CATÓLICA PORTO
BIOTECNOLOGIA

Development of oral care strips containing chitosan

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QUITORAL: DEVELOPMENT OF NEW CHITOSAN FORMULATIONS FOR ORAL MEDICINE

ON.2 O NOVO NORTE
PROGRAMA OPERACIONAL REGIONAL DO NORTE

QREN QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL
PORTUGAL 2007.2013

Fundo Europeu de Desenvolvimento Regional

Framework

ELIXIRS

ORAL STRIPS

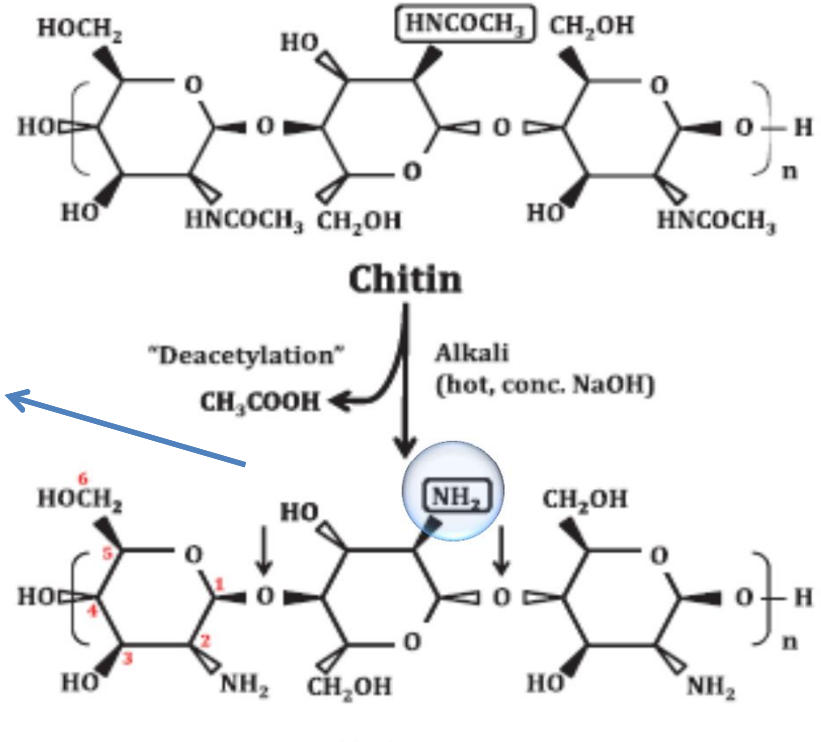
DEVELOPMENT OF NEW CHITOSAN FORMULATIONS FOR ORAL MEDICINE

DENTAL CONES

Oral strip – thin film prepared with hydrophylic polymers that rapidly dissolves on tongue or buccal cavity

