

**MICROBIAL ISOLATION AND CHARACTERIZATION OF
CARABAO-BASED PRODUCT (*ESPASOL DE LECHE*)
SOLD IN DAIRY COOPERATIVES
OF NUEVA ECIJA**

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ACCEPTANCE SHEET

This undergraduate thesis entitled "MICROBIAL ISOLATION AND CHARACTERIZATION OF CARABAO-BASED PRODUCT (*ESPASOL DE LECHE* SOLD IN DAIRY COOPERATIVES OF NUEVA ECIJA" prepared and submitted by LOIS MARIE A. SALMORIN in partial fulfillment of the requirements for the degree of BACHELOR OF SCIENCE IN BIOLOGY (MICROBIOLOGY), is hereby accepted.


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BIOGRAPHICAL SKETCH

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TABLE OF CONTENTS

	PAGE
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF APPENDICES	xi
LIST OF APPENDIX TABLES	xii
LIST OF APPENDIX FIGURES	xiii
ABSTRACT	xiv
INTRODUCTION	1
Background of the Study	1
Objective of the Study	3
Significance of the Study	3
Scope and Limitation of the Study	4
Time and Place of the Study	4
REVIEW OF RELATED LITERATURE	5
Milk and Milk Products	5
Dairy Processing	5
Microorganisms Associated with Spoilage of Dairy Products	6
Microorganisms Found in Rice-Flour Based Products	6
Identification of Food Borne Pathogens	8
Rapid Biochemical Test: API20E	9
Quality and Testing	10
MATERIALS AND METHODS	12
Collection of Samples	12
Evaluation of Abiotic Factors of Display Area	12
Preparation of Samples	12
Colony Count Determination	13
Test for the Presence of Coliform and <i>E. coli</i>	13
Isolation and Purification Method	14
Bacterial Identification	14
Cultural Characteristics	15
Morphological Characteristics	15

Gram Staining	15
Biochemical Test	16
Data Gathered	16
RESULTS AND DISCUSSION	18
Evaluation of Abiotic Factors of Display Area	18
Standard Plate Count of <i>Espasol de leche</i>	18
Cultural Characteristics of Bacterial Isolates	21
Morphological Characteristics of Bacterial Isolates	23
Biochemical Test	24
Dairy Processing Plant Audit and Cascading Report	26
SUMMARY CONCLUSION AND RECOMMENDATIONS	28
Summary	28
Conclusion	28
Recommendations	29
LITERATURE CITED	30
APPENDICES	33

LIST OF TABLES

TABLE		PAGE
1	Guideline levels for determining the microbiological quality of ready-to-eat foods	7
2	Standard for determining the microbiological quality of ready-to-eat foods	15
3	Microbiological count of <i>Espasol de leche</i> throughout three samplings.	19
4	Cultural characteristics of different bacterial isolates collected from <i>Espasol de leche</i>	21
5	Growth of the bacterial isolates in liquid medium collected from <i>Espasol de leche</i>	22
6	Gram staining results of the bacterial isolates collected from <i>Espasol de leche</i>	23
7	Biochemical analysis identification of the bacterial isolates using API 20 E™	25

LIST OF FIGURES

FIGURE		PAGE
1	EMBA plates negative for the presence of presumptive <i>E. coli</i>	20
2	Cultural characteristics of bacterial isolates collected from <i>Espasol de leche</i>	22
3	Growth of bacterial isolates in liquid medium collected from <i>Espasol de leche</i>	23
4	Gram staining of bacterial isolates collected from <i>Espasol de leche</i>	24

LIST OF APPENDICES

APPENDIX		PAGE
A	Standard Plate Count Analysis	34
B	Morphological and Cultural Characteristics of Isolates	37
C	Biochemical Test	38
D	Dairy Processing Plant Audit and Cascading Report	40
E	Experimental Procedures	41

LIST OF APPENDIX TABLES

APPENDIX TABLE		PAGE
1	Standard plate count of <i>Espasol de leche</i> during the 1st sampling – day 0.	34
2	Standard plate count of <i>Espasol de leche</i> during the 1st sampling– day 2.	34
3	Test for the presence of <i>E.coli</i> in <i>Espasol de leche</i> during the 1 st sampling	34
4	Test for the presence of coliforms in <i>Espasol de leche</i> during the 1 st sampling	34
5	Standard plate count of <i>Espasol de leche</i> during the 2nd sampling – day 0.	34
6	Standard plate count of <i>Espasol de leche</i> during the 2nd sampling – day 2.	34
7	Test for the presence of <i>E.coli</i> in <i>Espasol de leche</i> during the 2nd sampling.	35
8	Test for the presence of coliforms in <i>Espasol de leche</i> during the 2 nd sampling.	35
9	Standard plate count of <i>Espasol de leche</i> during the 3rd sampling– day 0.	35
10	Standard plate count of <i>Espasol de leche</i> during the 3rd sampling – day 2.	35
11	Test for the presence of <i>E.coli</i> in <i>Espasol de leche</i> during the 3rd sampling.	35
12	Test for the presence of coliforms in <i>Espasol de leche</i> during the 3rd sampling.	35
13	Interpretation of results for API 20 E TM	38

