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Effects of pre and post-harvest treatments on the phenolic compounds and antioxidant activity of different onion varieties

Thesis presented by
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Under the supervision of
Prof. Dr. Joseph Kerry
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To obtain the degree of
Doctor of Philosophy – PhD in Food Science and Technology

June 2019

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Declaration of Authenticity

I declare that this thesis is my own work and effort, and that it has not been submitted for any other degree, neither at Univerisity College Cork, UCC, Ireland nor elsewhere. Where other sources of information have been used, they have been acknowledged. I have read and understood the regulations of University College Cork concerning plagiarism.

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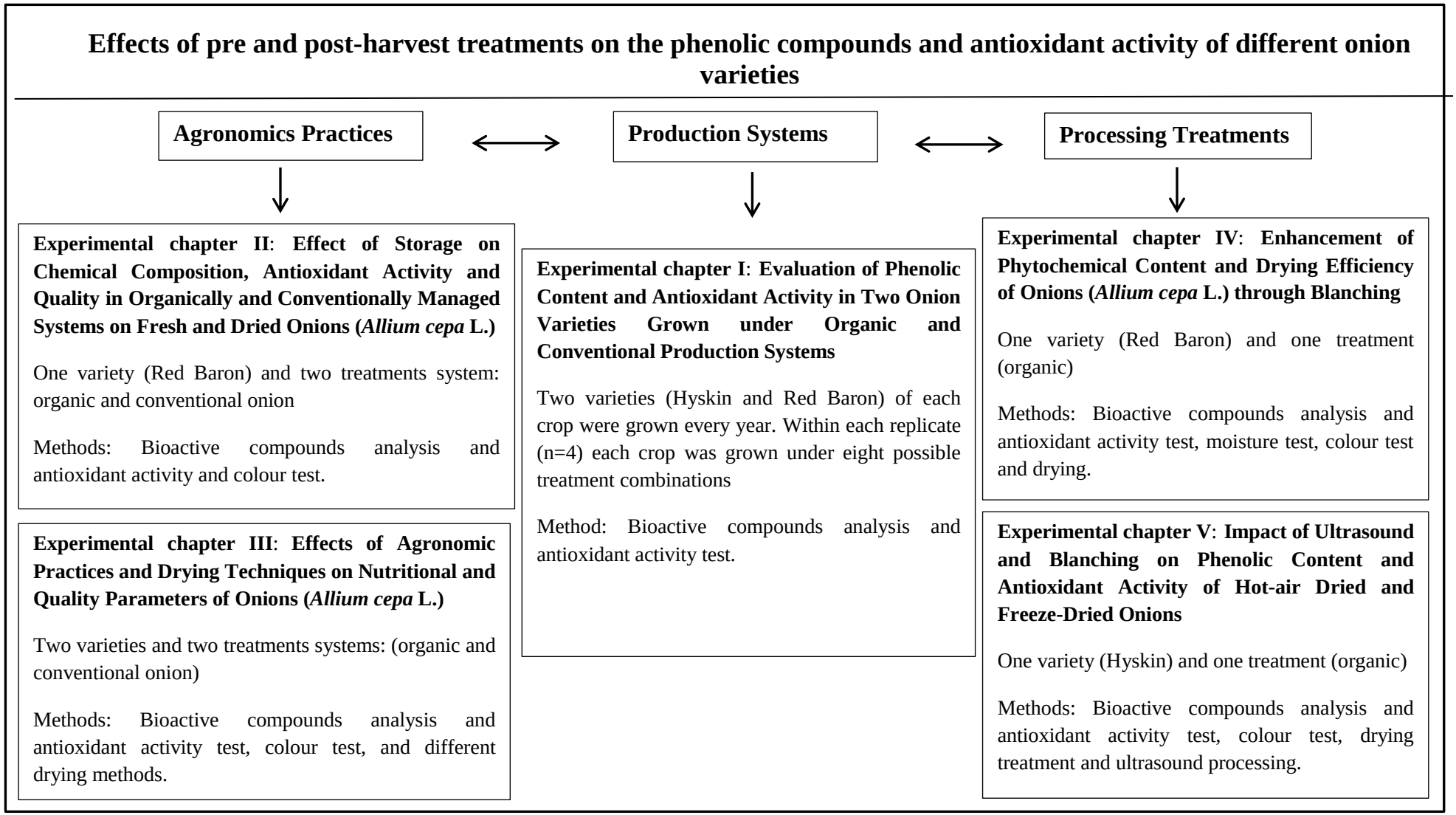
Date

Work Flow


Effects of pre and post-harvest treatments on the phenolic compounds and antioxidant activity of different onion varieties
Agronomics Practices
Production Systems
Processing Treatments
Experimental chapter II: Effect of Storage on Chemical Composition, Antioxidant Activity and Quality in Organically and Conventionally Managed Systems on Fresh and Dried Onions (Allium cepa L.)
One variety (Red Baron) and two treatments system: organic and conventional onion
Methods: Bioactive compounds analysis and antioxidant activity and colour test.
Experimental chapter III: Effects of Agronomic Practices and Drying Techniques on Nutritional and Quality Parameters of Onions (Allium cepa L.)
Two varieties and two treatments systems: (organic and conventional onion)
Methods: Bioactive compounds analysis and antioxidant activity test, colour test, and different drying methods.
Experimental chapter I: Evaluation of Phenolic Content and Antioxidant Activity in Two Onion Varieties Grown under Organic and Conventional Production Systems
Two varieties (Hyskin and Red Baron) of each crop were grown every year. Within each replicate (n=4) each crop was grown under eight possible treatment combinations
Method: Bioactive compounds analysis and antioxidant activity test.
Experimental chapter IV: Enhancement of Phytochemical Content and Drying Efficiency of Onions (Allium cepa L.) through Blanching
One variety (Red Baron) and one treatment (organic)
Methods: Bioactive compounds analysis and antioxidant activity test, moisture test, colour test and drying.
Experimental chapter V: Impact of Ultrasound and Blanching on Phenolic Content and Antioxidant Activity of Hot-air Dried and Freeze-Dried Onions
One variety (Hyskin) and one treatment (organic)
Methods: Bioactive compounds analysis and antioxidant activity test, colour test, drying treatment and ultrasound processing.

Publications and Presentations

Original Publications

1) **Feiyue REN**, Kim Reilly, Michael Gaffney, Joseph P Kerry, Mohammad Hossain, and Dilip K Rai (2017). Evaluation of polyphenolic content and antioxidant activity in two onion varieties grown under organic and conventional production systems. *Journal of the Science of Food and Agriculture*, *97*(9), 2982-2990.

2) **Feiyue REN**, Camila A Perussello, Zhihang Zhang, Michael T Gaffney, Joseph P Kerry, and Brijesh K Tiwari (2017). Effect of agronomic practices and drying techniques on nutritional and quality parameters of onions (*Allium cepa* L.) *Drying Technology, 36*(4), 435-447.

3) **Feiyue REN**, Camila A Perussello, Zhihang Zhang, Michael T Gaffney, Joseph P Kerry, and Brijesh K Tiwari (2017). Enhancement of phytochemical content and drying efficiency of onions (*Allium cepa* L.) through blanching. *Journal of the Science of Food and Agriculture*, *98*(4), 1300-1309.

4) **Feiyue REN**, Camila A Perussello, Zhihang Zhang, Joseph P Kerry, and Brijesh K Tiwari (2018). Impact of ultrasound and blanching on functional properties of hot-air dried and freeze dried onions. *LWT - Food Science and Technology, 87*,102-111.

5) **Feiyue REN**, Camila A Perussello, Zhihang Zhang, Joseph P Kerry, and Brijesh K Tiwari (2019). Factors influencing levels of flavonoids in onion during pre- and post-harvest food processing operations. A-Reveiw. Revision submission to *Food Research International*.

6) **Feiyue REN**, Kim Reilly, Camila A Perussello, and Joseph P Kerry (2019). Effect of storage on chemical composition and quality of organic and conventional onion. Prepare to submit *Journal of the Science of Food and Agriculture.*

7) **Feiyue REN**, Camila A Perussello, Zhihang Zhang, Joseph P Kerry, and Brijesh K Tiwari (2019). Effect of ultrasound pre-treatment on the drying kinetics and degradation kinetics of bioactive compounds and antioxidant activity of onion slices during the drying process. Prepare to submit *Ultrasonics Sonochemistry*.

8) Manuscript. Influence of variety on phenolic composition and antioxidant capacity: A study on different onion cultivars. Prepare to submit *Food Chemistry.*

9) Manuscript. Effect of different solvents on the extraction of phenolic compounds and antioxidant capacities from onion. Prepare to submit *Food Chemistry*.

10) Manuscript. Ultrasound-assisted extraction of phenolic from onion: optimization and comparison with conventional methods. Prepare to submit *Food Chemistry.*

Poster Presentations (Conference processings/Abstracts)

1) **Feiyue REN**, Zhihang Zhang, Joseph P Kerry, Brijesh K Tiwari (2016). Effect of blanching pre-treatments on total phenolics and flavonoids contents of hot air and freeze dried onion slices. Proceedings: 18th World Congress of Food Science and Technology (IUFoST), Dublin, Ireland.

2) **Feiyue REN**, Zhihang Zhang, Joseph P Kerry, Brijesh K Tiwari (2016). Effect of ultrasound and blanching pretreatments on total phenolics and flavonoids content of hot air and freeze dried onion slices. Proceedings: 2nd International Conference on ultrasonic-based applications: from analysis to synthesis, Caparica, Portugal.

Abstract

Onion (*Allium cepa* L.) is an important vegetable crop with an annual production of around 66 million tonnes worldwide. Onion and its by-products have the highest amount of quercetin content compared to other fruits and vegetables, which makes it a good free radical scavenging ability, contributing to its antioxidant capacity. Phytochemicals and antioxidants of onion are predominantly important for human health since phytochemicals have multiple compounds, including phenolics, flavonoids, anthocyanins, and quercetin. The total phenolic content of onions is not only higher than its fellow allium vegetables, such as garlic and leeks, but also higher than that of other common vegetables, such as carrots and red bell pepper.

The main objective of this study was to investigate phenolic contents and antioxidant activity of different onion varieties by using different pre-harvest (soil, sunlight, climate, and fertiliser) and post-harvest (processing and storage) methods. In order to achieve this, several experiments have been designed and carried out among different onion varieties, particularly between organic and conventional ones.

The first experimental chapter aimed to compare the total phenolic contents, total flavonoid contents, and antioxidant activity in onions grown under organic, conventional, and mixed cultivation practices in a multi-year experiment. ‘Hyskin’ and ‘Red Baron’ varieties had significantly higher phenolic contents and antioxidant properties in organic than in conventional production in most years. This study further investigated storage potential of organic and conventional onions at different storage conditions (-20 ^{o}C, 5 ^{o}C, and 25 ^{o}C with 60–75% relative humidity) for 10 weeks in the second experimental chapter. The findings suggested the fresh conventional onions were stored at -20 ^{o}C and 5 ^{o}C showed no significant quality (total phenolics, total flavonoids, flavonol, and antioxidant activity) loss. Meanwhile, the quality of dried organic onions remained stable during 10 weeks at -20 ^{o}C and 5 ^{o}C.

Moreover, the third and fourth experimental chapters aimed to evaluate the effects of food thermal processing technologies on organic onions nutritional (the levels of phenolic compounds, colour and antioxidant properties). More specifically, this study firstly investigated the quality change in onions dried by different drying methods (freeze-drying, hot-air drying, vacuum oven drying, and oven drying) in comparison with fresh samples. Results indicated that the dried onion showed significantly higher total phenolic contents and antioxidant activity than those of undried onions. In order to reduce the energy involved and reducing the drying time in onion drying methods, blanching as a pre-treatment method optimizes the onion drying process. Therefore, the combination of a fast blanching and hot-air oven drying (60 ºC) as a pre-treatment may be favorable since the application of heat treatment is the most common strategy for stabilising foods due to its capacity of destroying microorganisms and inactive enzymes. Since thermal processing, particularly blanching for a long time can negatively affect levels of phytochemicals in onions by experiencing a thermal breakdown or leakage of components, there is a growing interest in identifying new non-thermal strategies for the food industry.

Finally, the last part of this thesis aimed to investigate the effects of ultrasonic pre-treatment (non-thermal) and blanching prior to hot-air or freeze-drying of onions on the retention of phenolic compounds. These results showed that ultrasound pre-treatment is a potential alternative to conventional blanching pre-treatment in the different drying onion slices. It may be used in a combination with other processing techniques to obtain high nutritive dehydrated onions compared to that of the products dried without the pre-treatment.

In general, the research conducted in this thesis makes a notable contribution to the existing knowledge because it gives insights into pre and post- harvest conditions that contribute to high phenolic content and antioxidant activity in onions. The pre-harvest treatments and the novel approach to the post-harvest processing methods (blanching and ultrasound) can serve as ‘recommendations and guidelines’ for the

industry or the agriculture authorities at a national level.

Chapter 1
Literature Review

1.1 Introduction

The onion (*Allium cepa* L.) originated in central Asia and is one of the oldest cultivated plants, with cultivation records dating back to more than 4000 years. It is one of the most widely cultivated vegetable crops worldwide, after tomatoes, with an annual production estimated to be around 66 million tonnes (FAO, 2009), with 5.7 million tonnes of onions produced in Europe in 2013 (Eurostat, 2014). Onion bioactive compounds (i.e., phenolics) contribute to specific biological properties of onions. Table 1.1 highlights some of these biological properties.

When onions grow to an appropriate stage of maturity, they are harvested. The stage of maturity depends on the planting season, cultivar, market price, and conditions of the crop. Before harvest, onion bulbs along with their tops are pulled/lifted from under the soil in order to stop growth, and are usually kept/cured in the field for a few days to remove excess moisture from the outer skin and neck to reduce shrinkage, allowing for the colour development during storage. These fresh onions can then be directly supplied to the market, or further processed into different forms, for example, dried powders or flakes (Khan et al., 2016; Choi et al., 2017). Particularly, processing and storage steps deserve a pivotal relevance, since minor differences in the chemical and bioactive compounds of onions can lead to profound changes in their bioavailability and bioactive natural matrices (Pérez-Gregorio et al., 2014; Martins, Petropoulos, & Ferreira, 2016). Onion flavonoid effects of domestic treatments such as slicing chopping, shredding, peeling (Cantos et al., 2003; Berno et al., 2014; Islek et al., 2015), cooking (Rodrigues et al., 2009; Harris et al., 2015), or frozen (Ewald et al., 1999; Pérez-Gregorio et al., 2011b) were also investigated by a number of studies. Furthermore, onions could also be industrially processed. Industrial processing not only includes all domestic treatments but also includes thermal processing (roasting or boiling), freezing, drying, and packaging. The influence of these treatments on the onion flavonoid contents and profile will be further discussed in this thesis. It has been reported that the growing international

market for onion products has been developed with dehydrated methods such as powder, frozen, or canned onions (Arslan & Özcan, 2010). One of the drives to further process fresh onions into other forms was to reduce product loss (20% - 30%) during storage. Additionally, dehydrated products possess medicinal features, for containing higher concentrations of beneficial compounds than fresh onions (Lanzotti, 2006).

The food industry offers commercial onion powder as a nutraceutical or as a dietary supplement (Debnath, Hemavathy, & Bhat, 2002). Onion powders as spices may contain phenolic compounds and contribute to the intake of natural antioxidants, which can produce positive effects combining with other food (improve the antioxidant capacity and flavor) (Arslan & Özcan, 2010; Mitra, Shrivastava, & Rao, 2012; Sharma et al., 2015a).

Table 1.1 Reported bioactivity of phenolic in onions.

Reported bioactivities	**References**
Preventing cardiovascular diseases	Lamson and Brignall (2000); Ly et al. (2005); Siddiq et al. (2013); Pérez-Gregorio et al. (2014); Sharma et al. (2015a)
Antioxidant	
Anti-inflammatory	Hanahan and Weinberg (2000)
Anti-proliferative	Gee, Hara, and Johnson (2002) Yang et al. (2004)
Anti-angiogenic	Ly et al. (2005) Russo et al. (2012)
Pro-apoptotic	Lisanti et al. (2016) Sharma et al. (2016a)
Activating Immune destruction	Valentová et al. (2016) Fredotović et al. (2017)
Tumor promoting inflammation	Wang, Li, and Bai (2017) Murayyan et al. (2017)
Senescence induction and telomerase inhibition	Bahram-Parvar and Lim (2018)
Preventing the growth of tumors	Hanahan and Weinberg (2011)
Apoptosis autophagy	Hertog et al. (1993a); Arshad et al. (2017)
Reducing the risk of death from coronary heart disease	Kleemann et al. (2011)
Against arteriosclerosis	Rose et al. (2005); Wu et al. (2005); Santas, Almajano and Carbo (2010)
Antimicrobial activity against fungal, bacterial and viral infections.	Singh et al. (2009)
Anticarcinogenic and antimutagenic activities	Sanchez et al. (2007)
Anti-hypertensive effect and reduce blood pressure	Urios, Grigorova-Borsos, and Sternberg (2007); Akash, Rehman, and Chen (2014)
Anti-hyperglycemic or anti-diabetic potential and prevent advanced glycation of collagens, which contribute to the development of cardiovascular complications in diabetic patients	

1.2 Phenolic compounds in onions

Common fruits and vegetables that are abundant in phenolics include most berry crops, many tree fruit crops and onions. Onion was reported as one of the vegetables that contains the greatest amount of flavonoids, contributing to human daily diet (Hertog, Hollman, & Katan, 1993b).

Phenolic compounds comprise a wide variety of molecules that have a polyphenol structure (i.e., several hydroxyl groups on aromatic rings). Polyphenols are divided into several classes according to the number of phenol rings that they contain and the structural elements that bind these rings to each other (D'Archivio et al., 2007). They are important natural bioactive compounds found in onions and are widely recognized for their health benefits (Tiwari & Cummins, 2013). The total phenolic content is usually higher in red onions compared to white varieties.

1.2.1 Flavonoids in onions

Flavonoids are important polyphenols in foods and they are categorised by their chemical structure, namely: flavonols, flavones, flavanones, isoflavones, flavanols and anthocyanidins (Rice-Evans, 1995; Ignat, Volf, & Popa, 2011; Pérez-Gregorio et al., 2014) (Figure 1.1). Many studies have investigated the presence of flavonoids in onions (Pérez-Gregorio et al., 2010; Pérez-Gregorio et al., 2014; Arshad et al., 2017). At least 25 different flavonols have been characterized in onions (Slimestad, Fossen, & Vagen, 2007). There are seven major flavonol compounds in onions. They are (i) quercetin aglycone, (ii) quercetin monoglucoside, (iii) quercetin diglucosides, (iv) isorhamnetin, which is a methyl ether of quercetin, (v) isorhamnetin monoglucoside, (vi) rutin and (vii) kaempferol (Park & Lee, 1996). Kaempferol is detectable in certain onion varieties but it presents in much smaller quantities than quercetin (Bora & Sharma, 2009).

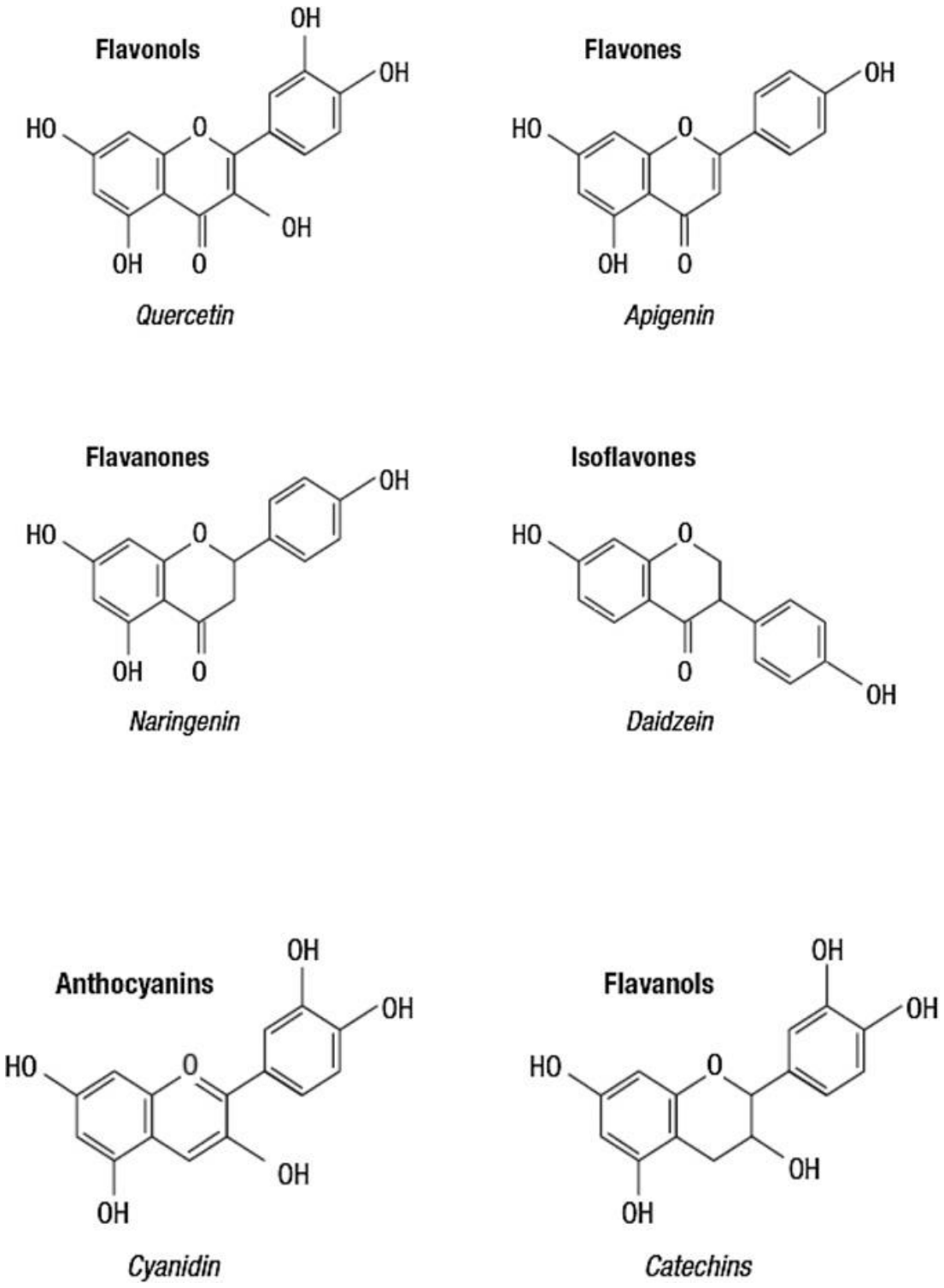


Figure 1.1 Chemical structures of flavonoids (Source: Ignat, Volf, & Popa, 2011)

Even though anthocyanins do not constitute a major flavonoid content in onions, they have frequently been reported to be presented in red onions. Slimestad, Fossen, and Vagen (2007) reported that there are at least 25 different anthocyanins in red onions,

with the quantitative content being approximately 10% (39 - 240 mg/kg fresh weight) of the total flavonoid content. Bystricka et al. (2013) also showed that many varieties of red onions covered red anthocyanins in the form of glycosides of cyanidin, peonidin, and pelargonidin. Anthocyanins are also reported to be a source of antioxidant activity (Geetha et al., 2011). Anthocyanin pigments, concentrated in the outer shell/skin of red onions, are only minor constituents of the edible portion.

Little has been known for the total amount of anthocyanins in red onions. Although some researchers (Ferreres, Gil, & Tomás-Barberán, 1999; Clifford, 2000) found about 250 mg/kg anthocyanins in red onions, however, they did not address which part of the onion they used, the edible part or the whole onion. Statistically, the amount of anthocyanins was found on the dry skin of red onions, ranging from a minimum 109 mg/100 g to a maximum 219 mg/100 g (Donner, Gao, & Mazza, 1997).

1.2.1.1 Quercetins in onions

Quercetin is the aglycone form of a number of flavonoid glycosides, such as rutin found in onions (Juergenliemk et al., 2003). Glycosides can be classified by the glycone, by the type of glycosidic bond, or by the aglycone. The aglycone part is the non-sugar group of a glycoside. Many plants store chemicals in the form of inactive glycosides. They can be activated by enzymatic hydrolysis, which causes the sugar part to be broken off, making the chemical available for use (Brito-Arias, 2007).

Quercetin diglucoside (Qdg) and monoglucoside (Qmg) account for up to 93% of the total flavonol content in onions (Lombard, Geoffriau, & Peffley, 2002). Sellappan and Akoh (2002) also reported that quercetin is the major flavonoid found in onion, present in the conjugated form, quercetin 4’ glucoside (4’Qmg), quercetin 3,4’ diaglucoside (3,4’Qdg), and quercetin. Slimestad, Fossen, and Vagen (2007) also agreed that quercetin and its derivatives were the most dominant flavonols found in studied onion cultivars. Similarly, Lombard et al. (2005) reported that 4’Qmg and

3,4'Qdg are the main flavonols present in onions, accounting for about 80 to 95% of the total flavonol content. Quercetin levels, to some extent, represents the flavonol or phenolic compounds present in onions.

1.3 Variation of phenolic contents among different crops and onion cultivars

Onions have the highest quercetin compounds among many other vegetables and fruits (Galdón, Rodríguez, & Romero, 2008). Onions contain 300 mg quercetin/kg fresh weight compared to 100 mg/kg fresh weight in kale, 40 mg/kg fresh weight in blackcurrants, and 30 mg/kg fresh weight in broccoli and apples (Mogren, Olsson, & Gertsson, 2006). However, the levels of phenolic compounds vary considerably between different onion cultivars. Onions provide a good example of the importance of a vegetable genotype on the content of phenolic compounds (Marotti & Piccaglia, 2002).

Colour is a phenotypical attribute and is closely related to the content of flavonols in onions (Lachman et al., 2003). Total flavonols contents in onions are usually higher in red onions, and lower in yellow or white varieties (Desjardins, 2008; Tedesco et al., 2015). In a detailed study of 55 onion cultivars, the level of quercetin was found to be the highest in red, pink, and yellow onion varieties (in the range of 54 – 286 mg/kg fresh weight (FW), whilst white onions contained low levels of quercetin (Patil, Pike, & Hamilton, 1995). Similarly, Dalamu et al. (2010) found that the quercetin content ranged from 22 to 895 mg/kg FW in 34 onion genotypes and hence it can be clearly seen that the quercetin content in onions varieties (Slimestad, Fossen, & Vagen, 2007).

However, colour may not be the only influencing factor affecting the total quercetin contents in different onion varieties (Patil & Pike, 1995). Crozier, Lean, McDonald, and Black (1997) reported that only 201 mg/kg FW quercetin was found in some parts of red onions, but a much higher quercetin content was observed in the whole

white onions (185-634 mg/kg FW). Marotti and Piccaglia (2002) also found higher levels of total flavonoids in golden onion varieties compared with red onions.

Long-day cultivars and short-day onion cultivars were studied by Okamoto et al. (2006), Yoo, Lee, and Patil (2010) and Petropoulos et al. (2015), who reported differences in quercetin content in these two cultivars. Lombard, Geoffriau, and Peffley (2004) also pointed out that the total quercetin content in long-day cultivars was also documented to be higher than in short-day cultivars and this difference did not depend on growing locations. Effects of onion bulb size on quercetin content were also investigated by some researchers. Lee et al. (2008) reported that small onions had higher flavonoid contents than larger onions. However, Patil, Pike, and Hamilton (1995) demonstrated that bulb size did not show any effect on the quercetin level. Mogren, Olsson, and Gertsson (2006) also reported that minor or no differences in quercetin glucoside content were observed between small- and large-sized onions.

1.3.1 Flavonoids distribution in onion tissue

Flavonoids in onions, mainly consisting of quercetins, accumulate to varying in plant tissues and levels determined in different plant parts are dependent upon environmental conditions (Hichri et al., 2011). Onion bulb skin (the non-edible dry peel) is richer in total flavonoids compared to the edible flesh (Hirota, Shimoda, & Takahama, 1998; Gulsen, Makris, & Kefalas, 2007; Nemeth & Piskula, 2007; Slimestad & Vagen, 2009; Pobłocka-Olech et al., 2016). Grzelak et al. (2009) determined a three-fold difference in flavonols present in the fresh outer scales of the studied onion compared to any other onion part. Lee et al. (2008) reported a decrease in the content of flavonoids in onion from the first to the seventh scale. Mogren, Olsson, and Gertsson (2006) claimed that about 90% of the total flavonols was concentrated and confined to epidermal tissue. Nemeth and Piskula (2007) and Slimestad and Vagen (2009) suggested that the higher flavonol contents in the outer

bulb scales, compared to in the inner scales, is due to cell aging. However, Beesk et al. (2010) found that the total flavonoid content in onions ranked as follows: middle layers > outer layers > inner layers.

Anthocyanin contents in onions were also studied and reported to be rich in the dry skin of onion bulbs (particularly in red and pink onion varieties). It is noteworthy that 63% of red onion anthocyanins are presented in the dry skin, which means that, after bulb peeling, only 27% of the total anthocyanins of red onion will be consumed (Gennaro et al., 2002).

1.4 Bioactivity of phenolic compounds in onions

Phenolic compounds are responsible for the major organoleptic characteristics of plant-derived foods, and also contribute to the nutritional qualities of fruits and vegetables (Parr & Bolwell, 2000). Phenolic compounds are known for their ability to provide a defence against the oxidative stress of oxidizing agents and free-radicals (Slusarczyk, Hajnos, Skalicka-Wozniak, & Matkowski, 2009). Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell, which can also be seen as a disturbance of the balance between oxidants and antioxidants caused by different factors such as aging, drug actions and toxicity and inflammation (Prakash, Singh, & Upadhyay, 2007). ROS can cause damage to important biomolecules, such as DNA, proteins, lipids, and carbohydrates, resulting in a variety of diseases (Prakash, Singh, & Upadhyay, 2007). Oxygen is a reactive species that has the ability to become part of potentially harmful or damaging molecules (free radicals). Free radicals cause healthy body cells to lose their function and structure. When the antioxidant capacity is limited, this damage can accumulate which leads to several diseases (Özyürek et al., 2014).

Antioxidants are natural substances (phenolics can act as reducing agents, metal chelators and singlet oxygen quenchers) that may prevent or delay some types of cell

damage and therefore protect the human body from free radicals. There are several species or molecules, which can be endogenous (internally synthesised) or exogenous (consumed), that are important in antioxidant defence and are considered as a biomarker of oxidative stress. Antioxidants can be regarded as either chain breaking antioxidants or preventive antioxidants (Özyürek et al., 2014). Antioxidants contribute to the suppression of oxidative stress and it is considered to be an effective way in preventing diseases caused by oxidative stresses (Özyürek et al., 2014). They are widely recognized for their health benefits, which include antioxidant, anti-inflammatory, antimicrobial and anticancer bioactivities (Ly et al., 2005; Paredes-Lopez et al., 2010; Tiwari & Cummins, 2013) and their protective effects against different degenerative pathologies such as cardiovascular and neurological diseases, and other dysfunctions based on oxidative stress (Griffiths et al., 2002).

Additionally, anthocyanins not only have strong biological functions such as anti-inflammatory and antioxidant activities (Kong et al., 2003), which are linked to the prevention of a number of degenerative diseases, but also provide sources of natural food dyes (Bleve et al., 2008).

Quercetin occurs at high levels in onions (Tiwari & Cummins, 2013). It is an effective scavenger of free-radicals and is also associated with antiviral, anti-inflammatory, antibacterial and muscle-relaxing properties (Jan et al., 2010). Table 1.1 lists some studies which have reported the potential health benefits of quercetin from onions. The same authors reported that there was substantial evidence demonstrating the chemo-preventive properties of quercetin against certain types of cancers, including; bladder, ovarian, breast, colon, stomach intestinal and lung (Harris et al., 2015). Quercetin has been shown to be a most effective inhibitor of peroxidation of membrane lipids, and thus can positively affect atherosclerosis (O'Reilly et al., 2001). Increased consumption of quercetin is associated with a reduced risk of prevalence of cardiovascular and other degenerative diseases (Erdman et al., 2007). Although quercetins have medicinal, pharmaceutical, and

nutritional properties (Hichri et al., 2011), it should also be noted that, at excessive doses, quercetins may be toxic to human health, as quercetins can also act as mutagens. Mutagens are pro-oxidants that can generate free-radicals, and act as inhibitors of key enzymes involved in hormone metabolism.

An integral part of the human diet is flavonols which are consumed daily by humans and for the determination of flavonol levels few efforts have been made. Intake of flavonoid varies from 2.6 to 68.2 mg daily as shown in the "Seven Countries Study" (Arshad et al., 2017). The daily intake of quercetin in the diet has been estimated to be about 5-40 mg/day (Hertog et al., 1995), although these levels can increase up to 200-500 mg/day in individuals who consume high quantities of fruits and vegetables (e.g., apples, onions, or tomatoes), which are rich in flavonols (Harwood et al., 2007). With regards to quercetin bioavailability, Hollman et al. (1995) showed that quercetin was indeed absorbed by the human body. The glycosides of quercetin (52%) are more efficiently absorbed than quercetin itself (24%) (Graefe et al., 2001) and the nature of the sugar residues in the glycosides appears to influence the extent of absorption. However, quercetin aglycone was absorbed more readily than glycosides (Wiczkowski et al., 2008). Quercetin aglycone seems to be more bioavailable than its glucosides (Hidalgo, Sanchez-Moreno, & de Pascual-Teresa, 2010).

1.4.1 Antimicrobial activity of onions

Pszczola (2002) highlighted the fact that onions have been used for centuries in several societies against fungal, bacterial and viral infections. Phenolic compounds in onions were reported to contribute to these activities (Griffiths et al., 2002). Due to the great antimicrobial activity that onions appear to possess, it is then not surprising that onion-derived phenolic compounds have been investigated for their antimicrobial properties, especially owing to the fact that these compounds appear to be relatively stable. Santas, Almajano, and Carbo (2010) investigated the antimicrobial activity of flavonol standards and ethyl acetate subfractions of

methanolic extracts of three Spanish onion varieties against three different bacterial strains. Among the onion extracts tested, ethyl acetate sub-fractions alone showed microbial inhibition.

It was reported that the major onion flavonoids possessed antiviral activity and enhanced the bioavailability of some antiviral drugs (Wu et al., 2005). Chen et al. (2011) found that shallots presented the highest antiviral activities, followed by onions. Given the high content of quercetin in onion, it was suspected that quercetin and its derivatives affected antiviral action.

Zohri, Abdel-Gawad, and Saber (1995) reported that onion extracts are effective against many yeast species, and their essential oils inhibit dermatophytic fungi. De Souza et al. (2010) demonstrated a relationship between the levels of total phenolics in onion and the antifungal activity tested against Rhizopus oryzae.

1.4.2 Antioxidant activity of onions

Antioxidants have the ability to protect organisms from diseases associated with oxidative stress, including cancer, cardiovascular diseases, inflammation, and other degenerative disorders (Ames, Shigenaga, & Hagen, 1993). Onions have shown antioxidant properties due to the presence of polyphenols (Stajner & Varga, 2003). Therefore, it is not surprising that onions are associated with health promoting properties and risk-reduction in terms of preventing human diseases (Sanderson, Mclauchlin, & Williamson, 1999). The antioxidant activity of phenolics in onions are principally derived from quercetin and its glycosides (Pérez-Gregorio et al., 2010). Owing to their redox properties, quercetin and its glycosides act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans et al., 1995). Their antioxidant activity is generally based on the number and location of hydroxyl groups present, as well as the presence of a 2-3 double bond (Rice- Evans & Miller, 1998).

Some research has highlighted the variation that occurs in antioxidant activity in onions, owing to the fluctuation in onion total phenolic content. Gokce et al. (2010) suggested that red onions had higher antioxidant activities than yellow and white onions. Bora and Sharma (2009) indicated that the dry outer layers of onion contained large amounts of quercetin, quercetin glycoside and their oxidative products, which are effective antioxidants against non-enzymatic lipid peroxidation and oxidation of low-density lipoproteins.

A good source of antioxidant polyphenols can be found in onion waste and by-products (Choi et al., 2015). Singh et al. (2009) pointed out that extracts from red onion peel contained large amounts of antioxidant polyphenols. Benite et al. (2011) reported a higher content of total phenolics and flavonoids from brown skin and tops-bottoms of industrial onion wastes had high antioxidant activity, among other bioactive compounds, and hence the wastes could be used as functional ingredients. A by-product developed by Roldan et al. (2008) derived from two Spanish onion cultivars that were stabilised by thermal treatments as shown to possess good antioxidant activities measured by the DPPH assay.

The antioxidant activities of onion extracts are influenced by onions selected, processing treatments used and extraction methods employed. Singh et al. (2009) used several methods to extract polyphenols and found that ethyl acetate (EA) extract obtained large amounts of polyphenols, and hence displayed a stronger antioxidant capacity. Roldan et al. (2008) found that processing of onion wastes to obtain a paste, followed by applying mild pasteurisation, were the best combination of processes to obtain an interesting stabilised onion by-product with good antioxidant activities. According to Lee et al. (2007), the levels of active compounds in onions increased after heating, since the antioxidant activities of the ethyl acetate fraction were higher in onions heated at 120 ^{o}C, 130 ^{o}C or 140 ^{o}C than in raw onions and the higher the processing temperature employed, the greater was the radical and nitrite scavenging activities observed. Similar results were found by Woo et al. (2007), who indicated

that the optimal heating time and temperature were 2 h at 130 ºC. In contrast, Khaki et al. (2009) employed acidified water/ethanol-based solvent extraction systems and reported that optimal extraction yields occurred at 6 h, whereas increasing temperature from 40 ºC to 60 ºC had a negative effect on yields.

1.4.3 Anticarcinogenic and antimutagenic activities

As highlighted previously, quercetin and its derivatives have been shown to exhibit anticancer properties, including activity against prostate, breast, skin, lung and liver cancers (Arung et al., 2011). Jeong et al. (2009) reported different anticancer activities from extracts from flesh and peel of white, yellow and red onion as a function of their total phenolics and flavonoids, as quercetin. In general, onion peel with the highest amounts of total phenolics and flavonoids inhibited the growth of several human cancer cell lines, including cells from both the stomach and colon, breast and prostate (Jeong et al., 2009), more efficiently than fresh onion flesh. Likewise, several studies have reported that quercetin enhanced the bioavailability of some anticancer drugs, such as Tamoxifen, a non-steroidal antiestrogen for the treatment and prevention of breast cancer, by promoting its intestinal absorption and reducing their metabolism (Wu et al., 2005).

1.4.4 Hypotensive and bradycardic effects

A study with several rat models of hypertension has indicated that quercetin and its methylated metabolite isorhamnetin, can reduce blood pressure (Sanchez et al., 2007). Moreover, Edwards et al. (2007) investigated the efficacy of supplementation of quercetin, rich in onions, on lowering blood pressure in hypertensive humans and demonstrated that 730 mg of quercetin per day could reduce systolic blood pressure by 7 mm Hg, diastolic blood pressure by 5 mm Hg, and mean arterial pressure by 5 mm Hg in hypertensive patients. Egert, Bosy-Westphal, and Plachta-Danielzik (2009) also found that quercetin reduced systolic blood pressure and plasma oxidised low-

density lipoproteins concentrations in overweight subjects with a high-CVD risk phenotype.

1.4.5 Anti-hyperglycemic and anti-diabetic potential

An investigation of the effects of quercetin on human diabetic lymphocytes showed an association between an increase in the protection against DNA damage from hydrogen peroxide at the tissue level and the number of consumed flavonols (mostly quercetin) from onions (Lean et al., 1999). Likewise, it has been reported that long-term absorption of quercetin could be useful to prevent advanced glycation of collagen, which contributes to the development of cardiovascular complications in diabetic patients (Urios, Grigorova-Borsos, & Sternberg, 2007).

1.4.6 Anti-platelet effect

Quercetin and its derivative showed their beneficial effects on cardiovascular health because of their antioxidant and anti-inflammatory activities (Kuhlmann et al., 1998), through the inhibition of lipid peroxidation and endothelial cell damage, which are involved in the early development of atherosclerosis (Kaneko & Baba, 1999). An in *vitro* study carried out by Janssen et al. (1998) showed that 2500 μmol/L quercetin isolated from onions inhibited platelet aggregation by 95-97%. However, an in *vivo* assay from the same authors with 18 human subjects ingesting 114 mg quercetin/day showed no significant effects. They finally concluded that necessary concentration levels of quercetin for the beneficial effects were too high to be obtained enough in daily dietary.

1.5 Factors and treatments influencing the phenolic compounds in onion production

Besides intrinsic characteristic such as the variety itself, many other factors influence the level of flavonoids and quercetin in onions, for example environmental conditions (e.g., soil type, sun exposure, and rainfall) and agronomic conditions (e.g.,

culture in greenhouses or fields, biological culture, hydroponic culture). Table 1.2 lists some factors and their effects on the quercetins content of onions during growing and storage.

Table 1.2 Studied factors to the content of quercetins during pre-harvest in onions.

Factors	Effect on quercetins content	References
Variety of onion	Rank of quercetins content: Red > Yellow > White	Lachman et al. (2003); Lombard et al. (2005) Kaur, Joshi, and Kapoor (2009); Yoo, Lee, and Patil (2010); Lu et al. (2011); Nile and Park (2013); Cheng et al. (2013); Lee, Patil, and Yoo (2015); Nayak, Liu, and Tang (2015); Kwak et al. (2017)
Bulb parts	Rank of quercetins content: small > large	Mogren, Olsson, and Gertsson (2006); Lee et al. (2008)
	Rank of quercetins content: dry outer skins > inner skins	Slimestad et al. (2007) Nencini et al., (2007); Kaur, Joshi, and Kapoor (2009); Grzelak et al. (2009); Benitez et al. (2011); Lee and Mitchell (2011); Albishi et al. (2013); Cheng et al. (2013); Yoo, Lee, and Patil (2013); Burri et al. (2017)
Organic/Conventional varieties onions	Rank of quercetins content: organic > conventional	Ren, Endo, and Hayashi (2001); Faller and Fialho (2010)
Light	Rank of quercetins content: Exposure to sun light during production period> less sun light during production period of onion	Rodrigues et al. (2011) Higashio et al. (2005)
	UV light lamps after harvest>no UV treated onion Fluorescent light after harvest > no UV treated onion	Lee et al. (2008)
Curing	Rank of quercetins content: after curing > at lifting	Lee et al. (2008)
	Rank of quercetins content: Field field curing > dark environment	Mogren, Olsson, and Gertsson (2006) Rodrigues et al. (2009)
Lifting	Rank of quercetins content: late lifting time > early lifting time	Mogren, Olsson, and Gertsson (2007) Downes, Chope, and Terry (2009)

The environmental factors that affect quercetin content in onions include sunshine radiation, temperature and climatic conditions within a geographical location (Riggi et al., 2013; Sharma et al., 2013; Tiwari & Cummins, 2013). Patil, Pike, and Hamilton (1995) showed that meteorological factors (including temperature and rainfall patterns) had a stronger influence on quercetin concentration in onion cultivars than soil factors or plant maturity. Mogren, Olsson, and Gertsson (2007) indicated a strong correlation between total radiation during lifting stages and the quercetin glucoside content of onions. However, Patil, Pike, and Hamilton (1995) reported no correlation between onion growth stage and quercetin content in onions. On the other hand, they showed a relationship between the quercetin content of onions and environmental factors such as location and soil type, which played a major role in affecting the levels of quercetins.

1.5.1 Light

Light can influence genetic expression that is relevant to enzymes participating in the phenylpropanoid pathway (Rodrigues et al., 2009). Light conditions during plant development and/or storage (in light or darkness) could play an important role in the total phenolic content (Rodrigues et al., 2009). Quercetins are strong UV-absorbing compounds, and can be accumulated mainly in the epidermal cells of plant tissues after UV-induction (Jaakola et al., 2004). Light stimulates the synthesis of quercetins, and L-phenylalanine ammonia-lyase (PAL) is the major inducible enzyme (Dioxn & Paiva, 1995). The formation of the quercetin glucoside (QG) is normally induced by UV light, which induces PAL activity up to 30-fold (Parr & Bolwell, 2000).

Yoo, Lee, and Patil (2013) exposed onions under different lights, and found that the synthesis of QG compounds is enhanced by UV light and, to a lesser extent, by visible light. In the sprouting leaves, 4’Qmg and 3,4’Qdg concentrations increased the most when exposed to UV light and, to a less extent, when exposed to visible

light; however, even the samples in dark conditions showed a slight increase in QG compounds.

Onions grown in full sunlight have been reported to contain higher levels of flavonoids (Rodrigues et al., 2011). A five-year study which examined the effect of climatic conditions on flavonoid contents in two Portuguese landrace onion varieties, total quercetin levels varied significantly between years, with highest levels quercetins observed in dry years (Rodrigues et al., 2011).

Mogren, Olsson, and Gertsson (2006) proposed that global radiation rather than temperature is the determining factor for quercetin glucoside biosynthesis in onions. Rodrigeus et al. (2011) pointed out that global radiation at the end of the onion production period seemed to be one of the major determinants of annual quercetin glucoside content in the onions. Mogren, Olsson, and Gertsson (2006) reported that, during a four-year study, the month with the lowest global radiation corresponded to the lowest levels of quercetin glucosides. Higashio et al. (2005) found that quercetin content in onion could be doubled after harvest using UV light lamps to irradiate the onion. Lee et al. (2008) conducted a similar study where they exposed onions to fluorescent light for 24 and 48 h, and showed that this induced time-dependent increases in quercetins content.

Light can also cause a stress signal enhancing flavonoid synthesis in some fresh cut foods (Cisneros-Zevallos, 2003). Under the light, an increase of flavonols (Lee et al., 2008) and quercetin (Higashio et al., 2007) has also been found in fresh-cut onions. Pérez-Gregorio et al. (2011a) also found that, with the light, total flavonoids in fresh-cut onions were increased by 58% and anthocyanins were increased by 39%.

1.5.2 Soil status

Patil, Pike, and Hamilton (1995) observed higher amounts of quercetin in onions grown in both clay and sandy loams soils with nitrogen limitation. However, the correlation between nitrogen stress and flavonols synthesis could not be verified due to the different growth stages of onions during experiments. On the other hand, they concluded that the site of growth, more so than the growth stage and soil type, is a major environmental factor in determining quercetin concentration in onion.

