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Research article

Study of inhibition of germination of potato by ethylene

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ABSTRACT

In Canada, the potato (*Solanum tuberosum*) is by far the most cultivated vegetable and plays a major nutritional role. However, during storage, the potato can easily undergo germination. In this study we have shown the inhibition potential of ethylene as an anti-germinative agent acting especially on phenols. In both varieties assayed (Yukon Gold and Russet Burbank) in this study, the ethylene treatment led to a decrease in total phenol concentration of about 20%. The analysis of potato extracts showed the decrease of specific phenol concentrations which was dependant on the time and temperature of extraction. Our hypothese that the transformation of phenols into phenolic ethyl ethers via possible radical mechanism were then formulated and confirmed by LC and LC/MS.

1. Introduction

The potato (*Solanum tuberosum*) is one of the most cultivated tubers in the world. The US and Canada are respectively the fifth- and fifteen-largest producers of potatoes in the world (FAOSTAT, 2014) and the potato industry is the important source of cash receipts from agriculture. In order to suit the local and export markets, many varieties of potato have been developed that match consumption and processing trends. During the potato storage and preservation processes, diverse losses may occur, in particular due to sprouting and germination.

Chloropham (Campbell et al., 2010) treatment is the most frequently applied of the many products used for germination control used in Canada. However, as it is a strong toxic chemical, the search for more ecologically friendly and less toxic treatments led to the use of ethylene in a potato chain production (Canaweed guides, 2015). Ethylene, a chemically neutral and more eco-friendly chemical, acts as a growth regulator (Foukaraki et al., 2016; Suttle, 1998) to control the ripening of tubers and inhibits the deterioration of plant tissue. The linkage between phenol levels and the germination of tubers was the object of several studies (Han et al., 2007; Mattila and Hellström, 2007; Maroun et al., 2013; Akyol et al., 2016; Kim et al., 2019; Ru et al., 2019) in which the effects of many chemicals were evaluated. These showed, however, common inconveniences that were already known and discussed, including toxicity (Wang et al., 2002; Liu et al., 2014; Mingchun et al., 2015; Iqbal et al., 2017).

In 2005 (Macheix et al., 2005), the use of ethylene as a germination inhibitor acting on potato phenols produced during these processes has been reported. In two other studies, by Parkizeh et al. (2011) and Rylski et al. (1974), some metabolites of ethylenization and the mechanism are discussed.

In this work, we report our results on the inhibition of germination in two popular Canadian varieties of potato, the Russet Burbank (RB) and Yukon Gold (YG), after treatment with ethylene. The mechanism of action of ethylene on alcohol extracts of potato was proposed from comparisons of the total phenol concentrations in the original potatoes before and after ethylene treatment of alcohol (ethanol or methanol) extracts. Particularly we used light of the use of model compounds of two popular, naturally occurring, potato phenols and their ethyl ethers with help of LC and LC/MS techniques. However, in both cases we used the activated phenols, with the electron withdrawing groups substituted in *para* position (carboxyl group or as in cinnamic acid system).

2. Material and methods

Both varieties of potato, Russet Burbank (RB) and Yukon Gold (YG), were purchased from the local Walmart in Moncton, NB, in autumn 2015. Both varieties, RB and YG, are produced in northwestern New Brunswick.

All organic and inorganic chemicals (solvents, phenols, their derivatives and standard gallic acid) were purchased from Aldrich

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Chemicals (Canada). Ethylene was purchased from Aldrich Chemicals Canada. We used a 250 mL lecture bottle of compressed gas.

2.1. Methods

2.1.1. Preparation of potato slurry

The potato extracts were produced according to the method of Singh (2010) as described in Liu et al. (2013), with some minor modifications.

2.1.2. Lyophilisation

2 kg of each variety of potato (RB, YG) were cut, crushed and frozen for 24 h and then freeze-dried to eliminate as much water as possible from the extract. This operation produced potato lyophilizate as a white–pale yellow powder.

