

Food preservation by high pressure

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Abstract Novel non-thermal food processing technologies aim to provide safe, high quality foods with desirable nutritional, physico-chemical and sensorial properties. More recently with the use of minimal processing treatment concepts have been added to the already existing food processing requirements. Some of them might be beneficial for the improvement of hygiene and the extension of shelf life. This presentation will focus on the current practice, the knowledge and future developments of high pressure processing (HPP). Hydrostatic high pressure technology is relatively new to food industry and is more and more considered as an alternative to traditional preservation methods like heat processing. Inactivation of bacteria, spores, virus has been demonstrated. Relevant aspects of the European legislation on novel foods will be discussed. International trends and recent developments in machinery will be reviewed.

Keywords High pressure processing · Inactivation of bacteria and their spores · Inactivation of virus · Preservation of food

Zusammenfassung Das wachsende Bewusstsein der Verbraucher hinsichtlich gesunder Ernährung

steht oft im Gegensatz zu der gleichzeitig gewünschten schnellen Verfügbarkeit frischer, verzehrfertiger Produkte. In der Lebensmittelindustrie sind daher Bestrebungen zu beobachten, dass über eine veränderte Herstellungsweise und dem Einsatz neuer Technologien die Qualität und Produktvielfalt gesteigert werden soll. In diesem Zusammenhang wird seit einigen Jahren die Hochdrucktechnologie bei der Haltbarmachung von Lebensmitteln eingesetzt. Die Wirksamkeit hoher hydrostatischer Drücke bei der Behandlung von Lebensmitteln beruht auf der durch technische Systeme erzwungenen Fluidkontraktion und den damit ausgelösten physikalischen und chemischen Veränderungen in den Mikroorganismen und Viren. Prinzipiell wird bei der Hochdruckbehandlung von Lebensmitteln im hydrostatischen System gearbeitet, d.h. die Kräfte im Inneren der Hochdruckanlage befinden sich im Gleichgewicht. Dies wird durch das Eintauchen der verpackten Produkte in ein druckübertragendes Fluid, im Normalfall Wasser, erreicht. Bei Behandlungsdrücken, die nicht selten bei 800 MPa liegen, werden Lebensmittel um bis zu einem Viertel ihres Volumens gestaucht. Diese Kompression ist reversibel, so dass am Ende der Behandlung das ursprüngliche Volumen wieder erreicht wird. Folgende Vorteile verbinden sich mit diesem alternativen, nicht-thermischen Pasteurisationsverfahren: (a) Niedrige thermische Belastung des Produkts, (b) Kurze Prozesszeiten (kleiner 5 Minuten pro Charge), (c) Automatisierbarkeit, (d) Geringer Energiebedarf (unter 20 kWh/t) und (e) Behandlung in der Endverpackung. Die Eignung des Hochdruckverfahrens muss sich in der Praxis an den Kosten und an den zur Verfügung stehenden Prozessalternativen orientieren. Ein Vergleich

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muss neben der Kosteneffizienz auch die mit der Erreichung des Prozessziels verbundenen Einbußen auf die Lebensmittelmatrix berücksichtigen. Grundlage der Entscheidung für eine bestimmte Technologie sollte daher immer eine am Einzelfall durchgeführte Kosten-Nutzen-Analyse sein. Die Intensität des Hochdruckverfahrens wird über die drei Prozessparameter Druck, Temperatur und Zeit gesteuert. Die damit erreichbare hohe Selektivität zeigt sich deutlich im Verlauf der Isokinetik-Linien in der Druck-Temperatur-Projektion des Inaktivierungsverhaltens von Bakterien, Sporen und Viren.

Abbreviations

HEPES	2-(4-(2-Hydroxyethyl)-1-piperazinyl)-ethansulfonsäure
HPP	High pressure processing
MPa	Mega-Pascal

1 Introduction

Novel food processing technologies aim to provide safe, high quality foods with desirable nutritional and physico-chemical properties. More recently with the emergence of functional foods and nutraceuticals as well as with the increased use of minimal processing, health aspects of foods as well as gentle treatment concepts have been added to the already existing food processing requirements. This necessitates careful process design and results from intensive kinetic and nutritional evaluations for process development and monitoring.

