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HIGH HOMOGENIZATION PRESSURES TO IMPROVE FOOD QUALITY, FUNCTIONALITY AND SUSTAINABILITY

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HIGH HOMOGENIZATION PRESSURES TO IMPROVE FOOD QUALITY, FUNCTIONALITY AND SUSTAINABILITY

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RESUMEN

El interés por la tecnología de alta presión de homogeneización ha crecido a lo largo de los años. Es una tecnología con bajo consumo de energía, que no genera altas emisiones de CO_2 ni efluentes contaminantes. Las principales aplicaciones alimentarias derivan de su efecto sobre el tamaño de partícula, causando una distribución más homogénea de las diferentes fases de un fluido (partículas, glóbulos, gotitas, agregados, etc.) y favoreciendo la liberación de componentes intracelulares; y su efecto sobre la estructura y configuración de componentes químicos como los polifenoles, y las macromoléculas como los carbohidratos (fibras) y las proteínas (también microorganismos y enzimas). Los desafíos del siglo XXI dirigen a la industria alimentaria a la obtención de alimentos con alta calidad nutricional y al aprovechamiento de los residuos para la obtención de ingredientes con propiedades específicas. Para este propósito, las tecnologías suaves y no térmicas, como la homogeneización por altas presiones, tienen un gran potencial. El objetivo de este trabajo es hacer una revisión bibliográfica que nos proporcione información de cómo la necesidad de combinar seguridad, funcionalidad y sostenibilidad en la industria alimentaria ha condicionado las aplicaciones de la tecnología de altas presiones de homogeneización en la última década.

PALABRAS CLAVE: alta presión de homogeneización; funcionalidad alimentaria; componentes bioactivos; desperdicio agroalimentario; sostenibilidad.

ABSTRACT

The interest in high homogenization pressure technology has grown over the years. It is a green technology with low energy consumption, not generating high CO2 emissions or polluting effluents. The main food applications derive from its effect on particle size, causing a more homogeneous distribution of fluid elements (particles, globules, droplets, aggregates, etc.) and favouring the release of intracellular components; and its effect on the structure and configuration of chemical components such as polyphenols and macromolecules such as carbohydrates (fibres) and proteins (also microorganisms and enzymes).The challenges of the 21st century lead food industry processing towards obtaining food with high nutritional quality and taking advantage of waste to obtain ingredients with specific properties. For

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this purpose, soft and non-thermal technologies such as high pressures homogenization have a huge potential. The objective of this work is to review how the need to combine safety, functionality and sustainability in food industry has conditioned the last decade applications of high-pressure homogenization technology.

KEYWORDS: High homogenization pressure; food functionality; bioactive components; agri-food waste; sustainability.

RESUM

Amb els anys, l'interès per la tecnologia d'alta pressió d'homogeneïtzació ha crescut. És una tecnologia ecològica amb baix consum d'energia, que no genera elevades emissions de CO_2 ni efluents contaminants. Les principals aplicacions alimentàries es deriven del seu efecte sobre la mida de les partícules, provocant una distribució més homogènia d'elements fluids (partícules, glòbuls, gotes, agregats, etc.) i afavorint l'alliberament de components intracel·lulars; i el seu efecte en l'estructura i la configuració de components químics com els polifenols i les macromolècules com els carbohidrats (fibres) i proteïnes (també microorganismes i enzims). Els reptes del segle XXI porten la indústria alimentària al processament cap a l'obtenció d'aliments amb alta qualitat nutritiva i l'aprofitament dels residus per obtenir ingredients amb propietats específiques. Per a aquest propòsit, les tecnologies suaus i no tèrmiques, com ara l'homogeneïtzació a altes pressions, tenen un potencial enorme. L'objectiu d'aquest treball és revisar com la necessitat de combinar seguretat, funcionalitat i sostenibilitat en la indústria alimentària ha condicionat a les aplicacions de la tecnologia d'homogeneïtzació d'alta pressió en l'última década.

PARAULES CLAU: Alta pressió d'homogeneïtzació; funcionalitat alimentària; components bioactius; residus agroalimentaris; sostenibilitat.

INTRODUCTION

In the homogenization process, a fluid is forced to pass through a gap, causing energy transformations that directly affect the dissolved, dispersed or emulsified components. The fluid undergoes mechanical (shear, hydrodynamic and cavitation effects) stress and an increase in temperature (thermal effect) of approximately 2-3 ° C for every 10 MPa of homogenization pressure (Augusto et al., 2018). These affect fluid structure and properties and also those of its constituent elements (particles, molecules, globules, droplets, aggregates, granules, etc.). The particle size decreases and a more homogeneous distribution of the elements is achieved, facilitating operations such as mixing and emulsification.

Initially the homogenization operation was introduced as a manufacturing step in the dairy industry. This operation reduced the size of the fat globules, increasing the stability of the emulsion and thus the physical and chemical stability of milk. It had a great impact on the quality of dairy products such as condensed milk, curd or ice cream. The applied pressure was less than 30

MPa and it was applied in one or two steps. However, technological development increased greatly having an impact on the design and geometry of the homogenization valves, which allowed working at higher pressures and with very short processing times (some seconds) (Osorio-Arias et al., 2020). High homogenization pressures operation was established at the beginning of the 2000's as an alternative non-thermal treatment in the food industry and applications were extended to industries other than dairy and to other fields such as textile or biotechnologic.

