

7URSLFDO -RXUQDO RH3KDUPDFHHSWLFHDEHU

,661 SULQW HOHFWURQL
(3KDUPDFRWKHUHS \ *URXS)DFXOWLQRI%KQUQDRLW8QLYHUMLMUED%HQ

\$YDLODEOH RQOLQH DW KWWS ZZ
KWWS G[GRL RUJ WMSU Y

2ULJLQDO 5HVHDFK \$UWLFQ

2SWLPL]D WosRiQunRtyrobutyricum HQFDSVXODWLRQ
E\ H[WUXVLRQ PHWKBGWDHJG]DKWLRQ RI WKH
IRUPXODWLRQ

0XKDPPDG 8PDU <D/TIQR EDQXQ 3H4KLSLQJ ;DDMLQJ 6XQ
<X\XH -LQXMLH /:HQMLQJ 7BRJ :DQJDLGRQJ :DQJ
:DQJ

¹College of Animal Science, Zhejiang University, Key Laboratory of Animal Nutrition and Feed Science (Eastern China), Ministry of Agriculture, Hangzhou 310058, ²Shanghai Zoo, 2381 Hongqiao Rd, Shanghai 200335, PR China

*For correspondence: Email: wangmq@zju.edu.cn; Tel: +86 571 88982112; Fax: +86 571 88982650

Sent for review: 23 March 2021

Revised accepted: 21 August 2021

Abstract

Purpose: To optimize the process parameters for the encapsulation of *Clostridium tyrobutyricum* (Ct) and to determine its in vitro characteristics.

Methods: The process parameters, including the concentration of the wall and hardening material, Ct to gelatin ratio and hardening time, were studied by single factor analysis, while optimization was performed by orthogonal experimental design for the encapsulation rate of Ct.

Results: Optimal conditions exhibited by orthogonal experimental design at a 92.17 % encapsulation rate with a viable count of 9.61 ± 0.06 lgCFU/g were: 6 % modified starch, 3 % sodium alginate, and 2 % CaCl₂ at a Ct to gelatin ratio of 1:1 with a hardening time of 30 min. The survival rates of encapsulated Ct were higher than free Ct in simulated gastric (6.22 %) and intestinal juices (15.55 %). Reduction in viable counts of Ct at 90 °C were higher for free cells (44.76 %) than encapsulated cells (28.09 %) after 30 min of heat treatment. Correspondingly, encapsulation boosted the capacity of Ct to withstand the strong acidic conditions of the stomach and improved the storage properties of Ct.

Conclusion: The results suggested that extrusion is a good technique for the encapsulation of Ct, as it enhances the viability of Ct during their transit through the gastrointestinal tract. Furthermore, encapsulation is favorable for Ct if planned for use in formulations where high temperature treatment is required.

Keywords: Encapsulation, Acid resistance, Bile salt tolerance, *Clostridium tyrobutyricum*, Extrusion, In vitro simulation, Temperature tolerance

7KLV LV DQ 2SHQ \$FFHV DUWLFQ WKDWRXVHQDQWIFKQDQJH RIRHDEGHZKLFUK WKHLU LQVWL
IRU DFFHV DQG GLVWULRQX\$WHWGLEXLQQLQG/HFHQVHWKH
KWWSFUHDWLYHFRPPRQVRUJOLFHQVHV E\ SHDQG\$FHWKHV%QIGVDSHW WYH 2
KWWS ZZZ EXGDSHVWRSHQDFFHVVLQLWLWVXQH RRVWUWLFHGHG ZKLFK GSHVWVULLEXWLRQ
UHSURGXFWLRQLQDQ\PHGLXP SURYLGHG\WFKHQLWJLQDO ZRUN LV SURSH

7URSLFDO -RXUQDO RI 3KDUPDFHXWLFDO 5HVHDFK\$UWLFQDOHQHGHGEL6HDFK 6FRSXV
,QWHUQDWLRQDO 3KDUPDFHXWLFDO \$EVWUDFWQ&KH RIRSHQ\$IEFVWUD(PV6 2 (FEDLFDQ
,QGH[OHGLFXV -RXUQDO6HHN -RXUQDO 8LWLRQLRQU\$HFRVUVR6FLSHQCF\$FIGHVV -RXUQDO
'2\$- \$IULFDQ -RXUQDO 2QOLQH %LRODQH ,QGHGQDWRIRKIDCEV2\$HDFWV

