

Influence of Sous Vide and Water Immersion Processing on Polyacetylene Content and Instrumental Color of Parsnip (*Pastinaca sativa*) Disks

ASHISH RAWSON,^{*,†,‡} ANASTASIOS KOIDIS,[†] DILIP K. RAI,[†] MARIA TUOHY,[‡] AND NIGEL BRUNTON[†]

[†]Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland, and [‡]Department of Biochemistry, National University of Ireland Galway, Galway, Ireland

The effect of blanching (95 ± 3 °C) followed by sous vide (SV) processing (90 °C for 10 min) on levels of two polyacetylenes in parsnip disks immediately after processing and during chill storage was studied and compared with the effect of water immersion (WI) processing (70 °C for 2 min.). Blanching had the greatest influence on the retention of polyacetylenes in sous vide processed parsnip disks resulting in significant decreases of 24.5 and 24% of faltarinol (**1**) and faltarindiol (**2**) respectively ($p < 0.05$). Subsequent SV processing did not result in additional significant losses in polyacetylenes compared to blanched samples. Subsequent anaerobic storage of SV processed samples resulted in a significant decrease in **1** levels ($p < 0.05$) although no change in **2** levels was observed ($p > 0.05$). **1** levels in WI processed samples were significantly higher than in SV samples ($p \leq 0.05$). **2** was particularly susceptible to aerobic storage following WI processing with losses of up to 70% occurring after 5 days storage. **1** type polyacetylene undergoes degradation such as oxidation, dehydrogenation when thermally treated forming oxidized form of **1** type molecules, in this case faltarindione, dehydrofaltarinol, dehydrofaltarinone. Thermal processing had a significant effect on instrumental color of parsnip samples compared to minimally processed in both SV and WI processed samples resulting in parsnip disks becoming darker, yellower and browner following processing and storage.

KEYWORDS: Polyacetylenes; parsnips; sous vide; processing; water immersion; color

INTRODUCTION

In recent years consumers have become aware of recommendations to increase their consumption of fruits and vegetables to help prevent the onset of free radical mediated diseases due to findings of large epidemiological studies (1–4). Evidence is accumulating that the protective effect of fruit and vegetable consumption may be in part due to the presence of biologically active secondary metabolites known as phytochemicals. To date research in this area has concentrated on well-known phytochemicals such as polyphenols, vitamin C, carotenoids, dietary fiber, selenium and folates (5–9). Despite the scientific progress, it is in many cases unclear which components are responsible for the health promoting properties of fruits and vegetables.

Parsnip (*Pastinaca sativa*) is a root vegetable of the family Umbelliferae that has been cultivated since Roman times for its long, fleshy, edible root. Parsnips can be eaten raw, boiled, roasted, fried or used in stews, soups, or casseroles, and they are a quite important part of the diet in the UK and Ireland (10). A number of recent studies have indicated that a group of C₁₇ acetylenes of the faltarinol type present in members of the

Apiaceae and Araliaceae families have cytotoxicity against human cancer cells (10–13).

Polyacetylene's natural role is to prevent fungal infections in the plant (14), and they also impart a bitter taste at high concentrations (15). Some members of the polyacetylene family are known to be potent skin sensitizers (16) and neurotoxic in high concentrations in mice (17). Newer scientific evidence, however, revealed that polyacetylenes possess antibacterial, anti-inflammatory and anti-platelet-aggregatory properties (11, 18–20). A previous study has shown that parsnip roots contain two polyacetylenes, namely, faltarinol (**1**) and faltarindiol (**2**) (21). Other studies have shown that faltarinol exhibited the highest biological activity of the two polyacetylenes (18, 21, 22). All the above studies categorize polyacetylenes as potential bioactive compounds that, within a balanced diet, might promote human health. Parsnips are normally thermally processed prior to consumption, and this has been shown to affect the level of polyacetylenes in vegetables (11). However, to date, there have been no reports on the effect of thermal processing on parsnip for their polyacetylene content.

Thermal processing of parsnips usually involves either water immersion (including boiling) or oven roasting. The increase in consumer demand for minimally processed refrigerated convenience

*Corresponding author. Phone: 00353863604659. Fax: 00353 1 8059550. E-mail: ashishrawson@gmail.com.

foods with characteristics closer to those of the fresh products has led to the development of technologies which can help retain potentially health promoting compounds in thermally processed foods. Refinement of vacuum packaging, pasteurization and chilled storage of various food products has given rise to a process known variously as sous vide, "cuisine en papillote sous vide", "cuisson sous vide" and sous vide cook–chill (23). Sous vide (SV) processing, as an alternative to boiling or water immersion (WI) treatments, uses milder temperatures ($\approx 90^\circ\text{C}$) than sterilization to heat process products in vacuum sealed pouches. This technology may be an effective strategy to minimize losses of thermally unstable compounds during processing (24) while also extending the shelf life (20 days at 4°C). SV products are stored in vacuum packed pouches following heating which may also serve to protect compounds susceptible to oxidation (25). Additionally, during processing and storage, color, as part of the sensory characteristics, may change in parsnips. As previously stated, the effect of a number of parameters in the polyacetylene levels and color of SV and WI processed parsnips has not been studied, hence the potential outcomes could lead to recommendations for processing and storage practices in households and industries aimed at minimizing losses of polyacetylenes in parsnips.

Thus, the aim of the present study is to compare the effect of SV processing on levels of polyacetylenes and color in parsnip disks in comparison to WI cooking immediately after processing and during chill storage.

MATERIALS AND METHODS

Chemicals. The following solvents were purchased from Fischer Scientific (Dublin, Ireland): acetonitrile (ACN) and water; they were of HPLC grade. Diatomaceous earth for accelerated solvent extraction was obtained from Dionex, Surrey, U.K.

Sample Preparation. Parsnips (*Pastinaca sativa* cv. Gladiator) were obtained from a local wholesaler (Sea View Ltd., Dublin, Ireland) and were minimally processed. Parsnip root samples with no visible damage were selected for the experiment. The roots were hand peeled and then sliced into disks (5 mm) using a Berkel 800 meat slicer (Berkel company, Indianapolis, IN). Only parsnips with root diameters in the range 60–85 mm were selected for the study.