2.1.3. Extraction with ethanol: water (50%:50%)

5 g of potato lyophilizate were suspended in 175 mL of 50% ethanol. The suspension obtained was homogenized for 30–40 min and incubated at 30 °C and 60 °C for 24 h to increase the solubility of the product in the solvent. The incubated mixture was centrifuged at 4°C/10,000 rpm/10 min. The resulting product (supernatant) was then filtered and concentrated by evaporation under vacuum.

2.1.4. Extraction with methanol: water (95%:5%)

50 g of potato lyophilizate was suspended in 350 mL of 95% methanol. This suspension was then incubated at 65 °C for 1 h. The homogenate was filtered and the resulting liquid filtrate was retained and set aside. Pellets were suspended again in 100 mL methanol 95% and subjected to the same treatment as before, i.e., incubated at 65 °C for 1 h and filtered. The combined mixture of both filtrates was centrifuged (10,000 rpm/10 min/4 °C) and evaporated to dryness as described above. A small amount of methanol was added to the dry extract to obtain a final potato extract. All potato extracts obtained in this manner were stored at -20 °C for further analysis.

2.1.5. Ethylenization, general procedure

The ethylene treatment of potato extracts was carried out by a splash system connected to an 250 mL ethylene lecture bottle. 20 mL aliquots of potato slurry in 95% methanol (as described in the previous section as containing 0.2 g of dry potato) were placed in a three-neck flask equipped with the splash system connected to the ethylene balloon filled with ethylene. The bubbling took place under a fume hood for 4–8 h at a pressure of 60 bars, corresponding to 800 psi at room temperature.

2.1.6. Preparation of Folin-Ciocalteu reagent

The Folin-Ciocalteu reagent was prepared in our laboratory, store at +4 °C and used as previously described (Folin and Ciocalteu, 1927).

2.2. Total phenol concentration

Potato extract (0.1 ml) was suspended in 7.9 ml of distilled water in the presence of 0.5 ml of Folin-Ciocalteu reagent. The suspension was mixed and rested for 5 min. A volume of 1.5 ml of Na_2CO_3 solution (20%, w/v) was added to the mixture. After stirring, the mixture was incubated at room temperature for 90 min. Its absorbance was determined at 765 nm using a spectrophotometer (Ultrospec 3000pro UV/Visble, Pharmacia Biotech, Texas, USA).

2.3. Gallic acid calibration curve

A calibration curve was plotted using different concentrations of gallic acid (0, 50, 100, 200, 300, 400 mg/L) $R^2 = 0.9946$, to determine the total phenolic compounds expressed in terms of gallic acid equivalents (SBE, in mg per 50 g of potato extract). This calibration curve is available on request from the corresponding author.

2.4. Liquid chromatography (LC) experiments

The extracts from the original potatoes and after the ethylene treatment were analysed on a Waters LCT-XE Premier Chromatograph, coupled to a UPLC Acquity Waters spectrometer. The column used was Acquity BEH C18 I*50 mm 1.7 μm. Elution (0.9 mL/min) was performed with a two-solvent system: Solvent A was water with 0.1% (v/v) of formic acid and Solvent B was acetonitryl with 0.1% of formic acid.

Negative ion mass spectrometry (NI-MS) was used to confirm the presence of the four following standards with masses corresponding to (M-H)- ions of m/z **1** (137), **2** (165), **3** (163) and **4** (191).*

Then the LC of extracts were swept in search of specific masses of the four standards (**1–4)** enabling the confirmation of presence of (M-H)- ions at a given retention time (Rt, min.). These experiments were confirmed again by recording LC after spiking the extracts with standards **1–4** (standards **3** and **4** run only for extracts after ethylenization). It was not possible to do a quantitative analysis of transformation of hydroxyl aryl acids into corresponding ethyl aryl ether because of the high complexity of the mixture (over 200 compounds, present in low concentrations) under analysis. However, the retention time for the standards **1–4** corresponded well to mass chromatogram searches for negative ions and confirmed the results from the spiking experiments.

