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# An investigation of microbial adhesion to natural and synthetic polysaccharide-based films and its relationship with the surface energy components

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**Abstract** In recent years, polysaccharide-based films have been developed for many applications. Some of these are in the pharmaceutical industry, where the adhesion of microorganisms to surfaces is a concern. After adhesion of a microorganism to a solid surface has taken place, the subsequent biofilm formed can act as a vehicle for spreading infections. The aim of this study is to compare the bacterial adhesion of *E. coli* and *S. aureus* from a contaminated solid model (Tryptone Soya Agar) to a range of polysaccharide-based films. These polysaccharide-based films consist of different natural starches (potato, cassava, wheat, pea and rice) and synthetic polymers hydroxyl-propyl cellulose (HPC) and carboxyl methyl cellulose (CMC)). The surface energy parameters of the films were calculated from the contact angle measurements by the sessile drop method. Apolar and polar liquids (water, formamide and hexadecane) and the Lifshitz-Van der Waals/acid-base (LW/AB) approach were used according to the method of Van Oss, Chaundhury and Good. The surface properties of the films were also correlated to the microbial adhesion. This indicated that, for both *E. coli* and *S. aureus,* the surface roughness did not affect the microbial adhesion. Only $\gamma_S^{AB}$ had any correlation with the microbial adhesion and $\gamma_S^{LW}$ was almost constant for all the various polysaccharide films tested. In addition, the electron—donor properties of the materials, exhibited via $\gamma_S^+$, were positively correlated with the adhesion of *S. aureus* but not with *E. coli*. This was in agreement with the results of the MATS (Microbial Adhesion To Solvents) test performed on the two bacteria. This revealed that only *S. aureus* presented an electron—acceptor characteristic.

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## 1 Introduction

Since the 1970s, biopolymers such as polysaccharides and proteins, have been studied as a potential alternative to synthetic polymers [1]. Polysaccharides are of significant interest, because they are made from renewable natural sources. As a consequence of their biodegradability, they promise an alternative solution to the environmental problem caused by plastic waste [2, 3]. In addition, starch is a relatively low cost choice as a foundation material for edible films and coatings, compared to protein and wax [4]. Recently, these biopolymers have been suggested as pharmaceutical coatings [5], packaging materials [6] and edible coatings in food [7, 8].

The microbiological safety is of paramount importance in many applications such as in the pharmaceutical and food processing and in packaging applications because many micro-organisms can contaminate the product. A number of these micro-organisms are human pathogens. The contamination and growth of such organisms can potentially pose a risk to human health. *Escherichia coli* (a Gram− bacterium) and *Staphylococcus aureus* (a Gram+ bacterium) are common contaminants. They cause numerous food poisoning

outbreaks [9] and hospital infections [10, 11] every year and sometimes this can lead to eventual death.

Microorganisms in the environment are predominantly present in the biofilm state [10, 12]. This consists of the cells being attached to a surface. The resistance of a microorganism, whilst in a biofilm state, can be remarkably higher than in the planktonic state where the cells are suspended in a liquid [13, 14]. In addition, contamination can often occur via the transfer of cells between two surfaces, when the two surfaces come into contact with each other [15]. Common sources of contamination are cutting surfaces [16] and the hands of operators [17].

Cell adhesion is the initial step in a biofilm formation [18]. Investigations into the factors which affect the attachment of cells to surfaces, such as the material's properties, have been widely pursued in an attempt to prepare materials capable of preventing, or significantly reducing, microbial adhesion [19, 20]. Both a thermodynamic approach [21] and the DLVO theory [22] have been successfully employed to explain the adhesion of cells to surfaces, demonstrating how surface energy parameters can have an effect on microbial adhesion.

To date, numerous studies have been conducted investigating the properties of polysaccharide-based films [23–27]. However, so far, most of the research has focused mainly on mechanical properties and the effect of various preparation methods [6]. The microbial adhesion to these materials remains unexplored. However, this area needs more in depth investigation, prior to using these films for applications such as edible coatings and packagings.

The primary purpose of this study was to screen a wide range of commercially available natural and synthetic polysaccharide-based films for their adhesive properties with *E. coli* and *S. aureus*. This will allow an assessment of their suitability in terms of their susceptibility to bacterial attachment. Secondly, the surface energy parameters ($\gamma_S^{TOT}$, $\gamma_S^{LW}$ and $\gamma_S^{AB}$ determined by $\gamma_S^+$ and $\gamma_S^-$) and the surface roughness (Ra) of the films were determined via contact angle measurements and atomic force microscopy (AFM) respectively. Subsequently, both these parameters were then correlated with microbial adhesion to the surfaces of the polysaccharide-based films, in order to identify which ones are primarily responsible for the observed adhesion. Knowledge of the relationship between microbial adhesion and surface properties of the film can be crucial. It will aid in choosing the most appropriate film for packaging applications or to develop and initially pre-screen investigational biopolymer films with the desired performance.