Sous Vide (SV) Processing of Parsnip Disks. Three batches (1.5 kg each) of peeled and sliced parsnips were subjected to blanching in water at 95°C for 90 s to inactivate enzymes. Samples (250 g) were vacuum packed in 20 cm \times 30 cm polyethylene bags (thickness, 75 μm ; gas permeability, 2.7 g/m^2 day, Packex Industries Ltd., Wicklow, Ireland) and heated in a retort unit (Barriquand Steriflow, Roanne, France) to a process equivalent of 90°C for 10 min ($P_{90} \geq 10$). This heat cycle approximated the minimum recommended SV pasteurization required to inactivate psychrotrophic *Clostridium botulinum* spores and give a chilled shelf life of 21 days (26, 27). Sample core temperature profiles and P_0 values were recorded during the process using an Ellab E-Val TM TM9608 data module (Ellab Ltd., Norfolk, U.K.). A Standard Ellab SSA-12080-G700-TS temperature probe was inserted through an Ellab GKM-13009-C020 packing gland (20 mm) into a 30 mm thick parsnip cylinder to monitor cook cycle. An Ellab SSR-60020-G700-TS water probe was used to record cook cycle water temperature. Temperature was recorded every 20 s, and the accuracy of temperature measurements was $\pm 0.1^\circ\text{C}$. Prior to any experiment, all Ellab unit probes were calibrated against a JOFRA (ATC-155B) calibration unit at temperatures from 40 and 100°C . All results associated with the calibration did not exceed $\pm 0.1^\circ\text{C}$. Following processing, samples were stored vacuum packed at 4°C for 20 days. Samples (≈ 250 g) were taken after 0, 5, 10, 15, and 20 days of storage. All samples (including minimally processed ones) were blast frozen (Avon Refrigeration Co., Bristol, U.K.), freeze-dried (model A6/14, Frozen in Time Ltd., York, U.K.), vacuum packed using a Vac Star S220 vacuum sealer (Vicquip Ltd., Dublin, Ireland) and stored in polythene bags at -20°C .

Water Immersion (WI) Cooking of Parsnips. Three sets (250 g each) of raw peeled and sliced parsnips were subjected to boiling in water

until a core temperature of 70°C was achieved. They were held at this temperature until they had reached a time–temperature ($P_{70} \geq 2$ min) equivalent to a six log reduction in numbers of vegetative cells of the target pathogen (*Listeria monocytogenes*). Sample core temperature profiles and P_0 values were recorded during the process as described above for SV processing. Following processing, the parsnip samples were stored at 4°C for 5 days. Samples (≈ 250 g) were taken after 0, 1, 3, and 5 days of storage, freeze-dried and stored at -20°C prior to extraction.

Extraction of Polyacetylenes. Extraction of polyacetylenes was performed using an ASE 200 Accelerated Solvent Extraction automated system (Dionex, Surrey, U.K.). Freeze-dried parsnip disks were milled to powder using a Kenwood grinder (Kenwood BL 430, Hertfordshire, U.K.), and 1 g was used as the extracting material. The remaining volume was filled with diatomaceous earth (Dionex, Surrey, U.K.). Acetonitrile was used as the extracting solvent, and an optimized protocol (extraction temperature, 80°C ; pressure, 1500 psi) was developed according to previous work from the authors (28). The acetonitrile extract was dried under N_2 at 37°C using Techne Sample Concentrator, Techne DRI-BLOCK DB-3D (Staffordshire, U.K.). The parsnip dry residue was rediluted in 1 mL of acetonitrile, filtered using an Acrodisc LC 25 mm syringe filter (Sigma Aldrich, U.K.) with 0.2 μm PVDF membrane and transferred to a 2 mL amber vial for reversed phase high performance liquid chromatography (RP-HPLC) analysis.

Determination of Polyacetylenes Using RP-HPLC. Separation and quantification of polyacetylenes was carried out by RP-HPLC using the method recommended by Zidorn and others (21) with some modifications (28). In brief, analysis was performed on an Agilent 1100 (Agilent Technologies, Boeblingen/Stuttgart, Germany) series HPLC system equipped with UV detector set at 205 nm. Separation were performed on a Luna C₁₈ 100A column (100 \times 4.6 mm 5 μm , Phenomenex, Cheshire, U.K.) at 40°C using the following solvent gradient: ACN–H₂O [0–5 min (20:80), 10 min. (50:50), 30 min (53:47), 45–50 min (65:35), 55–105 min (100:0)]. The flow rate was 1 mL/min and the injection volume 10 μL . Polyacetylenes were identified at 205 nm by peak addition of in-house polyacetylene standards and quantified using calibration curves of these standards (10–50 $\mu\text{g}/\text{mL}$). Replicate runs for each of the polyacetylenes gave R^2 ranging between 0.9997 and 0.9902. The in-house polyacetylene standards were isolated and purified from fresh carrots using a combination of solid/liquid extraction followed by column chromatography and subsequent preparative high performance chromatography (data not shown) as described by Hansen and others (11). The identity of the recovered polyacetylenes was confirmed by comparison of their high resolution NMR spectral data and their mass spectrometric spectra with literature values (21, 29). For all the samples involved in this study, the extraction and analysis was carried out after the end of the storage period simultaneously.

Liquid Chromatography/Time-of-Flight Mass Spectrometry. The LC–MS analysis was performed on a Q-ToF Premier mass spectrometer (Waters Corporation, Micromass MS Technologies, Manchester, U.K.) coupled to an Alliance 2695 HPLC system (Waters Corp., Milford, MA). The Q-ToF Premier is equipped with a lockspray source where an internal reference compound was introduced simultaneously with the analyte for accurate mass measurements. The LC separation of the compounds was achieved on a Phenomenex Luna 2.5 μm C₁₈ column (100 mm \times 2 mm; 2.5 μm particle size) using water (solvent A) and acetonitrile (solvent B). A stepwise gradient from 20% to 100% solvent B was applied at a flow rate of 0.250 mL/min for 35 min. Mass spectral data were acquired on a positive ionization mode for a mass range m/z 100 to 1000.

Measurement of Instrumental Color. Instrumental color values of the parsnip disks were measured using a Hunter-Lab DP-9000 color difference meter (Hunter Associates Laboratory, Reston, VA) fitted with a 2.5 cm diameter aperture. The instrument was calibrated using the black and white tiles provided. Color was expressed in Hunter Lab units L^* (whiteness or brightness), a^* (redness/greenness) and b^* (yellowness/blueness). Three replicate measurements were performed, and results were averaged. In addition, total color difference (ΔE) was calculated using the following equation, where L_0 , a_0 , b_0 are the minimally processed values for parsnips.

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

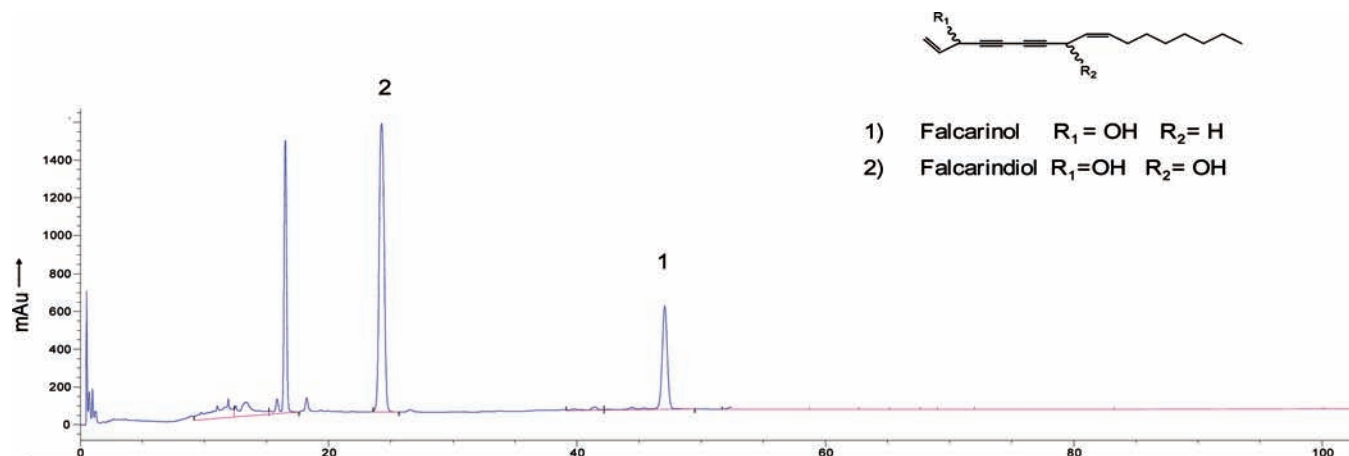


Figure 1. Sample HPLC chromatogram of a minimally processed freeze-dried parsnip extract *Pastinaca sativa*, cv. Gladiator.

