COMPARISON OF THERMAL STERILIZATION AND HIGH HYDROSTATIC PRESSURE-HHP ON FURAN FORMATION, MICROBIAL AND NUTRITIONAL QUALITY IN COMMERCIAL BABY FOODS

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COMPARISON OF THERMAL STERILIZATION AND HIGH HYDROSTATİC PRESSURE-HHP ON FURAN FORMATION, MICROBIAL AND NUTRITIONAL QUALITY IN COMMERCIAL BABY FOODS

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ABSTRACT

COMPARISON OF THERMAL STERILIZATION AND HIGH HYDROSTATIC PRESSURE-HHP ON FURAN FORMATION, MICROBIAL AND NUTRITIONAL QUALITY IN COMMERCIAL BABY FOODS

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Furan, which is a lipophilic contaminant and formed during heating process in foods, has been pointed out as a danger in baby foods since it has been classified as "possibly carcinogenic to human" by IARC (International Agency for Research on Cancer). Hence, a great concern has been addressed to the analysis of this substance in baby foods.

This study aims to prove that sterilization of baby foods is possible by high hydrostatic pressure (HHP) without allowing the formation of furan. Firstly, HHP treatments to two types of baby foods such as vegetable based baby food and fruit and cereal based baby food at 200, 300, 400 MPa, at 25^{o}C, 35 ^{o}C, 45 ^{o}C for 5, 10, 15 minutes were performed. Secondly, microbiological assays such as total mesophilic aerobic bacteria count (TMAB) and total yeast and mold (TYM) count were executed. Approximately, 6 log reduction in TMAB and TYM were seen in both types of baby foods at 400 MPa-45^{o}C-15 min. Thirdly, furan amount in pressurized samples, which was lower than 0.1ng/g, was determined by GC-MS for both types of pressurized baby food samples Then, optimum parameters of HHP process in the manner of microbial reduction were specified as 400 MPa- 45^{o}C-15 min when microbial reduction and furan amount both considered. Subsequently, shelf life of the HHP-treated samples with these optimum parameters was determined as 14 days for fruit based and 21 days for vegetable based baby food.

Key words: Baby food, sterilization, HHP, furan.

ÖZ

TİCARİ BEBEK MAMALARINDA FURAN OLUŞUMU, MİKROBİYAL VE BESİNSEL KALİTE PARAMETRELERİ ÜZERİNDEN ISIL STERİLİZASYON VE YÜKSEK HİDROSTATİK BASINÇ UYGULAMALARININ KARŞILAŞTIRILMASI

Kültür, Gülçin
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Lipofil bir bulaşan olan ve gıdalarda ısıtma işlemi sırasında ortaya çıkan furan, Uluslararası Kanser Araştırmaları Ajansı tarafından "insan için kanserojen olabilir" şeklinde sınıflandırıldığından, bebek mamalarında bir tehlike olarak dikkat çekmektedir. Bu nedenle, bebek mamalarında bu maddenin analizi büyük ilgi görmektedir.

Bu çalışmanın amacı furan oluşumuna mahal verilmeden bebek mamalarının yüksek hidrostatik basınçla (YHB) sterilize edilebileceğini kanıtlamaktır. İlk olarak, iki bebek maması türüne, 200, 300, 400 MPa, 25^{o}C, 35 ^{o}C, 45 ^{o}C 'de ve 5, 10, 15 dakika sürelerince yüksek hidrostatik basınç uygulaması gerçekleştirilmiştir. İkinci kısımda toplam mezofil aerob bakteri sayımı ve toplam küf ve maya sayımı analizleri yapılmıştır. Yaklaşık 6 logaritmik birimlik azalma her iki bebek maması türünde, her iki mikroorganizma için gözlemlenmiştir. Üçüncü olarak, basınçlanan örneklerdeki furan miktarı GC-MS ile 0.1 ng/g olarak belirlenmiştir. Daha sonra mikrobiyal azalma ve furan miktarı bir arada değerlendirilerek, YHB işleminin optimum parametreleri 400 MPa-45 ^{o}C ve 15 dakika olarak belirlenmiştir. Buna müteakip olarak belirlenen bu optimum parametrelerle YHB uygulanan örneklerin raf ömrü meyve bazlı bebek mamaları için 14 gün, sebze bazlı bebek mamaları içinse 21 gün olarak belirlenmiştir.

Anahtar Kelimeler: Bebek maması, sterilizasyon, YHB, furan.

To my family and my love…

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INTRODUCTION

1.1 History of HHP

Firstly, Pascal, who aimed to understand the studies on pressure of Galileo in 1640 and Toricelli in 1644, conducted a series of experiments to show the concept of pressure as a thermodynamic parameter later than for temperature. After the definition of pressure as a thermodynamic parameter, it was introduced in physics, hydrometallurgy, geochemistry and biology respectively (Demazeau & Rivalain, 2011).

The effects of liquid pressure were noted by three main scientists: Roger (1892, 1895), Büchner (1897) and Hite (Hite, 1899). Inactivation of bacteria by applying high hydrostatic pressure was first established by Roger (1892, 1895). It is stated in Demazeau and Rivalain (2011) study that, Roger also denoted two important phenomena about high hydrostatic pressure reaching approximately 300 MPa,

a) Behavior difference against pressure between microorganisms species,
b) Sensitivity difference against pressure between two bacterial forms of the same microorganisms which means spores are considerably more resistant to pressure than vegetative bacteria.

Hite was also a precursor in the development of high pressure. He started using high pressure for the preservation of foods (Hite, 1899). Again in the review written by Demazeau and Rivalain (Demazeau & Rivalain, 2011), in 1903 Choplin and Tamman established that a pressure approximately 300 MPa made some microorganisms go under a situation like fainting which they could not recover for a long time. And in 1918 Larson and his friends studied the effect of pressure in biology in terms of immunity and immunogenicity (Larson, Hartzell, & Diehl, 1918).

There were also studies conducted aiming to represent high pressure effect on proteins. First study on denaturation of proteins at cold temperature was performed by Bridgeman. As Demazeau and Rivalain reported, Bridgeman was first in this area. (Demazeau & Rivalain, 2011).

In the following years, between 1950 and 1985, the studies that had been conducted so far, repeated to find out the optimum HHP parameters for different issues (Demazeau & Rivalain, 2011).

According to Demazeau and his co-workers, after 1985, Japan became the destination in high pressure food processing research in the areas of compressibility of proteins and high

pressure bioscience (Kunihiko & Noguchi, 1979); (Gekko & Yamagami, 1991); (Demazeau & Rivalain, 2011); (Mozhaev, Heremens, Frank, Masson, & Balny, 1996).

1.2 Working principle of HHP

The effect of high pressure can be explained by two principles such as Le Chatelier Principle and Isostatic Rule. Firstly, in accordance with Le Chatelier Principle, a chemical system at equilibrium (phase transition, chemical reactivity, change in molecular configuration and chemical reaction) which is exposed to a change volume, concentration, temperature etc., behaves in a way to minimize the disturbance and finally new equilibrium is formed. Increasing temperature results in a volume increase. Also an increase in reaction rate occurs with increasing temperature with respect to Arrhenius' law (Smelt, 1998).

Secondly, in accordance with Isostatic Rule, Smelt (1998) points out in his study that pressure transmits instantaneously and uniformly independent of the size and the geometry of the food. The pressure is applied equally in all directions. This isostatic process is an advantage for solid foods to preserve their original shape (Smelt, 1998).

1.3 HHP Systems

A simple pressurization apparatus consists of a high-pressure steel cell where the sample is treated, a high pressure generating system, a temperature controller and there is also a loading system for the material to be treated. The sealing system of the container differs with respect to the type of application (Bertucco & Spilimbergo, 2001).

After putting samples to the container, the container is filled with a fluid that is responsible to transmit the pressure. This transmitting medium is generally water mixed with a little oil which is aimed as lubricant. Mixture of water and ethylene glycol with a weight ratio 3:1 is also used as pressure transmitting medium (Bertucco & Spilimbergo, 2001).

There are general compression methods: direct and indirect compression. In direct compression method, a piston coaxial with the container is required. The compressions are very fast when it is compared to indirect method. This method is very suitable for laboratory-scale plant since there is a sealing problem between the piston and the internal surface of the container. The indirect method is very common. This method consists of a pressure booster to pump the liquid from the pressure medium tank to the sample cell. Pumping stops when the target pressure is reached. There is also a third method which achieves to increase the pressure by heating medium. This is not an option in food industry but it is useful to prefer

this technique in processes like quartz production which both high pressure and high temperature are desired to success the process (Bertucco & Spilimbergo, 2001).

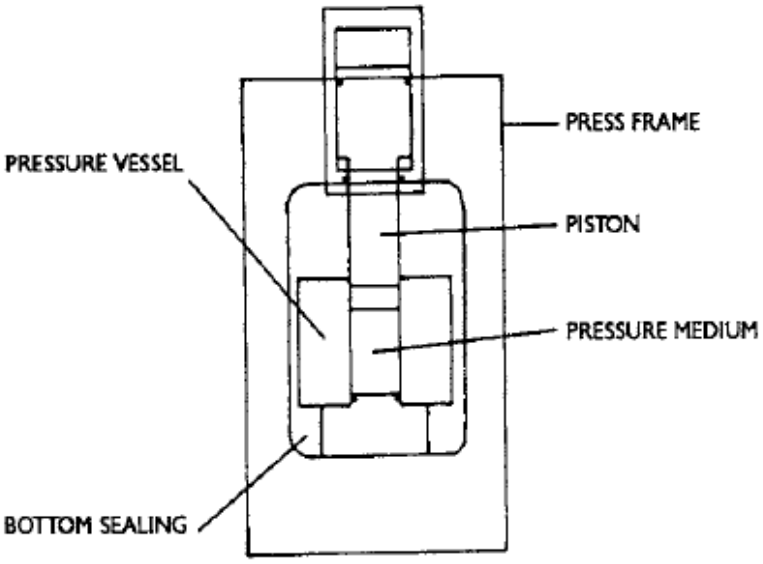

Figure 1.1 Schematic diagram for HHP which uses the direct method (Bertucco & Spilimbergo, 2001).

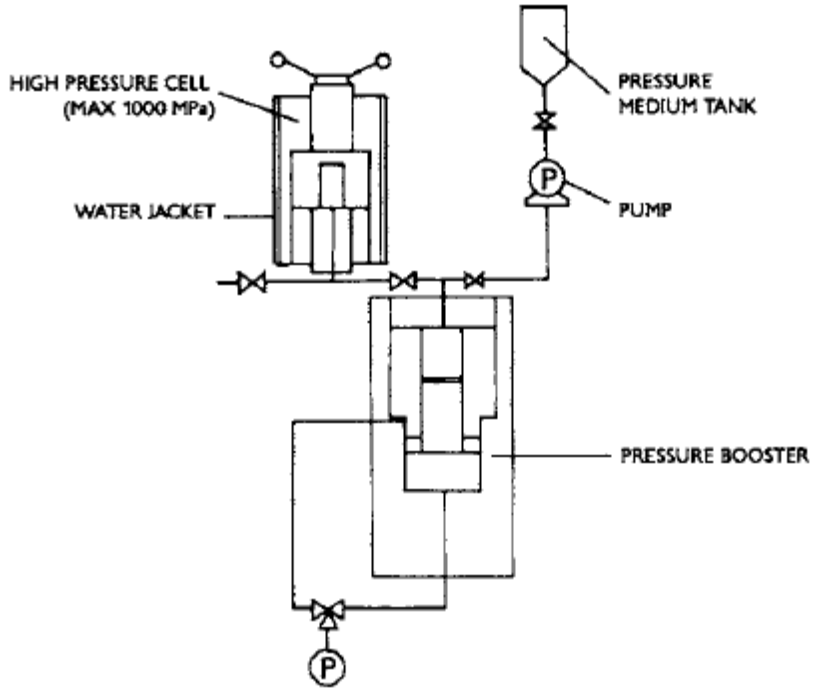

Figure 1.2 Schematic diagram for the HHP using the indirect method (Bertucco & Spilimbergo, 2001).

According to Bertucco and Spilimbergo, if only heating is needed, the temperature controller is only a simple electrical resistance, if it is not, a heat exchanger in a cell or heating/cooling jacket is used. For packaging, one would be careful to use a treatment –resistant packaging such as a plastic multi-layer envelope or aluminum foil. If rigid materials like glasses or metals are used, a compressibility space must be inside the packaging; otherwise the material could break (Bertucco & Spilimbergo, 2001).

Bertucco and his co-workers pointed out that the empty space in container should be minimized to reduce the stress through pressurization and depressurization steps. When high volumetric efficiency and therefore reduced cost per operating unit are taken into consideration, it is easily seen that the form of a container gains importance. Since the sample cells are generally cylindrical, the containers are hexagonal (Bertucco & Spilimbergo, 2001).

HHP is a batch process since the volume treated per unit time is a function of cycle time which is the summation of the times of each step such as sealing, pressurization, maintenance at the operating pressure, depressurization, opening, and unloading times, batch volume , number of cells which are used at the same time in parallel during the same cycle. A semi continuous process in HHP can be made by adding three or more pressure vessels in series. Figure 1.3 is an example of a semi continuous system (San Martin, Barbosa-Canovas, & Swanson, 2002).

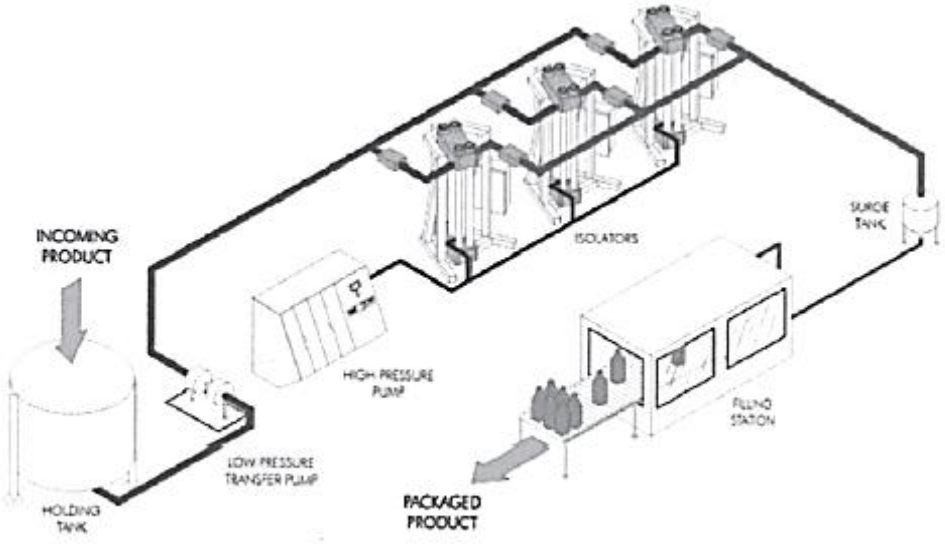

Figure 1.3 Semi-continuous HHP system (San Martin, Barbosa-Canovas, & Swanson, 2002).

When whole the process is taken into account in economic perspective, it is easily seen that the volumetric efficiency must be maximized to make the process economically applicable. In addition the temperature/pressure ratio must be at a level such that sample is not damaged. It is possible to reduce cycle time by minimizing loading and unloading times. This can be achieved by an automation system (Bertucco & Spilimbergo, 2001).

1.4 HHP Effect On Microorganisms

1.4.1 Vegetative Bacteria

It is well known that microorganisms are inactivated by HHP via abandonments of reproduction and survival skills. In order to achieve this, HHP destroys microbial membranes which are also responsible for the transport consisting of nutrient intake and disposal of cell waste. Leakage is easily understood from the intracellular fluid compounds which are extraordinarily found in the cell suspending fluid (FDA, 2011).

Cell envelope- related effects, cellular changes triggered by pressure, biochemical points and genetic mechanisms can be counted as the effects of high hydrostatic pressure. It is known

that pressure changes the cell morphology, and also increasing pressure can cause cell division. Pressure values between 100 and 300 MPa can ease spore germination, germination results with the vegetative cells which are more susceptible to environmental conditions (Gould & Sale, 1970).

Protein behaviors change with the variations in pressure in a particular way. High hydrostatic pressure can induce protein denaturation by changing its hydrophobicity (Jaenicke, 1981). Since enzymes are proteins, enzyme inactivation also occurs with this way. There are also other factors that cause enzyme inactivation such as changes in intramolecular structure and conformational changes at the active site (Suzuki & Suzuki, 1963). Substrate concentration, subunit structure of the enzyme and subunit concentration is also determined for the inactivation of enzymes under pressure (FDA, 2011). Since pressure has this effect on enzymes, it is established that microbial inactivation by pressure generally occurs via inactivation of enzymes, especially by inactivation of membrane-bound ATPases (Mackey, Forestiere, & Isaacs, 1995).

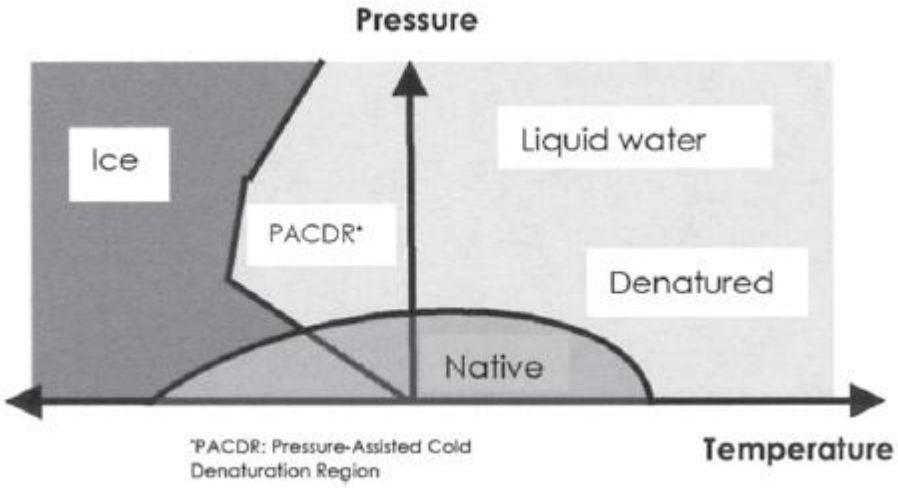

Figure 1.4 Pressure Effect on Protein Denaturation (San Martin, Barbosa-Canovas, & Swanson, 2002).

Pressure affects the membrane and changes the permeability. Damage in cell membranes causes inhibition of amino acid uptake mechanism with respect to denaturation of membrane protein. Membrane damage also cause loss of intracellular component from the cell. The amount of this loss shows the degree of death or injury (San Martin, Barbosa-Canovas, & Swanson, 2002).