The complex composition of most food and its large variety requires routes of processing which are both highly efficient in killing micro-organisms and flexible enough to retain the desirable attributes of the product. Despite the extensive knowledge in food preservation by heat treatment (Ramesh 1999; Larousse and Brown 1997) and despite continued attempts to improve the quality of processed foods (Durance 1997) there is still a need for technologies that minimise the heat effects on desired quality attributes of foods. Even intelligent concepts like e.g. high-temperature-short-time processing fail if heat transfer and/or heat penetration is limited by intrinsic physical properties of the product. Because the thermal energy which is required to kill the contaminating micro-organisms has to be conveyed across the product itself, the design of fast and uniform heating and cooling steps is one of the

primary challenges of industrial heat preservation. Heat can be transferred by conduction, convection, and radiation. Most of the in use thermal processing equipment (except few applications of microwave, inductive or ohmic heating) use systems where the heat is transferred across interfaces driven by a temperature gradient. On the product side only the convective heat transport can be enhanced by external measures, i.e. by forced agitation. The transferable heat flow and the required time to warm up the centre of a solid or highly viscous product by solely heat conduction is determined by the thermal diffusivity of the material.

Non-thermal processing technologies make use of other physical principles of transmitting the energy to the target structures within the product. One of those emerging processes which could serve as an alternative method for food preservation is the use of high hydrostatic pressure.

2 Processing technology

High pressure processing (HPP) of foodstuffs is used for the preservation and modification of foodstuffs. Thereby, foodstuffs are normally subjected for periods of a few seconds up to several minutes to hydrostatic pressures above 350 MPa. This treatment permits the inactivation of microorganisms and enzymes at low temperatures, whilst valuable low molecular constituents, such as vitamins, colours and flavourings, remain largely unaffected. The ability of hydrostatic pressures to inactivate microorganisms as well as to denature proteins was demonstrated about a hundred years ago (Knorr et al. 2006; Kessler and Horak 1981). Over the last decades process development has progressed rapidly and high pressure treated foodstuffs have been marketed in Japan since 1990 and in Europe and the United States since 1996 (Zhang et al. 1995; Morild 1981; Körmendy et al. 1998; Rizvi and Tong 1997). Without doubt, the preservation of foods is by far the largest commercial application of high hydrostatic pressure related to biological systems, and the application has steadily increased during the past 10 years. At present, 128 industrial installations exist with volumes from 55 to 420 litre and a total annual production volume of more than 200,000 tons. Almost half of it is meat, meat products, seafood or fish. The rest are plant based products like vegetable preparations or different kinds of fruit juices (Fig. 1).

Hydrostatic pressure is generated by increasing the free energy, e.g. by heating in closed systems or

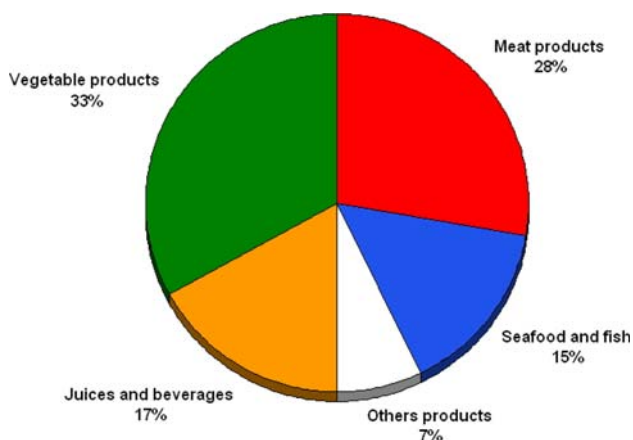


Fig. 1 Utilization of HPP preservation in different segments of food industry

by mechanical volume reduction. Industrial high pressure installations are operated batch wise and can reach pressures up to 800 MPa. The pressure is then kept constant for a designated time which ensures the success of the process, typically from several seconds to several minutes. From chemical industry, where pressure is widely used to increase the reaction yield, the technology has been transferred to food and biotechnology. In biological systems pressure higher than 400 MPa can lead to a reversible and irreversible cleavage of intermolecular and intramolecular bonds (Knorr et al. 2006). In this way structural changes in membranes as well as the inactivation of enzymes involved in vital biochemical reactions are the key targets of microbial kill by high pressure. The inactivation of virus is supposed to depend on the denaturation of capsid proteins essential for host cell attachment.

