ENDOTOXIN RESIDUES IN FOOD A REVIEW

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ABSTRACT

The initial section of this manuscript focus on the ultra-structure of a unique class of heat stable cell-bound lipopolysaccharides (endotoxin) produced by Gram-negative bacteria. Subsequently, this paper summarises literature on the human body's response when challenged with endotoxins present in food and further explores the influence of food manufacturing and storage practices on endotoxin production and release by bacteria commonly isolated from food. Finally, this paper presents a brief description on the methods applied by the food industry to quantify endotoxins.

Keywords: Lipopolysaccharide, Endotoxin, Food Processing

1. INTRODUCTION

Gramnegative bacteria produce a unique class of heat stable cell-bound macromolecules termed endotoxins (Rietschel et al. 1996). Initial chemical analyses of endotoxins indicated that they consist of polysaccharide and lipid subunits, from which the term lipopolysaccharide (LPS) is derived (Shear and Turner 1943). Unlike exotoxins, endotoxins are associated with the outer layer of the cell wall and are released in large amounts when the cell lyse to elicit various potent biological activities (Rietschel et al. 1996). The main differences between endo- and exotoxins are listed in Table 1.

Table 1. Fundamental properties of exo- and endotoxins (Brock and Madigan 1991).

Property	Endotoxin	Exotoxin
Chemical	Endotoxins are released during cell lyses – heat stable	Excreted during cell growth – generally heat labile
Mode of action / symptomology	Fever, diarrhoea, vomiting	Either cytotoxin, enterotoxin or neurotoxin with defined specific action on cells or tissue
Toxicity	Weakly toxic	Highly toxic
Immunogenicity	Relatively poor immunogen; immune response not sufficient to neutralise toxin	Highly immunogenic; stimulate the production of neutralising antibodies
Fever potential	Pyrogenic, often produce fever in host	Do not produce fever in host

LPS further constitutes the minimal immunologically active structure of Gram-negative bacterial cell walls (Wakabayashi et al. 1994). When released, LPS exerts a powerful pathophysiological effect in higher organisms and thus represents important virulence factors of Gram-negative bacteria. With the aim of controlling the endotoxic effects of LPS in the human body, various research groups have attempted to characterise the nature of the biologically active region of LPS. Subsequently it was shown that the lipid fraction of LPS, termed Lipid A, constitutes its endotoxic principle (Galanos et al. 1985). Rietschel et al. (1996) further demonstrated that certain humoral host factors interact with Lipid A, and that specific cellular receptors recognise Lipid A or LPS. During the activation of the cells (signal transduction) in the context of endotoxicity, the formation and secretion of bioactive primary mediators (TNFα, IL-1,6,8) by human monocytes, macrophages and vascular cells follows (Loppnow and Libby 1989, 1990; Galanos et al. 1992). It was also noted that the physiological host response to LPS is mediated by secondary mediators (PG, Txb2, LTC4, PAF, C5a, O2, NO, Elastase) which possess intrinsic biological activities (Dinarello et al. 1984; Beutlur and Cerami, 1989). These mediators are required in small amounts for the proper functioning of the immune system and its battle with invading micro-organisms. If, however, they are produced excessively, they cause toxic effects such as pyrogenicity, leucopenia, disseminated intravascular coagulation, multi-

organ-failure and ultimately irreversible septic shock (Rietschel et al. 1996) (Figure 1).

