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THE EFFECT OF HIGH-PRESSURE PROCESSING ON ENTERAL FOOD MADE FROM FRESH OR SEMI-FINISHED INGREDIENTS

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Abstract

The enteral feeding can be defined as a delivery of nutrients directly into the stomach, called also enteral nutrition. Dietetic products for enteral nutrition are a specific group of products designed to provide nutrients to the human body in case of various diseases and after surgery, when the daily intake of the product is affected. Today market offers special dietetic products, which are supplemented with synthetic vitamins and minerals, which bioavailability in the body is lower than that of natural organic complexes. Therefore it is important to develop special dietetic products from natural raw materials. The aim of this study was to analyse the effect of high-pressure processing on bioactive compounds in the enteral products. For this research enteral food was made using fresh or semi-finished fruit and vegetable juices. Products were processed applying high pressure, namely 400 MPa, 500 MPa and 600 MPa for 5 minutes at room temperature. All samples were tested for their content of vitamin C, total carotenes, anthocyanins, total phenols and antioxidant activity, as the control untreated enteral food samples were used. The obtained data showed that samples made from semi-finished juices have higher contents of vitamin C and total anthocyanins than samples prepared from fresh juices. Similarly this was observed with total phenol content where after high-pressure treatment in samples made from heated juices it was more stable and had in higher amounts than in samples from fresh ingredients. There were no significant differences in the content of bioactive compounds between products treated at different pressures.

Keywords: vitamin C, total carotene, total phenols, antioxidant activity.

Introduction

Enteral nutrition (EN) by means of oral nutrition supplements (ONS) and if necessary tube feeding (TF) offers the possibility of increasing or ensuring nutrient intake in cases where food intake is inadequate (Weimann et al., 2006). EN is only used for patients with a sufficient digestion where the food can be digested and nutrients assimilated in the body of a specially prepared diet (Rozenbergs, 2011). Enteral feeding is a method of supplying nutrients (oral food and fluids) using nasogastric, gastrostomy or jejunostomy feeding, which are sometimes referred to as enteral tube feeding (Jones et al., 2011).

There can be several types of EN, depending on the intended use and the specific needs of the patient, so creating foodstuff from natural ingredients can be challenging when it comes to producing microbially safe and stable products with necessary health attributes. Taking all of this into consideration high pressure processing (HPP) has emerged as a novel, additive-free food preservation technology that has been scientifically and commercially proven to be very useful (Barba et al., 2015).

High-pressure processing (HPP) is a method where very high pressure from 100 to 1000 MPa is used to process packaged food using liquids a medium to transmit pressure. Using HPP it is possible to process food in a wide range of temperaturesfrom-20 ºC to 100 °C for a certain time (typically from few seconds to 30 minutes). This allows eliminating harmful pathogens and microorganisms that are responsible for vegetative spoilage and to inactivate enzymes with minimal modifications in nutritional and sensory quality (Andrés et al., 2016, 2016b; Carbonell-Capella et al., 2013).

Aroma compounds, vitamins and minerals are rarely affected by HPP, because of their low molecular weight and low compressibility of covalent bonds, however this doesn't apply to macromolecules such as proteins and starch which can change their native structure during HPP similarly to thermal treatments (Barba et al., 2015). The aim of this study was to analyse the effect of high pressure processing on the shelf life of enteral products made from fresh and heated (semi-finished) fruit and vegetable juices.

Materials and Methods

Sample preparation

For the purpose of this research samples of the same recipe enteral food was prepared ensuring 100 kcal intake per 161.25 g of product by using juices from blackcurrant, beet, pumpkin, cabbage, Jerusalem artichoke and whey protein, canola oil, cod liver oil, iodized salt. For one part of the experiment fresh juices (obtained from raw fruits and vegetables grown in organic management system), for the other part juices previously vacuum cooked (prepared by Ltd 'KEEFA' 'Natural Food manufacturer' from the same raw material) were used. One average sample of each set of ingredients was made to be divided between 18 individually packaged 100 ml PP bottles (Kartell, Italy) for the HPP. Before applying the HPP each bottle was vacuum packaged in a polymer film (PA/PE) to prevent any product leakage during processing as a result of applied pressure.