HPP is a relatively young preservation technology (Fig. 2). Compared to other methods which are commonplace in food industry like e.g. fermentation, drying or heating there is less experience in the specific features of HPP. The high pressure process itself is characterised by 3 parameters: temperature T , pressure p , and pressure exposure time t . Compared to other processes like heat preservation which is based on two parameters only (T , t) the three parametric HPP offers a broad variability for process design. Table 1 shows typical processing parameters for traditional and novel preservation treatments. In a qualitative approach, process efficiency is assessed in terms of the lethality of the treatment and its structural impact on the food matrix. Evidently, those treatments which are powerful in killing microbes have usually a strong destructive effect on the integrity of the food matrix with severe consequences on quality and consumer acceptance.

Traditional products are usually preserved by traditional technologies which, to a large extent, meet the expectations of the consumers of those foods. On the other side traditional preservation strategies fail or are not applicable when new product developments are based on innovative or uncommon ingredient compositions. In those situations non-thermal technologies like irradiation, pulsed electric elds or HPP came into the focus. The justification for applying novel preservation concepts should be: high safety margins, superior quality and reasonable costs.

3 Physical and chemical background

Within the last 20 years a considerable knowledge on the impact of high pressure on microbes, virus, food constituents and food structures has been accumulated and many practical applications of high pressure technology in food industry and biotechnology took advantage from the substantial advances in biochemistry and biophysics which led to an improved understanding of the mechanistical background.

In complex matrices like food the desired effect of e.g. microbial inactivation may also produce biochemical changes which may affect the product properties in a negative manner. The suitable selection of the processing parameters temperature, time and pressure can ensure that the processing goal is reached without extensive detrimental effects.

Proteins which play a major role in the metabolic activity of all living cells are extremely susceptible to changes in the environment. The stability of the protein's molecular con guration in its functional form is determined by a narrow band of parameter settings which impact mainly on how the protein interacts with the solvent. In the case of water, which

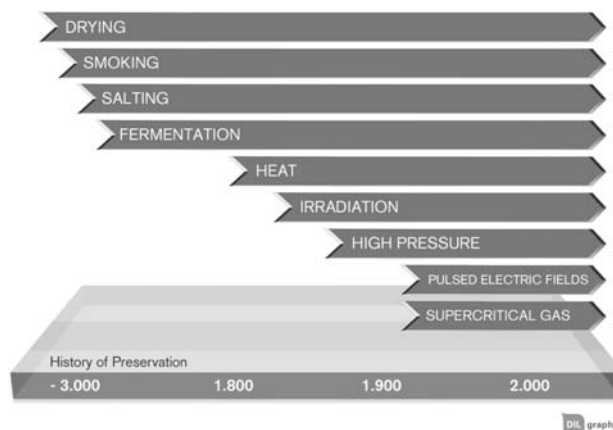


Fig. 2 History of food preservation methods

Table 1 Characteristic features of different preservation treatments

	Processing parameters	Processing intensity	Lethality	Structure impact	Cost [€/kg]
Drying	T, t	+	--	++	5
Smoking	c, T, t	+	+	++	2
Salting	c, t	-	+	++	1
Fermentation	b, T, t	--	+	+	2
Heat	T, t	+++	+++	+++	0.05
Irradiation	$\int w$	++	+++	++	0.3
High pressure	p, T, t	+	++	+	0.3
Pulsed electric fields	$E, \int w$	++	++	+	0.1
Supercritical gas	c, p, T, t	+	+	+	1

b microbial growth parameters, c chemical efficiency parameters, E electric field strength, $\int w$ specific total energy input, p pressure, T temperature, t pressure exposure time

is the natural protein environment a hydration shell is formed which itself is in unencing the intramolecular interaction. Likewise, ionizable groups in lateral positions produce conformational changes driven by the actual proton concentration and ionic strength. The losses in functionality of proteins in response to those perturbations is hence related to intramolecular reorientations or complete unfolding leaving the polypeptide chain in a random-coiled state.

