Functional food components prepared with assistance of high pressure treatment

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Summary

This paper reports on research results regarding the effect of different preservation methods (heat pasteurisation, high pressure pasteurisation and freezing) on the content of health important chemical components (vitamins, polyphenols) in the vegetable juices and their anti-mutagenic activity. The chilled cruciferous vegetable such as cauliflower, broccoli, red cabbage and Brussels sprouts were used for single sort vegetable juices preparation. These juices contain potentially large number of glucosinolates and their enzymatic (myrosinase) decomposition products (isothiocyanates) that are regarded as potentially anti-cancer anti-mutagenic agents provoking the decontamination processes in human body cells (chemoprevention). The paper is focused to the effect of preparation methods on sulforaphane content in broccoli juice and the high pressure treatment (time and level of pressure) on the sulforaphane content in the juice. The anti-mutagenicity of these juices is compared and effect of preservation treatment is evaluated. These juices can be used as components of functional foods (fruit-vegetable juices) having possible preventative anti-cancer and anti-mutagenic effects.

Introduction

The high pressure treatment of foods enables also the production of non-traditional products, Cheftel (1992), Earnshaw (1995) and Knorr (1995). Tewari et al (1999) published the comprehensive review of existing research results and industrial applications. There are already known commercial applications of high pressure pasteurisation of foods namely in Japan, USA, France, Spain and Germany. The commercial success of the high pressure treated products is related to their higher nutritive value, freshness and appearance. Due to the higher costs of high pressure processing in comparison with the existing heat treatment there is a chance to treat the high value and high quality original products with some health benefit by this process only. These demands are provided by the functional foods.

Typical examples of these products are juices prepared from cruciferous vegetables such as cauliflower, broccoli, red cabbage and Brussels sprouts. These vegetables contain high level of glucosinolates that react with enzyme myrosinase during processing and health active isothiocyanates can create. These substances have the antimutagenic effect, see e.g. Edenharder et al (1994). Sedmikova et al (1999) showed the high antimutagenic activity of the cauliflower juice preserved by freezing and high pressure pasteurisation whereas heat pasteurised juice exhibited no antimutagenic activity.

In broccoli juice it is well known isothiocyanate sulforaphane, Butz et al (1997). Butz and Tauscher (2000) showed that this substance is relatively stable during high pressure pasteurisation. This substance is the product of the reaction of glucoraphanine and myrosinase. Sulforaphane is known as the substance with anti-mutagenic and preventive anti-cancer activity, Fahey et al. (1997, 2001), Zareba and Serradelf (2004). Fahey et al (2002) showed that sulforaphane has also the antimicrobial activity against *Helicobacter pylori*.

Houska et al (2004) found that broccoli freezing before juice squeezing has a fatal effect on the sulforaphane content in the juice. Therefore, there is the need for research of preparation and preservation procedures assuring in the broccoli juice the highest possible level of the sulforaphane.

The main goal of this paper is to present some parameters influencing the sulforaphane concentration in prepared broccoli juice and to show the vitamin C, polyphenols content and antimutagenic activity of the juices prepared from different cruciferous vegetables and stabilised with different preservation methods (freezing, heat pasteurisation, high pressure pasteurisation).

Material and methods

All details of juice preparation and detailed description of experiments and procedures are given in the research report by Houska et al (2004).

Solid-phase extraction (SPE) of sulforaphane

LiChrolut Si columns (200 mg) were purchased by Merck (Germany). The original procedure was described by Bertelli et al. (1998) and the whole procedure with the minor modifications is as follows: activation of column with 5 ml of dichloromethane, sample application (juices were shaken with dichloromethane and concentrated dichloromethane extracts have been applied on SPE columns), washing of column with dichloromethane (5 ml), washing of column with ethylacetate (5 ml), column drying, elution of sulforaphane with methanol (3 x 1 ml) and eluate further concentrated if necessary.

Method of sulforaphane analysis in broccoli juice

High pressure liquid chromatograph Hewlett – Packard 1050 (USA) with the column Phenomenex Luna C18 (2), 3 μm, 2 x 150 mm, DAD detector Hewlett – Packard 1040A, mobile phase A = 5% acetonitrile + 0.15% trifluoroacetic acid, mobile phase B= 80% acetonitrile + 0.15% trifluoroacetic acid, gradient: 0 – 45%, B … 30 min has been used for sulforaphane analysis. Detection at 245 nm, flow rate 0.250 ml/min. By repeating analysis of the same broccoli juice sample the relative standard deviation 4.35 % was determined.

