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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- Literature searches found the major sources of polyacetylenes were in whole vegetables of carrot, celery and parsnip.
- Carrots cooked whole retain more polyacetylenes than those cut into disks or quarters and boiling time has little effect on retention.

Background

Nutritional factors have been shown to affect the risk of cancer. It is well known that the a higher intake of fruit and vegetables leads to a lower cancer risk and there is increasing evidence that certain fruit and vegetable groups have a protective effect against particular cancers. It is thought that the fibre, antioxidant, vitamin and mineral content of fruit and vegetables are the main factors that contribute to the anti-cancer effect, but current evidence has shown that these common constituents alone cannot explain the effect. Observational studies have found carrot consumption can lead to a lower incidence of cancer (Boggs *et al.*, 2010; Larsson *et al.*, 2010) and there is evidence from studies with isolated cells that suggests the polyacetylene (PA) class of compounds, first investigated in herbal medicines such as ginseng, but also found in root vegetables including carrots, have anti-cancer (Zidorn *et al.*, 2005) and anti-inflammatory properties (Alanko *et al.*, 1994). Animal studies have shown reduced levels of intestinal cancer when diets are supplemented with carrot (Kobaek-Larsen *et al.*, 2005; Saleh *et al.*, 2013). However, so far, there have been no studies into the health effects of PA intake in humans. The objective of the present study is to determine if consuming a portion of carrots can affect the biomarkers of cancer and inflammation in humans by examining:

1. The PA content of commonly eaten foods in a population from the North East of England and if there is any association between eating polyacetylene-rich foods and cancer incidence in people over 85 years old.
2. The effect of cooking techniques on PA concentration in carrots
3. The bioavailability (how much is digested and absorbed) of PA from cooked carrots and how much PA can be detected in the blood, urine and faeces after consumption by humans
4. The effect of a diet supplemented with carrots on biomarkers of cancer risk in humans (dietary intervention study)

Summary

Database of polyacetylene-containing foods

The database of foods has been compiled which contains the PA content of foods commonly eaten in a population in North East England. This database will allow the public to make choices about how they consume vegetables (fresh, cooked) and in what form (fresh, frozen, as part of a mixed ready meal, as part of a mixed home-made dish) to give them the greatest intake of PA. The database will be made available to other researchers to allow them to estimate the intake of PA in their study populations and to assess associations between consumption and disease state. A total of 17 different foods, with 124 independent samples were analysed (replicates from different supermarkets) and combined with values from previously published literature to complete the database. From the database, the highest concentrations of total PA were found in celery but the individual compounds of falcarinol, falcarindiol and falcarindiol-3-acetate were found in boiled parsnip (4.5mg/100g), raw celery (12.6mg/100g) and boiled carrot (1.6mg/100g) respectively. The lowest amounts were found in mixed meals as they are made of multiple ingredients. However, once portion size was taken into consideration, mixed meals achieved similar concentrations to some of the whole vegetables. Considering also how often the foods were eaten, by far the most important source of PA in this group was boiled carrot (0.631mg/day). Estimated daily intake of total PA in the Newcastle 85+ population was 1.03mg/day.

The data for cancer incidence from the cohort has been compiled and analysed to see if there are any associations between polyacetylene-rich food consumption and risk of cancer. Current analysis shows no association between polyacetylene-rich vegetable consumption and risk of cancer. The small sample size in this cohort may be the reason for the lack of association. Studies of this kind are usually performed on cohorts of tens or even hundreds of thousands of people, compared to the <800 people in this cohort, the likelihood of seeing an association was very small.

These results were shown at the Nutrition Society Summer conference, July 2016.

Preparation and cooking of carrots

Carrots have been prepared as either disks, quarters or whole then boiled and fried for different amounts of time.

At present we are unable to report the full results of the study as the PhD student aims to publish these first but the overall results are summarised below:

- boiling carrots retains more PA than frying them
- PA are more stable in oil than in water
- carrots cooked whole retain more PA than those cut into disks or quarters

The recommendation would be to cook the carrots whole rather than in disks or quarters for the best retention of PA. These results were shown at the HDC student conference 2015, Onion and Carrot Conference in November 2015, and the Nutrition Society Spring Conference, March 2016.

In vitro digestion, used to make an estimation of how much polyacetylene was available during digestion, has proved difficult to get useful results due to large variabilities in polyacetylene content in the digested matter. This experiment may be revisited in the 3rd year of the PhD if time permits.

Bioavailability of PA from carrots

The bioavailability trial recruited 6 healthy adults, aged between 18 and 30 years old. They provided urine and stool samples and a fasting blood sample before consuming a breakfast of carrots and bread and butter. They had blood samples taken up to 24 hours and provided further urine and stool samples up to 48 hours. The volunteers took part on 2 separate days, consuming different ‘doses’ of carrot on each day (100 or 250g). The last participant in the trial finished at the beginning of September 2016 and samples are being stored prior to analysis. A method is currently being developed to detect PA in biological samples. The analysis will allow us to determine where PA goes in the body: whether it remains in the gut or whether it is digested and absorbed by the body and detectible in the blood and urine. We can use this information to see if the concentrations that have been used in cell experiments to reduce inflammation and treat cancer cells, can also be seen in the body and therefore whether eating a normal amount of carrot could feasibly be affecting these biomarkers in humans.

Effect of supplementing the diet with carrots

This study recruited 39 healthy adults, aged over 45 years old. Each participant was randomised to eat either 100g white carrots plus 10g butter, or 3 oat cakes per day for 6 weeks. This was then followed by a 6-week ‘wash-out’ period where no carrots or oats were eaten (all other carrots, oat products and PA containing foods were also forbidden during the full 12-week period). Participants provided urine and stool samples at baseline, 6 and 12 weeks, and fasted blood samples were also taken at these time points. These samples will be analysed for inflammatory markers in blood and urine and cell damage in lymphocytes (white blood cells which can be used to assess the overall damage that our bodies experience on a day to day basis). The final participant finished the trial at the beginning of September and analysis of the biofluids is ongoing and predicted to be finished mid-October 2016.

Financial Benefits

The promotion of the health benefits of carrots, parsnips and other Apiaceae vegetable consumption could lead to a significant and sustained sales increase. Peer-reviewed scientific publications are required by EFSA (European Food Standards Agency) to substantiate the health claims of a food. This study aims to generate such publications.

Action Points

None

SCIENCE SECTION

Database of polyacetylene-containing foods

(from a draft manuscript for publication)

Introduction

While there is extensive knowledge about the content of other health beneficial compounds in the diet, little is known about the amount of polyacetylenes in specific foods and the diet as a whole. Assessing the dietary intake of polyacetylenes is important for determining how large the effect these compounds have on risk of disease. Vegetables have previously been analysed for polyacetylene content but often these foods were not prepared as they would be in the home and therefore do not truly reflect intake, and mixed meals, containing many ingredients, have not been studied. To estimate population intake, we need to know how much is in foods and how often these foods are eaten. The aim of this study is to compile a list of foods commonly eaten in a population in North East England and list their polyacetylene content. This will allow the public to make choices about how they consume vegetables (fresh, cooked) and in what form (fresh, frozen, as part of a mixed ready meal, as part of a mixed home-made dish) to give them the greatest intake of polyacetylenes.

Materials and methods

Data from the Newcastle 85+ study (Collerton *et al.*, 2007) was used to compile a list of the most commonly eaten polyacetylene-rich vegetables and mixed dishes that contain them. Foods were prepared according to their description in the food database. Foods were purchased from 3 different supermarkets, where possible. Cooked vegetables and retail meals were prepared according to the recommendations on packet instructions. Homemade meals were either prepared according to McCance and Widdowson's 'The Composition of Foods' (McCance and Widdowson, 2002) or, where not available, using recipes found on UK based websites (e.g. BBC Good Food).

Once prepared, the foods were immediately frozen. They were then freeze dried, extracted into ethyl acetate and analysed by high performance liquid chromatography (HPLC).

A systematic literature search was also performed to gather data on polyacetylene content of vegetables that had previously been analysed. A total of 25 papers were used to compile a list of polyacetylene concentrations and these data were combined with the newly analysed foods to compile a comprehensive database.

Results and Discussion

A total of 17 different foods with replicates gave a total of 12 independent samples. The results of the experiment are displayed in Table 1. Parsnip contained the highest total polyacetylene but for the individual polyacetylenes, raw carrot and boiled carrot had the highest concentrations of falcarinol and falcarindiol-3-acetate, respectively, and falcarindiol levels were highest in boiled celery and boiled parsnip. The lowest amounts were found in the mixed meals due to being made of multiple ingredients. Table 2 shows the full results of the literature search. Raw carrots were the most commonly analysed vegetable (n=136) but boiled carrot, parsnip, celery and fennel had also been analysed.

Table 1. Concentrations of polyacetylene measured in this experiment

		Falcarinol	Falcarindiol	Falcarindiol-3-acetate	Total Polyacetylene
Food		**Concentration (mg/Kg) fresh weight**			
Carrots, old, boiled in unsalted water	**Mean**	37.0	32.8	22.4	92.1
	SD	14.4	12.2	18.1	34.0
	SEM	3.4	2.9	4.3	8.0
	Range	14.30-51.59	18.54-64.96	4.17-64.55	44.17-148.16
Carrots, old, boiled in salted water	**Mean**	31.1	27.7	8.8	67.5
	SD	16.8	9.3	4.3	27.1
	SEM	9.7	5.4	2.5	15.6
	Range	13.67-53.82	15.02-37.07	5.55-14.91	50.11-105.79
Mixed vegetables, frozen, boiled in salted water	**Mean**	12.2	17.9	5.6	35.7
	SD	9.1	3.0	0.8	5.2
	SEM	3.0	1.4	0.3	2.6
	Range	4.84-15.39	8.42-11.00	1.74-4.09	15.90-29.84
Parsnip, boiled in unsalted water	**Mean**	9.5	23.7	0.9	34.1
	SD	4.2	7.6	0.5	12.0
	SEM	1.7	3.1	0.2	4.9
	Range	0.98-16.88	19.72-38.46	0.55-1.90	23.24-57.24
Beef stew	**Mean**	4.4	3.2	0.9	8.5
	SD	2.2	1.3	0.6	2.9
	SEM	0.8	0.5	0.2	1.1
	Range	5.85-13.26	7.09-18.54	0.92-2.03	2.63-10.13
Cottage/Shepherd's pie, chilled/frozen, reheated	**Mean**	1.9	0.1	0.0	2.0
	SD	0.6	0.0	0.0	0.7
	SEM	0.4	0.0	0.0	0.4
	Range	5.85-13.26	7.09-18.54	0.92-2.03	1.22-13.02
Carrots, fresh, steamed/micro	**Mean**	17.7	16.8	5.2	39.6
	SD	6.1	7.3	2.0	12.0
	SEM	2.2	2.6	0.7	4.3
	Range	2.48-23.58	6.51-21.52	0.92-5.51	18.53-42.79
Carrots, frozen, boiled in unsalted water	**Mean**	35.7	56.9	17.2	109.8
	SD	22.7	41.5	10.7	72.6
	SEM	8.0	14.7	3.8	25.7
	Range	2.48-63.19	7.55-131.82	4.58-36.21	15.20-231.22
Lentil soup	**Mean**	5.6	2.1	1.3	3.4
	SD	5.5	1.1	1.2	2.1
	SEM	1.9	0.4	0.4	0.8
	Range	0.78-2.14	0.61-1.63	0.07-0.60	1.46-4.37
Carrots, old, raw	**Mean**	64.9	34.2	20.1	119.3
	SD	21.7	7.4	7.3	35.2
	SEM	8.9	3.0	3.0	14.4

