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# Microbial inactivation and evaluation of furan formation in high hydrostatic pressure (HHP) treated vegetable-based infant food

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ABSTRACT

The inactivation of pathogenic and spoilage bacteria as well as the formation of food processing contaminants (e.g. acrylamide, furan, etc.) in infant foods is of utmost importance for industry, consumers as well as regulatory bodies. In this study, the potential of high hydrostatic pressure (HHP) for microorganism inactivation including total mesophilic aerobic bacteria (TMA) and total yeasts and molds (TYM) at equivalent processing conditions, as well as its effects on furan formation in vegetable-based infant food was evaluated. The process parameters evaluated were combinations of pressures (200, 300, and 400 MPa), temperatures (25, 35, and 45 °C), and treatment times (5, 10, and 15 min). Pressure, time and temperature had a significant influence on both TMA and TYM inactivation of vegetable-based infant foods, observing a significant reduction in both microbial populations when all the factors were increased, although the extent of reduction was clearly influenced by the type of microorganism. A synergism between pressure, time and temperature was observed for the reduction of both TMA and TYM populations and it was found that HHP at 400 MPa resulted in a complete inactivation of TMA as well as TYM after 15 min of treatment at 45 °C. The furan content in all HHP treated samples was found to be below the limit of detection. Thus, HHP treatment could be considered as a potential alternative to thermal processing of vegetable-based infant foods.

## 1. Introduction

Furan is a heterocyclic organic compound, consisting of a five-membered aromatic ring with four carbon atoms and one oxygen. The occurrence of furan in foods, the mechanisms leading to furan formation and analytical methods for detection have been reviewed in the past (Crews & Castle, 2007; Wenzl, Lachenmeier, & Gökmen, 2007; Van Lancker, Adams, Owczarek-Fendor, De Meulenaer, & De Kimpe, 2010). This compound is formed during thermal treatment of food products. Furan has been shown to be carcinogenic in animal laboratory studies by Gill et al. (2011) and the International Agency for Research on Cancer (IARC), thus constituting a chemical hazard (IARC, 2012). Per the toxicology and carcinogenesis studies of furan made by U.S. National Institutes of Health, furan is carcinogenic to rats and mice, showing a dose-dependent increase in hepatocellular adenomas and carcinomas (NTP, 1993). Furan induced carcinogenesis is suspected to be either genotoxic mediated (Burka, Washburn, & Irwin, 1991; Byrns, Predecke, & Peterson, 2002) or metabolite induced cell proliferation and uncoupling of mitochondrial oxidative phosphorylation (Kedderis & Ploch, 1999).

In the past, the US Food and Drug Administration (US FDA) had released a report on the occurrence of furan in many foods that are subjected to thermal processing, especially canned and jarred foods (US FDA, 2009). Coffee and ready-to-eat baby foods are reported to contain the high concentrations of furan among most ready-to-eat foods (EFSA, 2011). Historical FDA data on furan reveals that furan contents in

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commercially available infant foods typically range between 3.9 and 26.9 ppb (US FDA, 2009). It is interesting that the menace of furan formation is limited mainly to commercially sterilized baby foods, while freshly cooked home-made baby food is generally furan-free (Van Lancker et al., 2010). In light of this, the use of mild processing technologies, which can avoid the use of high temperatures, can be a useful tool to control furan level in baby foods (Barba, Terefe, Buckow, Knorr, & Orlien, 2015; Sevenich et al., 2013).

Among mild processing technologies, high hydrostatic pressure (HHP) processing has emerged as the most relevant one for food preservation mainly due to its ability to inactivate microorganisms (> 5-log reduction) (Baptista, Rocha, Cunha, Saraiva, & Almeida, 2016; Georget et al., 2015; Moreirinha, Almeida, Saraiva, & Delgadillo, 2016; Rendueles et al., 2011) and effectively control certain enzymes without destroying the nutritional and sensory components that are normally affected during heat treatment (Castro, Saraiva, Domingues, & Delgadillo, 2011; Misra, Kadam, & Pankaj, 2011; Terefe, Buckow, & Versteeg, 2014). A remarkable point for HHP processing is the use of 3-D thinking, meaning that it is possible to control three processing parameters (pressure, temperature and time), which offer a high versatility in the process design (Barba, Esteve, & Frígola, 2012; Barba, Parniakov et al., 2015; Barba, Terefe et al., 2015).

At this stage of development, there is a need to optimize HHP processing conditions to achieve a balance between safety, food quality, and health. Knowing the influence of HHP factors on microbial inactivation is of great importance for both food researchers and food industry as these are the key to develop innovative and effective processes and products. However, it is also necessary to evaluate the influence of process variables on the formation of processing contaminants (e.g. furan), which can have an important effect on infants' health and determine the consumer's acceptance.