## 2 Material and methods

The following five natural polysaccharides were used: cassava, pea, potato, wheat and rice. Cassava starch was supplied by AVEBE (Veendam, Netherlands). Pea, potato, rice and wheat were purchased from Sigma Aldrich Corp. (St. Louise, MO, USA). HPC (hydroxyl-propyl cellulose) was supplied by Hercules Corporation (USA) whilst CMC (carboxyl methyl cellulose) was purchased from Sigma Aldrich (UK).

### 2.1 Film preparation

For each polysaccharide, a suspension of 40.0 g/l in water was prepared, heated in a water bath at 90°C and stirred at 150 rpm for 2 h. On formation of a paste, the suspension was then equilibrated at 25° for 2 h, prior to pouring the paste into a polystyrene Petri dish for casting. Films with a thickness of about 100 μm were prepared and allowed to dry for 3 days at 27°C in controlled conditions (relative humidity 30%) [6].

### 2.2 Contact angle measurements

Contact angles were measured with a G40 goniometer (Krüss, France). Three probe liquids of different polarities were used for each biopolymer film: distilled water, formamide and hexadecane (Sigma Aldrich, UK). Contact angles at both the right and the left side were measured. The mean value of 10 readings was calculated for each film and for each liquid. These mean values were then used in equation [6] to determine the $\gamma^{LW}$, $\gamma^+$ and $\gamma^-$ components of the surface energy.

### 2.3 AFM imaging and roughness determination

AFM was used to assess the surface topographies and the surface roughness values for each of the biopolymer films. Two duplicate samples from three independent castings were studied. All AFM images were recorded in tapping mode using a Nanoscope IV (Digital Instruments) equipped with a silicone cantilever with a resonant frequency of 270 kHz. Scan rates were typically 1.0 Hz for all images taken and the typical scan time for a 10 × 10 μm image was 5 min.

### 2.4 Microorganism cultures and microbial adhesion to biopolymers

*E. coli* ATCC 25922 and *S. aureus* NCTC 6571 stock cultures were stored on Brain Heart Infusion (BHI) Agar (Oxoid, UK) at 4°C. A loopful of cells was used to inoculate 15 ml of fresh BHI broth. The cell count in both cases reached about $2 \times 10^9$ CFU/ml, after static incubation for 24 h at 37°C.

One hundred microliter of cell suspension, prepared as described, was spread onto the surface of the Petri dish ($\phi$ 10 cm) containing BHI Agar. This resulted in a surface cell concentration of around $3 \times 10^6$ CFU/cm$^2$. A biopolymer film piece (1 × 1 cm) was carefully deposited on to the Agar surface and a gentle pressure was applied for

2 s using tweezers. This allowed the whole surface of the biopolymer film to be in contact with the agar surface. The biopolymer was then removed and placed in a sterile universal bottle containing 1 ml of sterile Phosphate Buffer Solution (PBS). Bacterial cells were recovered by vortexing the mixture for 30 s and then serially diluting in PBS. The cell count was performed by plating 100 µl of the proper dilution on MacConkey Agar (Oxoid, UK) for *E. coli* and Mannitol Salt Agar (Oxoid, UK) for *S. aureus*. Colonies were then counted after incubation at 37°C for 24 h. Each experiment was performed in triplicate on three independent cultures and the results are presented as mean values ± 1 standard deviation.

### 2.5 Microbial adhesion to solvents (MATS)

The cell suspension, prepared as previously described, was centrifuged for 10 min at 6037 × *g* (HERMLE centrifuge Z-383K, LabPlant, Huddersfields, UK) at 4°C. After carefully removing the liquid, the cells were washed and centrifuged three times with 0.15 M NaCl. The cells were then diluted in NaCl (0.15 M) to reach a concentration of about $10^8$ CFU/ml. 4.0 ml of this cell suspension and 1.0 ml of one of the solvents were vortexed together for 1 min. The emulsion was left to stand for 15 min to allow the two phases to separate. The solvents used were: chloroform, hexadecane, ethyl-acetate and decane (Sigma, UK).

The absorbance of 1 ml of the aqueous phase was evaluated at 400 nm with a spectrophotometer (UV-1201, Shimadzu (UK), Milton Keynes). The affinity of the cells for each solvent was determined using the following equation:

$$\%\,\text{Affinity} = 100\left(1 - \frac{A}{A_0}\right) \tag{1}$$

where $A_0$ is the absorbance at 400 nm of the suspension before mixing and $A$ is the absorbance of the suspension after mixing with one of the solvents. This protocol was carried out on microbial cells that originated from four independent cultures and the results are presented as mean values ± 1 standard deviation.