The average retention times of model compounds, established as an average of two independent chromatogram runs, were as follow: **1** (1.27 min), **3** (2.21 min), **2** (1.83 min) and **4** (2.58 min).

3. Results and discussion

The total phenol concentration in extracts, as observed with or without ethylene treatment for two potato varieties, was determined by the Folin-Ciocіautelau method using a gallic acid calibration curve. The results obtained from the extraction with ethanol:water solvent system at two different incubation temperatures (30 °C and 60 °C) (Figure 1) showed a decreased phenol concentration after ethylenization for both varieties. As expected, at the higher incubation temperature the total phenol concentration rose. The ethylene treatment induced a greater reduction of phenol concentration for RB than for YG. The largest phenol content for this solvent system and the most pronounced difference between the original phenol concentration and that after ethylenization was observed for RB at 60 °C. It was, however, impossible to further use these extracts, such as in gas-chromatography experiments, because the very high content of carbohydrates rendered it difficult to identify single phenolic products on GC columns.

For this reason, methanol (95%) extraction of lyophilized potato was attempted at 65 °C for both varieties, with or without ethylenization, leading to the results shown in Figure 2.

The total phenol contents for YG were slightly higher than for RB. In both cases, the ethylene treatment led to a decrease in phenol concentration of about 20% (after ethylenization).

When methanol 95% (so with almost no water present during extraction) was used as the extraction solvent, with or without ethylene treatment, the same trend of reduced phenol concentration after the ethylenization as for the previous ethanol:water extraction solvent system was observed (at the same incubation temperature).

It is worth noticing at this point that in the literature an increased total phenol level under ethylenization of some plants was previously

* Several other compounds of interest were identified from LC/MS experiments, such as both 4-hydroxy aryl acids, 4-ethoxy aryl acids cinnamic acid and caffeic acid glucose conjugates. The LC of both extracts, original and after the ethylene treatment, or spiked with compounds **1–4** and other experimental data, together with all mass spectra data (NI-MS) and LC/MS of the extracts for both varieties of potato, are available from the corresponding author on request. The file also contains spectral material of other compounds identified from extracts (for example glucose conjugates.).

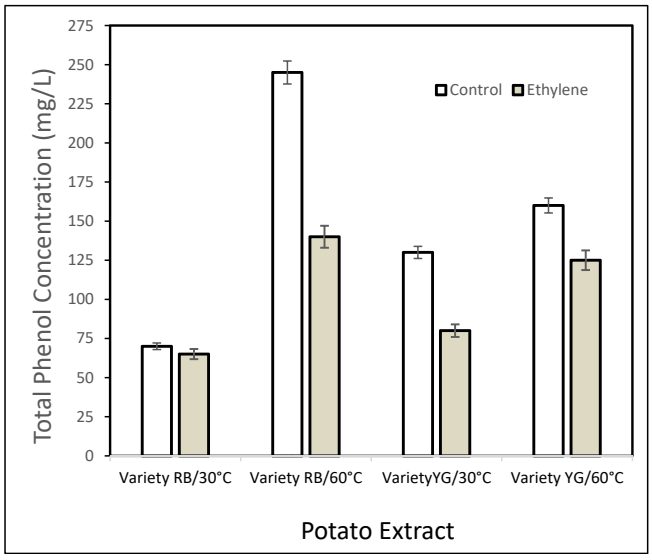

Figure 1. Total phenol concentration (mg/L) for ethanol-water (50:50) extracts for two varieties RB and YG at 30 °C and 60 °C; (White: original extract without ethylene treatment, Grey: after ethylene treatment).

