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ORIGINAL ARTICLE

Effect of high hydrostatic pressure on background microflora and furan formation in fruit purée based baby foods

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Abstract The baby foods industry is currently seeking technologies to pasteurize products without formation of processing contaminants such as furan. This work demonstrates the applicability of high hydrostatic pressure (HHP) as a non-thermal decontamination intervention for fruit purée based baby foods. HHP processing was evaluated at 200, 300, and 400 MPa pressures, for 5, 10 and 15 min of treatment times at 25, 35 and 45 °C. HHP application at 400 MPa, 45 °C for 15 min ensured complete inactivation (about 6 $\log_{10}$) of total mesophilic aerophiles, as well as yeasts and molds. No furan was detected in HHP processed products. Thus, the key advantage of HHP over thermal processing is the ability to achieve commercially acceptable microbiological inactivation while avoiding the formation of processing contaminants such as furan.

Keywords High pressure · Fruit purée · Mesophile · Yeast · Furan · Baby food · Infant

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Introduction

Infants are often vulnerable to infection due to immature internal organs and the lack of a developed immune system. It is undeniable that baby foods require considerably high levels of microbiological quality warranting a mandatory need for conforming to regulatory microbiological criteria due to their susceptibility to infections (Forsythe et al. 2005). Thermal processing of baby foods, although ensures microbiological safety, it is known to result in formation of processing contaminants such as furan. Historical FDA survey on furan levels in baby foods has revealed that commercially available baby foods typically contain between 3.9 and 100 ppb of furan (US FDA 2009); see Fig. 1. The European Food Safety Authority (EFSA) has expressed the opinion that furan possesses carcinogenicity attributable to genotoxic effects (EFSA 2011). Detailed reviews of furan in foods, the mechanisms underlying their formation and analytical methods for detection are available in literature (Crews and Castle 2007; Van Lancker et al. 2010).

Traditional food processing methods mainly prolonged shelf-life and ensured safety by relying on an interplay of heat and time while disregarding thermo-labile nutrients, vitamins and other components responsible for product flavor and taste, often with additional requirement of special packaging and/or additives. When considering

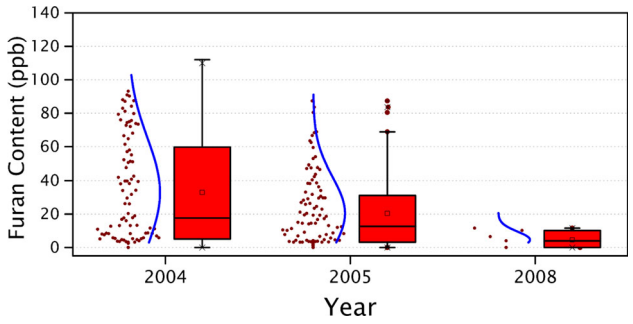

Fig. 1 Boxplot of year-wise furan content (in ppb) in baby foods based on data obtained from US FDA (2009). The raw data and a curve corresponding to normal distribution is also displayed to the left of each box

microbiological safety of foods, High Hydrostatic Pressure (HHP) processing as a novel non-thermal method has shown great potential in producing safer products while maintaining the natural characteristics of the food items (Rendueles et al. 2011). HHP is successfully applied on a commercial scale worldwide for *pascalization* of an array of foods such as fruit juices, sea foods, meat, fruit-vegetable products, ready-to-eat foods, salads and sauces and even pet foods (Misra et al. 2017).

The loss of viability of micro-organisms through HHP is often suggested as the result of a combination of injuries in the cell. The cell membrane is the main target of HHP, resulting in severe permeability modification and functionality disruption (Misra et al. 2017). Several recent studies continue to demonstrate the applicability of HHP for inactivation of a range of micro-organisms in several foods (Mukhopadhyay et al. 2017. However, most of the studies report one or two HHP conditions but there are not exhaustive studies evaluating the influence of the different HHP processing parameters (pressure, time and temperature).

HHP has been demonstrated as a feasible commercial process to obtain safe products. Therefore, evaluating the influence of process variables on spoilage microbial populations is a key factor in selecting microorganisms which can be used as a control parameters and defining treatment conditions to avoid the loss of important properties of foods and the formation of processing contaminants (e.g. acrylamide, HMF, furan, etc.) to obtain food products with utmost safety of the consumers.