### 2.6 Determination of surface free energy parameters

The total surface free energy $\gamma^{TOT}$ consists of two components:

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \tag{2}$$

$\gamma^{LW}$ is the apolar component of the surface free energy associated with Lifshitz-Van der Waals interactions, $\gamma^{AB}$ is the acid-base component of surface free energy. $\gamma^{AB}$ results from the electron-donor ($\gamma^-$) and electron-acceptor ($\gamma^+$) molecular interactions (i.e. Lewis acid-base interactions). The acid-base term is expressed as the product of the electron donor and electron acceptor parameters:

$$\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-} \tag{3}$$

The interfacial energy, $\gamma_{SL}$, is defined as [28]:

$$\gamma_{SL} = \left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_L^{LW}}\right)^2 + 2\left(\sqrt{\gamma_S^+\gamma_S^-} + \sqrt{\gamma_L^+\gamma_L^-} - \sqrt{\gamma_S^+\gamma_L^-} - \sqrt{\gamma_S^-\gamma_L^+}\right) \tag{4}$$

where the subscripts S and L refer to the solid and liquid phases, respectively. The Young equation can be combined with the Young-Dupre equation to yield Eq. 5.

$$\gamma_L(1 + \cos\theta) = \gamma_S + \gamma_L - \gamma_{SL} = -\Delta G_{SL} \tag{5}$$

Substituting the appropriate expressions then gives Eq. 6:

$$\gamma_L(1 + \cos\theta) = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^+\gamma_L^-} + \sqrt{\gamma_S^-\gamma_L^+}\right) \tag{6}$$

The equilibrium spreading pressure ($\pi_e$) was assumed to be negligible. A set of three simultaneous equations can then be solved to obtain the surface energy parameters of the solid, by using the known parameters of the three liquids and their contact angles on a solid. The surface energy parameters for the three probe liquids used in this work are shown in Table 1.

### 2.7 Statistical methods

The values of affinity towards solvents of the two microorganisms were compared with the Student's *t*-test. In assessing microbial adhesion, cell counts obtained from different polymer films were compared using the one-way ANOVA test followed post hoc by the Tukey's test for individual pairs of data sets. These analyses were performed using SPSS 14.0 software (SPSS Inc., Chicago, IL, USA). The correlation between surface energy parameters

**Table 1** Surface free energy parameters of the liquids at 20°C used for contact angle determinations

| Liquids | $\gamma^{TOT}$ (mJ/m$^2$) | $\gamma^{LW}$ (mJ/m$^2$) | $\gamma^{AB}$ (mJ/m$^2$) | $\gamma^+$ (mJ/m$^2$) | $\gamma^-$ (mJ/m$^2$) |
|---|---|---|---|---|---|
| Water | 72.8 | 21.8 | 51.0 | 25.5 | 25.5 |
| Formamide | 58.0 | 39.0 | 19.0 | 39.6 | 2.28 |
| Hexadecane | 27.2 | 27.2 | 0.0 | 0.0 | 0.0 |

and cell adhesion was tested with the chi-square test and the results expressed in term of the "goodness of fitness" value $R^2$ (Excel, Microsoft, USA).

## 3 Results and discussion

### 3.1 Surface properties of polysaccharide-based films

The contact angles for all three liquids on each polymer film are shown in Table 2. The contact angles for water are in the range 61–87°. Exceptions to this were found for cassava, which shows a remarkably lower contact angle (37°), and for pea and rice which show higher contact angles (104.7 and 103.7 respectively). The contact angles determined using water were always higher for each polysaccharide film than for those determined using formamide. In turn, the contact angles determined using formamide were always higher than those determined using hexadecane. Amylose and amylopectine are the main components of natural starches and they are present in a ratio of about 1:4. Lipids and proteins are minor constituents and their concentrations vary significantly between different starches. However, the concentration of lipids and proteins influences the hydrophobicity of the starches. Pea and rice are both starches which have lipids as a significant constituent [29]. These starches were found to exhibit higher contact angles with water (Table 2). This is in agreement with the hydrophobicity of the lipids. Wheat has 0.1% (w/w) of lipids [30] and it was found to have the third highest contact angle with water (Table 2). Cassava has the lowest ratio of amylose to amylopectin [29], which probably causes it to have the lowest contact angle with water. Amylopectine has a different structure compared to amylose and this causes it to have a greater affinity for water. Synthetic polysaccharides were generally found to exhibit hydrophobic behaviour. This could be attributable to the cellulose base of these polysaccharides.

