Food Chemistry

The conservative effects of lipopeptides from *Bacillus methylotrophicus* DCS1 on sunflower oil-in-water emulsion and raw beef patties quality

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ABSTRACT

Lipid oxidation was considered as a problem in food conservation. The present study aims to investigate the effect of lipopeptides DCS1 on the conservation of food models against lipid oxidation by determining the pri-mary and the secondary oxidation products. Lipopeptides DCS1 are able to preserve the nutritional properties of the emulsion during 23 days of storage, at a concentration of 0.0125% (w/w of emulsion), by slowing down the formation of hydroperoxides and malondialdehyde (MDA) compounds. The direct incorporation of lipopeptides in ground beef patties at a concentration of 0.5% (w/w of meat) was found to be more effective than gelatin film enriched with lipopeptides (2.5%, w/w of gelatin) as a coating, in inhibiting lipid oxidation. Furthermore, lipopeptides DCS1 are not toxic to human kidney cells HEK293 up to a concentration of 250 μg/ml. The results indicate that lipopeptides DCS1 are effective for the preservation of fatty foods against lipid oxidation.

1. Introduction

Lipid oxidation is recognized as a significant problem in food preservation and in the meat product processing. Oxidation is the most important cause of nutritional value and organoleptic quality of foods deterioration, such as oil-in-water (O/W) emulsions, which are used in the food, cosmetic and pharmaceutical industries (Dubuisson, Picard, Grisel, & Savary, 2018). The oxidation of lipid foods containing unsaturated or polyunsaturated fatty acids are mainly associated with the appearence of free radicals that cause the production of various low-molecular weight compounds such as aldhehydes and ketones, responsible for the development of off-flavors, making oil less acceptable or even unacceptable to consumers or for industrial use as a food ingredient. Lipid oxidation decreases the nutritional, organoleptic and palatability quality of food products, which may reduce their shelf life (Mc-Clements & Decker, 2000). In addition, the consumption of oxidized foods and toxic compounds causes serious diseases such as cancer, stroke, diabetes, cardiovascular diseases, etc. For this, in order to protect against these diseases, it is important to inhibit the formation of free radicals and lipids oxidation via the addition of antioxidants that can rapidly scavenge free radicals propagators of radical chains and/or species initiating oxidation (Schepens, Roelofs, Peters, & Wanten, 2006).

Hundreds of synthetic and natural compounds were tested for their effectiveness as free radical scavengers. Synthetic antioxidants, such as tertiary butyl hydroquinone (TBHQ), butylated hydroxytoluene (BHT), octyl gallate (OG), 2,4,5-trihydroxybutyrophenone (THBP), nordihydroguaiaretic acid (NDGA) and butylated hydroxyanisol (BHA) are used in edible vegetable oil to prevent rancidification, due to their high performance and low cost (Patil, 2013). However, some reports suggest that these chemical antioxidants have several disadvantages, they may be responsible for liver damage and carcinogenesis; hence the interest of using natural products in food industry has been increased (Kim, Kim, Lee, Yoo, & Lee, 2010).

The use of novel glycolipid and lipopeptide surfactants, produced by microorganisms, as functional ingredients in food products and

processing, and in pharmaceutical industries, is of great importance due to their interesting and unique properties such as low toxicity, functionality under extreme conditions and biodegradable nature (Cortés-Sánchez, Hernández-Sánchez & Jaramillo-Flores, 2013). Lipopeptides were described in several reports for their *in vitro* antioxidant properties; they exhibit a scavenging activity towards free radicals and reductive ability (Tabbene et al., 2012; Ben Ayed, Hmidet, Béchet, Jacques, & Nasri, 2017). They are often added to foods rich in polyunsaturated fatty acids to retard lipid peroxidation or oxidative rancidity.

Lipopeptides synthesized by *B. methylotrophicus* DCS1 are endowed with very interesting *in vitro* antioxidant activities (Jemil, Ben Ayed, Manresa, Nasri, & Hmidet, 2017). This paper discusses the effect of lipopeptides DCS1 incorporation in the composition of sunflower oil-in-water (10%, w/w of emulsion) emulsions and in raw beef patties, as protectors against oxidation reactions during conservation. Also a new type of active gelatin film enriched with lipopeptides DCS1 was used as coating for ground beef patties to retard lipid oxidation and extend shelf life.