As previously reported, HHP could be of significant benefit to baby foods industry as it could potentially lower the quantities of unwanted food processing contaminants as compared to thermal treatments (Sevenich et al. 2014; Palmers et al. 2015). This is due to the short processing times in HHP combined with much lower thermal loads. Scientific literature pertinent to the use of high pressure treatments as an alternative to thermal processing of baby foods is very scanty. Sevenich et al. 2014 evaluated the effects of high-pressure thermal sterilization (HPTS) on microbiological parameters and processing contaminants in baby foods. They demonstrated the inactivation of *Geobacillus stearothermophilus* and *Bacillus amyloliquefaciens*, over the temperature range 90–121 °C at 600 MPa. In addition, they found that HPTS allowed to reduce the amounts of furan between 81 and 96% in comparison to retorting. In another study, Palmers et al. (2015) showed that the faster heating and cooling rates during high-pressure high-temperature processing was responsible for lower furan contents in spinach purée than when retorted.

In the present study, HHP processing was employed near ambient temperatures to pasteurize fruit purée based baby food, and evaluate its consequence on the furan content. The main objective of this work was to understand the influence of pressure (200, 300 and 400 MPa), time (5, 10 and 15 min), and temperature (25, 35 and 45 °C) and their interactions on the inactivation of total mesophiles as well as yeast and mold, plus the furan formation. The choice of the microbial group is driven by the fact that mesophiles and molds are commonly associated with spoilage of fruit purées under commercial processing conditions. In addition, the experimental data for inactivation of total mesophiles as well as yeast and mold were evaluated using multiple regression to study the effect of individual parameters in a decoupled manner.

Materials and methods

Samples and treatments

The fruit-based baby food samples were a complex mixture of water, fruit purées (apple purée, banana purée, carrot purée, mango purée), grape juice concentrate, orange juice concentrate, oat flakes, rice starch, corn starch, and ascorbic acid (25 mg/100 g). The baby food samples were kindly supplied by baby foods division of Hero Group, Turkey (Herobaby 2017). The initial mesophilic aerophilic bacteria population was 6.18 $\log_{10}$, while that for total yeasts and molds was 5.9 $\log_{10}$. All samples for experiment were drawn from the same pool of product, thereby ensuring uniform initial microbial loads. For HHP application, the entrapment of air bubbles within samples is undesirable. Therefore, air trapped in the samples was excluded by vacuuming and the samples were carefully filled into 20 mL plastic tubes (LP Italiana SPA, Italy), with utmost care to avoid formation of air bubbles.

HHP treatment was performed in a 760.0118 type pressure equipment (SITEC-Sieber Engineering AG, Zurich, Switzerland) consisted of a pressure chamber of cylindrical design, two end closures, a means for

restraining the end closures and a pressure intensifier pump to generate and transfer high pressure for system compression, and also a temperature control device. The pressure 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 medium was heated up to the desired temperature prior to pressure application using the built-in system.

The increase in temperature originating from adiabatic heating was calculated to be between 4 and 5 °C. Pressurization was implemented to the samples at a pressure of 200, 300, 400 MPa, a temperature of 25, 35, 45 °C, and during 5, 10, 15 min. Come up and pressure release times should not be considered in HHP application time. HHP conditions were decided based on literature reports. Pressurization rates were 400 MPa/min for 200 MPa, 360 MPa/min for 300 MPa and 340 MPa/min for 400 MPa (Subasi and Alpas 2017). Pressure-treated samples were stored at − 18 °C until performing any chemical and microbiological analysis. Samples to be treated thermally were filled into jars at 80 °C and subsequently commercially sterilized at 105 °C for 10 min.

Microbiological enumeration

The reduction in background microflora, i.e. total mesophilic aerophiles and yeasts/moulds, primary contributors to spoilage of fruit based infant foods, caused by HPLC was studied. The microbiological enumerations were carried out as follows (Kultur et al. 2017).

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 of the suspension was 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. The experiment was repeated once more to determine the average.

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 of the suspension of was 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 (Nuve EN 120, Ankara, Turkey) at 25 °C for 120 h, colony enumeration was performed in duplicate, and average values reported.

