Toxicology Reports 2 (2015) 1164–1170

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Formulation of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*

Jenyffer M. Campos[a], Tânia L.M. Stamford[a], Raquel D. Rufino[b], Juliana M. Luna[b], Thayza Christina M. Stamford[a], Leonie A. Sarubbo[b,*]

[a] Universidade Federal de Pernambuco (UFPE), Rua Nelson Chaves, s/n, Cidade Universitária, Recife, PE, Brazil
[b] Centro de Ciências e Tecnologia, Universidade Católica de Pernambuco (UNICAP), Rua do Príncipe, Boa Vista, Recife, PE, Brazil

ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form 20 August 2015
Accepted 20 August 2015
Available online 24 August 2015

Chemical compounds studied in this article:
Glycerides (PubChem CID: 57.375.664)
Xanthan (PubChem CID: 7107)
Carboxymethylcellulose (PubChem CID: 6.328.154)
Starch solution (PubChem CID: 439.341)

Keywords:
Biosurfactants
Emulsion
Foods

ABSTRACT

Biosurfactants have a number of industrial applications due their diverse properties, such as emulsification, foaming, wetting, and surface activity. The aim of the present study was to produce a biosurfactant from *Candida utilis* and employ it in the formulation of a mayonnaise. The biosurfactant was produced in a mineral medium supplemented with glucose and canola waste frying oil at 150 rpm for 88 h. The product was biologically tested on rats and in different formulations of mayonnaise, which were submitted to microbiological evaluations. The biosurfactant was added to the diet of the rats for 21 days. Greater consumption was found of the experimental diet. Moreover, no changes were found in the liver or kidneys of the animals, demonstrating the absence of a toxic effect from the biosurfactant. Six different formulations of mayonnaise were prepared and tested regarding stability with the addition of carboxymethyl cellulose and guar gum (combined and isolated) after 30 days of refrigeration. The most stable formulation with the best quality was obtained with combination of guar gum and the isolated biosurfactant, with an absence of pathogenic microorganisms. In conclusion, the potential and innocuousness of the biosurfactant isolated from *C. utilis* indicates its safe use in food emulsions.

1. Introduction

Due to their physicochemical properties, microbial surface-active compounds are attractive for use in a wide variety of industrial and biotechnological applications as additives in foods, cosmetics, and detergent formulations [1,2]. Surfactants have been used in the food industry for centuries. The most useful property of these compounds is their ability to form stable emulsions, which improves the texture and creaminess of dairy products. Biosurfactants are also used to prolong the shelf life of products, solubilize vegetable oils, improve organoleptic properties in bakery and ice cream formulations and stabilize fats during the cooking process [3,4].

Naturally occurring surfactants, such a lecithin from egg yolk and various proteins from milk, are used in the preparation of food products, such as mayonnaise, salad dressing, and deserts. More recently, synthetic surfactants, such sorbitan esters and their ethoxylates and sucrose esters, have been used in food emulsions. Hence, the understanding of the formation, structure and properties of emulsions is essential to the stabilization of components in food products [5]. Campos et al. [6] recently published a review of the literature on the application of biosurfactants in the food industry due to their emulsifying properties, which contribute to consistency and texture, the solubilization of aromas and the stabilization of aerated systems.

A number of studies have reported food formulations involving gelled emulsions [7], dispersions [8], nanoemulsions [9], and emulsions in products such as mayonnaise and salad dressing. Emulsions have been developed with the inclusion of natural components, such as raw lentil powder [10], oatmeal flour [11], blends of gum arabic or propylene glycol alginate in admixture with xanthan [12], inulin [13,14], locust bean gum [15], and konjac glucomannan [16]. Emulsions also contribute to food preservation [17] and stability [18,19].

Due to the increasing use of surfactants, the identification of compounds with low toxicity and satisfactory surface activity properties is of considerable interest. Thus, the aim of the present study was to apply the biosurfactant produced from *Candida utilis* as a safe bioemulsifier in the formulation of a mayonnaise.

* Corresponding author. Fax: +55 81 21194000.
E-mail address: leonie@unicap.br (L.A. Sarubbo).

http://dx.doi.org/10.1016/j.toxrep.2015.08.009

2. Materials and methods

2.1. Microorganism

C. utilis (UFPEDA 1009) was obtained from the culture collection of the Department of Antibiotics of the Universidade Federal de Pernambuco, Brazil. The microorganism was maintained at 5 °C on yeast mold agar slants containing (w/v) yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), D-glucose (1.0%), and agar (2.0%). Transfers were made to fresh agar slants each month to maintain viability.

2.2. Substrates for biosurfactant production

All chemicals were of reagent grade. Canola waste frying oil and soybean frying oil were obtained from a local restaurant in the city of Recife (state of Pernambuco, Brazil), stored according to supplier's recommendations and used without any further processing.

2.3. Growth conditions

The microorganism inoculum was prepared by transferring cells grown on a slant to 50 ml of yeast mold broth. The seed culture was incubated at 28 °C and 200 rpm for 24 h. Slants of yeast grown in yeast mold broth were added to Erlenmeyer flasks with 100 ml of mineral media containing 0.1% NH_4NO_3, 0.01% KH_2PO_4, 0.5% $MgSO_4{\cdot}7H_2O$, 0.01% $FeCl_3$, 0.01% NaCl, 0.3% yeast extract, 6% glucose and 6% (w/v) canola waste frying oil as the carbon source [20]. The medium was sterilized by autoclaving at 121 °C for 20 min. The final pH of the medium was 5.7 and the surface tension prior to inoculation was 50 mN/m. The inoculum (1%, v/v) was added to the cooled medium at the amount of 10^8 cells/ml. Fermentation was carried out in Erlenmeyer flasks at 28 °C and 150 rpm for 88 h. Samples were withdrawn at regular intervals for analysis. All assays were carried out in triplicate and did not vary more than 5%.

