UNIVERSITI PUTRA MALAYSIA

EFFECT OF STORAGE TEMPERATURE ON BACTERIAL COUNT IN COMMERCIAL PASTEURISED MILK

MOHAMAD FIDDEZUAN AHMAD MURAD

FP 2017 114

EFFECT OF STORAGE TEMPERATURE ON BACTERIAL COUNT IN COMMERCIAL PASTEURISED MILK

MOHAMAD FIDDEZUAN B AHMAD MURAD

FACULTY OF AGRICULTURE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR DARUL EHSAN
2016/2017

EFFECT OF STORAGE TEMPERATURE ON BACTERIAL COUNT IN COMMERCIAL PASTEURISED MILK

BY
MOHAMAD FIDDEZUAN BIN AHMAD MURAD

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of SHW 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture (Animal Science)

Faculty of Agriculture
Universiti Putra Malaysia
2016/2017

ACKNOWLEDGEMENT

I would like to express my deepest appreciation to my supervisor Assoc. Prof. Dr. Halimatun Yaakub for her support throughout the process of finishing my final year project (SHW 4999). Your advice and support throughout my project experience have been crucial to the furthering of my education. Thank you for providing me all the sources and guides for me finishing this project. I am sorry for putting you down and cannot give full achievement towards this project.

I would also like to thank, Aminah Adnan and Ee Li Yen for their help with my work. And also Mrs. Siti Nur Alia Ramli, Mrs. Ezazura Abdul Rahim and Ms. Nurul Shuhada Adnan for helping me and guiding me during lab session.

For my parents Ahmad Murad Sulaimi and Salawati Jamaluddin, thank you for always supporting me in any field that I participate.

Finally, I would like to thank my friends Solehah Sairuddin, Azrin Arman, Raja Syukri Raja Ismail and others. “You helped me walk the fine line between too much fun and just enough. Without you, my degree experience would have been much duller”.

Thank you

TABLE OF CONTENT

LIST OF TABLES AND FIGURES

ABSTRACT

Awareness of consumer on proper handling and storing are not virtuous, milk are left at certain temperature without knowing the quality of the milk might be altered. A study is done, to investigate the effect of storage temperature and time on bacterial count of commercial pasteurise milk. This study had been conducted at Dairy Science Laboratory, Department of Animal Science, Faculty of Agriculture, UPM. For the first replication (n=8), total of eight commercial milk (300ml/pack) were used. These milk samples were stored in ice box (4ºC) before undergoing respective treatments; 4ºC (control), 21ºC (average air-condition room), 27ºC (average room temperature; humidity <70%) and 34ºC (average hot-day temperature). Factorial experiment, Completely Randomized Design (CRD) was used for this study. In determining the present of *Pseudomonas sp*, the colour of the agar change from pink to gold and brownish in colour. This indicating the presence of non-lactose fermentative bacteria which include *Salmonella* sp., *Shigella* sp., *Proteus* sp. *and Pseudomonas aeruginosa*. Second parameter are on the presence of the lactic acid bacteria in commercial milk. It shown that, there are significant effect of temperature and time on the growth of bacteria (P<0.05). Log mean of bacterial colony for temperature 4ºC, 23ºC, 27ºC and 34ºC are 4.81, 14.90, 20.06 and 30.70 respectively. It show, temperature 34ºC are highly significant compare to other value of treatment follow by temperature 27ºC, 23ºC, and 4ºC. It can be concluded that there is no significant effect of temperature and time on the growth of *Pseudomonas* sp. Also, temperature and time are highly significant to the growth of lactic acid bacteria in commercial milk

KEYWORDS: Non-lactose fermentative bacteria, Commercial milk, Lactic acid bacteria, *Pseudomonas* sp, Temperature

