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AGRARIAN SCIENCES

Influence of detergents and sodium hypochlorite on *Yarrowia lipolytica* biofilms in utensils used in industrial production of colonial cheese

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Abstract: The formation of microbial biofilms in materials used in the industrial production of dairy may lead to deterioration of these foods. *Yarrowia lipolytica* biofilms are widely found in dairy products and can modify the final characteristics of these products. Thus, this study investigated the effectiveness of hygienization by detergents and sodium hypochlorite on the formation of *Y. lipolytica* biofilms in different utensils usually employed during industrial cheese production, like polypropylene, hoses, and nylon/polyethylene. The utensils were sanitized using solutions of mild and alkaline detergents, and sodium hypochlorite, according to the cheese industry Standard Operation Procedure. Results showed that in all coupons there was biofilm formation with *Y. lipolytica* isolates. The contact angle measurements were favored to promote the adhesion of the biofilm in the evaluated surfaces. Even after treatment with sanitizers, a significant survival rate of planktonic cells was observed in all coupons tested. These results indicate that *Y. lipolytica* biofilms show a significant ability to adhere to polypropylene, presenting an important impact on the quality of colonial cheese.

Key words: *Yarrowia lipolytica,* biofilm formation, colonial cheese, hygienization, sanitizing.

INTRODUCTION

Yarrowia lipolytica is a nonpathogenic yeast easily found in nature and frequent object of study due to its capability of producing metabolites with intense secretory lipolytic and proteolytic activities (Fukuda 2013). Regarded as a safe yeast, *Y. lipolytica* is widely employed in industry and is closely linked to dairies (Coelho et al. 2010, Jean-Marc 2012, Zinjarde 2014). This yeast is constantly associated with high proportions of fat or protein in foods and has already been identified in different types of cheese, at the surface and inside, contributing towards the process of maturation during production. Additionally, fatty acids inside *Y. lipolytica* throughout volatile compounds can promote undesirable effects such as organoleptic modifications, adverse effects in texture and discoloring (Groenewald et al. 2014, Zinjarde 2014).

The colonial cheese is traditionally produced by cow milk and commercial rennet. This production takes place in small industrial scale, handmade, without standardization, taking a maximum of 30 days until maturation (Borelli et al. 2006, Koelln et al. 2009, Fava et al. 2012). These operational process steps are illustrated in Figure 1. Utensils constituted of porous material are usually employed in traditional milk processing and during cheese production (Birhanu et al. 2013). For instance,

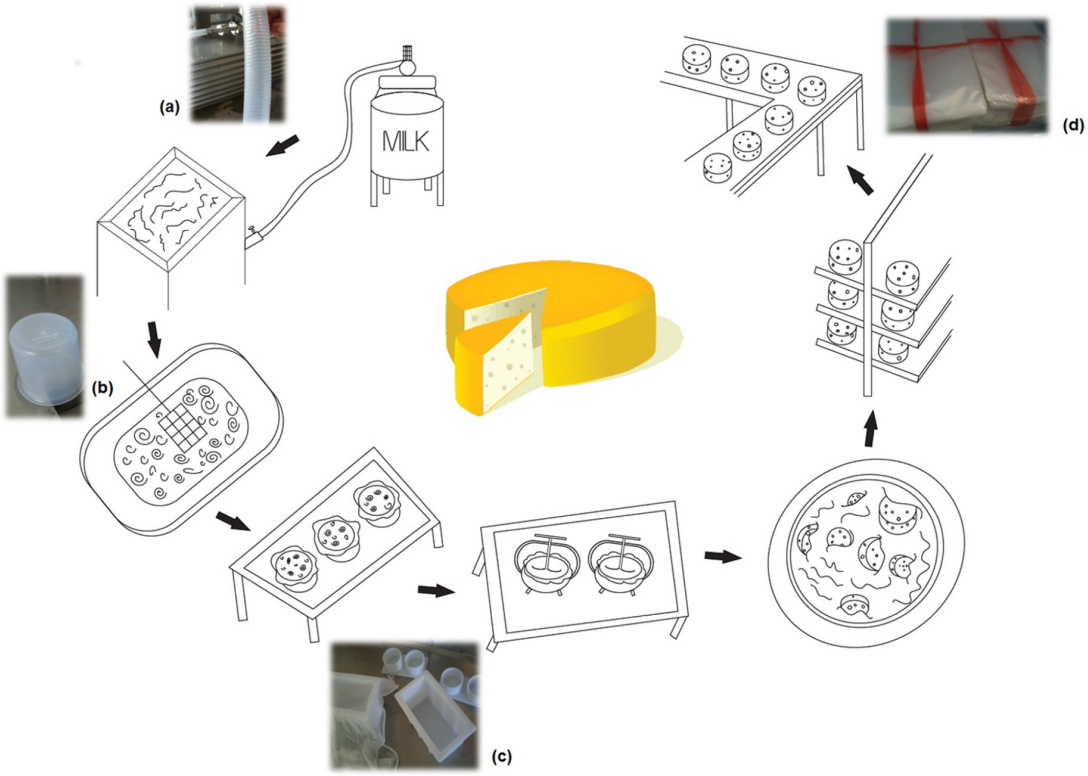

Figure 1. Illustration of the production process of colonial cheese and steps where utensils are employed: (a) hose used to transfer milk, (b) beaker used to transfer the curd, (c) mold used in cheese forming and (d) package employed in the vacuum packaging process.