2.4. Biosurfactant isolation

The broth after fermentation was initially filtered through a millipore membrane (0.45 μm) for the removal of the cells and then submitted to the extraction process described by Cameron et al. [21]. Three volumes of 95% ethanol containing 1% (v/v) acetic acid were added to the supernatant. The solution was cooled at 4 °C for 16 h and the precipitate was collected by centrifugation (10,000 × *g*). The crude biosurfactant was dried and stored at room temperature.

2.5. Biological characterization of biosurfactant

Biological analyses of the consumption of the isolated product were conducted to determine whether the biosurfactant was innocuous. Twenty-four juvenile Wistar rats (*Rattus norvegicus)* (mean age: 60 ± 2 days) were maintained in individual cages and distributed into two groups of 12 animals each (6 males and 6 females). The experimental group received a casein diet with the biosurfactant and the control group received the same diet without the biosurfactant. The diets were administered for 21 days. The macronutrient and micronutrient contents of the diets in both groups were calculated and balanced following the guidelines of the American Institute of Nutrition AIN (1993) [22]. The diets were prepared weekly in a laboratory. Twenty-five grams of ration were offered daily to each group in an amount capable of ensuring consumption *ad libitum*. Diet intake was quantified using the following equation:

$$C = \mathrm{DO} - (\mathrm{CR} + \mathrm{DR}) \quad (1)$$

Table 1
Additional ingredients incorporated into mayonnaise formulations.

Formulation	Ingredients tested
1	Carboxymethyl cellulose + guar gum
2	Carboxymethyl cellulose
3	Carboxymethyl cellulose + biosurfactant isolated from *C.utilis*
4	Guar gum
5	Guar gum + biosurfactant isolated from *C.utilis*
6	Biosurfactant isolated from *C.utilis*

in which *C* = weekly consumption of diet (g), DO = diet offered (g), CR = clean reject (g) and DR = dirty reject (g).

Body mass gain was measured weekly between 7 and 9 am using a digital electronic scale (Tecnal) with a 4-Kg capacity to plot the weight curve. The animals were weighed at the onset of the experiment and after each week for the determination of the feeding efficiency coefficient (FEC) using the equation proposed by Campbell [23]:

$$\mathrm{FEC} = \frac{(\mathrm{WW} - \mathrm{IW})}{\mathrm{TF}} \quad (2)$$

in which WW = weekly body weight, IW = initial weight and TF = total amount of food ingested weekly.

Euthanasia was performed on Day 21. The animals were anesthetized with a combination of ketamine and xylazine (p.c. 0.1 ml/g; volume 50 ml + 20 mg/body weight) through an intramuscular injection. After the verification of anesthesia, the kidneys and livers were collected and weight. The experimental protocol received approval from the Ethics Committee on Animal Experimentation of the Federal University of Pernambuco, Brazil (process n°23076.039279/2012-85).

2.6. Application of biosurfactant as food additive

The emulsifying property of the biosurfactant was tested in a mayonnaise with the following composition: 40% sunflower oil (Bunge), 40.3% water, 10% vinegar (commercially purchased), 4% egg powder (Naturovos LTDA, Brazil), 2% sugar, 2% salt, 1% mustard powder (all commercially purchased), and 0.5% instant starch (Unilever LTDA, Brazil). The biosurfactant isolated from *C. utilis* was used at a concentration of 0.7% with the addition of 0.2% guar gum and 0.2% carboxymethyl cellulose (Cromato Produtos Químicos LTDA, Brazil) in the formulation of six different mayonnaises (Table 1). The ingredients were mixed in a blender for one minute at room temperature. The mayonnaises were stored at 4 °C for one month for the visual inspection of appearance [24] and evaluated weekly regarding the stability of the emulsion.

2.7. Statistical analysis

The results were expressed as mean and standard error of the mean (SEM). Data analysis was performed with the aid of the Sigma Stat 3.5 software program. The Student's *t*-test was used for the comparisons, with p-values ≤ 0.05 considered statistically significant.

3. Results

3.1. Biological characterization of biosurfactant

Table 2 displays the composition of the diets offered to the two groups. It was necessary to calculate the proportion of the replacement of soybean oil with the biosurfactant, as the isolated biosurfactant is made up of 65.88% lipids and has a total caloric value of 646.88 Kcal/100 g. Thus, 6.07 g of isolated biosurfactant

Table 2
Centesimal composition of diets offered to control and experimental groups.

Ingredients (g/100 g diet)	Control		Experimental	
	Amount (g/100 g)	Energy (Kcal)	Amount (g/100 g)	Energy (Kcal)
Starch	61.9	247.60	59.9	239.60
Casein	14.0	48.0	14.0	48.0
Sucrose	10.0	40.0	10.0	40.0
Soybean oil	4.0	36.0	–	–
Biosurfactant	–	–	6.0	38.8
Fibers	5.0	–	5.0	–
Mineral mix (AIN-93 M)	3.5	–	3.5	–
Vitamin mix (AIN-93 M)	1.0	–	1.0	–
DL methionine	0.3	–	0.3	–
Choline bitartrate	0.3	–	0.3	–
Total	100.0	371.60	100.0	366.40

Table 3
Food intake, body mass gain and feeding efficiency of juvenile male and female animals fed control and experimental diets.

Variables/sex	Control group	Experimental group
Male	($n = 6$) (mean ± SD)	($n = 6$) (mean ± SD)
Body mass gain (g)	32.79 ± 37.35^a	29.10 ± 35.99^a
Diet intake (g)	299.27 ± 27.68^a	290.81 ± 11.42^a
FEC	-0.11 ± 0.08^a	0.01 ± 0.12^a
Liver mass (g/100 g BM)	3.09 ± 0.96^a	2.99 ± 0.63^a
Kidney weight (g/100 g BM)	0.96 ± 0.28^a	0.76 ± 0.16^a
Female	($n = 6$) (mean ± SD)	($n = 6$) (mean ± SD)
Body mass gain (g)	89.69 ± 16.51^a	109.79 ± 12.58^b
Diet intake (g)	270.03 ± 36.09^a	288.64 ± 18.93^a
FEC	0.34 ± 0.08^a	0.38 ± 0.04^a
Liver mass (g/100 g BM)	1.92 ± 0.21^a	2.27 ± 0.43^a
Kidney weight (g/100 g BM)	0.48 ± 0.04^a	0.50 ± 0.03^a

Abbreviations: SD = standard deviation; FEC = feeding efficiency coefficient; BM = body mass; Means on lines followed by different letters denote significant difference ($p \leq 0.001\%$, Student's *t*-test). Control group fed AIN-93G diet with addition of soybean oil; Experimental group fed AIN-93G diet with addition of biosurfactant.

were needed for the replacement of 4.0 g of soybean oil in the experimental group.