Analysis of furan

Untreated and HHP treated fruit purée based baby food were placed in vials (Supelco, Bellefonte, PA, USA) and held at 4 °C to minimize the loss of (highly volatile) furan. About 5 g of sample was put into 20 mL headspace vial and a Carboxen-PDMS SPME fiber (Supelco, Bellefonte, PA, USA) was introduced through the aluminium crimp seals with Teflon-faced silicon septa (Agilent, Waldbronn, Germany). The sample with Carboxen-PDMS SPME fiber was equilibrated to 40 °C in an incubator (for 30 min. After equilibration, SPME fiber was introduced into the injection port of GC and kept for 5 min for desorption. Chromatography was carried out on a capillary column (24 m × 320 μm, 20 μm) of HP-PLOT-Q connected to Agilent 5973N GC/MS (Agilent, USA). Helium gas at a rate of 2 mL/min (60 cm/s) was used as a carrier gas. Column temperature program was set as follows: 100 °C for 5 min, ramped to 200 °C at a rate of 10 °C/min, followed by holding at 200 °C for 15 min, resulting in a total analysis time of 30 min. Mass spectrometric scan was carried out within an m/z range of 20–200 AMU. Quantifier/qualifier ions for furan and d4-furan were set as 68/39 and 72/43 m/z. The elution time of furan was found to be 11 min. For calibration curve, external standard used was furan (minimum purity 99%, Fluka Chemie GmbH, Switzerland), and while 50 ng/g d4-furan (minimum purity 99%, Aldrich, USA) served as internal standard. For quantitation, a calibration curve of furan was prepared in the infant food covering the concentration range of 0–1000 ng/g. The limit of quantification (LOQ) for the method was found to be 1 ng/g.

Statistical analysis

Two independent treatments were carried out in this study and the mean results reported. A three-way analysis of variance (ANOVA) was applied to the results obtained to verify whether there were significant differences in the parameters studied in relation to treatment pressure and time, and to ascertain possible interactions between the factors (differences at $p < 0.05$ were considered significant). To capture the effects of the three parameters, viz. pressure (P), treatment time (t), and temperature (T) and

their interactions, the experimental data for inactivation of total mesophiles as well as yeast and mold was fitted to a quadratic function of the form-

$$Y = \beta_0 + \beta_1 P + \beta_2 t + \beta_3 T + \beta_4 Pt + \beta_5 PT + \beta_6 tT + \beta_7 P^2 + \beta_8 t^2 + \beta_9 T^2$$

where, β_i are the coefficients of the variables, with β_0 being the intercept. This allowed to include the purely quadratic effects as well as interaction terms. We employed a stepwise linear fitting routine, which allowed to retain only those terms which were significant ($p < 0.05$), whilst simplifying the models without losing the accuracy of predictions (MATLAB, The Mathworks, MA). The method uses a forward and backward stepwise regression to determine the final model. An analysis of the residuals revealed the suitability of the model for analysis of the experimental data.

Results and discussion

Microbial inactivation

The evolution of TMA count with time during HHP application for each of the temperatures studied is presented in Fig. 2. HHP resulted in significant inactivation of the total mesophilic aerophiles. Figure 2 reflects that the treatment temperature played an important role during the inactivation. The evolution of TYM count with time during HHP application for each of the temperatures studied is presented in Fig. 3. HHP was also effective in completely inactivating yeasts and molds in the baby formula. Unlike in the case of TMA inactivation, the effect of treatment time and other parameters is apparent in the case of TYM inactivation, due to more variability. In both cases, it can be observed that for the temperatures employed, an applied pressure of 400 MPa was effective in complete elimination of the microbiological contamination; therefore, same is recommended for practical applications.

The inactivation of micro-organisms following application of HHP is a function of all the processing conditions, namely pressure, time and temperature, besides known effects of food matrix and the class of microbe (Georget et al. 2015; Rendueles et al. 2011). Therefore, to understand the role of each HHP parameter and their interactions, the relative impact of HHP processing conditions (200, 300 and 400 MPa; 25, 35 and 45 °C; 5, 10 and 15 min) on microbial inactivation (TMA bacteria and TYM) was analysed using multiple linear regression (MLR). This technique allows to evaluate the independent contribution and interaction effects of more than one input variable; cf. Vazquez-Landaverde et al. (2007), and Zarate-Rodriguez et al. (2000).

