Contents of Sulforaphane and Total Isothiocyanates, Antimutagenic Activity, and Inhibition of Clastogenicity in Pulp Juices from Cruciferous Plants

Jiří TOTUŠEK[1], Jan TŘÍSKA[2], Danuše LEFNEROVÁ[1], Jan STROHALM[3], Naděžda VRCHOTOVÁ[2], Ondřej ZENDULKA[1], Jiřina PRŮCHOVÁ[3], Jana CHALOUPKOVÁ[1], Pavla NOVOTNÁ[3] and Milan HOUŠKA[3]

[1]Faculty of Medicine, Masaryk University, Brno, Czech Republic; [2]Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; [3]Food Research Institute Prague, Prague Czech Republic

Abstract

Totušek J., Tříska J., Lefnerová D., Strohalm J., Vrchotová N., Zendulka O., Průchová J., Chaloupková J., Novotná P., Houška M. (2011): **Contents of sulforaphane and total isothiocyanates, antimutagenic activity, and inhibition of clastogenicity in pulp juices from cruciferous plants**. Czech J. Food Sci., **29**: 548–556.

The consumption of *Cruciferous* vegetables is important for the prevention of cancerous diseases, particularly colorectal cancer. The effects of technological treatments (freezing, pasteurisation, high-pressure treatment) on the content of isothiocyanates, considered to be the active substance, were observed in single-species vegetable juices prepared from cruciferous vegetables (broccoli, cauliflower, Brussels sprouts, white and red cabbage). The contents of sulforaphane and total isothiocyanates were studied relative to the temperature, action period, and time delay after juice pressing. Sulforaphane and total isothiocyanates were determined by HPLC. Sulforaphane content in various parts of fresh broccoli was also assessed. Antimutagenic activity of the juices (frozen, pasteurised, and high-pressure treated) was evaluated using the Ames test and the following mutagens: AFTB1 (aflatoxin B1), IQ (2-amino-3-methyl-3H-imidazo-[4,5-*f*]quinoline), and MNU (2-nitroso-2-methylurea). Clastogenicity inhibition of the mutagens, in response to broccoli juice, as well as of pure sulforaphane, was observed using an *in vivo* experiment (the micronucleus test). It was shown that in terms of sulforaphane content, it is best to let broccoli juice stand for 60 min after pressing and pH adjustment. Sulforaphane content does not change under heating to 60°C. Its content decreases considerably (compared to fresh juice) with heating to higher temperatures than 60°C. High-pressure treatment preserves mutagenic inhibition to the same degree as juices freezing.

Keywords: cruciferous vegetables; juice; antimutagenic effects; sulforaphane; isothiocyanates

Fruits and vegetables are rich sources of vitamins, minerals, fiber, and many other biologically active compounds. These substances may exert effects on the detoxification enzymes, stimulate the immune system, reduce blood coagulation, modify hormonal metabolism, reduce blood pressure, and they may also provide antibacterial, antiviral, and antioxidative effects. Many of them are

Supported by the Ministry of Agriculture of the Czech Republic, Projects No. QF3287 and No. QH72149, Research Intention of FRIP CZ00027022-2 and Research Intention of ISBE AS CR, No. AV0Z60870520.

coloured; thus they provide coding that simplifies their selection. For example, red vegetables contain lycopene; yellow-green vegetables contain lutein and zeaxanthin; red-violet vegetables contain anthocyanins; orange-yellow vegetables contain flavonoids; green vegetables contain glucosinolates; and white-green vegetables contain alkyl sulfides. In particular, glucosinolates contained in cruciferous vegetable species, especially in broccoli, exhibit positive biological effects upon enzymatic decomposition to isothiocyanates through the action of myrosinase (Fahey *et al*. 2001; Kalač 2003; Kopec 2010).

