DEPARTMENT OF AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES

MASTER OF SCIENCE: FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

Influence Of Different Nitrogen Fertilization Rates On The Qualitative Performance Of Three Peach Cultivars

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I dedicate my thesis to my parents for their endless encouragement

Throughout my pursuit for education.

Abstract

Producing peach fruits (*Prunus persica L*) of high quality and with high nutritional value involves selecting the right cultivar and the optimum application of inputs during growing. This study evaluated the effects of using different concentrations of nitrogen fertilizer (60%, 80% and 100%) on the qualitative and nutritional performance of three different peach cultivars (Slapi, Romestar and Tardibelle) grown in the Marche region of Italy. It was found that peach cultivar strongly affected the qualitative parameters: for example, Slapi had the lowest significant average fruit firmness value (2.67 kg) as opposed to 4.99 kg in Romestar and 5.30 kg in Tardibelle. For the nutritional parameters, Romestar recorded the highest average values for both total antioxidant capacity (TAC) and total phenolic content (TPH), 8.38 mmol trolox eq/kg and 1216 mg GA/kg respectively, while Tardibelle recorded the lowest values (7.27 mmol trolox eq/kg and 1084 mg GA/kg, respectively). The qualitative parameters were less influenced by the nitrogen fertilization treatments. For the nutritional parameters, 80% treatment in Romestar cultivar recorded highest values in both fruit TAC and TPH (9.17 mmol Trolox eq/Kg fruit and 1268 mg GA/Kg fruit respectively), while 80% treatment of Tardibelle recorded the lowest fruit TAC and TPH (5.90 mmol Trolox eq/Kg fruit and 1025 mg GA/Kg fruit respectively). The findings derived from this study will help growers to select the most suitable combination of cultivar and fertilization treatment, to obtain the desired qualitative goals.

Keywords: *Prunus persica, peach cultivar, nitrogen fertilization, fruit quality, antioxidant capacity, total phenolic content*

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1. GENERAL INTRODUCTION

Output and quality of fruits are influenced by so many parameters. At the same time quality could be defined to suit a particular parameter more than the others. For this reason, the assessment of quality is most usually linked to the different uses. Generally, the qualitative parameters we tend to measure vary based on certain factors and methods used in growing the crop; cultivar, irrigation, crop load, canopy positioning, fertilization system, age, and condition of crops and farm practices (Gullo et al., 2014). To a primary consumer, a quality fruit is that which is safe to consume and offers certain health benefits. To the producer, quality entails input reduction with yet efficient crop production output. To this note, producing to meet quality standards requires alterations in fertilizer application and rates. Nitrogen is one of the most important determinants of plant growth and productivity (Othman and Leskovar, 2019). It can greatly affect tree growth, development, fruit production, and quality than any other element (Wang et al., 2007; Lu et al., 2009). Its application affect enzymatic stoichiometry and microbial resources in the soil as well as the absorption and allocation of other nutrients in the plant (Bilen and Turan, 2022; Yan et al., 2020; Yin et al., 2003). However, it is very often over- or under-applied for optimal fruit quality and this can affect the overall phytochemical composition of the produce. For efficient use of inputs and with increasing environmental concerns or the need for more ecologically friendly productions, it is therefore necessary to encourage utilization of natural inputs, and the optimization of inputs like Nitrogen fertilizers which might otherwise in excess cause nitrogen leaching in groundwater.

1.1. Background of Peach Plant

The peach (*Prunus persica*) plant belongs to the family of plant *Rosaceae* (Lu et al., 2003). This family is well represented with immense scientific and economic value. The peach family is large and also includes some large genera like *Prunus* (peach), *Malus* (apple), plum etc (Esmaeili et al., 2010). Plants of the *Rosaceae* family are widely grown

for their fragrance and beauty (Hafiz et al., 2015), and also well known for their tasty and fleshy fruit. These plants are widely cultivated. During the 16th century, the peach fruit was exported to America by Spain (Laura 2014).

Table 1: Peach classification. Source: Ravi et al., 2018

Kingdom	Plantae
Sub kingdom	Tracheobionta
Class	Megnoliopsida
Subclass	Rosidae
Order	Rosales
Family	Rosaceae
Subfamily	Amygyloideae (*Prunoideae*.)
Genus	*Prunus*
Species	*Prunus Persica*

The Mediterranean basins were found to provide suitable environmental conditions for peach trees. Earlier on, this fruit diffused into the North of the Roman Empire. Bakels and Jacomet in a review on luxury fruits introduced into Central Europe by Romans showed that peach was found in five Central European sites before 50 AD and between 50 and 100 AD in fifteen sites (Bakels and Jacomet 2003). Peach stone fruits were found in small archaeological channels dated (15-40 AD) in Modena, a rich colony in North Italy far from the capital Rome. This archaeological evidence lead to the suggestion of the following hypothesis; 'the introduction of peach in Italy can be dated to the Roman Republican age'. Emilia Romagna was one of the first three regions in Italy for the cultivation and production of peaches and has also obtained the Protected Geographical Indication (PGI) for peaches in a large area of Romagna (Della Casa 2008).

1.2. Cultivation

Peach fruits are one of the most popularly consumed fruits in the world due to their nutritional and economic value (Xiaoyong et al., 2015). Peach fruit is an important fruit crop and it is grown primarily in temperate zones at latitudes between 30 and 45 N and S. Its cultivation at higher latitudes is limited due to it hardy flower bud (about -23 to – 26 o C). China, USA, Italy, Spain and Turkey are the world most producing countries of *Prunus* and within this group, peaches/nectarines and plums are the most popular fruits with an annual production of about 17.5 and 9.7 million tons as of 2007 (Ariel et al., 2011). China is the world's largest producer of peach with about 15 million metric tons (Figure 1)

About 200 edible species of *Prunus* exist and are cultivated for fruit and seed. Just 20 are mostly cultivated in Europe, West Asia, Himalayas and India (Sumaira Aziz et al., 2012). Peaches were first introduced in China during the reign of King Kanishka by hostages of Chinese in the 1ST century AD and then spread throughout Europe by the Romans and the Greeks. Nectarines are also believed to have originated from Europe and then introduced to China (Ariel et al., 2011; Janick 2003).

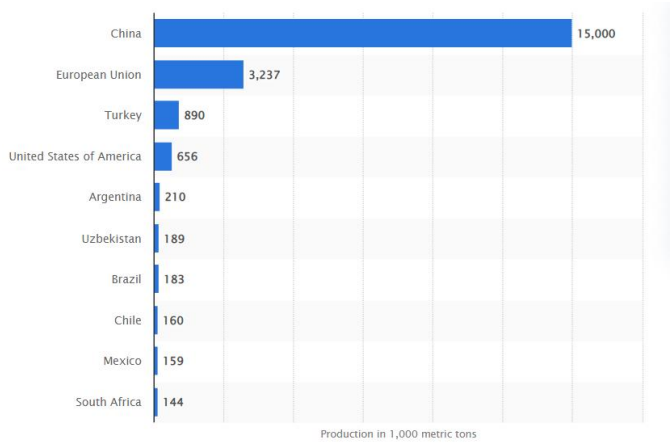

Figure 1: https://www.statista.com/statistics/739329/global-top-peaches-and-nectarines-producing-countries/

1.3. Botanical characterization

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Figure 2: A peach fruit

The peach tree is an evergreen deciduous tree up to about 10m height. It has a grayish bark or acuminate ashy glabrous. Commonly, it is known as "*Aaru*" and popularly called peach in English (Rakesh et al., 2011). It has pinkish-white sessile, pedicelled and short flowers, green coloured leaves which are useful as astringent, diuretic and laxative (Rakesh et al., 2011).