ABSTRAK

Kesedaran pengguna terhadap penjagaan dan penyimpanan susu masih tidak memuaskan. Susu diletakkan di kawasan yang berbeza dari segi suhu, yang mana kualiti asal susu tersebut mungkin akan berubah. Satu kajian telah dilakukan, bagi mengkaji kesan suhu penyimpanan dan masa pada kiraan bakteria di dalam susu komersial. Kajian ini telah dijalankan di Makmal Sains Tenusu, Jabatan Sains Haiwan, Fakulti Pertanian, UPM. Untuk replikasi yang pertama (n=8), sejumlah lapan susu komersial (300ml / pack) telah disediakan. Susu ini telah disimpan di dalam kotak simpanan ais (4ºC) sebelum menjalani rawatan masing-masing; 4ºC (kawalan), 21ºC (purata bilik penghawa dingin), 27ºC (purata suhu bilik; kelembapan <70%) dan 34ºC (Purata suhu panas hari). Faktorial eksperimen, Rekaan Rambang Sepenuhnya (CRD) telah digunakan di dalam kajian ini. Dalam menentukan kehadiran Pseudomonas sp., berlaku perubahan warna agar Mac Conkey dari merah jambu ke warna keemasan. Ini menunjukkan kehadiran bakteria fermentif bukan laktosa termasuk *Salmonella* sp., *Shigella* sp., *Proteus* sp. dan *Pseudomonas aeruginosa*. Parameter kedua adalah mengenai kehadiran bakteria asid laktik dalam susu komersial. Ia menunjukkan bahawa, terdapat kesan yang besar daripada rawatan suhu dan masa kepada pertumbuhan bakteria ($P < 0.05$). Log koloni bakteria untuk suhu 4ºC, 23ºC, 27ºC, dan 34ºC adalah 4.81, 14.90, 20.06 dan 30.70, masing-masing. Ia menunjukkan, Suhu 34ºC memberi kesan yang amat ketara terhadap nilai purata kiraan bakteria berbanding dengan nilai rawatan yang lain diikuti suhu 27ºC, 23ºC, dan 4ºC. Secara kesimpulan, tidak ada kesan yang ketara daripada rawatan suhu dan masa kepada pertumbuhan *Pseudomonas* sp. Selain itu, suhu dan masa adalah sangat penting kepada pertumbuhan bakteria asid laktik dalam susu komersial.

Kata-kata kunci: Bakteria Fermentif bukan laktosa, Susu komersial, Bakteria asid laktik, *Pseudomonas* sp, Suhu

1.0 INTRODUCTION

1.1 Background of Study

Cow's milk has long been measured as an extremely nutritious and valuable food for human consumption. Its various composition make it as an ideal medium for the bacteria to growth, and hence milk can be labelled as most perishable agricultural product as easily being contaminated (Bryan, 1983; Bramley & McKinnon, 1990; Heeschen, 1994). There are various kind of milk product that we can find at supermarket including Ultra-High Temperature (UHT) milk and High Temperature Short Time (HTST) milk. Both of this milk have undergone a heat-treatment process that could kill microbes, known today as pasteurisation, was introduced to further ensure milk safety. Pasteurisation requires heating milk to a specific temperature for a minimum period of time, and then quickly cooling it back down to refrigerated temperatures (4°C) (De Buyser *et al.*, 2001; Walstra *et al.*, 2006). Pasteurisation of raw milk is functioning in eliminating all but the thermoduric microorganisms of the genera *Microbacterium, Micrococccus, Streptococcus, Lactobacillus, Bacillus, Clostridium*, the corynebacterium, and irregularly some Gram-negative rods (Jay, 1996). Classic pasteurisation includes heating milk to 63°C for 30 minutes. Though, as pasteurisation become broadly recognized and dairy product become more industrialised, HTST (72°C for 15 seconds) and UHT (135°C for 2 seconds) become conventional (Mendelson 2011; Walstra *et al.*, 2006). Then, diverse in temperature and holding time upon heat-treatment bring different post-effect to the microbial growth and composition of the milk itself (© 2012 Department of Food, Nutrition, and Packaging Science). In addition, psychrotrophs, generally, *Pseudomonas* sp. are identified to be the

foremost factors of the shelf-life of pasteurised milk and refrigerated raw milk (Stevenson *et al.*, 2003).