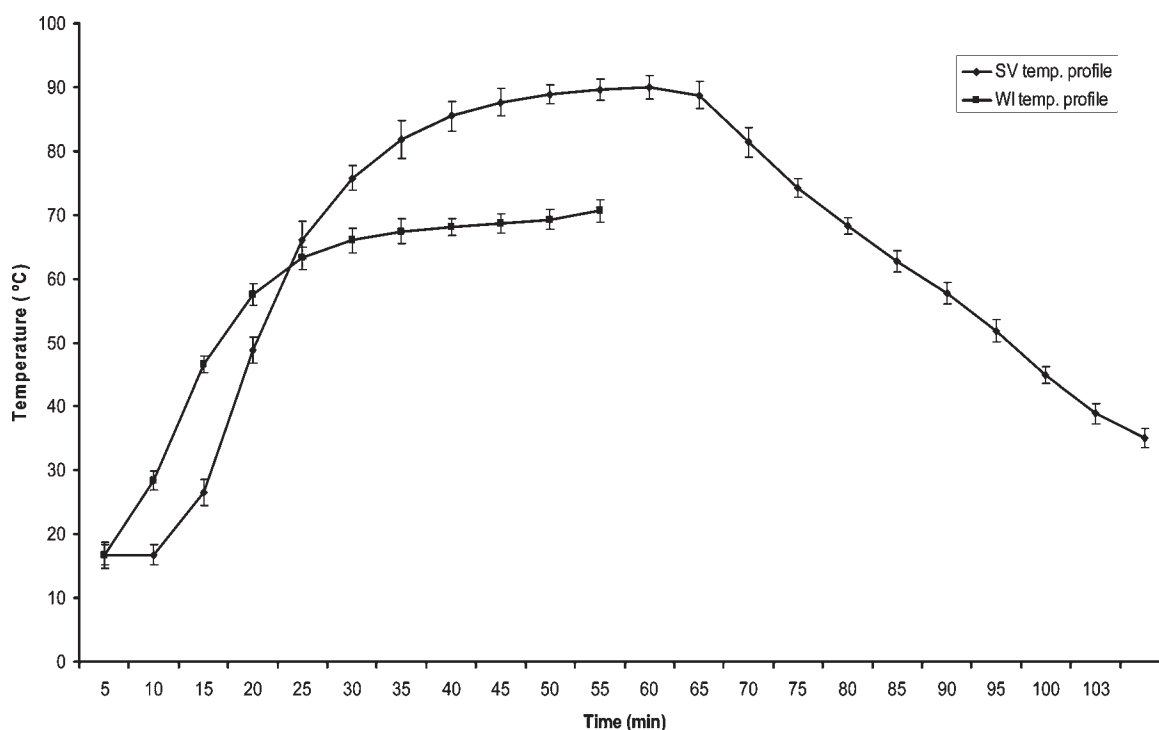


Figure 2. Time–temperature profile for parsnip disks during sous vide (SV) processing and water immersion (WI) processing.

Statistical Analysis. Differences were considered significant at $p < 0.05$. Analysis of variance (ANOVA) was performed using the GenStat Release 10.1 (PC/Windows XP).

RESULTS AND DISCUSSION

Effect of Thermal Processing on Polyacetylene Levels of Parsnip Disks. A typical HPLC chromatogram of a minimally processed freeze-dried acetonitrile extract from parsnip is illustrated in **Figure 1**. The peaks eluting at 24.74 and 47.64 min were identified as falcarindiol (**2**) and falcarinol (**1**) respectively on the basis of their retention on HPLC. These polyacetylenes were also identified in parsnips of other varieties grown or sold in Ireland (e.g., Gladiator, Dagger, Javelin, etc.; data not shown). The levels of compound **1** and **2** in minimally processed parsnip were found to be 7.42 and 4.90 mg/100 g fresh weight (FW). These levels are significantly lower than those demonstrated by Zidorn and others (21) of 29.20 and 105.28 mg/100 g FW of the sample for falcarinol (**1**) and falcarindiol (**2**) respectively. This is probably due to the fact that they were minimally processed, i.e., peeled

and sliced. Carrot peel has been shown to be particularly rich in polyacetylenes in previous studies (15, 30) although similar evidence for parsnips is not available. However, in the present study, parsnips were peeled and sliced in order to mirror the protocol followed in an industrial setting.

A typical time–temperature profile of WI and SV processed parsnip disks as monitored using the Ellab electronic temperature logging system is presented in **Figure 2**. Thermal processing had a significant ($p \leq 0.05$) effect on polyacetylene levels in parsnip disks as illustrated in **Figure 3**. The levels of falcarinol (**1**) for minimally processed, blanched, SV processed (day 0) and WI processed (day 0) were 7.42, 5.37, 5.63, and 6.61 mg/100 g FW. The corresponding values for falcarindiol (**2**) were 4.90, 3.75, 3.40, and 3.82 mg/100 g FW. Therefore, the initial step in SV processed samples, blanching (95 °C, 90 s) resulted in a reduction in polyacetylenes levels ($p < 0.05$) prior to SV or WI treatment. In fact, a reduction of 24.5 and 24% was observed in the levels of **1** and **2** in blanched samples compared to minimally processed disks. The degree of degradation for **1** following blanching is in