Application of fertiliser is one of the most dominant factors affecting the level of quercetins in onions (Mogren et al., 2008). Nitrogen, phosphorus and potassium are three major fertilisers required for optimum growth of plants, however, the amount and the method of fertiliser application also influence the level of quercetins. A decrease in soil nitrogen concentration may be associated with an increase in total quercetin concentration (Price, Bacon, & Rhodes, 1997), and a limited nitrogen supply was also reported to be associated with higher levels of phenolics in plants (Parr & Bolwell, 2000). However, either high or low levels of nitrogen fertilisers during growth of onions did not result in differences in quercetin content after field curing (Mogren et al., 2008).

Fertiliser application method and nitrogen source (organic and conventional fertilisers) can significantly affect bulb size, however without affecting total yield and quercetin content of dry bulbs (Mogren et al., 2008).

1.5.3 Agronomic conditions

Agronomic practices such as sowing date, fertilisation, irrigation, and subsequent harvesting would affect quercetins content in onions. Previous studies showed that organically grown onions had higher levels of flavonols and antioxidant capacity than conventionally grown onions (Ren et al., 2001; Faller & Fihlho, 2010).

However, Søltoft et al. (2010) found no significant differences between the conventionally and the organically grown onions in terms of quercetin content.

1.6 Changes in contents of the phenolic compounds in onions after cultivation

Onion bulbs are normally sown in March and generally mechanically harvested from late August to mid-September. Before harvest, they are lifted to stop their growth and cured by air drying at 25-28 ^{o}C and 65-75 % RH for ten days to six weeks in the UK and Ireland (Reilly et al., 2013).

Onion bulbs are ready for harvest as soon as the leaves of the plants (‘top’) start to recline (‘fall’). The usual practice is to harvest when 25-80% of tops have fallen, with consequently a significant effect on storage susceptibility and quality of the bulbs. Harvest stage can be essential for sprout incidence, since early lifting can result in lower sprouting percentage and better storage without negative effects on the initial quercetin content, which remains unchanged during storage (Mogren, Olsson, & Gertsson, 2006).

Quercetin content in onions increases after lifting and the lifting time would affect the increase. Mogren, Olsson, and Gertsson (2007) pointed out that an early lifting time of onions resulted in a reduced level of quercetin content in onions, probably due to low sprouting and lighter colour in the early lifted onions. On the other hand, late lifting (80% fallen leaves) resulted in up to 45% higher concentrations of quercetin glucosides compared to early lifting (50% fallen leaves).

1.6.1 Effect of curing on the phenolic compounds

Straight after harvest, bulbs have to be subjected to a drying process (‘curing’) in order to have their outer scales hardened and reduce skin cracks, and allow the necks to become narrower, thus inhibiting pathogen infections. Curing method (field curing or forced air curing) and conditions (temperature and relative humidity), as well as growing conditions and harvest stage, can be of major importance for maximum

quality of dry bulbs and minimum losses due to water losses and pathogen infections (Eshel et al., 2014).

Curing may result in an increase of flavonols in onions, although this phenomenon depends on the year and cultivars (Sharma et al., 2015b). Patil, Pike, and Hamilton (1995) indicated that onions cured in the field could accumulate more flavonols. Traditionally, in hot dry climates, onions can be left to cure in the field in windrows or mesh bags and this has been reported to be associated with the increase of quercetin content (Mogren, Olsson, & Gertsson, 2006). Rodrigues et al. (2009) also reported that field curing increased 4'Qmg and 3,4'Qdg (33–40%) content compared to levels at lifting, particularly when the flavonol concentrations were low at lifting. Light conditions (light or dark condition) during curing, however, did not affect flavonol and anthocyanin contents, regardless of skin colour and cultivar (Rodrigues et al., 2009).

The effect of cold storage combined with curing or post-curing treatment was studied by many researchers. Downes, Chope, and Terry (2009) cured two types of yellow onion and one kind of red onion at 20 ^{o}C, 24 ^{o}C or 28 ^{o}C for six weeks and then stored the onions at 1 ^{o}C for seven months. They found that levels of flavonols in Red onions cured at 20 ^{o}C decreased during cold storage for seven months.

The evolution of onion flavonols during storage after post-curing heat treatment at 36 ^{o}C for 24 or 96 hours was studied by Olsson, Gustavsson, and Vagen, (2010). Three onion varieties were cured in the field for two weeks and then heat treated, followed by subsequent cold storage at 2 ^{o}C for up to eight months. The levels of Q 3,4' D increased in the 24-hour. A lower content of total flavonols was found in the three onion varieties after eight months of cold storage following the 96-hour heat treatment, possibly due to negative effects of heat treatment on onion metabolism.

Price and Rhodes (1997) investigated levels of flavonols of two onion varieties ('Red Baron' and 'Crossbow') cured at 28 ^{o}C for ten days and stored for six months at $\leq$ 4

°C in dark, and found no change in the flavonols levels for the two varieties after storage.

1.6.2 Evolution of the phenolic compounds when sprouting

Post-harvest sprouting in onions would occur after long-term storage. Onions may be kept in cold storage at around 1-4 °C in the dark to induce dormancy and prevent sprouting, however, sprouting commonly initiates within one to three weeks after removal from cold storage (Sorensen & Grevsen, 2001). Benkeblia and Shiomi (2004) indicated that TPC in onions began to reduce when internal sprouting began, which can be caused by the temperature change (from cold to room temperature) during storage. In their study, the reduction also happened to onions stored in control conditions (in refrigerators) when internal sprouting began, after seven weeks of storage. Benkeblia et al. (2000) also reported that there was an inverse relationship between total phenolic content and sprout development.

However, conflicting results were reported by Sharma et al. (2014), who investigated the evolution of total flavonoid content (TFC) of onions during storage (post-storage) at room temperature and relatively humidity (RH) 60% - 80%, subsequent to a cold storage for eight months. They found that internal sprout was started within the 1st week of post-storage, but TFC increased during the post-storage time, and reached a maximum between the 4th and 8th weeks.

1.6.3 Evolution of the phenolic compounds during storage

Phenolic contents can be changed during storage (Dozio et al., 2015; Petropoulos et al., 2016; Petropoulos et al., 2017). Kevers et al. (2007) argued that, 23 days after purchasing, both antioxidant activity and the total flavonols of onions increased during storage and became 10 times higher than on purchase from the market. Sharma et al. (2014) also reported increased TPC and TFC during storage at room temperature after cold storage. However, due to sprouting and decay after four weeks

post-storage, they suggested the onions thereafter should be used only for, e.g. extraction of nutraceuticals. Rodrigue et al. (2010) stored Portuguese red and white onions after harvest for seven months under refrigeration (at 2 ^{o}C and 65% relative humidity) or under traditional bulk storage in the field. TFC in both of the two varieties increased up to 64% after six or seven months of storage, especially in the first three months (58% increase), irrespective of the storage temperature. Regarding anthocyanins, after seven months in both conditions (refrigeration and traditional treatment), the anthocyanin content was reduced by more than 40%. Elhassanneen and Sanad (2009) reported significant differences in flavonol content between white and red Egyptian onion varieties, with red varieties having a higher content of total flavonols, quercetin and quercetin glucosides after storage of three months period. Quercetin glycosides are not significantly affected by storage, however, the fact that these compounds are present mostly in the outer scales, which are severely affected by storage and usually discarded after the peeling of bulbs, could affect nutritional values and their intake on a daily basis (Lee & Mitchell, 2011).

It is believed that the phenolic compounds in onions could be influenced by storage conditions (Tiwari & Cummins, 2013). Storage conditions, like storage time, temperature and light have effects on the synthesis, retention or breakdown of quercetins. Sharma et al. (2015c) examined the effect of storage under aerobic and anaerobic conditions at ambient temperature, reporting that quercetin content increased significantly during anaerobic condition and that total phenolic and flavonoid contents were positively correlated with antioxidant activity. Light conditions and light quality during storage may affect quercetin and quercetin glucosides profile. Ko et al. (2015) examined the effect of five light wavelengths (dark, white, red, blue and UV-A light) for three days as a post-harvest treatment and found that white light treatment significantly increased quercetin glucoside content in peeled onion bulbs, as well as in bulb skins.

1.6.4 Effect of temperature during storage on phenolic compounds

The effects of storage temperature on phenolic compounds in onions have been investigated by a number of researchers. Patil, Pike, and Hamilton (1995) stored onion at 5 ^{o}C, 24 ^{o}C or 30 ^{o}C for up to five months, and an initial rise followed by a decline of quercetin levels. Changes were most pronounced under 24 ^{o}C treatment. They finally concluded that onion bulbs did not change in levels of quercetin content over the 5-month storage under controlled atmosphere (low temperature and low oxygen to ensure the least metabolism). Yoo, Lee, and Patil (2013) also reported that storage at 30 ^{o}C and 24 ^{o}C resulted in an increase of 3,4'Qdg, accompanied by a reduction of 4'Qmg. They did not observe significant changes in quercetin glucosides when bulbs were stored under controlled atmospheres; however, storage at 30 ^{o}C resulted in the increase by about 50% after three months of storage (Yoo, Lee, & Patil, 2013). Benkeblia (2000) evaluated total phenolics in onion bulbs during storage at 4 ^{o}C and 20 ^{o}C and observed a regular change in phenolics at both temperatures. Lachman et al. (2003) observed an increase in total flavonoids, especially at higher temperatures after 36 weeks of storage with red and yellow onion varieties. Ethylene was accumulated during onion storage which can stimulate the activity of PAL, a key enzyme in biosynthesis of phenolic compounds and accumulation of phenolic constituents (Benkeblia, 2000; Leja, Mareczeka, & Benb, 2003), which is in line with Rodrigues et al. (2010) who justify the significant increase in flavonols observed during storage. Benkeblia (2000) reported a positive relationship between PAL activity and total phenolic variations in long-term stored onion bulbs.

Most of the above mentioned studies were performed at elevated temperatures. On the other hand, the low temperature would minimise the growth of pathogens in the onions and also maintain bulb dormancy to delay physiological changes. Cold storage of onions was reasonably employed and its effect on phenolic compounds in onions (Gubb & MacTavish 2002; Brewster, 2008). Swedish onions showed a slight

decrease in QG during 1 °C storage (Mogren, Olsson, & Gertsson, 2007), while Polish onions stored at 1 °C maintained a nearly constant QG level (Grzelak et al., 2009). The QG concentration during storage showed different results. The level of 4'Qmg did not show any consistent changes, while 3,4'Qdg increased by 30-51% during eight months of storage at 2 °C in onions (Olsson, Gustavsson, & Vagen, 2010). In addition, Sharma et al. (2014) subjected onion bulbs after a storage period of eight months. From the results of their study it was supported that although under cold storage most physiological and enzymatic activities were delayed, a succession of internal changes was triggered, such as an increase in quercetin and quercetin glycoside contents, antioxidant activity and total phenolics (Sharma et al., 2014). These changes may be attributed to high respiration rates of bulb tissues, whereas infection by pathogens and sprouting incidence could play an important role in chemical composition changes (Chope, Terry, & White, 2007). Gennaro et al. (2002) investigated the effect of cold storage on total anthocyanins, and reported a total decrease of anthocyanins in red onions during the storage, with higher levels of loss of anthocyanins at 2 °C and 65% relative humidity.

Some researchers studied onion as a whole while others studied it as chopped one. Martinez et al. (2005) studied the effect of cold storage on chopped onions. They revealed that total quercetin (TQ) content increased by about 20% between 5 and 15 days at 4 °C but, after 30 days, the TQ content was similar to that of the control group. Similarly, chopped onions packed in polystyrene cups showed 28% and 58% increases in 3,4'Qdg and 4'Qmg in the dark and in the light, respectively, after 16 days at 1-2 °C (Pérez-Gregorio et al., 2011a).

All the aforementioned studies of cold storage of onions were above 0 °C. Pinho et al. (2015) evaluated the evolution of flavonoids of two Portuguese onion cultivars (Branca da Póvoa, white; and Vermelha da Póvoa, red) during storage below 0 °C, simulating domestically freezing conditions (–18 °C). Frozen portions of onions with different periods of domestic storage (3-5 months) at ambient temperature resulted in

increased flavonoid content when compared with the ones before freezing (portions from the same onions at room temperature). These results suggested that frozen storage of onions pieces positively affected flavonol metabolism. The authors therefore concluded that domestic freezing of onion portions extended onions shelf life and can be a good alternative to prevent the loss of unused fresh onions, preserving their antioxidant capacity.

1.6.5 Other storage technologies

Besides temperature and time control, many other storage technologies are employed to prolong onions or its products shelf life (Javier Moreno et al., 2006; Berno et al., 2014; Zudaire et al., 2017). Some studies have been carried out to investigate the effects of these technologies on the levels of phenolics, mainly flavonoid compounds, in onions during storage.

Drying technological developments are driven by consumers who demands for healthy, fresh-like, and convenient food. Hence, the effect of dehydration on onion quality was studied (Sahoo et al. 2015; Khan et al., 2016). Drying can prolong the shelf life of onions, and onions can be marketed as dried powder intended for culinary uses, by applying various drying processes (Alezandro et al. 2011; Mitra, Shrivastava, & Rao, 2015). Pérez-Gregorio et al. (2011a) pointed out that freeze-drying, as an innovative drying technique, used to dry chopped onions could prolong the shelf life of dried powder at room temperature for up to six months without significant quality losses in terms of antioxidant compounds (flavonols and anthocyanins), provided that they are stored under dark conditions and within air-tight containers. The freeze-drying process produces the highest-quality dried food product since the food structure is not damaged during sublimation, hence, it was verified that onion flavonoid content increases after the freeze-drying process (Pérez-Gregorio et al., 2011b). The stability of flavonoids of freeze-dried onion after long-term storage was mainly due to the inactivation of various enzymes, as well as

ethylene activity (Rodrigues et al., 2010). Moreover, the implementation of the freeze-drying technique itself resulted in an increase of the extractable flavonoids by 32 and 25% for flavonols and anthocyanins, respectively, because of the structural changes of bulbs tissues that made flavonoids more readily available (Pérez - Gregorio et al., 2011b).

The type of package could also be important for preserving fresh-cut onion slices' quality during storage. Pérez-Gregorio et al. (2011a) reported that storage in transparent polystyrene cups under light conditions resulted in increased total flavonols content, mostly due to the increased ethylene activity and the stimulating effect of the oxygen content of cups on the phenylalanine ammonia lyase activity. Marta et al. (2013) reported two different package systems for onion storage: the normal atmospheres (NA) and controlled atmosphere (CA) of the 4 compositions: (1) 5% CO_2 + 5% O_2, (2) 5% CO_2 + 2% O_2, (3) 2% CO_2 + 5% O_2, (4) 2% CO_2 + 2% O_2. They found that CA storage influenced the content of flavonoids in the bulbs. The highest contents of 3,4'Qdg and 4'Qmg showed increasing tendencies and the highest amounts of flavonoids in onion after storage at the gas composition of 5% CO_2 + 5% O_2.

Islek, Nilufer-Erdil, and Knuthsen (2015) investigated optimised storage conditions for sliced or fried onions, in terms of flavonoid in the onions during the storage. The studied conditions included atmospheric conditions (air, nitrogen and vacuum), temperature (ambient, +5 and -18 ^{o}C) and light (dark and light). They found that for sliced onions, dark conditions, in general, showed better flavonoids retain ability than light conditions for all atmospheric conditions, and that a nitrogen atmosphere gave the smallest losses of flavonoids. As for fried onions, they suggested vacuum drying storage conditions, as it caused a higher TFC, irrespective of light or dark conditions.

1.7 Variation of phenolic compounds during onion processing

Different processing treatments cause chemical and biochemical reactions in onion tissue. Such reactions could have an impact on the flavonoid structure, resulting in changes in the bioavailability and activity of these compounds (Rohn et al., 2007). In general, cooking of onions led to a decrease in total flavonol content in onions (Ioku et al., 2001), but these losses vary depending on the treatment conditions (Rodrigues et al., 2009). At the same time, quercetin in onion bulbs is remarkably resistant to degradation during many normal processing operations.

1.7.1 Minimal food processing

Many food processes like peeling, trimming, chopping, slicing, crushing, pressing and sieving of flavonoid-rich foods were studied (Ioannou et al., 2012). Processing is expected to affect content, activity and availability of bioactive compounds (Nicoli, Anese, & Parpinel, 1999). Table 1.3 lists the results of some studies on the effects of different minimal food processing on flavonoids and quercetins in onions. Ewald et al. (1999) pointed out that major losses of flavonoids took place during the pre-processing steps when parts of the product were removed, for example, onion trimming can result in 39% flavonoids loss and a 21% loss of total quercetin glucoside was found in onions after peeling (Gennaro et al., 2002). The similar trend (reduced to 29% – 36%) was found for the total antioxidant activity during minimal processing. Furthermore, Rodrigues et al. (2009) reported that chopping followed by refrigerated storage did not cause much change in the total levels of flavonols. Similar results were shown by Makris and Rossiter (2001), investigating flavonol content with the chopping of onion tissues. They, however, showed a loss of flavonol content ranging from 10.7% to 17.7% during prolonged maceration (5 h) of the onion bulb. However, that cutting increased flavonol content in fresh-cut onions (Pérez-Gregorio et al., 2011a). This could be caused by the fact that wounding enhances flavonol biosynthesis through the induction of Phenylalanine ammonia-lyase (PAL)

which is related to the wound-healing process in order to fight pathogen attack after tissue wounding (Tudela et al., 2002).

Table 1.3 Different minimal food processing and their effects on flavonoids content.

Minimal processing	Effects on flavonoids	References
Peeling	Reduction of 21% quercetins	Gennaro et al. (2002)
Chopping	No significant impact on quercetins content	Makris and Rossiter (2001); Rodrigues et al. (2009)
Maceration (5 h)	Loss of quercetins between10.7% to 17.7%	Makris and Rossiter (2001)
Cutting	Induction of flavonol biosynthesis	Pérez-Gregorio et al. (2011a); Ioannou et al. (2012); Bernaert et al. (2013)
Trimming	Losses of 39% flavonoids	Ewald et al. (1999)

1.7.2 Thermal processing

Heating can result in the oxidation, thermal degradation, and leaching of bioactive compounds of fresh vegetables (Kalt, 2005). Different heating conditions (e.g. heating duration and temperatures) have different effects on the antioxidant properties of vegetables. To obtain maximum health benefits, Faller and Fialho (2009) suggested that raw onions or moderately cooked onions are preferred

Tiwari and Cummins (2013) suggested that heating duration also had a strong influence on the stability of quercetins in onions. However, both losses and gains in phenolic compounds after heat treatment of onions have been reported by many researchers (Ewald et al., 1999; Makris & Rossiter, 2001; Rodrigues et al., 2009; Ozyurt et al., 2013; Harris et al., 2015; Islek et al., 2015; Juániz et al., 2016). Thermal processing procedures including boiling, frying, microwave heating and steam cooking could significantly degrade quercetin contents in onions (Juániz et al., 2016). Table 1.4 shows the influence of thermal processes on quercetin content of onions.

Table 1.4 Influence of thermal processes on quercetins content of onions.

Thermal processing	Effects on quercetins	References
Mild-heat for 1 min at 60°C	20% increase	Siddiq et al. (2013)
Griddled for 5 mins at 110°C	57.35% increase	Juániz et al. (2016)
Boiling for 5 mins at 100°C	18% decrease	Lombard et al. (2005)
Boiling for 10 mins at 100°C	more than 20% decrease	Gorinstein et al. (2009)
Boiling for 60 mins at 100°C	more than 20% decrease	Makris and Rossiter (2001)
Blanching for 1.5 mins at 100°C	10-25% decrease	Gorinstein et al. (2009)
Baking for 15 mins	7-30% decrease	Rodrigues et al. (2009)
Microwave cooking for 1 min	no significant effects on quercetins	Lee et al. (2008)
Microwave cooking (750w) for 4 mins	16-20% decrease	Lombard et al. (2005) Rodrigues et al. (2009)
Frying for 5 or 15 mins at 180°C	23-30% decrease	Price, Bacon, and Rhodes (1997)
Oven roasting for 15 or 30 mins at 180°C	No modification of the total levels of quercetins	Rodrigues et al. (2009)
Steaming	No significant effect on the content of quercetins	Lee et al. (2008)
Sautéing for 3 mins	no significant difference on quercetin	Lee et al. (2008)
Sautéing for 5 mins	25% increase 21% decrease	Lombard et al. (2005) Lee et al. (2008)

Rodrigues et al. (2009) reported that moderate microwave heating (450 W for 4 mins) did not affect flavonol contents, but intense microwave treatment (750 watts 4 mins) caused flavonol losses of 16% and 18% for 3,4'Qdg and 4'Qmg, respectively. Ewald et al. (1999) found that, after microwave cooking of onion with water, quercetin levels decreased considerably. In contrast, Ioku et al. (2001) revealed that microwave roasting without water was more favourable for the retention of quercetins in onion tissues.

Lee et al. (2008) reported that frying (180°C) decreased onion quercetins content by 25% to 33%. Price, Bacon, and Rhodes (1997) showed that 15 mins frying (180 °C) reduced levels of quercetin conjugates by 23% to 29%. Ewald et al. (1999) also found that frying (180 °C) for 5 mins in butter and rapeseed oil resulted in 24% and 39% losses of quercetin in onions, respectively. However, Rodrigues et al. (2009), in contrast, pointed out that frying with olive oil did not change the total levels of 3,4'Qdg and 4'Qmg.

Rodrigues et al. (2009) showed that oven roasting (180 °C) without water did not change the total levels of 3,4'Qdg and 4'Qmg. Rohn et al. (2007), on the other hand, observed that onion roasting for 60 mins at 180 °C led to the removal of sugar moiety resulting in the formation of 3,4'Qdg and 4'Qmg. They suggested that the sugar moiety attached at 3-position was more susceptible to thermal degradation compared to the sugar moiety attached at 4-position.

Boiling in water (100 °C) could cause a great loss of quercetins in onions as water soluble quercetins would migrate into cooking water during the boiling procedure (Rodrigues et al., 2009). Moreover, the level of quercetins in onions would decrease significantly with boiling time (Rodrigues et al., 2009). Boiling onions for 30 mins led to losses of quercetin glycosides, during which 37% 3,4'Qdg and 29% 4'Qmg leached to the boiling water without being degraded. A reduction of about 53% and 44% of 3,4'Qdg and 4'Qmg were reported during 60 mins of boiling of onions. Lombard et al. (2005) also reported 18% – 75% quercetin losses in onion boiled for 3 to 60 mins. Even though boiling onions would cause leach of the compounds, treating fresh-cut onion slices with hot water (50 °C) prior to storage resulted in lower weight losses and higher total phenolics comparing to a control to which no heat treatment was applied (Siddiq et al., 2013).

Steaming did not significantly affect quercetin contents of onions (Lee et al., 2008). Conversely, baking was found to increase quercetin glucosides in onions, as these compounds were concentrated in the tissues (Lombard et al., 2005). For anthocyanins, Rodrigues et al. (2009) suggested the level of decrease caused by cooking treatments was in the following order: frying > boiling > microwave roasting > oven roasting.

Sharma et al. (2015a) further investigated the effect of temperature on phenolic content and flavonoids in onion powders with different varieties. The heating temperature was scanned at 80 ^{o}C, 100 ^{o}C, 120^{o}C, and 150 ^{o}C for 30 mins each. The powders from all the varieties studied showed the same pattern in the heating effect: quercetin and its glucoside contents increased up to a certain temperature (e.g. 120 ^{o}C for the studied red-skinned variety) and then decreased at the highest temperature (e.g. 150 ^{o}C for the red-skinned variety).

The impact of cooking treatments (frying in olive oil, frying in sunflower oil and griddled) on phenolic compounds of onion was evaluated by Juániz et al. (2016). They found that all cooking treatments increase the concentration of flavonoid compounds in the onions. This is because thermal destruction of cell walls and sub cellular compartments during the cooking process released these compounds of onions. Griddle has a higher temperature in comparison with the frying process during treatment, and showed the highest amounts of phenolic compounds, by 57.35% compared to raw onions.

1.7.3 Non-thermal treatments

1.7.3.1 Ultraviolet (UV)

UV irradiation is currently used as a post-harvest treatment for sterilisation to inhibit sprouting and delay maturity (Higashio et al., 2007). Also, UV light could be used as a treatment in peeled or cut onions to increase quercetin content. Higashio et al. (2005) found that UV could not only reduce the incidence of spoilage moulds and survival of human pathogens, but also increase levels of quercetin and quercetin glucosides to fresh-cut onions. Rodov et al. (2010) found an increase in flavonol content of peeled onions treated with low (1.2 KJ/m^2) and medium (6.0 KJ/m^2) UV doses but a decrease in quercetin with high does (12 KJ/m^2).

1.7.3.2 High pressure

High pressure is more cost-efficient and environmental friendly with beneficial effects on bioactive content than other non-thermal treatment (Vikram, Ramesh, & Prapulla, 2005). Eduvigis et al. (2009) investigated the effects of high pressures (100–400 MPa) at (5-50 ^{o}C) for 5 mins on levels of flavonols content of onions. It

showed that processing onions at 400 MPa/5^{o}C could increase total phenol and flavonol by 33% Q 4’ G compared to untreated onions.

1.7.3.3 Chemical treatment of onions

Kamal et al. (2008) found that onion plants treated with benzothiadiazole (Bion) and dipotassium phosphate showed higher phenylalanine ammonia lyase (PAL) activity, PO activity, and phenolic contents than the untreated ones. They concluded that the application of simple chemical solutions could enhance phenolic compounds in onion plants.

Rodrigues et al. (2009) reported that ethylene treatments did affect the flavonol content of the edible portion of onions, with a significant increase. Heredia and Cisneros-Zevallos (2009) also found similar effects of exogenous application of ethylene on flavonol contents in two varieties of Portuguese onions. It was suggested that the significant increase in flavonols during storage could be caused by the action of ethylene (Leja, Mareczeka, & Benb, 2003). Ethylene can stimulate the activity of PAL, a key enzyme in the biosynthesis of flavonoid compounds (Leja, Mareczeka, & Benb, 2003), which is in response to biotic and abiotic stresses (Naoumkina et al., 2010).

1.8 Extraction of phenolic compounds from onions

Onions are a good resource of phenolic compounds, in particular, quercetin and its derivatives. The extraction of these compounds has been explored in a number of studies (Santas, Almajano, & Carbo, 2010; Sharma et al., 2016a; Singh, Krishan, & Shri, 2017; Viera et al., 2017). On the other hand, Sharma et al. (2014) suggested possible uses of sprouted and decayed onions as a source of quercetin and its glucosides. They further explained that, during post-storage, sprouted and decayed onions occurred in post-storage are usually unappealing to consumers and hence dumped as waste, however, during this period, there is an increase in the content of quercetin and its glucosides. Food-processing industry has further suggested the exploitation of onion waste as a food ingredient (Roldán et al., 2008), due to its associated health benefits (Corzo-Martínez, Corzo, & Villamiel, 2007; Manousaki, Jancheva, Grigorakis, & Makris, 2016).

The conventional way to extract flavonoids from plant material is SLE (solid liquid extraction) using organic solvents such as ethanol and methanol, either pure or mixed with water, although ethyl acetate, acetone and hexane have also been used to extract the compounds from the solid materials into the liquid solvents (Stalikas, 2007; Khiari, Makris, & Kefalas, 2008). Other normal physical measurements such as heating, boiling, pressing, blending, maceration, and mechanical fragmentation could be employed to assist the extraction, accelerating the extraction rate or increasing extraction ratio (Gorinstein et al., 2008). Rijke et al. (2006) employed ultrasonication, with water, methanol or acetonitrile, as a simple and easy method for flavonoids extraction. Those conventional methods could result in the degradation of some chemically sensitive phenols due to intensive mechanical disruption. In addition, the involvement of long extraction periods, severe heating and extensive use of organic solvents in the conventional extraction methods could lead to the release of oxidative enzymes that promote degradation (Zill-e-Human et al., 2011). Furthermore, the use of organic solvents, which are harmful both for the environment and for the persons working with them (Adekunte et al., 2010; Lindahl et al., 2013). Takahashi and Shibamoto (2008) reported extraction by steam distillation from onion sprouts and Singh et al. (2009) used a Soxhlet method for extraction from onion peel. An increased risk of degradation owing to long extraction times, and samples exposed to light and oxygen facilitates the degradation of flavonoids (Liazid et al., 2007).

In recent years, the use of new extraction techniques has increased, such as pressurised liquid extraction (PLE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurised hot water extraction (PHWE) (Ekman et al., 2013; Kumar et al., 2014). Table 1.5 lists some novel methods used in the extraction of phenolic compounds from onions. These novel methods are advantageous in comparison with conventional methods as the former requires shorter extraction periods and less organic solvents (Zill-e-Human et al., 2011).

PLE, as a novel method for extraction of flavonoids from onions, shows several advantages: simultaneous extraction, highly automated, small amounts of solvents are used, provide the cleanest extracts, and allow oxygen-sensitive flavonoids to be carried out in an inert atmosphere protected from light, however it can be time consuming (Søltoft et al., 2009).

Pressurised hot water extraction (PHWE) is an extraction technique that uses liquid water as extraction solvent at temperatures above the atmospheric boiling point of water (100 ºC /273 K, 0.1 MPa), but below the critical point of water (374 ºC /647 K, 22.1 MPa). PHWE has been used to extract quercetins from onion (Turner et al., 2006; Lindahl et al., 2013).

Lindahl et al. (2013) combined PHWE and enzymatic hydrolysis methods to exploit a continuous way of extraction of quercetin from onions. The optimised combined method achieved slightly higher extraction yield of quercetin within a much shorter period of time, compared to conventional methanol extraction. Compared to conventional extraction with acid-catalysed hydrolysis, the new method is more accurate. The continuous method combined extraction with hydrolysis in one step, which greatly reduced laboratory work.

Zill-e-Huma et al. (2009) used a microwave to assist extraction of polyphenols from onions. They regarded this novel method as a green technology. Chemat and Lucchesi (2006) suggested that microwaves accelerated the diffusion of secondary metabolites by increasing tissue softness and cell permeability. Microwaves also enhanced cell disruption due to their high penetration capacity, thereby increasing mass transfer within and outside the plant tissues.

Ultrasound processing on its own or in the combination with heat or pressure is an effective processing tool, which reduced processing time, achieved higher throughput, and lower energy consumption (Zenker, Heinz, & Knorr, 2003). Chemat, Zill-e-Huma and Khan (2011) reported that the application of ultrasound waves resulted in an increased yield of total phenolic content. Ultrasound-assisted extraction led to a yield of gallic acid equivalents (TPC) of 121±3.8 mg GAE/g dry weight, over 20% more than the yield by conventional maceration method (89.6 ± 2.3 mg GAE/g dry weight), in 30 mins of extraction.

Table 1.5 Previous studies on the use of novel techniques for extraction of onion bioactive compounds.

Extraction techniques	plant material	Extraction flavonoids	Solvent	Comments	References
ASE	Onion bulb sample	flavonoids	Methanol	Rapid, automatic, protected from light and no filtration necessary and low solvent consumption but possible degradation of compounds	Søltoft, Christensen, Nielsen, and Knuthsen (2009)
MAE	Edible of Yellow onion	Flavonoids	No water or solvent	Easy to handle and green, economical rapid produce, less energy but get a good percentage of yield, however extraction solvent must absorb microwave energy.	Zill-e-Huma et al. (2009); Zill-e-Huma et al. (2011); Kumar et al. (2014)
PHWE-EEH	Edible of Yellow onion	Quercetin glycosides	Water and ethanol	Higher quercetin extraction yield with short period of time and reduce the laboratory work and environmental impact. It is milder and more accurate.	Lindahl et al. (2013)
PLE	The edible portion of onion	Quercetin	Methanol	Allow extraction of oxygen sensitive flavonoids, highly automated method but limited application in the food industry due to the use of solvent	Søltoft, Christensen, Nielsen, and Knuthsen (2009)
SFE	Skins of red and yellow onions	Quercetin aglycone	Ethanol	Controlled pressure and temperature conditions for alternative of conventional methods, rapid , low solvent consumption but high cost and long extraction, many parameters to optimize	Martino and Guyer (2004)
SWE	Wastes of red and yellow onion Onion skin	Quercetin Isorhamnetin	Water	More green procedure as water but the temperature might cause degradation of compounds in onion	Turner et al. (2006) Ko et al. (2011); Lee et al. (2014); Tomšik et al. (2017); Munir et al. (2018)

UAE	Onion by-products	Flavonols	Water	Easy to use but high solvent consumption	Chemat et al. (2011); Jang et al. (2013); Katsampa et al. (2015)
PLP	Onion by-products	Flavonoids	Water	Environmentally friendly technologies capable of providing high-quality and high-activity extracts without any solvent toxicity.	Manohar et al. (2017)

Note: ASE - Accelerated solvent extraction, MAE - microwave assisted extraction, PHWE-EEH - Pressurized hot water extraction-extraction-enzymatic hydrolysis, PLE - pressurized liquid extraction, SFE - supercritical fluid extraction, SWE - subcritical water extraction, UAE - Ultrasound assisted extraction, PLP- Pressurized low polarity water extraction.

Subcritical water extraction (SWE) is increasingly used in the preparation of environmental samples, and the extraction of natural products from herbs, plants, and foodstuffs (Plaza et al., 2010). Subcritical water, also called pressurised (hot) water refers to water at a temperature between 100 and 374 ^{o}C and a pressure which is high enough to maintain the liquid state (below the critical pressure of 22 MPa) (Karakama, 2011). Ko et al. (2011) employed this technology to achieve quercetin yield over eight-, six-, and four-fold higher than those obtained by conventional extraction methods using ethanol, methanol and water at the boiling point, respectively. These results indicated that SWE is a highly efficient method for recovering a valuable flavonoid, and quercetin from onion skin and it is a potentially useful technique for the extraction of other flavonoids to be used in nutraceuticals.

1.9 Conclusions

Onion, as an important crop in the Allium family, contains a high amount of phenolics compounds, which to a great extent attributes to human overall dietary intake. Furthermore, it also contributes to human health for its antioxidant, anti-inflammatory, and anticancer features. The outer layers of the onion bulb are not edible and normally removed before the thermal treatment, however, it contains a wide range of flavonoid compounds. It is worth noting that the content of the flavonoid compounds can be different in different onions, but the most predominantly difference can be found in different cultivars. For example, red onion cultivars generally contain the highest level of flavonoids. In addition, agronomic practices (sowing date, fertilisation, and harvesting time) can also affect the flavonoid content in onions.

The storage of onions is a complex process that can be influenced by many factors. In general, these factors can be divided into three categories: pre-harvest conditions, post-harvest conditions, and its physiology. Pre-harvest conditions that affect storability of onion bulbs are related to genetic background and growing conditions

include cultivar, fertilisers, and harvest stage. In addition, storage condition (time, temperature, relative humidity, controlled atmospheres) are post-harvest factors, which are important for retaining the high quality of bulbs as well as prolonging postharvest life of onions. Optimised storage condition can subsequently be employed after processing to further reduce the loss of phenolics content in raw and processed onions. Some new techniques such as different drying methods (microwave, vacuum oven), which are applied in onion production have benefits in retaining quality, and bioactive compounds and increasing storage potential during storage.

For food processors, food processing operations can be the most effective and efficient way to reduce the loss of flavonoid compounds in onions. Onions should be cooked (thermal processing) under a suitable time and temperature, which can cause enzyme inactivation, leading to a reduction in the degradation of flavonoids. However, overcooking should be avoided since it can result in the destruction of flavonoids. Moreover, several non-thermal techniques such as ultrasound/sonication, ultraviolet (UV), and different packaging techniques have been mostly investigated regarding the enhanced extraction method of flavonoid compounds from onions.

Extraction of the phenolics compounds in onions is interested by many researchers, due to their potential health benefits. The new extraction techniques (pressurized liquid extraction, microwave assisted extraction, and supercritical fluid extraction) have been used in the extraction of phenolic compounds from onions. These novel methods are advantageous in comparison with conventional methods as the former requires a shorter extraction period and less organic solvents. Most of the studies so far have been performed at a laboratory scale, but further research is necessary to apply the knowledge to the industry needs, assessing the viability of the extraction methods economically.

Chapter 2

Evaluation of Phenolic Content and Antioxidant Activity in Two Onion Varieties Grown under Organic and Conventional Production Systems

Abstract

Onions contain a number of bioactive compounds, in particular polyphenols. They are a rich source of such compounds in the human diet and offer significant health benefits to the consumer. Demand for organic crops is steadily increasing partly based on the expected health benefits of organic food consumption. The current study examines the influence of organic and conventional crop management practices on the phenolic content of onion. We examined the effect of conventional, organic, and mixed cultivation practices on the content of total phenolics, total flavonoids and antioxidant activity in two varieties of onion grown over four years in a split-plot factorial systems comparison trial. Levels of total phenolics and total flavonoids showed a significant year on year variation and were significantly different between organic and conventional production systems. The levels of total phenolics, total flavonoids and antioxidant activity in general were significantly higher (p<0.05) under fully organic compared to fully conventional management.

Keywords: Onion (*Allium cepa* L.); Organic; Conventional; Phenolics; Flavonoids; Antioxidants.

2.1 Introduction

The demand for organic food products has increased rapidly during recent years, (FiBL, 2013) partially due to the notion that health benefits are linked with the consumption of organic foods. Organic food is perceived to be more nutritious, better tasting, and environmentally friendlier compared to conventionally grown crops (Wang et al., 2008). Organic crop production in Europe is controlled by EU Council Regulation No 834/2007. Certified organic producers must follow interpretations of the guiding EU legislation set down by, and inspected by, National certification bodies. In Ireland the main organic certification bodies are IOFGA (Irish Organic Farmers and Growers Association) and the Organic Trust, Dublin. Broadly organic crops cannot be genetically engineered, or treated with synthetic fertilisers, or synthetic pesticides. This raises a question if these restrictions of cultivation practices have any impact on plant metabolites, particularly secondary metabolites. Scientific studies have shown that organic cultivation directly impacts on the levels of secondary metabolites, mainly polyphenols, in fruits and vegetables (Asami et al, 2003; Barański et al., 2014). In addition to organic practices, the concentration of polyphenols in edible plants is affected by other factors such as cultivar and variety selection (Vågen & Slimestad, 2008), tissue maturity and damage at harvest: stress (pathogen infection and pest attack) (Dixon & Paiva, 1995; Ren et al., 2001), climate and soil microenvironment, fertiliser regime, temperature, irradiation, and post-harvest treatment (Manach, Scalbert, & Morand, 2004). Relative to conventional systems, organic systems may increase the exposure of crops to such stresses, thus inducing the synthesis of secondary metabolites (Manach, Scalbert, & Morand, 2004; Faller & Fialho, 2010). The polyphenols are ‘natural antioxidants’ and have received huge attention in recent times due to their diverse health enhancing properties by preventing oxidative damage to cellular macromolecules and organelles (Benbrook, 2005; Conklin, 2000; Zhou & Yu, 2006; Lee, Patil, & Yoo, 2015). Given that increasing evidence indicates a role for plant phenolics especially flavonoids in human health, efforts need to be directed in understanding the relationship between cultivation practices and phenolic levels in crops (Asami et al., 2003). There is a volume of scientific data in a relatively large number of studies showing the impact of the organic cultivation on the concentration of secondary metabolites with antioxidant activity, including a wide range of nutritionally desirable phenolics in

edible plants (Hallmann & Rembiałkowska, 2006; Cooper et al., 2011; Hajslova et al., 2013; Średnicka-Tober et al., 2013; Pérez-Gregorio et al., 2014; Barański et al., 2014; Valverde et al., 2015). The higher concentrations of a wide range of phenolics found in organic crops/crop-based foods may indicate the greatest potential nutritional benefits (Barański et al., 2014). However, there is little information on the impact of various cultivation practices on the production of secondary metabolites in onions, which are a major source of polyphenols in the human diet, and are globally an important agricultural product with annual production of 82.82 MT (FAO, 2012). It has been reported that onions (*Allium cepa* L.) make the greatest contribution of antioxidant flavonoids to the Western European diet by virtue of their content and their frequency of consumption (Hertog, Hollman, & Katan, 1992) and bioactive phenolic compounds found in onions are widely recognized as beneficial to health with the potential to protect the body from some degenerative diseases (Yin & Cheng, 1998; Sing et al., 2009; Pérez-Gregorio et al., 2010; Lu et al., 2011; Tiwari & Cummins, 2013; Wang, Li, & Bai, 2017). Many reports have indicated that onions have a wide range of beneficial properties for human health, such as anti-cholesterolaemic (Yin & Cheng, 1998; Bahram-Parvar & Lim, 2018), anti-mutagenic (Singh et al., 2009; Sharma et al., 2016a) and antioxidant capacity (Pérez-Gregorio et al., 2010; Lu et al., 2011; Sharma et al., 2015a). There is an increasing attention on the antioxidant content of onion because regular consumption of onions is associated with a reduced risk of neurodegenerative disorders, many forms of cancer, and cataract formation (Roldán et al., 2008).

The objective of this study was to compare the total phenolic contents, total flavonoid contents and antioxidant activity in onions grown under organic, conventional and mixed cultivation practices in a multi-year experiment. The onion trials described here are from a long-term systems comparison trial with samples harvest from research plots in 2010 to 2013 collection.

2.2 Materials and methods

2.2.1 Field trial experiments

Dr. Kim Reilly, Dr. James Grant, and Dr. Leo Finn designed the trial and cultivated the onion samples for over four years, taking into account of different factors such as

the climate change. They aimed to investigate the association between two different production systems (organic and conventional). Components such as soil management and pest control were measured in order to compare between organic and conventional systems on phytochemical accumulation, bioactive compounds, phenolic profile, and antioxidant activity. This experimental chapter was, therefore, developed from this previous research design. The onions analysed were from the systems field trial carried out at Teagasc, Kinsealy (53° 25N, 6° 10W), Dublin, Ireland. The soil type at this location was loam to clay loam (altitude, 28m O.D.; slope, 1°; moderately well drained). The field trial was a factorial split plot design with four replicates (n=4) and followed commercial vegetable production practices in Ireland. There were two levels of soil treatment, namely (i) organic soil treatment (OS) and (ii) a conventional soil treatment (CS); and two levels of pest-control, namely (i) an organic pest-control treatment (OP) and (ii) a conventional pest-control treatment (CP). Two varieties (V1=Hyskin, V2=Red Baron) of each crop were grown every year (Appendix 1). Within each replicate (n=4) each crop was grown under eight possible treatment combinations (V1+OS+OP, V1+OS+CP, V1+CS+OP, V1+CS+CP, V2+OS+OP, V2+OS+CP, V2+CS+OP, V2+CS+CP) giving a total of 32 plots per crop per year. The trial was set up in spring 2009 on land that had previously been under long standing grass for more than 10 years. Organic cultivation practices used were in compliance with EC1990/92, EC834/200719 and as described previously (Reilly et al., 2013). The organic soil (OS) treatments consisted the use of certified organic fertilisers; a 4-year horticultural crop rotation including a fertility building red clover ley (*Trifolium pratense*); and use of winter cover crops. In contrast the conventional soil (CS) treatment used mineral fertilisers and no set crop rotation (crops randomly allocated each year) with no winter cover crop. Equivalent rates of nitrogen (N), phosphorus (P) and potassium (K) were applied to both CS and OS treatments following a spring soil test and the rates applied were according to Teagasc recommendations for the crop (Coulter & Lalor, 2008). The fertiliser was applied as a mixture of calcium ammonium nitrate, single super-phosphate and sulfate of potash for the CS treatment; or Greenvale fertiliser (4.5:3:3) and ProKali (3:0:14) for the OS treatment. Conventional pest-control (CP) treatments comprised pesticide applications against weeds, pests and diseases typical of commercial vegetable production and in accordance with Alexander (2011, 2013).

Organic pest-control (OP) treatments comprised mechanical weed and pest-control methods, certified treatments of biological origin if required and provision of a refuge area to encourage beneficial insects. Applied inputs for onion cultivation in 2010-2013 are shown in Table 2.1 Additional information on the field trial layout is available at http://www.ipfn.ie/publications/agronomic.

For experimental plots, onions bulbs were harvested at commercial maturity stages from the internal rows with guard rows excluded. After harvesting, three disease free onions of similar size were taken as a composite sample from each plot. Samples for analysis were immediately refrigerated and then frozen at -20 ^{o}C within 24 hours of harvest. Frozen samples were freeze-dried in a large scale freeze drier (Cuddon Frozen Dry, Blenhein, New Zealand). Once freeze-dried, samples were vacuum packed in polypropylene bags and kept at -20 ^{o}C until analysis.

Table 2.1 Specific pest-control and soil treatment inputs used in the Teagasc Kinsealy Systems Comparison trial for onion cultivation 2010-2013.

PEST-CONTROL TREATMENT	Organic Pest-control (OP)	Mechanical weeding (hand hoeing). *Serenade[3] (10 L/ha)
	Conventional Pest-control (CP)	*Proplant[2] (10ml m^2 modular drench) , Roundup[1] (4L/ha) , Stomp[1] (3.3L/ha), CICP[1] (4.2L/ha), Defy[1] (3.3L/ha), *Totril[1] (1.8L /ha), Stratos Ultra[1] (4 L/ha), Penncozeb[2] (4.4 kg/ha). Folio Gold [2] (2L/ha), Amistar[2] (1L/ha).
SOIL TREATMENT	Organic Soil (OS)	Previous crop – broccoli Fertiliser (adjusted to) N 70 kg/ha P 20 kg/ha K 215 kg/ha Applied as Greenvale plant food (4.5:3:3) (pelleted chicken manure + calcified seaweed) and ProKali (3:0:14). A top dress equivalent to 35 kg/ha N, and contributing 25 kg/ha P and 24 kg/ha K was applied in June or July.
	Conventional Soil (CS)	Previous crop – broccoli / carrot / lettuce Fertiliser (adjusted to) - N 70 kg/ha P 20 kg/ha K 215 kg/ha Applied as CAN (27% N), single superphosphate (7.8%P) and sulphate of potash (42% K). A top dress equivalent to 35 kg/ha N, 25 kg/ha P and 24 kg/ha K was applied in June or July.

[1] Herbicide, [2] Fungicide. [3] Fungicide (certified organic). * Not applied in all years. Treatment codes: OP= organic pest-control, CP= conventional pest-control, OS=organic soil treatment, CS=conventional soil treatment.

2.2.2 Extraction and analysis of phenolic compounds

In each drying replicate, 100 g of onion slices were distributed uniformly as a thin layer onto stainless steel trays of size 20 × 10 cm and were dried by Freeze-drying. Freeze-drying was carried out in a Cuddon freeze-drier (FD80, Cuddon Freeze Dry, Blenheim, New Zealand) at a temperature of 40 ^{o}C and a pressure of 0.064 mBar for 72 h, according to the procedure described by Hossain et al. (2010).

