Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XII, Issue 3 – 2013, pag. 278 - 283

CALCIUM LACTATE INFLUENCE ON SOME NON-PATHOGENIC MICROORGANISMS

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Received August 29th 2013, accepted September 14th 2013

Abstract: *After calcium lactate was produced by chemical reaction between lactic acid and calcium carbonate from eggshells, it was tested on some non-pathogenic microorganisms. The influence of calcium lactate powder on non-pathogenic microorganisms varied as group and species. All the non-pathogenic bacteria growth was inhibited by calcium lactate, the inhibition diameter ranged between 1.92 ± 0.23 to 2.35 ± 0.12 cm. Among yeasts only Candida mycoderma and Pichia sp. growth was inhibited. Calcium lactate showed no influence on the studied moulds: Aspergillus niger, Penicillium sp. and Rhizopus sp. Calcium lactate is a substance that has an inhibition effect on some non-pathogenic micoorganisms. It can be used alone, without being combined with other substances, having an acidifying effect on the microorganism nutritive media.*

Keywords: *radial diffusion, cup plate method, bacteria, mould, yeast.*

1. Introduction

Lactic acid can be produced biochemically by fermentation or chemically by synthesis of petrochemical compounds [1]. According to Hofvendahl [2], the biotechnological production of lactic acid has the following advantages over the chemical synthesis: environment protection, the natural resources used for biotechnological production are renewable, the lactic acid isomer produced can be chosen because it is well known that only L(+)-lactic acid isomer is used in food industry and pharmaceutical compounds.

Calcium lactate is now used to preserve various foodstuffs and prolong shelf life of food. Its main applications include preservation of vegetables, fruits, meat and meat products, also being added in beverages, jelly, chewing gum, candy products [3-5]. Moreover, calcium lactate can be used in food industry as calcium source, dietary supplement, for preserving fresh food [6-7], as antioxidant and stabilizer [4, 8], as antimicrobial [5, 9-11].

It can be produced by chemical reaction between lactic acid and calcium carbonate. The calcium carbonate can be used as a pure, commercial substance, or can be obtained from egg shells or crustacean shells. It is recommended to use calcium from eggshells because eggshells are considered to be a waste product, and according to Omi [12], the calcium from eggshell powder is more easily absorbed by the rat small intestine than the commercial calcium carbonate.

Calcium lactate has antimicrobial activity against aerobic and anaerobic microorganisms that can be found in meat. Although calcium lactate, sodium lactate

and potassium lactate have some bactericidal activity, their ability to control bacteria and the pathogens from fresh and processed meat is due to their use together with other antimicrobial agents such as : malic acid, glutathione, acetyl-cysteine [13, 14]. The mixture of the previously mentioned substances acts upon *E. coli* O157: H7, mesophilic bacteria, psychrophilic bacteria, yeasts and moulds [14].

Calcium lactate activity on fruits and vegetables resulted in reduced water activity (for melon) when it was immersed in 2.5 % calcium lactate solution at 4 °C [7]. When applied at high temperature (50 ... 60 °C), there was diffusion of calcium in tissues [15] and the formation of calcium-bridges between pectin molecules.

The calcium lactate alone has some effect on the microorganisms and it can be used in combination with other substances such as sodium diacetate and sodium chloride.

The objective of this study was to produce calcium lactate by chemical reaction between lactic acid and calcium carbonate from eggshells powder. The calcium lactate thus produced was tested on various non-pathogenic microorganisms.

2.Experimental

2.1.Lactic acid solution

The lactic acid was produced by fermentation from Jerusalem artichoke flour, according to the method presented by Baston [16]. After having introduced the nutrients and optimized the fermentation parameters, we could increase the lactic acid yield from 4.5 g/l to 30 g/l. The lactic acid solution was then filtered by centrifugation with a centrifugal force of 6,026g for 10 minutes, using the centrifuge Universal 320R (Hettich GmbH, Germany). The solution was treated with active charcoal and then centrifuged again at 3,075g for 15 minutes.

2.2. Calcium lactate production

For calcium lactate production we used white eggshells that were initially cleaned by washing with water at 40-50 °C, then sanitized at 130 °C for 15 minutes using AE-110 Dry autoclave (Raypa, Spain) and dried at 50 °C for 60 minutes, using the STERICELL 111 (MMM Medcenter Einrichtungen GmbH, Germany) drying stove. The dried eggshells were then converted into powder and immersed in the lactic acid solution prepared as described above.

Two grams of eggshell powder were introduced into a 500 ml beaker together with 100 ml of lactic acid solution. After homogenization, the mixture was left for 12 hours at 25 °C for chemical reaction. To ease the gravimetric filtration and for pasteurization purpose, the mixture (calcium carbonate solution) was heated at 70 °C for 10 minutes and filtered on Whatman paper no. 1. The mixture was then freeze-dried using ALPHA 1-4 LD Plus (Martin Christ GmbH, Germany) lyophilization unit. The produced substance is calcium lactate powder, as can be seen in figure 1.

