TITLE

Evaluation of polyphenolic content and antioxidant activity in two onion varieties grown under organic and conventional production systems

RUNNING TITLE

Organic Conventional Onion

AUTHORS

Feiyue Ren[a, b¥], Kim Reilly[a¥], Michael Gaffney[a], Joseph P. Kerry[b], Mohammad Hossain[a*] and Dilip K. Rai[a]

*Corresponding author: mohammad.hossain@teagasc.ie.

¥ Joint first authors

[a] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland.

[b] University College Cork, Western Road, Cork, Ireland.

ABSTRACT

BACKGROUND: 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 bioactive polyphenolic content of onion.

RESULTS: 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

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.8138

antioxidant activity in generally were significantly higher ($p<0.05$) under fully organic compared to fully conventional management.

CONCLUSION: Organic cultivation practices resulted in significantly higher levels of potential bioactive compounds in onion.

Keywords: onion (*Allium cepa* L.); organic; conventional; phenolics; flavonoids; antioxidants.

INTRODUCTION

The demand for organic food products has increased rapidly during recent years,[1] 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.[2] Organic crop production in Europe is controlled by EU Council Regulation No 834/2007.[3] 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.[4, 5] In addition to organic practices, the concentration of polyphenols in edible plants is affected by other factors such as cultivar and variety selection,[6] tissue maturity and damage at harvest: stress (pathogen infection and pest attack),[7] climate and soil microenvironment, fertilizer regime, temperature, irradiation, and post-harvest treatment.[8] Relative to conventional systems, organic systems may increase the exposure of crops to such stresses, thus inducing the synthesis of secondary metabolites.[8] 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.[9-11] 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.[4] 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.[5, 12-17] The higher concentrations of a wide range of phenolics found in organic crops/crop-based foods may indicate the greatest potential nutritional benefits.[5] However, there is little information on the impact of various cultivation practices on the production of secondary metabolites in onion, which is a major source of polyphenols in the human diet, and is globally an important agricultural product with annual production of 82.82 MT.[18] 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[19] 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.[15, 20-24] Many reports have indicated that onions have a wide range of beneficial properties for human health, such as anti-cholesterolaemic,[21] anti-mutagenic,[22] and antioxidant capacity.[23, 24] 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.[25]

The objective of this study was to to compare the total phenolic contents, total flavonoid contents and antioxdiant 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 2014 collection.

MATERIALS AND METHODS

Chemicals

Gallic acid, methanol (MeOH), ethanol, Folin-Ciocalteau reagent, potassium acetate, Sodium carbonate (Na_2CO_3), Aluminium Chloride ($AlCl_3$), Acetate, Ferric Chloride, TPTZ (2,4,6-tripyridyl-5-triazine), Hydrogen Chloride (HCl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)

and DPPH (2,2-Diphenyl-1-picrylhydrazyl) were obtained from Sigma (Sigma Aldrich, Arklow, Ireland).

Field trial

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. 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,[26] EC834/200719[3] and as described previously.[27] 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.[28] Fertiliser was applied as a mixture of calcium ammonium nitrate, single super-phosphate and sulfate of potash for the CS treatment; or Greenvale fertilizer (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).[29] 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 2010-2014 are shown in Table 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 diseases 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 ℃ within 24 hours of harvest. Frozen samples were freeze dried in a large scale freeze drier (Frozen in Time Ltd. United Kingdom). Once freeze dried, samples were vacuum packed in poplypropylene bags and kept at -20 ℃ until analysis.

Extraction and analysis of phenolic compounds

Freeze dried onions were milled using a kitchen blender (Kenwood Limited, Havant, UK). The powdered onions (1g) were mixed with 10 ml of 80% methanol (MEOH) and homogenized with an Omni-prep multisample homogenizer (Omni International, Kennesaw, USA) at 24, 000 rpm. The homogenized sample suspensions were shaken overnight using a V400 Multitude Vortexer (Alpha Laboratories, North York, Canada) at 1,500 rpm at room temperature. The sample suspensions were then centrifuged for 20 min at 3,000 g (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK) and filtered through 0.22 μm polytera fluoethylene filters. The extracts were kept in -20℃ for subsequent analysis.

