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THE IMPACT OF THE HIGH-PRESSURE HOMOGENIZATION ON SOME MICROORGANISMS AND ENZYMES – A REVIEW®

Wpływ homogenizacji wysokociśnieniowej na wybrane mikroorganizmy i enzymy – przegląd publikacji®

The article presents the effect of high-pressure homogenization (HPH) on the reduction of microbial growth and changes in the activity of enzymes in food. The publications on the impact of HPH on the bacteria of the Alicyclobacillus, Escherichia and Lactobacillus genies, yeasts of the Zygosaccharomyces genus, as well as changes in the activity of the enzymes: alpha-amylase, amyloglucosidase, pectin methylesterase, glucose oxidase and neutral protease were reviewed.

Key words: High pressure homogenization, food, microorganisms, enzymes.

W artykule przedstawiono wpływ homogenizacji wysokociśnieniowej (HPH) na redukcję wzrostu drobnoustrojów oraz zmiany aktywności enzymów w żywności. Dokonano przeglądu publikacji dotyczących wpływu HPH na bakterie z rodzaju Alicyclobacillus, Escherichia oraz Lactobacillus, drożdży z rodzaju Zygosaccharomyces, a także zmiany aktywności enzymów: alfa-amylazy, amyloglukozydazy, metyloesterazy pektynowej, oksydazy glukozowej i neutralnej proteazy.

Słowa kluczowe: Homogenizacja wysokociśnieniowa, żywność, drobnoustroje, enzymy.

INTRODUCTION

High pressure homogenization (HPH) Emerged as a non-thermal technology to guarantee food safety, stability with a reduced sensory and nutritional damage [15], consists of pressurizing a fluid to flow quickly through a narrow gap valve, which greatly increases its velocity, resulting in depressurization with consequent cavitation and high shear stress. Thus particles, cells and macromolecules suspended in the fluid are subjected to high mechanical stress, becoming twisted and deformed [1]. In the pharmaceutical, cosmetic, chemical and food industries, HPH processing is used for the preparation and stabilization of emulsions and suspensions or for creating physical changes in products [4].

Moreover, a highly efficient technology for the preparation of drug nanosuspensions is high pressure homogenization (HPH), which achieves size reduction by the cavitation forces generated when drug dispersion is forced through a very narrow gap under extremely high pressure. The particle size of a nanosuspension manufactured by HPH is controlled by the homogenization pressure, the number of cycles, and the hardness of the drug particles [12].

A significant increase in the surface area accelerates the dissolution rates of the drugs, leading to improved bioavailability and rapid onset of action [12].

Several studies have evaluated the use of HPH for microbial inactivation in fruit products. The use of HPH as a partial or total substitute for the thermal processing of foods has been studied.

The aim of this reviewing study is presenting a microbial inactivation and enzymes activity changing effects, caused by the introduction into the manufacturing process the high-pressure homogenization operation.

THE HPH EFFECTS ON MICROBIAL GROWTH

A topic of great interest in food microbiology is the use of nonthermal methodologies for food preservation, i.e. approaches able to prolong food shelf life and inactivate foodborne pathogens and / or spoiling microorganisms without any significant increase in the temperature, in order to maintain the sensorial quality at acceptable levels. Some of these technologies are based on the use of high-pressure, ultrasound, pulsed electric fields or on the addition of natural antimicrobials [3].

High-pressure homogenization has been used to disrupt cells containing larger biospecies such as proteins, antibodies, vaccine particles, and DNA plasmids. In using disruptive fluid forces to break open cells containing such shear-sensitive entities, the potential exists for the product to be destroyed as it is released from the cells. In such a situation, it is likely that the concentration of intact and extracellular product in the homogenizer effluent could be maximized by exposing the cell-containing solution to the optimum combination of fluid dynamic forces inside the homogenizing valve [10]. This impact has been the most accurately documented for Alicyclobacillus, Escherichia and probiotical Lactobacilli bacterias and yeast of Zygosaccharomyces genus.

