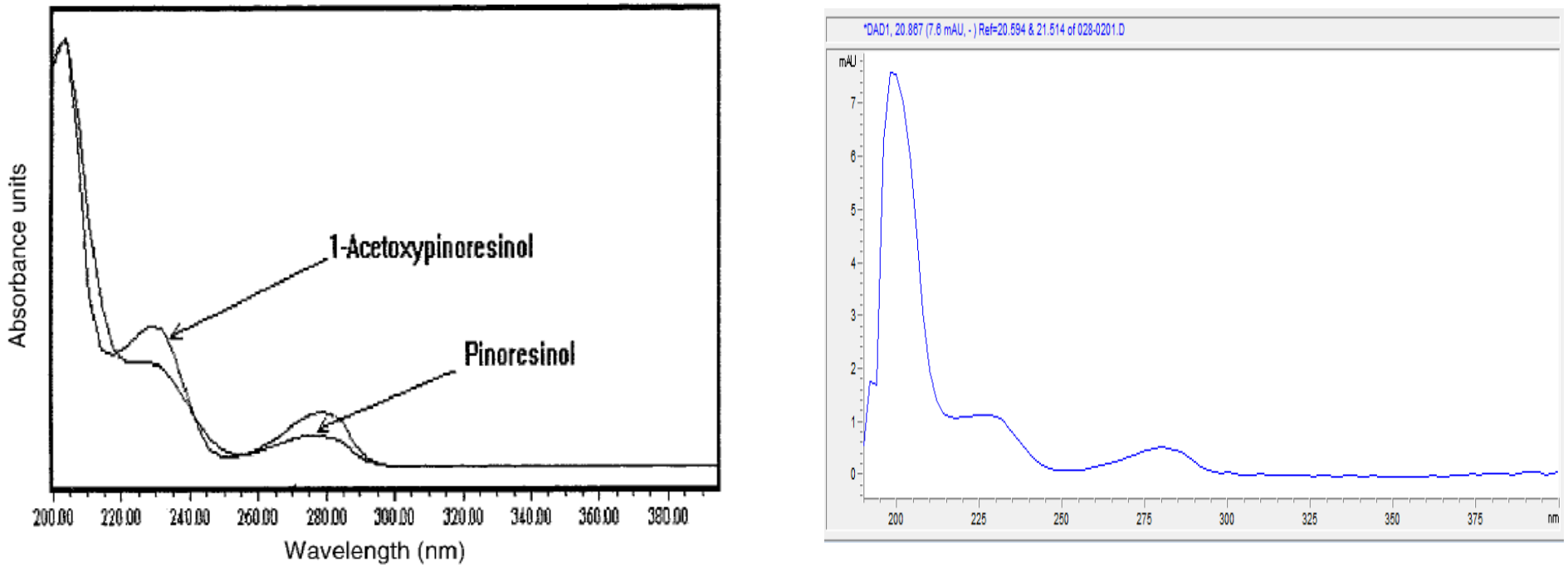


Figure 3.6: Pinoresinol HPLC spectrum, blue line is spectrum of pinoresinol in samples

Table 3.3: Common peaks with retention time found in ‘Quarto F1’ cocktail cucumber leaves from different RH conditions (60% RH and 90%RH), n = 9

Retention time	Peaks	60% RH	90% RH		
		Leaves	Leaves	SE	LSD
11.5	P1	1818.9a	1122.5b	131.67	S
12.5	P2	141.04a	71.475b	7.5488	S
13.5	P3	32.515a	15.35b	4.3347	S
14.8	P4	1178a	940.3a	198.3	NS
15.5	P5	164.26a	122.86b	9.5299	S
15.8	P6	722.16a	683.13a	74.757	NS
16	P7	1275.5a	1411.5a	203.71	NS
16.8	P8	432.72a	304.88b	36.247	S
17.5	P9	37.553a	28.553a	7.1314	NS
17.8	P10	33.425a	31.815a	10.273	NS
18.7	P11	41.758a	53.218a	16.67	NS
20.3	P12	202.92a	192.99a	45.51	NS
20.9	P13	65.293a	61.828a	10.815	NS
21.5	P14	174.1a	217.7a	29.448	NS
21.8	P15	242.07b	368.1a	49.929	S
22.5	P16	24.69a	35.417a	6.4105	NS
46.5	P17	412.51a	46.14a	342.81	NS
46.8	P18	149.86a	41.597a	83.054	NS

Means in rows not sharing similar letters differ significantly at P≤0.05; NS = Non-significant; S= Significant

For the fruits, the analysis was done for pulp and peel separately. All the polyphenols showed statistically significant different results in response to RH (Appendix 4). A higher contents of apigenin was observed in peel and pulp of fruits from moderate RH as compared to peel and pulp of fruits from high RH (Figure 3.8a). The luteolin was identified in pulp of fruits from 60% RH followed by pulp of fruits from 90% RH, while no significant difference between the two RH appeared (Figure 3.8b). It was observed that quercetin-3-glucoside only existed in the pulp of cucumber fruits and quercetin only existed in the peel of cucumber fruits. The concentration of both quercetin-3-glucoside and quercetin were significantly higher in pulp and peel of fruits from 60% RH as compared to 90% RH (Figure 3.8c and 3.8d). The pinoresinol contents was higher in peel of cucumber fruits as compared to pulp. Higher amount of pinoresinol was noticed in peel of 60% RH followed by peel of 90% RH, pulp of 60% RH and pulp of 90% RH (Figure 3.8e). Furthermore, the concentration of resveratrol was not significantly different in pulp and peel of fruits from moderate RH, but significantly different in pulp and peel of fruits from higher RH. A higher content of resveratrol was observed in pulp of fruits produced in 90% RH followed by pulp and peel from fruits produced in 60% RH and peel of fruits from 90% RH (Figure 3.8f). Two unknown compounds was also observed with different retention time. The first unknown compound was only found in peel of fruits, while the second unknown compound was noticed in pulp and peel of fruits. Unkown compound 1 was higher in peel of fruits from moderate RH as compared to high RH (Figure 3.8g). The unknown compound 2 was significantly higher in pulp as compared to peel, but no significant difference for both treatments, while higher contents was found in pulp of 60% RH (Figure 3.8h).

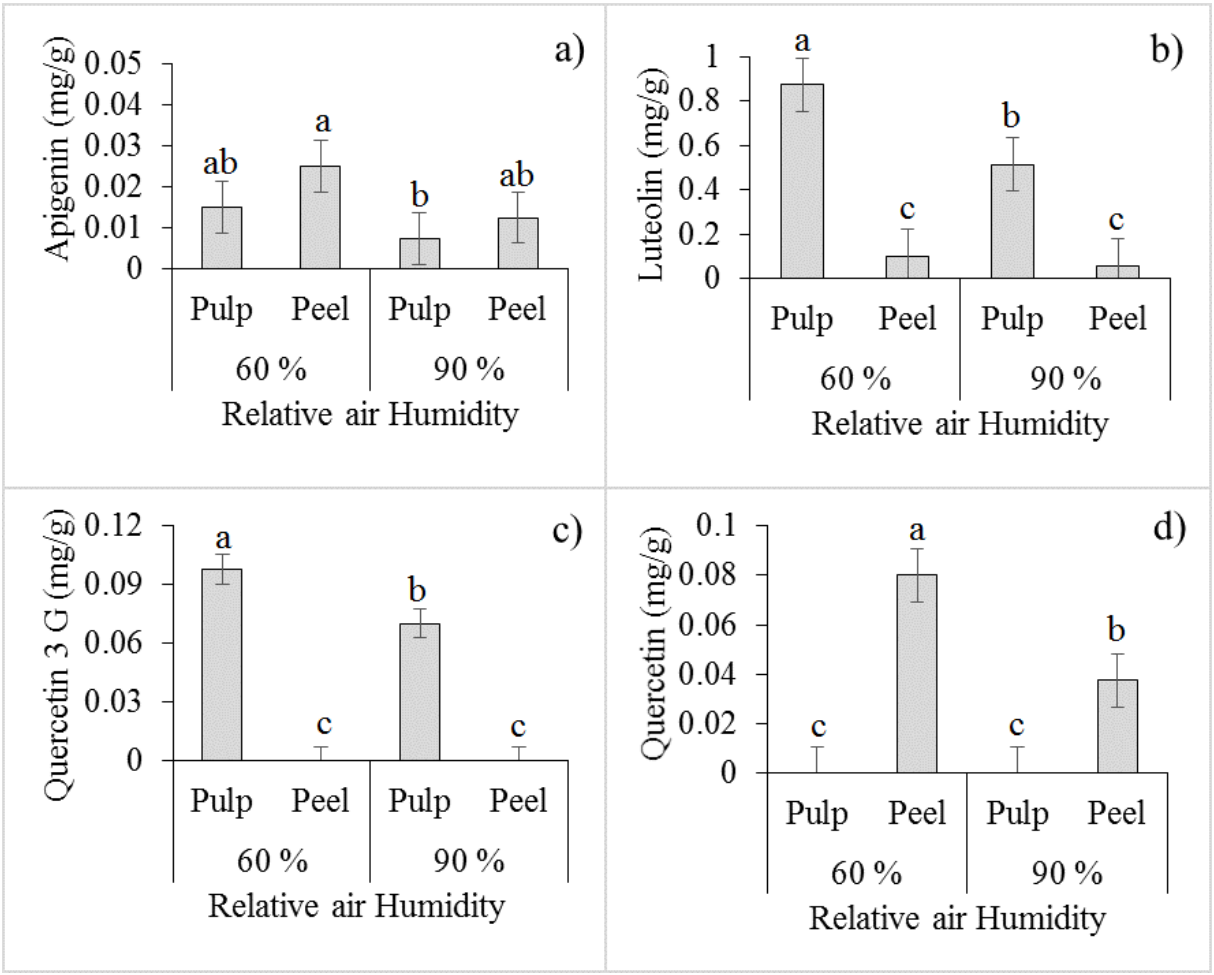


Figure 3.8: a) Apigenin (mg/g), (b) luteolin (mg/g), (c) quercetin 3 Glycoside (mg/g) and (d) quercetin (mg/g) in 'Quarto F1' cocktail cucumber fruit pulp and fruit peel from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 0.00623, 0.1213, 0.00747 and 0.0107, the different letters on the bars express significant difference, n=9

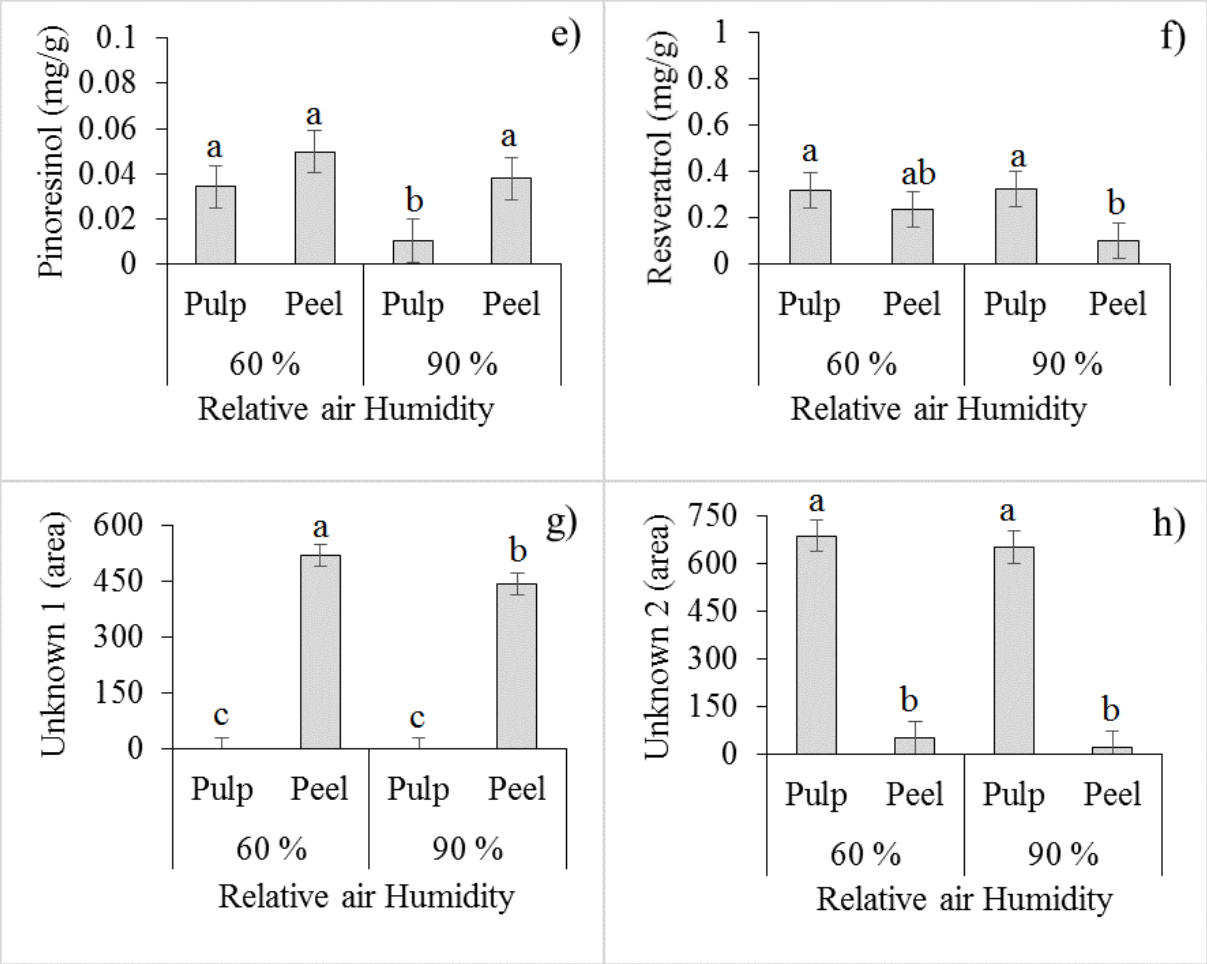
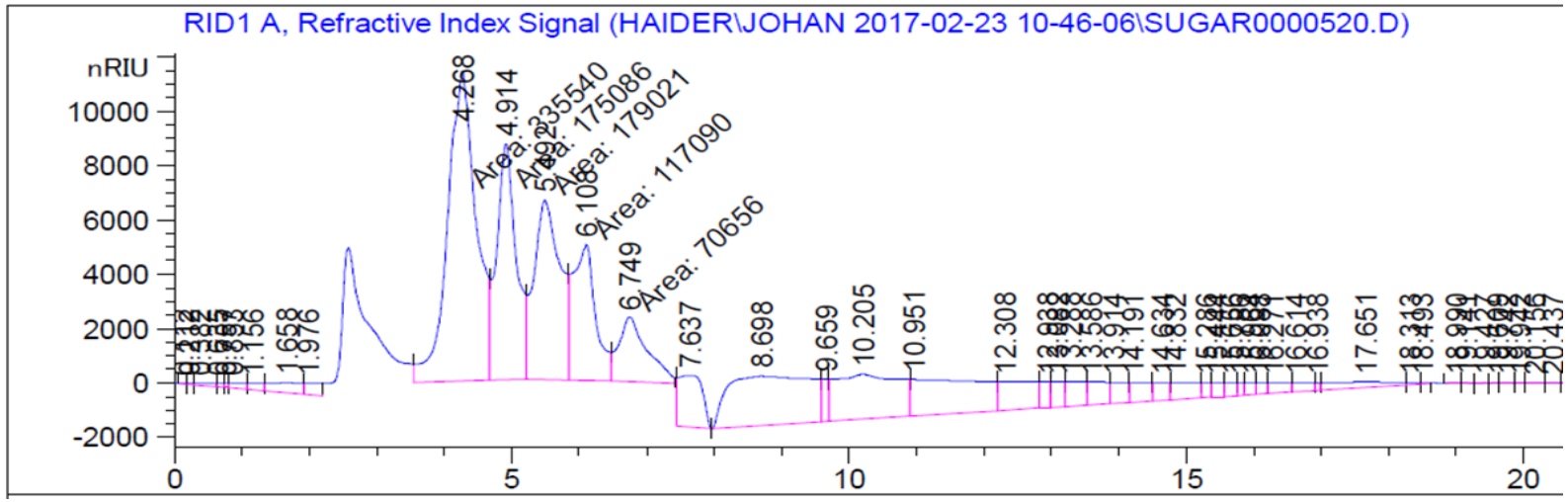


