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# Study of conditioning film of 316L stainless steel treated with two types of UHT milk using contact angle method and infrared spectroscopy

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**Abstract:** Surface coating during the manufacturing process has a significant impact on the effectiveness of cleaning and disinfection protocols and can promote biofilm development. The objective of this work was to study the influence of two milks (skimmed and whole milk) on the stainless steel 316L surface using two methods; contact angle and infrared spectroscopy. The surface properties of the support (Lifshitz-Van Der Waals component ($\gamma^{LW}$), electron donor ($\gamma^{-}$) and electron acceptor ($\gamma^{+}$)) were determined by the contact angle method. The constituents included in UHT milk and which cover the surface are revealed by the infrared spectroscopy. The results showed that the hydrophobicity of stainless steel 316L changes after treatment with either skimmed or whole UHT milk. Infrared spectroscopy showed that the biological elements fouling the steel surface were mainly lipids, proteins, and traces of fat, water and lactose.

**Keywords:** Conditioning film; Infrared spectroscopy; Contact angel; Surface properties

## 1. Introduction

In food processing environment, it is admitted that microbial adhesion is generally preceded by the adsorption of organic and inorganic molecules forming a conditioning film on equipment surfaces. The conditioning film provokes changes in the physicochemical properties of substrate surface (Chmielewski and Frank., 2003, Lorite *et al.,* 2011, Tankiouine et *al.,* 2020, Elfazazi et *al.,* 2021). Various techniques have been adopted to understand the mechanisms leading to the development of microbial adhesion in the industrial environments. Thus, many prevention and control strategies have been implemented to eliminate biofilms. This was carried out by the implementation of good hygienic practices, the hygienic design of equipment through the choice of materials and shapes, the selection of detergents and disinfectants, adequate hygiene procedures as well as systematic cleaning procedures.

Cleaning must remove all dirt from the surfaces of the equipment. Many works are interested in preventing or limiting biofouling on various surfaces. The modification of surface properties is therefore an appropriate way to achieve better fouling management. Most of them concern coatings of various materials, which can be roughly classified according to their wettability and which address the fouling problem by different mechanisms (Tankiouine et al., 2018, Patil et al., 2015) . First, hydrophilic coatings such as polymer brushes or hydrogels involving neutral (Caro *et al*., 2009, Khalil and Sonbol, 2014, Liu *et al*., 2019) or charged (Brady *et al*., 2000, Dat *et al*., 2010, Wu *et al*., 2015) polymers that are highly hydratable have excellent protein repellent properties. Their ability to tightly bind water molecules in well-organized structures allows them to act as a barrier and protect the substrate from protein adsorption (Krishnan *et al*., 2008, Mérian and Goddard, 2012): On the other hand, hydrophobic surface modifications, which usually involve silicone-based compounds (Dabagh *et al*., 2005, Liu *et al*., 2010) or fluorinated compounds (Barish and Goddard, 2013, Grimm *et al*., 2014, Oldani *et al*., 2015).

Spectroscopic methods are widely used for the qualitative and quantitative analysis of constituents of agri-food products. The coupling method of MIR (mid infrared spectroscopy) spectroscopy and PLS (Partial Least Squares Regression) is also applied, to determine the fatty acid profile in milk (Ferrand *et al*., 2011). The objectives of this study are, on the one hand, to contribute to the understanding of interracial phenomena, governing the biocontamination of materials used in the dairy industry, and to evaluate the impact of the pretreatment of various supports by UHT milk (whole, skimmed) on the surface properties of materials commonly used in the food industry.

Decantation is a process to separate mixtures by removing a liquid layer that is free of a precipitate. Decantation relies on gravity to pull precipitate out of solution. Decantation consists in eliminating sediments, turbidity and suspended solids in untreated sewage by sedimentation. This process is present in many industrial applications such as the sugar industry, mining industry and wastewater treatment.