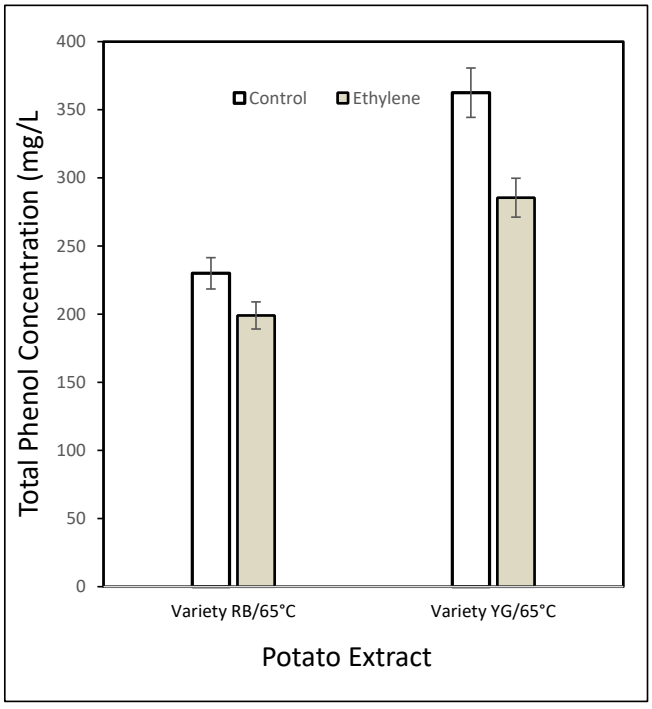

Figure 2. Total phenol concentration (mg/L) for 95% methanol extracts of RB and YG at 65°C. White: original extract without ethylene treatment, Grey: after ethylene treatment.

reported in sweet potatoes (Prange et al., 1998) and green beans (Liu et al., 2013). Small quantities of ethylene may also be produced by tubers (Wang et al., 2002; Liu et al., 2014; Mingchun et al., 2015; Iqbal et al., 2017). However, these results were reported for storage procedures, which were not what was reported on in this work. The in-lab simulation of ethylenization conditions such as the time of exposure and ppm count of ethylene presented here (see Experimental) are far from in-storage conditions of ethylenization. The ethylene in storage concentrations is usually maintained at 2–5 ppm under aerobic conditions for months (Belaid, 2015). The laboratory simulation of these conditions was reduced to an exposure time of a few hours but was strictly anaerobic in a pure ethylene atmosphere.

This last pair of extractions for both varieties of potato was subjected to liquid chromatography evaluation in order to elaborate the phenol fate hypothesis justifying this decreased concentration. We were inspired by the work of Singh (2011) and chose two specific phenolic aryl acids: 4-hydroxy benzoic acid (**1**) and 4-hydroxy cinnamic acid (**3**), following their transformation into the corresponding ethyl arylic ethers **2** and **4**.

Both phenolic acids were described as easily detectable under LC conditions. Consequently, two of their ethyl ethers, predicted to be metabolites according to this mechanism, were also detectable on the same LC column. Both the radical formation and quenching reactions may be assisted by enzymes present in the extracts as reported by Sonnewald and Sonnewald (2014). However, the mechanism of ethylene reaction in naturally occurring tuber phenols or polyphenols remains uncertain, despite different research studies conducted (in vitro or in storage) (Han et al., 2007).

In order to further confirm this hypothesis, we attempted spiking with standards of four metabolites **1–4** chromatograms. Figure 3 shows these experiments run for RB.

We observed and confirmed the presence of both phenolic acids **1** and **3** at lower retention times of 1.27 and 1.83 min respectively from LC, as well as by mass chromatograms. The intensity of the corresponding negative pseudomolecular ions (M-1)- cannot, however, be used as a quantitative measurement, as from TIC comparison of these ions. Also, their presence occurred in a highly crowded area with possible interferences with other isomass peaks, although this one is controlled by retention time (Rt).