Diphosphatidylglycerol which is responsible for the rigidity in membrane with calcium is high in bacteria. Because of this compound, bacteria are more susceptible to high hydrostatic pressure (Smelt, Rijke, & Hayhurst, 1994) . On the contrary, the compounds like docosahexaenoicacid (DHA) which increase the fluidity of membrane make the cell more resistant to pressure (Russell, Evans , ter Steeg, Helemons, Verheul, & Abee, 1995).

Pressure resistance varies in different strains of species and the stage of the growth of bacteria. It is more difficult to inactivate the bacteria cells at stationary phase than bacteria cells at exponential phase (FDA, 2011).

New HHP applications are derived from these destruction mechanisms of vegetative bacteria. To give an example, it is well known that the intracellular proteases of starter cultures are used in cheese ripening process. If we trigger the lysis of starter bacteria, the intracellular proteases will come up easily (FDA, 2011).

1.4.2 Spores

Spores are resistant to pressure. Applications up to 1200 MPa are needed to eliminate the spores. There is a multiple stage way to eliminate spores by pressure. At the first stage spore germination is triggered by low pressure values approximately 60 to 100 MPa. After germination, resultant vegetative cells are easier to handle. Since such applications are not feasible economically, it is needed a cooperation with low pH, reduced water activity and refrigeration (FDA, 2011).

1.4.3 Yeast and molds

Yeasts are not pathogens but their spoilage capability needs a major concern. It is possible to eliminate yeast in a few minutes with pressurization. Toxic mold growth is dangerous for health. Although vegetative forms of toxic molds are inactivated by pressurization at 300 MPa and 25 ^{o}C for a few minutes, ascospores need higher pressure-temperature and time combination like 600 MPa-60^{o}C-60 min. Ascospores of *Bsysochlamys nivea* and *Eupenicillium*. *B. nivea* need higher pressures and temperatures like 800 MPa and 70 ^{o}C to be inactivated. In elimination of ascospores, low water activities (aw=0.89) have a hurdle effect with pressurization (FDA, 2011).

Pressure effect is the same as the effects of high temperature and oxidative stress in yeasts. Cell membrane is the primary site affected from pressure (Iwahashi, Fuiji, Obuchi, Kaul, Sato, & Komatsu, 1993).

1.5 Hurdle Technology

There are challenges like spores and food enzymes in applications of HHP (Leistner & Gorris, 1995). HHP application (at generally reduced pressure levels < 15 MPa) with carbon dioxide is effective in inactivation of microorganisms. Processing time is very high in these applications due to diffusion of carbon dioxide into the cells. Increase in temperature can

lower the processing time. The availability of this technique is based on the antimicrobial effect of carbon dioxide (Haas, Prescott Jr., Dudley, Dik, Hintlian, & Keane, 1989); (Ballestra, Da Silva, & Cuq, 1996).

Irradiation is usually combined with high pressures. Either gamma irradiation alone or high hydrostatic pressure alone on staphylococci in lamb meat showed only 1 log reduction from 104 staphylococci/g. After combination, entire inactivation occurred (Paul, Chawla, Thomas, Kesavan, Fotedar, & Arya, 1997).

Antimicrobial compounds are also combined with high hydrostatic pressures to inactivate microorganisms. Lytic enzymes like lysozyme, antimicrobial chitosans and bacteriocins are used in cooperation with high hydrostatic pressure. For instance, where high pressure application alone up to 400 MPa is not sufficient for elimination of spores of *B. coagulans* 7050, combination of high pressure application of 400 MPa-70 ^{o}C for 30 min with pH 4.0 and 0.8 IU/mL nisin concluded with sterilization of spore crops containing 2.5 x 10^{6} cfu/mL. ((FDA, 2011); (Papineau, Hoover, Dietrich, & Farkas, 1991); (Roberts & Hoover, 1996)) Antimicrobial agents like pediocin AcH (Kalchayanand, Sikes, Dunne, & Ray, 1998), monoterpenes (Adegoke, Iwahashi, & Komatsu, 1997), polylysine, protamine, etiolated seedlings of adlay are used in combination with HHP (FDA, 2011).

1.6 Commercial HHP Products

Commercial food applications of HHP are based on the capability of killing spoilage microorganisms and relevant food borne pathogens in order to extend shelf life. High pressure processing is especially preferred since in addition to being microbiologically safe and therefore extended shelf life, sensorial properties of products such as original fresh taste, texture and nutritional content are retained. This is explained with the superior quality of the products when these products are compared to thermally processed ones (Patterson, Linton, & Doona, 2007).

Guacamole is a good example to high pressure processed foods. Guacamole has a short shelf life since enzymatic reaction and growth of microorganisms are dominant. Eliminating spoilage microorganisms by heat treatment is a good way to increase the shelf life of guacamole. But heat treatment also induces the disappearance of fresh green color (Patterson, Linton, & Doona, 2007).

Also in Turkey, there is a study on HHP processing of kiwifruit which is very beneficial for health and extremely nutritional because of its high vitamin C content and therefore its antioxidant capacity (Buzrul, Alpas, Largeteau, & Demazeau, 2008).

High hydrostatic pressure processing on vegetables and fruits has been tested worldwide since HHP has a harmless effect on freshness of the product. Mandarin and grapefruit juice in Japan, apple juice in Portugal, orange juice in France and United States, carrot juice and

broccoli- apple juice mixture in Czech Republic have been already on the shelves of markets (Martin, Barbosa-Cánovas, & Swanson, 2004).

HHP treatments at 500-600 MPa for a few minute are used for post packaging pasteurization of sliced meat products, fermented meats, whole and sliced cured ham, dry cured ham, pre-cooked chicken strips, cold cuts and also delicatessen products. The meat products are under dander of *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* spp. HHP treatments inactivate not only these food-borne pathogens, but also spoilage microorganisms. There is also an HHP pasteurized ham in Spanish markets. Pasteurization of ham with HHP extended its shelf life approximately 5 weeks (Patterson, Linton, & Doona, 2007).

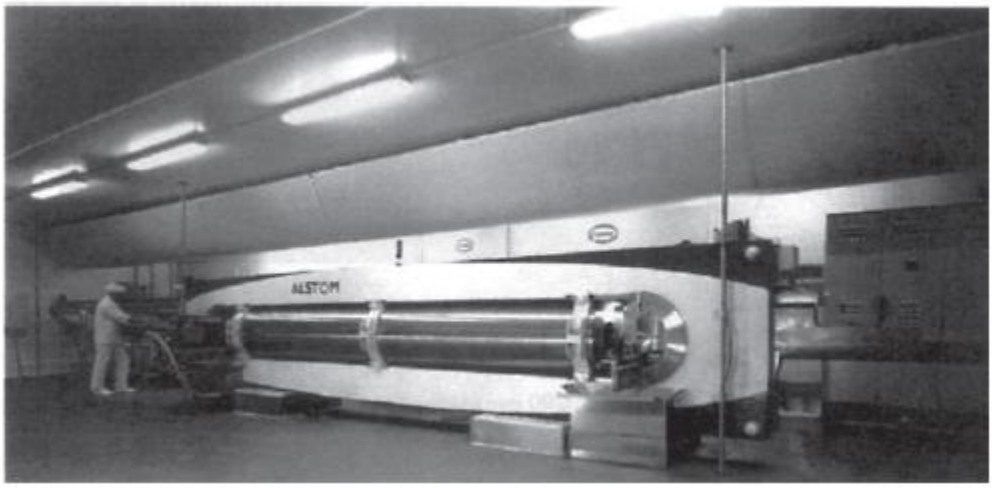

Figure 1.5 Industrial HHP System for ham processing in Spain (Martin, Barbosa-Cánovas, & Swanson, 2004).

HHP is very effective in eliminating *Vibrio parahaemolyticus* and *Vibrio vulnificus* which are located on raw oysters. After treatment, in addition to extended shelf life, the product retains the sensorial properties of a fresh oyster. In oysters, there is an adductor muscle, which gains resistance to shells to be closed tightly. HHP loosens this muscle and makes the shell open of its own Accord. This makes a significant increase the amount of meat taken from the shell. HHP is also used for increased microbial quality and product yield and various types of shellfish, such as mussels, Nephrops and lobsters (Murchie, et al., 2005).

1.7 Modeling approach of HHP

$$\ln\left(\frac{N_t}{N_o}\right) = -kt, \quad (1)$$

Where;

- N_F is the microbial load after pressurization (CFU/g or mL),
- N_o is the initial microbial load (CFU/g or mL),
- t is time (minutes or seconds),
- k is the inactivation rate constant (1/min or 1/s).

$$\log D_p = -mP, \quad (2)$$

Dp is the time (minutes or seconds) needed to eliminate 90% of initial microbial load at constant pressure.

Dp values (= 2.303/k) can be calculated from the inactivation rate constant (Guerrero-Beltrán, Barbosa-Cánovas, & Swanson, 2005).

Zp values (= −1/slope) is the pressure value needed to decrease *Dp value* for 1 log cycle. It is found plotting log *Dp* values as a function of pressure.

Where;

- *m* is slope (1/MPa),
- *P* is pressure (MPa),
- *Zp* (MPa) is the pressure alteration needed for a 10-fold alteration in inactivation or reduction time (Guerrero-Beltrán, Barbosa-Cánovas, & Swanson, 2005).

1.8 Furan

Furan, which is formed during thermal process is a lipophilic contaminant. As it is shown in Figure 1.6, furan is an aromatic and heterocyclic compound with a very low boiling point of 31.36 ^{o}C. Food products which are processed by the operations such as hot-air drying, baking, frying and roasting can contain furan. Cereal products, coffee, canned or jarred commercial foods are examples of food products which are subjected to those thermal treatments (Jestoi, Järvinen, Järvenpää, Tapanainen, Virtanen, & Peltonen, 2009); (EFSA, 2005); (FDA, 2004); (Zoller, Sager, & Reinhard, 2007).

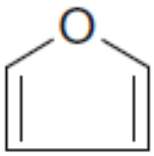

Figure 1.6 Chemical structure of furan.

There is not a single mechanism for the furan formation in foods. On the contrary, there are a lot of precursors and alternative routes. As a result of Strecker degradation, which is a non enzymatic browning involving reducing sugar only, furan is formed. Another non enzymatic browning with amino acids has also shown to produce furan and furan derivatives. These derivatives are also aroma compounds in latter parts of the reaction (Belitz, Grosch, & Schieberle, 2009). Ascorbic acid is also a good precursor of furan. It produces furan by using the same pyrolytic way with sugars almost in less harsh conditions (Becalski & Seaman, 2005); (Limacher, Kerler, Conde-Petit, & Blank, 2007). Furan is also formed after aldol condensation by the rearrangement of amino acids serine and cysteine. There is another pathway of furan formation which is very general for foods: furan is formed from oxidized polyunsaturated lipids by radical attack or lipoxygenase activity (Becalski & Seaman, 2005); (Perez Locas & Yaylayan, 2004).

Some of amino acids such as serine and cysteine are good at production of furan without any necessity of other sources. Acetaldehyde and glycolaldehyde give reactions like aldol condensation with resulting products like aldotetrose derivatives and consequently furan is produced. There are other amino acids such as alanine, threonine, and aspartic acid which are capable of producing furan but not alone . Since they can produce only acetaldehyde, they need cooperation of reducing sugars, serine or cysteine to produce glycolaldehyde (Perez Locas & Yaylayan, 2004).

1.8.1 Furan Formation Pathways

1.8.1.1 Furan formation through carbohydrate degradation

The resulting products of sugar degradation by roasting are formic acid and acetic acid. They are the signs of the split of C1 and/or C2 units from hexoses (Vranova & Ciesarova, 2009).

Furan formation occurs generally from rearrangement of C2 units such as acetaldehyde and glycol aldehyde. These products can be formed from not only amino acids, but also sugars (Limacher, J., Davidek, Schmalzried, & Blank, 2008).

Possible four pathways of carbohydrate metabolism are given in Figure 1.7. (Vranova & Ciesarova, 2009).

Intermediate reactive like 1-deoxy- and 3-deoxyosones are formed from the Maillard reactions of reducing hexoses with amino acids (Figure 1.8). The 1-deoxyosone needs alpha-dicarbonyl cleavage in order to form aldotetrose (Weenen, 1998). Alpha- dicarbonyl cleavage is not the unique pathway to produce aldotetrose. According to Perez and Yaylayan have ideas like there is also another way called retro-aldol cleavage that does not need amino acids for the reaction. (pathway B) Alpha- dicarbonyl cleavage produces aldotetrose to a greater extent when it is compared to retro- aldol cleavage. It is shown in the pathway C that 2-deoxy-3-keto-aldotetrose is formed after a dehydration reaction. Eventually, alpha-dicarbonyl cleavage occurs with 3-deoxyosone. The following reactions oxidation and decarboxylation gives 2-deoxyaldotetrose like shown in pathway D. The aldotetrose derivatives mentioned above have the ability to produce furan (Perez Locas & Yaylayan, 2004).

Pentoses can also give 3-deoxyosone derivatives by two pathways. One of them is a reaction which involves amino acids, the other one is dehydration process at the C-3 hydroxyl group (Weenen, 1998). The produced intermediate is capable of generating 2-deoxyaldotetrose which is one of the direct precursors of furan (Perez Locas & Yaylayan, 2004).

1.8.1.2 Ascorbic acid pathway

Ascorbic acid degradation products such as 2-deoxyaldoteroses, 2-furoic acid and 2-furaldehyde constitute the intermediates of furan. Furan formation from ascorbic acid degradation is easily understood from the identification of $13CO_2$ and H13COOH (Limacher, Kerler, Conde-Petit, & Blank, 2007).

Oxidation of ascorbic acid occurs very rapidly and dehydroascorbic acid is produced as an oxidation product. This oxidized from of ascorbic acid hydrolyzes in food into

2,3-diketogulonic acid (DKG). DKG transforms into aldotetrose which is known as precursor of furan (Vranova & Ciesarova, 2009).

In the presence of nonoxidative conditions, since it is impossible for ascorbic acid to produce DKG, ascorbic acid hydrolyzes and beta-elimination occurs (Niemela, 1987). Decarboxylation process follows beta-elimination to generate 3-deoxypentosulose. This intermediate (3-deoxypentosulose) produces furan by pursuing ribose pathway (Perez Locas & Yaylayan, 2004).

Dry heating makes dehydroascorbic acid cyclize, this hemiketal form prevents the formation of furan (Perez Locas & Yaylayan, 2004).

1.8.1.3 Formation of furan from polyunsaturated fatty acids (PUFA)

Becalski and Seaman pointed out that during thermal treatment at 118 ^{o} C for 30 min. polyunsaturated fatty acids produces furan (Becalski & Seaman, 2005). Also, throughout that study, it was concluded that linolenic acid and linoleic acid are both capable of producing furan but linolenic acid generates furan approximately 4 fold more than linoleic acid does. Besides ferric chloride stimulates furan formation. Triglycerides generated from linolenic or linoleic acids are also capable of producing furan, but this amount is less than fatty acids. 5 – pentylfuran has already been used as an indicator for rancidity (Yaylayan V. , 2006). Xu and Sayre (Xu & Sayre, 1998) reported that oxidation of PUFAs and occurrence of lipid peroxides are leading in harmful diseases in human body, off-flavors and rancidity in foods. PUFA can generate lipid hydro peroxides non-enzymatically by help of reactive oxygen species and also enzymatically by using lipoxygenases. Disruption of these peroxides under the catalization of metal ions results in production of alkenals such as 2-alkenals, 4-oxo-2-alkenals and 4-hydroxy-2-alkenals (Yaylayan V. , 2006). Cytotoxic form of 4-hydroxy-2-alkenals which is also known as 4-hydroxy-2-nonel (4-HNE) can play a role in modification of proteins, DNA and LDL. 4-hydroxy-2-alkenals can also form 5-pentlyfuran by following cyclization and dehydration steps this compound can turn in less toxic and more volatile formation (Perez Locas & Yaylayan, 2004).

1.8.1.4 Formation of furan from amino acids

Precursors like acetaldehyde and glycolaldehyde producing amino acids such as serine and cystein can form furan if they undergone aldol condensation of these two precursors and subsequent cyclization (Yaylayan V. , 2006). Aspartic acid, alanine and threonine are also able to produce only acetaldehyde which means that they need other compounds like reducing sugar, serine and cysteine to form glycolaldehyde. Serine decarboxylation and

ethanolamine formation finally result in production of acetaldhyde, whereas glycolaldehyde is produced through Strecker type reaction if there is reducing sugar or not (Yaylayan V. , 2003).

Aspartic acid and α-alanine can only generate acetaldehyde and therefore they need glycolaldehyde for the production of furan. α-alanine, generated from decarboxylation of ascorbic acid, can produce acetaldehyde by Strecker degradation. Threonine as a acetaldehyde producer needs sugar for generation of furan (Perez Locas & Yaylayan, 2004).

1.8.1.5 Formation of furan following exposure to ionizing radiation

Based upon the recent studies, ionizing radiation has also triggering effect on furan formation. Ionizing radiation found responsible of furan in apple and orange juices (Fan, 2005).There is a linear correlation between furan amount and radiation dose from 0 to 5 kGy. Moreover, studies indicated that in 3 days of storage of orange and apple juices following the irradiation treatment, furan levels carried on increasing. Irradiation has been known for its residual effect which can explain this increase in furan during early periods of storage (Fan, 2005).

Fan (2005) claimed in his studies that irradiation produces primary radicals such as hydrated electrons, hydrogen atoms, and hydroxyl radicals during radiolysis of water. Secondary radicals are generated from these primary radicals as a result of their reactions with food materials. Although radicals are not generally long-lived, it is known that some of the radical have longer lives. Stabilization of these compounds can cause the persistence of furan formation (Fan, 2005).

Irradiation also triggers the furan formation originating from ascorbic acid, fructose, sucrose and glucose. Comparison of thermal treatment to a 5 kGy irradiation process showed that the amount of furan is the same in both applications (Fan, 2005).