In many cases, the 'functional' state is considered as the native state whereas the 'unfunctional' state is referred to as the denatured state no matter which particular molecular structure they form. Nevertheless, there are means to discriminate between both states and thermodynamic potential functions like Gibbs free energy are particularly useful when the transition from the native to the unfolded conformation is under consideration. In those situations the difference in Gibbs free energy ΔG is reduced to zero which occurs only at distinct settings of the relevant physical and chemical parameters: $\Delta G = \Delta G_0 + f(T, p, \text{pH, co-solvents, ...})$. If all parameters apart from T and p are fixed, the slope of the phase boundary is described by the equation of Clausius-Clapeyron:

$$\frac{dp}{dT} = \frac{\Delta S}{\Delta V} \quad (1)$$

In those situations, the transition is accompanied by an exchange of latent heat with the environment. Generally, the existence of a phase transition is based on an enthalpic and an entropic contribution to the free energy function:

$$d(\Delta G) = \Delta V dp - \Delta S dT \quad (2)$$

An equation (Eyring equation) has been derived from the transition-state theory, relating pressure and the rate constant k of reactions under pressure using the activation volume ΔV^\ddagger as a parameter [7].

$$\left(\frac{\partial \ln k}{\partial p}\right) = -\frac{\Delta V^\ddagger}{RT} \quad (3)$$

The functional associations of pressure, temperature and reaction time are best presented by means of pressure–temperature diagrams (pT-diagrams), which show pressure-temperature combinations that will lead to a desired reaction (e.g. inactivation) rate constant. Thus, a database software was particularly designed to enable the user to call up pressure–temperature function equations for a number of microorganisms, enzymes and food constituents and to present them in pT-diagrams for predetermined treatment times or as kinetics under predetermined p-T conditions (Buckow and Heinz 2009).

4 HPP inactivation of vegetative bacteria

The main application of HPP in the food industry is for the extension of shelf-life or for the elimination of microbial pathogens. The viability of vegetative microorganisms may be affected by inducing structural changes at the cell membrane or by the inactivation of enzyme systems which are responsible for the control of the metabolic actions (Knorr and Heinz 2001). Typically, significant inactivation of vegetative bacteria, yeasts and moulds viruses can be observed within minutes at room temperature and pressures higher 300 MPa (Farkas and Hoover 2001). However, increasing the pressure to 700 MPa or higher most inactivation reactions are strongly accelerated.

So far there only a few studies reporting inactivation kinetics of vegetative microorganisms over a wide range of pressure–temperature combinations. Figure 3 exemplarily shows pressure–temperature

combinations that lead to 5 log reduction of several pathogenic and spoilage organisms within 5 min of treatment. It is generally accepted that pressure and temperature act synergistically on the destruction of vegetative bacteria in the high temperature domain, which is indicated by the left bend of isorate curves in Fig. 3. However, *L. casei* seems to represent an exception as counter-effects of pressure and temperature has been observed for its inactivation in 10 mM HEPES buffer (pH 5.3) (Sonoike et al. 1992). As can be seen from Fig. 3, pressure stability of microorganisms often appears to be maximal at 20–40 °C whereas stability is decreased at lower temperatures. This might be explained by the increase of water and cell cytoplasm compressibility with decreasing temperature (Bridgman 1912) and thus, an increased transfer of mechanical energy to the microbial cell. Assuming microbial cell death is initiated at a certain threshold of mechanical energy transferred into the cellular system, at low temperatures this lethal threshold is achieved at lower pressures than the pressure needed at higher temperatures (Lori et al. 2007).

5 HPP inactivation of bacterial spores

Bacterial spores are not by themselves an hazard to the food industry. It is the eventual germination, outgrowth, and proliferation of the organism which results in toxication or spoilage of food during the post-processing storage. Bacterial endospores, as

compared to vegetative cells, display a considerably higher resistance to temperature and high pressure. To cope with this potential hazard, three strategies are in use to minimise the risk of spore contamination:

1. Full inactivation in one step by severe temperature conditions or suitable pressure–temperature combinations,
2. Germination spores by temperature and/or pressure and inactivate them in a subsequent temperature or pressure/temperature treatment (milder than strategy 1),
3. Injury of spores by temperature or pressure/temperature treatment (milder than strategy 1) and prevent germination or outgrowth in the food by matrix inherent hurdles.