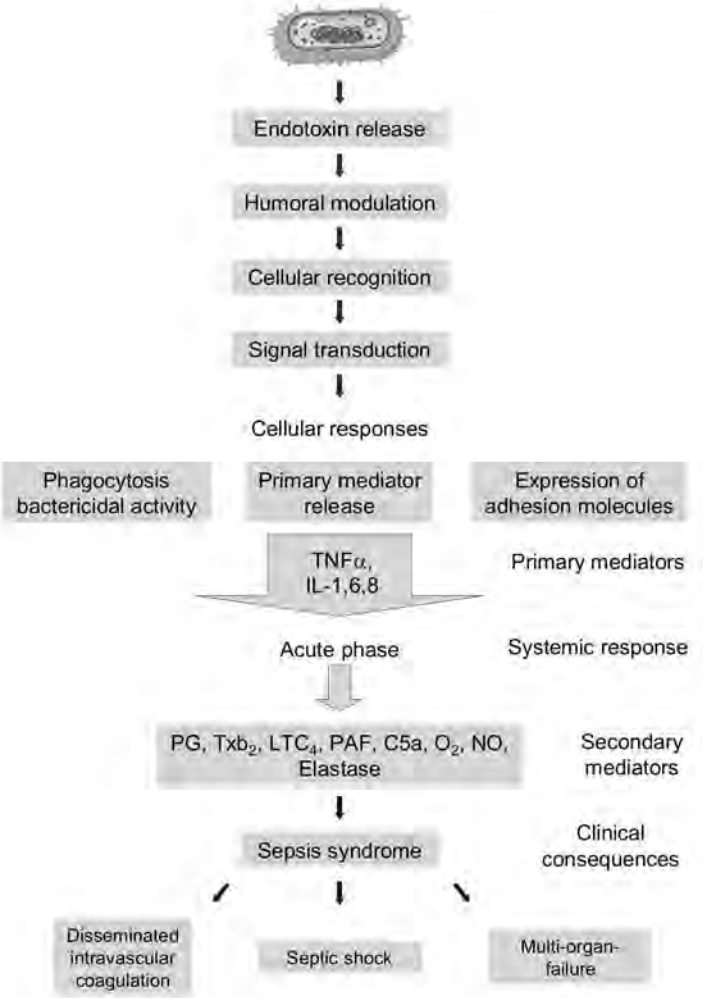


Figure 1. Intracellular pathways involved in endotoxin-induced signal transduction in monocytes (Rietschel et al. 1996).

This paper summarises the author's knowledge of host response when challenged with microbial endotoxins present in food. The author will also explore the influence of food manufacturing practices on endotoxin production and release by Gram-negative bacteria.

2. THE ENDOTOXIN

2.1 Structure

Numerous reviews on the composition of the LPS structure are available (Galanos et al. 1977; Wilkinson 1977; Lüderitz et al. 1982; Rietschel et al. 1982). According to Luderitz et al. (1982) the LPS of Salmonella is probably the most thoroughly investigated and typifies the architectural norm of these macromolecules. Within this LPS the polysaccharide portion consists of two regions, the O-specific chain and the core oligosaccharide that is covalently bound to the amphiphilic Lipid A region (Figure 2).

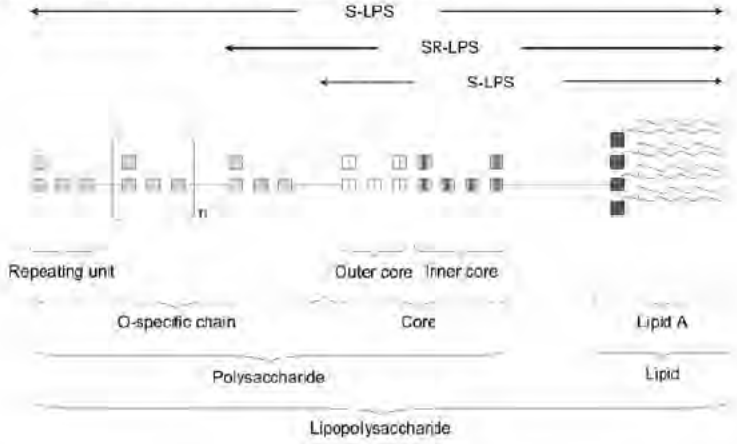


Figure 2. Schematic structure of a Salmonella LPS (Lüderitz et al. 1982; Rietschel et al. 1996).