The fruit has a unique shape and flesh color varies from yellow to red (Kim et al., 2009), and is less hardy when compared to pome fruits; it is soft at maturity and can be eaten fresh due to its short shelf-life. The shell is surrounded by a mesocarp (pit or stone) of a hardened endocarp with a seed inside. As a result of this, *Prunus* plants are also referred to as 'stone fruits'. Unlike in other fruits like almond where the edible portion consumed is the seed, in stone fruits, the edible portion consumed is the mesocarp and endocarp (Susan et al., 2005). Fruits are grouped based on the color of the flesh (yellow and white), stone adhesion to the flesh (freestone, semi-cling stone and cling stone), melting nature of flesh (melting and non-melting), and chilling requirement (EJ et al., 2001). Melting peaches are known for their juicy flesh, soft texture, good flavor, and sweet taste which makes them quite competitive in the fresh fruit market (Xiaoyon et al., 2015). Peaches predominant for fresh market are freestone peaches while those for processing are cling stone peaches. As the word 'cling'; the fruit clings to the flesh making it

difficult to separate the peach hence it is suitable for mechanical processing. The freestone cultivar separates easily from the peach. They are also consumed when dried similar to plums and apricots (V. Poonam et al., 2011).

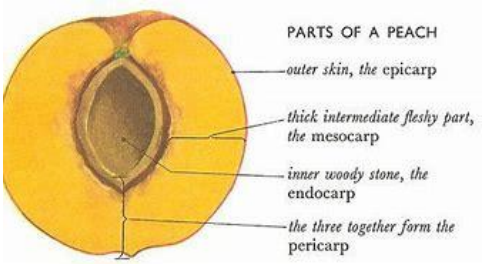

Figure 3: Labeled Transverse section of a peach

1.4. Market demand and trend for peach

The global fresh peaches market is projected to grow at an annual growth rate of 3.2% during the forecast period (2021-2026). According to the FAO, fresh peaches production is recorded at 23.9 million metric tons in 2016 and is increased to 25.7 million metric tons by 2019. As per the USDA, the per capita consumption of fresh peaches in the United States is declined from 2.73 pounds in 2016 to 2.13 pounds in 2019, which slightly affects the growth of the market.

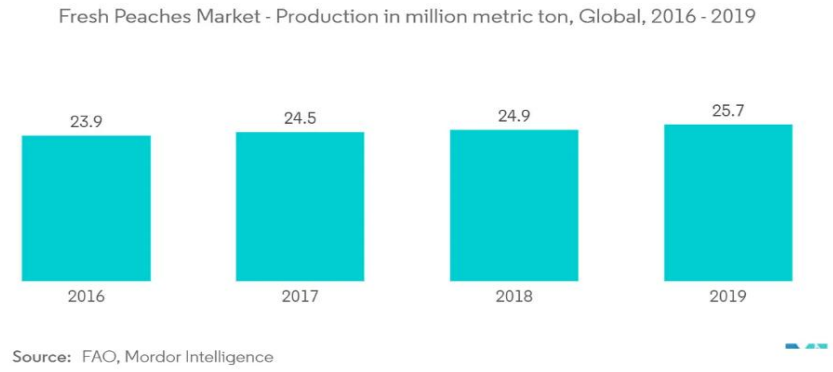

Figure 4: World annual peach production

Peaches were looked at as considered unhealthy fruits during the middle ages. Similar to other fruits, peaches were recommended to be taken at the beginning of meals since they

were light fruits and served as appetizers due to their sweet and tasty fragrance (Flandrin, 2003a). In the 16-17 century AD, peaches began to be more appreciated by consumers and served at the end of dinner (Flandrin, 2003b). Recently, increasing health consciousness is the main drive in trends in the Peach market. In the past few years, an increasing number of consumers, who lead a wellness-oriented lifestyle, are concerned with nutrition, fitness, stress, and the environment. The increasing health consciousness among consumers is fueling the peaches market, as peaches have many health benefits, which are related to the nutrients within the fruit, such as high levels of dietary fibers, low carbohydrate, phenolic compounds, vitamins, (vitamin C, vitamin A, vitamin E, and niacin), as well as minerals.

Owing to the demand for fresh peach in the market, the producers are focusing on the increase of fresh peach production which in turn drives the growth of the market. For instance, fresh peach production in Europe recorded 3.9 million metric tons in 2016 and increased to 4.2 million metric tons in 2019 (Mordor intelligence peach report, 2022)

Unfortunately, peach consumption is decreasing worldwide due to poor fruit nutritional and sensorial qualities which do not meet consumer expectations. This is largely due to not harvesting at optimal maturity or harvesting and allowing the fruits to ripen at home which leads to textural detriments that are a result of post-harvest disorders due to poor handling and storage practices (Kebede and Habtam Setu 2022). Consumers need to be educated on ripening and “ready to eat” fruits and on suitable storage conditions to be employed.

A great majority of peaches produced are consumed in the form of processed product (55%), while the remainder (45%) is consumed fresh (Boris Hayley and Henrich Brunke 2006). A great quantity of *Prunus* fruits harvested are processed to various food items like jam, canned, dried or roasted and consumed all year round. They are also processed into juices and or sliced and dried (Kim et al., 2009). Canned peaches account for 75% utilization, frozen peaches 17%, of processed peaches and dried peaches just 1.5% others are used for pickling, wine, baby food, and brandy account for 6%. (Economic

Research Service 2004). Peaches were pickled in vinegar in Roman times as a means of preservation to increase shelf-life (Laura 2014).

1.5. Quality of Peach fruit

Fruit quality is a property that depends mainly on the cultivar of fruit, but also on environmental conditions, packaging, and transportation to the consumer (Layne, 2007). Fruit quality can be evaluated through parameters such as soluble solids concentration, titratable acidity, flesh firmness, and flesh and skin color (Infante et al., 2008). At room temperature, peach fruit is perishable and deteriorates; cold storage or refrigerated storage are effective ways to prolong storage life (Aubert et al., 2014). Control atmosphere treatments have been widely used to extend shelf life and alleviate the chilling injury of fruits (Bodbodak et al., 2016). Effectively, metabolic activities are inhibited by low-temperature storage which widely occurs during postharvest storage and cold-chain transportation of horticultural crops. More so, the peach fruit is sensitive to cold storage, especially at mid-temperature (2.2–7.6 ℃), (Lurie et al., 2005; Xi et al., 2011). Peach chilling injury, including flesh browning, defect of ethylene synthesis, and loss of fruit scent and flavor greatly reduces the quality of this fruit (Lurie et al., 2005; Zhang et al., 2016; Akbudak et al., 2016). However, the effects of this controlled atmospheric environment on the flavor quality of peach and the related key metabolites are not clear.

Controlled atmosphere storage has been performed by mediating the atmospheric pressure of the storage micro environment, mainly including low oxygen and high carbon dioxide pressure to improve the shelf life of peaches while maintaining quality (Cano-Salazar et al., 2012; Mditshwa et al., 2018; Wright et al., 2015 Hongru et al., 2022). Different pressures of oxygen and carbon dioxide can introduce different physiological metabolisms such as anaerobic respiration, off-flavor, superficial scald incidence, and fruit softening (Wood et al., 2021; Ali et al., 2021). Control atmospheric environmental treatments have been developed to prolong the storage life of many

horticultural crops, including modified atmosphere with varying air permeability packages, dynamic controlled atmosphere storage, ultra-low oxygen treatments, and initial low oxygen stress atmosphere (Thewes et al., 2020; Wood et al., 2021; Ali et al., 2021; Anastasiadi et al., 2022;).

1.5.1. Sensorial Quality

The sensory quality of peach fruit is dependent on characteristics such as adequate flesh firmness of fruit, high sugar content, low acidity levels, and adequate fruit shape and color. The true sensory qualities of peach develop on delay in harvesting to attend full maturity (Mathias et al., 2008). This demand for high peach quality has been influenced by the health status of consumers (Agrianual, 2007). Peach qualities such as flavor, aroma, flesh firmness, and appearance, which include shape, size, and flesh color are key sensory qualities consumers demand from farmers (Francine et al., 2012).

The aroma and flavor of peaches are influenced by the amount of low vapor-pressure compounds that do not scent or smell like a peach flavor when tasted individually. However, only by identifying the right cultivar for the specific climatic condition and cultivation system, it is possible to reach the highest quality results for the market and the consumer, by expressing even the best sensorial and nutritional characteristics, associated with sweetness, acidity, sugar: acid ratio, and total phenolic content/ compounds in addition to textural characteristics.