The surface energy parameters are shown in Table 4. The value of $\gamma_S^{LW}$ was almost unchanged for each film (25.15–26.63 mJ/m$^2$). In contrast, $\gamma_S^{AB}$ varied in the range 0.22 mJ/m$^2$ for rice to 21.50 mJ/m$^2$ for cassava. $\gamma_S^{TOT}$ followed the same pattern for $\gamma_S^{AB}$, as a consequence of the almost constant $\gamma_S^{LW}$. Rice, wheat and HPC did not exhibit electron donor properties. This is shown by the fact that $\gamma_S^-$ was about 0 for these starches, whilst only rice and CMC had a $\gamma_S^+$ of almost 0.

The relationship between $\gamma_S^{TOT}$ and the contact angle for water showed that the contact angle decreases with increasing $\gamma_S^{TOT}$ (Table 2). This was expected as a consequence of the Young's equation.

AFM images of the starches are presented in Fig. 1. The surface roughness parameters (Ra) of the films were found

**Table 2** Contact angle (in °) of liquids at 20°C on natural and synthetic polysaccharide-based films

| Film material | Water | Formamide | Hexadecane |
|---|---|---|---|
| Cassava | 37.0 | 32.1 | 14.6 |
| Rice | 103.7 | 80.2 | 16.8 |
| Pea | 104.7 | 56.3 | 22.6 |
| Potato | 61.0 | 37.1 | 18.4 |
| Wheat | 86.0 | 68.8 | 22.5 |
| HPC | 71.0 | 63.0 | 21.6 |
| CMC | 87.0 | 32.0 | 13.0 |

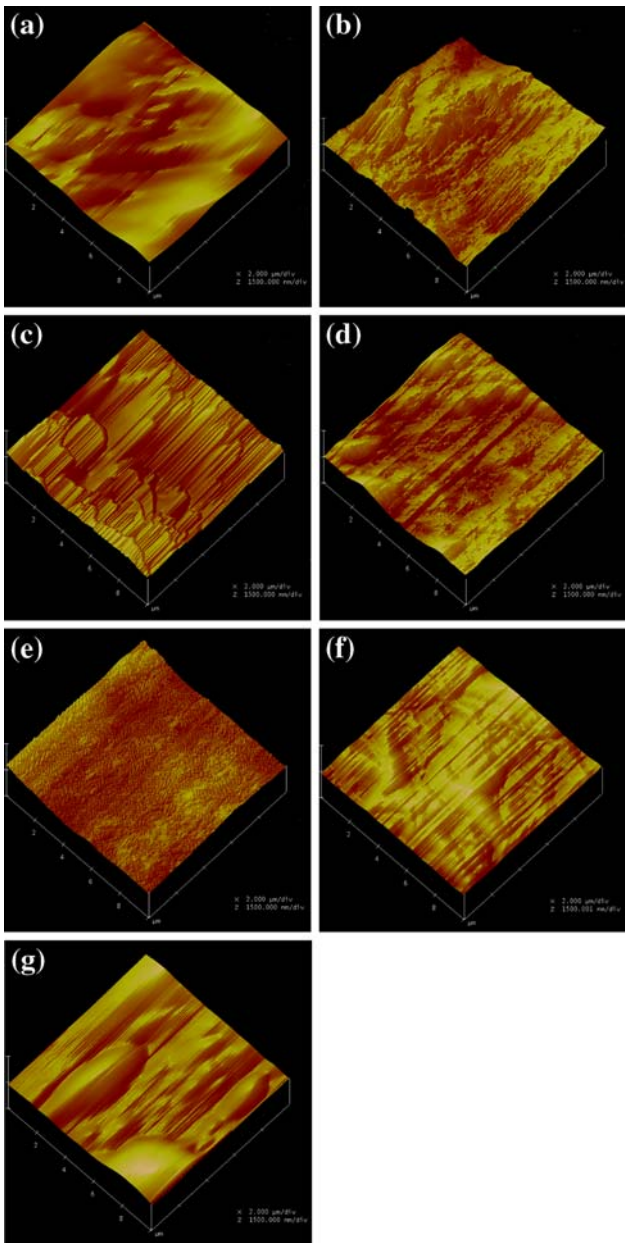


**Fig. 1** AFM images of the polysaccharide-based films. (**a**) Cassava, (**b**) Rice, (**c**) Pea, (**d**) Potato, (**e**) Wheat, (**f**) HPC, (**g**) CMC