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The impact of HPH on Alicyclobacillus acidoterrestris was shown in work [2]. This study focused on three different strains of A. acidoterrestris: DSMZ 2498, G4 and c8, isolated from a spoiled pear juice and soil, respectively. The strains were maintained at 4C on malt extract agar slants, acidified to pH 4Æ5 through a sterile solution of citric acid (acidified MEA) [2].

The results confirmed, that high pressure caused a low reduction of inoculated cell number for c8 strain at 1700 bar. G4 population, however, was reduced less at 500 and 1700 bar. The HPH processing caused a strong reduction of inoculated cells of DSMZ 2498 strain. The reduction of inoculated spores was low or low-to-moderate. The reduction of cells and spore number in function of homogenization pressure, is shown in fig. 1.

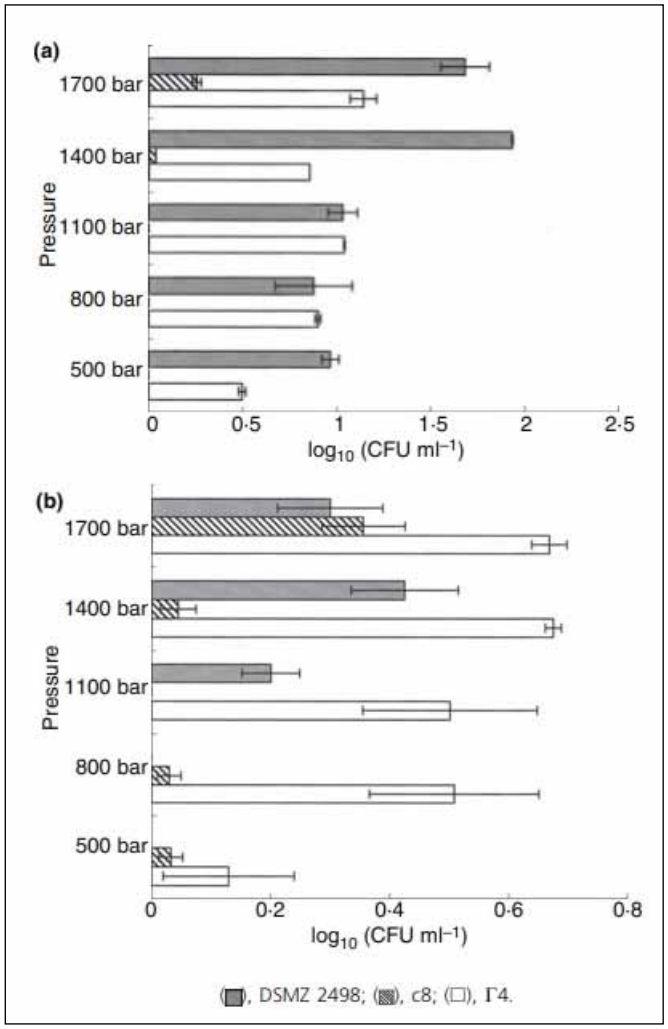

Fig. 1. Reduction of cell (a) and spore number (b) (log10 CFU ml-1) of Alicyclobacillus acidoterrestris in acidified malt extract broth (pH 4Æ5), processed through high-pressure homogenization.

Rys. 1. Zmniejszenie liczebności komórek (a) I przetrwalników (b) (log10 CFU ml-1) Alicyclobacillus acidoterrestris w zakwaszonym bulionie z ekstraktu słodowego (pH 4,5), poddane operacji homogenizacji wysokociśnieniowej.

Source: [2]

Źródło: [2]

Presented work provided useful information on the susceptibility of an emerging spoiling micro-organism, like A. acidoterrestris, to HPH and showed that this technique could be used in the food industry to reduce the thermal damage of fruit juices. Further investigations are in progress in order to clarify the way of action of HPH against A. acidoterrestris and the different mechanisms involved in the susceptibility /resistance of cells and spores. Moreover, the application of several successive rounds of HPH could have an additive effect on the reduction of viability and increase of susceptibility of A. acidoterrestris spores [2].

The next spoiling and very dangerous for food consumer is Escherichia coli bacteria.