The texture of fresh peaches is considered to be an important quality as flavor and aroma for consumer preference. Fruit maturity, environmental and cultural factors/practices, the chemical composition of the cultivar, and post-harvest handling methods of the fruits significantly influence the texture of fresh peaches (Nuzzi et al., 2015).

Peach pulp color ranges from greenish-white, cream-white, and cream red, while the peel at ripe can be orange-red, pink-red, medium red, dark red, and blackish-red in order of increased ripeness (Giovannini et al., 2013).

At maturity, the peach fruit undergoes a number of changes that may not necessarily be related to each other. During the last 3 to 4 weeks before the harvest period, the fruit grows rapidly, and the flesh softens and changes from green to yellow, and then finally to red; the sugars contained in the fruit increase, and the acid levels decline because of increasing respiration and conversion to sugars, with the fruit becoming more aromatic and flavor-able. The Ground color is usually used as an indicator of fruit maturity and firmness. Color should not be used as an index of firmness because fruit with similar ground color, but from trees with varying soil nitrogen status, had different flesh firmness.

Fruit size is highly valued at commercial levels in peach. Competition among fruit and among other sinks on a tree reduces the potential growth rate of the fruit. Hence, crop-load management strategies such as the removal of flowers or fruit (thinning) are often practiced by growers to optimize fruit size.

1.5.2. Nutritional Quality

Fruits are regarded as valuable food commodities with potential health benefits; They contain carbohydrates, antioxidants, minerals, and dietary fiber which contribute to the nutritional quality of the fruits (Wudineh et al., 2018). The natural antioxidant components, can contribute to decreasing the incidence of diseases such as cardiovascular diseases (Getaneh Seleshi, Kebede, 2019a; Gorinstein et al., 2004). Also, the presence of carotenoids and polyphenols e.g phenolics, flavonoids, anthocyanins, and phenylpropanoids help against the scavenging activities of free radicals (Gil et al., 2002). The major minerals are Pottasium, Phosphorus, Magnesium and Calcium. They are low in calories with approximately 30 calories per serving, yet add abundant flavor to a wide variety of foods.

The nutritional qualities of peach fruits are influenced by genotype and ripening stage, and by environmental conditions and orchard management practices. The redness of the fruit flesh selection indicates the highest levels of phenolic compounds (in

mesocarp/exocarp) and ascorbic acid. Total phenolic concentration was approximately three-fold higher in the exocarp than the mesocarp across all accessions of peaches in a study conducted by Sara et al. (2020); breeding selections generally reported higher levels of phenolic compounds than commercial cultivars (Sara et al., 2020).

Contents of organic acids, carbohydrates, and phenolic compounds in peach are not distributed evenly within different parts of fruits, but they are concentrated mostly in the epidermal and sub-epidermal layers of fruit (Kurz et al., 2012). The nutritional value of peaches per 100g serving can be seen on Table 2 below.

Table 2: Nutritional value of raw peaches per 100g. Source: (Gil et al., 2002, USDA, 2020)

Nutrient	**levels**	**Vitamins**	**levels**	**Minerals**	**Levels**
Energy	*165 KJ (39kcal)*	Vitamin A	*16 µg*	Calcium	*6 mg*
		Beta- carotene	*162 µg*		
Carbohydrates	*9.54 g*	Thiamine B1	*0.024 mg*	Iron	*0.25 mg*
Sugars	*8.39 g*	Riboflavin B2	*0.031 mg*	Magnesium	*9 mg*
Dietary fiber	*1.5 g*	Niacin B3	*0.806 mg*	Manganese	*0.061 mg*
Fat	*0.25 g*	Pantothenic acid B5	*0.153 mg*	Phosphorus	*20 mg*
Protein	*0.91 g*	Vitamin B6	*0.025 mg*	Potassium	*190 mg*
Water	*89 g*	Folate B9	*4 µg*	Sodium	*0 mg*
		Vitamin C	*6.6 mg*	Zinc	*0.17 mg*
		Vitamin E	*0.73 mg*		
		Vitamin K	*2.6 µg*		

1.6. Factors Affecting Peach Quality

Fresh and wholesome peaches are one of the most desirable fruits by consumers when allowed to ripen on the tree and harvested just prior to consumption. Producing peaches that have excellent and desirable color, flavor, and texture is a highly scientific process that requires the control of many environmental practices from the planting of the tree to harvesting and handling of the fruit after harvest. Introducing lower-quality peaches into the market mostly come about as a result of handling errors in harvesting/post-harvest handling or they could be due to members of the value chain trying to increase profits or lower shrinkage by cheating on the quality of the fruit (Winfree and McCluskey 2005).

As peach producers attempt to maintain market share and expand peach consumption, weaknesses have been noted. They have been stagnating to declining demand in certain countries, competition from other countries has increased due to the quality of peaches produced (Integrity Intellectual Property, Inc. 2009). There is extensive literature associated with supplying optimum quality peaches to consumers (Crisosto, 1994; Benjamin et al., 2014). Regardless of the reasons how and why inferior quality peaches end up on retail shelf, the consumer forms opinions or have different perceptions about the product and subsequently value chain members, notably the producer and production site to the retailer. Therefore consumers can only evaluate peach quality after the purchase has been made, the pre-purchase peach quality decisions can only be assessed in terms of the probability of being good or bad according to appearance. If the consumer experiences the good qualities of peaches, they will have a higher propensity to repurchase (Benjamin et al., 2014).

1.6.1. Factors related to the crop

1.6.1.1. Rootstock

Fruit composition can be affected by several factors including the rootstock (Forcada et al., 2013; Barreto et al., 2017). Barreto et al., (2017) observed that the quality of peaches throughout harvest is affected by the rootstock used in the orchard. Rootstocks may

affect the quality of fruits since they may interfere with water and nutrient absorption from the soil; this can then affect the vigor of the plants and the preservation of fruits (Martinez-Ballesta et al., 2010). Studies on orange (Hifny et al., 2012) storage have already shown that rootstocks can modify the physicochemical characteristics of the orange fruits. This fact has not been adequately investigated in peaches.

1.6.1.2. Genetics

Some peach cultivars develop more red color than others. Although the red color can be maximized for any cultivar by delaying harvest for a few days, the potential for red color differs for each cultivar. Poor coloring cultivars like the 'Loring' cultivar do not possess the genetic potential to develop the same level of red coloration as 'Redhaven'. The fruit colour is becoming more important characteristic for fruit breeders; so better coloring cultivars are often favored by consumers due to their attractiveness (Francine et al., 2012)

1.6.1.3. Climate

The Physicochemical characteristics of peaches can be influenced by the climatic conditions of the production area, altitude, temperature, rainfall, and relative humidity. Harsh climatic conditions will lead to the production of poor quality peaches with smaller fruit sizes, low degree of firmness, sometimes 'sun ripe', and/or easily affected by insect infestation, low total solute content and lesser nutritional content. Also, environmental factors like seasonal changes, sunlight, temperature, and humidity can influence tree growth, fruit load, and fruit development (Enrique et al., 2018).

1.6.1.4. Soil

Several studies found that the soil mineral element content is of significant importance to fruit quality. A multivariate analysis of soil nutrients and fruit quality of kiwi in an orchard revealed that the soluble sugar of kiwi fruit was mainly affected by the available soil potassium level and available sulfur in the soil and the titratable acid was mainly affected by the organic matter (Chen et al., 2021). In general, high nitrogen levels in

plants retard the development of red color in the fruit skin. Part of this can be attributed to increased levels of chlorophyll in the fruit skin and also due to increased shoot growth that shades the tree interior. Therefore, it is advisable judiciously apply nitrogen to maintain adequate shoot vigor for future cropping, without excess shade (Hailong et al., 2022). The nitrogen content of orchard soil has been found to directly affect the fruit quality and yield of peaches (Zhu et al., 2019). Also, there is a correlation between soil organic content, available potassium content, and peach fruit weight (Wang, Zhao, et al., 2021; Wang, Liu, et al., 2021).