2. Material and methods

2.1. Bacterial strain and lipopeptides production

Bacillus methylotrophicus DCS1 strain was used in this study as lipopeptides producer. The lipopeptides production and extraction were performed as described in our previous study (Jemil, Manresa, Rabanal, Hmidet, & Nasri, 2017).

2.2. Antioxidant properties of lipopeptides in model food emulsions

2.2.1. Removal of tocopherols from sunflower oil

Removal of tocopherols from sunflower oil was realized as described by Gallego, Gordon, Segovia, Skowyra, and Almajano (2013).

2.2.2. Preparation of emulsions and storage conditions

An oil-in-water emulsion was prepared as described by Gallego et al. (2013). Lipopeptides DCS1 were added to the emulsion and homogenized obtaining final concentrations of 0.00416% and 0.0125% (w/w of emulsion). The negative control was prepared substituting the sample with water and the positive control was prepared with trolox with a final concentration of 0.0022% (w/w of emulsion). All the emulsions were prepared in triplicate and incubated in an oven in darkness with constant elliptical movement at 30 ± 1 °C for 28 days.

2.2.3. Determination of primary oxidation products and measurement of pH

The primary oxidation products were monitored every 2 days by determining the peroxide value (PV) according to the method of Gallego et al. (2013). The pH of the emulsions was measured with a pH-meter (GLP21, Criston Instruments, Barcelona, Spain).

2.2.4. Determination of secondary oxidation products by thiobarbituric acid reactive substances (TBARS) method

An amount of each emulsion was taken and the TBARS reagent was added in the ratio 1:10. The TBARS assay was conducted as described by Gallego et al. (2013).

2.3. Retardation of lipid oxidation in ground beef patties using gelatin film enriched with lipopeptides

2.3.1. Gelatin film production

Films were prepared with type A gelatin (2 g porcine gelatin/100 g of filmogenic solution composed of ultrapure water + gelatin + sorbitol), which was hydrated for 10 min at 25 °C with stirring and then solubilized for 15 min at 55 °C in a thermostatic bath according to the method reported by Gallego, Gordon, Segovia, and Almajano (2016). After complete solubilization, sorbitol, the plasticizing agent, was added (20 g/100 g of gelatin) and the solution was stirred, then lipopeptides DCS1 (2.5%, w/w of gelatin) was added to the filmogenic solution. Similarly, a positive control film with the synthetic antioxidant BHA (0.2%, w/w of gelatin) and a negative control gelatin film without antioxidant product were prepared. The filmogenic solutions were spread in polyethylene plates (60 g filmogenic solution/plate), then they were dried in a ventilated oven (Environmental chamber model H110K-30DM; Seiwa Riko Co., Tokyo, Japan) at 30 ± 0.5 °C for 24 h.

2.3.2. Ground beef patties formulation and processing

A piece of ground meat (weighs 1 kg), taken from the round part of beef, was purchased fresh from the butchery. The ground meat was mixed with salt (1.5%, w/w) and divided into parts of 100 g. Some parts were mixed to homogeneity with compounds: positive control 0.5% (w/w) synthetic antioxidant (BHA), 0.5% (w/w) lipopeptides DCS1 and negative control (without antioxidant). After 3 min of kneading, each blend was flattened and formed into patties of 8–10 g of weight, 4 cm of diameter and 0.5 cm of thickness, using a round cutter as described by Ouerfelli, Bettaieb Ben Kaâb, and Almajano (2018). The other parts of ground meat were also formed into patties, then, each beef pattie was covered from the top and bottom with gelatin film: negative control gelatin film (without antioxidant), 0.2% (w/w of gelatin) BHA-gelatin film and 2.5% (w/w of gelatin) lipopeptides DCS1-gelatin film to finally obtain 6 different treated beef samples. Beef patties samples were placed in plastic trays, covered with cling film and stored in refrigerator at 4 ± 1 °C for 14 days. At each specified day (day 1, 3, 5, 7, 10, 12 and 14), beef patties were used for determination of TBARS and for pH and colour measurements.

