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MSc thesis:

Characterization of postharvest changes in onion and their relation with storage time

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Abstract

Onion (*Allium cepa* L.) is the most economically important *Allium* crop. The commercial cycle of onion starts with the harvest and expect for the bulbs a conservation in bulks or in storage area at controlled temperature and relative humidity. The storage period need a particular attention because coincides with the dormancy period, when plants stop their growth, so it is very important to prolong the storage potential as much as possible. The most important moment in onions cultivation is the moment when the dormancy start. Dormancy is a period where the biological activities of bulbs are reduced. This period starts when the climatic condition become unfavorable for the life of the bulbs, and is exactly when this period starts begin that the onions are harvested, because the consumption of energy and oxygen is low. Deterioration in store is largely due to resumption of growth. The long term aim of this project at Lund University in collaboration with Swedish Agricultural University is to find a compound that can be used as marker to determine the storage time of the onions. The objective of this MSc thesis was to develop methods and analysis to characterize onions during storage. An onion consists of different layers and they all differ from the outer shell to the center, so to monitor the ongoing biological activities in the onions during storage the three segments: outer layer, middle layer and the inner layer are studied. The results show that water activity show no correlation between samples according to harvest time. The results show some differences between growing fields, but there are similarities between the values of the bulbs from fields A and B, A and C and between C and D fields, the data show no significant differences in water activity trough the layers. The total soluble contents analysis indicates that there are not significant differences in onion according to the harvest time, while there any similarities in onion soluble content according to the analyzed layer, so it's possible to say that each layer has different sugars content. As of the field effect is possible to say that there are similarities between field A,B and D, but those fields are significantly different from field C. The texture analysis shows that the hardness decrease from the outer to medium layers and from medium to inner layers, the growing field and the harvest time are not relevant in modifying the texture, there are also no significant difference between growing fields and harvest time. The dry matter results suggest a greatly dependence from the harvest time, and in lesser extent from the growing field. The fructan assay results shows that the most influent factors in this assay are the growing fields and the onion layer. An histochemical analysis was made to research difference in starch. This results of this study shows that the quality parameter are mostly dependent the growing field this suggest a correlation between the quality of the crop and the culture methods. Not very influent proved to be instead the harvest time, showing that the onion can keep for a long period of time the quality parameters in an acceptable range.

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Onion

1. Introduction

The onion is a member of the genus *allium* and the *liliaceae* family. Onions are one of the worldwide most consumed crops, are used fresh or dry, milled in powder and used as a spice and are widely used also by the industry as an ingredient in preparation of meaty formulations or in canned and brined food. According to FAO data, the global production of onion from 1961 is more than quintupled reaching the highest point in 2011 with 85 008 230.23 tones of global produced onions. In 2011 the Swedish production of onion was 84 308 183.55 tones and the medium consumption per capita was: 4 390 kg circa (faostat3.fao.org.). In the same year the Italian production was 413 793 tones and the medium consumption per capita was 158, 26 kg. Based on FAO statistics for mean production, and using mean export prices as a measure of the relative values of different crops, averaged over the years 2002–2004 the value of world edible onion production was about 21% of the world tomato production, 54% of the world brassica production and 120% of the world barley production (FAO, 2007). Onion is one of the first domesticated and cultivated crop because of its growth facility and portability. Onion is a biennial crop, but is cultivated for markets as an annual crop. Onions are harvest after the bulb reaches the maturity and is undergoing in a phase called dormancy. During the dormancy the respiration and the metabolic rate is significantly reduced and there is no growth of the plants. During this period some post-harvest changes still occur and therefore is very important for the industry to monitor the changes in the product and to control the storage time itself. In this thesis is investigated the evolution of the dormancy period of onion monitoring some parameters that can work as a marker of the quality of onions and also as a marker of the ending of the dormancy period and the subsequent sprouting. The contribution of this thesis to general knowledge in the field is given by the study of the parameters and a speculated relation of this quality parameters with the probability of most lasting onion during the storage time.

1. Aims

The objective of this MSc thesis was to characterize the onions along storage in order to monitor the commercial duration of onions and find an index of the ending of the dormancy phase. Finding this index can open the way for more studies focused at obtaining knowledge about its interaction with the external environment (temperature, humidity, light, etc.) and with the other onion components. This knowledge can be used to predict the time that the stored product will take before becoming unacceptable. Better understanding of the mechanisms that regulate the physiological processes associated with storage life can be used also to obtain an elongation of the storage time with a reduction of waste and an increased monetary return for companies. The analysis conducted in this project have the objective evaluate quality parameters of onion and obtain data about the biochemical substances that can influence quality parameters, and try to find a correlation between the variation of the values and the onset of a vegetative growth that happen after the dormancy break. The aims of this project are to gain knowledge about why during the storage time some onion start to rot while other onion remain intact. We try to characterize onion from 4 different fields, and from 3 different weeks of storage, relating the studied properties of the raw material with the storage time and the changes that occur in the onion during the storage time.

2. Background

2.1 Onion

Onion has been eaten and cultivated since ancient times. An onion bulb is a storage organ, consisting of foliage leaf bases and swollen, bladeless inner sheaths (Figure 1). The *Allium* genus comprises over 700 botanical species distributed throughout the temperate, warm temperate, boreal and tropical (mountainous areas only) zones of the world, predominantly in the Northern Hemisphere. Today it is a worldwide consumed crop. Onion was one of the first cultivated crops because of its growing versatility and portability (Benkeblia. & Shiomi. 2004). From 1981 to 2012 the world production of onions has increased from 14 264 046.00 tones to 82 851 732.00 tones reaching the maximum value of 85008230.23 tones in 2011. As shown by these data the onion production increased 5 times from 1961 to 2013 (faostat3.fao.org).

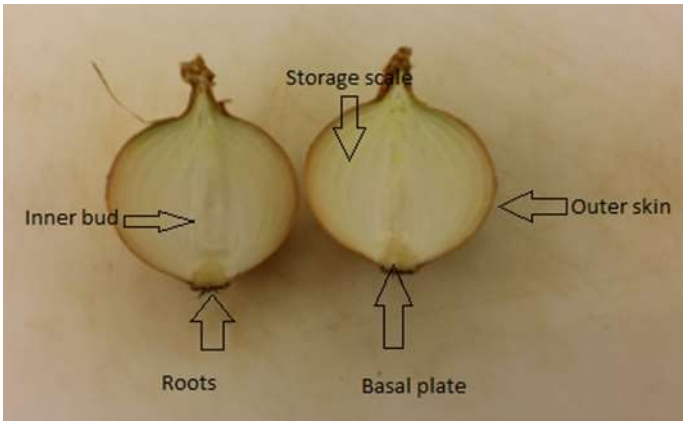


Figure 1. Inner scheme of onion *cv*. Renate

Many studies have reported beneficial effects of onions in the prevention and treatment of cancer (Gao *et al.*, 1999; Siess *et al.*, 1997), cardiovascular disease (Chen *et al.*, 2000), asthma (Dorsch, 1996), infections, and hyperlipidemia (Kumari *et al.*, 1995; Griffiths et al., 2002). Alliums have a long story of medicinal use and are ascribed to curing a wide range of ailments in traditional medical writings. Scientific studies have shown considerable pharmacological effects that have, in some cases, been attributable to specific molecular structures, mostly derived from the flavor sulfur compound. Three types of compounds found within alliums are of therapeutic interest. First are the substances derived from S-alk(en)yl- L-cysteine-sulfoxides (ACSOs) that are largely responsible for flavor and pungency. Second are the flavonoids, including quercetin, which is a powerful antioxidant and which is found in higher concentration in onion than in any other vegetable or fruit. Third, the fructo-oligosaccharides and fructans, soluble but non-digestible carbohydrates that promote a health-beneficial microbial flora in the lower digestive tract. There are three main types of onion: yellow, white and red. Yellow onions contain higher levels of quercetin as compared to red onions, whereas white onions have the lowest concentration. Quercetin has the highest radical scavenging activity due to the availability of phenolic hydrogen, which makes it a biomolecule of interest (Sharma., 2014). However, some onions have a strong, unpleasant, and long-lasting flavor that deters people from consuming them despite their health benefits. Flavor chemistry in onions is complex, unstable, and involves chain reactions. A study show that intact cells of onion have no odor, but when onion tissues are broken mechanically, the enzyme alliinase hydrolyzes a class of compound known as ACSOs, giving rise to pyruvic acid, ammonia, and numerous volatile sulfur compounds, which are principally responsible for the distinctive flavor (Lancaster and others 1998). From the ancient time onion, and more in general, vegetable members of the Allium genus are consumed for their strong taste, but only in recent time we have discovered the wide range of positive effects that this vegetables have on our organism (Randle, 1997; Griffiths *et al*., 2002). The compounds which are responsible for the flavor, coming from secondary metabolic pathways and their biosynthesis, involve the metabolism of sulfured amino acids (Jones *et al*., 2004). The sulphur is absorbed by the roots, reduced and stored into cysteine. Sulphur is present also in glutathione that is an antioxidant tripeptides formed by the plants as a defense. Glutathione after a conversion and the passage in other metabolic pathways terminate in the synthesis of ACSO who is a sulphured compound who constitutes from 1 to 5% of the dry weight of an onion bulb. The role of flavor compounds is not clearly understood but is suspected of being sulphur