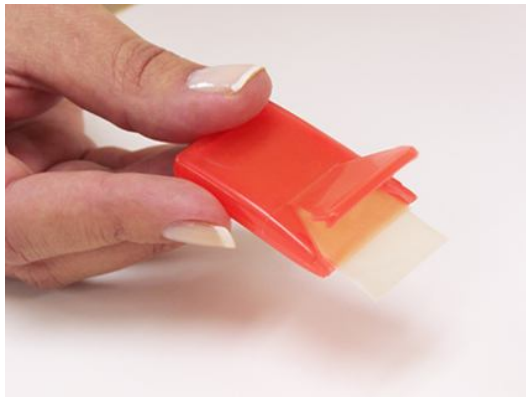
CHITOSAN

Binds to negatively charged surfaces such as oral mucosal



- ADHESIVE PROPERTIES
- FILM FORMATION
- HYDROHILIC
- ANTIBACTERIAL
- ANTIFUNGAL
- ANTI-INFLAMMATORY
- WOUNDHEALING

Main Objectives



★ **STABLE CHITOSAN ORAL STRIPS**

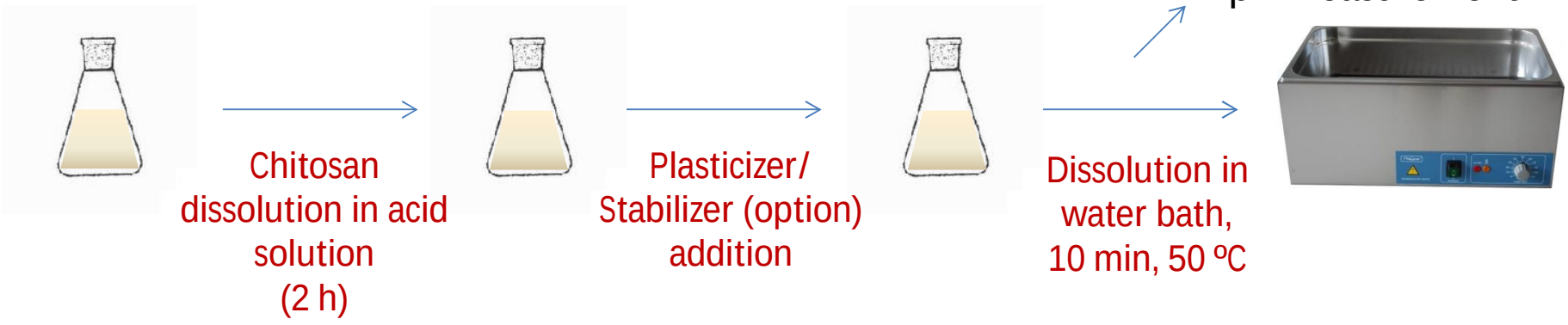
★ **ANTIMICROBIAL ACTIVITY**

★ **LOW ASTRINGENCY (NEGATIVE EFFECT OF CHITOSAN)**

★ **EASY TO USE AFTER MEALS**

•**Commercial strips** (to breath) with the composition: corn starch, xilitol (3.3 %); aspartame; carragen; citric acid; alcaçuz (0.05%); flavours

Oral strips production – *Solvent Cast Method*



- **SENSORIAL CONTROL FOR FLAVOUR AND ASTRINGENCY (GROUP PANEL).**
- **MACROVISUALIZATION OF THE MAIN PROPERTIES (STABILITY, APPEARANCE ETC.)**

Oral strips production: Factors

Chitosan:	• High, medium, low molecular weight (140, 300, 600 kDa, Sigma) • Concentrations 0.15-2% (m/v)
Acid solution:	• Citric and lactic • 2.0-0.5 % (v/v)
Plasticizers:	• Sorbitol, glycerol • Concentrations 5-40 % (v/v)
Stabilizing agents:	• Guar (0.25 and 0.5%, w/v) • Tween (0.5%, v/v)

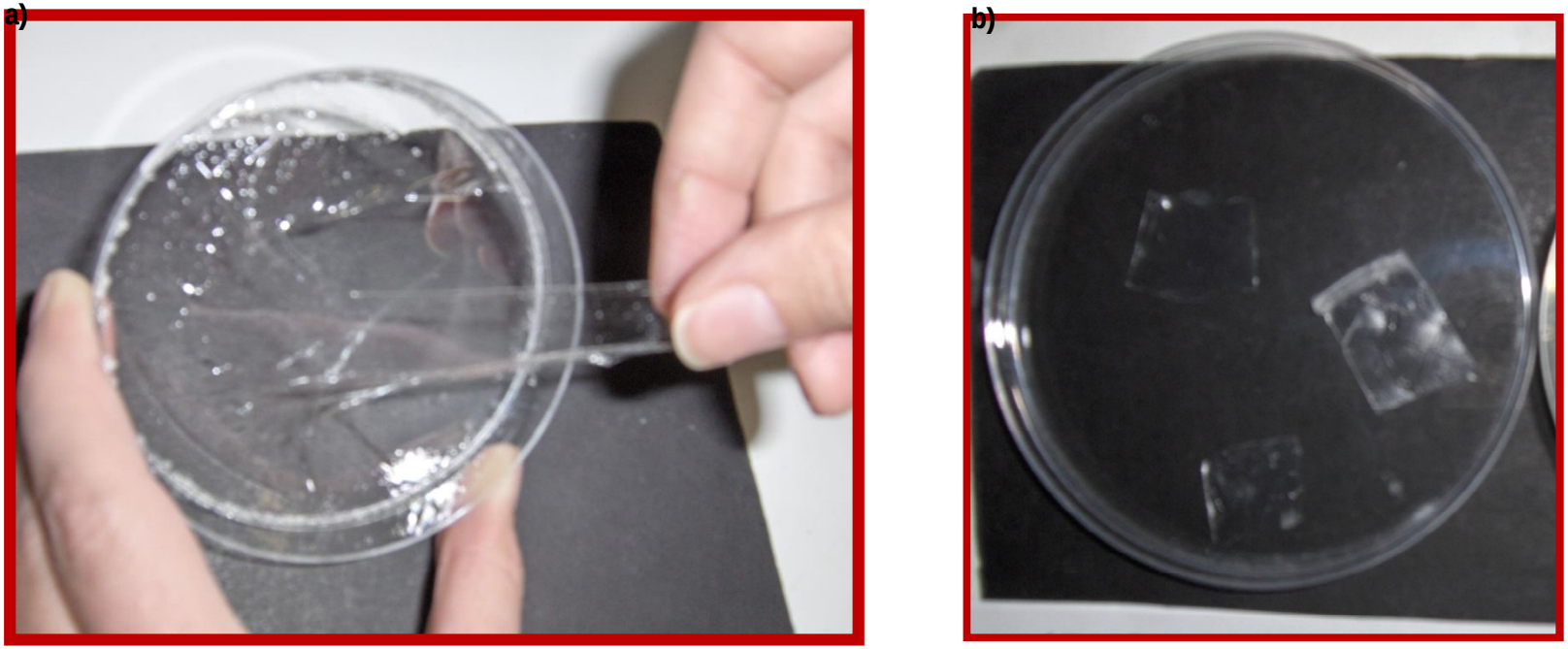
Oral strips production: Citric acid (1%)