Analysis of total phenolics

Total phenolics were determined using a modification of the Folin-Ciocalteau method.[30] 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 min at room temperature. Following this, the samples were centrifuged (Eppendorf, Centrifuge 5417R, Germany) at 17,900g for 3 min. The absorbance of the sample was read at 735 nm by spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan) using aqueous

gallic acid (10-400 mg l^{-1}) as a standard. Results are expressed as gallic acid equivalents on a dry weight basis (GAE mg g^{-1} DW). All measurements were carried out in triplicate.

Analysis of total flavonoids

Total flavonoid content was determined using the method described by Lin and Tang.[31] Briefly, 100 µl of methanolic extract was mixed with 300 µl of 95% ethanol, 40 µl of 10% aluminium chloride, 40 µl of 1.0 M potassium acetate and 520 µl of distilled water. After incubation at room temperature for 40 min, absorbance of the reaction mixture was measured against a blank at 415 nm using a spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan). Quercetin was used to develop a standard calibration curve and the total flavonoid content was expressed as milligrams of quercetin equivalents per gram dry weight (Quercetin mg g^{-1} DW).

Analysis of antioxidant activity

Assay for Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was carried out according to the method of Stratil *et al.*[32] 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℃. Appropriate dilutions of onion methanolic extracts were prepared by diluting 10-fold in 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. The tubes were vortexed and left at 37 ℃ for exactly 40 min, 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 mg Trolox equivalents per gram dry weight sample (Trolox mg g^{-1} DW). All measurements were carried out in triplicate.

Assay for DPPH antioxidant power

The DPPH scavenging activity assay was performed as per the method described by Goupy *et al.*[33] with a slight modification. 2, 2-diphenylpicrylhydrazyl (DPPH) was dissolved in methanol to a concentration of 0.238 mg ml^{-1} 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^{-1}) dissolved in methanol in appropriate dilution was used to make the standard curve. 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 with 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 a 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 (Trolox mg g^{-1} DW).

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 in SAS 9.1 software.

RESULTS AND DISCUSSION

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.[34] Levels of total free phenolics (TPC) in year 2010 were considerably higher

in 'Red Baron' with values ranging from 6.36±0.02 GAE mg g^{-1} DW to 7.75±0.1 GAE mg g^{-1} DW than 'Hyskin' which had TPC values in the range of 5.49 ±0.10 GAE mg g^{-1} DW to 7.21±0.01 GAE mg g^{-1} DW (Table 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.[35, 36] The finding of consistently higher levels of polyphenols in 'Red Baron' is therefore 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 has been reflected in the higher levels of total flavonoid content (TFC) values in 'Red Baron' than 'Hyskin' (Table 3). Although 'Red Baron' had higher TPC values than 'Hyskin' across the four year periods, the TPC data among the years in both the varieties were inconsistent. Data indicated that in 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 year 2011. We ascribe this result to increased stress (low temperature and more 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.[37] Following the year in 2011, the TPC values of both the varieties had increased significantly in year 2012 and 2013, with the highest values in the year 2012. This could be attributed to increased production of phenolics in response to stress caused by heavy rainfall and associated water logging of soils (Table 4) in year 2012. 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 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 year 2010. As shown in Tables 2 and 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 indicate 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 rates 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 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, Pand K; differences in the soil microbiome as well as other unknown differences that may be present. A number of other studies have shown total flavonoids decreased with increasing N application. For example Stewart *et al*.[38] found decreasing concentration of flavonoids when increasing N levels were applied in *Arabidopsis*. Groenbak *et al.*[39] also found a decrease in flavonoids with increased N for kale. Sander and Heitefuss[40] 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,[41] however it is not clear if this is simply a nutrient stess effect or if other factors including effect of the soil microbiome or other factors are involved. In onion an extensive previous study found that fertiliser type (mineral vs organic) and placement of fertilizer in onion had little effect on quercetin production.[42-44] A number of previous studies have indicated a significant genotype effect on total phenolic content and total flavonoids content profile in onion.[20, 45] 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[14] demonstrated that red onion grown organically contained more flavonoids compared with conventional samples. Ren *et al.*[46] reported that organically grown Welsh onion had higher levels of flavonols and antioxidant activity than conventional farmed ones. Faller and Fihlho[47] reported organic onion pulp had a higher antioxidant capacity than onions produced using conventional practices. Some research studies have also showed a slight yet significantly higher content of polyphenols in organic vegetables.[48] Organic black currants and tomatoes contained significantly more compounds with antioxidant properties in comparison with currants grown under conventional system.[49, 50] 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 polyphenolics, than plants grown conventionally,[51, 52] and the Cost-Benefit Hypothesis (CBH) and the Resource Availability Hypothesis (RAH) also designated by Growth Rate Hypothesis (GRH).[53-55] Where 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. [52] 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.[56]