Previously published epidemiological studies indicate a significant connection between an increased consumption of fruits and vegetables and a reduced risk of cancer and cardiovascular diseases. The consumption of Brassicaceae family (*Cruciferous*) vegetables (e.g. broccoli, cabbage, savoy cabbage, and Brussels sprouts) is associated with a reduced incidence of cancer, particularly colorectal cancer (Verhoeven *et al*. 1996; Sarikamis 2009). The mechanism of its effect involves the inhibition of proliferation and stimulation of apoptosis in tumor cells (Thornalley 2002; Keck & Finley 2004; Wang *et al*. 2004). Edenharder *et al*. (1995) tested antimutagenic activities of hexane, dichloromethane, acetone, and 2-propanol extracts from 12 vegetable species on *Salmonela typhimurium* TA 98 against the IQ mutagen and found that 96% of hexane extracts showed antimutagenic activity, which was increased after heating, particularly with broccoli and white and red cabbage. Antimutagenic effects can be reduced by heating and high pressure treatments. Butz *et al*. (1997) found that the antimutagenic characteristics of cauliflower declined with rising temperature, but they remained preserved after high pressure treatment. Sedmíková *et al*. (1999) and Houška *et al*. (2006) arrived at similar conclusions.

As mentioned by Mandelová and Totušek (2006), broccoli, being a representative of the cruciferous family, was found to be rich in vitamins C and E, minerals (potassium, magnesium, calcium, and iron), and secondary plant substances, for example glucosinolates. The latter compounds are present as ineffective precursors in the plant; however, they release isothiocyanates (for example, sulforaphane) upon hydrolysis catalysed by myrosinase, which is an enzyme found separately from glucosinolates in the live plant. The mechanisms of biological activity consist of inducing phase 2 biotransformation enzymes (i.e. glutathione transferase, epoxide hydrolase, NAD(P)H, quinone reductase, and glucuronyl transferase) which, at the mammalian level, support the antioxidative activity. These characteristics are attributed particularly to sulforaphane (4-methysulfinylbutylisothiocyanate). Its precursor, glucoraphanin, is abundant in broccoli and represents approximately 44–56% of the total glucosinolate content. Young sprouts of broccoli contain 20–50 times more glucoraphanin (and correspondingly sulforaphane) than mature plants. Also, the proportions of the individual glucosinolates differ according to the age of the plant. Indole glucosinolates predominate in adult plants, while the ratio of indole to other glucosinolates is about equal in broccoli sprouts. This is reflected in variations, particularly in the glucoraphanin content and in the content of sulforaphane resulting from hydrolysis, respectively. The main aim of this paper was to observe the effects of technological treatments (e.g. freezing, pasteurisation, high-pressure treatment) of single-species vegetable juices from cruciferous vegetables (broccoli, cauliflower, Brussels sprouts, white and red cabbage) on the contents of isothiocyanates as the effective substances, and on the antimutagenic activity of the juices produced.

MATERIAL AND METHODS

Equipment. pH-meter Sentron model 1001 (Sentron Technologies, Roden, the Netherlands); refractometer No. 139611 (Carl Zeiss, Jena, Germany); band press Voran 350 (Beskyd, Fryčovice, Czech Republic); hot air dryer MLW WS100 type 117-0100 (Medizin-LaborWerke, Berlin, Germany); high-pressure press type CYX 6/0103 (chamber volume 2 l) (ŽĎAS a.s., Žďár nad Sázavou, Czech Republic); high-pressure press type CYX 6/0410 (chamber volume 120 l) (ŽĎAS a.s., Žďár nad Sázavou, Czech Republic); liquid chromatograph Hewlett Packard HP 1050; microscope Olympus BX 60; colony counter Q count 530 (Spiral Biotech, Canada).

Chemicals. Aflatoxin B1 (AFTB1); 2-nitroso-2-methylurea (MNU), sulforaphane (all from Sigma-Aldrich, Prague, Czech Republic), 2-amino-3-methyl-3H-imidazo-[4,5-*f*]quinoline (IQ)(MP Biomedicals).

Preparation of juices. Single-species juices were prepared from cruciferous plants, namely white

and red cabbage, Brussels sprouts, cauliflower, and broccoli. The raw materials were all purchased locally (Interspar – cooled sales showcases) or were supplied by Beskyd Fryčovice a.s. (Czech Republic). The Champion, Green power (both Mipam bio s.r.o., České Budějovice, Czech Republic) or Twin Health presses were used to prepare the samples. The juices prepared in industrial conditions (mixed apple/broccoli juice) were pressed on a Rotor Vitamat Power Juicer type RVP (Rotor AG, Buttwil, Switzerland) or on a band press Voran 350 (Voran Maschine, HmbH, Pichl bei Wels, Austria). After pressing, the juices were mixed, acidified to pH 4 (optimum pH for releasing sulforaphane) using citric acid and allowed to stand for 60 min (at 20°C); subsequently, the juices were strained, measured, and sealed in containers. Afterwards, they were treated with high-pressure (500 MPa for 10 min), heat pasteurisation (temperature 80°C for 20 min), or frozen. Characteristics such as pH, refraction, yield, and time course of juice production (copied from industrial conditions) were followed during the preparation process. The samples were kept in a refrigerator at 5°C (heat and high pressure treated) or in a freezer at –18°C (untreated).