In paper [6] was described the inactivation of E. coli MG1655 by high-pressure homogenization at different pressures (100-300 MPa) and temperatures (5–50°C). It can be seen that at constant temperature inactivation increases with increasing pressure, and at constant pressure inactivation increases with increasing temperature. In addition, the influence of temperature increases at higher pressures. For example, at 100, 150, 200, 250, and 300 MPa the difference in inactivation between a treatment at 5 and 45°C was, respectively, 0.8, 1.2, 2.0, 2.2, and 2.7 log units [6] – showed on fig. 2.

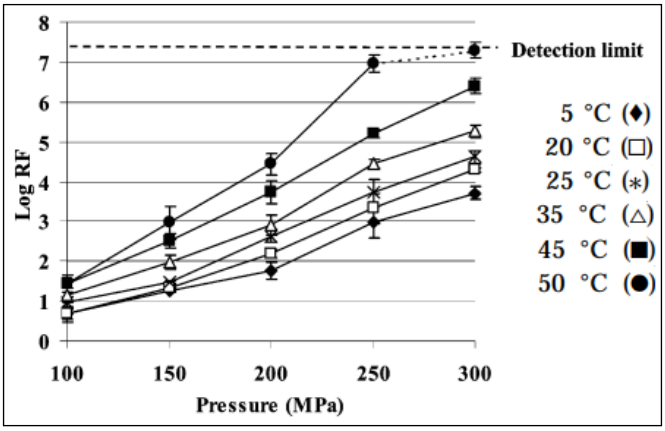

Fig. 2. Inactivation (log RF) of E. coli MG1655 by high pressure homogenization in function of pressure (MPa) at different process temperatures.

Rys. 2. Inaktywacja (logRG) E. coli MG1655 poprzez działanie HPH w funkcji ciśnienia (MPa) w odmiennych temperaturach prowadzenia operacji.

Source: [6]

Źródło: [6]

From these results it can be also concluded that temperature in the range where it does not by itself cause microbial inactivation (approximately 0–45°C) plays an important role in the inactivation of E. coli by highpressure homogenization. Although the exact cause of inactivation by high-pressure homogenization is not yet completely understood, it is clear that temperature has an influence on some of the proposed inactivation mechanisms, such as cavitation and turbulence. Fluid temperature has a dual effect on cell breakage due to hydrodynamic cavitation, which is defined as the dynamic process of gas cavity growth and collapse in a liquid. Cavities arise when the pressure in a liquid is lower than the vapor pressure of the liquid. At low temperature, liquids have a lower

vapor pressure and thus cavity formation will be reduced. On the other hand, the severity of cavitation increases at low temperature as a result of a more violent collapse when the vapor pressure is low [6].

The later experiments, described in [9] confirmed, that high-pressure homogenization is a promising technology, which may be an alternative to thermal Escherichia coli inactivation (by pasteurization) for apple juice and apple cider. Whereas homogenization pressures of 100 to 200 MPa cause microbial inactivation due to high pressure homogenization, homogenization pressures >250 MPa resulted in significant thermal inactivation. Homogenization pressures of >250 MPa resulted in greater than 7 log CFU/mL of E. coli K-12 inactivation in apple juice and apple cider mainly due to the thermal component of the high-pressure homogenization process. There were no significant ($P < 0.05$) 3-way interactions observed. However, significant ($P < 0.05$) 2-way interactions (pressure ∗ type of substrate and pressure ∗ chitosan concentration) were found during the study. The homogenization pressure was a critical factor in causing the inactivation and the incremental quantity of chitosan (2 types) acted synergistically with the pressure to give higher inactivation. Addition of chitosan (2 types) at 0.1% concentration resulted in enhancing E. coli K-12 inactivation in apple juice and apple cider up to 200 MPa. There was no significant ($P < 0.05$) effect of type of chitosan on the bacterial inactivation. Also, there was significantly ($P < 0.05$) higher inactivation in apple juice than apple cider using same homogenizing pressure. Future study will be carried out to evaluate the sensory and shelf-life studies to assess the impact of high-pressure homogenization on the apple juice [9].