storage molecules functioning as a defense against predators (Lancaster *et al*., 2001; Jones *et al*., 2004). Undamaged onion cells have no odor , but when onion cells suffer damage the alliinase, that is an enzyme that is restrained in the vacuoles, takes contact with the cytoplasm hydrolyze ACSO and this reaction form pyruvate, ammonia and unstable alk(en)yl sulphenic acids (Uddin and MacTavish, 2003). Those alk(en)yl sulphenic acids naturally condense in thiosulphinates providing a strong contribution to the perceived flavor (Briggs and Goldman, 2002). ACSOs are present in the onion in three different forms: methyl (MCSO), propyl (PCSO) and 1-propenyl (PRENCSO) cysteine sulphoxide. One of those, precisely PRENCSO, is the starting point for the formation of the lachrymatory factor (Lancaster *et al*., 1998; Kopsell *et al*., 1999).

3.2. Storage

Onions are harvested 90-120 days after the sowing. During the production, onions pass through different phases like curing, conservation in cold storage room or bulk then the last steps consist in the distribution to the markets and to final consumers. Onion are cured in order to remove water in excess, increasing the stability of the stored onion because decreases the amount of free water that is available for bacteria's development. Curing is performed in 2 ways: in the first way onions after the harvest are placed in a row on the ground and left to dry in the open air. In the second way onion are collected and placed in boxes in a warm and force ventilated room. Curing also close the neck of the bulb, which constitutes a physical barrier against pathogen attack like *botrytis allii*, the closed neck function also as a barrier against an excessive loss of water (Cools 2010). Curing permits to dry the outer skin which was in contact with the soil. Normally the curing process was performed directly in the field, but wet weather can provoke the discoloration of the skin. The curing process does not affect the amount of quercetin and quercetin 4-glucoside, that are very interesting components from the biological point of view (Downes et al 2009). Through the curing time the loss of water can be reduced with a careful choice of the harvest moment, the loss of water during this phase decrease with maturity at the harvest moment. This loss can be really high if onions are harvested too early: 10.1%, but decreases if onion are harvest at the right moment: 7.3%. Overripen onion has the lowest value of water loss: 5.9% at late harvest. The moisture content loss is also linked to the time of curing going from 7.2% at 24 h, to 8.3% at 48 h, to 9.3% at 72 h, to 10.3% at 96 h (B.W. Maw et al. 2005).

Onions are stored to preserve the quality of the onions. The storage is performed at low temperature in order to reduce the growth of microorganisms, but especially to delay as much as possible the onset of rooting and the subsequent sprouting (Sharma., et al., 2013). Vázquez- Gutiérrez (2014) found in a microstructural study that the onion cells release their cellular liquid into the extracellular space, increasing the release with storage time. For this reason the longer the storage time, the easier is to extract compounds of value like the phenolic compound (J.L. Vázquez-Gutiérrez et al. 2014).
Despite the importance of the storage in cold conditions for prolonging the storage time after the onion distribution to sellers or to the final consumers, the onions are keep at room temperature and not controlled humidity, this may results in the dormancy break and in bulb rot.

The most important factors that can lead to the deterioration of the bulbs are: respiration, resumption of growth and the attack by harmful microorganisms (Chope., et al 2006). A very important factor for storage life of the onion and for the dormancy time is the temperature: generally high and low temperatures inhibit the sprouting onset, an intermediate temperature instead promotes sprouting. There is also a different response at different temperatures depending on to the cultivars. The temperatures that need to be avoided to achieve a longer conservation are the temperatures between 10°C and 20°C. Particular attention needs to be placed at temperatures under 10°C and over 27°C because in that range of temperature, the water loss from the onions is larger.

When despite favorable conditions is not possible to see an appreciable growth of roots and the onset of sprout, is possible to say that the onion is in dormancy. During dormancy the plants do not grow and the rate of metabolic pathways just like the respiration rate is strongly reduced. Many cultivars have a short dormant period and the bulbs needs to be keep in an apparent dormancy phase to prolong the storage time, this apparent dormant phase is induced in the bulbs through a conservation in cold stores with a conditioned atmosphere. The sprouting indicate the end of the dormancy period. Is possible to see the onset of sprouting when instead of white layer leaves the leaf primordia start to produce green leaves that will come out from the neck (Abdalla & Mann, 1963). Storage life of onion is strongly determined by the onset of sprouting, the growth rate of the sprout and its suppression. Roots are another factor that can influence the stability during the conservation of onion. Miedema (1994 b.) found a correlation between the presence or absence of roots and the storage time, specifically found that: bulbs with roots (Figure 1A) sprout earlier during storage time than onion without roots (Figure 1B). It is suspected that the roots supply elements to the bulbs favoring the growth of the sprout.

Figure 1A. Rooted onion

Figure 1B. Unrooted onion

3.2.1 Changes in onion bulbs during storage and sprouting

During storage time, the water content is one the most important changes. The variations that take place inside the bulb are strictly linked to metabolic pathways, to respiration and to physiological state of bulbs.

With the increase of respiration level increase also the energy consumption. Respiration is expressed with Q10 value, this value represents a measure of the rate of changes in biological or chemical system as a consequence of increasing the temperature by 10 °C. This rate in dormant onion for the temperature range 10-30°C is of 1.3 (Brewster., 2008). Carter et al (1999) showed changes in the respiratory activity during storage time. At the harvest moment, in the middle of October, the metabolism is kept at low level and dormancy is ongoing. After 10/12 weeks (January) an increase in metabolic rate was detected. In March and April an increased metabolic activity was detected in the meristems of the growing shoot and in the sprout leaves. After the sprouting, the onions return to a lower level of metabolism. (Benkeblia *et al*., 2000).

The carbohydrates possible to find in the onion bulbs are glucose, fructose, sucrose and fructans. Fructans are a series of isomers made from many molecules of fructose (Darbyshire & Henry, 1978). These sugars constitute an high portion of the dry weight of the bulbs: 60/80% circa (Rutherford and Whittle, 1982). During storage at 15 °C the level of fructose increases during time, while the level of fructan decreases (Salama *et al*., 1990; Suzuki and Cutliffe, 1989; Ernst *et al*., 1998). Pak et al (1995) observed that a negative trend in concentration of fructan starts 2 weeks before harvesting, and which way depend from an utilization of the reserve carbohydrates in order to compensate the lower photosynthetic ability due to the fall of the leaves (Pak *et al*., 1995). Fructans are shown to have a tendency to decrease during storage at refrigerated conditions with or without modified atmosphere (Suzuki and Cutliffe, 1989; Pak *et al*., 1995; Ernst *et al*., 1998; Benkeblia *et al*., 2000), with a low concentration of oxygen. Fructose and glucose were found to higher concentrations is the outer layer with respect of the concentration in the inner layer of the bulbs (Darbyshire and Henry, 1978; Salama *et al*., 1990). The maximum soluble sugar content in the onion was found between week five and eight after the harvest day (Salama *et al*., 1990; Benkeblia *et al*., 2002). A link between carbohydrates content and storage life is only speculative, but not proved. Suzuki and Cutliffe (1989) found a positive relation between the presence of fructans and the percentage of onion still enough to be placed on market after 4 months of conservation at 6-10°C. Rutherford and Whittle found also a correlation between high level of fructose in onion from *cultivar* Robusta and a prolonged storage time at 4°C (Rutherford and Whittle, 1982).

Another class of compounds that have a deep effect on the storage time is the class of bioregulators and the most important of this compound is abscissic acid (ABA). ABA is a phytohormone and is biosintesis begins with the cleavage of a carotenoid with 40 carbon atoms and the formation of xanthoxin that is released in the cytoplasm and here converted by the abscissic alcohol in ABA (Cutler & Krochko, 1999; Taylor *et al*., 2005). ABA has various effects on the bulb, and many of them are related to the response to water and cold-stress, including bulb and seed dormancy, inhibition of

germination, stomatal closure and inhibition of cell elongation. The amount of ABA in the bulb is balanced by synthesis and degradation of this compound. But other factors like plant development, environmental conditions such as drought stress, and other growth regulators that can affect these equilibrium.