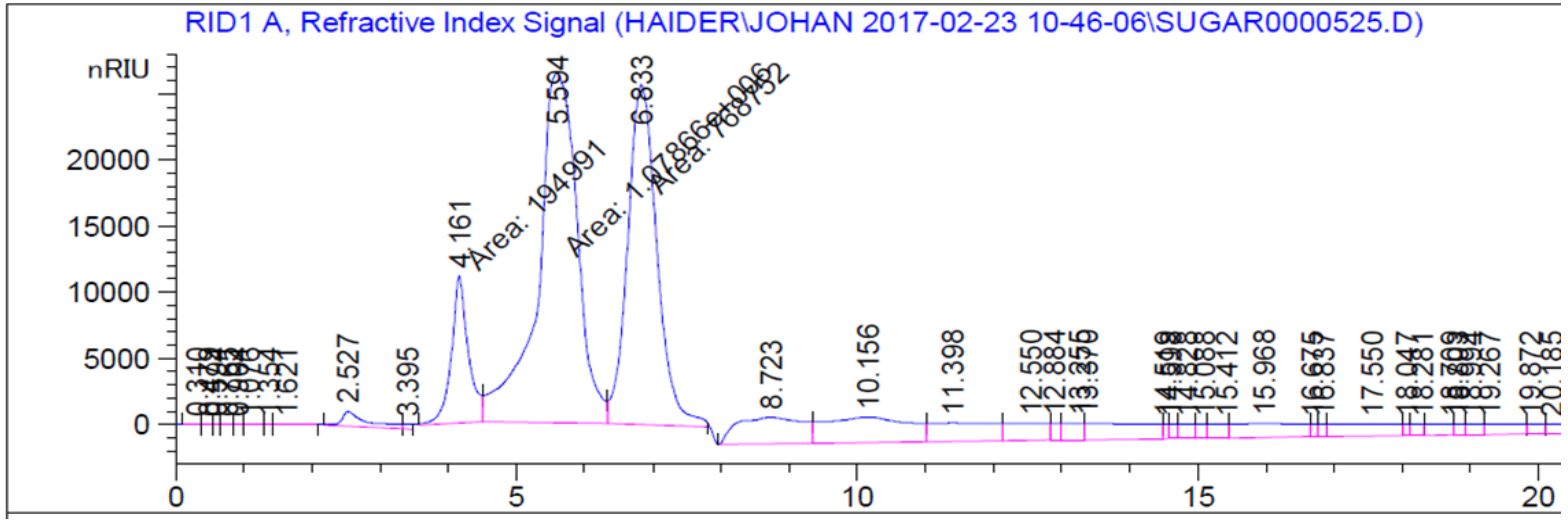
Figure 3.8: e) Pinoresinol (mg/g), (f) resveratrol (mg/g), (g) Unknown 1 (area) and (h) Unknown 2 (area) in 'Quarto F1' cocktail cucumber fruit pulp and fruit peel from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 0.00937, 0.0762, 28.923 and 50.645, the different letters on the bars express significant difference, n=9

3.1.2.1.3 Carbohydrates in cucumber plant leaves and fruits

Through HPLC analysis 6 different carbohydrates peaks in leaves samples, while 4 peaks in fruits samples were detected. On the basis of standard solution's retention time 6 peaks from leaves samples were found as fructose, glucose, raffinose, starch, stachyose and sucrose and 4 peaks of fruits samples were identified as fructose, glucose, starch and stachyose (3.10a and 3.10b).



3.10a: HPLC spectrum of sugars peaks separation in leaves samples.



3.10b: HPLC spectrum of sugars peaks separation in fruits samples.

3.1.2.1.3.1 Carbohydrates in Leaves

Among the leaves samples from different RH conditions, fructose, glucose and sucrose contents were non significantly different, while starch, stachyose and raffinose contents were significantly affected (Appendix 5).

The concentration of fructose in leaves was the same in both treatments (Figure, 3.11a), while the concentration of glucose was 16.8% higher in leaves from high RH as compared to leaves from moderate RH (Figure, 3.11b). The sucrose concentration in leaves from 60%RH was 15.4% higher than the samples from 90% RH (Figure, 3.11c).

Moreover, the concentration of starch in the leaves grown at 90% RH was 67% higher than the leaves from 60% RH (Figure, 3.11d). Whereas, the stachyose and raffinose concentration in leaves from 60% RH was more than double as compared to leaves from 90% RH (Figure 3.11e and 3.11f).

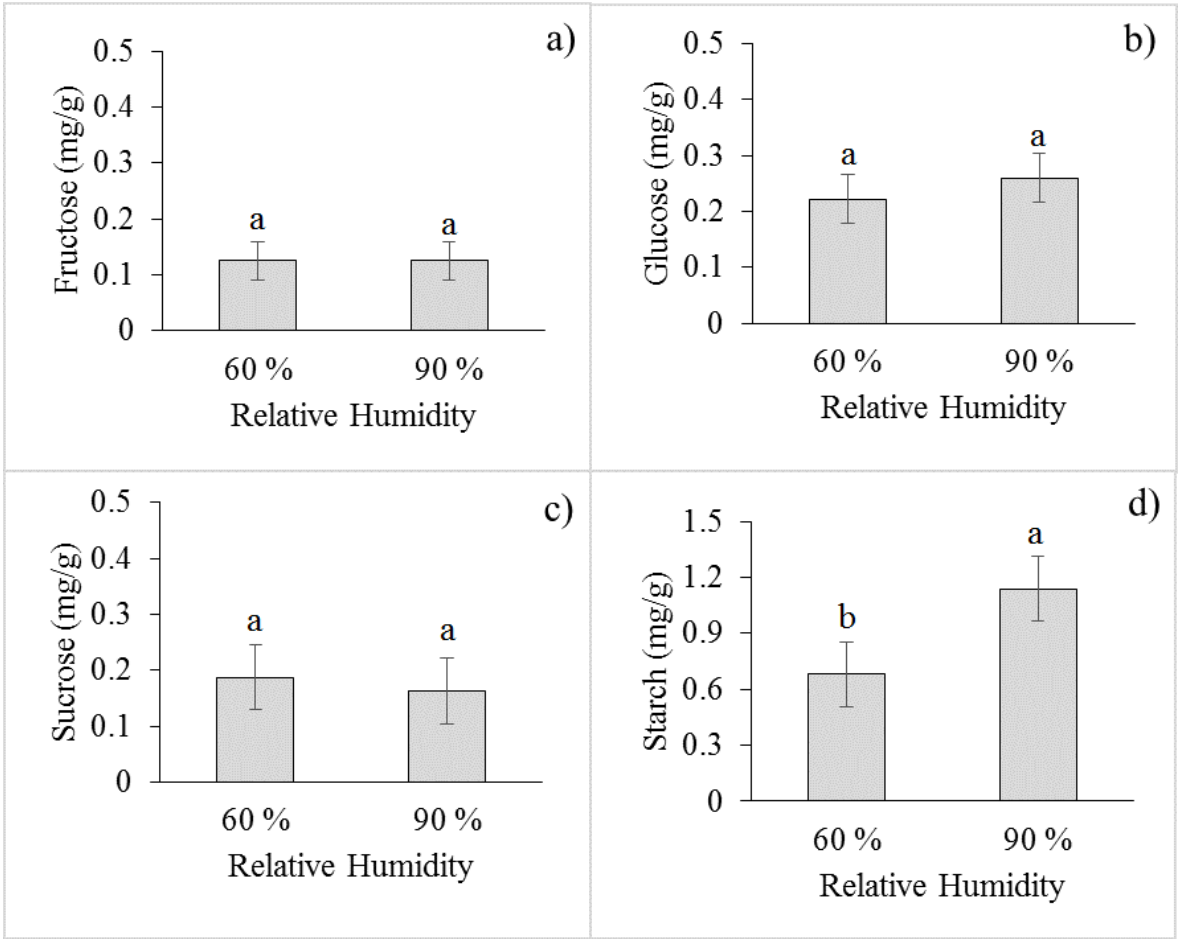


Figure 3.11: a) Fructose (mg/g), (b) Glucose (mg/g), (c) Sucrose (mg/g) and (d) Starch (mg/g) in 'Quarto F1' cocktail cucumber plant leaves from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 0.0342, 0.0429, 0.0586 and 0.1725, the different letters on the bars express significant difference, n=9

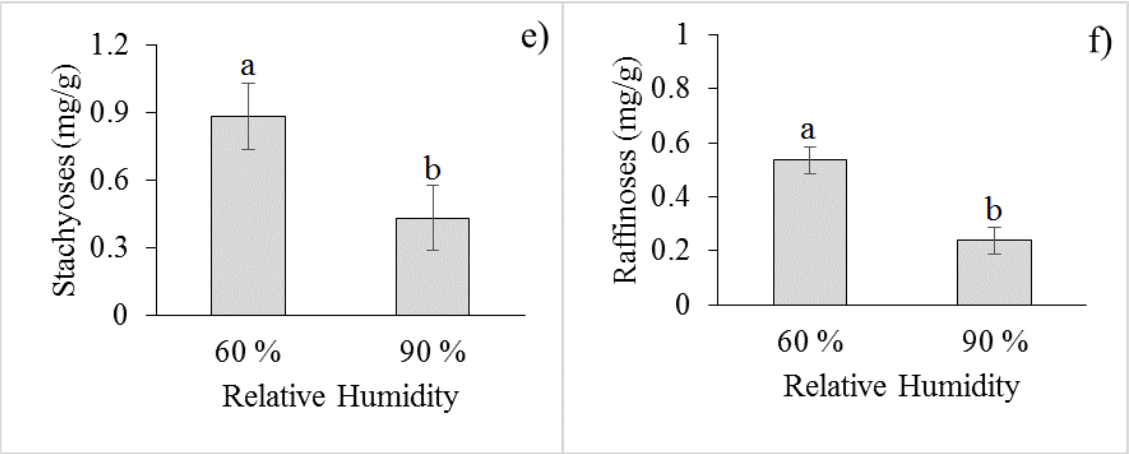


Figure 3.11: e) Stachyose (mg/g), and (f) Raffinose (mg/g) in 'Quarto F1' cocktail cucumber leaves from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 0.1456, and 0.05, the different letters on the bars express significant difference, n=9

3.1.2.1.3.2 Carbohydrates in Fruits

On the other hand, the contents of fructose, glucose and stachyose in fruits samples from different RH conditions were significantly different, while for starch highly significant difference was observed (Appendix 6).

The concentrations of fructose and glucose in fruits sample from 60% RH was 50.5% and 35% higher as compared to samples from 90% RH respectively (Figure 3.12a and 3.12b). High content of starch was found in cucumber fruit samples. However, the starch concentration in fruits sample from 60% RH was almost 3 times higher than the samples from 90% RH (Figure 3.12c). The concentration of stachyose showed an opposite trend as compared to the other carbohydrates. About 11% higher content of stachyose was found in the samples from 90% RH as compared to fruits samples from 60% RH (Figure 3.12d).

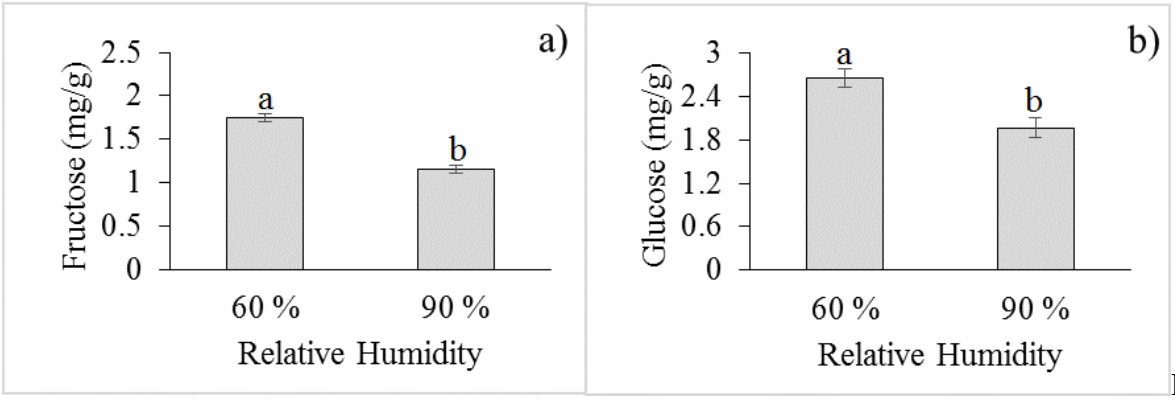


Figure 3.12: a) Fructose (mg/g) and (b) Glucose in 'Quarto F1' cocktail cucumber fruits from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 0.0437, and 0.1326, the different letters on the bars express significant difference, n=9

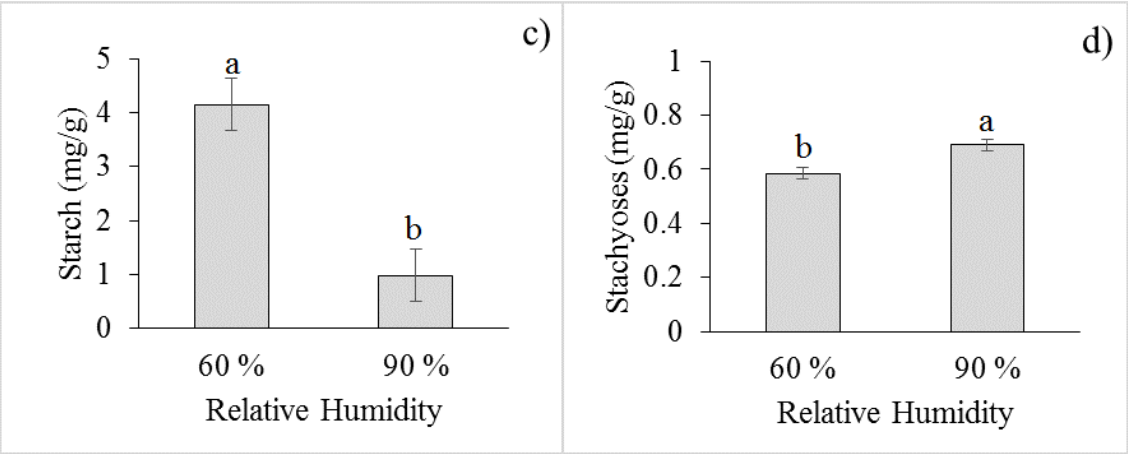


Figure 3.11: (c) Starch (mg/g) and (d) Stachyose (mg/g) in 'Quarto F1' cocktail cucumber fruits from different RH conditions (60% RH and 90%RH) Vertical bars represent SE± 0.4836, and 0.0214, the different letters on the bars express significant difference, n=9

3.1.3 Comparison of mineral contents in cucumber leaves and fruits from various RH conditions

3.1.3.1 Mineral contents in fruit

The results regarding total nitrogen, total carbon, carbon-nitrogen ratio, phosphorous, potassium, calcium, magnesium, manganese and molybdenum contents were significantly different in cucumber fruits from different RH conditions (Appendix 7).

Total nitrogen was highly significant between the treatments (Appendix 7). Higher total nitrogen contents were found in fruit samples from 60% RH followed by 90% RH with value of 3.02% and 2.48% respectively (Table 3.4). Total carbon content was higher in cucumber fruit samples from moderate RH than fruit from high RH. Furthermore, the carbon-nitrogen ratio (C: N) was higher in fruit samples of 90% RH as compared to fruit samples of 60% RH with value of 16.16 and 13.38 respectively (Table 3.4).

Pertaining to table (3.4), the phosphorous (P) and potassium (K) contents were higher in cucumber fruits from moderate RH as compared to high RH with value of 8.08 g/kg, 7.3 g/kg, 38 g/kg and 33.2 g/kg respectively (Table 3.4). Although boron (B) content was not affected significantly by different RH treatments. But the concentration of boron (B) in fruit samples from 60% RH was higher than the 90% RH (Table. 3.4).

However, the calcium (Ca) content was significantly different in cucumber fruits from both treatments, but cucumber fruits produced at 60% RH showed less Ca contents as compared to fruit grown at 90% RH (Table. 3.4). The cucumber fruits produced at high RH showed no Iron (Fe) contents, while 0.02 g/kg Fe content was found in fruits from moderate RH (Table 3.4).

Furthermore, the magnesium and manganese contents were significantly different in fruit samples in both RH treatments. Higher magnesium and manganese contents were found in fruit samples from 90% RH followed by fruit samples from 60% RH, with mean value of 4.5 g/kg, 0.04 g/kg and 4.08 g/kg, 0.03 g/kg, respectively (Table 3.4).

Pertaining to appendix (7) the molybdenum (Mo) content showed highly significant difference for both treatments. The molybdenum (Mo) content was higher in cucumber fruits produced at high RH as compared to fruit samples from moderate RH. While, the sodium (Na) and sulphur (S) contents in cucumber fruits were not significantly affected by different RH treatments (Table. 3.4).