	Range	13.03-47.43	17.53-64.22	22.16-58.06	52.71--169.71
Celery, boiled in salted water	**Mean**	21.7	81.1	0.4	103.2
	SD	10.7	36.6	0.6	43.4
	SEM	2.7	9.1	0.2	10.9
	Range	7.49-52.99	21.34-148.47	0.00-2.34	31.06-201.58
Carrots, canned, re-heated, drained	**Mean**	39.2	54.8	16.3	110.3
	SD	12.7	10.8	6.2	28.8
	SEM	4.5	3.8	2.2	10.2
	Range	1.96-62.39	13.68-72.41	3.92-27.39	19.56-162.19
Veg soup, instant/Powdered soup	**Mean**	8.7	3.1	0.9	4.0
	SD	3.2	2.6	0.4	2.6
	SEM	1.6	1.3	0.2	1.3
	Range	1.96-62.39	13.68-72.41	3.92-27.39	19.56-162.19

Table 2. Summary of full results of literature search

Food Description	n		Falcarinol	Falcarindiol	Falcarindiol-3-acetate	Total Polyacetylene
			Concentration (mg/Kg) fresh weight			
Carrots, old, boiled in unsalted water	**2**	**Mean**	11.5			11.5
		SD	2.0			2.0
		SEM	1.4			1.4
		Range	9.5-13.50			9.5-13.50
Celery, raw	**2**	**Mean**	46.3	166.3		212.5
		SD	34.8	62.8		97.5
		SEM	24.6	36.2		68.9
		Range	11.5-81.0	103.5-229.0		115.0-310.0
Parsnip, boiled in unsalted water	**2**	**Mean**	59.9	37.9		97.8
		SD	6.2	0.4		6.6
		SEM	4.4	0.2		4.6
		Range	53.70-66.10	37.50-38.20		31.06-57.24
Carrots, fresh, steamed/micro	**3**	**Mean**	12.0	25.0	8.7	23.2
		SD	1.8			16.7
		SEM	1.0			9.6
		Range	9.50-13.50	25.0	8.7	9.50-46.70
Carrots, frozen	**16**	**Mean**	40.6	18.2	13.4	68.3
		SD	15.6	12.3	4.7	27.4
		SEM	3.9	3.1	1.2	6.8
		Range	9.5-67.00	4.8-41.7	5.10-21.60	9.5-119.0
Carrots, old, raw	**136**	**Mean**	27.1	47.3	22.8	92.0
		SD	22.1	53.0	23.5	80.5
		SEM	1.9	4.5	2.0	6.8
		Range	0.6-155.3	0.00-347.5	0.70-109.00	12.8-484.60
		Median	23.3	36.4	12.3	71.6
Parsnip raw	**4**	**Mean**	119.4	351.1		470.5
		SD	113.6	453.2		563.3
		SEM	56.8	226.6		281.7
		Range	25.0-313.6	49.0-1130.9		114.2-1444.5
Fennel, fresh boiled and roasted	**4**	**Mean**	21.9	37.8	15.0	68.7
		SD	13.4	20.3	7.4	32.3
		SEM	6.7	10.1	3.7	16.1
		Range	7.0-40.0	11.6-61.0	4.3-15.0	23.1-92.9

Dry weights have been converted to fresh weights using the % dry weight from the cooking experiment – carrot 10%, parsnip 19.6% and celery 5%

The results of the literature search and experimental values from the literature were combined to create a full database, shown in Table 3. The combined data show the major sources of polyacetylenes were in the whole vegetables – carrot, celery and parsnip.

Table 3. Database of polyacetylene values

	Concentration (mg/Kg)			
	Falcarinol	**Falcarindiol**	**Falcarindiol-3-acetate**	**Total Polyacetylene**
Carrots, old, boiled (in salted or unsalted water)	23.10	30.41	16.12	46.37
Celery, raw	35.58	126.30	0.63	162.09
Parsnip, boiled in unsalted water	44.68	52.10	0.76	96.77
Carrots, fresh, steamed/micro	13.41	20.90	6.93	43.15
Carrots, frozen	31.10	25.93	13.21	61.55
Carrots, old, raw	23.97	35.42	14.85	69.39
Carrots, canned, reheated drained	39.18	54.82	16.31	110.31
Celery boiled	21.75	81.06	0.36	103.16
Mixed vegetables (in salted or unsalted water)	12.18	17.91	5.59	35.68
Lentil soup	5.57	2.12	1.26	8.94
Vegetable soup	12.30	8.66	2.20	23.16
Vegetable soup, canned	7.35	2.22	1.30	10.87
Instant soup powder	8.71	3.13	0.91	12.75
Vegetable soup, dried as served	8.71	3.13	0.91	12.75
Coleslaw (full or reduced fat)	2.52	1.41	0.27	4.20
Beef stew (made with regular or lean beef)	4.62	3.58	1.02	9.22
Cottage/shepherd's pie, chilled/frozen	1.86	0.14	0.05	2.05

The database can be used to assess the importance of each food as a source of polyacetylenes but it is important to know how much of each food is eaten as it is more likely that a person will eat 300g of soup than 300g of carrot. The intake from Newcastle 85+ study was used to estimate intake in a population. Figure 1 shows the estimated polyacetylene concentration in an average portion consumed. For ease of analysis and to make a more generalised view of the data, foods were grouped into catagories: raw and cooked vegetables (cooked carrots include boiled, microwaved, canned and frozen) soups (including dried soups mixes, canned and homemade soups) and mixed meals (including beef stew, cottage/shepherd's pie and coleslaw). These estimates show the highest concentration per portion are in the whole vegetables and especially those that have been most highly processed. Canned carrots have the highest per portion amount, followed by frozen carrots and boiled vegetables. Whilst the vegetables are higher in PA than the mixed meals, taking the portion size into consideration, the amount per portion of soups and mixed vegetables now becomes similar to some of the individual vegetables.

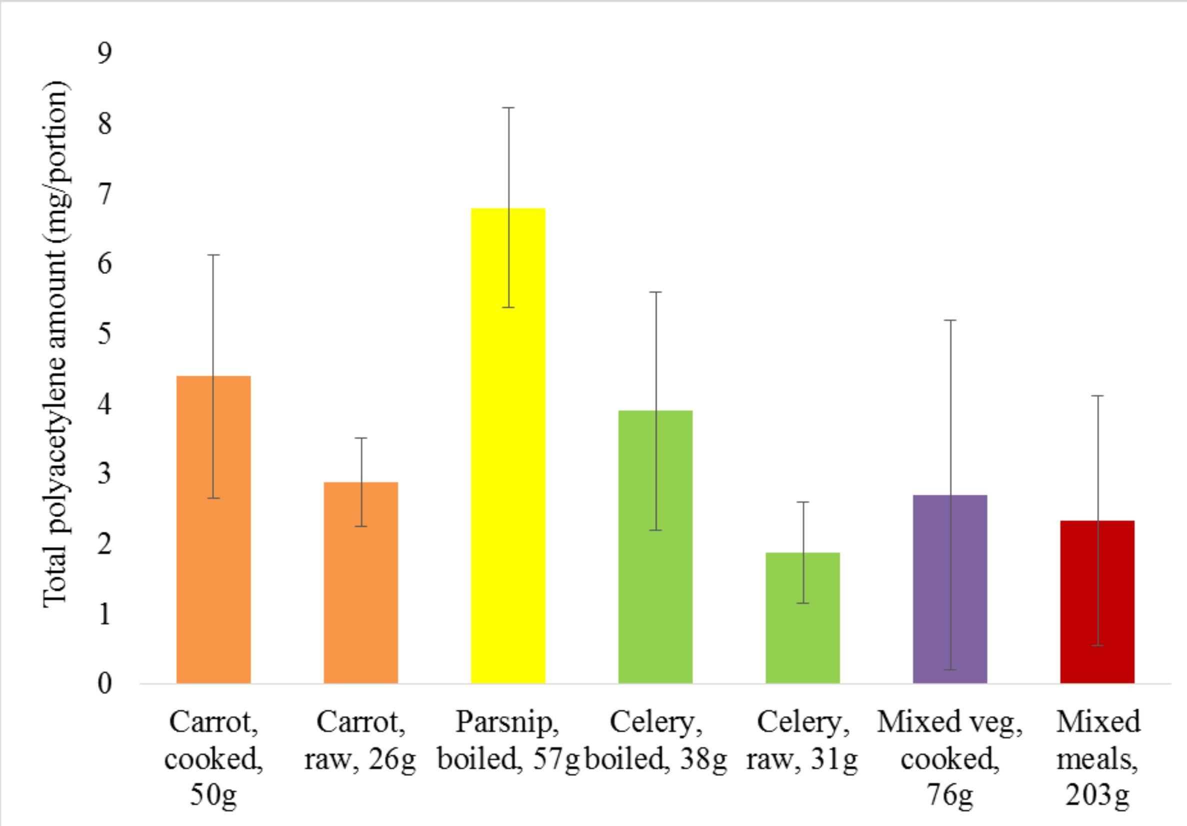


Figure 1. Amount of total polyacetylene per portion

Frequency of consumption is also important as eating a carrot every day would be more common than eating beef stew every day. Figure 2 illustrates the estimated intake per day. We can see that cooked carrots are by far the biggest contributor of polyacetylene to the diet in this population (0.63mg/day). This is followed by soups which were half the amount (0.30mg/day), then raw celery, boiled parsnip and mixed vegetables which were all 0.08-0.11mg/day and the other groups were <0.05mg/day. Using the database to estimate intake in each participant, the mean intake of total PA in this population was 1.57mg/day.

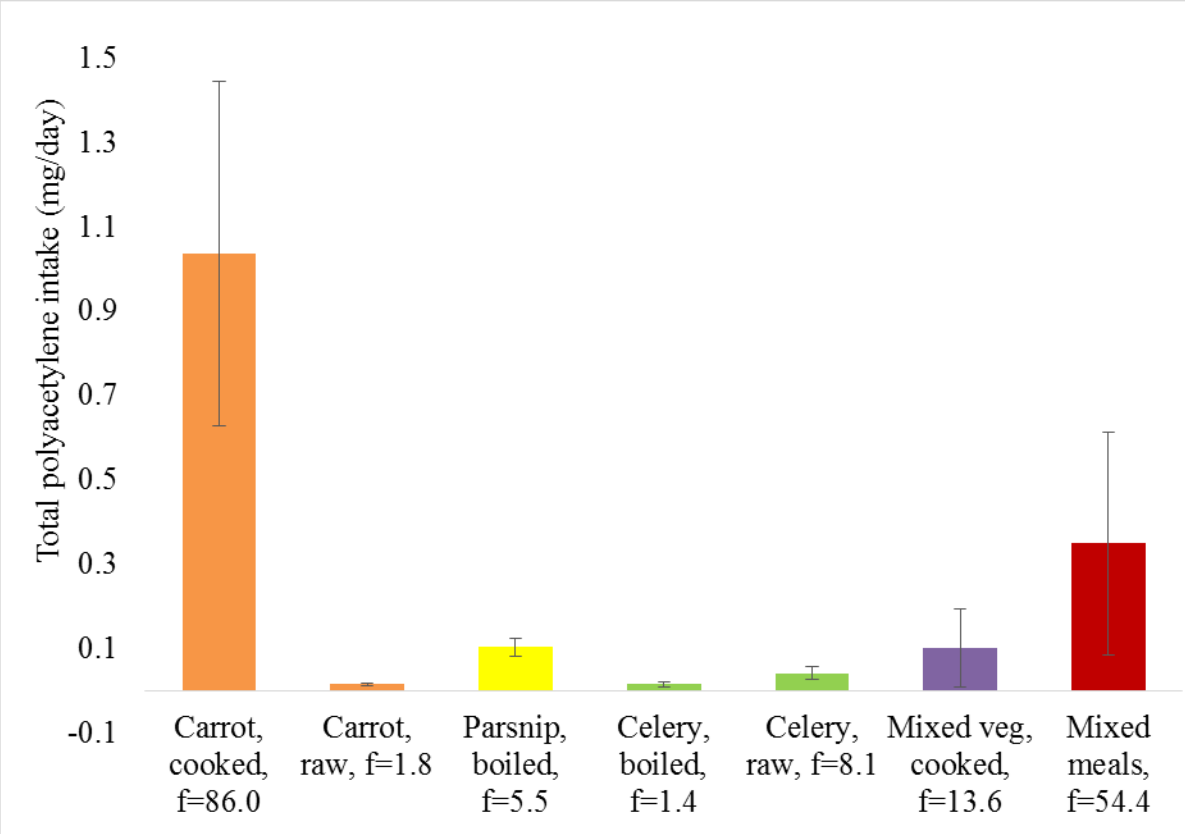


Figure 2. Estimated intake of polyacetylene per day

The data was also used in combination with cancer incidence information from the 85+ study cohort to determine if there is any association between polyacetylene-rich food consumption and risk of cancer.

