Faculty of Food Science

THESES OF THE DOCTORAL DISSERTATION

Development of quality characteristics of berries pasteurized by heat treatment or high hydrostatic pressure as a function of storage temperature

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1. BACKGROUND AND AIMS OF THE WORK

Research results of the last few decades highlight more and more the relationship between proper nutrition and healthy organism. Several scientific facts support that we can fight with better chances serious diseases threatening our civilized society, like cancer and cardiovascular diseases, if we change our diet in a right way.

Beside changing our rushing lifestyle, that includes more exercise and avoiding everyday stress situations, we can do much for our health by consuming less calories and more fruits and vegetables. With the increase of knowledge we can exceed empirical and traditional observations regarding beneficial effects of fruits and vegetables. Newer and newer fitonutrients become known and the mechanism of action of already known bioactive components becomes clear. Some examples: strong correlation was found between low vitamin C intake and cancer; Se was proved to strengthen the immune system. The phrases „free radical" or „ antioxidant" are familiar ideas. Synthetic processing and marketing of health promoting components mean considerable business prospects to pharmaceutical industry and producers and distributors of food supplements. However, research results have drawn the attention on the fact that excessive intake of synthetic antioxidants above the physiological requirement is rather harmful than beneficial, since when becoming a pro-oxidant they can damage the biomolecules of human organism. Thus consumption of natural antioxidants is preferred to synthetic supplements. Fruits are food materials containing considerable amounts of bioactive components and among them some berries have substantial natural antioxidant content.

Strawberry is the most widespread berry in the world. Its proportion of total berries reaches 60-65%. It contains high amounts of vitamin C, potassium, calcium, iron and phosphorus and the contents of anthocyanines, flavonoids and phenolic carbonic acids having significant antioxidant capacity are high in this berry. Raspberry has similarly favorable characteristics. It contains high quantities of vitamins C-, P- and B_9. These berries are popular not only because of these facts, but because they are very palatable (sugar-acid ratio, aroma compounds). Unfortunately, there is a declining tendency in strawberry growing in Hungary since the '80s, which can be explained by the decrease in its profitability and the narrowing of domestic and export markets. The same applies to our raspberry growing, which was considered a successful branch up to the beginning of the '90s. This tendency might be stopped or even reversed, since the consumption of ready-to-eat, minimally processed and additive free fruits and vegetables has rapidly increased in the developed countries in the last decade and a similar trend can be expected in Hungary as well.

In the production of minimally processed foods one has to keep in mind that products have to undergo processes, where no additives are used, shelf life is safely increased, and the complex characteristics of foods, first of all organoleptic properties, nutrients and vitamins are only minimally affected. Results published in studies about high hydrostatic pressure treatment are very promising, thus this method became an important research area. In contradiction with traditional heat treatment, where undesirable changes occur during processing, high hydrostatic pressure can better retain color and aroma compounds and vitamins. Although some products are already available on the market, much research is needed to completely unveil the mechanism of action of this technology, to find its application areas and to determine the conditions of safe food production. Investigation of the effect of storage conditions after treatment is an important but in the international literature less studied issue.

One has to keep in mind that investment costs of the technology are high, so it is expedient to use it only for high value added products.

On the long term, high hydrostatic pressure, as a food preservation technology, is advisable to be used only when products of better quality can be produced this way than with heat treatment having equivalent pasteurization effect and much lower investment costs. Thus complex comparative investigations are necessary to compare the effects of the two methods. Microbiological, physical and chemical examinations and organoleptic analysis have to be performed to get a comprehensive view. At the same time it is not enough to obtain a product which is very similar to the original one directly after the given treatment, since storage conditions might drastically alter the characteristics of the product. Therefore the effect of post-treatment storage conditions has to be studied. Since the potential of long-term storage is limited for the sake of keeping freshness, thus I considered sufficient to examine a relatively short-term storage period.

On the basis of the previously mentioned I wanted to answer the following questions:

1. What measure of high hydrostatic pressure treatment is needed to produce microbiologically stable strawberry and raspberry purées without heat effect during a relatively short-term storage period? How big heat load (temperature and time of heat treatment) can be regarded as the same pasteurization level from microbiological viewpoint?
2. Can it be proved that high hydrostatic pressure treatment affects organoleptic characteristics of strawberry and raspberry purées milder and whether possible changes can be detected by organoleptic analysis and instrumental methods?
3. Does high hydrostatic pressure technology decrease biological activity of strawberry and raspberry purées (vitamin C, total phenolic, and total anthocyanin content, antioxidant capacity) less than heat treatment having the same pasteurization effect?
4. Do changes appear or do the changes caused by the treatments increase after the treatments during the short term storage (4 weeks) at different temperatures (5, 10 and 20°C)? Can the products be kept at ambient temperature?