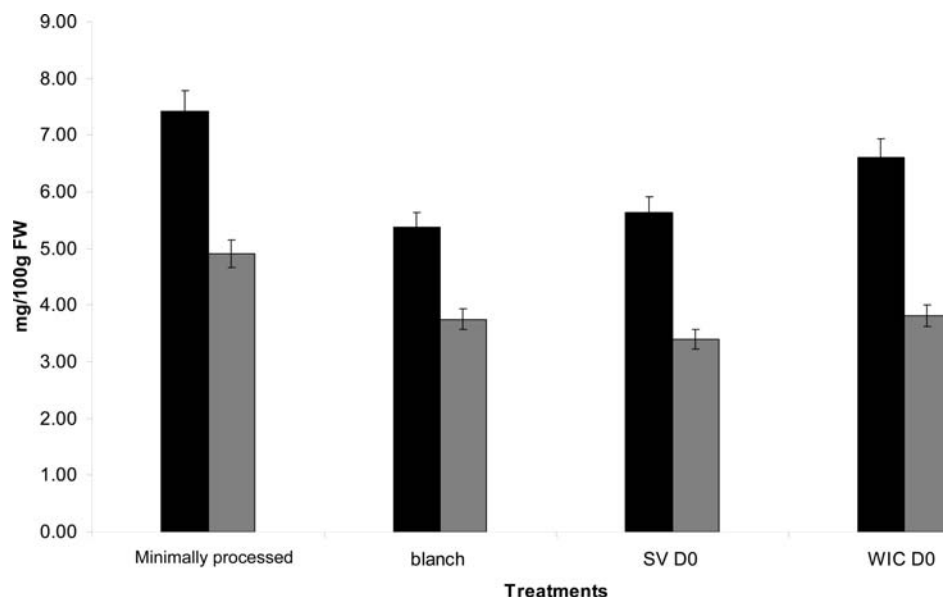


Figure 3. Effect of sous vide (SV) and water immersion processing on levels of polyacetylenes in parsnips (*Pastinaca sativa*, cv. Gladiator) disks at day 0 (immediately after thermal processing).

accordance with 50% loss reported by Hansen and others (11) after blanching (3 min, 100 °C) of carrots. Kidmose and others (31) also reported that blanching of carrots can result in a decrease in **2** and faltarindiol-3-acetate by 26.2% and 30.4%. The authors speculated that this was caused by degradation of heat sensitive polyacetylenes during blanching and/or conversion of **1** to **2** and faltarindiol-3-acetate. Optimizing the blanching step during SV processing could therefore reduce the losses of polyacetylenes.

In comparison to minimally processed samples, losses in SV processed samples were in the range of 24% and 30% for **1** and **2** respectively. Hansen and others (11) reported that boiling for longer periods (3–15 min) caused a further 20% loss of total **1** in carrot disks. However, in comparison to blanched samples no significant additional decrease in **1** or **2** occurred following SV processing ($p > 0.05$). This indicates that blanching was the major cause for the losses of polyacetylenes in parsnips rather than the SV processing itself. On average, WI processing resulted in a slightly lower loss of **2** as compared to the loss occurred due to SV processing, however, the difference was not significant ($p > 0.05$). **1** levels for WI processed samples were significantly higher than for SV processed samples ($p \leq 0.05$). However, it should be noted that SV processed samples had undergone a blanching step (95 °C, 90 s) prior to SV processing which was not the case for WI samples. To the best of our knowledge, to date, no other reports on the effect of SV processing on polyacetylenes have been published. Nonetheless, the technique has been examined for other potential bioactive compounds. For example, Patras and others (32) reported that the SV processing of carrot disks resulted in higher losses of polyphenols compared to WI processed samples. The authors commented that this may be due to the fact that SV processed samples received a more severe treatment in comparison to WI processed samples (90 °C for 10 min vs 70 °C for 2 min). This could also be the case in the present study. Stea and others (33) reported that SV processed broccoli had low levels of folate which they also attributed to an additional pretreatment with blanching. Higher losses of **2** also suggest that this compound may be more unstable to heat treatment than **1**. A similar trend was found in previous work in this laboratory related to water immersion processing of carrots (28).

Effect of Chill Storage on Polyacetylene Levels of Parsnip Disks.

Vacuum chill (4 °C) storage of SV processed parsnips discs (Figure 4) resulted in a significant decrease in the level of **1** ($\approx 13\%$ loss) after 5 days storage compared to SV processed samples immediately after processing ($p \leq 0.05$). This decrease continued following prolonged storage resulting in a total loss of $\approx 25\%$ by day 20 when compared to the levels of day 0 ($p \leq 0.05$). Concerning the levels of **1** in WI processed samples during storage, a decrease was also observed over time (Figure 5). For example, compared to samples immediately after processing (day 0), WI processed sample at day 5 showed a significant loss ($p \leq 0.05$) of $\approx 10\%$ in the levels of **1**.

In contrast to **1** levels, **2** levels in SV processed samples did not significantly decrease over the 20 day storage period when compared to day 0 samples. On average, losses were of the order $\approx 9.5\%$, however, the effect was not significant ($p > 0.05$). Aerobic storage following WI processing of parsnips disks did result in a significant decrease in the levels of **2** following 5 days chill storage ($p \leq 0.05$). In fact, total losses of **2** in these samples were severe (nearly up to 70% when compared to day 0). SV processed samples were stored under vacuum after processing whereas water immersion processed samples were stored in air, therefore the lower stability of **2** in WI samples could be due to oxidative degradation of this compound when stored in air. There have been no reports on the stability of polyacetylene compounds during chill storage (4 °C) of processed vegetables. Hansen and others (11) reported a reduction in **1** content in chill stored raw carrots. However, as the authors suggested, this reduction was probably due to changes in the balance between enzymatic degradation of **1** and its biosynthesis and these enzymes would presumably not have been active in the processed samples (11).

Additionally, Kidmose and others (31) reported that levels of **2** and faltarindiol-3-acetate were reduced in the blanched frozen carrots stored for 4 months because of degradation of heat sensitive polyacetylenes during blanching. Chill storage of thermally processed vegetables has been shown to result in a reduction in the levels of other bioactive compounds. For example, Patras and others (32) established that SV and WI processed carrot disks resulted in a decrease in levels of polyphenols during chill storage.

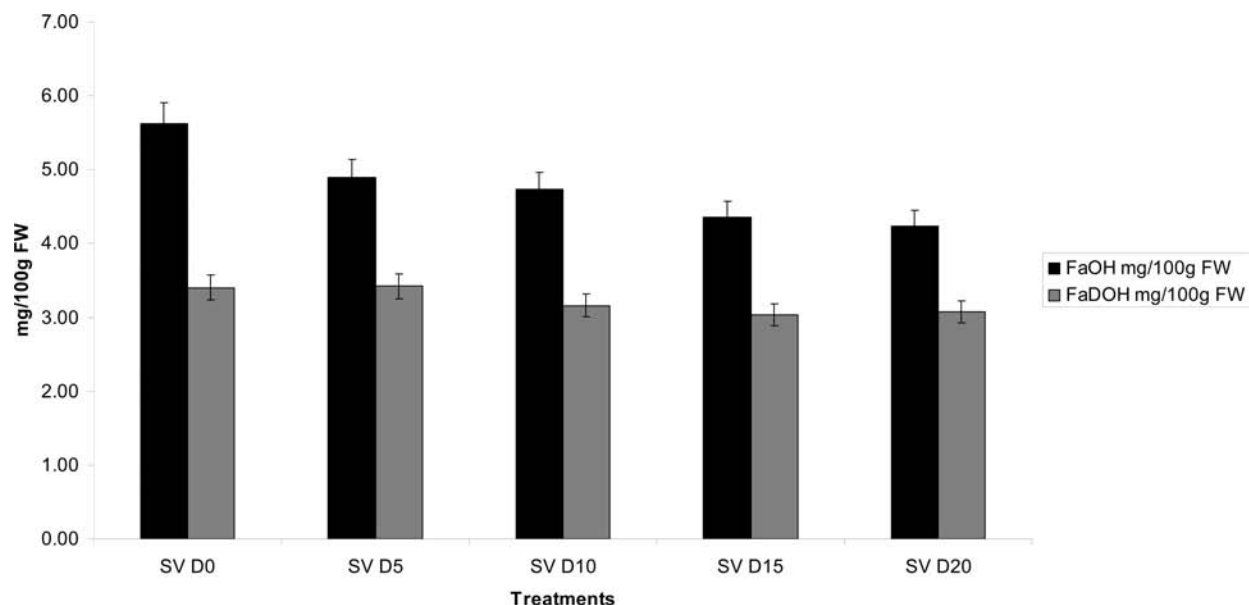


Figure 4. Effect of chill storage (4 °C) on levels of polyacetylenes in sous vide processed parsnip disks in vacuumized pouches.