Conclusions

- This study highlights the importance of the Apiacea vegetables, especially cooked carrots, in the diet as a source of polyacetylenes.
- The impact of these compounds on the inflammation and cytotoxic effects in humans needs further clarification but this study shows these vegetables contribute to the intake of these compounds.
- Further investigation into the retention of these compounds during cooking is important to ensure these beneficial phytochemicals are preserved.
- The database produced can be used for future research into polyacetylene intake in individuals and study populations.

Preparation and cooking of carrots

(from a draft manuscript for publication)

Introduction

The effect of cooking on the composition of the phytochemicals in vegetables is a complicated process. Heat degradation and leaching of phytochemicals into cooking fluid may decrease concentrations, and increases may occur due to the breakdown of the cell matrix and the release of phytochemicals from protein complexes (Aman *et al.*, 2005) leading to an increase in bioavailability. There is also a possibility of apparent increases in concentration due to leaching of soluble solids and changes in water content of vegetables after cooking. The aim of the current experiment was to explore the effect of cooking on phytochemical concentration when carrots were cooked for different amounts of time, cut into different shapes, and either boiled or fried.

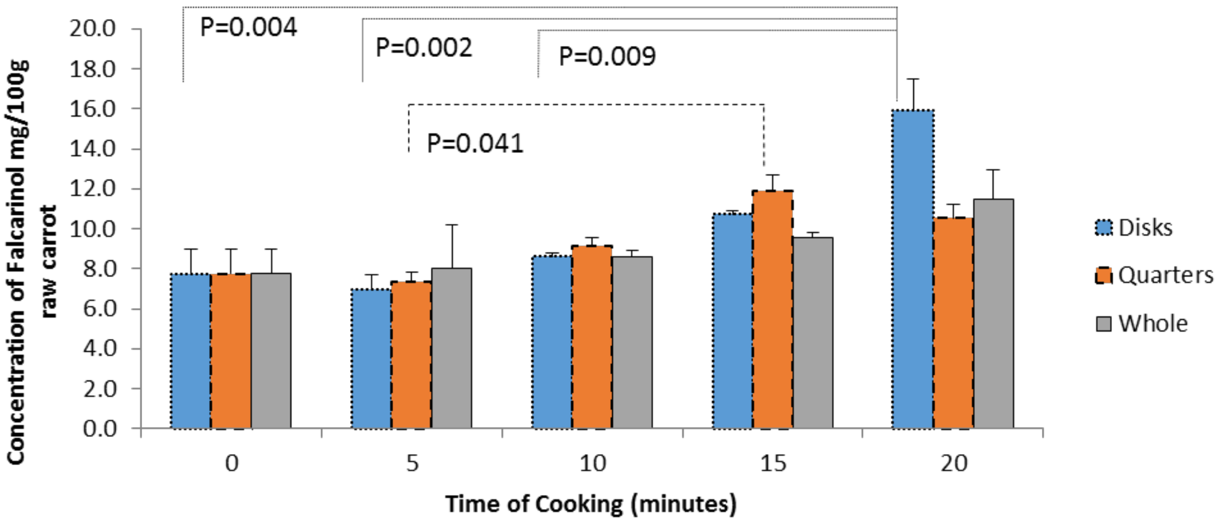
Materials and methods

British carrots were purchased in the local supermarket (Asda, UK). They were peeled, topped and tailed then cut into three different shapes/sizes: whole (W), quarters (Q) or disks (D). The (W) were left whole, (Q) were cut lengthways into 4 long quarters and (D) were cut into approximately 5mm rounds. Each type of carrot was both boiled (5, 10, 15 and 20 minutes) and fried (5, 8 and 10 minutes). Boiling was done inside a boil-in-the-bag with a ratio of 1:3 carrot to water and 160g carrot was fried in 30g oil. The water and oil were retained after cooking. Carrots were weighed before and after cooking and freeze dried for extraction of polyacetylenes. Water was also freeze dried and oil was measured directly for polyacetylenes. Analysis was done by HPLC.

Results and Discussion

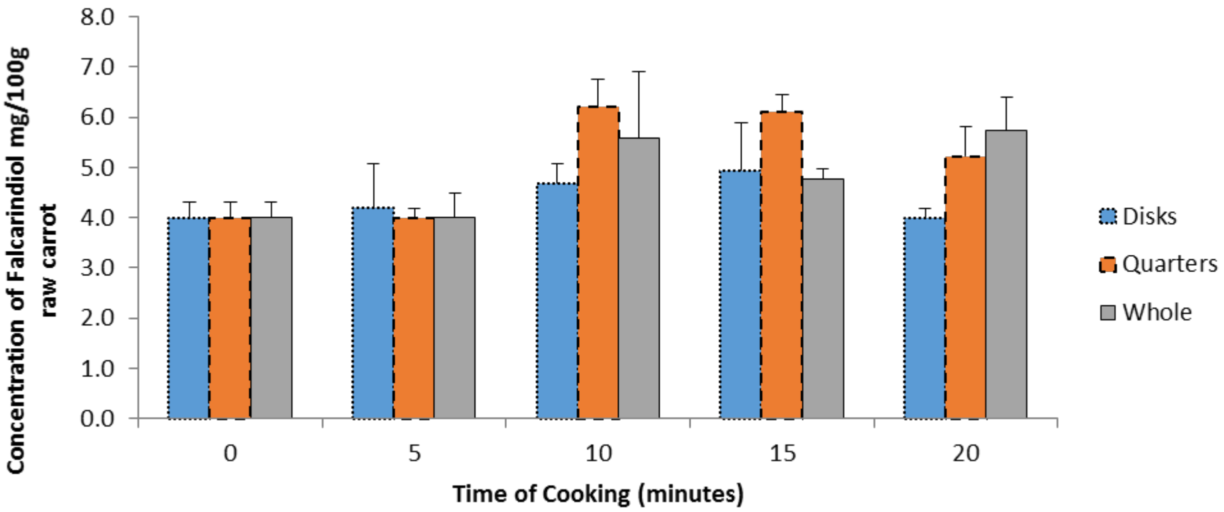
PA in boiled carrots were not significantly affected by the shape of the carrot during cooking – only FaD3Ac was higher in the D than the Q and W after 20 minutes of cooking – but there was a trend for increases in concentration over time, especially in the D and Q (as seen in figure 1). There were no significant changes in PA over time in W carrots such that they were no different to raw carrot at any time point.

A



P=0.004
P=0.002
P=0.009
P=0.041
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Concentration of Falcarinol mg/100g raw carrot
0
5
10
15
20
Time of Cooking (minutes)
Disks
Quarters
Whole

B



8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
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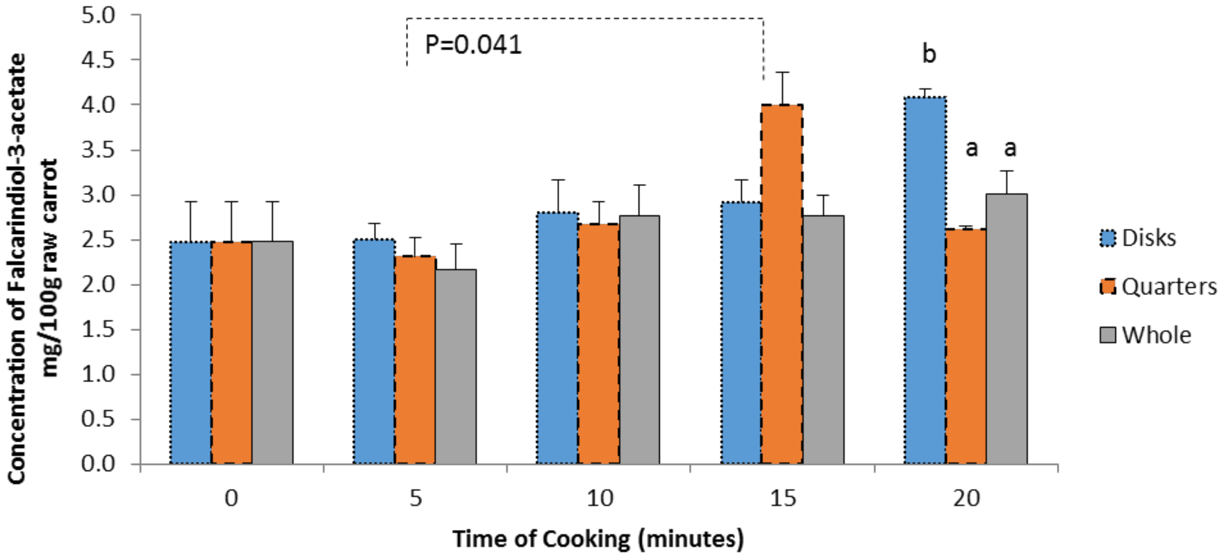
Concentration of Falcarindiol mg/100g raw carrot
0
5
10
15
20
Time of Cooking (minutes)
Disks
Quarters
Whole

C



D

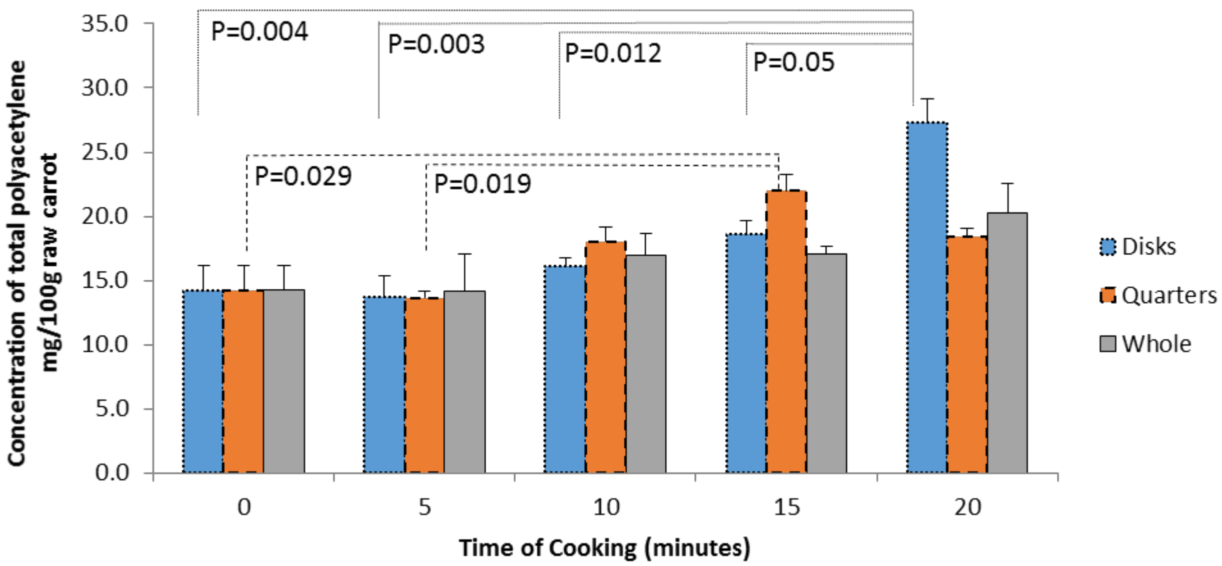
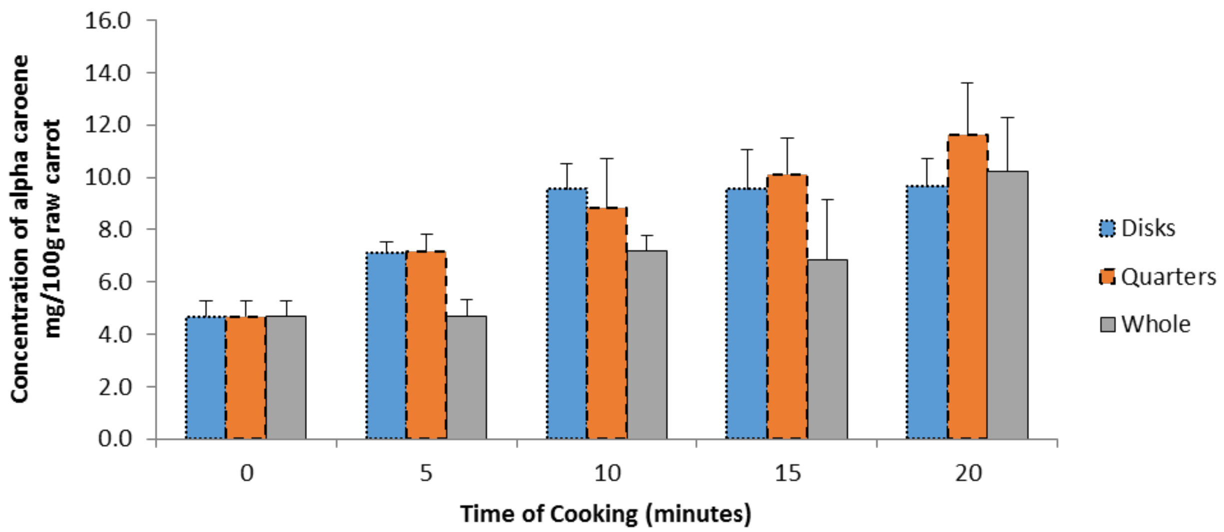