Anti-mutagenic activity of broccoli juice

Anti-mutagenic activity of the broccoli juice was studied under model conditions using bacterial strains of *Salmonella typhimurium* TA98 (Ames test). As model mutagen aflatoxin B1 was used in dose 4μg per plate. Tests were performed in the presence of metabolic activator S9 (mixture of the liver enzymes obtained from rats).

Instruments and machinery

Green power and Champion juicers (USA), Vitamat Power Juicer type RVP (Rotor AG, Switzerland), pH-meter SENTRON model 1001 (Netherlands), high-pressure equipment ŽĎAS, type CYX 6/ 0103 (Czech Republic) with chamber volume 2 litres were used.

Ascorbic acid analysis

The ascorbic acid was determined by titration method by 2, 6-di-chlorophenolindophenol (standard method described in Czech Standard ISO 6557/2). The potentiometric indication of equivalence point was used. Method had the variability coefficient of 7% of the mean value. Each result is the mean of two independent analyses that differ less than standard variability.

Total polyphenols

0.1 ml of the filtered vegetable juice was placed into 50 ml volumetric flask. After dilution with 20 ml of distilled water, 2.5 ml of Folin-Ciocalteu solution was added together with 7.5 ml of 20% sodium carbonate solution. The resulting blue coloured solution was very well mixed and flask filled-up to 50 ml by distilled water. The solution was kept standing for 2 hours and then the absorbance at 765 nm was measured and compared with blind experiment. Total polyphenols are calculated as amount of gallic acid in mg/l00 ml.

Selected phenolic acids

Selected phenolic acids have been analysed by high performance liquid chromatography with reversed phase column and gradient elution. Samples for analysis have been filtered over combined paper filter and over the membrane filter Whatman 0.45 μm PP. The mass balance was made for vegetable juices. Chromatographic conditions: column C18 Lichrospher 100RP-18;5 μm; Lichrospher Cart 250x4 mm. Mobile phase A - MetOH/H_2O (5/95), B - MetOH/H_2O (40/60), pH value at both mobile phases was adjusted to pH=2.5 by phosphoric acid (1/1 v/v). The flow rate was 1ml/min, injection volume 20 μl, detection at 280 nm. The following standards of ferulic, caffeic and chlorogenic acids, delivered by Fluka Chemie GmbH (Switzerland), have been used. Calibration curve was gained by means of different dilution of basic standard.

Preparation of juices from cruciferous vegetables for vitamins and antimutagenic activity

Chilled broccoli, Brussels sprouts, cauliflower, red cabbage has been delivered from Beskyd Frycovice Inc., Czech producer of fresh vegetable salads. The pasteurised frozen limet juice concentrate was used for pH=4 adjustment. The juice was prepared by using above mentioned juicers. One part of the same juice was frozen at -18°C, one part pasteurised by high pressure at 500 MPa for 10 minutes. Temperature of the juice was between 15-22°C, temperature of the water in pressure chamber changed between 12-20°C. Adiabatic compression heating of the juice can be regarded similar for water, about 15°C. Third part of the same juice batch was heat pasteurised at 80°C for 20 minutes in hot air sterilizer and chilled. Pressure and heat pasteurised juices were stored in the refrigerator at 4-6°C. Pressure treated samples have been stored in PET 100 ml bottles; heat pasteurised samples have been treated and stored in PA/PE cooking pouches (VAC STAR).

Preparation of broccoli juice for sulforaphane content (kinetics experiments)

The chilled broccoli from supermarket (imported from Poland) was juiced on Green power juicer. Received juice (about 800 ml) with pH=6.2 was mixed with citric acid and pH adjusted to pH=4. The first sample was taken from the batch. The batch was then chilled from 22 to 15°C within 30 minutes. Then every 60 minutes during 4 hours samples have been taken from the batch. Each sample was frozen after sampling. Next day all samples were thawed gently in the refrigerator and analysed for the sulforaphane content.