2.1.1 O-specific chain and core

The O-specific chain is generally composed of repeating oligosaccharide units (Rietschel et al. 1996). These units are usually made up of different constituents yielding a heteropolysaccharide. In certain organisms such as Legionella pneumophila serotype 1, however, the repeating units may contain an oligomer of up to 75 residues of a single sugar (5 - acetamidino - 7 - acetamidino - 8 - O acetyl - 3,5,7,9 tetradeoxy D glycero L - galactononulosonic acid; legionaminic acid) constituting a homopolymer (Knirel et al. 1994; Zähringer et al. 1995). The O-chain further contains the immunodeterminant structures (S-LPS) against which the anti-O antibodies are formed during infection or upon immunization (Galanos et al. 1977; Wilkinson 1977; Lüderitz et al. 1982; Rietschel et al. 1982). The O-chain is in addition characteristic of and unique to a given LPS and its bacterial origin (serotype). Consequently, in nature there are as many unique

LPS molecules as there are bacterial serotypes.
As mentioned, some LPS contain only a single repeating unit of the O-polymer; they are denoted as SR-LPS, whilst others have unsubstituted cores (R-LPS) and may lack units of the core oligosaccharide (Ratledge and Wilkinson 1988). LPS extracted even from wild-type bacteria frequently consist of a mixture of S-, SR- and R-LPS. The core region is relatively more conserved than the O-chain (highly variable in composition, structure and degree of polymerization) and consists of the O-chain proximal outer core and the Lipid A proximal inner core (Holst and Brade 1992) (Figure 2). Sometimes common elements are recognised within the core of different bacterial species, such as the presence of the pyranosidic hexoses D-glucose, D-galactose and 2-amino-2-deoxy-D-glucose (Süsskind et al. 1995). The inner core of all LPS-expressing Gram-negative bacteria contains a 2-keto-3-deoxyoctunate (Kdo). This common core structure is conserved in many bacterial strains as shown in Figure 3.

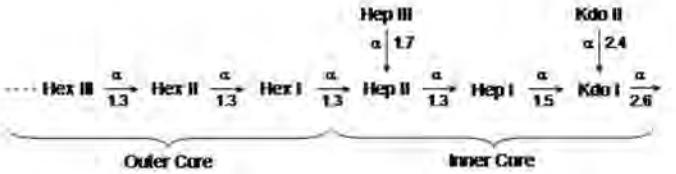


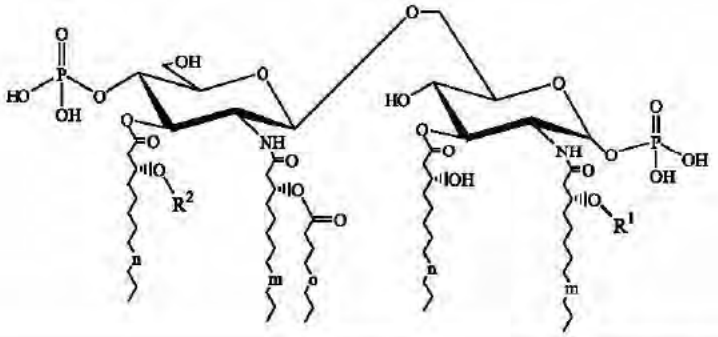
Figure 3. Common structure present in LPS core types of Escherichia coli and Salmonella enteritica. Hex, D-Glc, D-Gal, D-GlcNAc; Hep, L-glycero-D-manno-heptose; Kdo, 2-keto-3-deoxyoctulosonic acid. (Holst and Brade 1992).

2.1.2 Lipid A

The chemically most uniform part of the LPS is the Lipid A component (Rietschel et al. 1996). As mentioned the Lipid A has further been shown to constitute the endotoxic principle of LPS (Galanos et al. 1985). The fatty acid profiles of the LPS differ significantly from those of the phospholipids of the same species (Ratledge and Wilkinson 1988). Lipid A is not a normal glycerol lipid, but instead the fatty acids are connected by ester linkage to N-acetylglucoseamine (Figure 4). Fatty acids frequently found in the Lipid A are listed in Table 2 and include β-hydroxymyristic, lauric and palmitic acids (Ratledge and Wilkinson 1988).

Table 2. Fatty acid composition of Lipid A of selected Gram-negative bacteria (Ratledge and Wilkinson 1988).