Freeze-dried onions were milled using a kitchen blender (Kenwood Limited, Havant, UK). The powdered onions (1 g) were mixed with 10 ml of 80% methanol and homogenized with an Omni-prep multisample homogeniser (Omni International, GA, USA) at 24,000 rpm (Appendix 2). The homogenised sample suspension was shaken

8 hours with a V400 Multitude Vortexer (Alpha laboratories, North York, On, Canada) at 1500 rpm at room temperature. The sample suspension was then centrifuged for 20 mins at 3000 g (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK) and filtered through 0.22 μm polytetrafluoroethylene filters. The extracts were kept at -20 ^{o}C for subsequent analysis.

2.2.3 Analysis of total phenolics

Total phenolics were determined using a modification of the Folin-Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Briefly 100 μL of methanolic extract, 100 μL of MeOH, 100 μL Folin-Ciocalteau reagent and 700 μL of Na_2CO_3 were added to 1.5 mL microcentrifuge tubes and the samples were vortexed. The tubes were then left in the dark for 20 mins at room temperature. Following this, the samples were centrifuged (Eppendorf, Centrifuge 5417R, Hamburg, Germany) at 17,900 g for 3 mins. The absorbance of the sample was read at 735 nm by a spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan) using aqueous gallic acid (10-400 mg/L) as a standard (Appendix 3). Results are expressed as milligrams of gallic acid equivalents per gram on a dry weight basis (GAE mg/g DW). All measurements were carried out in triplicate.

2.2.4 Analysis of total flavonoids

Total flavonoid content was determined using the method described by Lin and Tang (2007). Briefly, 100 μL of methanolic extract was mixed with 300 μL of 95% ethanol, 40 μL of 10% $AlCl_3$, 40 μL of 1.0 M potassium acetate and 520 μL of distilled water. After incubation at room temperature for 40 mins, the absorbance of the reaction mixture was measured against blank at 415 nm using a spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan). Quercetin was used to develop a standard calibration curve (Appendix 3) and the total flavonoid content was expressed as milligrams of quercetin equivalents per gram dry weight (QE mg/g DW). All measurements were carried out in triplicate.

2.2.5 Analysis of antioxidant activity

2.2.5.1 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the method of Stratil, Klejdus, and Kuban (2006) with slight modification. The FRAP solution was freshly prepared on the day of use, by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively. Following this, the FRAP solution was heated, while protected from light, until it had reached a temperature of 37 ºC. Appropriate dilutions of onion methanolic extracts were prepared by diluting 10-fold in methanol. 100 µL of the diluted sample extract or for blank (100 µL methanol) and for Trolox standard curves 100 µL Trolox of appropriate concentration and 900 µL of FRAP solution were added into a micro-centrifuge tube (Appendix 3). The tubes were vortexed and left at 37 ºC for exactly 40 mins, and the absorbance was measured at 593 nm using spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan). The antioxidant activity of the samples was expressed in milligram Trolox equivalents per gram dry weight sample (TE mg/g DW). All measurements were carried out in triplicate.

2.2.5.2 DPPH antioxidant power assay

The DPPH scavenging activity assay was performed as per the method described by Goupy et al. (1999) with a slight modification. 2, 2-diphenylpicrylhydrazyl (DPPH) was dissolved in methanol to a concentration of 0.238 mg/mL in a conical flask. The reagent was prepared 2 hours prior to use, to ensure that the DPPH has fully dissolved and stabilised. The flask containing DPPH solution was covered with aluminium foil to protect from the light and stored in the refrigerator. For the actual measurement a 1 in 5 dilution of the DPPH stock was made using 10 ml of stock and making up to the 50 ml with methanol. Trolox (1-10 µg/mL) dissolved in methanol in appropriate dilution was used to make the standard curve (Appendix 3). This experiment was carried out in three replicates for both samples and standard. In each replicate, 500 µL from the appropriately diluted sample extract was added to 500 µL DPPH solutions. Experiments were carried out to determine the exact dilutions required. In the control, 500 µL of methanol was added in place of sample extract with an equal volume of DPPH solution. As for blank, 500 µL sample extract was

mixed with 500 μL methanol. The absorbance was measured at 515 nm by spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan). The radical scavenging activity was expressed in terms of mg Trolox equivalent per gram of dry weight (TE mg/g DW). All measurements were carried out in triplicate.

2.2.6 Statistical analysis

Statistical analysis was carried out using SAS 9.1 (Cary, NC). Total phenolic, total flavonoid, FRAP and DPPH data were analysed using an ANOVA mixed model containing a contrast code to compare the fully organic (OS+OP) and fully conventional (CS+CP) treatments as well the individual treatments and interactions. Pearson correlation coefficients were calculated between total phenolics, flavonoids and antioxidant activity using SAS 9.1 software.

2.3 Results and discussion

2.3.1 Total phenolic and total flavonoid content

The present study investigated the free phenolics of onion, as they constitute approximately 90% of the total onion polyphenols (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). Levels of total free phenolics (TPC) in the year 2010 were considerably higher in ‘Red Baron’ with values ranging from 6.36±0.02 GAE mg/g DW to 7.75±0.1 GAE mg/g DW than ‘Hyskin’ which had TPC values in the range of 5.49 ±0.10 GAE mg/g DW to 7.21±0.01 GAE mg/g DW (Table 2.2). ‘Red Baron’ consistently maintained higher levels of TPC across treatments and years ending in 2013. The levels of total phenolics and flavonoids reported here are in agreement with levels found in onion varieties in other studies (Shon et al., 2004; Rodrigues et al., 2010). The finding of consistently higher levels of polyphenols in ‘Red Baron’ is of relevance from a health perspective. ‘Red Baron’ is a deep red coloured onion while ‘Hyskin’ is a brown skinned, white fleshed onion. Thus, it is expected that ‘Red Baron’ would contain higher levels of anthocyanins (phenolic compounds) than its counterpart ‘Hyskin’. This reflected in the higher levels of total flavonoid content (TFC) values in ‘Red Baron’ than ‘Hyskin’ (Table 2.3). Although ‘Red Baron’ had higher TPC values than ‘Hyskin’ across the four years, the TPC data among the years in both the varieties were inconsistent. Data indicated that in the year 2010, a poor year for crop growth, total phenolic contents of ‘Red Baron’ across in treatments and

OS+CP treatment 'Hyskin' were higher than those of the year 2011. We ascribe this result to increased stress (low temperature and higher humidity) which might have caused a generalised increase in total phenolic content through up-regulation of the phenylalanine ammonia lyase (PAL), the key entry point enzyme for synthesis of phenolic compounds. This enzyme is well known to be up-regulated by stresses including UV light, low temperature, nutrient deficiency, wounding and pest or pathogen on attack (Winkler et al., 2007). Following the year in 2011, the TPC values of both the varieties had increased significantly in the year 2012 and 2013, with the highest values in the year 2012 and 2013. This could be attributed to increased production of phenolics in response to stress caused by heavy rainfall and associated water logging of soils (Table 2.4). These data showed the complexity of regulation of levels of bioactive compounds in crop plants, which may be affected by genotype and also respond differently to the plant's environment.

Mixed model ANOVA showed that total phenolics content in general was significantly (p<0.05) higher in samples grown under fully organic treatment (organic soil and organic pest-control; OS+OP) compared to samples grown under completely conventional treatment (CS+CP) except in 2010. This was expected as the organically grown onions were probably more exposed to pest stress than the conventionally grown ones. However, the responses of the onions in the year 2010 were different due to poor environmental conditions of the year. The environmental stress might have outweighed the pest stress giving irregular patterns in their phenolic contents in the year 2010. As shown in Tables 2.2 and 2.3 significant interactions among varieties (V), soil (S) and pesticide (P) types (VxP, VxS, SxP and VxSxP) were observed but were not consistent across years. In contrast, significant main effects for variety and soil treatment were observed in all years, with significant pest-control treatment effects observed in most years. These data indicated that variety and soil treatment have a major influence on total phenolic and flavonoid content in onion, with the increased levels found in the red variety 'Red Baron' and when onions are grown under the organic soil (OS) treatment. In our study, equivalent rate of nitrogen (N) was applied to both CS and OS treatments in order to minimise any nutrient stress effects in the OS treatment. However, it is important to note that mineral feriliser is more immediately available to the crop, as organic fertilzer requires a breakdown by soil processes and therefore may show slower

availability. The actual difference perceived by the crop between the CS and OS treatments include differences in plant available N, P and K; differences in the soil microbiome as well as other unknown differences that may be present. A number of other studies have shown that total flavonoid decreased with increasing N application. For example, Stewart et al. (2001) found decreasing concentration of flavonoids when increasing N levels were applied in *Arabidopsis*. Groenbak et al. (2014) also found a decrease in flavonoids with increased N for kale. Sander and Heitefuss (1998) also reported that increasing mineral N fertilization resulted in reduced concentrations of phenolic compounds in wheat leaves. There is increasing evidence that differences in fertilization regimens between organic and conventional production systems are associated with significantly higher phenolic concentrations in organic crops (Rühmann et al., 2002); however, it is not clear if this is simply a nutrient stress effect or if other factors including effect of the soil microbiome or other factors, are involved. In onions, an extensive previous study found that fertiliser type (mineral vs organic) and placement of fertiliser in onion had little effect on quercetin production (Mogren, Olsson, & Gertsson, 2006; Mogren et al., 2008). A number of previous studies have indicated a significant genotype effect on total phenolic content and total flavonoids content profile in onion (Tiwari & Cummins, 2013; Reilly et al., 2014). The two onion varieties in this study showed a different quantitative behaviour with regards to total phenolics and total flavonoids content under the same meteorological conditions. The content of these secondary metabolites are highly variable, not only depending on the meteorological conditions and production, but also the cultivar and post-harvest practices. Hallmann and Rembiałkowska (2006) demonstrated that red onions grown organically contained more flavonoids compared with conventional samples. Ren et al. (2001) reported that organically grown Welsh onions had higher levels of flavonols and antioxidant activity than conventionally farmed ones. Faller and Fihlho (2010) reported that organic onion pulp had a higher antioxidant capacity than onions produced using conventional practices. Some research studies have also shown a slight yet significantly higher content of polyphenols in organic vegetables (Mitchell et al., 2007). Organic black currants and tomatoes contained significantly more compounds with antioxidant properties in comparison with currants grown under the conventional system (Hallmann, 2012; Kazimierczak et al., 2008). The concepts of

measures of plant defense and photosynthesis explain two prominent mass-balance based hypotheses of secondary metabolite production and they are carbon-nutrient balance hypothesis (CNBH) and the growth-differentiation balance hypothesis (GDBH). Both hypotheses were based on the assumption that excess resources (i.e. nutrients and carbon) in plants were transferred into defences as the growth was restricted before photosynthesis. In addition, a key postulate of the CNBH and the GDBH is that defences will also be increased even under limited conditions of growth when photosynthesis continues to function at normal levels (Massad, Dyer, & Vega, 2012). Hypotheses for higher content of these compounds in organic products include the Growth-Differentiation Balance Hypothesis (GDBH), the Carbon Nutrient Balance Hypothesis (CNBH) which imply that organically grown plants will produce more bioactive compounds, including phenolics, than plants grown conventionally (Caris-Veyrat et al., 2004; Massad, Dyer, & Vega, 2012) and the Cost-Benefit Hypothesis-CBH and the Resource Availability Hypothesis-RAH also designed by Growth Rate Hypothesis-GBH (Coley, Bryant, & Chapin, 1985; Herms & Mattson, 1992; Barton, 2008). The growth is limited by deficiencies in carbon (C) or nitrogen (N) while rates of photosynthesis remain unchanged. The subsequent reduced growth results in the more abundant resource being invested in increased defense. Most support for these hypotheses comes from work with phenolics (Massad, Dyer, & Vega, 2012). Recently, a new quality concept for organic produce - the inner quality concept (IQC) – based on the balance between plant growth and differentiation has been discussed in the literature. The hypothesis of the IQC is that where growth and differentiation are optimally balanced or "integrated", integration results in higher crop quality including nutrient and bioactive content (Bloksma et al., 2007).

Brandt and Mølgaard (2001) had initially proposed that it was natural for plants cultivated organically to contain more phenolics and other secondary metabolites as defensive compounds. However, the opposite tendencies of higher contents of polyphenols in conventional products have also been observed (Anttonen & Karjalainen, 2006). Søltoft et al. (2010) also found no significant differences between conventionally and organically grown onions in the content of flavonoids.

Table 2.2 Onion total phenolic content under different management practices between 2010 and 2013.

	2010	2011	2012	2013
Treatment	GAE mg/g DW	GAE mg/g DW	GAE mg/g DW	GAE mg/g DW
V1+OS+OP	5.49±0.10	6.31±0.29	7.52±0.01	6.96±0.03
V1+OS+CP	7.21±0.01	6.42±0.07	7.13±0.02	7.09±0.01
V1+CS+OP	5.79±0.03	6.00±0.13	7.34±0.02	6.48±0.21
V1+CS+CP	5.64±0.07	5.29±0.18	7.21±0.03	6.37±0.27
V2+OS+OP	6.71±0.14	6.55±0.28	8.42±0.23	9.74±0.23
V2+OS+CP	7.75±0.01	6.49±0.24	8.34±0.02	9.55±0.05
V2+CS+OP	6.36±0.02	6.26±0.21	8.16±0.02	9.15±0.11
V2+CS+CP	7.08±0.03	5.82±0.11	7.65±0.03	9.33±0.05
Statistical significance ANOVA P value				
Rep	0.0372	0.0794	0.1465	0.9677
Variety	**<0.0001**	**<0.0001**	**0.0005**	**0.0005**
Soil	**<0.0001**	**<0.0001**	**0.0006**	**0.0002**
Pest	**0.0001**	0.1638	**0.0104**	0.9979
Variety*soil	0.0582	0.2541	**0.0026**	0.3487
Variety*pest	0.1123	0.8219	0.7943	0.9750
Soil*pest	**<0.0001**	**0.0084**	0.4691	0.7659
Variety*soil*pest	**<0.0001**	0.2873	**0.0125**	0.1645
Fully conventional vs. fully organic	**<0.0001**	**<0.0001**	**<0.0001**	**0.0077**

Total phenolic content in 2 varieties of onion grown under different management practices. Data shown are mean and standard error of the mean (n=4). Since the difference between years was significant data for individual years is shown separately.Treatment codes: V1= 'Hyskin', V2= 'Red Baron' OS=organic soil treatment, CS=conventional soil treatment, OP=organic pest-control, CP=conventional pest-control ANOVA P values in bold type are significant at $p<0.05$.

Table 2.3 Onion total flavonoid content under different management practices between 2010 and 2013.

	2010	2011	2012	2013
Treatment	QE mg/g DW	QE mg/g DW	QE mg/g DW	QE mg/g DW
V1+OS+OP	2.70±0.03	3.68±0.08	4.19±0.03	3.70±0.40
V1+OS+CP	2.80±0.07	3.59±0.07	3.92±0.12	4.15±0.15
V1+CS+OP	2.42±0.03	3.27±0.07	4.06±0.04	3.07±0.15
V1+CS+CP	2.70±0.06	3.02±0.04	3.79±0.04	3.30±0.08
V2+OS+OP	2.83±0.06	4.70±0.14	4.54±0.06	4.48±0.40
V2+OS+CP	3.17±0.30	4.65±0.12	4.26±0.08	4.24±0.06
V2+CS+OP	2.65±0.10	4.60±0.02	4.24±0.11	4.00±0.17
V2+CS+CP	2.97±0.50	4.64±0.03	3.89±0.12	4.16±0.03
Statistical significance ANOVA P value				
Rep	0.4437	0.4830	0.0652	0.1858
Variety	**0.0021**	**0.0005**	**<0.0001**	**0.0001**
Soil	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**
Pest	**0.0061**	**0.0159**	**0.0001**	0.0788
Variety*soil	0.9666	**0.0008**	**<0.0001**	**0.0015**
Variety*pest	**0.0110**	0.1406	0.0940	**0.0055**
Soil*pest	0.1451	0.7251	0.1235	0.4751
Variety*soil*pest	0.0714	0.2615	0.1852	**0.0177**
Fully conventional vs. fully organic	0.1315	**<0.0001**	**<0.0001**	**0.0005**

Total flavonoid content in 2 varieties of onion grown under different management practices. Data shown are mean and standard error of the mean (n=4). Since the difference between years was significant data for individual years is shown separately. Treatment codes: V1= 'Hyskin', V2= 'Red Baron' OS=organic soil treatment, CS=conventional soil treatment, OP=organic pest-control, CP=conventional pest-control ANOVA P values in bold type are significant at $p<0.05$.

Table 2.4 Climate conditions during onion crop production in growing season from March to September between 2010 and 2013.

Year	T	TM	Tm	PP	V	RA/SN	H
2010	10.0	12.4	4.1	465.5	17.4	153	81.9
2011	11.7	13.8	6.0	351.3	20.7	163	76.2
2012	11.2	12.9	5.7	560.0	19.9	156	76.9
2013	11.2	13.1	5.7	438.7	20.3	165	78.0

T = Mean temperature (℃), TM = Mean maximum temperature (℃), Tm = Mean minimum temperature (℃), PP = Total monthly precipitation amount (mm), V = Mean wind speed (Km/h), RA = Daily indicator for occurrence of rain or drizzle (total days), SN =Indicator for occurrence of snow or ice Pellets. H = Mean humidity (%). (Source from Climate Dublin Airport)

The red onion 'Red Baron' did accumulate lower amounts of flavonoids in 2010, the year with the lowest temperature. Temperature is one of the most important factors affecting flavonoid accumulation in plants. Low temperature results in a reduction in photosynthesis, which reduces the soluble sugar content of tissues and leads to a repression of genes that encode enzymes of the flavonoids biosynthetic pathway and to a reduction in substrates for flavonoid biosynthesis (Ubi, 2004). Our results show that variety, soil management and meteorological factors have a marked influence on

the content of flavonoids in onions. Total flavonoids varied significantly among seasons, with higher levels in 2011, which was warmer and drier than 2010, 2012 and 2013 (Table 2.3). We hypothesise that since environmental conditions in 2011 were more favourable (higher average temperature, less rainfall days and less humidity), PAL was not up-regulated, and this a greater proportion of phenolic synthesis would have shunted towards flavonoid synthesis. The higher levels of total flavonoids in 2012 and 2013 could be the result of higher temperature levels and lower humidity. Variability in total phenolic and total flavonoids content data is normally considered as the response of crops to different climatic conditions. Differences in onion total phenolic and total flavonoids content due to environmental conditions in particular temperature and humidity have been reported in other studies (Rodrigues et al., 2011). In the four seasons reported here, humidity and daily indicator for occurrence of rain or drizzle (total days), were similar in both years, but rainfall levels were higher in the year 2010 and 2012 relative to the year 2011 and 2013. The higher levels of flavonoids observed in 2011 are probably related to the lower rainfall and humidity during the growing season, as onion plants are exposed to sunlight longer that may have triggered the increased production of flavonoids. Vegetables grown in full sun have been reported to contain higher levels of flavonoids, and exposure to sunlight is known to enhance production of flavonols in onion (Rodrigues et al., 2011). These meteorological conditions can enhance secondary metabolism, favouring the synthesis of flavonoids. In contrast, in the years with the lowest soil and air temperatures, higher relative humidity and higher soil water availability (2010), onions accumulated less flavonoids.

Table 2.4 shows the climatic conditions for both years, with 2011 being on average slightly warmer and less humid with total monthly precipitation amount (mm) in rainfall (351.3) over 8 months in growing season. Responses to environmental effects seem to be variable depending on varieties. ‘Red Baron’ showed differences in total phenolic content in 2012 and 2013, while ‘Hyskin’ showed little difference between 2012 and 2013. In other crops, studies in controlled growing environments have found that heat stress increases the total flavonoids content, with diverse results reported for low temperatures (Nitithamyong et al., 1999). Drought stress seems to increase the total flavonoid content (Bejarano et al., 2000). Accumulation of phenolics and higher activity of biosynthetic enzymes in response to drought stress

have also been reported in other plants. Chaves, Escudero, and Gutierrez-Merino (1997) demonstrated that drought and high temperatures are correlated with the increase of the more methylated flavonoids. In water-stressed plants, there is a general increase in the levels of phenolic compounds (Abreu & Mazzafera, 2005). Wang and Zheng (2001) found a strong correlation between temperature and production of phenolic in strawberry fruits.

2.3.2 Antioxidant activity

As shown in Tables 2.5 and 2.6, FRAP and DPPH scavenging activities were generally significantly higher under fully organic cultivation (OS+OP) than fully conventional cultivation (CS+CP) except for DPPH in 2010. Significant interactions (VxP, VxS, SxP and VxSxP) were observed but were not consistent across years. In contrast significant main effects for variety (V) and soil treatment (S) were observed in all years, with significant pest control treatment (P) observed in most years. We therefore postulate that, in addition to variety, soil treatment has a strong influence on antioxidant activity in onions.

Prior et al. (1998) reported that flavonoid compounds play an important role in the antioxidant capacity as compared to other phenolics compounds. However, due to the complex nature of phytochemicals, the total antioxidant activities of vegetables cannot be evaluated by a single method (Chu, Chang, & Hsu, 2000). Thus, it has been recommended that two or more methods should always be employed to evaluate the total antioxidant activity of vegetables (Dalamu et al., 2010). Accordingly we have employed two methods to measure the antioxidant activity: the FRAP and DPPH assays. There was a positive correlation between antioxidant activity and values of total phenolics and total flavonoids in onion samples. The antioxidant activity values as measured by DPPH assay were always less than those obtained from FRAP. Similar findings were observed in previous studies (Hossain et al., 2010; Hossain et al., 2014). According to Wang, Cao, and Prior (1996), the content of a single specific antioxidant compound is important, but it is better to analyse the total antioxidant activity for the overall health potential. Wang, Cao, and Prior (1996) indicated that the antioxidant activity is strongly affected by the cultivars within a species, but it can also be affected by the cultivation condition of the plant for example, environmental and cultivation techniques. Individual

parameters are very important for further understanding and establishing the relationships among antioxidant activity, total phenolics and flavonoids. Therefore, the coefficient of correlation was also calculated. The positive correlation between phenolic contents, flavonoids content and antioxidant activity suggests that plant phenolics are primarily responsible for the antioxidant activity in onion. This is similar to previous results obtained by Santas et al. (2008) which showed a relatively strong positive correlation r^2 =0.78 between FRAP and total phenolics for two cultivated onions varieties. Similarly, Nencini et al. (2007) reported r^2 =0.46 between FRAP and total phenolic content, determined across several *Allium* species. Table 2.7 shows correlation analysis for total phenolics, total flavonoids, FRAP and DPPH indicating that antioxidant activity correlated well with total phenolics and flavonoids.

Table 2.5 Total antioxidant capacity (FRAP assays) under different management practices between 2010 and 2013.

	2010	2011	2012	2013
Treatment	Trolox mg/g DW	Trolox mg/g DW	Trolox mg/g DW	Trolox mg/g DW
V1+OS+OP	7.70±0.04	8.55±0.47	10.96±0.18	11.01±0.05
V1+OS+CP	9.32±0.09	9.12±0.15	10.40±0.06	11.86±0.07
V1+CS+OP	7.63±0.02	8.20±0.07	10.69±0.04	11.06±0.06
V1+CS+CP	9.18±0.06	7.40±0.26	10.45±0.03	10.86±0.02
V2+OS+OP	8.09±0.03	9.81±0.38	11.61±0.22	12.11±0.15
V2+OS+CP	10.40±0.05	10.20±0.20	10.92±0.02	11.96±0.01
V2+CS+OP	8.04±0.08	8.51±0.51	10.79±0.02	10.98±0.07
V2+CS+CP	10.00±0.06	8.22±0.19	10.60±0.03	11.62±0.16
Statistical significance ANOVA P value				
Rep	0.6652	0.6763	0.5858	0.6688
Variety	**<0.0001**	**0.0180**	**0.0004**	**<0.0001**
Soil	**0.0476**	**<0.0001**	**0.0150**	**0.0014**
Pest	**<0.0001**	0.8870	**0.0145**	**0.0349**
Variety*soil	0.2931	0.1872	**0.0093**	0.0520
Variety*pest	**<0.0001**	0.7392	0.8313	0.5457
Soil*pest	0.0628	**0.0331**	**0.0180**	0.2996
Variety*soil*pest	0.2330	0.3971	0.5745	**<0.0001**
Fully conventional vs. fully organic	**<0.0001**	**<0.0001**	**<0.0001**	**0.0043**

Antioxidant activity (FRAP) in 2 varieties of onion grown under different management practices. Data shown are mean standard error of the mean (n=4). Since the difference between years was significant data for individual years are shown separately. Treatment codes: V1= 'Hyskin', V2= 'Red Baron' OS=organic soil treatment, CS=conventional soil treatment, OP=organic pest-control, CP=conventional pest-control ANOVA P values in bold type are significant at $p<0.05$.

Table 2.6 Total antioxidant capacity (DPPH assays) under different management practices between 2010 and 2013.

	2010	2011	2012	2013
Treatment	Trolox mg/g DW	Trolox mg/g DW	Trolox mg/g DW	Trolox mg/g DW
V1+OS+OP	2.87±0.08	3.06±0.09	4.97±0.04	4.11±0.10
V1+OS+CP	3.78±0.03	2.85±0.13	3.96±0.05	4.83±0.11
V1+CS+OP	2.80±0.09	2.83±0.03	4.33±0.19	3.52±0.11
V1+CS+CP	3.05±0.09	2.54±0.12	3.93±0.08	3.55±0.11
V2+OS+OP	3.39±0.05	3.78±0.13	5.01±0.06	5.13±0.12
V2+OS+CP	4.03±0.05	2.97±0.16	4.52±0.01	5.19±0.09
V2+CS+OP	3.10±0.04	2.95±0.05	4.43±0.01	4.53±0.07
V2+CS+CP	3.03±0.03	2.90±0.02	2.73±0.16	5.11±0.10
Statistical significance ANOVA P value				
Rep	0.9781	0.8512	0.9912	0.4616
Variety	**0.0089**	**0.0415**	0.2407	**0.0032**
Soil	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Pest	**0.0036**	**0.0253**	**0.0013**	**0.0044**
Variety*soil	**0.0195**	0.1998	**<0.0001**	**<0.001**
Variety*pest	**0.0064**	0.2066	**0.0146**	0.5960
Soil*pest	**<0.0001**	**0.0243**	0.0537	0.4602
Variety*soil*pest	0.8102	**0.00071**	**<0.0001**	**<0.0001**
Fully conventional vs.fully organic	0.1859	**<0.0001**	**<0.0001**	**0.0009**

Antioxidant activity (DPPH) in 2 varieties of onion grown under different management practices. Data shown are mean standard error of the mean (n=4). Since the difference between years was significant data for individual years is shown separately. Treatment codes: V1= 'Hyskin', V2= 'Redbaron' OS=organic soil treatment, CS=conventional soil treatment, OP=organic pest-control, CP=conventional pest-control ANOVA P values in bold type are significant at $p<0.05$.

Table 2.7 Correlation analysis for total phenolics, total flavonoids, FRAP and DPPH with 'Red Baron' and 'Hyskin' varieties.

2010 Red Baron	Total flavonoids	DPPH	FRAP
Total phenolics	0.9815	0.6044	0.8259
Total flavonoids	-------	0.5183	0.8338
DPPH		------	0.2161
2011 Red Baron	Total flavonoids	DPPH	FRAP
Total phenolics	0.5841	0.3578	0.8195
Total flavonoids	-------	0.7272	0.6846
DPPH		------	0.2305
2012 Red Baron	Total flavonoids	DPPH	FRAP
Total phenolics	0.8822	0.9657	0.7645
Total flavonoids	-------	0.9263	0.8356
DPPH		------	0.5961
2013 Red Baron	Total flavonoids	DPPH	FRAP
Total phenolics	0.9495	0.6058	0.9313
Total flavonoids	-------	0.5224	0.8421
DPPH		------	0.8356
2010 Hyskin	Total flavonoids	DPPH	FRAP
Total phenolics	0.2874	0.9144	0.3786
Total flavonoids	-------	0.5617	0.5256
DPPH		------	0.5949
2011 Hyskin	Total flavonoids	DPPH	FRAP
Total phenolics	0.9502	0.8790	0.7812
Total flavonoids	-------	0.9078	0.8887
DPPH		------	0.6541
2012 Hyskin	Total flavonoids	DPPH	FRAP
Total phenolics	0.6510	0.8348	0.9430
Total flavonoids	-------	0.8305	0.8437
DPPH		------	0.9564
2013Hyskin	Total flavonoids	DPPH	FRAP
Total phenolics	0.8302	0.8441	0.515
Total flavonoids	-------	0.9545	0.6347
DPPH		------	0.8078

2.4 Conclusions

Although there have been several studies analysing fruits and vegetables produced under organic and conventional production systems, relatively few robustly designed field trial studies have compared phenolic content and antioxidant content in onion crops grown under conventional, organic and mixed systems. This study measured levels of total phenolics, total flavonoids and antioxidant activity in onions grown over four years using either conventional (CS+CP), organic (OS+OP) or mixed (OS+CP, CS+OP) treatments. Our data indicated that total phenolic and flavonoid content in onion was generally higher in 'Red Baron' red onions and was significantly higher in organic (OS+OP) compared to conventional (CS+CP) production in both varieties in most years. Significant year to year variation was also observed which we attribute to altered regulation of phenolic synthesis in different years due to meteorological conditions.

Chapter 3

Effect of Storage on Chemical Composition, Antioxidant Activity and Quality in Organically and Conventionally Managed Systems on Fresh and Dried Onions (*Allium cepa* L.)

Abstract

The objectives of this experiment were to investigate storage potential of organic onions and conventional ones under different storage conditions (-20 ^{o}C, 5 ^{o}C and 25 ^{o}C with 60–75% relative humidity). Quality changes (dry matter, phenolic compounds, colour and antioxidant activity) of onion bulb were investigated.

In the first experiment, after storing for 10 weeks at -20 ^{o}C and 5 ^{o}C, the quality of fresh conventional onions showed no significant loss. The phenolic compounds in fresh conventional onions increased significantly stored at 5 ^{o}C and they were found higher than in fresh organic onions at 5 ^{o}C. In the second experiment, the storage stability of dried onions was assessed. Compared to the dried conventional onions, the quality of organic ones was more stable during storage and it can be evidenced that the quality parameters (phenolics compounds and antioxidant activity) of dried organic onions stored after 10 weeks were observed at about the same level as the original ones, particularly at -20 ^{o}C.

Keywords: Antioxidant activity; Storage conditions; Colour; Phenolic compounds; Organic and Conventional onions.

3.1 Introduction

Onion is one of the most essential cultivated crop, which has been consumed globally. The worldwide production of onion has increased by 25% over the past decade with now the total production of 83 million tonnes, considering the second most important horticultural crop after tomatoes (FAO, 2013). Due to its storage characteristics and durability for shipping, onion has been traded more widely than any other vegetables (Griffiths et al., 2002). Therefore, onion (*Allium cepa* L.) storage has become an important issue mainly for providing products for fresh market, export, and processing. With an efficient storage technique, customers can benefit from having the same quality of onion throughout the year. The quality of onion after storage is of high importance for consumers and hence several factors have been considered for optimum quality by researchers, such as genotype, and pre-harvest and post-harvest conditions. However, the present study investigated the pre-harvest and post-harvest practices that have been adopted by large growers and food companies in order to maintain the onion quality during storage. A change in the quality of stored onions is generally due to high catabolism of substrates, primarily carbohydrates, and other phytochemicals (Mogren, Olsson, & Gertsson, 2007). The quality is highly affected by water loss, sprouting, and rooting incidence and changes in chemical composition. Sprouting and rooting are the main factors that limit their storage period causing storage losses and the decrease of the quality of stored onion bulbs (Adamicki, 2005; Ilić, Milenković, Djurovka, & Trajković 2009; Sharma et al., 2015d). Storage conditions play an important role in controlling sprouting, rooting, and transpiration rate of onion bulbs, which ultimately affect the physiology and phytochemical properties of onions (Adamicki, 2005). Moreover, according to Grevsen and Sorensen (2004), genotype and pre-harvest conditions such as propagation method and harvest stage can reduce sprouting after storage.

Storage of onion bulbs can affect chemical composition during storage, and many changes have been reported, such as changes in content of flavonols (Price, Bacon, & Rhodes, 1997; Coolong, Randle, & Wicker, 2008; Grzelak et al., 2009; Pérez-Gregorio, García-Falcón, & Simal-Gándara, 2011; Pérez-Gregorio, Regueiro, González-Barreiro, Rial-Otero, & Simal-Gándara, 2011; Sharma et al., 2014; Dozio

et al., 2015; Sharma & Lee, 2016b) and antioxidant activity (Gennaro et al., 2002; Kevers et al., 2007; Pinho et al., 2015).

To the best of our knowledge, very little work has been done relating to the stability of chemical composition with respect to the quality of fresh and dried organic and conventional onions upon storage. The integrated studies including production systems, postharvest handling practices (storage temperature), and levels of bioactive compounds with antioxidant activity in onion have not been studied comprehensibly. Apart from the proper storage condition, the selection of the production systems is crucial in the food industry and for the retailers of onion for retaining high quality until the product reaches the consumers. The aim of this study was to evaluate the effect of storage conditions applied to fresh and dried organic and conventional onions quality and health-promoting bioactive compounds with antioxidant activity. The three storage temperatures used in this study (-20 ^{o}C, 5 ^{o}C and 25 ^{o}C) are commonly used in onion production systems. Changes in concentration of bioactive compounds, antioxidant activity and quality features during onion storage were periodically monitored.

3.2 Materials and methods

3.2.1 Sampling

Organically and conventionally grown onions (variety Red Baron) were obtained from the Horticulture Development Department in Teagasc, grown as part of the Kinsealy systems experiment, based in Kinsealy, North Dublin, Ireland. The experiments were divided into two parts. The first experiment (E1) was conducted under different temperature conditions. Organic and conventional onions in two separate net bags of 20 kg in each were transported to the lab, and bulbs in a weight range of 150–250 g with no visible defects were chosen for the study. They were stored in dark storage rooms at -20 ^{o}C, 5 ^{o}C, and 25 ^{o}C with 60-75% relative humidity, respectively. The onions were tested at 0, 1, 3, 5 and 10 weeks after after harvest (upon their arrival at the lab). For each test, three organic and three conventional onions were randomly sampled, weighed, and labelled. Weight loss and dry matter were measured in each bulb at 0, 1, 3, 5 and 10 weeks during storage. All measurements were carried out in triplicate.

In the second experiment (E2), three organic onion bulbs and three conventional onion bulbs were randomly selected to produce two types of freeze-dried powder samples, which were then weighed into tightly closed pouches, sealed and stored at −20 ºC, 5 ºC, and 25 ºC for 10 weeks. Samples were removed for analysis on 0, 1, 3, 5 and 10 weeks. All measurements were carried out in triplicate.

3.2.2 Dry matter content

The percentage of dry onion bulbs was determined by drying chopped samples (1 cm long × 1 cm wide and thickness of approximately 5 mm) of approximately 25 g in an oven with air circulation first at 80 ºC for 24 h and then at 105 ºC for 2 h. All determinations were made in triplicate.

3.2.3 Preparation of extracts from dried onions

The methods of solid/liquid extraction have been described in section 2.2.2.

3.2.4 Total phenolic content (TPC)

The methods of total phenolics have been described in section 2.2.3.

3.2.5 Total flavonoid content (TFC)

The methods of total flavonoids have been described in section 2.2.4.

3.2.6 Analysis of antioxidant activity

3.2.6.1 Ferric reducing antioxidant power (FRAP) assay

The methods of antioxidant activity as measured by FARP assay have been described in section 2.2.5.1.

3.2.6.2 DPPH antioxidant power assay

The methods of antioxidant activity as measured by DPPH assay have been described in section 2.2.5.2.

3.2.7 Quercetin and its glycosides

Reversed phase high performance liquid chromatography (RP-HPLC) of the filtered sample extracts was carried out according to the method of Tsao and Yang (2003)

using HPLC-DAD system (Shimadzu SPD-M10A). Flavonols were separated on a ZORBAX SB-C18 column, 4.6 mm x 150 mm, 5 μm particle size (Agilent Technologies, CA, USA) and the target compounds were detected at 360 nm. The mobile phase consisted of HPLC grade water with 0.05% trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.05% TFA (solvent B). The gradient involved a linear increase in the amount of solvent B (%B), which was set as follow: 0-15 mins, 12-21%; 15-25 mins, 21- 100%; and re-equilibrated to 12% B for the last 25-35 mins at a flow rate of 1 mL/min. Samples (10 μL) were injected and the separation of analytes achieved at 30 °C. The data were processed using SHIMADZU EZ START Version 7.3 software and concentrations of quercetin and various quercetin glucosides were calculated against authentic calibration standards (Q 3 G, Q 4' G, Q 3,4' D and Q) (Appendix 4-6). All measurements were carried out in triplicate.

3.2.8 Colour

Three onion slices were randomly selected from fresh onions to determine their colour. The colour at both sides (internal and external) of each slice was measured at room temperature using a Hunter Lab D25A DP-9000 colorimeter (Hunter Lab, Reston, VA, USA) calibrated against a white and a black tile (illuminant D65 and 100 observer angle). Colour variables (L*, a* and b*) were recorded: brightness coordinate L* represents the whiteness value of a colour and ranges from black at 0 to white at 100; chromaticity coordinate a* indicates red when positive and green when negative; and b* indicates yellow when positive and blue when negative (Doymaz, Tugrul, & Pala, 2006). For colour differences during storage, total colour change, ΔE, was given by the equation below (Vega-Gálvez et al., 2012):

$$\Delta E=\sqrt[2]{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

where L_0^*, a_0^*, and b_0^* are the values for raw onion samples.

According to Chen and Mujumdar (2009) ΔE values within the range of 0–0.5 indicate trace colour differences, ΔE = 0.5–1.5 slight colour differences, ΔE = 1.5–3.0 noticeable colour differences, ΔE = 3.0–6.0 appreciable colour differences, ΔE = 6.0–12.0 large colour differences, and ΔE > 12.0 very obvious colour differences.

3.2.9 Statistical analysis

Statistical analysis was carried out using SAS 9.1 (SAS Institute, Cary, NC, USA). To test the effect of factors (storage temperature, treatment system, and time) and their interactions on each measured parameter, the data were analysed by analysis of variance (ANOVA). Significant differences were accepted at the minimum probability level of $p<0.05$. The data for the total phenolics, total flavonoids, quercetin and its derivatives are reported as mean values ± standard deviation, and comparisons among the mean values were evaluated by the Tukey's test. All measurements were carried out in triplicate of each sample. Correlation between variables and factors were also analysed by Principal Component Regression (PCR) using the Unscrambler Software, Version 10.3 (CAMO ASA, Oslo, Norway) to achieve an overview of the correlation between variables and their contribution to the variation of temperature, treatment and time.

3.3 Results and discussion

3.3.1 Weight loss and dry matter (DM) during storage of onion bulbs

In our experiments, onion bulb weight decreased during storage at -20 ^{o}C, 5 ^{o}C, and 25 ^{o}C respectively and the weight loss was observed to be the most significant at 25 ^{o}C (Table 3.1). This can be caused by desiccation, respiration and sprouting, which are all related to temperature. It is believed that higher storage temperatures result in higher activity of water and hence lead to quality loss. Similar results have been found in several studies (Kamerbeek, 1962; Ward, 1976; Ilić, Milenković, Djurovka, & Trajković 2009; Sharma et al., 2015b; Sharma and Lee, 2016b).

The bulbs remained intact and healthy until the 3rd week, when visible signs of sprouting and decay started to appear. From the 3rd to the 7th week, intense sprouting occurred and parallel to its signs of decay also became more evident. By the 10th week, the decay process reached its peak level and the onion bulbs lost their spherical shape and were reduced to roughly half of the initial size.

The weight loss during the 25 ^{o}C storage was higher than in cold storage (-20 ^{o}C and 5 ^{o}C) mainly due to the lower relative humidity and desiccation at 25 ^{o}C. As seen in Table 3.1, the weight losses during storage for organic onions in E1 were 0.4% to 1.0%

at -20 ºC, 1.2% to 2.6% at 5 ºC and 2.1% to 6.1% at 25 ºC. The weight losses for conventional onions at -20 ºC, 5 ºC and 25 ºC in E1 were 0.4% to 1.1%, 1.1% to 2.7%, and 2.0% to 6.3%, respectively, similar to the rates found for organic onions.

In general, the percentage weight loss was 2 to 4 times higher for the storage at 25 ºC comparing to -20 ºC and 5 ºC, regardless the onion type (conventional or organic). High temperatures promoted sprouting incidence, resulting in rapid water loss and quality degradation. Sprouting was observed at all storage temperatures, but the time and rate of sprouting were different for each temperature. Adamicki (2005) have reported that storage potential of dry onion bulbs is dependent on storage conditions, whereas Gubb and MacTavish (2002) and Ilić, Milenković, Djurovka, and Trajković (2009) suggested that apart from storage conditions, genotype could also affect storage potential.

In the present study, there were no drastic changes in DM in organic and conventional onions during storage at three different temperatures. Only relatively small DM fluctuations were observed at 25 ºC (Table 3.1) due to the dormancy breakage, which is indicated by the onset of inner sprouting. Similarly, Chope, Cools, Hammond, Thompson, and Terry (2012) and Sharma and Lee (2016b) reported that the storage conditions have a small impact on the DM change, however, cultivar (i.e. genotypes) and physiological parameters (dormancy and sprout of onion) can have a greater influence in DM.

Table 3.1 Changes in the dry matter (DM) and cumulative percentage weight loss in onion bulbs stored at -20 ℃, 5 ℃ and 25 ℃.

Onion Treatment	Week	DM % at -20 ℃	Weight Loss % at -20 ℃	DM % at 5 ℃	Weight Loss % at 5 ℃	DM % at 25 ℃	Weight Loss % at 25 ℃
OSOP	0	11.1±0.6^{a}	--	11.1±0.6^{a}	--	11.1±0.6^{a}	--
	1	11.1±0.4^{a}	0.4±0.1^{ab}	11.1±0.3^{a}	1.2±0.2^{b}	11.0±0.3^{a}	2.1±0.3^{d}
	3	11.1±0.3^{a}	0.6±0.1^{ab}	11.1±0.3^{a}	1.4±0.1^{b}	10.8±0.3^{a}	3.4±0.8^{c}
	5	11.0±0.3^{a}	1.0±0.1^{a}	11.3±0.1^{a}	1.9±0.2^{ab}	9.6±0.3^{b}	4.7±1.1^{b}
	10	11.0±0.3^{a}	1.0±0.2^{a}	11.4±0.9^{a}	2.6±0.3^{a}	10.7±0.4^{a}	6.5±0.7^{a}
CSCP	0	11.1±0.6^{a}	--	11.1±0.6^{a}	--	11.1±0.6^{a}	--
	1	11.2±1.2^{a}	0.4±0.1^{ab}	11.2±1.2^{a}	1.1±0.2^{b}	11.0±0.8^{a}	2.0±0.1^{d}
	3	11.2±1.1^{a}	0.6±0.1^{ab}	11.3±0.9^{a}	1.4±0.3^{b}	11.1±0.9^{a}	3.3±0.4^{c}
	5	11.1±1.0^{a}	1.1±0.2^{a}	11.5±1.0^{a}	2.2±0.1^{ab}	10.5±1.0^{ab}	4.3±0.9^{b}
	10	11.1±1.0^{a}	1.1±0.3^{a}	11.3±1.1^{a}	2.7±0.3^{a}	11.0±1.0^{a}	6.3±0.2^{a}

Organic onion (OSOP): Organic Soil (OS) + Organic Pest control (OP); Conventional onion (CSCP): Conventional Soil (CS) + Conventional Pest-control (CP). Values are expressed as mean ± standard deviation for triplicates (n=3). For each column, values followed by the same letter are not statistically different at $p<0.05$.

3.3.2 Changes in total phenolic compounds of fresh organic and conventional onions during storage

In E1, TPC was significantly different at 0 days after harvest in organic and conventionally grown onions (Table 3.2). Although the TPC degradation was minimal in both organic and conventional onions during storage at -20 ^{o}C, 5 ^{o}C and 25 ^{o}C (Table 3.2), these losses were significantly higher at 25 ^{o}C than at other temperatures (-20 ^{o}C and 5 ^{o}C).

Variability in TPC degradation and accumulation is a very common phenomenon observed during storage. In plants, TPC is the first line of defence against oxidative stress occurring due to increased respiration during storage.

In E1, TPC increased immediately in the first week of storage for the conventional onion (CSCP). However, the TPC levels returned to the original concentrations by the 10th week of storage. Prolonged storage periods would in theory decrease the TPC due to excessive free radical accumulation as a result of increased respiration. The results of this study demonstrated that the TPC in onions is highly influenced by various factors including production system and storage time and temperature.

Onions grown under a conventional system had higher TPC than organic onions after harvest (Table 3.2). In the case of organic samples, TPC decreased at 25 ^{o}C during 10 weeks, but it was relatively stable at -20 ^{o}C and 5 ^{o}C. Similar result was reported by Benkeblia (2000), suggesting that the phenolic content of onion decreased during storage at 20 ^{o}C due to sprouting.

3.3.3 Changes in total flavonoid compounds of fresh organic and conventional onions during storage

For the fresh conventional onions (CSCP) stored at all temperatures, total flavonoids content (TFC) have a similar trend to TPC: conventional onions showed a steep TFC increase followed by a decrease and then an increase in the final week of storage (Table 3.2). The total flavonoids fluctuates in fresh conventional onions during storage, and the reasons for the fluctuation can be the distribution of different glucosidases in different parts of onion, which change as per the storage temperature. The total flavonoids in conventional onion (CSCP) during storage was higher

compared to the fresh (harvest) one. This could be a result of the reduction of sugar content that can serve as substrates for the synthesis of phenolic compounds and hence an increased level of flavonoids is expected (Al-Weshahy, El-Nokety, Bakhete, & Rao, 2013). For the fresh organic onions (OSOP), storage at all temperatures showed no significant increase in total flavonoids, which can be ascribed to morphogenesis (sprouting) and external decay (microbial, fungal attack) of bulbs.

Table 3.2 Changes in total and individual phenolic compounds of organic and conventional onions during 10 weeks of storage.