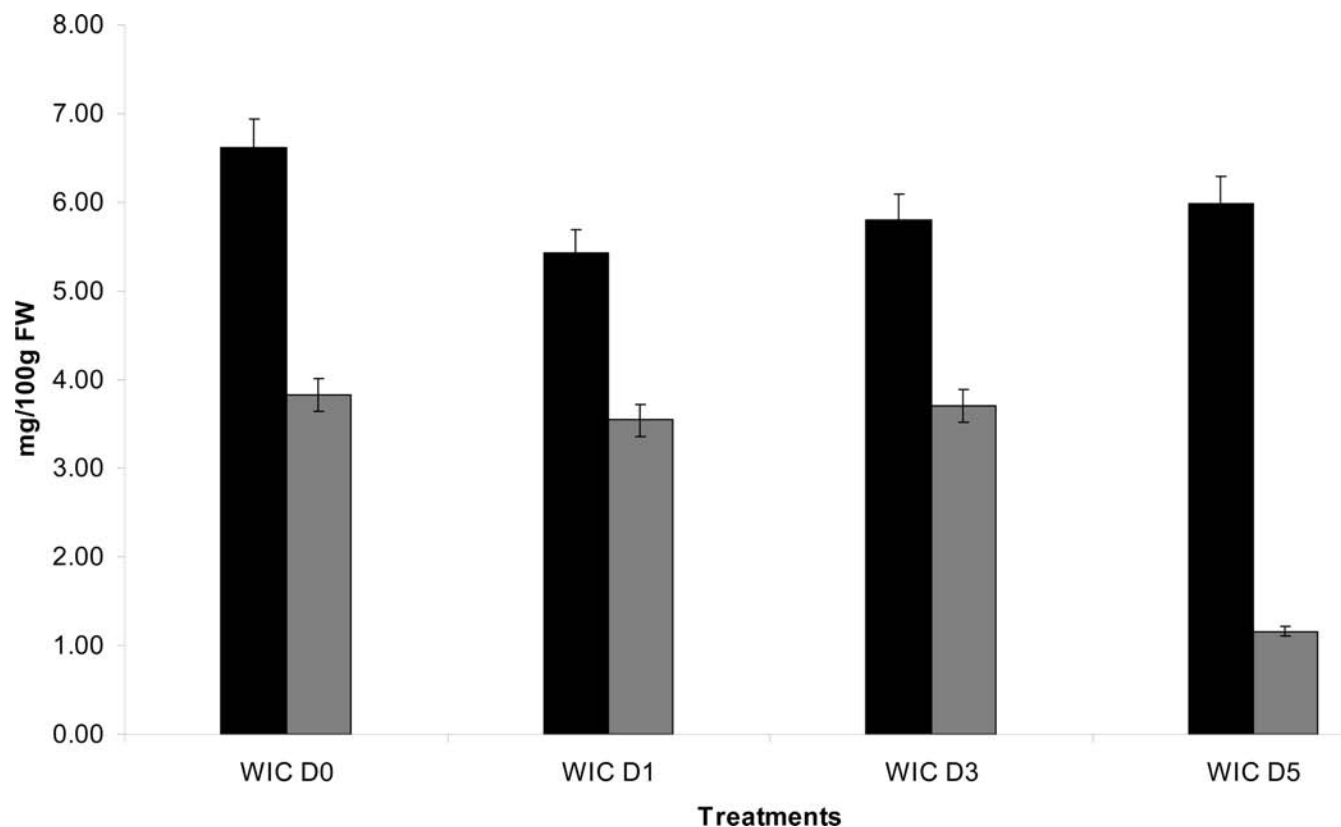


Figure 5. Effect of chill storage (4 °C) on levels of polyacetylenes in water immersion processed parsnip disks.

Effect of Thermal Treatment and Storage on Instrumental Color of Parsnip Disks. While the focus of the present study was to monitor the effect of thermal processing on levels of polyacetylenes in parsnip disks, we were also interested in the effect of thermal processing on levels of a commonly studied index of quality. Therefore instrumental color was monitored following thermal processing and during storage.

Hunter color values for minimally, WI and SV processed and stored parsnip disks are presented in Figures 6 and 7. Following thermal processing by both methods, L^* values (lightness) of

parsnip samples decreased, indicating that disks got darker following thermal treatment. In addition, there was a significant decrease in the L^* value from day 0 to day 5 for SV processed samples ($p \leq 0.05$). Further storage of the parsnip disks did not result in changes of the L^* parameter. In WI processed samples, L^* values remained constant during storage until day 5 ($p > 0.05$). To the best of our knowledge no other reports on the effect SV processing on instrumental color of parsnip disks have been published although some work has been done on carrot disks. Wierlen (34) reported that L^* values of carrot disks remained