Organism	3-Hydroxy fatty acids	2-Hydroxy fatty acids	Non-hydroxy acids
Escherichia coli	14:0 (60%)	-	12:0 (16%), 14:0 (20%), 16:0 (4%)
Salmonella spp.	14:0 (64%)	14:0 (5%)	12:0 (13%), 14:0 (11%), 16:0 (7%)
Shigella dysenteriae	14:0 (27%)	-	12:0 (10%), 14:0 (31%), 16:0 (13%)
Enterobacter cloacae	14:0 (52%), 13:0 (5%)	-	12:0 (4%), 14:0 (23%), 16:0 (3%)
Proteus mirabilis	14:0 (56%)	-	14:0 (35%), 16:0 (8%)
Yersinia pestis	14:0 (~75%)	-	16:0 (+)
Vibrio cholerae	14:0 (25%), 12:0 (36%)	-	14:0 (19%), 16:0 (16%), 16:1 (4%)
Pseudomonas aeruginosa	12:0 (34%), 10:0 (19%)	12:0 (14%)	12:0 (25%), 16:0 (6%)
Xanthomonas spp.	13:0 (17%), 12:0 (33%)	11:0 (6%)	11:0 (19%), 10:0 (8%)
Agrobacterium tumefaciens	16:0 (23%), 14:0 (77%)	-	(-)



	Nature of		Number of Carbon Atoms		
Organism	R^1	R ^{2}	m	n	o
Escherichia coli	H	14:0**	14	14	12
Haemophilius influenzae	H	14:0	14	14	14
Neisseria meningitidis	12:0*	H	14	12	12
Chromobacterium violaceum	12:0	H	12	10	12

*12:0, dodecanoic acid; **14:0, tetradecanoic acid

Figure 4. Chemical structure of the Lipid A component of various Gram-negative bacteria. (Rietschel et al. 1994; Zähringer et al. 1994))

2.2 Endotoxin - mode of action

A general overview on the host response when challenged with endotoxin is given in the remainder of this section.

2.2.1 Biological activity of endotoxin

Many papers describing the biological activity of endotoxin have been published during the last few decades and are reviewed by Rietschel et al. (1996). Included were comments that acute inflammatory response is not expected after the ingestion of food containing endotoxin. Several studies did however suggest otherwise. They emphasised that under naturally occurring enteroendotoxemic disease conditions in combination with thermal shock, alcoholic liver disease, and extra-intestinal invasion of Gram-negative bacteria, endotoxins readily enter the blood circulation from the intestinal tract to result in a similar biological response to when administered otherwise. Duncan and Morrison (1984) further report that bacterial endotoxin is selectively retained by macrophages and that post-phagocytic events that result in bacterial degradation are not accompanied by the degradation of endotoxin. Furthermore, although the endotoxin may be

modified by the macrophage, it retains its biological activity. As mentioned earlier, the resulting circulating endotoxin elicits cytokine-mediated host responses that include lethargy and hyperalgesia, as well as reduction in activity, grooming and feeding in test animals (Hart 1988; Kent et al. 1992). Results of several studies have also demonstrated a pronounced reduction in food intake after the administration of endotoxin (McCarthy et al. 1984, 1985; Langhans et al. 1990; Langhans et al. 1991; Langhans et al. 1993). Research on endotoxin associated-anorexia, anhedonia (inability to experience pleasure) and tissue damage has further emphasised the possible influence of this macromolecule on our daily life (van Deventer et al. 1993; Yirmiya 1996; Porter et al. 2000).

It is true that with an LD50 per mouse ranging from 200-400µg, endotoxin is not nearly as toxic as for instance the botulinum toxin (LD50 of 0.000025µg / mouse) (Brock and Madigan 1991). Intraperitoneally injected endotoxin at a concentration of 100µg / kg body wt is however sufficient to have an anorectic effect (Porter et al. 2000). At concentrations as low as 0.8ng / kg body wt, endotoxin administered to healthy human volunteers had no significant effect on water consumption, although food consumption was significantly reduced already in the first 0-4 hours after administration (Reichenberg et al. 2002). Again in human volunteers, endotoxin at a concentration of 0.4ng / kg body wt had a marked influence on nocturnal sleep patterns (Pollmacher et al. 1993). Low concentrations of endotoxin are also reported to influence verbal and non-verbal memory functions (Reichenberg et al. 2001).