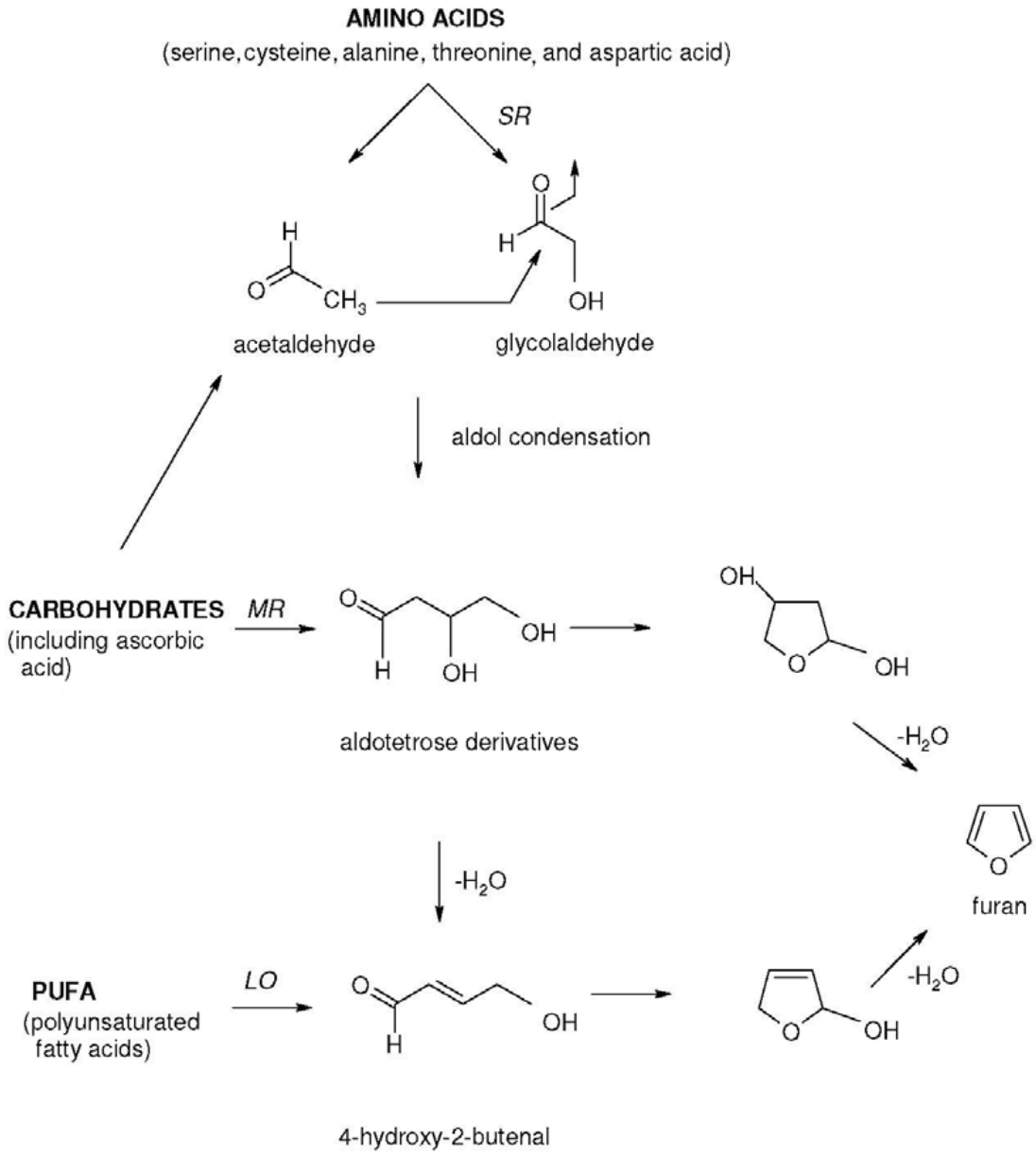

Figure 1.7 Furan pathways (Vranova & Ciesarova, 2009). SR: Strecker Reaction, MR: Maillard Reaction, LO: Lipoxgenase enzyme .

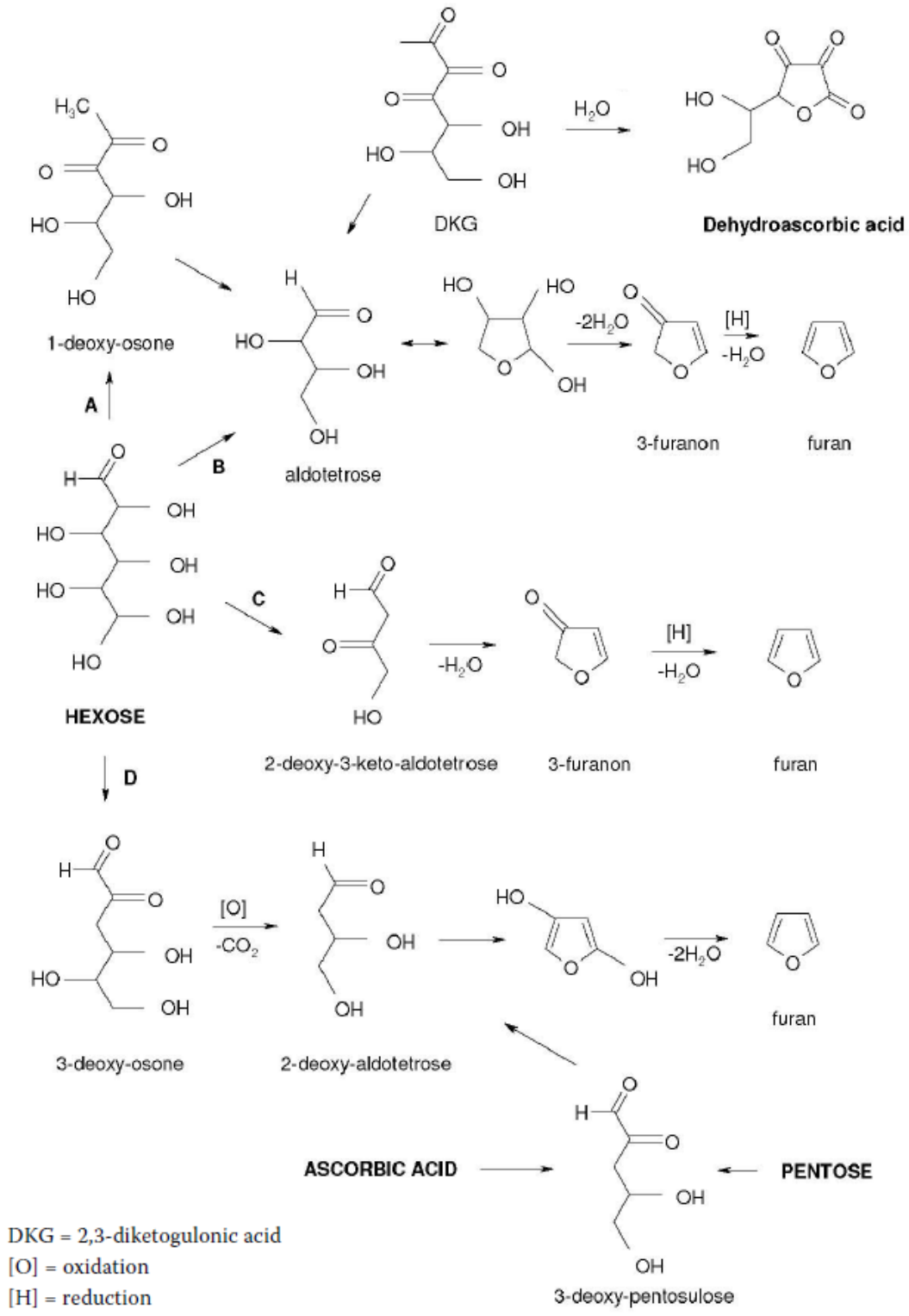

Figure 1.8 Furan formation originating from ascorbic acids and carbohydrates (Vranova & Ciesarova, 2009).

International Agency for Research on Cancer explained furan as a possible human carcinogen (Group IIB) (IARC, 1997). US Food and Drug Administration (US FDA) published a report in 2004 which includes the fact that furan occurred in canned and jarred foods whish are exposed to heat treatment. Baby foods, infant formulas, coffees, beers, soups, sauces, meats, fish, canned vegetables and fruits are the food products in which furan occurs as a result of thermal treatment (FDA, 2004). At the end of the research it is established that nearly all of the canned and jarred baby foods contained furan. Following FDA the European Food Safety Authority (EFSA, 2005) started a research on furan concentration levels for European baby food samples in addition to review on the analysis, occurrence, formation, exposure and toxicity of furan in foods (Vranova & Ciesarova, 2009).

There are three ideas which represent the carcinogenesis caused by furan. One of them is genotoxic mode of action which can be explained by the compound irreversibly bound to proteins and nucleosids, cis-2-butene-1,4-dial, which is generated by the metabolic activation with cytochrome P450 enzyme (CYP2E1) (Burka, Washburn, & Irwin, 1991) (Byrns, Predecki, & Peterson, 2002). This mechanism is not proved with in vitro and in vivo micronucleus experiments. The other ideas on carcinogenesis caused by furan metabolite induced cell proliferation and separation of mitochondrial oxidative phosphorylation (Vranova & Ciesarova, 2009).

In framework of studies on animals, it is well known that furan absorbance from the intestine and lung is very fast (Egle & Gochberg, 1979); (Burka, Washburn, & Irwin, 1991). Since furan has low polarity, it easily passes from biological membranes and goes into numerous organs by using this feature. After series of experiments on rats with repeated dosing, it is established that furan is accumulated in livers and kidney (Vranova & Ciesarova, 2009).

The substance which gives the toxic property to furan is cis-2-butene-1,4-dial attaches to proteins (Burka, Washburn, & Irwin, 1991) and nucleosides (Byrns, Predecki, & Peterson, 2002). One of the double bonds of furan is oxidized and forms an epoxide as an intermediate product. This process is continued by series of spontaneous reconversions and opening of ring (Vranova & Ciesarova, 2009). An enzyme called cytochrome P450 (CYP) has one of the main roles in toxicity originating from furan since it is functionary in furan biotransformation (Vranova & Ciesarova, 2009).

U.S. National Institutes of Health studied on furan in the perspective of its toxicological and therefore carcinogenic properties revealed that furan is uncomplicatedly carcinogenic to rats and mice. International Agency for Research on Cancer which is a foundation which works under World Health Organization classified furan as “possibly carcinogenic to humans” (Group 2B) in 1995 (IARC, 1997).

1.8.2 Determination of furan

The analysis of furan is very difficult since its boiling point is very low therefore it has a highly volatile nature. The technique which is generally used in furan determination is automated head-space extraction combined with gas chromatography- mass spectrometric determination (Becalski & Seaman, 2005); (Crews & Castle, 2007); (FDA, 2004). In recent years, a new method composed of sample extraction based on solid phase micro-extraction and a subsequent GC-MS assay has been come up (Bianchi, Careri, Mangia, & Musci, 2006); (Goldmann, Perisset, Scanlan, & Stadler, 2005).

Both techniques are very easy and they do not need expensive equipment for sample extraction/ concentration. These features make them convenient. Both techniques give substantial results on the condition that they are implemented correctly (Vranova & Ciesarova, 2009). Sample storage and preparation, attentive standard preparation and calibration using internal standard are very important steps in quality control.

Exposure temperature and time which are based on the food matrix, are very important in optimization of analysis of furan by SPME-GC/MS. Senyuva and Gökmen stated in their studies that food composition, analyte concentration and equilibration conditions must be taken into account when it comes to SPME analysis. In the same study, it was pointed out that furan response increased seriously with a change of 40 ^{o}C (40 to 80 ^{o}C) in equilibration temperature. This was explained by unsung furan response in headspace sampling (Şenyuva & Gökmen, 2005).

1.8.3 Furan in food

In the aim of monitoring furan in food, the European Commission Recommendation 2007/196/EC (2007/196/EC, 2007) proposed the Member States to collect data on food products which were exposed to thermal treatment. By this way it was planned to have a better prediction of dietary exposure.

20 countries of Europe have sent analysis results to European Food Safety Authority (EFSA) for furan amount in food so far. There are 5.050 results from the samples which were analyzed between 2004 and 2010. These 5.050 results were classified into 21 food categories. 5 of them are coffee and the remaining are non-coffee categories. In addition to this, 2 main categories (jarred baby food and other) were distinguished and sub-classified in subgroups for acquiring detailed information. With respect to the different types of raw coffee and coffee brews, the coffee category is also sub-classified (EFSA, 2005); (EFSA, 2010); (EFSA, 2011).

The highest furan content was observed in the five coffee categories when they were compared to the rest of the food groups with approximate values of 45 μg/kg for brewed coffee, 394 μg/kg for instant coffee powder, 1,936 μg/kg for roasted ground coffee, 2,016 μg/kg for non-specified coffee and 3,660 for roasted coffee beans. Mean values in the non-coffee categories were ranged between 3.2 μg/kg for infant formulae and 49 μg/kg for jarred baby food 'vegetables only' (EFSA, 2011).

Furan exposure for different populations was estimated by the combination of data obtained from the monitoring program which consisted of 28 surveys derived from the EFSA Comprehensive European Food Consumption Database about individual dietary information (EFSA, 2011).

The average furan intake among adults was estimated in the range of 0.03 and 0.59 μg/kg b.w. per day. Types of coffee brewing must be taken account as the main contributors to total furan exposure in adults with an average contribution of 88% across the surveys. In addition to coffee the major contributor, there are other contributors to furan exposure in adults such as ready-to-eat soups, sauces and fruit juices (EFSA, 2011).

Average furan intake among young people was estimated in the range of 0.02 and 0.13 μg/kg b.w. per day. Coffee is again the major contributor but it is not at the same level for the adult population. Cereal-based products, ready-to-eat soups, sauces, and fruit juice are the other contributors to furan exposure in young population (EFSA, 2011).

The average furan intake of children was in the range of 0.04 and 0.22 μg/kg b.w. per day and the 95th percentile between 0.09 and 0.46 μg/kg b.w. per day. Fruit juices, milk based products and cereal-based products are the major contributors to furan exposure in children population (EFSA, 2011).

There are only 2 surveys on food consumption information of infants (each of them in different countries). With respect to this limited data average exposure to furan for infants was estimated in the range of 0.09 and 0.22 μg/kg b.w. per day. When it comes to high exposure estimate, it was reported that 95th percentile of 0.97 μg/kg b.w. per day. In one country jarred baby foods was the main cause of furan exposure in infants, in the other country ready to eat soups became the major contributor. In both countries fruit juices and infant formula contributed the furan exposure value with a percentage of 8-10% (EFSA, 2011).

All of these predictions show that furan exposure are high among adults and infant as a result of increased consumption of jarred baby foods and coffee as major contributors. Children and teenagers stayed at the lower ranks in the list since their daily diet is deprived of those two major contributors (EFSA, 2011).

Sample description	Furan value (PPB)		Median (PPB)	Number of samples
	minimum	maximum		
Baby food in small glass jars	1	153	12	102
Fruit and vegetable juices for babies and young children	1	40	3	4
Coffee (drink)	13	146	74	9
Hot chocolate and malt beverage	< 2	< 2		2
Canned or jarred vegetables	< 2	12	3	15
Canned soups	19	43		2
Canned fruits	< 1	6		2
Tin containing meat	4	4		1
Tin containing meat and pasta	14	14		1
Sugo, tomato and Chilli sauces	< 4	39	6	13
Soy sauce, hydrolysed vegetable protein	18	91	50	7
Vegetables, fresh	< 1	< 2	< 1	7
Bread and toast	< 2	30	< 2	7
Whole milk UHT	< 0.5	< 0.5		1
Plum beverage	6	6		1
Beetroot juice with fruit juices (organic)	1	1		1
Potato flakes for mashed potatoes (flakes, not prepared)	< 5	< 5		1

Figure 1.9 Furan amounts in selected food samples (Vranova & Ciesarova, 2009).

1.9 Baby Food

Baby food industry is on a serious progress since baby food products' need is raising day by day. Parental concerns about nutrition are very high when it is compared with the past. Moreover working woman number is increased. Hence infant formulae and baby foods gained importance globally. Consciousness about infant or toddler health made baby food companies expand all over the world. These companies are competitors now in producing good quality baby food products and introducing consumers with innovations in baby food. Baby food market offers good and innovative products (BCC, 2012).

The market share percentage of baby food in 2010 was $27.1 billion globally. An increase to $28.2 billion is expected in 2011. It easily seen from the compound annual growth rate calculation resulting in 4.5 % that market share will reach $35.2 billion by the end of 2016. North America has the largest share in the global market and it seems that the situation will not change by the end of 2016 with a market share of $10.9 billion. The European market for baby foods in 2011 was $8.8 billion and it is expected to reach to $9.6 billion in 2016 (BCC, 2012).

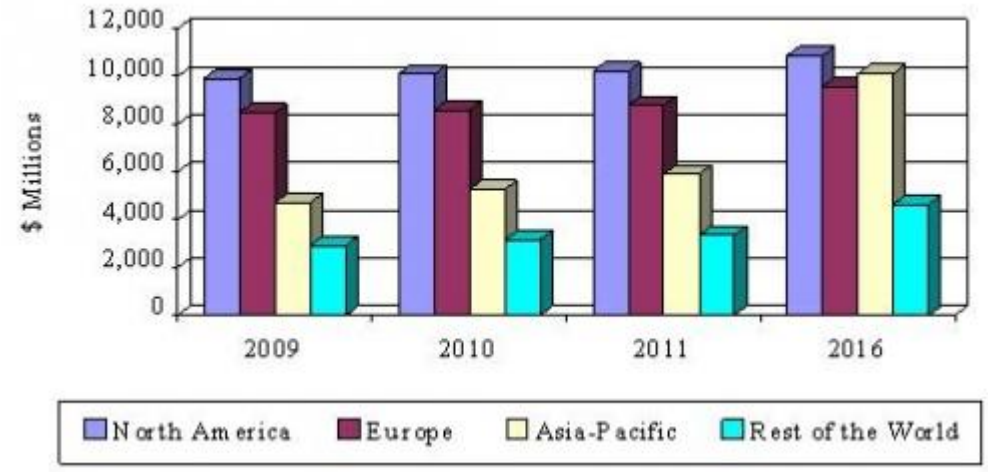

Figure 1.10 Global Values of the Baby Food Market, 2009-2016 ($ Millions) (BCC, 2012).

1.9.1 Baby Food Market in Turkey

Baby food market in Turkey reached 450 million TL in 2011. When this value is compared to the one in 2010, it is obvious that Turkey is over the world average with a 17 % growth rate (Erol, 2012).

The companies that has the main roles of baby feeding industry in Turkey are Danone (Bebelac, Milupa, Aptamil), Hero Gıda (Ülker Hero Baby), Eti (Cicibebe), Hipp, Sütaş and Pınar. Some of these firms serve only biscuits or follow-up milk, some of them offers a wide product portfolio (Erol, 2012).

Latest estimates show that, the number of baby and young children between the age of 0 and 3 is approximately 4, 7 million. Since the number of the children is very high compared with European countries, Turkey becomes a very attractive country in baby food market. On the other hand, the amounts of per capita consumption of a baby show that baby feeding industry in Turkey has just begun to grow. Growing expectations have two stands such as rareness of families who consumes industrial baby food and constraint of the amounts of per capita consumption of a baby. The nutrients for baby have to be different from the nutrients of adults in the manner of ingredients and quality. This fact should be emphasized in different channels in order to sustain the growth of baby food industry (Erol, 2012).

Bottled baby food has the highest growth rate with a percentage of 28 % among all baby food categories. This growth mainly arises from the studies on consciousness-raising about the harmful effects of cow milk in baby feeding and therefore stimulation of the mother to use follow-up milk for longer time periods (Erol, 2012).