Content of sulforaphane and total isothiocyanates. Total isothiocyanates and sulforaphane contents were determined as follows: 25 ml of broccoli juice was extracted with 10 ml of dichloromethane (DCM). The mixture was thoroughly shaken, then centrifuged, and the DCM fraction was removed. The rest of the juice was mixed with 5 ml of DCM, thoroughly and repeatedly shaken, then centrifuged. The DCM fractions were collected and this procedure was repeated three times. The DCM fractions were combined, filtered through cotton wool and dried over anhydrous Na_2SO_4. Sulforaphane was extracted and concentrated using an SPE column (Merck LiChrolut Si, content of the sorbent was 700 mg). Sulforaphane was then eluted with methanol (1.5 ml, 1 ml, 0.5 ml), the total volume of the eluate was deducted and the eluate was stored in a freezer. The sulforaphane content was measured using HPLC. The methanolic extract was analysed on a HP 1050 instrument using a Gemini C18 column, 110A, 5 µm, 2 × 150 mm and a DAD detector (HP1040A). The mobile phase was 80% acetonitrile in water. The detection was performed at 365 nm with a flow rate of 0.250 ml per minute. Qualitative and quantitative analyses of sulforaphane were performed using an external sulforaphane standard (Sigma-Aldrich, Prague, Czech Republic).

The determination of total isothiocyanates was performed according to the literature (Tříska *et al*. 2007): 1 ml of vegetable juice was mixed with 2 ml of 0.1M $Na_2B_4O_7$ (pH 9.3) and 0.2 ml of 10mM of 1,2-benzenedithiol. The mixture was incubated for 2 h at 60°C (shaken during incubation) and after cooling, it was extracted with hexane. 1 ml of hexane was added to the mixture, the mixture was shaken vigorously, and the extraction preceeded for 30 minutes. Next, the mixture was centrifuged and hexane was removed from the centrifugation fraction. 0.5 ml of hexane was added to the rest of the mixture, extracted for 15 min, and the hexane fraction was collected. 0.5 ml of hexane was added and the extraction was repeated two more times. The hexane fractions were combined and filtered through cotton wool with anhydrous sodium sulphate. Hexane was evaporated with nitrogen and the residue was then dissolved in 1 ml of methanol. The same procedure was done with the standard: instead of vegetable juice, phenylisothiocyanate was used. HPLC analyses were performed as above.

Determination of antimutagenic activity of single-species pulp juices upon high-pressure treatment, pasteurisation, or freezing. Antimutagenic activity of the samples of single-species pulp juices was evaluated using the Ames test for *Salmonella typhimurium* TA 98 and TA 100 (plate method). As reference mutagens were used 2-nitroso-2-methylurea (MNU) dosed at 100 µg per tray, 2-amino-3-methyl-3H-imidazo-(4,5-*f*)quinoline (IQ) dosed at 10 ng/tray, and aflatoxin B1 (AFTB1) dosed at 4 µg/tray. Metabolic activator S9 was added to AFTB1 and IQ (acting as indirect mutagens); MNU is a direct mutagen requiring no metabolic activation. The tests were performed in two independent experiments. The mutagenic inhibition of sulforaphane was tested similarly. The degree (I) of mutagenic inhibition was calculated according to the following formula:

$$I\ (\%) = 100 - ([R_t\ (\text{tested sample + mutagen})/R_t\ (\text{mutagen})] \times 100)$$

where:

R_t – number of revertant colonies

Evaluation scale of inhibition

0–20	negative
20–40	weakly positive
40–60	positive
60–90	strongly positive
90 and more	probably toxic

Determination of clastogenicity inhibition by broccoli juice *(test in experimental animals).* The inhibition of clastogenic effects of IQ and MNU mutagens by the action of pure sulforaphane and broccoli juice after high-pressure treatment (500 MPa for 10 min) was assessed using the *in vivo* micronucleus test.

