International Food Research Journal 19(1): 1-5 (2012)

MiniReview
The effect of high pressure homogenization on microorganisms in milk

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Abstract: Milk is a highly consumed product worldwide mainly because of nutrient content, but this rich composition associated to pH and water activity makes the milk an ideal medium for microbial growth. The thermal process is usually applied to guarantee microbial stability of milk, however, the heat promotes undesirable changes in the milk sensory and nutritional characteristics. Therefore, recent researchers have developed alternative non-thermal methods to obtain safe milk. High pressure homogenization (HPH) is based upon common milk homogenization processes, though it uses 10-15 times higher pressures, which makes the process able to promotes microbiological reduction. This review evaluated the application of HPH into fluid milk processing for microbiological reduction and also how the process condition (i.e. pressure, inlet temperature) and milk composition can affect the efficacy of this new process.

Keywords: Milk, dynamic high pressure, ultra-high pressure homogenization, non thermal treatment

Introduction

Milk plays a significant role for human nutrition and stands for one of the most frequently sold types of food worldwide. The nutritional composition, high water activity and neutral pH turn milk into an adequate media for microbial development which can lead growth of enterobacterias, lactic acid bacteria, *Pseudomonas*, *Staphylococcus* and *Listeria* (Walkling-Ribeiro *et al*., 2009) and also sporulated microorganisms as *Bacilli* and *Clostridia*, which are thermoresistant and important for milk deterioration. An initial contamination of 10^3 to 10^4 CFU/mL is expected to fresh milk; also, the fast generation time (around 20 minutes) of some contaminants results in a short shelf life for unprocessed milk.

Heat treatment in the main treatment applied to microbial stabilization of milk, since it is cheaper and easy to operate. It is applied as pasteurization or commercial sterilization through the ultra high temperature (UHT) technology but, although its benefits, the heat process affects vitamins (losses around 10% of folic acid and 15% of B-complex vitamins) and denatures proteins, resulting in the release of sulfured compounds (Gomes, 1995).

Therefore, the use of non-thermal technologies is being studied to milk stabilization. This technologies includes pulsed electric field (Walkling-Ribeiro *et al*., 2009), ohmic heating (Sun *et al*., 2008), high isostatic pressure and also high pressure homogenization (Ramaswamy *et al*., 2010). The last alternative is based on use of homogenizer at pressures 15 times higher then used for common milk homogenization, promoting microbial inactivation and changes on milk properties, also to the fat homogenization (Thiebaud *et al*., 2003; Briñez *et al*., 2007). The high pressure homogenization (HPH) in milk has been intensively studied in the last years and this review aimed to compile and discuss the main alteration caused by this process in the milk contaminants intentionally or not add on milk.

High pressure processing

High–pressure homogenization process is a non-thermal technology that was first developed in the mid-twentieth century. The technology is based on ordinary processes for the homogenization of dairy product and emulsions (Hayes and Kelly, 2003a), keeping the same operational principle (Diels and Michiels, 2006). The development of equipment items in the last 20 years led to the application of high pressures (10-15 times greater than the commonly applied pressures for milk homogenization), which allowed the use of such process for microorganism inactivation (Paquin 1999). HPH was mainly studied to stabilization of juices (Campos and Cristianini, 2007; Tribst *et al*., 2009a; Tribst *et al*., 2011) and milk (Hayes and Kelly, 2003a,b; Lanciotti *et al*., 2007).

During the process, the treatment fluid is forced under high pressures to pass through a narrow gap. Thus, it creates a fast acceleration (200 m/s at 340 MPa) (Floury *et al*., 2004) undergoing an extreme drop in pressure as the fluid exits the homogenization valve (Floury *et al*., 2004), which leads to inactivation of microorganisms (Fantin *et al*., 1996, Campos and Cristianini 2007, Tribst *et al*., 2008, Tribst *et al*.,