Currently, the existence of valves of different geometries has given rise to different equipment able to work at pressures higher than 400 MPa. Thus, a distinction is made among standard homogenization for pressures between 0 and 50 MPa, high pressure homogenization (HPH) for pressures between 50 and 300 MPa and ultra-high pressure homogenization (UHPH) for pressures equal to or greater than 400 MPa. Processing efficiency is modulated by applying various pressure ranges or combining a pressure value with a specific number of passes through the equipment (Barba et al., 2012; Bevilacqua et al., 2019). In addition, the possibility of operating in continuous conditions for a great diversity of pumpable fluids has made it possible to extend the application to activation/inactivation of enzymes, reduction of microbial load, mixing, dispersion, emulsification or encapsulation processes, cell breakage processes and modification of proteins or macromolecules to obtain ingredients or additives with differentiated properties.

Nowadays, concern about food functionality and sustainability has triggered research for increasing the bioavailability and bioaccesibility of active components and probiotics and for the extraction of macro and micro-molecules from food by-products. Moreover, the challenge of increasing the nutritional characteristics of food must be combined with a reduction of the environmental impact and food security. In this context, alternative, soft and non-thermal technologies such as high pressures homogenization have a huge potential. The objective of this work is to review how the need to combine safety, functionality and sustainability has conditioned the applications of high pressure homogenization technology in food.

MATERIAL AND METHODS

This bibliographic review covers the period from 2010 to early 2020. Online databases such as MEDLINE, Science Direct, Web of Science and Wiley Online Library have been used. The following keywords and combinations have been used as main search terms: high homogenization pressures, non-thermal technologies, food processing, encapsulation, functional food, bioactive components, probiotics, microbial load, enzyme inactivation and protein extraction.

RESULTS AND DISCUSSION

Last decade evolution and main applications

Publications in peer-reviewed journals show that the main applications of HPH in food have the following objectives:

- Conservation and safety by decreasing the microbial load and inactivating enzymes. It occurs as a consequence of thermal effect derived from mechanical stress or from structural changes in proteins.
- Recovery and extraction of proteins, fibrous materials and bioactive compounds of special interest (mainly polyphenols) and increase of the functionality considered in terms of technologic use (stabilization of emulsions and dispersions, flow capacity and viscosity modifications, emulsifying activity improvement...). Mechanical stresses and hydrodynamic effects induce cell disruption favouring the release of intracellular content or structural components of cell wall. Moreover, dispersed particles or fat droplets can be reduced in size and modified in structure.
- Increase of functionality in terms of health effect (increase bioaccessibility, bioavailability or probiotic effect). These effects result from favouring the release of bioactive compounds, modification of biopolymer structure and development of novel particle interactions and networking. Micro or nanocapsules can also be developed.

As it can be observed in Figure 1a, between 2000 and 2009 HPH were used mainly for the extraction of proteins, although a large number of research works were aimed to microorganisms and enzymes inactivation, contributing to food preservation and safety. The last decade (from 2010 until now) revealed a significant increase (74.87%) in the total number of scientific articles published. The main areas in which there has been an increase greater than the total value are related to the use of HPH for microorganisms inactivation, fibre extraction, and above all, that related to bioactive and probiotic components. Application of HPH to extract or increase functionality of food bioactive compounds and to improve probiotic effect grew by 89% and 87,9% respectively (figure 1a). The increasing interest of consumers and the food industry in improving the organoleptic and nutritional quality of foods, along with the concern for food waste valorization might explain this result.

Figure 1b. shows the evolution in the number of published research works related to HPH application with regard to the type of food. Although the majority of works belongs to the area of fruit juices, the largest growth has occurred in plant-based beverages and food waste. The huge increase in plant-based beverages consumption (Picart-Palmade et al., 2019) and the general concern about food processing waste related issues are responsible for this fact. Moreover, HPH technology is identified as a green technology, due to short processing times, low energy consumption, low CO2 emissions and the fact that it does not require polluting solvents.

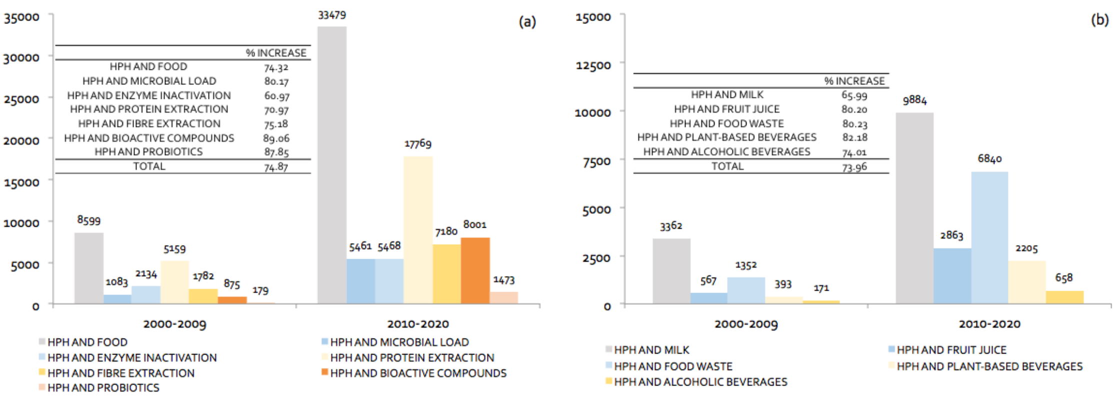

Figure 1. Number of scientific articles published regarding the main food application areas of HPH (a) and the different types of food to which it applies (b). % INCREASE has been calculated as the difference in the number of articles published between the periods 2010-2020 and 2000-2009, divided by the number of articles published in the period 2000-2009, expressed as percentage. (*Source: Science Direct. The following keywords and their combinations have been used as main search terms: high homogenization pressures, non-thermal technologies, food processing, encapsulation, functional food, bioactive components, probiotics, microbial load, enzyme inactivation, protein extraction, milk, fruit juice, food waste, plant-based beverages and alcoholic beverages).*

This increase in research works based on HPH technology is also due to the development of new homogenization equipment that works at elevated pressures (up to 400 MPa) and supports specific conditions. Since the invention of adjustable valves in 1930 (McClatchie, 1930), the possibilities of homogenization technology have increased. The geometry and design of the valve determines the mechanical effects on the treated fluid. In 1982 the invention of the Gaulin Micro-Gap valve (Pandolfe, 1982) greatly boosted the efficiency of the process, making high homogenization pressures possible in subsequent years and, more recently, the ultra-high homogenization pressures technology. In general, improvements have been obtained in all fields of application, making it an efficient tool with great potential for use in the food industry (Patrignani et al., 2020).