The experiment was performed on 7–10 week old maleility of the Faculty of Medicine, Masaryk University (Brno, Czech Republic). The animals were kept under standard laboratory conditions, with a 12-h light/dark cycle, at 22 ± 2°C. The animals were fed a complete laboratory mixture for SPF breeds of mice and rats; water was *ad libitum*. The animals were left undisturbed for one week before the experiment, to let them acclimatise.

The animals were divided into 4 groups of 8 mice each. Broccoli juice was given *per os* to animals in groups I, II, and IV for 14 days, in a dosage of 0.2 ml/10 g of body weight. On day 14, 20 mg/kg (0.2 ml/10 g) of IQ mutagen was given to group I and 50 mg/kg (0.2 ml/10 g) of MNU was given to group III; each compound was given as a single dose, one hour after the ingestion of broccoli juice. A dose of 7% dimethylsulfoxide (DMSO) (0.2 ml/10 g) was given to group IV; this substance was used to dissolve the mutagen. Group II was the control group, thus no application of mutagen or DMSO took place (the same procedure was also used for MNU).

Statistical evaluation. Two samples were prepared from each juice and each sample was analysed twice. The results of the chemical analyses of sulforaphane or isothiocyanates are given as the mean values of four results. The standard deviation (SD) and relative standard deviation (RSD) of chemical analyses results were predicted by repeated analysis of the same sample (standard error of the method). RSD for sulforaphane content was under 5%**,** RSD for total isothiocyanates content was under 12%**.** In special cases (mentioned in the text), the whole procedure, with new raw materials, was repeated after one year.

RESULTS AND DISCUSSION

The greatest number of experiments were performed with broccoli, considering its sulforaphane content and its significant biological effects. The pressing yield of broccoli juice from various plant parts and sulforaphane content were observed at the beginning of the experiments. During the preparation of raw materials, mass proportions were determined first for the stalk, rose, and unusable waste (i.e. leaves). The juice was prepared using a standard procedure, as described above, and placed in containers (100 ml in PA/PE bags). The first part of each sample was frozen and high-pressure treated (500 MPa for 10 min). The second part of each sample was heated-up to 60°C (during 2.5 min), then held at this temperature for 5 min and cooled to 10°C. Each sample was then frozen and kept at –18°C until analysed. The results are provided in Tables 1 and 2.

Based on the results in Tables 1 and 2, it can be stated that broccoli stalks, representing about 30% of the total mass, provide high pressing yields; however, the stalks had a very low sulforaphane content (5 times lower than the roses) under all experimental conditions. This means that the stalks are best suited for diluting the juice of the roses, thus increasing the overall sulforaphane yield from the entire plant. Furthermore, the results confirm the assumption that unlike with high-pressure treatment (500 MPa for 10 min), sulforaphane content shows no significant change on freezing (temperature –18°C), while heating to 60°C for 5 min leads to a partial reduction of sulforaphane content.

Sulforaphane content can be increased by incorporating a delay period after pressing, as illustrated in Table 3. After letting the samples stand, the samples were heated again to 60°C for 440 seconds. A standing time of 60 min proved optimal for sulforaphane formation and was used in the preparation of broccoli juice samples.

Besides broccoli and sulforaphane, other single-species vegetable juices from cauliflower, Brussels

Table 1. Mass proportions of broccoli parts and the characteristics of pressed broccoli juice

Raw material	Mass proportion (%)	Waste (%)	Pressing yield (%)	Pressing yield upon straining (%)	pH	Refraction (%)	Refraction upon straining (%)
Entire plant	95	5	51	37	6.3	6.9	6.2
Rose	64–73	1	48	29	6.4	8.2	7.6
Stalk	27–34	1	71	62	6.4	6.7	6.3

Table 2. Sulforaphane content in broccoli juice obtained from the roses, stalks and entire plants (pH 4; delay 60 min; strained; treated with freezing or high pressure 500 MPa for 10 min) and after preheating to 60°C for 5 min, treated with freezing or high pressure 500 MPa for 10 minutes