The second highest growth is in jarred baby food with a rate of 18 %. A significant rise in this category is expected in the future. Fruit puree, vegetable puree, soups, meals with chicken, milky desert, desert with yoghurt is in this category. Jarred baby food category becomes prominent with its' ease of use. Vegetables and fruits which are used in this type of baby food are in baby feeding standards which are also other outstanding features for this category (Erol, 2012).

Recent studies represent that although mothers know about the risk of pesticide in fresh fruits and vegetable, they are still against the packaged food and they are not sure about the right product. Consciousness- raising studies intended for parents is expected to increase the demand on baby food (Erol, 2012).

There are 3 main trends in baby feeding market such as:

a) Special products intended for the children of age 1 and 3

Dietary of young children between ages of 1 and 3 is important as much as infant feeding. They are still in growing period and therefore their vitamin, mineral, energy etc. demand is different from adults or elder children. As the entire world, in our country, Food Codex determines individual communiques for nutritional products of baby and young children between the ages of 0-3 (Erol, 2012).

b) Functional benefits

Probiotics and prebiotics have a positive impact on immune system of babies. Hence baby food which has these functional benefits shows a rising trend in baby food market (Erol, 2012).

c) Practical packages and out-of-home consumption

This category is very useful for the mothers who are social in their since these products offer ease of use wherever you are (Erol, 2012).

1.9.2 Regulation about baby food

1.9.2.1 EU Regulations

- *Commission Directive 2006/125/EC* is on processed cereal-based foods and baby foods for infants and young children (2006/125/EC, 2006).
- *Commission Directive 2006/141/EC* is on infant formulae and follow-on formulae (2006/141/EC, 2012).

1.9.2.2 Turkish Food Codex

In Turkish Food Codex there are 3 communiqués on babies and young children such as "Infant Formulae", "Follow-on Formulae", "Additional Foods for Infants and Young Children". All of them are prepared in the framework of EU adaptation. All the restriction is same as EU regulations numbered 2006/125/EC and 2006/141/EC (TGK, 2008); (TGK, 2008); (TGK, 2007).

1.10 Aim of the Study

Furan, which is a lipophilic contaminant and formed during heating process in foods, has been pointed out as a risk in baby foods since it has been classified as "possibly carcinogenic to human" by IARC (International Agency for Research on Cancer). Hence, a great concern has been addressed to the analysis of this substance in baby foods. EFSA and FDA is on alert about this subject, since there is a possibility for furan to accumulate in body and to reach to dangerous levels when one is exposed to furan from infancy.

Baby food market is growing day by day. HHP treatment which is a well-known non-thermal technique is a good advantage in sterilization or pasteurization of baby food since it does not result in undesired outcomes of thermal treatment. Moreover, it is known that HHP's detrimental effects on nutritional and sensorial quality of foods are dramatically less than those of other techniques (Dede, Alpas, & Bayındırlı, 2007).

This study aims to sterilize two types of baby food such as mixed vegetables and mixed fruits with vegetables with no allowance to the formation of furan. In addition, throughout this study, best conditions (P-T-t) were specified and shelf life of the samples which had been treated at these best conditions was also investigated.

CHAPTER 2

MATERIALS AND METHOD

2.1 Preparation of the samples

Baby food samples were obtained from a leading baby food producer in Turkey (Ankara, Turkey). There were two types of baby food such as mixed vegetables and mixed fruits with cereals.

The ingredients of vegetable based baby food are water, carrot, white cabbage, potato, marrow, rice flour, celery root, sugar, tomato juice concentrate, salt and sunflower oil.

The ingredients of fruit based baby food are fruit purees (apple puree, banana puree, carrot puree, mango puree) water, grape juice concentrate, orange juice concentrate, oat flakes, rice starch, corn starch, vitamin C (25 mg/100g).

Baby food samples were deaerated by advanced vacuuming technology. Therefore there is no need to food additives for preservation (Herobaby, 2013).

Half of the baby food samples were sterilized by thermal treatment, half of them were untreated so that we could implement high hydrostatic pressure to the same base used in pasteurization.

Unsterilized samples were filled to the plastic 20 ml plastic bottles (LP Italiana SPA, Italy). Since air bubbles in the bottles are undesirable in HHP process, filling process was carried out attentively.

Samples about to be treated thermally were filled to the jars at 80°C at the company. They were sterilized at 105°C for 10 minutes.

2.2 Pressurization of the samples

Pressurization of the samples was achieved by a 760.0118 type industrial high pressure system (SITEC CH-8124, Zürich, Switzerland) which is shown in Figure 2.1. The vessel where the pressurization occurs has a volume of 100 ml. Its inner diameter is 24 mm and length is 153 mm. Ethylene glycol was used as pressurization fluid since its viscosity is higher than water. This feature brings the possibility to avoid leakage of the liquid. In addition to this, ethylene glycol does not lose its liquidity at sub-zero temperatures; therefore performing experiments at sub-zero temperatures becomes possible (Buzrul, Alpas,

Largeteau, & Demazeau, 2008). This HHP system operates maximum 700 MPa and its operating temperatures are between -10 ^{o}C and 80 ^{o}C. This HHP equipment also has a heating- cooling system (Huber Circulation Thermostat, Offenburg, Germany) to control the required temperature. The temperature was measured by a thermocouple type K. Increase in temperature originating from adiabatic heating was calculated as 4-5 Pressurization was implemented to the samples at 200, 300, 400 MPa at 25, 35, 45 ^{o}C for 5, 10, 15 min. Come up and pressure release times should not be considered in HHP application time. HHP conditions were decided with respect to the literature research. Pressurization rates were 400 MPa/min for 200 MPa, 360 MPa/min for 300 MPa and 340 MPa/min for 400 MPa. Any vacuuming procedure was not implemented to the samples.

Pressure-treated samples had been stored at -18 ^{o} C till the chemical and microbiological analysis.

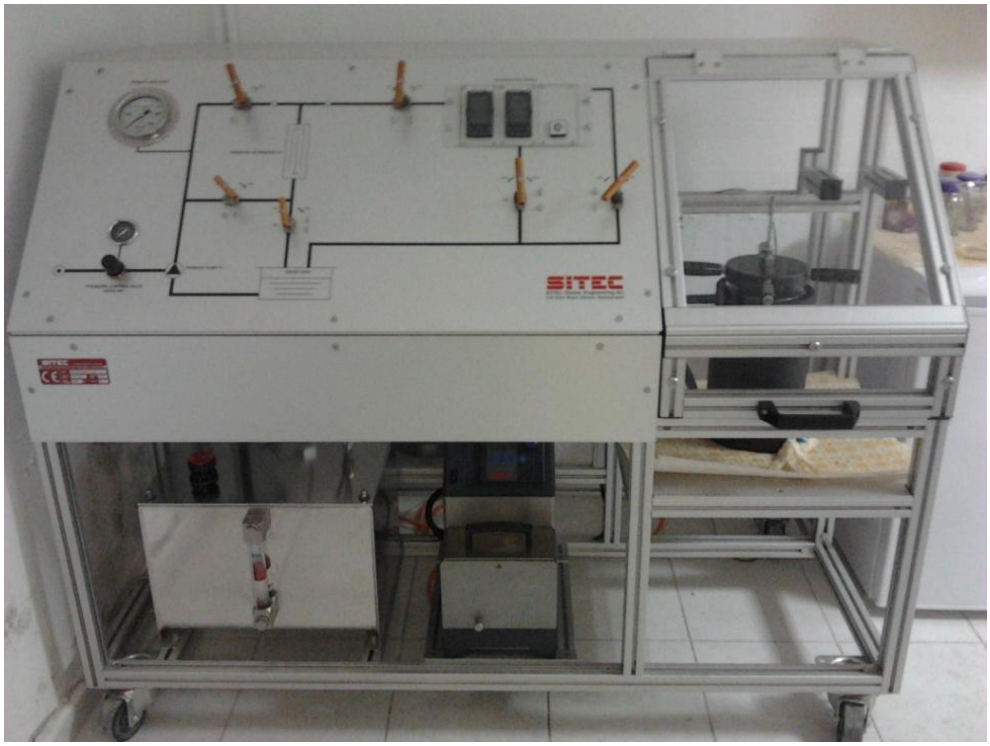

Figure 2.1 HHP equipment in laboratory scale.

2.3 Determination of Furan

All of the experiments were held in laboratory supervised by Prof.Dr. Vural Gökmen at Food Engineering Department of Hacettepe University.

The samples for the determination of furan were transferred to the vials (Supelco, Bellefonte, PA, USA) at 4 ^{o}C not to lose furan because of its high volatility. Aluminum crimp seals with teflon-faced silicon septa were used in analyzes (Agilent, Waldbronn, Germany).

5 g of sample was put in 20 ml headspace vial and a Carboxen-PDMS SPME fiber (Supelco, Bellefonte, PA, USA) was placed. The sample with Carboxen-PDMS SPME fiber is equilibrated in a 40 ^{o} C incubator for half an hour. After equilibration, SPME fiber was put into the injection port and was desorbed for 5 minutes. Chromatographic separation was achieved in capillary column (24 m x 320 um, 20 um) of HP-PLOT-Q connected to Agilent 5973N GC/MS (Agilent, USA). . Helium gas at a rate of 2 ml/min ((60cm/s) was used as a carrier gas. Column temperature program was set as follows; 100^{o}C'de 5 min; Temperature was quickly removed to 200 ^{o}C at a rate of 10^{o}C/min and became constant at 200^{o}C for 15 min (Total analysis time: 30 min.). Mass spectrometry was executed in the range of m/z 20-200 AMU scan. Quantifier/qualifier ions for furan and d4-furan are set as 68/39 and 72/43m/z.

The peak which proves the presence of furan in the samples was seemed after 11 minutes. Calibration curve was constituted after the injection of 100, 250,500, 1000 ng/g furan as external standard (minimum purity 99%, Fluka Chemie GmbH, Switzerland),and 50 ng/g d4-furan (minimum purity 99%, Aldrich, USA) as internal standard. The areas of the peaks were calculated and an equation was procured. Furan amount in the samples was calculated by using this calibration curve.

Figure 2.2 GC-MS Equipment

Figure 2.3 SPME device with microfiber used for GC injection.

2.4 Determination of Total Mesophilic Aerobic Bacteria

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 both types of baby food were surface plated on prepoured Plate Count Agar (Merck, Darmstadt, Germany) in three plates (0.3, 0.3 and 0.4 mL) . After the incubation at 37 ^{o}C for 48 h, colony enumeration was achieved. The experiment was repeated once more to determine the average.

2.5 Determination of Total Yeasts and Molds

The procedure is same as 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 both types of baby food were surface plated on prepoured 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 in order to avoid the bacterial growth (pH 3.5). After the incubation at 25 ^{o}C for 120 h, colony enumeration was achieved. The experiment was repeated once more to determine the average.

2.6 Shelf life analysis

Optimum pressure, temperature and time (P-T-t) for two types of baby food were determined with respect to the results where no growth on respective media was observed.

Both types of baby food were treated at this optimum P-T-t (400 MPa-45^{o}C-15 min), and stored at the conditions (25 ^{o}C) where industrial baby foods are also exposed to. It was planned to last for 60 days. Microbiological analysis was performed on 0th, 1st, 3rd, 5th, 7th, 14th, 21st, 28th, 35th, 42nd, 49th and 56th days of storage At each day of the analysis, 1 g of both baby food was suspended in peptone water and 1 mL of that suspension was surface plated on prepoured PCA in three plates (0.3, 0.3 and 0.4 mL). The plates were incubated at 37 ^{o}C for 48 h. It was planned for shelf life to last for 60 days. Shelf life analysis was ended where microbial growth was seen in two successive experiments.

2.7 Statistical analysis

MINITAB 16.0 implemented analysis of variance. The objective of this analysis was to test the effects of temperature, pressure and time on log reduction. Aiming to make paired comparisons between sample means, Tukey Test was used. Significance level was adjusted to 0.05.

CHAPTER 3

RESULTS AND DISCUSSION

This study is performed in order to represent that it is possible to sterilize baby foods with no allowance to furan formation.

In the first part of this study, two types of baby purees such as mixed vegetables and mixed vegetables with cereals were subjected to high hydrostatic pressure with pressure values between 200 – 400 MPa, temperature values between 25-45 ^{o} C and time values between 5-15 minutes.

In the second part of the study, experiments on furan determination were conducted in Food Engineering Department of Hacettepe University. Furan amount was determined through the instrument of GC-MS. Calibration curve was formed with respect to the technique namely "a fit for purpose". All of the experiments were done twice.

In the third part of the study, microbiological analysis was executed. Essential requirements on microbiological issues of baby food for infants and young children were specified in the communique on "Additional Foods for Infants and Young Children" which was partaking in Official Gazette of Republic of Turkey numbered 26,687 and dated November 1st, 2007 (KKGM, 2007). It is stated below:

Table 3.1 Essential requirements on microbiological issues of baby food for infants and young children (KKGM, 2007).

Microorganism	**Limit Value**
Total mesophilic aerobic bacteria (cfu*/g)	$1.0x10^4$
Coliform bacteria (cfu/g)	$2.0x10^1$
Escherichia coli (cfu/g)	None
Total yeast and mold (cfu/g)	$1.0x10^2$
Bacillus cereus (cfu/g)	$1.0x10^2$
Salmonella spp. (cfu/25g)	None
Listeria monocytogenes (cfu/25g)	None
Staphylococcus aureus (cfu/g)	None
Clostridium perfringens (cfu/g)	None

***cfu: colony forming unit**

With respect to this regulation, it was decided to determine total mesophilic aerobic bacteria count, coliform bacteria count and total yeast and mold count. During pre-studies of this research, even one coliform bacterium was not observed both in pressurized samples and unpressurized samples. Therefore coliform bacteria count was not executed in the manner of this finding. Total mesophilic aerobic bacteria count and total yeast and mold count were performed throughout this research.

Lastly, optimum pressure-temperature-time values were specified for the baby foods with respect to the results of furan and microbiological analysis. Since our aim was to prove that sterilization is possible with no allowance to furan formation, parameters which results in no microbial count were chosen for the shelf life analysis. Both types of baby foods were subjected to these optimum values and then shelf life analysis began. Pressurized samples were stored at ambient temperature. Storage conditions were same as the ones traditionally sterilized and sold in markets. Shelf life analysis was ended when microbial growth was confirmed in successive experiments.

3.1 Effect of Pressure, Temperature and Time on Inactivation of Mesophilic Bacteria

3.1.1 Mixed vegetables

HHP treatment applied at changing pressure, temperature and time on mixed vegetables type baby food is given in Figure 3.1.

Pegging the parameters respectively revealed the effect of pressure, temperature and time. It was observed that at a constant time and constant pressure, log reduction increases with increasing temperature significantly ($P<0.05$). As in the case of increasing temperature, increasing pressure increased log reduction significantly ($P<0.05$).

A log reduction of 6.28 was observed in fruit based baby foods at "4000 atm - 45 ^{o}C – 15 min".

Turkish Food Codex stated in the communique of "Additional Foods for Infants and Young Children" placed in Official Gazzette of Republic of Turkey numbered 26,687 and dated November 1st, 2007 that number of total mesophilic aerobic bacteria must be below 10^4 cfu/g (KKGM, 2007). Our data showed that even in minimum conditions that we applied (2000 atm - 25 ^{o}C – 5 min), number of total mesophilic aerobic bacteria was below the limit which means that HHP is very effective in inactivation of total mesophilic aerobic bacteria.

Longer treatment at constant temperature and constant pressure resulted in increased log reduction which also means that time has a significant effect on microbial inactivation

(P<0.05). A detailed analysis of the graph indicates that time effect increases at higher pressure and temperature. There is approximately a difference of 2.5 between "4000 atm - 45 ^{o}C – 10 min treated baby food" and "4000 atm - 45 ^{o}C – 15 min treated baby food". It can be explained with the synergistic effect of temperature and pressure. It is possible to say that pressure combined with mild heat treatment for 10 minutes makes the microorganisms much more sensitive and 5 minutes more treatment resulted in an increased effect of time. Pressure was found also effective since experiment conducted without pressurization at the same temperature and time (45 ^{o}C and 15 min.) revealed no decrease in microbial load.

Pilavtepe-Çelik et al. (2009) reported in their study which was about the investigation of media effect in inactivation of microbial load that, food systems can either protect or sensitize the microorganism. Throughout that study, two microorganisms such as *Escherichia coli* O157:H7 933 and *Staphylococcus aureus* 485 showed different resistances to pressure in carrot juices. It was also highlighted in that study that carrots have a protective effect on Gram negative bacteria. Since 1000 atm more was needed for eliminating all total mesophilic aerobic bacteria in vegetable based baby food when it is compared to fruit based ones, it is possible to say that percentage of Gram negative ones in total mesophilic aerobic bacteria were high.

Our sample also consists of white cabbage. There is also a study about HHP treatment on sauerkraut that established HHP reduced aerobic bacteria about 4-5 log cfu/g (Peñas, Frias , Gomez, & Vidal-Valverde, 2010).Throughout our study we also achieved to reduce total mesophilic aerobic count for about 6 log cfu/g.

Besides, statistical analysis done in MINITAB 16.0 shows that pressure, temperature and time and their binary combinations are efficient significantly *(P<0.05)* on inactivation of total mesophilic aerobic bacteria in vegetable based baby foods. General linear model implemented to the data is given in Appendix A.1.

No growth was observed at respective media where samples which were pressurized at "4000 atm- 45 ^{o}C-15 min" placed.

Error bars that are marked on the columns show standard deviations between two repetitions.

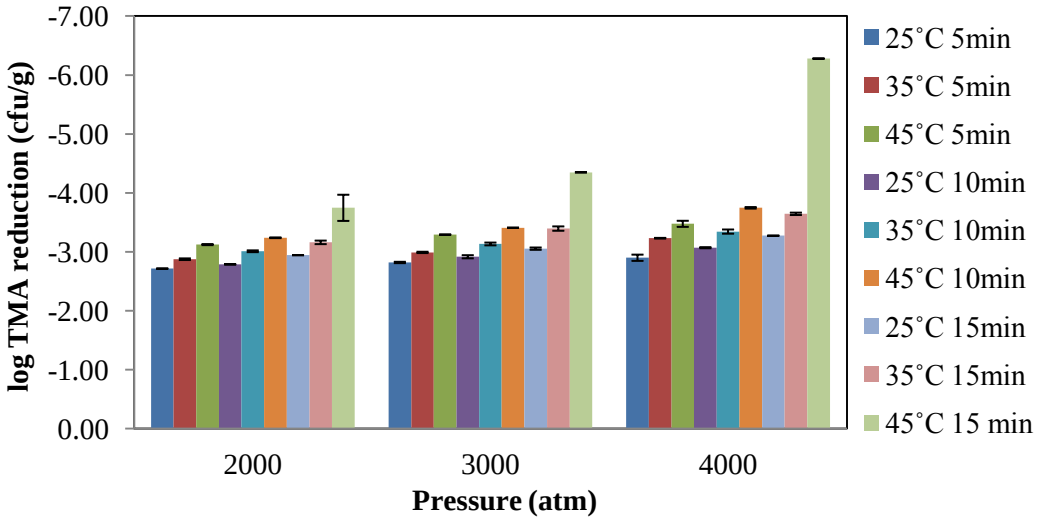

Figure 3.1 Effect of HHP on log reduction of TMA in vegetable based baby food.