The inactivation of microorganisms by high pressure homogenization is also possible for yeasts. The paper [11] describes the inactivation values of Zygosaccharomyces bailii in apricot and carrot juices after the HPH treatments. Zygosaccharomyces bailii inactivation was affected by the pressure applied. In both juices it increased with the number of passes at 100 MPa. The data of this experimental study show the potential of the HPH to modify the texture features of some vegetable and fruit juices and to reduce the cell loads and/or to avoid the proliferation of a spoilage microorganism such as Zygosaccharomyces bailii. However, the HPH technological potentialities were affected by the food matrix employed. In fact, repeated HPH treatments permitted to modify the structure of apricot juice, but they were insufficient to prevent the spoilage of the samples inoculated with Zygosaccharomyces bailii suggesting that, during storage, HPH needs to be supported by other hurdles such as low temperature. On the contrary, when applied for more than 4 cycles, the 100 MPa HPH treatment was able to significantly reduce the cell loads of the inoculated yeast in carrot samples and to prevent its proliferation during the 10 d of storage also at 25°C. This interesting effect on the microbial shelf life was not coupled with appealing modifications of the carrot juice microstructure. Thus, the experimental data suggest also that the multipass-HPH treatment has to be calibrated and combined with different hurdles in relation to the desired shelf life and to the final features to be imparted to the product. Although the scaling-up of multipass process is considered difficult because time and power consuming, it requires a time span comparable to that of single-pass treatments, due to the shortness of each pass (few milliseconds) [11].

Next, the spoilage microflora in beer can also be inactivated by HPH process, implemented to the production technology. The experiments conducted by Franchi and co-workers [8] enabled confirmation that HPH at 250 MPa can be used to inactivate the common beer spoilage microorganisms examined (fig. 3).

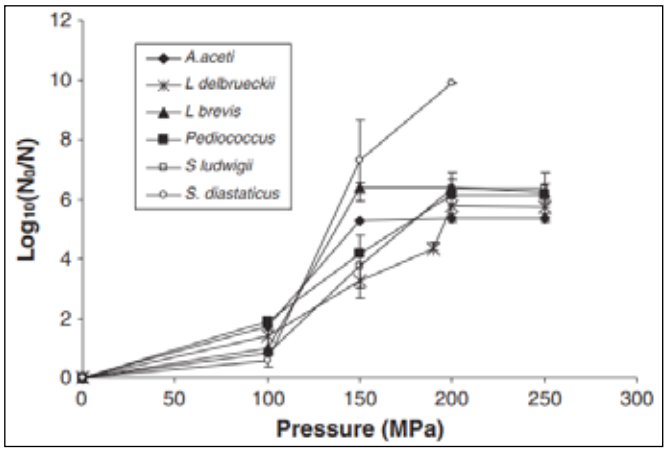

Fig. 3. Inactivation of beer spoilage microorganisms by high-pressure homogenization.

Rys. 3. Inaktywacja szkodliwych mikroorganizmów w piwie poprzez zastosowanie homogenizacji wysokociśnieniowej.

Source: [8]

Źródło: [8]

Moreover, the authors found, that by applying a multi-pass homogenization process (two or three consecutive treatments), it was possible to reduce the requirements of the pressure homogenization to 100–150 MPa. Similarly, this range of homogenization pressure is enough to obtain a stable beer if the HPH process is carried out at 50°C. Therefore, HPH is a promising non-thermal method to obtain microbial beer stability [8].

The experimental data that has been presented in this review yet refers only informations about HPH inactivation of spoilage or dangerous microflora in food products. But, it must also be borne in mind, the microbial inactivation by high-pressure homogenization treatment carries the risk of applies also to microorganisms, which the presence in the food product is beneficial from the point of view of the functionality of the food and the health of the consumer. However, the research results obtained so far do not support these concerns. As the example should be mentioned experiments described in paper [13]. Authors of this article found, that HPH treatment, as performed at 50 MPa, did not affect the viability of cells suspended in MRS medium. In fact, the treatment reduced the strain cell loads by less than 0·2 Log CFU per ml, which was not considered a significant result, confirming the tolerance to moderate pressure.

On the other hand, the severity of HPH treatment was chosen on the basis of previous works demonstrating that pressure level did not affect the cell's viability but, what is equally important, enhanced some probiotic and technological features. The ability to maintain good cell viability is

considered an indicator of probiotic capacity and is included in the selection criteria of innovative treatments that are performed to enhance the strain's probiotic properties [13].

