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Effect of processing on major flavonoids in processed onions, green beans, and peas

Catarina Ewald [a,1], Stina Fjelkner-Modig [b], Katarina Johansson [b], Ingegerd Sjöholm [a], Björn Åkesson [c,*]

[a] *Department of Food Engineering, Center for Chemistry and Chemical Engineering, Lund University, PO Box 124, S-221 00 Lund, Sweden*
[b] *Nestlé R&D Center Bjuv AB, PO Box 520, S-26725 Bjuv, Sweden*
[c] *Department of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund University, PO Box 124, S-221 00 Lund, Sweden*

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Abstract

The contents of flavonoids in onions, green beans, and peas have been analysed in relation to the effect of different heat treatments. Two major flavonoids were studied, quercetin and kaempferol. The identification and quantification of the flavonoids were performed with high performance liquid chromatography and UV detection. The greatest loss of flavonoids in onion took place during the pre-processing step where the onion was peeled, trimmed, and chopped before blanching. Blanched onion contained 25 mg quercetin and 0.35 mg kaempferol per 100 g edible part. Blanched green beans contained 1.3 mg quercetin and 0.24 mg kaempferol per 100 g, and blanched peas only 0.15 mg quercetin per 100 g. No kaempferol was detected in peas. Further cooking, frying or warm-holding for up to 2 h of the blanched vegetables, did not influence the flavonoid content. Onions in ready-made dishes and home-cooked food as well as green beans may be good dietary sources of flavonoids.

Keywords: Quercetin; Kaempferol; Antioxidant

1. Introduction

The beneficial health effects of antioxidants are of increasing interest to food manufacturers and, thus, it is important to study the content and structure of different antioxidants in foods. The stability of antioxidants during processing of fruits and vegetables is another important issue.

The major sources of dietary flavonoids in many countries are tea, onions and apples (Hertog et al., 1995). It has been implicated that flavonoids have an antioxidative effect (Bors, Heller, Michel, & Saran, 1990) and antioxidants may prevent or delay diseases like cardiovascular disease and cancer (Block, 1992; Block & Langseth, 1994; Gey et al., 1993; Renaud & de Lorgeril, 1992).

The phenolic compounds in foods include phenols, phenolic acids, hydroxycinnamic acid derivatives, and flavonoids (Decker, 1995). Quercetin and kaempferol are typical flavonols and their corresponding flavones are luteolin and apigenin (Hertog & Hollman, 1996). Flavonols and flavones are located predominantly in the leaves and in the outer parts of the plants, while only trace amounts can be found below the soil surface (Hertog & Hollman, 1996). In onions approximately 90% of the quercetin was localised in the first and second layers of the onion (Mizuno, Tsuchida, Kozukue, & Mizuno, 1992). Quercetin glycosides predominate in vegetables, but there are also glycosides of kaempferol, luteolin, and apigenin (Hertog & Hollman, 1996).

Although much remains to be learned about flavonoid content in different foods, even less is known about the effects of processing on flavonoid content. The aim of this study was, therefore, to analyse the content of some major flavonoids in onions, green beans, and peas before and after different heat treatments (blanching, water-cooking, cooking in a microwave oven, frying and warm-holding of the water-boiled sample at 60°C for 1–2 h). Onions contain high amounts of flavonoids (Hertog, Hollman, & Katan, 1992) and, therefore, most of the analyses were performed on onion samples.

[1] Present address: Frödinge Mejeri AB, S-598 95 Vimmerby, Sweden.
* Corresponding author.

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2. Materials and methods

2.1. Raw materials

The onions (*Allium* Cepa) were ecologically grown at the experimental farm of Nestlé R&D Center, Bjuv, Sweden and they were fresh when delivered. The green beans (*Phaseolus* spp) were grown in Norway. The peas (*Pisum sativum*), grown in the south of Sweden, were supplied by Svenska Nestlé AB, Bjuv, Sweden. Both the green beans and peas had been blanched and were frozen on arrival. All the crops were harvested at the optimal maturity.

2.2. Processing of vegetables

The blanched vegetables were used as reference samples in relation to further processed samples, except for the onion, where the raw vegetable was used as a reference sample. The raw onions were processed at a pilot plant, where they were peeled and washed before trimming and chopping. With the dicing equipment commonly used in industry, onions were chopped to 9.5×9.5×9.5 mm. Then they were steam-blanched, cooled in ice-water, and frozen in a flow-freezer. The green beans and the peas were blanched in water using standard procedures. All samples were kept in a freezer (−20°C) until further processing. Green beans and peas were processed according to the descriptions on commercially available packages. Onions were prepared according to commonly used kitchen methods. Details of the processing are shown in Table 1.