Similarly, in the ethylenization experiments both ethyl ether derivatives of these acids **2** (retention time of 2.2 min) and **4** (retention time of 2.6 min areas) were detected and confirmed from spiking with the synthesized compounds.

For the second variety of potato, YG, the same set of experimental results confirmed the previous pattern of transformations and reinforced the hypothesis of loss of phenol concentration due to the formation of ethyl ethers by ethylene action.

As is usual in such studies, many valuable metabolites were observed to be present from LC-MS experiments. These include sugar conjugates to both phenolic acids and their derivatives reported in this work, as well many other identified compounds. The conjugation to glucose, in particular, could also be considered as a potential reason for the modification of phenolic concentration. However, the fragility of glycoside bonds during fermentation in storage could in principle lead to the release of more phenols and the increase in this count could be incidental to their degradation. In parallel, the glycosides of phenolic acids can also undergo etherification under ethylene treatment. These comments, however, do not change the hypothesis of ethylenization advanced in this paper.

3.1. Statistical analysis

To analyze the results obtained in this study, the computer program PRISME 6 was used. An analysis of variance (ANOVA) was performed. An empirical threshold or confidence interval of 0.05 or 5% has been set. Thus, the significance level below 5% is considered statistically significant. Each experiment of this study was carried out in triplicate.

4. Conclusion

The results reported in this study confirmed first that the total phenol concentration in potato extracts is dependent on the temperature of incubation and nature of the solvent. The total phenol count, as determined by the Folin-Ciocautelau method, increased by up to three times if the temperature rose from 30 °C to 60 °C. This last temperature could be considered as optimal. Changing the extraction solvent system from water-ethanol to methanol 95% slightly increased the total phenol count.

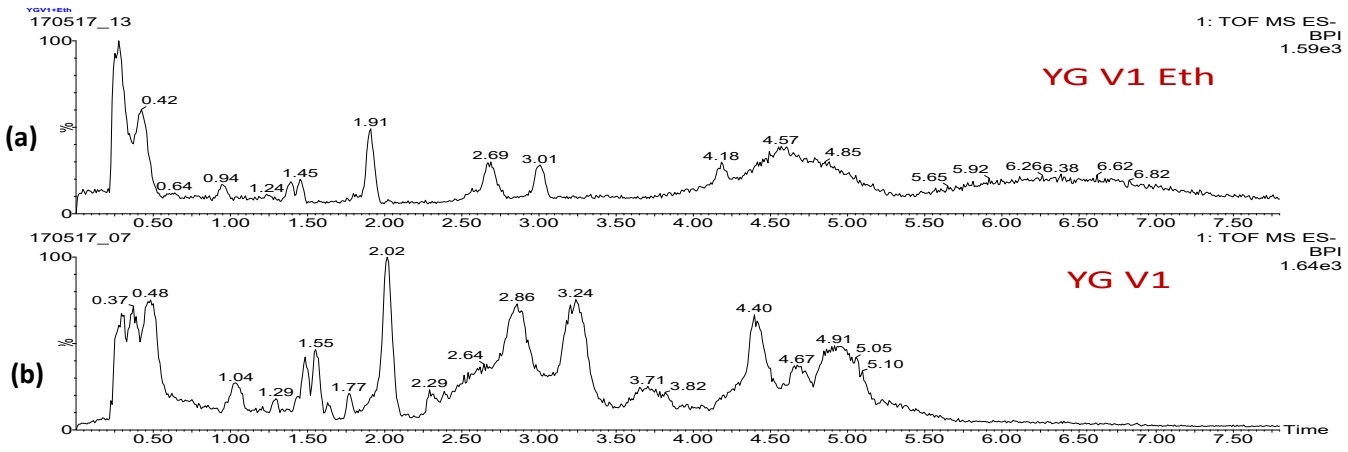

Figure 3. Example of LC of RB extracts (a) after ethylene treatment, (b) original extract without ethylene treatment.

The highest total phenol concentration was observed for YG in methanol at 65 °C, but for RB in water-ethanol at 60 °C.

