

Safety assessment of high pressure treated foods

(shortened version)

The DFG-Senate Commission for Food Safety (SKLM) considered the high pressure treatment of foodstuffs already in 1998 and published an opinion entitled "High pressure treatment of foodstuffs, particularly fruit juices". In view of the continued development of this technology, the extension of the types of product involved and the ongoing research in this field, the Working Group "Food technology and Food Safety" ¹⁾ of the SKLM has re-evaluated the microbiological, chemical, toxicological, allergological and legal aspects of the high pressure technology. On 06th December 2004 the SKLM agreed on the following opinion on the "Safety assessment of high pressure treated foods", the English version was adopted on 1th April 2005.

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1 Introduction

The high hydrostatic-pressure treatment (HHP pasteurisation) 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 150 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 [1, 2]. 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 [3-6].

[...]

2 Basis of the high pressure technology

Hydrostatic pressure acts equally at all points of the product. The efficacy of the pressure is thus independent of the geometry of the product, so that even in non-homogeneous food preparations all components experience a homogeneous treatment. In contrast, the thermal treatment of foodstuffs by pasteurisation processes is basically associated with large temperature gradients, in which heat-induced changes, such as denaturation, browning or film formation, may occur [8].

The basis of the efficacy of high-hydrostatic-pressure is Le Chatelier's principle. Reactions, conformational alterations or phase changes, that are associated with a volume reduction occur preferentially under pressure, while those accompanied by a volume increase are inhibited.

The increased pressure can be achieved by two differing technological processes. In the direct system a hydraulically driven piston is pushed into the pressure container and the volume is decreased. In the indirect system a pressure transferring medium is injected with the help of a pressure pump. Liquid foodstuffs can therefore be put directly into the pressure container, while packaged foodstuffs are treated by means of a pressure transferring medium (usually water).

[...]

3 Placing on the market of high pressure treated foodstuffs

Before high pressure treated foodstuffs can be introduced into the European Union's (EU) market it needs to be determined, whether they fall within the scope of Regulation (EC) No 258/97 which considers as novel

"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."

Before the Regulation (EC) No 258/97 came into force, high pressure pasteurised orange juice had been placed on the French market. Subsequently, national authorities of the EU member states that are responsible for the enforcement of the Regulation have examined applications for approval or requests regarding the legal status of the following high pressure treated products: fruit preparations (France), cooked ham (Spain), oysters (Great Britain),

fruits (Germany). In all cases, it was shown that the high pressure treatment has not caused significant changes in the composition or the structure of the products affecting their nutritional value, metabolism or the amounts of undesirable substances (see Fig.1).

Fig. 1: The placing on the market of high pressure treated foodstuffs

In France high pressure pasteurised **orange juice** had already been marketed before Regulation (EC) No 258/97 came into force.

In December 1998 the Groupe Danone submitted in accordance with Regulation (EC) No 258/97 an application for the placing on the market of high pressure treated **fruit preparations** to the French competent authority. Since high pressure treatment had been employed for the pasteurisation of orange juice but not for fruit preparations, the applicant considered the latter as a novel food ingredient in accordance with article 1, paragraph 2 f of Regulation (EC) No 258/97. However, the results of the studies that the applicant provided that the high pressure treatment does not cause no significant changes in the composition or structure of the fruit preparation, which might affect its nutritional value, metabolism or level of undesirable substances.

Having examined the dossier, the competent authority, the *Agence française de sécurité sanitaire des aliments* (AFSSA) arrived at the same conclusion and stated that the high pressure-treated fruit preparations, apart from a higher vitamin content in most cases, did not differ significantly from those that have been thermally pasteurised.

The European Commission decided in May 2001 to authorise the placing on the market of the high pressure pasteurised fruit preparations [65].

The competent authorities of the EG-Member States agreed in July 2001, that in future the national authorities should decide on the legal status of high pressure treated foodstuffs on the basis of appropriate data provided by the manufacturer. If the competent authority arrives at the decision, that the product does not fall within the scope of Regulation (EC) No 258/97 and thus can be marketed without approval, the Commission and the other Member States should be informed accordingly.

The competent authority of Spain informed in July 2001, that high pressure pasteurised **cooked ham**, and the British *Food Standards Agency* in August 2002, that high pressure treated **oysters** are not considered novel foods and could therefore be placed on the market without approval.