Chitosan type	Chitosan (%, v/v)	Plasticizer Type	% Plasticizer
Low MW Medium MW High MW	0.7 1.4 2.0	Sorbitol Glycerol	5 10 20 40

Observations:

- Chitosan - higher % - easy to dry and manipulated
- Glycerol – difficult to dry and manipulate, no stable strips
- Sorbitol –stability of the strip increase with % of sorbitol
- Sensorial control – astringency increases with the type of chitosan (low to high) and chitosan % (0.7 – 2 %)

Strips produced with glicerol and sorbitol



a) Glicerol and b) Sorbitol

New formulation with Sorbitol (40%)

Test	Chitosan MW	Chitosan (%, w/v)	Citric acid (%, w/v)
1	Low	0.7	2
2	Low	0.7	1
3	Low	0.7	0.5
4	Low	1.4	2
5	Low	1.4	1
6	Low	1.4	0.5
7	Medium	0.7	2
8	Medium	0.7	1
9	Medium	0.7	0.5
10	Medium	1.4	2
11	Medium	1.4	1
12	Medium	1.4	0.5

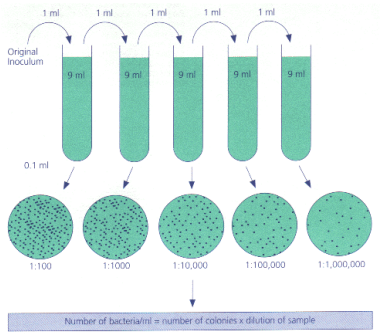
Antimicrobial activity – *in vitro* simulation of strip mastication

Simulated saliva inoculated with *Lactobacillus acidophilus* or *Streptococcus mutans* (10^6 cfu/ml)

Strip addition (ca. 0.1 g)

2 min, 37 ºC water bath, 200 rpm

pH control



Serial decimal dilutions and plating

Enumeration of bacteria

Results

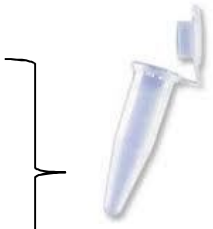
Test number	*S. mutans*		*L. acidophillus*	
	pH	Log (cfu/ml)	pH	Log (cfu/ml)
1	2.76 ± 0.014	<3	2.514 ± 0.0100	<3
2	2.75 ± 0.029	<3	2.710 ± 0.2900	4.10 ± 0.504
3	3.14 ± 0.010	<3	3.106 ± 0.0294	<3
4	3.43 ± 0.023	<3	3.478 ± 0.1937	<3
5	2.75 ± 0.045	<3	2.314 ± 0.0579	4.24 ± 0.753
6	3.54 ± 0.012	<3	3.507 ± 0.0972	<3
7	2.87 ± 0.029	<3	2.790 ± 0.0127	4.29 ± 0.245
8	3.10 ± 0.103	<3	3.224 ± 0.0103	4.67 ± 0.193
9	3.36 ± 0.139	<3	3.427 ± 0.0697	4.89 ± 0.872
10	2.33 ± 0.013	<3	2.195 ± 0.0497	4.82 ± 0.596
11	2.63 ± 0.169	<3	2.418 ± 0.0238	5.39 ± 1.208
12	3.16 ± 0.129	<3	2.959 ± 0.0384	5.30 ± 0.284
C1	4.99 ± 0.074	6.58 ± 0.896	6.035 ± 0.0590	6.20 ± 0.864
C2a	2.53 ± 0.023	6.29 ± 0.690	2.728 ± 0.1394	6.09 ± 1.203
C2b	2.84 ± 0.138	6.30 ± 1.028	2.918 ± 0.2946	6.30 ± 0.192
C2c	2.937 ± 0.539	6.39 ± 0.863	2.993 ± 0.2839	6.34 ± 0.134

Means (± SD) of pH and Log (cfu/ml) C1 – Control with no strip addition; C2a – Control with citric acid 2.0%; C2b – Control with citric acid 1.0 %; C2c – Control with citric acid 0.5 %