3.1.3.2 Mineral contents in leaves

Most of the mineral contents (total carbon, phosphorous, calcium, iron, magnesium, manganese, molybdenum and sulphur) in cucumber leaves were not significantly different for both treatments of RH. Only total nitrogen, carbon-nitrogen ratio, potassium, boron and sodium showed statistically significant results in response to different RH conditions (Appendix 8).

Total nitrogen content was higher in leaf sample from moderate RH (2.3 g/kg) than sample from high RH (1.8 g/kg) (Table 3.4). A higher contents of potassium and boron were found in leaf sample from moderate RH than samples from high RH with a mean value of 7.5 g/kg, 80.2 g/kg and 5.8 g/kg, 57.0 g/kg, respectively (Table 3.4). On the other hand, higher sodium content was noticed in leaf sample of high RH as compared to sample from moderate RH (Table 3.4).

Although total carbon, phosphorous, iron, calcium, magnesium, manganese, molybdenum and sulphur were significantly not different, but except calcium, magnesium and molybdenum, other minerals content were higher in leaf grown at moderate RH as compared to leaf sample of high RH (Table 3.4). On the other hand, calcium, magnesium, molybdenum and sulphur contents were higher in leaf sample from high RH than sample from moderate RH (Table 3.4).

Table 3.4: Comparison of mineral analysis (total nitrogen, total carbon, carbon nitrogen ratio, phosphorous, potassium, boron, calcium, iron, magnesium, manganese, molybdenum, sodium and sulphur) in 'Quarto F1' cocktail cucumber plant leaves and fruits from different RH conditions (60% RH and 90%RH), n = 9

Parameters	Cucumber Fruits				Cucumber Leaves			
	60% RH	90% RH	SE	LSD	60% RH	90% RH	SE	LSD
N (%)	3.02^{a}	2.48^{b}	0.0693	S	2.3a	1.8b	0.0729	S
C (%)	40.54^{a}	40.2^{b}	0.1077	S	35.8a	35.4a	0.4858	NS
C: N	13.38^{b}	16.16^{a}	0.3899	S	15.4b	19.6a	0.9684	S
P (g/kg)	8.08^{a}	7.3^{b}	0.2956	S	3.8a	3.5a	0.1954	NS
K (g/kg)	38^{a}	33.2^{b}	1.1136	S	7.5a	5.8b	0.4027	S
B (mg/kg)	25.8^{a}	25.6^{a}	1.1402	NS	80.2a	57.0b	4.2356	S
Ca (g/kg)	6.4^{b}	7.82^{a}	0.3247	S	52.2a	52.4a	3.1937	NS
Fe(g/kg)	0.02^{a}	0^{a}	0.02	NS	0.1a	0.1a	0.007483	NS
Mg (g/kg)	4.08^{b}	4.5^{a}	0.1772	S	15.0a	16.0a	1.9748	NS
Mn (g/kg)	0.03^{b}	0.04^{a}	0.002449	S	0.3a	0.2a	0.0231	NS
Mo (mg/kg)	11.18^{b}	17^{a}	0.8139	S	37.4a	49.4a	6.0679	NS
Na (g/kg)	0.44^{a}	0.48^{a}	0.0316	NS	0.2b	0.4a	0.0423	S
S (g/kg)	4.06^{a}	3.98^{a}	0.1703	NS	12.2a	11.1a	0.729	NS

Means in row not sharing similar letters differ significantly at $P \leq 0.05$; NS = Non-significant; S= Significant

3.1.4 Sensory analysis of cucumber fruits from various RH conditions

3.1.4.1 Determining the key attributes

The key attributes to describe the quality of cucumber fruits were identified by comparing scores and relations of all attributes in the biplot and analysing the data by PCA. All the attributes: sweetness, colour, texture, flavour, bitterness and water contents were well explained in component loading of first dimensions. With these nine attributes the percentage of variance was 66.27%.

The Biplot presented (Figure 3.13) below shows that the attributes formed clusters. Sweetness, colour, flavour, and texture formed a cluster together and bitterness and water contents formed a cluster as well. These two clusters were negatively correlated with each other, but detailed correlation are presented below.

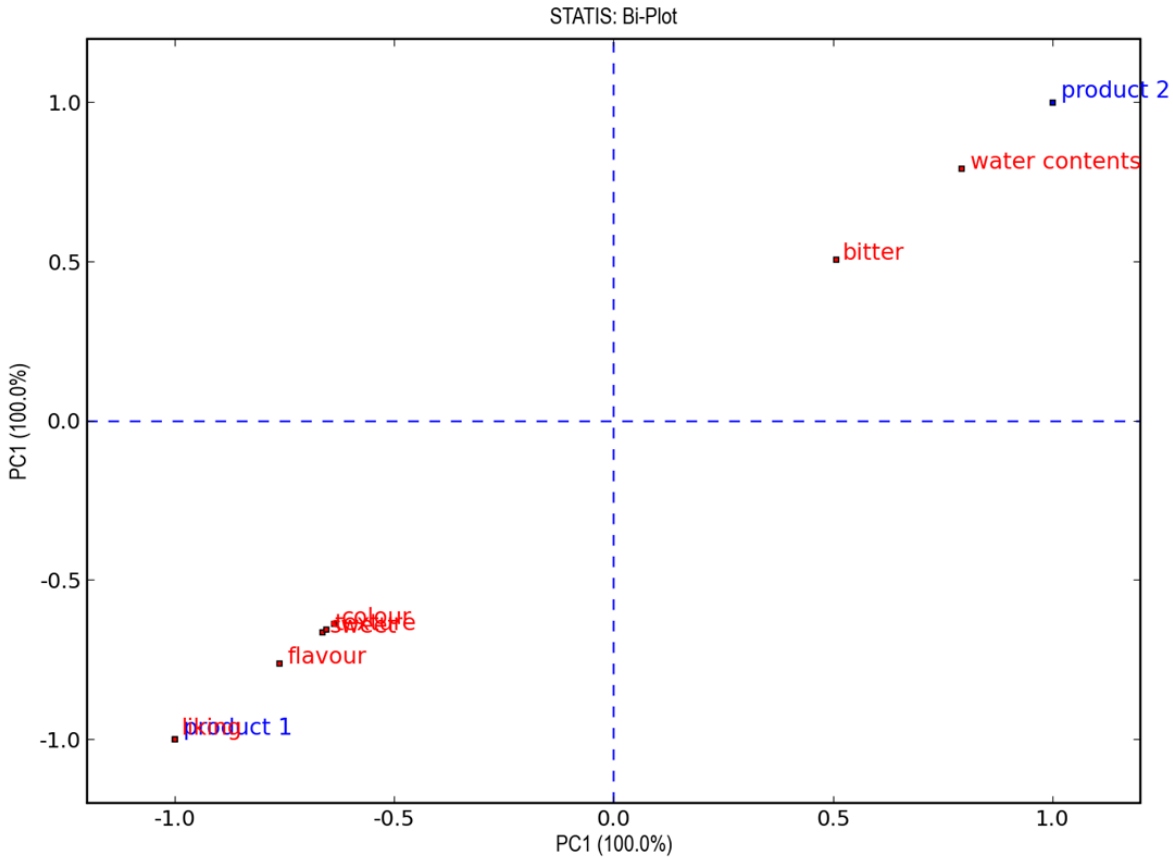


Figure 3.13: Biplot revealing clusters of the chosen attributes of 'Quarto F1' cocktail cucumber fruits from different RH conditions within 2 dimensions. Product 1 (fruits from moderate 60% RH) and Product 2 (fruits from high 90% RH).

3.1.4.2 Difference between Sensory perception of cucumber fruits from different RH conditions.

To test the sensory variables in cucumber fruits grown under different RH conditions, an ANOVA test was performed. The significant differences are shown in figure 3.14. The results regarding different sensory attributes for the various samples were significantly different (Figure 3.14). The red bars in figure (3.14) is representing the high level of significance at $p<0.001$.

Significant differences in pulp colour and texture was found between the RH treatments. A higher score was registered for cucumber fruits (7.34, 7.13) grown at moderate RH (60% RH) as compared to cucumber fruits (5.28, 5.01) from high RH (90% RH) (Figure 3.15).

Sweetness and flavor was also significantly affected by RH. Cucumber fruits from moderate RH (60% RH) had a higher score for sweetness (7.11) and flavour (7.31) as compared to fruits from high RH with a score of 4.97 and 4.86 respectively (Figure 3.15).

For bitter taste a significant difference was found. The fruits from high RH (90% RH) was more bitter in perception than the fruits sample from moderate RH (60% RH) with a score of 5.94 and 4.31 respectively (Figure 3.15).

Likewise, the score for water content was scored significantly different and higher (7.4) in fruits from high RH (90% RH) than the fruits (4.84) from moderate RH (60% RH) (Figure 3.15).

The overall liking for the samples also differed significantly. Higher liking score was given to fruits samples from moderate RH (60% RH) than the samples from high RH (90% RH) (Figure 3.15).

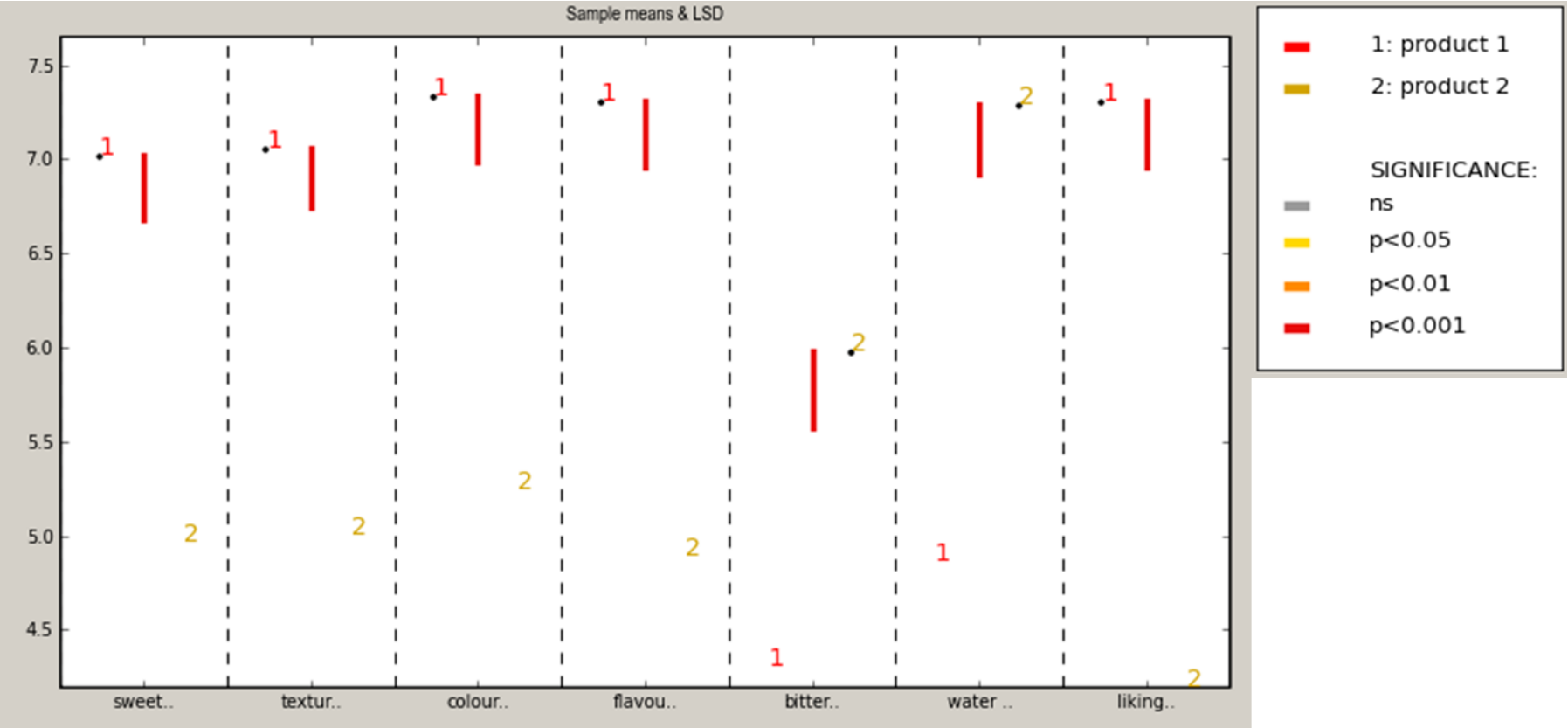


Figure 3.14: Sensory attributes (sweet taste, texture, pulp colour, flavor, bitterness, water contents and over all liking) in relation to Least Significant Difference for 'Quarto F1' cocktail cucumber fruits from different RH conditions. Product 1 (fruits from moderate 60% RH) and Product 2 (fruits from high 90% RH). The red colour of Bars present the level of significance.

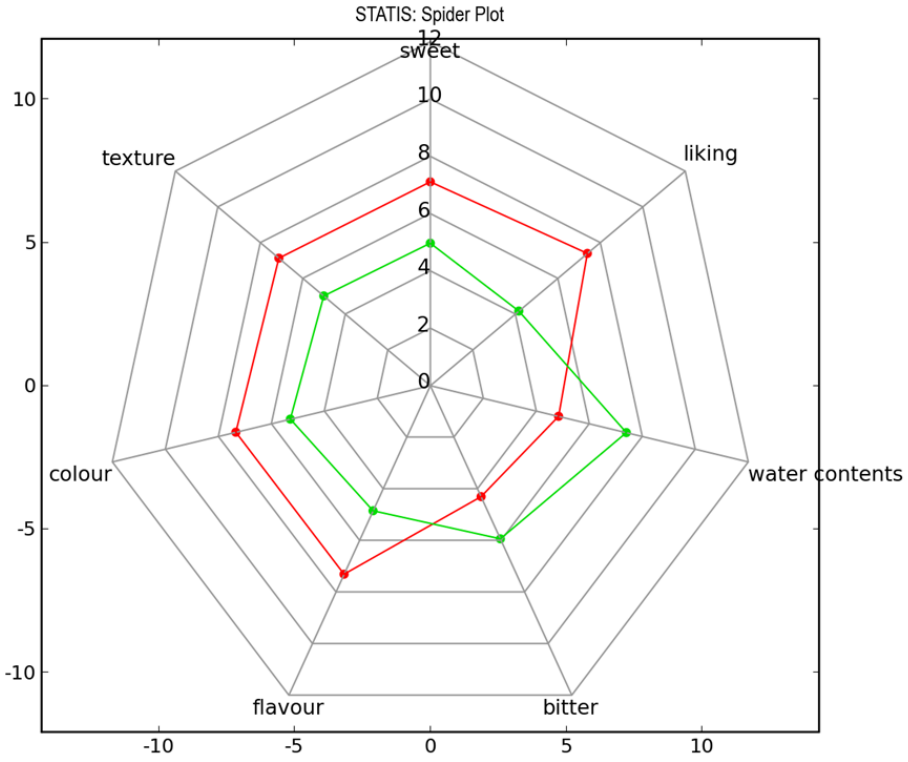


Figure 3.15: Spider plot explaining trend of sensory attributes (sweet taste, texture, pulp colour, flavor, bitterness, and water contents and over all liking) in relation to mean values of scoring by consumers. Red line (fruits from moderate 60% RH) and Green line (fruits from high 90% RH).

3.1.4.3 Correlation analysis between Sensory attributes, sugars and minerals contents in cucumber fruits sample

The correlations between the various sensory attributes are shown in Table (3.5) and several significant correlations were noticed.

Sweet taste of cucumber fruit and flavor was highly significant and positively correlated with starch content (R value 0.879, 0.887) and total nitrogen contents (R value 0.935, 0.804). For flavor perception total carbon contents (0.875) was found positively correlated. On the other hand, stachyose (-0.946, -0.828), carbon/nitrogen ratio (-0.936, -0.766) and calcium concentration (-0.67, -0.919) was negatively correlated with perception of sweet taste.

The texture of cucumber fruits was positively correlated with fructose (0.648), starch (0.867), total carbon (0.692) and total nitrogen (0.883) contents, while texture was negatively correlated with stachyose (-0.879), carbon/nitrogen ratio (-0.865) and calcium concentration (-0.773).