2.2.2 Endotoxin toxicity

As mentioned, the biological activities of endotoxin are well understood (Rietschel et al. 1996). Pollmacher et al. (1996) however identified a significant difference in human host response to endotoxin based on the time that the host is challenged. In this paper Pollmacher et al. (1996) demonstrate that the time of challenge (with endotoxin) during the day does not affect the endotoxin-induced cytokine formation. However, subjects who received endotoxin in the evening, when endogenous glucocorticoid levels were low, showed about twice the increase in rectal temperature as those who received endotoxins in the morning, when endogenous glucocorticoid levels were high. In addition, certain sections of the community i.e. the elderly, the young and those with compromised immune systems are more susceptible to infectious disease. It is also suggested that ageing is associated with an altered response to endotoxins that includes initial hyperactivity, prolonged inflammatory activity and prolonged fever response (Krabbe et al. 2001). The reaction of infants (2 years and younger) to endotoxin is also significantly higher than that of adults and exposure results in an increased risk of repeated wheeze (Bolte et al. 2003).

3. ENDOTOXIN PRODUCTION

Bacterial growth and endotoxin production

When Gram-negative bacteria multiply or die endotoxin is released in the free form or complexed to the outer membrane protein A (Rietschel et al. 1996). Since the early 1970s several papers have appeared on endotoxin release. Organisms from the genera Salmonella, Escherichia, Pseudomonas, Neisseria and Yersinia and related endotoxin release during growth have been the main focus (Rietschel et al. 1996). The majority of the evaluations showed that endotoxin biosynthesis underlies quantitative differences in the growth phases, resulting in changes in the endotoxin content of the cells. Cells producing S-LPS show a significant decrease in the percentage of endotoxin produced during the exponential growth phase (Weber-Frick and Schmidt-Lorenz 1988). In addition, these cells exhibit temporary, complete stagnation in endotoxin formation during the lag and stationary growth phases. Endotoxin production by SR-LPS mutants differs from the former in that the rate of endotoxin synthesis, and with it the percentage endotoxin content of the cells, is already detectable after 8 hours of growth and increases greatly in the exponential growth phase (Bartkova 1986).

Environmental factors and endotoxin production

The LPS from many Gram-negative bacteria constitutes a heterogeneous mixture of different molecules of different polysaccharide length and with variable levels of substitutions at specific sites (McGroarty and Rivera 1990). The size heterogeneity of LPS from genera that include Pseudomonas, Serratia and Salmonella has further been reported to be altered by growth temperature. Cadieux et al. (1983) for example reported in the early 1980s that Pseudomonas grown at 19°C produced LPS with a decreased content of O-chain polysaccharide as well as with a difference in the hexosamine:hexose ratio compared to cells grown at 25°C. These temperature-induced changes in LPS heterogeneity further affect various cell surface properties including bacteriophage-inactivation capacity, efficiency in plasmid transformation and biofilm formation. The Lipid A fraction also exerts thermal adaptation with a decrease in dodecanoic and hexadecanoic acids and an increase in the level of 3-hydroxydecanoate and 2-hydroxydodecanoate as growth temperatures decrease (Kropinski 1987).

Altering growth conditions, such as medium composition, also modulates the LPS chain length of E. coli and alters the sensitivity of the cells to neutrophil bactericidal protein (Weiss et al. 1986). Further, growth of Pseudomonas and E. coli in low magnesium or adaptive growth in polymyxin or aminoglycosides alters the sensitivity of the cells to EDTA and polymyxin (McGroarty and Rivera 1990; Bhatti et al. 1999). Growth under magnesium-depleted conditions further results in the decreased production of O-chain material where bacteria grown under iron-depleted conditions shows only minor changes in LPS expression (Nelson et al. 1991). Nitrogen-deficient/high-carbon conditions also result in increased

expression of the O-chain. It is further noted that near growth-limiting conditions that include high temperature, high concentrations of salt, sucrose or glycerol, low phosphate concentration and low pH alters the size heterogeneity of the LPS produced (McGroarty and Rivera 1990).