Brandt and Mølgaard[57] had initially proposed that it was natural for plants cultivated organically to contain more polyphenolics and other secondary metabolites as defensive compounds. However, the opposite tendencies of higher contents of polyphenols in conventional products have also been observed.[58] Soltoft *et al.*[59] also found no significant differences between conventionally and organically grown onions in the content of flavonoids.

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 reduction of 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.[60] 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 in 2010, 2012 and 2013 (Table 3). We hypothesise that since in 2011 environmental conditions were more favourable (higher average temperature, less rainfall days and less humidity), PAL was not up-regulated, 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 to be due to the crops response 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.[61] 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 year 2010 and 2012 relative to 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.[61] 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 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 month 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 increase the total flavonoids content, with diverse results reported for low temperatures.[62] Drought stress seems to increase the total flavonoid content.[63] Accumulation of phenolics and higher activity of their biosynthetic enzymes in response to drought stress have also been reported in other plants. Chaves *et al.*[64] 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.[65] Wang and Zheng[66] found a strong correlation between temperature and production of phenolic in strawberry fruits.

Antioxidant activity

As shown in Tables 5 and 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.*[67] 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.[68] Thus, it has been recommended that two or more methods should always be employed to evaluate the total antioxidant activity of vegetables.[69] 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.[70, 71] According to Wang *et al.*[72] 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 *et al.*,[72] 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.*,[73] 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.*[74] reported r^2 =0.46 between FRAP and total phenolic content, determined across several *Allium* species. Table 7 shows correlation analysis for total phenolics, total flavonoids, FRAP and DPPH indicating that antioxidant activity correlated well with total phenolics and flavonoids.

CONCLUSIONS

Although there are 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 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 we attribute to altered regulation of phenolic synthesis in different years due to meteorological conditions.

ACKNOWLEDGEMENTS

The authors thank Christy Roberts and Philip Dunne for assistance in implementing and maintaining the trials, and Dr Jim Grant for statistical input. Prof. Carlo Leifert is thanked for helpful discussion in planning the trial design. The Irish Department of Agriculture Fisheries and the Marine (FIRM

06/NITAFRC6) is gratefully acknowledged for financial support of this work. FR thanks Teagasc and the Walsh Fellowship programme for funding his work.