To the best of our knowledge, there is a lack of information in the available literature on the impact of HHP on microorganism inactivation/reduction on vegetable-based infant foods. Although, some previous studies have evaluated the formation/mitigation of furan and MCPD-esters in different food systems after applying HHP (Sevenich et al., 2013, 2014), the authors only evaluated the effect of a single pressure value (600 MPa) combined with very high temperatures (90, 105, 110, 115, and 121 °C). No attempts have been made to systematically evaluate the influence of HHP processing conditions (pressure, time and temperature) on microbial inactivation and furan formation/mitigation in vegetable-based infant foods. Therefore, in the present work, we focus on background microflora inactivation and furan formation in infant foods, because infants and toddlers are the most susceptible consumer groups (Suk, Murray, & Avakian, 2003) and because relatively high amounts of furan were previously detected in baby foods (Lachenmeier, Reusch, & Kuballa, 2009; US FDA, 2009).

In the present work, a systematic study was conducted to evaluate the effects of pressure, time and temperature on (*i*) total mesophilic aerobic (TMA) bacteria, (*ii*) total yeasts and molds (TYM), and (*iii*) furan formation, in vegetable-based infant foods. The results of our multi-parameter study were analysed using multiple linear regression to assess the relative contribution of each process variable.

## 2. Materials and methods

### 2.1. Samples and HHP treatments

The vegetable-based baby food was based on a mixture of water, carrot, white cabbage, potato, marrow (a sweet variety of zucchini), rice flour, celery root, sugar, tomato juice concentrate, salt and sunflower oil. The samples for experimentation were kindly supplied by baby foods division of Hero Group, Turkey (Herobaby, 2017). The initial mesophilic aerophilic bacteria population was 6.8 $\log_{10}$/g, while that for total yeasts and molds was 5.8 $\log_{10}$/g. All samples for experiment were drawn from the same pool of product, thereby ensuring uniform initial microbial loads. The samples were deaerated by vacuuming. For pressure treatment, samples were carefully filled into 20 mL plastic bottles (LP Italiana SPA, Italy) to avoid any air bubbles, undesirable in HHP process.

HHP treatment was performed in a 760.0118 type pressure equipment supplied by SITEC-Sieber Engineering AG, Zurich, Switzerland. The vessel had a volume of 100 mL with an inner diameter of 24 mm and length of 153 mm. A built-in heating-cooling system (Huber Circulation Thermostat, Offenburg, Germany) was used to maintain and control the required temperature. The temperature in the vessel was monitored using a type K thermocouple. The vessel was filled with a pressure transmitting medium consisting of distilled water.

The increase in temperature originating from adiabatic heating was calculated to be between 4 and 5 °C. The pressurization was applied to the samples at a pressure of 200, 300, 400 MPa, temperature of 25, 35, 45 °C, for 5, 10, 15 min. Come up and pressure release times were not considered for the HHP application times reported in the study. HHP conditions were decided with respect to the literature research (Barba, Koubaa, do Prado-Silva, Orlien, & de Souza Sant'Ana, 2017; Georget et al., 2015). Pressurization rates were 400 MPa/min for 200 MPa, 360 MPa/min for 300 MPa and 340 MPa/min for 400 MPa (Günlü, Sipahioğlu, & Alpas, 2014; Subasi & Alpas, 2017). Pressure-treated samples were stored at − 18 °C until performing the chemical and microbiological analysis. It may be noted that when time is not a constraint, performing microbiological analysis on the same day is recommended to avoid any sub-lethal injury to cells (Banwart, 1989; Jay, Loessner, & Golden, 2005); however, in the present study the relative recovery of TYM/TMA would be closely comparable for heat treated and HHP processing, thus sufficiently serving the objective of comparing the two processing approaches. Samples to be treated thermally were filled to the jars at 80 °C and subsequently pasteurized at 105 °C for 10 min. The setup of the experimental design is represented in Fig. 1.

### 2.2. Microbial enumeration

In this work, reductions in background microflora, i.e. total mesophilic aerophiles and yeasts/molds, resulting from HHP processing were studied, as these organisms are primary contributors to spoilage of vegetable based infant foods under most practical conditions. The microbiological enumerations were carried out as follows.

#### 2.2.1. Total mesophilic aerobic (TMA) bacteria

One gram of pressure-treated sample was suspended in 0.1% peptone water. Inoculations were performed from 1:10 dilution. 1 mL suspension of the baby food samples were surface plated on pre-poured Plate Count Agar (Merck, Darmstadt, Germany) in three plates (0.3, 0.3 and 0.4 mL). After the incubation at 37 °C for 48 h, colony enumeration was achieved.

#### 2.2.2. Total yeasts and molds (TYM)

The procedure was similar to the determination of total mesophilic aerobic bacteria except the medium and the incubation time. 1 g of pressure-treated sample was suspended in 0.1% peptone water. Inoculations were performed from 1:10 dilution. 1 mL suspension of the baby food samples were surface plated on pre-poured Potato Dextrose Agar (Merck, Darmstadt, Germany) in three plates (0.3, 0.3 and 0.4 mL). 14 mL of 10% tartaric acid was put to 1 L medium to avoid the bacterial growth (pH 3.5). After incubation at 25 °C for 120 h, colony enumeration was achieved.

### 2.3. Determination of furan content

Untreated, thermally- and HHP-treated vegetable-based baby food were transferred to vials (Supelco, Bellefonte, PA, USA) and kept at 4 °C to avoid any loss of furan due to its high volatility. Aluminium crimp