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2009b) and denaturation of enzymes (Lacroix *et al*., 2005). The mechanism for HPH-action is not thoroughly clear, but includes high–speed friction, cavitation collapse, strong impacts, turbulence (Middelberg, 1995; Klenig and Middelberg, 1998, Innings and Trägardh, 2007), and heating (Diels *et al*., 2004). Such effects result in cell wall rupture and cellular death (Diels *et al*., 2003; Diels *et al*., 2005). It is an effective process to inactivate vegetative bacteria (Wuytach *et al*., 2002; Campos and Cristianini 2007; Tribst *et al*., 2008; Tribst *et al*., 2009b), yeasts and moulds (Fantin *et al*., 1996; Tahiri *et al*., 2006; Campos and Cristianini 2007, Tribst *et al*., 2009a, Tribst *et al*., 2011). However, its low efficacy against bacterial spores turns the HPH into a treatment similar to thermal pasteurisation.

Some researchers showed that the process did not present sublethal effect against vegetative cells (Wuytach *et al*., 2002; Diels *et al*., 2005; Briñez *et al*., 2007). However, published studies recently revealed that a heat–resistant *A. niger* conidia (Tribst *et al*., 2009a) and *Bacillus cereus* and *Bacillus subtilis* spores (Chaves–López *et al*., 2009) were sensitised by HPH treatment followed by moderated thermal treatment. Adversely, no sensitisation for such microorganisms was observed when thermal treatment was performed before HPH, indicating that process combination order is important.

The heating effect during the HPH was intensively studied. The intense friction after homogenizer valve (Middelberg, 1995; Klenig and Middelberg, 1998; Innings and Trägardh, 2007) results in fluid heating (Floury *et al*., 2004). It is expected an increase on fluid temperature around 1.7 – 1.8ºC for milk and skim milk (Hayes and Kelly, 2003a; Hayes *et al*., 2005; Roach and Harte, 2008) at each pressure increment of 10MPa (Diels and Michiels, 2006). The residence time at high temperature, however, was reported as 0.7s (Campos and Cristianini, 2007), indicating that the heating involved in HPH process probably is not enough to promote microbial inactivation or physical-chemical changes in the product. Thus, the use of HPH for milk may be used as an alternative to thermal pasteurization as it causes microbial inactivation, minimizing undesired changes associated to the thermal treatment (i.e. vitamin loss and protein denaturation) (Pereda *et al*., 2008; Hayes *et al*., 2005).

Effect on milk contaminants

The effect of HPH on microbiological quality of milk was evaluated by some authors that studied the inactivation of native contaminants (Guerzoni *et al*., 1999; Hayes *et al*., 2005; Hayes and Kelly 2003a; Thiebaud *et al*., 2003) and the inactivation of important genera intentionally added to the milk (Lanciotti *et al*., 2007, Wuytach *et al*., 2002; Kheadr *et al*., 2002; Briñez *et al*., 2007).

The authors that evaluated the inactivation of native microflora of milk by HPH had obtained results similar to thermal pasteurization (Hayes *et al*., 2005), with 1, 2 and 3 decimal reductions when samples were treated at 100, 150 and 300MPa, respectively (Guerzoni *et al*., 1999; Hayes and Kelly 2003a; Thiebaud *et al*., 2003). It indicates that HPH milk can reach a similar shelf life of pasteurized milk, next to 1 or 2 weeks.

The effect of HPH in microorganisms intentionally inoculated in milk was evaluated by some researchers. Table 1 shows the pressure required to the inactivation of some microorganisms in milk.