Comparing to UHT milk, HTST milk mostly store on fridge shelf at the range of 4-6ºC of temperature. It is important to note that milk can become contaminated even after they have been pasteurised. For example, all pasteurised milk must be refrigerated. If the pasteurised milk is temperature-abused, it could become contaminated (© 2012 Department of Food, Nutrition, and Packaging Science).

Bacterial spoilage is the most momentous limiting factor in prolonging the shelf-life of HTST pasteurised milk beyond 14 days. Microbial growth and metabolism could abbreviate the shelf life of milk by producing undesirable changes in aroma and taste qualities that effect consumer satisfactoriness of the food product (Fromm and Boor, 2004). Currently, *Pseudomonas sp.* are the predominant microorganisms limiting the shelf life of processed fluid milk at 4°C (Boor *et al*.2001; Craven *et al.*, 1992; Ternstrom *et al*., 1993). In addition, *Pseudomonas sp*. have ability to grow to high numbers during refrigerated storage. Not only that, many of these strains produce heat-stable extracellular lipases, proteases, and lecithinases which in further contribute to the spoilage of milk (Champagne and Shah, 1994; Cousin *et al*., 1982; Sorhaugh *et al*., 1997). Degradation of milk components through numerous enzymatic activities can alleviate the shelf life of processed milk. For instance, from the digestion of casein by proteases, the flavour of the milk change to bitter and there are formation of clotting and gelation in milk. Besides that, Lipases hydrolyse tributyrin and milk fat to yield free fatty acids, which cause milk to taste rancid, bitter, unclean, and soapy. (Cousin, 1982; Cox *et al*., 1993; Shah *et al*., 1994).

Awareness of consumers on proper handling of milk upon usage are not virtuous. Milk are left wherever at improper temperature for a longer period which can lead to the spoilage of milk before the expired date. It shown, significant growth of psychrotrophs was observed when milk was stored above 4°C, whereas little or no growth was observed in milk stored below 4°C (O'Connell *et al.*, 2016).

1.2 Research Problem

Consumers lack of awareness in appropriate handling and storage of milks upon consumptions. The quality of pasteurised milks that left behind at improper temperatures or places might be altered. They placed the milks at different places upon arrival to their house event though milks is the last one to be picked. Additionally, consumers that do not have fridge or proper storage placed the milks at certain places where the temperature might be different depending on the weather or controlled temperature upon consumption. Hence, a study is done, to measure the quality of the milk at different storage temperatures and times.

1.3 Research Hypothesis

Refrigerated (4ºC) milk will have lower *Pseudomonas* sp. count compare to average air-conditioner temperature milk (21ºC) , average room temperature (27ºC) and average hot day temperature (34ºC) for both period of time (3h and 5h). Small amount of psychotropic bacteria are presence in milk at room temperature. The presence of lactic acid for both time of milk at average hot-day temperature are higher than the other samples.

1.4 Objectives of Study

General objective of this study is to investigate the effect of storage temperature (4°C, 21°C, 27°C, 34°C) and time (3 h, 5 h) of commercial pasteurised milk on bacterial count.

Specific objectives:

1. To determine the presence of psychotropic bacteria, *Pseudomonas* sp. in the milk
2. To determine the presence of lactic acid in the milk
3. To evaluate the quality of milk after undergoing the treatments

1.5 Significance of Study

This study practically help develop the consumer awareness on proper storage of pasteurised fresh milk at home.

7.0 REFERENCES

Barrefors, P., K. Granelli, L.A. Appelqvist, L. Bjoerck. 1995. Chemical Characterization of Raw Milk Samples with and Without Oxidative Off-Flavor. *J Dairy Sci*. 78(12):2691-2699.

Carpentier B., O. Cerf. 1993. Biofilms and their consequences, with particular reference to hygiene in the food industry. *J Appl Bact.* 75:495-511.