1.6.1.5. Stress Factors

In general, any type of stress (environmental, physiological, climatic, mechanical stress, etc) on the tree, which reduces photosynthesis, has a negative effect on red color development of the fruit and also the fruit size. Therefore, it is important to avoid the most common stress such as drought stress, and to protect leaves from insects and diseases infestation that damage leaves and reduce whole-tree photosynthesis (Toralles et al., 2004; Lurie and Crisosto 2005).

1.6.1.6. Light accessibility and availability

Light is important for fruit production in plants because crucial phases of the tree, as fruit growth and flower bud development, require carbohydrates that are produced by photosynthesis using ultraviolet rays from the sun in the leaves. Sunlight, therefore, provides the needed energy for photosynthesis by the plant. At least 30% full sunlight is needed for the final 6 weeks before harvest for maximum fruit size development. During the last 3 weeks to harvest, the surface colored red is not greatly affected by shade. Good peach red color development requires at least 23% full sun during the 6 weeks of maturity before harvest. Peach fruits with lower sugar content have lesser demand compared to peach fruits with higher sugar content. The total soluble solids concentration is the peach fruit quality characteristic mostly easily altered by stress. Development of high soluble solids requires more than 45% full sun during the last three

weeks before harvest and proper handling during flower blooming to maturity (Winfree and McCluskey 2005).

Fruit weight and soluble solids concentration are positively related to the amount of light intercepted by the fruit, but fruit firmness is negatively related to the amount of light intercepted by the fruit. This indicates that high light can advance fruit maturity. Also, the hue angle on the blush and the non-blush sides of the fruit is negatively related to the amount of light interception. The blush side of the fruit in high light will retain dark red color and the shaded fruits are reddish orange. The non-blush side of the fruit in high light will retain orange to reddish-orange coloration and the shaded fruit yellow to yellowish orange coloration (Nuzzi et al., 2015). Thinning is a cultural practice which greatly influence the amount of light which reaches the fruits. Thinning can be performed when the peach plant blooms or during early fruit development, and greatly affects the fruit size and sensory properties, which can lead to different market prices (Mary et al., 2020). The flesh firmness of peach fruit can also be influenced by thinning, as firmness is influenced by shade. Fruit receiving less than 45% full sun during the last 3 weeks before harvest will become softer than non-shaded fruit, and will differ in color from fruits that received full sun lights. This greatly reduces the sensorial attributes of the fruit as consumers prefer fruit with firmness.

1.6.2. Factors related to storage

1.6.2.1. Pulp browning

One of the factors that affect peach quality is pulp browning. Pulp browning cannot be observed on the external surface of the fruit, but when the fruit is cut for consumption. Cantillano et al., *(*2008) stated that, the more susceptible the fruits are to internal pulp browning, the shorter the storage life of the food. Pulp browning is normal in peaches after storage; compounds such as phenolic compounds may be related to this disorder (Lurie and Crisosto 2005). Therefore, not only classic technical losses but also variations in the nutritional contents for the compounds whose metabolism is specialized should be

evaluated. This is true in the case of peaches with yellow pulp; they have high antioxidant content (Santos et al., 2013).

1.6.2.2. Internal breakdown or chilling injury:

The major physiological cause of deterioration in peaches is a low-temperature or chilling injury problem during storage, generically called internal breakdown (IB). This disorder can manifest itself on the fruit as dry, mealy, or hard textured fruit, woolly, flesh or pit cavity browning, or flesh translucency from the flesh. A more concentrated red color development of the flesh which is usually radiating from the pit may be associated with this problem in some peach cultivars. In all of these cases, off-flavor or loss of flavor is the symptom that is evident. However, there is large variability in the internal breakdown vulnerability among peach cultivars (Carlos et al., 2000; Hongru et al., 2022).

Most of the mid-season and late-peach cultivated are most vulnerable to chilling injury. Chilling injury symptoms may develop faster and more intensely when fruit are stored at temperatures between about 2.2 ˚C and 7.6 ˚C than those stored at from 0 ˚C and below. In a study by Caroline et al., (2018) it was revealed that enzymatic peach browning of fruits was observed 21 days after storage in a cold chamber and a 3-days on the shelf, combined with a mass loss in peach fruits throughout the storage period. When the mass loss exceeds 10%, fruits show symptoms related to wrinkling (Crisosto et al., 2004), and the mass loss exceeded 10%, regardless of the rootstock at the end of the study. These results agree and were similar to an earlier report of Andrade et al. (2015), who observed a 33% mass loss in Maciel fruits 30 days after cold storage, followed by a 2-day commercialization shelf simulation. The peaches rot progressively and steadily throughout the storage period.

In general, peaches are harvested when they are well-mature and will ripen properly without exogenous ethylene application. However, ethylene application to fruit

harvested will ripen the fruit more uniformly without speeding up the rate of ripening (Gorny et al., 1998; Guoxiang et al., 2020).

1.6.2.3. Gray Mold:

This disease is caused by *Botrytis cinerea*. Gray mold can be a serious problem during wet spring weather which quickly grows on plants. It can occur during storage if gray mold contaminated the fruit during harvesting and handling wounds. It is recommended to avoid mechanical injuries and maintain good temperature management as effective control measures (Gorny et al., 1998; Carlos et al., 2000; Santana et al., 2011). The peach trees are also prone to diseases that do not directly affect the fruit most often, but can reduce the crop yield by partially defoliating the tree. However, these diseases can be controlled by the use of several fungicides (Santana et al., 2011)

Figure 5: *Botrytis cinerea* infected peach

1.6.2.4. Brown rot:

They are caused by *Monilinia fructicola*. Brown rot is the most important post-harvest disease of peaches. Brown rot infection begins during plant flowering and fruit rot may occur before harvest, but often occurs and account for post-harvest. Orchard sanitation is recommended to minimize infection sources, and pre-harvest fungicide application, and prompt cooling after harvest are also recommended. Also, post-harvest fungicide

treatment may be used to minimize brown rot (Crisosto et al., 1995; Carlos et al., 2000; Santana et al., 2011; Pinto et al., 2012).

1.6.2.5. Inking (black staining):

This is a cosmetic problem that greatly affects peach market acceptability by affecting only the skin of peaches. This inking disorder is characterized by black and/or brown spots or stripes on the peach. These symptoms usually appear generally 24-48 hours after harvest. Inking occurs as a result of mechanical damage which is mostly abrasion in combination with heavy metal (iron, copper, and aluminum) contamination. Black staining usually occurs during the harvesting and hauling operations, although it may occur in other steps during post-harvest handling of the fruits. Therefore, gentle fruit handling after harvest, short hauling, avoiding any foliar nutrient sprays within 15 days of the fruit harvest, and following the suggested fungicide spray before harvest interval guidelines are recommendations to reduce the inking of peaches (Carlos et al., 2000).

2. AIM OF STUDY

This work is a study carried out by the department of Agriculture, Food and Environmental Science of the Universita politecnica delle Marche. The study was performed using three prominent peach cultivars grown in the Marche region (Slapi, Romestar and Tardibelle).

The high number of new cultivars on the market makes their technical management and their quality performance identification difficult for both farmers and consumers. Compared to other fruits like strawberry and apples, limited studies and analyses have been performed on fruit quality (flesh firmness, soluble solids content, titratable acidity) and nutritional contents (antioxidant capacity and total phenolic content) for peach commercial cultivars.

Therefore, this work aims at determining quality (firmness, Titratable acidity and Soluble solid content) and nutritional variations (Total antioxidant capacity and total phenolic content) between peaches of three different cultivars grown with varied levels of nitrogen fertilization. In addition, the interrelationship among the parameters being studied would be evaluated by principal component analysis (PCA).

The results gotten would help indicate the peach cultivar which performed best in the quality or nutritional parameter considered and the nitrogen treatment used. This would be helpful especially for growers to identify which cultivar and level of input to use to obtain fruits with specific qualitative and nutritional properties as demanded by consumers and by so doing, the growers would save on over or under exploitation of inputs.