In Vitro Biofilms Assays

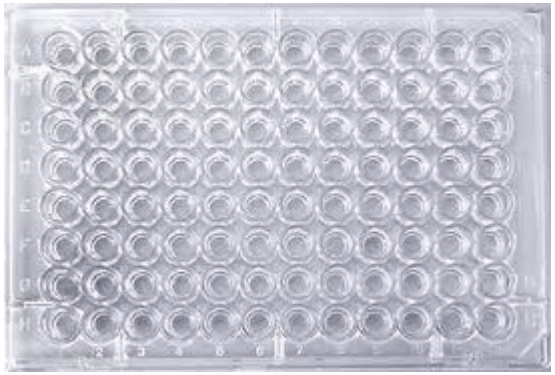
Methods

→ Inoculum at 2 %(v/v)
→ Medium with 5 % sucrose
→ Chitosan concentrations
→ Triplicate assays



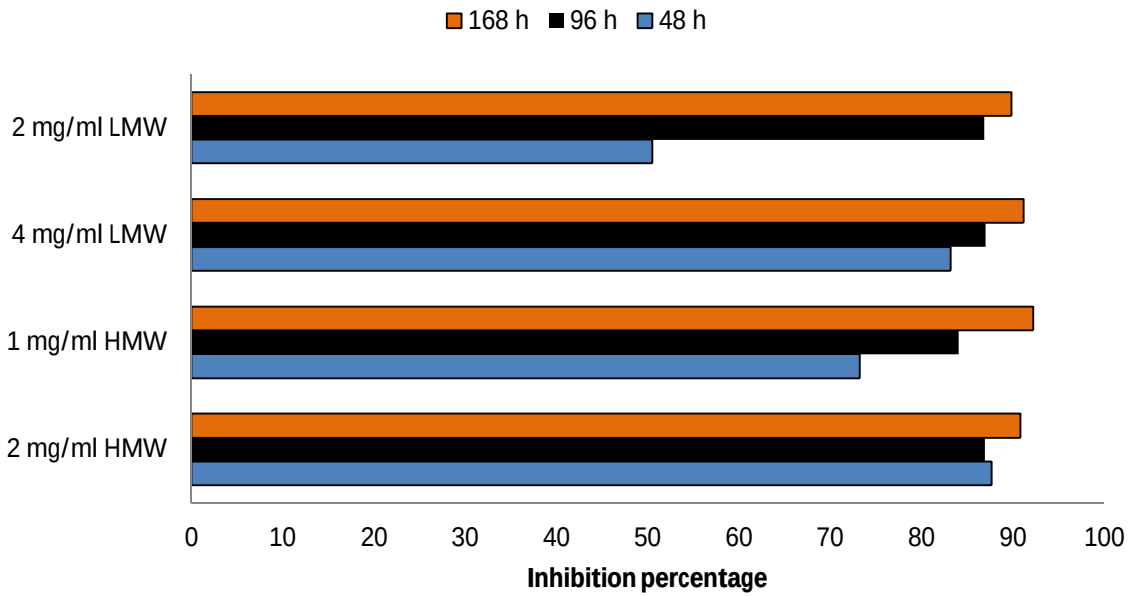
V =1ml

0,2 ml
(x3)



Incubation at 37 ºC

Streptococcus mutans



Results

48 h – MW and Concentration

96 h – HMW Concentration

168 h – No differences

Discussion and conclusions

- Low pH associated to the time of mastication is not enough to decrease significantly the initial bacterial counts, so the bacteriostatic/bactericide effect can be attributed to chitosan;
- *Streptococcus mutans* was the most sensitive bacterial strain
- Low MW chitosan showed better performance than medium MW chitosan;
- Sensorial control – presence of high astringency (Medium MW, high %, high citric acid %)

Low concentrations of chitosan permit the production of strips with in vitro antimicrobial and antibiofilm activity

Decrease chitosan and acid concentration!

Development of oral Strips

Reduced chitosan concentration

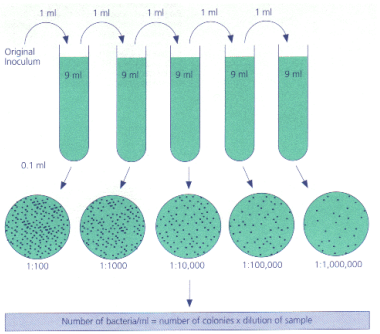
Test number	Chitosan type	Chitosan (%, m/v)	Citric acid (%, m/v)
1	Low	0.30	1.0
2	Low	0.15	1.0
3	Medium	0.30	1.0
4	Medium	0.15	1.0

Antimicrobial activity – *in vitro* simulation of strip mastication

Strip addition
(ca. 0.1 g)

n=3

pH control



Simulated saliva inoculated with *Lactobacillus acidophilus* or *Streptococcus mutans* (10^6 cfu/ml)