2.3.3. TBARS assay

The effect of lipopeptides on lipid oxidation of the chilled raw beef patties was evaluated during 14 days of storage at 4 °C by the determination of TBARS, assessed following the method described by Gallego, Gordon, Segovia, and Almajano (2015). TBARS determinations for each sample were performed in triplicate.

2.3.4. pH and colour measurements

Acidity of model meat products was measured directly using an Orion 3-Star pH Benchtop Meter (Thermo Fisher Scientific, Waltham, MA, USA).

The surface colour of model meat products was evaluated following the method of Skowyra, Janiewicz, Salejda, Krasnowska, and Almajano (2015). The evaluation was repeated after 3, 5, 7, 10, 12 and 14 days of cool storage in the darkness.

2.4. Cell line and culture

HEK293 cells (Human embryonic kidney 293 cells) were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% foetal bovine serum, 50 $IU.ml^{-1}$ penicillin, 50 mg/ml streptomycin at 37 °C in an humidified 5% CO_2 atmosphere (Elloumi-Mseddi et al., 2018).

2.5. Cytotoxicity assay

Cell viability of HEK293 cell lines was evaluated using the MTT assay as described by Mosmann (1983). 100 µl of lipopeptides DCS1, dissolved in distilled water, were added at concentrations ranging from 0.03 to 2 mg/ml in wells of 96 well-plate containing cells seeded at 8.10^4 cells/ml of medium. Control cells were supplemented with 100 µl distilled water. HEK cells were treated for 48 h. This test was conducted as reported by Jardak, Elloumi-Mseddi, Aifa, and Mnif (2017). The cell survival was expressed as follows:

$$(\%)\ \text{cell survival} = (A_1/A_0)_* 100$$

where A_0 is the control absorbance and A_1 the absorbance of the treated cells.

2.6. Statistical analysis

All experiments were run at least in triplicate. Data were expressed as means ± SD (standard deviation) and analyzed using IBM SPSS statistics ver. 20.0, professional edition. A one-way analysis of variance (ANOVA) was then performed and means comparison was carried out by Duncan's multiple range test to estimate the significance among the main effects at the 5% probability level.

3. Results and discussion

3.1. Antioxidant effects of lipopeptides DCS1 in stored emulsions

Sunflower oil is sensitive to oxidation and rancidity because of its high content of polyinsaturated fatty acids: linoleic acid (65.7%) and oleic acid (19.5%) (USDA National Nutrient Database for Standard Reference). Analyses of PV and TBARS, corresponding to the primary and secondary lipid oxidation products, respectively, were performed during 28 days to monitor the efficacy of lipopeptides DCS1 in inhibiting oil autoxidation.

3.1.1. Evolution of peroxide value

The development of primary oxidation of emulsions was monitored by the evolution of PV during storage to evaluate hydroperoxide formation (Fig. 1a). Primary degradation of lipids occurs due to the reaction between unsaturated fatty acids in oil and oxygen that form hydroperoxides. The time required for the emulsions to reach a peroxide value of 10 mg hydroperoxides/kg of emulsion was determined as a measure of stability. When the PV is greater than this value, the emulsion is in a oxidized state and starts to become rancid. This value was taken since the limit for products of edible fats (animal, plant and anhydrous), margarine, and fat preparations, to guarantee quality is < 10 mg hydroperoxides/kg (Azman, Segovia, Martínez-Farré, Gil, & Almajano, 2014).

The negative control emulsion (without antioxidant product) was oxidized first and the PV value increased rapidly during storage, reaching more than 10 mg hydroperoxides/kg of emulsion after only 4 days of storage, followed by the positive control, trolox (0.0022%, w/w) which reach a level of deterioration after 6 days. The emulsion containing lipopeptides DCS1 with a concentration of 0.00416% was not oxidized during the first 14 days, reaching a value of 6.7 mg hydroperoxides/kg of emulsion on the 14th day, after this period, the PV increased and the emulsion was oxidized. While the emulsion containing lipopeptides DCS1 with a concentration of 0.0125% remains stable until the 26th day of storage with a PV value of 8.7 mg hydroperoxides/kg of emulsion. It can be concluded that increasing the concentration of DCS1 lipopeptides from 0.00416% to 0.0125% improves the durability of the emulsion from 14 to 26 days (Fig. 1a).