Preservation and safety

HPH treatment for enzyme and microbial inactivation is one of the technologies that have been used in recent years as an alternative to thermal processes that, in most cases, cause undesirable effects such as non-enzymatic browning, cooked flavour or valuable components degradation. It has been demonstrated that HPH treatment at processing pressures higher than 100 MPa contributes to microbial load reduction and enzyme inactivation. As previously stated the heating derived from homogenization (a rise of about 2.5 °C per 10 MPa) together with structural modifications of cell wall are the main effects responsible for microorganisms or enzymes inactivation. Final impact of HPH on microorganism viability or enzyme activity depend on several factors such as processing pressure, microbial strain or enzyme and food matrix.

In general, it has been verified that gram-negative bacteria exhibit a greater susceptibility to inactivation by HPH than gram-positive ones, due to the reduced content of peptidoglycan in the cell wall that makes it thinner and therefore easier to disrupt. Fungi and yeasts seem to have a susceptibility intermediate in between gram-positive and gram-negative bacteria, probably due to their wall structure, which is thicker but more complex than that from gram-positive bacteria (Balasubramaniam et al., 2016). For bacterial spores inactivation, pressures up to 400 MPa and additional hurdles are required (Patrignani et al., 2020).

Table 1. Research works evaluating the decrease in microbial load in different food products by HPH.

PRODUCT	TREATMENT	TERMS	MICROBIOLOGIC CONTROL	RESULTS	REFERENCE
Fruit juices (apricot and carrot)	HPH + rapid cooling	100 MPa (1-8 passes)	*Zygosaccharomyces bailii 45*	The juice type affected the yeast fate (growth or death) and viscosity change after HPH treatment	(Patrignani et al., 2020)
Mango nectar	HPH + thermal shock	200 MPa 10-20 s at 60-85 ° C	*A. niger* (COI 4573)	The combination of HPH with subsequent thermal shock was efficient in inactivating the heat resistant mould in the mango nectar	(Tribst et al., 2011)
Banana juice	HPH + rapid cooling	0, 150, 200, 300 and 400 MPa	Total mesophilic bacteria	Pressures greater than 200 MPa were required to obtain a reduction of 4 logarithmic units	(Calligaris et al., 2012)
Apricot juice	HPH+ citral + rapid cooling	100 MPa (1,3,5 and 8 passes)	*Saccharomyces cerevisiae* SPA	Decrease of the viability of the yeasts following a linear tendency with the pressure. Improvement of the antimicrobial effect by adding citral	(Tabanelli et al., 2013)

Mango juice (*Mangifera indica L.*)	HPH + heat treatment	40-190 MPa (1-5 passes)	Total plate count, moulds and yeasts	Complete inactivation of moulds and yeasts was achieved by 1 and 3 passes at 190 MPa and 60 °C, while the total plate count was less than 2.0 log CFU/ mL	(Guan et al., 2016)
Mulberry juice (*Morus atropurpurea* Roxb.)	HPH + heat treatment + Addition of Dimethyl Dicarbonate (DMDC)	200 MPa (1-3 passes)	Total count, yeast, mould and lactic acid bacteria	Combination treatment with 3 passes at 200 MPa and 250 mg DMDC/ L, decreased total count to the level reached by heat treatment at 95 ° C	(Yu et al., 2016)
Lupine based drinks	HPH + refrigeration	50, 100 and 175 MPa (2,4,6 passes)	Total bacterial count, moulds and yeasts. *Bacillus cereuses* and coliform bacteria	At 175 MPa yeasts, moulds and coliforms were completely eliminated with 2 and 4 passes	(Xia et al., 2019)
Granada juice	HPH + low temperature pasteurization	100, 150 MPa (10 passes) 55 or 65°C for 15 s	*Escherichia coli* (ATCC 26) and *Saccharomyces pastorianus* (ATCC 42376)	HPH at 150 MPa followed by a low heat intensity at 65 ° C for 15 s showed a reduction of 3 log CFU/ mL	(Benjamin & Gamrasni, 2020)
Skim milk	Heat treatment + HPH	100-300 MPa	*Bacillus stearothermophilus* ATCC 7953 and *Clostridium sporogenes* PA 3679	The efficacy of HPH is similar to pasteurization and must be combined with other conservation technique	(Pinho et al., 2011)

Milk	Heat treatment + HPH	300 MPa	Spores of *B. cereus*, *B. lincheniformis, B. sporothermodurans, B. coagulans, B. stearothermophilus, and B. subtilis*	Sterility at 300 MPa can be achieved with an initial milk temperature of 85 ° C	(Amador Espejo et al., 2014)
Skim and whole milk concentrates	Heat treatment + HPH	Skim milk: 0,20,50,70, 100,120 and 150 MPa. Whole milk: 0,20,30,35 and 40 MPa.	Total count, coliforms, enterobacteriaceae, moulds and yeasts and *Staphylococco*	HPH at 120 MPa completely inactivates the microbial load of milk concentrates	(Mercan et al., 2018)
Almond beveages	Heat treatment + HPH	200, 300 MPa (1,2 passes)	*Micrococcaceae, Bacillus cereus* and Mesophilic aerobic bacterias	Complete elimination of microbial growth when working with the highest pressure and with an inlet temperature of 65- 75 ° C	(Valencia-Flores et al., 2013)
Rice drink	HPH+ sonication	50-100 MPa (1-3 passes)	*Lactobacillus Plantarum , Lactobacillus Casei , y Bifidobacterium Animalis*	Reduction and elimination of post-acidification by lactic acid bacteria.	(Bevilacqua et al., 2016)
Tiger nuts' milk beverage	HPH + refrigeration	200 and 300 MPa	Psychrotrophic bacteria, Lactobacilli, Enterobacteriaceae and faecal coliforms	Improved shelf life and microbial inactivation compared to other heat treatments	(Codina-Torrella et al., 2018)
Lagger beer	HPH + lysozyme addition	0-300 MPa	*Lactobacillus brevis* (CCT 3745)	The inhibitory concentration of lysozyme against *L. brevis* was 100 mg/ L. HPH at 100, 140, and 150 MPa promoted decimal reductions of 1, 3, and 6 in microbial counts	(Franchi et al., 2011)