LIST OF APPENDIX FIGURES

APPENDIX FIGURE		PAGE
1	SPC plates during the 1 st sampling (Day 0)	36
2	Test for the presence of coliforms during the 1 st sampling	36
3	Test for the presence of <i>E. coli</i> during the 1 st sampling	36
4	Streak inoculation of bacterial isolates in nutrient broth	37
5	Identification of isolate 1 via API 20 E TM	39
6	Identification of isolate 2 via API 20 E TM	39
7	Identification of isolate 5 via API 20 E TM	39
8	Absence of protective personal equipment of the dairy cooperative's staff	40
9	Areas in need of cleaning, maintenance, and repair	40
10	Storage of dry goods and temperature sensitive materials	40
11	Collection of sample	41
12	Serial dilution and plating of sample	41
13	Streak point inoculation	42
14	One point inoculation	42
15	Gram staining procedure	42
16	Biochemical test procedure	43
17	Evaluation of temperature and relative humidity	43

ABSTRACT

SALMORIN, LOIS MARIE A., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2018, MICROBIAL ISOLATION AND CHARACTERIZATION OF CARABAO-BASED PRODUCT (*ESPASOL DE LECHE*) SOLD IN DAIRY COOPERATIVES OF NUEVA ECIJA**

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The detection and identification of food borne pathogens found in surfaces that come into contact with food are an important component of any integrated program to ensure the safety of foods throughout the food supply chain. The study was conducted to isolate and characterize different bacterial organisms present in *Espasol de leche* sold in dairy cooperatives of Nueva Ecija.

Espasol de leche was collected from a dairy cooperative in Nueva Ecija during the month of March and April and was evaluated using standard plate count as well as the presumptive test for the presence of *E. coli* and coliforms. Sampling was performed three times, with each sampling observed during the freshly produced (day 0) and during its 2nd day of storage.

The standard plate count of *Espasol de leche* in cfu/g, within three samplings have all satisfied the given standards for ready-to-eat-foods. No growth of presumptive Enteric bacteria and coliforms were also observed. It was concluded that the product was under the "Good" category of microbiological quality.

Distinct bacterial colonies were then isolated and were observed based on its morphological and cultural characteristics. A total of five bacterial isolates were subjected to one point inoculation, nutrient broth, Gram staining, and biochemical testing.

Results showed that three bacterial colonies were Gram positive and two were Gram negative. Using API 20 E[™] system of identification, three bacterial isolates were identified as *Pseudomonas aeruginosa*, *Pasteurella pneumotropica/Mannheimia haemolytica*, and *Pseudomonas luteola* which are mostly contaminants found in the environment. Routine quality check for conformance to good handling and manufacturing practices of dairy cooperatives are highly recommended.

LITERATURE CITED

- Casalta, J. P., Fournier, P. E., Habib, G., Riberi, A., & Raoult, D. (2005). Prosthetic valve endocarditis caused by *Pseudomonas luteola*. *BMC Infectious Diseases*, 5(1), 82.
- Chandan, R.C., Kilara, A., & Shah, N.P. (2009). Dairy Processing and Quality Assurance. John Wiley & sons.
- Chaturvedi, M., Kumar, V., Singh, D. & Kumar, S. (2013). Assessment of microbial load of some common vegetables among two different socioeconomic groups. *International Food Research Journal*, 20(5), 2927-2931.
- Collins, M. T., & Swanson, E. C. (1981). Use of the API 20E system to identify non-Enterobacteriaceae from veterinary medical sources. *American Journal of Veterinary Research*, 42(7), 1269-1273.
- Edinger, R.C., Migneault, P.C., & Nolte, F.S. (1985). Supplementary rapid biochemical test panel for the API 20E bacterial identification system. *Journal of Clinical Microbiology*, 6, 1063-1065.
- Food and Agriculture Organization of the United Nations (2014). Milk and milk products. Retrieved October 23, 2017, from <http://www.fao.org/dairy-production-products/products/en/>
- Food and Agriculture Organization of the United Nations (2014). Quality and Testing. Retrieved October 23, 2017, from <http://www.fao.org/dairy-production-products/products/quality-and-testing/en/>
- Food and Drug Administration (2013). Revised Guidelines for the Assessment of Microbiological Quality for Processed Food. Retrieved May 26, 2018, from [http://www.fda.gov/ph/attachments/article/71149/Annex%20I%20%20FC%202013-010%20Microbiological%20Standard%20for%20P rocessed%20Food.pdf](http://www.fda.gov/ph/attachments/article/71149/Annex%20I%20%20FC%202013-010%20Microbiological%20Standard%20for%20P%20rocessed%20Food.pdf)
- Food Safety Authority of Ireland (FSAI). (2016). Guidelines for the interpretation of results of microbiological testing of ready-to-eat foods placed on the market (revision 2). Retrieved November 23, 2017, from www.fsai.ie/publications_GN3_microbiological_limits/
- Gadberry, J. L., Zipper, R., Taylor, J. A., & Wink, C. (1984). *Pasteurella pneumotropica* isolated from bone and joint infections. *Journal of Clinical Microbiology*, 19(6), 926-927.
- Guillermet, F. N., & Desbresles, A.M.B.. (1971). A propos de l'utilisation d'une micromethode d'identification des enterobacteries. *Revue de l'Institut Pasteur de Lyon*. 4, 71-78.