## 2. Methodology

### *2.1. Cleaning of coupons surfaces*

The solid support selected for this study was the 316L stainless steel. Before being coated with milk, the stainless steel was cut into 1 $cm^2$ coupons and soaked for 15 min in 70% (Vol/Vol) of ethanol solution. The coupons were then rinsed with distilled water and autoclaved for 15 min at 120 °C.

### *2.2. Treatment of stainless-steel coupons with milk*

After being cleaned the stainless steel coupons were pot in Petri dishes with 10 ml of each milk (skimmed and whole milk) for 3h at 25°C. Then, the coupons have been recovered and rinsed three times with distilled water.

### *2.3. Contact angle measurements*

The hydrophobicity of stainless steel was estimated by contact angle with water (θw). The Lifshitz–van Der Waals ($\gamma^{LW}$) component, the electrons donor ($\gamma^-$), the electrons acceptor ($\gamma^+$) and all surface energies of stainless steel were also assessed by contact angle using the approach proposed of (van Oss *et al.,* 1988). In this approach, the contact angle θ, measured with a pure liquid (L) is expressed in the equation1:

$$\cos\theta = -1 + 2\frac{\sqrt{\gamma_S^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}}{\gamma_L} \qquad \text{Eq. 1}$$

The surface hydrophobicity was evaluated through contact angle measurements and by the approach of Van Oss (Oss, *et al.* 1988). In this approach, the degree of hydrophobicity of a given material is expressed as the free energy of interaction between two entities of that material when immersed in water (w): $\Delta G_{iwi}$. If the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic ($\Delta G_{iwi} < 0$), conversely, for a hydrophilic material, $\Delta G_{iwi} > 0$ (Elgoulli, *et al.* 2021; Bennouna, *et al.* 2022). $\Delta G_{iwi}$ is calculated through the surface tension components of the interacting entities, according to the following formula Equation 2:

$$\Delta G_{iwi} = -2\gamma_{iw} = -2\left[\left((\gamma_i^{LW})^{\frac{1}{2}} - (\gamma_w^{LW})^{\frac{1}{2}}\right)^2 + 2(\gamma_i^{+}\gamma_i^{-})^{\frac{1}{2}} + (\gamma_w^{+}\gamma_w^{-})^{\frac{1}{2}} - (\gamma_i^{+}\gamma_w^{-})^{1/2} - (\gamma_w^{+}\gamma_i^{-})^{1/2}\right] \quad \text{Eq. 2}$$

### *2.4. Surface analysis by infrared spectroscopy (MIR)*

A Bruker "Vector 22" spectrophotometer instrument equipped with a DTGS detector, a Globar source (IR) and a KBr Germanium separator, was used to record the MIR spectra of 316l stainless steel supports treated with two types of UHT milk. The samples were scanned 98 times in the spectral range of 4000 $cm^{-1}$ to 600 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The reference was measured before each sample analysis. The 316l stainless steel surfaces are placed directly on the central surface of the diamond. Measurements are made at room temperature. The software used in this study allows the automatic acquisition of spectra without any form of computer manipulation that can affect the quality of the results. The Fourier transform is automatically calculated by the software before the spectra are acquired. The ATR plate is cleaned with an ethanol solution, which allows the ATR to dry. The cleanliness was verified by taking a reference spectrum and comparing it with the previous reference spectrum.

## 3. Results and discussion

### *3.1. Physicochemical characteristics of stainless steel treated and untreated with different types of milks*

The treatment of the 316l stainless steel surface with milk changes the degree of hydrophobicity of the surface both qualitatively and quantitatively. Based on the qualitative approach, the contact angle relative to the water gives us information on the hydrophobicity, which changes completely when the surface is treated with skimmed or whole milk **Table 1**.