The statistical models applied to the inactivation cases were statistically significant ($p < 0.05$). The values of coefficients of regression (R^2) of 0.84 and 0.66 for TMA bacteria and TYM, respectively were acceptable. The lower value of R^2 for TYM was due to the variability in the data coupled with involvement of three different independent process variables. This is also evident from earlier reports on inactivation of TMA and TYM during high pressure application with the three independent variables selected in the study (Penas et al. 2008; Sevenich et al. 2014). Another parameter that provides helpful insight into the goodness of a fit is the root mean squared error (RMSE)

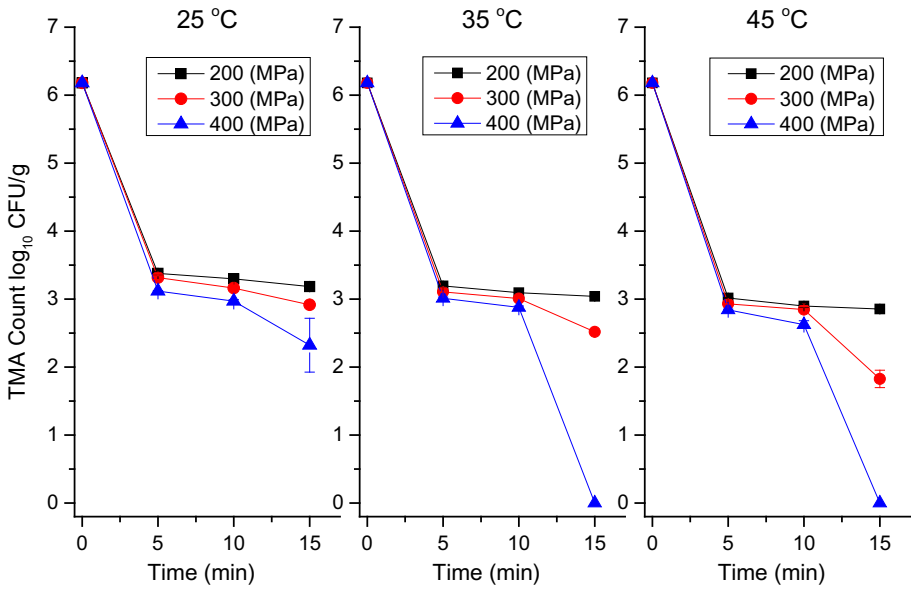

Fig. 2 The inactivation of total mesophilic aerobic (TMA) bacteria in the baby food during HHP processing. Error bars represent standard deviations

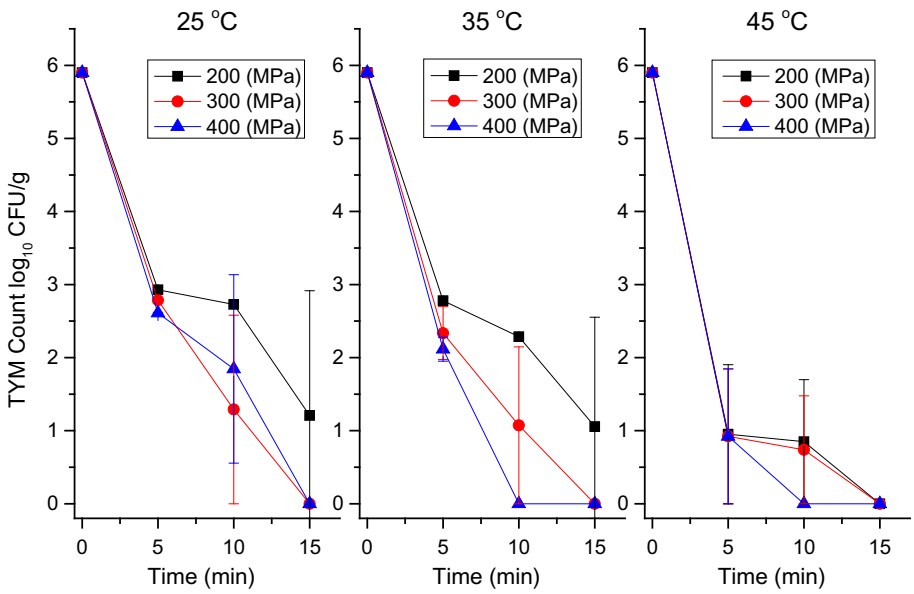

Fig. 3 The inactivation of total yeasts and molds (TYM) in the baby food during HHP processing. Error bars represent standard deviations

with low values of 0.28 for the TMA count and 0.73 for the TYM count.