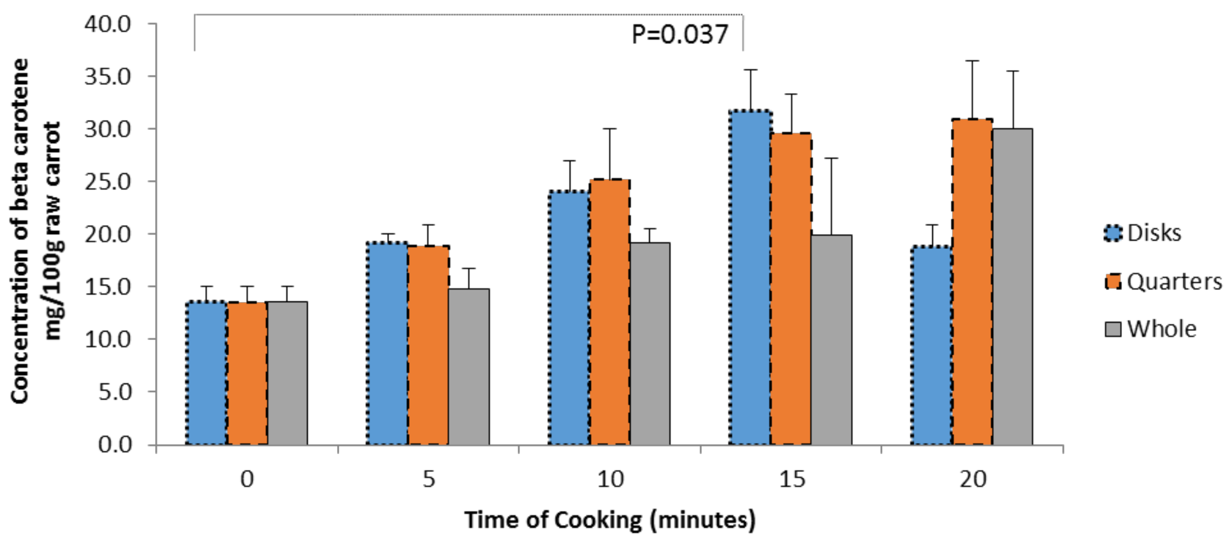
Figure 1. Concentrations of falcarinol (A), falcarindiol (B), falcarindiol-3-acetate (C) and total polyacetyelene (D) in carrot after boiling. Letters over columns signify differences between shapes. P values signify differences between time points.

For carotenoids, as with PA, the general trend was for the larger surface areas to have higher concentrations of carotenoids but this was only significant at 20 minutes at which time D had higher concentrations of lutein than W (figure 2). The differences in concentration due to cooking shape became clearer when looking at changes over cooking time. Q carrots increased in lutein and total carotenoid and D increased in concentrations of β carotene, lutein and total carotenoid over cooking time. W carrots had no significant differences in concentrations over cooking time in any of the carotenoids and, like the PAs, carotenoid concentrations in W were not significantly different to raw. The carotenoid concentration most affected by cooking was lutein. This could be due to the structure or distribution of lutein in the carrot and how the softening affects the release and subsequent extraction of the compound which could differ from α and β-carotene.

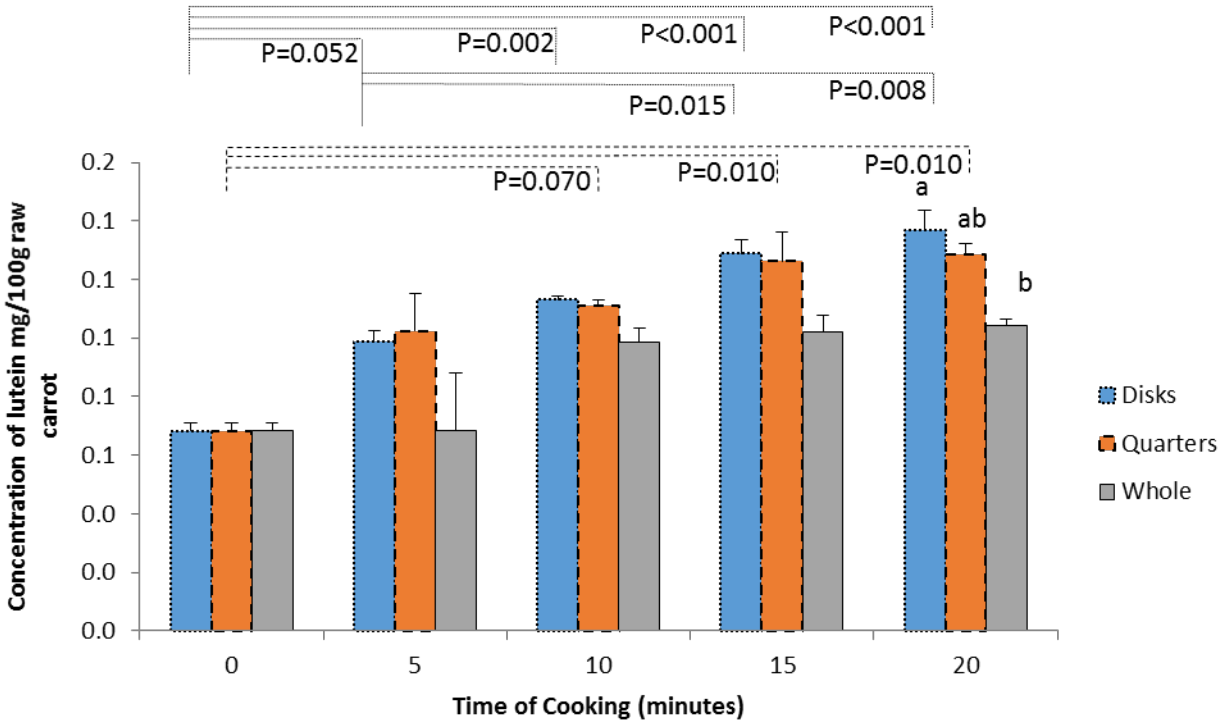
A



B



C



D

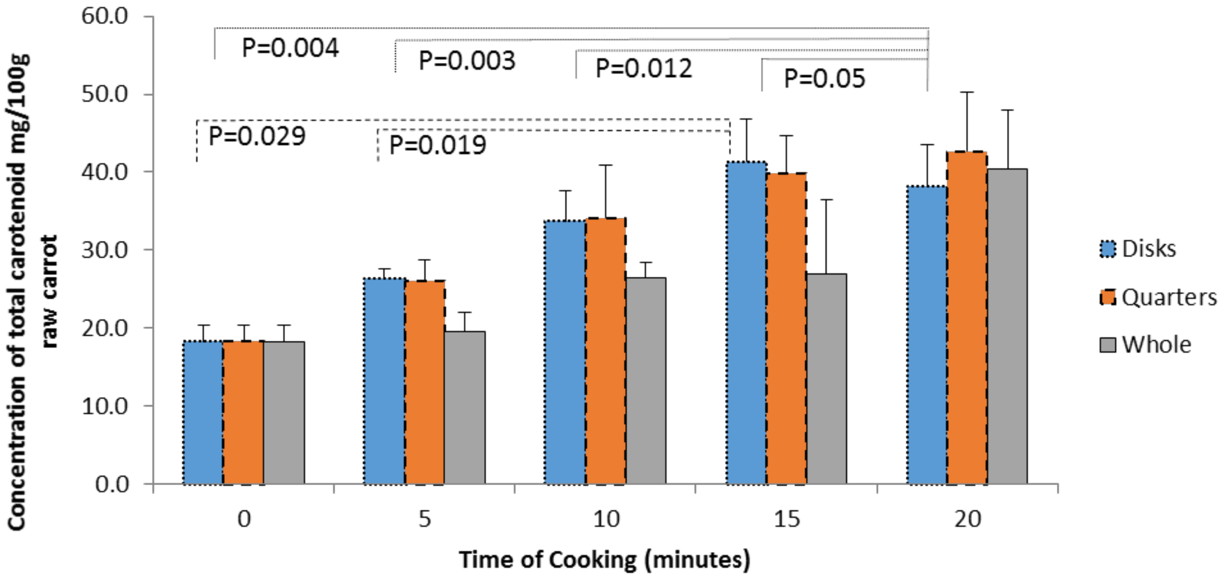
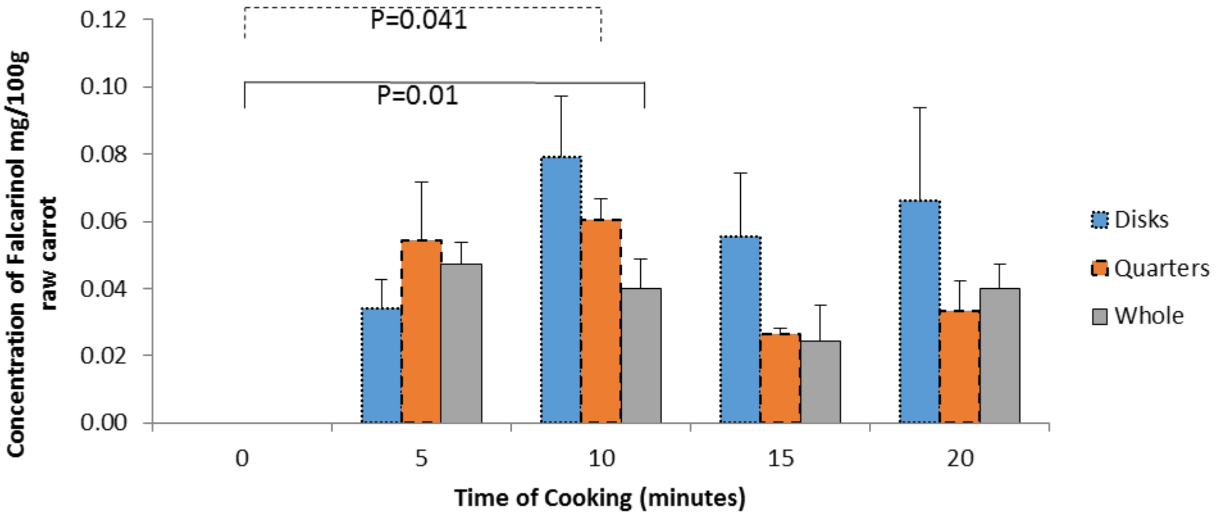


Figure 2. Concentrations of α-carotene (A), β-carotene (B), lutein (C) and total carotenoid (D) in boiled carrot. Letters over columns signify differences between shapes. P values signify differences between time points.