Champagne, C. P., R. R. Laing, D. Roy, and A. A. Mafu. 1994. Psychrotrophs in dairy products: their effects and their control. *Crit. Rev. Food Sci. Nutr.* 34:1–30.

Chapman, K., L.J. Whited, K.J. Boor. 2006. Sensory threshold of light-oxidized flavor defects in milk. *J Food Sci.* 67(7):2770-2773.

Clark, S., M. Costello, M.A. Drake, F. Bodyfelt. 2009. The sensory evaluation of dairy products. 73-128 in *Fluid milk and cream products*. Second edition. Clark, S., Costello, M., Drake, M. A., Bodyfelt, F, ed. Springer Science and Business Media, New York, NY.

Cox, J. M. 1993. The significance of psychrotrophic pseudomonads in dairy products. *Aust J Dairy Technol.* 48:108–113.

Cousin, M. A. 1982. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. *J Food Prot.* 45:172–207.

Cousins, C. M., and A. J. Bramley. 1981. The microbiology of raw milk, p. 119–163. In R. K. Robinson (ed.), *Dairy Microbiology*, vol. 1. Applied Science Publishers, Englewood, N.J.

Cousin, M. A. 1982. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. J. Food Prot. 45:172–207.

Craven, H. M., and B. J. Macauley. 1992. Microorganisms in pasteurised milk after refrigerated storage. 1. Identification of types. *Aust J Dairy Technol*. 47:38–45.

Dairy Microbiology. from http://dairyextension. foodscience.cornell.edu/sites/ dairyextension.foodscience.cornell.edu/files/shared/Diary Microbiology.pdf, Retrieved April 15, 2016.

Dunkley W.L., J.D. Franklin, R.M. Pangborn. 1962. Influence of Homogenization, Copper, and Ascorbic Acid on Light-Activated Flavor in Milk. *J Dairy Sci.* 45(9):1040-1044.

Francis Group. Early, Ralph. *The Technology of Dairy Products.* New York, NY: Thompson Science, 1998.

Fromm. H.I., Boor. K. J. 2004. Characterization of pasteurised fluid milk shelf-life attributes. *J Food Sci.* 69: 207-214

Food standard Agency & Food Hygiene Regulation 2006, Milk Storage and Temperature Control. from https://www.coolmilk.com/files/Milk storage and temperature control sheet embedded v2.pdf, retrieved April 20, 2016.

Garcia, M. L., B. Sanz, P. Garcia-Collia, and J. A. Ordonez. 1989. Activity and thermostability of the extracellular lipases and proteinases from pseudomonads isolated from raw milk. *Milchwissenchaft* 44:547–549.

Griffiths, M. W., J. D. Phillips, and D. D. Muir. 1984. Post-pasteurization Contamination:

The major cause of failure of fresh dairy products. *Hannah Res.* 1984:77–87.

Gruetzmacher, T.J., R.L. Bradley Jr. 1998. Identification and control of processing variables that affect the quality and safety of fluid milk. J. Food Prot. 62:625-631.

Hickey, C. D., Sheehan, J. J., Wilkinson, M. G., & Auty, M. A. E. 2015. Growth and location of bacterial colonies within dairy foods using microscopy techniques: a review. Frontiers in Microbiology, 6, 99. http://doi.org/10.3389/fmicb.2015.00099

Huck, J.R., B.H. Hammond, S.C. Murphy, N.H. Woodcock, K.J. Boor. 2007. Tracking spore-forming bacterial contaminants in fluid milk-processing systems. *J Dairy Sci.* 90:4872-4883.

Huck, J.R., M. Sonnen, K.J. Boor. 2008. Tracking heat-resistant, cold-thriving fluid milk spoilage bacteria from farm to packaged product. *J Dairy Sci*. 1218-1228.