The obtained results suggest that lipopeptides DCS1 are able to delay and inhibit the formation of hydroperoxides, at low concentrations, during the storage time of the emulsions. The amino acid composition contributes greatly to the antioxidant properties (Malomo, He, & Aluko, 2014). In our previous study (Jemil et al., 2017); the structural characterization and identification of cyclic lipopeptides produced by *B. methylotrophicus* DCS1 strain were investigated. A variety of amino acids was detected in crude lipopeptides DCS1, the majority of them are present in the composition of lipopeptides belonging to surfactin, iturin and fengycin families, they are: Glx, Asx, Tyr, Leu, Thr, Pro, Ser, Val, Ala and Ile with molar ratios of 19.5%, 14.74%, 8.45%, 8.33%, 7.93%, 7.49%, 6.29%, 5.69%, 4.49% and 3.12%, respectively. These results showed clearly that DCS1 lipopeptides are rich on hydrophobic amino acids (alanine, valine, leucine, isoleucine, proline and tyrosine). Rajapakse, Mendis, Byun, and Kim (2005) reported that high radical scavenging activities of peptides are related to the high hydrophobicity. Also, Nam, You, and Kim (2008) showed that aromatic amino acids like Tyr, which is present with one residue in the structure of iturin lipopeptide variants and with two residues in the structure of fengycin isoforms, contribute to the antioxidant potency of peptides because they act as hydrogen donors. Furthermore, the antiradical activity can be assigned to the presence of the hydrocarbon fatty acid chain in lipopeptide structure (Ben Ayed et al., 2017) and some active residues in the peptide ring containing amine and hydroxyl groups which react with the free radicals of polyunsaturated fatty acids by giving a hydrogen atom converting them to more stable products, thereby terminating the radical chain reaction (Khantaphant & Benjakul, 2008).

3.1.2. Evolution of pH over time

The pH of the emulsions was measured as a parameter to investigate its correlation with PV values (Fig. 1b). Following the level of primary oxidation, the pH decreased in accordance with oxidation. The pH of the negative and positive control emulsions decreased as the PV increased from the first days, the decrease of pH is significant in the first 12 days, then it was nearly constant. Many antioxidants have the disadvantage of being less effective as antioxidants when the pH is low, such as the trolox. The high values of pH were observed in the emulsion enriched with 0.0125% (w/w) lipopeptides DCS1, followed by that enriched with 0.00416% (w/w) lipopeptides DCS1. From the 12th day of storage, a difference between the pH values of these two emulsions was observed, this result is correlated with that of the PV. We have not observed a decrease in pH values in the emulsion containing 0.0125% (w/w) lipopeptides DCS1, this is explained by the low values of PV. While the pH of the emulsion with a concentration of 0.00416% (w/w) lipopeptides DCS1 decreased slightly from the 16th day of storage (Fig. 1b). This pH drop is caused by the increase of the PV of the emulsion from the 16th day. It could be concluded that the pH remains almost stable and does not decrease when the hydroperoxides content in the emulsion is very low and the lipid oxidation is very slow. When the oxidation of the fatty acids in the emulsion is accelerated, the pH decreases.

Lipid hydroperoxides are highly unstable that break down easily, decomposition products are acidic such as the formation of ketones, esters, alcohols, epoxides and organic acids that leads to changes in the pH (Skowyra, Falguera, Gallego, Peiró, & Almajano, 2014). The levels of some metal compounds in sunflower oil such as iron (0.26 ppm) and copper (5.2 ppb), will promote oxidation wich affects the pH (Choe & Min, 2006). Furthermore, the redox state of metals and chelation capacity of antioxidants are among the parameters that affect the rate of emulsion oxidation (Decker, Warner, Richards, & Shahidi, 2005).