Figure 1.The freeze drying vials with calcium lactate powder

The purity of the calcium lactate was determined according to Food Chemical Codex [17]. The calcium lactate purity was of 88 ± 2.35 %. The pH of calcium lactate was determined for the 10 % solution of calcium lactate using S20 pH-meter (Mettler Toledo, Spain).

2.3.Testing the antimicrobial activity of calcium lactate

For the bacteria growth we used the Plate Count Agar (PCA) media from Liofilchem, Italy, and for all the moulds and yeasts, except *Saccharomyces cerevisiae*, we used the Malt Extract Agar (MEA) media from Scharlau Chemie, Spain. For *Saccharomyces cerevisiae* we used Sabouraud Agar (Scharlau, Spain). We made a slant culture in the tubes. Using a sterile loop we have drawn the following microorganism colonies:

-bacteria: *Bacillus (B. subtilis), Sarcina* sp., *Lactobacillus* sp.

-yeasts: *Saccharomyces (S. cerevisiae), Candida (Candida mycoderma), Pichia* sp., *Rhodotorula* sp., *Torulopsis* sp.

-moulds: *Aspergillus (A. niger), Penicilium* sp., *Rhizopus* sp.

The studied microorganisms were provided by the collection of microorganisms of Bioaliment platform.

Using a BF 4000 oven (Binder, Germany) at 37 °C for bacteria and CLW 32 (POL-EKO Aparatura, Poland) oven at 25 °C for moulds and yeasts, we incubated the pure culture colonies on slant culture media. *Lactobacillus* was anaerobically incubated. With a sterile loop, 9 ml of sterile suspension were taken from every slant media and every 1 ml of this suspension was inoculated in Petri dishes with nutritive media characteristic to the type of microorganism. The Petri dishes were incubated according to Tofan [18]. The purity of pure microorganism was verified according to Tofan [18].

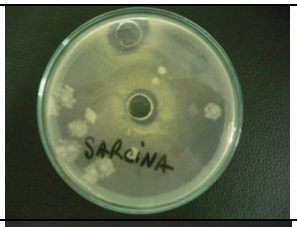
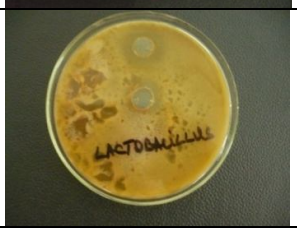
As regards the antimicrobial activity of calcium lactate we used agar well diffusion method according to Toba [19]. The well diameter was of 10 mm. The calcium lactate powder was sterilized using an ultraviolet light lamp. We used the calcium lactate powder to fill every well. The Petri dishes were incubated as mentioned above, for every type of microorganism. We worked in duplicate with one more repetition of the experiment. We used Microsoft Excel software from Microsoft Office 2003 to determine the mean value and standard deviation.

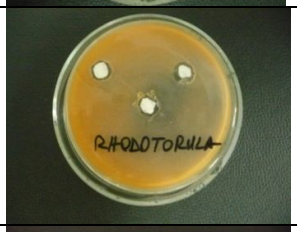
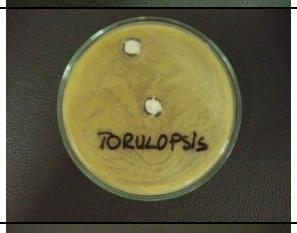
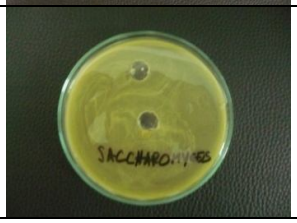
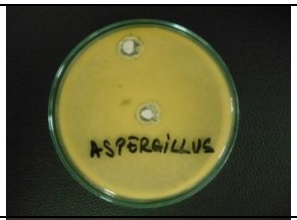
3. Results and Discussion

The results of the incubation period for every type of microorganisms are shown in table 1.

Table 1

The calcium lactate effect on tested microorganisms

Microorganism	Calcium lactate action
Bacteria	
Sarcina sp.	
Bacillus subtilis	
Lactobacillus sp.	

Yeasts	
Pichia sp.	
Rhodotorula sp.	
Torulopsis sp.	
Candida mycoderma	
Saccharomyces cerevisiae	
Moulds	
Aspergillus sp.	
Rhizopus sp.	
Penicilium sp.	

As can be seen in table 1, some of the non-pathogenic microorganisms studied were affected by calcium lactate, whereas others were not.
All the studied bacteria showed a zone of inhibition, the extent of this zone being different, as can be seen in figure 2.