Storage at -20 ℃	Week	TPC	TFC	Storage at 5 ℃	Week	TPC	TFC	Storage at 25 ℃	Week	TPC	TFC
OSOP	0	7.37±0.42^{b}	2.65±0.28^{c}	OSOP	0	7.37±0.42^{cd}	2.65±0.28^{c}	OSOP	0	7.37±0.42^{b}	2.65±0.28^{bc}
	1	7.35±0.40^{b}	2.64±0.30^{c}		1	7.71±0.12^{c}	2.95±0.23^{c}		1	6.69±0.09^{c}	2.14±0.09^{cd}
	3	7.30±0.34^{b}	2.63±0.28^{c}		3	6.72±0.17^{d}	2.69±0.05^{c}		3	6.26±0.16^{cd}	2.03±0.10^{cde}
	5	7.33±0.42^{b}	2.65±0.28^{c}		5	6.49±0.39^{de}	2.56±0.08^{cd}		5	6.03±0.31^{d}	1.73±0.32^{de}
	10	7.31±0.42^{b}	2.65±0.28^{c}		10	7.30±0.40^{cd}	2.70±0.30^{c}		10	5.56±0.14^{e}	1.46±0.26^{e}
CSCP	0	8.72±0.17^{a}	3.73±0.05^{b}	CSCP	0	8.72±0.17^{b}	3.73±0.05^{b}	CSCP	0	8.72±0.17^{a}	3.73±0.05^{b}
	1	8.90±0.11^{a}	4.78±0.09^{a}		1	9.14±0.08^{a}	4.92±0.05^{a}		1	8.82±0.11^{a}	4.82±0.09^{a}
	3	7.88±0.23^{b}	3.96±0.13^{b}		3	8.54±0.16^{b}	4.71±0.06^{a}		3	8.29±0.12^{a}	3.90±0.11^{ab}
	5	7.51±0.16^{b}	3.75±0.07^{bc}		5	7.74±0.28^{bc}	3.83±0.05^{b}		5	7.35±0.21^{b}	3.63±0.07^{bc}
	10	8.88±0.10^{a}	4.85±0.10^{a}		10	9.10±0.10^{a}	4.90±0.15^{a}		10	8.79±0.01^{a}	4.80±0.10^{a}

Organic onion (OSOP): Organic Soil (OS) + Organic Pest control (OP); Conventional onion (CSCP): Conventional Soil (CS) + Conventional Pest-control (CP). TPC: Total phenolics content (mg of gallic acid equivalents per g of dry weight). Total flavonoids content (mg of quercetin equivalents per g of dry weight). Values are expressed as mean ± standard deviation on a dry weight basis in triplicate (n=3). For each column, values followed by the same letter are not statistically different at $p<0.05$.

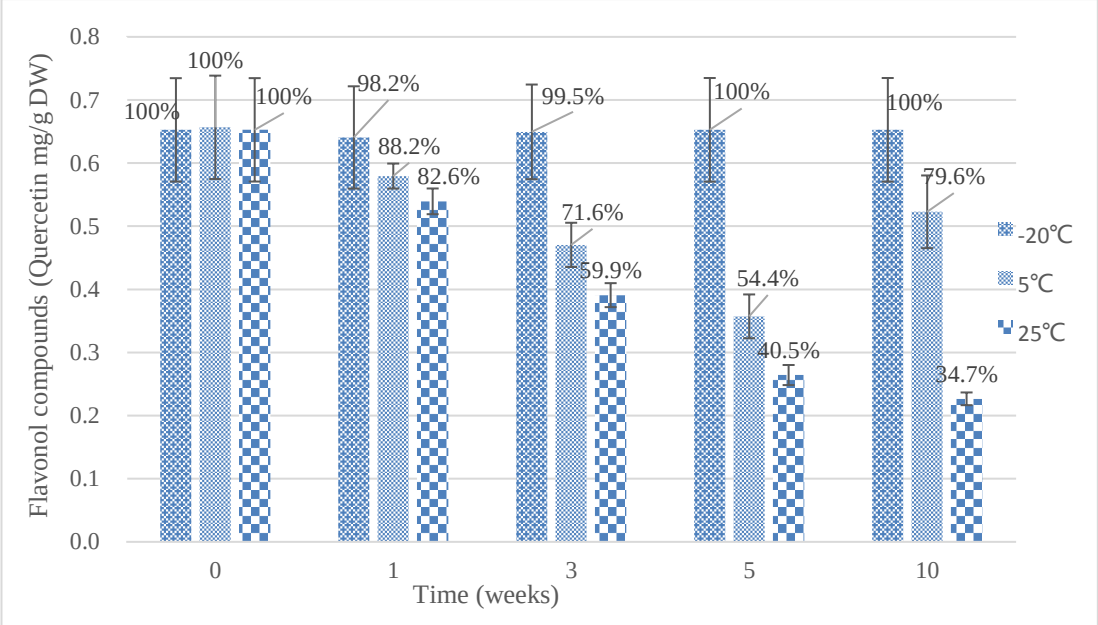
3.3.4 Changes in flavonols (quercetin and its glucosides) of fresh organic and conventional onions during storage

The flavonols (quercetin and its glucosides) changes for organic onions (OSOP) stored at 5 ºC and 25 ºC can also be divided into two stages. At the first stage (from the time of harvest to 1st week), the flavonols levels gradually decreased and there was no sprouting. At the second stage, from 3rd to 10th weeks, the onion decaying intensified and the flavonols content decreased continually. Gennaro et al. (2002) reported that the flavonoid in red onion tends to decrease after 6 weeks storage especially at 20 °C and 30 °C. However, the storage at -20 ºC prevented changes in flavonols levels, remain unchanged from harvest until 10 weeks of storage (Figure 3.1). In addition, low temperatures positively affect the biosynthesis of phenolic compounds and induce flavonoid accumulation in response to a biotic stress (Cisneros-Zevallos, 2003; Cantos et al., 2003).

For the fresh conventional onion (CSCP) stored at all temperatures, the content of flavonols (total quercetin and its derivatives) in general increased in the initial weeks and then decreased during the later weeks of post-storage. Benkeblia and Shiomi (2004) reported the levels of total phenolics increase during the 5 week-storage, and decrease after 7 weeks when internal sprouting began.

Based on our results, we can divide the post-storage changes of conventional onion bulbs (CSCP) into two stages. The first stage starts at the 1st week, with the flavonols levels increasing gradually with a simultaneous increase in total phenolics and flavonoids and the onion bulb remaining intact. The internal sprouting was observed within the 1st week and visible signs of sprouting appeared after the 3rd week. The second stage starts from the 10th week, during which sprouting becomes more intense and the maximum level of total phenolics and flavonoids with high antioxidant activity occurred. That is why end consumers prefer to use onion bulbs at the first stage of post-storage, after which onion bulbs are discarded as waste due to sprouting and decay. However, from the second stage, these waste conventional onions can be used as raw material for commercial flavonols extraction, as the onion bulbs exhibit the highest level of flavonols during this period. The findings showed an increase in the content of flavonols during post-storage, suggesting the possible use of sprouted and decayed onion as a source of quercetin and its glucosides.

(a)



(b)

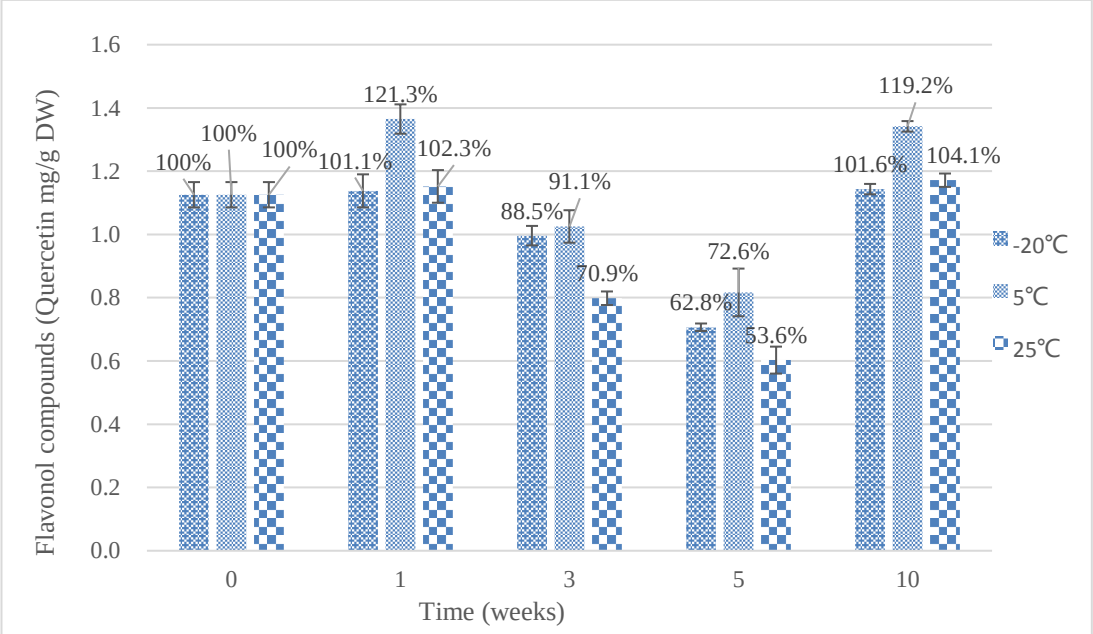


Figure 3.1

a - Effect of storage time at different temperatures on flavonols (quercetin and its glucosides) for storage fresh organic onion (OSOP).

b - Effect of storage time at different temperatures on flavonols (quercetin and its glucosides) for storage fresh conventional onion (CSCP).

3.3.5 Colour changes in fresh organic and conventional onions during storage

Organic and conventional onions in general were not significantly different regarding flesh and skin colour. Conventional onions had a slightly brighter red coloured flesh compared to organic onions. ΔE colour values before and after the 10 weeks storage changed significantly, with flesh and skin colour differences being higher at 25 ^{o}C compared to -20 ^{o}C and 5 ^{o}C. According to Chen and Mujumdar (2009), such colour differences were visually obvious (ΔE values: > 12.0) (Table 3.3). Furthermore, Downes, Chope, and Terry (2009) suggested that skin colour is highly prone to change when high storage temperatures applied, since flavonols content in skin may change. However, Eshel et al. (2014) did not found significant changes in hue angle values on skin of onion colour after five months of storage.

3.3.6 Influence of storage temperature and time on phenolic compounds in freeze-dried onions

Degradation of TPC was observed in E2 for dried conventional onions stored at all temperatures, while TPC remained more stable in organic onions (except 25 ^{o}C). In E2, dried organic onions showed significantly higher levels of TFC over conventional onions after harvest (Table 3.4). The TPC and TFC levels of dried onions decreased immediately in the first week of storage, but then reached stable concentrations afterwards until the 10th week.

Initial decrease in the phenolic compounds may be related to their stability during storage. Due to oxidative and enzymatic effects, polyphenols can easily breakdown to subunits. The decrease in phenolic compounds was related to temperature, with the maximum decrease at 25 ^{o}C. This suggests that the breakdown process in onions is prominent at higher temperatures. An increase in phenolic compounds during storage has been also reported in many vegetables and fruits including red beet, carrot, onion, and potato (Kevers et al., 2007; Koca & Karadeniz, 2008; Rodrigues et al., 2010; Al-Weshahy, El-Nokety, Bakhete, & Rao, 2013). The increase in phenolic compounds can be considered a response to the stress caused during storage.

Table 3.3 Flesh and skin colour of fresh organic and conventional onions during 10 weeks of storage at -20 ℃, 5 ℃ and 25 ℃.

Storage at -20 ℃	Flesh ΔE	Skin ΔE	Storage at 5 ℃	Flesh ΔE	Skin ΔE	Storage at 25 ℃	Flesh ΔE	Skin ΔE
OSOP	7.48±0.20[a]	13.10±0.51[a]	OSOP	14.70±1.02[b]	10.17±0.74[a]	OSOP	22.05±0.06[a]	21.34±3.22[a]
CSCP	5.65±0.07[b]	12.85±1.95[ab]	CSCP	15.05±0.30[ab]	9.85±0.55[ab]	CSCP	17.48±0.10[b]	16.79±0.73[b]

Organic onion (OSOP): Organic Soil (OS) + Organic Pest control (OP); Conventional onion (CSCP): Conventional Soil (CS) + Conventional Pest-control (CP).

ΔE = colour change (dimensionless). Values are expressed as mean ± standard deviation for triplicates (n=3).

For each column, values followed by the same letter are not statistically different at $p<0.05$.

Table 3.4 Changes in total and individual phenolic compounds of dried organic and conventional onions during 10 weeks of storage at -20 ℃, 5 ℃ and 25 ℃.

Storage at -20 ℃	Week	TPC	TFC	Storage at 5 ℃	Week	TPC	TFC	Storage at 25 ℃	Week	TPC	TFC
OSOP	0	7.07±0.12^{a}	3.57±0.02^{a}	OSOP	0	7.07±0.12^{a}	3.57±0.02^{a}	OSOP	0	7.07±0.12^{a}	3.57±0.02^{a}
	1	6.03±0.40^{c}	3.19±0.10^{c}		1	6.29±0.30^{bc}	3.32±0.11^{bc}		1	6.59±0.21^{b}	3.15±0.09^{bc}
	3	6.07±0.34^{c}	3.21±0.07^{c}		3	6.30±0.30^{bc}	3.37±0.13^{bc}		3	6.59±0.21^{b}	3.16±0.10^{bc}
	5	6.10±0.41^{c}	3.25±0.09^{c}		5	6.33±0.31^{bc}	3.39±0.19^{bc}		5	6.51±0.11^{b}	3.19±0.15^{bc}
	10	7.07±0.12^{a}	3.57±0.02^{a}		10	6.64±0.29^{ab}	3.48±0.09^{ab}		10	6.45±0.20^{b}	3.20±0.05^{bc}
CSCP	0	7.0±0.20^{a}	3.60±0.08^{a}	CSCP	0	7.0±0.20^{a}	3.6±0.08^{a}	CSCP	0	7.0±0.20^{a}	3.6±0.08^{a}
	1	5.24±0.32^{d}	2.30±0.19^{d}		1	5.80±0.40^{c}	3.07±0.05^{d}		1	5.36±0.15^{c}	2.36±0.31^{d}
	3	5.24±0.32^{d}	2.30±0.19^{d}		3	5.85±0.47^{c}	3.10±0.06^{d}		3	5.39±0.17^{c}	2.46±0.22^{d}
	5	5.20±0.30^{d}	2.33±0.21^{d}		5	5.81±0.41^{c}	3.0±0.03^{d}		5	5.40±0.11^{c}	2.40±0.30^{d}
	10	6.57±0.21^{b}	3.41±0.27^{b}		10	6.14±0.28^{bc}	3.25±0.03^{bcd}		10	5.30±0.10^{c}	2.31±0.20^{d}

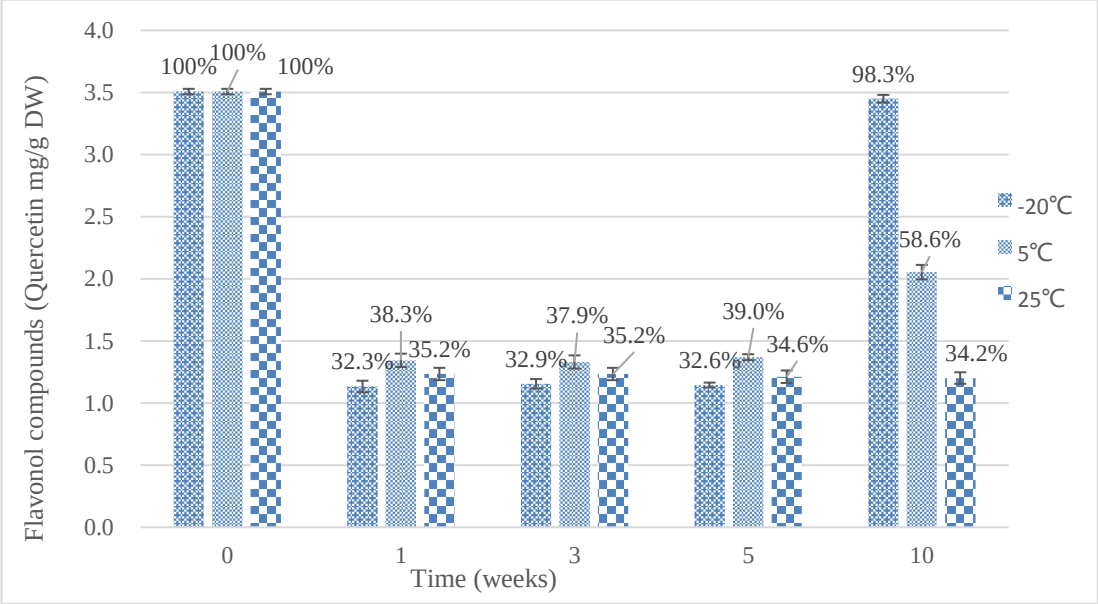
Organic onion (OSOP): Organic Soil (OS) + Organic Pest control (OP); Conventional onion (CSCP): Conventional Soil (CS) + Conventional Pest-control (CP). TPC: Total phenolics content (mg of gallic acid equivalents per g of dry weight) Total flavonoids content (mg of quercetin equivalents per g of dry weight). Values are expressed as mean ± standard deviation on a dry weight basis in triplicate (n=3). For each column, values followed by the same letter are not statistically different at $p<0.05$.

An increase in phenylalanine ammonialyase (PAL) activity may result in an increased level of phenolic compounds in dried onions at -20 ^{o}C (Shetty, Randhir, & Shetty, 2005). The increase of PAL leads to a low level of polyphenol oxidase activity, which may reduce the oxidation of phenolic substrates to quinines, and hence the levels of these compounds during storage increase (Leja, Mareczek, & Ben, 2003). In our study, we used freeze-dried onion samples, meaning that most of the water had been removed previously. However, it is possible that the residual moisture was sufficient to maintain the enzyme activity, leading to an increase in TFC. A similar result was observed in the storage of dehydrated plum, berries and potato (Del Caro et al., 2004; Michalczyk, Macura, & Matuszak, 2009; Al-Weshahy, El-Nokety, Bakhete, & Rao, 2013).

3.3.6.1 Long-term stability of quercetin and its glucosides

Freeze-dried onion powders were stored at -20 ^{o}C, 5 ^{o}C and 25 ^{o}C to investigate the stability of flavonols (quercetin and its glucosides) during storage. The levels of flavonols in dried organic and conventional onions significantly decreased during the first week of storage, which may due to the possible breakdown of the phenolic compounds at an early stage. At the third week of storage, the result further indicates that the flavonols become stable in dried organic and conventional onions for more than 5 weeks at room temperature (25 ^{o}C) and refrigerated temperatures (5 ^{o}C). Interestingly, the levels of flavonols increased significantly at the 10th week at -20 ^{o}C particularly in dried organic onion (Figure 3.2). It might be that the enzymes maintain their activity during storage and result in an increase in TFC, despite that it was at −20 °C and the moisture level was very low in the freeze-dried samples.

(a)



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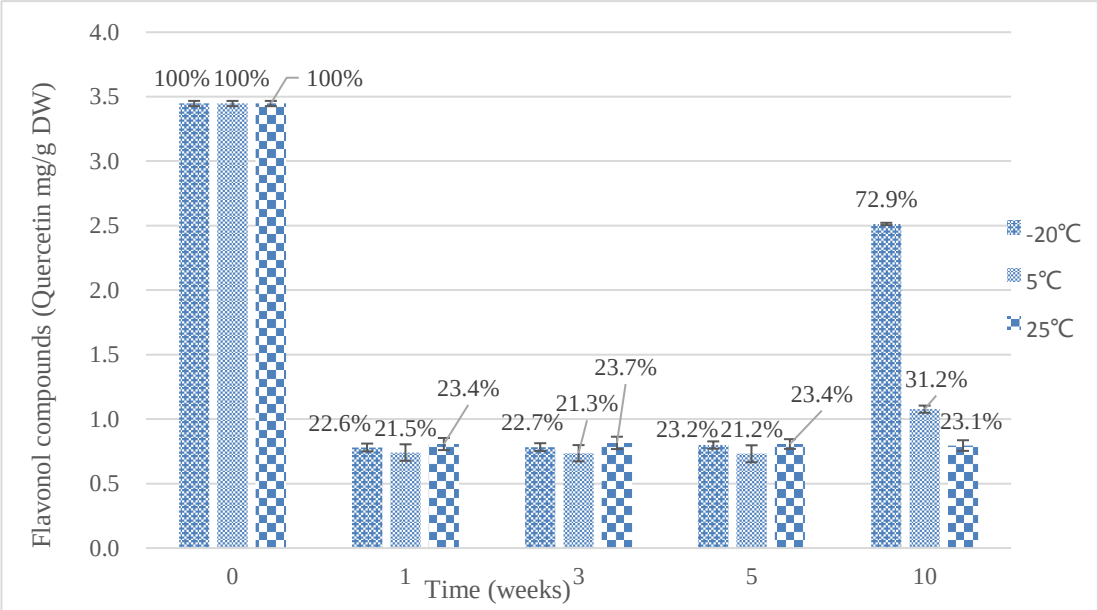


Figure 3.2

a - Effect of storage time at different temperatures on flavonols (quercetin + quercetin glucoside) for dried organic onion (OSOP).

b - Effect of storage time at different temperatures on flavonols (quercetin + quercetin glucoside) for dried conventional onion (CSCP).

3.3.7 Antioxidant activity of freeze-dried samples during storage

Principal component regression (PCR) of the whole data set shows that temperature and storage time affects the behaviour of the phenolic compounds (Figure 3.3).

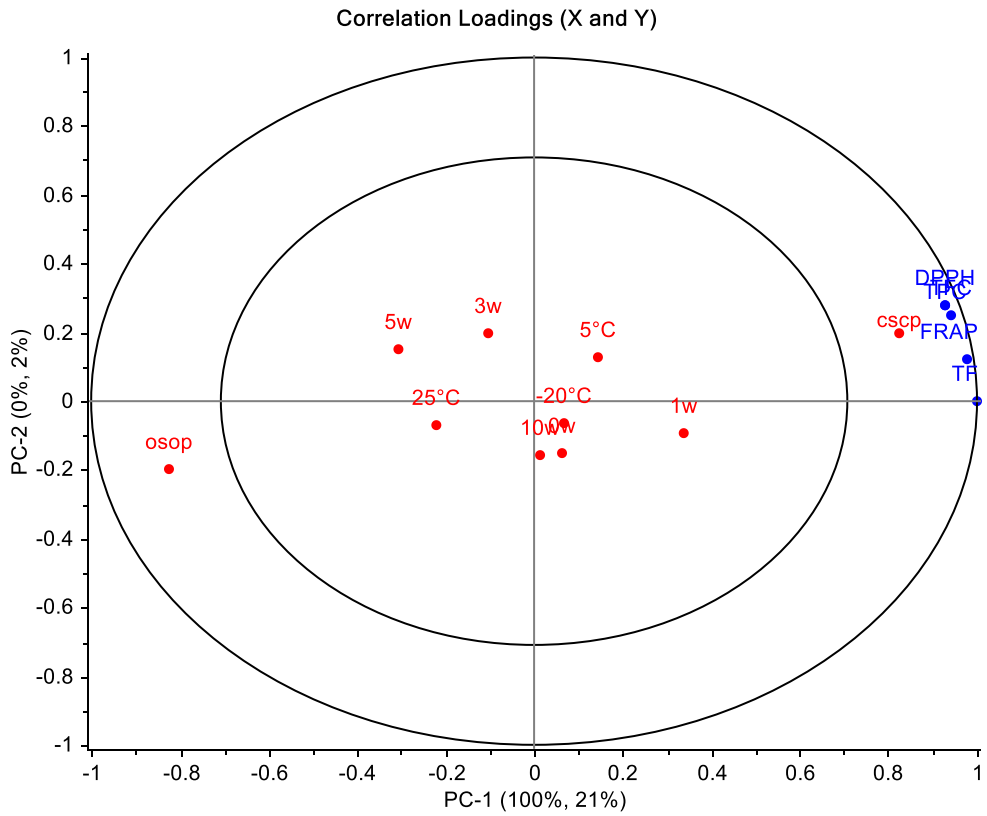


Figure 3.3 Principal Component Regression (PCR) biplot of PC1 versus PC2 for storage fresh onion. The model was derived from total and individual phenolics, flavonoids and antioxidant activity in the X-matrix, and temperature, time and treatment (organic (OSOP) and conventional (CSCP)) in the Y-matrix. Total phenolic content (TPC); Total flavonoid content (TFC); Total flavonol content (TF); Antioxidant activity (DPPH and FRAP); Organic soil (OS); Conventional soil (CS); Organic pest-control (OP); Conventional pest-control (CP).

The two onion treatments were placed in the diagonally opposite quadrant, indicating their opposite relationship of response to some parameters. The antioxidant capacities (DPPH and FRAP) of CSCP (conventional treatment) onions stored at 5 ^{o}C were located in the same quadrant and thus were considered positively correlated

with each other. CSCP (conventional treatment) onions stored at 5 ºC were negatively correlated with OSOP (organic treatment) onions stored at 25 ºC, which were located in diagonally opposed quadrants. The levels of flavonols located close to CSCP onions in the plot had a positive association with said samples at 5 ºC. Temperature plays an important role on the flavonoid content and antioxidant capacity of the onions, particularly at 5 ºC storage.

The second principal component distinguished the freeze-dried onions according to treatment (OSOP and CSCP) and also storage temperature and time. PC1 and PC2 explained 100% of the X matrix's variance and 20% of the Y matrix's variance (Figure 3.4). When the PCR was applied separately to all storage temperatures and times, the differences between organic and conventional dried onion samples could be easily observed. As noted previously, some individual flavonoids had higher concentrations at certain storage conditions for the fully organic treatment (OSOP). The contents of bioactive compounds and antioxidant activities were placed between the inner and outer ellipses, indicating that the antioxidant activity correlated well with total phenolics in E2.

In E2, the antioxidant activity of freeze-dried organic onion samples decreased after one week of storage and then remained unchanged until the 10th week followed by a significant increase at -20 ºC and 5 ºC. Due to the synergistic interaction between antioxidant activity and phenolic content, (Leo et al., 2008), the increase in antioxidant activity up to 10 weeks storage might be attributed to the production of phenolics in response to stress caused during -20 ºC and 5 ºC storage.

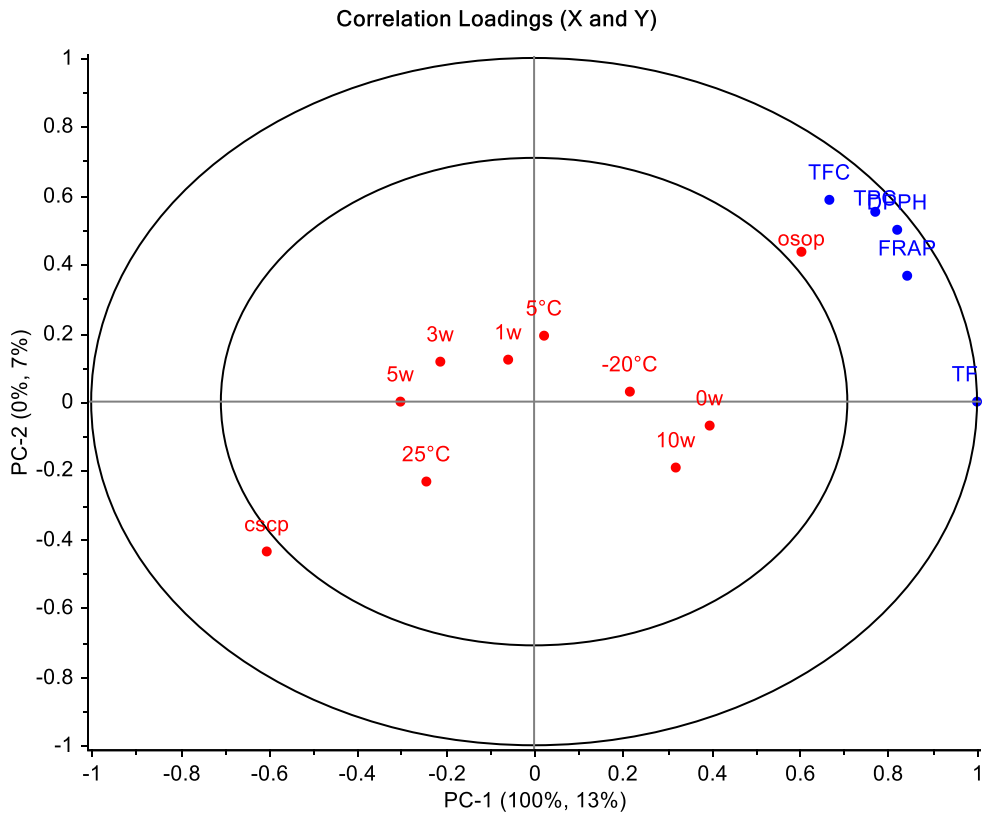


Figure 3.4 Principal Component Regression (PCR) biplot of PC1 versus PC2 for freeze-dried onions. The model was derived from total and individual phenolics, flavonoids and antioxidant activity in the X-matrix, and temperature, time and treatment (dried samples with organic (OSOP) and conventional (CSCP), respectively) in the Y- matrix. Total phenolic content (TPC); Total flavonoid content (TFC); Total flavonol content (TF); Antioxidant activity (DPPH and FRAP); Organic soil (OS); Conventional soil (CS); Organic pest-control (OP); Conventional pest-control (CP).

3.4 Conclusions

The aim of the present study was to evaluate the effects of storage conditions for fresh and dried organic onions in comparison with conventional onions on their quality, chemical composition and antioxidant activity. Three storage conditions were tested: storage temperature, time, and cultivar, which can influence the quality of the onion samples.

This study suggested that organic and conventional onion as a valuable source of phenolic compounds and antioxidant properties requiring proper storage conditions (-20 ^{o}C and 5 ^{o}C) to maintain the best quality for marketability. The results showed that phenolic contents and antioxidant activity were higher in fresh conventional onions than in fresh organic onions during storage at different temperatures (-20 ^{o}C, 5 ^{o}C and 25 ^{o}C).

After stored for 10 weeks, the percentage of flavonols in dried organic onions was remained the same at refrigerated (-20 ^{o}C). This is attributed to the enzymatic inactivation resulting from the freeze-drying process. Hence, freeze-drying is a good alternative to produce onion powder with an extended shelf-life and high nutritional quality.

Furthermore, significant differences in the parameters assessment have been found in fresh and dried organic and conventional onions. This may bring values to the food industry to adjust onion production systems for producing different onion products.

Chapter 4

Effects of Agronomic Practices and Drying Techniques on Nutritional and Quality Parameters of Onions (*Allium cepa* L.)

Abstract

The effects of four drying treatments (freeze-drying, hot air-drying, oven drying and vacuum oven drying) on bioactive compounds (total phenolics, total flavonoids and quercetins), colour and antioxidant capacity of organic and non-organic onions of two varieties (Red Baron and Hyfort) subjected to two agronomic treatments (organic and conventional) were investigated. The average final dry weight of the samples was 9.82 ± 0.41 %. After drying, there was a significant increase in total phenolics, total flavonoids and total quercetin and antioxidant capacity in comparison with fresh onion samples, which suggested that drying can improve the extractability of phenols and accordingly the antioxidant activity of onions. Different drying techniques also resulted in different fractions of individual quercetins. Dried organic onions had higher levels of bioactive compounds and antioxidant capacity than dried non-organic onions for a same variety. The highest antioxidant capacity displayed by freeze-dried and hot air-dried organic Red Baron onion is in agreement with their higher phenolic and flavonoid contents compared to all other samples.

Keywords: Antioxidant; Bioactive compounds; Colour; Drying methods; Organic onions.

4.1 Introduction

Onions correspond to the third world's highest production amongst the seven major vegetables consumed worldwide. The four major onion producing countries are China, with the largest production of 3.93 million tonnes, followed by India with 3.35 million tonnes, the USA with 2.45 million tonnes and Turkey with 1.55 million tonnes (Kumar, Hebbar, & Ramesh, 2006).

Onions are a good source of polyphenols such as flavonoids. It has been reported that onions are one of the vegetables which make the greatest contribution of antioxidant flavonoids to the Western European diet by virtue of their high content and frequency of consumption (Hertog, Hollman, & Katan, 1992; Arslan & Özcan, 2010). Many reports have indicated that onions have a wide range of beneficial properties for human health, such as anti-cholesterolaemic (Yin & Cheng, 1998), anti-mutagenic (Singh et al., 2009), and antioxidant capacity (Wang et al., 2011; Pérez-Gregorio et al., 2011; Russo et al., 2012; Sharma et al., 2015a; Valentová et al., 2016). There is an increasing attention on the antioxidant content of onions because the regular consumption is associated with reduced risk of neurodegenerative disorders, many forms of cancer, and cataract formation (Roldán, Sánchez-Moreno, de Ancos, & Cano, 2008). Phenolic compounds, important natural bioactives found in onions, are widely recognized for their health benefits regarding the potential to protect the body from some diseases (Tiwari & Cummins, 2013).

Conventional agricultural practices utilize high-yield crop cultivars, chemical fertilisers and pesticides, irrigation, and mechanization. Although conventional practices result in reliable high-yield crops, there is concern regarding the negative biological and environmental consequences and long-term sustainability associated with these practices. Organic food is perceived to be more nutritious, better tasting, and environmentally friendlier compared to conventionally grown crops. Relative to

conventional systems, organic systems may increase the exposure of crops to stresses, thus inducing the synthesis of secondary metabolites (Asami et al., 2003).

Onions are widely used in both fresh and dried forms. Dried onions, a product of considerable importance in world trade, can be marketed in several forms: flaked, minced, chopped and powdered (Sarsavadia, 2007; Arslan & Özcan, 2010; Sahoo et al., 2015; Khan et al., 2016). They are used as a flavour additive in a wide variety of food formulations such as comminuted meats, sauces, soups, salad dressings and pickle relishes, dry soup mixes, cheeses, crackers and other snacks and special food products (Arslan & Özcan, 2010; Mitra, Shrivastava, & Rao, 2012). Drying is one of the most widely used methods for vegetable preservation. It can inhibit enzymatic degradation and limit microbial growth (Doymaz & İsmail, 2011). Drying can also prolong shelf life, and reduce packaging cost, shipping weights and environmental impacts. Dried foods can be easily reconstituted without substantial loss of flavour, taste, colour and aroma (Sarsavadia, 2007; Pérez-Gregorio et al., 2011b). Effective drying of onion not only prolongs the shelf life of the final product but also stabilises its healthy compounds.

Several commercial drying technologies can be used to dry onions. The objective of this study was to investigate variations of quality regarding colour, antioxidant capacity and content of bioactive compounds (such as phenolics, flavonoids and quercetins) in onions dried by different drying methods (freeze-drying, hot-air drying, vacuum oven drying and oven drying) in comparison with fresh samples. The information obtained can aid food professionals in choosing the most appropriate drying technology to minimize quality degradation in terms of the investigated parameters.

4.2 Materials and methods

4.2.1 Agronomic practices

Two different varieties of onion (Red Baron and Hyfort) were used. They were cultivated by Teagasc, Kinsealy located in the North County Dublin, Ireland. Essentially agricultural management was composed of two aspects – soil treatment (how the soil is fertilized and managed) and pest-control (how biological pests such as weeds, insects, and microbial diseases are managed). Therefore, conventional agriculture consists of conventional soil (CS) treatment with conventional post-control (CP); while organic agriculture consists of organic soil treatment (OS) with organic pest-control (OP). Thus, two agronomic treatments were employed (OSOP and CSCP) to obtain completely organic and non-organic onions.

As published in detail by Reilly et al. (2013) the organic soil (OS) treatments comprehended the use of certified organic fertilisers, winter cover crops, and a 4-year horticultural crop rotation including a fertility building red clover ley (*Trifolium pratense* L.). In contrast, the conventional soil (CS) treatment used mineral fertilisers and no set crop rotation (crops randomly allocated each year) with no winter cover crop. Equivalent rates of nitrogen (N), phosphorus (P) and potassium (K) were applied to both CS and OS treatments following a spring soil test and the rates applied were according to Teagasc recommendations for the crop. The fertiliser was applied as a mixture of calcium ammonium nitrate, single super-phosphate and sulfate of potash for the CS treatment Greenvale fertiliser (4.5:3:3) and ProKali (3:0:14) were used in the OS treatment. Conventional pest-control (CP) treatments comprised pesticide applications against weeds, pests and diseases typical of commercial vegetable production and in accordance with Alexander (2011, 2013). Organic pest-control (OP) treatments comprised mechanical weed and pest-control methods, certified treatments of biological origin if required, and provision of a refuge area to encourage beneficial insects.

4.2.2 Drying

After harvest, healthy, disease-free onions of six replicates were chosen for each variety and each treatment. The onions were peeled, had both ends removed, and were cut into slices of approximately 1 cm long × 1 cm wide and thickness of approximately 5 mm.

In each drying replicate, 50 g of onion slices were distributed uniformly as a thin layer onto stainless steel trays of size 20 × 10 cm and were dried using one of the following four drying methods: freeze-drying, vacuum drying, hot air drying or oven drying. Freeze-drying was carried out in a Cuddon freeze-drier (FD80, Cuddon Freeze Dry, Blenheim, New Zealand) at a temperature of 40 ^{o}C and a pressure of 0.064 mBar for 72 h, according to the procedure described by Hossain et al. (2010). Vacuum drying was performed at 60 ^{o}C and 600 mBar for 16 h in a vacuum oven (VD 115, Binder, UK). Hot air drying was conducted in a constant air temperature oven (SG96⁄06⁄333, Gallenkamp, UK) at 60 ^{o}C for 16 h at an air velocity of 0.3 m/s. To determine the final moisture content, samples were oven dried (VD 53, Binder, UK) at 60 ^{o}C for 8 h, following the AOAC method 920.87.

4.2.3 Colour measurement

Three onion slices were randomly selected from fresh or dried samples to determine their colour. Instrumental colour at both (internal and external) sides of each slice was measured using a Hunter Lab D25A DP-9000 (Hunter Lab, Reston, VA, USA) calibrated against a white and a black tile (illuminant D65 and 100 observer angle). All onion samples from each triplicate were evaluated for colour at room temperature. Colour variables (L*, a* and b*) were recorded. The colour brightness coordinate L* indicates the whiteness of a sample and ranges from black at 0 to white at 100. The chromaticity coordinate a* indicates redness when positive and greenness when negative, while b* indicates yellowness when positive and blueness

when negative (Doymaz, Tugrul, & Pala, 2006). Colour change, ΔE, was given by the equation below (Vega-Gálvez et al., 2012):

$$\Delta E = \sqrt[2]{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

where L_0^*, a_0^*, and b_0^* are the values for raw onion samples.

Browning of samples was assessed using the Browning Index (BI), which was calculated using the equations below (Ruangchakpet & Tanaboon, 2007):

$$BI = 100(x - 0.31)/0.17 \quad (2)$$

where

$$x = (a^* + 1.75L^*)/(5.645L^* + a^* - 0.3012b^*) \quad (3)$$

4.2.4 Preparation of extracts from fresh and dried onions

Extracts from fresh or dried onions were prepared for further total phenolics content, total flavonoids content, and antioxidant analyses. The fresh onions were chopped into small pieces and then blended into semi-paste by a kitchen blender (BL335, Kenwood Limited, UK). As for the dried onions, they were blended directly with the kitchen blender. The blended samples (1 g) were immediately mixed with 10 mL of methanol (80%) and homogenised at 24,000 rpm using an Omni-prep multi-sample homogeniser (Omni International, USA). The homogenized sample suspension was shaken overnight with a V400 Multitude Vortexer (Alpha laboratories, North York, Canada) at 1500 rpm at room temperature. The sample suspension was then centrifuged (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK) at 3000 g for 15 mins and immediately filtered through 0.22 μm polytetrafluoroethylene filters. The extracts were kept at -20 ^{o}C until further analysis.

4.2.5 Analysis of total phenolics (TPC)

The methods of total phenolics have been described in section 2.2.3.

4.2.6 Analysis of total flavonoid content (TFC)

The methods of total flavonoids have been described in section 2.2.4.

4.2.7 Analysis of antioxidant activity

4.2.7.1 Ferric reducing antioxidant power (FRAP) assay

The methods of antioxidant activity as measured by FARP assay have been described in section 2.2.5.1.

4.2.7.2 DPPH antioxidant power assay

The methods of antioxidant activity as measured by DPPH assay have been described in section 2.2.5.2.

4.2.8 Assessment of quercetin and its glycosides

Separation and quantification of flavonoid were carried out by RP-HPLC using the method, as outlined previously in section 3.2.7.

4.2.9 Statistical analysis

All analytical measurements were made in triplicate. The data were analysed using the general linear models (GLM) procedure of SAS 9.1 (Cary, NC, USA). The model included the fixed effects of variety, treatment, drying method and their interactions. Multiple comparisons were adjusted by the Tukey-Kramer test with a 95% significance level ($p<0.05$). Pearson's correlation coefficients between TPC, TFC, individual quercetin content, antioxidant capacity (FRAP and DPPH) and scale colour parameters were calculated using the CORR procedure from SAS 9.1.

4.3 Results and discussion

4.3.1 Effect of drying methods on TPC and TFC

Drying is a frequently used method for conserving food. During drying of onions, retaining their phenolic contents is a significant issue because phenolic complexes play an important role in human health. Drying at temperatures higher than 60 ^{o}C is regarded as unfavourable due to the possibility of inducing oxidative condensation or decomposition of thermo labile compounds (Asami et al., 2003). As hot air and oven drying at 60 ^{o}C are recommended to dry onions to obtain a finished product of acceptable quality (Mitra, Shrivastava, & Rao, 2015), 60 ^{o}C was set as the drying temperature in all experiments except freeze-drying. The onion slices were dried from around 90% to 10% w.b. As shown in Table 4.1, there was a low variation of dry weight between different onion varieties and drying methods. The average dry weight (of all samples/treatments) was 9.82 ±0.41 % w.b. The results of antioxidant capacity, total phenolic content and flavonoids content were all calculated by dry weight.

TPC of fresh Red Baron (OSOP, CSCP) and Hyfort (OSOP, CSCP) was 6.85 ± 0.12, 5.76 ± 0.16 mg/g DW and 5.68 ± 0.14, 5.54 ± 0.38 mg/g DW, respectively. Figure 4.1 shows a considerable increase of TPC in dried samples in relation to their fresh counterparts, which is ascribed to the concentration effect caused by the water removal and also by the liberation of phenolic compounds from the matrix during the drying process. The differences attributed to different drying methods were significant (p<0.05) according to the three-way ANOVA analysis. Red Baron onions clearly had higher TPC than Hyfort onions dried by the same method, and OSOP treated onions showed higher TPC in comparison with their CSCP treated counterparts. According to the ANOVA analysis, the two differences were significant (p<0.05). Organically grown onions were produced without or with very little pesticides, causing higher pathogenic pressures, which may explain the higher

total phenolic content levels found in the OSOP samples. Higher TPC in onions cultivated by organic agronomic treatment than by conventional treatment was also reported by Asami et al. (2003).

Drying may enhance the release of bound phenolic compounds as a result of the breakdown of cellular constituents (Alfaro et al., 2014; Suna et al., 2014). Drying of the onion samples rendered the plant tissue more brittle, which in turn resulted in increased and faster cell wall breakdown during the blending and homogenisation steps of the extraction procedure. These broken cell walls could release and liberate more phenolic compounds from the matrix into the solvents during the overnight extraction process (Hossain et al., 2010; Suna et al., 2014), lead to a higher extractability of polyphenols. Serratosa et al. (2011) also found an increase of phenolic contents of red grape due to drying, and proposed that the increase was a result of (a) water evaporation, (b) improved extraction from skins, and (c) compound hydrolysis and biosynthesis. Hossain et al. (2010) suggested another possible reason; they proposed that the fresh samples might lose antioxidant compounds due to enzymatic degradation during processing as the enzymes are still active in fresh samples. The dried samples avoided this loss, as the enzymes were inactivated due to decreased water activity and thus were retained high antioxidant capacity and total phenols in the extracts. The total phenolic contents of the dried samples of Red Baron (OSOP), for instance, were 10.54 ± 0.72, 10.48 ± 0.48, 10.20 ± 0.31 and 9.08 ± 0.21 mg/g DW for freeze-drying, hot-air drying, vacuum oven drying and oven drying, respectively. The TPC difference between fresh and dried onions was similar to the range reported by Arslan and Ozcan (2010) and Priecina and Karklina (2014). The TPC varied from 2.23 mg/g DW to 3.69 mg/g DW, with an increase of 1.46 mg/g DW. As for the Red Baron onion with CSCP treatment, the increase varied more than the OSOP counterpart, from 1.02 to 4.46 mg/g DW (variation of 3.46 mg/g DW). For the Hyfort (OSOP, CSCP) onions, the increase variations were even greater, from 0.53 to 4.34 mg/g DW (variation of 3.81 mg/g

DW for OSOP) and from 0.35 to 4.10 mg/g DW (variation of 3.75 mg/g DW for CSCP), respectively. A larger variation means a greater effect of the drying method on the TPC increase. In general, the variation for Red Baron onions, irrespective of the agronomic treatment, was smaller than for the Hyfort onions. This means that the effects caused by different drying methods depend on the variety, which implies that there is an interaction between drying method and vegetable variety as confirmed by the statistical analysis (p<0.05). The results also imply that TPC in the dried Red Baron onions were more stable than in the Hyfort onions, given the lower variation ascribed do different drying methods. Furthermore, it is evidence that the Red Baron onions are suitable for drying in terms of availability of TPC in the final dried products irrespective of the drying method used. There were significant differences (p<0.05) of TPC between freeze-dried, hot-air dried, vacuum-oven dried and oven dried samples. Among these drying methods, TPC in the dried samples followed the order: freeze-drying > hot air drying > vacuum oven drying > oven drying. The highest TPC in freeze-dried samples could be ascribed to the development of ice crystals within the plant matrix during the freezing step. Ice crystals could result in a greater rupturing of plant cell structure, which may allow for better solvent access and extraction (Keinänen & Julkunen-Tiitto, 1996). Wojdyło, Figiel, and Oszmiański (2009) analysed phenolics in strawberries dehydrated by different methods and found that the freeze-dried samples showed the highest concentration of phenolic compounds.

As for the total flavonoids content (TFC), its variation was similar to TPC, as shown in Figure 4.2. TFC of fresh Red Baron (OSOP, CSCP) and Hyfort (OSOP, CSCP) was 1.96 ± 0.09, 1.36 ± 0.04 mg/g DW and 1.18 ± 0.05, 1.14 ± 0.03 mg/g DW, respectively. TFC in Red Baron onions were significantly higher (p<0.05) than in the Hyfort counterparts. OSOP had higher TFC than CSCP. There were clear significant increases of TFC (p<0.05) as a result of drying in comparison with their fresh counterparts. Different drying techniques had different effects on the TFC of dried

onions. The variations for the Red Baron onions (OSOP, CSCP) were 2.27 and 2.76 mg/g DW, respectively. As for the Hyfort (OSOP, CSCP) onions, the variations were much smaller, 1.4 and 1.28 mg/g DW, respectively, which implies an interaction between drying methods and onion variety on the increase of TFC. However, contrary to the variation for TPC, TFC in Red Baron onions revealed a lower stability during drying than in the Hyfort onions.