At present the database only considers those published results that reach spore inactivation by a one step process (strategy 1). Spores of *Clostridium botulinum* and *Bacillus species* are the key bacteria for the safety or the spoilage of low acid (heat treated) preserved goods. These spores have shown remarkable tolerance to pressures above 1,000 MPa at room temperature (Margosch et al. 2004, 2006). On the other hand, many other bacterial endospores, which are relevant to food are inactivated at pressures 600 MPa or greater in combination with initial temperature above 60 °C (Heinz and Knorr 2002). Often the required inactivation temperature and/or time is lowered by combination with pressure as indicated in the pressure-temperature plain of Fig. 4 for a number of bacterial spores.

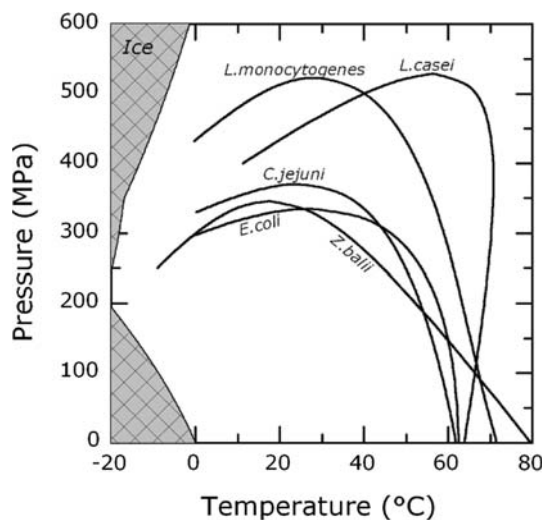


Fig. 3 Pressure–temperature isorate diagram for 5 log inactivation of *C. jejuni* (Lori et al. 2007), *E. coli* and *L. casei* (Sonoike et al. 1992), *L. monocytogenes* 74903 (Lori 2008) and *Z. bailii* (Reyns et al. 2000) after 5 min isothermal/isobaric treatment

6 HPP inactivation of virus

Viruses, regardless of their type of envelope, show a wide range of sensitivities in response to high hydrostatic pressure (Koutchma et al. 2005). It has been suggested that virus inactivation by high pressures is due to denaturation the capsid proteins essential for host cell attachment to initiate infection but leaves the actual capsid and RNA intact (Khadre and Yousef 2002; Kingsley et al. 2002). For protein unfolding it has already been stated that high pressure can not be seen independently from the temperature at which the treatment is performed. Thus, it is not surprising that also the pressure stability of viruses is greatly affected by the process temperature (Fig. 5). However, in contrast to proteins, pressure induced stabilization of viruses towards heat inactivation is not a general

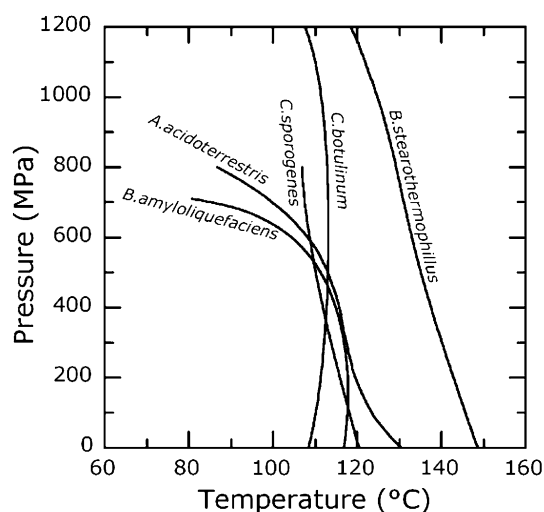


Fig. 4 Pressure–temperature isorate diagram for 5 log inactivation of *A. acidoterrestris* (Ardia et al. 2003), *B. amyloliquefaciens* (Rajan et al. 2006), *B. stearothermophilus* (Ardia 2004), *C. botulinum* (Margosch et al. 2006) and *C. sporogenes* (Koutchma et al. 2005) after 5 min isothermal/isobaric treatment