Other, different and interesting data are given from the work relating the microbial inactivation with homogenizer valve shape [7]. In the microbial inactivation tests were used two different lab-scale high pressure homogenizers: a nm-GEN 7400 series system by Stansted Power Fluids (Stansted, UK) and a NanoDeBee 45 by Bee International (South Easton, MA 02375, USA), both equipped with a single pressure intensifier. The homogenizers are characterized by significantly different valve geometries, one based on an adjustable conical piston valve (indicated as SFP) and the other based on an 130 lm orifice gap (indicated as NDB) (fig. 4).

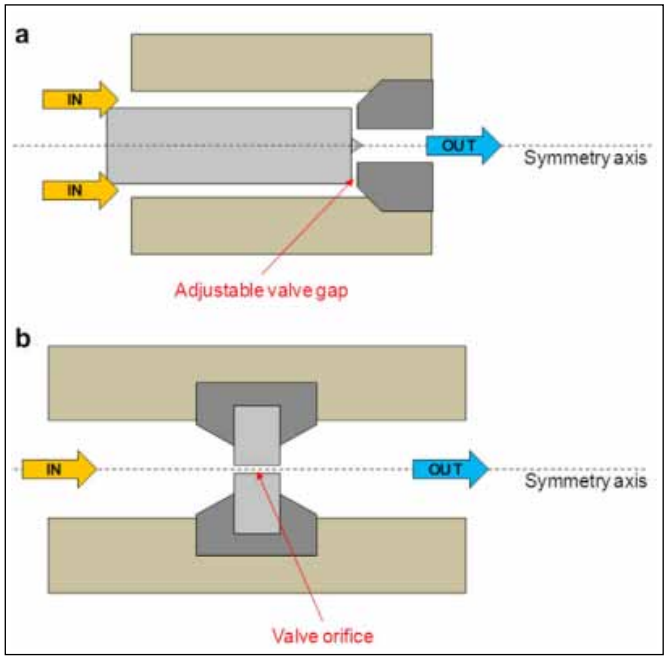

Fig. 4. Schematics of the homogenization valves tested: (a) Stansted Fluid Power (SFP) valve geometry; (b) Nano De Bee 45 (NDB) valve geometry.

Rys. 4. Schematy używanych zaworów homogenizujących: a) geometria Stansled (SPF); geometria Nano De Bee 45 (NDB).

Source: [7]

Źródło: [7]

The kinetics of microbial inactivation by high pressure homogenization (HPH) significantly depended on the geometry of the disruption chamber, with the piston valve resulting more efficient than the orifice valve. Tests conducted on E. coli, L. delbrueckii and S. cerevisiae, clearly showed that similar level of inactivation were always attained at lower pressures and less number of passes when using a piston valve, likely due to the higher probability of mechanical interaction of microbial cells with valve surfaces in such configuration. In the piston valve, the opening through which the microbial suspension is forced to flow is an annular section, whose gap (3–14 lm) is comparable in size with microbial cells. In contrast, in the orifice valve, the characteristic dimension (130 lm) is significantly larger. In addition, the analysis of the fluid-mechanical stresses occurring in the valve through adimensional numbers, such as Reynolds, Weber, Capillary and Cavitation numbers, showed that turbulence and shear and elongational stresses predominate in the orifice valve, while cavitation, which is hence likely to significantly contribute to microbial inactivation, rules in the piston valve. Finally, in order to develop a predictive tool of cell lethality upon HPH treatments, an empirical power law equation (Weibull model) was successfully tested against the experimental data of inactivation of the different microorganisms at varying pressure and number of passes in both valve geometries, resulting in a highly accurate data fit [7].

In terms of impact on microbial disruption, the most important distinctive feature between the two valve geometries is undoubtedly the characteristic size of the valve, which is much larger for the round orifice of NDB (diameter of 130 lm) than for the annular flow section of SFP geometry, characterized by a very thin gap (from 14 to 3 lm), comparable in size with microbial cells (5 lm for yeast cells, 0.1–1 lm for bacteria). The second most important distinctive feature may instead be considered the mean valve velocity, which is much higher for NDB valve (up to 400 m/s), than in the SFP valve (50 m/s) [7].

