Adhesion of *Staphylococcus aureus* on stainless steel treated with three types of milk

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**A B S T R A C T**

*Staphylococcus aureus* has the ability to adhere and to form biofilm on inert surface such as stainless steel commonly used in food industry. The biofilm formed on the surface of milk processing equipments could be a source of dairy products contamination. This contamination causes a food poisoning. In this paper the *S. aureus* adhesion on stainless steel treated by three types of milk (ultrahigh-temperature (UHT)-treated milk; UHT skimmed milk, UHT semi-skimmed milk) was investigated.

Stainless steel was exposed to three types of milk with a different amount of fat component. Contact angles measurements were used to determine the surface physicochemical properties of substratum treated with the three milk products. The hydrophobicity and electron acceptor properties of stainless steel seem to be decreasing with the amount of fat component present in milk but its electron donor property increase with this component. The ability of *S. aureus* to adhere to stainless steel treated and untreated with milk was also examined. Treatment with the three types of milk reduces bacterial attachment. On treated substratum, the adhesion extent was affected by the type of milk and consequently by the amount of fat component. The lower and the higher adhesion were obtained when the steel was treated by the UHT semi-skimmed milk and UHT skimmed milk respectively. The correlation between physicochemical properties and *S. aureus* adhesion show that this latter was controlled by hydrophobicity and electron donor properties.

The findings of this work can contribute to develop strategies for prevent *S. aureus* adhesion on stainless steel and biofilm formation. Also they could be taken into account in cleaning and disinfection procedures.

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**1. Introduction**

*Staphylococcus aureus* is a gram positive which causes gastroenteritis resulting from the consumption of contaminated food. Food that is frequently responsible for staphylococcal food poisoning include meat products, poultry and eggs products, milk and dairy products. *S. aureus* is being occasionally found in food processing plant and have the ability to adhere to inert surface (Hamadi et al., 2005; Oulahal, Brice, Martial, & Degraeve, 2008) and consequently form biofilms (Marques et al., 2007).

Bacterial contamination adversely affects the quality functionality and safety of products by the dairy industry. When contamination of dairy products occurs evidence suggests that biofilm on the surface of milk processing equipment are a major source (Flint, Bremer, & Brooks, 1997; Koutzayiotis, 1992). As established biofilm are difficult to eradicate, prevention of the microbial adhesion is an alternative approach to control biofilm formation. Microbial adhesion to surface is one of the first stages in the development of biofilm. This process depends on cell surface properties, substratum surface properties and surrounding medium characteristics.

Milk is an example of heterogeneous soil, being a complex mixture of numerous types of proteins, fats, minerals and endogenous microorganisms of milk. In the food processing environment, it is accepted that microbial adhesion is generally preceded by the adsorption of organic and inorganic molecules forming a conditioning film. This latter alter the physicochemical properties of...
substratum surface (Chmielewski & Frank, 2003; Lorite et al., 2011). Better understanding and hence optimal control of biofilm problem seen best accomplished by focusing attention on the early events that take place on the food contact interface which lead to bacterial attachment and biofilm formation. So, a detailed description of the surface properties after conditioning with food such milk could assist in designing or modifying substrate inhibitory to bacterial adhesion. A large number of studies have assessed the effect of milk proteins on physicochemical properties especially hydrophobicity and on microbial adhesion. However, it has remained unclear the role of other components such as fats in adhesion process.

The mechanisms of attachment under different conditions need to be established. In particular the effect of conditioning layers of adsorbed organic as found in a food processing environment requires a deeper study and the role of this film in bacterial adhesion need to be expended. However the significance of our study originates from the fact that we employed essentially three types of milk with a different amount of fat component. Therefore, the purpose of this study was to evaluate the physicochemical properties of stainless steel coated with three milk types differing in amount of fat components at 25 °C. The study of S. aureus adhesion to substratum under these conditions was another objective of our works. Moreover the role of substratum properties in the adhesion process was analyzed. Stainless steel was selected in this work because it has been the most widely used material in the construction of food processing equipments due to its mechanical strength, corrosion resistance and its resistance to damage caused by the cleaning process.