PVC, hoses, beaker, spatulas, polypropylene molds and nylon/polyethylene wrapping.

In the food industry, material and utensils that make up the contact surface with food exert a significant effect on the level of connection with the creation of biofilms (Van Houdt & Michiels 2009, Sokunrotanak et al. 2013). Biofilms can be defined as sessile communities of surface-attached cells encased in an extracellular matrix. This phase is followed by an intermediate state where the irreversibly attached cells form microcolonies on the surface of interest (Kiedrowski & Horswill 2011). The adherence of microorganism cells on the surfaces shows similarity to a physical-chemical process, resulting from the interaction between electrostatic forces and hydrophilic and hydrophobic interactions (Giaouris et al. 2012). In dairy production, the formation of microbial biofilm in different types of materials is very common (Galinari et al. 2014). Highly structured biofilms with sessile cells show greater resistance to antibiofilm treatment compared to planktonic cells (Bergamo et al. 2014).

When the hygienization procedure is not performed effectively, disinfectants do not penetrate through the biofilm matrix, avoiding the destruction of their living cells (Simões et al. 2010). To prevent biofilm maturation, the correct frequency of disinfection and sanitation must follow strict definitions in food processing. Moreover, the cleaning time and the type of sanitizing product are essential to prevent the formation of microbial biofilm (Van Houdt & Michiels 2009, Fouladynezhad et al. 2013). The food industry employs different categories of sanitizing products and chemical disinfectants to prevent the formation of these biofilms.

Regardless of the precise operation of the hygiene process in the industry, the materials employed during the process hold distinct characteristics that may ease up the formation of biofilms. *Y. lipolytica* is widely used in food industry and can have a significant impact on the final quality of the cheese. Moreover, there are few pieces of research regarding the formation of biofilms by this yeast. Thus, the aim of this study was to investigate the effectiveness of hygienization in the formation of *Y. lipolytica* biofilms in different utensils usually employed during industrial cheese production, like polypropylene, PVC, and nylon/polyethylene. Also, the Time-Kill Assay, Sessile Drop Method (SDM) and Emulsification Index (E24) tests were performed to evaluate the effectiveness of sanitizers in inhibiting the growth of *Y. lipolytica* planktonic cells.

MATERIALS AND METHODS

Microbial strains

This study employed six *Y. lipolytica* strains isolated from cheese marketed as colonial cheese (QU22, QU77, QU13, QU69, QU16, and QU50). Mattanna et al. (2014) performed the molecular identification through the sequencing with the domain D1/D2 of the great sub-unity (26S) ribosomal DNA using initiators NL-1 NL-4. All these isolates have 99% of sequence identity with *Y. lipolytica* type strains.

Biofilm formation assay on utensils

This methodology was conducted in accordance with Flach et al. (2014) and Bergamo et al. (2014) using different coupons: molds used for cheese production (polypropylene), hose (spiral PVC), beaker (polypropylene) and vacuum packaging (nylon/polyethylene), cut into shapes of 1 x 1 cm^2. Before the experiments, a coupon sterilization process took place using ultraviolet radiation exposure in Biological Security Cabin Class II type A (Veco, Campinas, Brazil) for the period of 1 h.

Y. lipolytica isolates were grown on Sabouraud Dextrose Agar (SDA) with chloramphenicol (Himedia, Mumbai, India) during 24 h at 32 °C. Young cultures were added to 5 mL of Tryptone Soya Broth (TSB - Himedia, Mumbai, India) originating a suspension with 10^6 CFU/mL, incubated at 32 °C for 24h. Then, 1 mL of this suspension was transferred for 9 mL of Peptone water 1 % (Merck, Darmstadt, Germany). Subsequently, the coupons were added to this solution and incubated for 96 h at 35 °C. Finally, the coupons were washed three times with peptone water for removal of poorly adhered cells and were added to another flask containing 50 mL of this solution. The adhered cells were released from the coupon by sonication at a frequency of 40 KHz (Unique, Indaiatuba, Brazil) for 10 min. Decimal dilutions were spread on SCA plates for assessment of microbial growth.

Evaluation of the antibiofilm activity of sanitizers in utensils

Y. lipolytica isolates were inoculated in TSB medium and incubated at 35°C for 24 h. After that, 1 mL of these cultures was added to 9 mL of sterile peptone to obtain the solution test, resulting in 10^6 CFU/mL. The utensils were submitted to a hygienization process, using the following sanitizing solutions according to the cheese Industry Standard Operation Procedure (SOP): mild detergent (3 %) for 5 min, alkaline detergent (6 and 8 %) for 10 min, sodium hypochlorite (1 and 1.5 %) for 10 and 20 min, followed by three washes using sterile distilled water. Then, the utensils were immediately immersed in the described fungal suspension for 96 h. Coupons were washed three times with peptone water to remove weakly adhered

cells. Finally, the samples were sonicated for 10 min in 50 mL of peptone water to collect the biofilm for quantification. Decimal dilutions of sonicated peptone water were spread on SCA plates for assessment of microbial growth. This methodology followed Bergamo et al. (2014).