3.1.2 Mixed fruits with cereals

Effects of pressure, temperature and time on total mesophilic aerobic bacteria count are same as in mixed vegetables type baby food. Log reduction increases with increasing temperature and pressure significantly (P<0.05). They are stated in Figure 3.2.

A log reduction of 6.18 was observed in fruit based baby foods at "3000 atm - 45 ^{o}C – 15 min".

Our data, again showed that even in minimum conditions that we applied (2000 atm - 25 ^{o}C – 5 min), number of total mesophilic aerobic bacteria was below the limit which means that HHP is very effective in inactivation of total mesophilic aerobic bacteria similar to vegetable based baby food.

Needed pressure for total sterilization was 3000 atm in fruit based baby foods which is lower than vegetable based ones. This can be explained by low pH of fruit based baby foods (pH 3,75). Bayındırlı et al. (2006) established in their studies that HHP treatment combined with heat treatment to fruit juices with a pH value below 4.5 has a significant effect for inactivation of microorganisms and enzymes. The microorganisms used in that study such as *Staphylococcus aureus* 485, *Escherichia coli* O157:H7 93 and *Salmonella enteritidis* FDA were classified as resistant to pressure relatively by Alpas et al. (1999). It was also preseen throughout in the study conducted by Bayındırlı et al. (2006) that microorganisms which are less resistant could be inactivated by combination of low pH, heat and pressure treatment. In our study, total mesophilic aerobic bacteria are easily eliminated. Fruit based baby food needed 1000 atm pressure less to be totally sterilized. Besides Hayashi (1992) and Mermelstein (1998) reported that HHP treatment was used in preservation of low pH low protein food products (Alpas, Kalchayanand, Bozoğlu, Sikes, Dunne, & Ray, 1999). Alpas et al. (1999) indicated in their study that a combination of heat, pressure and low pH could

be considered as an effective elimination method for foodborne pathogens such as *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* O157 :H7 *and Salmonella*. Our study also established the differences in log reduction of total mesophilic aerobic bacteria between fruit based baby food and vegetable based baby food.

It is observable from the graph that at constant pressure and temperature, longer treatment also resulted in higher log reduction significantly ($P<0.05$).

A detailed analysis of the graph indicates that time effect increases at higher pressure and temperatures similar to vegetable based ones. There is approximately a difference of 2.63 between "4000 atm - 45 ^{o}C – 10 min treated baby food" and "4000 atm - 45 ^{o}C – 15 min treated baby food". It can be explained with the synergistic effect of temperature and pressure. It can be seen from the graph that longer time periods like 15 minutes caused much more log reduction differences in increasing time and temperature.

Also, statistical analysis done in MINITAB 16.0 establishes the fact that pressure, temperature and time and their binary combinations are efficient significantly *(P<0.05)* on inactivation of total mesophilic aerobic bacteria in fruit based baby foods. General linear model implemented to the data is given in Appendix A.2.

No growth was observed in respective media at 3000 atm - 45 ^{o} C -15 min".

Error bars that are marked on the columns show standard deviations between two parallels.

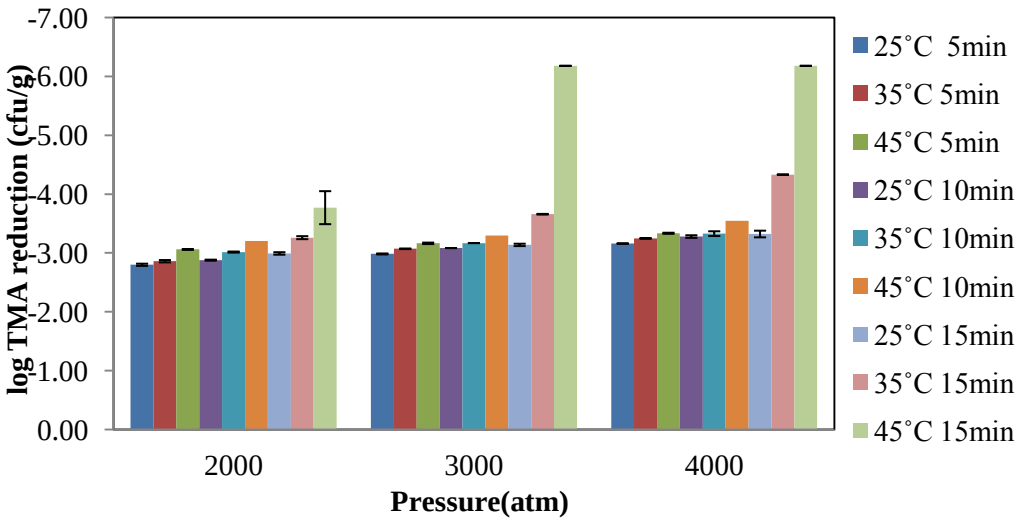

Figure 3.2 Effect of HHP on log reduction of TMA in fruits based baby food.

3.2 Effect of Temperature and Pressure on Inactivation of Yeasts and Molds

Yeasts and molds just like vegetative cells can be considered as sensitive to pressure which also means that it is possible to eliminate those type of microorganisms with pressure values between 300-600 MPa (Knorr, 1995).

Yeasts are one of the most important causes for spoilage of foods with low pH and high sugar and salt concentration ((Praphailong & Fleet, 1997); (Marx, Moody, & Bermudez-Aguirre, 2011)). There are studies in the literature conducted to establish the inactivation effect of HHP on yeasts (Basak, Ramaswamy, & Pierte, 2002); (Goh, Hocking , Stewart, Buckle, & Fleet, 2007). A study about orange juice conducted by Başak et al. (2002) reported that it is possible to get a 3 log reduction of yeasts at 250 MPa. pH, type of microorganism, composition of the medium and HHP parameters effect the inactivation of yeasts (Marx, Moody, & Bermudez-Aguirre, 2011). Another study executed by Goh et al. (2007) pointed out that *S. cerevisia* in sucrose solutions with different brixes resulted in different log reduction at the same HHP parameters applied.

Food matrix does also effect the pressure resistance on fungal cells. For example, denser sugar concentration means higher resistance of fungal cells (Goh, Hocking , Stewart, Buckle, & Fleet, 2007); (Marx, Moody, & Bermudez-Aguirre, 2011).

3.2.1 Mixed vegetables

In our study, it is obvious that a 6 log reduction was achieved in vegetable based baby foods. Effect of pressure, temperature and time can be seen on Figure 3.3. It seems that microbial load is decreased with increasing temperature and pressure ($P<0.05$).

As is seen on the graph, longer time periods so as 15 minutes brought increased effects of temperature and pressure on reduction of microbial load. For sure, it can be explained again the synergistic effect of temperature and pressure. It is possible to say that pressure combined with mild heat treatment for 10 minutes makes the microorganisms much more sensitive and 5 minutes more treatment resulted in an increased effect of time.

On the other hand, statistical analysis showed that pressure, temperature and time have significant effect on inactivation of total yeasts and molds individually ($P<0.05$). Considering their binary combinations, analysis indicated that combinations such as “pressure-temperature” and “temperature-time” were insignificant ($P>0.05$) on inactivation of yeasts and molds where “pressure-time” combination is significant ($P<0.05$). Implementation of general linear model is given in Appendix A.3 in detail. So it can be concluded from this situation that, independent from temperature, longer time periods showed increased effect of pressure which was resulted in higher log reduction.

No growth was observed on the respective media at “4000 atm - 35 ^{o} C -15 min”.

Error bars marked on the columns show standard deviation between two repetitions.

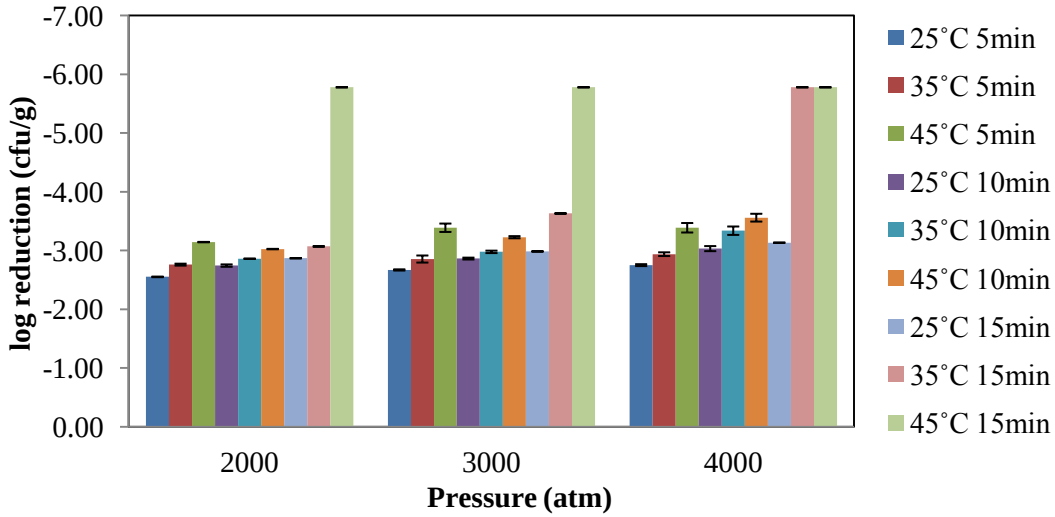

Figure 3.3 Effect of HHP on log reduction of total yeasts and molds in vegetable based baby food.

3.2.2 Mixed fruits with cereals

Approximately 6 log reduction was achieved in fruit based baby foods. Effect of pressure, temperature and time can be seen on Figure 3.4. The effects are similar in vegetable based baby foods. Similar to previous one, increasing time resulted in increased effects of temperature and pressure on reduction of microbial load.

In addition to this, statistical analysis done in MINITAB 16.0 represented that pressure, temperature and time and their binary combinations are efficient significantly on inactivation of total mesophilic aerobic bacteria in fruit based baby foods ($P<0.05$).General Linear Model is given in Appendix A.4.

Considering pH, it is stated in the literature that, it is possible for *S. cerevisia* to grow at pH 1.8 (Deak & Beuchat, 1996). There is also another study conducted by Praphailong et al. (1997) which is about pH effects on behaviors of yeasts in high sugar or salt concentrations.

It is possible to say for our data that low pH supported the inactivation of microorganisms which is obvious when two types of baby foods are compared to each other in the manner of log reduction of total yeasts and molds. On the other hand, according to the shelf life analysis, yeasts and molds appeared first, since they are capable of growing at low pH (FAO, 1998). It is observable from the results that shelf life of fruit based baby food lasts less than

vegetable based ones since sugar content of baby foods are higher than the others. Because of this, microorganisms find suitable medium to grow and multiply.

No growth was observed on respective media at "3000 atm-45^{o}C-10 min", also at 45 ^{o}C for 10 and 15 minutes at every pressure values.

Error bars marked on the columns show standard deviation between two repetitions.

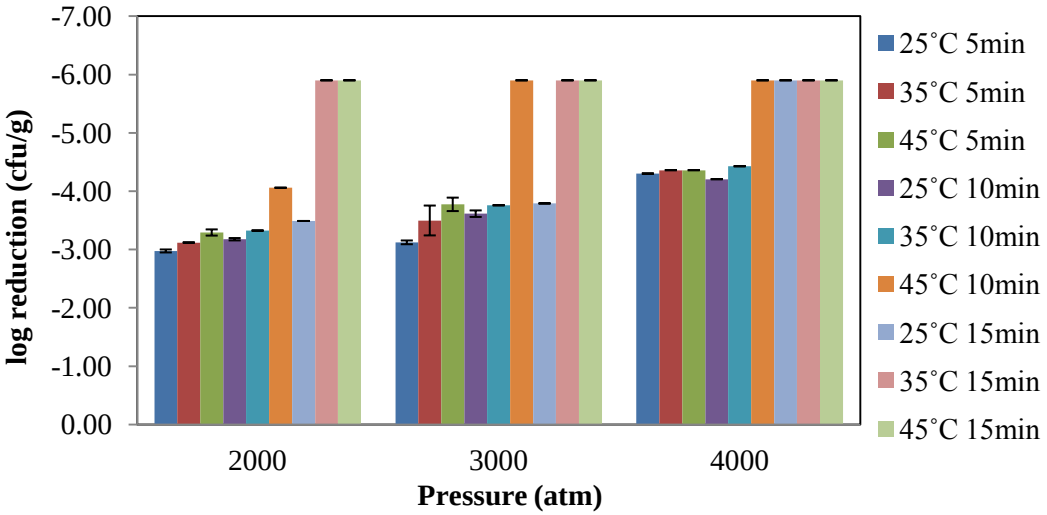

Figure 3.4 Effect of HHP on log reduction of TMA in fruit based baby food.

3.3 Effect of Type of Baby Foods on Logarithmic Reduction of Total Mesophilic Aerobic Bacteria

Log reduction of total mesophilic aerobic bacteria in different types of baby foods at constant time and temperature are stated below in Figures 3.5 through 3.13. Log reduction in fruit based baby foods are more than vegetable based ones in all temperature and time values with the exception of "45 ^{o} C-5 minutes" and "45 ^{o}C-10 minutes" treatment. Also, one way ANOVA was executed to data of total mesophilic aerobic bacteria count to establish the effect of type of baby food on microbial inactivation. It is given in Appendix A.5. Statistical analysis pointed out that type of baby food is significant in microbial reduction ($P<0.05$). After all, difference in log reduction of total mesophilic aerobic bacteria in different types of baby foods can be explained with the pH value of baby foods. Fruit based baby food has a pH of 3.75, and vegetable based baby food has a pH of 4.95. It is possible that low pH value can create a synergism in this type baby food which means low pH acted as an additional agent on microbial reduction.

At the end of the HHP treatment both types of baby foods became totally sterilized.

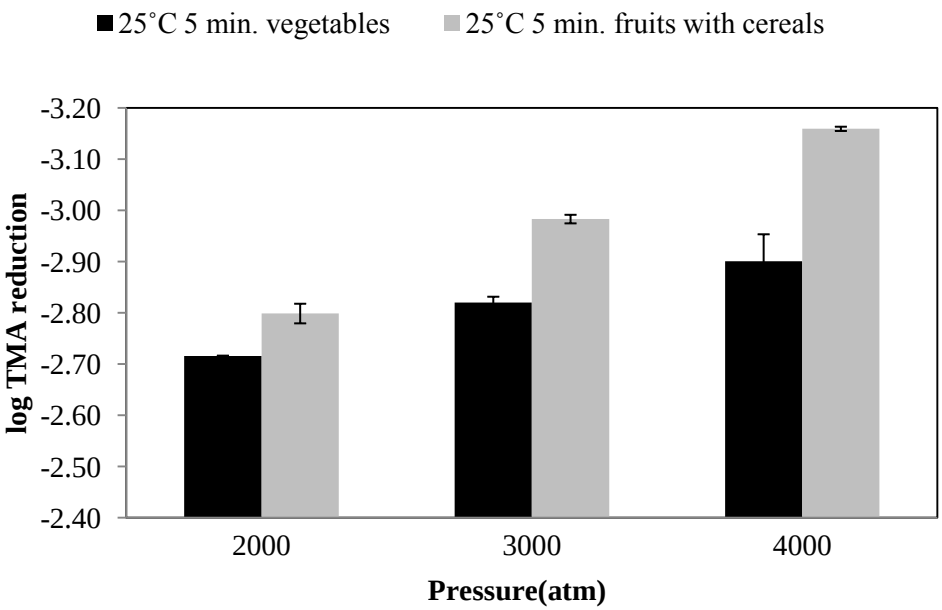

Figure 3.5 Effect of types of baby foods on log reduction of TMA at constant temperature (25 ^{o}C) and constant time (5 min.).

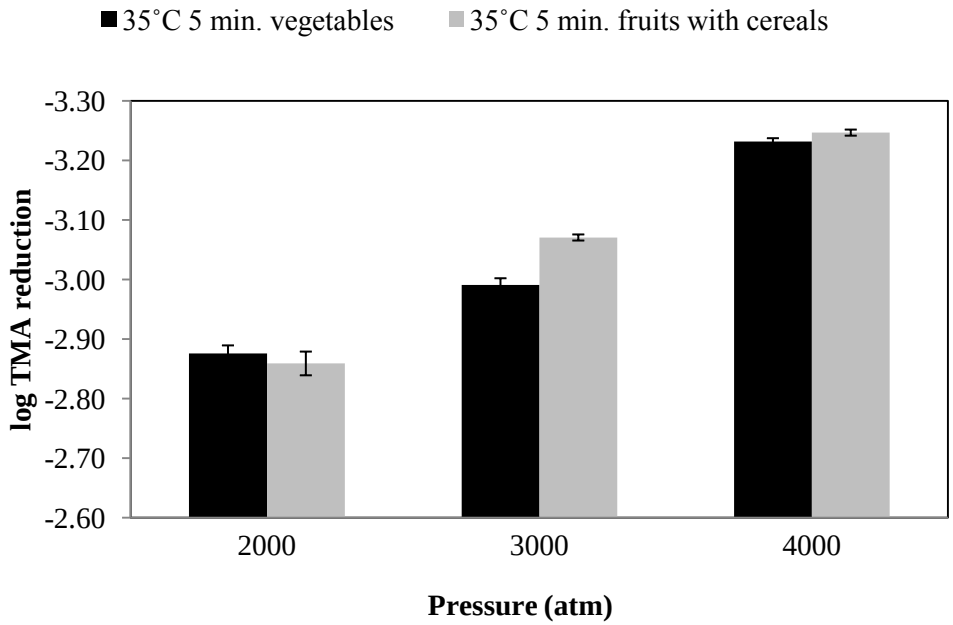

Figure 3.6 Effect of types of baby foods on log reduction of TMA at constant temperature (35 ^{o}C) and constant time (5 min.).