Plant part	Final treatment	Sulforaphane content (μg/ml)	
		no preheating	upon preheating
Rose	M	25.06	21.44
Stalk	M	5.66	3.98
Entire plant	M	13.77	12.54
Rose	T	23.42	22.94
Stalk	T	5.07	4.60
Entire plant	T	14.42	12.71

M – final treatment by freezing; T – final treatment by pressure

Table 3. The effect of various time delays (standing time), after pressing, on the sulforaphane content in broccoli juice

Sample labeling	Delay (min)	Sulforaphane content (μg/ml)
Without standing time	0	35.75
Standing time	30	40.51
	60	45.81
	90	34.07

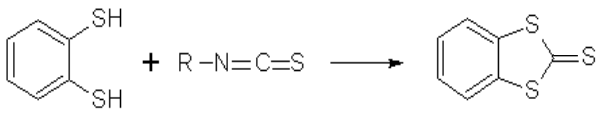
sprouts, and white and red cabbage were also studied. In total five kinds of vegetable juices were analysed, including broccoli, in order to compare the treatment methods (e.g. freezing, pasteurisation and pressurisation). Before the individual determinations, the juices were strained, sometimes they were also filtered (depending on the analysis methods), and sulforaphane and total isothiocyanates were determined using HPLC. Isothiocyanates (ITCs) determination was based on their reaction with 1,2-benzenedithiol, in which

Table 4. Comparison of sulforaphane and isothiocyanate content in individual single-species juices, treated with high pressure, freezing and pasteurisation

Raw material	Final treatment	Sulforaphane content (μg/ml)		Isothiocyanates content (μmol/l)	
		experiment 1	experiment 2	experiment 1	experiment 2
White cabbage	M	2.77	0.63	7.02	1.00
	P	2.54	0.57	5.99	2.57
	T	2.70	0.62	4.21	1.30
Broccoli	M	7.77	14.25	10.68	1.45
	P	8.87	15.67	5.54	1.94
	T	8.24	14.64	11.27	1.87
Red cabbage	M	8.94	4.35	9.81	1.58
	P	7.99	4.92	5.39	2.05
	T	10.47	4.98	3.85	1.92
Cauliflower	M	0.66	1.61	25.92	0.79
	P	0.69	0.98	16.15	2.80
	T	0.64	1.29	10.92	1.04
Brussels sprouts	M	4.74		29.64	
	P	4.38		24.67	
	T	4.90		21.46	

M – final treatment by freezing; P – final treatment by pasteurisation; T – final treatment by pressur

a cyclocondensation product (1,3-benzenedithiol-2-thione) is formed according to the following equation (Zhang *et al*. 1996).



Based on the literature and Choi *et al*. (2004), who studied the reactivity of 5 various isothiocyanates (*R* = propyl-, butyl-, benzyl-, phenetyl-, and 4-methoxyphenyl-), it can be expected that various isothiocyanates react with the aforementioned compound with minimum yield of 90%, thus the amount of cyclocondensation products corresponds to the total amount of isothiocyanates (expressed as molar concentration). Thanks to its spectral characteristics (ε = 23 000 l·mol^{-1}·cm^{-1} at a wavelength of 365 nm), this compound can be measured using spectrophotometry or HPLC. Its determination using GC–MS has been described in the literature as well.

It follows from Table 4 that the highest sulforaphane content was found in juices from broccoli and red cabbage, red cabbage being a considerable surprise, since this fact has not been previously mentioned in the literature. The differences caused by various methods of juices treatment were not significant. Cauliflower juice showed the lowest sulforaphane content. High annual variations also follow from the data (one year elapsed between experiments 1 and 2).

It is apparent that the juices from Brussels sprouts and cauliflower contain the highest quantities of total isothiocyanates (ITCs). The lowest content of total ITCs was found in the juice from white cabbage. It is of interest that except for broccoli, the trend was that total ITCs quantities decline after the pressure treatment, unlike with freezing, which can probably be explained by the different chemical structures of the other ITCs. In broccoli, where the highest quantities of ITCs are found in the form of sulforaphane, the trend was that the content was preserved even after the pressure treatment.