3. MATERIALS AND METHODS

3.1. Plant Material and field Trial

The site for the field trial was conducted on 'Fratelli Boni' farm, in Colli al Metauro, PU, Italy - 43°44'25.6"N 12°54'44.6"E. It is a flat land area characterized by medium soil texture. For this study, were grown three peach cultivars, namely;

- Slapi (medium-early ripening cultivar)
- Romestar (medium ripening cultivar) and
- Tardibelle (late ripening cultivar)

The cultivars were all planted in 2008 with a plant density of 4 by 3 and a total of 833 plants per hectare.

The rootstock used was GF677, with a free pot training system. The irrigation system consisted of polyethylene pipes placed along farm rows 60 cm off the ground surface and from which were positioned 3m static sprinkler systems flowing at 40 l/hour, covering an area equivalent to an irrigation diameter of 3 meters. The injection of fertilizers (Fertigation) was ensured by 3 Dosatron® D20s with the injection of the stock solution at 0.3, 0.37, and 0.5% in the different treatments. In March, organic mineral fertilization (Belfrutto MB 5-10-15, SCAM, Italia) was done, followed by fertigation with calcium nitrate (YaraLiva Calcinit 15, 5-0-0). For each of the 3 cultivars, the three fertilization levels tested were referred to as 60%, 80%, and 100%. The amount of nitrogen in each level was as follows: 40, 50, and 60 $kg \cdot ha^{-1}$ N.

Nine plants were considered (3 for each cultivar) for each nitrogen treatment, with each corresponding to a plot. The experimental design was arranged as a split-plot, randomized complete block design with three replicates. The fruits were harvested 2 to 3 times during the harvesting periods. 30 fruits each were harvested, 24 of which were preserved and sampled for acidity, sugar content, and firmness. The other 6 were

preserved, methanolic extraction done and subsequent qualitative analysis of the anti-oxidative and total phenolic content was done.

Table 3: Peach cultivars and their harvest periods

Cultivar	**Harvest dates**
Slapi	27/07/2021 30/07/2021
Romestar	12/08/2021 19/08/2021 26/08/2021
Tardibelle	16/09/2021 23/09/2021

3.2. Measuring Qualitative Parameters (Firmness, Soluble solids and Titratable acidity)

3.2.1. Fruit Firmness

Harvesting time was established following a maturation index and based on the flesh firmness, which was measured using a manual penetrometer with an 8-mm diameter tip. The penetrometer measures the resistance of the fruit to the force applied by the penetrometer. The fruits sampled at each harvest were perforated, after the removal of the peel, in 2 diametrically opposed points. Data were expressed in kilograms (kg).

3.2.2. Soluble Solids Content (SSC)

Fruit Soluble Solids Content was measured with a digital temperature compensation refractometer N-1E (Atago, Tokyo, Japan). At each harvest, the juice was extracted and centrifuged (BOSCH, Stuttgart, Germany). From this juice, one or two drops were

dropped on the refractometer prism for reading. The measurement was expressed in °Brix.

3.2.3. Titratable Acidity

Fruit Titratable Acidity was determined from 10 ml of the same juice extracted for the Soluble Solids Content analysis, diluted with distilled water, and titrated with 0.1N NaOH solution, until pH 8.2, and expressed as % of Malic Acid Equivalents (% MAE).

3.3. Measuring Nutritional parameters (TAC and TPH)

The nutritional parameters were investigated using extracts from the fruits. These extracts were gotten in a two-step extraction procedure and then subsequently stored in the refrigerator. The process was performed with keenness to limit light oxidation as much as possible.

3.3.1. Materials used

- The peach cultivars (Slapi, Romestar and Tardibelle)
- Neoprene gloves, Isothermal container
- Methanol and Acetic acid
- Fume cupboard, MilliQ water
- Methanol glass bottle (2.5L volume) with dispenser
- 250mL glass flasks
- Pipettes, Scotch (to label flasks), plastic film, Aluminium foil, Cutting board, Knives
- 50mL falcon (2 per 250mL glass flask)
- Ultra-turrax homogenizer (Janke & Kunkel, IKA-Labortechnik)

- Centrifuge (run at 4000rpm for 10min)
- Amber glass vials in polystyrene container

3.3.2. Extraction Process

The first of the two-phase extraction consisted of crushing the peach samples and extracting the limpid, the second was a purification phase by using a centrifuge. The procedures are detailed below

Samples were collected from the fridge in the order in which they were harvested and preserved. Codes were assigned to them with those from the first harvest/cultivar being Slapi of 27/07/2021. Slapi-1 to Slapi-9 was coded D-4-1 to D-4-9, the next set was Romestar of 12/08/21 with Romestar-1 continuing with the code D-4-10.

Figure 6: Stored cultivar sample and its label

3.3.2.1. First extraction Process (Solid-Liquid extraction)

- Prepare 250mL conical glass flasks, as many as the number of samples to be prepared for the day, labeling them with their sample code on a scotch tape and covering them with Al foil to control light entry and impromptu oxidation;
- In a 1L cylinder, make a solution of 80:20:1 (800ml methanol, 200ml MilliQ water and 10ml of acetic acid)

- Prepare a glass bottle with the dispenser: unscrew the dispenser and pour the contents of the cylinder into the glass bottle. Screw the dispenser back onto the glass bottle;
- Set the dispenser to the maximum (50mL) and press the dispenser piston, pouring the 50mL of 80:20:1 solution inside the exuberant falcon. Screw back the falcon and store it under the hood;
- Prepare a station with a cutting board, scale, pliers, and knife;
- Take the bag with the peaches of the "first sample" from the freezer, transport them to the station in an isothermal container;
- Take out 3 to 4 fruits from each bag and cut 3 random cloves from each, then continuously cut to reduce the size and mix them for representative samples, then put and weigh 10g in a conical flask wrapped in an Aluminium foil and placed on an electronic balance with 0.1g margin of error;
- Register in the notebook the date of collection and the parcel number (next to the identification code);
- Carry to the fume cupboard and add 100mL (50mL+50mL falcon) of 80:20:1 solution to the flask. Close the flask with a plastic film;
- When 4 flasks accumulate under the hood, homogenize the contents of each flask using the Ultra-Turrax homogenizer;

Figure 7: Samples stored in the refrigerator

- After homogenizing, store the flasks in the refrigerator (4°C) for 48h, for the second part of the extraction;
- Perform the same process for all other flasks.

3.3.2.2. Second Extraction Process

- Set the centrifuge to 4000rpm for 10 minutes, 4°C;
- Prepare twice as many falcons (test tubes) as we have flasks (falcons wrapped with aluminum foil). The contents of each conical flask will be poured into test tubes for centrifuge;
- Take out the first 4 flasks from the refrigerator (in order of identification code) and bring them under the fume cupboard. Shake each and pour contents into the labeled test tubes;
- Place the 8 test tubes inside the centrifuge supports, balancing the weights on each opposite arm of the centrifuge (maximum 1g difference between one arm and another);

- After the centrifuge, cover the test tubes with Al foil and take to the hood cupboard where we also have polystyrene container with labeled vials. We would use 6 vials per sample;

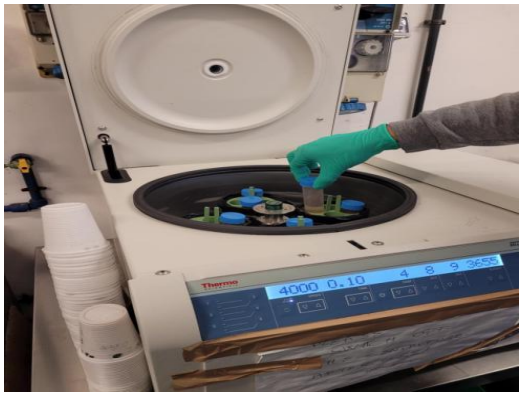

Figure 8: Placing of samples in the centrifuge

- With a Pasteur pipette, pour about 2ml of the contents of the first test tube of sample 1 into each of 6 vials, then 2ml from the second test tube to make about 4ml per vial. Make sure the pipette does not go to the bottom of the test tube where there is solid matter. Do the same for the other 3 samples. A total of 24 vials, for the first 4 samples;
- Store the polystyrene containing the filled vials in the freezer;
- Do the same for the remaining flasks.