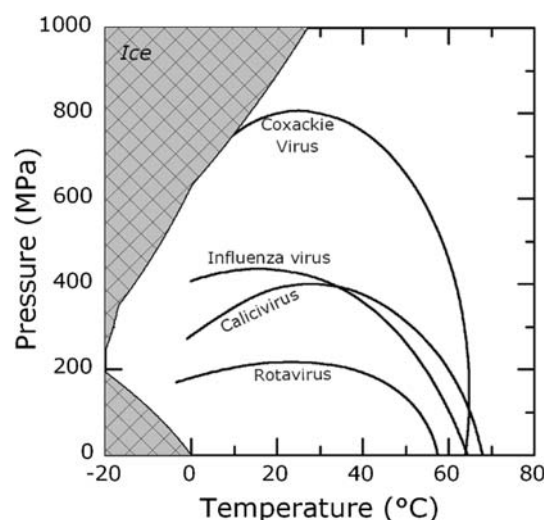


Fig. 5 Pressure–temperature isorate diagram for 5 log inactivation of Avian influenza virus (in chicken meat slurry) (Isbarn et al. 2007), Feline calici virus (in eagle medium) (Buckow et al. 2008), Coxsackie virus and Rota virus (Isbarn 2008) after 1 min isothermal/isobaric treatment

phenomenon, but has been observed in isolated cases (Müller-Merbach and Hinrichs 2006). On the other hand, a number of reports have indicated that the dissociation and denaturation of proteins and viruses by pressure is promoted by low temperatures (Buckow et al. 2008; Kingsley et al. 2004). Such behaviour is exemplarily shown in Fig. 5 for selected viruses and might be explained by an increased exposure of nonpolar protein side chains to water at low temperatures. This leads to enhanced interactions of nonpolar groups causing partial denaturation of proteins at elevated pressures (Grove et al. 2006).

7 HPP modification of food constituents (starch, protein, fat)

The primary structure of low molecular weight molecules such as vitamins, peptides, lipids, and saccharides is rarely affected by high pressure because of the very low compressibility of covalent bonds at pressures <2,000 MPa (Gross and Jaenicke 1994; van den Broeck et al. 1998; Oey et al. 2006; Cheftel and Culioli 1997). On the other hand, certain macromolecules, such as starches, can change their native structure during HPP, in a manner analogous to thermal treatments (Cheftel 1992; Heremans 1982). For example, starch granule solutions can form very smooth starch pastes, which can be used to replace fat in reduced energy foods (Stute 1997; Stute et al. 1996). Starch granules solutions can form a weak gel

due to pressure induced swelling of the granule (Stolt et al. 2000). Therefore, the occurrence of intermediate degradation levels of the lamellar crystalline regions of the starch granule can be anticipated, which is a possible reason for the significant difference, e. g. in viscosity between starch gels formed at different pressure/temperature conditions.

The pressure range in which gelatinization occurs is specific for each starch and is partly dependent on its crystalline structure, e. g. B-type starches are more resistant to pressure than A- and C-type starches (Stute et al. 1996; Stolt et al. 2000; Bauer and Knorr 2005; Muhr and Blanshard 1982; Blaszcak et al. 2005) and the proportion of amylose and amylopectin (Blaszcak et al. 2005; Blaszcak et al. 2007). Usually the extent of gelatinization reached depends on pressure level, treatment temperature and processing time (Stolt et al. 2000). The pT diagram of Fig. 6 shows a compilation of phase transition lines (native/gelatinized) of different starch-water suspensions. It is evident that the gelatinization temperature of starch granules is decreased when the pressure exceeds a specific threshold level. However, the pressure effect is by far smaller when the treatment temperature is approaching the gelatinization temperature at atmospheric pressure. Wheat starch is different in that sense because the temperature required to irreversibly change the native granule structure is already decreased markedly when pressure is increased by 50 MPa (Douzals et al. 2001).

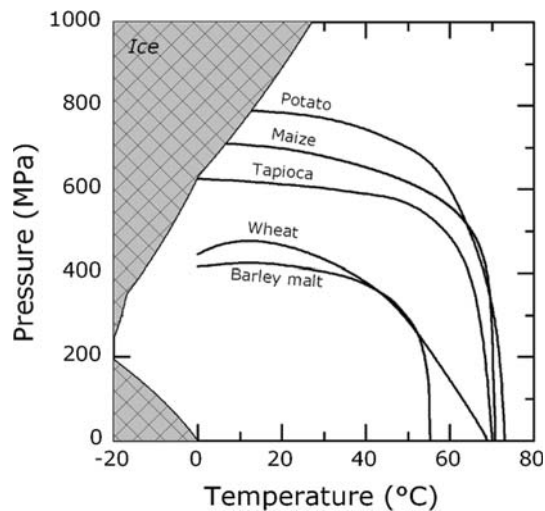


Fig. 6 Pressure–temperature phase diagram for complete gelatinization of starch granules from barley malt (Heinz et al. 2005), maize (Buckow et al. 2007), potato (Rumpold 2005), rice (Rubens and Heremans 2000), tapioca (Rumpold 2005) and wheat (Bauer and Knorr 2005) after approximately 15 min isothermal/isobaric treatment