Time-kill assay

The Time-kill assay evaluated the sanitizing efficacy (fungicidal activity) against *Y. lipolytica* plantain cells according to Abreu et al. (2011), with modifications. The suspensions were prepared from isolates of *Y. lipolytica* containing 10^6 CFU/mL and the following sanitizers were used: sanitizing solutions, mild detergent (3 %), alkaline detergent (6 and 8 %), sodium hypochlorite (1 and 1.5 %). The experiments were conducted using the ratio of 1.5 mL of sanitizing product to 0.5 of fungal inoculum. The contact times were 5, 10, 15, 20 and 25 min. Then, 1 mL of each fungal suspension was added to 9 mL of Peptone Water (1%) to obtain dilution 10^{-3}. After each contact time, 0.1 mL of this suspension was seeded in SDA and incubated for 24 h at 32 °C for determination of the number of CFU/mL.

Sessile drop method (SDM)

According to Locatelli et al. (2004), a drop with 20 µL of TSB containing a inoculum with 10^6 CFU/mL was carefully deposited above the coupons surfaces for later assessment of the contact angle of the drop on the surface. A Canon® Powershot SD200 digital camera captured the images showing the drops on the coupons surfaces after 5 sec of touchdown to enhance the drop surface stability. The measurement of the contact angle values occurred observing the straight-line inclination formed between the contact base radius and the height of the drop, supported by Image J software, and after three consecutive measurements (Skolodowska et al. 1999).

Emulsification index (E_{24})

The measurement of emulsification activity was performed according to Cooper & Goldenberg (1987). The fungal suspension of Y. *Y. lipolytica* in 10^6 CFU/mL was added to 4 mL of xylene in TSB. After stabilization of the mixture, the emulsification index evaluation was performed by dividing the height of the emulsion layer by the total height of the mixture, multiplying by 100.

Statistical analysis

Statistical analysis of the results included mean ± DP and variance through ANOVA. In groups where significant statistical differences were found, the Turkey test was used along with the 't' test with a significance level $p < 0.05$. Mean values of Kolmogorov-Smirnov (KS) together with the log (CFU/cm^2) results were applied to all isolates of *Y. lipolytica* in the study, in which all variables presented normality in its distributions. All analysis was processed by using software IBM SPSS Statistics v. 22.

RESULTS

In this study, all strains were able to form biofilms on mold, hose, beaker and wrapping (Table I). The counting of adherent cells ranged from 3.95 to 6.20 log CFU/cm^2, with higher biofilm formation in the mold. Strain QU16 was the strongest biofilm formers in the mold, hose and beaker: 6.23, 5.87, 5.83 (log CFU/cm^2), respectively. The strain QU50 was the strongest biofilm formers on wrapping: 6.27 log CFU/cm^2. Strain QU22 was the weakest biofilm former on the mold, hose and beaker: 5.62, 3.95, 5.27 (log CFU/cm^2), respectively. Strain QU69 was the weakest biofilm former on wrapping: 5.11 log CFU/cm^2. However, there was no significant difference (p <0.05) between the mold and the other coupons.

Table I. Counting of sessile cells of *Yarrowia lipolytica* isolates adhered to coupons during the biofilm formation process.

	Log CFU/cm²*					
	QU16	**QU50**	**QU69**	**QU77**	**QU22**	**QU13**
Mold (polypropylene)	6,23±0,03	5,89±0,09	5,73±0,02	5,72±0,01	5,62±0,06	5,68±0,58
Hose (PVC spiral)	5,87±0,12	5,78±0,17	5,60±0,05	5,02±0,05	3,95±0,35	4,95±0,06
Becker (polypropylene)	5,83±0,06	5,48±0,14	5,60±0,06	5,71±0,07	5,27±0,09	5,73±0,09
Wrapping (nylon/polyethylene)	5,30±0,18	6,27±0,02	5,11±0,06	5,20±0,04	5,53±0,14	5,45±0,02

***Yarrowia lipolytica* isolates: QU16, QU50, QU69, QU77, QU22 and QU13; *Average values ± SD.**

The evaluated biofilm inhibition on the utensils were statistically significant (p <0.05) among the evaluated treatments. In the mold, significant results were observed with the use of mild detergent (3%) (p = 0.002, t = 6.022, r = 0.813, IC = 0.281, 0.699) and sodium hypochlorite (1 %) for 10 min (p = 0.005, t = 4.798, r = 0.677, IC = 0.186, 0.617). The alkaline detergent (6 %) was the only one that did not present statistical significance in the results for this coupon. The beaker coupon was analyzed using only the mild detergent (3 %) in the period of 5 min and presented less statistical significance in the values comparing to the others in this study (p = 0.036, t = 2.839, r = -0.808, IC = 0.047, 0.953) (Figure 2). The hose coupons presented significant results with the sanitizing product sodium hypochlorite (1 %) in the period of 10 min (p= 0.001, t= 6.869, r= 0.843, IC= 0.688, 1.512). Sodium hypochlorite (1.5 %) in the period of 20 min presented similar results (p = 0.004, t = 5.188, r = 0.418, IC = 0.974, 2.890) (Figure 3 and Figure 4).