Body mass gain of the animals was calculated by measuring food intake and calculating the FEC for each sex separately (Table 3).

A significant difference in body mass gain was found between the females in the experimental and control groups ($p < 0.05$), whereas no significant difference was found between the males. Moreover, no significant differences between groups were found regarding food intake, FEC, relative mass of the liver or relative mass of the kidneys. From the data in Table 3, one may infer that the animals in the experimental group consumed a mean of 17 g of biosurfactant by the end of the experiment.

3.2. Application of biosurfactant as food additive

Fig. 1 displays the different formulations of mayonnaise and their behavior throughout the four-week evaluation. In the second week, Formulation 6 exhibited aqueous separation at the base of the recipient, which was more evident at the end of the experiment. Moreover, all formulations, except Formulation 5, underwent some degree of destabilization, as evident by the accumulation of liquid. Fig. 2 shows the most stable (Formulation 5) and least stable (Formulation 6) mayonnaises. Upon opening the recipients at the end of the experiment, Formulations 1, 2, and 3 exhibited a fluid consistency, whereas Formulation 5 exhibited a creamy, uniform, stable consistency.

4. Discussion

4.1. Biological evaluation of biosurfactant

Prior to the authorization of a food additive for use, it must undergo an adequate toxicological evaluation, taking into account any cumulative, synergic, and protective effects stemming from its use. Certain tests, such as an acute toxicity test and allergy tests, are needed to determine the effects of a given compound. A potential food component that is acutely toxic to a considerable extent obviously cannot be used. Thus, it is necessary to determine the LD50, which is an acute dose that is lethal to half of the exposed animals. (Range-finding studies (e.g., a one-week multiple dose feeding study in rats) are necessary to ensure that the ingredient proposed for use in food has an adequately low degree of acute toxicity [5]).

In the present study, oral dosages of the purified biosurfactant were administered to the rats in the experimental group incorporated into the ration. The animals were continuously and carefully observed for one month with regard to changes in weight, food intake, and excretion as well as signs of physical illness and changes in sensory aspects and normal activities. All aspects were recorded as normal in both the experimental and control groups and no observable, measurable ill effects or deaths occurred during the experiment. Based on food intake, each animal in the experimental group consumed approximately 0.6 g of biosurfactant per day, which is equivalent to 3600 mg/kg of initial body mass. Thus, the acute oral LD50 (lethal dose) of the purified biosurfactant is >3600 mg/kg of body mass. In a previous study with rats, Saravanakumari and Mani [25] administered subcutaneous doses of purified biosurfactants and determined the acute, oral LD50 to be >5000 mg/kg of body weight. The animals were observed for two months with no signs of toxicity, demonstrating that the biosurfactants could be considered safe for oral applications.

With regard to allergy, some food additives may cause intolerance reactions in certain individuals with symptoms similar to genuine allergic reactions. Thus, there is a need to study these end points in the testing of food ingredients. However, there is no animal model or *in vitro* test system currently available that unequivocally reveals intolerance. For food components, long-term studies may not always be necessary. In the guidelines of the Scientific Committee for Food of the European Union, a decision point approach is recommended [5]. In the present study, no allergic phenomena were found in either group, such as reddening, inflammation, and irritation in the tail region. Moreover, no deaths occurred during the toxicity testing.

Food ingredients may be additives after receiving approval from the US Food and Drug Administration (FDA) for specific uses or when having the status of "generally recognized as safe" (GRAS). A substance may be GRAS if its general recognition of safety is