2. MATERIALS AND METHODS

Deep-frozen raspberry and strawberry were thawed then sieved and purées were prepared. Sugar content of the samples was adjusted to 20 ref % by the addition of granulated sugar and filled into plastic bottles. A part of the samples was heated in an Armfield FT40 processing vessel until 80°C - 5 min heat equivalent was reached. After heat treatment, samples were rapidly cooled in ice-water. The remaining part of the samples was pressurized in a "Food Lab900" high hydrostatic pressure equipment at 600 MPa for 5 min. To preclude heating up of samples, 4°C water was circulated in the wall of the high pressure equipment. Berry purées were stored at three different temperatures (5, 10 and 20°C) for 4 weeks to study the effects of storage conditions (temperature and time). Samples were examined on the 0th, 14th and 28th days of storage.

In the **microbiological examinations** total aerobic cell count, yeast and mould counts were examined. Total aerobic cell count was determined by the traditional pour plate method while yeast and mould counts were determined on RBC agar (Rose-bengal Chloramphenicol) by surface streaking. In the **organoleptic evaluation** by profile analysis panelists had to qualify the samples on an unstructured scale, while when the preference method was used, they could give maximum 9 scores for each of the four organoleptic characteristics (color, smell, taste and texture). Scores were evaluated by Kramer's ranking method. Changes in the texture of liquid berry purées were examined by a rotational viscometer and the Ostwald-Waele model was fitted to the data. Instrumental analysis of volatile compounds was performed by an **electronic nose** (NST 3320, Applied Sensor Technology). Taste characteristics were measured by α-ASTREE **electronic tongue** (Alpha MOS, Toulouse). Data of electonic nose and tongue were computed by discriminant analysis (SPSS 11.0). Color measurements were performed by Minolta CR-200 type tristimulus colorimeter suitable for reflective color measurements. Samples were compared according to the color difference (ΔE^*_{ab}) calculated from the lightness value (L*), red-green hue (a*) and yellow-blue hue (b*). Total phenolic and anthocyanin content, and antioxidant capacity of berry purées were determined by spectrophotometric methods. Vitamin C content and determination of the individual anthocyanin and other phenolic compounds were performed by high performance liquid chromatography. In the framework of Marie Curie scholarship programme I had the opportunity to study the pressure and heat inactivation kinetics of polyphenol oxidase enzyme of strawberry in the Laboratory of Food Technology in the Catholic University of Leuven. Fresh strawberry (*Fragaria ananassa, cv Elsanta*) was purchased at a local supermarket. The method of SERRADELL and co-workers (2000) was used with slight modifications in the enzyme extraction and cleaning process. After enzyme extraction, kinetic parameters (k, E_a) of heat inactivation of PPO isolated from strawberry were determined in isothermal inactivation experiments. Enzyme solutions were filled in glass capillaries then various heat treatments were performed in the temperature range of 50-65°C for different incubation times in a temperature controlled water bath. Following treatment, samples were placed in ice-water until activity measurements. Kinetic parameters of combined heat and pressure treatments of PPO isolated from strawberry were determined in isotherm-isobaric inactivation experiments through the measurement of residual enzyme activity. Measurements were carried out in a temperature-controlled (-20 °C 100 °C) high pressure (max. 1000 MPa) multi-vessel equipment (HPIU-10.000, Resato). Treatments were done in the pressure range of 100-750 MPa and in the temperature range of 10-65°C. Flexible micro-tubes were used in the high pressure experiments. After treatments, PPO activity was determined by spectrophotometry. Kinetic parameters of the two-phase model describing the heat inactivation of the enzyme and the modified Hawley model describing the combined heat and pressure dependence were estimated by non-linear regression.

3. RESULTS

Based on the results of my own and international ivestigations, treatments at 600 MPa for 5 min without heat and at 80°C for 5 min have microbiologically the same pasteurizing effect. The applied treatment levels have ensured microbiological stability of sweetened strawberry and raspberry purées for the desired period (4 weeks) even under ambient conditions.

Once the food safety requirements have been fulfilled, the next question is, what are the organoleptic characteristics of the product like. Results of the organoleptic analysis performed directly after the treatments showed that neither HHP treatment nor heat treatment caused any pronounced differences in the organoleptic characteristics of strawberry and raspberry purées, thus similar pasteurizing effect could be proved, too. Results of instrumental meaurements supported this finding as well. According to texture analyses by rotational viscometer, direct effect of treatments was neglectable. No decided differences could be found in the consistency factors, determined by the Ostwald-Waele model, of the control, HHP and heat treated samples on the 0th day. Taste and smell characteristics were tested by chemical sensor arrays (electronic tongue and nose), too. Sensor arrays seemed to be more sensitive than human senses, since in some cases differences were found between the samples even directly after the treatments as well. Electronic nose separated untreated, HHP and heat treated samples more decidedly from each other than electronic tongue did. Heat treated and pressurized samples showed different characters for both instruments and both berries, which is indicated by their positions compared to each other and the control samples in the discriminant space determined by multivariate statistical method. Regarding changes in bioactive compounds (anthocyanins, total phenolics and vitamin C content) of berry purées we found slight changes (mostly decrease) in the amounts of those components as an effect of the treatments, but significant changes occurred during storage.