2 min, 37 ºC water bath, 200 rpm

Serial decimal dilutions and plating

Enumeration of bacteria

Results

Test	Thickness (mm)	*S. mutans*			*L. acidophilus*		
		Weight (g)	pH	Log (cfu/ml)	Weight (g)	pH	Log
1	**0.20 ± 0.04**	**0.16 ± 0.09**	**3.39 ± 0.03**	**3.38 ± 0.37**	**0.18 ± 0.04**	**3.55 ± 0.10**	**4.12 ± 0.90**
2	0.18 ± 0.02	0.17 ± 0.01	3.51 ± 0.01	4.40 ± 0.02	0.19 ± 0.09	3.51 ± 0.24	4.67 ± 0.13
3	0.21 ± 0.09	0.19 ± 0.05	3.40 ± 0.02	4.12 ± 0.10	0.17 ± 0.01	3.51 ± 0.47	4.34 ± 0.59
4	0.20 ± 0.01	0.17 ± 0.04	3.48 ± 0.08	4.39 ± 0.79	0.19 ± 0.03	3.56 ± 0.89	4.89 ± 0.20
C1	**NA**	**NA**	**5.02 ± 0.23**	**5.78 ± 0.24**	**NA**	**5.95 ± 0.12**	**5.89 ± 0.26**
C2	**NA**	**0.20 ± 0.09**	**3.24 ± 0.45**	**5.50 ± 0.90**	**0.20 ± 0.08**	**3.12 ± 0.46**	**5.50 ± 0.89**

Means (± SD) of pH and log cfu/mL C1 – Control with no strip addition C2 – Control with citric acid 1.0%;

Discussion and conclusions

- Low concentrations of chitosan can be used, exhibiting 2-3 log cycles reduction
- Sensorial control – slight persistence of astringency – attributed to the interaction citric acid + chitosan

- ***Use of low chitosan concentrations of low MW – 0.15 and 0.30 % (w/v)***
- ***Change of acid solution to dissolve chitosan, from citric to lactic acid***
- ***Use of lower concentrations of sorbitol to optimize the strip drying and to avoid precipitation problems.***

Oral strips production - Lactic acid

Chitosan type	Chitosan (%, m/v)	Lactic acid (%, v/v)	Sorbitol (%, m/v)
Low	0.3	0.1	10
Low	0.15	0.1	10

Lower sorbitol concentration improved appearance and reduced formation of precipitates;

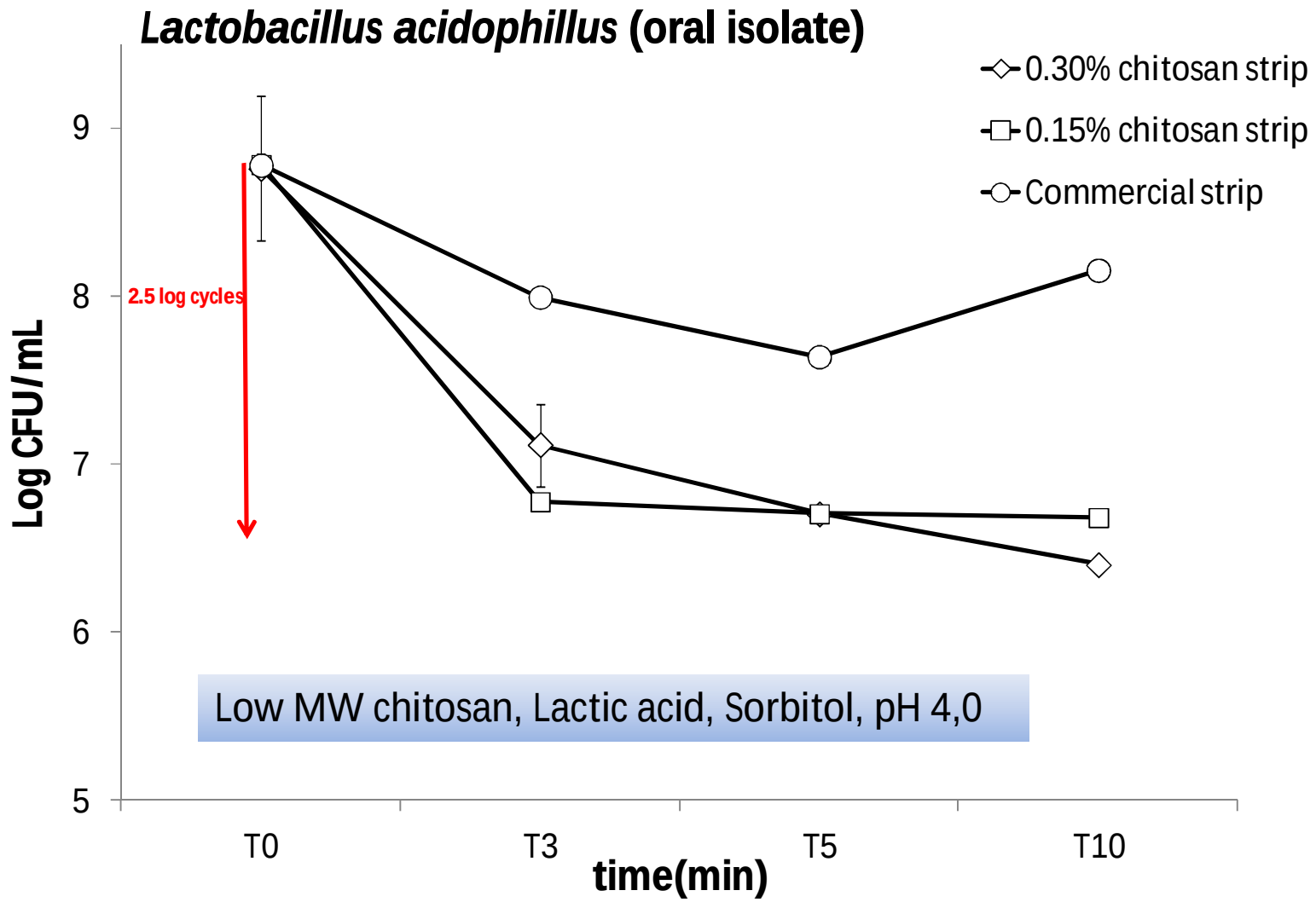
Use o lactic acid at low content increase the pH of films and reduced sensory astringency