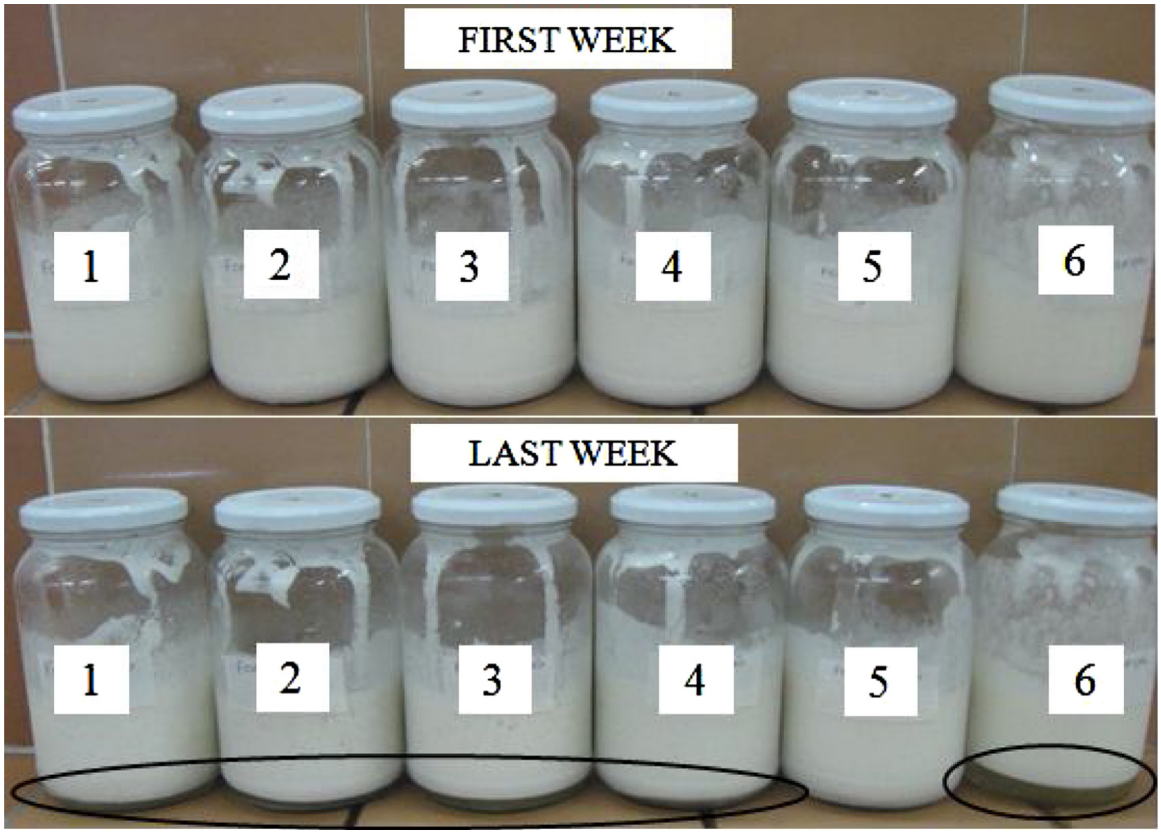

Fig. 1. Emulsion stability of mayonnaises formulated with different combinations of carboxymethyl cellulose, guar gum and biosurfactant (0.7%) during four weeks of refrigeration.

based on the views of qualified experts [5]. *C. utilis* is on the list of the Code of Federal Regulations, which derives partially from FDA regulations, with Title 21 (21CFR-§172.590), which includes approved food additives, substances with confirmed GRAS status and substances that the FDA has listed as GRAS based on a history of safe use in foods. Moreover, microorganisms and microbial-derived ingredients may be the subject of a GRAS notice [26].

The safety of *Yarrowia lipolytica* and *Candida tropicalis* has been extensively tested since the 1960s. Acute (3–6 weeks), subchronic (90 days), and chronic (1.5–2 years) toxicity has been assessed mainly using rats and mice, although guppies, chickens, and quail have been used from time to time. Dried biomass of *Y. lipolytica* (and *C. tropicalis* in parallel studies) grown on pure n-paraffin was fed at dietary levels of 10%, 20% or 30% to groups of 30 male and 30 female rats in a two-year study and to groups of 10 male and 20 female rats in a reproduction study over three generations. Each study included two control groups—one on feed similar to the yeast diet, but with soybean meal substituting the yeast, and the other on the institute's stock diet. In the two-year study, the yeast had no adverse effect on mortality, rate of body mass gain, hematology, urine composition or kidney function. In a 90-day study on rats from the third generation, no changes attributable to the yeast were apparent for any of the parameters investigated. It was concluded that the yeast did not exert any harmful effect on rats at dietary levels up to 30% for two years and over three generations. In the CIVO studies, about 50,000 test animals were used and no adverse effects were found [27].

However, few studies are found regarding the use of biosurfactants tested on rats. Most biological tests of biosurfactants are carried out for the potential use of these compounds in the pharmaceutical, biomedical, and medical fields [28,29] and are conducted *in vitro* [30]. However, the literature describes the nutritional and toxicological effects on laboratory animals of genetically modified foods [31], new food products [32] or isolated components of foods [33].

In the present study, the biosurfactant was produced using waste canola frying oil as the carbon source. The isolated product exhibited a high lipid content and clear aroma of fried food. The experimental diet was consumed in a similar amount to that in the control group, with substantial body mass gain in both males and females (Table 3). Tong et al. [34] also report no significant differences in food intake and body mass gain among male rats ($n = 27$) that consumed experimental diets with the addition of 7% oat oil and rice bran oil in place of soybean oil (control). Ohara et al. [35] tested the replacement of the lipid in a standard diet with an experimental diet containing 10% (w/v) canola oil and found that the experimental diet had 20% more total calories in comparison to the control. This did not occur in the present study, as the total calories were similar in the experimental and control diets. Poudyal et al. [36] found no significant differences in food intake between control and experimental groups for rations with the addition of macadamia oil, safflower oil, and flaxseed oil.

No statistically significant differences between the control and experimental groups were found regarding the mass of the liver or kidneys in either males or females (Table 3). These findings are in agreement with data described by Qi et al. [33], who tested the subchronic toxicity of a polysaccharide (ulvan) extracted from green algae, which is used as a food source in many parts of the world. The relative mass of the liver was 2.42 to 2.78 g/100 g of body mass in males and 2.59 to 3.76 g/100 g of body mass in females. Moreover, the kidneys were smaller in males than in females (0.56 to 0.59 g/100 g of body mass and 0.66 to 0.84 g/100 g of body mass, respectively). The authors suggest that high doses of ulvan may cause liver damage in females. In the present study, both the liver

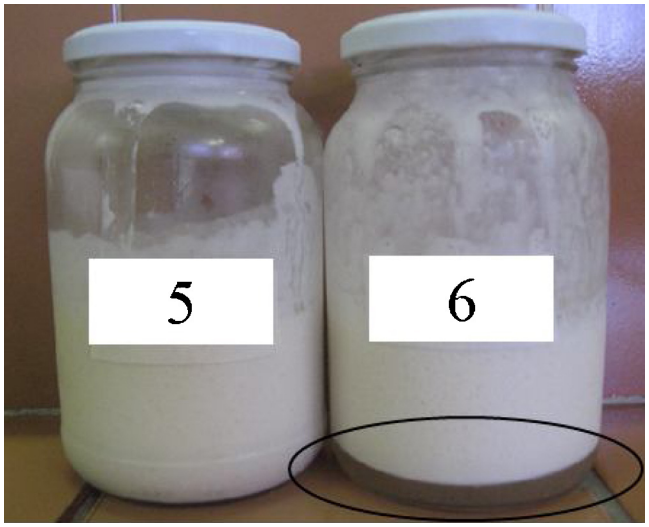

Fig. 2. Most stable emulsion (Formulation 5: guar gum + biosurfactant isolated from *C. utilis*) and least stable emulsion (Formulation 6: biosurfactant isolated from *C. utilis*).

and kidneys were smaller in females than in males, which is in agreement with data described in previous studies [37].