, 1752'8&7, 21

in vitro et al

6WDWLWVWLFDO DQDO\VLV

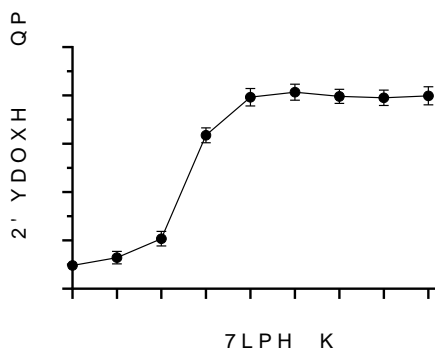
in vitro

p

5(68/76

%DFWHULDO JURZWK FXUYH

Ct,



)LJXUH

2SWLPLJHG PLFURHQFDSVXODWLRQ SURFHVV

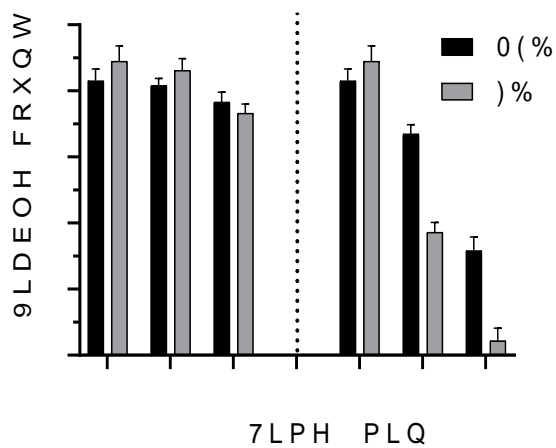
7DEOH

Ct

)DFWRUV								
1R	1D	DOJLQDWH	Z Y	0	VWDUZKY	Z Y	Ct	JHODWLRQ	UDWH
							Y	&D&O	

Note:

In vitro JDVWURLQWHVWLQDO VLPXODWLRQ



)LJXUH

%LOH VDOW WROHUDQFH

Lactobacillus acidophilus & 2 1 & / 8 6 , 2 1
 Bifidobacteria Lactobacilli
 Ct Ct
 Ct Ct
 casei Lactobacillus Lactobacillus Ct
 Lactobacillus acidophilus
 ' (& / \$ 5 \$ 7 , 2 1 6
 Ct Acknowledgement