Use of lactic acid producing films with low pH may reduce antimicrobial activity – lower protonation of NH_2 from chitosan

IN VITRO ANTIMICROBIAL TESTS
Comparison with commercial strips

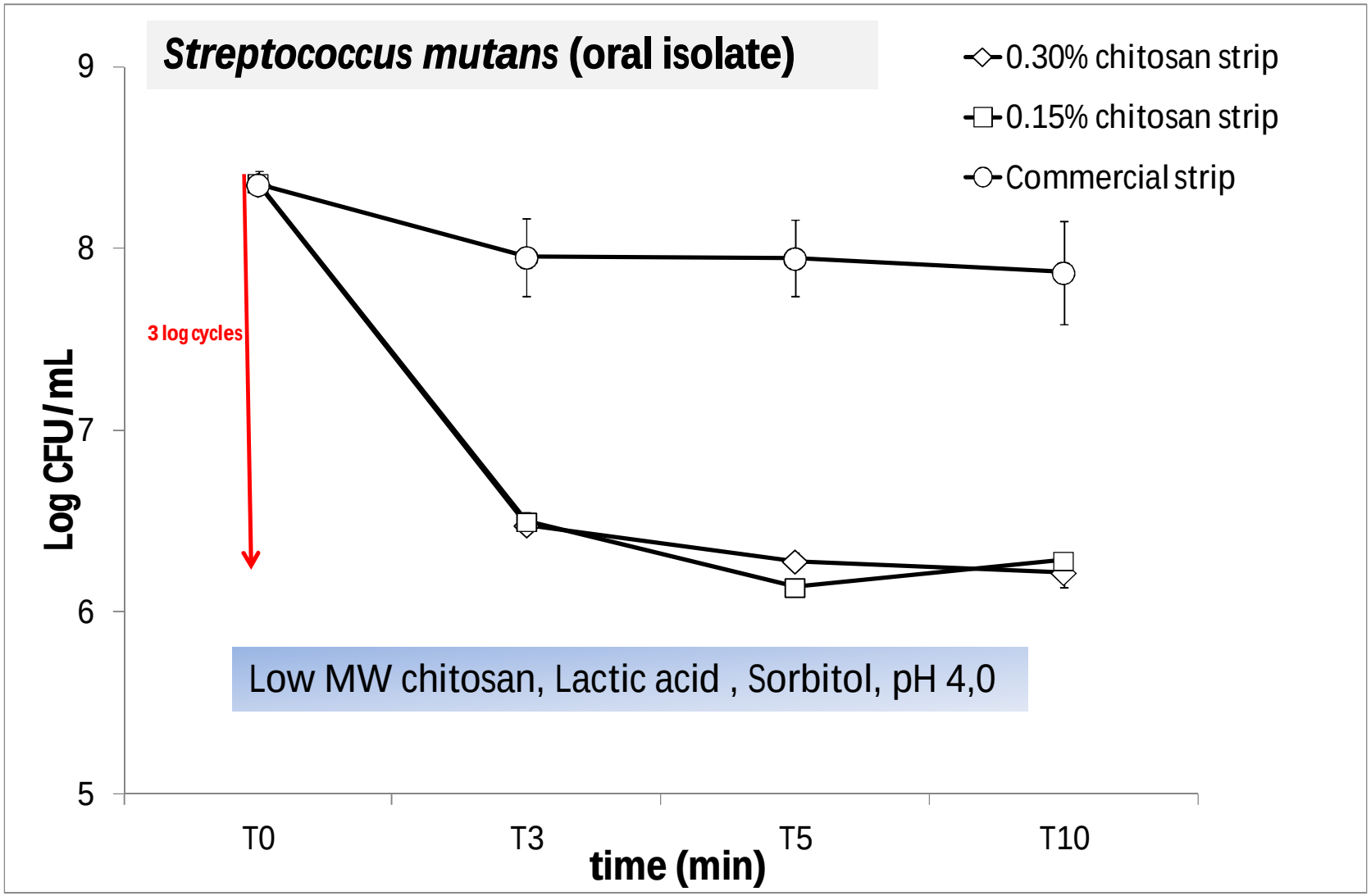
In vitro antimicrobial tests

Comparison with commercial strips



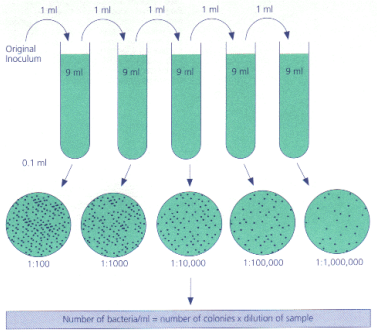
In vitro antimicrobial tests