Antimutagenic activity of single-species pulp juices was assessed using the Ames test; AFTB1 mutagenic inhibition was tested using Aflatoxin B1 (4 μg/tray) and *Salmonella typhimurium* TA 98, with metabolic activator S9. The assessment was performed in two independent experiments. A tabular presentation of the results is given in Table 5.

Table 5. AFTB1 mutagenic inhibition induced by single-species pulp juices

Pulp juice	Treatment	Mutagenicity inhibition
Cauliflower	high pressure	positive to weakly positive
	freezing	positive to weakly positive
Red cabbage	high pressure	positive to weakly positive
	freezing	weakly positive
Brussels sprouts	high pressure	strongly positive to positive
	freezing	positive to weakly positive
White cabbage	high pressure	negative
	freezing	negative

High-pressure treatment provided positive effects in juices from cauliflower, red cabbage, and Brussels sprouts. The juice from white cabbage did not provide any evidence for AFTB1 mutagenic inhibition, and its treatment with heat pasteurisation caused no inhibition either.

IQ mutagenic inhibition was tested using IQ mutagen (10 ng/tray) and *Salmonella typhimurium* TA 98 with metabolic activator S9. The assessment was done in two independent experiments and the tabular presentation of the results can be seen in Table 6.

Neither method of treatment (high pressure or freezing) had an impact on the strongly positive to positive mutagenic inhibition of the IQ mutagen; heat pasteurisation completely suppressed the inhibition in all juices.

MNU mutagenic inhibition was tested using MNU (100 μg/tray) and *Salmonella typhimurium* TA 100, but without the metabolic activator S9. The determination was performed in two independent experiments. The tabular presentation of the results can be seen in Table 7.

Table 6. IQ mutagenic inhibition induced by single-species pulp juices

Pulp juice	Treatment	Mutagenicity inhibition
Broccoli	high pressure	strongly positive
	freezing	strongly positive
Cauliflower	high pressure	strongly positive
	freezing	strongly positive
Red cabbage	high pressure	strongly positive
	freezing	strongly positive
Brussels sprouts	high pressure	strongly positive to positive
	freezing	strongly positive to positive
White cabbage	high pressure	strongly positive
	freezing	strongly positive

Table 7. MNU mutagenic inhibition induced by single-species pulp juices

Pulp juice	Treatment	Mutagenicity inhibition
Broccoli	high pressure	strongly positive to positive
	freezing	strongly positive to positive
Cauliflower	high pressure	strongly positive to positive
	freezing	positive to weakly positive
Red cabbage	high pressure	positive to weakly positive
	freezing	positive to weakly positive
White cabbage	high pressure	positive to weakly positive
	freezing	positive to weakly positive
Mixed fruit-vegetable juice	high pressure	strongly positive to positive
	freezing	positive to weakly positive

The comparison of antimutagenic activities of single-species vegetable juices is presented in Table 8.

It is apparent from Table 8 that a high degree of inhibition of both mutagens was shown in the majority of frozen juices (except white cabbage for mutagen AFTB1). On the other hand, heat pasteurisation, with the exception of broccoli and Brussels sprouts, eliminated the ability to inhibit mutagens AFTB1 and IQ; although there was a substantial reduction in the inhibition ability even with the exceptions mentioned. High-pressure treatment preserved in all cases the same degree of inhibition seen after freezing. In red cabbage and Brussels sprouts (AFTB1) and white cabbage (IQ mutagen), the level of inhibition in the pressurised juices was slightly higher than in frozen ones. The results for MNU, as a direct mutagen, were similar. The pasteurised samples showed virtually no inhibition of mutagenicity except for broccoli, which was weakly positive. High-pressure treatment, in all cases, preserved the same degree of mutagenic inhibition as freezing.

Clastogenicity (*in vivo* micronucleus test) was studied during a two-week administration of broccoli juice to the experimental laboratory animals. The results demonstrated a statistically significant reduction in the number of micronuclei induced by the application of both mutagens IQ and MNU. The results are presented in Table 9.