4.2. Application of biosurfactant as food additive in mayonnaise formulations

According to the Brazilian Health Surveillance Agency [38], mayonnaise is a creamy product in the form of a stable oil-in-water emulsion made from vegetable oil(s), water, and eggs, with the possible addition of other ingredients that do not alter the basic constitution of the product. The addition of food dyes is prohibited and 0.5% is the maximum permitted amount of starch. Emulsions are colloids, which are heterogeneous blends composed of miniscule particles suspended in an immiscible material.

An emulsifier is a substance that allows the formation or maintenance of a uniform blend of two or more immiscible phases in a food product [39]. In other words, an emulsifier is an amphipathic agent that alters the properties of foods so that they can be blended in the form of an emulsion. An emulsifier has molecules with one polar extremity that is attracted to water and another polar extremity attracted to oil. Thus, by definition, the biosurfactant produced in the present study acts as an emulsifier. Biosurfactants can either stabilize (emulsifiers) or destabilize (de-emulsifiers) an emulsion. High molecular mass biosurfactants are generally better emulsifiers than low molecular mass biosurfactants [40]. The present biosurfactant has a high molecular weight (data not shown).

Brazilian regulatory norm n° 372 [41], which stipulates that the use of additives should be limited to specific foods under specific conditions at the lowest possible concentration to achieve the desired effect [39], allows the use of additives with a stabilizing function in mayonnaise (Table 4).

There is no defined limit for the addition of a biosurfactant as a food additive. As a concentration of 0.7% (w/v) was used in the mayonnaise formulations, the consumption of a soup spoon (15 g) by an adolescent weighing 50 kg would be equivalent to 0.10 g of biosurfactant, which would correspond to 2 mg/kg of body weight. This is a far lower dose in comparison to that administered to the laboratory animals (3600 mg/kg), which offered no acute risk to the group studied.

For food additives, the Joint Expert Committee on Food Additives formed by the US Food and Agriculture Organization and the World Health Organization decided long ago that an acceptable daily intake (ADI) should be established that would provide "an adequate margin of safety to reduce to a minimum any hazard to health in all groups of consumers." The threshold dose for the most critical effect in a test is the highest exposure level without adverse (toxicologically important) effects, which is denominated the "no observed adverse effect level" (NOAEL). Risk assessment is carried out by determining the NOAEL, which is the highest dose in the most sensitive animal species that causes no toxic effects. The NOAEL is then divided by a safety factor to set an ADI level. The ADI is an estimate of the amount of a food additive expressed on the basis of body weight that can be ingested daily over a lifetime with no appreciable health risk. Safety factors are used to set an ADI that provides an adequate safety margin for the consumer by assuming that a human is tenfold more sensitive than the test animal. A food additive is considered safe for its intended use if the human intake figure is less than or equivalent to the ADI. The ADI is usually derived from the results of lifetime studies in animals and therefore relates to lifetime use in humans. This provides a sufficient safety margin so that there is no particular concern if a human is exposed to levels higher than the ADI for short periods provided that the average intake over longer periods does not exceed it [5].

Commercial-scale biosurfactant production has already been undertaken in the USA, particularly for rhamnolipids, at a company producing food additives (Jeneil Biotech, Milwaukee, USA; www.jenielbiotech.com), with no reported problems. Other biosurfactants, which are produced by yeasts, do not have pathogen issues and the commercial-scale production of sophorolipids is also already underway in Asia [1].

Soybean oil and sunflower oil are among the most commonly used vegetable oils in the production of mayonnaise. Other types have also been used in this product, such as palm oil [42]. An egg yolk is a natural emulsion of oil in water in combination with proteins, lecithins, and other phospholipids. As an emulsion, the yolk forms the nucleus in which the mayonnaise emulsion is made, affecting the viscosity and strength of the final emulsion.

Mustard powder is among the ingredients in the formulation of mayonnaise. The seeds of white or yellow mustard (*Brassica alba*) contain special compounds denominated glucosinolates, which characterize the flavor of mustard products. Glucosinolates are degraded into isothiocyanates by the enzymatic action of plant-specific myrosinase or intestinal flora in the body to yield allyl isothiocyanate, which has shown remarkable results in inhibiting the growth of food-borne pathogens and cancer cells [43,44]. Mustard powder has functional properties, such as emulsifying, stabilizing, agglutinating, conserving, and antioxidant actions. In products such as mayonnaise and salad dressing, mustard powder is used at concentrations of 0.2–0.4% of the total as an additional emulsifier and stabilizer and confers aroma to the product. As a stabilizer, the fine mustard particles accumulate on the oil/water interface, forming a physical barrier against the breakdown of the emulsion (www.portalsaofrancisco.com.br). As a conservative, the bioactivities of glucosinolates exhibit antifungal, antibacterial, and antioxidant functions [45] due to the presence of sinigrin, sinalbin, allyl isothiocyanate, and benzyl isothiocyanate [46]. Other condiments, such as ginger powder, have been added to mayonnaise to extend the shelf life to a long as 20 weeks [47].

The addition of vinegar in mayonnaise reduces the pH of the medium, thereby inhibiting the growth of pathogenic microorganisms, as reported by Shen et al. [48] in the control of the growth of *Listeria monocytogenes* in salads. The pH of a mayonnaise formulated by Kishk and Elsheshetawy [47] lowered from 4.3 to 3.5 after four weeks. Likewise, the use of gums, such as gum arabic, can affect the growth and *in situ* production of bacteriocin-like inhibitory substances from *Lactobacillus curvatus/sakei* ACU-1, *Listeria innocua* ATCC 33.090 and *Staphylococcus aureus* FBUNT [17].

Table 4
Additives with stabilizing function approved for use in mayonnaise.