3.2.2 Pre-harvest factors affecting storage life

The factors that influence onions storage are various, and it is very important to consider the factors that can affect the life of onion during the storage even before harvesting. These factors can be nutrition in the field, ambient temperature during the development of the bulbs, maturity of the onions at harvest, method of harvest itself and the administration of maleic hydrazide to the onions.

The nitrogen input is also very important, Sorensen & Grevsen (2001) found that onion mature later in deficiency of nitrogen and also the harvest day needs to be moved forward to allow the onions to mature. The yield was also reduced with respect to the onion with a normal intake of nitrogen. Another microelement that is really important for the onion cultivation is sulphur. Sulphur nutrition has a impact on dry matter and quality of the onions bulbs. Specifically a high intake of sulphur in onion provoke firmer bulbs and higher fresh weight. Onions growing in field with a deficiency of water mature before and have a higher dry matter, but they have also a reduced yield (Sorensen & Grevsen, 2001). A high level of water deficiency (75%) in the first 25 centimeters of soil can reduce the sprouting, but involves also a reduced storage life.

Onions are sensible to the season during which they grow. Rutherford and Whittle (1982) found that onions have different storage potential even if the other parameters such as: drying process, storage conditions and cultural regimes are the same. High temperature during the season of growth reduce the shelf-life of the bulbs (Rutherford and Whittle, 1982; Wheeler *et al*., 1998; Sorensen and Grevsen, 2001).

The moment of sprout can be delayed using maleic hydrazide. Maleic hydrazide is an isomer of the amino acid Uracil that is sprayed on the onions before harvest and results in a sprouting inhibition during storage (Sorensen and Grevson, 2001). The working method of maleic hydrazide is based on a reduction of the mitotic index (evolution of cellular division) of the bulbs. Maleic hydrazide has no effect on sugars and organic acids (Salama *et al*., 1990). To achieve longer conservation time, is fundamental to harvest in the right moment. Europe and U.S.A have the concordant opinion that the right moment for onion harvest is when 80-90 of the tops fall down, with this method a little of the yield is lost in favor of more intact skins (Gubb and MacTavish, 2002).

Bulbs harvested before the commercial maturity result in not dormant bulb, having the highest metabolism rate, consequently these bulbs are unsuitable for storage. Early harvested onions have not reached the maximum size and a reduced yield is obtained. Komochi (1990) showed, in order to maximize the time of sprouting in store, it is best to harvest before the bulbs have reached their maximum weight, probably when 50–80% of the plants have reached the stage of ‘soft-necks’ and foliar collapse (Komochi, 1990).

The harvesting process can also cause a great stress to the onion bulbs and, as a consequence, it is possible that onions could be wounded or damaged facilitating pathogen or pests attack. Damage localized on the basal plate can also cause accelerated sprout growth. Physical damage must be minimized especially for less pungent onion, because they are more sensitive to external attack and accelerated sprout.

3.2.3 Post-harvest factors affecting storage life

Harvest means extracting the onion from the ground, and therefore to separate the bulbs from the source of nutrition that the soil is. But the onion is still metabolically active, and metabolic changes still occur. In absence of the nourishment given by the soil, the metabolic changes energy for these changes needs to come from the stocks that the onion accumulates. The metabolic changes consume energy, affecting the internal reserve of nutrients and reducing the yield and the nutrition value of the bulbs. Many strategies can be adopted in order to decrease the effect that these changes have on the product. These strategies can be: drying, irradiation and nitrous oxide treatments, but is also fundamental to control the storage conditions.

After the harvest, curing and drying, allow the onion to maintain high quality, maintaining firmness, color and flavor. Drying form an outer barrier that protects the onion from diseases and pests attack (Gubb & MacTavish, 2002). This skin protect the onion also from the loss of water, but especially from fungal attack, as they cause rot to the neck, to the basal plate and also to the inner part of the onion resulting in soft rot (Maude *et al*., 1984 Fenwick and Hanley, 1985). Curing is complete when the necks are dried and close (O'Connor, 1979). The time that the curing needs to be completed is dependent on: temperature, humidity and also from the maturation stage of the onion. Curing is normally performed in bulks with 30°C forced air for 3-5 days. After this stage onions are cooled at 24°C and the relative humidity is maintained to 70-75% to complete the process.

The trend of the markets and the increasingly high request from the consumers for a product with a lower content of chemicals found a response in irradiation of the onion. Irradiation extends the stability and delay the onset of sprouting. The effect of ionizing radiation (^{60}Co source at a dose of 150 Gy) is to reduce the respiration rate. This effect might be caused bythe destruction of meristems and the death of the sprouts. The destruction of the meristems and of sprout cause a deceleration in the respiration process. (Benkeblia *et al*., 2002).

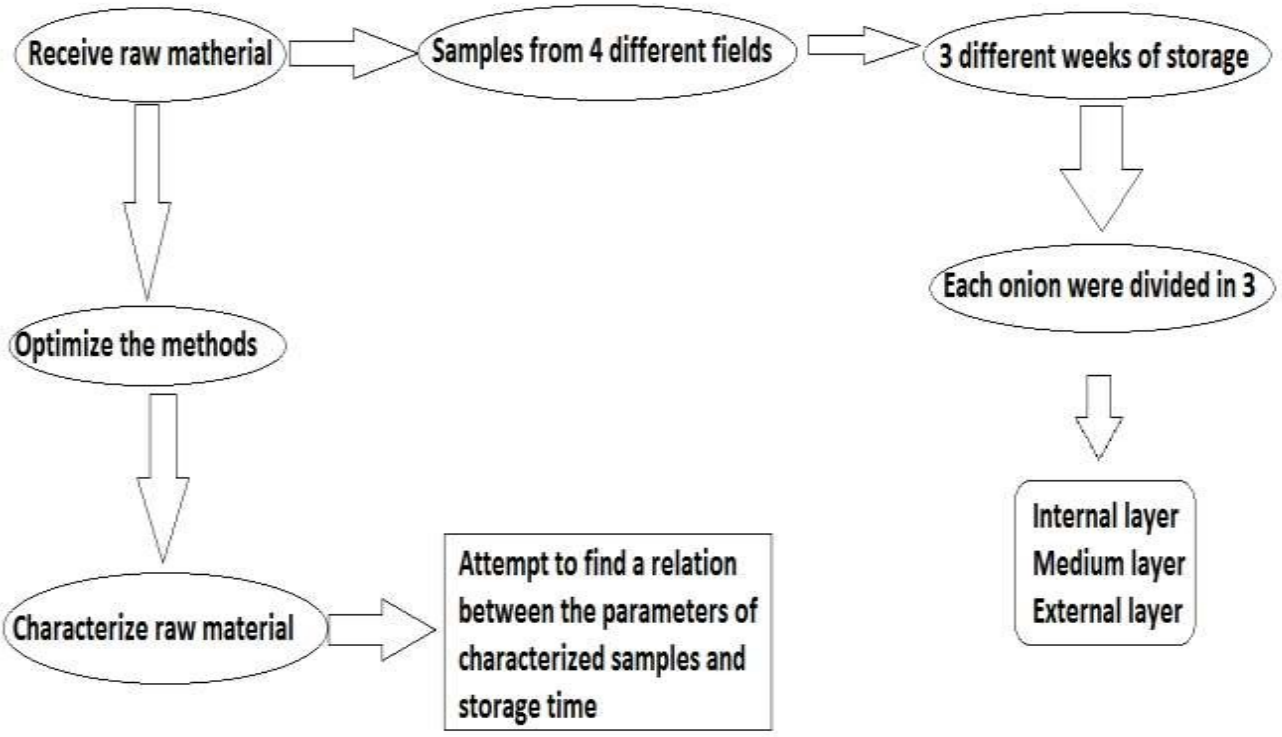
One of the most influent parameters in conservation is surely the temperature, which has a deep impact on the dormancy time and, therefore, on the storage time itself. Generally speaking, low and high temperature inhibits the sprouting onset but temperatures between 10°C and 20°C promote the sprouting. The choice of temperature has to be careful because in this range the sprouting is disadvantaged but the loss of water is greater (Abdalla and Mann, 1963; Brewster, 1977b; Miedema, 1994a; Ernst *et al*., 1999).