Regarding the time-kill assay, it was observed that there was no inhibition of growth in none of the tested times for the mild detergent at concentration of 3 % (Figure 4), except for isolate QU16. However, little change occurred in the course of time for the other isolates. It was also observed that there was no statistical significance (p < 0.05) in the results when comparing times 5 and 25 min (p = 0.650, t = 0.483, r = 0.650, IC = - 0.622, 0.425). However, the sanitizing products alkaline detergent 6 and 8 % and sodium hypochlorite 1 and 1.5 % were effective in inhibiting the growth of the other isolates of *Y. lipolytica,* in all assays.

The comparison between the angle of the water drop, Broth TSB drop and the angle of the inoculum 10^6 CFU/mL did not present statistical significance in the results (p > 0.05). However, there were significant differences (p < 0.05) among the different coupons. With the contact angle measurements, a higher angle value of the hose coupon (overall average of 65.2° ± 6.5°) demonstrated a less wetting surface property when comparing to other coupons (Table II). When relating all contact angles of the studied cultures, the coupon beaker presented significance in results (p < 0.05) compared to mold, hose and packaging, therefore presenting an enhanced wetting property. All the material culture angles presented significance in results (p < 0.05).

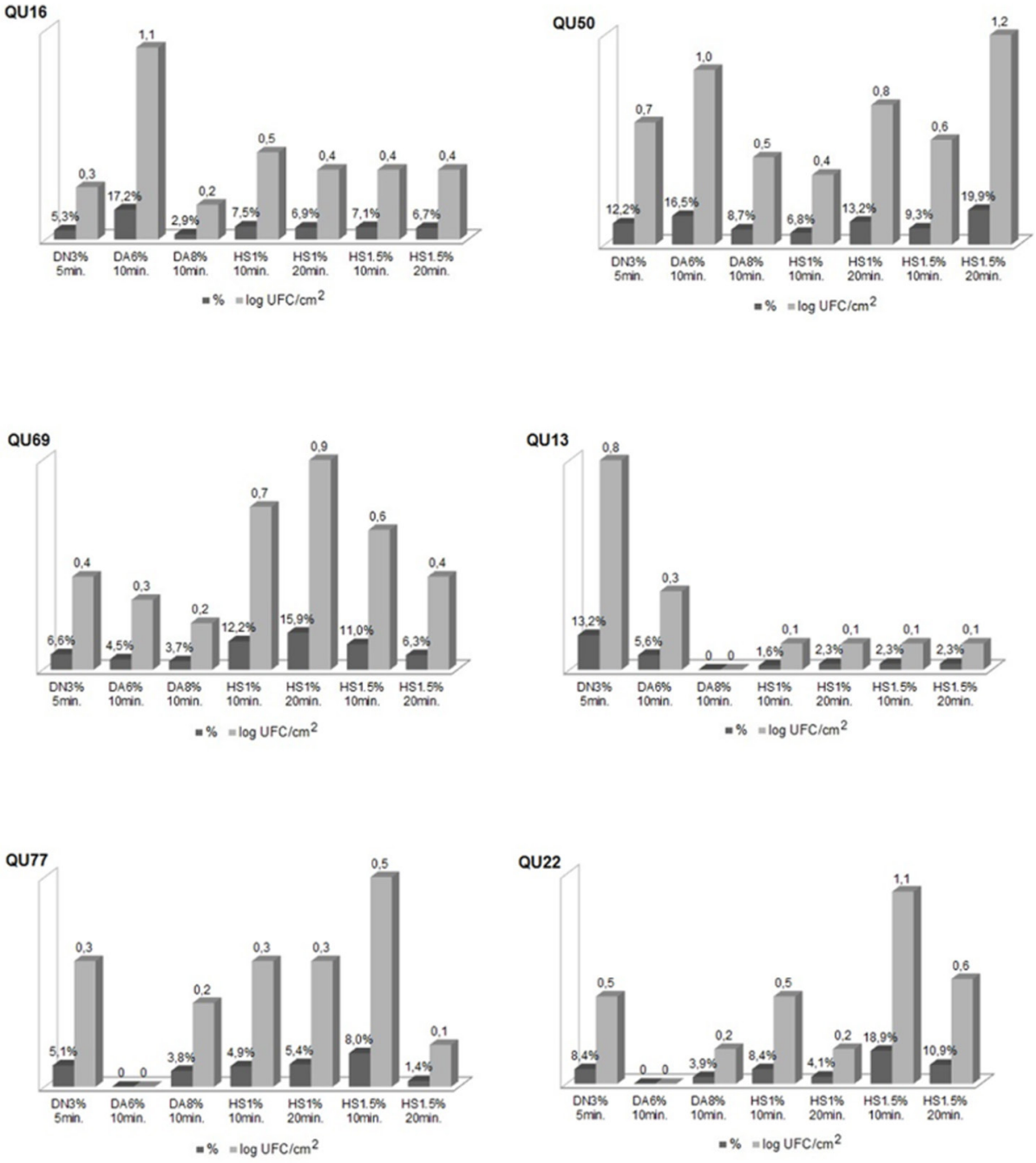

Figure 2. Survival of (a) QU16, (b) QU50, (c) QU69, (d) QU13, (e) QU77, (f) QU22 *Yarrowia lipolytica* isolates (log CFU/cm^2) on the mold coupon before and after application of sanitation product (DN 3 %) mild detergent 03 - 05 min, (DA6 %) and (DA8 %) alkaline detergent and 8 % - 6% - 10 min (HS 1 %) sodium hypochlorite 1 % - 10 to 20 min. and (HS1,5 %) sodium hypochlorite 1.5% - and 10-20 min.