3.1.3. Evolution of TBARS values

The TBARS method is widely used for determining the oxidation of fats and oils in foods (Mendes, Cardoso, & Pestana, 2009; Wenjiao, Yongkui, Yunchuan, Junxiu, & Yuwen, 2014). At the last week of emulsions storage, the oxidation was also monitored by determining the secondary oxidation products formation using the TBARS method (Fig. 2). MDA compounds are formed when the concentration of hydroperoxides is appreciable in sunflower oil (Guillén & Cabo, 2002). Hydroperoxides decompose to form secondary oxidation products, which are responsible for the off-flavor, the rancid odor and undesirable taste of oxidized edible oil (Pangloli, Melton, Collins, Penfield, & Saxton, 2002).

According to analyses, TBARS values of negative control emulsion are very high. The TBARS values of all the treated emulsions including 0.0022% trolox, 0.00416% and 0.0125% lipopeptides DCS1, were significantly lower ($p < 0.05$) than those of the non-treated emulsion during the 4th week of incubation. The TBARS values of emulsion containing trolox (0.0022%) are significantly lower ($p < 0.05$) than

those of emulsion containing lipopeptides with 0.00416% concentra-tion. In this case, there is no correlation with the results obtained in peroxide value. Lipopeptides DCS1 with the concentration of 0.0125%contributed a significant lipid stability, which is stronger than that at the concentration of 0.00416% during storage as shown by the lowest TBARS values. TBARS values of emulsion containing 0.0125% lipo-peptides DCS1 were the lowest values, increasing from 0.49 to 1.96 (mg MDA/kg emulsion) from day 20 to day 27 (Fig. 2).

The acceptable limits of TBARS value in fat products was set at 1.0 mg MDA/kg (Nollet & Toldra, 2011). As conclusion, lipopeptides DCS1 at a concentration of 0.0125% (w/w) are able to preserve the nutritional properties of the emulsion during 23 days of storage.

3.2. Study of the oxidative stability of raw beef patties enriched with lipopeptides DCS1

3.2.1. Lipid oxidation

A direct method via the determination of species reacting with thiobarbituric acid (TBARS) was used to evaluate the effectiveness of the antioxidant compounds in preventing oxidative degradation of li-pids with the production of compounds, such as aldehydes and con-jugated hydroperoxides.

The antioxidant effects of DCS1 lipopeptides and the synthetic an-tioxidant BHA in ground beef patties (0.5%, w/w) compared with the negative control are illustrated in Fig. 3a. The TBARS index revealed that the secondary oxidation of beef patties without antioxidant com-pound increased progressively as storage advanced. DCS1 lipopeptides showed effective antioxidant activity against lipid oxidation. In fact, the TBARS content of the beef patties treated with lipopeptides was almost similar to that of the patties treated with BHA and increased slightly over time of storage. The inhibitory effect of MDA formation observed with DCS1 lipopeptides is greater than that observed with the negative control. Fig. 3a shows that after 12 days of storage, the TBARS value of the beef pattie without antioxidant compound reached a value of 2.8 mg MDA/kg meat, whereas the TBARS values of patties enriched with 0.5% DCS1 lipopeptides and BHA were 0.35 and 0.25 mg MDA/kg meat, respectively, significantly less than the control. It was noted that the beef patties containing DCS1 lipopeptides and BHA did not reach a value of 1.5 mg MDA/kg meat by the end of the long storage period (day 14). According to Martínez, Cilla, Beltrán, and Roncalés (2006), an index of 1.5 mg MDA/kg is closely related to perceptible and un-acceptable off-odour of meat. It can be concluded that DCS1 lipopep-tides are natural antioxidants which could be considered as alternatives to synthetic antioxidants for the preservation of meat against lipid oxidation for a long period.

Fig. 3b shows TBARS values of beef patties covered by active films. They were very affected by the storage time and increased continuously up to 14 days of storage. TBARS values at the third day of storage of control, DCS1 lipopeptides and BHA were 1.3 ± 0.05, 1 ± 0.012 and 0.9 ± 0.028 mg MDA/kg meat, respectively and increased to 3.1 ± 0.015, 3 ± 0.03 and 2.28 ± 0.37 mg MDA/kg at the end of storage (day 14).