REFERENCES

1. FiBL, Data on organic agriculture 2005-2013. The Organic-World.net website mainained by the Research Institute of Organic Agriculture (FiBL), Frick, Switzerland. Data available at http://www.organic-world.net/statistics/.
2. Wang SY, Chen C-T, Sciarappa W, Wang CY and Camp MJ, Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *J Agric Food Chem* **56**:5788-5794 (2008).
3. EU Council Regulation No 834/2007 of 28th June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91 *Official Journal of the European Union* 20.7.(2007).
4. Asami DK, Hong Y-J, Barrett DM and Mitchell AE, Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *J Agric Food Chem* **51**:1237-1241 (2003).
5. Barański M, Średnicka-Tober D, Volakakis N, Seal C, Sanderson R, Stewart GB, Benbrook C, Biavati B, Markellou E and Giotis C, Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *Br J Nutr* **112**:794-811 (2014).
6. Vågen IM and Slimestad R, Amount of characteristic compounds in 15 cultivars of onion (*Allium cepa* L.) in controlled field trials. *J Sci Food Agric* **88**:404-411 (2008).
7. Dixon RA and Paiva NL, Stress-induced phenylpropanoid metabolism. *Plant Cell* **7**:1085 (1995).
8. Manach C, Scalbert A, Morand C, Rémésy C and Jiménez L, Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**:727-747 (2004).
9. Conklin KA, Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. *Nutr. Cancer* **37**:1-18 (2000).
10. Zhou K and Yu L, Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. *LWT-Food Sci Technol* **39**:1155-1162 (2006).
11. Benbrook CM, Elevating Antioxidant Levels in Food through Organic Farming and Food Processing., Ed (2005).
12. Reilly K, Chapter nine: On-farm and fresh produce management. In: Tiwari BK, Brunton N, Brennan C, Eds. Handbook of plant food phytochemicals: sources stability and extraction, Wiley-Blackwell Publishing Co. (ISBN: 9781444338102).pp.201-235 (2013).
13. Valverde J, Reilly K, Villacreces S, Gaffney M, Grant J and Brunton N, Variation in bioactive content in broccoli (*Brassica oleracea var. italica*) grown under conventional and organic production systems. *J Sci Food Agric* **95**:1163-1171 (2015).
14. Hallmann E and Rembiałkowska E, Zawartość związków antyoksydacyjnych w wybranych odmianach cebuli z produkcji ekologicznej i konwencjonalnej. *J Res Appl Agric Eng* **51**:42-46 (2006).
15. Pérez-Gregorio M, Regueiro J, Simal-Gándara J, Rodrigues A and Almeida D, Increasing the added-value of onions as a source of antioxidant flavonoids: A critical review. *Crit Rev Food Sci Nutr* **54**:1050-1062 (2014).
16. Cooper J, Sanderson R, Cakmak I, Ozturk L, Shotton P, Carmichael A, Haghighi RS, Tetard-Jones C, Volakakis N and Eyre M, Effect of organic and conventional crop rotation, fertilization, and crop protection practices on metal contents in wheat (*Triticum aestivum*). *J Agric Food Chem* **59**:4715-4724 (2011).
17. Średnicka-Tober D, Barański M, Gromadzka-Ostrowska J, Skwarło-Sońta K, Rembiałkowska E, Hajslova J, Schulzova V, Çakmak I, Öztürk L and Królikowski T, Effect of crop protection and

fertilization regimes used in organic and conventional production systems on feed composition and physiological parameters in rats. *J Agric Food Chem* **61**:1017-1029 (2013).
18. FAO, Food and Agriculture Organization of the United Nations. (2012). accessed at: http://faostat.fao.org/site/339/default.aspx
19. Hertog MG, Hollman PC and Katan MB, Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* **40**:2379-2383 (1992).
20. Tiwari U and Cummins E, Factors influencing levels of phytochemicals in selected fruit and vegetables during pre-and post-harvest food processing operations. *Food Res Int* **50**:497-506 (2013).
21. Yin M-C and Cheng W-S, Antioxidant activity of several Allium members. *J Agric Food Chem* **46**:4097-4101 (1998).
22. Singh BN, Singh B, Singh R, Prakash D, Singh D, Sarma B, Upadhyay G and Singh H, Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. *Food Chem Toxicol* **47**:1161-1167 (2009).
23. Lu X, Wang J, Al-Qadiri HM, Ross CF, Powers JR, Tang J and Rasco BA, Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. *Food Chem* **129**:637-644 (2011).
24. Pérez-Gregorio RM, García-Falcón MS, Simal-Gándara J, Rodrigues AS and Almeida DP, Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. *J Food Comp Anal* **23**:592-598 (2010).
25. Roldán E, Sánchez-Moreno C, de Ancos B and Cano MP, Characterisation of onion (Allium cepa L.) by-products as food ingredients with antioxidant and antibrowning properties. *Food Chem* **108**:907-916 (2008).
26. EU,Council Regulation No. 2092/91 of 24th June on organic production of agricultural producrs and indications referring thereto on agricultural products and foodstuffs. *OJL 198 227 p1* (1991).
27. Reilly K, Cullen E, Lola−Luz T, Stone D, Valverde J, Gaffney M, Brunton N, Grant J and Griffiths BS, Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop. *J Sci Food Agric* **93:**3700-3709 (2013).
28. Coulter BS and Lawlor S (Eds), Major and Micro Nutrient Advice for Productive Agricultural Crops, 3rd Edition, pp.15-16. Johnstown Castle, Wexford, Ireland (2008).
29. Alexander S, Approved Pesticides for use on vegetable crops Teagasc, Kinsealy, Dublin 17 (2011).
30. Singleton VL, Orthofer R and Lamuela-Raventos RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Method Enzymol* **299:**152-178 (1999).
31. Lin J-Y and Tang C-Y, Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem* **101**:140-147 (2007).
32. Stratil P, Klejdus B and Kuban V, Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. *J Agric Food Chem* **54**:607-616 (2006).
33. Goupy P, Hugues M, Boivin P and Amiot MJ, Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *J Sci Food Agric* **79:**1625-1634 (1999).
34. Acosta-Estrada BA, Gutiérrez-Uribe JA and Serna-Saldívar SO, Bound phenolics in foods, a review. *Food Chem* **152**:46-55 (2014).
35. Shon MY, Choi SD, Kahng GG, Nam SH and Sung NJ, Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food Chem Toxicol* **42**:659-666 (2004).
36. Rodrigues AS, Pérez-Gregorio MR, García-Falcón MS, Simal-Gándara J and Almeida DP, Effect of post-harvest practices on flavonoid content of red and white onion cultivars. *Food Control* **21**:878-884 (2010).