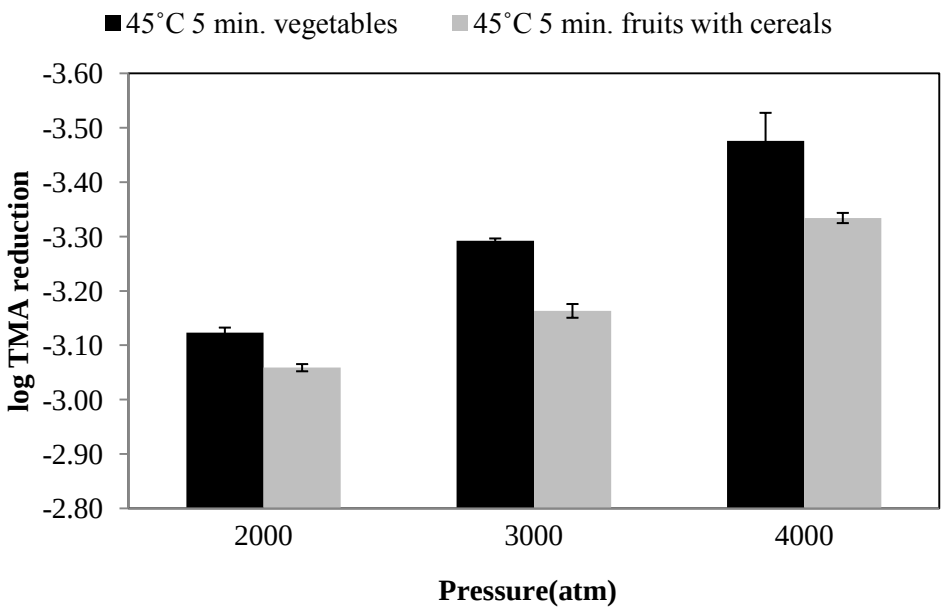

Figure 3.7 Effect of types of baby foods on log reduction of TMA at constant temperature (45 ^{o}C) and constant time (5 min.).

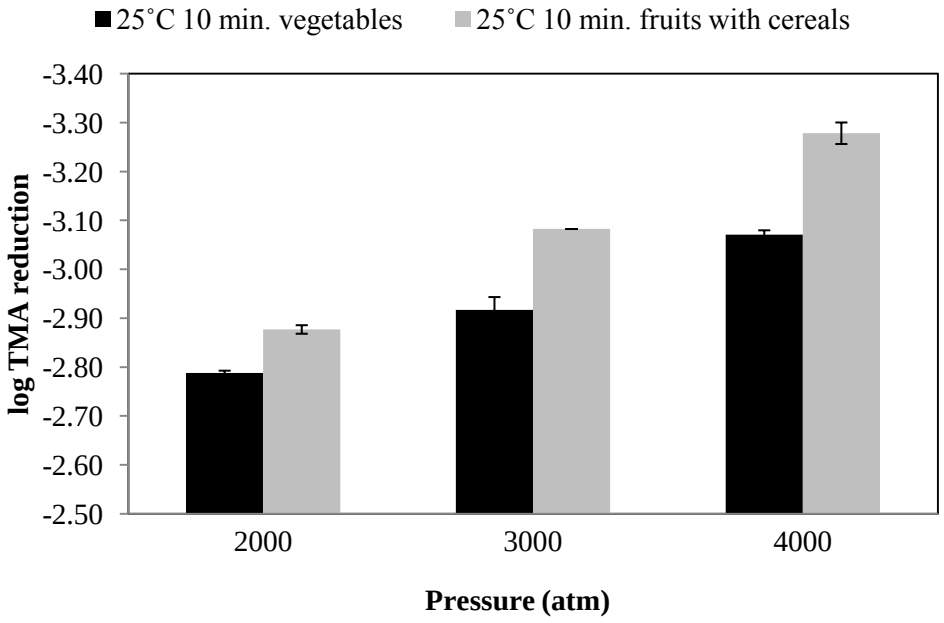

Figure 3.8 Effect of types of baby foods on log reduction of TMA at constant temperature (25 ^{o}C) and constant time (10 min.).

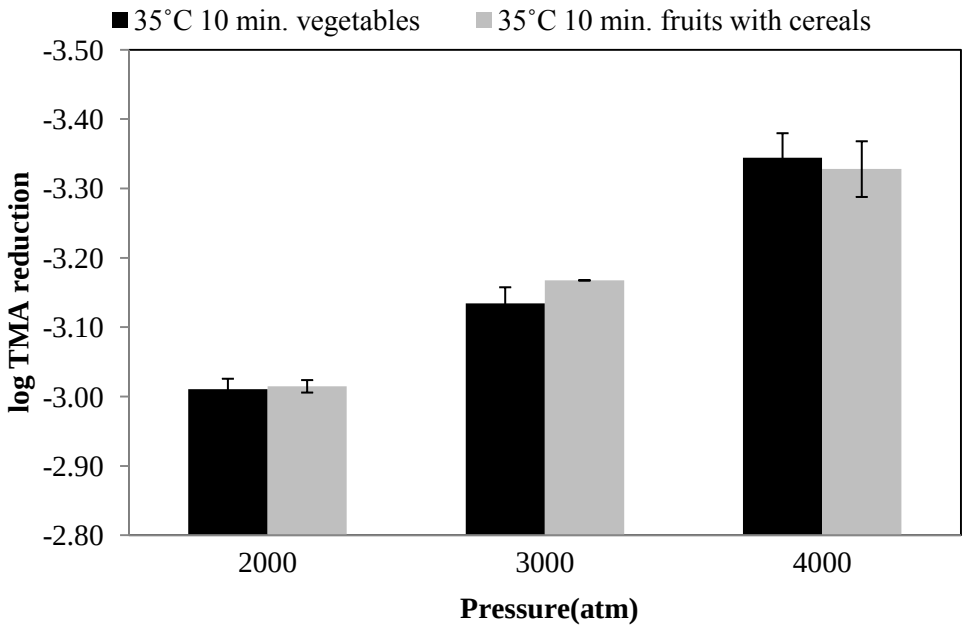

Figure 3.9 Effect of types of baby foods on log reduction of TMA at constant temperature (35 ^{o}C) and constant time (10 min.).

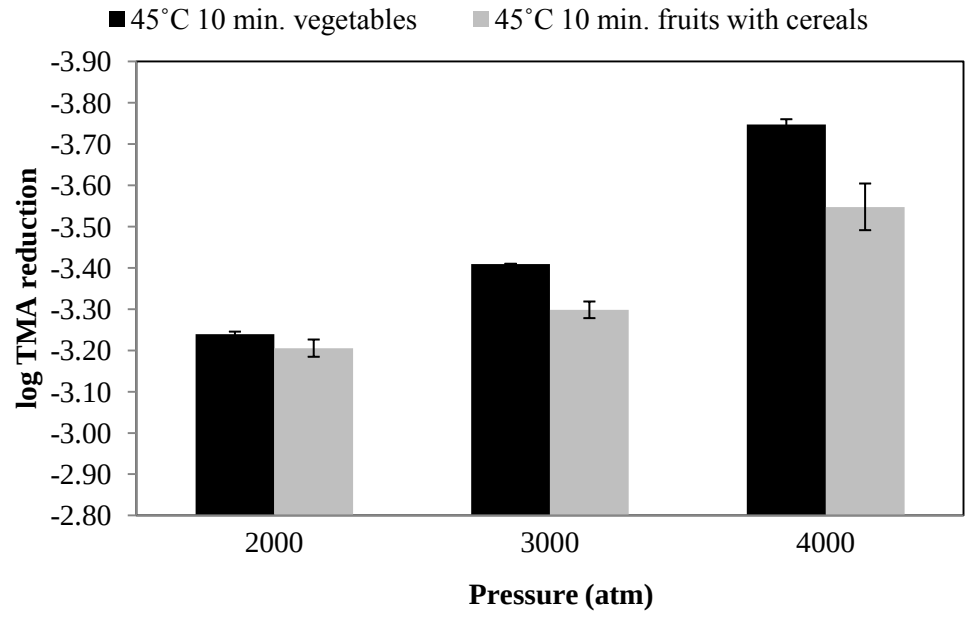

Figure 3.10 Effect of types of baby foods on log reduction of TMA at constant temperature (45 ^{o}C) and constant time (10 min.).

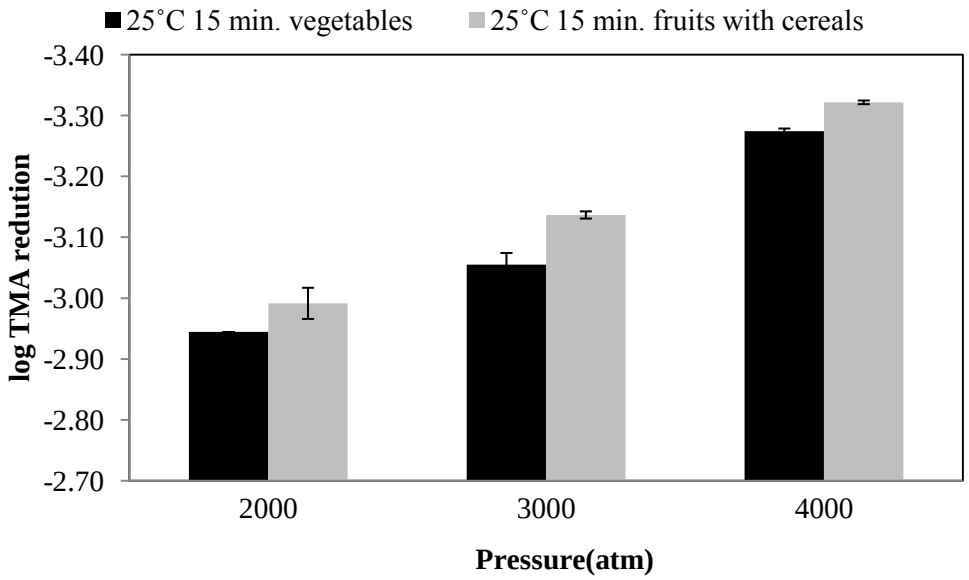

Figure 3.11 Effect of types of baby foods on log reduction of TMA at constant temperature (25 ^{o}C) and constant time (15 min.).

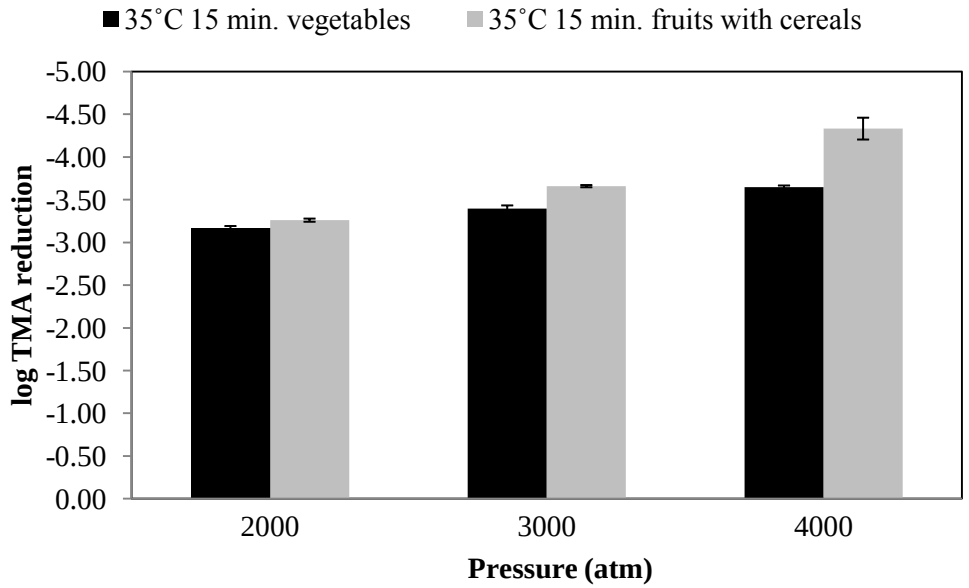

Figure 3.12 Effect of types of baby foods on log reduction of TMA at constant temperature (35 ^{o}C) and constant time (15 min.).

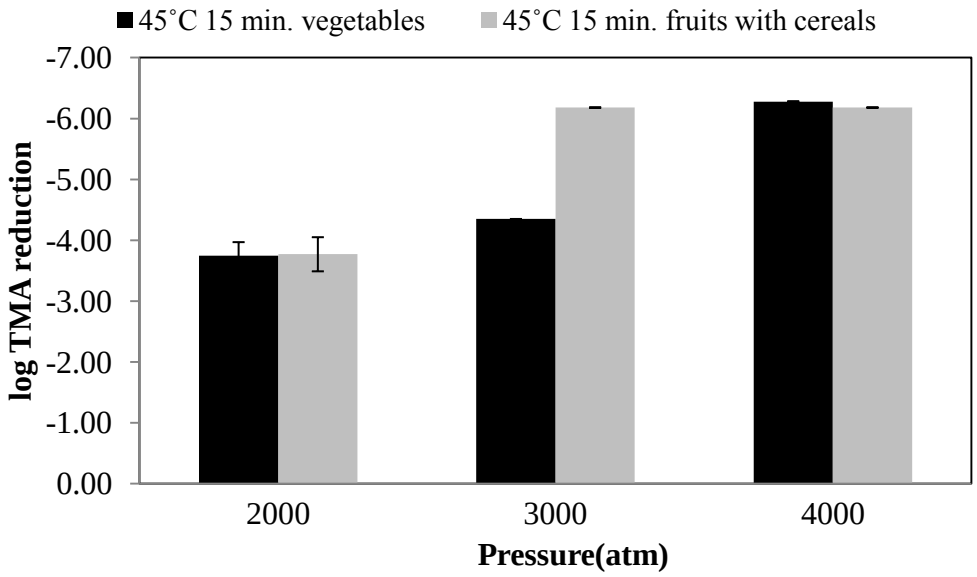

Figure 3.13 Effect of types of baby foods on log reduction of TMA at constant temperature (45 ^{o}C) and constant time (15 min.).

3.4 Effect of Types of Baby Foods on Logarithmic Reduction of Total Yeasts and Molds

Log reduction of total yeasts and molds in different types of baby foods at constant time and temperature are stated below in Figures 3.14 through 3.22. At all temperature and pressure values fruit based baby foods showed much more log reduction than vegetable based ones without any exception. Also, one way ANOVA was executed to data of total yeasts and molds count to establish the effect of type of baby food on microbial inactivation. Detailed analysis of variance is given in Appendix A.2. Statistical analysis pointed out that type of baby food has a significant effect in microbial reduction ($P<0.05$). This can be explained again with low pH.

Lowering the pH represents a synergistic effect on microbial load reduction.

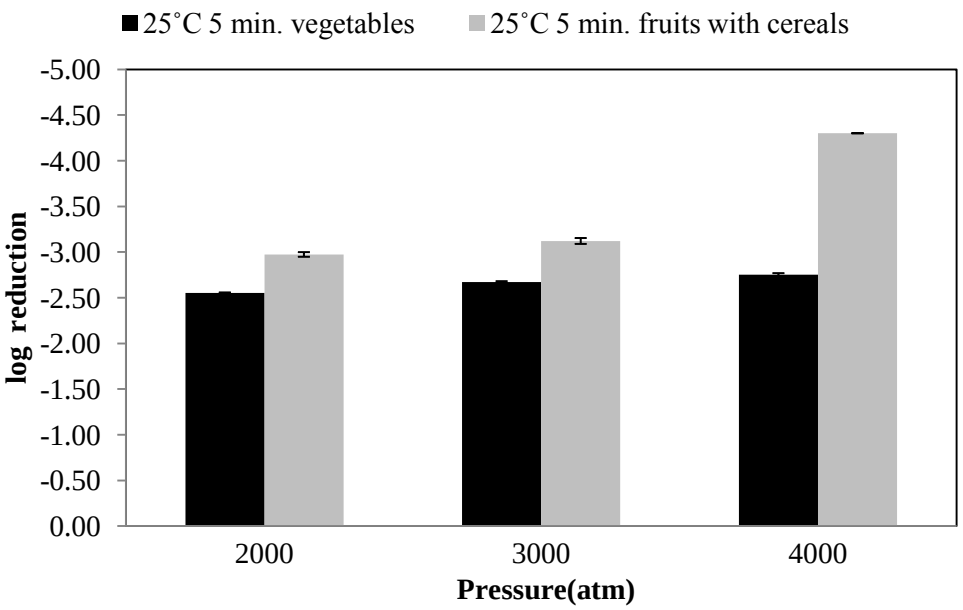

Figure 3.14 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (25 ^{o}C) and constant time (5 min.).

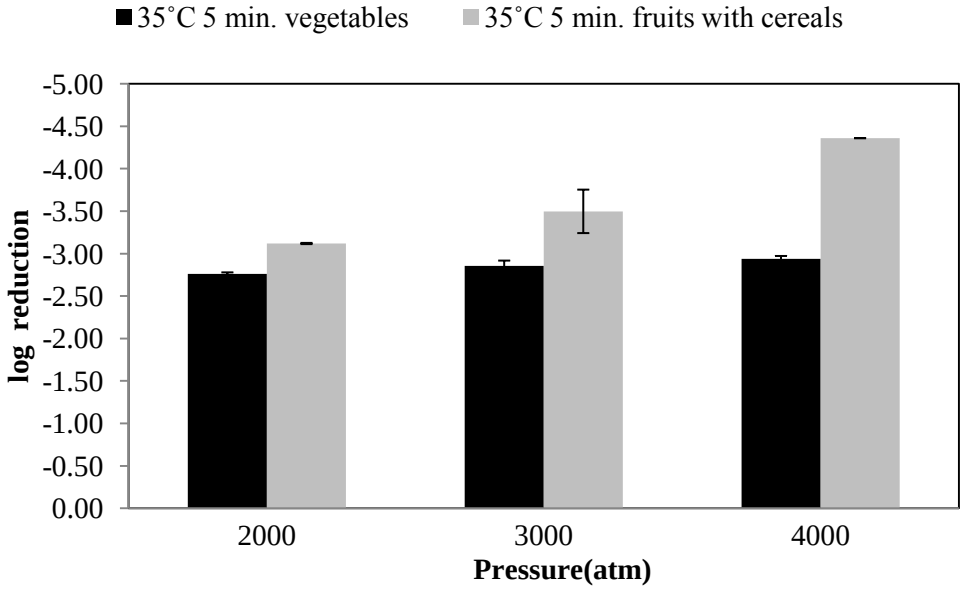

Figure 3.15 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (35 ^{o}C) and constant time (5 min.).

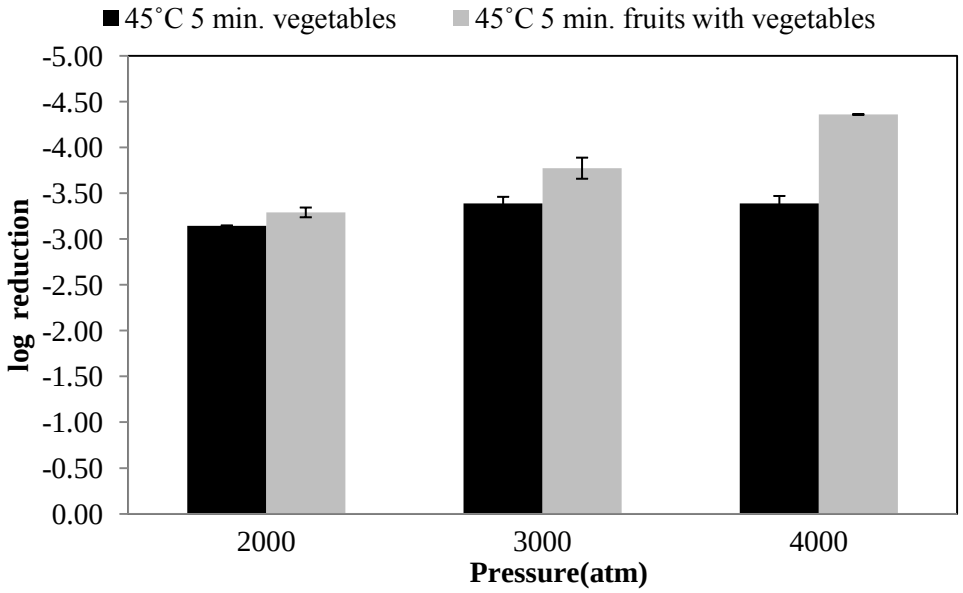

Figure 3.16 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (45 ^{o}C) and constant time (5 min.).