Iklim Malaysia - Malaysian Meteorological Department. (n.d.). from http://www.met.gov.my/web/metmalaysia/education/climate/generalclimateofmalaysia, Retrieved October 09, 2016,

Jay, J.M. *Modern food microbiology* (5th Ed.). New York, Chapman&Hall, 1996, 137–141, 328–342, 347–352.

Keynan, A., Z. Evenchik, H.O. Halvorson, J.W. Hastings. 1964. Activation of bacterial endospores. *J Bacteriol.* 88(2):313-318.

Malini, A., Deepa, E., Gokul, B., & Prasad, S. 2009. Nonfermenting Gram-Negative Bacilli Infections in a Tertiary Care Hospital in Kolar, Karnataka. *J Lab Physic*, 1(2), 62–66. http://doi.org/10.4103/0974-2727.59701

Mayerhof, H. J., R. T. Marshall, C. H. White, and M. Lu. 1973. Characterization of a heat stable protease of *Pseudomonas fluorescens* P26. *Appl Microbiol*. 25:44–48.

Ministry of Science, Technology and the Environment. 2000. Malaysia Initial National Communication. Report submitted to the United Nations Framework Convention on Climate Change.

Odongo, N. O., Lamuka, P. O., Matofari, J. W., & Abong, G. O. 2016. Risk factors associated with the post-harvest loss of milk along camel milk value chain in Isiolo County, Kenya. *African J Agric Res*, 11(8), 674-682.

Olsen., S. J., M. Ying., M. F. Davis., M. Deasy., B. Holland., L. Iampietro., C. M. Baysinger., F.Sassano., L. D. Polk., B. Gormley., M. J. Hung., K. Pilot., M. Orsini., S. Van Duyne., S. Rankin., C. Genese., E. A. Bresnitz., J. Smucker., M. Moll., and J. Sobel. 2004. Multidrug-resistant Salmonella Typhimurium infection from milk contaminated after pasteurization. *Emerg Infect Dis.* 10:932-5.

Oliver. S.P., Jayarao, B.M., Almeida, R.A. 2005. *Dairy Processing Handbook,* Page: 19. Foodborne pathogens in milk and the dairy farm environment:

Oliver. S.P., Jayarao. B.M., Almeida. R.A. 2005. Dairy Processing Handbook,Food safety and public health implications. Foodborne pathogens and disease 2:115-129

Petrus, R. R., de Alvarenga Freire, M. T., de Camargo Setogute, L., & Higaho, V. M. 2011. Effect of pasteurization temperature and aseptic filling on the shelf-life of milk/Efeito da temperatura de pasteurizacao e do envase asseptico na vida de prateleira de leite. Alimentos e Nutricao. *Brazilian J Food Nutr.* 22(4), 531-539.

Robertson, G. L. 2009. *Shelf life of milk.* Broken Sound Parkway, NNW: Taylor

Shafii, H. 2012. *Keselesaan terma rumah kediaman dan pengaruhnya terhadap kualiti hidup penduduk* (Thermal comfort of house and it's influence on people's quality of life). Geografia: *Mal J Soc Space*, 8(4), 28-43.

Shah, N. P. 1994. Psychrotrophs in milk. Milchwissenchaft 49:432–437.

Sorhaug, T., and L. Stepaniak. 1997. Psychrotrophs and their enzymes in milk and dairy products: quality aspects. *Trends Food Sci. Technol.* 8:35–40.

Sohrabvandi S, Mortazavian AM, Dolatkhahnejad MR, Monfared AB. Suitability of MRS-bile agar for the selective enumeration of mixed probiotic bacteria in presence of mesophilic lactic acid cultures and yoghurt bacteria. *Iran J Biotechnol.* 2012; 10:16–21.

Ternström., A., A. M. Lindberg., and G. Molin. 1993. Classification of the spoilage flora of raw and pasteurised bovine-milk, with special reference to *Pseudomonas* and *Bacillus*. *J Appl Bacteriol*. 75:25–34.

Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiol Mol Biol Rev.*, 64(4), 655-671.