The atmosphere inside the storage room, is also fundamental to maintain high level of quality of the bulbs (Gubb & MacTavish, 2002). Low quantities of oxygen in the storage room inhibit sprouting, decrease the weight loss, and results in a lower onset of neck rot. Nevertheless, is important to choose carefully the composition of the atmosphere because too low levels of oxygen may cause: a higher number of sprouting after the removal of the onion from the storage room, growth of anaerobic fermenting bacteria giving rise to off-odors and rupture of the tissues. An atmosphere in the storage room composed of 5% CO2 and 3% O2 has been found to give a good percentage of marketable bulbs while keeping the quality (Adamicki and Kepka, 1974; Smittle, 1988).

In the storage room is also possible to use nitrous oxide. The effect of nitrous oxide is to inhibit the use of oxygen by the mitochondria, arrest the growth of bacteria and fungi and it has also anti-ethylene effect.

The humidity level in the storage environment can also have strong effect on the conservation time. The level of humidity in the storage room need to be intermediate between a high value, where the bacteria growth is promoted and a low value where the onion quickly loses water (Hole *et al.*, 2000). The outer skins that work as a barrier against water loss tend to crack and fall off at values of relative humidity lower than 55%, and microorganism attack is promoted at values higher than 80%.

4 Experimental overview



5 Material and methods

5.1 Raw material

Onions (*Allium cepa L. cv. Renate*) were provided by Almhaga Grönsaker Ab. Höllviken (Figure 2). The onions were harvested in the autumn 2014. They were collected from 4 different fields (A, B, C and D). The samples were harvest and stored in the company in bulk at 4°C and delivered to the laboratory at 3 different time points: week 4th after harvest, week 11th after harvest and week 17th after harvest. After delivery of the onions to our laboratory, they were stored at 4°C and used in the experiments within 4-5 days.

Figure 2. Onion samples

5.2 Raw material characterization

5.2.1 Water activity

Water activity of onions was measured with an Aqua (Aqua Lab Technologies USA) instrument which measures the dew point of the head air of the samples. The samples were cut with alligator cutter. The samples were kept wrapped with parafilm before the analysis. Two grams of the samples were used for the analysis

Figure 3. Onion samples divided in layers

5.2.2 Total soluble solids

Total soluble solids (TSS) were measured with a refractometer (Hanna instruments, USA). One drop of liquid was extracted from onions with a manual press and analyzed. The TSS content is expressed in °Brix.

5.2.3 Texture analysis

The texture analysis of onions was performed with a texture analyzer (Texture Technologies Corp, Hamilton, USA) equipped with a 25 kg load cell. The instrument was equipped with a knife probe at a speed of 2 mm/s and 90% of deformation of the samples. The texture analysis were conducted in 2 different ways: in the first way onion layers were cut in 3 cm wide slices and placed in a texture analyzer as shown in figure 4 A. In the second way onion were cut in two halves and each half was analyzed as shown in figure 4 B. Three onion layers outer, medium and internal layer were analyzed. From the graph obtained were extracted different parameters: the area and the gradient of the graph that are proportional to hardness of the onions and inversely proportional to elasticity of onions.

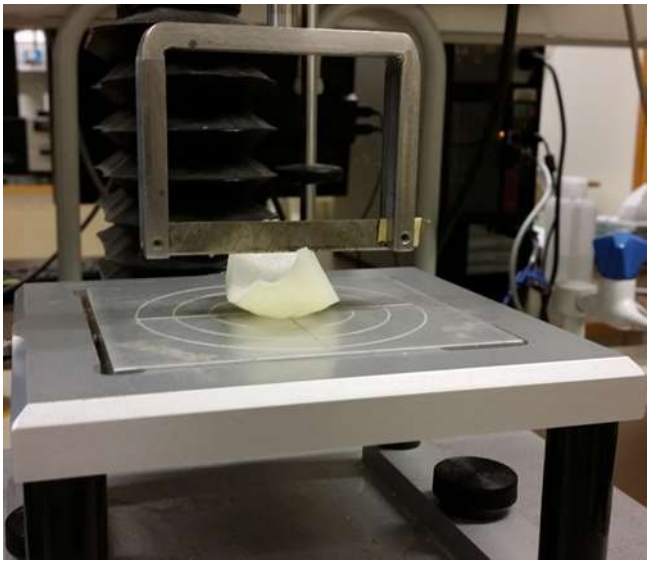


Figure 4 A 3 cm wide onion sample from external layer under the knife probe

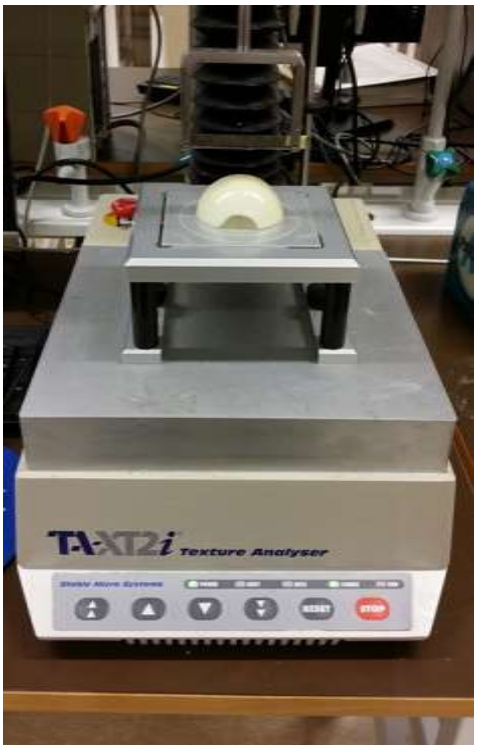


Figure 4 B Half leaf onion samples under the knife probe

5.2.4 Dry matter

The onions were divided into three layers: internal, medium, and external. The onions were cut with a manual chopper. Five grams of the sample were placed in metal containers and three drying protocols were used: I) the onion samples were placed in a regular oven (Horo, Gemini Bv, Netherlands) at 70°C for 24h and then the temperature was increased to 105°C for 1 h, II) the onion samples were placed in a vacuum oven (Gallenkamp, Fistreem,UK) at 100°C for 16 h at 1020 bars, III) the onion samples were placed in a vacuum oven (Gallenkamp, Fistreem,UK) at 70°C for 16 h at 1020 bars. Once dried, the onions were placed in a desiccator until the samples were cool. The dry matter percentage was calculated with the equation:

$$DM = (Md/Mw) \times 100$$

where *DM* is the dry matter, *Md* is the mass of a dry sample and *Mw* is a mass of wet sample.

5.3 Analysis of components of interest

5.3.1 Fructan

To analyze fructan the following chemicals were used: sodium maleate buffer, sodium acetate buffer, PAHBAH (reducing sugar assay reagent), sodium hydroxide and acetic acid. Sodium maleate buffer was prepared by dissolving 11.6 g of maleic acid in 900 ml distilled water and adjusting pH to 6.5. Sodium acetate buffer was prepared by dissolving 5.8 ml glacial acetic acid in 900 ml of distilled water and the pH was adjusted to 4.5. PAHBAH (sugar reducing assay reagent) was prepared mixing 2 solutions: the first one containing 10 g of p-hydroxybenzoic acid hydrazide, 10 ml of concentrated hydrocloric acid and water adjusting to 200 ml. The second one containing
24.9 g of trisodium citrate dissolved in 500 ml of water, 2.2 g of calcium chloride dehydrate, 40 g of sodium hydroxide the volume was adjusted to 2 l. These solutions were mixed immediately before the use. Sodium hydroxide 50 mM solution was prepared by dissolving 2.0 g of sodium hydroxide in 900 ml of distilled water. A solution of acetic acid was also prepared dissolving 11.5 ml of glacial acid acetic in 600 ml of deionized water and adjusting to 1 l . Before the analysis, onions were freeze dried for 7 days in a freeze drier at 200 mBars, following a temperature program starting from -20°C and increasing the temperature of 1°C per hour until the temperature reached the 20°C, then the temperature was maintained stable until the end of the 7 days of drying. The freeze dried samples were milled in a mortar until an homogenous powder was obtained, then 1.0 g was weighted and placed in 80 ml of hot distilled water (80°C). The onion powder dispersed in water was placed on a magnetic stirrer and stirred for 15 min with heating at 80°C. The solution was cooled to room temperature and the volume was adjusted with deionized water to 100 ml. The solution was filtered and 0.2 ml of filtered solution was moved in a glass test tube. Then 0.2 ml of diluted sucrose/amylase solution was added in the tube and the samples were incubated for 30 min to 40°C. After this procedure, 0.2 ml of alkaline borohydrate solution was added into the solution and the mix was stirred vigorously and incubated for 30 min at 40°C in order to reach the complete reduction of sugars. Then 0.5 ml of acetic acid was added to the solution and the solution was stirred. 0.2 ml was transferred into 3 glass tubes. 0.1 ml of fructanase solution was added to

two of that tubes and in third tube 0.1 ml of 0.1 M sodium acetate was added in order to obtain a blank sample. The tubes were sealed with parafilm and incubated at 40°C for 30 min to achieve the complete hydrolysis of fructan in D-fructose and D-glucose. A 5 ml of PAHBAH working reagent (solution 1 and 2 mixed) was added to all the tubes, a reagent blank were prepared mixing 0.3 ml of sodium acetate buffer and 5.0 ml PAHBAH working reagent. Then the samples and the blank were incubated in boiling water for 6 min. After that the tubes were placed in cold water for 5 min and the absorbance was measured at 410 nm against the reagent blank. The final concentration of fructan was calculated with Mega-Calc™, a program downloaded from the reagents producer's site.