3.3.3. Determination of Peach Total Antioxidant Capacity (TAC)

Assessed by the Trolox Equivalent Antioxidant Capacity (TEAC) method.

The reaction is based on the ability of substances contained in the fruit, extracted by the previously described method, to quench a radical solution. The pre-formed radical solution possesses a blue/green coloration; it is generated through the radicalization of ABTS-+ by potassium persulfate (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid). The radical cation possesses the maximum absorbance at 734 nm. The radical is quenched in the presence of hydrogen ion donor antioxidants causing discoloration of

the solution. This decolorization is determined as a function of concentration and calculated as a function of Trolox (external standard with increasing concentration) reactivity by linear regression (Miller et al., 1993; King et al., 1999).The standard scale is obtained by reacting ABTS and sodium persulfate solution with Trolox (a water-soluble Vitamin E analog) at increasing concentrations for a total volume of 10 ml.

The samples are diluted 1:20 with phosphate buffer. The sample thus diluted is reacted 1:20 with the radical solution and kept in the dark for six minutes. After six minutes, the solution is read by the spectrophotometer. The higher the antioxidant capacity, the more the color will tend toward white. Calculation of the percentage of radical inhibition:

$$\%inhibition = \frac{Abs_{blank} - Abs_{sample/s\tan dard}}{Abs_{blank}} \times 100\%$$

The calibration curve allows us to use linear regression to evaluate the TAC of the extracts (Δ A = ac + b, c = Trolox concentration mmol/l, Δ A = % inhibition, a = % slope, b = % intercept).

$$TEAC - Value\ (mg\ Trolox\ eq/kg\ Fruit) = \frac{(\Delta A - b) \times F}{a \times E}$$

Δ A = % inhibition

a = slope

b = intercept

F = dilution factor (20)

E = sample weight [kg/L extracting agent]

TEAC value is expressed as [mmol Trolox equivalents/kg].

3.3.4. Determination of Phenolic content using Folin ciocalteau reagent

The Total phenolic assay not only determines the content of phenol compounds but also other reducing agents like ascorbic acid because the basic mechanism is a redox reaction. The chemical composition of the Folin reagent is heteroposphospho-tunstane molybdate. Molybdenum is easily reduced in this complex. An electron transfer reaction occurs between the reducing compounds and the Mo(VI) under alkaline conditions with the production of a blue coloration with maximum absorbance at 760nm.

The phenolic content is expressed by linear regression calculated as a function of the Gallic acid calibration curve (external standard) at increasing concentrations.

Materials

- Spectrophotometer, stopwatch, vortex;
- Folin Ciocalteau reagent, 20% w/V Sodium Carbonate solution, Gallic acid.

The Gallic acid standard solution is prepared by diluting in water and making concentrations of the dilution range from 5mg to about 70mg Gallic acid/L in a 10ml volumetric flask (e.g 50ul Gallic acid in 9.95ml water for the first and 700ul in 9.3ml water for 70mg).

Sample preparation

- The sample is also diluted in a 1:20 ratio with distilled water;
- Test tubes are filled with 7ml of milliQ water. 1 ml of the diluted sample is reacted in the test tube with 500 µl of Folin Ciocaltou's reagent. After stirring by means of a mechanical agitator (vortex), the solution is left to react for 3 minutes, then 1.5 ml of Sodium Carbonate solution is added. After the addition of carbonate, the solution is left to react in darkness for 60 minutes after which the absorbance at 760 nm is measured on a spectrophotometer.

- The Gallic acid standards are measured like the samples above, and the calibration is repeated when a new Folin Ciocaltou's reagent is used.

The calibration is calculated by linear regression (ΔA = ac + b, c = Gallic Acid concentration mg/l, ΔA = absorbance, a= slope, b= intercept)

$$TP(mg\ GallicAcid\ eq/kg\ Fruit) = \frac{(\Delta A - b) \times F}{a \times E}$$

ΔA = **A** sample/standard

a = slope, b = intercept

f = dilution factor (20)

E = sample weight [kg/L extracting agent]

Results are expressed as mg Gallic Acid equivalent/kg fruit

3.4. Statistical Analysis

The fruits' qualitative and nutritional parameters were analyzed in triplicate for each sample. The data analysis was done using one-way analysis of variance (ANOVA) using the cultivar and the Nitrogen treatment levels as independent variables. Significant differences within samples were calculated according to Fisher tests (Least Significant Difference, LSD). Principal component analysis (PCA) was then used to evaluate the levels of association among the productive, qualitative, and nutritional parameters, and among the input variables; cultivars and nitrogen fertilization treatments. In the PCA bi-plot, the parameters and variables closest to each other in the same geometric plane are considered to be interrelated, and consequently, the parameters and the genotypes that are distant from each other are not related or are distantly related. The greater the distance of a vector from the origin of the axis, the higher the correlation of the variable with the PC represented in that axis. All analyses were performed using the software STATISTICA 7.0 (StatSoft. Tulsa, USA). Differences were considered significant for $p \leq 0.05$.

3.5. Results and Discussion

3.5.1. Qualitative and Nutritional parameters

Variance analysis of the qualitative parameters (firmness, soluble solids content, Acidity) and nutritional parameters (total antioxidant capacity, TAC and total phenolic content, TPH) indicated that all these parameters were statistically influenced by the cultivar, $p<0.01$. On an individual level, The nitrogen fertilization treatments influenced the firmness to a certain degree ($p<0.05$), but had no significant influence on the other qualitative parameters or any of the nutritional parameters, meaning a great degree of variance is down to the genotypes. The combined interaction of cultivar and Nitrogen treatment had a significant influence on all parameters except the fruit Titratable Acidity which showed no significant influence. The data is presented in table 4.

Table 4: Multivariate test analysis (ANOVA). Data refers to average fruit firmness, soluble solids, Titratable acidity, TAC and TPH ** = significant differences for $p < 0.01$; * = significant differences for $p < 0.05$; n.s. = non-significant differences

Factor	Firmness	Soluble Solids	Acidity	TAC	TPC
Treatment	*	ns	ns	ns	ns
Cultivar	**	**	**	**	**
Treatment*Cultivar	**	**	ns	**	**

3.5.1.1. Firmness

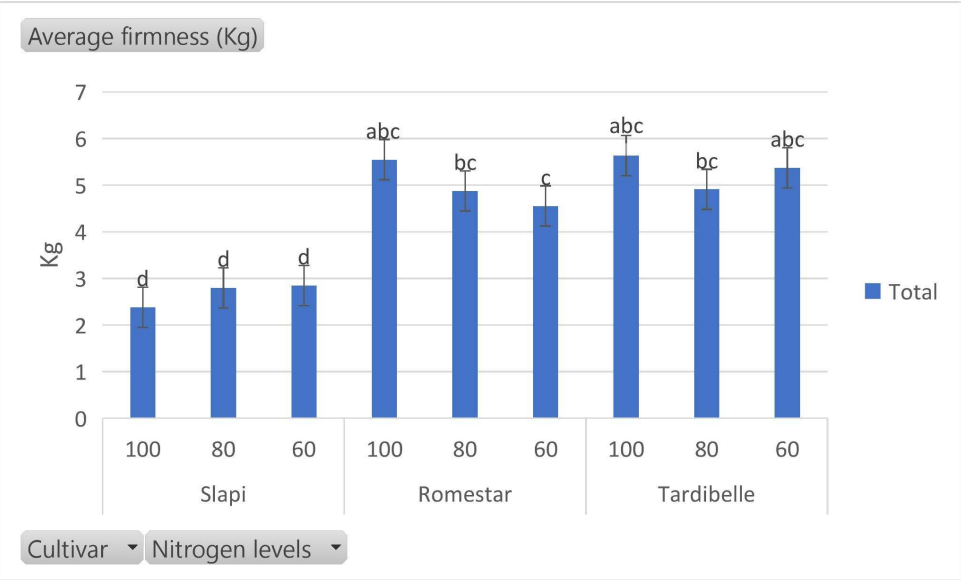

Figure 9: Average data of firmness ± standard error for each cultivar grown in different nitrogen treatments (N100, N80, N60). Values indicated with different letters express statistical differences for P<0.05, LSD test.