THE HPH EFFECTS ON ENZYMES ACTIVITY

Some of the effects on enzymes activity causing by high-pressure homogenization were presented by researching teams under the leadership of Alline Tribst and Jose Carbonell [5, 14, 15, 16, 17, 18].

Tribst and co-authors determining the effect of the HPH on the activity and stability of a neutral protease from B. subtilis [15]. The results obtained for the native enzyme and for the pre-heated native enzyme (data not shown) showed no significant differences between them, indicating that the initial heating was not able to partially inactivate the enzyme. The activity of homogenised protease (0 bar) at a high inlet temperature showed a slight enzymatic activity reduction in all evaluated conditions. At 2000 bar, no differences were observed in the enzymatic activity at 55 C, when compared with the non-heated enzyme [15].

These results also demonstrated that the combination of homogenisation and heating can be used in some cases, when enzyme inactivation is desirable. It is interesting to observe that the combination of a mild thermal process and a HPH is also a promising method for the microorganisms inactivation [15].

It was concluded that the HPH can promote reversible or irreversible changes in the B. subitilis neutral protease activity, promoting activation, inactivation and even changing enzyme optimum temperature. The obtained results highlight the HPH as an interesting tool to improve enzyme commercial applications [15] (fig. 5).

Other experiments of this researchers were aimed of determining the effect of the HPH on the activity and the stability of a fungi α-amylase [17].

The activity of high pressure homogenized α-amylase at different pHs presented similar results to the previously obtained for the native enzyme, with a maximum activity at a pH of 5.8 and with same activity at pH levels of 4.0, 5.5

and 6.7. Therefore, no differences were observed between the native and the homogenized α-amylase at each evaluated pH. Consequently, the HPH did not change the activity and/or the stability of the studied fungi α-amylase.

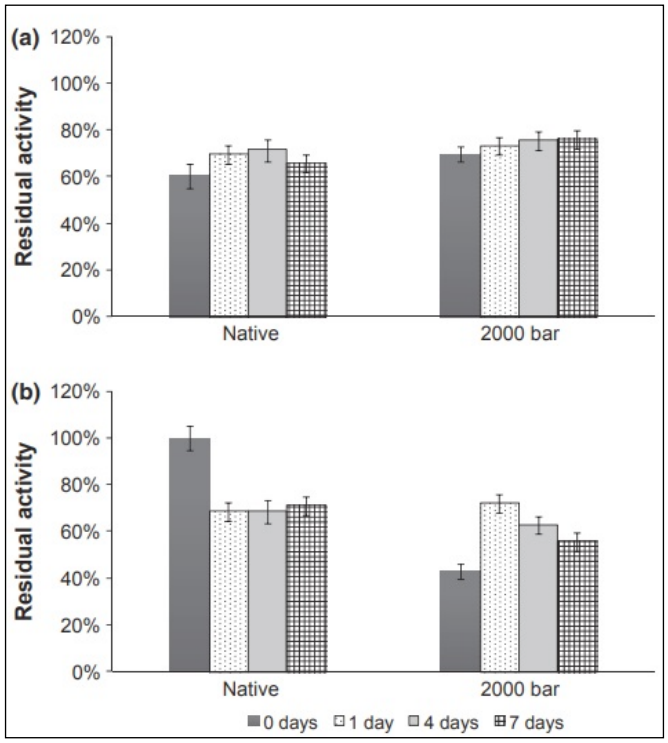

Fig. 5. Stability of native and high pressure homogenised (2000 bar) protease stored at pH 7.5 and 8 C for 1 week. Activity measured at 20 C (a) and 55 C (b).

Rys. 5. Stabilność proteazy w formie natywnej oraz po homogenizacji ciśnieniowej (2000 bar) przechowywanej w pH 7.5 oraz 8 przez 1 tydzień.