**Table 3** Surface roughness values for natural and synthetic polysaccharide-based films

| Film material | Surface roughness Ra (nm) |
|---|---|
| Cassava | 148.61 |
| Rice | 121.45 |
| Pea | 43.43 |
| Potato | 68.79 |
| Wheat | 89.76 |
| HPC | 33.78 |
| CMC | 109.35 |

to be in the range 33.78–148.61 nm and are shown in Table 3. The measurements for Ra, from different castings of the same polysaccharide, showed errors below 5%. Cassava, rice and CMC presented the roughest surfaces, whilst pea and HPC were the smoothest. The surface roughness values appear to correlate with the average granule size of the starch, as reported by Copeland [31]. No apparent correlation was found between surface roughness and the contact angle for water. However, in this case, the contact angle of any liquid depends not only on the surface roughness, but also on the composition of the starch. For example, cassava and rice have a similar Ra (Table 3). However, their contact angles for water are at the extremes of the range (Table 2).

### 3.2 Microbial adhesion to solvents (MATS)

MATS is a partitioning method based on a comparison of the affinity of microbial cells towards different organic solvents [32]. The solvents comprise of two monopolar (either electron donor or electron acceptor) and two non polar solvents. The solvents chosen for this work include chloroform (electron acceptor)—hexadecane (non polar) and ethyl-acetate (electron donor)—decane (non polar). These have been used in previous studies [33, 34]. Comparisons have been made between a polar and a non polar solvent. If the affinity towards the electron donor solvent is higher than the affinity for the non polar solvent, then the cell surface has electron acceptor characteristics. In the same way, if the affinity towards the electron acceptor solvent is higher than the affinity for the non polar solvent, then the cell surface has electron donor properties. The higher the affinity towards hydrophobic solvents (decane and hexadecane), the higher the surface hydrophobicity of the cells.

The results of the MATS investigation are shown in Fig. 2. They show that *S. aureus* has a high affinity towards the two apolar solvents (58% for decane and 40% for hexadecane). This means that *S. aureus* is significantly more hydrophobic than *E. coli* ($P < 0.01$) which shows a low affinity to both solvents. *S. aureus* presents a markedly

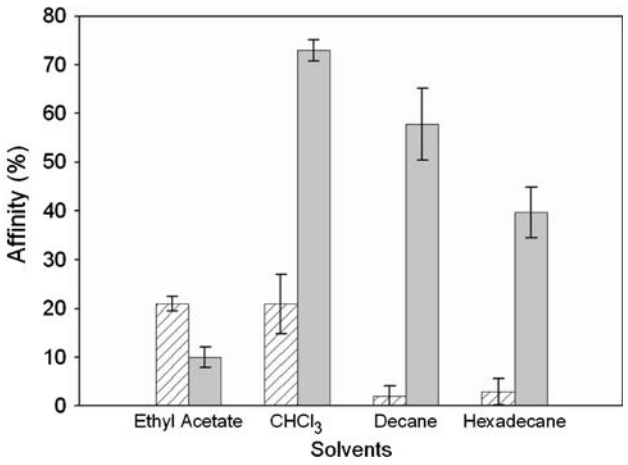


**Fig. 2** Affinity of *E. coli* ▨ and *S. aureus* ▬ to organic solvents

higher affinity towards chloroform (73%), than hexadecane (40%). This indicates that this bacterium has an affinity towards electron donor materials ($P < 0.05$). The low affinity towards ethyl acetate compared to that for decane represents a low level of attraction towards electron acceptor materials ($P < 0.01$). The affinity of *E. coli* towards ethyl acetate and chloroform was 21% and 22% respectively. As these values are higher than the affinity towards the apolar solvents ($P < 0.05$) this indicates a moderate affinity for either electron acceptor or donor materials.

The variation in the surface properties of the bacterial cells can be attributed to the different constituents, such as the fatty acid composition of the cell surface [35]. Both bacteria are grown in the same stationary phase. Therefore, differences caused by different growth rates are unlikely [34].

### 3.3 Bacterial adhesion to polysaccharide-based films

The contamination system in this study it is the same as that proposed and used by various authors [15, 36]. It was chosen to mimic the behaviour of a contamination occurring from a solid substrate.

The adhesion of *S. aureus* was found to vary between $1.1 \times 10^5$ and $2.8 \times 10^6$ CFU/cm$^2$, whilst the adhesion of *E. coli* varied between $1.5 \times 10^5$ and $1.4 \times 10^6$ CFU/cm$^2$ (Figs. 3–6). According to the Tukey's test the number of *S. aureus* cells attached to HPC and cassava films was significantly higher than the number of cells recovered from potato and wheat films ($P < 0.05$). Furthermore the adhesion to CMC, pea and rice films constituted another group of results ($P < 0.05$). For *E. coli* the ANOVA test revealed that only the adhesion to rice films was significantly different ($P < 0.05$) than the cell count on the other films.