2. Materials and methods

2.1. Bacterial strains, growth conditions and preparation of microbial suspension

The bacterial strain used in this study was S. aureus ATCC 25923. The strain was cultured in Luria Bertani broth at 37 °C for 24 h. After culture, the cells were harvested by centrifugation for 15 min at 8400 × g and were washed twice with and resuspended in KNO3 solution with ionic strength 0.1 M. The physicochemical properties of this strain were measured by contact angle measurements. The results were published previously (Hamadi & Latrache, 2008).

2.2. Cleaning of stainless steel coupons

The solid support selected for this study was stainless steel 304. Before being coated with milk, the sample was cut into 1 cm × 1 cm coupons and cleaned by soaking for 15 min in 70% (Vol/Vol) ethanol solution. The coupons were then rinsed with distilled water and autoclaved at 120 °C for 15 min.

2.3. Treatment of stainless steel coupons with milk

The cleaned stainless steel was placed into a Petri dish and 10 ml of following milk types: ultrahigh-temperature (UHT)-treated milk; UHT skimmed milk, UHT semi-skimmed milk was added. The sample was allowed to contact milk for 3 h at 25 °C. After contact time, the coupons steel was rinsed three times with distilled water. The composition of the three types of milk is presented in Table 1. These types of milk don’t have the same amount of fat component as reported in Table 1.

2.4. Contact angle measurements

Contact angle measurements were performed using a goniometer (GBX instruments, France) by the sessile drop method. One drop of a liquid was deposited onto a dry stainless steel uncoated and coated with milk. Three to six contact angle measurements were made on substratum surface for all probe liquids including water, formamide and diiodomethane.

The Lifshitz-Van der Waals ($\gamma_{lw}$), electron donor ($\gamma_-$) and electron acceptor ($\gamma_+$) components of the surface tension of bacteria and for stainless steel were estimated from the approach proposed by Van Oss (Van Oss, Good, & Chaudhury, 1988). In this approach the contact angles ($\theta$) can be expressed as:

$$\cos \theta = -1 + 2 \sqrt{\frac{\gamma_{lw} \gamma_{iw}}{\gamma_{lw} \gamma_{iw} + \gamma_{lw} \gamma_{L} + \gamma_{iw} \gamma_{L}}}$$

The Lewis acid-base surface tension component is defined by:

$$\gamma_{LB}^{AB} = 2(\gamma_{lw} \gamma_{S})^{1/2}$$

The surface hydrophobicity was evaluated through contact angle measurements and by the approach of Van Oss (Van Oss, 1995; Van Oss et al., 1988). In this approach, the degree of hydrophobicity of a given material ($\delta$) is expressed as the free energy of interaction between two entities of that material when immersed in water ($\omega$): $\Delta Giwi$. If the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic ($\Delta Giwi < 0$); conversely, for a hydrophilic material, $\Delta Giwi > 0$. $\Delta Giwi$ is calculated through the surface tension components of the interacting entities, according to the following formula:

$$\Delta Giwi = -2 \gamma_{lw}$$

$$= -2 \left[ \left( \left( \gamma_{iw} \right)^{1/2} - \left( \gamma_{lw} \right)^{1/2} \right)^2 + 2 \left( \gamma_{lw}^2 - \gamma_{iw} \gamma_{lw} \right) \right]$$

2.5. Adhesion experiments

Ten millimetres of bacterial suspension containing $10^5$ CFU ml$^{-1}$ was incubated in a Petri dish containing stainless steel coupons treated by milk for 3 h at 25 °C. After 3 h of incubation at 25 °C, the coupons were then rinsed three times with sterilised distilled water to remove the nonadhering bacteria. The coupons were immersed in a test tube containing physiological water (NaCl: 9 g/l). Bacterial cells were detached from the inert support by using a sonication bath (ultrasonic) for 5 min. CFUs were counted by using the serial dilution technique of the bacterial suspension obtained after sonication. Counts were determined on Luria Bertani agar after incubation for 24 h at 37 °C. Each experiment was performed in triplicate.