There is an apparent increase in concentration over boiling time of both types of phytochemical measured even when calculating the concentration accounting for the loss of dry matter. It is unlikely that the carrot is still metabolically active and producing these compounds during the cooking process but many other studies have also found the same increases in concentration with polyacetylenes (Rawson *et al.*, 2010) and other phytochemicals (Puupponen-Pimiä *et al.*, 2003; Imsic *et al.*, 2010; Chiavaro *et al.*, 2012; Bongoni *et al.*, 2014) which has been attributed to the ease of extractability of phytochemicals with the breakdown of cell matrix during the cooking process. However, this is not consistent and some studies have found decreases in phytochemicals during cooking (Sulaeman *et al.*, 2001; Hansen *et al.*, 2003). Whilst some studies do not account for the loss of sugars in the calculation of change in concentrations, some of those that did also resulted in increased concentrations (Khachik *et al.*, 1992).

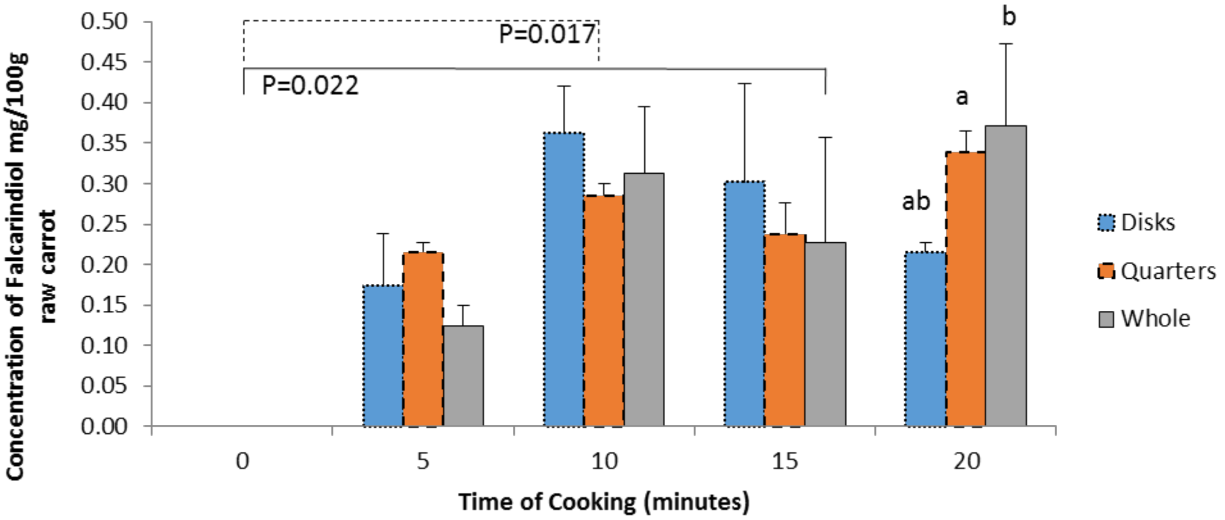
In water, again the shape had little effect on increase in concentration of PA during cooking, only FaDOH was higher in W compared to D and Q after 20 minutes (figure 3) but increased cooking time led to increased concentrations in the cooking water indicating that PA moved into water over the cooking period. The major changes occurred in the first 5-10 minutes as you would expect from a change from zero. Concentrations from D and Q were higher in water than those from W in the early stages of boiling but the difference in concentrations were not maintained over the full 20 minute cooking period, decreasing from peak concentrations at 5 minutes. Carotenoids in the water from boiling, despite the orange colour of the resulting fluid, were unable to be detected and were assumed to be completely degraded.

A



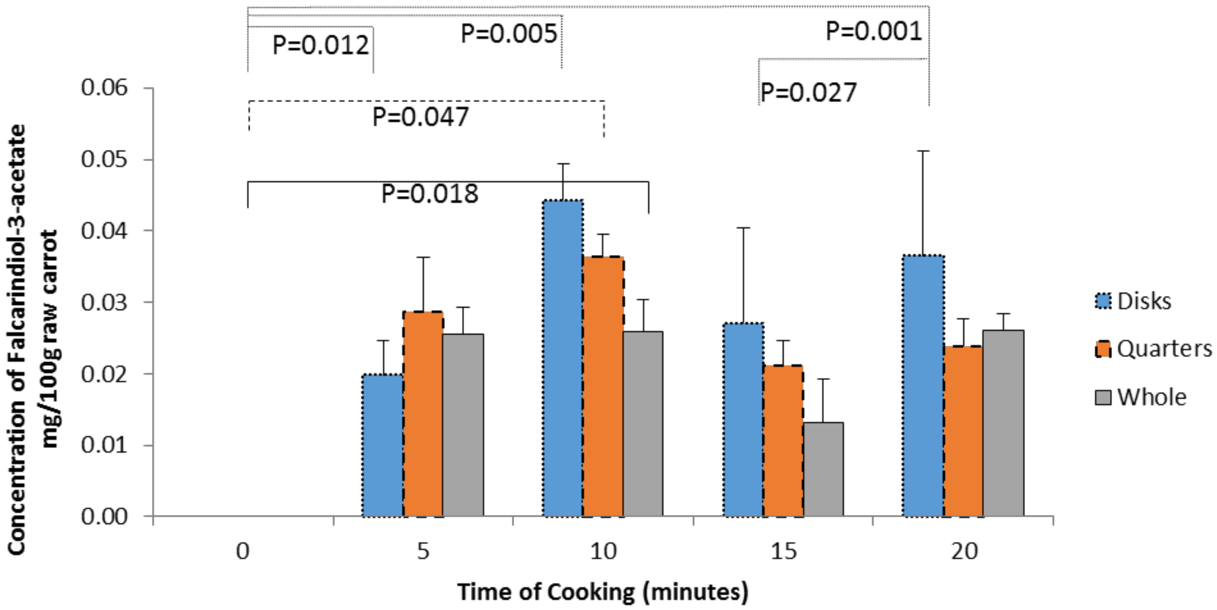
0.12
0.10
0.08
0.06
0.04
0.02
0.00
P=0.041
P=0.01
Concentration of Falcarinol mg/100g raw carrot
0
5
10
15
20
Time of Cooking (minutes)
Disks
Quarters
Whole

B



0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
P=0.017
P=0.022
ab
a
b
Concentration of Falcarindiol mg/100g raw carrot
0
5
10
15
20
Time of Cooking (minutes)
Disks
Quarters
Whole

C



D

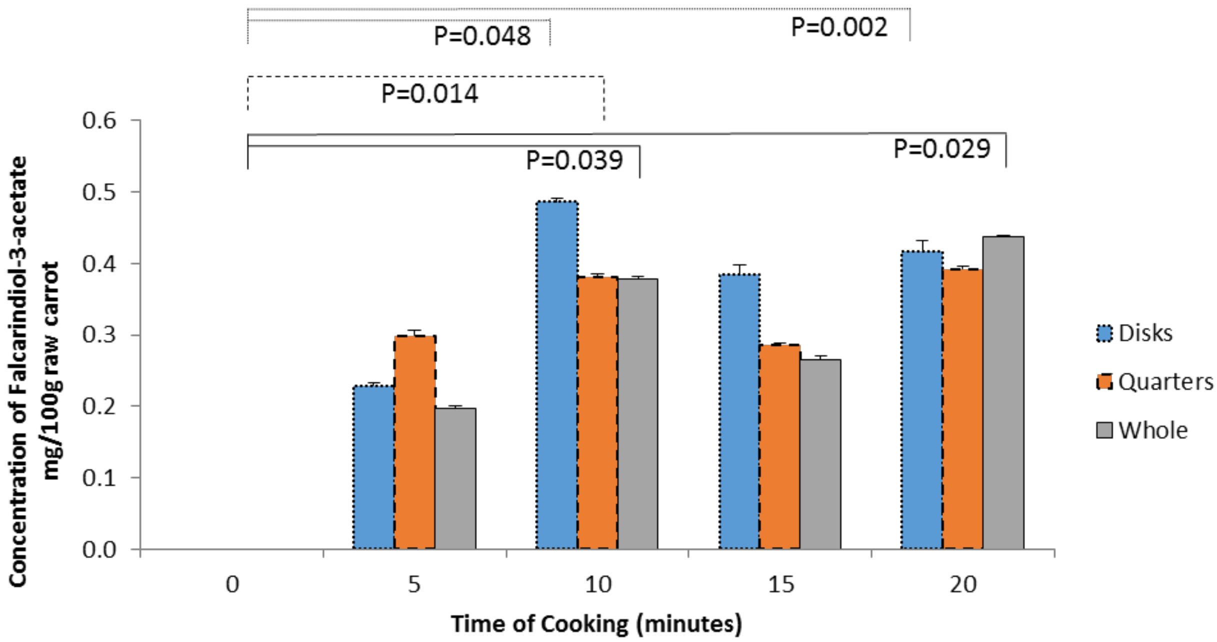


Figure 3. Concentrations of falcarinol (A), falcarindiol (B), falcarindiol-3-acetate (C) and total polyacetyelene (D) in water from boiling. Letters over columns signify differences between shapes. P values signify differences between time points.

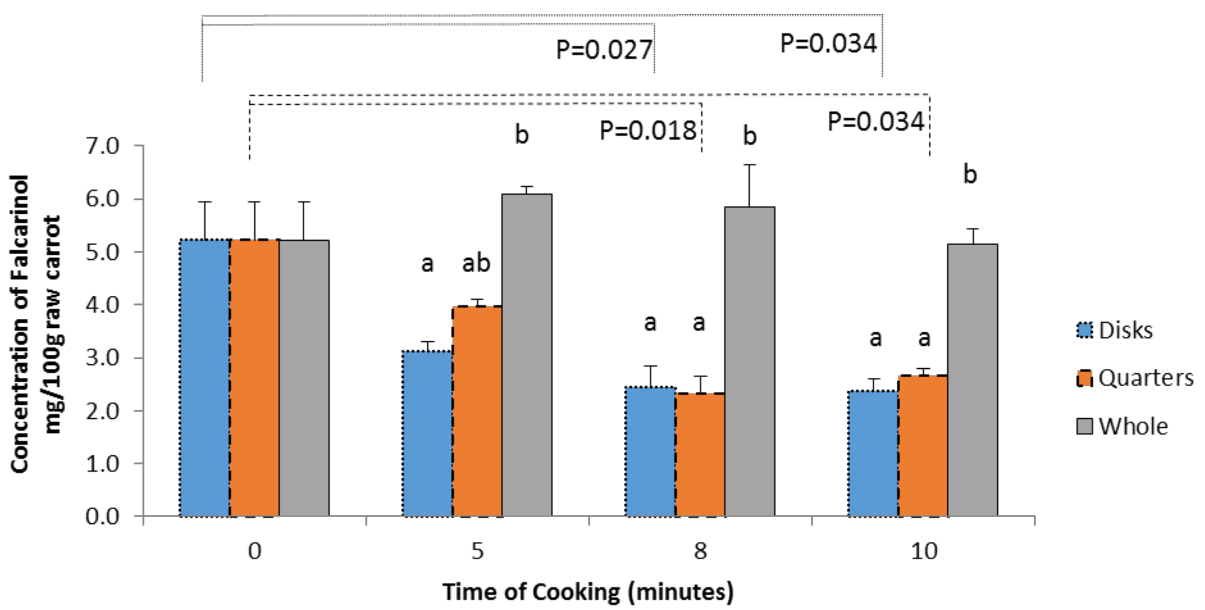
The initial rise in PA in water over the first 10 minutes followed by a reduction in concentration in subsequent times could be due to degradation of compounds in an aqueous environment. Both PA and carotenoids are known to degrade easily by oxidation and the carotenoids have been shown to be much less stable as a purified compound compared to when it is bound inside the vegetable. We would expect an increase in phytochemicals in cooking water at 5 minutes due to leaching of compounds as the cells on the surface of the carrot break open and leach their contents into the water. It would also be reasonable to expect the concentrations to continue to increase over cooking time as more of the cells break open over cooking time. However, this is not seen which suggests the initial release into the water could be attributed to the mechanical release of phytochemicals, that is, the damage to cells during peeling and cutting of the carrot, releasing PA which is then washed into the liquid. This would explain the difference between shapes according to the surface area. It could also be a combination of mechanical cutting and leaching followed by degradation. More falcarindiol (~5-8%) leaches into water than other PAs (~0.2-1.8% dependant on time and shape) and into oil (13% FaDOH vs 9-11.5% others in D and Q. W had much lower % of leaching). Falcarindiol is found mainly in the surface tissue of the root (Czepa and Hofmann, 2004) and thus would be closer to the water exposed edge of the carrot and therefore lost more easily than the FaOH and FaD3Ac which are more even spread throughout the root. FaDOH is also the most polar of the compounds and therefore more attracted to the aqueous environment of the water.

