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Survival of *Candida tropicalis* and *Lactobacillus plantarum* starter culture after using protective agent and drying

1*Maneesri, J., 2Masniyom, P. and 1Liming, M.

[1]*Department of Food Science and Nutrition, Faculty of Science and Technology, Prince of Songkla University, Pattani 94000, Thailand*
[2]*Department of Technology and Industry, Faculty of Science and Technology, Prince of Songkla University, Pattani 94000, Thailand*

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Abstract

Yeast *Candida tropicalis* and lactic acid bacteria *Lactobacillus plantarum* were isolated from Ka-nom Tuay-fu as traditional starter culture in Thailand. The isolates were used on a labolaratory-scale process. Effect of temperature drying (40, 50 and 60ºC) on the survival and activity of *C. tropicalis* and *L. plantarum* were investigated. Rice flour was used as supporting material. The temperature drying had affect cell viability, moisture content, water activity and drying time. The cell viability of starter cultures have significantly affect under different temperature. The dry condition at 40ºC for 16 hr was obtained the high cell viability of *C. tropicalis* and *L. plantarum* with $7x10^6$ and $5x10^6$ CFU/5g, respectively. The starter culture powder can dehydrated to low of the moisture content at 10% and water activity 0.4. Sucrose and glucose solution were used as protective agent at concentrations of 0-15%(w/v). Glucose 15%(w/v) showed superior protective on recovery cell survival of *C. tropicalis* and *L. plantarum* up to 99 and 90%, respectively. The result revealed the optimal condition of drying process can be used for preparation of the Ka-nom Tuay-fu starter culture.

Introduction

Thai steam bread or a fermented spongy rice cake is one of the traditional fermented food and a few maker in Thailand as Ka-nom Tuay-fu. The production is still homemade. It is made from rice flour, palm sap or palm sugar, water and Loog-pang Khaomak which are major ingredients. Loog-pang Khaomak is a traditionally mixed starter culture with variety of microorganisms whose strains have the highest growth rate and predominant activities during fermentation. A significant disadvantage in use Loog-pang Khaomak for Ka-nom Tuay-fu is difficultly control the quality of products. All time processes was about 2 days. So far, the baking powder or baker's yeast had been used and obtained the spongy texture, but a specific flavor-acidity flavor from lactic acid bacteria has not attained yet. The process of Ka-nom Tuay-fu was include to crush the rice, mix the ingredients, ferment with natural inoculum and steam. The steam bread products was a sweet with a little sour taste and good smell. It used as a popular dessert in the south of Thailand. Our research has been isolate and achieve the new cultures have important adventages, such as a specific flavor and a spongy texture. It has been selected a proper strains with good ability and preparation of pure inoculum for steam bread, mainly yeast *Candida tropicalis* and lactic acid bacteria *Lactobacillus plantarum* (Maneesri and Masniyom, 2015). Both strains were studied the optimum of freeze drying process and can used for starter culture preparation. Rice flour was used as supporting material. Moreover, sucrose and glucose solution were used as protective agent. Freeze drying process has effect on cell viability. But the cost is higher than other drying methods (Liming *et al.*, 2014). In the other fermentation food, "sourdough bread" is well know that using the mixed starter culture containing lactic acid bacteria (LAB) and yeasts (Caballero *et al*., 1995; Gobbetti *et al.*, 1995; Linko *et al.*, 1997; Meignen *et al.*, 2001). The pure cultures expose a remarkable improvement such as fermentation time reduction and desirably sensorial quality. The wet pure culture is incompatible with the commercial one and unfavorably applied in fermentation and storage. Therefore, desiccation has been the preferable method to prepare the starter cultures has to reach the targets as viability, vitality, storage stability and handling. (Santivarangkna and Foerst, 2015)

In order to minimize damage during drying, protective agents are added to the cell suspension before drying and has the proposed: preferential hydration, water replacement and glass transition

*Corresponding author.
Email: *jaruwan.ma@psu.ac.th*

(Santivarangkna *et al.*, 2008). Protective agents have been used in several researches such as triols, polyalcohols, monosaccharide, disaccharide and polysaccharide (Hubalek, 2003). The type and concentration of protective agent depends mainly on the microorganism. The survival of low temperature drying methods of *L. plantarum* CIF17AN2 as showed at 45ºC.The survival of freeze dried *C. sake* cells was increased to 30-40% by using 5% or 10% glucose or 10% sucrose (Abadiasand *et al*., 2001). Glucose has been used to improve the survival cells at concentration 1-18% and 1-68% for sucrose (Hubalek, 2003). It is well know that drying process is the common technology and reduce cost. However, little information regarding the optimal process in Ka-nom Tuay-fu starter powder has been reported. Therefore, it is necessary to investigate the viability of *C. tropicalis* and *L. plantarum* starter culture in the different temperatures and protective agents during drying.

Materials and Methods

Microorganism

C. tropicalis TISTR 5922 and *L. plantarum* TISTR 2083 were isolated from Loog-pang Khaomak used as Ka-nom Tuay-fu production that it were obtained from Pattani province, Thailand (Maneesri and Masniyom, 2012). Both strains were collected at Thailand Institute of Scientific and Technological Research (TISTR), sub-cultured every month on Yeast Peptone Dextrose (YPD) agar and Man Rogosa and Sharpe (MRS) agar, respectively and stored at 4ºC until used.

Cell preparation

C. tropicalis and *L. plantarum* were respectively cultured in 500 ml flask containing 300 ml of YPD broth and MRS broth. The culture conditions to obtain the logarithmic phase were 30ºC, 110 rpm and 12 hr for *C. tropicalis* and 21 hr for *L. plantarum.* Cells were harvested by centrifugation at 9,000 xg for 10 min at 4ºC. The supernatant was decanted and the cell paste was resuspended in 30 ml distilled water. This process was twice repeated to collect the cell for subsequent mixing with distilled water or protective agents.