5.4 statistical analysis

Data from the measurements of water activity, total soluble solids content, texture, dry matter and fructan were analyzed by Student's t-test and ANOVA.

6. Results

6.1 Water activity

All the results of measuring the water activity are presented in Figure 5. The differences between samples are very low and provoked by the field more than the onion layer or time. As shown by the results, there is no correlation between samples according to harvest time (fig. 5b). The results show that there are no significant differences between fields A and B, A and C and between C and D fields. The data show no significant differences in water activity trough the layers.

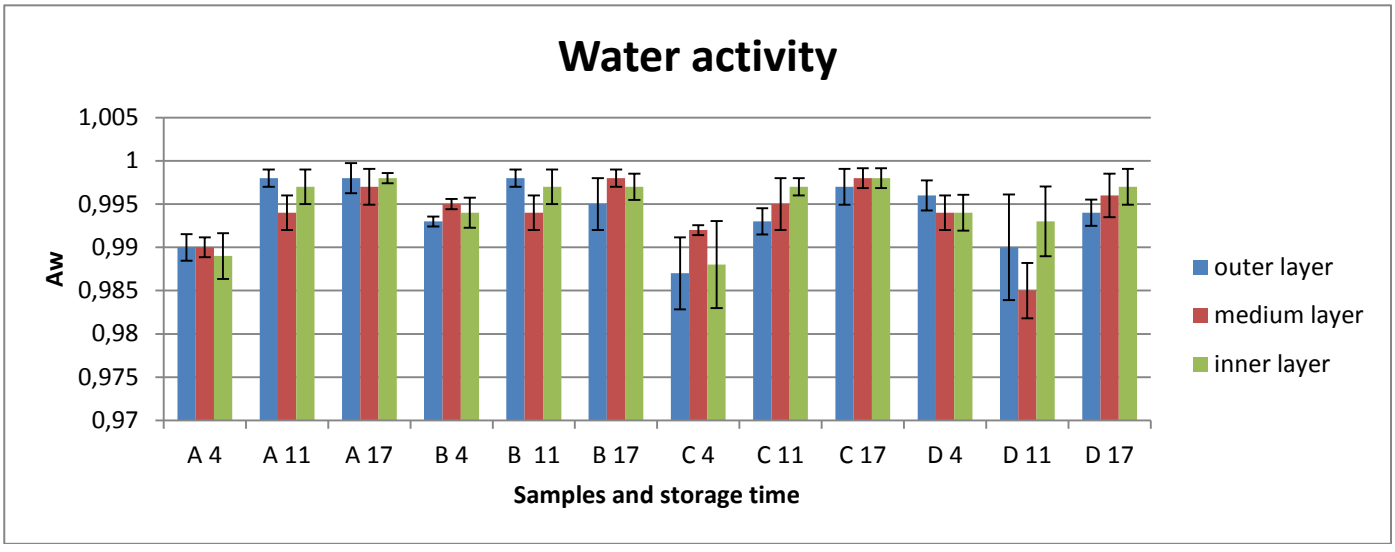


Figure 5. Water activity of onions during 17 weeks of storage

6.1.1 Total soluble content

The total soluble solids in the onions (Fig. 6) is greatly dependent on the analyzed layers and in lesser extent to the growing field, the harvest date is not relevant. There are not significant differences in onion according to the harvest time, but there are significant differences in onion total soluble solid content according to the analyzed layer. As of the field effect is possible to say that there are not significant differences between field A, B and D but those fields are significantly different from field C.

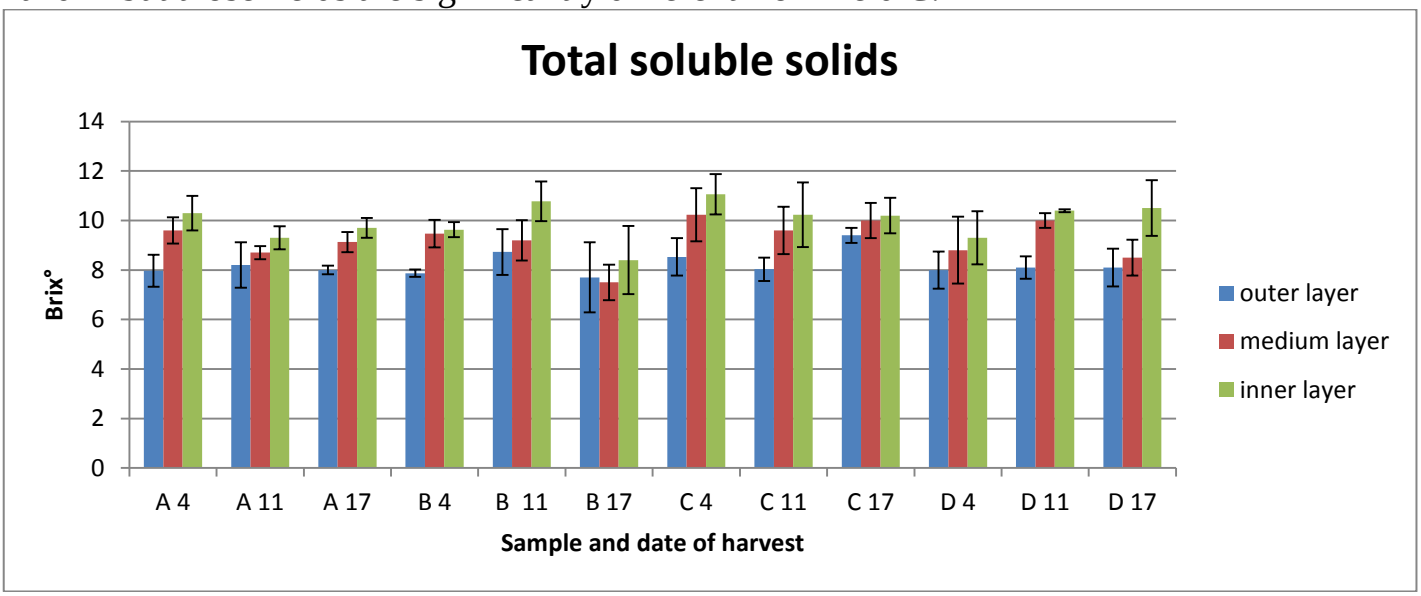


Figure 6. Total soluble solids of onions during 17 weeks of storage

6.1.2 Texture analysis of onions at 4 and 11 weeks of storage

The texture analysis graphics show that the hardness decrease from the outer layer to medium layer and from medium layer to internal layer, and this is confirmed by the ANOVA that also shows that the growing field and the harvest time are not relevant (figure 7).