The lack of degradation and even increase in concentration of PA and carotenoids during boiling leads us to the conclusion that the compounds are very heat stable, retained within the vegetable, and even stable and detectible in the cooking fluid, regardless of shape or for how long we boil carrots. There does not appear to be any reason why phytochemicals in the whole carrot would be more degraded and they are not found in higher concentrations in the water than in the D and Q. Therefore, the higher surface areas of the other shapes are more likely facilitating the release of compounds from the cellular matrix or complexes within the cell during cooking. Whole carrots will be staying more intact under the hydrothermal conditions, softening less and retaining more sugar than D and Q. This would lead to apparent lower concentrations but it is more likely that the compounds are still within the carrot and the method of extraction is unable to detect the bound PA within the cells. Improvements in extractability and therefore availability of the compounds are one of the reasons that it is commonly thought that cooked carrot would have improved digestibility when consumed, and therefore increased bioavailability, but this has not been tested in vivo for these compounds.

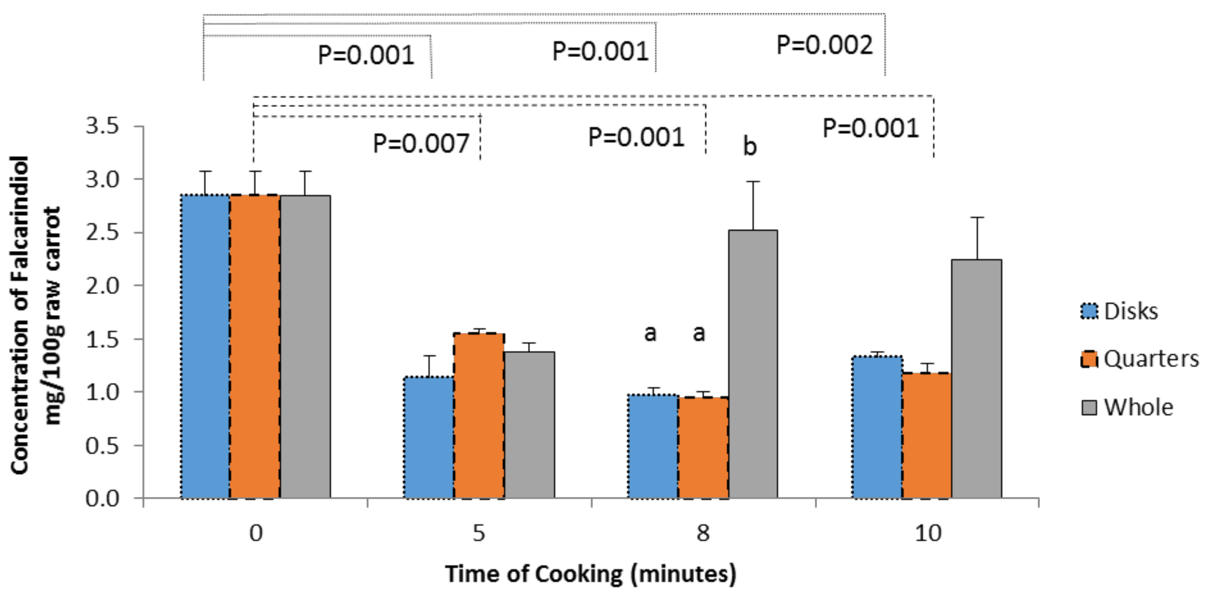
Phytochemicals in carrot and cooking oil during frying:

PA and carotenoids were much less retained in the fried than the boiled carrots. Unlike the boiled carrot, the shape appears to be important for retention of both PA and carotenoids during frying. For the PA there were large reductions in concentration in the first 5 minutes of cooking (figure 4) in D and Q with all PAs being lower than raw (except FaOH which is significantly lower from 8 minutes) and only minor losses occurred from the carrot after this time. W carrots retained more PA and carotenoids than D and Q during frying and subsequently they were not different in concentration from raw carrot.

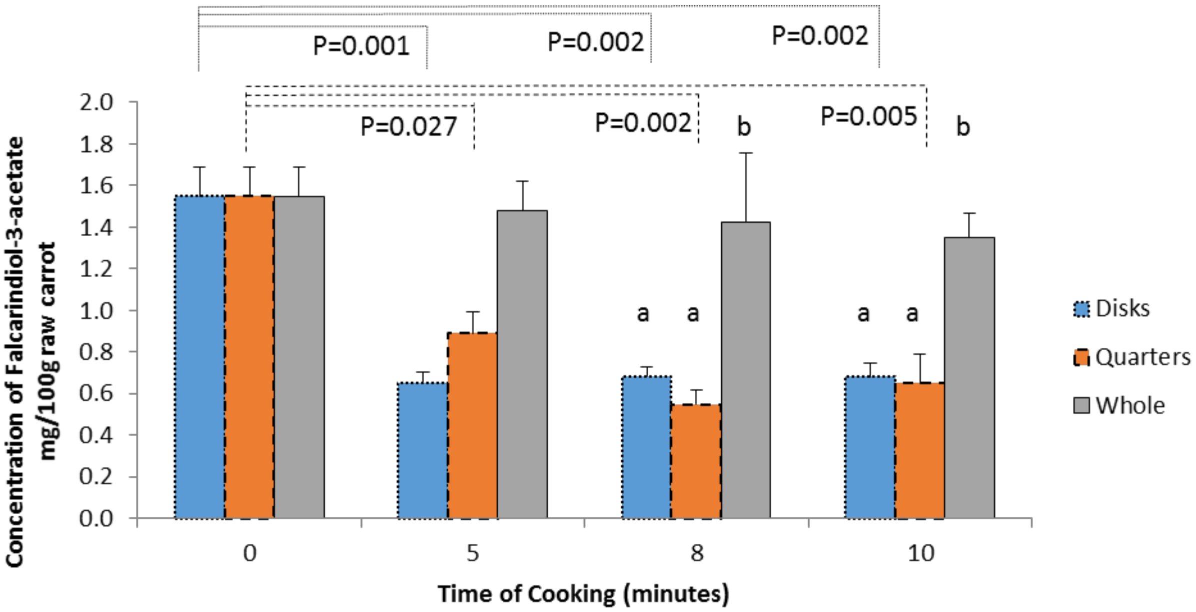
A



B



C



D

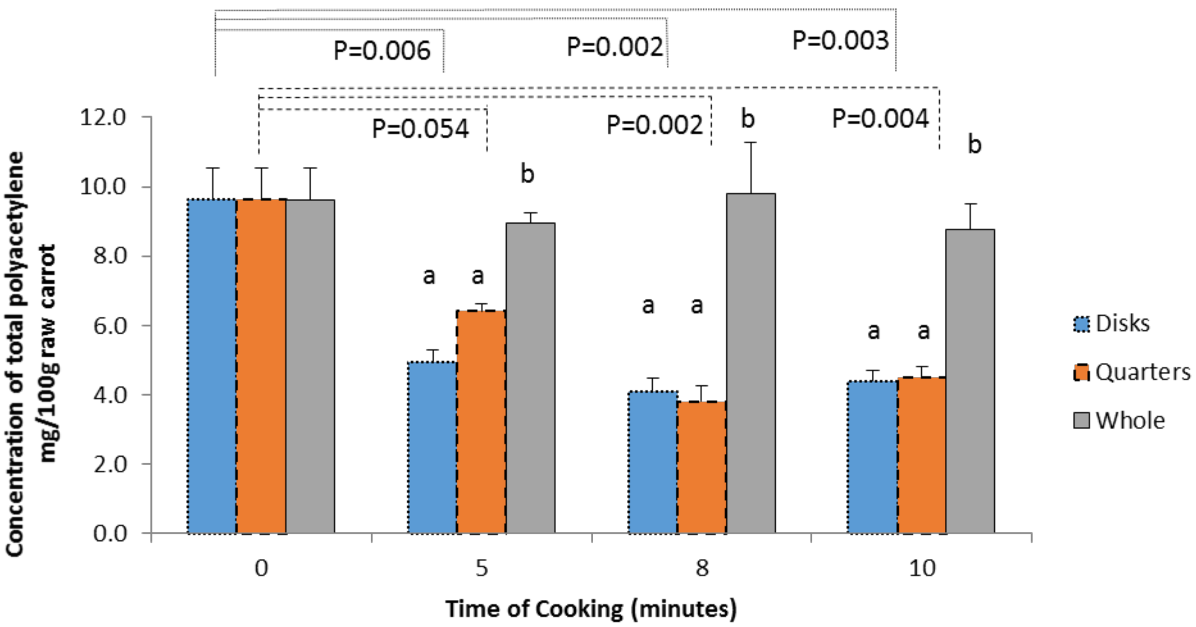
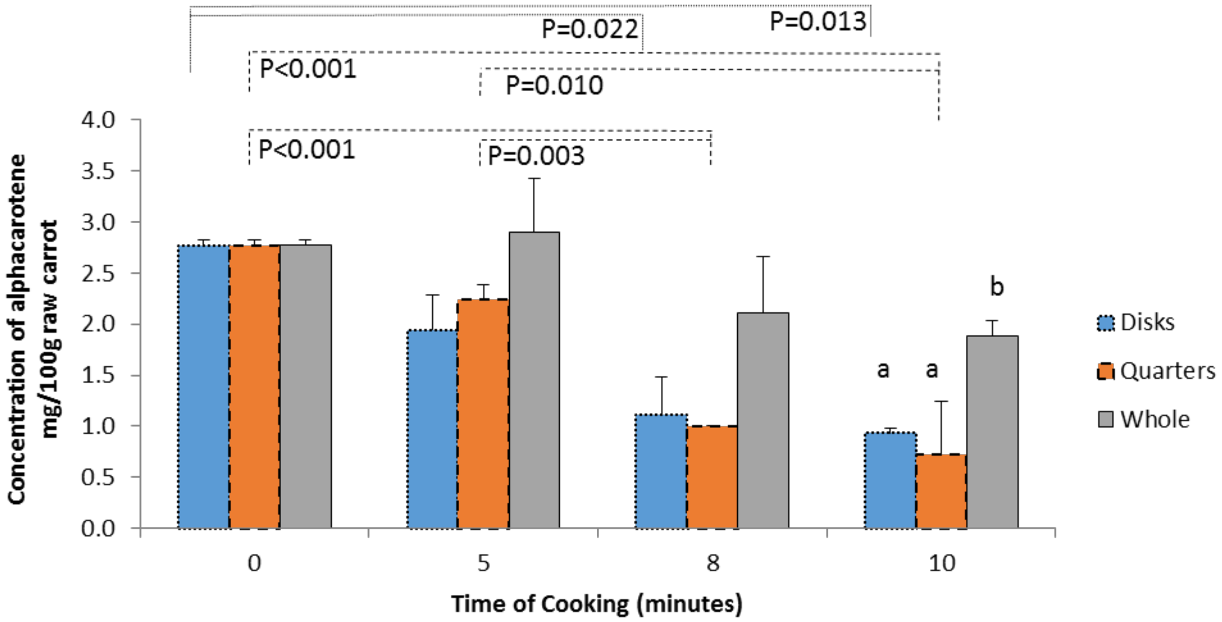