et al

Bifidobacteria Bifidobacterium bifidum
 Lactobacillus acidophilus

et al

et al

Lactobacillus

Ct

5 () (5 (1 & (6

1. Alagawany M, Abd El-Hack ME, Farag MR, Sachan S, Karthik K, Dhama K. The use of probiotics as eco-friendly alternatives for antibiotics in poultry nutrition. *Environ Sci Pollut Res* 2018; 25: 10611-10618.
2. Chandramouli V, Kailasapathy K, Peiris P, Jones M. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. *J Microbiol Methods* 2004; 56: 27-35.
3. Se JK, Seung YC, Sae HK, Ok-Ja S, Shik S, Dong SC, Hyun JP. Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. *LWT-Food Sci Technol* 2008; 4: 493-500.
4. Annan NT, Borza AD, Truelstrup HL. Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Res Int* 41: 184-193.
5. Bajracharya P, Islam MA, Jiang T, Kang SK, Choi YJ, Cho CS. Effect of microencapsulation of *Lactobacillus salivarius* 29 into alginate/chitosan/alginate microcapsules on viability and cytokine induction. *J Microencapsul* 2012; 29: 429-436.
6. Lee KY, Heo TR. Survival of *Bifidobacterium longum* immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. *Appl Environ Microbiol* 2000; 66: 869-873.
7. Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int J Food Microbiol* 2000; 62: 47-55.
8. Truelstrup HL, Allan WPM, Jin YL, Paulson AT. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiol* 2002; 19: 35-45.
9. Yaqoob MU, Wang B, Pei X, Xiao Z, Wanjiang S, Jin Y, Liu L, Wenjing T, Wang G, Wang H, et al. Microencapsulation of *Clostridium tyrobutyricum* by spray drying method and its characteristics in-vitro. *Pak Vet J* 2020; 40(4): 419-424.
10. Mokarram RR, Mortazavi SA, Najafi MBH, Shahidi F. The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Res Int* 2009; 42: 1040-1045.
11. Etchepare MA, Raddatz GC, Flores EMM, Zepka LQ, Jacob-Lopes E, Barin JS, Grosso CRF, Menezes CR. Effect of resistant starch and chitosan on survival of *Lactobacillus acidophilus* microencapsulated with alginate. *LWT-Food Sci Technol* 2010; 65: 511-517.
12. Liserre AM, Maria IR, Bernadette DGMF. Microencapsulation of *Bifidobacterium animalis* subsp. *lactis* in modified alginate-chitosan beads and evaluation of survival in simulated gastrointestinal conditions. *J Food Biotechnol* 2007; 21: 1-16.
13. Ding WK, Shah NP. Acid, bile, and heat tolerance of free and microencapsulated probiotic Bacteria. *J Food Sci* 2007; 72: 446-450.
14. Mandal S, Puniya AK, Singh K. Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298. *Int Dairy J* 2006; 16: 1190-1195.
15. Iyer C, Kailasapathy K. Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under in vitro acidic and bile salt conditions and in yogurt. *J Food Sci* 2005; 70: 18-23.
16. Guerin D, Vuilleumard JC, Subirade M. Protection of bifidobacteria encapsulated in polysaccharide-protein gel beads against gastric juice and bile. *J Food Prot* 2003; 66: 2076-2084.
17. Picot A, Lacroix C. Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *Int Dairy J* 2004; 14: 505-515.
18. Sun W, Griffiths MW. Survival of bifidobacteria in yoghurt and stimulated gastric juice following immobilization in gellan-xanthan beads. *Int J Food Microbiol* 2000; 61: 17-25.
19. Le-Tien C, Millette M, Mateescu MA, Lacroix M. Modified alginate and chitosan for lactic acid bacteria immobilization. *Biotechnol. Appl Biochem* 2004; 39: 347-354.
20. Trindade CSF, Grosso CRF. The effect of the immobilization of *Lactobacillus acidophilus* and *Bifidobacterium lactis* in alginate on their tolerance to gastrointestinal secretions. *Milchwissenschaft* 2000; 55: 496-499.
21. Krasaekoopt W, Bhandari B, Deeth H. Review: evaluation of encapsulation techniques of probiotics for yogurt. *Int Dairy* 2003; J13: 3-13.
22. Hansen LT, Allan-Wojtas PM, Jin YL, Paulson AT. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiol* 2002; 19: 35-45.
23. Khalf M, Dabour N, Kheadr E, Fliss I. Viability of probiotic bacteria in maple sap products under storage and gastrointestinal conditions. *Bioresour Technol* 2010; 101: 7966-7972.
24. Pirarat N, Komkiew P, Channarong R, Nantarika C, Ei LO, Takayuki K, Masashi M. Viability and morphological evaluation of alginate-encapsulated *Lactobacillus rhamnosus* GG under simulated tilapia gastrointestinal conditions and its effect on growth performance, intestinal morphology and protection against *Streptococcus agalactiae*. *Anim Feed Sci Technol* 2015; 207: 93-103.
25. Albertini B, Vitali B, Passerini N. Development of microparticulate systems for intestinal delivery of *Lactobacillus acidophilus* and *Bifidobacterium lactis*. *Eur J Pharm Sci* 2010; 40: 359-366.
26. Fan Z, Xiao YL, Hyun JP, Min Z. Effect of microencapsulation methods on the survival of freeze-

- dried Bifidobacterium bifidum. J Microencapsul 2013; 30: 511-518.
27. Tang J, Gong G, Su H, Wu F, Herman C. Performance evaluation of a novel method of frost prevention and retardation for air source heat pumps using the orthogonal experiment design method. Appl Energy 2016; 169: 696-708.
28. Feng Z, Niu W, Cheng C, Liao S. Hydropower system operation optimization by discrete differential dynamic programming based on orthogonal experiment design. Energy. 2017; 126: 720-732.