Table 3.5: Correlation analysis of sensory attributes with sugars and mineral contents of 'Quarto F1' cocktail cucumber fruits from different RH conditions (60% RH and 90%RH)

		Glucose	Fructose	Starch	Stachyose	Tot. C %	Tot. N %	K g/kg	Ca g/kg	C:N
sweet	Pearson Correlation	0.337	0.544	,879**	-,946**	0.559	,935**	0.468	-,670*	-,936**
	Sig. (2-tailed)	0.341	0.104	0.001	0.000	0.093	0.000	0.173	0.034	0.000
texture	Pearson Correlation	0.403	,648*	,867**	-,879**	,692*	,883**	0.438	-,773**	-,865**
	Sig. (2-tailed)	0.248	0.043	0.001	0.001	0.027	0.001	0.205	0.009	0.001
colour	Pearson Correlation	0.249	0.498	,826**	-,792**	,727*	,809**	0.405	-,777**	-,794**
	Sig. (2-tailed)	0.489	0.143	0.003	0.006	0.017	0.005	0.246	0.008	0.006
flavour	Pearson Correlation	0.292	0.551	,887**	-,828**	,875**	,804**	0.396	-,919**	-,766**
	Sig. (2-tailed)	0.412	0.099	0.001	0.003	0.001	0.005	0.258	0.000	0.010
bitter	Pearson Correlation	-0.413	-0.591	-,814**	,718*	-0.543	-,765*	-,657*	,757*	,735*
	Sig. (2-tailed)	0.235	0.072	0.004	0.019	0.105	0.010	0.039	0.011	0.015
water contents	Pearson Correlation	-,777**	-,907**	-,875**	,729*	-0.397	-,847**	-0.487	0.630	,833**
	Sig. (2-tailed)	0.008	0.000	0.001	0.017	0.256	0.002	0.153	0.051	0.003
liking	Pearson Correlation	0.327	0.569	,872**	-,819**	,802**	,792**	0.455	-,913**	-,763*
	Sig. (2-tailed)	0.357	0.086	0.001	0.004	0.005	0.006	0.187	0.000	0.010

Values with * and ** present level of significantly at P≤0.05; * = significant; **= Highly Significant

The liking of pulp colour was also found positively correlated with starch (0.826), total carbon (0.727) and total nitrogen (0.809) contents. Colour was negatively correlated with stachyose (-0.792) and calcium (-0.777) concentration in pulp (Table 3.5).

Correlations between carbohydrates and minerals content, bitterness and juicy contents showed opposite behavior than sweetness, texture and colour attributes. Bitterness was positively correlated with stachyose concentration (0.718). Carbon/nitrogen ratio (0.735) and calcium contents (0.757). In opposite way, a negative correlation of bitterness with starch contents (-0.814) was found. Just like bitterness, water contents perception in pulp was also positively correlated with stachyose (0.729) and total nitrogen (0.833) contents and negatively correlated with starch concentration (-0.875). But water contents also had highly negative correlation with glucose (-0.777), fructose (-0.907) and total nitrogen contents (-0.847) (Table 3.5).

Through correlation analysis. It was found that the sweet perception, pulp texture, pulp colour, flavor and bitterness were key drivers of liking. The flavor of fruit showed a highly significant correlation with liking of fruit (Table 3.5).

3.2 Experiment 2

3.2.1 Respiration of cucumber fruits from different treatments and different storage period under temperature condition

The CO_2 production of cucumber fruits packed in MAP bags did not differed significantly for the various storage duration under low temperature storage condition. While treatment wise, CO_2 production differed significantly. The comparison of various removals with different treatments also showed significant difference (appendix 8). Higher CO_2 production rate was observed in Non ABA treated fruits (4.8%) with 21 days in storage followed by Non ABA treated fruits (4.3%) with 14 days in storage, ABA treated fruits (3.425% and 2.8%) with 21 days in storage and 14 days in storage (Figure 3.16).

On the other hand, the O_2 concentration was significantly different in the storage durations, but not significantly different between treatments. The combined effect of different treatments under the two removals showed significant difference (Appendix 10). The higher O_2 concentration was measured in bags of ABA treated fruits (16.05%) and non ABA treated cucumber fruits (15.35%) at removal 1, while at removal 2 ABA treated fruit (14.45%) respired less as compared to non ABA treated fruits (13.85) (Figure 3.16).

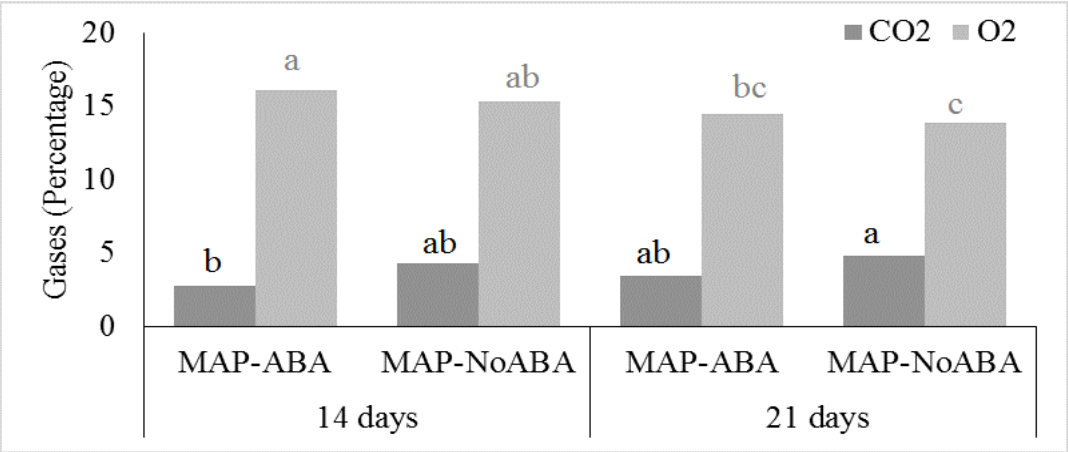


Figure 3.16: Comparison of CO2 and O2 production in ABA and Non ABA treated cucumber fruits under MAP bagged at 11±1°C temperature for storage duration of 14 days (Removal 1) and 21 days (Removal 2), the different letters on the bars express significant difference, n=15

3.2.2 Physical weight loss (percentage) in cucumber fruits from different treatments at different removals kept at different stages (various temperature conditions)

The physical fruit weight loss of cucumber fruits with different treatments at different stages stored for 14 and 21 days was statistically significant different (Appendix 9).

A significantly higher fruits weight loss was observed in non ABA treated – Plastic foil wrapped (T4) cucumber fruits followed by ABA treated – Plastic foil wrapped (T3), non ABA treated – MAP bagged (T2) and ABA treated – MAP bagged (T1) fruits (Figure 3.17).

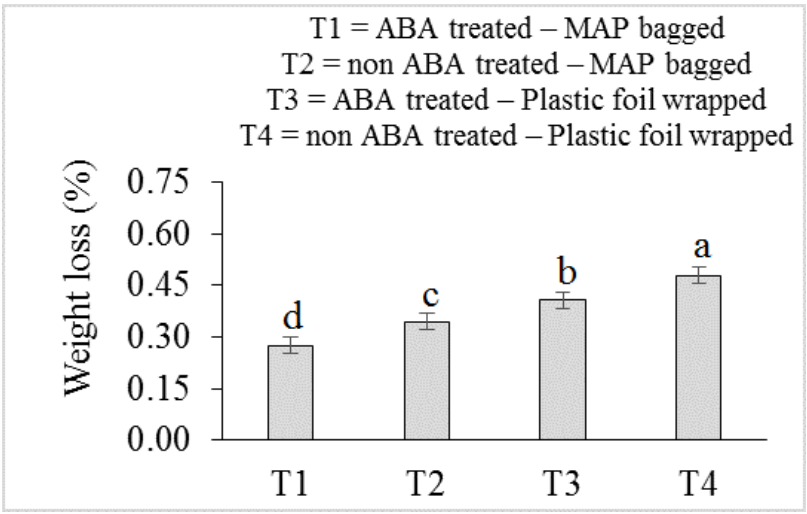


Figure 3.17: Physical weight loss (percentage) of cucumber fruits from different treatments. Vertical bars represent SE± 0.0241, the different letters on the bars express significant difference, n=15

On the other hand, the highest fruits weight loss was found at ambient temperature (25°C) (stage 2) as compared to other stages with temperature of 11°C and 6°C (Figure 3.18).

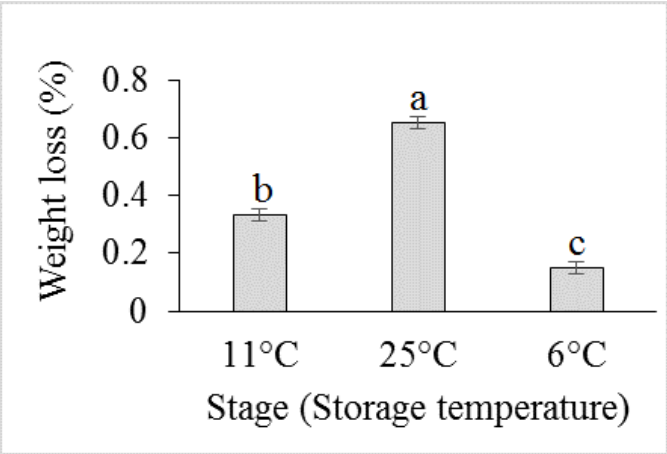


Figure 3.18: Physical weight loss (percentage) of cucumber fruits from different storage and shelf stages S1 (11±1°C: 90% RH), S2 (ambient conditions) and S3 (6±1°C: 90% RH). Vertical bars represent SE± 0.0209, the different letters on the bars express significant difference, n=15

Physical weight loss in cucumber fruits was higher at removal of 21 days than 14 days (Figure 3.19).

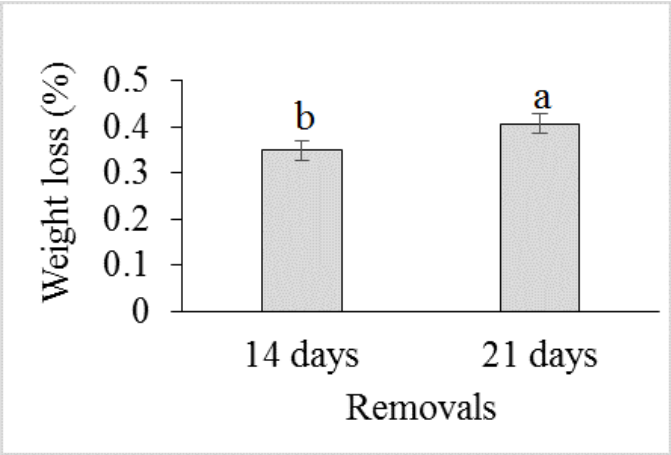


Figure 3.19: Physical weight loss (percentage) of cucumber fruits from different storage periods of 14 days (Removal 1) and 21 days (Removal 2). Vertical bars represent SE± 0.0206, the different letters on the bars express significant difference, n=15

The comparison of physical weight loss in cucumber fruits with different treatments, storage stages and storage period showed significant difference (Appendix 11). An overall higher weight loss was noticed in non ABA treated – Plastic foil rapped (T4) cucumber fruits at ambient temperature (25°) from storage duration of 14 days followed by T4 at ambient temperature (25°) from storage duration of 21 days (Table 3.6).

Table 3.6: Physical weight loss (percentage) in cucumber fruits with different treatments, kept at different stages (S1=11±1°C: 90% RH, S2=ambient conditions and S3=6±1°C: 90% RH) and removed at different storage period of 14 days and 21 days,

treatments	14 days			21 days		
	11°C	25°C	6°C	11°C	25°C	6°C
ABA treated – MAP bagged	0.20^{ijkl}	0.46^{ef}	0.05^{m}	0.27^{hij}	0.59^{cd}	0.09^{lm}
non ABA treated – MAP bagged	0.21^{ijkl}	0.70^{abc}	0.10^{lm}	0.27^{hij}	0.66^{abcd}	0.16^{ijklm}
ABA treated – Plastic foil wrapped	0.33^{gh}	0.70^{abc}	0.14^{klm}	0.43^{fg}	0.63^{bcd}	0.23^{hijk}
non ABA treated – Plastic foil wrapped	0.40^{fg}	0.76^{a}	0.15^{jklm}	0.56^{de}	0.75^{ab}	0.27^{hi}
Mean SE	SE± 0.0591					

Means in columns and rows not sharing similar letters differ significantly at $P \leq 0.05$, n=15

3.2.3 Disease incidence (%) in cucumber fruits from different treatments at different removals kept at different stages (various temperature conditions)

The disease incidence (percentage) in different treatments and combined effect with different stages (shelf temperature) and removals were significantly different (Appendix 10).

Higher percentage disease incidence was found in treatment 4 (non ABA treated – Plastic foil wrapped). As par combined effect of removals, stages and treatments, high disease incidence (18%) was observed in non ABA treated – Plastic foil wrapped cucumber fruits at 6°C and removal 2 (21days) followed by non ABA treated – Plastic foil wrapped cucumber fruits at 6°C, removal 1 (14 days) and non ABA treated – MAP bagged, 11°C, removal 1 (14 days) with percentage index of 9 % and 5% respectively (Figure 3.20).

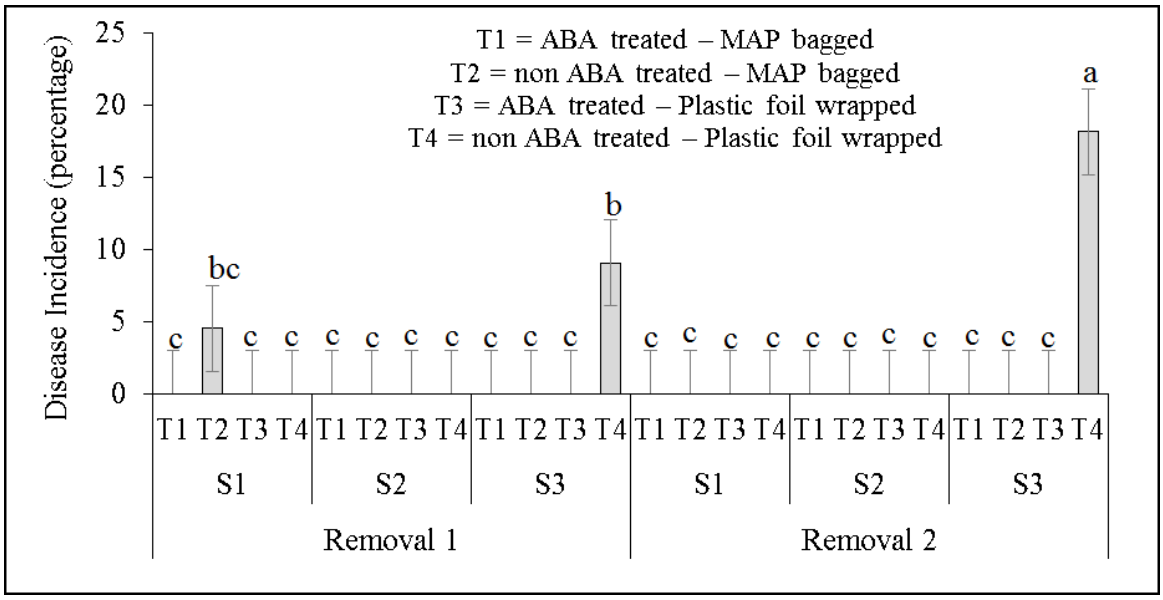


Figure 3.20: Comparison of percentage disease incidence in different treatments of cucumber fruits at S1 (11±1°C: 90% RH), S2 (ambient conditions) and S3 (6±1°C: 90% RH) from storage period of 14 days (Removal 1) and 21 days (Removal 2). Vertical bars represent SE± 2.9844, the different letters on the bars express significant difference, n=15

3.2.4 Skin shriveling in cucumber fruits from different treatments at different removals and different at different stages (various temperature conditions)

The shriveling of cucumber fruit skin from different treatments, at various stages and different removals was significantly different (Appendix 11).

Maximum fruit skin shriveling (Score 2.75) was observed in non ABA treated – Plastic foil wrapped cucumber fruits, while skin shriveling was not found in ABA treated – MAP bagged fruits (Table 3.7).

Table 3.7: Fruit skin shriveling in cucumber with different treatments, kept at different stages (S1=11±1°C: 90% RH, S2=ambient conditions and S3=6±1°C: 90% RH) and removed at different storage period of 14 days and 21 days.

treatments	14 days			21 days		
	11°C	25°C	6°C	11°C	25°C	6°C
ABA treated – MAP bagged	1^{e}	1^{e}	1^{e}	1^{e}	1^{e}	1^{e}
non ABA treated – MAP bagged	1^{e}	1.25^{de}	1.65^{de}	1^{e}	1.7^{cde}	1.7^{cde}
ABA treated – Plastic foil wrapped	1^{e}	1.4^{de}	1.75^{cd}	1^{e}	1.8^{bcd}	1.8^{bcd}
non ABA treated – Plastic foil wrapped	1^{e}	2.75^{a}	2.5^{ab}	1^{e}	2.4^{abc}	2.55^{a}
Mean SE	SE± 0.3596					

Means in columns and rows not sharing similar letters differ significantly at $P \leq 0.05$, n=15

No shriveling was observed in any treatment at 11°C in both 14 days and 21 days storage period. But non ABA treated – MAP bagged, ABA treated – Plastic foil wrapped, and non ABA treated – Plastic foil wrapped cucumber fruits from 14 days and 21 days storage period showed shriveling at 25°C and 6°C (Table 3.7).