After ethylenization, in both solvent systems and for both YG and RB, a decrease of around 20% in the total phenol concentration was observed. This level of decrease seemed to vary only a little between the two varieties of potato.

As expected, the extraction system combining water with an organic solvent led to an important co-extraction of sugar moieties phenol conjugates. The total methanol extract using this method was cleaner and was used in the further LC and mechanism studies.

The hypothesis for the possible transformation of phenols into their ethyl ethers under ethylene treatment was then advanced. It was supported by LC experiments with spiking of synthetic pairs of model compounds. It was already reported that the two parent phenolic acids **1** and **3** were present in the extracts (Singh, 2011; Singh et al., 2011). When the ethylenization of potato slurry is performed with trace quantity of TEMPO, a common radical generator, the important increase of ethyl phenolic ethers (**2** and **4**) is observed. These results also support the hypothesis of the radical mechanism of ethyl phenolic ether formation discussed in this study. However, we did not run the NMR or FTIR of extracts because of the simultaneous present of many phenolic ethers in this mixture.

The transformation of phenols into their corresponding ethyl ethers cannot give a high yield, even in presence of entire, not fractionated potato slurry. This transformation alone cannot be responsible for the decreased phenol concentration after ethylenization, especially for the example with decomposition of potato sugar conjugates, as this acts to increase phenol count.

In a real-life potato storage situation, the ethylene concentration is between 2 to 10 ppm in a closed but still aerobic atmosphere. The suggested increase of this concentration above 5 ppm, or even 20 ppm previously suggested (Rylski et al., 1974), would probably further increase the phenol ethyl ether formation but may create construction (airtight storage building) and security problems for storage with higher concentration of this gas.

The additional proof of this hypothesis could, however, be obtained only through costly experiments under a perdeuterated ethylene atmosphere. Because of the very complex nature of the mixture analysed, the quantitation of these transformations cannot be foreseen.

Declarations

Author contribution statement

Etienne DAKO: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Christopher K. JANKOWSKI: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yves-Marie GNIMASSOU: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Diane LEBEAU: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Akyol, H., Riciputim, Y., Capanoglu, E., Caboni, M.F., Verardo, V., 2016. Phenolic compounds in the potato and its byproducts: an overview. Int. J. Mol. Sci. 17, 835–853.

Belaid, D., 2015. Techniques de culture et de conservation de la pomme de terre en Algérie. Edition 2015. Collection. Science et Techniques Agronomiques, p. 51.

Campbell, M.A., Gleichsner, A., Alsbury, R., Horvath, D., Suttle, J., 2010. The sprout inhibitors chlorpropham and 1,4-dimethylnaphthalene elicit different transcriptional profiles and do not suppress growth through a prolongation of the dormant state. Plant Mol. Biol. 73 (1-2), 181–189.

Canaweed guides, 2015. L'éthylène (phythormone). En ligne. https://www.cannaweed.com/guides/_/culture-avanc-e/experiences-articles-scientifiques-et-publications/l%C3%A9thyl%C3%A8ne-phythormone-r308. Consulté le 12/01/18.

FAOSTAT. En ligne. www.fao.org/faostat/fr/#home. Consulté le 04/12/19.

Folin, O., Ciocalteu, V., 1927. On tyrosine and tryptophane determinations in proteins. J. Biol. Chem. 73, 627–650.

Foukaraki, S.G., Cools, K., Chope, G.A., Terry, L.A., 2016. Impact of ethylene and 1-MCP on sprouting and sugar accumulation in stored potatoes. Postharvest Biol. Technol. 114, 95–103.

Han, X., Shen, T., Lou, H., 2007. Dietary polyphenols and their biological significance. Int. J. Mol. Sci. 8 (9), 950–988.

Iqbal, N., Khan, N.A., Ferrante, A., Trivellini, A., Francini, A., Khan, M.I.R., 2017. Ethylene role in plant growth, development and senescence: interaction with other phytohormones. Front. Plant Sci. 8, 475–493.