Pilsen beer	Heat treatment + HPH	100, 150, 200 and 250 MPa (1-3 passes)	*Lactobacillus del brueckii*	It is possible to inactivate the most common microorganisms of beer deterioration at 250 MPa. The effect increases with increasing number of passes.	(Franchi et al., 2013)
Wine	Chemical treatment + HPH	0, 50, 100 and 150 MPa	*Saccharomyces bayanus*	HPH at 150 MPa the best treatment by inducing yeast autolysis, also suitable to accelerate sur lie maturation	(Comuzzo et al., 2015)

Significant work has been performed on the application of HPH to reduce microbial load in fruit juices. In this type of food, it has been observed that the presence of some aroma compounds and essential oils can greatly influence the effect of HPH treatment. Patrignani et al. (2013) studied the effect of number of passes and citral addition on spoilage microbiota of apricot juice when subjected to HPH at 100 MPa. Their results showed that yeast cell viability decreased with the increase of passes and the relationship between both variables followed a linear trend. Moreover, the citral addition enhanced the effect of HPH, increasing the storage time by 6-8 days. To analyse the effect of the food matrix, the same authors compared the effect of HPH treatment at 100 MPa on viability loss of S. cerevisiae 635 inoculated at a level of about 6.0 Log10 cfu/mL in apricot juice and carrot juice. In apricot juice a significant viability decreases (2.2 Log10 cfu/ml) was obtained with only four repeated passes at 100 MPa. A further increase of the number of passes at 100 MPa did not significantly increase the effectiveness of HPH treatment. Concerning carrot juice, eight repeated passes at 100 MPa were unable to completely inactivate the inoculated cells. They concluded that because of the higher viscosity and sugar content, apricot juice required more passes in HPH treatment to reduce yeast load (Capra et al., 2009) On the contrary, Zygosaccharomyces bailii 45 exhibited the same susceptibility to HPH treatment in both juices. Eight passes at 100 MPa allowed a yeast inactivation higher than 2.5 log CFU/mL regardless of the juice considered (Patrignani et al., 2010). Nevertheless, (Benjamin & Gamrasni, 2020) established that HPH treatment at 100 and 150 MPa was not sufficient to reduce total bacteria and yeast count in pomegranate juice and it needed to be combined with a thermal treatment at 65 °C for 15 s, to achieve the same effect than pasteurization at 75 °C.

Besides fruit juices, plant-based beverages are complex dispersions with suspended proteins and oil droplets that require a homogenization stage to stabilize them and extend its commercial life. HPH can be applied at pressures higher than 100 MPa using multiple passes for these purposes along with microbial cells destruction (Bevilacqua et al., 2016; Codina-Torrella et al., 2018). Valencia-Flores et al. (2013) compared the effect on bacterial growth of HPH at 200-300 MPa and soft temperature inlet (55-75 °C) with conventional pasteurization treatment (90 °C, 90 s) in almond beverage. They showed that 200 MPa and an inlet temperature of 55 °C of inlet temperature was enough to improve conventional pasteurization effect on microbiological quality.

Moreover, beer is another beverage susceptible to be treated by HPH. Some research works established it was possible to completely inactivate the microorganisms in addition to improving the colour of the beer by HPH at pressures between 200-300 MPa and with 1 to 3 passes. The addition of antimicrobials as lysozyme enzyme (50 mg/L) had a synergistic effect, reducing the pressure needed to 100-150 MPa. However, HPH treatment could result in greater values for turbidity and it would be necessary to perform another stabilization treatments to minimize the negative effects (Franchi et al., 2011; Franchi et al., 2013).

HPH treatment has also been used to modulate the activity of various enzymes. This treatment can increase or decrease the enzyme activity depending on processing conditions (pressure and number of passes), homogenizing valve structure, specific enzyme, pH, temperature and food matrix. Since enzymes are a complex type of globular protein, mechanical forces and cavitation effects associated to HPH treatment result in conformational and structural changes modifying enzyme activity and stability. The main modifications in the enzyme are linked to changes in the quaternary, tertiary and even secondary structure. Formation or interruption of hydrogen bonds, Van der Waals, hydrophobic and electrostatic interactions can occur increasing the number of hydrophobic sites, revealing amino acid and sulfhydryl groups and thus, accelerating, delaying or impeding enzyme- substrate interaction(Franchi et al., 2013). Furthermore, the magnitude of the changes induced by the HPH treatment will determine their reversibility or irreversibility. dos Santos Aguilar et al. (2018) mentioned that protein denaturation can be reversible at 100 MPa and irreversible above 200 MPa.