In Germany an application for the examination of the legal status of high pressure preserved fruits was submitted to the then existing *Bundesinstitut für Verbraucherschutz und Veterinärmedizin* (BgVV). The BgVV came in March 2001 to the decision that the high pressure treatment does not cause significant changes in the composition or the structure of the fruits, which affect their nutritional value, metabolism or level of undesirable substances. The European Commission and the EU-Member States were informed accordingly by the BgVV.

4 Safety evaluation

Guidance on the types and extent of information that are likely to be required to establish the safety of foods which have been subjected to a process not currently used in food production can be found in the Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and food ingredients [9].

In the case of high pressure treated foodstuffs information on the specification of the origin and the composition of the product and on the technical details of the applied process, the apparatus and equipments as well as packaging materials is considered necessary to predict whether the potential of the process to introduce physical, chemical and/or biological changes in the food might have an impact on essential nutritional, toxicological and microbiological parameters. This includes a description of storage conditions of the food before and after application of the process. The food to which high pressure has been applied should be compared either to untreated counterparts or to counterparts which have been processed in a related traditional manner, e.g. thermal heating, with regard to the chemical composition and/or structure of inherent nutrients and toxicants of the food, taking into consideration the information available in the scientific literature. In particular, evidence should be provided, that an adequate destruction of health-relevant microorganisms has been achieved.

[...]

4.1 Microbiological aspects

Vegetative cells of bacteria relevant for foodstuffs are destroyed by hydrostatic pressures ranging from 150-800 MPa. Evidence for this derives from numerous investigations including pathogenic microorganisms. The inactivation kinetics for microorganisms under pressure show a steady decrease which, as in thermal processing, may tail off. It is not yet clear as to whether this represents a pressure-resistant sub-population. The survival of vegetative cells during and after high pressure treatment strongly depends on the matrix of the foodstuff [10-15].

[...]

Bacterial endospores, as compared to vegetative cells, display a considerably higher resistance to high pressure. Spores of *Clostridium botulinum* and *Bacillus* species are key bacteria for the safety or the spoilage of low acid (heat treated) preserved goods; these tolerate pressures above 1000 MPa at room temperature. By combined pressure/temperature treatment an inactivation of such food-relevant bacterial endospores is possible. In principle the required inactivation temperature and/or time is lowered by combination with pressure [11, 18-21]. Bacterial endospores can, however, be protected under certain pressure/temperature combinations through high pressure treatment against thermal inactivation [22]. This has to be taken into account, particularly in the development of rapid processes with very high pressures and temperatures. It is not possible to make extrapolations on the basis of data from conventional systems.

The pressure tolerance of bacterial endospores varies within species and strains, and within strains it is also dependent on sporulation conditions. Among the examined endospore formers the spores of *Bacillus amyloliquefaciens* showed the highest resistance to pressure. In a carrot pulp matrix these spores are inactivated at 800 MPa and 80°C within 50 min. by 5 log. The endospores of *C. botulinum* type A, B and F were inactivated at 600 or 800 MPa within a few minutes by more than 5 log [23].

In principle, **viruses** can also be inactivated by high pressure. The multiplicity of virus types and their structures is, however, so large as to make it impossible to formulate a general statement at the present time. An increased risk compared to untreated foodstuffs is presently not recognisable.

Conclusion: The microbiological safety of high pressure treated foodstuffs can be evaluated by following established criteria. A case-by-case evaluation is necessary by using realistic numbers of the relevant bacterial species. A specific microbiological risk to health from high pressure treatment is not discernible according to the existing state of knowledge. However the inactivation by high pressure treatment of undesirable microorganisms present in a raw material has to be examined in each individual instance. A global assessment is not possible on the basis of conclusions drawn from the experience of the behaviour of substances and microorganisms during thermal processes. For the development and evaluation of processes it is therefore necessary to characterise the hygiene-relevant key organisms.

4.2 Chemical aspects

In principle, those chemical reactions are accelerated under pressure for which the reaction and activation volumes are negative. Examples of such reactions are the formation of covalent bonds by cycloadditions and the formation of ions e.g. through dissociation. Under

realistic production conditions cycloadditions of appropriate reaction partners have not been observed in the food matrix [24, 25]. Homolytic -cleavage (radical formation) is, however, inhibited by pressure. Interactions between the dissolved species and the solvent influence the partial volumes and thereby the reactivity [6, 26, 27].