2.3. Flavonoid analysis

2.3.1. Sample preparation

After different heat treatments, the vegetables were frozen in a convective Frigoscandia freezer. When frozen, the samples were moved to a freeze-dryer and lyophilised for at least 4 days. The samples were then ground to pass a 0.425-mm sieve and stored at −20°C until analysis.

2.3.2. Standards

The five flavonoids used as standards were three flavonols, quercetin, kaempferol, and myricetin, and two flavones, luteolin and apigenin. They were purchased from Fluka (myricetin 70050, quercetin dihydrate 83370, kaempferol 60010, and apigenin 10790) and from Roth (luteolin 5801). The standards were dissolved in methanol to a concentration of 500 μg ml^{-1} as stock solution and stored at 4°C. Calibration standards with concentrations between 0.1 and 25 μg ml^{-1} were prepared by diluting an aliquot of the stock solution with 20 ml 62.5% aqueous methanol with 2 mg ml^{-1} of the antioxidant *tert*-butylhydroquinone (TBHQ), 5 ml 6 M HCl, and methanol up to 50 ml.

2.3.3. Extraction and hydrolysis

The standard procedure for hydrolysis of quercetin in fruits and vegetables was as follows: the freeze-dried sample (0.5 g) was hydrolysed in 40 ml of 62.5% aqueous methanol with 2 mg ml^{-1} of TBHQ and 10 ml of 6 M HCl at 90°C with reflux for 2 h. The acid concentration was thus 1.2 M HCl during hydrolysis. For peas, instead, 1 g of freeze-dried sample was analysed due to their low content of quercetin. Green beans required a higher acid concentration (2 M HCl) for complete hydrolysis and 0.5 g freeze-dried sample was hydrolysed in 33.3 ml of 62.5% of aqueous methanol with 2 mg ml^{-1} of TBHQ and 16.7 ml of 6 M HCl at 90°C with reflux for 2 h. Methanol was added to all samples to 100 ml after hydrolysis, and the samples were finally sonicated for 5 min to remove oxygen before injection.

2.3.4. HPLC

The analytical method used was modified from a published method (Hertog, Hollman, & Venema, 1992). The samples were separated on a reversed phase column, an Inertsil ODS-2 (4.6×150 mm; 7-μm particle

Table 1
Preparation of onions, green beans and peas

Sample	Preparation	Onions	Green beans	Peas
0	Raw	Peeled, cut		
1	Blanched	Steam	Water	Water
2	Boiled in water, 3 min	500 g + 500 g water	300 g + 400 g water	300 g + 400 g water
3	Microwave (650 W)	300 g + 30 g water, 3 min	300 g + 30 g water, 2×5 min	300 g + 30 g water, 2×2.5 min
4	Pan-fried, rape-seed oil, 5 min	300 g + 15 g oil	300 g + 15 g oil	300 g + 15 g oil
5	Pan-fried, butter, 5 min	300 g + 15 g butter	300 g + 15 g butter	300 g + 15 g butter
6	Boiled in water, 3 min warm-holding 60°C, 1 h	500 g + 500 g water	300 g + 400 g water	300 g + 400 g water
7	Boiled in water, 3 min warm-holding 60°C, 2 h	500 g + 500 g water	300 g + 400 g water	300 g + 400 g water

size) manufactured by HiCHROM, UK. The mobile phase consisted of 30% of acetonitrile in 0.025 M KH_2PO_4 (pH 2.4) with a flow rate of 1.3 ml min^{-1}. The column was placed in a column oven set at 30°C. The HPLC system consisted of a Varian 5500 HPLC and a Varian 9090 autosampler and the injection volume was 20 μl. The compounds were detected by a UV-vis detector set at 370 nm. All five standards could be separated and quantified, the average retention times being myricetin 3.2, luteolin 5.4, quercetin 5.8, apigenin 9.5 and kaempferol 11.1 min but only quercetin and kaempferol could be detected in the vegetables. The quantification of flavonoids in unknown samples was based on an external standard.

2.4. Dry matter content

The dry matter contents of raw and processed vegetables were determined by drying 1–5 g of sample at 70°C in a vacuum oven for 48 h.