Samples were **stored** at 5, 10 and 20°C for 4 weeks after the various treatments. Changes in organoleptic properties have already showed that minimally processed berry purées can not be stored at ambient temperature in spite of being microbiologically acceptable. Storage at 20°C caused significant changes in both strawberry and raspberry purées, independent of the type of treatment (HHP or heat) before 14-28 days of storage. Organoleptic scores of refrigerated samples of the two berries were different. For strawberry purées, HHP samples stored at 5 and 10 °C got the highest scores (positive consumer acceptance) followed by the heat treated refrigerated samples. Raspberry samples separated from each other according to the storage temperature. Samples stored at the lowest temperature got the highest scores independent of the type of treatment. This hints at the fact, that treatments and storage conditions had different effects on the raw material, that is, the measure of the effects of treatments and storage conditions have to be investigated for each new raw material/product. Results of texture analyses by rotational viscometer of both berries had similar tendencies during storage. While neither time nor temperature of storage caused definitive changes in the texture of heat treated samples, viscosity of pressurized samples changed significantly as a function of storage temperature. Texture was more jelly-like (higher consistency factor) at lower storage temperature. This phenomenon could be observed already on the 14th day and hasn't changed by the 28th day. Electronic nose and electronic tongue were suitable to discriminate heat treated and pressurized samples on the individual storage days. Based on the separation, the preservation method itself had stronger effect than storage temperature on the characteristics measured by instruments in case of samples having the same storage time. Data of instrumental measurements obtained at different times can be reliably merged in one model only for the electronic nose. Sensor responses of electronic nose showed stronger linear correlation with the data of organoleptic evaluation during PLS calibration than those of electronic tongue. Results of tristimulus color measurement of berry purée samples showed that L* lightness value of any of the berries under investigation didn't change significantly during storage, while red (a*) and yellow (b*) hues decreased depending on the storage temperature. Biggest changes were observed in samples stored at ambient temperature and the smallest changes in samples stored at 5°C. Color difference (ΔE^*) values showed that pre-storage treatments caused changes only in some refrigerated samples. Dynamics of color changes was different for the two fruit species. Regarding changes in bioactive components (anthocyanin, total phenolic and vitamin C contents) during storage we found that storage temperature played significant role. Very rapid decrease took place in samples stored at ambient temperature compared to refrigerated samples. This observation affirms that these products in themselves are not shelf stable, cold chain has to be sustained. Differences were detected between the refrigerated samples as well, but in a lesser extent than in samples stored at 20°C. All the three components correlated with the factors a* and b* of color measurement that affirms direct (e.g. anthocyanins) or indirect (e.g. vitamin C) effect on the color of berries. Among flavonoid compounds determined by HPLC separation, anthocyanins decreased, non-anthocyanin compounds showed unlike changes as an effect of the treatment itself, treatment time and temperature. Bioactive components had stronger – weaker correlation with antioxidant capacity, too. The strongest correlation was found with total phenolic content and the weakest one with anthocyanin content. Antioxidant capacity of all the samples decreased during storage; however, treatment method had stronger influence than storage temperature. Antioxidant capacity of HHP treated samples decreased more rapidly and in higher extent than that of heat treated ones. This might be attributed to the incorporated air. Enzyme examinations completed my measurements. Polyphenol oxidase enzyme was isolated from strawberry then heat inactivation (50-65°C) and pressure inactivation (10-50°C, 100-750 MPa) kinetic studies were performed. It appeared that PPO extract, having appropriate enzyme activity, could be prepared only by ionic solvent (supplemented by salt). It was proved in the heat inactivation experiments, that heat inactivation could be modelled by a two-phase kinetic relation, namely there is a heat stable fraction beside the easily inactivated fraction. Results of inactivation experiments in the low heat and broad pressure range showed that at temperatures higher than 50°C and 200 MPa pressure, rate of inactivation increased, indicating a synergistic effect between heat and pressure. However, at similarly high temperature (>50°C) but at pressures lower than 200 MPa, antagonistic effect could be observed between pressure and heat. In this range, at constant temperature, rate of inactivation decreased by increasing pressure. These results indicate that the combination of mild heat – higher pressure is better for PPO inactivation than pressure treatment without heating.