Preparation of broccoli juice for study of influence of high pressure pasteurisation on sulforaphane content

The chilled broccoli from supermarket (imported from Spain) was juiced on Green power juicer (initial temperature of the juice 21°C). Received juice (about 800 ml) with pH=6.2 was mixed with citric acid and pH adjusted to pH=4. This juice was placed into the refrigerator for 3 hours and then filled into 100 ml PET bottles and stored overnight for 18 hours in the refrigerator at 4-6°C. Then samples have been thermostated to 6.8-7.6°C and pressure treated with different pressures for different holding times (350, 400, 450 a 500 MPa for 3, 5, 7 and 10 minutes). One sample has not been treated for comparison reason. After pressure treatment the samples have been stored at temperature 8-10°C. All samples have been frozen and stored at -18° after finishing the treatment. Before sulforaphane analysis the samples have been gently thawed in the refrigerator.

Results and discussion

Vitamin C

The results of analysis of vitamin C are given in Table 1. It is apparent from this table that the highest vitamin C content was found in the juice from red cabbage. Broccoli juice exhibited nearly half vitamin C content of red cabbage juice; about 30 mg per 100 g of the juice. Cauliflower and Brussels sprouts exhibited lowest vitamin C content. The preservation method influenced substantially the vitamin C content only in the case of cauliflower juice. In the case of Brussels sprouts juice the frozen juice exhibited lowest vitamin C content but this result cannot be simply explained to be caused by described treatment.

Table 1 Vitamin C contents in juices of cruciferous vegetables preserved by different methods (in mg/100g of the juice)

Treatment	Cauliflower juice	Broccoli juice	Red cabbage juice	Brussels sprouts juice
Heat pasteurised	7.9	28.4	54.3	8.6
High pressure	7.4	29.4	58.7	2.9
Frozen	14.4	32.3	56.9	1.3

Total polyphenols

The highest content of polyphenols was found in juices prepared from red cabbage and Brussels sprouts; see Table 2. The total polyphenol content is not dependent on the preservation method.

Table 2 Total polyphenols contents in juices of cruciferous vegetables preserved by different methods (in mg/100g of the juice)

Treatment	Cauliflower juice	Broccoli juice	Red cabbage juice	Brussels sprouts juice
Heat pasteurised	130	136	275	260
High pressure	130	137	280	270
Frozen	130	136	280	270

Selected phenolic acids

The results of analyses are given in Table 3. Cauliflower and Brussels sprouts juices exhibited only chlorogenic acid. Broccoli juice contained chlorogenic and ferulic acids. Red cabbage juice exhibited all analysed acids; it corresponds with the highest total polyphenols content. The preservation methods did not influence substantially the analysed components with exception of heat pasteurised cauliflower juice. This juice contained the highest level of the chlorogenic acid. The chlorogenic acid content was lowest in heat pasteurised red cabbage and Brussels sprouts juices. The ferulic acid content was lowest in heat pasteurised broccoli and red cabbage juices whereas high pressure treated and frozen juices showed nearly the same contents of this acid.

Table 3 Selected phenolic acids content in juices of cruciferous vegetables preserved by different methods (in mg/100 ml of the juice)

Treatment	Cauliflower juice	Broccoli juice	Red cabbage juice	Brussels sprouts juice
Chlorogenic acid				
Heat pasteurised	9.03	1.03	0	0.48
High pressure	2.19	1.14	0.73	1.48
Frozen	2.12	1.28	0.71	1.56
Ferulic acid				
Heat pasteurised	0	0.33	3.58	0
High pressure	0	0.58	3.96	0
Frozen	0	0.41	4.11	0
Caffeic acid				
Heat pasteurised	0	0	0.56	0
High pressure	0	0	0.57	0
Frozen	0	0	0.56	0

Antimutagenic activity of juices

The biological activity tests have been made in the two independent experiments; in each experiment three replications have been made. Results of experiments are given in Tables 4, 5, 6 and 7 for broccoli, cauliflower, red cabbage and Brussels sprouts juices, respectively. The inhibition rate was calculated from number of revertants R_t by the relation

$$\text{Inhibition rate [\%]} = 100 - [(R_t \text{ (juice + mutagen)} / R_t \text{ (mutagen)}) .100]$$

The inhibition rate is ranged by the following way: 0 – 20 % negative, 20 – 40% weak positive, 40 – 60 % positive, 60 – 90% strongly positive, > 90% probably toxic

Table 4 Results of Ames test for frozen, heat pasteurised and high pressure pasteurised broccoli juice

	Frozen		Heat pasteurised		High pressure pasteurised	
Dilution coefficient of the juice	R_t	Inhibition[%]	R_t	Inhibition [%]	R_t	Inhibition [%]
1	119	35	89	75	54	82
1	84	54	75	79	53	82
2	71	61	101	71	177	61
2	52	72	138	61	128	57
5	81	56	181	49	287	37
5	85	54	192	45	278	39
10	89	52	241	32	312	31
10	88	52	174	51	353	22
1 (without mutagen)	30 26		34 25		22 30	
Negative control	37		38		39	
Positive control	184		352		299	

Remark: dilution 1= not diluted, valid for dose of 4µg of aflatoxin B1

The results given in Table 4 for broccoli juice show that heat and high pressure pasteurised juices presented strongly positive inhibition rate of the used mutagen. Frozen juice showed positive inhibition rate.