High-pressure processing

The HPP was carried out using ISO-Lab High Pressure Pilot Food Processor (S-FL-100-250-09-W, Stansted Fluid Power Ltd., Essex, UK) in a 2.0 L pressure vessel. A propylene glycol, water mix (1:2 v / v) was used as the pressure transmitting liquid (Kirse et al., 2015).

Both of the experimental groups were subjected to high pressure processing under 400 MPa, 500 MPa and 600 MPa for 5 minutes at room temperature. Due to pressure increase the product temperature increased by

15 °C during pressurization at 400 MPa, by 17 °C at 500 MPa and by 20 ºC at 600 MPa.

After product processing, samples (Table 1) were stored at room temperature in direct light to observe the changes of bioactive compound content during storage. Obtained results were compared depending on the type of used ingredients for the preparation of products and the selected HPP modes. For initial comparison of the HPP impact on both group samples control tests were done with samples without the use of the HPP.

Table 1

Abbreviations used in sample identification

Sample abbreviation	Type of juices	Applied HPP
Fresh	Fresh	not applied
Heated	Heated	not applied
I AS	Fresh	400
II AS	Fresh	500
III AS	Fresh	600
I AP	Heated	400
II AP	Heated	500
III AP	Heated	600

The quality changes of the samples during the storage were evaluated by detection of vitamin C, soluble solids content, pH value, total carotenes, total anthocyanins, total phenols, and antiradical activity. The microbial safety was tested with the detection of total plate count, coliforms, molds, and yeasts.

Microbiological analyses

Microbiological testing of enteral food was completed using 90 ml of 0.5% sterile peptone water solution to which 10 ml of enteral food was added and mixed. The mixture was pour-plated in duplicate for determination of total plate count (TPC) according to standard LVS EN ISO 4833:2003 (Ref. No. 01-14, Sharlau, nutrient agar, incubation at 30 °C for 72 h); Coliforms according to standard LVS ISO 7251:2005 (Ref. No. 401460, Sharlau ENDO agar, incubation at 37 ºC for 24 h); mold fungi and yeast cells according to standard ISO 21257-2:2008 (Ref.No.01-111, MRS agar, incubation at 27 ºC for 48 h (yeast cells) and 5 to 7 days (mold fungi).

Microbiological safety of enteral food was evaluated according to the guidelines by Cabinet of Ministers, Latvia regulation No 441/2016 for Vegetable jams, purees and similar products which sets allowed limits for TPC at $5 \cdot 10^3$ CFU g^{-1}; presence of Coliforms per 1 g of product is not allowed; Mold fungi and yeast cells no more than 50 CFU g^{-1}.

Soluble solids content

The soluble solids content (Brix%) was measured with digital refractometer Refracto 30GS (Mettler Toledo, Japan) using standard method ISO 2173:2003 Fruit and vegetable products - Determination of soluble solids - Refractometric method. Measurements were carried out in five replications.

pH

pH was measured by pH-meter (Lutron electronic enterprise CO., Ltd., UK) using standard method LVS ISO 5542:2010. Measurements were carried out in two replications.

Vitamin C

Content of vitamin C was determined according to iodine method as described by (Kerch et al., 2011). This method determines L-ascorbic acid, which is the reduced form of ascorbic acid. Measurements were carried out in four replications.

Total carotenes

Total carotenes were analysed by spectrophotometric method using UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK), at 440 nm described by Kampuse et al. (2015). The content of carotenes (mg 100 g^{-1}) was calculated in four replications.

Total anthocyanins

Total anthocyanin content was determined by spectrophotometric method according to (Moor et al, 2005), detected on spectrophotometer Jenway 6705 at wavelength of 540 nm. Measurements were carried out in two replications.

Total phenol content

Total Phenol content was determined according to the Folin-Ciocalteu method (Yu et al., 2003) with modifications: to 0.5 mL of extracted sample add 2.5 mL of 0.2N Folin-Ciocalteu reagent, that has been diluted ten times with distilled water; after 5 minutes 2.0 mL of 7.5% $NaCO_3$was added; the resulting solution was mixed and allowed to stand for 30 minutes at 18±1 °C in a dark place; absorption was read at 760 nm using JENWAY 6300 (Banoworld Scientific Ltf., UK) spectrophotometer (Priecina et al., 2014).