4. NEW SCIENTIFIC RESULTS

1. I have established, that high hydrostatic pressure treatment of sweetened (20 ref%) strawberry and raspberry purées at 600 MPa for 5 min ensured microbiological stability for at least 28 days even at ambient temperature. Regarding its effect on microbiological and organoleptic characteristics, this pressure treatment is equivalent to the effect of heat treatment at 80 °C for 5 min.

2. I have demonstrated that textural changes in sweetened (20 ref%) strawberry and raspberry purées pressurized at 600 MPa for 5 min were strongly influenced by the post-treatment storage temperature. Decreasing storage temperature caused increasing viscosity even after 14 days of storage that practically hasn't changed by the 28th day of storage. On the other hand, viscosity of heat treated (80°C, 5 min) berry purées was not influenced either by storage temperature or storage time.
3. I have found out, that chemical sensor arrays are suitable for monitoring the changes in complex volatile compounds and taste characteristics taking place during storage in heat treated (80°C, 5 min) and pressurized (600 MPa, 5 min) sweetened (20 ref%) strawberry and raspberry purées. I proved by means of canonical discriminant analysis models that heat treatment and pressure treatment affect volatile components and taste characteristics of fruit purées in a different way.
4. Based on the changes in antioxidant capacity (measured by FRAP method) of treated and stored berry purées I have proven that oxidative changes take place during post-treatment storage more rapidly in pressurized (600 MPa, 5 min) sweetened (20ref%) strawberry and raspberry purées irrespective of storage temperature than in heat treated (80°C, 5 min) purées.
5. I have found out that from strawberry isolated polyphenol oxidase extract, dissolved in phosphate buffer, consists of a heat stable fraction and a fraction that can be easily inactivated by heat, thus heat inactivation of the enzyme extract can be described by a two-phase kinetic model.
6. Based on the kinetic analyses of combined heat (10-50°C) and pressure (100-750 MPa) treatments, I have established that combinations of pressures not higher than 200 MPa and temperatures higher than 50°C have antagonistic effect, and combinations of pressures higher than 200 MPa and temperatures higher than 50°C have synergistic effect on inactivation rate of polyphenol oxidase enzyme dissolved in phosphate buffer.

5. SUGGESTIONS

In the Ph.D. study, storage experiments of sweetened heat treated (80°C, 5 min) and by high hydrostatic pressure treated (600 MPa, 5 min) strawberry and raspberry purées were performed. I have experienced in most cases that applied treatments didn't directly deteriorate quality, that is, they can be considered as minimal processes. Both treatments ensured microbiological stability of the products even after 4 weeks of storage at ambient temperature. However, organoleptic and analytical examinations (anthocyanin, phenolic and vitamin C content, antioxidant capacity) proved that none of the treatments could produce shelf stable products, thus refrigeration is necessary. Characteristics of strawberry and raspberry samples have changed on various occasions in a different way during storage. This finding highlights the **necessity of testing each new product before introduction according to its reaction to high pressure treatment. Tests should include investigation of changes during post-treatment storage.** Differences could be found between physical preservation methods in several occasions, which were well detected by chemical sensor arrays. It is important from measurement technical viewpoint that data obtained by the electronic nose could be more reliably merged in one model than those of electronic tongue and their correlation with organoleptic scores was stronger. This finding hints at the more limited applicability of electronic tongue. Antioxidant capacity (FRAP) decreased more rapidly in HHP treated samples than in pressurized ones independently of storage temperature, which suggests that more intense oxidative processes took place in the pressurized samples. Thus, HHP treatment preserved quality worse than heat treatment under the applied conditions. This could be attributed to inadequate enzyme inactivation and the substantial amount of air incorporated during purée preparation. Thus, **one has to keep in mind during processing and later examinations that incorporated air should be removed and air-tight packaging should be used**. Since a part of the changes traces back to enzymatic processes, so **enzyme inactivation studies are necessary to estimate shelf life of pressurized products.** Therefore I have conducted combined heat and pressure inactivation experiments with polyphenol oxidase enzyme extract isolated from strawberry. The two-phase inactivation mechanism detected during heat inactivation experiments indicates the presence of a heat stable enzyme fraction, thus the increased heat demand of its inactivation has to be taken into consideration in the design of heat treatment processes. Based on the kinetic analysis of high pressure treatment combined with heat, **pressurizing at temperatures higher than ambient temperature but lower than that of traditional heat treatment is suggested to achieve better keeping quality of products made of berries.** Complex survey including several characteristics has to be done to see how much temperature increase enhances inactivation of polyphenol oxidase and other enzymes without triggering significant heat damage processes.

6. PUBLICATIONS RELATED TO THE THESIS

Publications in journals

IF publications

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