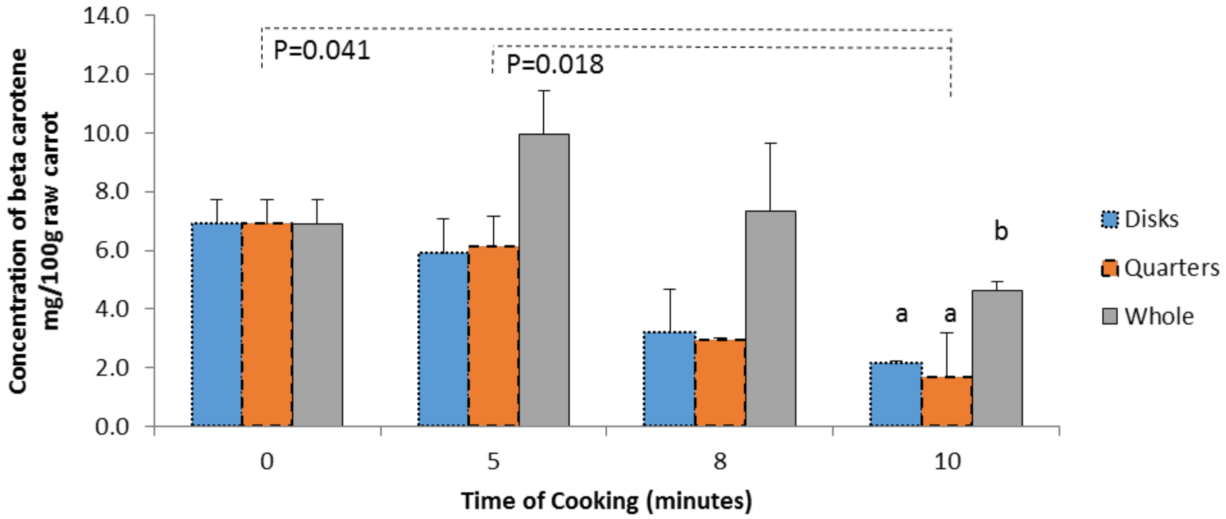
Figure 4. Concentrations of falcarinol (A), falcarindiol (B), falcarindiol-3-acetate (C) and total polyacetyelene (D) in carrot after frying. Letters over columns signify differences between shapes. P values signify differences between time points.

For the carotenoids, the W carrots also retained significantly more than the D and Q but only after 10 minutes (figure 5). This suggests the smaller surface area of W carrot protects phytochemicals from losses during frying (due to degradation or leaching).

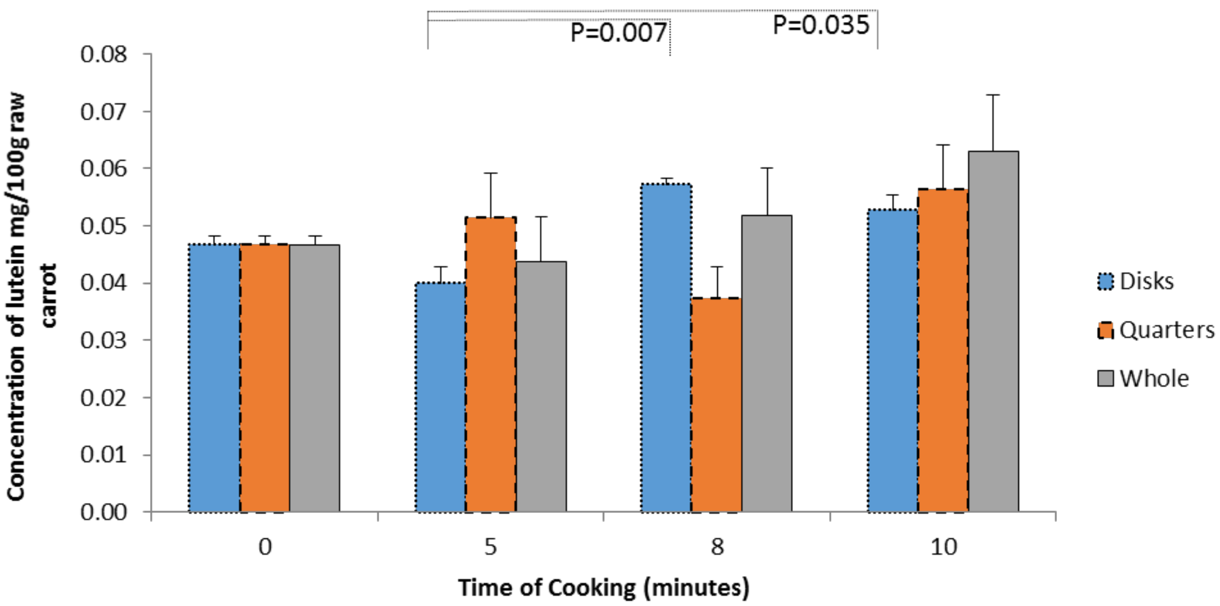
A


P=0.022
P=0.013
P<0.001
P=0.010
P<0.001
P=0.003
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Concentration of alphacarotene mg/100g raw carrot
a
a
b
Disks
Quarters
Whole
0
5
8
10
Time of Cooking (minutes)

B


14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
P=0.041
P=0.018
Concentration of beta carotene mg/100g raw carrot
a
a
b
Disks
Quarters
Whole
0
5
8
10
Time of Cooking (minutes)

C



D

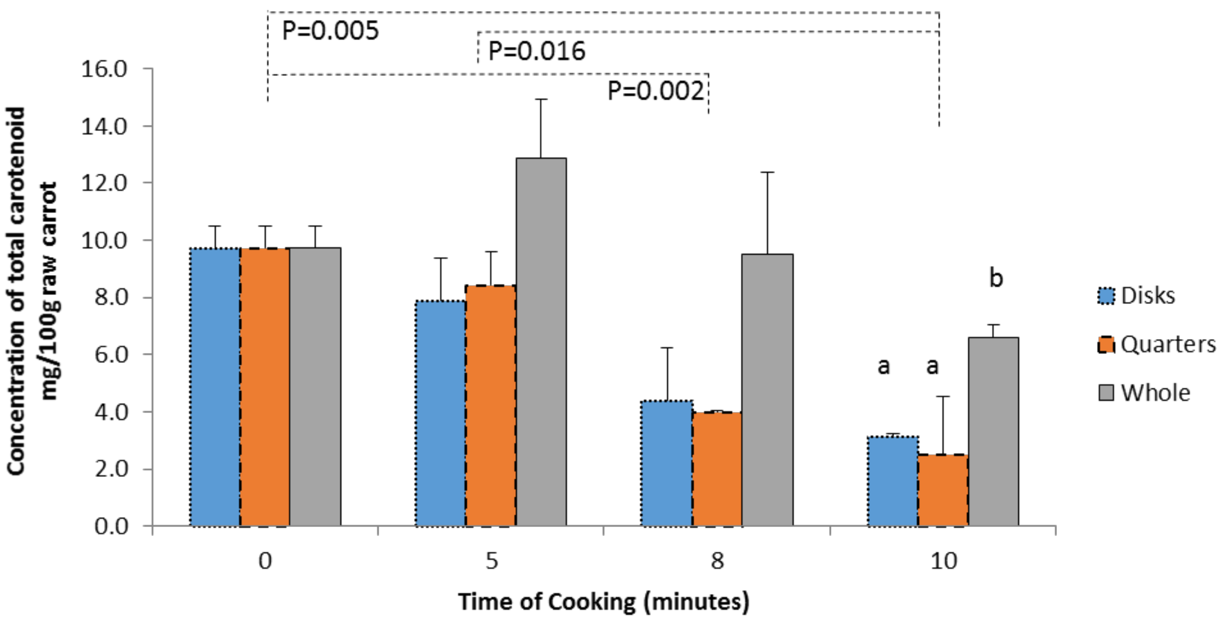
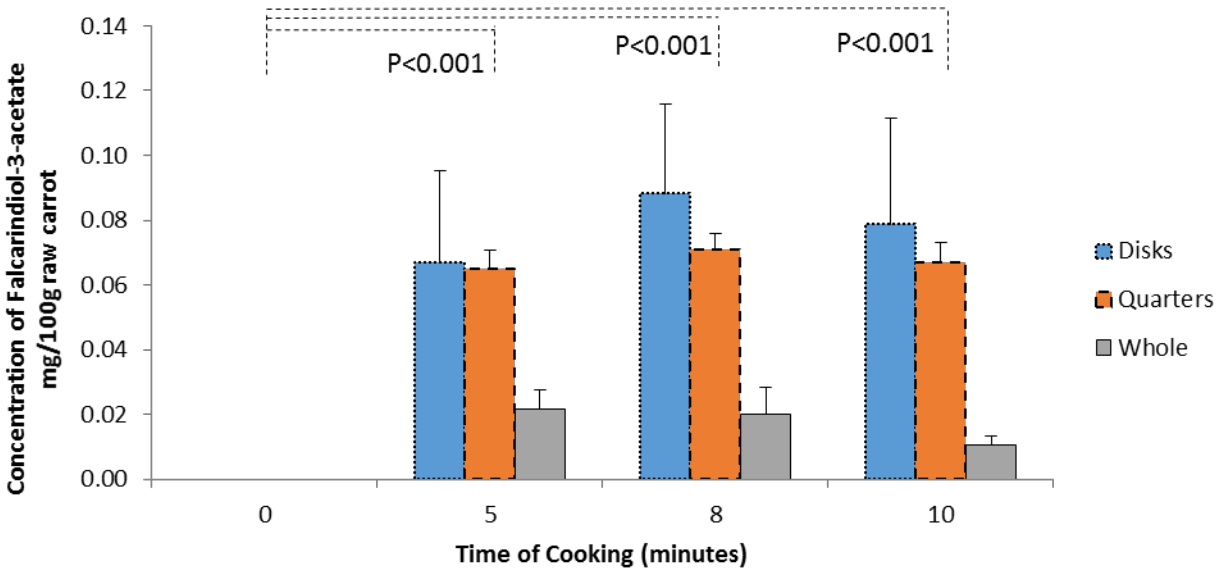


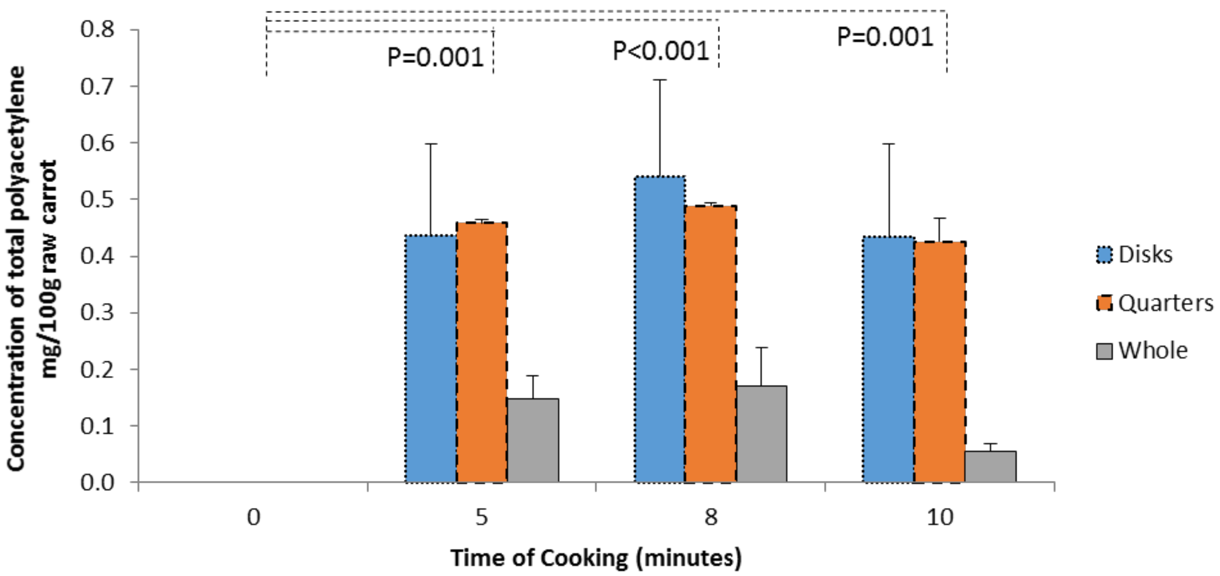
Figure 5. Concentrations of α-carotene (A), β-carotene (B), lutein (C) and total carotenoid (D) in carrot after frying. Letters over columns signify differences between shapes. P values signify differences between time points.