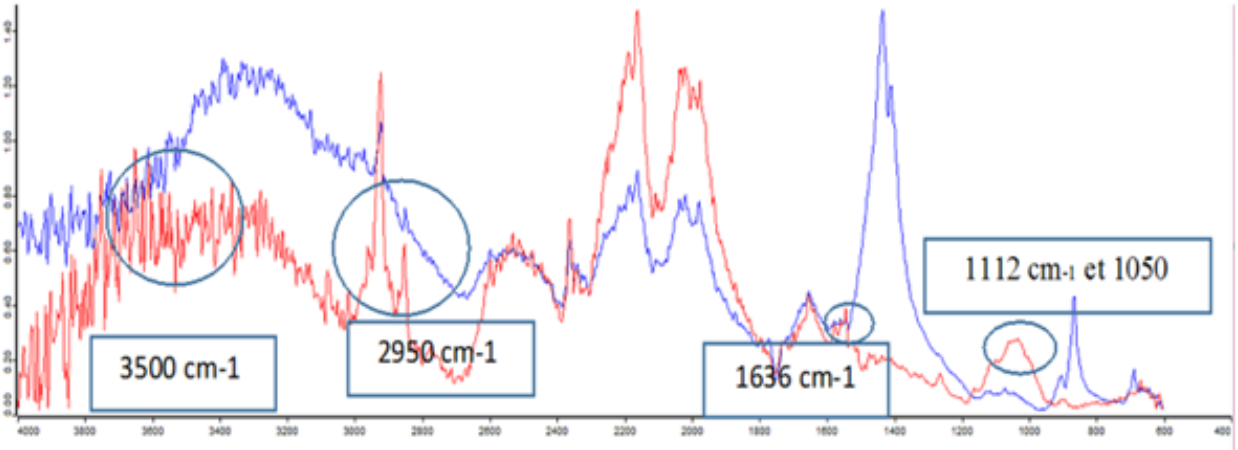
**Table 1.** Surface tension components and free energy interaction of stainless steel treated and no treated with various milks.

| **Samples** | **Contact angle (°)** | | | **Surface tensions (mJ/m²)** | | | **$\Delta G_{iwi}$ (mJ/m²)** |
|---|---|---|---|---|---|---|---|
| | **Θ Diiodomethane** | **Θ Formamide** | **Θ Water** | $\gamma^{LW}$ | $\gamma^{+}$ | $\gamma^{-}$ | |
| **316l untreated** | 60 | 69 | 62 | 27 | 0.3 | 27.8 | 3.6 |
| **316l whole** | 76.1 | 59.7 | 82,6 | 19.5 | 4.3 | 3.9 | -36.5 |
| **316l skimmed** | 73.7 | 25.2 | 85,9 | 20.9 | 0.7 | 7.2 | -39.5 |