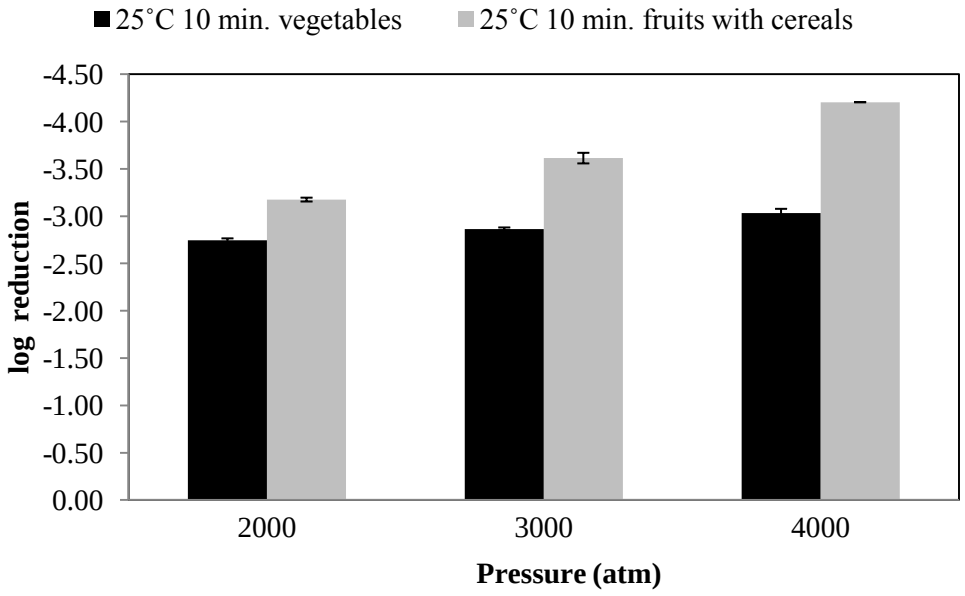

Figure 3.17 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (25 ^{o}C) and constant time (10 min.).

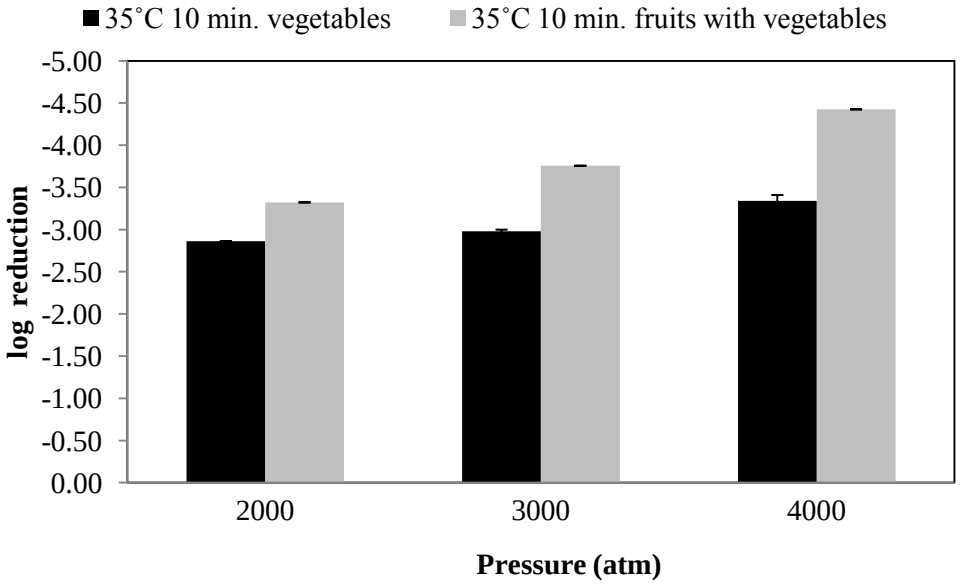

Figure 3.18 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (35 ^{o}C) and constant time (10 min.).

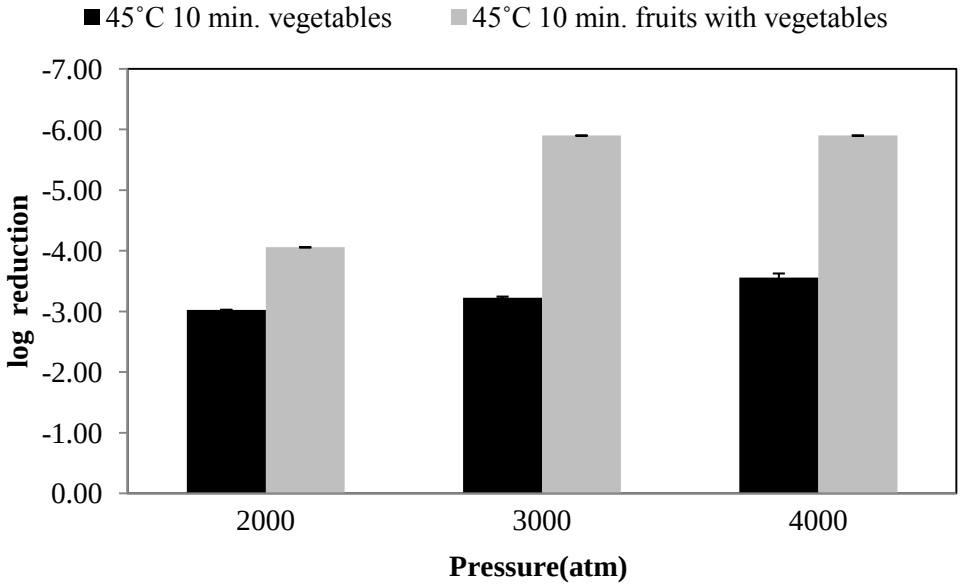

Figure 3.19 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (45 ^{o}C) and constant time (10 min.).

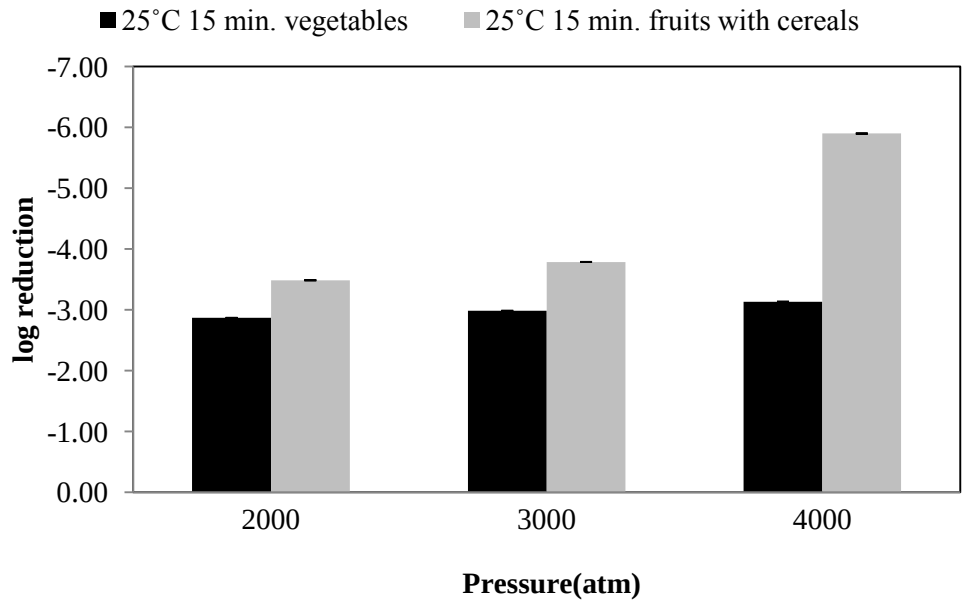

Figure 3.20 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (25 ^{o}C) and constant time (15 min.).

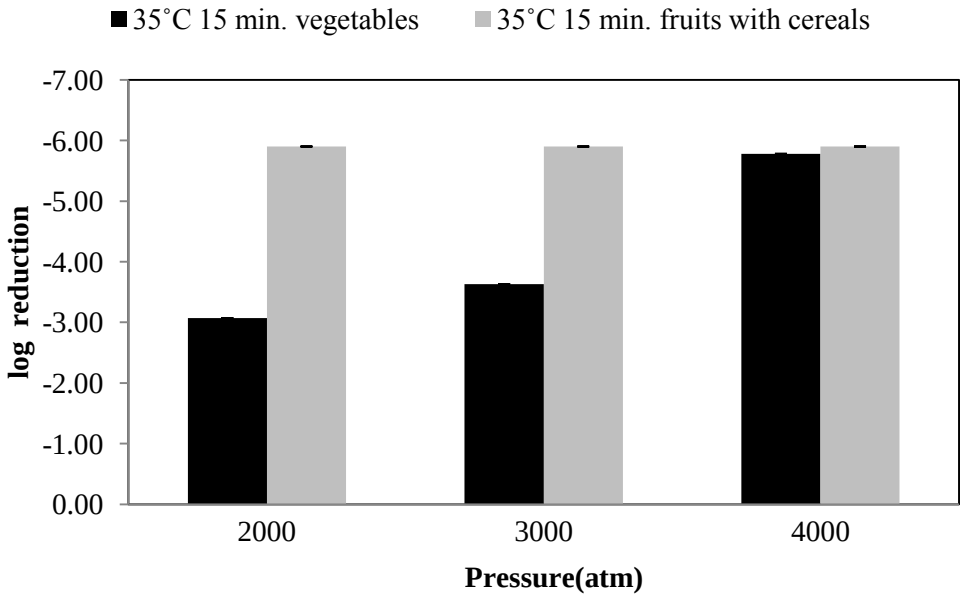

Figure 3.21 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (35 ^{o}C) and constant time (15 min.).

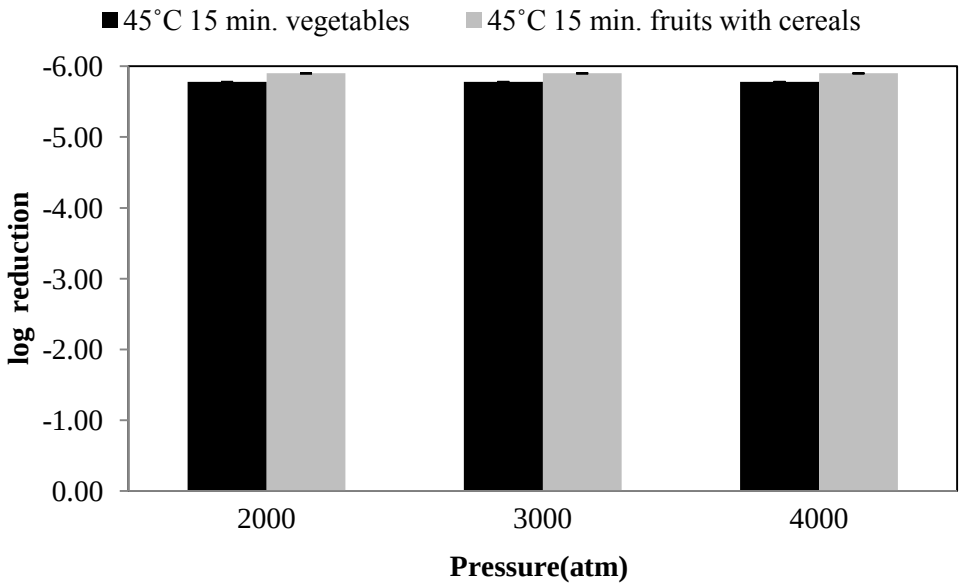

Figure 3.22 Effect of types of baby foods on log reduction of total yeasts and molds at constant temperature (45 ^{o}C) and constant time (15 min.).

3.5 Comparison of Furan Amounts in HHP-Treated Baby Food Samples and Thermal Treated Baby Food Samples

Determination of furan is known with its difficulty since its volatility is significantly high. Also, attention must be paid on homogenization of the sample; hence the amount of furan can be different in different parts of the sample vessel. Homogenization can result in the loss of furan since a little heat is generated and surface area is increased during blending process. Increase in surface area can cause increase in evaporation. Blending process and transfer of the baby food samples to the vials for the determination of furan were held at 4 ^{o}C not to lose furan because of its high volatile structure. The vials are sealed hermetically as soon as possible not to allow any loss of furan.

All experiments on furan were performed in duplicate. Furan amount was determined by GC-MS. A sample chromatogram is given in Fig 3.23. Calibration curve for furan determination is given in Appendix B.

3.5.1 Furan Amount in Vegetable Based Baby Food Samples

EFSA published a report in 2005 about the furan amounts ranged from non-detectable to 112 ng/g (EFSA, 2005).

Wegener et al. (2010) reported in their studies that juices, which are produced for babies, had the highest level of furan: 40 ng/g. Also in the same study, there is relevance between furan amount, pH and thermal treatment. Carrot juices with higher pH levels resulted in higher furan amounts. Juices with highest pH levels also need sterilization, which refers to thermal treatment at higher temperatures. This situation leads furan formation at the end. In the same study, it is also pointed out that beta-carotene seems to be a source furan formation (Wegener & Lopez-Sanchez, 2010).

Furan amount in vegetable based baby foods, which were commercially sterilized at 105 ◦C for 10 minutes is 5.84 ng/g. Furan amount of vegetable based baby foods, which were exposed to high hydrostatic pressure treatment, is given in Table 3.2. When they were compared to each other, it is easily seen from the tables that HHP-treatment did not let the formation of furan.

Considering the ingredients of vegetable based baby foods, it can be said that ascorbic acid pathway or carotenoid pathway could be followed for the formation of furan in conventionally sterilized baby food. Carotenoid pathway could be possibly followed as a result of carrot content which is famous for its' vitamin A content. Since furan is formed during higher heat treatments, it was impossible for furan to form during HHP treatment. Therefore, it would not be right to say that one parameter is significant to another in furan formation. By the way, there is not a regular data that shows the effects of these parameters because of impossibility of furan formation.

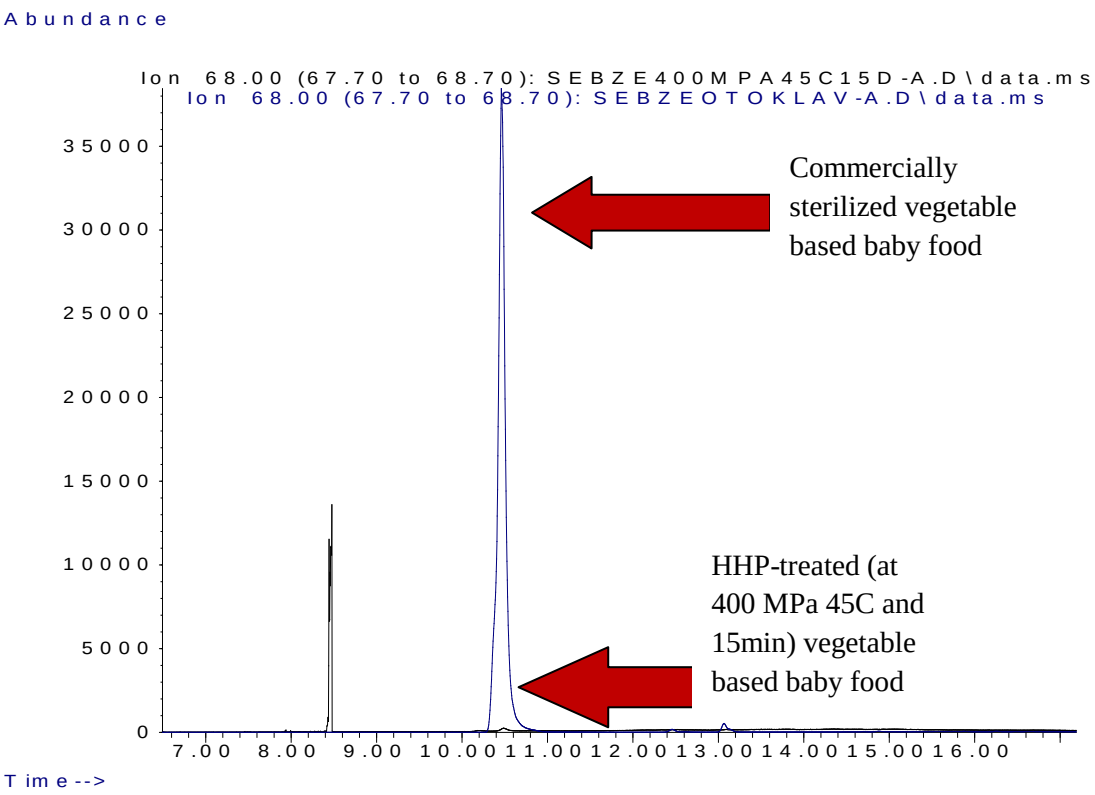

Figure 3. 23 GC-MS chromatogram overlay of the samples. One of them was treated at 400 MPa-45C-15 min, the other one was commercially sterilized.

Table 3.2 Furan amount in HHP treated and commercially sterilized vegetable based baby food.

