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Full Length Research Paper

The influence of food preservation methods on *Escherichia coli*, *Salmonella enteritidis* and *Pseudomonas aeruginosa* lipopolysaccharide composition and liberation

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***Escherichia coli*, *Salmonella enteritidis* and *Pseudomonas aeruginosa* were subjected to pasteurization, ultra high temperature (UHT) treatment and sodium benzoate preservation to determine the effect of these treatments on lipopolysaccharide (LPS) structure. *S. enteritidis* was the only bacterium that showed an overall decrease in LPS liberation after subjection to the heat treatments. Pasteurization of *E. coli* resulted in changes in LPS composition, increased LPS liberation, abundance and allocation; while the same treatment applied to *P. aeruginosa* caused a decrease in the release of LPS from the outer membrane and noticeably influenced the component distribution measured in the supernatant of treated cells. Significant changes were evident in the lipid A, core and O-chain components of the LPS structure. Although no trends could be established, certain components like D- xylose (X), octadecanoic acid ($C_{18:0}$) and D-ribopyranose (RP) were subject to change in all three organisms tested and alteration in the distribution of D-fructopyranose (FP), L- leucine (L) and phenylalanine (PA) was unique to *P. aeruginosa*. These alterations occurred in the centres where endotoxicity, phylogenetic relationships and serotype classification are established.**

Key words: Pasteurization, lipopolysaccharides, heat treatment, foodborne pathogens, gas chromatography-mass spectrometry.

INTRODUCTION

Designated to the Gram-negative group of bacteria, *Escherichia coli, Salmonella enteritidis* and *Pseudomonas aeruginosa* are organisms frequently connected with food contamination. Apart from their association with diseases such as haemorrhagic colitis and haemolytic uraemic syndrome in humans (*E. coli),* foodborne toxic salmonellosis (*S. enteritidis)* and infections like folliculitis and infantile diarrhoea (*P. aeruginosa*), they also share a characteristic membrane composition (Molina et al., 1991; Baylis et al., 2004; Whiteman and Bickford, 2007). This membrane consists of a tri-laminar cell wall consisting of a cytoplasmic membrane, peptidoglycan layer and outer membrane. Three quarters of the outer membrane layer consists mainly of lipopolysaccharides (LPS) that are essential and differ in structure and composition among bacterial serotypes. LPS mainly consists of a lipid core (lipid A) linked to the O-antigen with a 2-keto-3-deoxyoctonate (KDO) and is also described as an endotoxin. It further determines the essential physicochemistry of these organisms and subsequently has relevance in the food industry as they are implicated in bacterial resistance to sanitation efforts (Ferris and Beveridge, 1986). For effective preservation and sanitation the food industry relies on a combination of intrinsic, extrinsic and implicit inhibitory and lethal factors (hurdles) which, when applied simultaneously or sequentially, are intended to curtail or

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inactivate the development or survival of contaminating pathogenic micro-organisms.

Common hurdles employed for food preservation include physical elements such as refrigeration, modified atmosphere packaging, heat treatments and physicochemical factors like water activity, pH and preservatives (Leistner, 2000). However, as these conditions represent extensions of the natural variation that a micro-organism may encounter, it is not surprising that many bacteria are capable of mounting an adaptive response (Davidson and Harrison, 2002).

As the LPS constitutes molecular interface between foodborne micro-organisms and the external environment, adaptation or response to stresses/preservation efforts by alteration of the LPS structure have been reported (Wilkinson, 1996; Venter et al., 2006). Though the LPS is constructed to function as a selective barrier that in its normal state controls the entry of low molecular hydrophilic molecules into the cell, but excludes hydrophobic antibiotics and detergents, the micro-organisms, if still viable, alters the molecule's configuration and composition to survive unfavourable conditions (Snyder et al., 1999). The most frequent alterations reported include branching of the O-chain and substitution as well as addition or deletion of saccharides in the polymer (Reeves, 1992; Thomsen et al., 2003). Subsequent studies revealed that these molecular responses by bacteria could enhance their ability to form biofilm and resistance to antibiotics and disinfectants (Banemann et al., 1998; Bower and Daeschel, 1999; Davey and O'Toole, 2000).

Since heat processing is of paramount importance to the food industry, several studies have focused on the heat shock response and its impact on thermo-tolerance of foodborne pathogens (Gross, 1996; Yura and Nakahigashi, 1999; Ramos et al., 2001). Although the LPS of Gram-negative foodborne pathogens is extremely heat stable and remains intact and toxic even after ordinary steam sterilization and retorting, little information is available on heat induced structural adaptation and liberation of the LPS into the direct external environment (Abraham et al., 2009). Moreover, as beverages such as milk, generally subjected to pasteurization and UHT, might contain low/no viable potential pathogenic micro-organisms, the influence on LPS structure and the amount of free LPS liberated (exposing the endotoxic centre) into the environment is not known (Murinda et al., 2002).