Measurements were carried out in six replications from two separately weighed samples.

Antiradical scavenging activity (DPPH)

The antiradical scavenging activity of extracts was determined on the radical scavenging ability in reacting with stable 2,2-diphenil-1-picrylhydrazyl (DPPH) free radical according to researchers group (Yu et al., 2003) with modifications: to 0.5 mL of extracted sample 3.5 mL freshly made DPPH solution was added (4 mg of DPPH reagent was dissolved in 100 ml pure ethanol);the mixture was shaken and kept in the dark place at 18±1 °C for 30 min; absorbance was measured at 517 nm using JENWAY 6300 Spectrophotometer (Priecina et al., 2014).

Measurements were carried out in six replications from two separately weighed samples.

Statistical analysis

The obtained data was processed using 'Microsoft Office Excel' 2007 version, differences between the results were analysed using ANOVA: Two-factor with replication. The obtained results are presented as their mean with standard deviations. Differences among results were considered to be significant if p-value$<\alpha_{0.05}$.

Results and Discussion

Effect of the HPP and storage on microbial safety

Microbial counts were evaluated for both types of ingredients high pressure processed samples and also to control samples without HPP.

No coliforms were found in the evaluated samples, also mold fungi where only detected in control samples made from fresh juices (2 CUF g^{-1}) before storage.

Control samples weren't tested during storage, only their initial results were used for evaluation of the efficiency of the HPP. The total plate count is shown in Table 2 where the mean value of untreated fresh juice samples was $3.3 \cdot 10^2$ CUF g^{-1} and for semi-finished juices 5 CUF g^{-1}. The testing showed that after the HPP all samples were microbiologically safe and with both types of used ingredients (fresh and semi-finished) the applied pressures (400 MPa, 500 MPa, 600 MPa) were sufficient for reduction of microbial activity. This coincides with findings of Andrés et al. (2016) on microbial shelf life on refrigerated milk- and soy-smoothies. However during the four weeks of storage only samples made from semi-finishedingredients stayed microbiologically safe, but sampeles from fresh juices after the week 1 started to show contamination, which gradually grew and at the week 2 exceeded the allowed yeast cell count 50 CUF g^{-1} and became unsuitable for further testing Table 2.

The highest TPC after the week 1 was in the sample I AS treated at 400 MPa – 62 CUF g^{-1} and the lowest in sample III AS (500 MPa pressure) 36 CUF g^{-1} but no significant difference was found between the applied pressure effect on TPC.

Table 2

Total plate count, CUF g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	$3.3 \cdot 10^2$	NA	NA	NA	NA
Heated	5	NA	NA	NA	NA
I AS	ND	62	$1.1 \cdot 10^2$	NA	NA
II AS	ND	59	95	NA	NA
III AS	ND	36	$1.02 \cdot 10^2$	NA	NA
I AP	ND	ND	ND	ND	ND
II AP	ND	ND	ND	ND	ND
IIIAP	ND	ND	ND	ND	ND

ND – not detected, NA – not analysed

As mentioned before only the growth of yeast cells (Table 3) was observed in enteral food samples during storage where the mean value of untreated fresh ingredient EN food was $8.4 \cdot 10^2$ CUF g^{-1} and for heated ingredient EN food 8 CUF g^{-1}. The highest yeast cell count was in the sample I AS (HPP at 400 MPa) after week 1 it was 39 CUF g^{-1}, after the week 2 cell count grew up to $1.09 \cdot 10^2$ CUF g^{-1}. A similar change was detected with the rest of the samples.