It is worthwhile mentioning that the TPC and TFC increase is not a result of moisture removal only, since the final weight – and accordingly moisture content – of all samples was very similar (Table 4.1), and all determinations were compared in dry weight, while the increment of total phenolics and total flavonoids was significantly different depending on the drying method (Figures 4.1 and 4.2). This implies that each drying technique has a different effect on the extractability of phenolic compounds and flavonoids, which in turn depends strongly on the type of damage caused to the plant tissue.

Table 4.1 Dry weight percentage (%) of onion slices after freeze-, hot air-, vacuum oven-, and oven drying.

Sample	Freeze-drying	Hot-air Drying	Vacuum drying	Oven drying
Red Baron OSOP	9.70±0.23[a]	10.05±0.31[a]	9.85±0.35[a]	10.29±0.36[a]
Red Baron CSCP	9.40±0.26[a]	9.53±0.34[a]	10.02±0.37[a]	10.07±0.31[a]
Hyfort OSOP	9.27±0.37[b]	9.38±0.42[a]	10.17±0.41[a]	9.96±0.45[a]
Hyfort CSCP	10.02±0.45[a]	9.53±0.40[a]	9.62±0.36[a]	9.72±0.48[a]

For each row, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation on dry weight (%) for n=6.

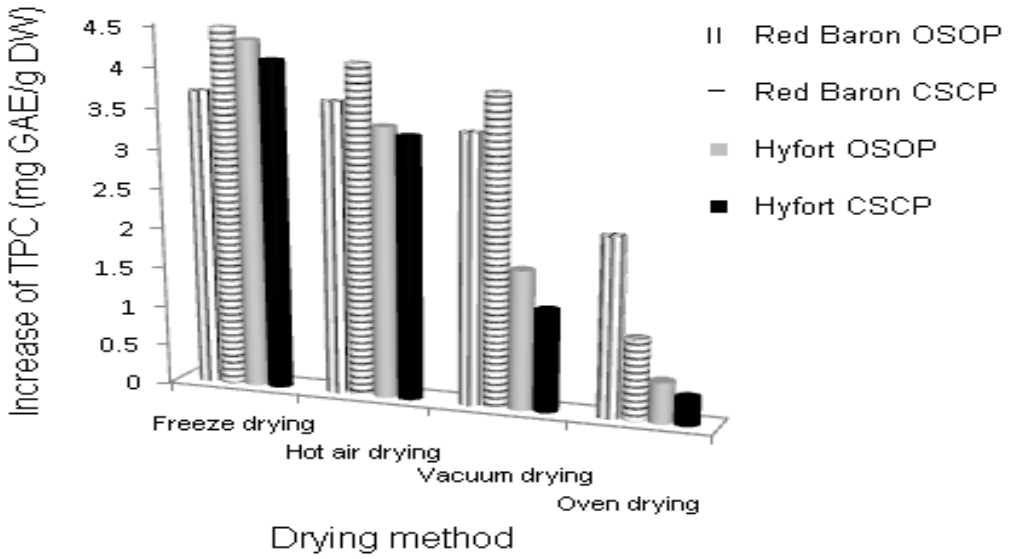


Figure 4.1 TPC increase as a result of drying for a different variety of onions cultivated under OSOP and CSCP agronomic treatments.

*TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight). TPC of fresh Red Baron (OSOP, CSCP) and Hyfort (OSOP, CSCP) was 6.85 ± 0.12, 5.76 ± 0.16 mg/g DW and 5.68 ± 0.14, 5.54 ± 0.38 mg/g DW, respectively.

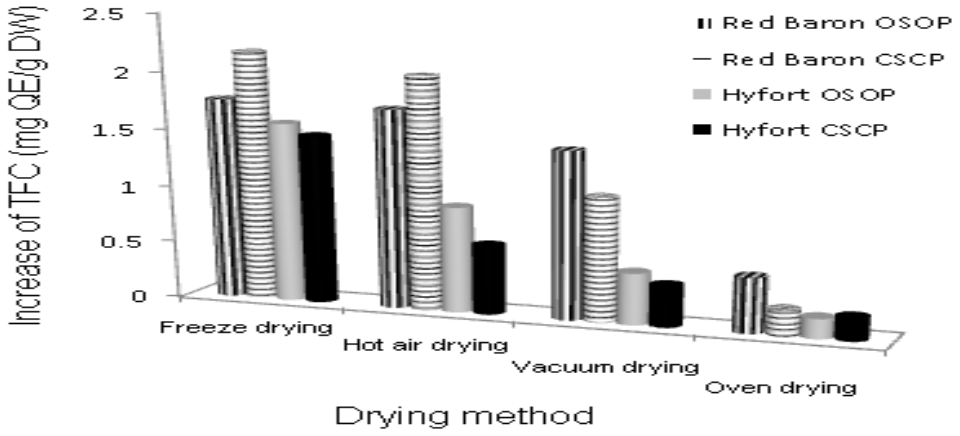


Figure 4.2 Effect of drying method on the increase of TFC in dried onions for different varieties and agronomic treatments.

*TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight). TFC of fresh Red Baron (OSOP, CSCP) and Hyfort (OSOP, CSCP) was 1.97 ± 0.09, 1.36 ± 0.04 mg/g DW and 1.19 ± 0.05, 1.14 ± 0.03 mg/g DW, respectively.

4.3.2 Quercetin content in fresh and dried onions

The HPLC results for quercetin and their statistical analysis are listed in Table 4.2A and 4.2B. The total content of quercetin in onion was as high as 300 mg/kg, considerably higher than in many other fruits and vegetables (Hollman & Arts, 2000). In dried red onion, the concentration of quercetin was found to be as high as 2.1% w/w. In our study, the content of total quercetin varied from 448 to 14,086 mg/kg sample. Quercetin and its derivatives were reported as main antioxidant components in onions (Benítez et al., 2011). Some authors previously identified Q 3,4' D and Q 4' G as the major quercetin derivatives in the mature onion bulb; these components account for about 93% of the total flavonols (Pérez-Gregorio et al., 2011b). Flavonols are also found in the flesh scale tissue, where they account for a yellow colour if their concentrations are high enough. In the present study, similar results were observed. Total quercetin content could be regarded as the sum of the three components (Q 4' G, Q 3,4' D and Q). Contents of quercetin varied from one component to another for the same sample. Q 4' G showed a much higher level than Q 3,4' D, and Q often occupied a very small percentage (0.07%-15.06%) of the total quercetin content.

The quercetin content depended especially on variety, and Red Baron showed a considerably higher total quercetin content than the Hyfort onion. For a same variety of onion dried by the same method, organic treatment (OSOP) generally resulted in a significantly higher content than the conventional treatment (CSCP) regarding the same quercetin component. The higher contents were ascribed to the high pathogenic pressure of the plants in the organic agronomic environment.

The statistical analysis showed a significant effect (p<0.05) of the drying methods on the quercetin content, as indicated in Table 4.2A and 4.2B. Drying resulted in considerably higher total quercetin in comparison with fresh onions, which is in agreement with the results of TPC aforementioned. The individual quercetin compounds also varied according to the different drying technologies. The content of Q 4' G varied significantly, irrespective of the onion variety, in a similar order: hot-air drying (60 °C) > freeze-drying > vacuum drying (60 °C) > oven drying (60 °C) > fresh. Nonetheless, the contents of Q 3,4' D followed a different order: freeze-drying > hot-air drying (60 °C) > vacuum drying (60 °C) > oven drying (60 °C) > fresh. It is interesting that the contents of quercetin generally varied in a different order: freeze-drying < vacuum drying (60 °C) < fresh < hot-air drying (60 °C) < oven drying (60 °C). From the above results, it can be seen that different drying methods lead to varying responses of Q 3,4' D, Q 4' G and Q.

The highest Q 4' G contents occurred in hot-air drying, while oven drying resulted in the highest Q contents in comparison with the other drying methods. Fu (2004) also observed similar results when comparing the contents of Q 4' G, Q 3,4' D and Q between freeze-dried, vacuum dried and hot air dried onions. The author suggested that the prolonged heating during hot-air drying activated hydrolytic enzymes that led to a higher level of quercetin aglycone in hot-air drying; this enzyme activity was confirmed by many other researchers (Fu & Huang, 2003). However, the activated hydrolytic enzyme cannot explain why the content of Q in freeze-dried and vacuum-dried onions was even lower than in fresh onions (Table 4.2A and 4.2B). A possible reason is related to the porous structure of the dried onions generated during vacuum oven drying or freeze-drying. After drying under vacuum, when the vacuum was broken, oxygen could have filled into the pores in the dried onions, which would accelerate oxidation, in particular of sensitive components, during storage of the samples before quercetin determination.

Table 4.2A Quercetin content in fresh and dried onions (Red Baron).

Variety	Treatment	Drying method	Q 4' G (µg/g sample)	Q 3'4 D (µg/g sample)	Q (µg/g sample)
Red Baron	OSOP	Fresh	160.23±40.60^{e}	53.73±5.70^{e}	10.24±0.60^{d}
		Freeze-drying	903.83±60.67^{a}	503.42±10.35^{a}	1.41±0.02^{d}
		Hot-air drying	954.26±69.97^{a}	403.19±20.39^{b}	26.72±2.85^{c}
		Vacuum oven	512.40±70.46^{c}	222.89±12.46^{c}	3.75±0.43^{d}
		Oven drying	678.67±32.33^{b}	42.63±1.59^{ef}	97.21±8.93^{a}
	CSCP	Fresh	96.70±7.34^{ef}	53.75±7.03^{e}	1.10±0.07^{d}
		Freeze-drying	585.97±22.36^{bc}	473.97±14.64^{a}	1.20±0.02^{d}
		Hot-air drying	624.57±33.6^{b}	178.83±11.92^{d}	9.80±0.50^{d}
		Vacuum oven	236.35±11.64^{d}	177.98±5.96^{d}	1.20±0.05^{d}
		Oven drying	265.13±92.15^{d}	19.88±0.71^{f}	50.50±5.72^{b}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation on a dry weight basis for n=6. Q 4' G = Quercetin 4'glucoside; Q 3'4 D = Quercetin 3,4' diglucoside; Q = Quercetin.

Table 4.2B Quercetin content in fresh and dried onions (Hyfort).

Variety	Treatment	Drying method	Q 4' G (µg/g sample)	Q 3'4 D (µg/g sample)	Q (µg/g sample)
Hyfort	OSOP	Fresh	58.32±3.11^{e}	19.20±1.39^{f}	7.80±0.04^{c}
		Freeze-drying	351.22±32.81^{a}	210.83±10.15^{bc}	0.48±0.03^{f}
		Hot-air drying	197.19±8.72^{cd}	246.05±12.46^{ab}	2.35±0.71^{d}
		Vacuum oven	223.65±23.54^{bc}	223.18±10.56^{ab}	2.08±0.12^{de}
		Oven drying	150.39±11.56^{d}	30.68±1.63^{f}	19.89±1.10^{a}
	CSCP	Fresh	38.41±1.68^{e}	55.09±1.44^{de}	0.93±0.03^{ef}
		Freeze-drying	290.40±11.73^{ab}	190.09±12.09^{c}	0.35±0.02^{f}
		Hot-air drying	243.41±12.99^{bc}	279.69±13.09^{a}	8.11±0.70^{c}
		Vacuum oven	198.15±6.03^{cd}	52.14±2.05^{de}	0.55±0.06^{f}
		Oven drying	134.15±10.53^{d}	64.35±2.88^{d}	17.02±0.58^{b}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation on a dry weight basis for n=6. Q 4' G = Quercetin 4'glucoside; Q 3'4 D = Quercetin 3,4' diglucoside; Q = Quercetin.

Quercetin aglycones are the more active compounds (Abascal, Ganora, & Yarnell, 2005), so they would be more readily oxidized in comparison with glycoside. On the other hand, oven drying and hot-air drying would lead to structure collapse, which avoids the accelerated oxidation occurring in the porous dried onions. The accelerated oxidation of Q in freeze-dried and vacuum oven dried samples probably led to the lower level of Q found. This implies that in order to retain as much aglycone as possible, onions dried by freeze-drying or vacuum oven drying should be kept away from contact with oxygen after drying. This could be done using nitrogen in the vacuum chamber during breakage of the vacuum, thus reducing the presence of oxygen in the pores. Another solution would be to vacuum pack the dried product.

4.3.3 Effects of drying methods on antioxidant assay

Several analytical methods have been developed to determine the antioxidant capacity of natural substances *in vitro*. However, the antioxidant activity of plant extracts cannot be evaluated using one method due to the complex composition of the phytochemical and oxidative processes (Inchuen, Narkrugsa, & Pornchaloempong, 2010). In fact, the antioxidant activity may be attributed to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition-metal ion catalysts (Mao et al., 2006). Therefore, at least two methods should be employed to evaluate the total antioxidant activity (Inchuen, Narkrugsa, & Pornchaloempong, 2010). In our study, DPPH radical scavenging and ferric-reducing antioxidant potential (FRAP) methods were used to evaluate the antioxidant activity (results summarized in Table 4.3A and 4.3B). All the studied extracts (from fresh and dried onion samples) were able to scavenge the DPPH free radical to different extents. Table 4.3A and 4.3B shows that almost all dried onions displayed higher antioxidant capacities, in terms of DPPH and FRAP, than their fresh counterparts, irrespective of the drying method employed.

Table 4.3A Antioxidant activity determined as per DPPH and FRAP assays in fresh and dried onions (Red Baron).

Variety	Treatment	Drying method	FRAP (mg TE/g DW)	DPPH (mg TE/g DW)
Red Baron	OSOP	Fresh	10.96±0.09^{e}	7.82±0.14^{e}
		Freeze-drying	14.43±0.11^{a}	11.35±0.04^{a}
		Hot-air drying	14.27±0.14^{ab}	11.32±0.08^{a}
		Vacuum oven	13.95±0.22^{b}	11.12±0.10^{ab}
		Oven drying	13.21±0.10^{d}	10.80±0.21^{bc}
	CSCP	Fresh	10.72±0.12^{e}	6.83±0.05^{g}
		Freeze-drying	14.03±0.24^{ab}	11.26±0.01^{a}
		Hot-air drying	13.83±0.15^{bc}	10.67±0.15^{c}
		Vacuum oven	13.43±0.19^{cd}	10.18±0.09^{d}
		Oven drying	10.67±0.12^{e}	7.29±0.18^{f}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation on a dry weight basis for n=6.

Table 4.3B Antioxidant activity determined as per DPPH and FRAP assays in fresh and dried onions (Hyfort).

Variety	Treatment	Drying method	FRAP (mg TE/g DW)	DPPH (mg TE/g DW)
Hyfort	OSOP	Fresh	10.23±0.26^{d}	6.73±0.04^{f}
		Freeze-drying	13.91±0.19^{a}	11.06±0.13^{a}
		Hot-air drying	13.20±0.25^{b}	10.53±0.17^{b}
		Vacuum oven	11.13±0.24^{c}	8.36±0.09^{d}
		Oven drying	10.11±0.21^{d}	7.07±0.16^{f}
	CSCP	Fresh	9.13±0.13^{e}	4.67±0.20^{h}
		Freeze-drying	13.53±0.26^{ab}	10.21±0.15^{b}
		Hot-air drying	13.03±0.12^{b}	9.58±0.07^{c}
		Vacuum oven	10.85±0.16^{c}	7.64±0.03^{e}
		Oven drying	9.75±0.03^{d}	5.11±0.19^{g}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation on a dry weight basis for n=6.

TPC results aforementioned revealed that drying could effectively increase extractability of phenolic compounds from onions and their resultant apparent TPC, in comparison with fresh onions. Polyphenols are one of the main antioxidant compounds in onions. Higher TPC in the dried onions reasonably increased the antioxidant activity of the dried onions. Table 4.3A and 4.3B also indicate that the different drying methods result in significantly different antioxidant capacity (DPPH and FRAP) of onion (p<0.001). The antioxidant activity of flavonoids is generally governed by its chemical structure. The activity increases with increasing the number of hydroxyl (OH^-) groups replaced on the B ring, especially at C-3', and decreases rapidly as the number of hydroxyl groups decreases (Ratty & Das, 1988).

The antioxidant capacities in terms of DPPH or FRAP in the dried onions were in the order freeze-drying> hot-air drying > vacuum drying > oven drying, which is similar to the trend found for TPC, TFC and quercetin content. FRAP and DPPH results were significantly correlated with TPC (correlation coefficients 0.98 and 0.99, respectively) and TFC (correlation coefficients 0.86 and 0.83, respectively). The tendency observed for the total flavonoid content had an association with TPC because flavonoids belong to the phenolic group. Likewise, the effect of different drying conditions on the total flavonoid content was similar to TPC since flavonoids belong to an assembly group of natural compounds with variable phenolic structure (Nijveldt et al., 2001). In addition, Q 4' G and Q 3,4' D were significantly and positively correlated with FRAP and DPPH (r^2 = 0.77, 0.76, and 0.77, 0.75 respectively). Significant correlations between TPC and FRAP were reported for both cultivated and wild onions. Santas et al. (2008) reported a correlation coefficient (r^2) of 0.78 between FRAP and phenolic content in two cultivated onions. Similarly, Nencini et al. (2007) reported r^2 = 0.46 between FRAP and phenolic content determined for several *Allium* species (*A. neapolitanum Cyr., A. roseum L., A. subhirsutum L*). Not only the drying methods exhibited effects on antioxidant capacities of dried onions, but also variety and agronomic treatment showed

significant influence, as shown in Table 4.3A and 4.3B. As expected, Red Baron onions had higher antioxidant capacity than Hyfort onions, and the OSOP treatment resulted in a higher capacity than CSCP. As a consequence, Red Baron onions with agronomic treatment OSOP were the richest sources for antioxidants among the four different types of raw material. On the other hand, both variety and agronomic treatment interacted with the drying method (Table 4.3A and 4.3B), which implies that the extent of the effect of drying method varied depending on the variety and treatment.

4.3.4 Colour assessment

The chromatic coordinates L* (brightness-darkness), a*(redness-greenness) and b* (yellowness-blueness) have been widely used to describe colour during thermal processing of food products as they can reflect information on some specific chemical components present (Bahloul et al., 2009).

The colour of onions at their internal side (Table 4.4A and 4.4B) was considerably different from the external one (Table 4.5A and 4.5B). Therefore, the colour parameters for both sides of fresh and dried onion must be measured to avoid introduction of variation into the data, hindering the statistic conclusions.

For the external side of onion samples, the difference between the cultivars was found to be significant (p<0.001) (Table 4.5A and 4.5B), as expected. L* means varied from 43.09 to 46.68 for Red Baron onions, and from 75.19 to 76.36 for Hyfort onions. The a* values varied from 16.1 to 17.34 for the Red Baron onions, and -5.30 to -5.31 for Hyfort onions. The b* values ranged from -4.73 to -5.63 (Red Baron onions), and 20.41 to 20.45 (Hyfort onions). These results were similar to the values reported by Gokce et al. (2010). Significant differences (p<0.001) were also found for the internal side. As for the agronomic treatment, its effect on the colour of dried samples was also significant (p<0.001), as revealed in Tables 4.4 and 4.5.

Table 4.4A Effects of different drying methods on the colour of onion slices (Red Baron) at their internal side.

Variety	Treatment	Drying method	ΔE	L*	a*	b*
Red Baron	OSOP	Fresh	---	63.14±0.31^{e}	0.51±0.02^{f}	1.76±0.04^{g}
		Freeze-drying	16.70±1.02^{b}	77.70±0.53^{b}	-1.15±0.05^{g}	3.13±0.05^{f}
		Hot-air drying	13.82±0.14^{d}	73.91±0.01^{c}	1.51±0.01^{d}	10.23±0.03^{b}
		Vacuum oven	7.49±0.21^{e}	57.29±0.25^{f}	1.34±0.03^{e}	6.37±0.02^{d}
		Oven drying	12.56±0.05^{d}	55.44±0.02^{g}	7.14±0.01^{a}	8.76±0.05^{c}
	CSCP	Fresh	---	62.41±0.25^{e}	0.65±0.01^{f}	2.72±0.04^{g}
		Freeze-drying	19.08±0.47^{a}	81.43±0.90^{a}	-1.67±0.03^{h}	5.73±0.11^{e}
		Hot-air drying	13.08±0.25^{d}	53.23±0.10^{g}	2.08±0.01^{c}	12.74±0.06^{a}
		Vacuum oven	5.65±0.07^{f}	67.07±0.06^{d}	0.75±0.01^{f}	5.28±0.12^{e}
		Oven drying	16.75±0.55^{b}	47.60±0.05^{h}	6.10±0.01^{b}	8.50±0.09^{c}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation for n=6. ΔE = colour change.

Table 4.4B Effects of different drying methods on the colour of onion slices (Hyfort) at their internal side.

Variety	Treatment	Drying method	ΔE	L*	a*	b*
Hyfort	OSOP	Fresh	---	58.37±0.25^{c}	-0.53±0.04^{e}	3.34±0.12^{e}
		Freeze-drying	29.46±0.15^{a}	87.43±1.05^{a}	-2.63±0.12^{f}	6.90±0.26^{d}
		Hot-air drying	7.84±0.00^{g}	55.76±0.01^{c}	2.03±0.04^{c}	9.47±0.90^{c}
		Vacuum oven	22.60±0.02^{b}	79.00±0.01^{b}	0.39±0.01^{d}	11.19±0.03^{b}
		Oven drying	16.50±0.02^{d}	49.44±0.01^{d}	6.39±0.07^{a}	15.42±0.02^{a}
	CSCP	Fresh	---	54.48±0.30^{c}	-0.34±0.02^{e}	2.99±0.04^{e}
		Freeze-drying	27.76±0.41^{a}	82.25±0.13^{a}	-1.36±0.01^{f}	6.86±0.01^{d}
		Hot-air drying	13.31±0.12^{f}	56.79±0.10^{c}	0.16±0.02^{d}	16.05±0.02^{a}
		Vacuum oven	18.14±0.00^{c}	70.53±0.08^{b}	-0.31±0.01^{e}	10.59±0.01^{b}
		Oven drying	14.98±0.01^{e}	55.20±0.01^{c}	3.64±0.02^{b}	17.19±0.01^{a}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation for n=6. ΔE = colour change.

Table 4.5A Effects of different drying methods on the colour of onion slices (Red Baron) at their external side.

Variety	Treatment	Drying method	ΔE	L*	a*	b*
Red Baron	OSOP	Fresh	---	46.68±3.25^{b}	16.1±0.97^{a}	-5.63±0.48^{g}
		Freeze-drying	8.75±0.74^{e}	47.21±2.34^{a}	5.40±0.38^{e}	-4.84±0.45^{f}
		Hot-air drying	11.68±6.15^{c}	41.93±2.51^{d}	7.87±0.32^{d}	1.17±0.79^{c}
		Vacuum oven	13.10±0.51^{b}	43.07±0.09^{c}	5.83±0.71^{e}	1.56±0.08^{c}
		Oven drying	17.98±3.22^{a}	34.19±3.16^{f}	13.30±1.29^{b}	7.00±0.70^{a}
	CSCP	Fresh	---	43.09±2.76^{c}	17.34±1.56^{a}	-4.73±0.44^{f}
		Freeze-drying	8.71±2.17^{e}	43.67±1.08^{c}	8.69±0.10^{c}	-3.86±1.10^{f}
		Hot-air drying	9.85±0.55^{d}	39.71±2.38^{e}	9.27±0.11^{c}	-0.56±0.07^{d}
		Vacuum oven	11.85±1.95^{c}	40.05±0.05^{d}	6.05±0.05^{e}	-2.62±0.05^{e}
		Oven drying	17.19±0.73^{a}	36.04±1.31^{f}	14.66±0.58^{b}	4.38±0.08^{b}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation for n=6. ΔE = colour change.

Table 4.5B Effects of different drying methods on the colour of onion slices (Hyfort) at their external side.

Variety	Treatment	Drying method	ΔE	L*	a*	b*
Hyfort	OSOP	Fresh	---	76.36±0.71^{b}	-5.30±0.53^{g}	20.45±1.99^{e}
		Freeze-drying	12.96±0.94^{d}	82.75±0.5^{a}	-10.08±0.26^{h}	30.67±0.96^{c}
		Hot-air drying	18.94±2.85^{b}	70.31±0.55^{c}	5.64±0.60^{c}	33.79±2.60^{b}
		Vacuum oven	20.63±1.96^{b}	69.48±0.67^{c}	0.92±0.02^{f}	38.38±0.60^{a}
		Oven drying	38.87±2.87^{a}	40.76±0.26^{f}	7.98±0.15^{b}	15.42±0.64^{f}
	CSCP	Fresh	---	75.19±1.15^{b}	-5.31±0.36^{g}	20.41±1.06^{e}
		Freeze-drying	12.08±1.94^{d}	80.20±2.36^{a}	-11.37±0.60^{h}	29.37±0.35^{c}
		Hot-air drying	18.39±1.09^{b}	61.27±1.18^{e}	4.57±0.70^{d}	27.25±1.58^{d}
		Vacuum oven	17.50±0.05^{c}	64.38±0.73^{d}	3.43±0.05^{e}	31.24±0.26^{c}
		Oven drying	39.30±2.88^{a}	40.66±2.82^{f}	11.06±0.07^{a}	13.71±0.39^{f}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation for n=6. ΔE = colour change.

Tables 4.4 and 4.5 show that freeze-drying generally resulted in significant higher lightness, lower yellowness b* and lower a* values than the other drying methods. L* values of fresh onion slices decreased due to drying (except freeze-drying), for both external and internal sides. Oven dried samples had the lowest L* value, which means they were the darkest ones among all dried onions. This may be ascribed to the high temperature during oven drying (Sumnu, Turabi, & Oztop, 2005). The oven drying method resulted in the highest a* value (redness) on both internal and external sides when compared to the other drying methods. Pott et al. (2005) reported that high temperatures resulted in a noticeable increase in redness in mango slices. The dried samples Red Baron OSOP and CSCP exhibited dark colour, and the oven-dried onions showed a darker colour (a*) than all others. The highest darkness and redness of the oven-dried onions is associated with caramelization during the Maillard reaction, enzymatic degradation and pigment loss during drying (Arslan & Özcan, 2010).

In most dried products, product colour is preferred to remain unchanged after drying, and higher L* values are usually desirable in the dried products (Doymaz, Tugrul, & Pala, 2006). All dried onions had lower luminosity (L*) than fresh samples, except the freeze-dried onions, which displayed the highest brightness (L*). The total colour change (ΔE) (external side) for freeze-drying was the smallest among all drying methods tested, probably due to the reduction of the intensity of browning reactions, thus minimizing the colour damage (Krokida & Maroulis, 1999). Unlike ΔE for the external side, the largest ΔE for the internal colour was observed in freeze-dried samples, in comparison with onions dried by the other drying methods ($p<0.05$). The large ΔE is a result of the considerable increase of L* value of the freeze-dried onions, as shown in Table 4.5A and 4.5B. López et al. (2010) reported that the colour changes ascribed to the thermal treatment may be caused not only by the non-enzymatic browning reaction, but also by the destruction of pigments present in the food.

In most red fruits and vegetables, the intensity of red tone is a good indicator of high antioxidant capacity, as redness implies a high concentration of anthocyanins (Çelik et al., 2008; Özgen, Serçe, & Kaya, 2009). Most of the colour parameters did not show a significant correlation (p>0.05) with the antioxidant capacity (DPPH and FRAP). However, the internal L* value was significantly (p<0.05) correlated with the antioxidant capacities, even though the relationships were not strong, with correlation coefficients of 0.47 and 0.48 for ΔDPPH and ΔFRAP, respectively (Table 4.6).

Table 4.6 Correlation coefficients (r) for colour coordinates, TPC, TFC, quercetins (Q 4' G, Q 3'4 D and Q) and antioxidant capacity (FRAP and DPPH) of onion.

Analysis	L_I	a_I	L_E	a_E	b_E	ΔBI_I	ΔBI_E	ΔE_I
TPC	0.49*	-0.27	-0.09	-0.06	-0.16	-0.56*	-0.67*	-0.72*
TFC	0.56*	-0.32	-0.22	0.01	-0.44*	-0.52*	-0.72*	-0.52*
Q 4 G	0.29	0.03	-0.33	0.17	-0.31	-0.1	-0.45	-0.30
Q 3'4 D	0.71	-0.45*	0.02	-0.10	-0.22	-0.64	-0.6	-0.43*
Q	-0.37	0.77***	-0.45*	0.51*	-0.16	0.79*	0.59*	0.22
ΔFRAP	0.48*	-0.29	-0.23	-0.02	-0.15	-0.65*	-0.37	-0.65*
ΔDPPH	0.47*	-0.33	-0.18	-0.13	-0.19	-0.64*	-0.35	-0.67*

L_I, a_I, b_I stand for internal L, a and b coordinates, respectively; L_E, a_E, b_E means stand for external L, a and b coordinates; ΔBI_I = Internal Browning Index change; ΔBI_E = External Browning Index change; ΔE_I = Internal colour change.

The results given in Table 4.6 show that the change of browning index (ΔBI_I and ΔBI_E) is highly correlated with quercetin, with coefficients of 0.79/0.59 in the two sides of the onion slices. The high levels of quercetin after drying are correlated with browning degree in dried samples, thus suggesting that quercetin concentration might contribute to the colour of onion. In addition, there was a negative correlation between ΔBI_I and ΔBI_E with TPC and TFC, which leads to the assumption that the brown index is highly related to a lower phenolic concentration in dried onions. The correlations between a* values (for both internal and external sides) and quercetin aglycone were high as well. Gokce et al. (2010) also correlated onion colour with total phenolics. Although significant (p<0.05), the correlations did not rise above

0.50 (Table 4.6). The lack of strong correlations could be attributed to the fact that the total phenolic content in onions covers not only quercetin but also isorhamnetin, kaempferol, and other phenolic acids, such as gallic acid and ferulic acid. These compounds probably do not contribute to colour changes of dried onion slices (Georgé et al., 2005). In our results, a significant negative correlation (-0.65/-0.64) was found between ΔIBI, ΔFRAP and ΔDPPH, which is an evidence that the variation of colour in onion slices indicates a change in antioxidant activity.

4.4 Conclusions

Drying of onion has been found to be a very useful technique for increasing the phenolic compounds and antioxidant capacity of the extracts. All four drying methods exhibited strong influences on the colour, total phenolic content, quercetin content, flavonoid content and antioxidant capacity of dried samples. Among the drying techniques tested, freeze-drying and hot-air-drying were found to be the best methods, which showed higher total phenolics and levels of quercetins as determined by HPLC. Therefore, freeze-drying and hot-air drying are the best techniques in terms of extractability of phenolic compounds from different varieties of onion. Although freeze-drying had a better performance than the other drying methods regarding the preservation of the phenolic bioactive compounds, it is a costly procedure, which limits its usage in the food industry. The use of adequate (60 ^{o}C) temperature for hot-air drying may also ensure the preservation of these compounds, which can be applicable due to the acceptable final levels of bioactive compounds and its low cost. The different drying methods showed a positive effect on the antioxidant activity due to the increase in both extractability and concentration of phenolic compounds, which have a strong correlation with antioxidant activity. Higher levels of bioactive compounds were found in organically grown onions (Red Baron and Hyfort) dried with different drying methods as compared to those produced by conventional cultivation.

Chapter 5

Enhancement of Phytochemical Content and Drying Efficiency of Onions (*Allium cepa* L.) through Blanching

Abstract

This study investigated the effect of blanching (60 ºC, 70 ºC and 80 ºC for 1, 3, 5 and 10 mins) combined with oven drying at 60 ºC on the phenolic compounds, antioxidant activity and colour, and on the thin layer drying characteristics (drying time, drying rate constant, effective moisture diffusivity and activation energy) of onion slices. Blanching at lower temperature and shorter time provided a better preservation of phenolics, flavonoids and colour. The loss of antioxidant activity and bioactive compounds might be related to the migration or leaching of components into the water. Blanching at 60 ºC for 3 mins or at 70 ºC for 1 min prior to drying onion slices increased their levels of bioactive compounds and antioxidant activity in comparison with the control samples and other blanching temperatures and times. Eighteen thin layer drying models were evaluated. The goodness of fit was assessed based on the coefficient of determination (R^2), root mean square error (RMSE) and reduced chi- square error (χ^2). Modified Page and Two-term exponential models were found to best represent the drying data. The effective diffusivity ranged from 3.32×10^{-11} m^2/s (control sample) to 5.27×10^{-11} m^2/s, 5.01×10^{-11} m^2/s, and 4.74×10^{-11} m^2/s for onions blanched at 60 ºC, 70 ºC and 80 ºC, respectively. The activation energy ranged from 2.367 to 9.779 kJ/mol. The higher activation energy was observed for the control (unblanched) sample with 9.779 kJ/mol and slightly lower values (2.367 kJ/mol and 2.832 kJ/mol) were found for 1 min and 3 mins-blanched samples, confirming the higher drying efficiency as a result of the blanching pre-treatment.

Keywords: Antioxidant; Blanching; Colour; Phenolic compounds; Drying efficiency.

5.1 Introduction

Onion (*Allium cepa* L.), one of the most widely grown and consumed vegetables in the world, is known to contain high levels of bioactive with protective effects against different degenerative diseases (Pérez-Gregorio et al., 2011b; Bahram-Parvar & Lim, 2018). Dried onions are used as a food ingredient in various food formulations including soups, sauces, salad dressings, sausage and other convenience foods (Kaymak-Ertekin & Gedik, 2005; Arslan & Özcan, 2010; Mitra, Shrivastava, & Rao, 2012; Sharma et al., 2016a).

Hot water blanching is commonly used in the food processing industry as an essential thermal treatment carried out prior to many preservation processes such as drying, canning and freezing, and largely determines the product quality. The main objectives of blanching are: to inactivate enzymes to prevent possible deterioration reactions, to reduce microbial load to prolong shelf-life, eliminate air in the intracellular space to increase the rate of heat and mass transfer, and prevent oxidation (Behsnilian & Mayer-Miebach., 2017; Wang et al., 2017). The quality of blanched products depends significantly on the blanching time and temperature, and also on the physical and chemical properties of the vegetable to be blanched. Industrial blanching processes involve treating fruits and vegetables with steam or hot water for 1 to 10 mins at temperatures ranging from 70 to 95 ^{o}C (Morales, Chandia, & Cisneros, 2002).

Drying of materials with high moisture content involves complex processes of simultaneous heat and mass transfer. A number of studies have been conducted on drying kinetics of various fruits and vegetables, so that preservation can be achieved by reducing the moisture content with minimal loss in nutrients. Several phenomena related to heat and mass transfer is involved in drying processes. The kinetics of mass transfer (mainly water) during drying depends on temperature, relative humidity, air flow rate, product thickness, load density and product shape (Olivas et

al., 1999). The predominant mechanism in food drying processes is the diffusion of water from as well as within the food to the surface in contact with the drying air. Modelling of the drying process is an efficient tool in the prevention of product deterioration, energy consumption, equipment stress and product yield (Olivas et al., 1999; Mitra, Shrivastava, & Rao, 2012; Sahoo et al., 2015). A number of empirical equations have been proposed to describe drying processes, modelling kinetics and design of drying systems (Kiranoudis, Maroulis, & Marinos-Kouris, 1992). These equations derive a direct relationship between the change in moisture content and the drying time, and are strongly related to Fick's second law of diffusion (Akpinar, 2006).

There is a dearth of literature on the effect of the combination between blanching temperature and blanching time on the phenolic compounds, antioxidant activity, colour and drying of onions. There are also few reports concerning the drying characteristics of onions pretreated by blanching. Therefore, the main objective of this study was to investigate the effect of blanching temperature-time combinations on the bioactive compounds and the overall quality of onion slices.

In spite of onions' high phenolic content and antioxidant properties (Gökçe et al., 2010; Pérez-Gregorio et al., 2014; Sharma et al., 2015a), these properties have not been studied in freshly cut onion thin slices. As a survey of literature shows that the interest in the role of antioxidants in human health has been increasing, it is important to test appropriate processing such as blanching and drying to thinly sliced onions. Onion slices are highly susceptible to oxidation, therefore a pretreatment before drying is necessary to reduce the changes in the phytochemicals to obtain a stable product.

The present study investigated the thin layer drying characteristics of onion slices in a tray dryer regarding the effect of blanching temperature and time. The best mathematical models to obtain the characteristic drying curves were selected. The

effect of temperature and blanching time on the diffusion coefficient, activation energy, phenolic content, antioxidant activity and colour was also evaluated.

5.2 Materials and methods

5.2.1 Materials

Organically grown onions (variety Red Baron) were obtained from the Horticulture Development Department in Teagasc, grown as part of the Kinsealy systems experiment, based in Kinsealy, North Dublin, Ireland. The onions were grown to organic standards, according to the methodology previously described in Reilly et al. (2013). Fresh onion slices (1 cm long × 1 cm wide and thickness of approximately 5 mm, total 2 kg) were prepared. A sample of 50 g was blanched in 100 mL of distilled water in a beaker using a temperature controlled water bath (DK-420 Glufex Medical and Scientific, England). The samples were blanched at 60 ºC, 70 ºC and 80 ºC for 1, 3, 5 and 10 mins. After they were removed from the water bath, the samples had the excess water removed with tissue paper. A further 50 g of unblanched sliced onions were used as a control. All measurements were carried out in triplicate.

Control and blanched onion slices were dried in an oven at 0.3 m/s and 60 ºC for 8 h. The hot air-drying operation temperature (60 ºC) was chosen based on the literature (Mitra, Shrivastava and Rao, 2012) and preliminary experiments, for a better preservation of sensory and nutritional properties. Samples were weighed at intervals of 1 h during drying until the equilibrium moisture content was obtained about (7.0 ± 0.4%) for all the samples (blanched and unblanched/control), hence the dry weight was the same for all samples (blanched and control). The moisture contents of both fresh and dried samples were determined according to AOAC (2005) (protocol number 930.15). All measurements were carried out in triplicate.

5.2.2 Preparation of extracts from dried onions

The methods of solid/liquid extraction have been described in section 2.2.3.

5.2.3 Total phenolic content (TPC)

The methods of total phenolics have been described in section 2.2.3.

5.2.4 Total flavonoid content (TFC)

The methods of total flavonoids have been described in section 2.2.4.

5.2.5 Total anthocyanin content (TAC)

The total anthocyanin content in onion was determined by the pH differential method of Huang et al. (2009) with some modifications. In summary, 1 mL of onion extract samples were dissolved in a 0.2 mol/L potassium chloride buffer, pH 1.0, making up to 25 mL. Then, another 1 mL of anthocyanin extract was dissolved in a 0.2 mol/L sodium acetate buffer, pH 4.5, up to 25 mL. Sample spectral absorbance measurements (OD) were read at 525 and 700 nm. The total anthocyanin content of the diluted samples was then calculated as follows:

$$TAC\ \left(\frac{mg}{L}\right) = \left[(OD_{525} - OD_{700})_{pH1.0} - (OD_{525} - OD_{700})_{pH4.5}\right] \times 449.2 \times \frac{1000}{26900} \times DF \quad (1)$$

where: 449.2 is the relative molecular mass of cyanidin-3-glucoside, 26900 is the molar absorptivity, and DF is the dilution factor.

5.2.6 Analysis of antioxidant activity

5.2.6.1 Ferric reducing antioxidant power (FRAP) assay

The methods of antioxidant activity as measured by FARP assay have been described in section 2.2.5.1.

5.2.6.2 DPPH antioxidant power assay

The methods of antioxidant activity as measured by DPPH assay have been described in section 2.2.5.2.

5.2.7 Assessment of quercetin and its glycosides in the extract using HPLC

Separation and quantification of flavonoid were carried out by RP-HPLC using the method, as outlined previously in section 3.2.7.

5.2.8 Colour measurement

The colour was measured using the method described in section 3.2.8.

5.2.9 Drying kinetics

Eighteen commonly used empirical models were evaluated to describe the drying kinetics of onion slices. In these models, MR represents the dimensionless moisture ratio, which is defined as:

$$MR = \frac{M - M_e}{M_0 - M_e} \quad (2)$$

where M is the moisture content (kg/kg d.b.) of the product after time t (h), M_0 is the initial moisture content of the product (kg/kg d.b.) and Me is the equilibrium moisture content (0.08 kg/kg d.b).

The regression analysis was performed using a procedure of SPSS 20.0. The fitness of each model was evaluated based on the root mean square error (RMSE), chi-square (χ^2) and correlation coefficient (R^2), which are the major criteria used for selection of the best model to describes drying data. The predicted moisture ratio was compared to the experimental moisture ratio using root mean square error and chi-square as shown below (Addo, Bart-Plange, & Boakye, 2009).

In order to evaluate the goodness of fit of the simulation provided by the models, different statistical parameters are used. In this study, the reduced chi-square (Doymaz, 2007) was calculated, where N is the total number of observations, Z is the number of model parameters, *i* MR*expi* are the experimental moisture ratio values and MR*pred,i* are the predicted moisture ratio values. These modules have been used

in the literature to evaluate the goodness of fit of different mathematical models (Başlar et al., 2014).

$$\text{RMES} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(MR_{exp,i} - MR_{pred,i})^2} \quad (3)$$

$$X^2 = \frac{\sum_{i=1}^{N}(MR_{exp,i} - MR_{pred,i})^2}{N-z} \quad (4)$$

5.2.10 Determination of moisture diffusivity

Fick's second law of diffusion, which characterizes moisture migration during thin layer drying of food materials, was used to calculate the effective moisture diffusivity, where Deff is the effective moisture diffusivity (m^2/s) and L is half the thickness of the sample (m) (Gupta et al., 2014):

$$MR = \frac{8}{\pi^2}\exp\left(-\frac{\pi^2 D_{eff} t}{4L^2}\right) \quad (5)$$

5.2.11 Activation energy

The Arrhenius equation was used to describe the temperature dependence of the effective diffusivity:

$$D_{eff} = D_0 \exp\left(-\frac{E_a}{RT}\right) \quad (6)$$

where D_0 is the pre-exponential factor of the Arrhenius equation (m^2/s), E_a is the activation energy (kJ/mol), R is the universal gas constant, 8.314 J/mol·K, and T is the absolute temperature (K). The activation energy is determined from the slope of the Arrhenius plot, ln (D_{eff}) vs. 1/T (Gupta et al., 2014).

5.2.12 Statistics

All measurements were carried out in triplicate and the results are presented as means ± standard deviation. The data were analysed using the general linear models (GLM) procedure of SAS 9.1 (Cary, NC, USA). Tukey-Kramer test was applied for multiple comparisons among means at a 95% significance level (p<0.05).

5.3 Results and discussion

5.3.1 Effects of blanching temperature and time on phenolic components

TPC in the onion slices was significantly affected (p<0.05) by both blanching temperature and time (Table 5.1).

Table 5.1 Phenolic content, flavonoid content, anthocyanin content and antioxidant activity of blanched and unblanched onion slices.

Blanching temperature (°C)	Blanching time (min)	TPC	TFC	TAC	FRAP	DPPH
Control	0	7.59±0.37^{b}	2.26±0.04^{bcd}	1.15±0.20^{c}	12.40±0.12^{bc}	8.00±0.23^{b}
60	1	7.60±0.20^{b}	2.84±0.13^{ab}	0.88±0.19^{d}	12.43±0.12^{bc}	8.09±0.26^{b}
	3	9.31±0.19^{a}	3.01±0.49^{a}	1.55±0.14^{b}	13.69±0.11^{a}	8.76±0.07^{a}
	5	7.08±0.11^{bc}	2.76±0.33^{ab}	0.77±0.06^{d}	11.98±0.11^{cd}	7.36±0.14^{cd}
	10	5.79±0.30^{de}	1.84±0.06^{cde}	0.51±0.04^{de}	11.29±0.16^{def}	7.20±0.10^{d}
70	1	9.65±0.29^{a}	3.21±0.05^{a}	1.98±0.05^{a}	13.53±0.15^{a}	8.62±0.23^{a}
	3	8.91±0.21^{a}	2.86±0.17^{ab}	0.75±0.15^{d}	12.33±0.13^{bc}	7.92±0.15^{b}
	5	7.43±0.42^{b}	2.38±0.09^{bc}	0.58±0.08^{de}	11.82±0.11^{cde}	7.26±0.11^{d}
	10	5.91±0.20^{de}	1.67±0.15^{de}	0.47±0.05^{de}	11.19±0.13^{ef}	7.03±0.17^{d}
80	1	7.07±0.16^{bc}	2.28±0.22^{bcd}	1.08±0.17^{c}	12.03±0.23^{cd}	8.02±0.24^{b}
	3	6.46±0.29^{cd}	1.72±0.13^{de}	0.85±0.06^{d}	11.33±0.24^{def}	7.80±0.19^{bc}
	5	5.55±0.40^{ef}	1.79±0.11^{cde}	0.54±0.13^{de}	10.58±0.32^{g}	7.13±0.46^{d}
	10	5.11±0.19^{f}	1.51±0.17^{e}	0.53±0.15^{de}	10.57±0.34^{g}	6.50±0.37^{e}

The results are expressed in mean ± standard deviation for analysis triplicates (n=3). Means in the same columns with different superscript letters are significantly different according to the Tukey's test (p<0.05). TPC: Total phenolic content (mg GAE/g DW); TFC; Total flavonoid content (mg Quercetin/g DW); TAC: Total anthocyanin content (mg cyanidin/g DW); Antioxidant activity: FRAP (mg Trolox/g DW); Antioxidant activity: DPPH (mg Trolox/g DW).

The blanching of onion slices for 3 mins at 60 ºC and for 1-3 mins at 70 ºC significantly (p<0.05) increased the TPC compared to the control sample. The TPC increase at the conditions mentioned might be attributed to the inactivation of oxidative enzymes and the induced structural changes leading to improved release of extractable and non-extractable phenolic compounds (Renard, 2005). Wolfe and Liu (2003) reported similar findings for the short-time (10 s) blanching of apple peels with subsequent drying, which resulted in better retention of phenolic compounds.

However, a higher blanching temperature (80 ºC) and longer blanching times (5-10 mins) at any temperatures were detrimental to the TPC. Jaiswal, Gupta and Abu-Ghannam, (2012) reported similar conclusions about the effects of blanching time on the degradation of the total phenolic content of York cabbage. Amin and Lee (2005) applied blanching methods for red, green, mustard, Chinese and Chinese white cabbage for 5, 10 and 15 mins. A significant (p<0.05) reduction in the total phenolic content was observed irrespective of cabbage type. Losses in phenolic content are attributed to the disruption of the plant tissue due to the heating effect, leading into polyphenols leaching out into the blanching water environment (Gonçalves et al., 2010; Martínez et al., 2013). Furthermore, the reciprocal inter conversion of insoluble phenolics into more soluble forms can also occur, which may lead to additional losses in polyphenols. The phenolic content losses upon blanching could also be attributed to their respective solubility and stability, which are highly influenced by the type of blanching environment (hot water) and sample: blanching environment volume ratio. It is also worth noting that free polyphenols leach out faster in water as compared to bound polyphenols (Abu-Ghannam & Jaiswal, 2015).