Another popular method of food preservation entails the addition of chemical preservatives such as sodium benzoate to, for instance, fruit juice concentrates at a concentration of 0.1 to 0.2% (El-Shenawy and Marth, 1988; Russell, 1991; Stevens et al., 1991). Considering that these concentrates and, therefore, also the preservative are diluted; reducing its effectiveness and creating an environment, though non-optimal, for static contaminants present to resume proliferation.

Bacterial destruction via thermal and non-thermal preservation during food processing storage is well understood and readily cited (Hubbert et al., 1996; Barbosa-Canovas et al., 1997). Nevertheless, the consequent changes in LPS structure and release from the bacterial outer membrane during these processes are poorly described. This paper considers the unexplored influences of selected extrinsic stresses implemented by the food industry and by the consumer on the three mentioned foodborne pathogens. The main objective is to critically assess changes in the LPS release and ultra structure by evaluating alterations in distribution and location of its numerous components that occurred during heat and preservation treatments.

MATERIALS AND METHODS

Culture preparation and treatment parameters

E. coli O111, *S. enteritidis* ATCC 13076, and *P. aeruginosa* ATCC 27853 were inoculated into 50 ml nutrient broth (Merck, SA) at a concentration of *ca.* 10^3 cfu/ml. The pure cultures were further propagated by agitation at 35ºC for 12 to 20 h. Cell growth was monitored by means of optical density ($OD_{610\ nm}$), harvested in the late exponential growth phase and subsequently subjected to laboratory simulated heat treatments. The parameters of the most commonly employed treatments, in particular the dairy industry, were applied. These included pasteurization at 72°C for 30 s with post-treatment cooling to 5°C and ultra high temperature (UHT) treatment at 145°C for 4 s, with a pre-treatment conditioning to 85°C for 15 min and post-heat treatment conditioning to 20°C. Treated cultures were then centrifuged at 12 000 g for 5 min followed by LPS extraction from both the pellet and supernatant.

Filter sterilized sodium benzoate (Cape Food Ingredients, SA) suspended in deionized water (pH 6.12) was added to 50 ml nutrient broth to a final concentration of 0.1%. This amounted to 50% of the lethal dose, which ensured subsequent LPS production in the test organisms. *E. coli*, *S. enteritidis*, and *P. aeruginosa* were inoculated into the 50 ml sodium benzoate containing nutrient broth at a concentration of *ca.* 10^3 cfu/ml. Cultures were propagated at 35ºC with agitation for 12 to 20 h, harvested in the late exponential phase by centrifugation at 12 000 g for 5 min and LPS was extracted from the cell pellet. Throughout the study bacterial viability was assessed by means of the plate-count method on nutrient agar. Control samples represents cultures similarly prepared, but not exposed to any of the treatments. All data are representative of at least duplicate experiments.

LPS extraction and derivatisation

LPS extraction was performed as described by Venter and Lues (2003). Samples were dried with a rotary evaporator with added 2-propanol (1 ml), reconstituted in 200 µl chloroform: methanol mixture (1:2) and transferred to reaction vials. The samples were subsequently dried under a nitrogen stream. Prior to GC-MS analysis the LPS was methanolyzed and trifluoroacetylated according to the methods described by Jantzen et al. (1972) and Bryn and Jantzen (1982).

Gas chromatography and mass spectrometry (GC-MS)

For the GC-MS analysis of the fatty acid methyl esters and trifluoroacetylated sugars, a ZB-1, fused dimethyl polysiloxane 30 m (length) × 0.25 mm (internal diameter) column was used on a

Table 1. Lipopolysaccharide liberation in response to heat treatments, expressed as the loss of β-hydroxy fatty acids.

β-hydroxy fatty acids [a]	Control	Pasteurization	UHT
E. coli	1323	2825	1190
S. enteritidis	6595	2235	5790
P. aeruginosa	10480	2398	13565

[a] β-hydroxy fatty acids were expressed as MS signal/ml supernatant.

Finnigan Focus GC (Separations, SA) with helium as carrier gas (constant flow – 3.0 ml/min) and operated in a split-less injection mode. The column temperature was programmed for an initial temperature of 40 to 90ºC at a rate of 8ºC/min and then to a final temperature of 90 to 280ºC at 10ºC/min. The run duration was 20 min and sample size 2 µl. The column was linked to a Finnigan Focus DSQ mass spectrometer for mass detection of fragments with an m/z less than 1000. Mass analysis was performed at 70 eV with an ion source temperature of 200ºC. Integration of the peaks was performed on the TIC using X-calibur version 1.4 software (Finnigan). All experiments were conducted in at least duplicate.