3. Results

3.1. Physicochemical characteristics of stainless steel treated and untreated with various types of milks

Table 2 presents the surface physicochemical characteristics of stainless steel before treatment with various types of milk. Water
contact angle can be used as a qualitative indication of the cell surface hydrophobicity (Oliveira, Azeredo, Teixeira, & Fonseca, 2001). According to Vogler (1998), hydrophobic surface exhibits water contact values higher than 65°; lower values indicate hydrophilic characteristics. Based on the approach of Van Oss (1997), it is possible to determine the absolute degree of hydrophobicity of any substance (I) vis-à-vis water (w), which can be accurately expressed in internationally applicable system of units. According to the approach of Van Oss, the stainless steel surface was hydrophilic (ΔG > 0) and it was predominantly electron donors (high value of γ⁺) with low electron acceptor (low value of γ⁻).

The surface tension values have been measured after treatment of the samples with three types of milk at 25 °C. The results are presented in Table 2. A large change of physic-chemical properties was observed regardless of the type of milk. Important variations in electron donor were detected after contact of the steel with various types of milk. Whatever, the treatment conditions are, the electron donor for stainless steel decrease after the treatment. The level of this reduction depends on the type of milk used for treatment. It is very higher when the sample is treated with UHT milk. Conversely slight changes were observed for electron acceptor characteristics after treatment with three types of milk. The level of these characteristics after treatment seem to be depend on the amount of fat component contained in the milk. The electron donor and the electron acceptor of steel after treatment increase and decrease respectively with the decrease of the amount of fat component (Table 2). The results also, show that the steel surface hydrophobicity decrease after treatment with different milk. After treatment, the stainless steel hydrophobicity decreases with the amount of fat component (Table 2). Finally, we can suggest that there is a parallelism between the amount of fat component of milk and all physicochemical properties of stainless steel.

3.2. Influence of different types of milk on S. aureus adhesion to stainless steel

Fig. 1, shows the adhesion of S. aureus on steel both treated and untreated with different types of milk. We observed that S. aureus adheres readily on substratum whatever the conditions of work. Treatment of stainless steel with the three types of milk inhibits S. aureus adhesion. This inhibition was greater when the substratum was treated with semi skimmed milk. On the treated substratum surface, the total number of adherent bacteria depends on the type of milk. The adhesion is maximal on the steel treated with a lower amount of fat component (skimmed milk) whereas it is minimal when it treated with a moderate amount of this component (semi skimmed milk) (Fig. 2).

Based on the results obtained here we suggest that the reduction and the augmentation of adhesion could be dependent on the amount of fat component.

4. Discussion

Milk is a highly perishable commodity which can be frequently in contact with surface equipment during its processing and storage. In the food processing environment, surface is rapidly coated with an organic conditioning film (Chmielewski & Frank, 2003). This film can occur within seconds of exposure to an aqueous environment (Mitteleman, 1998). In dairy operations this film might consist of proteinaceous components of milk and milk products (Mitteleman, 1998). Once a material surface is exposed to an aqueous medium with nutrients, its interfacial properties are often modified by the surrounding fluid through the adsorption of organic compounds (Fletcher, 1996; Frank & Chmielewski, 2001; Marshall, 1996; Shakerifar, 2010; Sheng, Ting, & Pekonen, 2008; Somers & Wong, 2004).

In the first part of this work our objective was to determine the physicochemical properties of stainless steel and glass treated and untreated with three types of milk (UHT milk, UHT skimmed milk, semi UHT skimmed milk). Our data showed an important modification of the surface properties of stainless steel after treatment with three types of milk. Several works (Bower, McGuire, & Daesel, 1996; Dat, Hamanaka, Tanaka, & Uchino, 2010; Rubio et al., 2002; Szlavik et al., 2012; Yang, McGuire, & Kolbe, 1991) have reported that milk and milk compounds alter substratum properties. Yang et al. (1991) used contact angle methods to measure the change in hydrophilic—hydrophobic balance exhibited by a number of different materials following adsorption of β-lactoglobulin. They found that adsorption of β-lactoglobulin rendered hydrophilic surfaces more hydrophobic and hydrophobic surfaces more hydrophilic. Rubio et al. (2002) reported that the adsorption of bovine serum albumin (BSA) leads to important variations in the electron donor characteristics of stainless steel and chromium surfaces. Szlavik et al. (2012) demonstrated that the electron donor property increases and the hydrophilic character decreases after coating glass with homogenized milk. In this study the use of the three types of milk with different amount of fat component, allowed us to evaluate the role of fat component in physicochemical properties of substratum. We concluded that lower hydrophobicity and higher electron donor property for steel could be due to a lower amount of fat component. In contrast, higher electron acceptor property could be related to a higher amount of fat component. However, the
modification observed on Stainless steel properties after treatment with all types of milk might be due to milk components including proteins and fat adsorbed on these substrata. As reported previously (Rosmaninho et al., 2007) the adsorption of milk compounds follow different process and different kinetics. Others works found that the amount and the structure of adsorbed proteins depend on the nature (Rubio et al., 2002) and the surface energy of substrate (Rosmaninho et al., 2007). On the other hand, Rubio et al., 2002 found a modification in the structure of the proteins adsorbed on the surface. So, the variation observed in the level of physico-chemical properties could be related to the type and the kinetics of adsorbed component on the surface.