Water soluble **vitamins**, such as vitamin C, the vitamins B1, B2, B6 and folic acid [28], appear to be not or only little affected by pressure treatment under realistic production conditions. Changes are noticed in model systems rather than in the food matrix which exerts a protective effect. This holds also for fat soluble vitamins, such as vitamin A, vitamin E [29] and vitamin K as well as provitamin A [30, 31]. Chlorophyll is stable under pressure at low temperatures [32-34].

[...]

Contradictory statements exist regarding the **oxidation of fats** in foodstuffs through high pressure treatment. Such changes are often not clearly distinguished from changes occurring during storage [36, 37]. Residual enzymatic activities, fatty acid spectrum, water content, pH-values, degree of oxidation before pressure treatment, pro- and antioxidants all have a decisive influence on the pressure-induced changes in lipids and the progress of oxidation during storage. Structural changes in the cell membrane up to destruction of the cellular agglomerate and decompartmentation all have an effect on the oxidation of lipids.

Carbohydrates are largely insensitive to pressure. Methylglycosides may be hydrolysed under pressure into the aglycone and MeOH (the activation volume is slightly negative). Disaccharides are stable (the activation volume is slightly positive). At pressures above 1000 MPa solvolysis reactions of the glycosidic bonds can occur [6]. Polysaccharides can, however, be influenced as concerns their water binding and gel forming properties [38]. These changes relate, however, to the functional properties and do not involve structural alterations.

The primary structure of **proteins** is not affected by pressure. Pressure influences the quaternary structure of the protein through hydrophobic-interactions, the tertiary structure through reversible unfolding, and the secondary structure through irreversible unfolding. Pressure-induced gels have rheological properties different from heat-induced gels. The break-down of pressure-modified proteins by proteases is increased, which probably points to a higher water binding capacity.

Of special interest is the behaviour of prion proteins. High pressure treatment of hamster and cattle prion proteins reduces their resistance to proteolysis [42, 43].

Pressure treatment can affect not only the activity but also the substrate specificity of **enzymes**. A partial inactivation is also possible. Reactivation of the enzyme activity, e.g.

during storage, can possibly lead to the formation of undesirable substances. In some cases an increase under pressure in the activity of enzymes has been observed, which could produce off-flavours during the build-up phase of the pressure. Only very few data exist on the substrate specificity of enzymes in the field of foodstuffs. Thus peroxidases are inactivated at low pressures in the presence of some substrates but not of others [45, 46]. As yet, the formation of toxic compounds as a consequence of a changed substrate specificity under pressure has not been observed.

Studies have shown that the **antioxidative** and **antimutagenic potential** of fruit and vegetable juices remains intact after high pressure treatment, although it is often lost through heat treatment [47].

If high pressure is used on foodstuffs it must be examined whether, under the chosen processing conditions, peptides may be formed, which after oral uptake might be biologically active. In general those peptides with pyroglutamate (2-oxyprolin) at the N-terminal are more resistant to breakdown by peptidases. Such substances are occasionally biologically active. Under conditions of elevated pressure as well as of elevated temperature, the conversion of glutamine into pyroglutamate (2-oxyprolin) is favoured [39-41].

[...]

Conclusion: High pressure treatment can cause chemical changes in foodstuffs, which involve preferentially those reactions and conformational changes, that are associated with a reduction in volume. The vitamins, colours and flavourings so far examined remain largely unaffected in comparison with conventional thermal processes.

4.3 Toxicology

The required extent of toxicological investigations depends on the type of changes induced by the high pressure treatment [7, 9].

If it can be shown by appropriate analytical studies, that the high pressure treatment causes none or no significant changes in the chemical composition and/or the structure of the foodstuff ingredients, then the product may be judged to be substantially equivalent to the corresponding conventionally treated comparison product and therefore can be accepted without further investigations. This is the case with the presently permitted products (see annex). If however any evidence appears that such changes occur, then an evaluation of the safety to health becomes necessary. The toxicological studies needed depend on the nature of changes induced by the high pressure treatment, the expected magnitude of the consumption of these products, and the resulting exposure of the consumer to the ingredients concerned.

Furthermore, it must be ensured, that components of the packaging do not transfer into the foodstuff in concentrations relevant for health. The corresponding migration limits for packaging components must be met.

From the investigations of high pressure treated foodstuffs so far carried out no evidence has appeared for an increased toxicological potential compared to unprocessed or thermally preserved foodstuffs. But, present toxins may not be eliminated in the same way as in a thermal process.