The results presented in (Table 1) show also that stainless steel 316l had a very remarkable hydrophilicity (ϴ= 62°<65°). The treatment of the surface by skimmed UHT milk and whole UHT milk for 3 hours, completely reverses its hydrophobicity where it becomes relatively hydrophobic (ϴ= 85.9°>65°, ϴ= 82.6°> 65°). The contact angle method gave us very detailed results in terms of hydrophobicity and electrons donor/acceptor properties for the 316l stainless steel support, treated and untreated with both types of milk (skimmed milk and whole milk). The hydrophobicity changes significantly depending on the type of milk involved. In industry, especially dairy, it is accepted that microbial adhesion is affected by organic and inorganic molecules that adsorb and form a conditioning layer and changes the physicochemical characteristics of the carriers (Chmielewski and Frank, 2003, Lorite *et al.,* 2011). These films are composed by molecules of dairy origin where the most notable are proteins, fats and carbohydrates (Mittelman 1987) Thus, many works (Fletcher 1996, Fard 2010, Frank and Chmielewski, 2001, Eileen *et al.,* 2004) have affirmed that once the organic molecular compounds are in contact with the supports subject to microbial adhesion, their physicochemical characteristics are modified by adsorption and fixation (by low energy bonds) of these chemical compounds.
Food-equipment contact is unavoidable in the food industry. Milk is an easily perishable product due to contamination. In the dairy industry, the formation of biofilms on processing equipment is very common. According to (Chmielewski and Frank, 2003), the chemical constituents of food are easily and quickly adsorbed on the surfaces of the equipment. Many studies (Fletcher 1996, Fard 2010, Frank and Chmielewski, 2001, Eileen B *et al.*, 2004, Sheng Ting and Pehkonen, 2008, Tankiouine et al., 2020) have reported that in the dairy industry, once a material is in contact with processing equipment, the equipment acquires new energy properties. Our results showed a very significant change in the hydrophobicity of 316L steel. In the literature it is widely reported that milk modifies the surface properties (Dat *et al.,* 2010, Bower *et al.,* 1996, Rubio *et al.,* 2002, Szlavik *et al.,* 2012). Yang *et al.* (1991) reported that adsorption of β-Lactoglobulins from milk makes a hydrophilic surface more hydrophobic and a hydrophobic surface more hydrophilic. In our results, we noted a change in the hydrophobic character of 316L stainless steel towards a more hydrophilic character. This is probably due to the adsorption of β-Lactoglobulins on the surface of 316L stainless steel. The electron donor property of stainless steel surfaces and chromium also changes after adsorption of bovine serum albumin (Rubio *et al.,* 2002). In addition, it has been reported that contact between homogenized milk and the glass surface makes the glass surface more electron donor and less hydrophilic (Szlavik *et al.,* 2012). In this study, we studied the effect of two milks with different fat concentrations, which allows us to conclude the effect of lipids on the surface properties of steel. The results showed that the traces of fat influence the surface properties of steel, which is in accord with the work of (Hamadi *et al.,* 2014) who found that the surface properties of steel are strongly influenced by the concentration of milk fat. Many works have reported that hydrophobicity is related to the concentration of protein or carbon in the form of hydrocarbon compounds, while hydrophilicity is related to the presence of oxygen and polysaccharides exposed on the surface (Latrache *et al.,* 2002, Cowan *et al.*, 1992, Cuperus *et al.*, 1993, Dufrêne and Rouxhet, 1996). Beyond that, the results obtained can be explained by the intense adsorption of milk proteins and sugars. There were those who detected changes in the structures of the adsorbed proteins (Rubio *et al.,* 2002). On the other hand, this is probably due to the fact that the adsorption followed different processes (Rosmaninho *et al.*, 2007). Other work has reported that the structure of adsorbed proteins depends on the nature (Rubio *et al.*, 2002) and surface energy of the substrate (Rosmaninho *et al.*, 2007) . Thus, the quantities and structure of the adsorbed compounds (proteins and carbohydrates) is probably at the origin of the physicochemical changes observed in the 316L stainless steel surface.

*3.2. Physicochemical characteristics of stainless steel treated and untreated with different types of milks using infrared spectroscopy*

All the spectra are characterized by several bands characteristic of the interaction of infrared radiation with the different milk components (Figure1). We noticed the appearance of bands corresponding to the hydroxyl groups [OH], located around 3500 $cm^{-1}$ and 1636 $cm^{-1}$. The elongation vibrations which are asymmetrical at 2955 $cm^{-1}$, 2925 $cm^{-1}$, 2872 $cm^{-1}$ and 2854 $cm^{-1}$. These vibrations, which are characteristic only of trace amounts of fat, are attributed to the [C-H] bonds of the methyl [-$CH_3$] and methylene [-$CH_2$] (groups.




Stainless steel 316l untreated
Stainless steel 316l treated with skimmed UHT milk

**Figure 1.** Mean infrared spectra of 316L steel before and after treatment with skimmed UHT milk.

In agreement with (Rosmaninho *et al.*, 2007), strong absorptions were observed at 2900 $cm^{-1}$ and 2800 $cm^{-1}$, respectively, corresponding to elongation vibrations of the [C-H] bond of the [CH2] and [CH3] groups. The two bands, located around 1650 $cm^{-1}$ and 1545 $cm^{-1}$, are particularly characteristic of the amide functions of proteins. The first characteristic band of the amide function I, concerns the elongation vibrations of the [C=O] bond conjugated with the deformation vibrations of the [N-H] bond. Whereas the second band, which concerns the elongation vibrations of the [C-N] bond conjugated to the [N-H] bond deformation vibrations, is characteristic of the amide function II of proteins (Lerma-García *et al.,* 2010). The bands at about 1112 $cm^{-1}$ and 1050 $cm^{-1}$ are dominated by [C-O] bond elongation vibrations (Reynolds *et al.,* 2006).