37. Winkler S, Faragher J, Franz P, Imsic M and Jones R, Glucoraphanin and flavonoid levels remain stable during simulated transport and marketing of broccoli (*Brassica oleracea* var. *italica*) heads. *Postharvest Biol Tec* **43:**89-94 (2007).
38. Stewart A, Chapman W, Jenkins G, Graham I, Martin T and Crozier A, The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. *Plant Cell Environ* **24:**1189-1197 (2001).
39. Groenbaek M, Jensen S, Neugart S, Schreiner M, Kidmose U and Lakkenborg Kristensen H, Influence of cultivar and fertilizer approach on curly kale (*Brassica oleracea L. var. sabellica*). 1. Genetic diversity reflected in agronomic characteristics and phytochemical concentration. *J Agric Food Chem* **62**:11393-11402 (2014).
40. Sander JF and Heitefuss R, Suceptibility to Erysiphe graminis f. sp tritici and phenolic acid content of wheat as influenced by different levels of nitrogen fertilization. *J Phytopathol* **146**:495-507 (1998).
41. Rühmann S, Leser C, Bannert M and Treutter D, Relationship between growth, secondary metabolism, and resistance of apple. *Plant Biol* **4:**137-143 (2002).
42. Mogren L, Quercetin Content in Yellow Onion (*Allium cepa* L.): Effects of cultivation Methods, Curing and Storage. Doctoral thesis Swedish University of Agricultural Sciences, Faculty of Landscape Planning, Horticulture and Agricultural Science, Department of Crop Science, Alnarp, Acta Universitatis Agriculturae Sueciae, 2006: 96 ISSN 1652-6880, ISBN 91-576-7145-1.
43. Mogren L, Gertsson U, Olsson ME, Effect of cultivation factors on flavonoid content in yellow onion (*Allium cepa* L.). ISHS Acta Horticulturae 765: XXVII International Horticultural Congress - IHC2006: International Symposium on Plants as Food and Medicine: The Utilization and Development of Horticultural Plants for Human Health.
44. Mogren LM, Caspersen S, Olsson ME and Gertsson UE, Organically fertilized onions (*Allium cepa* L.): Effects of the fertilizer placement method on quercetin content and soil nitrogen dynamics. *J Agric Food Chem* **56**:361-367 (2007).
45. Reilly K, Valverde J, Finn L, Gaffney M, Rai DK and Brunton N, A note on the effectiveness of selenium supplementation of Irish-grown Allium crops. *Irish J Agr Food Res* **53:**91-99 (2014).
46. Ren H, Endo H and Hayashi T, Antioxidative and antimutagenic activities and polyphenol content of pesticide−free and organically cultivated green vegetables using water−soluble chitosan as a soil modifier and leaf surface spray. *J Sci Food Agric* **81:**1426-1432 (2001).
47. Faller A and Fialho E, Polyphenol content and antioxidant capacity in organic and conventional plant foods. *J Food Compos Anal* **23:**561-568 (2010).
48. Mitchell AE, Hong Y-J, Koh E, Barrett DM, Bryant D, Denison RF and Kaffka S, Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *J Agric Food Chem* **55**:6154-6159 (2007).
49. Kazimierczak R, Hallmann E, Rusaczonek A and Rembiałkowska E, Antioxidant content in black currants from organic and conventional cultivation. *J Pol Agric* Univ **11:**28-33 (2008).
50. Hallmann E, The influence of organic and conventional cultivation systems on the nutritional value and content of bioactive compounds in selected tomato types. *J Sci Food Agric* **92:**2840-2848 (2012).
51. Caris-Veyrat C, Amiot M-J, Tyssandier V, Grasselly D, Buret M, Mikolajczak M, Guilland J-C, Bouteloup-Demange C and Borel P, Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *J Agric Food Chem* **52**:6503-6509 (2004).
52. Massad TJ, Dyer LA and Vega G, Costs of defense and a test of the carbon-nutrient balance and growth-differentiation balance hypotheses for two co-occurring classes of plant defense. *PLoS One* **7**:e47554 (2012).
53. Herms DA and Mattson WJ, The dilemma of plants: to grow or defend. *Q. Rev. Biol* **67:**283-335 (1992).
54. Barton KE, Phenotypic plasticity in seedling defense strategies: compensatory growth and chemical induction. *Oikos* **117**:917-925 (2008).
55. Coley PD, Bryant JP and Chapin FS, Resource availability and plant antiherbivore defense. *Science* **230**:895-899 (1985).