4. ENDOTOXINS AND FOOD-BORNE PATHOGEN SURVIVAL

In an interesting paper, Cefali et al. (2002) speculates that Gram-negative bacteria produce LPS to survive within their environment and to resist macrophage attack. This statement is supported by several papers that elaborate on the role of LPS composition in the response of bacteria to external threats such as osmotic stress, temperature, various chemical compounds as mentioned in section 3.2. Currently little is known about the role of the LPS molecule in the survival of Gram-negative bacteria in food and the food processing environment. Bacteria grown on complex media under laboratory conditions do however provide some insight as to what may be expected when bacteria proliferates in food and related environments. For instance, significant changes in the steric structure and the quantity of LPS produced by E. coli are noted when cells are grown at sub-optimal growth temperatures in complex media, comparable to low temperature storage (Weber-Frick and Schmidt-Lorenz 1988b). Yersinia grown under similar conditions on solid media produces in addition more virulent LPS than when grown in a broth, comparable to solid versus liquid food (Bakholdina et al. 2001). It is further proposed by Bakholdina et al. (2001) that the composition of bacterial growth medium significantly influences bacterial virulence and that bacterial growth in complex media renders them more resistant to osmotic stress, comparable to different food types (Gauthier et al. 1989). With an increase in LPS production at sub-optimal growth temperatures it is not surprising that E. coli and Pseudomonas are found to be 50 times less susceptible to bactericidal agents than when grown at higher temperatures (Parte and Smith 1994). Hasegawa et al. (1996) furthermore demonstrated that a strong relation exists in drug-resistance patterns of clinical isolates of Pseudomonas aeruginosa and their O-chain length. Bacterial membrane and LPS response to environmental stress conditions is further emphasised in a paper by Cefali et al. (2002), who demonstrates that Pseudomonas (usually rod shaped cells) are capable of rearranging cellular and membrane components to attain a coccoid stress resistant form.

5. ENDOTOXINS IN FOOD

The quantity of endotoxin released during bacterial lyses and growth differs from bacterial strain to strain. In general, 0.1-1mg/kg cells (wet weight) endotoxin is released when bacterial cells rupture (Guard-Petter et al. 1999). Strains of E. coli release quantities of endotoxin amounting to 3,0 12,0ng/ml, for an inoculum of 1 3 x 108 CFU (Demonty and De Graeve 1982). Likewise a study by Cadieux et al. (1983) revealed that Pseudomonas aeruginosa liberates endotoxin at a constant rate during log-phase growth equivalent to 1.3 2.2ng/108 cells. With these quantities as the benchmark it could be calculated that endotoxin is liberated in

notable quantities in all food and dairy produce during normal Gram-negative bacterial growth and lyses (Table 3); hence the question, how does food processing and storage influence the quantity and virulence of endotoxin present and consequently the safety of food?

Table 3. Estimated endotoxin quantities in food and dairy produce.

Product	Quantity Escherichia coli isolated	Estimated average endotoxin content due to normal growth	Reference
Raw milk from street vendors	$1x10^5$/ml	0.0075ng/ml	Brayan et al. 1997
Ready-to-eat food	$1x10^5$/ml	0.0075ng/ml	Fang et al. 2002
Eggs	$2.7x10^6$/g	0.2025ng/ml	Imai 1980
Red meat	$1x10^5$/100cm^2	0.0075ng/ml	Gill et al. 1996
Raw milk from urban settlements	$1x10^9$/ml	75ng/ml	Lues et al. 2003

5.1 Endotoxins in the food industry

It is globally accepted that consumer food safety is non-negotiable. Therefore food safety is defined as "all conditions and measures that are necessary during the production, processing, storage, distribution and preparation of food to ensure that it is safe, sound, wholesome and fit for human consumption" (Miller 1984). The food industry is in constant battle with inevitable microbial contamination of raw material, processing machinery, workers, etc. and upholds the aforementioned conditions and measures by manipulating intrinsic and extrinsic factors in food products and the processing environment to be bactericidal or bacteriostatic.