The best gelatin film regarding lipid oxidation retardation is the one prepared with 0.2% BHA. The highest MDA concentration was obtained in the patties covered with gelatin film without antioxidant compound, followed by those covered by gelatin film treated with DCS1 lipopep-tides (2.5%). Their TBARS content are close indicating that lipopeptides incorporated in gelatin film are not very efficient in delaying lipid oxidation. From the results obtained, the conclusion is that the direct incorporation of antioxidants into meat is more effective in preserva-tion against lipid oxidation than using gelatin-based antioxidant packaging.

3.2.2. Changes in pH values of raw beef patties

Changes in pH values of raw beef patties enriched with lipopeptides DCS1, as well as those enriched with BHA and the non-enriched patties, during storage period are represented in S1. The pH values of the en-riched raw beef patties were significantly higher ($p < 0.05$) than those of non-enriched samples during 14 days of refrigerated storage. The difference in pH values was not significant in the same sample from the beginning to the end of refrigerated storage, indicating no production of basic compounds in the meat. The difference in pH values was not significant between the different samples coated with gelatin-films enriched with BHA, DCS1 lipopeptides and the negative control-film and in the same sample during storage period.

Muela, Sañudo, Campo, Medel, and Beltrán (2010) reported that the high degradation of proteins in muscle tissues can cause a high spoilage of microorganisms, resulting the production and the accumulation of amines, ammonia and other basic substances that could be responsible for the increase of the pH of the meat and its products.

3.2.3. Colour changes

The first impression consumers have of any meat product is its colour and thus colour is of extreme importance because it could be a good sign of the quality and freshness of meat. Colour acceptability decreases throughout storage time.

Instrumental colour parameters measured on the surface of the ground beef patties during 14 days of refrigerated storage are illustrated in S2. The most important colour parameter of meat products is the Redness *(a*)* value. The Redness values of ground beef patties enriched with BHA were significantly higher ($p < 0.05$) than those of the ne-gative control samples and the samples enriched with lipopeptides DCS1 during 14 days of refrigerated storage. The Redness of the dif-ferent samples decreased significantly ($p < 0.05$) during storage days. The colour of beef patties with lipopeptides DCS1 is darker than the other samples because of the lipopeptides powder colour. From the 10th day of storage, the redness values of beef patties enriched with lipo-peptides DCS1 were significantly higher ($p < 0.05$) than those of the negative control.

During all the storage period, the Yellowness *(b*)* values of samples enriched with BHA were significantly higher ($p < 0.05$) than those of the negative control and the samples enriched with lipopeptides DCS1. We notice that the Yellowness *(b*)* values decreased significantly over time for all samples tested. The difference in Lightness *(L*)* values was not significant ($p < 0.05$) between the different samples from the be-ginning to the end of refrigerated storage (S2a).

As shown in S2b, the values of Redness *(a*)*, Yellowness *(b*)* and Lightness *(L*)* of the different samples coated with gelatin-films en-riched with BHA, DCS1 lipopeptides and the negative control-film were lower than those of samples enriched with BHA, DCS1 lipopeptides and the negative control, respectively, at the 14th day of storage, indicating that the direct incorporation of bioactive compounds in ground beef patties is more effective than the application of gelatin film enriched as a coating in inhibiting deterioration of beef patties quality.

3.3. Cytotoxicity assay

Mitochondria play a key role in cellular respiration and energy production, which is essential for cell growth and proliferation and for cell function. As a measure of cell viability, the mitochondrial activity of HEK293 cells after exposure to lipopeptides DCS1 for 48 h was de-termined using the MTT assay. Cells without treatment with lipopep-tides were considered as a control with 100% cell viability. Results of lipopeptides cytotoxicity assay are expressed as percentage (%) of cell viability based on increasing lipopeptides DCS1 concentration (30–250 μg/ml) (Fig. 4). As indicated in Fig. 4, cell viability decreases significantly ($p < 0.05$) with increasing lipopeptides concentration from 98% at a concentration of 30 μg/ml to 74% at a concentration of 62 μg/ml. From a concentration of 62 μg/ml lipopeptides DCS1, there is no significant difference between cell viability percentages. The results obtained suggest that lipopeptides DCS1 at concentrations ranging from 30 to 250 μg/ml are nontoxic, since cell viability is greater than 50%.