INS	Additive	Function	Product	Maximum limit g/100 g
401	Sodium alginate	Stabilizer	Mayonnaise	0.10
402	Potassium alginate	Stabilizer	Mayonnaise	0.10
407	Carrageen (and its sodium, ammonium and potassium salts)	Stabilizer	Mayonnaise	0.10
410	Jataí gum, alfarroba	Stabilizer	Mayonnaise	0.10
412	Guar gum	Stabilizer	Mayonnaise	0.10
413	Gum adragant	Stabilizer	Mayonnaise	0.10
414	Gum arabic, acacia	Stabilizer	Mayonnaise	0.10
415	Xanthan gum	Stabilizer	Mayonnaise	0.10
440	Pectin, amidated pectin	Stabilizer	Mayonnaise	0.10
460	Microcrystalline cellulose	Stabilizer	Mayonnaise	0.10
466	Sodium carboxymethyl cellulose	Stabilizer	Mayonnaise	0.10

Source: Adapted from Campos et al. [6].
INS- International Numbering System.

Besides their antimicrobial properties, starches, and non-starch hydrocolloids or gums are often used as texturizers in the food industry as well as to stabilize emulsions (Table 4). In the present study, both guar gum and carboxymethyl cellulose were tested at concentrations of 0.2% as stabilizers in the formulations of mayonnaise. The most stable mayonnaise (Formulation 5) was made with a combination of guar gum and the biosurfactant (Figs. 1 and 2) due to the stabilizing capacity of the former and the emulsifying action of the latter. In contrast, Formulation 6 had no stabilizing agent and a clear segregation of the two phases occurred. In relatively stable emulsions, the separation of a layer (oil in the case of mayonnaise) has also been reported in alginate emulsions after 30 days of evaluation [7]. In contrast, the brewer's yeast β-glucan was found to stabilize an emulsion for 91 days at 4 to 6 °C and can be used to replace fat in mayonnaise [49].

Alimi et al., [13] tested inulin between 0 and 10% (w/v) and achieved the best results at a concentration of 5% (w/v). The use of 0–1% (w/v) locust bean gum offers important information regarding the influence of hydrocolloids on the properties of multicomponent model emulsions [15]. Guar and xanthan gum can affect the rheological properties of starch, which is also used in mayonnaise, inducing indirect effects by better preserving the granular structure [18]. Paraskevopoulou et al. [12] found that polysaccharides (gum arabic and propylene glycol alginate) were able to increase the viscosity of the product. A micrometer-scale konjac gel not surpassing 30% has demonstrated good potential for use as a fat analog in mayonnaise [16].

The most complex colloids and emulsions are found in food and food products, which are difficult to stabilize due to the large number of microstructures made up of combinations of proteins, carbohydrates, and lipids. The nearly infinite number of combinations are organized and arranged in very complex internal microstructures with various types of assemblies, such as dispersions, emulsions, foams, gels, etc. [5]. As mentioned above, the addition of the biosurfactant alone (Formulation 6) was not capable of maintaining the emulsion for 30 days. Sophorolipids from *T. bombicola* have been shown to reduce surface and interfacial tensions, but are not good emulsifiers [50]. In contrast, liposan does not reduce surface tension, but has been successfully used to emulsify edible oils [51]. Polymeric surfactants offer additional advantages, as they coat droplets of oil to form stable emulsions [40].

Unlike the present study, the few papers in the literature reporting the application of a biosurfactant in the formulation of new food products only offer inferences regarding probable use. In a pioneering study similar to the present investigation, Shepherd et al. [52] successfully used an extracellular carbohydrate-rich compound from *C. utilis* as an emulsifying agent in salad dressing formulations. The literature also describes other biosurfactants produced by yeasts with emulsifying properties [21,24,51,53–56].

5. Conclusion

Based on the present findings, the biosurfactant produced by *C. utilis* in a medium containing waste canola frying oil demonstrated innocuousness in acute toxicity tests and can therefore, be used in small concentrations in mayonnaise formulations. The best stabilizer was guar gum. In combination with the biosurfactant, these two substances conferred stability to the emulsion for a period of 30 days. Thus, the biosurfactant produced by *C. utilis* could be produced and used on an industrial scale as a promising new ingredient in the food industry.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was funded by the Brazilian fostering agencies Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE), the Research and Development Program of the National Agency of Electrical Energy (ANEEL), the National Council for Scientific and Technological Development (CNPq) and the Coordination for the Improvement of Higher Level Education Personnel (CAPES). The authors are grateful to the laboratories of the Center for Sciences and Technology of the *Universidade Católica de Pernambuco*, Brazil.

References

[1] R. Marchant, I.M. Banat, Microbial biosurfactants: challenges and opportunities for future exploitation, Trends Biotechnol. 11 (2012) 558–565.
[2] M. Pacwa-Plociniczak, G.A. Plaza, S. Piotrowska-Seget, S.S. Cameotra, Environmental applications of biosurfactants: recent advances, Int. J. Mol. Sci. 12 (2011) 633–654.
[3] I.M. Banat, A. Franzetti, I. Gandolfi, G. Bestetti, M.G. Martinotti, L. Fracchia, T.J. Smyth, R. Marchant, Microbial biosurfactants production, applications and future potential, Appl. Microbiol. Biotechnol. 87 (2010) 427–444.
[4] M. Nitschke, S.G.V.A.O. Costa, Biosurfactant in food industry, Trends Food Sci. Technol. 18 (2007) 252–259.
[5] I. Kralova, J. Sjöblom, Surfactants used in food industry: a review, J. Dispers. Sci. Technol. 30 (2009) 1363–1383.
[6] J.M. Campos, T.L.M. Stamford, L.A. Sarubbo, J.M. Luna, R.D. Rufino, I.M. Banat, Microbial biosurfactants as additivies for food industries, Biotechnol. Prog. 29 (2013) 1097–1108.
[7] A.C.K. Sato, K.E.F.P. Moraes, R.L. Cunha, Development of gelled emulsions with improved oxidative and pH stability, Food Hydrocoll. 34 (2014) 184–192.