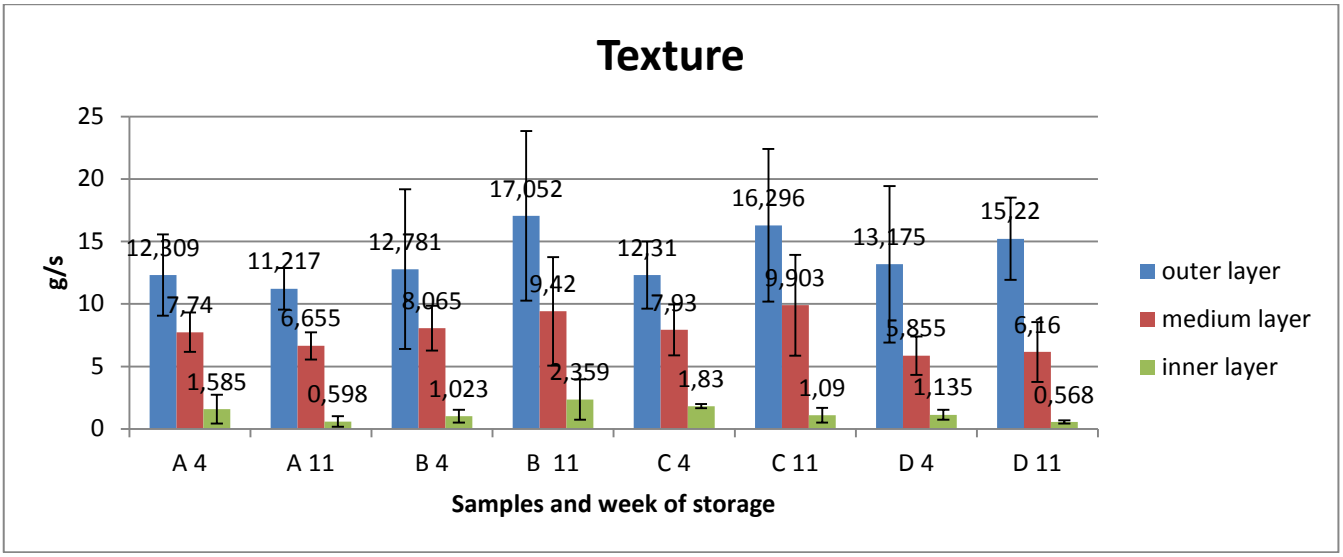


Figure 8. Results from first method of texture analysis

6.1.3 Texture analysis 2nd week

This analysis was conducted just on onions from 17th week of storage, so is not possible to compare the samples for the time factor, is possible instead to notice that is more relevant the difference between the different layers than the difference trough the fields.

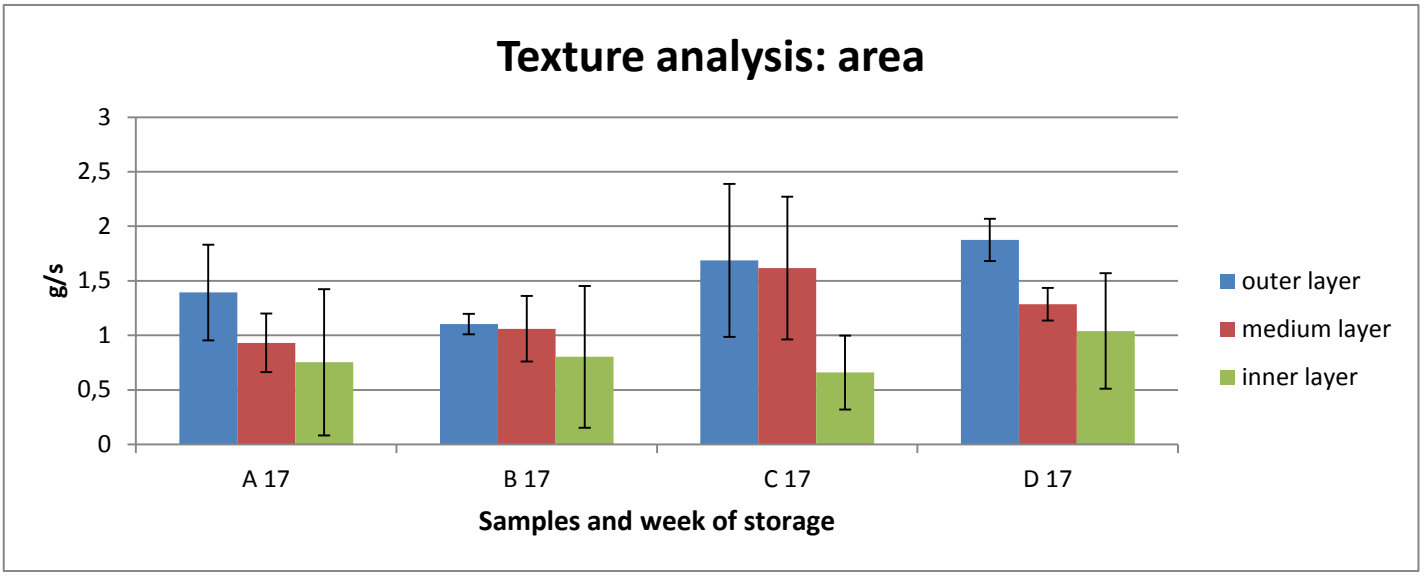


Figure 9. Results from second method of texture analysis, area

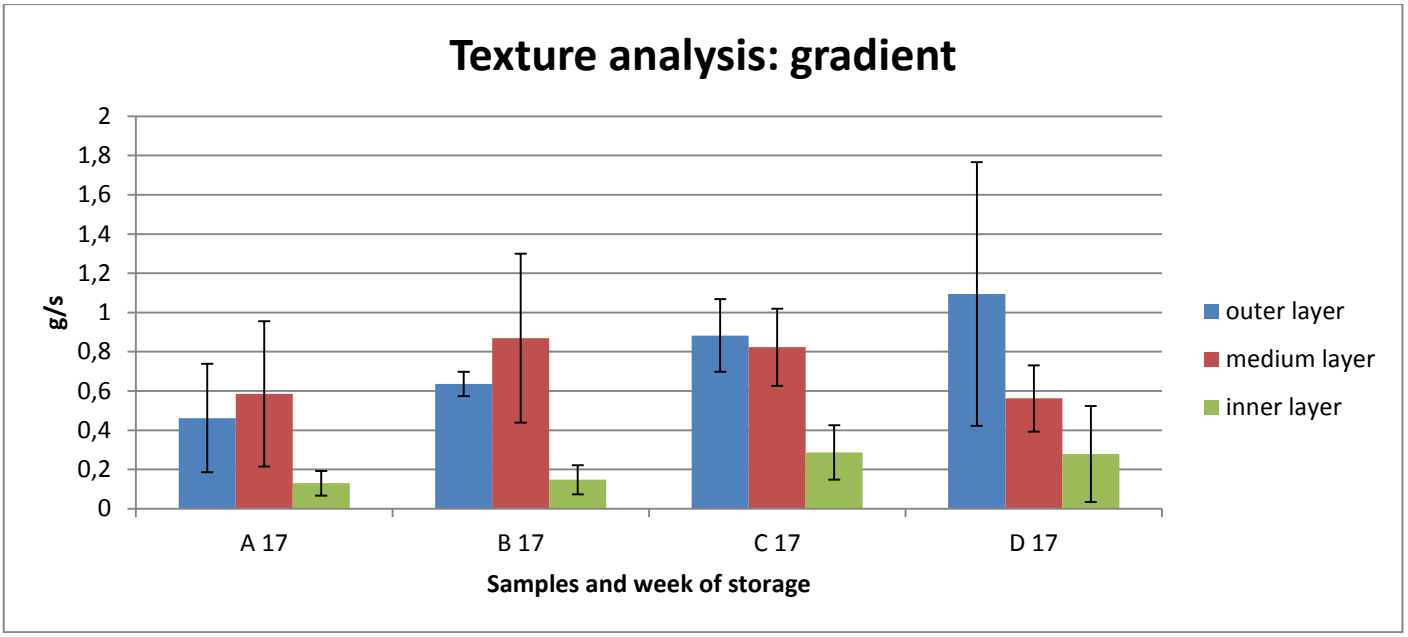


Figure 9a. Results from second method of texture analysis, gradient

6.1.4 Dry matter

The dry matter results (Fig. 9) show a greatly dependence from the harvest time, and in lesser extent from the growing field. The harvest time of onion give shows no significant differences between 4th and 17th week of storage, significantly different from this data is the data of 48th week of 2014. The growing fields show differences between field A and fields B, C and D that are similar amongst them. The comparison of the dry matter percentage of different layers shows that the value is different for each layer, specifically it increase from inner to medium layer and from medium layer to outer layer.