In the case of juices, the main alteration reactions are caused by polyphenoloxidase responsible for browning and oxidation reactions. It was possible to inactivate it with homogenization pressures of 80-150 MPa (Bot et al., 2018; S. Plazzotta & Manzocco, 2019). On the other hand, α-amylase, whose use in recent years has been increasing since it reduces the starch content of beverages to avoid turbidity and gelatinization, is resistant to HPH (Tribst & Cristianini, 2012b). A similar resistance has been observed on Pseudomonas fluorescens protease when HPH at 100-150 MPa was applied to reduce its proteolytic rate (Oliveira et al., 2018).

Table 2. Research works evaluating enzyme activity modulation by HPH.

PRODUCT	ENZYMES	TREATMENT	EFFECT	REFERENCE
Commercial enzymes	Glucose oxidase	50, 100, 150 MPa	Decrease in enzyme activity at 50 MPa. Improvement in activity and stability at 100 and 150 MPa	(Tribst & Cristianini, 2012a)
Commercial enzymes	Amyloglucosidase, Glucose oxidase, Neutral protease	Amyloglucosidase, neutral protease: 150, 200 MPa (3 passes). Glucose oxidase: 100, 150 MPa (3 passes)	Improvement of enzymatic activity	(Franchi et al., 2013)
Fruit juices	α-amilase	0, 40, 80, 120 and 150 MPa	Stability of the enzyme	(Tribst & Cristianini, 2012b)
Apple juice	Polyphenoloxidase	150 MPa (10 passes)	Inactivation	(Bot et al., 2018)
Lettuce waste juice	Polyphenoloxidase	80 MPa (1 pass) and 150 MPa (1-10 passes)	Inactivation	(Plazzotta & Manzocco, 2019)
Peanut protein	Alcalase	0, 1, 40 and 80 MPa	Increased enzymatic hydrolysis	(Dong et al., 2011)
Chicken egg white	Lysozyme muramidase	40, 80, 120, 160 and 190 MPa	Activation and increase of enzymatic activity	(Tribst et al., 2017)
Raw skim milk	Alkaline phosphatase and lactoperoxidase	100, 150, 200, 250 and 300 MPa	Decrease and inactivation of alkaline phosphatase. Increased activity of lactoperoxidase.	(Pinho et al., 2011)
Milk	Protease *Pseudomonas fluorescens*	100 and 150 MPa	Decreased proteolytic rate	(Oliveira et al., 2018)

On the other hand, HPH can also be applied to enhance enzyme activity. Some authors have applied HPH to increase the activity of enzymes involved in the shelf life or processing of several food matrices. Lysozyme and lactoferrin have increased their antimicrobial activity against L. monocytogenes by HPH at 100 MPa in milk (Patrignani et al., 2007; Patrignani et al., 2020). Pinho et al. (2011) obtained a rise on enzymatic activity of lactoperoxidase in skim milk at pressures between 100 and 250 MPa. In contrast, if the homogenization pressure increased up to 300 MPa a reduction of around 30% in enzyme activity was detected. In another work, defatted peanut flour was dispersed in distilled water and pH adjusted, and further subjectedto HPH treatment at 40 and 80 MPa. After that, the peanut protein was recovered from the dispersed solution by an acid precipitation and re-dispersed in distilled water. The HPH treatment increased the extraction yield and the hydrolysis of the peanut protein isolates by endogenous enzymes. DPPH radical scavenging and hydroxyl radical scavenging activities were also increased (Dong et al., 2011).

It is evidenced that costs, versatility and performance improvement of enzymatic processes can be achieved when activity of commercial enzymes is increased by HPH. In particular, Tribst et al. (2013) improved the activity of amyloglucosidase, glucose oxidase and neutral protease, at HPH between 100-150 MPa and non- optimum temperatures. Commercial enzymes derived from fungi and available as powders were diluted in acetate buffer solutions and then subjected to HPH treatment. They observed an uneven effect of the number of passes. Only one pass was required to increase activity of amyloglucosidase and neutral protease while no effect was observed in subsequent passes, successive steps continued to increase the enzyme activity of glucose oxidase. The energy involved in the molecular changes associated to the increase in enzyme activity might be responsible for it.

Extraction and technological functionality improvement of proteins, fibrous materials and bioactive compounds

HPH is being used in the last years to contribute to food process sustainability (Picart-Palmade et al., 2019). In this area, HPH has been applied for valorisation of agrifood by-products with two different objectives: to increase extractability of intracellular or cell wall structural components and to improve technologic functionality of biomolecules from food by-products. Most agri-food wastes or by-products are rich in fibrous material and, in some cases, in proteins or bioactive compounds of interest for the food industry to be used as food ingredients (Zhu et al., 2016), or either to be used as environmentally safe and sustainable packaging materials (Flôres et al., 2017). HPH induces cells disruption favouring the release of structural and intracellular content and consequently its valorisation.

The main kind of products in which HPH is applied to extract fibres, proteins or bioactive compounds are solid by- products such as pomace from fruit or vegetable juicing, fruits or vegetables peels, minimal processing waste and vegetal parts of plants or cereal seed hulls. In this cases, the solid wastes need to be fluidized by diluting them in water or another solvent. In other cases, an extraction method is applied and the extracted phase further subjected to HPH. (Xie et al., 2018) extracted pectin form potato peel by HPH at 200 MPa. They obtained improvements in the viscosity, emulsifying properties, degree of esterification and physicochemical characteristics. Therefore they recommend the use of HPH to obtain pectin that could be used as an ingredient acting as a stabilizing agent or a thickener in food manufacturing. Similarly, Fayaz et al. (2019) showed that HPH favours the release of okara proteins and soluble fibre. Soy okara was dispersed in deionized water at 10 g/100 g concentration and pre-homogenized with a high-speed blender. After that, an homogenization pressure of 150 MPa for 5 passes made it possible to extract proteins with a yield of 90%.(Wang et al.,

2018) applied HPH to make edible and biodegradable films for food packaging from a type of edible fungus of the variety Flammulina velutipes. Wu et al. (2020) demonstrated the possibility of using HPH treatment to make biodegradable biopolymer films from pomelo peel.