[8] V. Samavati, Z. Emam-Djomeh, A. Mehdinia, Thermodynamic and kinetic study of volatile compounds in biopolymer based dispersions, Carbohydr. Polym. 99 (2) (2014) 556–562.
[9] R. Adjonu, G. Doran, P. Torley, S. Agboola, Whey protein peptides as components of nanoemulsions: a review of emulsifying and biological functionalities, J. Food Eng. 122 (2014) 15–27.
[10] Z. Ma, J.I. Boye, J. Fortin, B.K. Simpson, S.O. Prasher, Rheological, physical stability, microstructural and sensory properties of salad dressings supplemented with raw and thermally treated lentil flours, J. Food Eng. 116 (4) (2013) 862–872.
[11] F. Mantzouridou, A. Karousioti, V. Kiosseoglou, Formulation optimization of a potentially prebiotic low-in-oil oat-based salad dressing to improve *Lactobacillus paracasei* subsp. *paracasei* survival and physicochemical characteristics, Lebensm. Wiss. Technol. 53 (2) (2013) 560–568.
[12] D. Paraskevopoulou, D. Boskou, A. Paraskevopoulou, Oxidative stability of olive oil–lemon juice salad dressings stabilized with polysaccharides, Food Chem. 101 (3) (2007) 1197–1204.
[13] M. Alimi, M. Mizani, G. Naderi, A.M. Mortazavian, M.B. Moghadam, Development of low-fat mayonnaise containing combined mixtures of different types of inulin, J. Food Agric. Environ. 11 (1) (2013) 99–104.
[14] F. Mantzouridou, A. Spanou, V. Kiosseoglou, An inulin-based dressing emulsion as a potential probiotic food carrier, Food Res. Int. 46 (1) (2012) 260–269.
[15] C. Chung, B. Degner, D.J. McClements, Understanding multicomponent emulsion-based products: influence of locust bean gum on fat droplet—Starch granule mixtures, Food Hydrocoll. 35 (2014) 315–323.
[16] J. Li, Y. Wang, W. Jin, B. Zhou, B. Li, Application of micronized konjac gel for fat analogue in mayonnaise, Food Hydrocoll. 35 (2014) 375–382.
[17] M.P. Castro, N. Palavecino, C. Herman, M. Cayré, C.A. Campos, Influence of several gums on the growth and the production of a bacteriocin like substance from *Lactobacillus curvatus*/*sakei* ACU-1, Food Control 28 (1) (2012) 52–54.
[18] B.B. Heyman, W.H. De Vos, F. Depypere, P.V.D. Meeren, K. Dewettinck, Guar and xanthan gum differentially affect shear induced breakdown of native waxy maize starch, Food Hydrocoll. 35 (2014) 546–556.
[19] N.A.S. Neta, J.C.S. Santos, S.O. Sancho, S. Rodrigues, L.R.B. Gonçalves, L.R. Rodrigues, J.A. Teixeira, Enzymatic synthesis of sugar esters and their potential as surface-active stabilizers of coconut milk emulsions, Food Hydrocoll. 27 (2012) 324–331.
[20] J.M. Campos, T.L.M. Stamford, L.A. Sarubbo, Production of a bioemulsifier with potential application in the food industry, Appl. Biochem. Biotechnol. 172 (2014) 3234–3252.
[21] D.R. Cameron, D.G. Cooper, R.J. Neufeld, The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier, Appl. Environ. Microbiol. 54 (6) (1988) 1420–1425.
[22] P.G. Reeves, F.H. Nielsen, G.C. Fahey, Purified diers for laboratory rodents: final report of the american institute of nutrition and hoe writing committee on the reformulation of the American Institute of Nutrition (AIN) -76[a] rodent diet, J. Nutr. 123 (1993) 1939–1951.
[23] J.A. Campbell, Method for determination of PER and NPR. In: Food and nutrition board. Committee on Protein Quality. Evaluation of protein quality. Washington: [s.n.] (1963) 31–32.
[24] H. Torabizadeh, S.A. Shojaosadati, H.A. Tehrani, Preparation and characterization of bioemulsfier from *Saccharomyces cerevisiae* and its application in food products, Lebensm. Wiss. Technol. 29 (1996) 734–737.
[25] P. Saravanakumari, K. Mani, Structural characterization of a novel xylolipid biosurfactant from *Lactococcus lactis* and analysis of antibacterial activity against multi-drug resistant pathogens, Bioresour. Technol. 101 (2010) 8851–8854.
[26] Food and Drug Administration. (2010) http://www.fda.gov/food/ingredientspackaginglabeling/gras/microorganismsmicrobialderivedingredients/default.htm
[27] M. Groenewald, T. Boekhout, C. Neuvéglise, C. Gaillardin, P.W.M. van Dijck, M. Wyss, Yarrowia *lipolytica*: safety assessment of an oleaginous yeast with a great industrial potential, Crit. Rev. Microbiol. 1 (2013) 1–20.
[28] A.J. Cortés-Sánchez, H. Hernández-Sánchez, M.E. Jaramillo-Flores, Biological activity of glycolipids produced by microorganisms: new trends and possible therapeutic alternatives, Microbiol. Res. 168 (2013) 22–32.
[29] H. Sikorska, W. Smoragiewicz, Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections, Int. J. Antimicrob. Agents 42 (2013) 475–481.
[30] C. Burgos-Diaz, R. Martin-Venegas, V. Martinez, C.E. Storniolo, J.A. Teruel, F.J. Aranda, A. Ortiz, A. Manresa, R. Ferrer, A.M. Marques, In vitro study of the cytotoxicity and antiproliferative effects of surfactants produced by *Sphingobacterium detergens*, Int. J. Pharm. 453 (2013) 433–440.
[31] Y. Zhu, X. He, Y. Luo, S. Zou, X. Zhou, K. Huang, W. Xu, A 90-day feeding study of glyphosate-tolerant maize with the G2-aroA gene in Sprague–Dawley rats, Food Chem. Toxicol. 51 (2013) 280–287.