Comparison with commercial strips



Procedure for in vivo tests

Collection of sample from teeth and tongue individually (n=6)



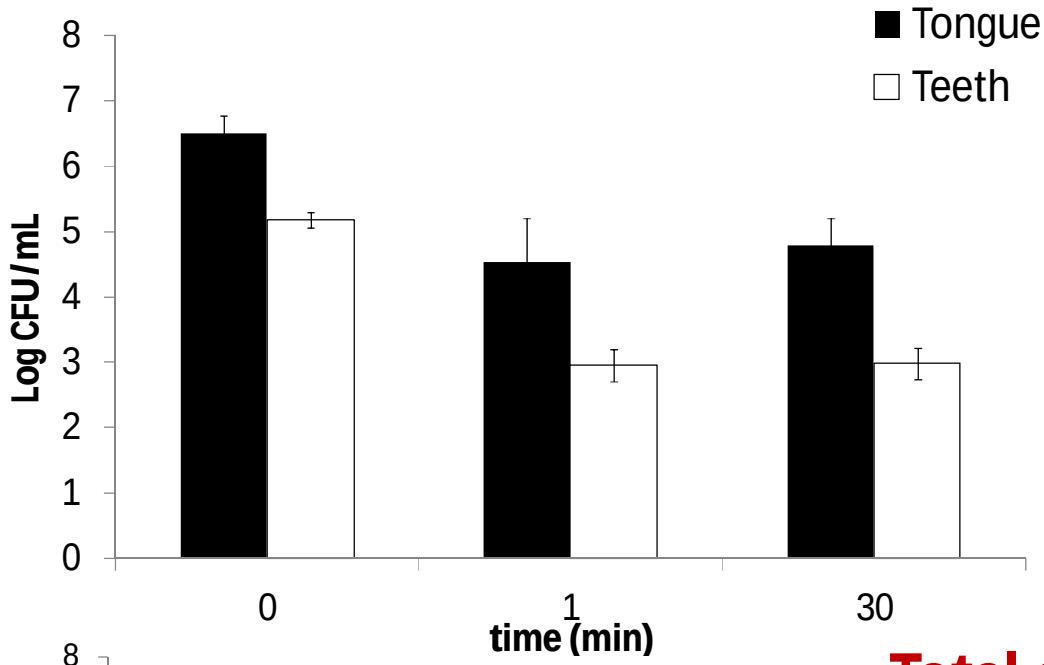
Serial decimal dilutions and plating

t= 1 and 30 min

Collection of sample from teeth and tongue

In vivo antimicrobial tests

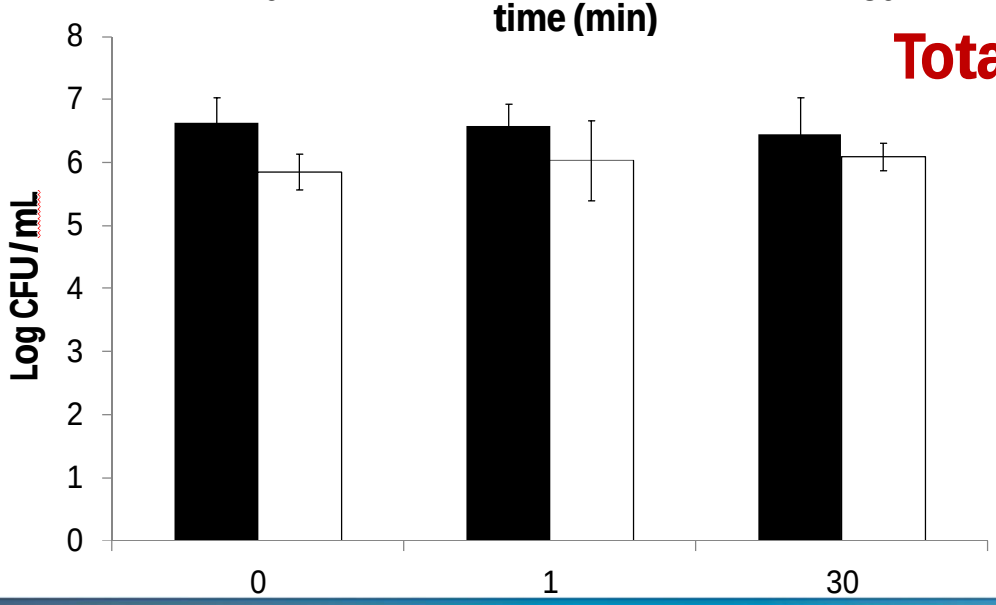
Strip based on chitosan (0.3%)