From figure 9, fruits of Slapi cultivar had lower values compared to Romestar and Tardibelle (average 2.67 kg as opposed to 5.00 kg and 5.30 kg respectively) but no significant difference in the Nitrogen treatment levels except when 100% was used which showed a decrease in firmness. In Romestar fruits, the firmness decreased with decreased treatment while Tardibelle had varied responses to the different treatment levels, recording its lowest value at treatment 80 (4.91 kg).

3.5.1.2. Titratable acidity

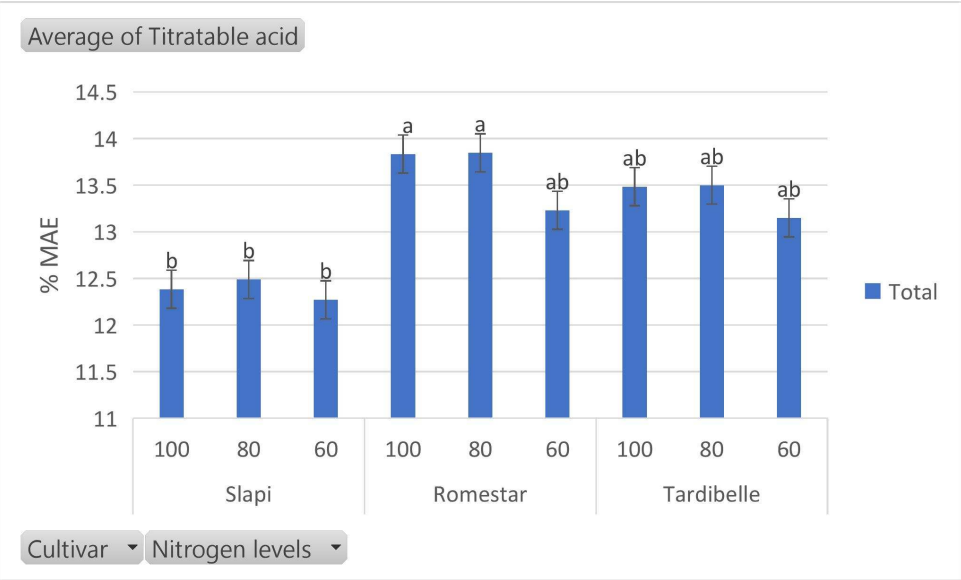

Figure 10: Average data of titratable acidity ± standard error for each cultivar grown in different nitrogen treatments (N100, N80, N60). Values indicated with different letters express statistical differences for P<0.05, LSD test.

Fruits of Romestar cultivar showed the highest acid levels with Tardibelle following closely, while Slapi had significantly lower levels than them (figure 10). However in all cultivars, the 100% and 80% treatments seemed to be almost inseparable while the 60% treatment had a significantly lower acidity of the fruit, for example dropping from 13.85, and 13.83 to 13.23 Malic Acid Equivalents (MAE) in Romestar.

3.5.1.3. Soluble Solid Content (SSC)

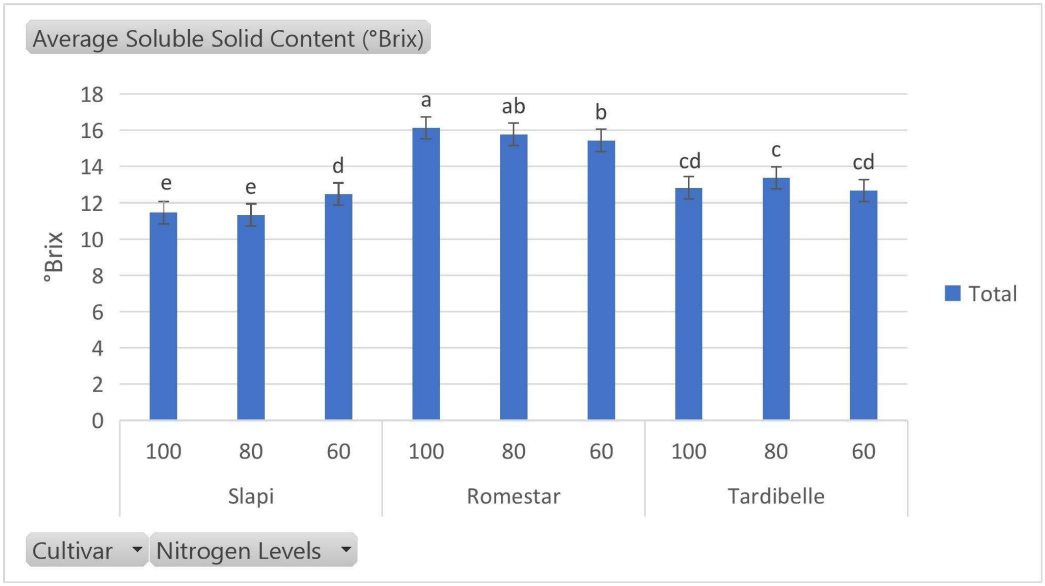

Figure 11: Average data of SSC ± standard error for each cultivar grown in different nitrogen treatments (N100, N80, N60). Values indicated with different letters express statistical differences for P<0.05, LSD test.

The different Nitrogen treatments had no significant difference within each cultivar but however, Romestar still had significantly higher values of fruit SSC (100% treatment highest with 16.1 °Brix), and 80% treatment of Slapi recorded the lowest at 11.33 °Brix, figure 11.

3.5.2. Nutritional Parameters

3.5.2.1. Total Anti-oxidant Capacity TAC

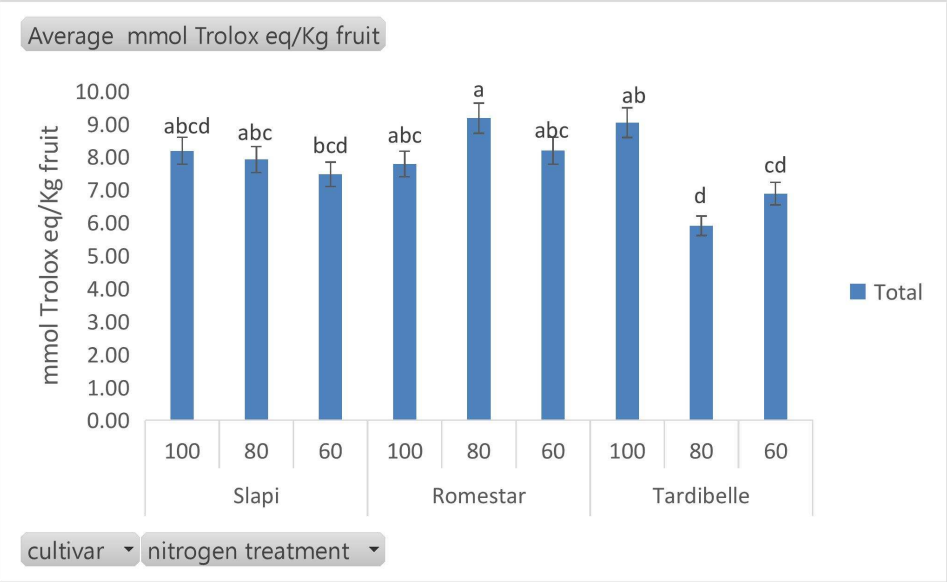

Figure 12: Average data of TAC ± standard error for each cultivar grown in different nitrogen treatments (N100, N80, N60). Values indicated with different letters express statistical differences for P<0.05, LSD test.

The antioxidant capacity of the fruit varied with different Nitrogen treatments as well as with the cultivars (figure 12). The highest fruit anti-oxidant capacity was noticed at Romestar 80% treatment (9.17 mmol Trolox eq/Kg fruit) which was not significantly different from Tardibelle 100% (9.04 mmol Trolox eq/Kg fruit), while the lowest value was noted to be Tardibelle 80% (5.90 mmol Trolox eq/Kg fruit). Fruit of Romestar 100%, 60% and Slapi 100%, 80% were all significantly related. In the Slapi cultivar, there was a slight decrease in fruit antioxidant capacity as the Nitrogen levels reduced, more so from 80% treatment to 60%. While in Tardibelle cultivar, a reduction in Nitrogen levels from 100% treatment significantly reduces the total antioxidants by at least 31% (from 9.04 to 6.88 mmol Trolox eq/Kg fruit).