Table 3

Yeast cell count, CUF g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	$8.4 \cdot 10^2$	NA	NA	NA	NA
Heated	8	NA	NA	NA	NA
I AS	ND	39	$1.09 \cdot 10^2$	NA	NA
II AS	ND	37.5	95	NA	NA
III AS	ND	36	98.5	NA	NA
I AP	ND	ND	ND	ND	ND
II AP	ND	ND	ND	ND	ND
IIIAP	ND	ND	ND	ND	ND

ND – not detected, NA – not analysed

The effect of the HPP and storage on soluble solids and pH

Both soluble solids and pH showed no significant change after the HPP or storage, however enteral food made from fresh juices had higher content of soluble solids 12 Brix% on average than those made from semi-finished juices, which on average was 11 Brix%. Similarly, enteral food from fresh juices had pH 5, but from semi-finished juices pH 4.5. These findings coincide with other researcher findings (Andrés et al., 2016; Landl et al., 2010) of no significant change in pH and soluble solids during refrigerated storage of the HPP treated samples.

Effect of the HPP and storage on vitamin C content

The content of vitamin C (Table 4) in the sample without high pressure processing made from fresh ingredients was 25±3.46 mg 100 g^{-1}, but in sample made from semi-finished ingredients 28.2±1.37 mg 100 g^{-1}.

The high pressure processing initially shows better vitamin C retention in the samples made from semi-finished ingredients but after 2 weeks of storage the loss of vitamin C on average was 50%. However samples made from fresh ingredients showed a 33 to 65% decrease right after applying HPP. This could be explained with the enzymatic activity in fresh juices where enzymes keep deteriorate ascorbic acid contrary to semi-finished ingredients where enzymatic inactivation was achieved by heat treatment. The statistical analysis showed a significant difference in content of vitamin C between samples made from different groups of ingredients ($p<0.05$) and a slight difference between the applied pressure ($p=0.003$) for samples before storage.

Table 4

Content of vitamin C, mg 100 g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	25.0±3.5	NA	NA	NA	NA
Heated	28.2±1.4	NA	NA	NA	NA
I AS	17.4±1.3	20.5±2.9	11.4±1.5	NA	NA
II AS	15.9±2.8	19.2±1.1	13.7±1.4	NA	NA
III AS	11.1±1.8	16.2±2.5	14.5±1.4	NA	NA
I AP	29.8±1.0	25.5±1.1	14.6±1.4	14.6±1.4	13.4±1.4
II AP	33.3±2.2	27.4±2.5	13.3±1.4	13.3±1.4	12.8±2.1
IIIAP	30.8±0.1	29.5±1.3	13.5±1.4	14.5±1.4	13.3±1.2

NA – not analysed

The highest vitamin C content was 33.3±2.24 mg 100 g^{1} in the sample II AP (made from semi-finished ingredients processed at 500 MPa) but the lowest in the sample III AS (fresh ingredients, processed at 600 MPa) 11.1±1.83 mg 100 g^{-1}. It is partially possible to link the obtained data to other studies on this subject however the storage in those was mostly refrigerated. For example Andrés et al. (2016) also show a more rapid loss of vitamin C, but on the day 30 (32% for sample processed at 450 MPa and 36% for 600 MPa). The statistical analysis showed a significant loss of vitamin C during storage for samples from both ingredient groups ($p<0.05$) and also between applied pressures for samples made from fresh ingredients (I AS, II AS, III AS) $p=0.006$, these results are consistent with Andrés et al. (2016), Oey et al. (2008) who also reported the enhanced degradation rate of vitamin C by increased pressure.

The effect of the HPP and storage on total carotene content

Similarly to the content of vitamin C, also content of total carotenes (TC) show a significant difference between enteral food samples made from fresh juices which is lower and samples made from semi-finished juices. The lowest amount of TC (Table 5) was in I AS (0.15±0.01 mg 100 g^{-1}), but the highest in the sample processed at the same pressure made from semi-finished ingredients I AP (0.44±0.01 mg 100 g^{-1}) immediately after processing. No significant difference was found between samples of fresh juice enteral food and semi-finished ingredient EN food without the HPP treatment, but such was found in all samples after the HPP ($p<0.05$). EN food made from fresh juices showed a significant degradation of TC which isn't supported by other author findings, but the samples made from semi-finished ingredients didn't show such degradation.