56. Bloksma J, Northolt M, Huber M, van der Burgt GJ and van de Vijver LPL, A new food quality concept based on life processes. In "Handbook of Organic Food Safety and Quality," eds. J. Cooper, U. Niggli, and C. Leifert, pp. 53-73, Woodhead Publishing, Cambridge, UK (2007).
57. Brandt KM, J. P, Organic agriculture: Does it enhance or reduce the nutritional value of plant foods. *J Sci Food Agric* **81:**924-931 (2001).
58. Anttonen MJ and Karjalainen RO, High-performance liquid chromatography analysis of black currant (Ribes nigrum L.) fruit phenolics grown either conventionally or organically. *J Agric Food Chem* **54**:7530-7538 (2006).
59. Soltoft M, Nielsen J, Holst Laursen K, Husted S, Halekoh U and Knuthsen P, Effects of organic and conventional growth systems on the content of flavonoids in onions and phenolic acids in carrots and potatoes. *J Agric Food Chem* **58**:10323-10329 (2010).
60. Ubi BE, External stimulation of anthocyanin biosynthesis in apple fruit. *J Food Agric Environ* **2**:65-70 (2004).
61. Rodrigues AS, Pérez-Gregorio MR, García-Falcón MS, Simal-Gándara J and Almeida DPF, Effect of meteorological conditions on antioxidant flavonoids in Portuguese cultivars of white and red onions. *Food Chem* **124**:303-308 (2011).
62. Nitithamyong A, Vonelbe JH, Wheeler RM and Tibbitts TW, Glycoalkaloids in potato tubers grown under controlled environments. *Am J Potato Res* **76:**337-343 (1999).
63. Bejarano L, Mignolet E, Devaux A, Espinola N, Carrasco E and Larondelle Y, Glycoalkaloids in potato tubers: the effect of variety and drought stress on the α-solanine and α-chaconine contents of potatoes. *J Sci Food Agric* **80:**2096-2100 (2000).
64. Chaves N, Escudero JC and Gutierrez-Merino C, Role of ecological variables in the seasonal variation of flavonoid content of Cistus ladanifer exudate. *J Chem Ecol* **23**:579-603 (1997).
65. Abreu IN and Mazzafera P, Effect of water and temperature stress on the content of active constituents of Hypericum brasiliense Choisy. *Plant Physiol. Biochem* **43:**241-248 (2005).
66. Wang SY and Zheng W, Effect of plant growth temperature on antioxidant capacity in strawberry. *J Agric Food Chem* **49**:4977-4982 (2001).
67. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W and Krewer G, Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. *J Agric Food Chem* **46**:2686-2693 (1998).
68. Chu YH, Chang CL and Hsu HF, Flavonoid content of several vegetables and their antioxidant activity. *J Sci Food Agric* **80:**561-566 (2000).
69. Dalamu, Kaur C, Singh M, Walia S, Joshi S and Munshi A, Variations in phenolics and antioxidants in Indian onions (*Allium cepa* L.) Genotype selection for breeding. *Nutr Food Sci* **40:**6-19 (2010).
70. Hossain M, Barry-Ryan C, Martin-Diana AB and Brunton N, Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. *Food Chem* **123**:85-91 (2010).
71. Hossain MB, Camphuis G, Aguiló-Aguayo I, Gangopadhyay N and Rai DK, Antioxidant activity guided separation of major polyphenols of marjoram (Origanum majorana L.) using flash chromatography and their identification by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J Sep Sci* **37:**3205-3213 (2014).
72. Wang H, Cao G and Prior RL, Total antioxidant capacity of fruits. *J Agric Food Chem* **44**:701-705 (1996).
73. Santas J, Carbo R, Gordon M and Almajano M, Comparison of the antioxidant activity of two Spanish onion varieties. *Food Chem* **107**:1210-1216 (2008).
74. Nencini C, Cavallo F, Capasso A, Franchi GG, Giorgio G and Micheli L, Evaluation of antioxidative properties of Allium species growing wild in Italy. *Phytother Res* **21:**874-878 (2007).