Broccoli juice administered *per os*, using a probe, produced a statistically significant ($P < 0.01$) reduction in the number of micronuclei in group I compared to group III (single administration of IQ only). The numbers of micronuclei showed no statistically significant difference between group II (administered broccoli juice only) and group IV

Table 8. Summary of results from the Ames test for vegetable juices with AFTB1, IQ and MNU mutagens (results of two independent experiments are provided for AFTB1 and IQ; all the presented results are mean values from four trays)

Mutagen	Juice	Inhibition/Selection for coefficient of dilution 1 (%)		
		frozen	pasteurised	treated with high pressure
AFTB1	broccoli	70/64	31/39	71/75
	cauliflower	52/45	0/0	44/55
	red cabbage	39/26	10/0	50/50
	brussels sprouts	53/42	0/0	74/70
	white cabbage	0/0	0/0	0/0
IQ	broccoli	79/86	41/–	81/81
	cauliflower	92/–	0/–	90/–
	red cabbage	77/81	0/–	79/85
	brussels sprouts	83/83	35/0	70/85
	white cabbage	80/81	2/-	90/84
MNU	broccoli	64	36	72
	cauliflower	56	0	68
	red cabbage	53	22	58
	brussels sprouts	–	–	–
	white cabbage	43	0	48
	apple – broccoli	62	19	65

Table 9. The effect of broccoli juice on formation of micronuclei induced by the IQ mutagen and MNU

Group	Applied substances	MNPCE/1000 PCE	No. of animals	Mean ± SD
IQ mutagen				
I.	broccoli (14 × 0.2 ml/10 g) + IQ (1 × 20 mg/kg)	3, 3, 3, 2, 3, 4, 2	7	2.86 ± 0.69*
II.	broccoli (14 × 0.2 ml/10 g)	2, 3, 1, 3, 2, 2, 1, 3	8	2.13 ± 0.83
III.	IQ (1 × 20 mg/kg)	6, 8, 8, 5, 5, 5, 6, 7	8	6.25 ± 1.28**
IV.	7% DMSO (1 × 0.2 ml/10 g)	1, 2, 3, 3, 2, 3, 1, 2	8	2.13 ± 0.83
MNU				
I.	broccoli (14 × 0.2 ml/10 g) + MNU (1 × 50 mg/kg)	8, 7, 7, 6, 8, 8, 8, 7	8	7.38 ± 0.74*
II.	broccoli (14 × 0.2 ml/10 g)	2, 2, 3, 3, 3, 0, 2, 3	8	2.25 ± 1.04
III.	MNU (1 × 50 mg/kg)	17, 16, 20, 21, 20, 16, 16, 18	8	18.0 ± 2.07**
IV.	7% DMSO (1 × 0.2 ml/10 g)	1, 2, 3, 2, 0, 2, 3, 2	8	1.88 ± 0.99

MNPCE – number of micronuclei in polychromatic erythrocytes; PCE – polychromatic erythrocytes; SD – standard deviation; *$P < 0.01$ – significantly lower number of micronuclei relative to IQ; **$P < 0.01$ – significantly higher number of micronuclei relative to controls (7% DMSO)

(single administration of 7% DMSO). A statistically significant difference in the number of micronuclei was found between group III (single administration of IQ only) and group IV (7% DMSO) at a significance level of $P < 0.01$. The results provide evidence for the suppressive action of broccoli juice on the clastogenic effect of the IQ mutagen.

In animals assigned to group I (administration of broccoli juice and MNU), a statistically significant ($P < 0.01$) reduction in the number of micronuclei was found compared to group III animals (administration of MNU only), however, there was a statistically significant ($P < 0.01$) increase in micronuclei compared to group II (administration of broccoli juice only). In group III, where only the mutagen was applied, a statistically significant ($P < 0.01$) difference was seen when compared to group IV (7% DMSO). Micronuclei numbers showed no statistically significant difference between group II (application of broccoli juice only) and group IV (application of 7% DMSO). The results offer evidence for the suppressive action of broccoli juice on clastogenic effects of MNU.

Note: Broccoli juice was administered to male BALB-C mice for 14 days at a dosage of 0.2 mg/10 g of the body weight. Percentage weight increments in the course of the first week were 8.15%, 7.94%, 6.59%, and 5.95% (in the stated order). After 14 days, these increments were 0.71%, 2.79%, 5.84%, and 10.15%, respectively. The effect of broccoli juice was not statistically significant ($P > 0.05$).