3. Results and discussion

3.1. Methodological considerations

To check the repeatability of the method, two different batches of onions, processed in the same way, were prepared. Duplicate samples were prepared on each occasion for hydrolysis, except for the raw onion, where two single hydrolyses were performed. Since the replicate onion samples coincided very well, duplicate samples were performed on green beans and peas. Generally the duplicate samples coincided very well. The differences between the two batches of the onions were not higher than the differences between duplicate samples. The coefficient of variation for quercetin content in all processed onion samples was approximately 10% and for kaempferol it was somewhat higher indicating good repeatability. The fact that the deviations were higher for kaempferol content was probably due to its lower concentrations. The precision for quercetin analysis was lower for peas, especially among the samples containing fat, which could be explained by the small amount of quercetin found. On the other hand, the duplicate samples for peas coincided very well except for the samples containing fat.

Flavonoids occur as glycosides in vegetables and fruits but, since reference compounds of the glycosides were not commercially available, the samples had to be hydrolysed to the aglycone form of the flavonoids, in order to be identified and quantified. The hydrolysis was performed in boiling 1.2/2.0 M HCl in 50% aqueous methanol (v/v). The flavonol 3-*O*-glycosides are completely hydrolysed within a few minutes whereas the hydrolysis of the flavonol 3,7- and 4′-*O*-glucuronides takes 60–250 min (Hertog et al., 1992). The method can be optimised for each vegetable or fruit and for each flavonoid as made by Hertog et al. This was accomplished by altering the acid concentration during the hydrolysis and the time for extraction.

3.2. Flavonoid content and processing

The results on quercetin and kaempferol content in the vegetables are presented in Tables 2–4 (mean value of all replicate samples). The content of quercetin in raw onion, blanched beans, and peas was 41, 1.3 and 0.15 mg/100 g, respectively. For kaempferol, its content in raw onion and blanched beans was 0.96 and 0.24 mg/100 g. No kaempferol was detected in the peas. The measured level of quercetin in onion was similar to the level previously reported (35 mg/100 g fresh edible part) (Hertog et al., 1992). When different processing steps for onions were compared, the only significant losses of flavonoids took place during the peeling and trimming process (39%). Since quercetin previously has been found to be heat-stable (Mizuno et al., 1992) the losses may depend on the peeling and trimming procedure of the onions. The onions were of different size and they were peeled very unequally which means that several layers were peeled off from some onions and only one

Table 2
Flavonoid content of onions

Sample [a]	Quercetin mg/100 g mean (range)	*n*	Kaempferol mg/100 g mean (range)	*n*	Dry matter content g/100 g
0 Raw	41 (39–42)	2	0.96 (0.87–1.10)	2	10.4
1 Blanch	25 (21–29)	12	0.35 (0.14–0.43)	8	10.3
2 Boil	22 (20–25)	10	0.30 (0.27–0.36)	6	8.4
3 Mw	24 (21–27)	8	0.29 (0.22–0.39)	6	10.2
4 Fry, RSO	25 (22–29)	6	0.41 (0.27–0.45)	4	17.7
5 Fry, butter	31 (27–37)	6	0.39 (0.37–0.46)	4	16.0
6 60°C, 1 h	20 (18–24)	6	0.26 (0.22–0.30)	4	8.2
7 60°C, 2 h	21 (20–23)	6	0.31 (0.27–0.33)	4	8.4

[a] For explanation of sample abbreviations see Table 1.

Table 3
Flavonoid content of green beans

Sample [a]	Quercetin mg/100 g mean (range)	*n*	Kaempferol mg/100 g mean (range)	*n*	Dry matter content g/100 g
1 Blanch	1.3 (1.3–2.8)	6	0.24 (0.20–0.25)	4	7.5
2 Boil	1.0 (1.0–1.0)	2	0.20 (0.19–0.20)	2	6.7
3 Mw	1.4 (1.4–1.4)	2	0.31 (0.29–0.33)	2	8.0
4 Fry, RSO	1.1 (1.1–1.2)	2	0.24 (0.23–0.26)	2	9.0
5 Fry, butter	1.5 (1.5–1.5)	2	0.28 (0.26–0.30)	2	10.0
6 60°C, 1 h	1.1 (1.1–1.1)	2	0.23 (0.21–0.24)	2	8.0
7 60°C, 2 h	1.2 (1.2–1.3)	2	0.25 (0.25–0.26)	2	7.0

[a] For explanation of sample abbreviations see Table 1.