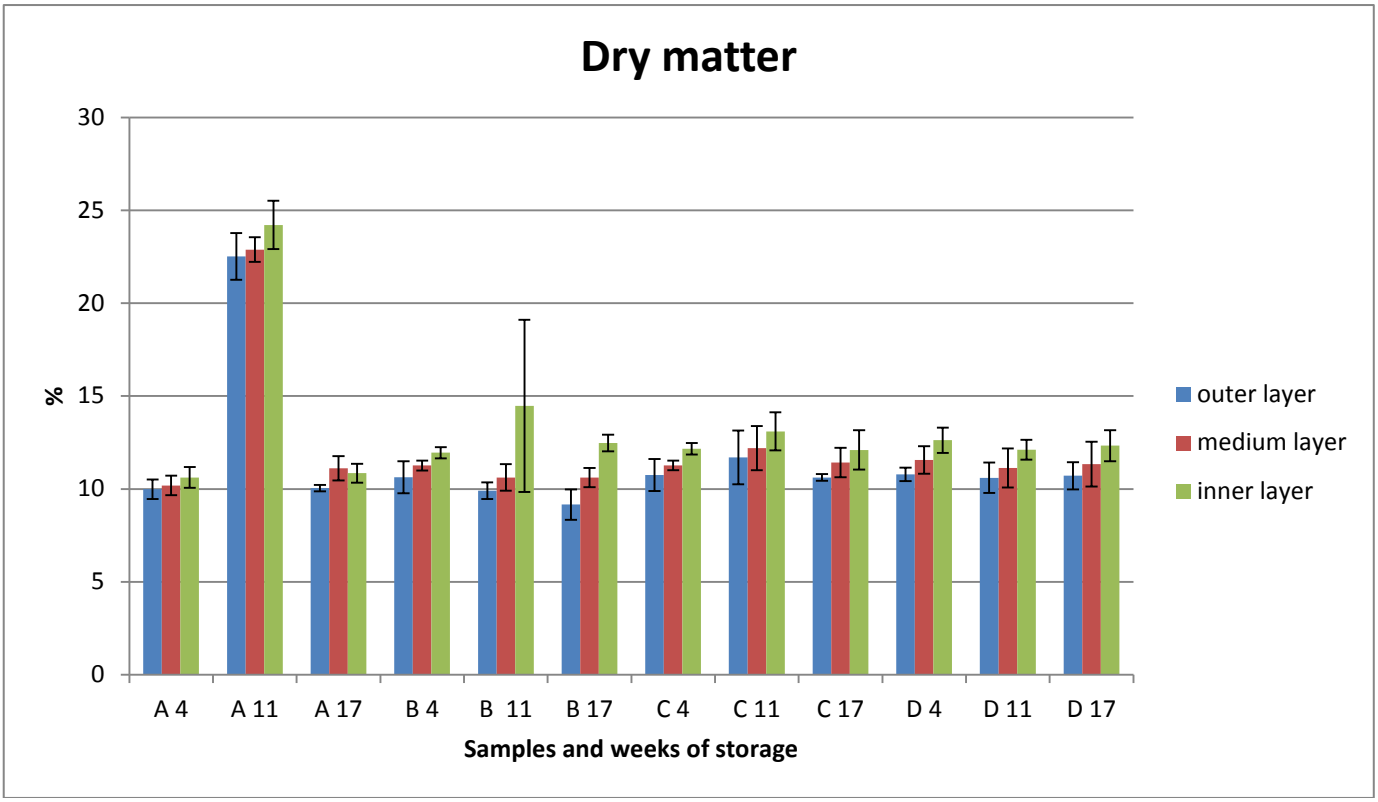


Fig 9. Dry matter results

6.6 Fructan content

The fructan assay results are shown in Figure 10, and from the ANOVA is possible to say that the most influent factors in this assay are the growing fields and the layer. The ANOVA allowed also to say that there no significant differences in onion samples according to harvest time. Is possible to notice according to the layer that there is no significant difference between medium and inner layer, but the outer layer has a significant lower value.

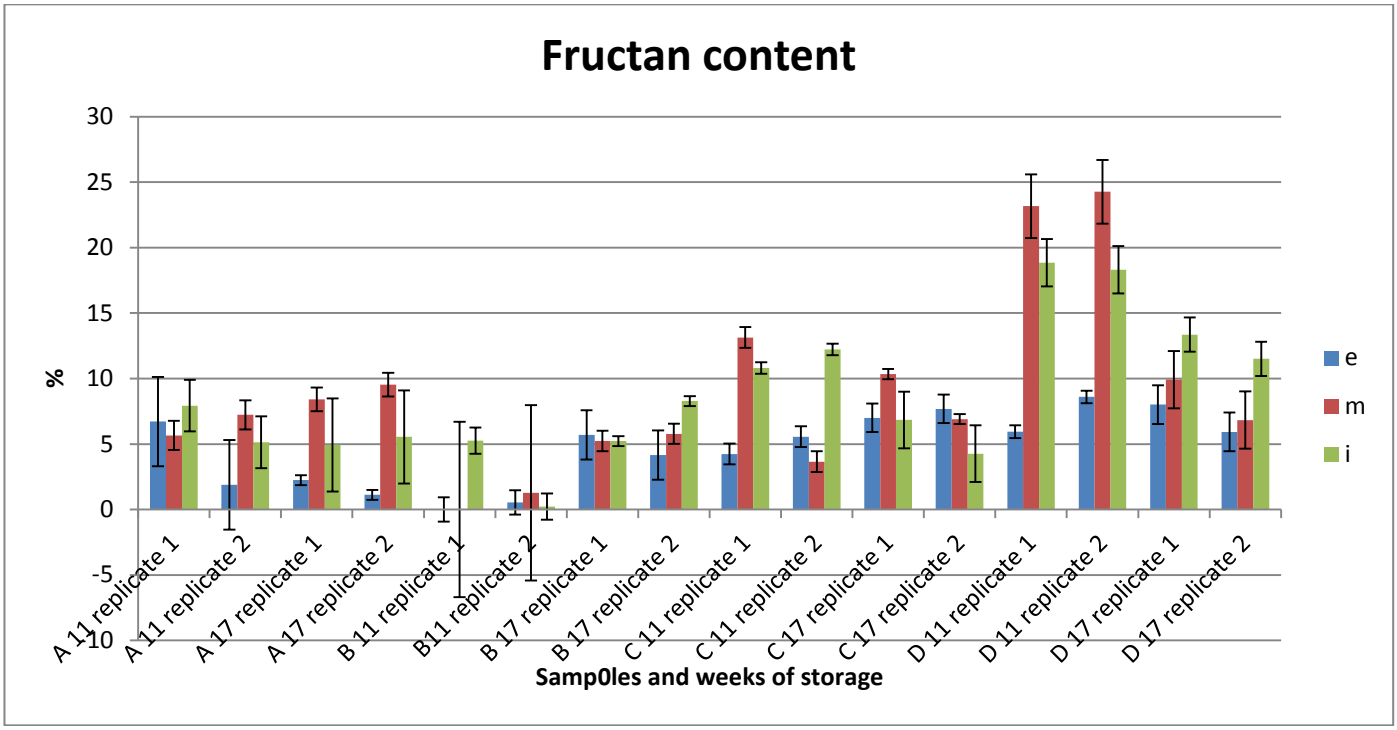


Figure 10. Fructan results

7. Discussion

The long term aim of this study is to find a marker that can be used to predict with a high level of precision the storage potential that an onion can have. To estimate the time in which the onion will expire can greatly reduce the waste with benefits at every level of the supply chain. Onions are one of the most important world crop and a reduction of waste may mean also a greater availability of food for underdeveloped economies, this can result in more food for poor people and a decrease in cultivated areas, a greater availability in cultivable soil. This study focusses on testing the different aspects of onion in order to reach a better comprehension of the crop and of the mechanism that can take place inside the bulbs during the conservation. Water activity is a stable parameter and is influenced in really small way from the harvest time. Total soluble content is also function of the analyzed onion layer and in lesser extent of the fields. From these results is possible to conclude that different field can affect the presence of sugar in a small way, but there is an intrinsic difference between layers in onion and this is expected because the concentration of sugars grow going through the layers, with the inner layer with the highest level of sugars. This is expected because the highest concentrations of sugar is where the sprouting begins. The dry matter is one of the most important parameters in onion conservation, because it's strictly bound to the onions quality level. As shown by the data dry matter is function of the harvest time; Dry matter decrease during time, making the onion more and more unacceptable during time. Fructan is a sugar made of a repetition of fructose molecules and is also positive correlated with abscissic acid (ABA) that is a growing hormones which it is strictly correlated to the storage potential, with this in mind result really important to find an easy and fast method to control it. The results obtained by Chope *et al* on onion of the same cultivar says that dry weight of onion change significantly during storage time, but

the results obtained in this study does not show a significant difference in dry matter in weeks 4 and 17 of conservation. Chope *at al* find also that the concentration of total soluble solids vary significantly during the storage time, this is in contrast with our results that show no significantly difference in total soluble solids during the storage. Regarding fructan Chope *et al* found a statistically significant negative correlation between storage time and fructan, this data is not confirmed by our results, but our result take in exams a period of time that can be too short to see an appreciable difference.