8 Legislative aspects

Prior to introducing novel foods to the market food companies need to get an approval that those products are in compliance with the food law. With regard to the manufacturing process the question may arise whether a technology that can be considered as “novel”, is necessarily producing “novel food” within the meaning of the law. The “Novel Foods Regulation” (Regulation (EC) No 258/97) defines novel food as a food, that does not have a significant history of consumption within the European Union (EU) before the 15th of May, 1997. Such foods are subject to a pre-market safety assessment, before a decision is made on EU-wide authorisation.

The original intention of the Novel Foods Regulation was to introduce a legal framework for foods and food ingredients containing or consisting of genetically modified organisms (so called GMO). For the first time, this legal framework provided the possibility to allow GMO in food stuffs. Because of the fact that GMO were (and are) politically highly disputed, the European legislator tried to “hide” this new approach of allowing GMO and used the term of “Novel Foods”, regulating not only GMO, but also other kinds of novel foods like — for example — foods consisting of micro-organisms, fungi or algae. Nowadays, GMO are not subject of the Novel Foods Regulation any more. Today, GMO form a separate legal category and they are regulated by separate provisions.

What concerns processing aspects, the legislations defines Novel Foods as follows:

Article 1

(f) foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances.

If a food falls under the definition of novel food, the person responsible for placing it on the market has to apply for an authorisation. Only if a food was commercialised in at least one Member State before the 15th of May, 1997, it can be marketed elsewhere in the EU under the “principle of mutual recognition”, and the Novel Foods Regulation does not apply.

In order to be granted the authorisation, the applicant submits a request to the Member State in which the product is to be placed on the market for the first time. The request shall contain the necessary information, including a copy of the studies which have been carried out and any other material which is available to demonstrate that the food complies with the demanded criteria. These criteria are:

Article 3

Foods and food ingredients falling within the scope of this Regulation must not:

- *present a danger for the consumer,*
- *mislead the consumer,*
- *differ from foods or food ingredients which they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer.*

Furthermore, the applicant has to provide an appropriate proposal for the presentation and labelling of the food.

Once the application has been accepted, the Member State has 90 days to produce an initial opinion. This opinion is then circulated in all EU Member States, who are then given a further 60 days period to comment or make a reasoned objection. If there are no objections, the novel food will be authorised (or rejected) at the end of the 60 days in line with the initial opinion. Otherwise, a decision on the authorisation will be taken by a vote among Member States at the Standing Committee on the Food Chain and Animal Health. If necessary, the European Food

Safety Authority will first be asked for its opinion on any outstanding safety questions.

The Novel Foods Regulation includes a simplified procedure for marketing certain types of novel food or novel food ingredient in the EU, if it is considered “substantially equivalent” to an existing food or food ingredient that is already marketed within the EU. In these cases, the company can submit a notification to the European Commission after obtaining an opinion on equivalence from an EU Member State.

In the UK for example, it is the Food Standards Agency who is the Competent Authority. If a company wants to benefit from the simplified procedure, it must provide the Competent Authority with specific data when requesting such an opinion. The company’s application dossier should show how the novel food or novel food ingredient may be substantially equivalent to an existing food or food ingredient as regards to its: (a) composition (such as the source organism and preparation method), (b) nutritional value, (c) metabolism, (d) intended use (such as a food ingredient or supplement) and (e) level of undesirable substances (such as contaminants, mycotoxins and allergens).

9 Conclusions

HPP is a process that can inactivate microorganisms, spores and virus at low or moderate temperatures whilst retaining sensory and nutritional properties. This ‘novel’ non-thermal technology has the potential to be used in the development of a whole new generation of value added foods.

Although food safety issues and the achievable extension of shelf-life and the legislative situation need to be inspected case-by-case the existing experimental data can be helpful in exploring potential fields of application for high pressure processing. HPP is not likely to replace all traditional processing methods, but it may complement such methods and find niche applications. In addition, novel physico-chemical and sensory properties obtained from this technology offer exciting opportunities for industry.

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