Table 1. Microbial inactivation at different pressure in milk

Microorganism	Pressure	Inlet Temperature	Decimal Reduction	Reference
Bacillus subtilis A2 (vegetative cell)	130MPa	NI	2.90	Vannini *et al.*, 2004
Coliformes	150MPa	45ºC	> 1.12	Hayes *et al.*, 2005
Coliformes	200MPa	55ºC	>4.10	Smiddy *et al.*, 2007
Escherichia coli 555	130MPa	NI	0.30	Vannini *et al.*, 2004
E. coli MG1655	300MPa	25ºC	3.50	Diels *et al.*, 2005
Lactobacilli	200MPa	55ºC	> 3.80	Smiddy *et al.*, 2007
Lactobacillus arizonensis 21	150 MPa	10ºC	1.50	Lanciotti *et al.*, 2007
Lactobacillus casei 28	150 MPa	10ºC	0.60	Lanciotti *et al.*, 2007
Lactobacillus pentosus 57	150 MPa	10ºC	1.00	Lanciotti *et al.*, 2007
Lactobacillus plantarum 58	150 MPa	10ºC	0.60	Lanciotti *et al.*, 2007
Listeria innocua ATCC 33090	300MPa	24ºC	1.80	Picart *et al.*, 2006
Listeria monocytogenes CCUG 15526	400MPa	NI	7.95*	Roig-Sagués *et al.*, 2009
Listeria monocytogenes Scott A	130MPa	NI	1.03	Vannini *et al.*, 2004
Listeria monocytogenes Scott A	100MPa	2-4ºC	1.20	Iucci *et al.*, 2007
Micrococcus luteus ATCC 4698	300MPa	24ºC	2.50	Picart *et al.*, 2006
Proteus vulgaris PV1	130MPa	NI	1.80	Vannini *et al.*, 2004
Pseudomonas	200MPa	55ºC	> 4.90	Smiddy *et al.*, 2007
Pseudomonas fluorescens ATCC 13525	300MPa	24ºC	3.70	Picart *et al.*, 2006
Pseudomonas fluorescens AFT36	200MPa	45ºC	6.00	Hayes *et al.*, 2005
Pseudomonas putida 754	130MPa	NI	2.42	Vannini *et al.*, 2004
Psychrotrophs	200MPa	55ºC	> 4.60	Smiddy *et al.*, 2007
Salmonella enteritidis E4	130MPa	NI	1.40	Vannini *et al.*, 2004
Staphylococcus aureus	150MPa	45ºC	> 2.71	Hayes *et al.*, 2005
Staphylococcus aureus	200MPa	55ºC	>2.90	Smiddy *et al.*, 2007
Staphylococcus aureus ATCC 13565	300MPa	20ºC	4.00	Briñez *et al.*, 2007
Staphylococcus aureus ATCC 13565	300MPa	6ºC	3.35	Briñez *et al.*, 2007
Staphylococcus aureus ST1	130MPa	NI	1.94	Vannini *et al.*, 2004
Staphylococcus carnosus CECT 4491	300MPa	20ºC	3.34	Briñez *et al.*, 2007
Staphylococcus carnosus CECT 4491	300MPa	6ºC	-0,01	Briñez *et al.*, 2007

* milk with 15% of fat, ** NI = not identified

The process conditions are also important in the effectiveness of HPH. Pressures up to 150 MPa is not able to promote more than 2 decimal reduction of different microorganisms (Vannini *et al*., 2004; Hayes *et al*., 2005, Lanciotti *et al*., 2007; Iucci *et al*., 2007), while pressure at 200, 300 and 400 MPa are required to reach high level of microbial inactivation (Briñez *et al*., 2007; Smiddy *et al*., 2007; Diels *et al*., 2005; Picart *et al*., 2006; Roig-Sagués *et al*., 2009). Therefore, the application of pressures above 200 MPa probably represents a safe process.

It was previous reported that the inactivation behavior of HPH is linear (Vannini *et al*., 2004) however, many researchers find a non-linear performance of microbial inactivation by HPH (Fantin *et al*., 1996; Wuytack *et al*., 2002; Campos and Cristianini, 2007; Tribst *et al*., 2008; Tribst *et al*., 2009a). This can explain the high level of microbial inactivation reached at pressures of 300 MPa or higher.

The inlet temperature was also essential to microbial inactivation. According to Briñez *et al*. (2007) increase on decimal reduction of 0.65 and 3.35 for *S. aureus* and *S. carnosus* respectively, were obtained when the HPH milk treatment was carried out at an initial temperature of 20ºC instead of 6ºC. Similarly, it was noticed that processes conducted at 45-50ºC yielded greater microbial inactivation (Diels *et al*., 2003).