0 min = before strip consumption
1 and 30 min= 1 and 30 min after strip consumption

Total anaerobic bacteria

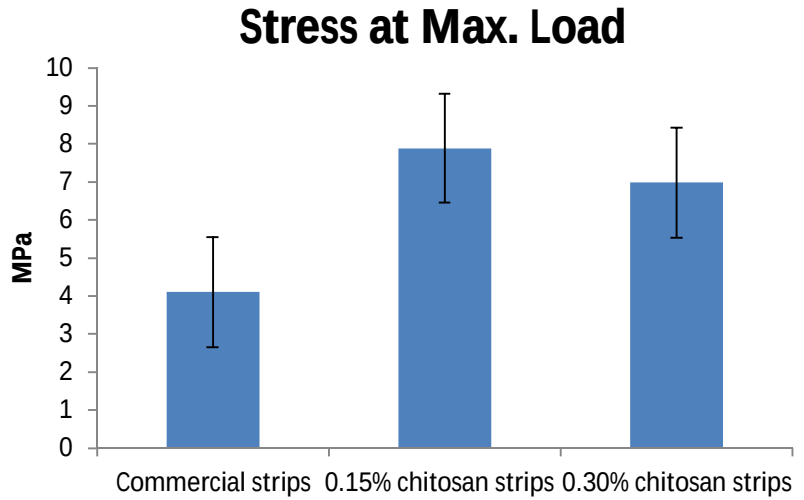
Commercial strip

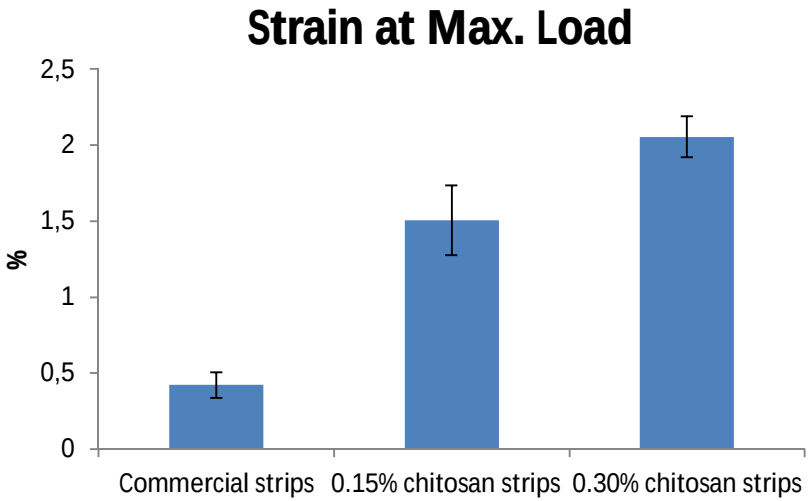


Functionality tests

- **Tensile strength**
- **Elongation**

Speed 2.5 mm/s
Force 100 N
n=5





Functionality tests

- Adhesiveness
- Resilience

- Texturometer TAX-T2
- Measure of Adheseviness and Resilience

Strip	Adhesiveness (g)	Resilience
0.30 %	0.249±0.0590	1.667±0.0441
0.15 %	0.255±0.0359	1.655±0.0562
Commercial	0.209±0.0183	1.674±0.0361

In terms of **adhesiveness**, the new strips with chitosan presented a slight higher adhesiveness than the commercial ones ($P<0.05$);
In terms of **resilience** the three strips tested are similar.

General conclusions

Oral strips can be produced successfully using chitosan showing textural properties similar or better to those commercialized for fresh breath;

Final formulation of Oral strips containing chitosan was produced with confirmed antimicrobial activity - demonstrated in vitro and in vivo;

High sensory acceptability with lower % of Low MW chitosan, with lower % sorbitol and lactic acid

Acknowledgments

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Thank you for your attention!

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