Basit, Rasool, Naqvi, Waseem, and Aslam (2018) reported that the cy-totoxicity test of biosurfactants from *B. cereus* towards the BHK-21 cell line revealed a 63% cell viability at a concentration of 10 mg/ml and these biosurfactants are considered nontoxic since the percentage of cell survival is >50%.

4. Conclusion

Lipopeptides DCS1 were used as protectors against oil oxidation (oil-in-water emulsion) and fat in beef meat with great results, the durability of the emulsion and beef meat increased significantly relative to oxidation. The results of this study suggest that lipopeptides DCS1 are potential source of natural antioxidants and can be successfully used to decrease lipid oxidation and improve the shelf life of the emulsion and beef meat. This is the first work describing the effect of lipopeptides in the stability of oil-water emulsion and the conservation of ground beef patties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influ-ence the work reported in this paper.

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FIGURES

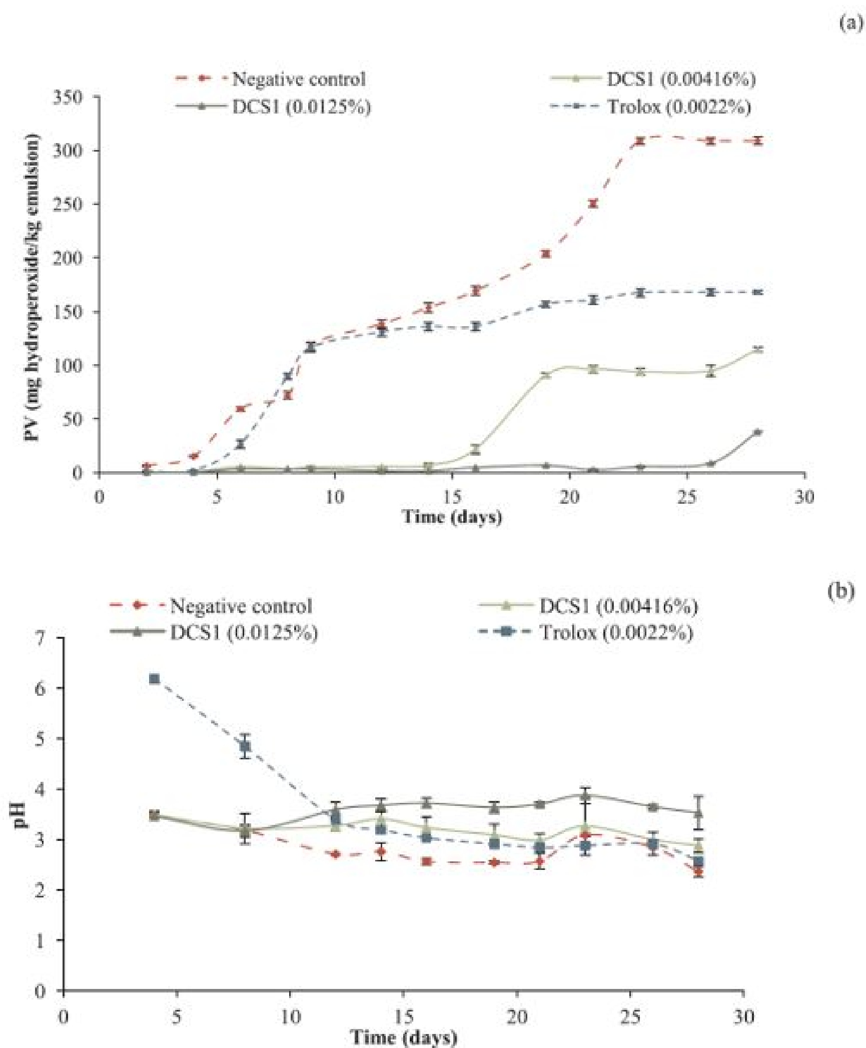


Fig. 1. Changes in PV (a) and pH evolution (b) of emulsions conserved with lipopeptides DCS1. Trolox was used as positive control.