Evaluating the obtained data no direct coherence of total carotenes change during storage can be lined, but it does show a tendency of degradation with every week of storage and increasing pressure for semi-finished ingredient EN food samples. The uneven data from this study could be indicative of an uneven distribution of oil and oil-soluble ingredients during filling. Barba et al. (2015) also reported that the HPP treatment can increase extractable carotenoid amount in plant-based products explaining it with the permeabilization of the plasma membrane cell and denaturation of the carotenoid-binding protein induced by the HPP (400-600 MPa/20-25ºC/2–5 min).

The effect of the HPP and storage on total anthocyanin content

Evaluating total anthocyanin content a significant difference ($p<0.05$) between fresh and semi-finished ingredient enteral food samples and a slight difference between the applied pressure ($p=0.03$) was detected before storage and also during storage for all samples ($p<0.05$). This however does not apply to semi-finished ingredient enteral foods that show the highest total anthocyanin content from 2.25±0.02 mg 100 g^{-1} (II AP) to 2.38±0.01 mg 100 g^{-1} (III AP) which in all samples similarly decreased during storage Figure 1.

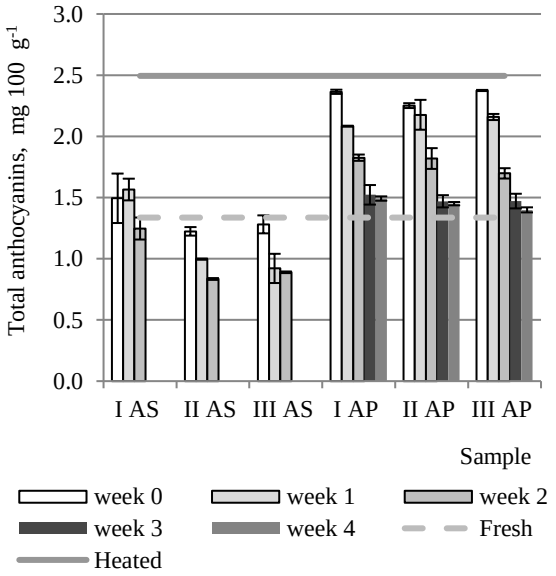

Figure 1. Content of total anthocyanins, mg 100 g^{-1}

There was a significant difference between processing pressures on fresh juice samples. The more divers' changes with these samples could also be explained with one medium weight difference and some authors do mention that some enzymatic activity is still present in products even after the HPP treatment which makes these samples unstable during storage (Denoya et al., 2016).

Table 5

Content of total carotene, mg 100 g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	0.44±0.00	NA	NA	NA	NA
Heated	0.44±0.02	NA	NA	NA	NA
I AS	0.15±0.01	0.20±0.03	0.16±0.02	NA	NA
II AS	0.18±0.04	0.16±0.01	0.19±0.02	NA	NA
III AS	0.21±0.02	0.13±0.00	0.16±0.02	NA	NA
I AP	0.42±0.02	0.44±0.01	0.37±0.01	0.37±0.02	0.24±0.01
II AP	0.25±0.01	0.38±0.03	0.35±0.01	0.38±0.01	0.32±0.04
IIIAP	0.35±0.00	0.32±0.00	0.34±0.01	0.37±0.00	0.33±0.00

NA – not analysed

Table 6

Content of total phenols, mg GAE 100 g^{-1}

Sample	Storage time				
	Before storage	**week 1**	**week 2**	**week 3**	**week 4**
Fresh	52.15±4.16	NA	NA	NA	NA
Heated	49.62±3.15	NA	NA	NA	NA
I AS	36.08±6.37	48.57±7.45	38.91±3.51	NA	NA
II AS	34.56±5.20	50.82±6.98	47.33±0.98	NA	NA
III AS	33.52±4.34	46.76±3.13	50.40±4.33	NA	NA
I AP	49.201±2.29	55.95±4.47	39.87±4.01	36.61±2.49	31.07±3.50
II AP	45.981±2.84	54.06±4.13	48.70±3.48	33.03±3.91	28.07±3.23
IIIAP	46.492±2.33	51.28±4.29	47.59±4.87	32.78±3.92	28.61±3.93

NA – not analysed

The effect of the HPP and storage on total phenol content

The total phenol content (Table 6) after HPP showed a significant difference between used ingredient groups, but an increase of total phenols content was detected during storage, which has been reported also by Andrés et al. (2016), however samples made from fresh juices do not show the same tendency. Barba et al. (2015) on extraction of polyphenols using the HPP treatment similarly to total carotene and also anthocyanin content suggests, that this type of treatment compared to conventional methods can be able to enhance mass transfer processes within plant cellular tissues, as the permeability of cytoplasmatic membranes can be affected. Having said that the initial analyses of bioactive compounds could show less total phenols content, because they have not been fully extracted, compared to storing them for a week (Barba et al. 2015).