The results of the infrared spectrum transformed in an oven show that INOX 316L allows to discern elongation vibrations at 2955 $cm^{-1}$, 2925 $cm^{-1}$, 2872 $cm^{-1}$ and 2854 $cm^{-1}$ when treated with whole UHT milk (Figure2), in the first part of the spectrum. These vibrations, characteristic of the fat, are attributed to the [C-H] bonds of the methyl [-$CH_3$] and methylene [-$CH_2$] groups. The spectral band at 1746 $cm^{-1}$ is also dominated by the reactivity of the [C=O] bond of the ester function (Karoui and De Baerdemaeker, 2007) present in the triglycerides of the fat fraction. In addition, to a lesser extent, the carbonyl function of carboxylic acids and proteins. The two bands, located around 1650 $cm^{-1}$ and 1545 $cm^{-1}$, are particularly characteristic of the amide functions of proteins. Moreover, the appearance of the bands corresponding to [OH] hydroxyl groups, located around 3500 $cm^{-1}$ and 1636 $cm^{-1}$. As regards the bands located at the regions 900-1200 $cm^{-1}$, they characterize the [C-O-C C-O-P], [P-O-P] bonds

of the oligo and polysaccharides. These findings are in good agreements with recent works (Gieroba *et al.,* 2020; Espinosa-Velázquez, *et al.,* 2017).

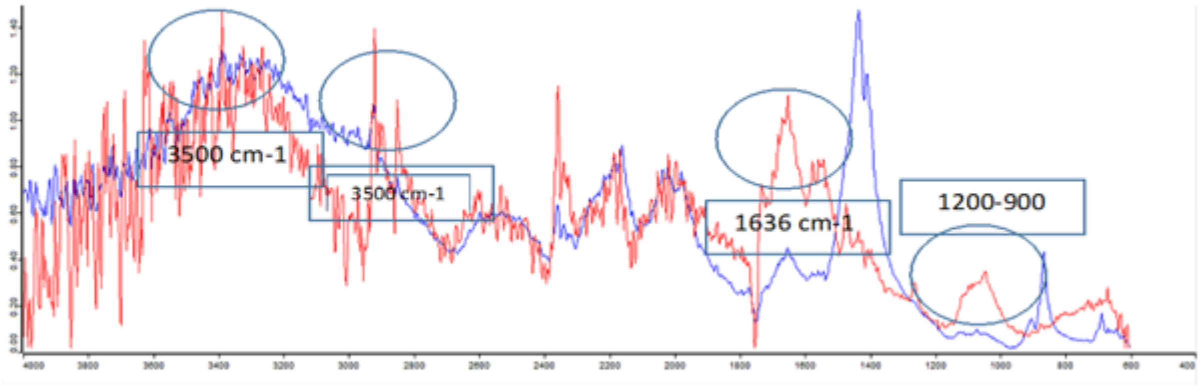



**Figure 2.** Mean infrared spectra of 316L steel before and after treatment with whole UHT milk.

The physicochemical and chemical analysis of the surface of stainless steel 316l, by the two types of UHT milk (skim, whole), shows that the fouling makes the surface hydrophobic whatever the type of milk, and that according to the analysis by infrared spectroscopy due to the appearance of methyl and methylene groups, amide functions, and proteins, the bond [C-O] and hydroxyl grouping [OH] as also found by Krishnan *et al.,* 2021.

## Conclusion

Our study has shown that the hydrophobicity of surface of 316L stainless steel changes either qualitatively or quantitatively after treatment with UHT milk (skimmed and whole milk). The results of infrared spectroscopy show that the elements causing this change could be phospholipids, traces of fat, caseins and lactose. Therefore, this study could be used to develop strategies to control the equipment surfaces' hygiene, especially in the dairy industry, and to rationalize cleaning and disinfection protocols.

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