The selection of food preservation methods is guided by many factors, the major one being that the preservation method should have as little impact as possible on the product character. To achieve this, a decision is made between thermal and non-thermal preservation or a combination of both (hurdle technology). Bacterial destruction in food resulting from high temperature or thermal treatment is well understood and readily cited (Hubbert et al. 1996). Consequent

endotoxin release during this process on the other hand is poorly understood. As well as this, reviews on non-thermal food preservation methods that include high hydrostatic pressure, pulsed electric fields, oscillating magnetic fields, light pulses, ionizing irradiation, the use of chemicals and bacteriocins as preservation aids, and hurdle technology, report on various kinetic models of bacterial destruction within both solid and liquid food during processing without mentioning much about endotoxin release (Barbosa-Canovas et al. 1997). Again a parallel needs to be drawn between food preservation processes and clinical information to highlight the importance of possible endotoxin release due to food preservation. The pharmaceutical industry currently invests more resources into quality control and product safety than the food industry. It is therefore not surprising that they have subsequently recognised endotoxin resulting from bacterial lyses in their products as a true threat to the consumer. Their exploration into sterilization methods that result in the least amount of endotoxin being released from intact bacterial cells recommended that non-bacteriolytic sterilization methods such as UV-irradiation should be the method of choice (Koga et al. 1992). It is not suggested that UV-irradiation should be the method of choice in food preservation but in the light of the facts as mentioned it is clear that the selection criteria for food preservations methods should consider endotoxin release in the destruction of Gram-negative bacterial cells.

5.2 Endotoxins in the dairy industry

The dairy industry is influenced by endotoxins in an even more pronounced way. Endotoxin induced mastitis (inflammation of the mammary gland of cows) results from both bacterial infection and free LPS. Infections with vegetative coliform occur frequently in cattle across the globe (Jones 1990). Consequent treatment of these infections constitutes administration of various bactericidal and bacteriostatic agents. Though the relief of symptoms is readily obtained, secondary inflammation with a more severe physiological response than the former sometimes occurs as a direct result of endotoxins liberated by lysed bacterial cells (Jones 1990). In addition, regular treatment of dairy cows with anti-microbial agents such as polymyxin, results in adaptive changes rendering the pathogens more resistant (McGroarty and Rivera 1990). As mentioned, such alterations are the consequence of changes in the outer membrane LPS which not only affects the permeation of these drugs but also causes the bacteria to be more virulent (Bakholdina et al. 2001).

In both the dairy and food industry the formation of biofilm in tubing and processing machinery is recognised as a notable product safety hazard. Biofilm formation is a complex process (O'Toole and Kolter 1988; O'Toole et al. 2000), whose initial step is the adhesion of bacteria to a surface (Marshall 1986; Rijnaarts et al. 1995, 1996). LPS play an important role in this process as it can establish strong short-range interactions with solid surfaces via the formation of hydrogen bonds (Williams and Fletcher 1996; Jucker et al. 1997; Jucker et al. 1998; Rocchetta et al. 1999). In addition, biofilm are considered to be highly resistant to antimicrobial agents (Spoering and Lewis 2001). Subsequently

machinery and CIP cleaning systems require several sanitation processes that usually include detergents, acids and oxidative agents to limit biofilm formation, again destroying bacterial cell walls to release endotoxin.