Model fitting for total mesophilic aerobic (TMA) bacteria

For the inactivation data of total mesophilic aerobic (TMA) bacteria, the fitting routine allowed to eliminate several terms, with the simplified model represented as

$$TMA = \beta_0 + \beta_1 P + \beta_2 t + \beta_3 T + \beta_4 Pt + \beta_5 PT + \beta_6 tT + \beta_8 t^2 \ldots \quad (1)$$

The values of the significant coefficients are provided in Table 1. The interpretation of coefficients in Eq. (1) is *ceteris paribus.* In addition, the contribution of each of the three variables independently, towards the total mesophilic bacteria reduction effect is depicted in Fig. 4. This plot shows the estimated effect on the TMA bacteria inactivation from changing each HPP process variable, minimizing the effects of the other variables. The treatment time was found to have the most significant effect on the inactivation of TMA bacteria, followed by temperature and pressure, when keeping remaining factors at minimum. There could be several reasons for the treatment time being a governing parameter, including a variability in bacterial populations for sensitivity to pressures, mixed bacterial populations in samples, and/or yield points of the bacterial adaptation to the pressure stress making them more susceptible (Chen et al. 2005). From Fig. 4 it can be concluded that keeping all other parameters at minimum, an increase in treatment time from 6.6 to 15 min would result in an average decrease in the TYM population by $\sim$ 1.0 $\log_{10}$. Likewise, treatment times below 6.6 min will not cause any considerable effects on the microbial populations; this is evident from the statistically significant value of quadratic coefficient for time (t) in Eq. 1. It may be noted that while the predicted individual contributions may be low, it is their interactions that make HHP more effective.

Model fitting for total yeast and mold (TYM)

The simplified model for inactivation of yeast and mold was found to be-

$$TYM = \beta_0 + \beta_1 P + \beta_2 t + \beta_3 T + \beta_4 Pt \ldots \quad (2)$$

where, the coefficient estimates are summarized in Table 1. The contribution of each of the three variables towards a reduction in yeasts and mold population is depicted in Fig. 5. From Fig. 5 it can be noted that the treatment time was found to have the most significant effect on the inactivation of TMA bacteria, followed by pressure and temperature. An increase in treatment time from 5 min to 15 min would result in a decrease in the TYM population by $\sim$ 2 $\log_{10}$, given other factors are maintained at minimum. However, increasing the temperature from 25 to 45 °C would contribute to a decrease in TYM by less than 1 $\log_{10}$. The absence of quadratic terms in Eq. (2) signified that even the minimum values of the studied parameters played a role in the microbiological inactivation.

Influence of pressure, time and temperature per the targeted microbial population

A three-way analysis of variance (ANOVA) was conducted to compare the effect of HHP processing parameters (pressure, time and temperature). As can be seen in Figs. 2,

Table 1 Values of the significant coefficients of the quadratic model fitted to the TMA bacteria and TYM inactivation data

Coefficients	Total mesophilic aerobes		Total yeast and mold	
	Estimate	SE	Estimate	SE
Intercept (β_0)	− 6.989	0.895	2.465	1.097
Pressure (β_1)	− 0.006	0.002	− 0.013	0.003
Time (β_2)	0.566	0.0.87	− 0.395	0.093
Temperature (β_3)	0.087	0.022	− 0.040	0.012
Pressure × time (β_4)	− 0.0004	0.000	0.001	0.000
Pressure × temperature (β_5)	− 0.00015	0.000	–	–
Time × temperature (β_6)	− 0.007	0.001	–	–
Pressure^2 (β_7)	–	–	–	–
Time^2 (β_8)	− 0.012	0.003	–	–
Temperature^2 (β_9)	–	–	–	–
R^2	0.84		0.66	
RMSE	0.28		0.73	