Looking at the oil, the oil from W carrot frying had lower mean concentrations of PA than from oil from D and Q carrot but these are not significantly different between shape (figure 6). Only the oil from cooking the Q significantly increased in PA concentrations from raw. Despite similar mean concentrations from D carrots, the large variation of the data means no significant differences are seen from raw. The oil from W carrots was much lower in concentration and was also no different from raw. As with the water, no carotenoids were detected in the oil after cooking.

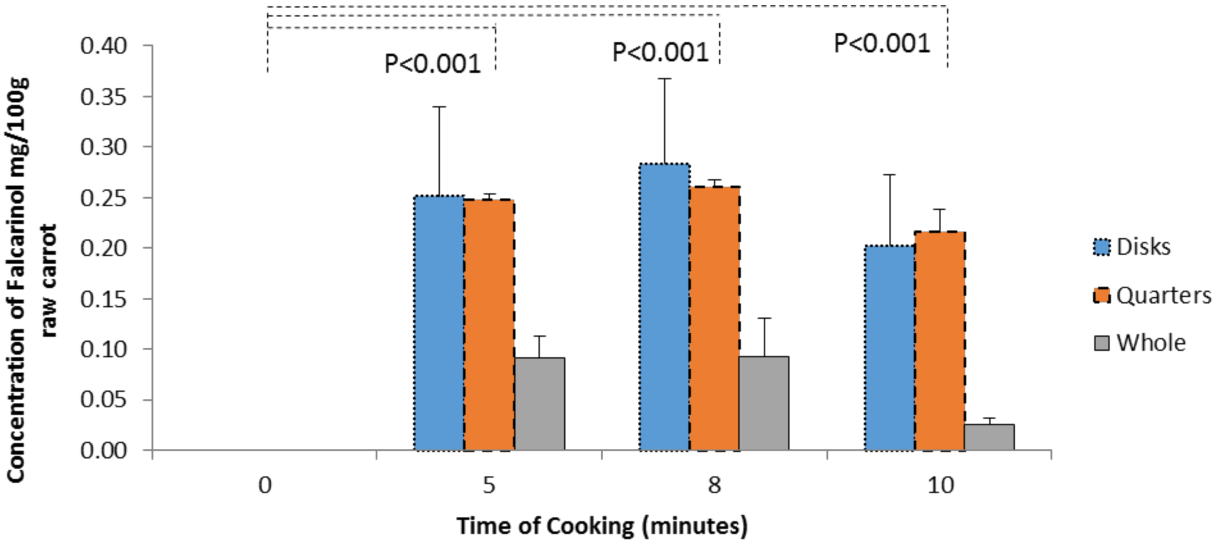
A



B



C



D

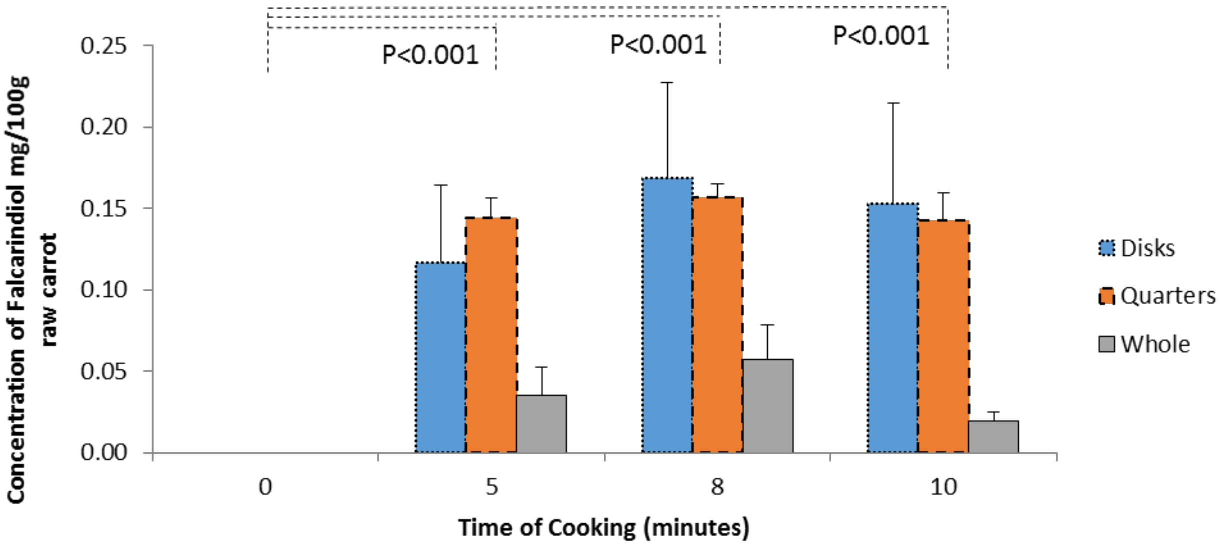


Figure 6. Concentrations of falcarinol (A), falcarindiol (B), falcarindiol-3-acetate (C) and total polyacetyelene (D) in oil from frying. Letters over columns signify differences between shapes. P values signify differences between time points.

Both sets of compounds are more soluble in fats than in water so they should more readily leach into oil than the water. PA in the oil is around 9-13% of the amount in the carrot from disks and Q, compared to the 0.5-5% in water in the same shapes.

A common recommendation for improving the nutritional impact of a food is to incorporate the fluid from cooking into the dish to retain phytochemicals. For water this would increase intake by less than 5% but for oil it is more important with around 10% of phytochemicals being exchanged into the oil. However, changing the shape of the carrot during frying could mitigate the losses as only 2% of PAs were leached into oil when carrots were cooked whole.

Conclusions

Boiling time has little effect on phytochemical retention suggesting PA and CA are reasonably heat stable at this temperature. Longer cooking times may even 'increase' concentration of PA and CA due to compositional changes in the carrot as well as increased extractability of compounds which may have effects on digestibility.

Fried carrots retain much less PA and CA than boiling. This could be due to a combination of leaching of compounds and heat damage. These losses could be mitigated by changing the shape of carrot to W during cooking.

Keeping cooking water and oil to incorporate into a dish may increase intake of PA, but carotenoids appear to be degraded once it has left the carrot.

Bioavailability Study

(taken from an Acta Horticulurae article that is pending publication)

Introduction

PA have previously been shown to be bioavailable in humans in 2 small trials using carrot juice (Hansen-Møller *et al.*, 2002; Haraldsdóttir *et al.*, 2002). Whilst carrot juice is a convenient way to administer an accurate dose, carrots are more commonly consumed as a whole vegetable in a typical diet. This study will investigate the bioavailability from carrot, including fibre and other components of the whole vegetable that may affect the release of phytochemicals.

In epidemiological studies, carrot intakes have been associated with a lower risk of colorectal (Franceschi *et al.*, 1998; Slattery *et al.*, 2000), breast (Boggs *et al.*, 2010) and bladder cancer (Zeegers *et al.*, 2001); therefore blood, urine and faecal samples will be analysed to see if PA are present in these bio-fluids. *In vitro* studies have shown that polyacetylenes can inhibit the growth of colon cancer cells (Sun *et al.*, 2010) and leukaemia cells (Zaini *et al.*, 2012), and it has been demonstrated that polyacetylene compounds can be absorbed by cells of the intestine (Lee *et al.*, 2013). The anti-cancer effect of carrots has also been demonstrated *in vivo* in mouse (Saleh *et al.*, 2013) and rat models (Kobaek-Larsen *et al.*, 2005) that are predisposed to intestinal cancer. It is therefore possible that one or several carrot components may be affecting risk of colon cancer and thus it is worth examining faeces/faecal water to allow us to determine whether epithelial cells in the digestive tract are exposed to concentrations of PAs that have been shown to affect the viability of colon cancer cells *in vitro*.

The aim of this study is to determine whether eating an amount of carrot can lead to the appearance of polyacetylenes (PA) in the biofluids and whether they are adequately absorbed and detectable in blood, and urine and whether they remain in the gut and are detectable in the faeces. The secondary aim of the study is to determine if there is a detectable difference between biofluid concentrations of polyacetylenes after the high 'dose' versus the low 'dose' of carrots.

Methods and Materials

Participants were asked to avoid eating PA containing foods (such as carrots, parsnips etc.) for 3 days prior to each test session. They came to the test centre, fasted (no food or drink for 12 hours) having eaten a standard meal (tomato pasta bake with brocolli) the night before and provide a stool sample from that morning (or the night before) and 24 hour urine from the day before. They had a cannula fitted and a fasting blood sample was taken. The participant then consumed a meal containing a specific amount of carrots - randomised on either high or normal ‘dose’ of boiled carrot: 100g and 250g - with bread and butter and had further blood samples taken at 1, 2, 4, 5, 6 and 8 hours. The participant consumed standard meals for lunch (tomato soup, cheese and tomato sandwich, cereal bar and apple), snack (banana) and dinner (cheese pizza with cauliflower) and returned the next morning for a further fasted blood sample. Faecal and urine samples were collected on the test day and up to 48 hours after the test day. A gap of at least 1 week was allocated between testing sessions then the participants repeated the methodology with the alternative ‘dose’ of carrot. Plasma, faeces and urine was frozen and stored until analysis for PA by LCMS (Liquid Chromatography-Mass spectrometry) at a later date. All foods provided were tested for polyacetylenes to ensure they were absent. An additional portion of carrots was prepared on each testing day for analysis of polyacetylene content.

Results

6 healthy adults were recruited to the study and all samples have been collected. The method for extracting and analysing polyacetylenes from plasma samples is still ongoing and a method for urine and faecal analysis will also be developed if time permits.

Results will be analysed to determine if concentrations of polyacetylene are seen in the body at similar concentrations that have been shown to have anti-inflammatory and anti-cancer effects in cell studies. It will also be possible to see how long the polyacetylenes remain in the body and therefore estimate the length of exposure to these compounds. The two doses will be compared to see if there is a dose effect of the carrots – i.e. will eating more carrot lead to more compound in the biofluids.

Dietary Intervention Study

(taken from a Acta Horticulurae article that is pending publication)

Introduction

The effect of polyacetylenes on humans has not been tested *in vivo* so it is unknown whether the biological actions seen in isolated cells and animal studies will also be seen in humans. 'Biomarkers' can be used to measure the risk for certain diseases such as measuring damage to DNA and inflammatory markers to predict risk of cancer (Mayne, 2003). The human dietary intervention trial will test the effects of eating PA-rich root vegetables on these biomarkers.

Methods and Materials

A randomised parallel design was used, involving consumption of either 100g cooked white carrots (with 10g of butter) or a portion of oat biscuits (matched to carrots for sugar, fibre and fat) per day for 6 weeks. All other PA containing foods and oats were avoided during this period. A wash-out period of 6 weeks followed when no supplemental foods were eaten, and participants continued to exclude PA containing foods and oats. At the beginning and end of the intervention and wash out periods, blood, urine and faecal samples were collected to see the effect on inflammatory markers and DNA damage in lymphocytes. Dietary intake was monitored throughout to check compliance to the intervention, wash out and baseline diet.

Measured biomarkers will be compared between control and intervention periods and correlated with changes in PA concentration as a result of the intervention.

Results

50 volunteers underwent screening and 39 healthy adults, aged 45 years or older were recruited to take part. There were 3 drop outs so that 36 people completed the trial. All the samples have been collected and are frozen, awaiting analysis. The samples will be analysed for inflammatory markers in blood and urine and cell damage in lymphocytes. The final participant finished the trial at the beginning of September and analysis of the biofluids is currently ongoing, predicted to be finished mid-October 2016.

Knowledge and Technology Transfer

N/A

Glossary

Bioavailability – the amount of a substance which enters the circulation when introduced to the body.

Dietary Intervention study – a study designed to modify the diet from a person's normal diet in order to study the outcome.

Polyacetylene – a naturally occurring plant compound and natural pesticide. They are long chain organic polymers formed from the biosynthesis of fatty acids.

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