Table 5 Results of Ames test for frozen, heat pasteurised and high pressure pasteurised cauliflower juice

	Frozen		Heat pasteurised		High pressure pasteurised	
Dilution coefficient of the juice	R_t	Inhibition[%]	R_t	Inhibition [%]	R_t	Inhibition [%]
1	32	83	114	68	151	57
1	75	72	66	81	150	50
2	46	75	161	54	214	28
2	79	70	127	64	198	34
5	56	70	179	49	230	23
5	57	79	163	54	239	20
10	82	55	192	45	241	19
10	111	58	211	40	244	18
1 (without mutagen)	27 22		27 23		37 36	
Negative control	37 29		39		39	
Positive control	184 267		299		299	

Remark: dilution 1= not diluted, valid for dose of 4µg of aflatoxin B1

The results given in Table 5 for cauliflower juice show that the heat pasteurised and frozen juices presented strongly positive and positive inhibition rate of the used mutagen.

High pressure pasteurised juice showed positive or weak positive inhibition rate (dependent on dilution of the juice).

Table 6 Results of Ames test for frozen, heat pasteurised and high pressure pasteurised red cabbage juice

	Frozen		Heat pasteurised		High pressure pasteurised	
Dilution coefficient of the juice	R_t	Inhibition[%]	R_t	Inhibition [%]	R_t	Inhibition [%]
1	88	67	467	0	338	12
1	92	66	323	8	312	19
2	110	59	385	0	309	20
2	118	56	330	6	231	40
5	154	42	405	13	332	14
5	124	54	493	0	543	0
10	189	29	277	21	374	3
10	162	39	366	0	442	0
1 (without mutagen)	23 23		31 35		34 35	
Negative control	29		37		38	
Positive control	267		352		385	

Remark: dilution 1= not diluted, valid for dose of 4µg of aflatoxin B1

The results given in Table 6 valid for red cabbage juice show that frozen and high pressure pasteurised samples presented strongly positive or positive inhibition rate of the used mutagen. Heat pasteurised juice did not exhibit the inhibition effect.

Table 7 Results of Ames test for frozen, heat pasteurised and high pressure pasteurised Brussels sprouts juice

	Frozen		Heat pasteurised		High pressure pasteurised	
Dilution coefficient of the juice	R_t	Inhibition[%]	R_t	Inhibition [%]	R_t	Inhibition [%]
1	253	50	1317	neg.	214	72
1	-	-	1440	neg.	186	76
2	446	12	967	neg.	232	70
2	-	-	900	neg.	212	73
5	690	neg.	747	3	633	18
5	-	-	607	21	630	18
1 (without mutagen)	39		44 53		43 29	
Negative control	39		44		44	
Positive control	507		773		773	

Remark: dilution 1= not diluted, valid for dose of 4µg of aflatoxin B1

The results given in Table 7 valid for Brussels sprouts juice show that high pressure pasteurised samples presented strongly positive inhibition rate even in double dilution by distilled water. Heat pasteurised juice showed no inhibition rate. The number of revertants was higher than positive control! The frozen juice exhibited the positive inhibition rate.

Kinetics of sulforaphane content in broccoli juice

It is apparent from Table 8 that sulforaphane content is increasing during first 180 minutes of the chilled storage since juice squeezing. The reaction of glucoraphanine with myrosinase is probably finished during this time. The prepared juice has to be filled into the bottles and high pressure pasteurised or frozen to keep the sulforaphane content relatively stable. The additional experiment with frozen juice stored at -18°C for 7 days showed that sulforaphane content decreased during this storage time to the level 6.6μg/ml. It represents the 89.6% of the original value.