Kim, J., Soh, S.Y., Bae, H., Nam, S.-Y., 2019. Antioxydant and phenolic contents in potatoes (Salanum tuberosum L.) and micropropagated potatoes. Appl. Biol. Chem. 62, 17–25.

Liu, H.K., Cao, Y., Huang, W.N., Guo, Y.D., Kang, Y.F., 2013. Effect of ethylene on total phenolics, antioxidant activity, and the activity of metabolic enzymes in mung bean sprouts. Eur. Food Res. Technol. 237 (5), 755–764.

Liu, M., Diretto, G., Pirrello, J., Roustan, J.P., Li, Z., Giuliano, G., Regad, F., Bouzayen, M., 2014. The chimeric repressor version of an Ethylene Response Factor (ERF) family member, Sl-ERF.B3, shows contrasting effects on tomato fruit ripening. New Phytol. 203, 206–218.

Macheix, J.-J., Fleuriet, A., Jay-Allemand, C., 2005. Les composés phénoliques des végétaux : Un exemple des métabolites secondaires d'importance économique. Collection biologie, Presses Polytechniques et Universitaires Romandes (PPUR), p. 192.

Maroun, R.G., Louka, N., Karam, M., Amir-Aslani, A., 2013. Enjeux et opportunités des composes phénoliques. Stratégie pour l'Innovation, Spectra Analyse N293, 19–21.

Mattila, P., Hellström, J., 2007. Phenolic acids in potatoes, vegetables, and some of their products. J. Food Compos. Anal. 20 (3-4), 152–160.

Mingchun, L., Pirrello, J., Chervin, C., Roustan, J.-P., Bouzayen, M., 2015. Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation. Plant Physiol. 169, 2380–2390.

Pakizeh, M., Kuliev, A.A., Mammadov, Z.M., Ardjmand, M., Hasani, M., 2011. Effect of ethylene on potato sprouts and NADPH activity. World Appl. Sci. J. 14, 67–71.

Prange, R.K., Kalte, W., Daniels-Lake, B.J., Liew, C.L., Page, R.T., 1998. Using ethylene as a sprout control agent in stored 'Russet Burbank' potatoes. J. Am. Soc. Hortic. Sci. 123, 463–469.

Ru, W., Pang, Y., Gan, Y., Liu, Q., Bao, J., 2019. Phenolic compounds and antioxidant activities of potato cultivars with white, yellow, red and purple flesh. Antioxidants 8, 419–429.

Rylski, I., Rappaport, L., Pratt, H.K., 1974. Dual effects of ethylene on potato dormancy and sprout growth. Department of Vegetable Crops, University of California, Davis, California Plant Physiol. 53 (4), 658–662.

Singh, A., Sabally, K., Kubow, S., Donnelly, D.J., Gariepy, Y., Orsat, V., Raghavan, G.S.V., 2011. Microwave-assisted extraction of phenolic antioxidants from potato peels. Molecules 16, 2218–2222.

Singh, A., 2010. Optimization of Microwave-Assisted Extraction of Antioxidants from Potato Peels. Master Thesis. *M*cGill University, Quebec, Canada, p. 108.

Singh, P.P., 2011. Subcritical Water Extraction of Functional Ingredients and Glycoalkaloids from Potato Peel. University of Alberta, Edmonton, Alberta, Canada, p. 178. Master of Science in Food Science and Technology, Master Thesis.

Sonnewald, S., Sonnewald, U., 2014. Regulation of potato tuber sprouting. Planta 239, 27–38.

Suttle, J.C., 1998. Involvement of ethylene in potato microtuber dormancy. Plant Physiol. 118, 843–848.

Wang, K.L.-C., Li, H., Ecker, J.R., 2002. Ethylene biosynthesis and signaling networks. Plant Cell S131–S151.