In this work, the maximum loss of phenolics was observed at 80 ºC for 10 mins, with the TPC reduced by 34.23%. In fact, high temperatures and long blanching times can lead to loss of phenolic compounds due to thermal degradation, leaching or diffusion of components into water and enzymatic oxidation (Gonçalves et al., 2010). Enzymes such as phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO)

play an important role during the phenol synthesis in plants. PAL is the first key enzyme in the biosynthesis of phenolic components. The increased activity of PAL leads to an increase in the synthesis of phenolics (Luo et al., 2012). As these enzymes get inactivated during heat treatments, there should be a consequent reduction in the phenolic components after heat treatments. However, there is a series of factors affecting the TPC of blanched samples, not only related to enzymatic activity. In our results, blanched onion slices in general showed lower TPC than control samples, except at 60 ºC for 3 mins and at 70 ºC for 1-3 mins. This result can be explained by several possibilities: the release of high amounts of antioxidant compounds (total phenolic content) due to thermal destruction of cellular and sub-cellular compartment walls, production of strong antioxidants and radical elimination by thermal chemical reaction, suppression of oxidation capacity of antioxidants due to thermal inactivation of oxidative enzymes, and/or the production of new non-nutrient antioxidants or the formation of new compounds such as Maillard reaction products with antioxidant capacity (dos Reis et al., 2015).

The total flavonoid content (TFC) in the onion slices was also significantly affected (p<0.05) by the blanching time and temperature (Table 5.1). The TFC increased after 1-5 mins of blanching at 60 ºC and 70 ºC, which can be attributed either to the better extractability of flavonoids as a result of the cell disruption or to the reduced rate of polyphenol degradation. On the other hand, blanching at 80 ºC reduced the TFC, similarly to what occurred to the phenolic compounds.

The anthocyanin content significantly increased after blanching at 60 ºC for 3 mins and at 70 ºC for 1 min. All the other conditions led to a TAC reduction. Although anthocyanins degrade with blanching at high temperatures, the drying air temperature of 60 ºC was reported as the optimum for the retention of most phenolic compounds (Katsube et al., 2009; Wiriya, Paiboon, & Somchart, 2009). Mild heat treatments (at approximately 60 ºC) can inactivate degradation enzymes such as polyphenol oxidase and glycosidase resulting in higher rates of polyphenols. Anthocyanins,

phenolic compounds and flavonoids degrade enzymatically, which catalyse the hydrolysis of anthocyanins to yield free sugars and aglycone (Lohachoompol, 2007). However, blanching may also cause excessive leaching of this pigment. In addition, heating can also encourage cellular fluids containing phytochemicals to diffuse from the plant cell to the water media. Thus, the anthocyanin content after blanching is the net result of combined increased in extractability, degradation and leaching. Wahyuningsih (2008), for instance, recorded a decreased in the anthocyanin content of red turi (*Sesbania grandiflora* (L.) Pers.) flower, which was ascribed to the leaching of anthocyanin into the blanching media. Khanal, Howard and Prior (2010) found that the anthocyanin content was significantly degraded at high drying temperatures (more than 60 ºC) in grape and blueberry pomace. Generally, drying processes can induce undesirable effects on the profiles of plant phytochemicals. Therefore the importance of optimizing drying processes and pre-treatments of plant materials destined to the recovery of bioactive compounds (Dai & Mumper, 2010).

5.3.2 Effects of blanching temperature and time on individual phenolic compounds

The concentration of individual phenolic compounds significantly increased after blanching at 60 ºC for 3 mins and 70 ºC for 1 min followed by drying when compared with unblanched samples. Dried blanched onion slices featured a significant increase in the concentration of individual phenolic compounds such as quercetin and its glucosides, since they are sub-groups of phenolic compounds, which was probably caused by the inactivation of degradation enzymes such as polyphenol oxidase and peroxidase (Renard et al., 2001).

Blanching of dried onion slices at 60 ºC for 3 mins and 70 ºC for 1 min caused a significant increase in most of the analysed quercetin compounds compared to the control (Table 5.2). Both blanching temperature and time influence the contents of Q, Q 4’ G and Q 3,4’ D. While blanching is detrimental to Q 3,4’ D regardless of its

conditions, blanching temperatures of up to 70 ºC applied for 1-5 mins increase the Q 4' G levels. The quercetin content, for its turn, is benefited from the application of blanching at any temperature and time duration, with optimum results at 70 ºC for 1-3 mins.

Table 5.2 Quercetin content of blanched and unblanched onion slices.

Blanching temperature (℃)	Blanching time (min)	Q 4' G	Q 3,4' D	Q
Control	0	812.08±97.80^{c}	1090.17±200.53^{a}	25.40±2.39^{f}
60	1	936.51±34.60^{b}	87.80±1.97^{b}	139.42±25.60^{c}
	3	1124.32±97.73^{a}	114.75±14.43^{b}	226.20±24.29^{b}
	5	961.53±29.97^{ab}	77.79±6.81^{b}	141.96±14.23^{c}
	10	310.49±17.18^{ef}	11.01±2.61^{b}	46.96±3.38^{ef}
70	1	981.50±16.91^{ab}	180.06±4.64^{b}	392.31±23.51^{a}
	3	940.73±44.16^{b}	175.75±14.72^{b}	362.03±24.85^{a}
	5	826.29±44.28^{bc}	145.83±30.87^{b}	228.79±34.38^{b}
	10	346.77±83.01^{e}	63.65±14.18^{b}	46.38±10.44^{ef}
80	1	740.90±123.48^{cd}	100.98±17.17^{b}	126.25±10.94^{cd}
	3	625.57±42.51^{d}	83.75±6.91^{b}	181.14±14.09^{bc}
	5	339.49±44.11^{e}	49.22±13.22^{b}	77.74±11.82^{de}
	10	294.87±42.38^{ef}	30.93±6.15^{b}	45.49±15.46^{ef}

The results are expressed in mean ± standard deviation for triplicates (n=3). The data is expressed as µg/g expressed on a dry weight basis (DW). Means in the same columns with different superscript letters are significantly different according to the Tukey's test (p<0.05).

There were no significant changes in the Q 3,4' D of onions during different blanching treatments followed by drying. After further heating, the values of Q 3,4' D decreased compared to the control samples. This phenomenon is ascribed to the leaching of Q 3,4' D into the water. Due to the enhanced water solubility, the additional hydroxyl group is assumed to support leaching of the former compound into the blanching water. Accordingly, the lesser gain of some phenolic compounds upon extended water-blanching compared to the control may be attributed to enhanced leaching of Q 3,4' D into the blanching water. Therefore, the leaching effect of blanching is assumed to be more decisive than the degradation or release of the phenolic compounds. On the other hand, the increases observed in the Q 4' G and Q contents appeared to occur at the expense of Q 3,4' D, which is in agreement with the study by Price and Rhodes (1997), where decreases in Q 3,4' D were quantitatively explained by increases in the Q 4' G and quercetin contents. This was as result of the conversion of Q 3,4' D in Q 4' G and further breakdown of Q 4' G in quercetin aglycon by enzymatic hydrolysis of glucosides during blanching (Pérez-Gregorio et al., 2010).

5.3.3 Effects of blanching temperature and time on antioxidant activity

The antioxidant activity of fresh and blanched samples is presented in Table 5.1. The blanching pre-treatment in general lowered the antioxidant activity of the onion slices, especially as blanching temperature and time increased (p<0.05). However, onions blanched at 60 ºC for 3 mins and 70 ºC for 1 min after drying resulted in a significant increase of the antioxidant activity in comparison with the control. Similar conclusions on drying and blanching of apple pomace were published by Heras-Ramírez et al. (2012) who reported that blanched apple pomace showed higher antioxidant activity than the unblanched peels. The increase in total phenolic contents and antioxidant capacities during blanching may be mainly ascribed to the increase of the contents of individual phenolic compounds. Furthermore, synergistic and additive effects of phenolic compounds may enhance the antioxidant activity

(Eberhardt, Lee, & Liu, 2000). In fact, some studies have reported an increase in the antioxidant content derived from structural changes in tissues that may release bound antioxidant polyphenols (Renard, 2005), resulting in an increase of antioxidant activity despite the thermal treatments applied to the food materials (Xu & Chang, 2009). On the other hand, thermal processes may also induce chemical changes of phenolics resulting in the formation of degradation products, which may retain or even feature a higher antioxidant activity (Buchner et al., 2006). Chantaro, Devahastin, and Chiewchan (2008), for instance, observed that the drying of carrot peels led to the reduction of antioxidant capacity with correlation to the loss of phenolic.

5.3.4 Colour analysis

The colour parameters of fresh and blanched onion slices are shown in Table 5.3. Blanching temperature and time had a significant effect on the colour of the dehydrated onion slices (p<0.05). All the blanched-dried samples had a lower luminosity compared to the unblanched-dried samples (Table 5.3), which decreased further as blanching time and temperature time increased. The same trend was observed for the a* and b* coordinates, meaning that higher temperatures and blanching times result in slightly greener/yellower onion slices rather than redder/bluer. At 80 ºC for 10 mins, excessive loss in the natural pigments and decreased lightness were observed. This might be the result of the non-enzymatic browning and caramelization due to the high temperature, similarly to the results found in carrots by Gonçalves et al. (2010).

5.3.5 Effects of blanching temperature and pretreatment time on drying kinetics of onion slices

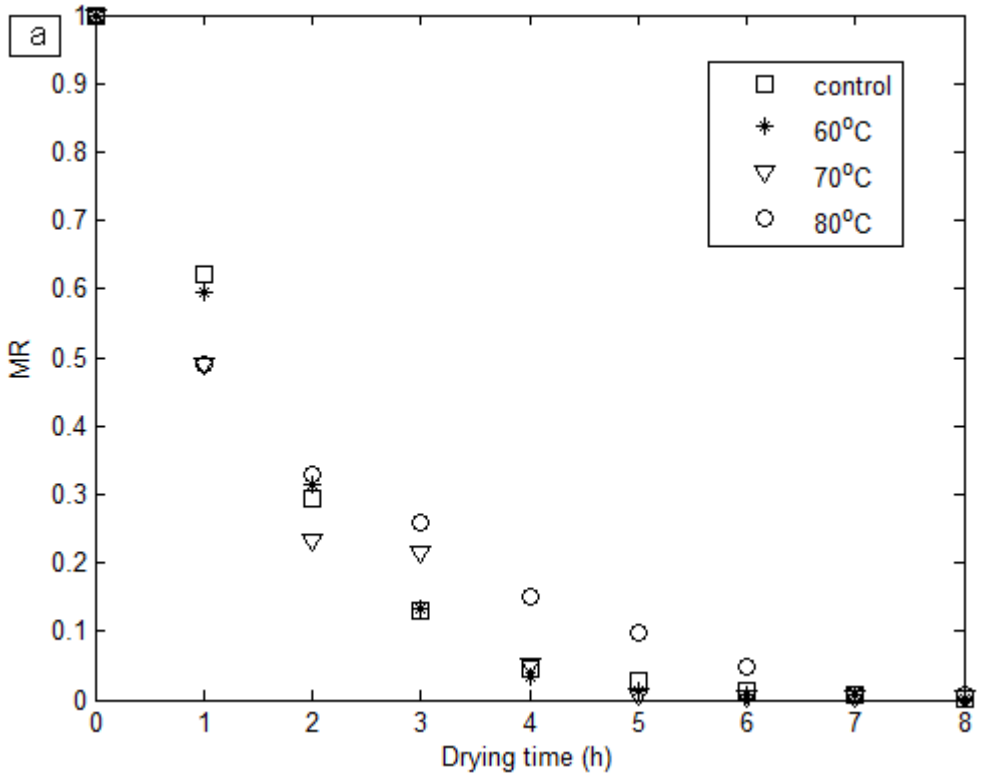
The moisture ratio (MR) of the blanched and unblanched onion slices decreased continuously with drying time (Figures 5.1a-d). This continuous decrease in moisture ratio indicates a diffusion controlled internal mass transfer. This is in agreement with

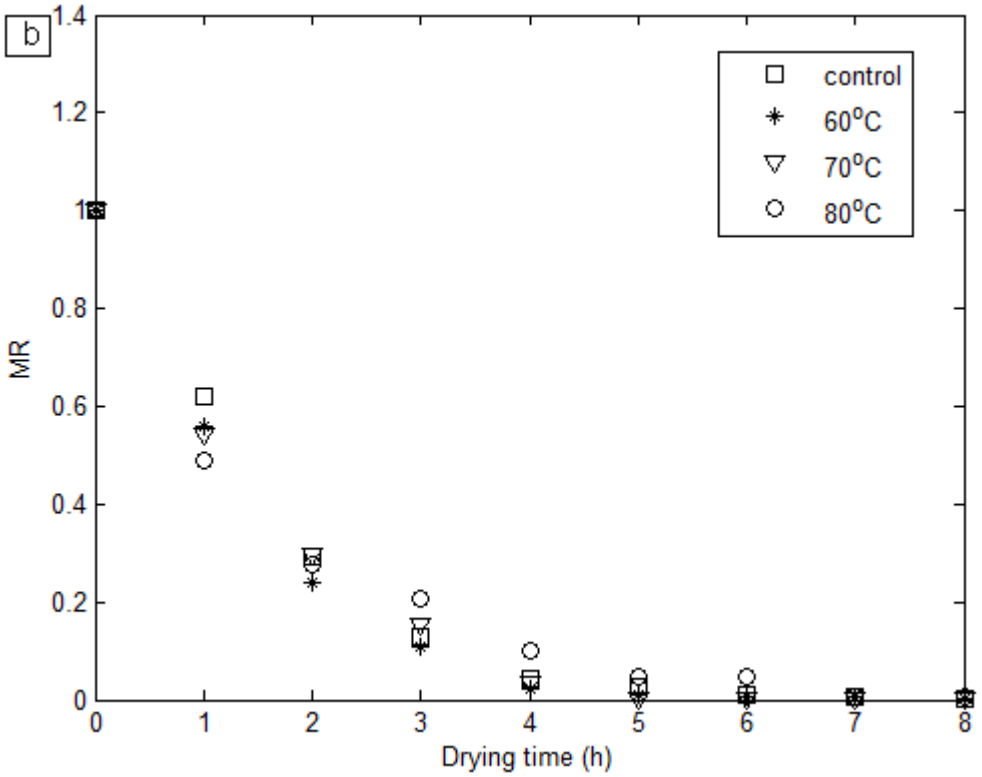
the observations of Kingsly et al. (2007) regarding the drying properties of blanched onion, figs and peach. The blanching pre-treatment increased the drying rate of onion samples. The drying rates of unblanched and blanched onions were initially high as a result of the great initial amounts of free water, but decreased rapidly to almost the same rate in the course of drying.

Table 5.3 Effect of blanching temperature and time on luminosity (L*) and colour coordinates (a* and b*) of onion slices.

Blanching temperature	Blanching time (min)	L*	a*	b*
Control	0	46.70±3.45^{a}	7.34±1.33^{a}	-0.29±0.42^{g}
60 ^{o}C	1	42.57±1.33^{b}	6.28±0.55^{b}	2.34±0.48^{f}
	3	40.99±1.12^{c}	5.69±0.12^{cd}	3.10±0.08^{e}
	5	40.90±1.82^{c}	5.40±0.99^{cd}	3.39±0.12^{de}
	10	39.57±1.11^{c}	4.62±0.32^{e}	3.53±0.72^{d}
70 ^{o}C	1	40.27±1.32^{c}	5.82±0.04^{c}	6.27±1.77^{b}
	3	38.64±2.31^{d}	5.39±0.19^{cd}	6.62±0.50^{b}
	5	37.79±1.91^{d}	5.24±0.30^{cd}	8.17±0.03^{a}
	10	35.52±4.20^{e}	4.38±0.69^{ef}	8.37±0.43^{a}
80 ^{o}C	1	38.88±1.33^{d}	5.80±0.99^{c}	4.55±0.35^{c}
	3	35.70±1.55^{e}	5.39±0.89^{de}	6.29±0.42^{b}
	5	34.27±1.26^{ef}	4.11±0.84^{f}	8.31±0.37^{a}
	10	32.83±1.97^{g}	4.08±1.49^{f}	8.42±0.22^{a}

L* ranges from 0 (black) to 100 (white), a, from -60 (green) to 60 (red), and b, from -60 (blue) to 60 (yellow). The results are expressed in mean ± standard deviation for triplicates (n=3). Means in the same columns with different superscript letters are significantly different according to the Tukey's test (p<0.05).


a
control
60°C
70°C
80°C
MR
Drying time (h)


b
control
60°C
70°C
80°C
MR
Drying time (h)

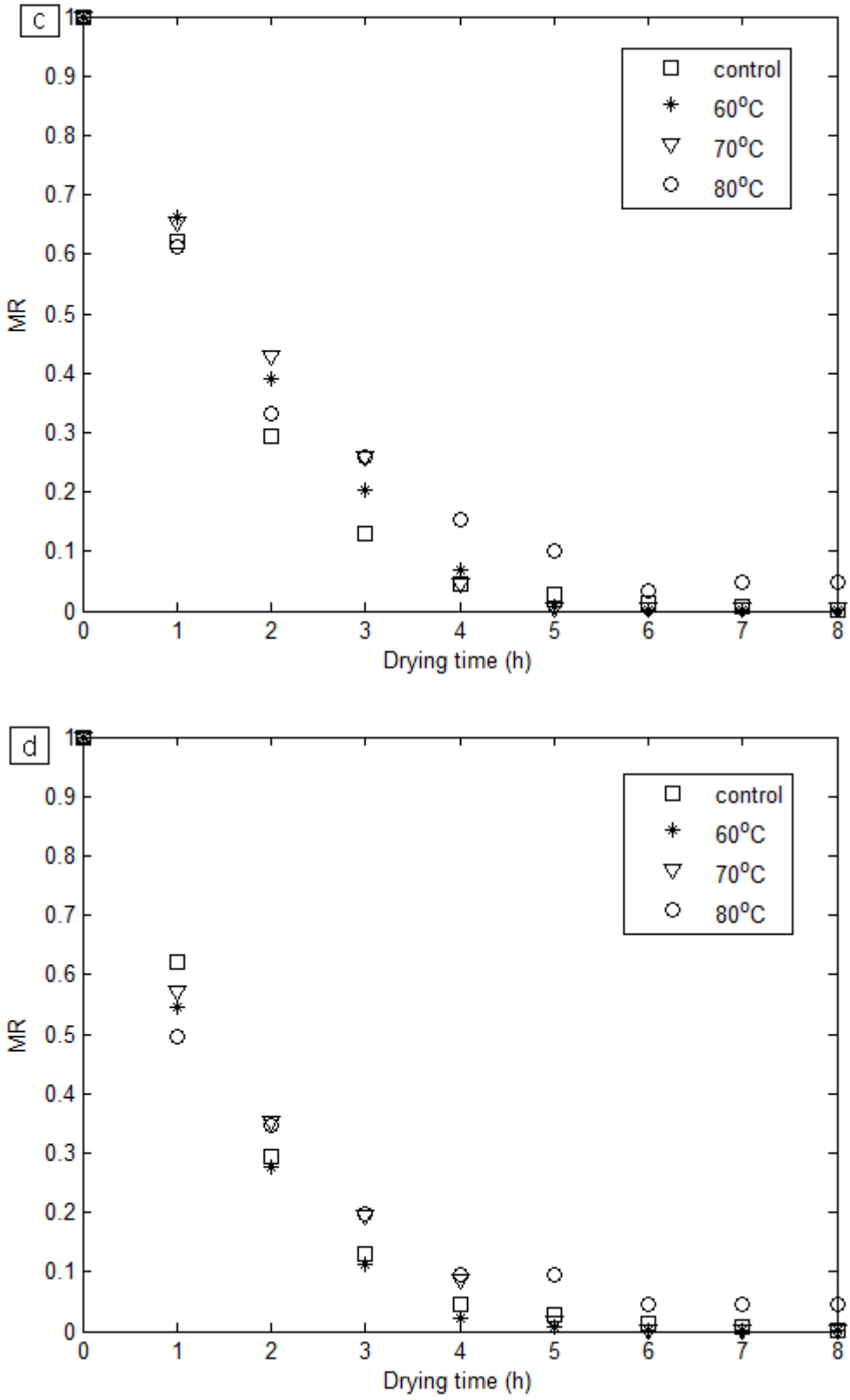


Figure 5.1 Moisture content versus drying time of onion slices blanched for: (a) 1 min, (b) 3 mins, (c) 5 mins, and (d) 10 mins.

At any blanching temperature (60 ºC, 70 ºC, 80 ºC), the initial drying rate increased as the blanching time increased from 3 to 10 mins. However, there was a progressive drop in the drying rate at a higher blanching time than at a lower blanching time, resulting in a longer drying time. This drop in the drying rate at a higher blanching time may be ascribed to the gelatinization of carbohydrates (Maté et al., 1998), which increased as blanching time increased, thus leading to lower rates of moisture transport from inside the blanched onions to their surface during drying.

In addition, at all each blanching times (1, 3, 5 and 10 mins), the initial drying rate increased as the blanching temperature increased from 60 to 80 ºC (Figures 5.1a-d). However, there was a more gradual decrease in the drying rate for samples blanched at higher temperatures, probably due to the greater extent of the carbohydrate gelatinization, which seems to affect the mobility of water during the drying and reduced water diffusively of onion slices (Maté et al., 1998).

5.3.6 Evaluation of the models

The purpose of testing different models was to compare the drying efficacy between unblanched (control) and blanched samples. The experimental moisture content obtained during the drying experiments was converted to moisture ratio (MR) and then fitted to the 18 different models (Table 5.4). Based on the statistical results of reduced chi-square (χ^2), root mean square (RMSE) and correlation coefficient (R_2), the Two-term model had the best performance for the unblanched samples (Table 5.5), while the Modified Page and the Two-term exponential model had the best fit for the blanched onions (Table 5.6). According to Table 5.6, the χ^2 and RMSE values were very low for all blanching conditions, with R^2 value between 0.927 and 0.999, indicating an excellent fit. Table 5.7 shows the constants of the two models aforementioned.

Table 5.4 Drying models proposed by various authors and tested in this work.

Model	Expression	Reference
Newton	$MR=\exp(-kt)$	Wang et al. (2007)
Page	$MR=\exp(-kt^n)$	Akoy (2014)
Modified page	$MR=\exp(-(kt)^n)$	Vega et al. (2007)
Henderson and Pabis	$MR=a\exp(-kt)$	Hashim et al. (2014)
Logarithmic	$MR=a\exp(-kt)+c$	Kaur and Singh (2014)
Two-term	$MR=a\exp(-k_1t)+b\exp(-k_2t)$	Sacilik (2007)
Two-term exponential	$MR=a\exp(-kt)+(1-a)\exp(-kat)$	Yaldiz and Ertekin (2001)
Midilli and others	$MR=a\exp(-kt)+bt$	Ayadi et al. (2014)
Parabolic	$MR=a+bt+ct^2$	Daghbandan et al. (2006)
Wang and Singh	$MR=1+at+bt^2$	Omolola et al. (2014)
Verma and others	$MR=a\exp(-kt)+(1-a)\exp(-gt)$	Akpinar (2006)
Modified Midilli and others	$MR=a\exp(-kt)+b$	Gan and Poh (2014)
Demir and others	$MR=a\exp(-kt)^n+b$	Demir et al. (2007)
Approximation of diffusion	$MR=a\exp(-kt)+(1-a)\exp(-kbt)$	Yaldyz and Ertekyn (2007)
Silva and others	$MR=\exp(-at-bt)$	Pereira et al. (2014)
Peleg model	$MR=1-t/(a+bt)$	Da Silva et al. (2015)
Hii and others	$MR=a\exp(-k_1t^2)+b\exp(-k_2t^2)$	Kumar et al. (2012)
Aghbashlo and others	$MR=\exp(k_1t/1+k_2t)$	Aghbashlo et al. (2009)

Table 5.5 Goodness of fit of different drying models applied to unblanched dried onion slices.

Model	χ^2	RMSE	R^2
Newton	1.20E-03	0.033	0.990
Page	5.30E-02	0.203	0.893
Modified Page	1.38E-04	0.010	0.999
Henderson and Pabis	1.26E-03	0.031	0.990
Logarithmic	9.61E-02	0.253	0.993
Two-term	2.90E-05	0.004	0.999
Two-term exponential	1.37E-03	0.033	0.990
Midilli and others	3.58E-03	0.049	0.993
Parabolic	9.19E-01	0.783	0.972
Wang and Singh	6.54E+01	7.134	0.990
Verma and others	1.57E-02	0.102	0.994
Modified Midilli and others	1.92E-03	0.036	0.993
Demir and others model	6.62E-02	0.192	0.664
Approximation of diffusion	1.47E-03	0.038	0.950
Silva and others	6.00E+01	5.100	0.939
Peleg	5.74E+01	2.134	0.949
Hii and others	3.90E-05	0.054	0.980
Aghbashlo and others	7.98E-05	0.040	0.980

Reduced chi square (χ^2); Root mean square error (RMSE); Regression coefficient (R^2).

Table 5.6 Goodness of fit of the best models evaluated in this work to describe the dying kinetics of blanched dried onion slices.

Model	Blanching time (min)	Blanching temperature (°C)	χ^2	RMSE	R^2
Modified Page $MR=\exp(-(kt)^n)$	1	60	1.29E-04	0.010	0.999
		70	1.35E-03	0.032	0.991
		80	3.90E-03	0.055	0.993
	3	60	8.61E-05	0.008	0.999
		70	3.70E-04	0.017	0.998
		80	5.04E-04	0.020	0.997
	5	60	8.28E-04	0.025	0.984
		70	4.16E-03	0.057	0.995
		80	1.12E-03	0.029	0.99
	10	60	1.63E-04	0.011	0.999
		70	4.73E-04	0.019	0.997
		80	4.36E-03	0.058	0.991
Two-term exponential $MR=aexp(-kt)+(1-a)exp(-kat)$	1	60	1.52E-04	0.011	0.999
		70	1.37E-03	0.033	0.990
		80	8.59E-03	0.082	0.927
	3	60	6.16E-05	0.007	1.000
		70	3.47E-04	0.016	0.997
		80	3.88E-04	0.017	0.998
	5	60	4.71E-04	0.019	0.997
		70	1.93E-03	0.039	0.988
		80	6.30E-03	0.070	0.948
	10	60	1.88E-04	0.012	0.999
		70	4.52E-04	0.019	0.997
		80	8.82E-04	0.026	0.992

Reduced chi square (χ^2); Root mean square error (RMSE); Regression coefficient (R^2).

Table 5.7 Constants of the Modified Page model and the Two-term exponential model applied to blanched dried onion slices.

	Blanching temperature (℃)	Blanching time (min)	k	n
Modified Page	Control	0	0.562	1.267
	60	1	0.573	1.248
		3	0.643	1.230
		5	0.524	1.184
		10	0.638	1.196
	70	1	0.679	0.927
		3	0.611	1.106
		5	0.563	1.100
		10	0.549	1.095
	80	1	0.640	0.830
		3	0.643	0.837
		5	0.489	0.821
		10	0.467	0.800
	Blanching temperature (℃)	Blanching time (min)	k	a
Two-term Exponential	Control	0	0.608	1.058
	60	1	0.858	1.832
		3	0.969	1.848
		5	0.573	1.889
		10	0.895	1.736
	70	1	0.819	0.626
		3	0.799	1.609
		5	0.704	1.847
		10	0.687	1.555
	80	1	0.401	1.051
		3	2.450	0.208
		5	0.375	1.057
		10	0.778	0.519

k, n, a are empirical coefficients retrieved from drying experimental data.

5.3.7 Effective moisture diffusivity and activation energy

The effective moisture diffusivity (D_{eff}) during drying and the activation energy were determined by the Fick's diffusion model and the Arrhenius model, respectively, and the results are shown in Table 5.8. The effective diffusivity ranged between 3.32 × 10^{-11} m^2/s to 5.27 × 10^{-11} m^2/s, 5.01 × 10^{-11} m^2/s, and 4.74×10^{-11} m^2/s for the samples blanched at 60 ºC, 70 ºC and 80 ºC, respectively. The effective diffusivity was higher for longer blanching times. An increase in moisture diffusivity was observed for all blanched samples in comparison to the control (unblanched). Agarry, Durojaiye and Afolabi (2005) reported that blanching prior to drying improves the effective moisture diffusivity as a result of the high draining of additional water absorbed during blanching. An Arrhenius-type equation was used to calculate the activation energy. The natural logarithm of D_{eff} as a function of the reciprocal of absolute temperature was plotted for the blanched samples (Figure 5.2). The activation energy ranged from 2.367 to 9.779 kJ/mol. The highest activation energies were observed for the control (unblanched) sample (9.779 kJ/mol). The slightly lower activation energy of pre-treated onions compared to untreated samples is an indication that less energy is used during drying of onions subjected to blanching. The fact that water travels faster in pre-treated samples indicates that blanching can be used as a pre-treatment to optimize the drying process of onion in terms of energy demand (Maté et al., 1998). The cell wall rupture ascribed to blanching results in high internal mass transfer during drying and thus had higher moisture diffusivities. In fact, it has been reported that blanching generally increases water diffusion from within the product to its surface during drying of fruits (Kingsly et al., 2007) Similar results of the influence of blanching pre-treatment on moisture diffusivity during air drying were reported in apricots (Pala, Mahmutoğlu, & Saygi, 1996).

Table 5.8 Kinetic parameters of unblanched and blanched dried onion slices.

Blanching temperature (^{o}C)	Blanching time (min)	Deff (m^2/s)	Ea (KJ/mol)
Control	0	3.32E-11	9.779
60		4.69E-11	
70	1	4.57E-11	2.367
80		4.47E-11	
60		4.97E-11	
70	3	4.78E-11	2.832
80		4.67E-11	
60		5.27E-11	
70	5	5.01E-11	5.194
80		4.74E-11	
60		5.03E-11	
70	10	4.81E-11	5.212
80		4.52E-11	

Deff: Effective diffusivity; Ea: Activation energy

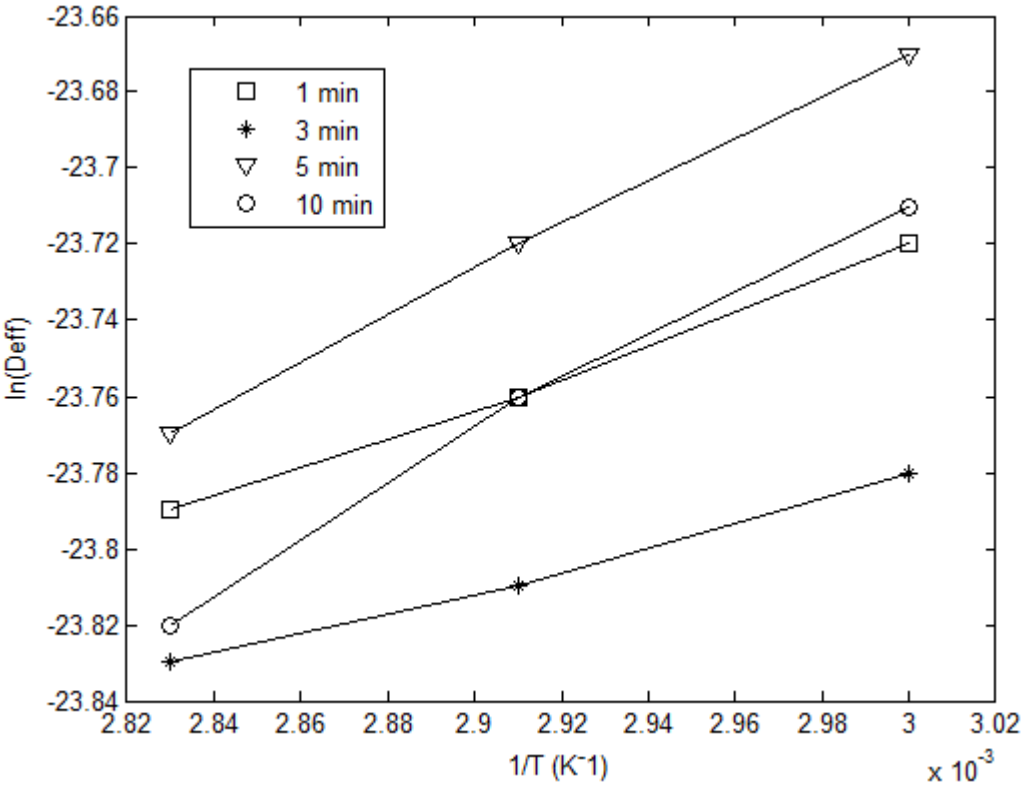


Figure 5.2 Arrhenius plot of the activation energy for blanched samples from 1 to 10 mins.

5.4 Conclusions

Hot water blanching is currently the most preferred pre-treatment for fruits and vegetables due to the low capital costs and blanching uniformity. Short-time water-blanching was found to be a suitable initial step in the production of onion slices. Blanching affects individual phenolic compounds, the total phenolic content and the antioxidant capacity, and proved to be a suitable method regarding the retention of polyphenols and antioxidant capacity, since minor destruction of tissue may better protect secondary metabolites from degradation.

Pretreatments such as blanching are effective in retarding the oxidation reactions by native enzymes present in onion slices. This method increases the phytochemical constituents of the onion slices responsible for its antioxidant activity and total phenolics. From the current study, it can be deduced that blanching at 70 ºC for 1 min and at 60 ºC for 3 mins followed by drying are the optimum process conditions.

The combination of blanching and hot air oven drying treatments led to a higher recovery of phenolic compounds and enhanced the antioxidant capacity. For better recovery of bioactive phenolic compounds from onion slices, the combination of blanching with short time and hot air oven drying (60 ºC) as a pre-treatment may be favourable.

The use of blanching as a pretreatment during drying of onions is also recommended because it reduces the time and rate of drying. Reduction in time and rate of drying results in dried products of higher quality. In addition, since the use of blanching as a pretreatment optimizes the drying process of onions in terms of energy utilization, it is recommended for use by local processors in order to reduce the energy demand involved in onion drying. Energy reduction during drying will reduce the production cost, which will accordingly result in higher earnings by the processors.

Chapter 6

Impact of Ultrasound and Blanching on Phenolic Content and Antioxidant Activity of Hot-air Dried and Freeze-Dried Onions

Abstract

The aim of this study was to investigate the effect of ultrasonic pre-treatment and blanching prior to hot-air or freeze-drying of onions on the retention of bioactive compounds (total phenolics, total flavonoids, and quercetin). Onion slices were treated with ultrasound at 20 kHz or blanching with hot water at 70 ^{o}C for 1, 3 or 5 mins. The colour change was similar between blanched and US-treated dried onions. The ultrasound treatment improved the retention of bioactive compounds (especially quercetin) and accordingly the antioxidant activity in onion slices dried either by freeze-drying or hot-air drying. The US-freeze-dried onions had a higher retention of quercetins compared to the US-hot air dried ones. Blanching for 1 min also resulted in a substantial increase of phenolic content and antioxidant activity in comparison to control dried onion slices. These results are ascribed to the destruction of the original tissue structure and thus higher extraction ability of the studied phytochemicals due to blanching and ultrasound. The short time ultrasound treatment provides valuable co-products in water because phenolic and soluble plant material are transferred from onion into the water. This study shows that ultrasound pretreatment is a potential alternative to conventional blanching treatment in the different drying of onion slices.

Keywords: Ultrasound treatment; Thermal blanching; Antioxidant activity; Colour.

6.1 Introduction

Onions find widespread usage in both fresh and dried forms. Dried onions are a product of considerable importance in the world trade. They are found in different forms: flaked, minced, chopped and powdered, which are of extensive demand in several parts of the world, such as UK, Japan, Russia, Germany, Netherlands, and Spain (Sarsavadia et al., 1999; Arslan & Özcan, 2010).

Sonication is a promising non-thermal technology in the food industry (Tiwari et al., 2010; Jang et al., 2013; Katsampa et al., 2015). Ultrasound treatment (US treatments) are used to induce desirable chemical and physical changes in foods and can support several processes, such as drying, osmotic dehydration, extraction, mixing, emulsification, filtration, crystallization, thawing and freezing (Marcuzzo et al., 2010). Ultrasonic waves cause rapid compressions and expansions to plant cells, which leads to the formation of bubbles in the sonicated sample and its surroundings. The resulting rapid and short pressure and temperature shifts in the product lead to changes of viscosity and surface tension, destroying cell walls, forming microscopic channels and free radicals, and producing sonochemicals. Scientific evidence exists to support both the positive and the negative impacts of US on the retention of bioactive compounds in various fruit and vegetables, although the particular effect depends on the process conditions and specificity of the material involved (Gamboa-Santos et al., 2014; Mieszczakowska-Frąc, Dyki, & Konopacka, 2016). Advantages of power ultrasound include a reduction in processing time, the effective removal of occluded oxygen in juices, and lower energy consumption (Knorr et al., 2004).

The responses of plants to abiotic stresses, such as US, associated with the production of stress signalling molecules (i.e. reactive oxygen species – ROS) activate the expression of genes involved in the primary and secondary metabolism of the plant (Jacobo-Velázquez, González-Agüero, & Cisneros-Zevallos, 2015). These genes are associated with an increase in the activity of enzymes related to the

biosynthesis of secondary metabolites and with the accumulation of secondary metabolites (Jacobo-Velázquez, González-Agüero, & Cisneros-Zevallos, 2015). For this reason, US can be used as an approach to increase the extractability of bioactive compounds (Nowacka & Wedzik, 2016; Rombaut et al., 2014), for instance, found a 12.5% higher extractability of carotenoid from carrots after the application of US at 21 kHz. Ultrasound has also shown higher extraction rates of phenolic compounds from carrot pomace and strawberries (Jabbar et al., 2015). Power ultrasound has also potential as a means of preservation due to the microbial inactivation ascribed to cavitation, as the resulting pressure shifts contribute to cell disruption. Ancillary chemical effects, such as the formation of free radicals as a consequence of the sonochemical reaction, also contribute to the microbial cell disruption (Kadkhodaee & Povey, 2008).

The most popular drying methods for onions are hot-air drying and freeze-drying. Hot-air drying involves exposure of the product to be dried to a continuously flowing hot air stream. It produces dehydrated products with a shelf life of up to one year, but their quality is usually lower than that of the original foodstuff (Ratti, 2001). Freeze-drying is based on dehydration by sublimation of water from a frozen product. Due to the absence of liquid water and the low temperatures required for freeze-drying, most of the deterioration and microbiological reactions are retarded resulting in a final product of high quality (Rawson et al., 2011). However, the quality of a dehydrated product depends also on the pre-treatments employed before drying (Negi & Roy, 2000). Hot-water blanching (heating of a product with hot water for a short period) has also been reported to lower drying time up to a certain operation temperature. Similarly to other thermal processes, blanching affects the concentration of some bioactive compounds in vegetables (Rawson et al., 2011).

Give the possible detrimental effect on blanching on the quality of onions, there is a need to develop alternative pre-treatment method such as: ultrasound treatment,

which is an emerging and promising alternative technology for food processing to possible replace blanching. Despite the fact that power ultrasound has been extensively reviewed in fruit, their effects on quality parameters have not been extensively studied in onions.

The present study investigated the effect of ultrasonic and blanching pre-treatments prior to hot-air drying and freeze-drying on the retention of bioactive compounds (total phenolics, total flavonoids, individual flavonoids, colour and antioxidant activity) of onions.

6.2 Materials and methods

6.2.1 Sample preparation

Organically grown onions (variety: Hyskin) were obtained from the Horticulture Development Department in Teagasc, grown as part of the Kinsealy systems experiment, based in Kinsealy (53° 25N, 6° 10W), Dublin, Ireland and stored at 4 ^{o}C prior to analysis. Fresh organic onions (variety: Hyskin) in net bags of 20 kg were transported to the lab (2015). Several organic onion bulbs with no visible defects were chosen by myself. The organic onions were grown to organic standards (Organic cultivation practices used were in compliance with EC1990/92, EC834/200719), according to trial previously described in experimental chapter 1. After hand-peeling, onions were vertically sliced (5 mm thickness) using a Berkel 800 meat slicer (Berkel company, Indiana, USA).

6.2.2 Ultrasound and blanching pre-treatments

One kg of fresh organic onion slices (1 cm long × 1 cm wide and thickness of approximately 5 mm) were obtained from onion bulbs (variety: Hyskin). In each treatment, 50 g of onion slices were mixed with 100 mL of distilled water at 70 ^{o}C in a 200 mL beaker.

Ultrasound (20 kHz) was irradiated to 50 g of onion slices mixed with 100 mL of water at 70 ^{o}C with an ultrasonic probe (Ø19 mm) connected to an ultrasonic generator (VC 1500, Sonics and Materials Inc., USA). The energy input was controlled by setting the amplitude of the sonicator probe. Extrinsic parameters of amplitude (power output of 40%, 60% and 80%, equivalent to 24.4, 42.7 and 61.0 µm) and processing time (1, 3 and 5 mins) were varied with a pulse duration of 5 s on and 5 s off. Ultrasound conditions have been chosen in parameters of amplitude (power output of 40%, 60% and 80%, equivalent to 24.4, 42.7 and 61.0 µm) in the last experiment chapter. Due to the range of amplitude chosen at the minimum, middle and maximum, it is much clearer to observe and compare the variation among individual flavonoids with ultrasound conditions. The ultrasound probe was submerged to a depth of 25 mm into the sample. All treatments were carried out in triplicate. The ultrasound densities ranged between 0.06 and 0.59 W/mL.

For the blanching pre-treatment, carried out alternatively to the-US treatment, 50 g of onion slices were mixed with 100 mL of distilled water at 70 ^{o}C for 1, 3 and 5 mins. All treatments were carried out in triplicate.

6.2.3 Preparation of extracts from dried onions

Control, sonicated and blanched slices were either freeze-dried or hot-air dried. Hot-air drying of sonicated, blanched and untreated (control) samples was carried out in a laboratory scale hot-air dryer (SG96⁄06⁄333, Gallenkamp, UK) at 60 ^{o}C and 0.3 m/s for 8 h. Pre-treated and control samples of 50 g were placed in a perforated basket (300 x 400 mm; perforation size of 5 x 5 mm), which was inserted in the drying chamber. Each sample was dried separately. Freeze-drying was carried out in a Cuddon freeze-drier (FD80, Cuddon Freeze Dry, Blenhein, New Zealand) at 0.064 mbar for 72 h. After freeze-drying or hot-air drying, the samples were vacuum-packed in polypropylene bags and stored at -20 ^{o}C until analysis.

The leaching water resulting from the ultrasound and blanching pre-treatments were also freeze-dried or hot-air dried, according to the drying method selected for the onion slices. The dry weights were used to calculate the transfer of material from the onions into the cooking water. For this, the dried onions were blended by a kitchen blender (Kenwood Ltd, Havant, UK). Then, 1 g of the blended sample was mixed with 10 mL of methanol (80%) and homogenised at 24,000 rpm using an Omni-prep multi-sample homogeniser (Omni International, USA). The homogenized sample suspension was shaken overnight with a V400 Multitude Vortexer (Alpha laboratories, North York, Canada) at 1500 rpm at room temperature. The sample suspension was centrifuged (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK) at 3000 g for 15 mins and immediately filtered through 0.22 μm polytetrafluoethylene filters. The extracts were kept at -20 ^{o}C until further analysis.

6.2.4 Analysis of total phenolics (TPC)

The methods of total phenolics have been described in section 2.2.3.

6.2.5 Analysis of total flavonoid content (TFC)

The methods of total flavonoids have been described in section 2.2.4.

6.2.6 HPLC analysis of the extracts

Separation and quantification of flavonoid were carried out by RP-HPLC using the method, as outlined previously in section 3.2.7.

6.2.7 Colour

The colour was measured using the method described in section 3.2.8.

6.2.8 Analysis of antioxidant activity

6.2.8.1 Ferric reducing antioxidant power (FRAP) assay

The methods of antioxidant activity as measured by FARP assay have been described in section 2.2.5.1.

6.2.8.2 DPPH antioxidant power assay

The methods of antioxidant activity as measured by DPPH assay have been described in section 2.2.5.2.

6.2.9 Statistical analysis

All experiments were carried out in triplicate and average values were reported as means ± standard deviation. The experimental data were statistically analysed using the software SAS V.9.1 (SAS Institute, NC, USA). The Tukey-Kramer test was applied for multiple comparisons among means at a 95% significance level (p<0.05).

6.3 Results and discussion

6.3.1 Change of total phenolics content

The ultrasound and blanching treatments influenced the total phenolic content (TPC) of onion slices (Table 6.1). Blanching applied for 1 min and ultrasound applied for 1-3 mins in general increased the TPC of dried onions. After 3 mins of ultrasound treatment at 42.7 μm and 61.0 μm, for example, there was a 17%-21% TPC increase in freeze-dried onions (p<0.05). Samples treated by ultrasound at 61.0 μm for 1 min followed of hot-air drying had a 10% increase (p<0.05) in relation to the untreated dried samples. The application of sonication techniques to assist in the extraction of bioactive compounds is widely reported (Keenan et al., 2012).

Samples subjected to UFD (ultrasound + freeze-drying) at 24.4 μm for 3 mins and UHD (ultrasound + hot-air drying) at 61.0 μm for 1 min resulted in greater retention

of phenolics than samples blanched for the same time. Also, blanching caused phenolics to leach into the cooking water nearly 1-3 times more than during the ultrasound treatment (Table 6.1). In agreement with this finding, Rawson et al. (2011) reported higher retention of carotenoids and polyacetylenes in dried carrots subjected to a 10 mins-pre-treatment with a US-probe under pulsed mode than in dried carrots blanched at 80 ^{o}C for 3 mins. The blanched freeze-dried (BFD) and blanched hot-air dried (BHD) (3 and 5 mins) samples had lower retention of phenolics compared to the control (p<0.05). Turkmen, Sari, and Velioglu (2005) also reported that blanching decreased the total phenolics in squash, peas and leek.