3.2.5 Ion leakage (percentage) in cucumber fruits from different treatments at different removals and different at different stages (various temperature conditions)

The ion leakage for peel of cucumber fruits from different treatments, storage duration, stages and their comparison showed significant difference (Appendix 12).

Highest ion leakage was observed in non ABA treated – Plastic foil wrapped (T4). Followed by ABA treated – Plastic foil wrapped (T3), ABA treated – MAP bagged (T1) and non ABA treated – MAP bagged (T2). While ion leakage of ABA treated – MAP bagged and non ABA treated – MAP bagged cucumber fruits were non-significantly different from each other. (Figure 3.21).

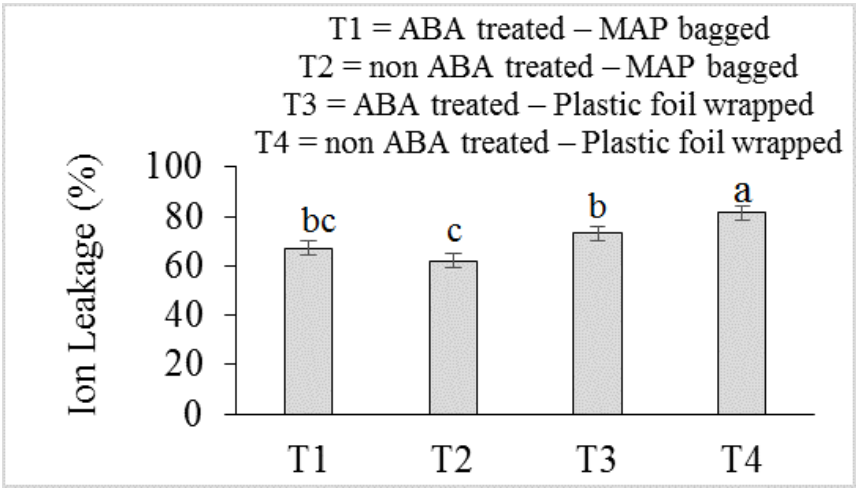


Figure 3.21: Ion Leakage (percentage) of cucumber fruits from different treatments. Vertical bars represent SE± 3.0145, the different letters on the bars express significant difference, n=15

The ion leakage was higher in cucumber fruits at 6±1°C: 90% RH than 11±1°C: 90% RH and 25±1°C The ion leakage was differed non-significantly in fruits from 11±1°C: 90% RH and 25±1°C (Figure 3.22).

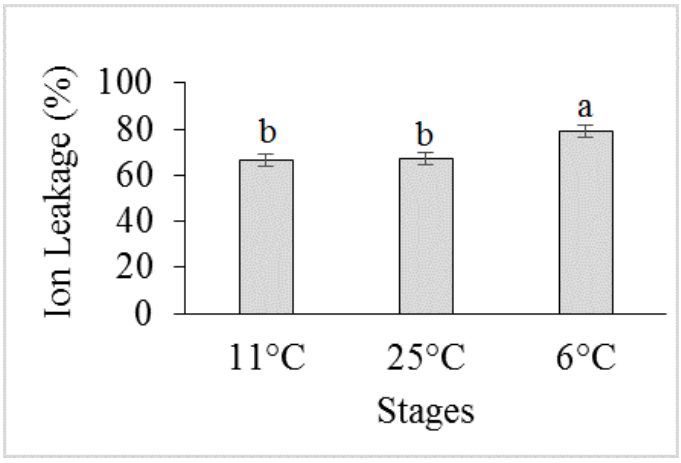


Figure 3.22: Ion Leakage (percentage) of cucumber fruits from different Stages (keeping conditions). Vertical bars represent SE± 2.6107, the different letters on the bars express significant difference, n=15

Furthermore, the ion leakage was more in fruits samples from 21 days storage period as compared to 14 days storage period (Figure 3.23).

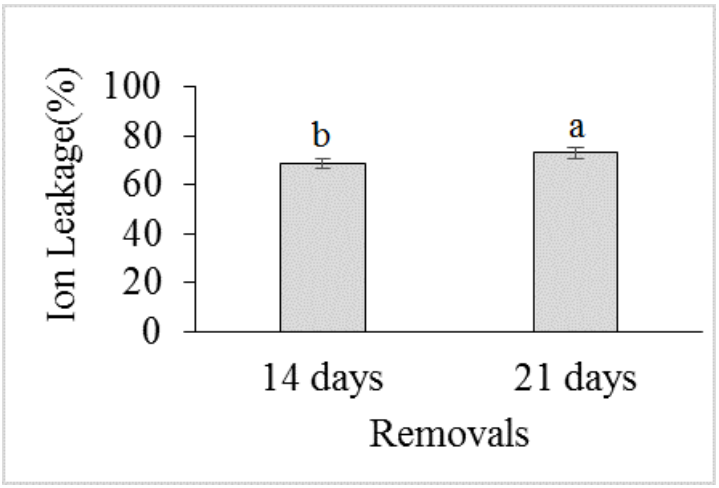


Figure 3.23: Ion Leakage (percentage) of cucumber fruits from different storage durations. Vertical bars represent SE± 2.1316, the different letters on the bars express significant difference, n=15

The combined effect of treatments, different stages (keeping conditions) and storage duration showed significantly affected ion leakage cucumber fruit peel (Appendix 14).

Maximum ion leakage (91.5%) was observed in non ABA treated – Plastic foil wrapped cucumber fruits from 21 days storage duration kept at 6°C (stage 3) as compared to all other combinations. While minimum ion leakage (53.4%) was found in non ABA treated – MAP bagged fruits from 14 days storage duration kept at 25°C (stage 2) (Table 3.8).

Table 3.8: Ion Leakage (percentage) in cucumber fruits with different treatments, kept at different stages (S1=11±1°C: 90% RH, S2=ambient conditions and S3=6±1°C: 90% RH) and removed at different storage period of 14 days and 21 days

treatments	14 days			21 days		
	11°C	25°C	6°C	11°C	25°C	6°C
ABA treated – MAP bagged	62.5^{ghij}	55.0^{ij}	$69.1^{cdefghi}$	70.9^{cdefgh}	64.6^{efghij}	80.6^{abcd}
non ABA treated – MAP bagged	60.0^{ghij}	53.4^{j}	70.9^{cdefgh}	58.4^{hij}	58.6^{hij}	71.4^{cdefgh}
ABA treated – Plastic foil wrapped	$70.0^{cdefghi}$	$69.1^{cdefghi}$	80.9^{abcd}	$63.4f^{ghij}$	74.6^{bcdefg}	80.8^{abcd}
non ABA treated – Plastic foil wrapped	$65.7^{defghij}$	78.6^{abcdef}	89.3^{ab}	79.5^{abcde}	83.9^{abc}	91.5^{a}
Mean SE	SE± 7.384					

Means in columns and rows not sharing similar letters differ significantly at $P \leq 0.05$, n=15

3.2.6 Comparison of anti-oxidants capacity (FRAP) and total phenolics in cucumber fruits from different treatments at different removals

The anti-oxidants and total phenolics concentration was significantly different for various treatments with different storage time (Appendix 13).

Higher anti-oxidants concentration was observed in ABA treated – MAP bagged (T1) fruits samples followed by ABA treated – Plastic foil wrapped (T3), non ABA treated – MAP bagged (T2) and non ABA treated – Plastic foil wrapped (T4). The T1, T2 and T3 was significantly different from T4, but no significant difference found between them (Figure 3.21).

The effect of removal on anti-oxidants concentration was observed higher at storage time of 14 days as compared to 21 days (Figure 3.21).

On the other hand, the total phenolics were differed significantly for different treatments with various storage duration (Appendix 14).

Higher total phenolics contents (15.147 mg/L) were observed non ABA treated – MAP bagged (T2) fruits, while least concentration of total phenolics was noticed in non ABA treated – Plastic foil wrapped (T4).

The storage period influenced the total phenolics contents in cucumber fruits. Higher total phenolics were found in fruits sample from storage period of 14 days (removal 1) as compared to storage period of 21 days (removal 2) (Figure 3.21).

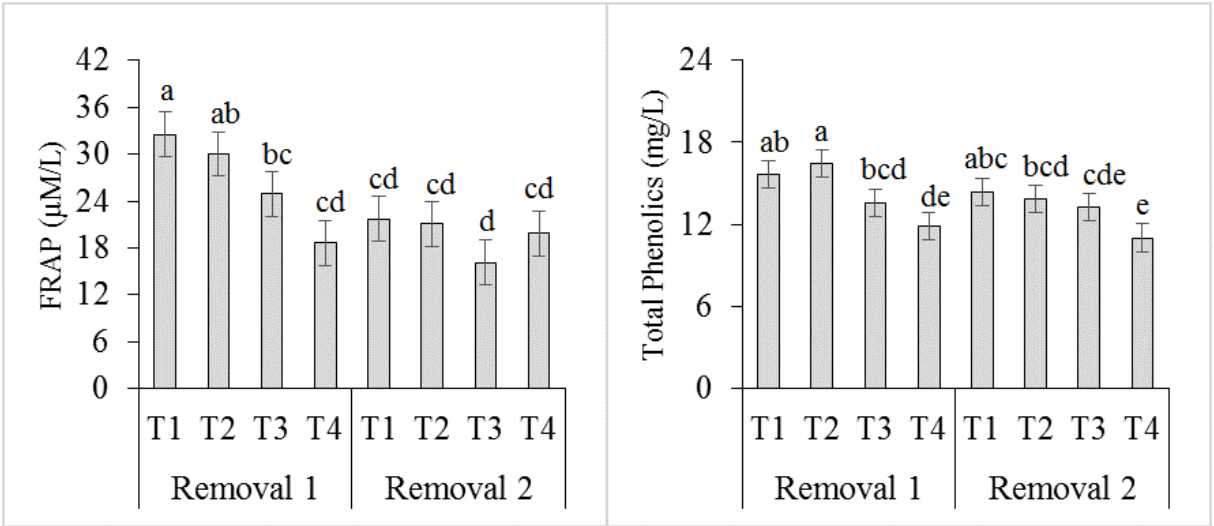


Figure 3.21: Comparison of Anti-oxidants and total phenolics contents in T1 (ABA treated – MAP bagged), T2 (non ABA treated – MAP bagged), T3 (ABA treated – Plastic foil wrapped) and T4 (non ABA treated – Plastic foil wrapped) of cucumber fruits from storage period of 14 days (Removal 1) and 21 days (Removal 2). Vertical bars represent SE± 2.8584 and 1.0062, the different letters on the bars express significant difference, n=15

4. Discussion

4.1 Experiment 1

4.1.1 Plant growth and morphology

Cucumber plants did not show difference in morphology in response to different RH conditions after first the two week under experimental condition. However, at the time of harvest, all growth and morphological characters such as average leaf area, relative chlorophyll contents, number of leaves, shoot length, fruits diameter and fruit length except total number of fruits showed significant response to various relative air humidity conditions. Previously, many studies also reported significant effect of high relative air humidity on increased plant height (Hoffman et al., 1971; Mortensen, 1986; Mortensen and Gislerød, 1990; Mortensen and Fjeld, 1998; Mortensen and Gislerød, 1999; Mortensen, 2000; Torre and Fjeld, 2001). In our study, an increase in shoot length was observed with increased humidity, which supported earlier findings (Hoffman et al., 1971; Mortensen, 1986; Mortensen and Fjeld, 1998; Mortensen, 2000).

In most of studies, it has been observed that leaf area increased with increase of RH (Van de Sanden & Veen, 1992; Roriz et al., 2014). Increased leaf length under high RH is a possible response of increased cell expansion in differential epidermal cell (Murphy et al., 2013). But Innes, (2015) found that leaf expansion in terms of leaf length and leaf area was independent of RH level. In this study, the largest leaf area was observed in plants from moderate relative air humidity (60% RH). Similar results have been reported for cucumber plants previously (Van de Sanden, 1985; Jakobsen, 2016). Thus, leaf area response to RH is probably species dependent.

Few studies have reported effects of RH on chlorophyll contents. Only one study stated an increase in relative chlorophyll contents in response to increases RH (Jeon et al., 2006). On the other hand, Innes, (2015) and Jakobsen, (2016) found lower chlorophyll contents in plants under high RH compared to moderate RH. Studies on mineral relationship and chlorophyll contents have been reported, specifically involvement of iron (Fe) and nitrogen (N) (Roriz et al., 2014; Zheng, 2010). Vasconcelos and Grusak (2014) also stated that iron (Fe) deficiency is characterized to cause significant decrease in leaf chlorophyll contents. In this study, same kind of findings (Fe deficiency and decrease in relative chlorophyll content) were observed at high relative air humidity (90% RH), which are totally opposite to the results of Jeon et al. (2006).

4.1.2 Fruit growth and morphology

In previous studies, fruit size (fruit diameter, fruit length and weight) was reported to increase in response to high RH (Bakker, 1991). Furthermore, the numbers of fruits were also founf to be higher under high relative humidity (Bakker et al., 1987). In our study, under elevated RH the increase in number of fruits as well as fruit size was found, which agree with previous studies. The possible reason for big fruit size can be increased cell expansion and more turgid cells under elevated RH (Bakker, 1991). However, bigger fruits are also reported as a result of changes in the hormonal content and/or balance. Higher gibberellin or auxin content increase fruit size (Crane, 1964; Ozga et al., 2003).

4.1.3 Antioxidant capacity and polyphenols concentration

A lot of studies have been done on effect of RH on other physiological process and very less consideration have been given to effect of RH on polyphenols production. Guichard et al. (1999) stated that net water accumulation in fruits was reduced under low relative humidity, which might promote the production of polyphenols in the fruits. In our study, 3 different polyphenols (apigenin, luteolin and resveratrol) were identified, while in fruits samples 6 polyphenols (apigenin, luteolin and quercetin 3 glycoside, quercetin, pinoresinol and resveratrol) were identified. Previously apigenin, luteolin, quercetin (Hertog, et al., 1992; Chu, et al., 2000; Lugast and Hovari, 2000) and pinoresinol (Peñalvo, et al., 2005; Milder, et al., 2005; Peñalvo, 2007) were identified in fresh cucumber fruits. While in this study, a new polyphenol called resveratrol was found in cucumbers. Previously resveratrol has been reported in grapes (Bavaresco, et al., 2002), strawberry (Ehala, et al., 2005) and peanut (Lee, et al., 2004).

Except apigenin in leaf, all other polyphenols were significantly higher in cucumber leaf and fruits samples taken from low relative air humidity. Our findings agree with Guichard et al. (1999) statement.

4.1.4 Carbohydrates concentration

Previously, a lot of studies have reported effect of temperature and light on photosynthesis and carbohydrates production in plants (Taji et al. 2002). In our study, the quantities of fructose, sucrose and glucose were not significantly different in leaves from different relative air humidity conditions. However, glucose content was higher in leaves produced in 90% RH. Glucose is product of photosynthesis process. So, this finding supports Bakker (1991) statement that more photosynthesis take place at high RH. Sucrose contents

was higher in leaf sample from 60% RH. Some recent studies have reported the involvement of sucrose breakdown in stomatal movement (Daloso et al., 2015; Horrer et al., 2016) and decrease of stomatal conductance under low relative air humidity (Torre et al. 2003). Starch is known as energy storage compound in plants. At higher RH plant produce more glucose (Bakker. 1991) and store that glucose in form of starch (Pfister and Zeeman, 2016). Our study agree with these finding because we found significantly higher starch contents in leaf samples from higher RH. Furthermore, stachyose and raffinose contents were significantly higher in leaf sample from moderate relative air humidity. The amount of stachyose sugar was almost double in leaves from 60% RH. Stachyose and raffinose are well known primary soluble carbohydrates and also considered as transport sugars in cucumbers (Handley et al., 1983), while stachyose concentration increase during leaf expansion (Pharr and Sox, 1984). So, in our studies, higher leaf area under moderate relative air humidity can be linked to higher stachyose concentration.

In cucumber fruit, significantly higher fructose, glucose and starch was found in the samples from moderate relative air humidity, while stachyose was significant higher in fruit samples from 90% RH. Glucose, starch and stachyose concentration in fruit samples showed totally opposite trend than leaf samples for different relative humidity conditions. As mentioned earlier that stachyose sugars are involved in leaf expansion and it might be possible that expanded cucumber fruit size from higher relative humidity conditions is also associated with higher stachyose sugars in fruits.