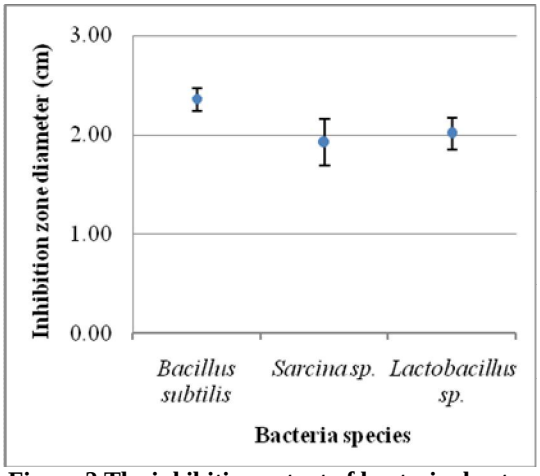


Figure 2.The inhibition extent of bacteria due to calcium lactate influence

Of all the non-pathogenic bacteria studied, we can state that *Bacillus subtilis* registered the longest diameter, followed by *Lactobacillus.* The inhibition of bacteria was due to the pH of the calcium lactate that diffused into the culture media and changed the PCA pH value. The PCA pH media was of 7.0 ± 0.2.
An interesting fact occurred in the case of moulds, namely calcium lactate did not develop any inhibition zone in all the studied moulds. It seems that these ones

have the capacity to adapt to pH change of the culture media and develop colonies.

As regards yeasts' inhibition, two of the studied species were affected only, as shown in figure 3.

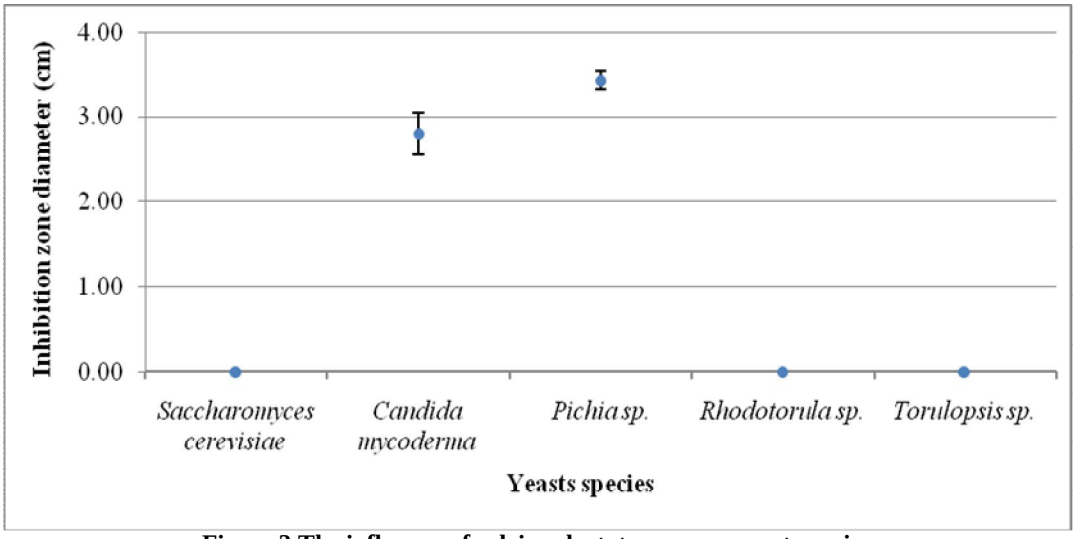


Figure 3.The influence of calcium lactate on some yeast species

As seen in figure 3, only *Candida mycoderma* and *Pichia* were affected by calcium lactate. They were more susceptible than the other studied yeasts to even calcium or carbonate ions added in the nutritive media, or to pH change of the culture media (MEA). The pH of the MEA culture media was of 5.4 ± 0.2 and for Sabouraud Agar was 5.6 ± 0.2.
Taking into consideration the fact that the calcium lactate produced had a pH value of 4.3 ± 0.13, we can state that the calcium lactate influence is due to the acidifying activity on nutritive media.

4. Conclusion

Calcium lactate is a substance that has an inhibition effect on some non-pathogenic micoorganisms. Eleven microorganisms (bacteria, yeasts and moulds), were subjected to calcium lactate activity. All the bacteria were inhibited by calcium lactate. Only *Candida mycoderma* and *Pichia* sp. yeasts were inhibited. Calcium lactate showed no influence on the studied moulds: *Aspergillus niger*, *Penicillium* sp. and *Rhizopus* sp. Calcium lactate as antimicrobial substance can be used alone, without being combined with other substances, having an acidifying effect on the microorganism nutritive media.
Further studies are needed to observe the effect of calcium, and lactate ions on various microorganisms.

5. Acknowledgments

This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project „Organizing the national interest postdoctoral school of „Applied biotechnologies" with impact on Romanian bioeconomy", project co-financed by the European Social Fund through the Sectoral Operational Programme Human Resources Development 2007-2013.

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