The presence of a specific fatty acid in LPS which is not found in phospholipids (i.e. 3-hydroxy myristic and 3-hydroxy lauric acids) makes it possible to quantify LPS release by means of GC-MS. The characteristic β-hydroxy fatty acids for the Enterobacteriaceae - *Escherichia*, *Salmonella* and *Pseudomonas* are β-hydroxy tetradecanoic and dodecanoic acids (Moss and Dees, 1975; Saraf et al., 1997). The fraction of cellular LPS released by UHT and pasteurization treatment was determined by comparing the amount of LPS in the supernatant with the corresponding amount of cellular LPS (pellet) of the control.

RESULTS AND DISCUSSION

The influence of pasteurization and UHT on the liberation of 3-OH fatty acids from cellular outer membrane

LPS molecules are liberated from the bacterial outer membrane during multiplication, death, and lysis of the whole cells, as well as from constant sloughing off, in a manner similar to the human body shedding hair. Therefore, it would be erroneous to only consider the intact LPS when quantifying the influence of preservation treatments on whole LPS. The authors opted to express the quantity of LPS release as the ratio between the amount of β-hydroxy fatty acids (main constituent of the stable Lipid A fraction of the LPS) present in the supernatant of the control set (no preservation induced) and the heat-stressed supernatant of the organisms in question (Table 1). No common trend as a result of pasteurization or UHT treatment was observed among the organisms tested.

It was evident; however, that pasteurization resulted in a decreased β-OH fatty acid presence in the supernatants of *S. enteritidis* and *P. aeruginosa.* A substantial increase was visible in the case of *E. coli* where the ratios of β-OH fatty acid (FA) in the supernatant of pasteurized cells compared to that of the control cells were 2.14. UHT treatment of the same organisms had no significant influence on either *E. coli* or *S. enteritidis*, where only slightly less β-OH FA was present in the supernatant. *P. aeruginosa* responded to the UHT treatment with an increased β-OH FA ratio (1.3).

Changes in the whole LPS composition subsequent to heat treatments

Apart from evaluating LPS release, the structure composition of both the O-chain and lipid A is also relevant as it would provide a clearer understanding of, for instance, the accuracy that could be expected from the application of immunodiagnostic kits for the rapid identification of the three mentioned organisms. The structure of the membrane associated LPS (pellet) as well as the supernatant fraction (released/liberated LPS) of *E. coli*, *S. enteritidis* and *P. aeruginosa* were analyzed with a GC-MS after pasteurization and UHT treatments. The untreated controls represent LPS extracted from each strain grown under the same conditions as previously described and harvested in the same growth phase. Discussion of the data was done under the presumption that the LPS components detected in the pellet is membrane associated and those detected in the supernatant were liberated or shed from the bacterial cells. Although lysis of some cells was likely to have occurred, the centrifugation speed concentrated such debris to the pellet. For the purpose of this study heat dissociation of the polysaccharides from the lipid fraction was not taken into account, since independent duplicate experiments demonstrated that core and O-chain saccharides were detected in the membrane associated fraction of all the strains tested.

From the range of compounds identified on the LPS molecules the most prominent compounds were selected to illustrate changes in LPS structure of *E. coli, S. enteritidis* and *P. aeruginosa* brought on by pasteurization or UHT treatment (Figures 1 and 2). The results obtained for the LPS composition were expressed as MS Signal/ml, since each component had a unique MS spectrum and total ion chromatogram. The authors further sufficed with the direct correlation between compound quantity and the total ion count expressed in this paper as MS signal (Venter et al., 2006). A procedure error of less than 5% was upheld, confirming the report by Bohin et al. (2005).