4.1.5 Nutrients concentration

High RH affects stomatal regulation, photosynthesis and water evapotranspiration and may influence uptake and translocation of minerals as shown in Gislerod et al. (1987). In this study total nitrogen, carbon-nitrogen ratio, potassium, boron and sodium contents in leaves were significantly affected by RH (Table 3.4).

Normally minerals uptake by plants are passive, which means they are transported in the transpiration stream. Thus, the macro nutrients uptake and transport within plant normally benefit from water flux and high transpiration rate. High RH reduces transpiration rate (Lihavainen, et al., 2016). Gislerod, et al. (1987) reported that the transpiration rate in *Euphorbia pulcherrima*, *Begonia* x *hiemalis*, *Lycopersicon esculentum* and some other plants was decreased significantly at high RH. They also found a decrease in the macro nutrients contents with increasing RH, similarly as observed in our study, i.e. nitrogen and potassium

contents in cucumber leaf were significantly reduced at high RH (Table 3.4). These findings are also the same as in the study of Tromp and Oele (1972).

After Carbon, nitrogen (N) is the most important element required by plants for normal growth and development (Hawkesford et al., 2012). Plants grown under high RF often develop chlorotic leaves. According to Scheible et al. (2004), nitrogen deficiency or restriction will lead to suppression of genes involved in photosynthesis and chlorophyll synthesis. So, less nitrogen content, low relative chlorophyll and carbohydrates contents in leaves (except starch) and fruits from high RH in our findings, support previous studies of Hawkesford et al. (2012).

Previously, it was reported that the potassium is reciprocal to calcium and magnesium. So, if plants contain more potassium, the calcium and magnesium contents will be reduced (Lucas and Scarseth, 1947). This was also the result from our study, that we found higher potassium contents and lower calcium and magnesium contents in leaves at moderate RH (Table 3.4). The content of boron was also higher in leaves and fruits from moderate as compared to high RH (Table 3.4). This result is an agreement with the findings of Krug, et al. (2013). Except the leaf samples of *Lycopersicon esculentum*, leaves from other plants species such as *Euphorbia pulcherrima* and *Begonia* x *hiemalis* showed no significant effect of RH on magnesium contents (Gislerod, et al., 1987) as observed in our study. Although iron contents were not different significantly in cucumber leaf and fruits from different RH conditions. But higher contents of iron was observed in fruit and leaf from moderate RH. Previously, similar findings are reported by Roriz, et al. (2014).

4.1.6 Sensory analysis of fruits from different relative air humidity conditions

The results regarding sensory perception in comparison of cucumber fruit grown under different relative humidity conditions were significantly different for all attributes. Previously a couple of studies have been conducted about sensory perception of organically and conventionally grown cucumber (Zhao, et al., 2007) and the effect of time of harvest in different cultivars of cucumber on sensory perception of fruits (Pevicharova and Velkov, 2009). But the influence of relative humidity on fruit quality of cucumber or any other fruit and vegetable have never been investigated. In our study, cucumber fruits harvested from 60% RH was found to be better in sweet taste perception, good in texture and pulp colour, contained low water contents and felt less bitter in an organoleptic analysis. Sweet perception and good texture was positively correlated with starch contents. The fruits from moderate humidity was sweeter and the possible reason of this sweet perception might be due to the

higher glucose contents in fruits (Figure 3.11). We also found a positive correlation between the colour of the cucumber fruit and total nitrogen and starch contents in fruits. Jasso-Chaverria et al. (2005) reported the same kind of relation of total nitrogen and cucumber fruit colour. Bitterness of cucumber fruits from high RH was positively correlated with stachyose and negatively correlated with starch contents. In our study very less starch contents were observed in fruits sample from higher RH. So, it can be concluded that starch in cucumber fruits reduces the bitter taste and stachyose increase the bitter taste perception in cucumber fruits. A highly positive correlation was also observed between flavor of cucumber fruit, fruit colour, fruit texture, and sweet taste perception with over all liking.

4.2 Experiment 2

Besides preharvest conditions, postharvest factor also affects the cucumber fruit quality. Although quality of fruit cannot be improved after harvesting. But the duration of quality acceptability by consumers can be increased through adopting appropriate techniques.

The objective of this experiment was to analyze the individual as well as combined effect of packaging materials and exogenous application of ABA on quality of cucumber fruits at different temperature conditions of supply chain such as grower to retailer (11 ± 1°C), retailer to (25 ± 1°C) and consumers (6 ± 1°C).

4.2.1 MAP bags influence on cucumber fruit respiration and physical weight loss

The respiration rate of the fruits and vegetables depends on storage environment (temperature, RH and gases) and the process of respiration can be slowed down by using the MAP bags (Martínez-Ferrer et al., 2002). However, the CO_2 concentration was not significantly different for 14 days and 21 days storage time. The MAP bagged fruits with the exogenous application of ABA showed slightly less CO_2 concentration than normal MAP bagged fruits, but the effect was not significantly different (Figure 3.16). Our results support previous results (Martínez-Ferrer et al., 2002).

The weight loss of fruits also depends on temperature and is indirectly associated with respiration rate. Although MAP bags slow down the respiration rate, but they also reduce weight loss because they are selective permeable for water vapor (Kudachikar, et al., 2011). In our study, MAP bags reduced weight loss effectively (Figure 3.17).

4.2.2 MAP bags influence on disease incidence and chilling injury in cucumber fruits

In previous studies, it has been reported that the fruits and vegetables keep continue their physiological process after harvest and under favorable conditions these process get speeded up and move towards senescence (Kader, et al., 1989). They respire and breakdown the complex substrates of and produce energy (Fonseca et al., 2002) and get sensitive to stresses. Due to high sensitivity and favorable conditions (temperature and RH) the probability of disease incidence increases (Rehman, et al., 2015). Kader et al. (1989) stated that the MAP bagged fruits and vegetables get additive to stresses. In our study, higher disease incidence was observed in non ABA treated – plastic folio wrapped fruits and no disease was found in ABA treated – MAP bagged fruits (Figure 3.20). So far, our findings are agree with the hypothesis of Kader et al., (1989).

Postharvest chilling injury is considered as one major physiological stresses in cucumber fruits, and it occurs at temperature ±6°C (Fukushima and Tsugiyamato, 1977). Kader et al. (1989) stated that the MAP bags increase the ability of fruits and vegetables to bear the stress conditions such as chilling injury (Ion leakage). In our study, we found the higher percentage of ion leakage in non-MAP bagged fruits as compared to other treatments (Figure 3.21). Furthermore, higher anti-oxidant capacity was also found in ABA treated-MAP bagged and non ABA treated-MAP bagged fruits (Figure 3.24). This means that the cucumber fruits can conserve better their water, antioxidant capacity and better tolerate chilling temperature when stored in MAP bags compared to polyethylene wrapping.

Conclusion

The hypothesis that the relative air humidity during plant production and fruit development effects the plant growth and fruit quality was confirmed. The high RH resulted tall plants, smaller, but more number of leaves and bigger fruits. While moderate RH produced plants with larger leaf area, more relative chlorophyll contents, as well as higher carbohydrates, minerals and polyphenol contents in cucumber leaves and fruits. The fruits from moderate RH were better in sensory perception. In short, the effect of moderate RH in controlled condition showed promising results for plant growth, internal and external quality of fruit. On the other hand, the MAP bagged fruits showed best results in terms of physical weight loss, fruits respiration, ion leakage, antioxidant capacity and total phenolics contents. MAP bagged fruits maintained their quality even under low temperature storage condition. Further investigation with combination of these two experiment may provide better result about storage life of cucumber fruits grown at different relative humidity conditions.

References

ABELLANOSA AL, PAVA HM. 1987. Introduction to Crop Science. CMU, Musuan, Bukidnon: Publications Office. p. 23-64.

Ahmed, M., Hamid, A., and Akbar, Z. (2004). Growth and yield performance of six cucumber (*Cucumis sativus* L.) cultivars under agro-climatic conditions of Rawalakot, Azad Jammu and Kashmir [Pakistan]. International Journal of Agriculture and Biology (Pakistan).

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols, 2(4), 875-877.

Alam, M. A. (2016). Night time temperature and day time irradiance on photosynthesis and growth of cucumber: Potential and possibilities for energy saving (Master's thesis, Norwegian University of Life Sciences, Ås).

Anonymous, (2014a). World Bank Organization. Retrieved on 25th September, 2017 from; http://databank.worldbank.org/data/reports.aspx?source=2&country=NOR

Anonymous, (2014b). Fresh Plaza. Retrieved on 25th September, 2017 from; http://www.freshplaza.com/article/140058/Norway-Fruit-production-up-30-procent-in-2014

Anonymous, (2017). Cucumber nutrition facts. Retrieved on 29th September, 2017. Available online at: http://www.nutrition-and-you.com/cucumber.html

Autio, W. R., & Bramlage, W. J. (1986). Chilling sensitivity of tomato fruit in relation to ripening and senescence. Journal of the American Society for Horticultural Science, 111(2), 201-204.

Ayer-Le Lièvre, C., Lapointe, F., & Leibovici, M. (1995). Avian olfactory neurogenesis. Biology of the Cell, 84(1-2), 25-34.

Bakker, J. C. (1991). Analysis of humidity effects on growth and production of glasshouse fruit vegetables (Doctoral dissertation, Bakker).

Bakker, J. C., Welles, G. H. M., and van Uffelen, J. A. M. 1987. The effects of day and night humidity on yield and quality of glasshouse cucumbers. J. Hort. Sci. 62: 363–370.

Bavaresco, L., Fregoni, M. A. R. I. O., Trevisan, M. A. R. C. O., Mattivi, F., Vrhovsek, U., & Falchetti, R. (2002). The occurrence of the stilbene piceatannol in grapes. VITIS-GEILWEILERHOF-, 41(3), 133-136.

Beuchat, C.R. (1998) Surface decontamination of fruits and vegetables eaten raw: A review. Food Safety Unit. World Health Organisation, WHO/FSF/FOS/98.2.

Bordbar, S., Anwar, F., and Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods—a review. Marine drugs, 9(10), 1761-1805.

Bot, G. P. A. 2003. The solar greenhouse; technology for low energy consumption. Acta Hort. 611: 61–69.

Bourn, D. and Prescott, J., 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Critical reviews in food science and nutrition, 42(1), pp.1-34.

Cabrera, R. M., & Saltveit, M. E. (1990). Physiological response to chilling temperatures of intermittently warmed cucumber fruit. Journal of the American Society for Horticultural Science, 115(2), 256-261.

Carins Murphy, M. R., Jordan, G. J., & Brodribb, T. J. (2014). Acclimation to humidity modifies the link between leaf size and the density of veins and stomata. Plant, Cell & Environment, 37(1), 124-131.

Chu, Y. H., Chang, C. L., & Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. Journal of the Science of Food and Agriculture, 80(5), 561-566.
Cockshull, K. E., and Ho, L. C. 1995. Regulation of tomato fruit size by plant density and truss thinning. J. Hort. Sci. 70(3): 395–407.
Crane, J. C. (1964). Growth substances in fruit setting and development. Annual Review of Plant Physiology, 15(1), 303-326.
Cuartero, J., and Fern´andez-Munoz, R. 1999. Tomato and salinity. Sci. Hortic.78: 83–125.
da Graça Campos, M., & Markham, K. R. (2007). Structure information from HPLC and on-line measured absorption spectra: flavones, flavonols and phenolic acids. Imprensa da Universidade de Coimbra/Coimbra University Press.
da Graça Campos, M., & Markham, K. R. (2007). Structure information from HPLC and on-line measured absorption spectra: flavones, flavonols and phenolic acids. Imprensa da Universidade de Coimbra/Coimbra University Press.
Daloso, D. M., Antunes, W. C., Pinheiro, D. P., Waquim, J. P., AraÚJo, W. L., Loureiro, M. E., ... & Williams, T. C. (2015). Tobacco guard cells fix CO2 by both Rubisco and PEPcase while sucrose acts as a substrate during light-induced stomatal opening. Plant, cell & environment, 38(11), 2353-2371.
DEVLIN R. 1975. Plant Physiology. New York, NY: D. Van Nostrand Company. 600 p.
Dorais, M., Papadopoulos, A., and Gosselin, A. 2001. Greenhouse tomato fruit quality. Hort. Reviews 26: 239–319.
Eagleman, J. R. (1985). Meteorology, Ch. 3. Wadsworth, Belmont California.
Edmond, J. B., Senn, T. L., Andrews, F. S., & Halfacre, R. G. (1975). Fundamentals of horticulture (No. 4th ed.). McGraw-Hill, Inc..
Ehala, S., Vaher, M., & Kaljurand, M. (2005). Characterization of phenolic profiles of Northern European berries by capillary electrophoresis and determination of their antioxidant activity. Journal of agricultural and food chemistry, 53(16), 6484-6490.
Ennis, D.M. and O'sullivan, J., 1979. Cucumber Quality-A Review. Journal of Food Science, 44(1), pp.186-189.
FAOSTAT, (2014). Worldwide Cucumber production by continents. Retrieved on 25th September, 2017 from; http://www.fao.org/faostat/en/#data/QC/visualize
FAOSTAT, (2014b). Top four vegetable crops of Norway by annual production. Retrieved on 25th September, 2017 from: http://www.fao.org/faostat/en/#data/QC
Fern, K. (1997). Plants for a future. Edible and Useful Plants for a Healthier World. Perm. Publ.
Fonseca, S. C., Oliveira, F. A., & Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. Journal of food engineering, 52(2), 99-119.
Ford, M. A., & Thorne, G. N. (1974). Effects of atmospheric humidity on plant growth. Annals of Botany, 38(2), 441-452.
Fordham, M. C., Harrison-Murray, R. S., Knight, L., & Evered, C. E. (2001). Effects of leaf wetting and high humidity on stomatal function in leafy cuttings and intact plants of Corylus maxima. Physiologia Plantarum, 113(2), 233-240.
Fricke, A., and Krug, H. 1997. Influence of humidification and dehumidification on greenhouse climate as well as water relations and productivity of cucumber. II. Influences on plants. Gartenbauwiss. 62(6): 241–248.

Fukushima, T., & Tsugiyama, T. (1977). Chilling-injury in cucumber fruits. II. Chemical analyses of leakage substances and anatomical observation of symptoms. Scientia Horticulturae, 6(3), 199-206.
Garden, M. B. (1893). Missouri Botanical Garden. Board of Trustees.
Geissler, T. 1985. Gem¨useproduktion unter Glas und Plasten. VEB Deutscher Landwirtschaftsverlag—Berlin, p. 279.
Gine-Bordonaba, J., Cantín, C. M., Echeverría, G., Ubach, D., and Larrigaudière, C. (2016). The effect of chilling injury-inducing storage conditions on quality and consumer acceptance of different Prunus persica cultivars. Postharvest Biology and Technology, 115, 38-47.
Gislerod, H. R., Selmer-Olsen, A. R., & Mortensen, L. M. (1987). The effect of air humidity on nutrient uptake of some greenhouse plants. Plant and Soil, 102(2), 193-196.
Gislerød, H. R., & Mortensen, L. M. (1990). Relative humidity and nutrient concentration affect nutrient uptake and growth of Begonia× hiemalis. HortScience, 25(5), 524-526.
Gislerød, H. R., & Nelson, P. V. (1989). The interaction of relative air humidity and carbon dioxide enrichment in the growth of Chrysanthemum× morifolium Ramat. Scientia Horticulturae, 38(3-4), 305-313.
Grange, R. I., & Hand, D. W. (1987). A review of the effects of atmospheric humidity on the growth of horticultural crops. Journal of Horticultural Science, 62(2), 125-134.
Gross, K. C., & Pharr, D. M. (1982). Cucumber fruit sucrose synthase isozymes. Phytochemistry, 21(6), 1241-1244.
Grubben, G. J. (2004). Plant Resources of Tropical Africa (PROTA) (Vol. 1). Prota.
Gruda, N. (2005). Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. Critical Reviews in Plant Sciences, 24(3), 227-247.
Guichard, S., Gary, C., Longuenesse, J. J., & Leonardi, C. (1999). Water fluxes and growth of greenhouse tomato fruits under summer conditions. Acta Horticulturae, 223-230.
Handley, L. W., Pharr, D. M., & McFeeters, R. F. (1983). Carbohydrate changes during maturation of cucumber fruit implications for sugar metabolism and transport. Plant physiology, 72(2), 498-502.
Heino, P., Sandman, G., Lång, V., Nordin, K., and Palva, E. T. (1990). Abscisic acid deficiency prevents development of freezing tolerance in Arabidopsis thaliana (L.) Heynh. Theoretical and Applied Genetics, 79(6), 801-806.
Hendrix, J. E. (1982). Sugar translocation in two members of the Cucurbitaceae. Plant Science Letters, 25(1), 1-7.
Hertog, M. G., Hollman, P. C., & Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. Journal of agricultural and food chemistry, 40(12), 2379-2383.
Holder, R., and Cockshull, K. E. 1990. Effects of humidity on the growth and yield of glasshouse tomatoes. J. Hort. Sci. 65(1): 31–39
Horrer, D., Flütsch, S., Pazmino, D., Matthews, J. S., Thalmann, M., Nigro, A., ... & Santelia, D. (2016). Blue light induces a distinct starch degradation pathway in guard cells for stomatal opening. Current Biology, 26(3), 362-370.
Hovenden, M. J., Vander Schoor, J. K., & Osanai, Y. (2012). Relative humidity has dramatic impacts on leaf morphology but little effect on stomatal index or density in