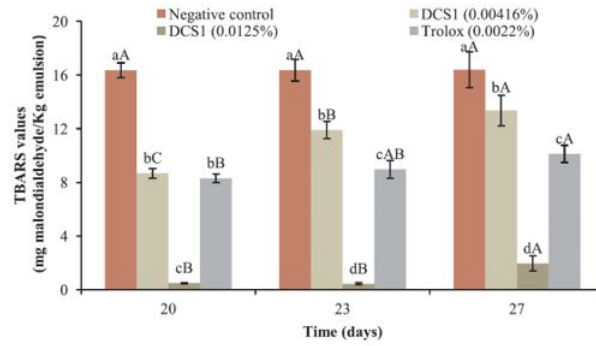
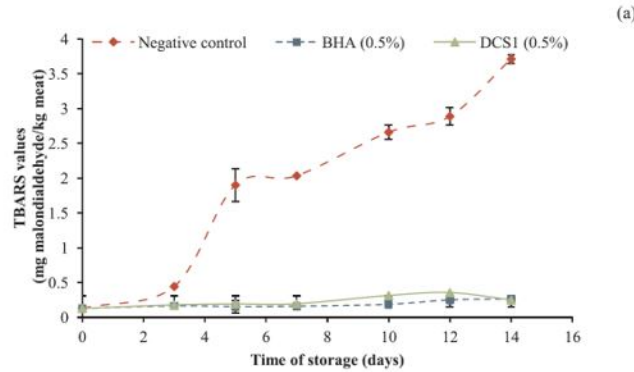


Fig. 2. TBARS values of emulsions con-served with lipopeptides DCS1. Trolox was used as positive control. a, b, c Different letters in different samples within the same period of storage indicate significant differ-ences ($p < 0.05$). A, B, C Different letters in different periods of storage within the same sample indicate significant differences ($p < 0.05$).

(a)



(b)

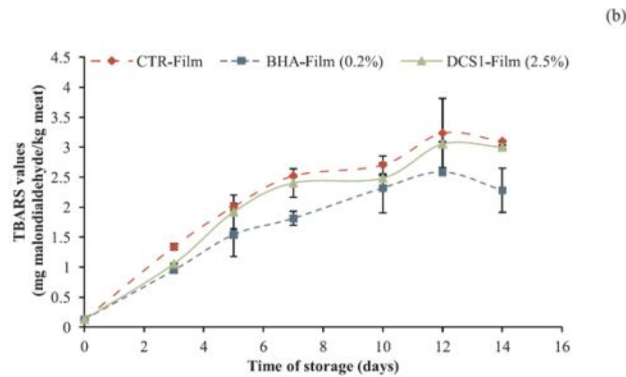


Fig. 3. TBARS values of raw beef patties formulated with lipopeptides DCS1 (0.5%). BHA (0.5%) was used as positive control (a). TBARS values of raw beef patties covered with gelatin films containing lipopeptides DCS1 (2.5%). BHA-gelatin film (0.2%) was used as positive control (b). Values presented are the mean of triplicate analyses (n = 3) and are expressed as mean value ± standard deviation.

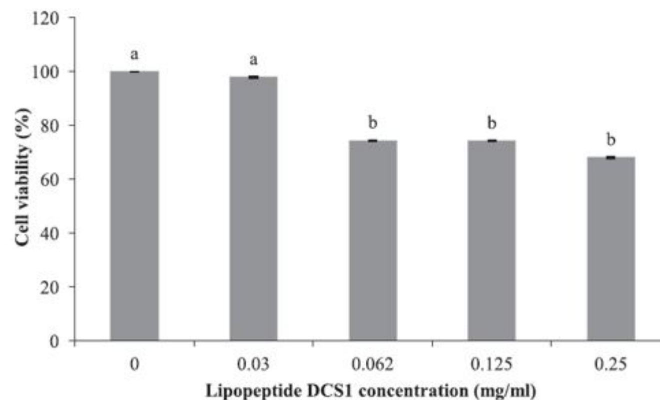


Fig. 4. Cytotoxicity effect of lipopeptides DCS1 on HEK293 cells line for 48 h. Each point represents a mean ± standard deviation of six replicates per con-centration. a, b, c Different letters in different lipopeptide DCS1 concentrations indicate significant differences ($p < 0.05$).

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