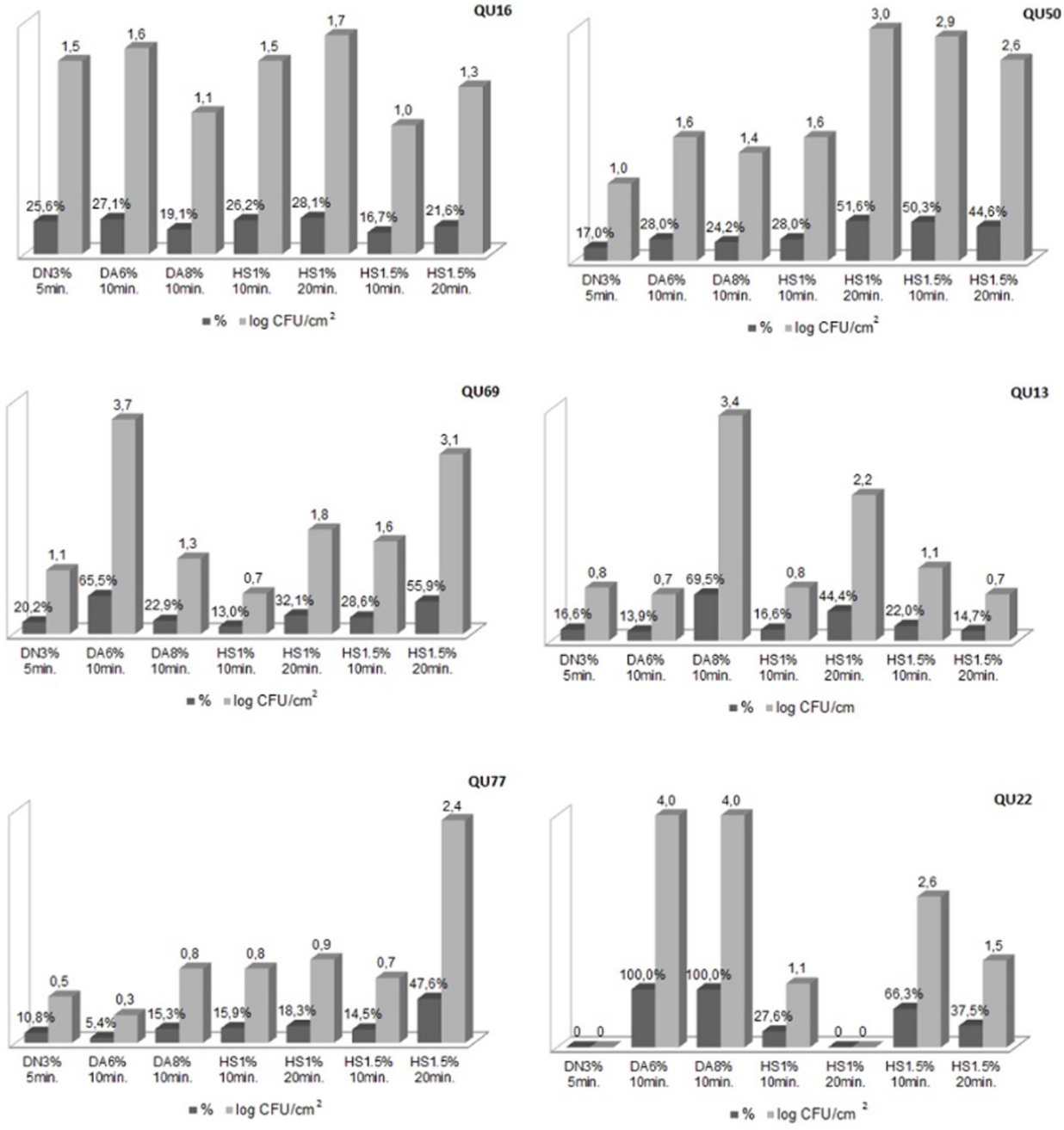

Figure 3. Survival of (a) QU16, (b) QU50, (c) QU69, (d) QU13, (e) QU77, (f) QU22 *Yarrowia lipolytica* isolates (log CFU/cm^2) on the hose coupon before and after application of sanitation product (DN 3%) % mild detergent 03-05 min, (DA6 %) and (DA8 %) alkaline detergent and 8 % - 6 % - 10 min (HS1 %) sodium hypochlorite 1 % - 10 to 20 min and (HS 1,5 %) sodium hypochlorite 1.5 % - and 10-20 min.