3.5.2.2. Total Phenolic Content (TPH)

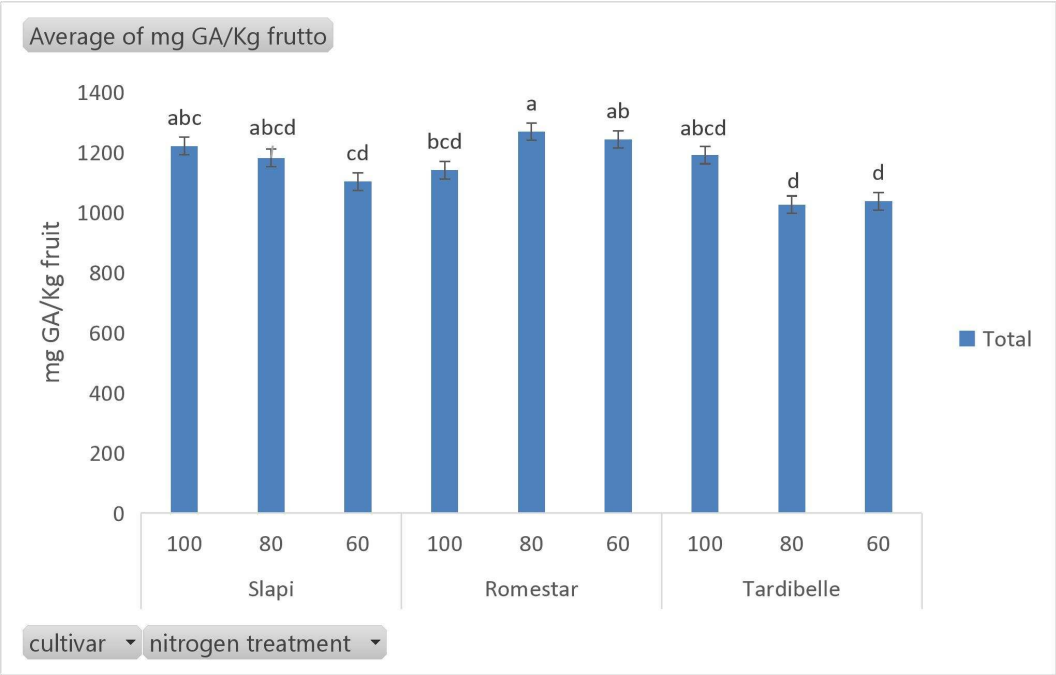

Figure 13: Average data of TPH ± standard error for each cultivar grown in different nitrogen treatments (N100, N80, N60). Values indicated with different letters express statistical differences for P<0.05, LSD test.

Just like for fruit antioxidant capacity, the Phenolic content showed the highest levels in the fruit of Romestar 80% (1268 mg GA/Kg fruit) with no significant difference to Tardibelle 100% (1190 mg GA/Kg fruit) and Romestar 60% (1242 mg GA/Kg fruit). Tardibelle 80% also recorded the lowest values (1025 mg GA/Kg fruit). Slapi continued to show a decrease in Phenolic content as the Nitrogen fertilization levels decrease. The trend with Tardibelle was the same with a drop of about 15% in phenolic content from higher levels of fertilization (from 100 to 80 and 60).

3.5.3. Principal Component Analysis

The Principal Component Analysis factor 1 and 2 explains 91.69% of the data variation. It all but confirmed the close proximity and strong correlation between the fruit content of Phenolic compounds, the anti-oxidative properties, and their quality variation

dependability on cultivar. These results are confirming that these parameters have likely effects on the nutritional quality of the fruit as a whole (Scalzo et al., 2005).

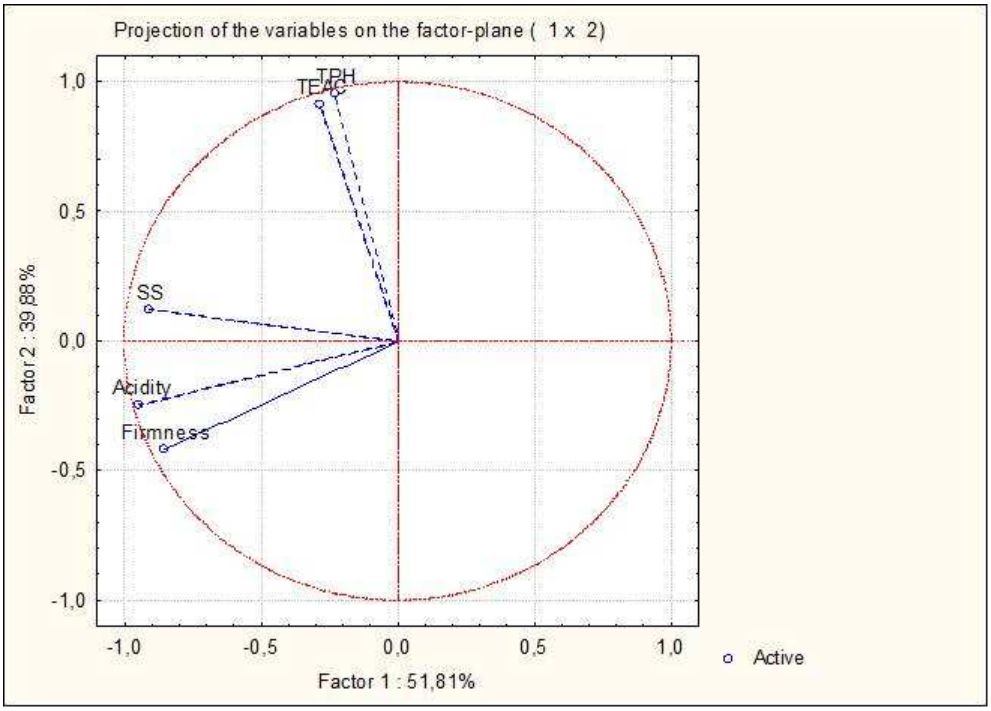

Figure 14: Principal Component Analysis of the various parameters

The nutritional parameters were at the top of the upper left quadrant. The other 3 parameters congregated close to each other at the center-left quadrants with acidity and firmness rather closer to each other than with SSC; this could be explained by the fact that a fruit's acidity and firmness reduces as ripening proceeds while its sugar content increases due to increase respiration and subsequent conversion of polysacharides to simple sugars.

4. Conclusion

The qualitative parameters (firmness, Titratable acid, and SSC) showed more variance from one cultivar to the other while they were less influenced by the fertilization treatments. A common trend was observed in the fruit titratable acid levels across all cultivars: at the higher nitrogen levels, all fruits had the same level of acidity while it was reduced in fruits at 60% which resulted in a drop in the fruits' titratable acid levels, and Slapi 60% eventually recorded the lowest value of 12.27 % MAE.

Generally, the three cultivars responded differently to the Nitrogen treatment levels. Overall, these data suggest that the concentration of health-benefiting compounds such as polyphenols and antioxidants can be altered with cultural practices such as fertilization rates.

Romestar fruits recorded the highest average values in all the nutritional and qualitative parameters observed except in the case of firmness, where Tardibelle had the highest average values. Slapi fruits recorded the lowest mean values across all the fruit qualitative parameters investigated, the most noticeable was in firmness where the average firmness was about 50% less than that of the other cultivars. For the nutritional parameters, the lowest average values were recorded by fruits of the late mature cultivar, Tardibelle, due to a significant drop in values when the Nitrogen levels were reduced from 100. With these outcomes, it is therefore imperative that manipulations to cultural practices such as reduced N fertilization or cultivar maturity can be easily done without any added cost to obtain fruit with higher health benefits. The grower can simply pick out the cultivar and Nitrogen level that would yield optimum results, for example, growing Tardibelle at 100 nitrogen yields optimum results of firmness, this could be good for a farmer looking to supply fruits to the international market or far-off from production. Romestar would be the perfect choice if nutritional properties are of high importance to the consumer.

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