8. Conclusions

This study in conclusion has contributed to general knowledge about this crop exploring the quality parameters that can determine a quality decline in onion. In this study is establish how and if the quality parameter changes dependent on the growing fields. Is known from the literature that onions with an high levels of dry matter and fructan, but with low level of total soluble solids at the beginning of harvest increase the onion possibility of have a long storage life. The results that we have obtained are too few to find a strong correlation between the studied parameter and the storage life, but the data are promising and allow me to speculate that onions from fields A and C and from week 11 of storage have the highest possibility of during more than onions from other fields and storage time.

9. Future work

Longer time range is required to find the relation between storage time and the measured characteristics. Further experiments can monitor the concentration of bulb softening enzymes such as PME and PG or plant hormones such as ABA.

10. Appendix

10.1 Water activity tables

Source	DF	Anova SS	Mean Square	F Value	Pr > F
time	2	0,00046	0,000228	40,37	<.0001
field	3	9E-05	3,01E-05	5,33	0,0022
layer	2	2E-05	9,95E-06	1,77	0,1785
time*field*layer	12	5,6E-05	4,65E-06	0,83	0,6243
time*field	6	0,00046	7,66E-05	13,59	<.0001
time*layer	4	0,00012	2,88E-05	5,11	0,0011
field*layer	6	7,7E-05	1,28E-05	2,26	0,0466

Table 1. Results of the statistical analysis of water activity data

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**time**
A	0,997	36	3
B	0,9944	36	2
C	0,992	36	1

Table 2. Statistical differences between samples according to storage time

Means with the same letter are not significantly different.				
t Grouping		**Mean**	**N**	**field**
	A	0,9958	27	2
	A			
B	A	0,9947	27	1
B				
B	C	0,9941	27	3
	C			
	C	0,9933	27	4

Table 3. Statistical differences between samples according to growing field

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**layer**
A	0,9951	36	3
A			
A	0,9942	36	1
A			
A	0,9941	36	2

Table 4. Statistical differences between samples according to analyzed layer

10.2. Total soluble solids tables

Source	DF	Anova SS	Mean Square	F Value	Pr > F
time	2	2,6057	1,3029	2,11	0,1285
field	3	11,974	3,9915	6,47	0,0006
Layer	2	55,484	27,742	44,96	<.0001
time*field*layer	12	8,1424	0,6785	1,1	0,3739
time*field	6	17,325	2,8876	4,68	0,0005
time*layer	4	3,0531	0,7633	1,24	0,3029
Field*layer	6	1,3987	0,2331	0,38	0,8909

Table 5. Results of the statistical analysis of total soluble solids

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**field**
A	9,6963	27	3
B	9,1074	27	4
B			
B	8,9815	27	1
B			
B	8,8111	27	2

Table 6. Statistical differences between samples according to growing field

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**time**
A	9,2778	36	2
A			
A	9,2389	36	1
A			
A	8,9306	36	3

Table 7 Statistical differences between samples according to storage time

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**layer**
A	9,9833	36	3
B	9,2306	36	2
C	8,2333	36	1

Table 8. Statistical differences between samples according to analyzed layer

10.3 texture analysis tables

Source	DF	Anova SS	Mean Square	F Value	Pr > F
time	1	14,546	14,546	1,38	0,2464
field	3	41,29	13,763	1,3	0,2844
layer	2	1881,8	940,92	89,06	<.0001
time*field*layer	6	10,748	1,7913	0,17	0,9837
time*field	3	29,847	9,9491	0,94	0,4279
time*layer	2	20,021	10,011	0,95	0,3948
field*layer	6	30,199	5,0332	0,48	0,8225

Table 9. Results of the statistical analysis of texture analysis

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**time**
A	8,0449	36	2
A			
A	7,146	36	1

Table 10. Statistical differences between samples according to storage time

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**field**
A	8,45	18	2
A			
A	8,229	18	3
A			
A	7,019	18	4
A			
A	6,684	18	1

Table 11. Statistical differences between samples according to growing field

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**layer**
A	13,795	24	1
B	7,7165	24	2
C	1,2744	24	3

Table 12. Statistical differences between analyzed layer

10.3.1 Second way of texture analysis tables

Source	DF	Anova SS	Mean Square	F Value	Pr > F
time	0	0	.	.	.
field	3	1,1557	0,3852	1,77	0,1793
Layer	2	2,9758	1,4879	6,84	0,0044
field*layer	6	0,9196	0,1533	0,71	0,6485

Table 13. Results of the statistical analysis from second way of texture analysis

Level of field	N	TextNew	
		Mean	Std Dev
1	**9**	1,0257	0,5102
2	**9**	0,9894	0,3872
3	**9**	1,3209	0,7112
4	**9**	1,4002	0,4723

Table 14. Statistical differences between samples according to growing field

Level of layer	N	TextNew	
		Mean	Std Dev
1	**12**	1,5148	0,4756
2	**12**	1,2236	0,4302
3	**12**	0,8138	0,5016

Table 15. Statistical differences between analyzed layer

10.4 Dry matter tables

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Time	2	296,85	148,43	117,61	<.0001
Field	3	216,62	72,206	57,22	<.0001
Layer	2	59,734	29,867	23,67	<.0001
time*field*layer	12	8,3066	0,6922	0,55	0,8752
time*field	6	686,69	114,45	90,69	<.0001
time*layer	4	5,1611	1,2903	1,02	0,4017
field*layer	6	11,626	1,9376	1,54	0,179

Table 16. Results of the statistical analysis of dry matter

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**time**
A	14,621	36	2
B	11,145	36	1
B			
B	11,064	36	3

Table 17. Statistical differences between samples according to storage time

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**field**
A	14,713	27	1
B	11,695	27	3
B			
B	11,465	27	4
B			
B	11,233	27	2

Table 18. Statistical differences between samples according to growing field

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**Layer**
A	13,254	36	3
B	12,123	36	2
C	11,452	36	1

Table 19. Statistical differences between analyzed layer

10.5 Fructan assay tables

Source	DF	Anova SS	Mean Square	F Value	Pr > F
time	1	13,928	13,928	3,12	0,0899
field	3	589,65	196,55	44,08	<.0001
layer	2	174,57	87,286	19,57	<.0001
time*field* layer	6	140,21	23,368	5,24	0,0014
time*field	3	209,55	69,852	15,67	<.0001
time* layer	2	27,354	13,677	3,07	0,0651
field* layer	6	96,512	16,085	3,61	0,0108

Table 20. Results of the statistical analysis of fructan assay

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**time**
A	7,9431	24	2
A			
A	6,8657	24	3

Table 21. Statistical differences between samples according to storage time

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**field**
A	12,889	12	4
B	7,7225	12	3
C	5,5317	12	1
D	3,4742	12	2

Table 22- Statistical differences between samples according to growing field

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**layer**
A	8,8348	16	2
A			
A	8,6694	16	3
B	4,7091	16	1

Table 23. Statistical differences between analyzed layer

10.6 Appendix 1: method test for dry matter

Dry matter was measured in 3 ways for establishing the best method. In the first was used a vacuum oven at 100°C for 16 h, in the second a vacuum oven at 70°C for 16 h and in the last a regular oven was used at 70°C for 24 h and 105°C for 1 h more in order to evaporate the residues water. After this three different trials when the data was compared (figure 5) we decide to use the vacuum oven at 70°C because the onions were less burned so did not show the problem of sugar caramelization that can give artifacts and can conduct to a misinterpretation of the data.

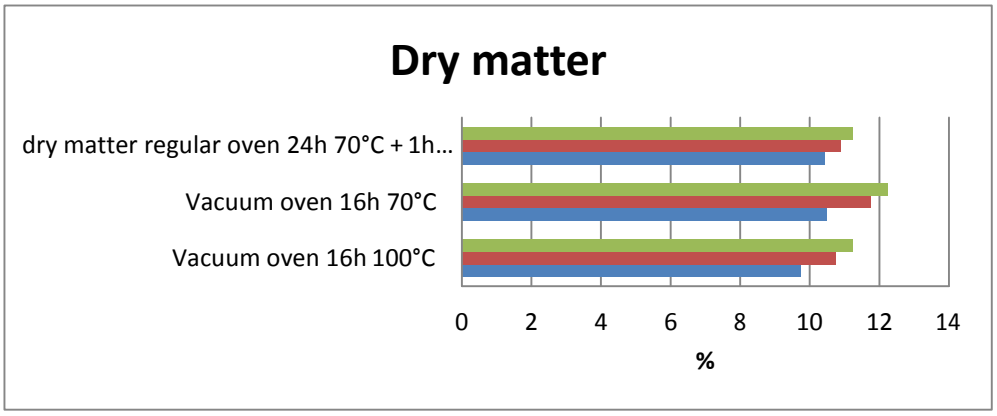


Figure 5. Dry matter % of onion dried in 3 different ways

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