4.4 Allergenicity

The assessment of the influence of high pressure treatment on the allergenicity of foods should be performed by comparison with traditional food technological processes, in particular heat treatment. Allergenicity can be altered after technological processing (e.g. by formation of new allergens or -epitopes).

Many technological, particularly thermal, processes result in a partial inactivation of the allergenic potential [48, 49]. Studies of high pressure processes performed to date point in the same direction. Although the thermal treatment of foodstuffs causes drastic structural and chemical changes of food constituents, there is very little evidence for an increase in allergenicity from food processing [50-54]. An increase of the allergenic potential through high pressure treatment of foods is therefore unlikely, but cannot be entirely excluded because of the few studies carried out so far.

Up to now very few studies have been performed on the influence of high pressure treatment on allergenicity. Jankiewicz et al. found reduced IgE reactivity of an extract from celery tuber which had been treated with high pressure at 600 Mpa, when compared to untreated celery [49]. In particular, the IgE-binding capacity of the major allergen was reduced. RLB 2H3 cells (rat basophil leukaemia cells), passively sensitised with celery-specific IgE, showed a more than 50% reduced release of the mediator β -hexosaminidase. Therefore, the allergenic potential of the high pressure treated vegetable was graded between that of raw and of cooked celery. Another mechanism which might explain reduced allergenicity of a food after high pressure treatment has been described by Kato et al. [55]. It was shown, that the major allergens of rice are released from the grains after high pressure treatment at 500 Mpa in a liquid medium. Presently attempts are being made to use this observation for industrial production of hypoallergenic rice.

[...]

Experiments with the recombinant major allergen from apple revealed by CD-spectroscopy changes in the secondary structure [56]. Here a decrease of the α -helical regions and an increase of the β -sheet structures were found. Subsequent to high pressure treatment, apples were tolerated in challenge tests without symptoms by 5 individuals allergic to apples. Moreover, the major allergens of apple and celery were also found to be susceptible conventional treatment processes. Since this is in contrast to other allergens such as those from peanuts, it is not possible to draw general conclusions from these experiments.

Conclusion: The changes induced in foodstuffs by high pressure are from the allergological point of view relatively minor as compared to those occurring during thermal processing. Up to now no evidence is available that the allergenic potential of foodstuffs might be increased by high pressure treatment. Results of presently available studies suggest that high pressure treatment might be used for the specific reduction of the allergenicity of certain protein families.

4.5 Requirements for packaging and storage

Packaged foodstuffs are treated by means of a pressure-transmitting medium (usually water), which results in special demands on the packaging. Packaging materials sustain a mechanical strain during the high pressure treatment. At a pressure of, for example, 600 MPa (22°C) the volume of water or of liquid foodstuffs decreases by about 15%. Packaging suitable for high pressure must therefore be able to withstand without any damage the elastic deformation caused by this volume change [57, 58]. After high pressure treatment packaging materials must retain their barrier properties against gases (oxygen, water vapour, carbon dioxide), as these properties are needed in order to maintain product quality and shelf life expectancy [59, 60].

[...]

Conclusion: On the basis of these experimental results it is generally accepted that plastic foils and other packaging materials may be used in the high pressure treatment of foodstuffs. The suitability of the packaging must be examined case-by-case to ensure that the quality of the foodstuff is maintained during the storage period.

5. Research needs

5.1 Microorganisms

A large data base on the behaviour of **vegetative cells** under high pressure shows that the risks from spoilage organisms or pathogenic bacteria are in principle as manageable as in

thermal processes. The intentional utilization of synergistic or antagonistic effects of high pressure treatment with the foodstuff matrix requires an in-depth understanding of the mechanisms of the pressure-induced inactivation of bacteria. The inclusion of microbial sub-populations, especially in the case of pathogenic bacterial strains, is as necessary as the consideration of eukaryotic microorganisms and their spores, for which only few investigations exist.

The heat resistance of **endospores** does not correlate with their pressure resistance. For the evaluation of high pressure processes it is necessary to identify key organisms for the pressure inactivation of bacterial endospores including the participation of hygiene-relevant microorganisms and spores. Special interest should be directed toward the pressure-tolerant fraction of microorganisms, the behaviour of microorganisms and spores in processes with high compression rates, and the potential stabilisation of bacterial endospores in combined pressure-thermal processes. Experience exists regarding the behaviour of microbial toxins in thermal processes. However, these do not permit any extrapolation to their behaviour in high pressure treatment. Therefore data on the inactivation of bacterial toxins require to be determined.