Source: [15]

Źródło: [15]

The combination of the HPH and a high inlet temperature again caused no changes in the α-amylase activity. Thus, it can be determined that the HPH at the evaluated conditions was not able to cause significant changes in the α-amylase. This result can be useful for industries that intend to use HPH with products containing α-amylase, since the results indicate, with no doubt, that the homogenization process did not affect the activity and stability of the enzyme. This is mainly interesting to some juice industries that apply α-amylase for juice clarification and viscosity and they can use HPH as a non-thermal process to stabilize juices microbiologically and physically, through particle size reduction.

Summarizing, the α-amylase activity and stability were not affected by the high pressure homogenization up to 1500 bar and the homogenization at a high temperature also caused no changes in the enzyme activity. Therefore, it can be concluded that the fungi α-amylase is stable under high pressure homogenization up to 1500 bar [17] (fig. 6).

A significant part of researches of the 'Tribst group' was the experiments aimed at determining the temperature impact on the activity of glucose oxidase, amyloglucosidase and neutral protease after HPH operation [14, 16, 18].

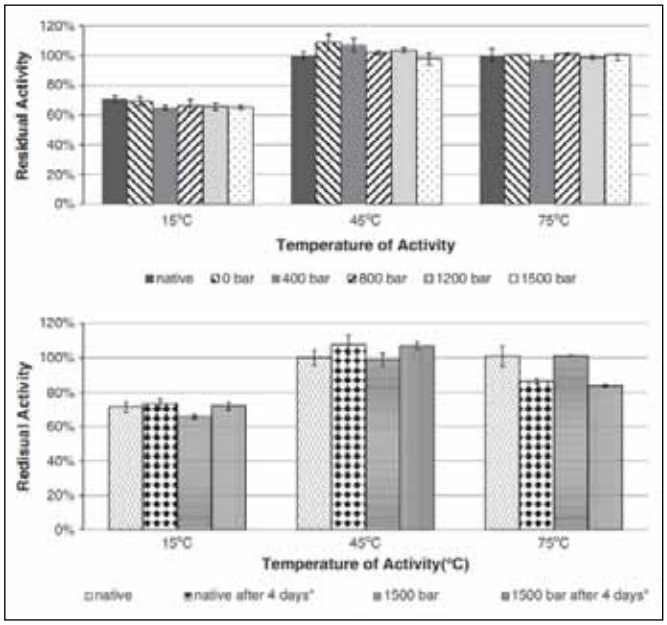

Fig. 6. Up – α-amylase activity at different temperatures after homogenization;
Down – Effect of refrigerated storage on the stability of the homogenized α-amylase.

Rys. 6. Góra – Aktywność α-amylazy w różnych temperaturach po homogenizacji;
Dół – Wpływ przechowywania chłodniczego na stabilność homogenizowanej α-amylazy.

Source: [17]

Źródło: [17]

There were investigated, at the optimum temperatures, only amyloglucosidase showed a slight improvement in activity and only after one pass at 200 MPa. This may indicate that the configuration of native enzyme is the best one to react at the optimum temperature, since this optimum condition was chosen based on the enzyme reaction of the native form. To the contrary, improvements in activity at non-optimum temperatures were observed for all enzymes, and the maximum enzyme activity increase occurred after only one pass for amyloglucosidase and neutral protease and after three passes for glucose oxidase [14, 18].

High pressure homogenization is able to alter the glucose oxidase activity and increase its residual relative activity at high temperature after homogenization at pH 5.7 and 150 MPa. Additionally, the HPH can cause an increment up to 400% on glucose oxidase stability as evaluated after 24 h storage at 8°C, as compared to the native one stored under the same conditions. Therefore, the HPH may be an interesting tool to increase glucose oxidase relative stability, improving the potential applications of glucose oxidase in food industry [16].

Moreover, the homogenization at high inlet temperature was highly deleterious for amyloglucosidase activity at all evaluated conditions, with activity loss higher than 90%. Considering that reached temperature during the process and HPH at 2000 bar (Fig. 3) were individually not able to promote this level of enzyme inactivation, it was concluded that homogenization associated to temperature had a synergistic effect on amyloglucosidase inactivation.