CONCLUSIONS

The results of this paper provide evidence that juices from cruciferous vegetable species treated with high pressure exhibit similar levels of sulforaphane and total isothiocyanates as frozen juices. Biological activity, expressed by antimutagenicity, as assessed using the Ames test, is preserved after pressure pasteurisation of the juices or after freezing, while heat pasteurisation causes a substantial reduction or complete elimination of biological activity. The clastogenicity test, assessed using the micronucleus test, showed that broccoli juice causes a statistically significant suppression of the effects of mutagens IQ and MNU. High-pressure pasteurisation technology should be applied during the preparation of foods based on cruciferous vegetable species in order to retain the positive benefits coming from the biologically active substances.

References

Butz P., Edenharder R., Fister H., Tauscher B. (1997): The influence of high pressure processing on antimutagenic activities of fruit and vegetable juices. Food Research International, **30**: 287–291.

Choi M.M.F., Shuang S., Lai H.Y., Cheng S.C., Cheng R.C.W., Cheung B.K.B., Lee A.W.M. (2004): Gas chromatography-mass spectrometric determination of total isothiocyanates in Chinese medicinal herbs. Analytica Chimica Acta, **516**: 155–163.

Edenharder R., Leopold C., Kries M. (1995): Modifying action of solvent extracts from fruit and vegetable residues on 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline (MeIQX) induced mutagenesis in *Salmonella typhimurium* TA-98. Mutation Research-Genetic Toxicology, **341**: 303–318.

Fahey J.W., Zalcmann A.T., Talalay P. (2001): The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, **56**: 5–51.

Houška M., Strohalm J., Kocurová K., Totušek J., Lefnerová D., Tříska J., Vrchotová N., Fiedlerová V., Holasová M., Gabrovská D., Pauličková I. (2006): High pressure and foods-fruit/vegetable juices. Journal of Food Engineering, **77**: 386–398.

Kalač P. (2003): Funční potraviny kroky ke zdraví. Dona Publishers, České Budějovice.

Keck A.S., Finley J.W. (2004): *Cruciferous* vegetables: cancer protective mechanism of glucosinolate hydrolysis products and selenium. Integrative Cancer Therapies, **3**: 5–12.

Kopec K. (2010): Zelenina ve výživě člověka. Grada Publishers, Prague.

Mandelová L., Totušek J. (2006): Antimutagenní aktivita brokolicové šťávy ošetřené vysokým tlakem. Česká a slovenská hygiena, **1**: 6–10.

Sarikamis G. (2009): Glucosinolates in crucifers and their potential effects against cancer: Review. Canadian Journal of Plant Science, **89**: 953–959.

Sedmíková M., Turek B., Bárta I., Strohalm J., Šmerák P., Houška M., Müllerová J. (1999): Evaluation of the antimutagenic activity of pressure treated and heat pasteurized cauliflower juice. Czech Journal of Food Sciences, **17**: 149–152.

Thornalley P.J. (2002): Isothiocyanates: mechanism of cancer chemo-preventive action. Anticancer Drugs, **13**: 331–338.

Tříska J., Vrchotová N., Houška M., Strohalm J. (2007): Comparison of total isothiocyanates content in vegetable juices during high pressure treatment, pasteurization and freezing. High Pressure Research, **27**: 147–149.

Verhoeven D.T.H., Goldbohm R.A., van Poppel G., Verhagen H., van den Bandt P.A.. (1996): Epidemiological studies on brassica vegetables and cancer risk. Cancer Epidemiology, Biomarkers & Prevention, **5**: 733–748.

Wang L., Liu D., Ahmed T., Chung F.L., Conaway C., Chiao J.W. (2004): Targeting cell cycle machinery as a molecular mechanism of sulforaphane in prostate cancer prevention. International Journal of Oncology, **24**: 187–192.

Zhang Y., Wade K.L., Prestera T., Talalay P. (1996): Quantitative determination of isothiocyanates, dithiocarbamates, carbon disulfide, and related thiocarbonyl compounds by cyclocondensation with 1,2-benzenedithiol. Analytical Biochemistry, **239**: 160–167.

Received for publication October 13, 2010
Accepted after corrections January 15, 2011

Corresponding author:

Ing. Milan Houška, CSc., Výzkumný ústav potravinářský Praha, v.v.i., Radiová 7, 102 31 Praha 10, Česká republika
tel.: + 420 296 792 306, e-mail: m.houska@vupp.cz