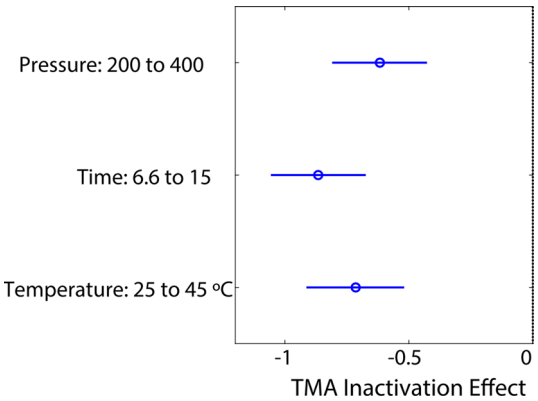

Fig. 4 Contribution of the variables towards inactivation of the total mesophilic aerobic bacteria. The width of the horizontal lines represents the confidence interval for the effect on the TMA in each variable

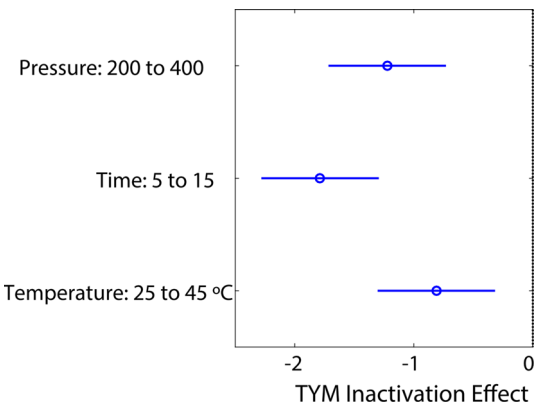

Fig. 5 Contribution of the individual variables towards inactivation of the total yeast and mold. The width of the horizontal lines represents the confidence interval for the effect on the TYM in each variable

3 and 4, all the studied HHP factors had a significant ($p < 0.05$) impact on TMA and TYM inactivation of the fruit-based infant foods. Overall, the number of TMA and TYM was decreased when pressure, treatment time and temperature were increased, being especially important temperature increase to reduce the number of microbial population. This fact can be attributed to a synergism between pressure, time and temperature, which is of a great importance to optimize HHP processing conditions. It should be noted that from a commercial point of view as well as for consumer acceptance, increasing pressure, time and temperature can lead to increased cost for manufacturers and the nutritional and quality attributes of these food products can be compromised. Moreover, as can be expected TMA bacteria seemed to be less sensitive to the HHP conditions. These results are in close agreement to those reported by Georget et al. (2015) and Rendueles et al. (2011), when they reviewed the HHP inactivation of TMA and TYM. They observed a higher TMA bacteria resistance to pressure compared to TYM, although there is a lack of information comparing equivalent HHP processing conditions on the same food matrices. From a mechanistic standpoint, pressures at a sufficiently high level, induces enzyme inactivation, membrane protein denaturation and cell membrane rupture caused by a phase transition of the membrane and its fluidity, thus resulting in inactivating the microorganisms (Georget et al. 2015).

Furan content

The furan contents of all the samples evaluated were found to be below the limit of quantification (i.e. 1 ng/g) and therefore not detected, indicating that the HHP process conditions employed did not induce furan formation. It is well established that furan formation occurs under high

temperature conditions, such as those employed during thermal processing (Crews and Castle 2007. The absence of furan is likely due to the near ambient temperatures employed in this study. Our results are also consistent with earlier reports of significant reduction in furan formation in HHP processed fish (Sevenich et al. 2013), and baby food purée (Sevenich et al. 2014), respectively.

Conclusion

High hydrostatic pressure application in the order of 400 MPa to fruit based baby foods could guarantee a microbiologically safe product, while still employing short treatment times of up to 15 min and near ambient temperatures between 25 and 45 °C. HHP treatment conditions of 400 MPa for 15 min at 45 °C allowed to eliminate total mesophilic aerobic (TMA) bacteria and total yeast and mold (TYM) by $\sim$ 6 $\log_{10}$ population, without causing furan formation. In summary, this study deduced the essential HHP process conditions for pasteurizing baby foods without inducing furan formation (i.e. below the limit of quantification of 1 ng/g). Thus, HHP is recommended as a potential technology for pasteurization of baby foods while overcoming the challenge of processing contamination, as is encountered with thermal processing, where furan content in the range of 4–100 ng/g is reported. In order make this technology ready for implementation in baby foods industry, further validation at pilot scale is recommended.

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