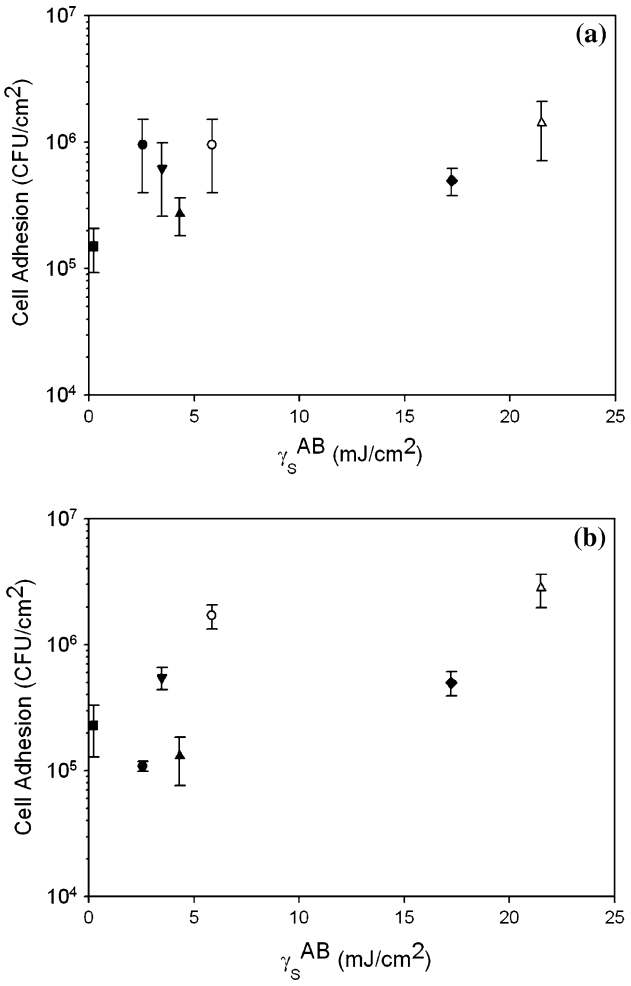


**Fig. 3** The relationship between the microbial adhesion of (**a**) *E. coli* (**b**) *S. aureus* with $\gamma_s^{AB}$ of the polysaccharide-based films. Δ Cassava, ■ Rice, ▲ Pea, ◆ Potato, ▼ Wheat, ○ HPC, ● CMC

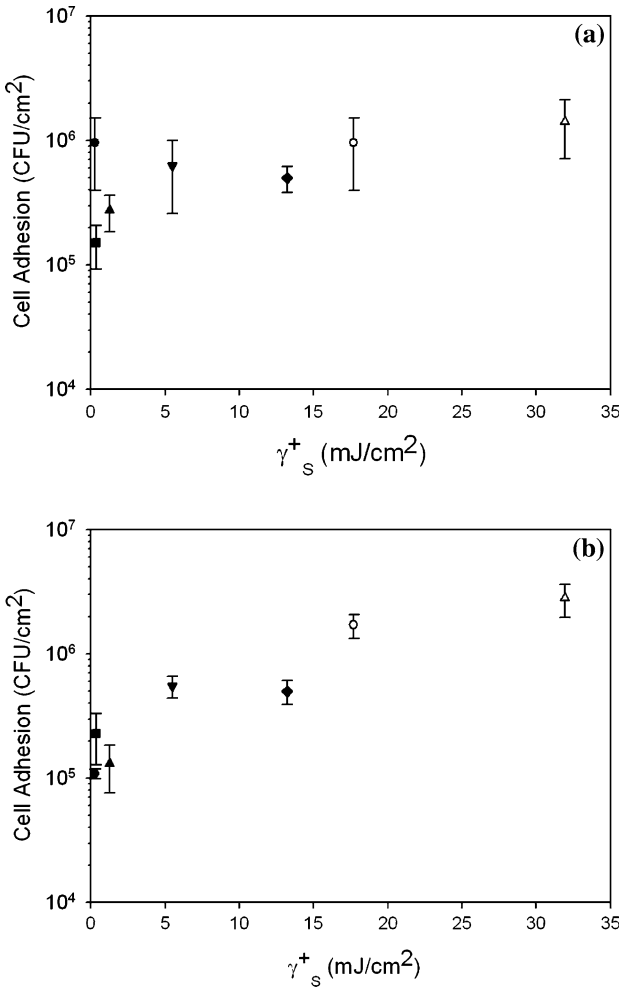


**Fig. 4** The relationship between the microbial adhesion of (**a**) *E. coli* (**b**) *S. aureus* with $\gamma_s^{+}$ of the polysaccharide-based films. Δ Cassava, ■ Rice, ▲ Pea, ◆ Potato, ▼ Wheat, ○ HPC, ● CMC