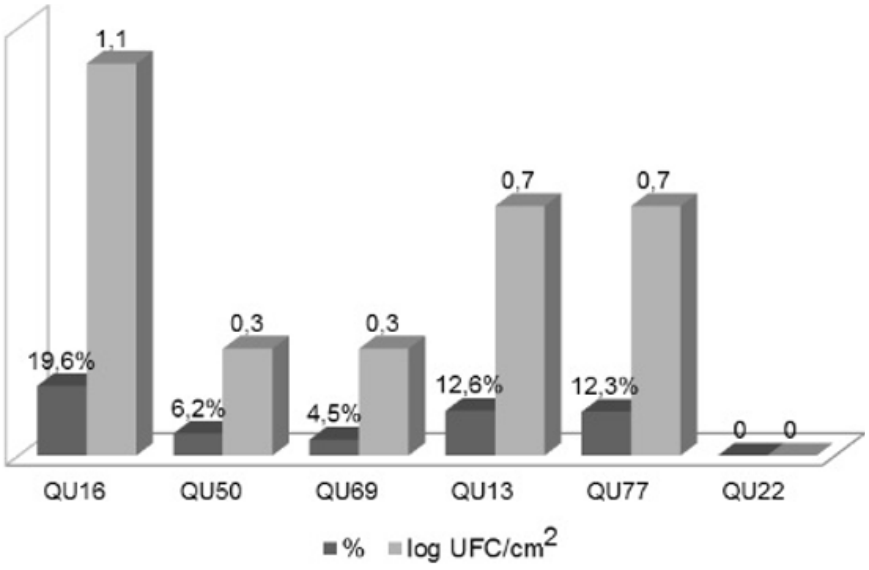

Figure 4. Survival of QU16, QU50, QU69, QU13, QU77, QU22 *Yarrowia lipolytica* isolates (log CFU/cm^2) on the beaker coupon before and after application of sanitizing product (DN3 %) % mild detergent 03-05 minutes.

Table II. Sessile drops angle measurements in degrees on the coupons mold, hose and packaging.

	Angle Measurements(°°)*							
	Water	**Broth TBS**	**QU16**	**QU50**	**QU69**	**QU77**	**QU22**	**QU13**
Mold (polypropylene)	72,7±0,26	68,4±0,33	59,2±0,32	60,7±0,17	62,1±0,58	47,6±0,32	62,3±0,82	59,6±0,54
Hose (PVC spiral)	77,5±0,26	69,3±0,12	72,6±0,25	63,6±0,26	57,5±0,32	65,6±0,26	66,3±0,40	62,3±0,20
Becker (polypropylene)	60,9±0,04	61,2±0,77	49,5±0,14	48,7±0,30	52,8±0,04	34,4±0,42	34,9±0,23	52,5±0,31
Wrapping (nylon/ polyethylene)	62,8±0,18	64,8±0,82	58,6±0,18	61,8±0,18	57,7±0,06	67,4±0,04	59,1±0,14	59,8±0,02

***Yarrowia lipolytica* isolates: QU16, QU50, QU69, QU77, QU22, QU13; Broth TSB: Tryptic Soy Broth; *Average values ± SD.**

Table III. Emulsification indexes values in percentage of *Y. lipolytica* isolates.

Isolates	E_{24} ± SD
QU16	88,3 ± 3,05
QU50	49,7 ± 1,53
QU69	83,0 ± 4,58
QU77	72,3 ± 2,89
QU22	86,3 ± 1,79
QU13	74,7 ± 3,20