Otherwise, HPH reduces particle size and structure of macromolecules modifying solubility, interaction properties, viscosity, or other physic-chemical properties. Saricaoglu et al. (2019) improved the functionality of proteins from hazelnut industry by HPH at 100 MPa and 1 pass. The homogenization pressure decreased particle size of proteins, increasing zeta potential and water solubility. Emulsifying and sparkling properties were improved too. Hua et al. (2017) demonstrated a microstructural change of tomato waste fibres by applying HPH at 100 MPa and 10 passes. The authors transformed around 8% of the insoluble fibres into soluble ones.(Xu et al., 2015) indicated that for the preparation of soluble peach fibre from fresh peach marc it must be dispersed in 3 times the volume of deionized water, thus improving the efficiency of cellulose hydrolysis. For pectin extraction from milled dried lemon peel, variations in dilutions change the properties of the pectin extracted, resulting in residues with different pectic characteristics (Willemsen et al., 2017). Discarded external lettuce leaves were dispersed in hydroalcoholic solutions and polyphenols extracted with ethanol to get good phenolic extraction yields (Plazzotta & Manzocco, 2018).

Table 3. Research works aimed to the extraction and improvement of technological functionality of proteins, fibres or bioactive compounds from agri-food wastes by HPH.

SUSTRATE	COMPONENT	TREATMENT	OBJECTIVE	REFERENCE
Sweet potato leaves	Flavonoids	100 MPa (2 passes)	Strengthens the antioxidant activities of the flavonoid	(Huang et al., 2013)
Potato peel	Biopolymer film	150 MPa	Extraction	(Rommi et al., 2016)
Peach pomace	Soluble fibres	140 MPa (4 passes)	Significantly improved the efficiency of cellulase hydrolysis in the preparation of soluble fibres and a high binding capacity for sodium cholate and cholesterol.	(Xu et al., 2015)
Potato peel	Phenolic acids	159 MPa (2 passes) + NaOH treatment	Improved extraction and release of total phenolic content and total flavonoid content	(Zhu et al., 2016)
Desmodesmus sp. F51	Carotenoids	69 - 276 MPa (1-4 passes)	Extraction	(Xie et al., 2016)
Dry tomato residue waste	Fibres	100 MPa (10 passes)	Improved the soluble fibre content and its oil holding capacity	(Hua et al., 2017)

Citrus peel	Fibres	90, 160 MPa (2 passes)	Improvement of physical, chemical and functional properties including surface area, water holding capacity, texture and viscosity	(Zhu et al., 2018)
Lemon peels fibre	Pectin	20 and 80 MPa	Extraction	(Willemsen et al., 2017)
Soybean	Protein	100 MPa	Extraction	(Preece et al., 2017)
Hazelnut oil industry by-products	Hazelnut meal proteins	0, 25, 50, 75, 100 and 150 MPa	Improves functional (solubility, emulsifying and foaming properties) and rheological properties of proteins	(Saricaoglu et al., 2018)
Black cherry tomato waste	Pectin	0, 40, 80, 120 and 160 MPa (2 passes)	Increase the esterification degree of pectins	(Zhang et al., 2018)
Carrot processing waste	Biodegradable composite films were prepared	138 MPa (7 passes)	Extraction	(Otoni et al., 2018)
Lettuce waste	Polyphenols	50, 100 MPa	Extraction	(Plazzotta & Manzocco, 2018)
Potato peel	Pectin	200 MPa	Increased galacturonic acid content, viscosity and emulsifying properties. Decreased esterification degree and molecular weight	(Xie et al., 2018)
Broccoli seeds	Sulforaphane	20-160MPa (1-5 passes)	Increases the extraction yield	(Xing et al., 2019)
Agri-food waste (tomato peel, coffee beans)	Application for structuring peanut oil	70 MPa (3 passes)	Replacing part of the lipids with water and low calorie fibres.	(Mustafa et al., 2018)
Edible mushroom by-products	Biodegradable edible film	100 MPa (3 passes)	Improve tensile strength, elongation at break, water vapor permeability, oxygen barrier and thermal stability.	(Wang et al., 2018)
Grape seeds, tomato stem, walnut shells, coffee	Polyphenolic compounds and antioxidants	20, 50, 100, 120 MPa	Extraction	(Griffin et al., 2018)
Soybean okara	Proteins and soluble fibers	50, 100, 150 MPa (1 pass) 150MPa (5 pases)	Extraction	(Fayaz et al., 2019)
Sugar palm	nanofibrillated cellulose	50 MPa (3 passes)	Extraction	(Ilyas et al., 2019)

Tomato peels	Bioactive compounds: proteins, polyphenols, lycopene	100 MPa (1-10 passes)	Increased release of intracellular compounds (proteins, sugars, anioxidants)	(Jurić et al., 2019)
Pomelo peel	Biopolymer film	20, 40, 60 and 80 MPa (10 passes)	Improve mechanical properties, microstructure, optical and barrier properties	(Wu et al., 2020)
Soybean meal	Resins	20 MPa	Extraction	(Zhang et al., 2020)