[32] J.T.M. Ngatchic, S.D. Sokeng, N.Y. Njintang, T. Maoundombaye, J. Oben, C.M.F. Mbofung, Evaluation of some selected blood parameters and histopathology of liver and kidney of rats fed protein-substituted mucuna flour and derived protein rich product, Food Chem. Toxicol. 57 (2013) 46–53.
[33] H. Qi, X. Liu, K. Wang, D. Liu, L. Huang, S. Liu, Q. Zhang, Subchronic toxicity study of ulvan from Ulva pertusa (*Chlorophyta*) in Wistar rats, Food Chem. Toxicol. 62 (2013) 573–578.
[34] L.-T. Tong, K. Zhong, L. Liu, L. Guo, L. Caob, S. Zhou, Oat oil lowers the plasma and liver cholesterol concentrations by promoting the excretion of faecal lipids in hypercholesterolemic rats, Food Chem. 142 (2014) 129–134.
[35] N. Ohara, Y. Naito, K. Kasama, T. Shindo, H. Yoshida, T. Nagata, H. Okuyama, Similar changes in clinical and pathological parameters in Wistar Kyoto rats after a 13-week dietary intake of canola oil or a fatty acid composition-based interesterified canola oil mimic, Food Chem. Toxicol. 47 (2009) 157–162.
[36] H. Poudyal, S.A. Kumar, A. Iyer, J. Waanders, L.C. Ward, L. Brown, Responses to oleic, linoleic and α-linolenic acids in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats, J. Nutr. Biochem. 24 (2013) 1381–1392.
[37] B.I. Onyeanusi, A.A. Adeniyi, C.G. Onyeanusi, J.O. Ayo, C.S. Ibe, A study of the kidney of the Wistar rat in Northern Guinea Savannah Zone: the morphometric aspect, Pak. J. Nutr. 8 (7) (2009) 1040–1042.
[38] Brasil, Resolução RDC. n° 276–Agência Nacional de Vigilância Sanitária–Ministério da Saúde, de 26 de setembro de 2005.
[39] Brasil, Portaria n° 540–Agência Nacional de Vigilância Sanitária–Ministério da Saúde, de 27 de outubro de 1997.
[40] K. Muthusamy, S. Gopalakrishnan, T.K. Ravi, P. Sivachidambaram, Biosurfactants: properties, commercial production and application, Curr. Sci. 94 (6) (2008) 736–747.
[41] Brasil,Portaria n° 372–Agência Nacional de Vigilância Sanitária–Ministério da Saúde, de 26 de abril de 1999. http://www.anvisa.gov.br/legis/portarias/372_99.htm
[42] M. Chandrasekaran, A.H. Bahkali, Valorization of date palm (Phoenix dactylifera) fruit processing by-products and wastes using bioprocess technology—Review, Saudi J. Biol. Sci. 20 (2013) 105–120.
[43] D. Nadarajah, J.H. Han, R.A. Holley, Use of mustard flour to inactivate *Escherichia coli* O 157:H7 in ground beef under nitrogen flushed packaging, Int. J. Food Microbiol. 99 (2005) 257–267.
[44] R. Tsao, Q. Yu, I. Friesen, J. Potter, M. Chiba, Factors affecting the dissolution and degradation of oriental mustard-derived sinigrin and allyl isothiocyanate in aqueous media, J. Agric. Food Chem. 48 (5) (2000) 1898–1902.
[45] A.P. Vig, G. Rampal, T.S. Thind, S. Arora, Bio-protective effects of glucosinolates—A review, Lebensm. Wiss. Technol. 42 (10) (2009) 1561–1572.
[46] S. Herzallah, R.A. Holley, Determination of sinigrin, sinalbin, allyl- and benzyl isothiocyanates by RP-HPLC in mustard powder extracts, LWT- Food Sci. Technol. 47 (2012) 293–299.
[47] Y.F.M. Kishk, H.E. Elsheshetawy, Effect of ginger powder on the mayonnaise oxidative stability, rheological measurements, and sensory characteristics, Ann. Agric. Sci. 58 (2) (2013) 213–220.
[48] C. Shen, I. Geornaras, P.A. Kendall, J.N. Sofos, Antilisterial activities of salad dressings, without or with prior microwave oven heating, on frankfurters during simulated home storage, Int. J. Food Microbiol. 132 (1) (2009) 9–13.
[49] G. Marinescu, A. Stoicescu, L. Patrascu, The preparation of mayonnaise containing spent brewer's yeast β-glucan as a fat replacer, Rom. Biotechnol. Lett. 16 (2) (2011) 6017–6025.
[50] D.G. Cooper, D.A. Cavalero, The effect of medium composition on the structure and physical state of sophorolipids produced by *Candida bombicola* ATCC 22214, J. Biotechnol. 103 (2003) 31–41.
[51] M.C. Cirigliano, G.M. Carman, Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica*, Appl. Environ. Microbiol. 50 (1985) 846–850.
[52] R. Shepherd, J. Rockey, I.W. Sutherland, S. Roller, Novel bioemulsifiers from microorganisms for use in foods, J. Biotechnol. 40 (1995) 207–217.
[53] E. Rosenberg, E.Z. Ron, High- and low-molecular-mass microbial surfactants, Appl. Microbiol. Biotechnol. 52 (2) (1999) 154–162.
[54] J.A.T. Barriga, D.G. Cooper, E.S. Idziak, D.R. Cameron, Components of the bioemulsifier from *S.cerevisiae*, Enzyme Microb. Technol. 25 (1–2) (1999) 96–102.
[55] L.A. Sarubbo, M.C.R. Marçal, A.L.F. Porto, G.M. Campos-Takaki, The use of babassu oil as substrate to produce bioemulsifiers by *Candida lipolytica*, Can. J. Microbiol. 45 (1999) 1–4.
[56] L.A. Sarubbo, M.C. Marçal, M.L.C. Neves, M.P.C. Silva, A.L.F. Porto, G.M. Campos-Takaki, Bioemulsifier production in batch culture using glucose as carbon source by *Candida lipolytica*, Appl. Biochem. Biotechnol. 95 (2001) 59–67.