***Yarrowia lipolytica* isolates: QU16, QU50, QU69, QU77, QU22 and QU13.**

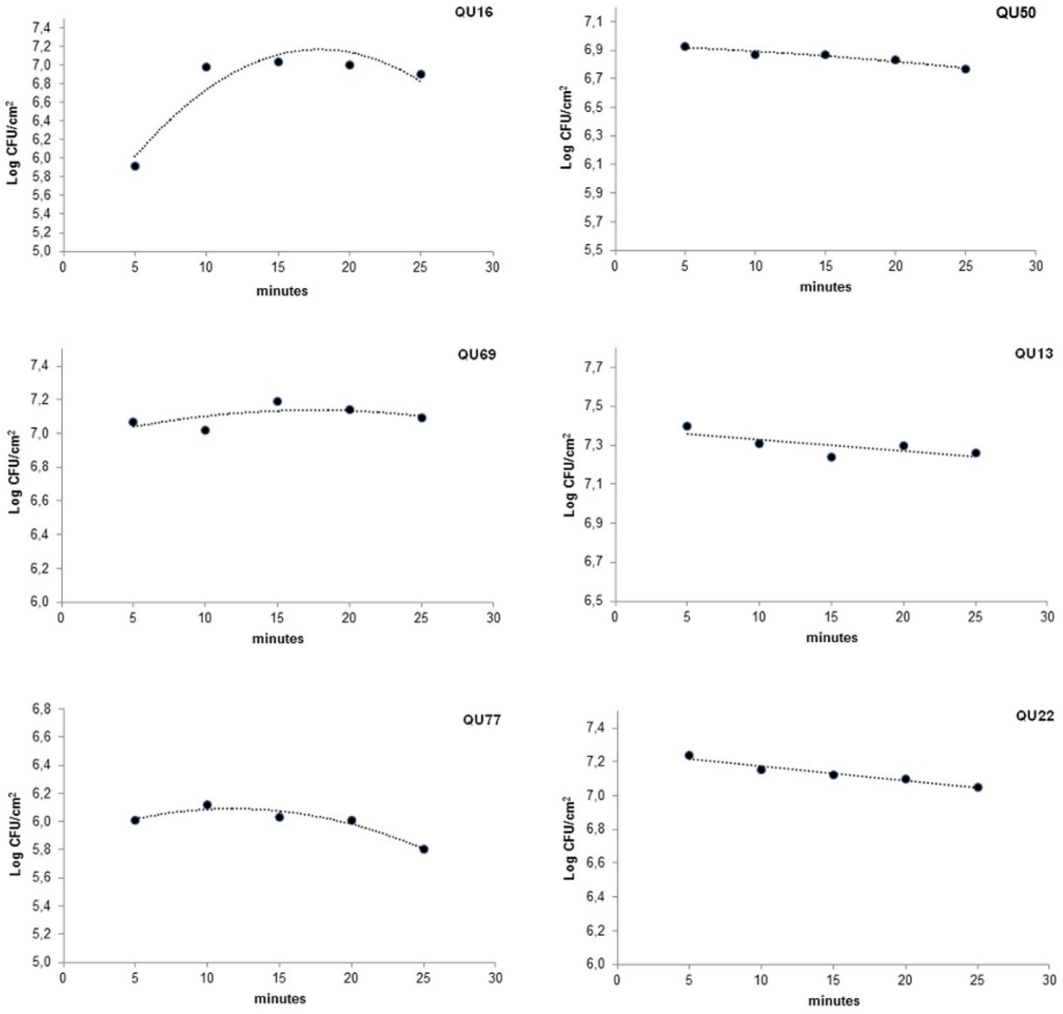

Figure 5. ***Yarrowia lipolytica*** **isolates used in the assay with mild detergent (3 %): QU16, QU50, QU69, QU77, QU22, QU13 at 5, 10, 15, 20, 25 min.**

The emulsification indexes (E_{24}) (Table III) ranged from 49.7 to 88.3 % in isolates of *Y. lipolytica* and bio-emulsification was demonstrated for all tested isolates. Strain QU50 presented the lower E_{24} result with 49.7 % index. Consequently, it obtained a higher significance ($p < 0.05$) in the results when compared to the values obtained for the other isolates (QU16 $p < 0.000$, QU69 $p < 0.000$, QU22 $p < 0.000$, QU13 $p < 0.001$ e QU77 $p < 0.002$).

DISCUSSION

The strains presenting the strongest biofilm formation capacity on the coupons were from *Y. lipolytica*. Corroborating with our findings of Montel et al. (2014), *Y. lipolytica* is a biotechnologically relevant fungus capable of colonizing utensils used during the manufacture of colonial cheese. In addition, the presence of yeasts biofilms in artisanal cheese can lead to spoilage of the product, resulting in discoloration, gas production, undesirable flavor

and changes in texture, as reported by Galinari et al. (2014). An investigation was performed to study biofilm formation on these materials, like stainless steel, rubber, silicon, glass, plastic, wooden surfaces and milking equipment. Molds, yeasts, and bacteria are the dominant microorganisms in this segment with stable formation of biofilms for an extended period (Montel et al. 2014). Biofilm formation by yeast can act in farm cheese in two ways, inducing product deterioration, creating undesirable flavor and discoloring the final product or can generate beneficial effect through proteolytic and lipolytic enzymes flavor enhancing during maturation.

Brugnoni et al. (2012) and Rosa et al. (2015) obtained yeast cell counting with results higher than 6.0 log (CFU/cm^2) and 7.0 log (CFU/cm^2) respectively. They also observed equal values between the counts in which there was no significant difference ($p > 0.05$), results that match our findings of 6.27 and 6.23, for instance, when coupons' surface adhesion was evaluated. It is also clear that the strain of this yeast presents itself in biofilm growing mode under certain conditions. The same strain is associated with food deterioration, including cheese varieties that develop the tyrosine-processing capability, promoting the change in its coloration (Zinjarde 2014). Galinari et al. (2014) tested biofilm formation with yeasts in wooden utensils employed in cheese production and observed lower resulting values when compared to the results of this study.

Many studies reported the diversity of biofilm-forming microorganisms isolated from diverse areas of the food industry. In contrast to what was presented in our study, we can relate bacteria adherence with significant results found by Beltrame et al. (2014) and Santos Junior et al. (2014), respectively, in which maximum counts of 6.92 log (CFU/cm^2) were observed in polyethylene coupons and, 6.17 log (CFU/cm^2) in solid polypropylene surfaces.

The production of cheese marketed as colonial also uses several sanitizing products employed in the food industry. In these areas, sanitation takes place by using the sanitizing products tested in our experiments, varying only the concentration and the amount of time the product is applied. Figures 2, 3 and 4 demonstrate survival rate of isolates of *Y. lipolytica* in all coupons. Based on these results, we can notice that the mold coupon was the most adherence favorable material, followed by beaker and hose coupons, respectively. The sanitizing product alkaline detergent had the better efficiency (Figure 3) in hose coupons with QU22 strain. Also in this coupon, the effect of sanitizer sodium hypochlorite showed higher reduction of biofilm. The coupon received treatment in the concentration of 1 and 1.5% (Figure 3) in the period of 10 and 20 minutes.