Table 8 Sulforaphane content changes during chilled storage since broccoli juice squeezing

Sample number	Sulforaphane content (μg/ml)	Relative sulforaphane content (%)	Time since squeezing (minutes)
1	7.41	100	0
2	7.85	105.98	60
3	7.76	104.70	120
4	7.90	106.68	180
5	7.31	98.68	240

Influence of high pressure treatment on sulforaphane content in broccoli juice

Results given in Table 9 show that pressure and holding time do not influence the sulforaphane content in the broccoli juice. The Grubbs statistical test of outlayers was made and sample number 5 was excluded from data set. The mean value of sulforaphane content without outlayer sample is 16.2μg/ml. The standard deviation calculated from data has value 0.77μg/ml and reliability interval (for importance level α=0.05) has value 0.42μg/ml. The relative standard deviation is 0.77/16.2*100=4.7%. This value is fully comparable with relative standard deviation of the repeated analysis of the same sample (see above mentioned value 4.35%). This is the evidence that changes in sulforaphane content caused by high pressure treatment are negligible and within the error of the analytical procedure.

Table 9 Sulforaphane content in the broccoli juice as a function of pressure and holding time

Sample number	pressure (MPa)	Holding time (s)	Sulforaphane content (μg/ml)	Remark
1	0	0	17.21	Not pressure treated
2	0	0	14.73	
3	350	180	16.51	
4	350	300	14.96	
5	350	420	18.93	excluded outlayer
6	350	600	16.38	
7	400	180	15.81	
8	400	300	16.44	
9	400	420	17.52	
10	400	600	17.29	
11	450	180	16.31	
12	450	300	15.93	
13	450	420	15.91	
14	450	600	15.89	
15	500	180	16.04	
16	500	300	15.16	
17	500	420	16.41	
18	500	600	16.65	

Conclusions

Sulforaphane content grows in the pulp broccoli juice during 180 minutes after squeezing. The high pressure treatment does not influence the sulforaphane content in this juice in the pressure range 350-500 MPa and holding times 3-10 minutes.

Vitamin C content is known as the most sensitive substance on procedure of juice squeezing and preservation method. The freezing and high pressure pasteurisation kept the higher levels of the vitamin C content in the broccoli and red cabbage juices. The cauliflower and Brussels sprouts juices are not rich sources of the vitamin C and their preservation methods provided inconsistent results that cannot be simply explained.

Red cabbage and Brussels sprouts juices are rich sources of the total polyphenols. Cauliflower and broccoli juices exhibited nearly half content of total polyphenols. The preservation methods did not show any significant effect on the total polyphenols content.

Cauliflower and Brussels sprouts juices exhibited only chlorogenic acid. Broccoli juice contained chlorogenic and ferulic acids. Red cabbage juice exhibited all analysed acids; it corresponds with the highest total polyphenols content. The preservation methods did not influence substantially the analysed components with exception of heat pasteurised cauliflower juice. This juice contained the highest level of the chlorogenic acid. The chlorogenic acid content was lowest in heat pasteurised red cabbage and Brussels sprouts juices. The ferulic acid content was lowest in heat pasteurised broccoli and red cabbage juices whereas high pressure treated and frozen juices showed nearly the same contents of this acid.

Antimutagenic activity of cruciferous vegetable juices treated by different preservation methods was evaluated. The influence of the preservation method is not the same for different juices. For undiluted juices the Table 10 was prepared for inhibition rate comparison.

Table 10 Comparison of inhibition rates (%) for undiluted juices

Preservation method	Cauliflower juice	Broccoli juice	Red cabbage juice	Brussels sprouts juice
Frozen	72-83	35-54	66-67	50
Heat pasteurised	68-81	75-79	0-8	negative
High pressure pasteurised	50-57	82	12-19	72-76

For cauliflower juice the highest inhibition rate was found for frozen and heat pasteurised juices but high pressure pasteurised juice showed lower inhibition rate.

For broccoli juice the highest inhibition rate was found for the high pressure and heat pasteurisation.

For red cabbage juice the highest inhibition rate was found for frozen juice. Heat pasteurised red cabbage juice does not practically inhibit the used mutagen.

For Brussels sprouts juice the highest inhibition rate was found for high pressure pasteurisation. The heat pasteurisation juice showed negative inhibition rate (number of revertants was higher than positive control).

The absolute highest inhibition rate was presented for the frozen cauliflower juice and for the high pressure pasteurised broccoli juice. The high pressure pasteurisation can be regarded as effective preservation method that saves the antimutagenic activity of most juices of cruciferous vegetables (with exception of red cabbage).

These juices can be used as components of the functional foods preserved by high pressure pasteurisation.

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