Also, the activity evaluation after one day of storage at 8°C showed that this inactivation was not reversible, since the relative activity at 65°C of sample homogenized and stored at pH 4.3 was 9.4±1.3%, with no significant difference with sample activity just after homogenization. Therefore, it can be concluded that homogenization at 65°C was deleterious for the enzyme activity. In contrast, the results highlighted that HPH of amyloglucosidase at high inlet temperatures can be a very interesting way to inactivate the enzyme at the end of the hydrolysis process without using heat.

High pressure homogenization was able to relatively keep or increase the amyloglucosidase activity immediately after homogenization, depending on the pH of homogenization and the temperature of activity. Best results were obtained at 80°C, which is very interesting especially when amyloglucosidase is applied in starch saccharification process, which requires enzyme active at higher temperatures, for improving time and energy economy [18].

HPH affected the activity of amyloglucosidase, glucose oxidase and neutral protease, particularly with respect to improving it at nonoptimum temperatures. For amyloglucosidase and neutral protease, the main effects of homogenization were observed after only one pass, indicating that the energy gain of the enzyme under this condition was sufficient to affect the maximum molecular changes caused by homogenization. To the contrary, the continuous improvement in the activity of glucose oxidase can be attributed to the additional molecular change caused by each homogenization pass. Therefore, HPH can be applied to improve enzyme activity and the efficacy of multiple passes is dependent on the kind of enzyme [14]. The optimal conditions to improve activity of investigated enzymes are presented in fig. 7.

Enzyme	Process conditions		Temperature of activity measurement (°C)	[a]Activity increase (%)
	Pressure (MPa)	Number of passes		
AMG	200	1	80	7.5%
GO	150	3	75	78%
Neutral protease	200	1	20	12%

[a] compared with native enzyme activity measured at the same conditions of the HPH enzyme.

Fig. 7. Conditions of HPH process for maximum enzyme activity increase.

Rys. 7. Warunki operacji HPH do uzyskania maksymalnego wzrostu aktywności enzymów.

Source: [14]

Źródło: [14]

The last of presented in this work research is the investigation in the influence of high pressure homogenization and pulp reduction on residual pectinmethylesterase activity, conducted by Carbonell and co-authors [5].

The experiments conducted shows pectinmethylesterase activities found in fresh and homogenized juices. As expected, pectinmethylesterase activity monotonically decreased as a function of the pulp content reduction and the increase of the homogenization temperatures. Hence, LPJs homogenized at 68°C had a residual pectinmethylesterase activity near 10% of the initial value showed by fresh juice, on the contrary to what happened with the homogenized WJ at the same temperature that reached a residual pectinmethylesterase value of about 25%. Moreover, residual pectinmethylesterase activity was increased by reducing the homogenization temperature (such increase was proportional in all assayed juices). These results confirm the intimate relationship between pectinmethylesterase activity and pulp content of juices (mentioned in the introduction section) and clearly indicate that for a considered HPH treatment, once knowing the residual pectinmethylesterase activities of whole juices it would be feasible to predict the residual activities of their pulp reduced derived products [5].

As a conclusion – homogenization at 150 MPa and 68°C preserved acceptability and cloudiness of Lane Late juice for at least 3 months of refrigerated storage at 3°C even with a high residual pectinmethylesterase activity. This methodology can be an interesting alternative to traditional heat treatments above 85°C used by citrus industry that promote losses in the acceptability of commercial orange juices [5].

CONCLUSIONS

The presented research results confirm the high usefulness of the high-pressure homogenization operation. These results show the additional benefits of HPH – causing a decrease in microorganisms number (also pathogenic) in the food products, and thus increasing its safety for the consumer. In addition, it has been shown to reduce the enzymatic activity, which consequently improves the quality of the product by increasing its stability.

PODSUMOWANIE

Przedstawione rezultaty prac badawczych potwierdzają wysoką użyteczność operacji homogenizacji wysokociśnieniowej. Rezultaty te unaoczniają dodatkowe korzyści płynące z HPH – powodowanie spadku liczby mikroorganizmów (także chorobotwórczych) w produkcie spożywczym, a tym samym zwiększenie jego bezpieczeństwa dla konsumenta. Ponadto wykazano zmniejszanie aktywności enzymatycznej, co w konsekwencji powoduje poprawę jakości produktu poprzez zwiększenie jego stabilności.

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