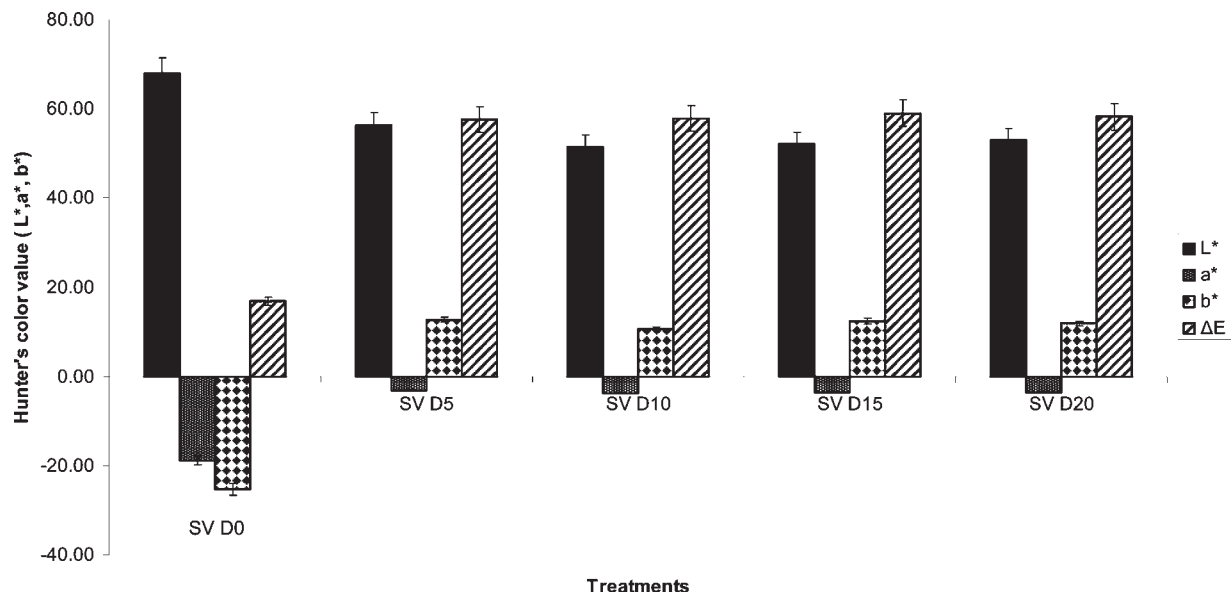


Figure 6. Effect of sous vide processing on instrumental color values (L^* , a^* , b^* , ΔE) of parsnip disks (*Pastinaca sativa*, cv. Gladiator).

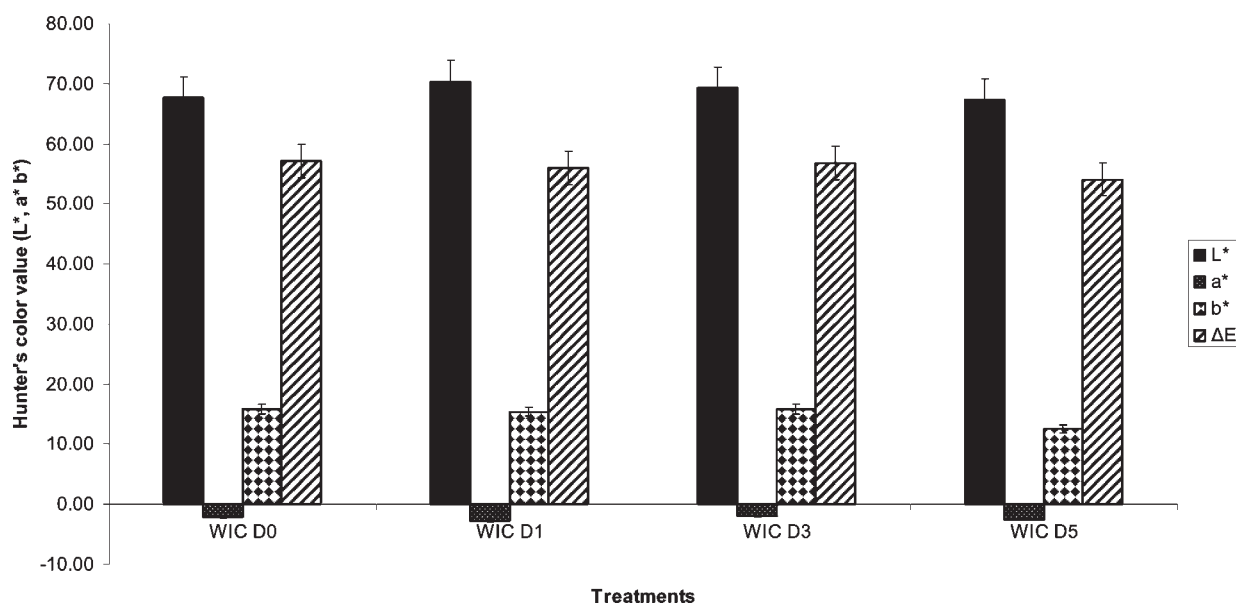


Figure 7. Effect of water immersion processing on instrumental color values (L^* , a^* , b^* , ΔE) of parsnip disks (*Pastinaca sativa*, cv. Gladiator).

unaffected during storage of SV processed samples. In addition, Araya and others (35) reported that L^* values decreased immediately after thermal processing in comparison to minimally processed samples in carrot disks, in SV processed samples L^* values did not change over time, while in WI processed samples L^* diminished during storage.

The values of a^* (positive = redness, negative = greenness) increased for SV processed parsnip discs during storage from day 0 to 5 ($p < 0.05$), but no significant change was found thereafter. This indicates that during the first 5 storage days parsnip disks became less green. For WI processed samples there was a significant increase in a^* value ($p \leq 0.05$) immediately after processing (day 0); thereafter values remained constant during the storage period of 5 days. Patras and others (32) reported a decrease in the a^* values for both SV and WI processed carrot disks at day 0 and during storage compared to minimally processed samples. Werlien (34) also reported that a^* value remained unchanged during storage of SV and WI processed carrots.

The values of b^* (positive = yellowness, negative = blueness) increased after thermal processing, indicating that there was a significant increase in yellowness after blanching and SV processing ($p \leq 0.05$). b^* values decreased markedly during storage from day 0 to day 5 (49.7%) for SV processed samples and thereafter remained relatively constant. In the case of WI processed parsnip samples, a significant increase in b^* values after WI processing when compared to minimally processed samples (57.5%, $p \leq 0.05$) was observed; thereafter values remained constant up to 3 days storage but decreased after 5 days ($p \leq 0.05$).

Thermal processing had a significant effect on total color difference (ΔE) of parsnip samples compared to minimally processed, both SV and WI processed samples ($p \leq 0.05$) as illustrated in Figures 6 and 7. However, for SV processed samples, the magnitude of change in ΔE was much greater during the first five days of chill storage than it was following either of the thermal processing steps. No significant changes in ΔE occurred in SV processed parsnip disks after 5 days chill storage. The most considerable change in ΔE values for WI samples occurred