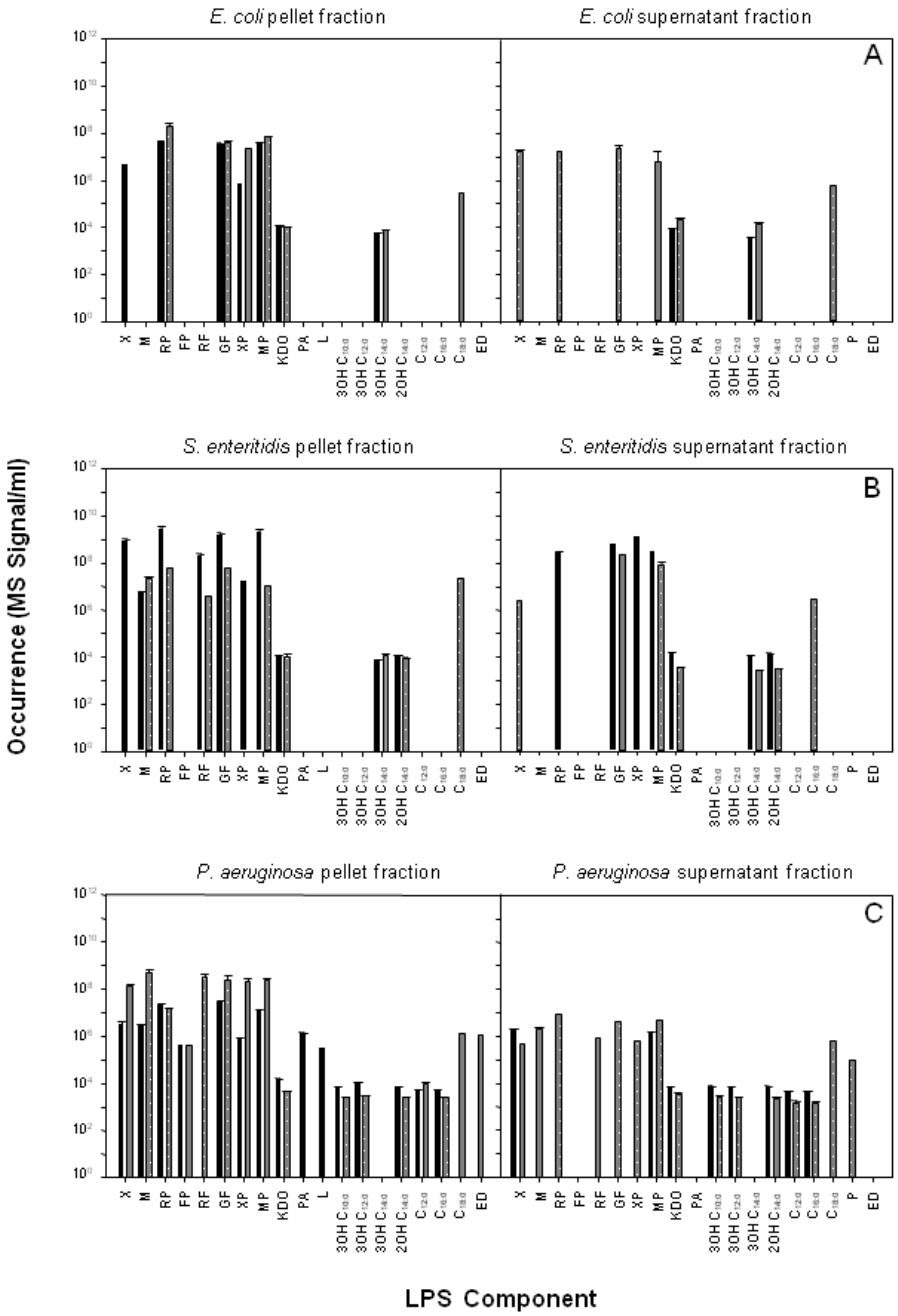

Figure 1. Selected LPS component distribution of the pellet and supernatant fractions of *E. coli* (A), *S. enteritidis* (B) and *P. aeruginosa* (C) grown in nutrient broth (control) (■) and treated with pasteurization (▨). The results are expressed as the MS signal/ml for the following saccharides, amino acid, fatty acids sugar-and ethandimine derivatives: D-xylose, 2, 3, 4, 5-tetrakis (trifluoroacetate) [X], α-L-mannopyranose 6-deoxy tetrakis (trifluoroacetate) [M], d-ribopyranose, tetrakis (trifluoroacetate) [RP], D-fructopyranose, pentakis (trifluoroacetate) [FP], d-ribofuranose, tetrakis (trifluoroacetate) [RF], D-galactofuranose, pentakis (trifluoroacetate) [GF], methyl 2,3,4-tri-O-trifluoroacetyl α-d-xylopyranoside [XP], D-mannopyranoside, methyl, tetrakis (trifluoroacetate) [MP], 2-keto-3-deoxy-octulosonic acid [KDO], phenylalanine , 2-trifluoromethyloxazolinone [PA], L-leucine, N-(trifluoroacetyl)-, butyl ester [L], 3-hydroxydecanoic acid, methyl ester [3OH $C_{10:0}$], 3-hydroxydodecanoic acid, methyl ester [3OH $C_{12:0}$], 3-hydroxytetradecanoic acid, methyl ester [3OH $C_{14:0}$], 2-hydroxytetradecanoic acid, methyl ester [2OH $C_{14:0}$], dodecanoic acid, methyl ester [$C_{12:0}$] , hexadecanoic acid, methyl ester [$C_{16:0}$], octadecanoic acid, methyl ester [$C_{18:0}$], proline, N-(trifluoroacetyl)-, methyl ester [P] and 1, 2-ethandimine, N, N'-ditrifluoroacetyl [ED].