6. METHODS FOR ENDOTOXIN QUANTIFICATION IN FOOD AND DAIRY PRODUCE

Several methods have been developed during the last few decades for the quantification and qualification of endotoxins in both food and dairy products. Ironically these methods resulted from the quest to enumerate endotoxins as a rapid biomarker for Gram-negative bacteria and not for the sake of quantifying endotoxin. These methods could further be categorised under pyrogen detection, immunological, chromatographic and electrophoretic methods. For many decades the screening for endotoxin was accomplished by injecting the substance thought to contain endotoxin into rabbits. Consequent increase in rectal temperature constituted a positive test (Blechova and Pivodova 2001). Immunological test procedures and the more popular Limulus test, employing amoebocyte lysate (LAL) obtained from the horseshoe crab, gradually replaced the rabbit pyrogen method. The Limulus method is not only faster but proved to be more accurate and sensitive (Blechova and Pivodova 2001). Direct evaluation of the endotoxin concentration in dairy products with the Limulus test had already been successfully done in the early 1980s when (Hansen et al. 1982) succeeded in the detection of less than 1ng endotoxin / ml of milk. Applications for the LAL method grew to include many food and dairy products, as well as water. In poultry carcasses for instance this method succeeded in the detection of 20 Gram-negative cells per gram (Weber-Frick and Schmidt-Lorenz 1989). On the down side however, Weber-Frick and Schmidt-Lorenz (1988) has warned that alterations in the O-chain morphology (as discussed in section 3) result in variable accuracy of the LAL test when enumerating endotoxins. Consequently, gel-electrophoresis is also used to evaluate O-chain morphology (Hodgson et al. 1999) as well as for the detection of unique fatty acids originating from the Lipid A fraction, mass-spectrometry (Maitra et al. 1986; Venter et al. 1997). Finally, in the ongoing search for rapid endotoxin detection methods Venter and Lues (2003) established that capillary electrophoresis could be applied to detect endotoxin in food and to evaluate O-chain composition for etiological purposes.

7. CONCLUSION

From the cited literature it is clear that the survival strategy of Gram-negative bacteria is founded on physical and biochemical adaptation that coincide with the production and modification of the LPS macromolecule. The consequent modified endotoxin alters the bacterial virulence that subsequently increases the host response when challenged. If the host is immunocompromised, like the current circa 40 million people living with HIV in the world, or falls into the elderly or infant category of the population, the response is expected to be even worse. In addition, the majority of populations in third-world countries struggle daily with

issues that include food security, disease and war and in turn pay little attention to food safety. A high frequency of stress-related diseases on the other hand typifies survival in first-world countries. Subsequent alcohol and anti-microbial agent abuse and microbial invasion of the digestive tract open the portals for and increase the virulence of Gram-negative bacteria and/or endotoxin in the host. Stress related symptoms in many cases include anhedonia, amended sleep patterns and a reduction in food intake: all symptoms also induced by endotoxin. As luck would have it a busy lifestyle usually results in having ones main meal for the day in the evening the time when endotoxin induced-fever is most pronounced.

Endotoxins are as abundant and diverse as bacterial serotypes in nature and even more so where bacterial proliferation is sustained. Although no formal risk assessment has been conducted to date on endotoxins in food (especially in third-world countries), many groups across the globe have been quantifying Gram-negative pathogens in food and dairy produce for decades. Though not mentioned, the liberation of endotoxin by these pathogens into food, in turn, is inevitable and cannot be ignored. It is true that pathogenic vegetative bacterial cells pose a larger threat to human and animal health than free endotoxins. But, as mentioned, endotoxins present in food are mainly recognised as biomarkers and not for the threat they pose. Therefore the author is of the opinion that in compliance with food safety obligations, food producers, authorities and consumers should consider endotoxins in the development of food preservation processes, government policies (in first and third-world countries) and bio-security programs. The question is not whether endotoxins are toxic and present in food but whether endotoxin presence is caused by the diseases and processes of our time or whether it is a result of these. Fortunately all is not too grim: a recent paper by Starkie et al. (2003) provide the first experimental evidence that physical activity ("non-damaging" exercise) mediates anti-inflammatory activity and inhibits endotoxin-induced biological reactions. Sugiyama (1993) moreover shows that histatins (histidine-rich polypeptides) secreted in human saliva of healthy individuals, inhibit and reverse the anti-complement action of endotoxin. Therefore until the food and dairy industry respond to the threat of endotoxins the message should be conveyed that it is healthy in more than one way to chew food properly and to exercise regularly.

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