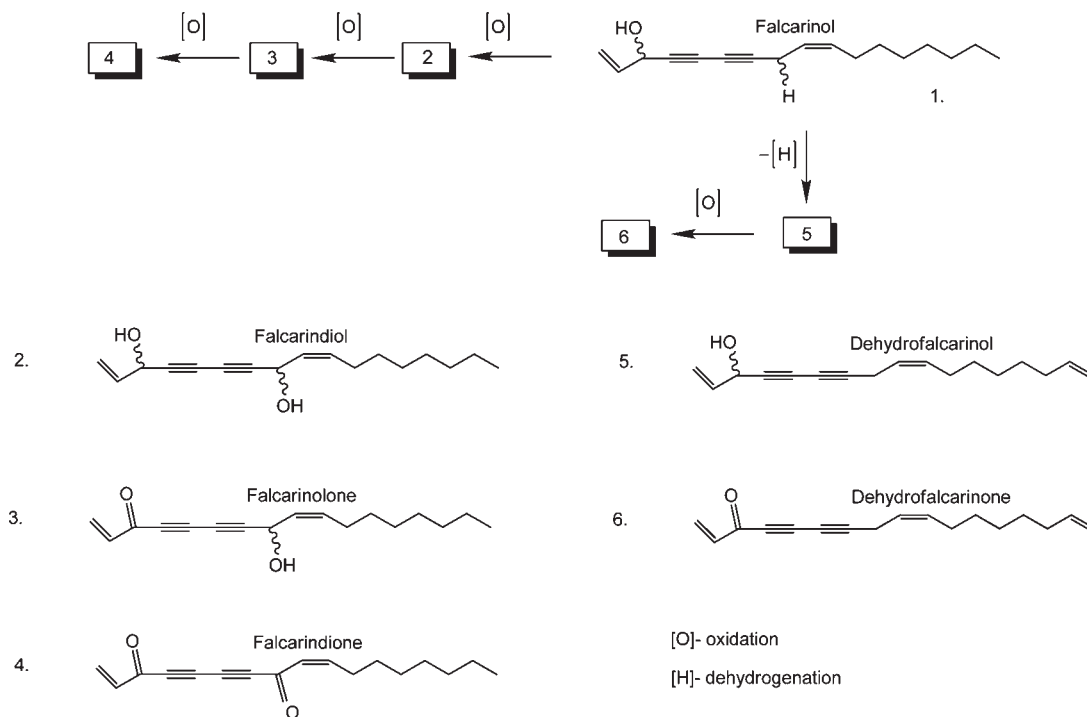


Figure 8. Degradation mechanism for falcarinol type molecules.

following processing which was mainly accounted for by a^* and b^* values changing from negative to positive, i.e., samples becoming more red or less green and more yellow or less blue in color (Figure 7). In contrast, no significant changes in ΔE values of WI processed samples were found to occur during chill storage ($p \geq 0.05$).

Degradation Mechanism of Polyacetylenes Due to Thermal Treatment and Its Effect on the Color. Effect of thermal treatment on falcarinol type polyacetylenes may be oxidation and/or dehydrogenation which may lead to acetylenes of the falcarinol type as outlined in Figure 8. Crude extracts from parsnip for minimally and thermally processed (SVD0) were analyzed on LC-MS to investigate the presence of possible degradation product of falcarinol type polyacetylenes. Bohlmann and others (36–38) have suggested several degradation pathways and may include oxidation, dehydrogenation, acetylation and loss of water.

Accurate mass measurement on the LC-QTOF-MS revealed a compound with a mass of 277.1213 and fits well with the empirical formula of $C_{17}H_{18}O_2Na$ (mass error < 5 ppm), and this empirical formula in turn could correspond to falcarindione, a possible oxidation product of falcarinol type polyacetylene. This mass was not present in the extracts from minimally processed parsnip sample. In addition the presence of a compound with a mass of 243.1754 fitted well with the empirical formula $C_{17}H_{22}O$ (mass error ~ 2 ppm). This formula and mass fit well with dehydrofalcarinol, a dehydrogenation product of falcarinol type polyacetylene. Molecular ion abundance for this mass was almost double in the thermally processed sample as compared to the minimally processed samples of parsnips. Further oxidation of dehydrofalcarinol may result in formation of ketone at carbon 3 position which is dehydrofalcarinone, a compound with an exact mass of 239.1425 which fitted well with the empirical formula $C_{17}H_{18}O$ (mass error < 5 ppm) detected on LC-MS. Similarly to the molecule mentioned earlier, this mass was found in both minimally and thermally processed samples; however, the abundance was about two-thirds higher in the thermally processed sample.

A possible explanation for the color change in parsnip may be due to the fact that falcarinol type polyacetylenes in their isolated forms are yellow colored oily liquids. Thermal processing may result in the production of yellow colored falcarinol type molecules resulting in an increase in yellowness in thermally processed parsnips.

In conclusion blanching prior to sous vide processing had the greatest influence on the retention of polyacetylenes in parsnip disks. Subsequent SV processing did not result in additional significant losses in polyacetylenes compared to blanched samples. Liquid chromatography and accurate mass mass spectrometry revealed that degradation of falcarinol type polyacetylene following thermal processing may be a result of oxidation and dehydrogenation, forming oxidized form of falcarinol type polyacetylene, i.e., falcarindione, dehydrofalcarinol, dehydrofalcarinone. Anaerobic storage of SV processed samples resulted in a decrease in falcarinol (1) levels ($p < 0.05$) but no change in falcarindiol (2) levels ($p > 0.05$). On average, WI processing resulted in a nonsignificant but lower loss of 2 as compared to SV. 1 levels for WI samples were significantly higher than for SV samples. The higher losses of polyacetylenes in SV compared to WI processed samples were probably due to the combination of the inclusion of a blanching step in SV processing and the higher end point temperature. However, given the longer shelf life afforded by SV processing in comparison to WI processing (5 days vs 20 days), the SV technology may be the better option for retaining polyacetylenes. 2 was particularly susceptible to aerobic storage following WI processing with losses of up to 70% occurring after 5 days. Thermal processing had a significant effect on total color difference (ΔE) of parsnip samples in both SV and WI processed disks. However, for SV processed samples the magnitude of change in ΔE was much greater during the first 5 days of chill storage than it was following either of the thermal processing steps. In general both SV and WI processing performed equally with regard to color retention with parsnip disks becoming darker, yellower and browner following processing and storage.