Increase of bioavailability and encapsulation of bioactive compounds

In the last decade, many studies have been carried out to demonstrate that the application of HPH in liquid foods can produce a modification in the bioaccessibility (i.e. fraction of an ingested nutrient that is released from the food matrix and made available for intestinal absorption) or bioavailability (i.e. fraction of an ingested nutrient that is absorbed in the intestine and incorporated into the bloodstream) of its bioactive compounds. In most studies, an increase in the bioaccessibility of phytochemicals is observed due to their release within the structure of the food in which they are found. In other cases a modification of its biological functionality occurs due to some change in its chemical structure. (Zhou, 2019) carried out an interesting review that demonstrates these effects in three bioactive components: carotenoids, phenolic compounds and vitamin C. The review shows that fruit juices (carrot, tomato, orange, apple and berries) are the most common food in which HPH increase bioaccessibility of bioactive compounds. HPH decreases the particle size of suspended pulp, increases cloud stability and thus the availability of bioactive components. Treatment with HPH in mandarin juices increased the bioaccessibility of total carotenoids by five times, although in the case of flavonoids no such drastic changes were observed. Therefore, the HPH treatment was recommended for the production of tangerine juices that promote health, mainly through the improvement of the bioaccessibility of its carotenoids (Sentandreu et al., 2020). Quan et al. (2020) established that bioaccessibility improvement could be conditioned by food matrix. They observed that HPH at 250 MPa favoured the release from cell walls and increased the content of total phenolic compounds in kiwi and pomelo juices, but it had a negative effect on its bioaccessibility (in vitro) as a consequence of a major degradation along the digestion process. Conversely, the addition of skimmed or whole milk to the juices had no significant effect on total phenolics content but increased phenolic bioaccessibility in kiwi juice and pomelo juice from 21.6% to 37.8% and 60.1% to 63.3%, respectively. Similarly, Alongi et al. (2019) showed that chlorogenic acids bioaccessibility (in vitro) increased from nearly 25% to more than 50% by adding milk with different fat content to coffee and applying HPH (50-150 MPa). They observed the pressure required was lower the lower the fat percentage and they attributed the effect to chlorogenic acids micellarization, a phenomenon that reduced their susceptibility to degradation during digestion. Sometimes, positive effect of HPH could be observed after storage. Betoret et al. (2017) found in low pulp mandarin juice that, despite the suspended pulp increased with HPH and trehalose addition, flavonoid hesperidin initially decreased but resulted in less flavonoid degradation during storage.

HPHs have also been applied, with a much smaller number of published articles, to non-dairy vegetable based beverages. Although in some cases no significant improvements in the nutritional characteristics were detected, in others, such as almond or soy beverages, a reduction in antinutrients was achieved (Munekata et al., 2020). Denaturation, aggregation and chemical modification of proteins may change their allergic potential. Toro- Funes et al. (2014) demonstrated an increase of 40% in the extractability of phytosterols from almond milk subjeted to a HPH of 300 MPa (6 passes). However, the content of tocopherol and polyamines such as spermidine were reduced by up to 90%. The application of HPH for kefir production from hazelnut beverage achieved improvements in the total content of phenolic compounds and antioxidant capacity, causing a reduction in the content of lactic and citric acid (Atalar, 2019).

Improvement in the bioavailability of bioactive components by HPH is also possible in solid foods. HPH (10-20 cycles and 100- 200 MPa) was used to fabricate fermented soybean powder into an aqueous nanosuspension, favouring in-vitro release of isoflavones from nanosuspension (Kapoor et al., 2014).

The other way in which HPH can contribute to an improvement in the nutritional properties of foods is by using this technology for the encapsulation of bioactive components. In this way it is possible to increase its stability, conservation and controlled delivery in the target site increasing food functionality. HPH produces intense disruptive forces that break up particles into smaller sizes favouring encapsulation of specific components in a suitable media. Mechanical stress, heating associated and emulsifier interactions can affect the effectiveness of the process and therefore the activity and bioaccessibility of the bioactive compound.

Many studies have investigated nanoencapsulation of curcumin by HPH at 40-100 MPa. Results showed that the emulsifier type had an influence in curcumin bioaccessibility (Jiang et al., 2020). Frank et al. (2012) studied the degradation of anthocyanins from bilberry extract by subjecting them to temperature and mechanical stress similar to the process of emulsification and encapsulation by HPH. The HPH were applied with a simple pass and in the pressure range between 30 and 150 MPa. Thereafter the samples were immediately cooled to 298K. The results showed no significant influence of mechanical stresses associated to HPH on anthocyanin stability, even at high-pressure treatment up to 150 MPa. The combination temperature–time was the main parameter affecting anthocyanin degradation.

A great interest has been shown in applying HPH as encapsulation technique in bacteria with probiotic effect. Patrignani et al. (2017) underlined the applicative potential of HPH microencapsulation of probiotic microorganisms to produce fermented milk with improved functionality and with enhanced sensory properties. They established 50 MPa and 5 passes as adequate conditions to produce stable microcapsules of Lactobacilli with high yield and viability during storage. Moreover, microencapsulation of adjunct bacteria reduced acidity of fermented milk. Calabuig-Jiménez et al. (2019) microencapsulated *L. salivarius* spp. *salivarius* in alginate coatings by HPH at 70 MPa. A positive effect of microcapsules was observed when evaluating the survival of the probiotic strain on simulated gastrointestinal conditions.

Table 4. Research works in which HPH treatment was applied to encapsulate.

COMPONENT ENCAPSULATED	MATRIX	CONDITIONS	RESULTS	REFERENCE
Lactobacillus paracasei A13 and *Lactobacillus salivarius* subsp. *salivarius* CET 4063	Fermented milk	50 MPa (5 passes)	The microcapsules presented high yields in terms of trapped viable cells and acceptable sizes. Furthermore, microencapsulation caused the decrease in acidity in fermented milk.	(Patrignani et al., 2017)
Phenolic compounds and anthocyanins from blueberry pomace	-	50- 200 MPa	The encapsulation efficiency, size and charge characteristics of the emulsion droplets were affected by HPH.	(Bamba et al., 2018)
Lactobacillus salivarius spp. *Salivarius*	Mandarin Juice	70 MPa (2 passes)	Improving the survival of probiotics with the use of alginate as a coating	(Calabuig-Jiménez et al., 2019)
Phenolic powder from strawberry pomace	-	50 and 70 MPa (3, 5, 7 passes)	High encapsulation efficiency	(Cilek Tatar et al., 2019)
L. salivarius spp. Salivarius	Mandarin juice impreganted in apple	70 MPa (2 passes)	The final count of *L. salivarius spp. Salivarius* encapsulated was high enough to exert a potential beneficial effect	(Betoret et al., 2019)