In the second part of this study, adhesion of S. aureus was investigated on stainless steel treated and untreated with the three types of milk. The results show that S. aureus adheres readily on clean substratum (untreated). These findings are similar to those obtained by others authors (Hamadi et al., 2005; Herrera, Cabo, Gonzalez, Pazos, & Pastoriza, 2007; Marques et al., 2007; Oulahal et al., 2008). After treatment of stainless steel, the extent of adhesion decreases with the milk whatever the type of this milk. Conflicting results about the role of milk or milk components on bacterial adhesion are available in literature. Some works (Al-Makhlafl, McGuire, & Daeschel, 1994; Barnes, Lo, Adams, & Chamberlain, 1999; Flint, Plamer, Bloemen, Brooks, & Crawford, 2001; Helke, Somers, & Wong, 1993; Pakar, Flint, Palmer, & Brooks, 2001; Rubio et al., 2002; Wong, 1998) found that bacterial adhesion was inhibited or reduced by preconditioning with whole milk or milk components (proteins). However, in the presence of whey proteins, an increase in the attachment of several milk-associated microorganisms to stainless, rubber and glass surfaces was observed by Speers and Gilmour (1985). Other researches (Peng, Tsai, & Chou, 2001) found that there is no difference in the number of spores adhering to stainless steel in the presence of whole milk and diluted milk. The apparent conflict in these reports and in our work may be related to the type of milk used, the type of strains studied and the nature of substratum.

The studies realized on the effect of milk components on bacterial adhesion were focused on the effect of proteins and they reported that these components could reduce or augment bacterial adhesion. The obtained results here show that, the fat component could also reduce bacterial adhesion. Finally we can conclude that it is difficult to establish a relationship between bacterial adhesion and the effect of milk components.

It is well known that bacterial adhesion to inert surface depends on both bacterial cell and substratum surfaces properties such as hydrophobicity, surface charge and electron donor-electron acceptor. In order to get insight into the adhesion process of S. aureus to substrate we have examined the role of physicochemical properties in this process. While a positive correlation between hydrophobicity, electron donor/electron acceptor properties and bacterial adhesion was previously reported (Hamadi et al., 2005, 2008; Henriques, Azeredo, & Oliveira, 2004; Silva, Teixeira, Oliveira, & Azeredo, 2008), other researchers were unable to establish a relation between bacterial adhesion and physicochemical properties (Flint et al., 1997; Oliveira et al., 2006; Teixeira, Silva, Aradjo, Azeredo, & Oliveira, 2007). Our results show that a good correlation was obtained between S. aureus adhesion on Stainless steel and its hydrophobicity (Fig. 2a), its electron donor (Fig. 2b).

These findings suggest that S. aureus adhesion on Stainless steel could be controlled by hydrophobicity and electron donor properties.

5. Conclusion

An understanding of the effect of film formed on Stainless steel, commonly used in food processing environment, is essential in order to find ways to prevent contamination and to develop strategies. In this regard, the results of this study indicate that treatment of substratum with three types of milk differing in the amount of fat component alter strongly their properties and reduces S. aureus adhesion. The steel hydrophobicity decreases with the amount of fat component. Also, the extent of adhesion on treated substratum was influenced by the amount of fat component present in the milk. Thus fat component should be considered as an important factor which could affect bacterial adhesion and this requires a further study in order to understand its role in microbial adhesion. Finally, the results obtained here show that it is necessary to take into account the fat component in cleaning and disinfection procedures.

References