Table 4
Flavonoid content of peas

Sample [a]	Quercetin mg/100 g mean (range)	*n*	Dry matter content g/100 g
1 Blanch	0.15 (0.14–0.16)	2	19
2 Boil	0.092 (0.083–0.10)	2	17
3 Mw	0.098 (0.094–0.10)	2	18
4 Fry, RSO	0.16 (0.13–0.20)	2	27
5 Fry, butter	0.16 (0.16–0.16)	2	26
6 60°C, 1 h	0.092 (0.085–0.10)	2	18
7 60°C, 2 h	0.12 (0.11–0.12)	2	18

[a] For explanation of sample abbreviations see Table 1.

layer from others. Consequently the losses of flavonoids were very high in the onions where several layers were peeled off since 90% of the quercetin has been reported to be in the first and second layer of the onion (Mizuno et al., 1992). The small changes of flavonoid content at further processing of all onion samples were within the observed biological variation among samples (Table 2). There was a tendency that the onion samples having been held warm for 1 and 2 h, respectively, had the smallest flavonoid amounts. The samples cooked in microwave oven had lower losses than the samples cooked in water.

Regarding kaempferol, the losses during blanching of the raw onions were even larger than for quercetin (64 vs 39%). The onions containing fat had higher amounts of kaempferol than the reference sample, which was due to their higher dry matter content including the fat, probably an effect due to evaporation. The only consistent tendency of further processing effects was that the two samples, having been held warm, showed the greatest losses of kaempferol.

For blanched green beans different further heat treatments had no consistent effects on quercetin and kaempferol content (Table 3). The observed changes were within the observed biological and experimental variation. For peas, boiling gave limited reduction in quercetin content but further warm-holding had no effect (Table 4). Thus, similar effects of heat treatments were observed for onions, green beans and peas.

3.3. Biological and preventive effects of flavonoids

Flavonoids have been found in various fruits, vegetables, nuts, seeds, grains, spices, and herbs as well as in tea, cocoa, and wine (Kandaswami & Middleton, 1994). They are known to have a number of biological effects (Singleton, 1981; Havsteen, 1983; Middleton & Kandaswami, 1992, 1994). Flavonoids can function as metal chelators and reducing agents, as chain-breaking antioxidants, as scavengers of reactive oxygen species, and as quenchers of the formation of singlet oxygen (Kandaswami & Middleton, 1994). Also plants use flavonoids for protection against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Larson, 1988). The protective effects of fruits and vegetables in the diet against chronic diseases probably arise from a combination of several components of the food (Knekt, Järvinen, Reunanen, & Maatela, 1996; Williamson, 1996).

3.4. Intake and bioavailability of flavonoids

From a nutritional point of view, it is interesting that flavonoids may be more efficient antioxidants than tocopherols (Vinson, Dabbagh, Serry, & Jang, 1995). The recommended intake of vitamin E in the Nordic countries is 8–10 mg day^{-1} for adults. As a comparison, an intake of only 10 g of onion would provide approximately 4 mg of quercetin and <0.01 mg of alpha-tocopherol. A portion of 100 g of green beans would provide 1.3 mg of quercetin, but only 0.1 mg of alpha-tocopherol. In red wine, quercetin levels of 4-16 mg l^{-1} have been reported (Hertog, Hollman, & van de Putte, 1993) and a 0.2-liter glass of wine would thus contain 0.8–3.2 mg quercetin.

For such comparisons it is also important to consider the absorption and metabolism of flavonoids, which is not well known. Recently Hollman, de Vries, Van

Leeuwen, Mengelers and Katan (1995) found that the absorption in ileostomy subjects of approximately 0.1 g of quercetin glycosides from fried onion (52%) was higher than that from pure quercetin aglycone (24%) and pure quercetin rutinoside (17%). The study also indicated that the colon is not essential for quercetin absorption, but further studies are necessary to explain the mode of absorption for different forms of flavonoids. Interestingly, different quercetin glycosides were demonstrated in human plasma very recently (Paganga & Rice-Evans, 1997).

4. Conclusion

The present study shows that onions are a better source of quercetin and kaempferol than green beans and peas. It is shown that pilot plant peeling and blanching of onions reduced flavonoid content to approximately half the starting level, probably due to loss of the outer flavonoid-rich onion layer. Further processing by cooking, frying, and warm-holding of blanched onion, beans, and peas had only small effects on flavonoid content. It is concluded that onions in ready-made dishes and home-cooked food, and also beans, may be important dietary sources of flavonoids.

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