Table 1 Specific pest-control and soil treatment inputs used in the Teagasc Kinsealy Systems Comparison trial for onion cultivation 2010-13

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

Table 2 Onion total phenolic content under different management practices between 2010 and 2013

	2010	2011	2012	2013
Treatment	GAE mg g^{-1} DW	GAE mg g^{-1} DW	GAE mg g^{-1} DW	GAE mg g^{-1} 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±0.133	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 3 Onion total flavonoid content under different management practices between 2010 and 2013

	2010	2011	2012	2013
Treatment	QE mg g^{-1} DW	QE mg g^{-1} DW	QE mg g^{-1} DW	QE mg g^{-1} DW
V1+OS+OP	2.7±0.03	3.68±0.08	4.19±0.03	3.70±0.4
V1+OS+CP	2.8±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±0.039	3.07±0.15
V1+CS+CP	2.70±0.06	3.02±0.04	3.79±0.036	3.30±0.077
V2+OS+OP	2.83±0.06	4.70±0.14	4.54±0.058	4.48±0.4
V2+OS+CP	3.17±0.3	4.65±0.12	4.26±0.08	4.24±0.06

V2+CS+OP	2.65±0.1	4.60±0.02	4.24±0.11	4.00±0.17
V2+CS+CP	2.97±0.5	4.64±0.033	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 4 Climate conditions during onion crop production in growing season from March to September between 2010 and 2013. T = Mean temperature (°C), TM = Mean maximum temperature (°C), Tm = Mean minimum temperature (°C), PP = Total monthly precipitation amount (mm), V = Mean wind speed (Km h^{-1}), RA = Daily indicator for occurrence of rain or drizzle (total days), SN =Indicator for occurrence of snow or ice Pellets. H = Mean humidity (%)

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

Table 5 Total antioxidant capacity (FRAP assays) under different management practices between 2010 and 2013

	2010	2011	2012	2013
Treatment	Trolox mg g^{-1}DW	Trolox mg g^{-1}DW	Trolox mg g^{-1}DW	Trolox mg g^{-1}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	10.36±0.05	10.15±0.2	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 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 6 Total antioxidant capacity (DPPH assays) under different management practices between 2010 and 2013

	2010	2011	2012	2013
Treatment	Trolox mg g^{-1} DW	Trolox mg g^{-1} DW	Trolox mg g^{-1} DW	Trolox mg g^{-1} 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 7 Correlation analysis for total phenolics, total flavonoids, FRAP and DPPH with 'Red Baron' and 'Hyskin'

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