The effect of the HPP and storage on antiradical activity (DPPH)

Similarly to total phenol content DPPH appears to be stable up until the second week when its activity starts to drop. The obtained data is shown in Figure 2.

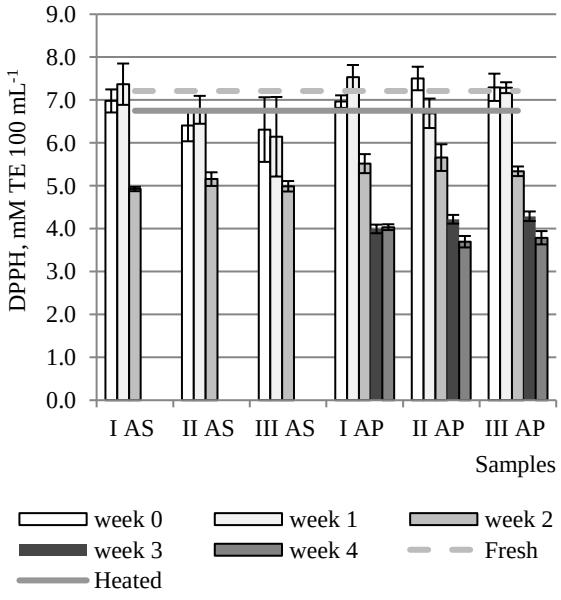

Figure 2. Antiradical activity (DPPH) of EN foods during storage, mM TE 100 mL^{-1}

The antiradical activity of EN foods after the HPP isn't significantly different from samples without applied HPP and no significant difference was found between the applied pressures ($p<0.05$). Although a significant change was observed during storage where the DPPH gradually decreased which coincides with findings of Andrés et al. (2016) and Oey et al. (2008). The highest mean of DPPH was found in the semi-finished ingredient sample processed at 500 MPa before storage II AP 7.50±0.27 mM TE 100 mL^{-1}, which also had the lowest antiradical activity after storing the sample for 4 weeks (3.70±0.13 mM TE 100 mL^{-1}).

The data analysis of several bioactive compounds to some enteral food samples showed a higher content after storing samples for one week than it was determined before storage. These findings do not fully coincide with other author findings on bioactive compound changes during storage and does suggest the need of additional testing. The sample preparation technique of one average sample volume and division between separate packages could cause an uneven distribution of ingredients that can impact the outcome of tested compounds. In literature it has also been mentioned that HPP improves the extraction of bioactive compounds from plant cellular tissues, as the permeability of cytoplasmatic membranes are affected, as this isn't an instant occurrence it may take some time to be fully detectable (Barba et al. 2015).

Conclusions

After this preliminary research it can be concluded that additional research needs to be done, to provide more data on the HPP treatment impact on enteral foods made from different ingredients and the shelf life of these products. The obtained data showed different results for the tested bioactive compounds which not always were compatible with findings of other authors. For the further research it can be suggested to evaluate and make some additional changes in the sample preparation to ensure greater reliability on the obtained data.

Over all the research showed that samples prepared from vacuum heated (semi-finished) ingredients were more stable after high pressure processing and were microbiologically safe for 4 weeks when they were stored at room temperature in direct light. Samples made

from fresh juices showed bigger variation in contents of bioactive compounds during storage, but similarly to semi-finished samples didn't show significant changes with the applied pressure. Also samples from fresh juices in these conditions kept their shelf life only for one week before they were deemed to be ineligible for the further research.

Vitamin C, total carotene and total phenol content in enteral food samples made from semi-finished juices slightly decreased with the increased pressure.

Initial results also show that after using the HPP at 400 MPa, 500 MPa and 600 MPa it is possible to obtain microbiologically safe products.

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