Nothofagus cunninghamii (Nothofagaceae). Australian Journal of Botany, 60(8), 700-706.
Innes, S. N. (2015). Effects of UV radiation and air humidity on morphology, stomatal function and photosynthesis of Euphorbia pulcherrima (Master's thesis).
Jakobsen, S. B. (2016). Vekst, transpirasjon og absisinsyreregulering i Cucumis sativus: betydningen av luftfuktighet og blått lys (Master's thesis, Norwegian University of Life Sciences, Ås).
Jasso-Chaverria, C., Hochmuth, G. J., Hochmuth, R. C., & Sargent, S. A. (2005). Fruit yield, size, and color responses of two greenhouse cucumber types to nitrogen fertilization in perlite soilless culture. HortTechnology, 15(3), 565-571.
JC Bakker. (1995). Greenhouse climate control: an integrated approach. Wageningen Academic Pub.
Jeon, M. W., Ali, M. B., Hahn, E. J., & Paek, K. Y. (2006). Photosynthetic pigments, morphology and leaf gas exchange during ex vitro acclimatization of micropropagated CAM Doritaenopsis plantlets under relative humidity and air temperature. Environmental and Experimental Botany, 55(1), 183-194.
Jiang, Y., and Joyce, D. C. (2003). ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. Plant Growth Regulation, 39(2), 171-174.
Julkunen-Tiitto, R., Rousi, M., Bryant, J., Sorsa, S., Keinänen, M., & Sikanen, H. (1996). Chemical diversity of several Betulaceae species: comparison of phenolics and terpenoids in northern birch stems. Trees-Structure and Function, 11(1), 16-22.
Kader, A. A., Zagory, D., Kerbel, E. L., & Wang, C. Y. (1989). Modified atmosphere packaging of fruits and vegetables. Critical Reviews in Food Science & Nutrition, 28(1), 1-30.
Kinoshita, T., Nishimura, M., and Shimazaki, K. I. (1995). Cytosolic concentration of Ca2+ regulates the plasma membrane H+-ATPase in guard cells of fava bean. The Plant Cell, 7(8), 1333-1342.
Krug, B. A., Whipker, B. E., McCall, I., & Frantz, J. (2013). Elevated relative humidity increases the incidence of distorted growth and boron deficiency in bedding plant plugs. HortScience, 48(3), 311-313.
Kudachikar, V. B., Kulkarni, S. G., & Prakash, M. K. (2011). Effect of modified atmosphere packaging on quality and shelf life of 'Robusta'banana (Musa sp.) stored at low temperature. Journal of food science and technology, 48(3), 319-324.
Lee, S. S., Lee, S. M., Kim, M., Chun, J., Cheong, Y. K., & Lee, J. (2004). Analysis of trans-resveratrol in peanuts and peanut butters consumed in Korea. Food research international, 37(3), 247-251.
Leuschner, C. (2002). Air humidity as an ecological factor for woodland herbs: leaf water status, nutrient uptake, leaf anatomy, and productivity of eight species grown at low or high vpd levels. Flora-Morphology, Distribution, Functional Ecology of Plants, 197(4), 262-274.
Lihavainen, J., Ahonen, V., Keski-Saari, S., Kontunen-Soppela, S., Oksanen, E., & Keinänen, M. (2016). Low vapour pressure deficit affects nitrogen nutrition and foliar metabolites in silver birch. Journal of experimental botany, 67(14), 4353-4365.
Lixandru, M. (2014). Properties and benefits of Cucumbers. Retrieved on 19 September, 2017. Available online at: https://www.natureword.com/properties-and-benefits-of-cucumbers/
Lu, A., Huang, L., Chen, S. K., and Jeffrey, C. (2011). Cucurbitaceae. Flora of China, 19, 1-56.

Lucas, R. E., & Scarseth, G. D. (1947). Potassium, calcium, and magnesium balance and reciprocal relationship in plants. Journal of the American Society of Agronomy.
Lugast, A., & Hovari, J. (2000). Flavonoid aglycons in foods of plant origin I. Vegetables. Acta Alimentaria, 29(4), 345-352.
Malik, M.N. and Bashir, E., 1994. Horticulture: National Book Foundation. Islamabad, Pakistan, 633.
MANAKER GH. 1981. Interior Plantscapes: Installation, Maintenance, and Management. Englewood Cliffs, NJ: Prentice-Hall, Inc. 283 p.
Mao, L., Pang, H., Wang, G., and Zhu, C. (2007). Phospholipase D and lipoxygenase activity of cucumber fruit in response to chilling stress. Postharvest Biology and Technology, 44(1), 42-47.
Martínez-Ferrer, M., Harper, C., Pérez-Muntoz, F., & Chaparro, M. (2002). Modified atmosphere packaging of minimally processed mango and pineapple fruits. Journal of Food Science, 67(9), 3365-3371.
McCollum, T. G., and McDonald, R. E. (1991). Electrolyte leakage, respiration, and ethylene production as indices of chilling injury in grapefruit. HortScience, 26(9), 1191-1192.
Miao, M., Xu, X., Chen, X., Xue, L., & Cao, B. (2007). Cucumber carbohydrate metabolism and translocation under chilling night temperature. Journal of plant physiology, 164(5), 621-628.
Milder, I. E., Arts, I. C., van de Putte, B., Venema, D. P., & Hollman, P. C. (2005). Lignan contents of Dutch plant foods: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. British Journal of Nutrition, 93(3), 393-402.
MILLER GT Jr. 2001. Environmental Science: Working With the Earth. 8th ed. Pacific Grove, CA: Brooks/Cole. 549 p.
Mortensen, L. M. (1986). Effect of relative humidity on growth and flowering of some greenhouse plants. Scientia horticulturae, 29(4), 301-307.
Mortensen, L. M. (1987). CO2 enrichment in greenhouses. Crop responses. Scientia Horticulturae, 33(1-2), 1-25.
Mortensen, L. M. (2000). Effects of air humidity on growth, flowering, keeping quality and water relations of four short-day greenhouse species. Scientia horticulturae, 86(4), 299-310.
Mortensen, L. M., & Fjeld, T. (1998). Effects of air humidity, lighting period and lamp type on growth and vase life of roses. Scientia horticulturae, 73(4), 229-237.
Mortensen, L. M., & Gislerød, H. R. (1990). Effects of air humidity and supplementary lighting on foliage plants. Scientia Horticulturae, 44(3-4), 301-308.
Mortensen, L. M., & Gislerød, H. R. (1999). Influence of air humidity and lighting period on growth, vase life and water relations of 14 rose cultivars. Scientia Horticulturae, 82(3), 289-298.
Mortensen, L.V. 1987. CO2 enrichment in greenhouses. Crop responses. Review article. Sci. Hortic. 33(1–2): 1–25.
Mulholland, B. J., Fussell, M., Edmondson, R. N., Basham, J., and McKee, J. M. T. 2001. Effect of VPD, K nutrition and root-zone temperature on leaf area development, accumulation of Ca and K and yield in tomato. J. Hortic. Sci. Biotech. 76(5): 641–647.
Nonnecke, I.L., 1989. Vegetable production. Springer Science and Business Media.
Ozcelik, N., and Akilli, M. 1999. Effects ofCO2 enrichment on vegetable growth, yield and quality of greenhouse—grown tomatoes in soil and soilless cultures. Acta Hort. 486: 155–160.

Ozga, J. A., Yu, J., & Reinecke, D. M. (2003). Pollination-, development-, and auxin-specific regulation of gibberellin 3β-hydroxylase gene expression in pea fruit and seeds. Plant Physiology, 131(3), 1137-1146.
Peñalvo, J. L., Adlercreutz, H., Uehara, M., Ristimaki, A., & Watanabe, S. (2007). Lignan content of selected foods from Japan. Journal of agricultural and food chemistry, 56(2), 401-409.
Peñalvo, J. L., Haajanen, K. M., Botting, N., & Adlercreutz, H. (2005). Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry. Journal of Agricultural and Food Chemistry, 53(24), 9342-9347.
Peryam, D. R., & Pilgrim, F. J. (1957). Hedonic scale method of measuring food preferences. Food technology.
Pesis, E., Aharoni, D., Aharon, Z., Ben-Arie, R., Aharoni, N., and Fuchs, Y. (2000). Modified atmosphere and modified humidity packaging alleviates chilling injury symptoms in mango fruit. Postharvest biology and technology, 19(1), 93-101.
Pevicharova, G., & Velkov, N. (2009). Sensory analysis of cucumber varieties at different harvest times II. Pickling cucumbers. Journal of Central European Agriculture, 10(3), 289-295.
Pfister, B., & Zeeman, S. C. (2016). Formation of starch in plant cells. Cellular and Molecular Life Sciences, 73(14), 2781-2807.
Pharr, D. M., & Sox, H. N. (1984). Changes in carbohydrate and enzyme levels during the sink to source transition of leaves of Cucumis sativus L., a stachyose translocator. Plant science letters, 35(3), 187-193.
POINCELOT RP. 1980. Horticulture: Principles and Practices. Englewood Cliffs, NJ: Prentice-Hall, Inc. p. 87-119.
Reed, J. W., Nagpal, P., Poole, D. S., Furuya, M., and Chory, J. (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. The Plant Cell, 5(2), 147-157.
Rehman, A., Malik, A. U., Ali, H., Alam, M. W., & Sarfraz, B. (2015). Preharvest factors influencing the postharvest disease development and fruit quality of mango. Journal of Environmental and Agricultural Sciences, 3, 42-47.
Reznicek, A. A., Voss, E. G., and Walters, B. S. (2011). Michigan flora online. University of Michigan. Disponible en ligne à: michiganflora. net/home. aspx.[Visité le 12-01-16].
Riesmeier, J. W., Willmitzer, L., & Frommer, W. B. (1994). Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning. The EMBO Journal, 13(1), 1.
Rocculi, P., Del Nobile, M. A., Romani, S., Baiano, A., & Dalla Rosa, M. (2006). Use of a simple mathematical model to evaluate dipping and MAP effects on aerobic respiration of minimally processed apples. Journal of Food Engineering, 76(3), 334-340.
Roriz, M., Carvalho, S. M., & Vasconcelos, M. W. (2014). High relative air humidity influences mineral accumulation and growth in iron deficient soybean plants. Frontiers in plant science, 5.
Saltveit, M. E. (2002). The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. Postharvest Biology and Technology, 26(3), 295-304.

Sarhan, T. Z., & Ismael, S. F. (2014). Effect of Low Temperature and Seaweed Extracts on Flowering and Yield of Two Cucumber Cultivars (Cucumis sativus L.). International Journal of Agricultural and Food Research, 3(1).
Scheible, W. R., Morcuende, R., Czechowski, T., Fritz, C., Osuna, D., Palacios-Rojas, N., ... & Stitt, M. (2004). Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. Plant physiology, 136(1), 2483-2499.
Schroeder, J. I., and Hagiwara, S. (1989). Cytosolic calcium regulates ion channels in the plasma membrane of Vicia faba guard cells. Nature, 338(6214), 427-430.
Serrano, M., Martínez-Madrid, M. C., Pretel, M. T., Riquelme, F., & Romojaro, F. (1997). Modified atmosphere packaging minimizes increases in putrescine and abscisic acid levels caused by chilling injury in pepper fruit. Journal of agricultural and food chemistry, 45(5), 1668-1672.
Shewfelt, R. L. 1999. What is quality? Postharvest Biology and Technology 15: 197–200.
Skálová, H., Moravcová, L., Dixon, A. F., Kindlmann, P., and Pyšek, P. (2015). Effect of temperature and nutrients on the growth and development of seedlings of an invasive plant. AoB Plants, 7.
Spomer, G. G. (1979). Prospects of hormonal mechanisms in cold adaptations of arctic plants. In Comparative Mechanisms of Cold Adaptation (pp. 311-322).
SSB, (2015). Horticultural production. Retrieved on 25th September, 2017 from: http://www.ssb.no/en/jord-skog-jakt-og-fiskeri/statistikker/hagebruk/aar/2016-06-08
Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., ... & Shinozaki, K. (2002). Important roles of drought-and cold-inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana. The Plant Journal, 29(4), 417-426.
Tibbitts, T. W. (1979). Humidity and plants. BioScience, 29(6), 358-363.
Tibbitts, T. W., & Bottenberg, G. (1976). Growth of lettuce under controlled humidity levels. J. Amer. Soc. Hort. Sci, 101(1), 70-73.
Tognoni, F., Pardossi, A., and Serra, G. 1999. Strategies to match greenhouses to crop production. Acta Hort. 481: 451–461.
Torre, S., Fjeld, T., & Gislerød, H. R. (2001). Effects of air humidity and K/Ca ratio in the nutrient supply on growth and postharvest characteristics of cut roses. Scientia Horticulturae, 90(3), 291-304.
Torre, S., Fjeld, T., Gislerød, H. R., & Moe, R. (2003). Leaf anatomy and stomatal morphology of greenhouse roses grown at moderate or high air humidity. Journal of the American Society for Horticultural Science, 128(4), 598-602.
Tromp, J., & Oele, J. (1972). Shoot growth and mineral composition of leaves and fruits of apple as affected by relative air humidity. Physiologia Plantarum, 27(2), 253-258.
Van de Sanden, P. A. C. M. (1985). EFFECT OF AIR HUMIDITY ON GROWTH AND WATER EXCHANGE OF CUCUMBER-SEEDLINGS. PRELIMINARY REPORT. In Symposium Greenhouse Climate and its Control 174 (pp. 259-268).
van de Sanden, P. A., & Veen, B. W. (1992). Effects of air humidity and nutrient solution concentration on growth, water potential and stomatal conductance of cucumber seedlings. Scientia horticulturae, 50(3), 173-186.
Vasconcelos, M. W., & Grusak, M. A. (2014). Morpho-physiological parameters affecting iron deficiency chlorosis in soybean (Glycine max L.). Plant and soil, 374(1-2), 161-172.