P(MPa)	T(^{o}C)	T(min)	Furan amount (ng/g)
200	25	5	0.065 ± 0.009
300	25	5	0.071 ± 0.09
400	25	5	0.051 ± 0.022
200	35	5	0.055 ± 0.003
300	35	5	0.045 ± 0.026
400	35	5	0.035 ± 0.006
200	45	5	0.020 ± 0.003
300	45	5	0.022 ± 0.013
400	45	5	0.021 ± 0.005
200	25	10	0.028 ± 0.011
300	25	10	0.072 ± 0,001
400	25	10	0.030 ± 0.002
200	35	10	0.059 ± 0.010
300	35	10	0.025 ± 0.005
400	35	10	0.070 ± 0.019
200	45	10	0.039 ± 0.013
300	45	10	0.038 ± 0.011
400	45	10	0.043 ± 0.008
200	25	15	0.040 ± 0.003
300	25	15	0.030 ± 0.000
400	25	15	0.033 ± 0.006
200	35	15	0.035 ± 0.000
300	35	15	0.036 ± 0.005
400	35	15	0.031 ± 0.004
200	45	15	0.029 ± 0.006
300	45	15	0.052 ± 0.019
400	45	15	0.041 ± 0.018
	105	**10**	**5.839 ± 2.366**

Table 3.3 Furan exposure for babies

Months	Body weight (kg)	Intake of baby foods (g/day)	Mean furan value (ng/g)	Exposure/day (µg/kg body weight.day)	Total exposure (µg/kg body weight)
3	5.8	67	5.84	0.067	6.072
6	7.5	195	5.84	0.152	27.331
9	8.6	234	5.84	0.159	42.904
12	9.4	208	5.84	0.129	46.521

Data for body weights and intake of baby foods were retrieved from DONALD (Dortmund Nutritional Anthropometrical Longitudinally Designed) study (Kersting, Alexy, Sicher-Hellert, Manz, & Schoch, 1998). Since there is no study published on toxic effect of furan on humans, it is not proper to evaluate these exposure scenarios in toxicological manner. Daily exposure values were found below 0.22 µg/kg body weight.day which was stated in EFSA report on exposure values published in 2011 (EFSA, 2011).

3.5.2 Furan Amount in Fruit and Cereal Based Baby Food Samples

Furan amount in fruit based baby foods which were commercially sterilized at 105 ^{o} C for 10 minutes is 0.074 ng/g. Furan amounts in processed baby foods reported in the study of Bianchi et al. (2006) were in the range of 2.78 ng/g and 140.9 ng/g. Fruit based ones that were also placed in the same study were in the range of 2.78 ng/g and 4.3 ng/g. It was observable from the data that all fruit based samples had lower furan content with respect to vegetable based ones. It was explained with differences in thermal treatment whereas fruit based baby food generally pasteurized and vegetable based ones sterilized (Bianchi, Careri, Mangia, & Musci, 2006). This comment also matches with the comments of Wegener et al. (2010) about the same subject.

Investigations about furan in baby food samples of Finnish market (Jestoi, Järvinen, Järvenpää, Tapanainen, Virtanen, & Peltonen, 2009) revealed that furan amounts in Finnish markets ranged from 4.7 ng/g to 9.3 ng/g. The lowest one is a banana and peach puree and the highest one is made of vegetables, rice and pork meat. It is essential to give a great concern to composition of baby food while considering the formation pathway and amount of furan (Ruiz, Santillana, Nieto, Cirugede, & Sanchez, 2010).

Furan amount with an average value of 28 µg/kg throughout baby food samples collected from all over the world was involved in report published in 2004. The amounts obtained in our HHP-treated samples are considerably lower than the amounts that are stated in that report (FDA, 2004).

Table 3.3 shows the furan amounts of HHP treated samples. There is approximately no furan formation even in thermally sterilized ones. This situation can be explained again with low

pH of this type of baby foods. Since throughout our experiments fruit based baby foods and vegetable based ones were undergone same thermal treatment, it will not be true to evaluate these results with different heating values as in the studies of Bianci et al. (2006) and Wegener at al. (2010). For fruit based baby foods, especially fortified with organic acids, it was also known from the literature that furan formation following sugar or ascorbic acid pathway is difficult at low pH (Becalski & Seaman, 2005); (Limacher, Kerler, Davidek, Schmalzried F., & Blank , 2008). This explains low pH situation in our data more likely. Study on determination of furan amount in baby foods bought from Spanish markets also resulted in supportively (Ruiz, Santillana, Nieto, Cirugede, & Sanchez, 2010). In addition, throughout that study, it was concluded that lowest furan concentrations was seen in fruit based baby food, the highest ones were observed in meat or vegetable based baby food. Similar to vegetable based baby foods, there is not a correlation between HHP parameters and furan amount since it was impossible for furan to form at those temperature values. Also it was resulted from our study that pressure, itself does not induce furan formation process.

Our fruit based baby food sample was fortified with 25 mg/100 g ascorbic acid. Since ascorbic acid pathway is very important in furan formation, the contribution of the ascorbic acid to furan amount at the end of the thermal treatment was also researched in this study and a study on this issue was found. Owczarek-Fendor et al. (2010) reported that ascorbic acid concentrations ranged from 0.1 mg/g to 4.5 mg/g were negligible in furan formation whereas 18.0 mg/g of ascorbic acid resulted in a significant increase in furan content in foods. In our sample, there is only 0.25 mg/g ascorbic acid, which accounts for negligible in furan amount at the end of the thermal treatment.

Table 3.4 Furan amount in HHP-treated and commercially sterilized fruit based baby foods.

P(MPa)	T(°C)	T(min)	Furan amount (ng/g)		
200	25	5	0.056	±	0.038
300	25	5	0.016	±	0.016
400	25	5	0.012	±	0.012
200	35	5	0.013	±	0.004
300	35	5	0.016	±	0.005
400	35	5	0.016	±	0.008
200	45	5	0.023	±	0.013
300	45	5	0.012	±	0.012
400	45	5	0.016	±	0.004
200	25	10	0.009	±	0.009
300	25	10	0.014	±	0.014
400	25	10	0.066	±	0.049
200	35	10	0.049	±	0.029
300	35	10	0.020	±	0.020
400	35	10	0.051	±	0.030
200	45	10	0.115	±	0.030
300	45	10	0.018	±	0.018
400	45	10	0.032	±	0.006
200	25	15	0.117	±	0.014
300	25	15	0.139	±	0.047
400	25	15	0.089	±	0.024
200	35	15	0.010	±	0.001
300	35	15	0.012	±	0.012
400	35	15	0.006	±	0.006
200	45	15	0.005	±	0.005
300	45	15	0.004	±	0.004
400	45	15	0.003	±	0.003
	105	**10**	**0.074**	**±**	**0.006**

3.6 Shelf Life Analysis

Optimum pressure, temperature and time (P-T-t) for two types of baby food were determined with respect to the results of microbiological assays as 4000 atm- 45^{o} C- 15 minutes. At this optimum P-T-t, the number of both mesophilic aerobic bacteria and yeast and molds was zero in both types of baby foods.

The samples were treated with these optimum parameters and stored at room conditions (25 ^{o}C) where industrial baby foods are also exposed to.

In successive microbiological experiments, first growth in PCA medium was seen in fruit based baby foods at the 14th day of storage. At that day experiment was repeated and after two days incubation, we became sure that shelf life analysis ended up at 14th day of storage. Shelf life of vegetable based baby food lasted for 21 days.

These results are lower than we expected since commercial baby foods have a shelf life of two and a half year. Baby foods on the market are exposed to commercial sterilization which occurs at 105 ^{o}C for 10 min. Also they are placed in a jar; caps of the jars are sealed hermetically. There is also a vacuum effect while they are sealing which means that there is no way for the microorganism to place in the jar. We actually do not have chance to achieve that much professional sealing throughout our experiment. This shows importance of packaging in determination of shelf life. When it comes to importance of type of baby food considering shelf life, it is observable from the results that shelf life of fruit based baby food lasts less than vegetable based ones since sugar content of baby foods are higher than the others. Because of this, microorganisms find suitable media to grow and multiply.

Table 3.5 Appearance of microorganisms on three plates during shelf life in vegetable based baby foods.

Days/Type of microorganism	**0-21st**	**23rd**	**25th**
Mesophilic aerobic bacteria	(-);(-);(-)	(-);(+);(+)	(+);(+);(+)
Yeasts and molds	(-);(-);(-)	(-);(+);(+)	(+);(+);(+)

Table 3.6 Appearance of microorganisms on three plates during shelf life in fruit based baby foods.

Days/Type of microorganism	**0-12th**	**14th**	**16th**
Mesophilic aerobic bacteria	(-);(-);(-)	(-);(-);(-)	(-);(+);(+)
Yeasts and molds	(-);(-);(-)	(-);(-);(+)	(+);(+);(+)

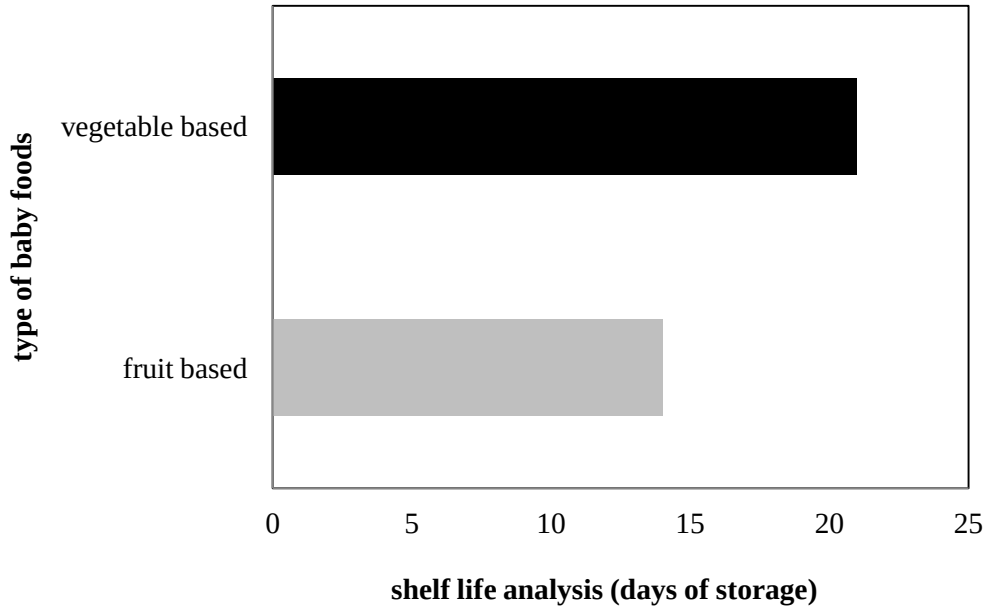

Figure 3.24 Results of shelf life analysis in different types of baby foods.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

It can be concluded from this study that it is possible to sterilize vegetable and fruit based baby food without allowing furan formation by HHP. From microbiological point of view vegetable based baby food got sterilized with HHP treatment at 4000 atm-45 ^{o} C-15 minutes. For fruit based baby foods, it can be concluded that no growth was observed at "3000 atm-45^{o}C-10 min".

When it comes to furan amount in those baby foods after HHP treatment, it has no obstacle to say that fruit based baby foods do not let the furan formation because of their low pH even in sterilized samples. Considering the difference between furan amounts in HHP treated and thermally treated baby foods, it is clearly seen that there is a significant difference in furan amounts at the end of those operations. HHP treatment does not let the occurrence of furan since treatment temperature does not reach that much higher value, although ascorbic acid content is very high in those kinds of baby foods. Besides HHP parameters do not have significant effects on furan formation.

Shelf life analysis ended up before the day we expected. Therefore the importance of packaging procedure drew attention, since we are not capable of sealing the caps of vessels hermetically. Efficient packaging of HHP treated foods can be another research area connected to this subject.

Because of being classified as "possibly carcinogenic to humans" by IARC, furan has the potential to cause cancer by accumulation in the body. For this reason, a big importance has been put on this issue by food safety authorities. Consisting of furan is unfavorable for baby foods. So an alternative method for sterilization has been needed urgently.

This study established that this alternative route could be HHP- treatment undoubtedly; having no detrimental effects on antioxidant capacity.

Considering HHP-treatment as an alternative method in term of avoiding furan formation, this study is expected to light the way for implementation on other foods, known for their furan content such as, ready to drink coffee etc.

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APPENDIX A

RESULTS OF ANOVA PERFORMED BY MINITAB

1. General Linear Model of total mesophilic aerobic bacteria versus P (atm); T (C); t (min) for vegetable based baby foods

Table A. 1 Parameters of HHP-treatment.

Factor	Type	Levels	Values
P(atm)	fixed	3	2000; 3000; 4000
T(C)	fixed	3	25; 35; 45
t(min)	fixed	3	5; 10; 15

Table A. 2 Analysis of Variance for total, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
P(atm)	2	9155833	9155833	4577917	275.03	0.000
T(C)	2	25618478	25618478	12809239	769.55	0.000
t(min)	2	10655233	10655233	5327617	320.07	0.000
P(atm)*T(C)	4	713589	713589	178397	10.72	0.000
P(atm)*t(min	4	227567	227567	56892	3.42	0.018
T(C)*t(min	4	409922	409922	102481	6.16	0.001
Error	35	582578	582578	16645		
Total	53	47363200				

S = 129.016 R-Sq = 98.77% R-Sq(adj) = 98.14%

2. General Linear Model of total mesophilic aerobic bacteria versus P (atm); T (C); t (min) for fruit based baby foods

Table A. 3 Parameters of HHP-treatment.

Factor	Type	Levels	Values
P(atm)	Fixed	3	2000; 3000; 4000
T(C)	Fixed	3	25; 35; 45
t(min)	Fixed	3	5; 10; 15

Table A. 4 Analysis of Variance for total, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
P(atm)	2	6268070	6268070	3134035	448.97	0.000
T(C)	2	5362715	5362715	2681357	384.13	0.000
t(min)	2	6176904	6176904	3088452	442.44	0.000
P(atm)*T(C)	4	712219	712219	178055	25.51	0.000
P(atm)*t(min)	4	316763	316763	79191	11.34	0.000
T(C)*t(min)	4	453719	453719	113430	16.25	0.000
Error	35	244315	244315	6980		
Total	53	19534704				

S = 83.5489 R-Sq = 98.75% R-Sq(adj) = 98.11%

3. General Linear Model of total yeasts and molds versus P(atm); T(C); t(min) for vegetable based baby foods

Table A. 5 Parameters of HHP Treatment.

Factor	Type	Levels	Values
P(atm)	fixed	3	2000; 3000; 4000
T(C)	fixed	3	25; 35; 45
t(min)	fixed	3	5; 10; 15

Table A. 6 Analysis of Variance for total, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
P(atm)	2	1231411	1231411	615706	27.90	0.000
T(C)	2	2904744	2904744	1452372	65.81	0.000
t(min)	2	4767678	4767678	2383839	108.02	0.000
P(atm)*T(C)	4	73111	73111	18278	0.83	0.516
P(atm)*t(min)	4	180844	180844	45211	2.05	0.109
T(C)*t(min)	4	575911	575911	143978	6.52	0.000
Error	35	772383	772383	22068		
Total	53	10506083				

S = 148.553 R-Sq = 92.65% R-Sq(adj) = 88.87%

4. **General Linear Model of total yeasts and molds versus P(atm); T(C); t(min) for fruit based baby foods**

Table A. 7 Parameters of HHP Treatment.

Factor	Type	Levels	Values
P(atm)	Fixed	3	2000; 3000; 4000
T(C)	Fixed	3	25; 35; 45
t(min)	Fixed	3	5; 10; 15

Table A. 8 Analysis of Variance for total, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
P(atm)	2	780559	780559	390280	51.21	0.000
T(C)	2	378559	378559	189280	24.84	0.000
t(min)	2	893559	893559	446780	58.62	0.000
P(atm)*T(C)	4	187396	187396	46849	6.15	0.001
P(atm)*t(min)	4	457963	457963	114491	15.02	0.000
T(C)*t(min)	4	90630	90630	22657	2.97	0.003
Error	35	266748	266748	7621		
Total	53	3055415				

S = 87.3005 R-Sq = 91.27% R-Sq(adj) = 86.78%

5. One way ANOVA to determine the effect of type of baby foods on total mesophilic aerobic count

Table A. 9 Analysis of Varience / Total mesophilic aerobic bacteria versus type of baby food.

Source	DF	SS	MS	F	P
Type	1	5566948	5566948	8,82	0,004
Error	106	66897904	631112		
Total	107	72464852			

S = 794,4 R-Sq = 7,68% R-Sq(adj) = 6,81%

Table A. 10 Means and standard deviations / Total mesophilic aerobic bacteria versus type of baby food.

Level	N	Mean	StDev
Fruits with cereals	54	975,9	607,1
Vegetables	54	1430,0	945,3

Pooled StDev = 794,4

Table A. 11 Grouping Information Using Tukey Method for mesophilic aerobic bacteria.

Type	N	Mean	Grouping
Vegetables	54	1430,0	A
Fruits with cereals	54	975,9	B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of type

Individual confidence level = 95,00%

6. One way ANOVA to determine the effect of type of baby foods on total yeasts and molds

Table A. 12 Analysis of Varience / Total yeasts and molds versus type of baby food.

Source	DF	SS	MS	F	P
Type	1	3857112	3857112	30,15	0,000
Error	106	13561498	127939		
Total	107	17418610			

S = 357,7 R-Sq = 22,14% R-Sq(adj) = 21,41%

Table A. 13 Means and standard deviations / Total yeasts and molds versus type of baby food.

Level	N	Mean	StDev
Fruits with cereals	54	158,1	240,1
Vegetables	54	536,1	445,2

Pooled StDev = 357,7

Table A. 14 Grouping Information Using Tukey Method for yeasts and molds.

Type	N	Mean	Grouping
Vegetables	54	536,1	A
Fruits with cereals	54	158,1	B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of type

Individual confidence level = 95,00%

APPENDIX B

CALIBRATION FOR DETERMINATION OF FURAN

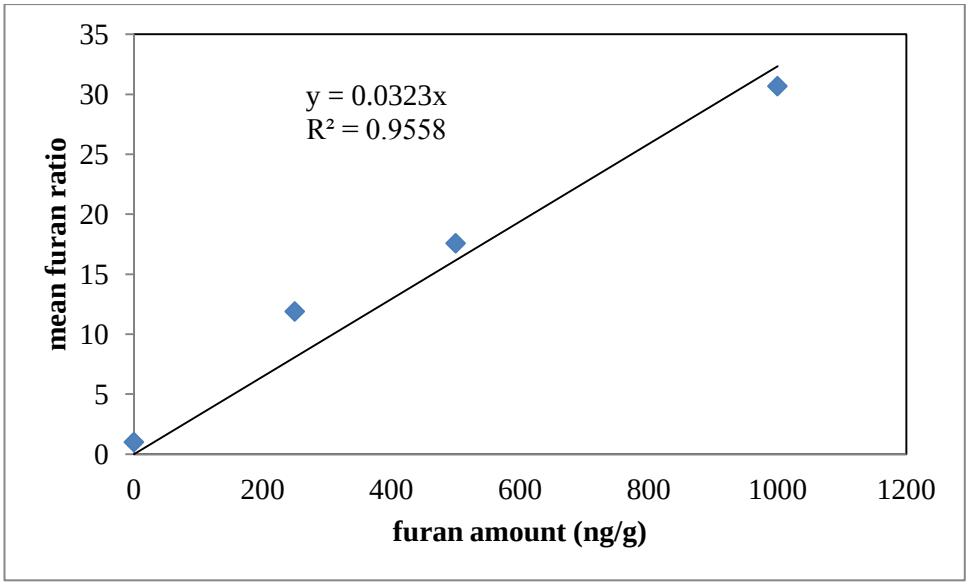

Figure B. 1 Calibration Curve for Furan Determination.