The effect of $\gamma_S^{LW}$ on adhesion could not be investigated, as the value of this parameter was almost the same between the different films (Table 4). The adhesive results for *E. coli* compared to the $\gamma_S^{AB}$ and $\gamma_S^-$ values of the films are shown in Figs. 3a and 4a. The results show a minimal effect of these parameters on the microbial adhesion ($R^2 < 0.26$). However, Fig. 3b shows the positive relationship of $\gamma_S^{AB}$ on *S. aureus* adhesion as this was found to increase with increasing values of $\gamma_S^{AB}$ ($R^2 > 0.46$). Even more pertinent is the relationship between the adhesion of *S. aureus* and the value of $\gamma_S^+$ shown in Fig. 4b ($R^2 > 0.85$). This is also in agreement with the result from the MATS test for *S. aureus* (Fig. 2) which indicates an increased affinity towards chloroform and thus, an affinity towards electron donor materials (represented by elevated $\gamma_S^+$).

It was anticipated that no relationship between $\gamma_S^-$ and the microbial adhesion ($R^2 < 0.09$) would be found, as both bacteria did not show an affinity towards electron acceptor materials (Fig. 5). However, Liu et al. 2008 [37] reported a negative correlation between $\gamma_S^-$ and the adhesion of *P. aeruginosa*. However, this could be explained by the high value of $\gamma_S^-$ for this microorganism reported by the same authors.

The relationship between microbial adhesion and the surface roughness of the substrate is often ambiguous. Some authors suggest that rougher surfaces increase bacterial adhesion [38]. However, others do not report such a correlation [39]. These discrepancies could be attributed to the fact that often processes intended to alter the surface roughness, also alter the surface energy parameters [37]. In this work, both the roughness and the surface energy parameters of the film surfaces depend on the composition of the polysaccharide (Tables 2 and 3). There is no effect from surface roughness on the adhesion ($R^2 < 0.02$) of either *E. coli* or *S. aureus* (Fig. 6). However, a correlation has been observed for $\gamma_S^{AB}$. These results suggest that the surface energy has more relevance in regards to microbial adhesion than surface roughness.

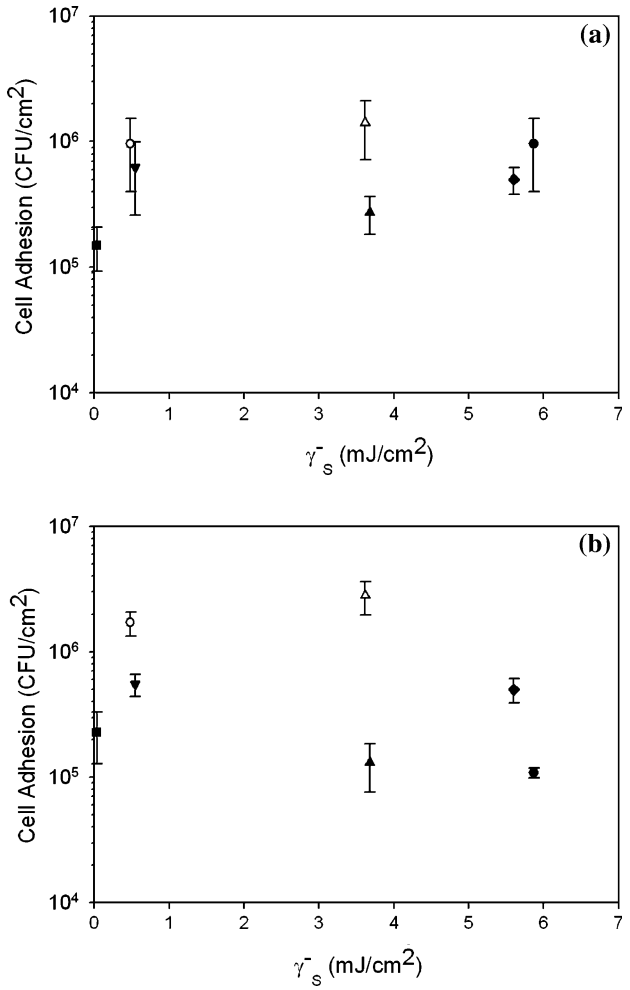


**Fig. 5** The relationship between the microbial adhesion of (**a**) *E. coli* (**b**) *S. aureus* with $\gamma_s^-$ of the polysaccharide-based films. Δ Cassava, ■ Rice, ▲ Pea, ◆ Potato, ▼ Wheat, ○ HPC, ● CMC