HPH treatments at pressure levels below 100 MPa, considered as sublethal pressures, are applied to microbial cultures: initiators, co-initiators, or probiotics and yeasts, in order to produce cultures with improved functional, technological and sensory properties. The use of strains belonging to the genus *Bifidobacterium* and *Lactobacillus* predominate as probiotics, and to a lesser extent Enterococcus, Streptococcus and Saccharomyces (Siroli et al., 2020b). The bacterial cells response to mechanical stress induced by HPH modifying their metabolic activity and membrane composition. Therefore technological and functional properties such as fermentation kinetic, enzymatic activities, hydrophobicity or resistance to gastrointestinal digestion can be improved.

Table 5. Research works in which HPH treatment was applied to probiotic cells.

FOOD MATRIX	MICROBIAL STRAIN	CONDITIONS	RESULTS	REFERENCES
Yogurt	*L. Delbrueckii* ssp. *bulgaricus* LB- 12, S. *Salivarius* ssp. *thermophilus* ST-M5 and *L. acidophilus* LA-K	0, 3.45, 6.90, 10.34 and 13.80 MPa	Improved tolerance to acid and bile	(Muramalla & Aryana, 2011)
-	*L. acidophilus Dru* y *L. paracasei* A13	0.1 and 50 MPa	Increased probiotic characteristics in vivo, there was no modification in the interaction of lactobacilli with the small intestine	(Tabanelli et al., 2012)
-	*Lactobacillus paracasei* A13, *Lactobacillus acidophilus* 08 and Dru, *Lactobacillus delbrueckii* spp. *lactis* 200	50 MPa	Increased functional characteristics depending on the type of strain	(Tabanelli et al., 2013)
Fermented milks	*Lactobacillus rhamnosus* BFE5264, *L. delbrueckii* spp. *bulgaricus* FP1 and *Streptococcus thermophilus* LI3	60 MPa	Reduced product clotting time and increased viability of the probiotic strain	(Patrignani et al., 2016)
Cacciotta cheese	*Lactobacillus paracasei* A13	50 MPa	Increase in quality and decrease in cheese maturation time.	(Burns et al., 2015)
Mandarin juice	*L. salivarius* spp. *Salivarius*	0, 20 and 100 MPa	Improvement of cellular hydrophobicity	(Betoret et al., 2017)
Clementine juice	*L. salivarius* spp. *Salivarius*	25, 50, 100 and 150 MPa	Improvement of the antioxidant properties of the juice.	(Barrera et al., 2019)
Fresh Culture (1% v/v)	*Lactobacillus paracasei* A13	50, 150, 200 MPa	Increase the unsaturation in membrane fatty acids.	(Siroli et al., 2020a)

Siroli et al. (2020a) reported that the main regulatory mechanism that probiotic lactobacilli adopt to counteract pressure stress is the modification of the composition of membrane fatty acids. Specifically, they observed an increase of unsaturated fatty acids when HPH at 100 and 150 MPa was applied to *Lactobacillus paracasei* A13. Considering that the increase of unsaturation level is a key mechanism to compensate for the oxidative damages induced by physico-chemical stressors in microbial cells, they concluded that HPH at sublethal pressures is useful to improve activity of some Lactobacillus species.

Lanciotti et al. (2007) studied the effect of HPH between 50 MPa and 100 MPa on fermentation kinetics, metabolic profile and enzymatic activity of four species of Lactobacilli involved in dairy product fermentation and ripening. Although the results varied according to the species, they documented no significant effect on cell viability, an increased proteolytic activity and positive changes in fermentation dynamics. The resistance to simulated gastric conditions, hydrophobicity and auto-aggregation capacity resulted also strain-dependent for *L. acidophilus* Dru and *L. paracasei* A13 when subjected to HPH at 50 MPa. HPH increased the three properties for *L. paracasei* A13 while reduced them for L. acidophilus Dru. Authors attribute the differences to the compositional and structural differences in the cellular outer structures, thus suggesting the HPH effects on macromolecules and their interactions with the gut immune cells have a key role in the probiotic effect. The same authors noted that HPH treated *L. paracasei* cells modified their interaction with the small intestine of mice, inducing a higher IgA response compared to untrated *L. paracasei* cells (Tabanelli et al., 2013; Tabanelli et al., 2012). Betoret et al. (2017) demonstrated the improvement in the hydrophobicity of *Lactobacillus salivarius* spp. salivarius added to mandarin juice with trehalose when HPH at 0, 20 and 100 MPa were applied.

CONCLUSIONS

Although in the beginning the application of high homogenizing pressures was aimed at homogenizing and increasing the stability of emulsions such as milk, the advances in valve design and equipment allowed for an increase in working pressure extending the scope of application.

In the last decade, it has grown by more than 80% the number of research works related to the implementation of HPH in the process of extracting bioactive components from agri-food wastes, to improve bioavailability and probiotic properties of bioactive components and microorganisms and also as an encapsulation technique. At the same time, progress has been made in the application of HPH to reduce the microbial load or modulate the activity of some enzymes.

The general mechanisms responsible for the effect of HPH are known, but the final effect is largely conditioned by the type of valve, pressure applied, number of passes, nature of the components and macromolecules, and food matrix. For this reason research is needed for each specific application.

Results published in the last decade have shown HPH as a non-thermal technology suitable to accomplish the food industry's objectives of quality and safety, functionality and sustainability.

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