LITERATURE CITED

- (1) Patras, A.; Tiwari, B. K.; Brunton, N. P.; Butler, F. Modelling the effect of different sterilisation treatments on antioxidant activity and colour of carrot slices during storage. *Food Chem.* **2009**, *114*, 484–491.
- (2) Gundgaard, J.; Nielsen, J. N.; Olsen, J.; Sørensen, J. Increased intake of fruit and vegetables: estimation of impact in terms of life expectancy and healthcare costs. *Public Health Nutr.* **2003**, *6*, 25–30.
- (3) Maynard, M.; Gunnell, D.; Emmett, P.; Frankel, S.; Smith, G. D. Fruit, vegetables, and antioxidants in childhood and risk of adult cancer: the Boyd Orr cohort. *J. Epidemiol. Community Health* **2003**, *57*, 218–225.
- (4) Trichopoulou, A.; Naska, A.; Antoniou, A.; Friel, S.; Trygg, K.; Turrini, A. Vegetable and fruit: the evidence in their favour and the public health perspective. *Int. J. Vitam. Nutr. Res.* **2003**, *73*, 63–69.
- (5) Patras, A.; Brunton, N. P.; O'Donnell, C.; Tiwari, B. K. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci. Technol.* **2010**, *21*, 3–11.
- (6) Tibble, D. L. Further evidence of the cardiovascular benefits of diets enriched in carotenoids. *Am. J. Clin. Nutr.* **1998**, *68*, 521–522.
- (7) Williamson, G. Protective effects of fruits and vegetables in the diet. *Nutr. Food Sci.* **1999**, *1*, 6–10.
- (8) Gardner, P. T.; White, T. A. C.; McPhail, D. B.; Duthie, G. G. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.* **2000**, *68*, 471–474.
- (9) Sánchez-Moreno, C.; Plaza, L.; De Ancos, B.; Cano, P. Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juice. *J. Sci. Food Agric.* **2003**, *83*, 430–439.
- (10) *Britannica Concise Encyclopedia*; Encyclopedia Britannica Inc.: 2006.
- (11) Hansen, S.; Purup, S.; Christensen, L. Bioactivity of falcarinol and the influence of processing and storage on its content in carrots (*Daucus carota* L.). *J. Sci. Food Agric.* **2003**, *83*, 1010–1017.
- (12) Kobaek-Larsen, M.; Christensen, L. P.; Vach, W.; Ritskes-Hoitinga, J.; Brandt, K. Inhibitory effects of feeding with carrots or (–)-falcarinol on development of azoxymethane-induced preneoplastic lesions in the rat colon. *J. Agric. Food Chem.* **2005**, *53*, 1823–1827.
- (13) Metzger, B. T.; Barnes, D. M.; Reed, J. D. Purple carrot (*Daucus carota* L.) polyacetylenes decrease lipopolysaccharide-induced expression of inflammatory proteins in macrophage and endothelial cells. *J. Agric. Food Chem.* **2008**, *56*, 3554–3560.
- (14) Olsson, K.; Svensson, R. The influence of polyacetylenes on the susceptibility of carrots to storage diseases. *J. Phytopathol.* **1996**, *144*, 441–447.
- (15) Kreutzmann, S.; Christensen, L. P.; Edelenbos, M. Investigation of bitterness in carrots (*Daucus carota* L.) based on quantitative chemical and sensory analyses. *LWT Food Sci. Technol.* **2008**, *41*, 193–205.
- (16) Murdoch, S. R.; Dempster, J. Allergic contact dermatitis from carrot. *Contact Dermatitis* **2000**, *42*, 236–236.
- (17) Wittstock, U.; Hadacek, F.; Wurz, G.; Teuscher, E.; Greger, H. Polyacetylenes from water hemlock, *Cicuta virosa*. *Planta Med.* **1995**, *61*, 439–445.
- (18) Bernart, M. W.; Cardellina, J. H.; Balaschak, M. S.; Alexander, M. R.; Shoemaker, R. H.; Boyd, M. R. Cytotoxic falcarinol oxylipins from *Dendropanax arboreus*. *J. Nat. Prod.* **1996**, *59*, 748–753.
- (19) Miyazawa, M.; Shimamura, H.; Bhuvra, R. C.; Nakamura, S.; Kameoka, H. Antimutagenic Activity of Falcarindiol from *Peucedanum praeruptorum*. *J. Agric. Food Chem.* **1996**, *44*, 3444–3448.
- (20) Young, J. F.; Duthie, S. J.; Milne, L.; Christensen, L. P.; Duthie, G. G.; Bestwick, C. S. Biphasic effect of falcarinol on CaCo-2 cell proliferation, DNA damage, and apoptosis. *J. Agric. Food Chem.* **2007**, *55*, 618–623.
- (21) Zidorn, C.; Joehrer, K.; Ganzera, M.; Schubert, B.; Sigmund, E.; Mader, J.; Greil, R.; Ellmerer, E.; Stuppner, H. Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. *J. Agric. Food Chem.* **2005**, *53*, 2518–2523.
- (22) Matsunaga, H.; Katano, M.; Yamamoto, H.; Fujito, H.; Mori, M.; Takata, K. Cytotoxic activity of polyacetylenes compounds in *Panax-Ginseng*. *Chem. Pharm. Bull.* **1990**, *38*, 3480–3482.
- (23) Church, I. J.; Parsons, A. L. Review: sous vide cook – chill technology. *Int. J. Food Sci. Technol.* **1993**, *28*, 563–574.
- (24) Schellekens, M.; Martens, T. Sous-Vide State of the Art. Commission of the European Communities Directorate General XII, *Research and Development. Publication* 1992, N1 EUR 15018 EN, Brussels.
- (25) Creed, P. M. The sensory and nutritional quality of 'sous vide' foods. *Food Control* **1995**, *6*, 45–52.
- (26) Gaze, J. E.; Brown, G. D. Determination of the heat resistance of a strain of non-proteolytic *Clostridium botulinum* type B and a strain of type E, heated in cod and carrot over the temperature range 70 to 90 °C; Technical Memorandum no. 592; Chipping Campden: Campden & Chorleywood Food Research Association (CCFRA); U.K., 1990.
- (27) Gould, G. W. Sous vide foods: conclusions of an ECFF botulinum working party. *Food Control* **1990**, *10*, 47–51.
- (28) Rawson, A.; Koidis, A.; Patras, A.; Tuohy, M. G.; Brunton, N. P. Modelling the effect of water immersion thermal processing on polyacetylene levels and instrumental color of carrot disks. *Food Chem.* **2010**, *121*, 62–68.
- (29) Czepa, A.; Hofmann, T. Quantitative studies and sensory analysis on the influence of cultivar, spatial tissue distribution and industrial processing on the bitter off – taste of carrots (*Daucus carota* L.) and carrot products. *J. Agric. Food Chem.* **2004**, *52*, 4508–4514.
- (30) Baranska, M.; Schulz, H. Spatial tissue distribution of polyacetylenes in carrot root. *Analyst* **2005**, *130*, 855–859.
- (31) Kidmose, U.; Hansen, S. L.; Christensen, L. P.; Edelenbos, M.; Larsen, E.; Norbek, R. Effect of genotype, root size, storage, and processing on bioactive compounds in organically grown Carrots (*Daucus carota* L.). *J. Food Sci.* **2004**, *69*, S388–S394.
- (32) Patras, A.; Brunton, N.; Butler, F. Effect of water immersion and SV processing on antioxidant activity, phenolic carotenoid content and color of carrot disks. *J. Food Process. Preserv.* **2009**, *10*, 420–423.
- (33) Stea, T. H.; Johansson, M.; Frølich, W. Retention of folates in cooked, stored and reheated peas, broccoli and potatoes for use in modern large-scale service systems. *Food Chem.* **2006**, *101*, 1095–1107.
- (34) Werlein, H. D. Comparison of the quality of sous-vide and conventionally processed carrots. *Z. Lebensm.-Unters. -Forsch.* **1998**, *207*, 311–315.
- (35) Araya, X. I. T.; Smale, N.; Zabarar, D.; Winley, E.; Forde, C.; Stewart, C. M.; Mawson, A. J. Sensory perception and quality attributes of high pressure processed carrots in comparison to raw, sous-vide and cooked carrots. *Innovative Food Sci. Emerging Technol.* **2009**, *10*, 420–433.
- (36) Bohlmann, F.; Burkhardt, F.; Zdero, C. *Naturally Occurring Acetylenes*; Academic Press: London, 1973.
- (37) Hansen, L.; Boll, P. M. Polyacetylenes in araliaceae: Their chemistry, biosynthesis and biological significance. *Phytochemistry* **1986**, *25*, 285–293.
- (38) Christensen, L. P.; Brandt, K. Bioactive polyacetylenes in food plants of the Apiaceae family: Occurrence, bioactivity and analysis. *J. Pharm. Biomed. Anal.* **2006**, *41*, 683–693.

Received for review February 7, 2010. Revised manuscript received May 17, 2010. Accepted May 23, 2010. This project is funded under the Food Institutional Research Measure (FIRM, ref. 363 06/RTD/AFRC/518) by the Irish Department of Agriculture, Food and Fisheries (DAFF).