The main effect of elevated inlet temperature of milk in the homogenizer is the fluid viscosity reduction (Diels *et al*., 2003; Briñez *et al*., 2007), which affect the microbiological inactivation due to the changes in the flow through the homogenization valve (Diels and Michiels, 2006), enhancing turbulence and cavitation effects (Diels *et al*., 2005). Other possible explanation to the phenomena of higher inactivation at higher inlet temperature is the change in physical properties of bacteria membrane, reducing its selectivity (Vachon *et al*., 2002). Meanwhile, as the main inactivation mechanism through HPH is cell disruption instead of membrane modifications, it is more likely that the first hypothesis is correct (Diels and Michiels, 2006).

The milk composition seems to be important to determine the efficiency of HPH microbial inactivation. The results obtained by Briñez *et al*. (2007), Diels *et al*. (2005) and Roig-Sagués *et al*. (2009) demonstrated that microbial inactivation is dependent of fat concentration, being higher at high fat. This contrary the effect expected due to the viscosity enhance caused by high fat content, but can be partially explained considering two hypothesis. The first that attribute higher temperature during depressurization to milk with high fat content, which promotes a thermal inactivation also to mechanisms involved in HPH process and the second (most probably) that justify that at high fat content occurs a change of lypoproteins of cell membrane to triglycerides, resulting in loss of selective cell permeability during HPH process.

The application of HPH combined with other process has been tested aiming to increase the microbial inactivation obtained just with homogenization. The combined use of HPH and lysozyme, lactoferrin and lactoperoxidase system indicates that the antimicrobial effects were enhanced and/or accelerated by HPH treatment (Vannini *et al*., 2004; Iucci *et al*., 2007), resulting in reduction of the resistance to HPH process between 3 and 65% for ten bacteria previous added by lysozyme (Vannini *et al*., 2004). Similar results were obtained to lactoperoxidase system (Vannini *et al*., 2004) and lactoferrin (Iucci *et al*., 2007). Results obtained by Diels *et al*. (2005), however, indicates that no sensitization of *Escherichia coli* to nisin, lysozyme, and lactoperoxidase occurred when the antimicrobials was added after phosphate buffer treatment by HPH at 100 or 300 MPa. It probably signify that the synergic effect between HPH and antimicrobials happen just at pressurization/depressurization time.

In addition to the microorganisms, the enzymes produced by them also affect milk shelf life, mainly lipases and proteinases, which have high thermal resistance ($D_{140ºC}$=1min – Cromie, 1992) and are produced by psychrothropic bacteria such as *Pseudomonas* (Nielsen, 2002). *Pseudomonas* at counts of 10^7 CFU/mL is able to produce enough protease to degrade milk after thermal treatment (Shah, 1994). Pressure up to 150 MPa was not able to reduce the Pseudomonas proteinase activity (Hayes *et al*., 2005), even when 100 MPa is applied 10 times consecutively. Elevate pressure can promotes some reduction of this enzyme activity, around 20 and 30%, when milk is treated at 200 MPa and 250 MPa, respectively (Hayes *et al*., 2005). It indicates that the milk initial quality is very important to the milk shelf life stability.

The evaluation the shelf life of milk produced by HPH at 200 or 250 MPa indicates that the product presented stability similar to heat pasteurized milk, around 1 (Smiddy *et al*., 2007) or 2 weeks (Pereda *et al*., 2008) at refrigerated conditions. The end of shelf life was established when the total bacteria counts reached 10^5 CFU/mL (Smiddy *et al*., 2007) or when the maximum limit of psychrotrophic established by the European legislation was reached (Pereda *et al*., 2008). The differences obtained by the authors may

be due to the microorganisms under evaluation and/or due to the efficacy of each equipment.

Conclusions

HPH is effictive for the substitution of thermal pasteurization treatment in addition to being used as a pre-processing of milk aimed at extending its shelf-life when used as a raw material for industries such as cheese, UHT milk, and yogurt. The efficacy of the HPH treatment is function of milk fat content, inlet temperature and reached pressure, being major when associated at high inlet temperature, high pressure level and to high fat content. Also, it was observed that the process was not able to inactivate heat resistant enzymes produced by psychotropic microorganism, requiring a good initial quality of milk to obtain stable milk during its storage.

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