Changes detected in the LPS ultra structure of cells subjected to pasteurization

Pasteurization resulted in an increase in the overall abundance of LPS and liberation of certain constituents in both *E. coli* and *P. aeruginosa* compared to the controls (Figure 1 (A, C)), with the opposite evident in the case of *S. enteritidis* (Figure 1(B)). All three strains showed the gain of octadecanoic acid ($C_{18:0}$) in the membrane associated fraction (pellet), which was also liberated from *E. coli* and *P. aeruginosa*, while hexadecanoic acid ($C_{16:0}$) was liberated from *S. enteritidis*. Pasteurization triggered the liberation of saccharides D-xylose (X), D-ribopyranose (RP), D-galactofuranose (GF) and D-mannopyranose (MP) in *E. coli*, while the absence of D-xylose (X) represents the only change that occurred in saccharide distribution of the membrane associated fraction (Figure 1(A)). Liberation of D-ribopyranose (RP) and α-D-xylopyranoside (XP) did not take place when *S. enteritidis* was subjected to pasteurization; in fact this constituent no longer formed part of the LPS structure and as noted in *E. coli*, D-xylose (X) was not detected in the pellet of *S. enteritidis* either (Figure 1(B)). The effect of pasteurization on the LPS composition of *P. aeruginosa* was visible in the liberation of saccharides α-L-mannopyranose (M), D-ribopyranose (RP), D-ribofuranose (RF), D-galactofuranose (GF) and α-D-xylopyranoside (XP) and amino acid proline (P) from treated cells. D-ribofuranose (RF) was exclusively detected in the treated cells in both supernatant and membrane associated fractions, while sugar ethandimine derivative 1, 2-Ethandimine (ED) was noted only in the pellet.

Amino acid components phenylalanine (PA) and L-leucine (L) were only present in the membrane associated fraction of control cells and did not form part of the LPS structure of cells subjected to pasteurization (Figure 1(C)). The occurrence of unusual sugars and amino compounds in the O-chain are characteristic of *P. aeruginosa* LPS (Pier et al., 1981; Rowe and Meadow, 1983; Kulshin et al., 1991). Since our analysis identified these compounds only in *P. aeruginosa*, we can say with certainty that they originated from the LPS and not medium contaminants or proteinase K digestion of other membrane associated proteins.

Changes detected in LPS ultra structure after UHT treatment

The LPS composition of *E. coli* was marginally influence by exposure to ultra high temperature, noticeable in the liberation of saccharides D-xylose (X), D-galactofuranose (GF) and D-mannopyranose (MP). In the membrane associated fraction the complete loss of α-D-xylopyranoside (XP) and addition of octadecanoic acid($C_{18:0}$) was apparent (Figure 2(A)). *S. enteritidis* LPS liberated D-xylose (X), but not D-ribopyranose (RP) or α-D-xylopyranoside (XP) as was noted in the control cells. Hexadecanoic acid ($C_{16:0}$) was present in the pellet fraction (Figure 2(B)), a compound not detected in treated *E. coli* or *P. aeruginosa*. UHT treatment had a pronounced effect on the LPS composition of *P. aeruginosa* where D-xylose (X), α-L-mannopyranose (M), D-fructopyranose (FP), D-galactofuranose (GF) α-D-xylopyranoside (XP) and L-leucine (L) were lost from the membrane associated fraction and 1, 2- Ethandimine (ED) was the only addition (Figure 2(C)). Saccharides α-L- mannopyranose (M), D-ribopyranose (RP) and D-galactofuranose (GF) were liberated from the treated cells, but was not detected in the supernatant fraction of the control cells.

The effects of sodium benzoate preservation on the LPS composition

Cells challenged with the preservative sodium benzoate showed different transient responses to the treatment (Figure 3). *E. coli* cells showed an overall increase in LPS content and D-ribofuranose (RF), hexadecanoic acid ($C_{16:0}$) and octadecanoic acid ($C_{18:0}$) formed part of the membrane associated fraction of the treated cells, but not the control (Figure 3(A)). Saccharides D-xylose (X) and α-D-xylopyranoside (XP) were no longer present in the membrane fraction of the challenged cells. A different pattern was noted for *S. enteritidis,* where an overall decrease in the total LPS was noticed, although the expression trend of LPS components were similar to that of the control cells (Figure 3(B)). *P. aeruginosa* responded with a general decrease in the sugars, fatty acids and amino acids. When considering the LPS variation, D-ribofuranose (RF) and octadecanoic acid ($C_{18:0}$) were present in the treated cells, but absent from the control LPS. The saccharide constituent D-fructopyranose (FP) and the amino acid components phenylalanine (PA) and L-leucine (L) no longer formed part of the membrane fraction LPS of the sodium benzoate treated cells (Figure 3(C)).

LPS are known heat stable antigens, their structure and configuration are used for detection and identification of pathogens and food safety measurements, like the use of sanitizers, and they can cause ultra structure alterations in certain foodborne bacteria (Wilkinson, 1996; Venter et al., 2006). The importance of LPS and the evidence reported on structural alterations in response to environmental stresses proves the relevance of investigating the influence of standard practice heat treatments and chemical preservation on the ultra structure of foodborne pathogens *E. coli*, *S. enteritidis* and *P. aeruginosa*. Although the method of analysis employed could not elaborate on molecular association and linkage among the constituents, definite changes in the abundance and localization of individual constituents were documented in all three organisms tested.