Since all samples were hot-air dried under the same condition (60 ^{o}C and 0.3 m/s), blanching led to a major loss of phenolics by leaching than ultrasound, but there were minor losses during dehydration. Lower amounts of phenolics were detected after drying since the prior blanching and the 3 mins-ultrasound treatment were very severe. The main mechanism involved in the loss of phenolics during US treatment might be the formation of microchannels during cavitation, which facilitates the transport of food constituents, especially soluble nutrients (Mothibe et al., 2011). In fact, Opalić et al. (2009) reported that prolonged US pre-treatment in samples with the same geometry led to a decrease in total phenolics and flavonoids and accordingly in the antioxidant capacity of dried apples. The degradation trend during ultrasonic processing may be also related to the formation of free radicals, resulting in a potential increase in the oxidation pathways (Pétrier, Combet, & Mason, 2007). The degradation observed in the present study may point to additional contributory factors. Due to the relatively high temperature and longer holding time (after 5 mins-ultrasound treatment) lead to oxidative and thermal degradation. Furthermore, the ultrasound probe had direct contact with the sample, with the vessel opened to the atmosphere (i.e., it was not a closed system). Therefore, oxidation could freely occur at the liquid/atmosphere interface during processing. This effect would be increased in samples processed for longer periods.

Table 6.1A Influence of ultrasound and blanching treatments followed of drying on the total phenolics content (TPC), total flavonoid content (TFC) and antioxidant activity of onion slices.

Freeze-Drying	TPC	Retention (%)	TFC	Retention (%)	FRAP	Retention (%)	DPPH	Retention (%)
Control	9.21±0.82^{cdef}	---	4.10±0.08^{bcd}	---	11.05±0.99^{c}	---	4.42±0.82^{bc}	---
UFD 24.4 µm 1 min	9.65±0.24^{bcd}	104.87%	4.19±0.18^{abc}	102.08%	11.58±0.29^{bc}	104.87%	5.21±0.84^{abc}	117.98%
UFD 42.7 µm 1 min	9.48±0.40^{bcde}	102.99%	4.13±0.07^{abcd}	100.59%	11.38±0.48^{bc}	102.99%	5.12±0.83^{abc}	115.87%
UFD 61.0 µm 1 min	9.31±0.37^{cdef}	101.13%	4.15±0.03^{abcd}	101.15%	11.17±0.44^{bc}	101.13%	5.03±0.8^{abc}	113.78%
Blanching 1 min	9.22±0.10^{cdef}	100.18%	4.16±0.10^{abcd}	101.27%	11.07±0.12^{c}	100.18%	4.98±0.84^{b}	112.71%
UFD 24.4 µm 3 mins	11.18±1.27^{a}	121.41%	4.47±0.15^{a}	108.93%	13.41±1.52^{a}	121.41%	6.04±0.89^{a}	136.59%
UFD 42.7 µm 3 mins	10.81±0.43^{ab}	117.48%	4.42±0.24^{ab}	107.65%	12.98±0.52^{a}	117.48%	5.84±0.88^{ab}	132.16%
UFD 61.0 µm 3 mins	9.76±0.56^{abc}	106.06%	4.27±0.56^{abc}	104.06%	11.72±0.68^{bc}	106.06%	5.27±0.85^{abc}	119.32%
Blanching 3 mins	8.19±0.11^{defg}	88.96%	3.81±0.11^{bcde}	92.83%	9.83±0.14^{d}	88.96%	4.40±0.76^{bc}	100.08%
UFD 24.4 µm 5 mins	8.09±0.07^{efg}	87.91%	3.76±0.06^{cdef}	91.71%	9.71±0.09^{d}	87.91%	4.37±0.75^{abc}	98.90%
UFD 42.7 µm 5 mins	7.68±0.06^{g}	83.45%	3.49±0.10^{ef}	84.96%	9.22±0.07^{de}	83.45%	4.15±0.70^{c}	93.88%
UFD 61.0 µm 5 mins	7.33±0.14^{g}	79.61%	3.15±0.06^{f}	76.75%	8.79±0.17^{e}	79.61%	3.96±0.63^{c}	89.56%
Blanching 5 mins	7.86±0.15^{fg}	85.41%	3.57±0.30^{def}	86.98%	9.43±0.18^{de}	85.41%	4.25±0.71^{c}	96.08%

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for triplicates (n=3). TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight). TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight). FRAP and DPPH = Antioxidant activity (Trolox mg/g DW).

*Blanching was carried out at 70 ºC, Hot-air drying at 60 ºC and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

Table 6.1B Influence of ultrasound and blanching treatments followed of drying on the total phenolics content (TPC), total flavonoid content (TFC) and antioxidant activity of onion slices.

Hot-Air Drying	TPC	Retention (%)	TFC	Retention (%)	FRAP	Retention (%)	DPPH	Retention (%)
Control	7.76±0.39^{abc}	---	3.34±0.36^{bcde}	---	9.31±0.47^{b}	---	3.82±0.67^{bc}	---
UHD 24.4 µm 1 min	6.50±0.37^{def}	83.84%	3.35±0.20^{bcde}	100.12%	7.80±0.45^{ef}	83.84%	3.36±0.70^{de}	87.93%
UHD 42.7µm 1 min	7.67±0.47^{abc}	98.88%	3.66±0.18^{bc}	109.43%	9.20±0.56^{bc}	98.88%	3.96±0.73^{b}	103.70%
UHD 61.0 µm 1 min	8.58±0.44^{a}	110.65%	4.34±0.27^{a}	130.04%	10.30±0.53^{a}	110.65%	4.43±0.87^{a}	116.05%
Blanching 1 min	7.93±0.14^{ab}	102.24%	3.90±0.31^{b}	116.71%	9.52±0.17^{b}	102.24%	4.09±0.78^{ab}	107.23%
UHD 24.4 µm 3 mins	6.69±0.65^{cde}	86.26%	3.45±0.34^{bcd}	103.15%	8.03±0.78^{e}	86.26%	3.45±0.69^{cd}	90.47%
UHD 42.7 µm 3 mins	7.34±0.26^{bcd}	94.58%	3.79±0.35^{bc}	113.57%	8.80±0.31^{cd}	94.58%	3.79±0.76^{bc}	99.19%
UHD 61.0 µm 3 mins	7.74±0.27^{abc}	99.83%	3.83±0.14ab	114.63%	9.29±0.33^{b}	99.83%	4.00±0.79^{b}	104.70%
Blanching 3 mins	6.23±0.17^{def}	80.27%	3.10±0.33^{cdef}	92.67%	7.47±0.21^{fg}	80.27%	3.21±0.62^{de}	84.19%
UHD 24.4 µm 5 mins	5.50±0.37^{f}	70.94%	2.70±0.17^{f}	80.85%	6.60±0.45^{h}	70.94%	2.84±0.54^{f}	74.40%
UHD 42.7 µm 5 mins	6.34±0.26^{def}	81.69%	2.88±0.08^{def}	86.35%	7.60±0.3^{ef}	81.69%	3.27±0.58^{de}	85.67%
UHD 61.0 µm 5 mins	7.25±0.23^{bcd}	93.46%	3.34±0.27^{bcde}	100.11%	8.70±0.27^{d}	93.46%	3.74±0.68^{bc}	98.02%
Blanching 5 mins	5.93±0.14^{ef}	76.46%	2.77±0.32^{ef}	82.84%	7.12±0.17^{g}	76.46%	3.06±0.55^{ef}	80.19%

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for triplicates (n=3). TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight). TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight). FRAP and DPPH = Antioxidant activity (Trolox mg/g DW).

*Blanching was carried out at 70 ºC, Hot-air drying at 60 ºC and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

6.3.2 Change of total flavonoids content

There was a significant difference of TFC (p<0.05) between ultrasound-treated and blanched onions after drying compared to dried samples without pre-treatment, considering either freeze-dried or hot-air dried (Table 6.1).

TFC in dried (freeze-drying and hot-air drying) onion slices treated with ultrasound for 1-3 mins in general increased compared to the control dried samples. Lower ultrasound amplitudes (24.4 μm) with freeze-dried samples and highest amplitude (61 μm) with hot air dried samples resulted in better retention of TFC compared to other no other ultrasound treatment or no pre-treatment dried samples (Table 6.1). This increase in the retention of TFC may arise from an increase in the extractability of the compounds. Improved extraction efficiency following sonication has been attributed to the propagation of ultrasound pressure waves, induced cavitation and high shear forces resulting in increased mass transfer (Rawson et al., 2011). There was also a significantly (p<0.05) higher retention of flavonoids in UFD (24.4 μm for 3 mins) and UHD (61.0 μm for 1 min) than BHD (1, 3 and 5 mins) samples. Regarding blanching, the higher the process time, lower was the retention of flavonoids.

6.3.3 Changes of quercetin and quercetin glucosides

The levels of the 3 major quercetins – quercetin 3,4'diglucoside (Q 3,4' D), quercetin 4'glucoside (Q 4' G), and quercetin (Q) – in dried onions are presented in Figure 6.1-6.3.

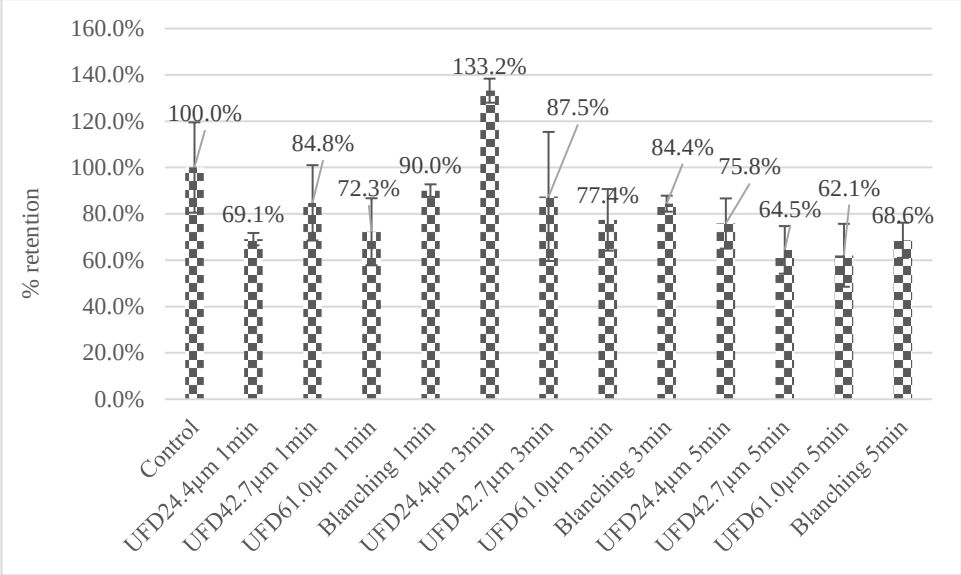
In general, the retention levels of Q 3,4' D and Q for US-freeze-dried and US-hot air dried samples were higher compared to the samples dried without any pre-treatment. This can be ascribed to the increased extractability induced by cavitation of US-treated samples (Rawson et al., 2011).

In BFD and BHD onions slices (1 min), the retention levels of Q were higher compared to the control (p<0.05). Blanching in fact does not always result in the destruction of bioactive compounds. In some cases, thermal treatments can induce the formation of novel compounds and improve the antioxidant capacity (Xu &

Chang, 2008). Bunea et al. (2008) suggested that the increase in the concentrations of certain bioactive compounds after thermal treatment may be explained either by their better release from the food matrix as a result of breakdown of supramolecular structures containing functional groups or their thermal stability. However, in BFD and BHD samples (3 and 5 mins), the retention levels of Q were lower compared to the control (p<0.05). This is most likely due to the relatively high temperatures required for blanching (70 ^{o}C sustained for 3-5 mins), which could lead to oxidative and thermal degradation (Rawson et al., 2010).

Regarding the freeze-drying, the ultrasound treatment at 24.4 μm for 3 mins resulted in significantly higher retention levels of Q 3,4' D and Q compared to BHD (1-5 mins) samples. With regard to the hot-air drying, there were significantly higher retention levels of Q 4' G and Q after US treatment at 61.0 μm for 1 min compared to BHD (1-5 mins) samples.

(a)



(b)

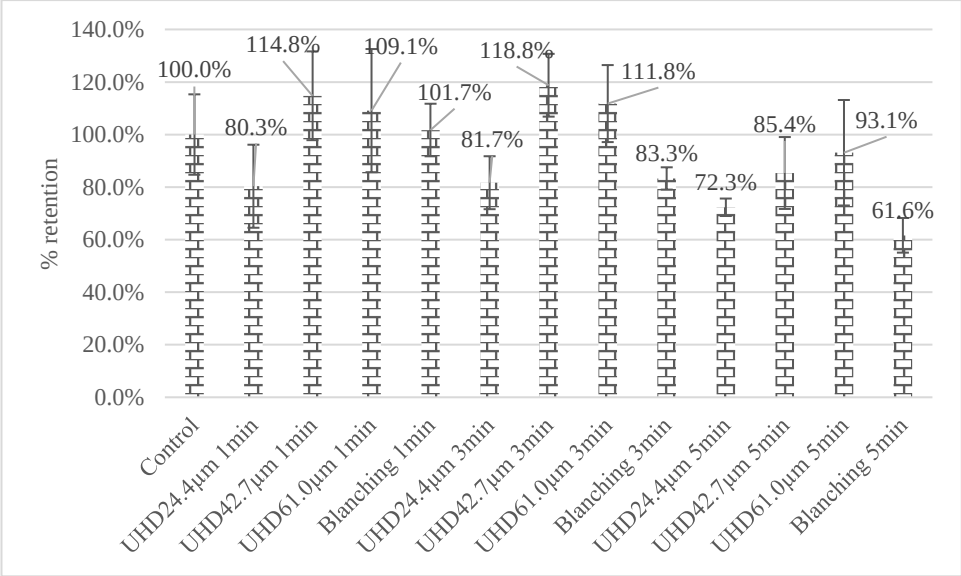
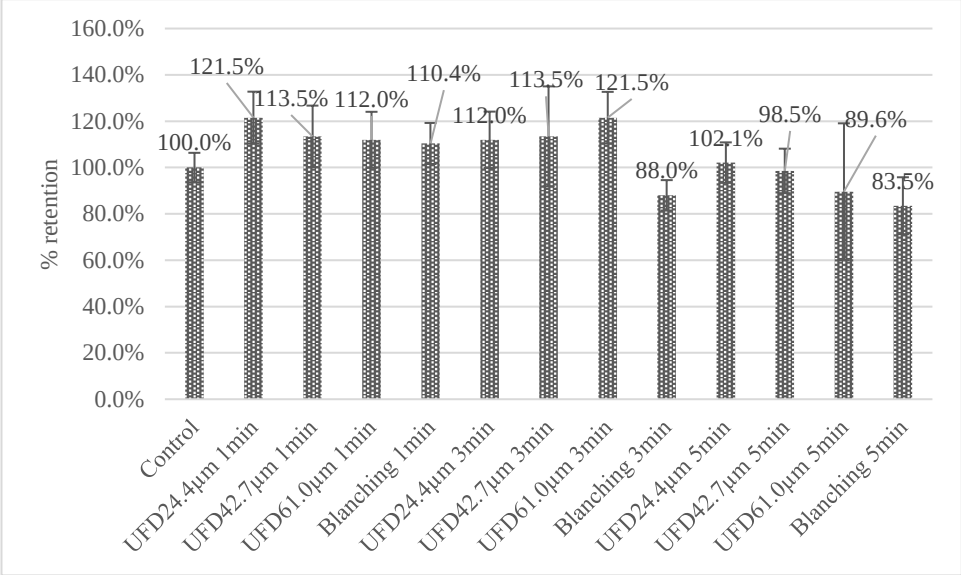


Figure 6.1 Retention of Quercetin 3,4' Diglucoside in different pretreatment with (a) freeze-drying and (b) hot-air drying.

(a)



(b)

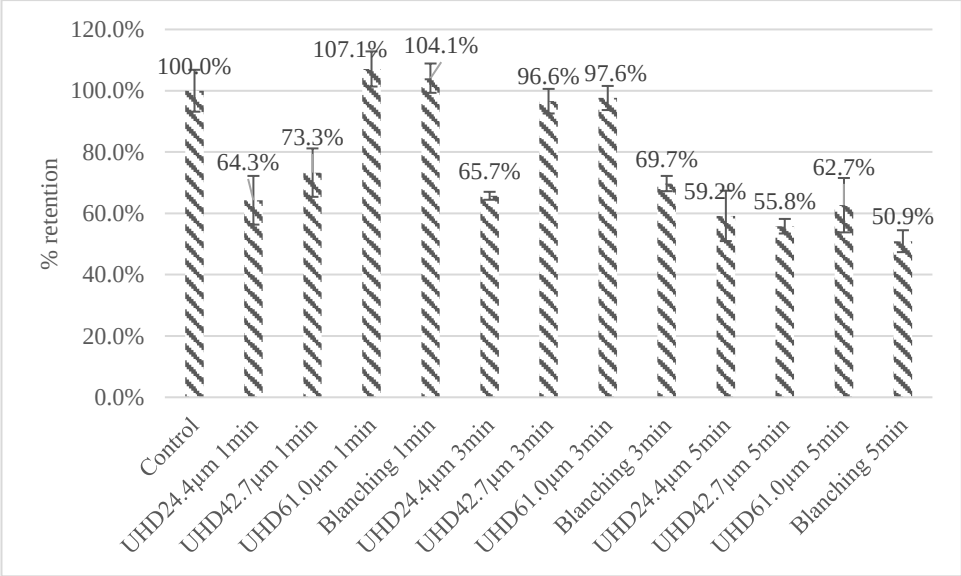
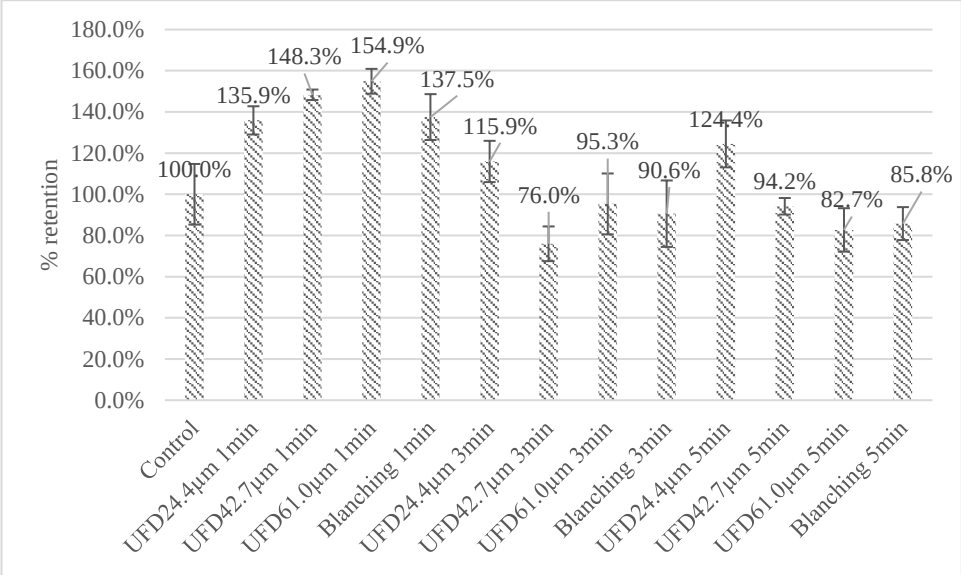


Figure 6.2 Retention of Quercetin 4'Glucoside in different pretreatment with (a) freeze-drying and (b) hot-air drying.

(a)



(b)

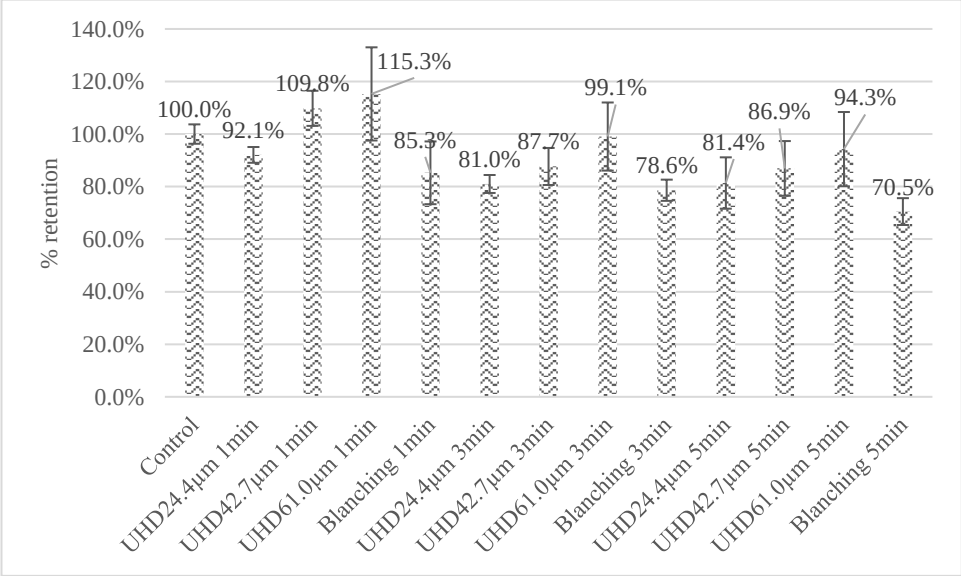


Figure 6.3 Retention of Quercetin in different pretreatment with (a) freeze-drying and (b) hot-air drying.

6.3.4 Change of antioxidant activity during pre-treatment

The antioxidant activity of pre-treated and untreated (control) dried onion slices are presented in Table 6.1. Sonicated samples processed at the highest amplitude (61.0 µm) for the longest time (5 mins) and then freeze-dried as well as sonicated samples processed at the lowest amplitude (24.4 µm) for 5 mins and then hot-air dried had the lowest (p<0.05) antioxidant activity. Generally, onions sonicated at lower amplitudes followed of freeze-drying had the highest antioxidant activity (FRAP and DPPH), while longer US-times reduced the antioxidant activity (Table 6.1).

The DPPH and FRAP values were similar and indicate that blanching generally resulted in lesser preservation of antioxidant compounds compared to fresh and sonicated samples. The exception was the 1 min-blanching, which resulted in enhanced antioxidant activity. Some studies have suggested that blanching is generally regarded as being destructive to antioxidant components (Krishnaswamy & Raghuramulu, 1998). On the contrary, Halvorsen et al. (2006) reported increased antioxidant activity for several vegetables such as carrots, spinach, mushroom, asparagus, broccoli and cabbage after thermal treatment. Dewanto, Xu and Liu (2002) found similar results in thermally processed tomatoes compared with fresh controls. These authors hypothesised that higher antioxidant activities may be related to an increase in extractability of antioxidant components following thermal processing.

6.3.5 Phenolic compounds and antioxidant activity in water

Blanching retained greater amounts of phenolic compounds than ultrasound (p<0.05). The losses could be attributed to water soluble phenolics leaching into the cooking water as well as breakdown of phenolics during thermal processing. These significant losses could be attributed to water soluble phenolics leaching and transferred into the cooking water as well as breakdown of phenolics during thermal processing, which rendered water a good source of dietary phenolics (Table 6.2).

However, degradation of phenolics in onion slices may be a more serious problem than leaching. The percentage of phenolics undergoing degradation during the US-treatment was higher than the percentage loss to the cooking water. These results suggest that the degradation of phenolics after sonication was greater than the losses

due to leaching. Some authors have indicated that pressure-cooking enhanced the antioxidant composition and palatability of vegetables (Xu & Chang, 2009). However, higher power could result in greater degradation (Hiemori, Koh, & Mitchell, 2009).

Table 6.2 Effect of ultrasound and blanching treatments followed by drying on the bioactive compounds and antioxidant activity of the leaching water from onion slices.

Treatment	TPC	TFC	Q 3,4' D	Q 4' G	Q	FRAP	DPPH
UFD 24.4 µm 1 min	0.66±0.03^{e}	0.22±0.01^{b}	10.43±0.31^{def}	55.56±5.42^{de}	4.42±0.71^{cd}	0.81±0.04^{d}	0.47±0.03^{abcd}
UFD 42.7 µm 1 min	0.96±0.01^{d}	0.24±0.01^{b}	11.83±0.13d^{e}	61.97±1.24^{d}	4.58±0.26^{cd}	0.79±0.06^{d}	0.46±0.11^{bcd}
UFD 61.0 µm 1 min	1.31±0.07^{c}	0.26±0.00^{b}	17.67±0.04^{d}	92.31±1.31^{c}	4.71±0.47^{c}	0.78±0.05^{d}	0.45±0.12^{bcd}
Blanching 1 min	1.52±0.02^{a}	0.71±0.29^{a}	225.05±3.00^{a}	408.37±2.50^{a}	63.00±0.92^{a}	1.10±0.05^{a}	0.60±0.08^{a}
UFD 24.4 µm 3 mins	0.43±0.02^{g}	0.06±0.01^{c}	3.67±0.15^{gf}	32.31±2.20^{f}	1.24±0.12^{cde}	0.78±0.18^{d}	0.45±0.16^{bcd}
UFD 42.7 µm 3 mins	0.53±0.01^{f}	0.06±0.00^{c}	3.83±0.31^{gf}	35.6±5.94^{f}	1.58±0.83^{cde}	0.93±0.06^{bc}	0.54±0.12^{ab}
UFD 61.0 µm 3 mins	0.63±0.02^{e}	0.09±0.01^{c}	7.43±0.02^{efg}	41.97±1.84^{ef}	1.67±0.14^{cde}	0.91±0.08^{c}	0.53±0.15^{abc}
Blanching 3 mins	1.35±0.02^{b}	0.24±0.01^{b}	208.38±3.60^{b}	325.03±12.43^{b}	38.05±3.38^{b}	0.98±0.07^{b}	0.53±0.12^{abc}
UFD 24.4 µm 5 mins	nd	nd	nd	nd	nd	nd	nd
UFD 42.7 µm 5 mins	nd	nd	nd	nd	nd	nd	nd
UFD 61.0 µm 5 mins	nd	nd	nd	nd	nd	nd	nd
Blanching 5 mins	1.34±0.03^{c}	0.21±0.00^{b}	175.5±1.60^{c}	310.70±19.10^{b}	35.1±1.58^{b}	0.94±0.10^{bc}	0.51±0.06^{abc}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for triplicates (n=3). TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight). TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight). Q 3,4' D = quercetin 3,4'glucoside (µg/g); Q 4' G = quercetin 4'glucoside (µg/g); Q = quercetin (µg/g).

*Blanching was carried out at 70 ^{o}C, Hot-air drying at 60 ^{o}C and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

6.3.6 Flavonoids in water

The total flavonoid content in the cooking water revealed a trend similar to that described for the TPC (Table 6.2). The flavonoid losses could be a result of degradation or decomposition of flavonoids (Ioannou et al., 2012). The ultrasound treatment resulted in a higher percentage of flavonoids being degraded than retained in the cooking water ($p<0.05$). There was a transfer of especially Q 3,4' D and Q 4' G from onions to water. This suggests that the decrease of flavonoid during ultrasound was predominantly caused by the breakdown of flavonoids rather than their leaching. Higher ultrasound amplitudes and longer time resulted in greater leaching of flavonoids.

6.3.7 Quercetin and its glucosides in water

The amounts of quercetin 3,4’ diglucoside and quercetin 4’ glucoside were also measured in water after ultrasound and blanching treatments (Table 6.2). In the blanching water, about half of the quercetin 3,4’ diglucoside was in the form of quercetin 4’ glucoside. In the US-treatment water, the quercetin 4’glucoside fraction was greater than the quercetin 3,4’ diglucoside one. Hirota, Shimoda, and Takahama (1998) observed that the monoglucoside derivative was oxidized more rapidly than its diglucoside form during cooking, and that the difference in the stability between mono and diglucoside was due to the presence or absence of a hydroxyl group at the C-3 position in the glucosides. As the antioxidant power of flavonols substantially depends on the catechol group in the B-ring and on the 3-hydroxyl group (Rodrigues et al., 2009), the monoglucoside is likely to have a higher antioxidant capacity than the diglucoside, since in the latter these two basic functions are blocked. In this work, there was a lower content of flavonols in water, which was however enriched with antioxidant monoglucoside forms.

Free quercetin was found in the onion slices but only in very small amounts in the cooking water (Table 6.2), which may correspond to its poor solubility in water and/or stronger binding to plant structures than its glycoside forms. Quercetin was not detected in water after the 5 mins-ultrasound treatment, indicating that this compound is not prone to leaching.

6.3.8 Antioxidant activity in water

The blanching water had high antioxidant (Table 6.2), especially for the 1 min-treatment, followed by 3 mins. The cooking water from US-treated onions had low values of antioxidant activity according to both assays. The sum of antioxidant activity of the cooked onion and cooking water is different from the antioxidant activity of fresh samples, which may suggest losses in the antioxidant activity due to breakdown or degradation of antioxidant compounds.

6.3.9 Effects of ultrasound and blanching on colour

Colour has a major impact on the acceptance of a product by the consumer (Kalt, 2005). Fresh onions were characterized by high luminosity (L* = 74.24 ± 2.15), with a tendency to green and yellow (a* = -6.23 ± 0.53 and b* = 22.79 ± 2.8, respectively) (Table 6.3). The L* of dried samples ranged from 58.3 to 93.74, b* varied from 23.7 to 33.98, and a* varied from -9.73 to -4.36, indicating the dried onions had more intense green and yellow tones than the fresh ones. All dried samples were characterized by high ΔE values, regardless of the ultrasound and blanching conditions (Table 6.3).

Although luminosity was similar for fresh, blanched-dried and US-dried onions, sonicated samples had a higher colour difference (ΔE) than blanched ones ($p<0.05$). The longer the sonication time (and blanching time as well), the higher was the colour difference, regardless of the ultrasound amplitude. The use of ultrasound as a pre-treatment to onions contributed to a significant colour change. UFD and UHD (highest amplitude applied for 5 mins) samples showed significantly ($p<0.05$) higher ΔE compared to other amplitudes and to BFD and BHD samples. These changes can be explained by the formation of free radicals and sonochemicals as a result of cavitation (Bermúdez-Aguirre, Mobbs, & Barbosa-Cánovas, 2011), which may influence the food properties. The change of coordinate a*, in specific, can be linked to the formation of colour compounds (Vadivambal & Jayas, 2007) related to non-enzymatic browning during treatment. The greatest colour change for the samples treated by ultrasound is also ascribed to the presence of air during processing,

leading to enzymatic browning. In the case of blanching, the colour was better preserved as the contact between samples and air was limited.

The colour of vegetables is determined by natural colour compounds that can be oxidized during the pre-treatment, and the most important factor accelerating degradation is high temperature and presence of oxygen. Enzymatic browning also plays an important role in colour change due to the brown pigments formed from colourless polyphenols (Maskan, 2001). Table 6.4 shows that the b* chroma was correlated to TPC and Q 4' G at 5% significance (Table 6.4) in the hot-air drying, but the colour coordinates had no correlation with the bioactive compounds in freeze-drying.

Table 6.3A Colour of freeze-dried and hot-air dried onion slices subjected to blanching and ultrasound pre-treatments.

Freeze-Drying	L*	a*	b*	ΔE
Control	74.24±2.15^{e}	-6.23±0.53^{a}	22.79±2.80^{c}	---
UFD 24.4 µm 1 min	80.80±0.60^{cd}	-8.84±0.62^{ef}	31.07±2.17^{a}	10.88±1.13^{g}
UFD 42.7 µm 1min	81.51±1.21^{bcd}	-9.21±0.19^{fg}	29.78±1.66^{ab}	11.51±1.02^{g}
UFD 61.0 µm 1min	92.41±0.66^{a}	-9.01±0.43^{fg}	29.25±0.78^{ab}	19.47±0.50^{c}
Blanching 1 min	86.51±0.38^{bc}	-7.08±0.05^{b}	25.72±0.60^{c}	12.64±0.47^{e}
UFD 24.4 µm 3 mins	81.5±1.54^{bcd}	-8.98±0.83^{ef}	31.80±1.09^{a}	11.90±1.15^{fg}
UFD 42.7 µm 3 mins	82.35±1.32^{bcd}	-9.32±0.21^{gh}	29.98±0.93^{ab}	12.27±0.82^{ef}
UFD 61.0 µm 3 mins	92.41±0.30^{a}	-8.21±0.13^{de}	29.25±0.06^{ab}	19.31±0.16^{c}
Blanching 3 mins	89.34±0.61^{bc}	-7.28±0.18^{bc}	27.61±0.50^{ab}	15.97±0.43^{d}
UFD 24.4 µm 5 mins	91.85±0.45^{a}	-9.30±1.04^{hi}	32.80±2.07^{a}	20.49±1.19^{b}
UFD 42.7 µm 5 mins	91.51±1.18^{ab}	-9.73±0.63^{i}	33.97±5.83^{a}	20.87±2.55^{b}
UFD 61.0 µm 5 mins	93.74±0.11^{a}	-7.97±0.45^{cd}	33.82±4.76^{a}	22.47±1.74^{a}
Blanching 5 mins	88.06±0.80^{ab}	-7.44±0.20^{bc}	29.93±0.60^{abc}	15.60±0.53^{d}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for triplicates (n=3). *Blanching was carried out at 70 ºC, Hot-air drying at 60 ºC and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

Table 6.3B Colour of hot-air dried onion slices subjected to blanching and ultrasound pre-treatments.

Hot-air Drying	L*	a*	b*	ΔE
Control	74.24±2.15^{c}	-6.23±0.53^{b}	22.79±2.80^{de}	---
UHD 24.4µm 1 min	85.80±1.61^{b}	-7.84±0.51^{cd}	30.07±0.98^{a}	10.06±1.03^{k}
UHD 42.7 µm 1 min	82.50±0.36^{b}	-8.21±0.08^{d}	28.78±0.16^{ab}	10.74±0.20^{i}
UHD 61.0 µm 1 min	90.41±0.09^{a}	-6.18±0.08^{b}	28.25±0.51^{ab}	17.06±0.23^{c}
Blanching 1 min	59.10±0.34^{e}	-6.04±0.29^{b}	23.71±0.78d^{e}	15.50±0.45^{f}
UHD 24.4 µm 3 mins	85.98±0.88^{b}	-7.98±0.48^{cd}	30.51±0.65^{a}	10.46±0.67^{j}
UHD 42.7 µm 3 mins	82.85±1.02^{b}	-8.62±0.03^{de}	28.98±0.91^{ab}	10.87±0.65^{h}
UHD 61.0 µm 3 mins	90.94±1.37^{a}	-6.43±0.51^{bc}	28.52±0.76^{ab}	17.66±0.88^{b}
Blanching 3 mins	58.29±0.46^{e}	-5.85±0.22^{b}	25.60±0.22^{bc}	15.70±0.33^{e}
UHD 24.4 µm 5 mins	86.28±0.95^{b}	-8.40±0.28^{d}	31.05±1.86^{a}	14.76±1.03^{g}
UHD 42.7 µm 5 mins	83.15±0.86^{b}	-8.82±0.38^{de}	29.28±1.29^{a}	15.72±0.84^{e}
UHD 61.0 µm 5 mins	91.34±2.36^{a}	-6.74±0.05^{bcd}	28.85±1.18^{ab}	18.15±1.20^{a}
Blanching 5 mins	64.40±0.88^{d}	-4.36±0.38^{a}	29.29±1.10^{ab}	15.93±0.79^{d}

For each column, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for triplicates (n=3). *Blanching was carried out at 70 ºC, Hot-air drying at 60 ºC and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

Table 6.4 Correlation matrix of colour and chemical indices of freeze-dried and hot-air dried onion slices.

Freeze-Drying	TPC	TFC	Q 3,4' D	Q 4' G	Q	L	a	b
TPC	1.00	0.83	0.63	0.58	0.21	-0.55	-0.06	-0.23
TFC		1.00	0.52	0.66	0.34	-0.50	-0.03	-0.33
Q 3,4' D			1.00	0.19	0.09	-0.57	-0.01	-0.31
Q 4' G				1.00	0.47	-0.22	-0.20	-0.04
Q					1.00	-0.11	-0.13	-0.08
L*						1.00	-0.07	0.45*
a*							1.00	-0.54*
b*								1.00
Hot-air Drying	TPC	TFC	Q 3,4' D	Q 4' G	Q	L	a	b
TPC	1.00	0.79	0.75	0.83	0.75	0.17	0.05	0.46*
TFC		1.00	0.68	0.81	0.64	0.23	-0.05	-0.27
Q 3,4' D			1.00	0.77	0.67	0.30	-0.25	-0.23
Q 4' G				1.00	0.65	0.01	-0.05	0.52*
Q					1.00	0.51	-0.17	-0.06
L*						1.00	-0.44*	0.64
a*							1.00	-0.32
b*								1.00

Chromameter describes colour in three coordinates: L, lightness, from 0 (black) to 100 (white); a, from -60 (green) to 60 (red); and b, from -60 (blue) to 60 (yellow). TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight); TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight); Q 4' G = quercetin 4'glucoside (µg/g); Q 3,4' D = quercetin 3,4'glucoside (µg/g); Q = quercetin (µg/g). * Represents significance at 5%.

6.4 Conclusions

Blanching and ultrasound treatments significantly affected the colour, TPC, TFC, individual phenolic compounds and antioxidant activities of onion slices dried either by freeze-drying or hot-air drying. In this work, ultrasound has been identified as an alternative pre-treatment to blanching regarding the enhancement of functional properties in onions. The ultrasound-treatment applied for 1 min at any amplitude (24.4-61.0 μm) increased (1%-20%) the content of phytochemicals and the antioxidant activity of freeze-dried and hot-air dried onions. Sonicated samples with short time showed higher retention of phenolic compounds, flavonoids and quercetin, and thus featured higher antioxidant activity than blanched ones. However, sonication with longer time (5 mins) had a deleterious effect (more than 10% degradation) on bioactive compounds and antioxidant activity. As the leaching water from onions treated with ultrasound and blanching contained high amounts of antioxidants, it may be considered a valuable co-product for the food industry. Further research is required to optimize retention of bioactive by varying ultrasonic processing parameters such as power level, treatment time and temperature, which is to be successfully implemented and important implications in the food industry.

Chapter 7
General Discussion and Conclusions

General Discussion and Conclusions

An increasing awareness of health issues is driving the onion market where the growth of the onions and their diversification have been significantly increased worldwide. A large amount of onions has been consumed every year due to its numerous benefits for human health and its taste. Onions have been perceived as healthier and having more nutritional functions than other mainstream equivalent vegetables. In this regard, the main objective of this thesis was to investigate the development of onion products and a wide range of enhanced onion products that can reflect the market and consumers' needs. The current research encompasses information on several parameters including productions systems, temperature, and storage conditions, which can influence onions' bioactive compounds. This study also provides a comprehensive profiling of the chemical compounds during the storage of onions. Further experiments aim to research a cost-effective extraction method for quercetin and other relevant compounds that would ensure a minimum wastage of onions in the industry as well as in the market. In addition, the underlying mechanisms that cause these phenolic variations were due to plant nutrition, environmental factors, biotic, and abiotic stress, plant growth, and the association between them need to be addressed in further studies. The thesis was broadly divided into two sections. The first section, which consists of chapters 2-3, aimed to investigate how pre-harvest (agronomics) affects the bioactive content in onions. The second section focused on how processing affects onion quality, which can be found in chapters 4-6.

More specifically, in experimental chapters, the results on agronomic practices are useful for different stakeholders, for example, onion farmers who want to grow organic onions with enhanced levels of phytochemicals or quality; consumers who are interested in buying onions perceived as 'healthier'; onion breeders who aim to produce new onion varieties with higher levels of some particular compounds meanwhile maintaining the levels of phenolics; government and professional parties who are marketing added-value for onion varieties. The environmental effects were partially explained in some cases by climate data and soil characteristics, but other factors considered in this study can also influence variation among trial sites, such as

pressure from pests or pathogens. Besides environmental factors, post-harvest treatments had important effects on the data presented here. These treatments included storage, handling, and other processing methods such as blanching and ultrasound treatments. These treatments influence the final phytochemical and nutritional content of consumed onions, depending on the particular types of compounds that need to be considered, as outlined in the introduction chapter. More specific discussion of each experimental chapter is introduced below:

In Chapter 2,'Hyskin' and 'Red Baron' (modern commercially grown varieties) were selected as the main samples to develop bioactive enriched crops, as they are an excellent source of flavonoids. Although there are several studies on the analysis of fruits and vegetables produced under organic and conventional production systems, relatively few robustly designed field trial studies have compared phenolic contents and antioxidant contents in onion crops grown under conventional, organic, and mixed systems. This study measured levels of total phenolics, total flavonoids, and antioxidant activity in onions grown over four years using either conventional (CS+CP), organic (OS+OP) or mixed (OS+CP, CS+OP) treatments. Our data indicated that total phenolic and flavonoid contents in onion was generally higher in red onion 'Red Baron' and was significantly higher in organic (OS+OP) compared to conventional (CS+CP) production in both varieties in most years. Significant year to year variation was also observed, which is attributed to altered regulation of phenolic synthesis in different years due to meteorological conditions. Nowadays, organic agriculture is increasing in terms of the area of production and number of operators. A wide set of solutions have been proposed which, although valid with respect to organic certification standards, still need scientific assessment concerning claims of sustainability and high quality production. More in-depth analyses may relate the organic vs. conventional comparison to the more general issue of pre-harvest effects on postharvest performance of onions. In this respect, the balance between secondary metabolic pathways seems to be an important aspect resulting from the complex connection among genotype, environment, and agricultural practices which lead to differences in quality and postharvest performance of different types of onions. Organic onions are mainly purchased for their safety and absence of synthetic pesticide residues. Furthermore, a diet based on organic products claims to provide

health benefits due to the high nutritional compounds that are more concentrated in organic products compared to conventional ones. Possible explanations for the effects of organic farming practices on nutritional quality and postharvest performance are the following: (i) organic amendments provide a high input of exogenous organic matter and of nutrients for a long period; in contrast, mineral fertilisers, allowed only in conventional farming systems, are highly concentrated in nutrients that are directly available for root uptake in a shorter time period; (ii) the use of synthetic pesticides (only possible in conventional agriculture) slows down defence mechanisms against pathogens, with the consequence of favoring secondary metabolism.

In Chapter 3, in this study the storage period was extended up to the time point where bulbs were unmarketable due to early sprouting, however, if the storage was for short terms and stored at either -20 ºC or 5 ºC, onion bulbs' shelf life can be prolonged. On the other hand, there are several other parameters could influence onion quality during storage, which can be optimized to allow onions to exhibiting a particular set of characteristics. The result showed that the conventional onions can be considered as a ''storage" onion by retaining its marketability and nutritional quality for up to 10 weeks, significantly longer than the organic onion. But in the E2 experiment, one of the interesting observations was that the quality parameters (phenolics compounds and antioxidant activity) of dried organic onion powder were at the same level to the one stored for 10 weeks at -20 ºC. It was suggested that breakdown products (powder) may have potential antioxidant properties, which can keep the product quality during storage but this remains to be confirmed by future studies.

For the post-harvest treatments, with an easier management of the equipment, thermal processing (blanching, hot-air drying) has been favored by the important technological developments over the last few years. However, heat processing particularly under extreme conditions, may lead to onions' chemical and physical changes that impair the organoleptic properties and reduce the content or bioavailability of some bioactive compounds. Therefore, there is a demand for adopting mild processing technologies such as power ultrasound. In the past decade, novel technologies such as ultrasound treatment have become established. In addition

to their possible beneficial effects on nutritional and bioactive content, many of these novel technologies are more cost-efficient and environmental friendly for obtaining premium quality foods and this has led to their revival and commercialization. On the other hand, this study also assessed the effects of common manufacturing techniques on the retention of bioactive compounds in onion products in order to recommend practices and protocols to maximize their retention. Many investigations into the effect of thermal processing of the content of well-known phytochemicals such as polyphenols have been carried out. However, relatively little is known about the effect of new and well-established processing methods on onion bioactive compounds.

Dried onions are considerably important in the world trade and are made in several forms: flaked, minced, chopped and powdered. They are used as flavor additives in a wide variety of food formulations such as comminuted meats, sauces, soups, salad dressings and pickle relishes (Kumar, Hebbar, & Ramesh, 2006). In Chapter 4, the drying of onions was found to be a very useful technique for increasing the number of phenolic compounds and antioxidant capacity of the extracts. All four drying methods (freeze-drying, hot-air drying, vacuum oven drying and oven drying) were tested and they exhibited a different level of influence on the colour, total phenolic content, quercetin content, flavonoid content and antioxidant capacity. Among the drying techniques tested, freeze-drying and hot air-drying were found to be the best techniques in terms of extractability of phenolic compounds from different varieties of onion. Although freeze-drying had a better performance than the other drying methods regarding the preservation of the phenolic bioactive compounds, it is a costly procedure, which limits its usage in the food industry. The use of adequate (60 ^{o}C) temperature for hot-air drying may also ensure the preservation of these compounds, which can be applicable due to the acceptable final levels of bioactive compounds and its low cost. The different drying methods showed a positive effect on the antioxidant activity due to the increase in both extractability and concentration of phenolic compounds, which have a strong correlation with antioxidant activity. With the use of different drying methods, higher levels of bioactive compounds were found in organically grown onions (‘Red Baron’ and ‘Hyfort’) than in conventional cultivation. In addition, the fresh samples had the lowest values for all the parameters

tested and enzymatic degradation of polyphenols and poor efficiency of extraction from the fresh samples could be responsible for these results.

In Chapter 5, thermal pre-treatments, such as blanching, showed to be effective in retarding the oxidation reactions by native enzymes presented in onion slices. Onions are often sold as ingredients in other products, in particular dehydrated soups. They could also be sold in a dehydrated form as a healthy snack. To offset enzyme-induced quality loss, onion slices are blanched prior to drying. This blanching step is usually performed using simple water immersion. From the current study, it suggested that blanching at 70 ºC for 1 min and at 60 ºC for 3 mins, followed by drying are the optimum process conditions. A short-time water-blanching was found to be a suitable initial step in the production of dried onion slices. Blanching affected the total phenolic content, individual phenolic compounds and the antioxidant capacity, and proved to be a suitable method regarding the retention of polyphenols and antioxidant capacity at some operational conditions, since minor destruction of tissue may better protect secondary metabolites from degradation. The combination of blanching and hot air oven drying treatments (60 ºC) with a short time as a pre-treatment may be favoured, which led to a higher recovery of phenolic compounds and enhanced the antioxidant capacity. The use of blanching as a pre-treatment in the drying of onions is also recommended because it reduces the drying time and the energy demand involved in onion drying. This will lower the production cost and result in higher profits by the processors.

Most food processors do not have access to the sophisticated analytical equipment to determine levels of phenolics and optimize their procedures for the compounds retention. In this study, mathematical modelling and graphical model performance indices indicated a good predictive performance on phenolic levels. The models could aid food processors in the optimization of critical operation parameters for desired product quality attributes. The model has a high R^2 value and low *RMSE* values indicating a good correlation with the experimental data at 95% confidence level. The findings suggested that Modified Page and Two-term exponential model distribution could be employed to describe the changes in the levels of phenolics during blanching of onions.