- Holmes, B., Willcox, W.R., & Lapage, S.P. (1978). Identification of Enterobacteriaceae by the API 20E system. *Journal of Clinical Pathology*, 31, 22-30.
- Institute Of Food Technologies/Food And Drug Administration Of The U.S. Department Of Health And Human Services (If/Fda). (2001). Evaluation and definition of potentially hazardous foods. Retrieved November 23, 2017, from <http://www.fda.gov/Food/FoodScienceResearch/ucm094141.htm>
- Khan, A. A., & Cerniglia, C. E. (1994). Detection of *Pseudomonas aeruginosa* from clinical and environmental samples by amplification of the exotoxin A gene using PCR. *Applied and Environmental Microbiology*, 60(10), 3739-3745.
- Kvenberg, J. E., & Schwalm, D. J. (2000). Use of microbial data for hazard analysis and critical control point verification—Food and drug administration perspective. *Journal of Food Protection*, 63(6), 810-814.
- Ledenbach, L. H., & Marshall, R. T. (2009). Microbiological spoilage of dairy products. *Compendium of the Microbiological Spoilage of Foods and Beverages*. Springer New York, 41-67.
- Little, C. L., Omotoye, R., & Mitchell, R. T. (2003). The microbiological quality of ready-to-eat foods with added spices. *International Journal of Environmental Health research*, 13(1), 31-42.
- López-Campos, G., Martínez-Suárez, J. V., Aguado-Urda, M., & López-Alonso, V. (2012). *Microarray detection and characterization of bacterial foodborne pathogens*. Springer Science & Business Media.
- Pal, M., Mulu, S., Tekle, M., Pintoo, S.V., & Prajapati, J.P. (2016). Bacterial Contamination of Dairy Products. *Beverage and Food World*, 3, 40-43.
- McLean, S.K., Dunn, L.A. & Palombo, E.A. (2010). A pilot study of the microbiological quality of culturally diverse, ready-to-eat foods from selected retail establishments in Melbourne, Australia. *Foodborne Pathogens and Disease*, 7(5), 585-588.
- Muir, D. D. (1996). The shelf-life of dairy products: 1. Factors influencing raw milk and fresh products. *International Journal of Dairy Technology*, 49(1), 24-32.
- NSW Food Authority (2017). Rice-based Dessert: a Survey. Retrieved November 23, 2017, from www.foodauthority.nsw.gov.au/_Documents/.../rice_based_desserts_survey.pdf
- NSW Food Authority (2009). Microbiological quality guide for ready-to-eat-foods. Retrieved November 23, 2017, from www.foodauthority.nsw.gov.au/_Documents/scienceandtechnical/microbiological_quality_guide_for_RTE_food.pdf

- Okahisa, N., Inatsu, Y., Juneja, V.K. & Kawamoto, S. (2008). Evaluation and control of the risk of foodborne pathogens and spoilage bacteria present in Awa-Uirou, a sticky rice cake containing sweet red bean paste. *Foodborne Pathogens and Disease*, 5(3), 351-359.
- Philippine National Standard/ Food and Drug Administration. (2011). Recommended code of practice for the processing and handling of ethnic flour-based confectioneries (Polvoron, Piaya and Barquillos)
- Rice, J. A., Carrasco-Medina, L., Hodgins, D. C., & Shewen, P. E. (2007). *Mannheimia haemolytica* and bovine respiratory disease. *Animal Health Research Reviews*, 8(2), 117-128.
- Shayegani, M., Maupin, P.S., & Meglynn, D.M. (1978). Evaluation of the API 20E system for identification of Nonfermentative Gram-Negative Bacteria. *Journal of clinical microbiology*, 539-545.
- Swanson, E. C., & Collins, M. T. (1980). Use of the API 20E system to identify veterinary Enterobacteriaceae. *Journal of Clinical Microbiology*, 12(1), 10-14.
- The University of Adelaide. (2016). Identification and Antifungal Susceptibility of Medically Important Fungi. Retrieved September 5, 2017, from <http://www.mycology.adelaide.edu.au/descriptions/>
- United States Food and Drug Administration (2004). GMPs - Section Two: Literature Review of Common Food Safety Problems and Applicable Controls. Retrieved October 27, 2017, from <https://www.fda.gov/Food/GuidanceRegulation/CGMP/ucm110911.htm>
- Verhaeg, D. (2016). Preventing Microbiological Contamination with Product Inspection Equipment. Retrieved October 25, 2017, from: <https://www.foodmanufacturing.com/article/2016/10/preventing-microbiological-contamination-product-inspection-equipment>