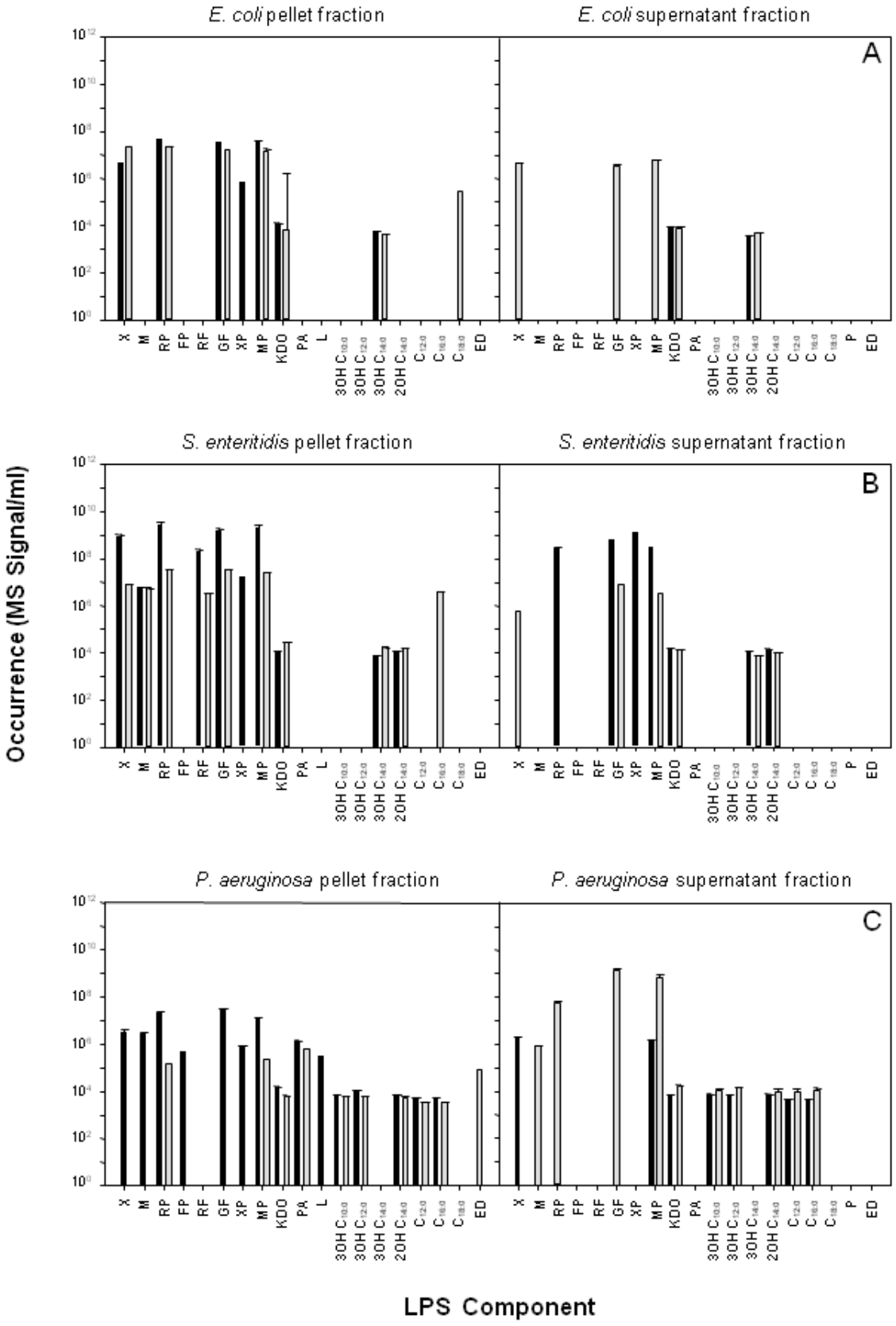

Figure 2. Selected LPS component distribution of the pellet and supernatant fractions of *E. coli* (A), *S. enteritidis* (B) and *P. aeruginosa* (C) grown in nutrient broth (control) (■) and treated with UHT (□). The results are expressed as the MS signal/ml. X-axis abbreviations were explained in the legend of Figure 1.