Similarly no investigations are available on the inactivation or infectivity of foodstuff-transmitted **viruses** after high pressure treatment.

5.2 Chemical aspects

In the high pressure treatment of foodstuffs attention must be paid to certain reactions of food ingredients, that could lead to chemical changes. This holds in particular for the following reaction types: *dissociation* of organic acids and amines, the reversibility and reactivity of the dissociated species; *cyclisation reactions*: reactions of quinones with dienes (Diels-Alder) as well as 2+2 cycloadditions; formation of ammonium, sulfonium and phosphonium-salts, reversibility and reactivity of ions formed under pressure; *hydrolysis reactions* of ethers, esters, acetals and ketals. There is a need to clarify whether these reactions really play a role in foodstuffs.

The possible formation of bioactive peptides following the application of high pressure to protein-rich foodstuffs requires to be investigated. In the case of the already demonstrated transformation of glutamine into 2-oxyproline or of glutamate into pyroglutamate at the N-terminus of peptides it is also necessary to determine the influence of different neighbouring amino acids on the reaction-rate. Possibly further investigations may become necessary, e.g. of the absorption of such modified peptides from the gastro-intestinal tract and of their biological effects.

The reactions of certain amino acids in peptides and proteins to form succinimide structural elements are examples of non-enzymatic cyclisation reactions. Ring opening may lead to the formation of possibly undesirable derivatives. The deamidation reaction is a known modification of peptides and proteins. Here the investigation of its dependence on process parameters is required.

There exist numerous cyclic dipeptides, e.g. also with diketopiperazine structure, which represent bioactive substances. The pressure dependence of the cyclisation of di- and oligopeptides with a modified C-terminus requires investigation.

The effect of high pressure on the conformation of proteins in appropriate systems requires detailed investigation. Of special interest are protein-fibrils from β -sheets associated through wrongly folded protein aggregates, which appear in certain diseases such as the TSE-diseases. Despite the known pressure stability of β -sheets the use of a pressure of several hundred MPa already reduces the resistance of infective hamster-prion proteins (brain homogenates) to proteolysis.

Contradictory statements on the oxidation of fats in foodstuffs through high pressure treatment require clarification.

5.3 Allergenicity

The effects of high pressure treatment should not be examined exclusively *in vitro* but also *in vivo* by skin tests and challenge experiments. Model studies on the influence of high pressure treatment on the allergenicity of food proteins should concentrate on foods with known allergenic potential, and which are suitable for the application of high pressure technology. Only patients with confirmed food allergy, preferably by means of a double-blind challenge test, should be included in the clinical part of such studies. Foods from which purified, preferably recombinant, allergens are available should be selected in order to facilitate structural investigations. Moreover, the use of pure allergen molecules would permit to examine in models the interactions with constituents of the food matrix under controlled conditions. Studies in food allergic patients can of course only cover the effects of the high pressure treatment after sensitisation has already occurred. Possible alterations of the sensitising potential should be studied in selected animal species, preferentially using validated mouse models as well as cell culture systems.

5.4 Packaging

Concerning the effects of pressure on components of packaging materials it should be, for example, examined whether the physico-chemical properties of polymers change so much under pressure as to result in an accelerated diffusion of plasticisers, such as phthalates.

There is also a dearth of investigations on the behaviour of the residual monomers and volatile organic substances under high pressure. Similarly, product-specific attention should be paid to possible sensory changes.

5.5 Research needs of the processing technology

[...]

In the construction as well as in the choice of materials it is necessary to meet the special requirements of plants for the treatment of foodstuffs. From the point of view of process technology the construction of pressure containers and compression aggregates for pressures greater than 1000 MPa is also of special interest, because a shortening of process times or an optimisation of the process outcome can be achieved. The pressure-transferring medium in combination with the compression aggregate, the pressure container and the flowpipe system have a decisive influence on the speed and uniformity of the pressure build up.

6 Final comments

Hitherto, investigations on high pressure treated foodstuffs have not revealed any evidence of any microbial, toxicological or allergenic risks as a consequence of high pressure treatment. However, these findings do not suffice for a general evaluation, because they derive from only a few already marketed products. At present it is necessary, when a new product category is involved, always to carry out an individual case-by-case examination of high pressure treated foodstuffs. In the future it would be desirable to develop product- and process-specific test parameters, in order to be able to carry out any future safety evaluation of high pressure treated foodstuffs according to recognised standard criteria.

8. References

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