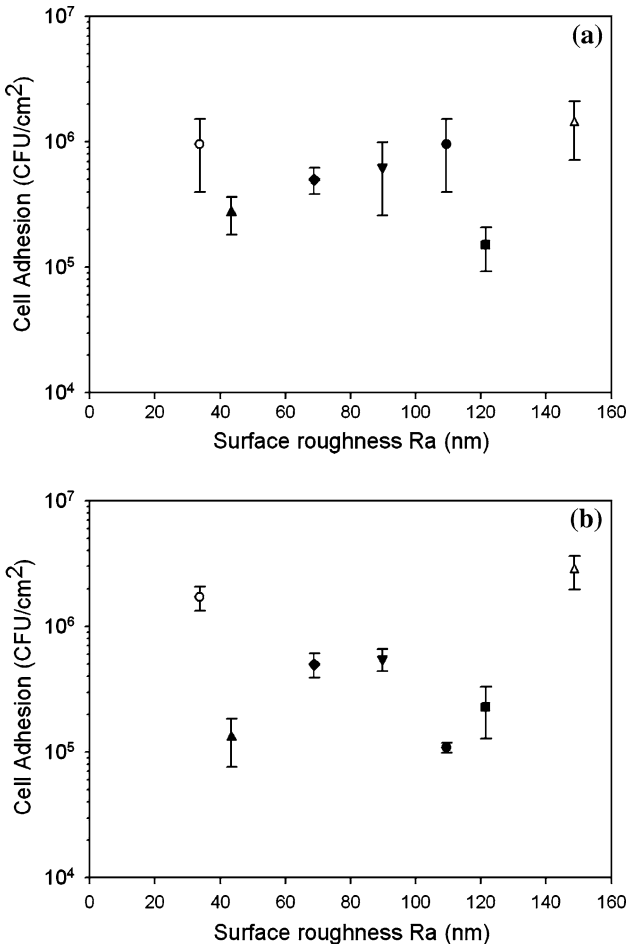


**Fig. 6** The relationship between the microbial adhesion of (**a**) *E. coli* (**b**) *S. aureus* with the surface roughness of the polysaccharide-based films. Δ Cassava, ■ Rice, ▲ Pea, ◆ Potato, ▼ Wheat, ○ HPC, ● CMC

## 4 Conclusions

The surface properties of natural and synthetic polysaccharide-based films have been investigated and shown to significantly affect the adhesion of two of the most common sources of food-borne diseases and causes of infections in home and community settings [40]. Of the three components of the surface energy, only $\gamma_S^{LW}$ remained unchanged between the polysaccharides that were tested. The other two ($\gamma_S^-$ and $\gamma_S^+$) varied, but only $\gamma_S^+$ was found to have an effect on the adhesion of *S. aureus*. This is attributable to the electron acceptor characteristics of this bacterium, as proven by the MATS protocol. Surface roughness was found to have no influence on microbial adhesion. The value of $\gamma_S^{AB}$ was found to correlate with the adhesion of both microorganisms. Overall, the surfaces with the lowest values of $\gamma_S^{AB}$ (such as: rice and pea) gave

**Table 4** Surface energy parameters for natural and synthetic polysaccharide-based films

| Film material | $\gamma_S^{LW}$ (mJ/m$^2$) | $\gamma_S^+$ (mJ/m$^2$) | $\gamma_S^-$ (mJ/m$^2$) | $\gamma_S^{AB}$ (mJ/m$^2$) | $\gamma_S^{TOT}$ (mJ/m$^2$) |
|---|---|---|---|---|---|
| Cassava | 26.33 | 31.93 | 3.62 | 21.50 | 47.82 |
| Rice | 26.05 | 0.36 | 0.03 | 0.22 | 26.27 |
| Pea | 25.15 | 1.26 | 3.68 | 4.31 | 29.46 |
| Potato | 25.82 | 13.23 | 5.60 | 17.22 | 43.04 |
| Wheat | 25.16 | 5.48 | 0.54 | 3.46 | 28.62 |
| HPC | 25.33 | 17.70 | 0.48 | 5.85 | 31.17 |
| CMC | 25.16 | 0.27 | 5.87 | 2.54 | 27.71 |

the lowest count for the adhesion of *E. coli* and *S. aureus*. This indicates that these polysaccharides are more suitable for applications, such as those in the packaging and pharmaceutical industry, where microbial contamination must be kept to a minimum. It has also been found that cassava seems to be less suited for such applications.

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