Wang, C. Y. (1987). Changes of polyamines and ethylene in cucumber seedlings in response to chilling stress. Physiologia Plantarum, 69(2), 253-257.
Wang, C. Y. (1993). Approaches to reduce chilling injury of fruits and vegetables. Hort. Rev, 15, 63-95.
Webb, J. A., & Gorham, P. R. (1964). Translocation of photosynthetically assimilated C14 in straight-necked squash. Plant physiology, 39(4), 663.
Zagory, D., & Kader, A. A. (1988). Modified atmosphere packaging of fresh produce. Food Technol, 42(9), 70-77.
Zhao, X., Chambers, E., Matta, Z., Loughin, T. M., & Carey, E. E. (2007). Consumer sensory analysis of organically and conventionally grown vegetables. Journal of Food Science, 72(2).
Zheng, S. J. (2010). Iron homeostasis and iron acquisition in plants: maintenance, functions and consequences. Annals of botany, 105(5), 799-800.
Zomlefer, W.B. (1994). Guide to Flowering Plant Families. Chapel Hill: The University of North Carolina Press

Appendix

Appendix 1

Completely Randomized AOV for FRAP

Source	DF	SS	MS	F	P
treatment	1	736.16	736.164	9.69	0.0144
Error	8	608.04	76.005		
Total	9	1344.20			

Grand Mean 17.900 CV 48.70

Appendix 2

Completely Randomized AOV for total

Source	DF	SS	MS	F	P
treatment	1	7.5690	7.56900	5.82	0.0424
Error	8	10.4120	1.30150		
Total	9	17.9810			

Grand Mean 7.8700 CV 14.50

Appendix 3

Completely Randomized AOV for Apigenin

Source	DF	SS	MS	F	P
treatment	1	0.00781	0.00781	1.89	0.2181
Error	6	0.02477	0.00413		
Total	7	0.03259			

Grand Mean 0.4163 CV 15.44

Completely Randomized AOV for Luteolin

Source	DF	SS	MS	F	P
treatment	1	0.07031	0.07031	83.1	0.0001
Error	6	0.00508	0.00085		
Total	7	0.07539			

Grand Mean 0.2863 CV 10.16

Completely Randomized AOV for Resveraterol

Source	DF	SS	MS	F	P
treatment	1	1.74845	1.74845	27.6	0.0019
Error	6	0.38015	0.06336		
Total	7	2.12860			

Grand Mean 1.9700 CV 12.78

Appendix 4

Analysis of Variance Table for Apigenin7

Source	DF	SS	MS	F	P
replicati	3	0.00085	2.833E-04		
organ	1	0.00023	2.250E-04	2.89	0.1232
treatment	1	0.00040	4.000E-04	5.14	0.0495
organ*treatment	1	0.00003	2.500E-05	0.32	0.5846
Error	9	0.00070	7.778E-05		
Total	15	0.00220			

Grand Mean 0.0150 CV 58.79

Analysis of Variance Table for Luteolin7

Source	DF	SS	MS	F	P
replicati	3	0.05100	0.01700		
organ	1	1.51290	1.51290	51.43	0.0001
treatment	1	0.16403	0.16403	5.58	0.0425
organ*treatment	1	0.09922	0.09922	3.37	0.0994
Error	9	0.26475	0.02942		
Total	15	2.09190			

Grand Mean 0.3875 CV 44.26

Analysis of Variance Table for Q3G

Source	DF	SS	MS	F	P
replicati	3	0.00007	0.00002		
organ	1	0.02806	0.02806	250.94	0.0000
treatment	1	0.00076	0.00076	6.76	0.0287
organ*treatment	1	0.00076	0.00076	6.76	0.0287
Error	9	0.00101	0.00011		
Total	15	0.03064			

Grand Mean 0.0419 CV 25.25

Analysis of Variance Table for Quercetin

Source	DF	SS	MS	F	P
replicati	3	0.00082	0.00027		
organ	1	0.01381	0.01381	60.43	0.0000
treatment	1	0.00181	0.00181	7.91	0.0203
organ*treatment	1	0.00181	0.00181	7.91	0.0203
Error	9	0.00206	0.00023		
Total	15	0.02029			

Grand Mean 0.0294 CV 51.46

Analysis of Variance Table for U7

Source	DF	SS	MS	F	P
replicati	3	2997	999		
organ	1	919825	919825	549.80	0.0000
treatment	1	5841	5841	3.49	0.0945
organ*treatment	1	5841	5841	3.49	0.0945

Error	9	15057	1673		
Total	15	949561			

Grand Mean 239.77 CV 17.06

Analysis of Variance Table for U8

Source	DF	SS	MS	F	P
replicati	3	6199	2066		
organ	1	1598581	1598581	311.63	0.0000
treatment	1	4193	4193	0.82	0.3895
organ*treatment	1	78	78	0.02	0.9044
Error	9	46168	5130		
Total	15	1655219			

Grand Mean 354.51 CV 20.20

Analysis of Variance Table for pinoresin

Source	DF	SS	MS	F	P
replicati	3	0.00016	0.00005		
organ	1	0.00185	0.00185	10.51	0.0101
treatment	1	0.00126	0.00126	7.16	0.0253
organ*treatment	1	0.00014	0.00014	0.82	0.3892
Error	9	0.00158	0.00018		
Total	15	0.00500			

Grand Mean 0.0331 CV 40.04

Analysis of Variance Table for resveratr

Source	DF	SS	MS	F	P
replicati	3	0.03175	0.01058		
organ	1	0.09000	0.09000	7.74	0.0213
treatment	1	0.01690	0.01690	1.45	0.2587
organ*treatment	1	0.01960	0.01960	1.69	0.2265
Error	9	0.10465	0.01163		
Total	15	0.26290			

Grand Mean 0.2475 CV 43.57

Appendix 5

Completely Randomized AOV for Fructose

Source	DF	SS	MS	F	P
Treatment	1	0.00000	0.00000	0.00	1.0000
Error	6	0.01400	0.00233		
Total	7	0.01400			

Grand Mean 0.1250 CV 38.64

Completely Randomized AOV for Glucose

Source	DF	SS	MS	F	P
Treatment	1	0.00281	0.00281	0.76	0.4156
Error	6	0.02208	0.00368		
Total	7	0.02489			

Grand Mean 0.2413 CV 25.14

Completely Randomized AOV for Raffinose

Source	DF	SS	MS	F	P
Treatment	1	0.17701	0.17701	35.4	0.0010
Error	6	0.02997	0.00500		
Total	7	0.20699			

Grand Mean 0.3862 CV 18.30

Completely Randomized AOV for Stachyose

Source	DF	SS	MS	F	P
Treatment	1	0.40500	0.40500	9.55	0.0214
Error	6	0.25435	0.04239		
Total	7	0.65935			

Grand Mean 0.6575 CV 31.31

Completely Randomized AOV for Starch

Source	DF	SS	MS	F	P
Treatment	1	0.41861	0.41861	7.03	0.0379
Error	6	0.35707	0.05951		
Total	7	0.77569			

Grand Mean 0.9112 CV 26.77

Completely Randomized AOV for Sucrose

Source	DF	SS	MS	F	P
Treatment	1	0.00125	0.00125	0.18	0.6843
Error	6	0.04115	0.00686		
Total	7	0.04240			

Grand Mean 0.1750 CV 47.32

Appendix 6

Completely Randomized AOV for Fructose

Source	DF	SS	MS	F	P
Treatment	1	0.68445	0.68445	179	0.0000
Error	6	0.02295	0.00383		
Total	7	0.70740			

Grand Mean 1.4500 CV 4.27

Completely Randomized AOV for Glucose

Source	DF	SS	MS	F	P
Treatment	1	0.95220	0.95220	27.1	0.0020
Error	6	0.21095	0.03516		
Total	7	1.16315			

Grand Mean 2.3125 CV 8.11

Completely Randomized AOV for Stachyose

Source	DF	SS	MS	F	P
Treatment	1	0.02205	0.02205	24.1	0.0027
Error	6	0.00550	0.00092		
Total	7	0.02755			

Grand Mean 0.6375 CV 4.75

Completely Randomized AOV for Starch

Source	DF	SS	MS	F	P
Treatment	1	20.0978	20.0978	43.0	0.0006
Error	6	2.8062	0.4677		
Total	7	22.9040			

Grand Mean 2.5650 CV 26.66

Appendix 7 Leaves Minerals

Completely Randomized AOV for B

Source	DF	SS	MS	F	P
Treatment	1	1345.60	1345.60	30.0	0.0006
Error	8	358.80	44.85		
Total	9	1704.40			

Grand Mean 68.600 CV 9.76

Completely Randomized AOV for C N ratio

Source	DF	SS	MS	F	P
Treatment	1	43.4306	43.4306	18.5	0.0026
Error	8	18.7548	2.3444		
Total	9	62.1854			

Grand Mean 17.518 CV 8.74

Completely Randomized AOV for Ca

Source	DF	SS	MS	F	P
Treatment	1	0.100	0.1000	0.00	0.9516
Error	8	204.000	25.5000		
Total	9	204.100			

Grand Mean 52.300 CV 9.66

Completely Randomized AOV for Fe

Source	DF	SS	MS	F	P
Treatment	1	0.00004	4.000E-05	0.29	0.6075
Error	8	0.00112	1.400E-04		
Total	9	0.00116			

Grand Mean 0.0820 CV 14.43

Completely Randomized AOV for K

Source	DF	SS	MS	F	P
Treatment	1	7.2250	7.22500	17.8	0.0029
Error	8	3.2440	0.40550		
Total	9	10.4690			

Grand Mean 6.6100 CV 9.63

Completely Randomized AOV for Mg

Source	DF	SS	MS	F	P
Treatment	1	2.5000	2.50000	0.26	0.6263
Error	8	78.0000	9.75000		
Total	9	80.5000			

Grand Mean 15.500 CV 20.15

Completely Randomized AOV for Mn

Source	DF	SS	MS	F	P
Treatment	1	0.00025	0.00025	0.19	0.6761
Error	8	0.01064	0.00133		
Total	9	0.01089			

Grand Mean 0.2490 CV 14.65

Completely Randomized AOV for Mo

Source	DF	SS	MS	F	P
Treatment	1	360.00	360.000	3.91	0.0834
Error	8	736.40	92.050		
Total	9	1096.40			

Grand Mean 43.400 CV 22.11

Completely Randomized AOV for Na

Source	DF	SS	MS	F	P
Treatment	1	0.10000	0.10000	22.4	0.0015
Error	8	0.03576	0.00447		
Total	9	0.13576			

Grand Mean 0.2580 CV 25.91

Completely Randomized AOV for P

Source	DF	SS	MS	F	P
Treatment	1	0.36100	0.36100	3.78	0.0878
Error	8	0.76400	0.09550		
Total	9	1.12500			

Grand Mean 3.6500 CV 8.47

Completely Randomized AOV for S

Source	DF	SS	MS	F	P
Treatment	1	2.9160	2.91600	2.19	0.1767
Error	8	10.6280	1.32850		
Total	9	13.5440			

Grand Mean 11.660 CV 9.89

Completely Randomized AOV for Tot carbon

Source	DF	SS	MS	F	P
Treatment	1	0.52900	0.52900	0.90	0.3714
Error	8	4.72000	0.59000		
Total	9	5.24900			

Grand Mean 35.610 CV 2.16

Completely Randomized AOV for Tot nitrogen

Source	DF	SS	MS	F	P
Treatment	1	0.65025	0.65025	49.0	0.0001
Error	8	0.10624	0.01328		
Total	9	0.75649			

Grand Mean 2.0710 CV 5.56

Appendix 7 Minerals in fruits

Completely Randomized AOV for B

Source	DF	SS	MS	F	P
Treatment	1	0.1000	0.10000	0.03	0.8651
Error	8	26.0000	3.25000		
Total	9	26.1000			

Grand Mean 25.700 CV 7.01

Completely Randomized AOV for C nitrogen ratio

Source	DF	SS	MS	F	P
Treatment	1	19.3210	19.3210	50.8	0.0001
Error	8	3.0400	0.3800		
Total	9	22.3610			

Grand Mean 14.770 CV 4.17

Completely Randomized AOV for Ca

Source	DF	SS	MS	F	P
Treatment	1	5.04100	5.04100	19.1	0.0024
Error	8	2.10800	0.26350		
Total	9	7.14900			

Grand Mean 7.1100 CV 7.22

Completely Randomized AOV for Fe

Source	DF	SS	MS	F	P
Treatment	1	0.00100	0.00100	1.00	0.3466
Error	8	0.00800	0.00100		
Total	9	0.00900			

Grand Mean 0.0100 CV 316.23

Completely Randomized AOV for K

Source	DF	SS	MS	F	P
Treatment	1	57.6000	57.6000	18.6	0.0026
Error	8	24.8000	3.1000		
Total	9	82.4000			

Grand Mean 35.600 CV 4.95

Completely Randomized AOV for Mg

Source	DF	SS	MS	F	P
Treatment	1	0.44100	0.44100	5.62	0.0452
Error	8	0.62800	0.07850		
Total	9	1.06900			

Grand Mean 4.2900 CV 6.53

Completely Randomized AOV for Mn

Source	DF	SS	MS	F	P
Treatment	1	9.000E-05	9.000E-05	6.00	0.0400
Error	8	1.200E-04	1.500E-05		
Total	9	2.100E-04			

Grand Mean 0.0370 CV 10.47

Completely Randomized AOV for Mo

Source	DF	SS	MS	F	P
Treatment	1	84.6810	84.6810	51.1	0.0001
Error	8	13.2480	1.6560		
Total	9	97.9290			

Grand Mean 14.090 CV 9.13

Completely Randomized AOV for Na

Source	DF	SS	MS	F	P
Treatment	1	0.00400	0.00400	1.60	0.2415
Error	8	0.02000	0.00250		
Total	9	0.02400			

Grand Mean 0.4600 CV 10.87

Completely Randomized AOV for P

Source	DF	SS	MS	F	P
Treatment	1	1.52100	1.52100	6.96	0.0298
Error	8	1.74800	0.21850		
Total	9	3.26900			

Grand Mean 7.6900 CV 6.08

Completely Randomized AOV for S

Source	DF	SS	MS	F	P
Treatment	1	0.01600	0.01600	0.22	0.6511
Error	8	0.58000	0.07250		
Total	9	0.59600			

Grand Mean 4.0200 CV 6.70

Completely Randomized AOV for Tot carbon

Source	DF	SS	MS	F	P
Treatment	1	0.28900	0.28900	9.97	0.0135
Error	8	0.23200	0.02900		
Total	9	0.52100			

Grand Mean 40.370 CV 0.42

Completely Randomized AOV for Tot nitrogen

Source	DF	SS	MS	F	P
Treatment	1	0.72900	0.72900	60.7	0.0001
Error	8	0.09600	0.01200		
Total	9	0.82500			

Grand Mean 2.7500 CV 3.98

Appendix 8

Analysis of Variance Table for CO2

Source	DF	SS	MS	F	P
replicati	1	0.02531	0.02531		
removal	1	0.63281	0.63281	2.69	0.1996
treatment	1	4.13281	4.13281	17.56	0.0248
removal*treatment	1	0.00781	0.00781	0.03	0.8670
Error	3	0.70594	0.23531		
Total	7	5.50469			

Grand Mean 3.8313 CV 12.66

Analysis of Variance Table for O2

Source	DF	SS	MS	F	P
replicati	1	0.24500	0.24500		
removal	1	4.80500	4.80500	31.68	0.0111
treatment	1	0.84500	0.84500	5.57	0.0994
removal*treatment	1	0.00500	0.00500	0.03	0.8675
Error	3	0.45500	0.15167		
Total	7	6.35500			

Grand Mean 14.925 CV 2.61

Appendix 9

Analysis of Variance Table for Wt

Source	DF	SS	MS	F	P
replicati	1	0.01050	0.01050		
Stage	2	2.10625	1.05313	301.84	0.0000
removal	1	0.04025	0.04025	11.54	0.0025
treatment	3	0.27559	0.09186	26.33	0.0000
Stage*removal	2	0.01913	0.00956	2.74	0.0855
Stage*treatment	6	0.05268	0.00878	2.52	0.0508
removal*treatment	3	0.00916	0.00305	0.87	0.4685
Stage*removal*treatment	6	0.02634	0.00439	1.26	0.3147
Error	23	0.08025	0.00349		
Total	47	2.62015			

Grand Mean 0.3773 CV 15.66

Appendix 10

Analysis of Variance Table for Diseased

Source	DF	SS	MS	F	P
replicati	1	1.721	1.7214		
Stage	2	106.728	53.3640	5.99	0.0080
removal	1	1.721	1.7214	0.19	0.6643
treatment	3	170.420	56.8068	6.38	0.0026
Stage*removal	2	24.100	12.0499	1.35	0.2783
Stage*treatment	6	402.812	67.1353	7.54	0.0001
removal*treatment	3	32.707	10.9023	1.22	0.3235
Stage*removal*treatment	6	44.757	7.4595	0.84	0.5537
Error	23	204.849	8.9065		
Total	47	989.816			

Grand Mean 1.3256 CV 225.13

Appendix 11

Analysis of Variance Table for Shrivelli

Source	DF	SS	MS	F	P
replicati	1	0.1302	0.13021		
Stage	2	5.3262	2.66312	20.59	0.0000
removal	1	0.0352	0.03521	0.27	0.6068
treatment	3	6.5506	2.18354	16.88	0.0000
Stage*removal	2	0.0329	0.01646	0.13	0.8811
Stage*treatment	6	3.3688	0.56146	4.34	0.0045
removal*treatment	3	0.1456	0.04854	0.38	0.7716
Stage*removal*treatment	6	0.2788	0.04646	0.36	0.8971
Error	23	2.9748	0.12934		
Total	47	18.8431			

Grand Mean 1.4688 CV 24.49

Appendix 12

Analysis of Variance Table for FRAP

Source	DF	SS	MS	F	P
replicati	1	38.007	38.007		
removal	1	187.827	187.827	22.99	0.0020
treatment	3	123.848	41.283	5.05	0.0358
removal*treatment	3	138.802	46.267	5.66	0.0275
Error	7	57.194	8.171		
Total	15	545.679			

Grand Mean 23.097 CV 12.38

Appendix 13

Analysis of Variance Table for phenols

Source	DF	SS	MS	F	P
replicati	1	3.2220	3.2220		
removal	1	6.4009	6.4009	6.32	0.0401
treatment	3	35.6851	11.8950	11.75	0.0040
removal*treatment	3	2.8890	0.9630	0.95	0.4663
Error	7	7.0870	1.0124		
Total	15	55.2840			

Grand Mean 13.750 CV 7.32