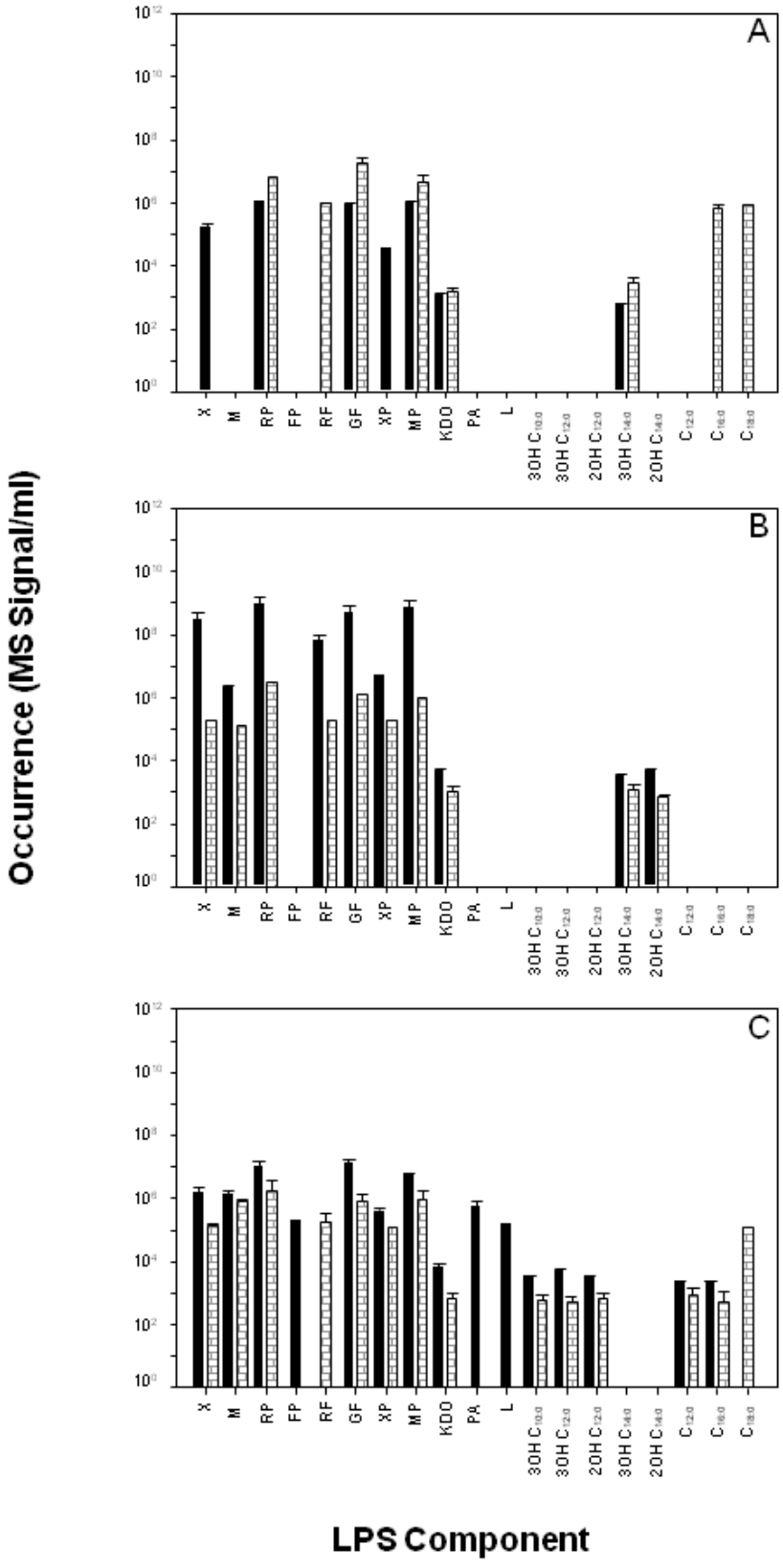

Figure 3. Selected LPS component distribution of *E. coli*, *P. aeruginosa*, and *S. enteritidis* grown in nutrient broth (■) and nutrient broth with added sodium benzoate as preservative (▭). The results are expressed as the MS signal/ml. X-axis abbreviations were explained in the legend of Figure 1.

Lipid A

Lipid A is the bioactive component of LPS that is also known as endotoxin, and its structure is fairly conserved among different pathogenic bacteria (Raetz and Whitfield, 2002). The presence of 3-hydroxytetradecanoic acid (3OH $C_{14:0}$) as a conserved feature of enterobacteria (Wilkinson, 1996) was observed for the *E. coli* and *S. enteritidis* strains tested. The toxicity of lipid A moieties depend on the number and the nature of fatty acids (Caroff et al., 2002). Numerous researchers have reported on changes in the Lipid A structure and configuration in different Gram-negative bacteria including *E. coli* and *Salmonella tyhimurium* (Van Alphen et al., 1979; Guo et al., 1997) and the effect thereof on the toxicity of the subsequent molecule. Considering the data presented in this study, it is evident that changes occurred in the lipid A moiety of all the organisms investigated. Octadecanoic acid ($C_{18:0}$) was detected in the membrane bound LPS or the liberated fraction (or both) in all the bacteria tested following exposure to pasteurization and UHT treatments. In the cases of *E. coli* and *P. aeruginosa* this fatty acid was also detected after treatment with sodium benzoate. $C_{16:0}$ is generally considered to be a characteristic component of *Salmonella* endotoxin, to a lesser extent of *P. aeruginosa* and absent from *Escherichia* (Kropinski et al., 1987; Karibian et al., 1993; Tanamoto and Azumi, 2000). The present study reported $C_{16:0}$ as a component of the LPS of *P. aeruginosa* and its presence was largely unaffected by exposure to the different treatments. This fatty acid did not form part of the LPS of the control *S. enteritidis* strain, but was detected in the membrane associated fraction after exposure to UHT treatment, as well as in *E. coli* cells cultivated in the presence of sodium benzoate. Lipid A of some pathogenic bacteria can be modified by the addition of a palmitoyl group suggested to result in a more tightly packed LPS, thereby increasing the integrity of the outer membrane (Guo et al., 1997; Guo et al., 1998; Trent, 2004). Strengthening of the outer membrane does not offer any explanation for the addition of $C_{16:0}$ to the LPS of *S. enteritidis* subjected to UHT treatment, since survival of the bacteria is unlikely. It is, however, tempting to speculate on the contribution it could make to the survival of *E. coli* in food products containing sodium benzoate as preservative, especially in sub-lethal doses. There is of course the necessity for viability correlation that cannot be provided at present.