According to Van Houdt & Michiels (2009) and Mogotsi et al. (2014), among the many sanitizing products available, active chlorine is probably the most used compound, and sodium hypochlorite proved to be an active oxidizing agent that can destroy protein cell activity. However, penetration only happens completely when they are in a de-ionized state. Moreover, for better disinfection and efficacy, cleaning agents such as detergents appear in combination with a chlorine-based solution.

In contrast, if we compare the sanitizing products' effect on strains of *Y. lipolytica* there are no data regarding resistance of its adherence cells. Even so, there are other studies describing the action of disinfectants on yeast. Sodium hypochlorite and 70 % alcohol obtained effective action against a mixture of planktonic yeasts (Théraud et al. 2004). This solution reduced the adhesion of all strains of *Candida albicans*. For the majority of other species of

Candida (non-*albicans*), the increase in the rate of blastospores against hyphae on polystyrene did not show cause-effect over the production or in the proteinase enzyme activity (Webb et al. 2007). Biofilms formed by strains of *C. albicans* were eradicated when exposed to sodium hypochlorite for 30 minutes in concentrations of 1:32 or higher (Dahlan et al. 2011). Finally, IIknur et al. (2012) demonstrated biofilm reduction against the control group in species of *Candida* exposed to polystyrene. However, no tested disinfectant completely removed the biofilm.

The time-kill curve was used to determine the fungicidal activity of sanitizing products on isolates of *Y. lipolytica*. This study demonstrates results similar to those presented by Brugnoni et al. (2012), using the sanitizing products hypochlorite against strains of yeast *C. krusei*, *Zygosaccharomyces* sp., *K. marxianus* and R. *mucilaginosa*. It was also noticed that for planktonic cells the reduction happened by using a lower concentration of sodium hypochlorite (0.02 %) in all tested strains. However, in studies with Gram-negative bacteria, lower effectiveness is noticeable in comparison to our study with yeasts. When using chlorine based sanitizing, the time-kill curve showed that an average of 83 min of action of sodium hypochlorite (0.02 %) (Sukplang & Thongmme 2014) and 60 min of action of sodium hypochlorite (0.05 %) is needed for an effective sanitizing to take place. (Mazolla et al. 2006).

The contact angle of the sessile drop served as a method to characterize the hydrophobicity/hydrophilicity of the surfaces. The relation between hydrophobicity and biofilm formation, and the correlation among them are, in most cases, clear and with physical-chemical surface properties regulating the initial adhesion of microorganisms. Thus, the hydrophobic characteristics of the biofilm make its adhesive properties attach easily to the surfaces of material (Tarifa et al. 2013, Cappitelli et al. 2014). The free surface energy defined by the roughness of the material can also influence the formation of biofilm (Flausino et al. 2014). Lehocký et al. (2007) emphasize in their study that yeast cells play a significant role in adhesion as well as the substrate's surface. According to this concept, *Y. lipolytica* is an yeast capable of connecting only to very hydrophobic surfaces. As in our study, other results were favorable regarding the angle measurements with yeasts, promoting microbial adhesion. Gole et al. (2002) observed hydrophobic regions with contact angles of drops measured up to 105° in *Y. lipolytica* at the tested material surface. Gallardo-Moreno et al. (2004) with strains of *Candida parapsilosis* with measurements from 15° to 92°, also highlighted the contact angle measurements. The hydrophobicity of yeasts positively related to the adhesion rate of the tested material and with different levels of biofilm formation (Tarifa et al. 2013).

Many studies have demonstrated the formation of emulsification. Fontes et al. (2012) reported that strains of *Y. lipolytica* presented emulsification indices up to 68.0 and 70.2 %. Souza et al. (2012) also achieved good emulsifier production in their results with *Y. lipolytica* in the presence of seawater. To improve the production of biosurfactants, Fontes et al. (2010) achieved a better result of emulsification index (67.7 %) in a blend media with ammonium sulfate and yeast extract. Emulsification activity was also detected with *Y. lipolytica* in culture media in the study performed by Amaral et al. (2006).

Most emulsified hydrocarbons degrading microorganisms produce biosurfactants. The cell surface hydrophobicity is also an important aspect of the microbial cell adhesion to surfaces. Therefore, there is direct correlation between hydrophobicity, biosurfactant production, and microbial adherence (Youssef et al. 2004,

Coimbra et al. 2009). All the isolates, excluding QU50 isolate, displayed relatively significant emulsification capabilities, with rates above 70%.

Groenewald et al. (2014) describe that, due to its lipolytic and proteolytic activities, *Y. lipolytica* strains has been widely employed in maturation or contributed to organoleptic characteristics, although it does trigger deterioration in some types of cheese. These quality altering effects include non- standard flavor, undesirable texture, surface browning and biogenic amine formation that contributes to product decomposition.

There are few studies on biofilm formation by *Y. lipolytica*. The results showed that *Y. lipolytica* isolates from colonial cheese shows significant ability to adhere to polypropylene. However, tests with sanitizers were not able to inactivate all adherent cells. Hydrophilic capability and bioemulsifier production were observed within these isolates.

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