In Chapter 6, results in the same onion varieties showed that blanching and ultrasound treatments significantly affected the colour, TPC, TFC, individual phenolic compounds and antioxidant activities of onion slices dried either by freeze-drying or hot-air drying. The ultrasound has emerged as a promising technique for retaining bioactive components. With an increasing knowledge on the possible negative impact brought by traditional thermal processing techniques on thermally-labile, health-promoting compounds, a range of non-thermal alternative processing strategies have emerged. A decrease in the levels of polyphenols during thermal treatment may be due to heat-induced chemical oxidation and leaching of water-soluble polyphenols during blanching. The fact that levels of total phenols and the majority of individual polyphenols decrease are correlated with antioxidant activity values. It suggests that phenolics may be the major contributor to the total antioxidant activity of the samples tested. Indeed this technology is now the most widely employed in the industry. As suggested by various authors, improved extraction efficiency following sonication can be attributed to the propagation of ultrasound pressure waves, including cavitation resulting in increased mass transfer (Vilkhu et al., 2008). Some authors have suggested that the degassing effect observed under sonication can enhance diffusion into pores on the surface and may explain the enhanced extractability (Simal et al., 1998). The research in this thesis has shown that ultrasound pre-treatment is a potential alternative to conventional blanching treatment in the preparation of desiccated forms of onions to enhance the functional properties. The US-treatment that applied for 1 min or 3 mins at any amplitude (24.4-61.0 μm) increased the content of phytochemicals and the antioxidant activity of freeze-dried and hot-air dried onions. Sonicated samples showed a higher retention of phenolic compounds, flavonoids, and quercetin, and thus featured higher antioxidant activity than blanched ones. At last, the leaching water from onions treated with ultrasound and blanching contained high amounts of antioxidants and it may be considered as a valuable co-product for the food industry.

To sum up, this thesis has shown some of the possibilities for a new generation of processed onion products. Onions are known as an excellent source of phenolics including important phytochemicals such as flavonoids. It has been demonstrated that onion products can be used as carriers for functional ingredients and that

processing techniques, especially non-thermal, may offer alternatives to traditional heat treatments. In summary, the research reported in this thesis has shown that phenolic compounds are significantly affected by different types of processing. Novel processing technology, such as ultrasound, has a great potential to retain the levels of these important compounds in processed onions. Processing treatments, in particular non-thermal techniques, such as ultrasound processing, applied in this thesis may be of interest to producers who are looking for exploiting the future market. The emergence of non-thermal processing technologies has given processors the option to produce shelf stable products while preserving heat labile components.

Phenolic compounds and flavonoids were found to contribute to the antioxidant activity of onions. A strong correlation was found among phenolic and antioxidant activity, which suggest that the large contribution of phenolic compounds in the samples to the full antioxidant presented by onion extracts. Thus, phenolic compounds can be regarded as determinants of the antioxidant activities of onion powder. The correlation found between phenolics and antioxidant activity implies that onion varieties with higher levels of phenolics become more popular due to perceived 'healthy' properties. This must also be considered by scientists aiming at increasing the phenolic content of onions, either through breeding or genetic engineering. Furthermore, one of the features of the onions is to control certain diseases. This notion has been supported by some epidemiological studies demonstrating that fruits and vegetables are associated with various health benefits.

In addition, future optimisation of the processed onion products may be required to maximise their quality, which could be adopted in the food industry. These new approaches and techniques included in this thesis showed the accumulation of many years of research and development in this field. Many of them are not only used in the laboratory context, but also are able to be applied in the pilot- or full- scale industrial operations. This study offers the consumer a new range of healthier and convenient onion products that can be purchased on the market.

Limitations of This Study

This thesis aimed to study the effects of pre-harvest and post-harvest treatments on onion phenolic compounds and antioxidant activity (in vitro studies) in order to increase or maintain those contents in the onion products. The study found a strong correlation between phenolic and antioxidant activity, suggesting that onion varieties with higher levels of phenolics have higher antioxidant activity. Higher antioxidant activity, therefore, can bring potential health benefits and nutritional values to human. However, there were some limitations:

- One of the main limitations was that the DPPH and FRAP antioxidant methods used in this study were both based on electron transfer assays. However, there are some disadvantages in choosing the methods that share the same mechanism due to the single approach.

  If different antioxidant assays with different mechanisms were used in the experiments, different fractions of the antioxidant molecules reacting were able to be observed. It can be a more holistic approach for measuring onion antioxidant capacity. For example, the Oxygen Radical Absorbance Capacity (ORAC) and Total Radical Trapping Antioxidant Parameter (TRAP) assay are based on hydrogen atom transfer, which has a different mechanism with DPPH and FRAP. The use of ORAC and TRAP can enrich the antioxidant capacity assay in the future study.

- The DPPH, FRAP ORAC, and TRAP were widely used in conventional in vitro antioxidant assays since they are easy to use and have high reliability. However, there is a lack of studies adopting them in vivo antioxidant assays and they are criticised for the following reasons.

  Conventional in vitro antioxidant assays measure chemical reactions only, and these reactions cannot be equal to the activity in vivo, as the former cannot account for the bioaccessibility and bioavailability and metabolism of the antioxidant compounds under physiological conditions.

  The best in vivo antioxidant measures should come from animals or human bodies; however, they can be of high cost and time consuming, hence they are not ideal for initial antioxidant screening of foods. Alternatively, in vitro methods for simulating

human digestive tract have been extensively used since they are rapid, safe and easy to perform. In vitro methods, simulating the digestion and absorption processes and measuring the concentration of a nutrient (e.g. phenolics or flavonoid) from the onion extract that has been digested and absorbed (Kamiloglu, Boyacioglu, & Capanoglu, 2013).

- This thesis lacks knowledge of the bioaccessibility and bioavailability of the concentration of phenolics (e.g. flavanols, anthocyanins, or organosulfur compounds) from onion extracts. The bioaccessibility and bioavailability can be evaluated using cell lines, Caco-2 as the most commonly used one, which is obtained from a human colon carcinoma (Gonçalves et al., 2019). Caco-2 cell cultures model provides a cost-effective and relatively fast approach to address issues of uptake and transport of various antioxidants and metabolism (Kamiloglu et al., 2013; Hithamani, Kizhakayil, & Srinivasan, 2017).
- In addition, different drying methods (freeze-drying, hot-air, oven, and vacuum oven) were used to generate onion slices and this study only compared phenolic compounds and antioxidant activity. The optical examination could be further used to evaluate microstructure in onion slices.

Suggestions for Future Work

Health care has gained much attention worldwide, food scientists, medical experts, and biologists have been trying to investigate further on how onions can benefit human's health. The use of advanced technology in developing functional and new onion products has great prospects. This section introduces four future research directions:

- Due to the single approach used by DPPH and FRAP in this study, future work can focus on the use of ORAC and TRAP assays. Comparative studies of DPPH, FRAP, ORAC and TPRA assays for the determination of antioxidant activity in organic and conventional onions will be evaluated.

- This study was limited to conventional in vitro antioxidant assays. Future work could develop a cellular antioxidant activity (CAA) assay in onion study. It has been served as a more appropriate method to measure antioxidant activity than the conventional antioxidant activity methods to assess the absorption and mechanism of antioxidants in cells (Wolfe & Liu, 2007). In addition to antioxidant capacity, it is also important to evaluate the bioaccessibility and bioavailability of antioxidant compounds such as phenolics, flavonoids, and quercetins present in onion products. This will provide valuable data to indicate the biological relevance of these compounds with nutrition uptake and human health in the future. Furthermore, the impact of the bioaccessibility and bioavailability of the phenolic compounds from onions will be evaluated. Specifically, future research will focus on the intestinal uptake performance of phenolics using Caco-2 human intestinal cells and mimick human digestion to assess phenolics (flavonoid and quercetin) absorption with fresh organic and conventional onions.

- Little has been known on the changes in bioaccessibility and bioavailability of phenolics (flavonoids and quercetins) and antioxidant activity during onions processing in previous studies. Processing onions into different products may bring changes in bioaccessibility and bioavailability of onion antioxidants. In the future, we will first investigate the effects of different food processing techniques (thermal, non-thermal) on the bioaccessibility and bioavailability of dried onions compared to fresh ones. Secondly, phenolics and organosulfur compounds from conventional and

novel extracted onions will be compared regarding their bioaccessibility and bioavailability.

Moreover, processing conditions could be further optimised in order to obtain studied compounds with the highest yield and purity. It can reduce industrial cost. Also, to understand how processing affects the concentrations and activity of these compounds, providing emphasis on the bioaccessibility and bioavailability of these valued onion products.

- With regard to the measurement of phenolics content and antioxidant activity, future studies could compare the effect of non-thermal technology such as high-pressure processing (HPP) with other traditional processing technologies (thermal technologies) and secondly, compare HPP and non-thermal processing technologies (ultrasound). Different processing methods can damage onion tissues, causing cell shrinkage and wrinkles on the onion surface. Future studies could compare firstly between untreated raw and treated onion samples and secondly among different processing methods, to evaluate the extent to which the microstructure of onions has been changed after the treatments.

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Appendices

Appendix 1. Influence of Variety on Phenolic Composition and Antioxidant Capacity: A Study on Different Onion Cultivars.

The varietal survey in 26 bulb onion varieties for phenolic content and antioxidant activity were evaluated (the content is from my paper manuscript 'Influence of variety on phenolic composition and antioxidant capacity: A study on different onion cultivars.'). This experiment was conducted to justify the use of 'Hyskin' and 'Red Baron' varieties as the commercial bulb onions used in the following experimental chapters for comparing among different processing treatments.

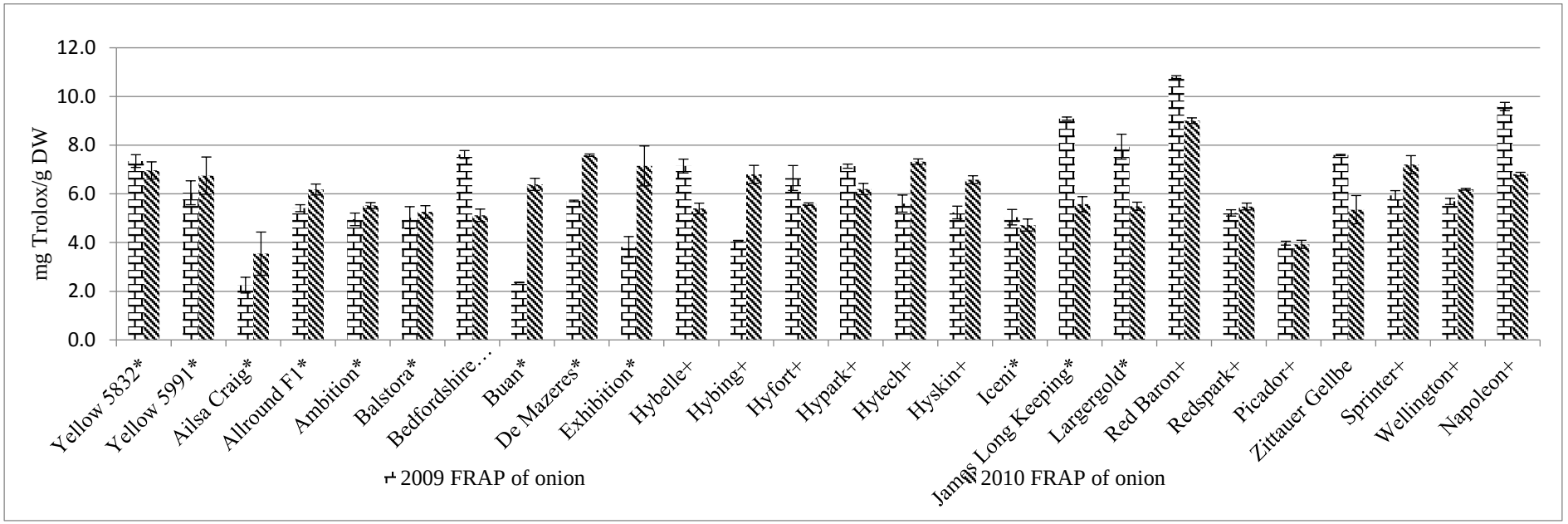


Figure 1A FRAP of bulb onions in 2009 and 2010. *Represents heritage and traditional varieties. + Represents modern and commercial varieties.

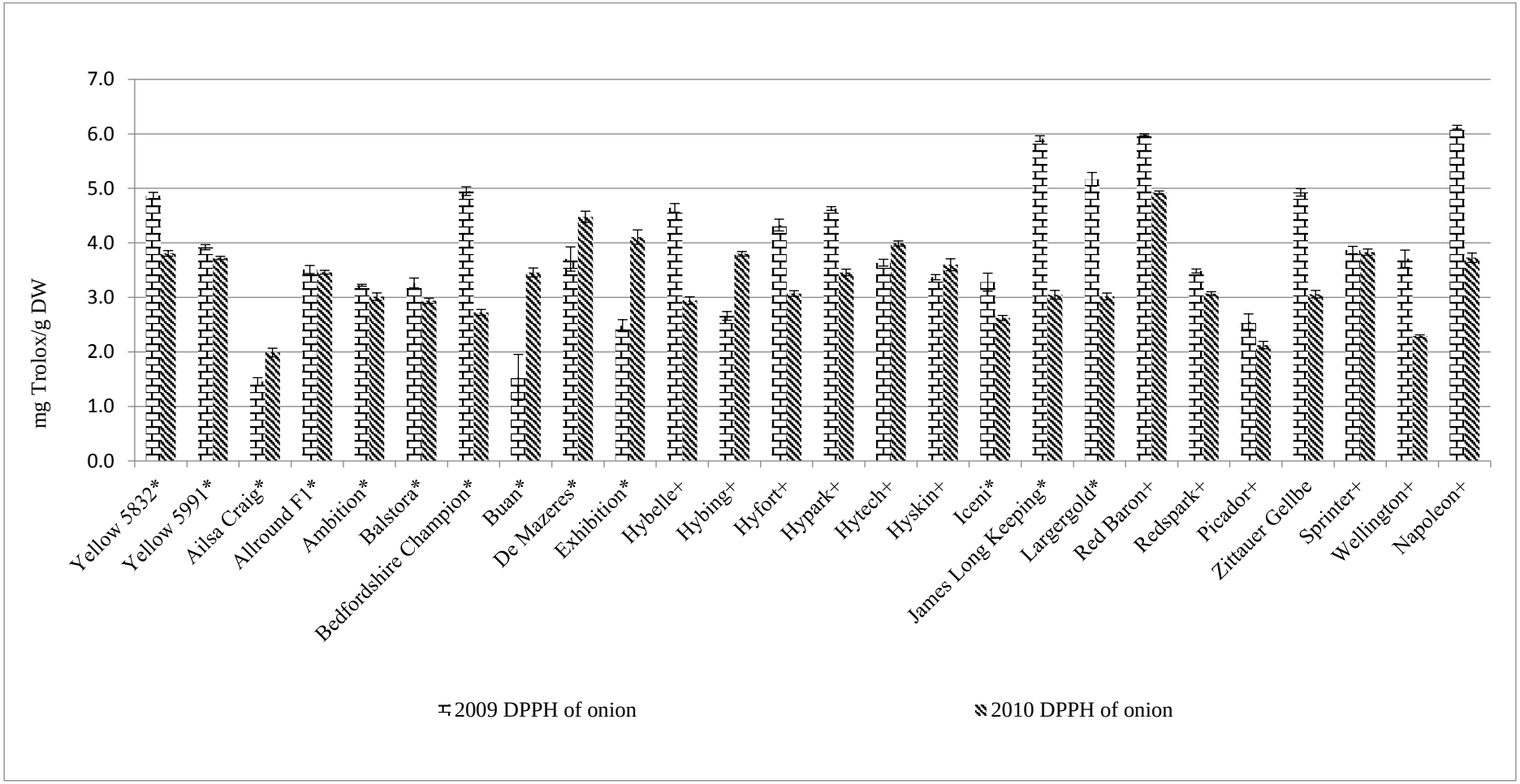


Figure 1B DPPH of bulb onions in 2009 and 2010. *Represents heritage and traditional varieties. + Represents modern and commercial varieties.

Appendix 2. The optimisation of different solvents, different solvent concentration, and different extraction time used for the extraction of phenolic, flavonoids, and antioxidant activity was carried out.

In order to select the best solvent combination for the extraction of phenolic compounds from onions, a preliminary solid–liquid extraction was carried out in onion samples by using different solvent combinations, i.e. (i) 100% distilled water, (ii) 100% methanol-water (10%, 20%, 40%, 60%, 80% and 100% methanol), (iii) ethanol–water (10%, 20%, 40%, 60%, 80% and 100% ethanol), and (iv) acetone–water (10%, 20%, 40%, 60%, 80% and 100% acetone). For the second experiment, 40%-80% methanol were selected to extract dried onion samples for different times (0.5 h, 1 h, 2 h, 4 h, 8 h, 18 h, 24 h).

The result of this study showed that the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA) were maximised using the 80% methanol for eight hours as the extraction solvent. This has been applied in the solvent that I chose in 'material and methods' throughout all chapters in my thesis. (The content is from my paper manuscript 'Effect of different solvents on the extraction of phenolic compounds and antioxidant capacities from onions').

Principal component regression of the whole data set shows that the most common pattern in phenolics and antioxidant reflecting the differences between types of solvent and percentage of solvent (Figure 2A). Antioxidant capacity (DPPH and FRAP) with 60% and 80% were located in the same quadrant of plot and were considered positively correlated with each other while they were negatively correlated with 10 % and 20%, which were located in diagonally opposed quadrant of plot probably due to the low antioxidant capacity. TPC and TFC with methanol were located in the same quadrant of plot and were considered positively correlated with each other while they were negatively correlated with acetone and water, which were located in diagonally opposed quadrant of plot probably due to the low content of the studied components. Secondly, almost no distinction could be made between the methanol and ethanol, with the exception of water and acetone. It appears that acetone and water were not as efficient as methanol for the extraction of phenolic compounds given their physical distance from the phenolic variables seen in Figure 2A.

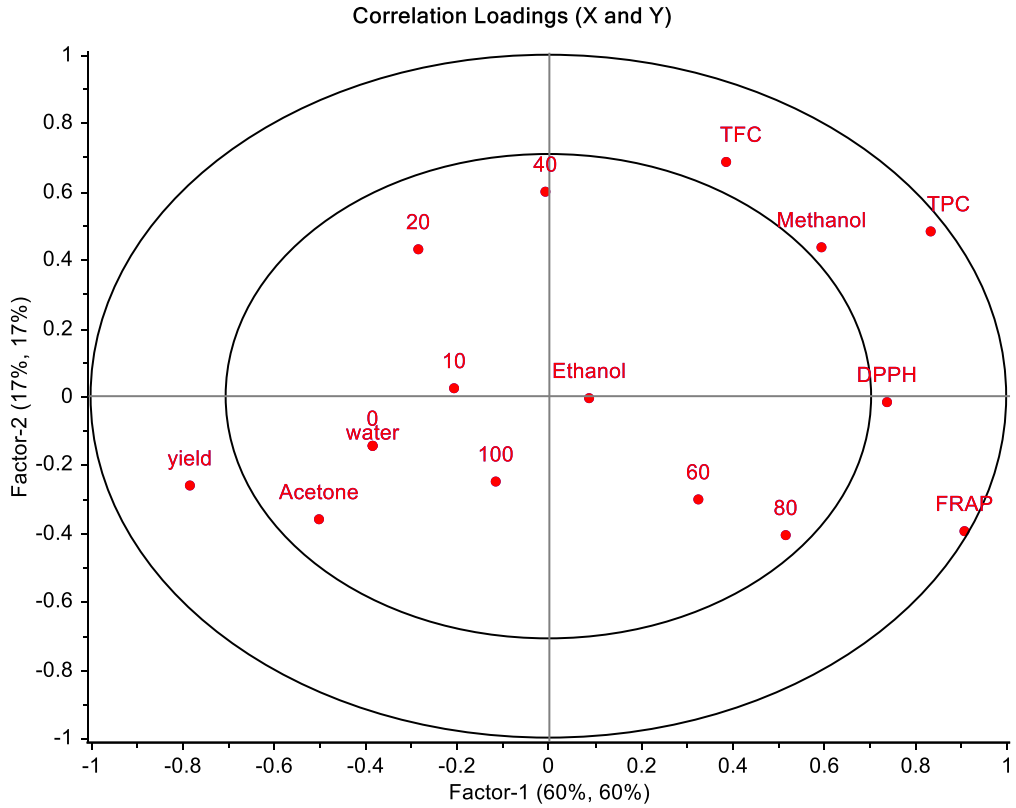


Figure 2A Principal Component Regression (PCR) biplot of PC1 versus PC2. The model was derived from total phenolic, flavonoids, antioxidant activity in the X-matrix and different solvents and percentage in the Y-matrix. Total phenolic content (TPC); Total flavonoid content (TFC); Antioxidant Activity (FRAP and DPPH).

The different percentage (40%-80%) of methanol onion extract and time were evaluated on the context of antioxidant activity and phenolic compounds. Figure 2B was formed by different extracts according to different time. It was possible to discriminate methanol–water mixtures (40%-80%) and extraction time (0.5-24 hours). TFC with 80% methanol at extraction 8 hours were located in the same quadrant of plot and were considered positively correlated with each other, due to their higher values of TFC and antioxidant capacity by DPPH and FRAP assays.

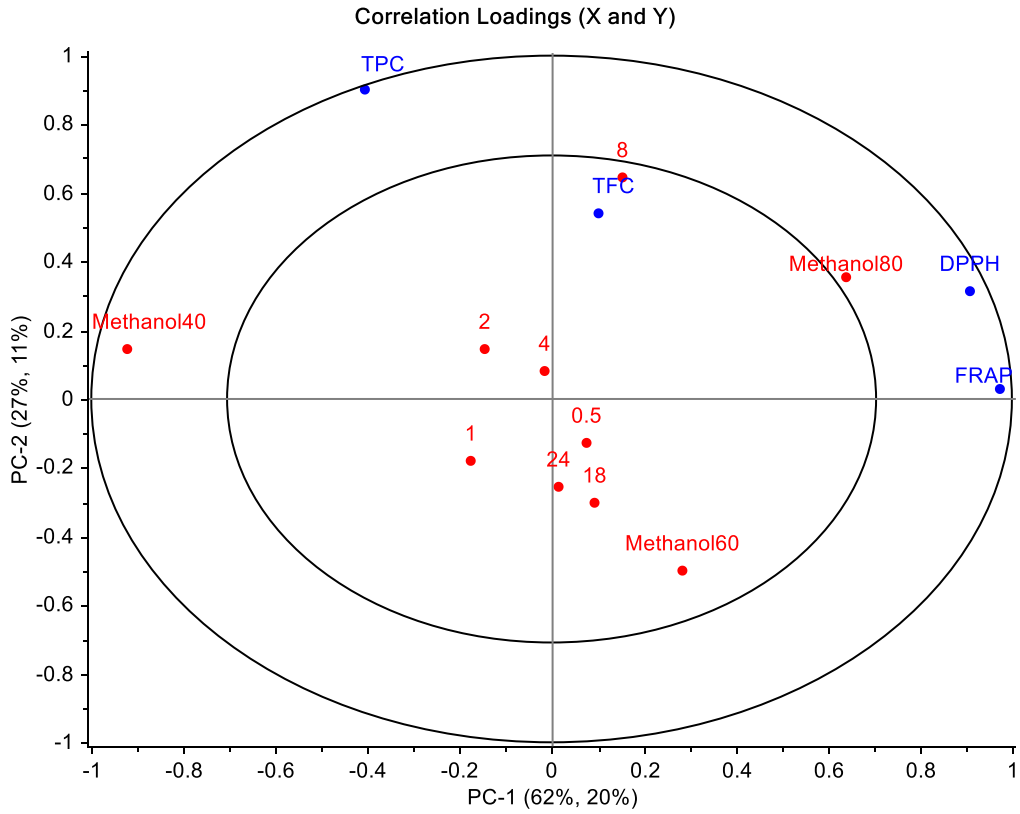


Figure 2B Principal component regression of onion in different methanol percentage and extraction time. The model was derived from total phenolic content (TPC), total flavonoids content (TFC), antioxidant activity (FRAP and DPPH) in the X-matrix and different methanol percentage and extraction time in the Y-matrix.

Appendix 3. Calculation of Total Phenolic Content, Total Flavonoid Content and Antioxidant (FRAP and DPPH) Assay.

The absorbance of the onion extract was compared with standard curves for estimating the concentration of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (FRAP and DPPH) in the onion samples. Based on different standard curves, the content of TPC, TFC and antioxidant activity (FRAP and DPPH) can be calculated in the onion samples, respectively.

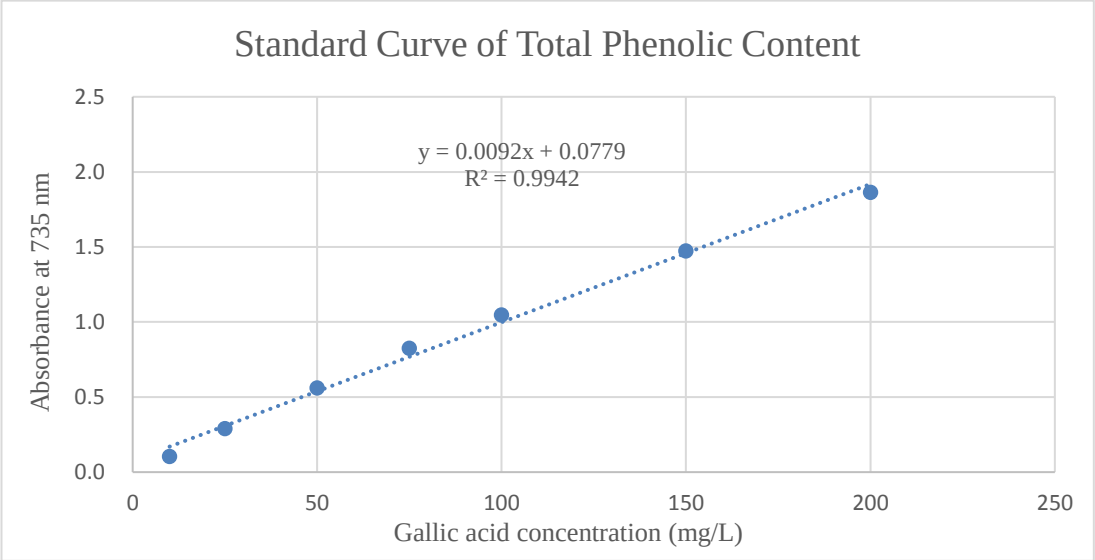


Figure 3A The TPC of the onion samples were quantified according to the gallic acid standard curve ($y = 0.0092x + 0.0779$; $R^2 = 0.9942$) and expressed as milligrams of gallic acid equivalents per gram dry weight sample (GAE mg/g DW).

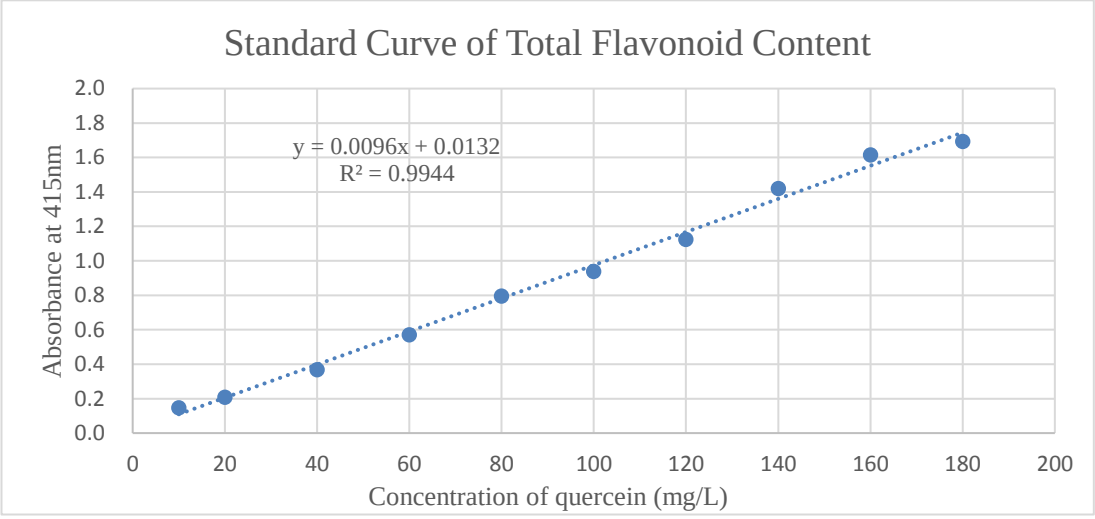


Figure 3B The TFC of the onion samples were quantified according to the quercetin standard curve ($y = 0.0096x + 0.0132$; $R^2 = 0.9944$) and expressed as milligrams of quercetin equivalents per gram dry weight sample (QE mg/g DW).

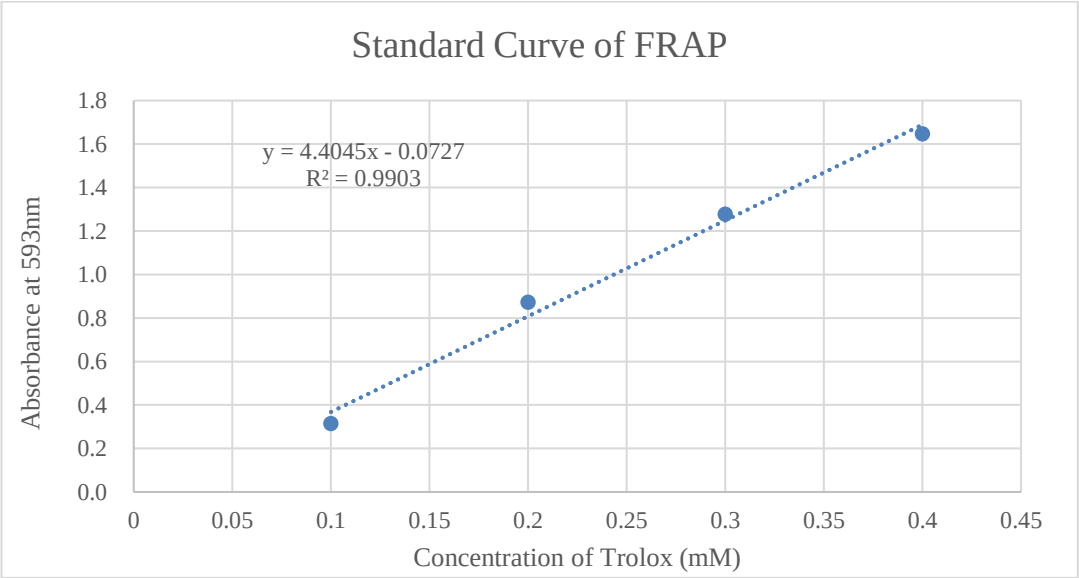


Figure 3C The FRAP of the onion samples were quantified according to the trolox standard curve (y = 4.4045x - 0.0727; R^2 = 0.9903) and expressed as milligram of trolox equivalents per gram dry weight sample (TE mg/g DW).

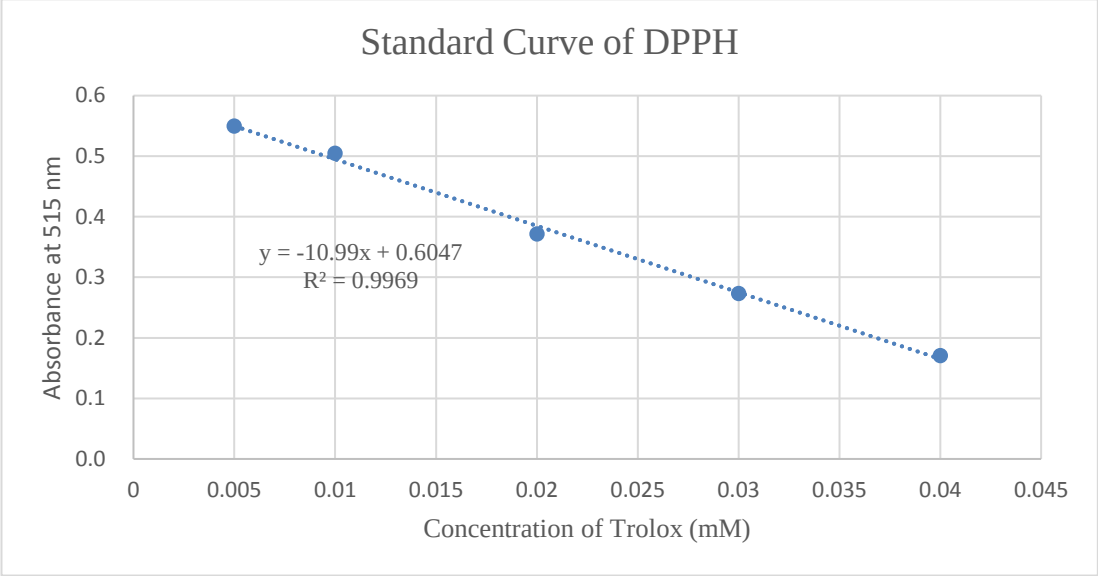


Figure 3D The DPPH of the onion samples were quantified according to the trolox standard curve (y = -10.99x + 0.6047; R^2 = 0.9996) and expressed as milligram of trolox equivalents per gram dry weight sample (TE mg/g DW).

Appendix 4. Parameters of the Calibration Curve, Regression Equation, Correlation Coefficient (R^2) of Individual Flavonoids for HPLC Method Validation.

The concentration of individual flavonoid compounds in onion samples was determined by the equation obtained from individual flavonoids standard curve. Based on different standard curves, individual flavonoids contents were calculated in the onion samples respectively.

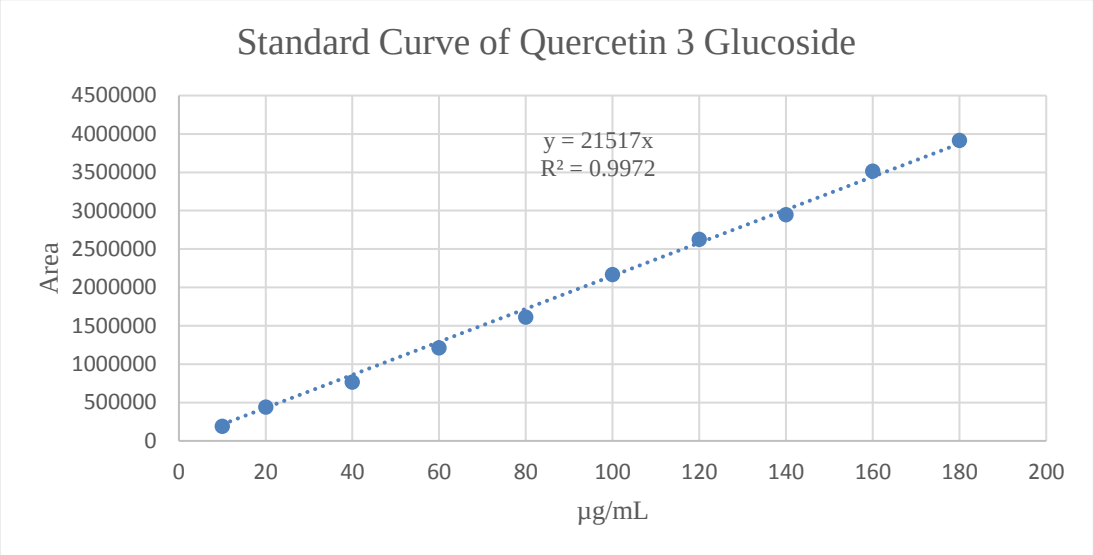


Figure 4A The content of Q 3, G in the onion samples were quantified according to the standard curve (y = 21517x; R^2 = 0.9972) and expressed as microgram per millilitre dry weight sample (µg/g DW).

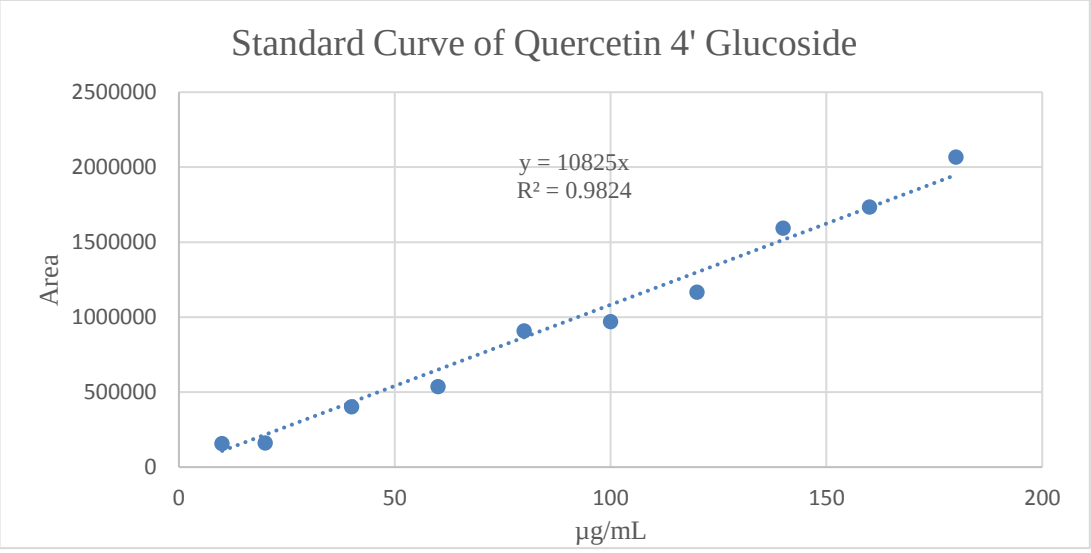


Figure 4B The content of Q 4’ G in the onion samples were quantified according to the standard curve (y = 10825x; R^2 = 0.9824) and expressed as microgram per millilitre dry weight sample (µg/g DW).

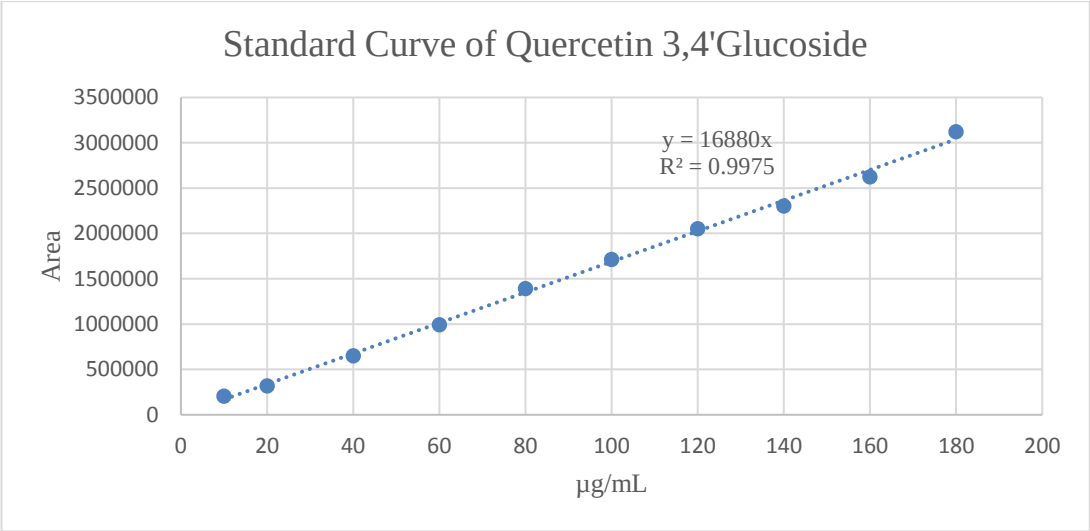


Figure 4C The content of Q 3,4' D in onion samples were quantified according to the standard curve (y = 16880x; R^2 = 0.9975) and expressed as microgram per millilitre dry weight sample (µg/g DW).

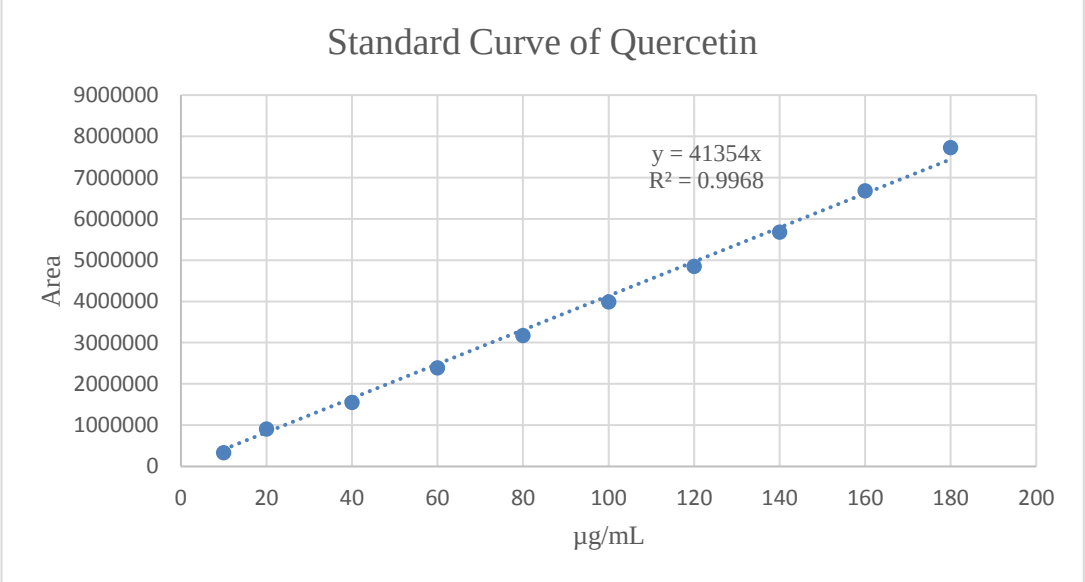
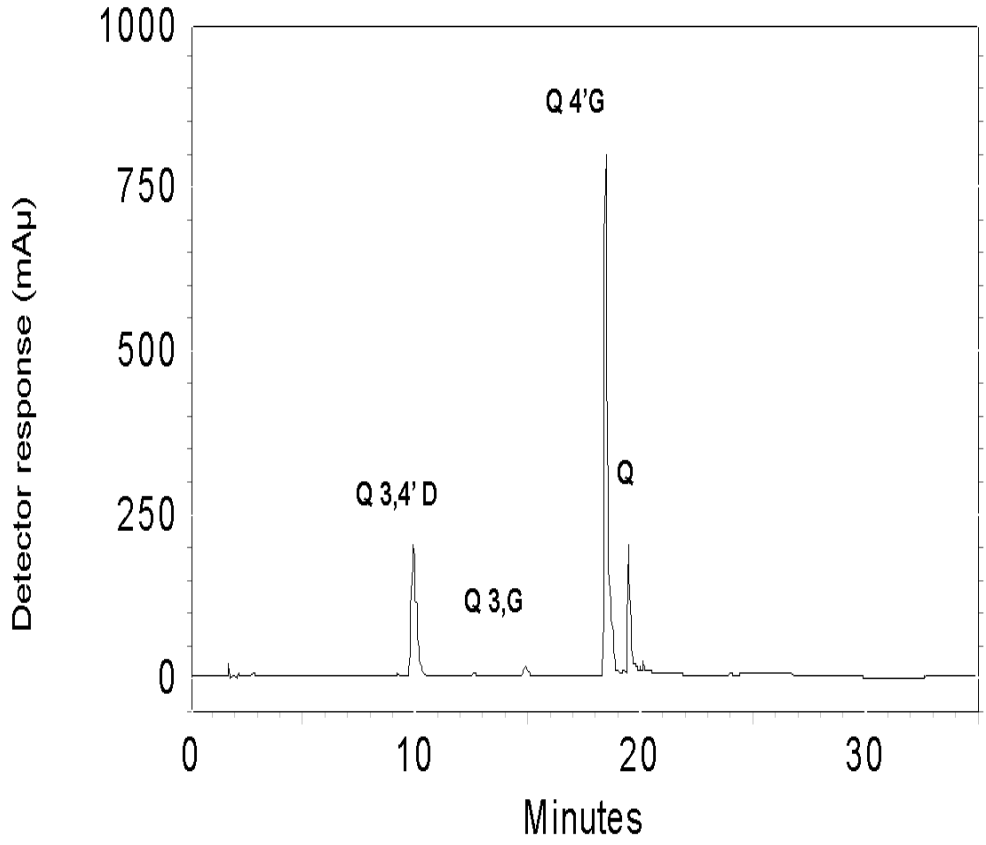


Figure 4D The content Q in the onion samples were quantified according to the standard curve (y = 41354x; R^2 = 0.9968) and expressed as microgram gram per millilitre dry weight sample (µg/g DW).

Appendix 5. High-Performance Liquid Chromatography of Flavonoids in Onion Extracts. The Compounds Quantified were Quercetin 3 4' Diglucoside (Q 3,4' D), Quercetin 3 Glucoside (Q 3, G), Quercetin 4' Glucoside (Q 4' G), Quercetin (Q).

The individual flavonoid compounds were separated in high-performance liquid chromatography (HPLC) according to different polar in onion extracts. Based on different retention time shown on HPLC, individual flavonoids can be identified.



Appendix 6. Method validation for analytical methods on phenolic compounds and antioxidant activity

The limit of detection (LOD) and relative standard deviation (RSD) of the TPC, TFC, individual TFC and antioxidant activity methods were calculated. The results show the LOD and RSD have good precision to analyse the extract of samples for phenolic compounds and antioxidant activity.

The first table shows the parameters of detected at wavelength, retention time and regression coefficient (R^2) of individual flavonoids for HPLC method validation.

The second table shows the parameters of limit of detection (LOD) and relative standard deviation (RSD) of phenolic compounds and antioxidant activity.

a)

Name of the Standard	Detected at Wavelength λ nm	Retention Time (min)	Regression Coefficient (R^2)	LOD (μg/mL)	RSD (%)
Quercetin 3,4' Glucoside	360	9.80	0.99	0.001	0.1
Quercetin 4' Glucoside	360	18.86	0.98	0.002	0.4
Quercetin 3 Glucoside	360	14.86	0.98	0.003	0.2
Quercetin	360	19.50	0.99	0.003	0.6

b)

Name of the Standard	LOD (mg/L)	RSD (%)
Total Phenolic Content	0.01	0.8
Total Flavonoid Content	0.01	0.5
Antioxidant Activity Assay	LOD (mg/L)	% RSD
FRAP	0.01	0.23
DPPH	0.01	0.9

The precision of phenolic compounds and antioxidant activity was determined from six replicates carried out for each validation level. The precision (measured as the percentage relative standard deviation (%RSD)) was less than 1% for all phenolics compounds and antioxidant activity assay, which provided good precision to analyse the extract of samples.