O-chain

The polysaccharide division of LPS consists generally of two distinct regions, a core oligosaccharide containing 10 to 12 sugars, and a polysaccharide chain of repeating units, the O-specific chain. The core is covalently bound through an acidic sugar 3-deoxy-D-manno-oct-2-ulopyranosonic acid (Kdo) to the lipid A. The core oligosaccharide region of LPS displays conserved features and variations seem to reflect phylogenetic relationships (Wilkinson, 1996). Although most of the biological activities have been associated with the lipid moiety of the LPS molecule, Caroff and Karibian (2003) demonstrated that the role of the polysaccharide moiety is not negligible. Rocchetta et al. (1999) suggested a contribution to virulence by the saccharide portion of *P. aeruginosa* and Muroi and Tanamoto (2002) observed a similar trend in various *Salmonella* strains. It is evident from the results that numerous changes occurred in core saccharide distribution of LPS in *E. coli*, *S. enteritidis* and *P. aeruginosa* strains tested. No distinct trends were observed in the retention, shedding or complete absence of certain saccharides, but alterations unique to a specific strain or occurring in all three, were observed. Shedding of D-ribopyranose (RP) occurred in treated *E. coli* and *P. aeruginosa* cells while this saccharide was retained in *S. enteritidis* membrane fractions after both heat treatments. No distribution changes, only relative abundances, were noted for heat treated and sodium benzoate preserved *S. enteritidis* and *P. aeruginosa*, while liberation was evident in heat treated *E. coli* cells. D-fructopyranose (FP) was a saccharide component unique to *P. aeruginosa* and while there were no changes in distribution observed after pasteurization, this substituent was no longer part of the LPS make up of UHT or sodium benzoate treated *P. aeruginosa* cells. Amino acids that form part of the unique LPS of *P. aeruginosa* like L-leucine (L) was absent from all treated cells and similar results were evident for phenylalanine (PA).

The O-chain forms the basis of serotype classification and these chains protect the bacteria from the effect of numerous antibiotics (Banemann et al., 1998; Caroff et al., 2002). The variation in the sugar content are known to contribute to a wide variety of antigenic types of *Salmonella* and *E. coli* as well as several other strains of Gram-negative species (Lüderitz et al., 1982). The O-chain consists of repetitive subunits responsible for much of the immuno-specificity of the bacterial cell and can influence serum resistance through structure and chain length changes (Rocchetta et al., 1999; Caroff and Karibian, 2003). Similar to the saccharide and amino acids that form part of the core LPS, changes in the O-chain composition of heat and preservative treated *E. coli*, *S. enteritidis* and *P. aeruginosa* were also documented. D-xylose (X) no longer formed part of the membrane associated LPS of pasteurized *E. coli* or *S. enteritidis*, and disappeared from the *P. aeruginosa* LPS altogether after UHT treatment. α-D-xylopyranoside (XP) was not part of the LPS structure of *S. enteritidis* after heat treatments and was lost from UHT and sodium benzoate treated *E. coli*. D-galactofuranose (GF) is a constituent of the core and O-chain structure of all three organisms and its distribution and liberation was affected by the heat treatments. Occurrence of α-L-manno-

pyranose (M) was not influenced in treated *S. enteritidis* cells, but the distribution of this saccharide differed in the case of heat treated *P. aeruginosa*. Although it is understandable that the ability of foodborne pathogens to react and respond to changes in their surroundings is crucial for their survival, the effect of such changes in LPS composition, especially in the core oligosaccharides used for phylogenetic typing, cannot be overlooked. Rapid serotyping kits which assess LPS structure/presence for the verification of various foodborne bacteria are readily available and applied broadly. It can be argued that most of these techniques require culturing prior to analysis, which should allow cells to adapt their LPS structure. In the case of sodium benzoate or sanitizer treatment, however, sub-lethal doses of the preservative or cleaning agent will remain in the tested sample and will have an influence on the LPS structure. Arguably, since the LPS structure repair/reconfiguration time is yet to be determined, the possibility that preservation methods may influence accurate verification should be considered.

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