Protective agent

D-glucose and sucrose were two types of protective agent and distilled water was used as a control treatment for comparison. Protective agents were prepared at 5, 10 and 15% (w/v) concentrations and subsequently autoclaved at 121ºC for 15 min before use.

Sample preparation for experiments

Cell paste obtained as described above was resuspended in distilled water. Cell suspension of *C. tropicalis* and *L. plantarum* were contained approximately 10^6-10^7 CFU/ml and 10^8-10^9 CFU/ml, respectively. The suspension (5 ml) was transferred and mixed with 5 g sterilized flour in petri dish to make the starter culture. Starter culture was dried by hot air oven at 40ºC, 50ºC and 60ºC, the sample was tested every 4 hour for 24 hr.

The cell viability of both treatments were determined before and after drying by pour plate method. The percentage of cell viability was expressed as ratio between the total number of viable cells after drying and total number of viable cells before drying, which subsequently multiplies with one hundred.

Storage experiments

Starter culture powder of *C. tropicalis* and *L. plantarum* were filled about 5 g in plastic bag (NY/LLDPE size 90x75 mm.), aluminium foil (ALU/LLDPE size 90x75 mm) with vacuum or non vacuum with stored at 4ºC and room temperature kept at 30ºC.The sampling was done every 15 days up to 90 days storage. At every sampling point was measured the cell viability, water activity and moisture content. The percentage of survival was calculated from the viable cell counts before and after storages at each sampling time.

Determination of water activity and moisture content

Moisture content was determined according to the method of AOAC (2000) and water activity measured with water activity meter (Aqua Lab, 4TE).

Statistical analysis

The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$). All analyses were run in triplicate.

Results and Discussion

Effect of temperature drying

Drying condition is related to the temperature and time drying on the cell survival which leads to an economic. The temperatures were varied to investigate their effects on the survival cells of *C. tropicalis* and *L. plantarum* starter culture. After

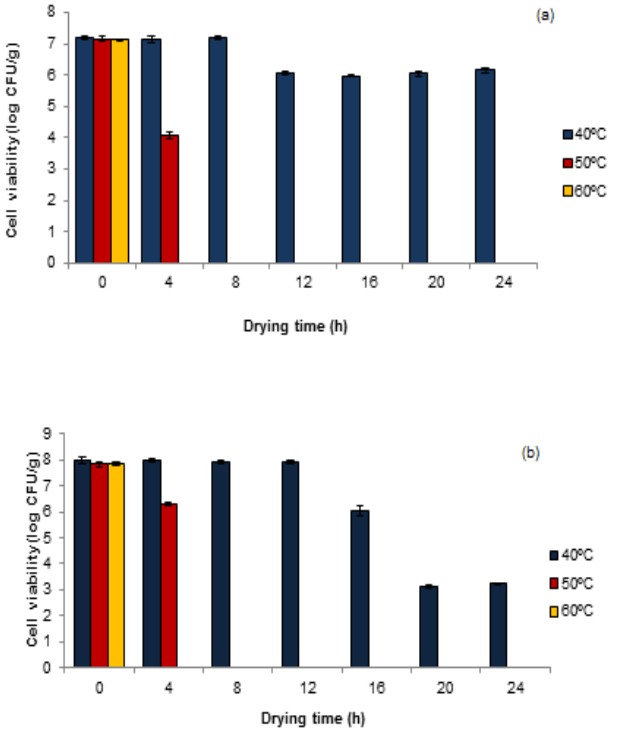

Figure 1. Cell viability of C. tropicalis (a) and *L. plantarum* (b) starter during to drying at 40, 50 and 60 ºC

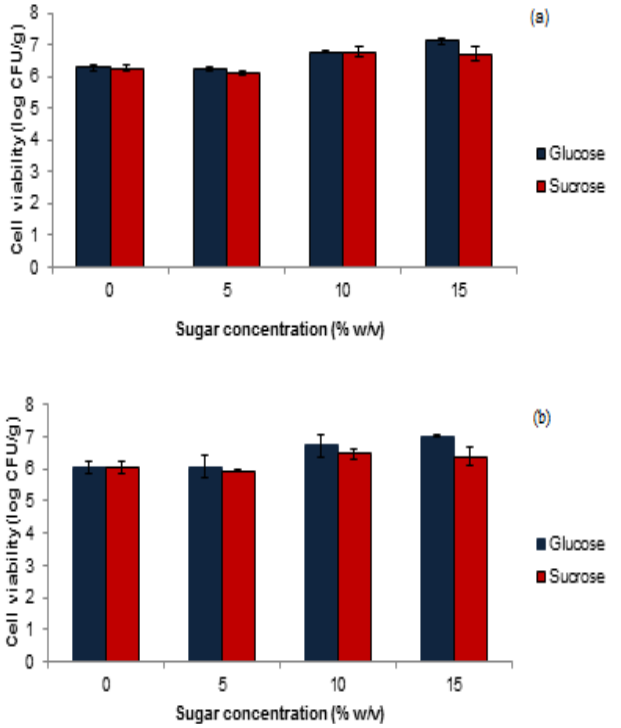

Figure 2. Cell viability of C. tropicalis (a) and *L. plantarum* (b) starter with a protectant agent as glucose and sucrose during drying at 40 ºC for 16 hr

drying, the viability of *C. tropicalis* and *L. plantarum* were drastically decreased and no cells at 60ºC for 4 hr (Figure. 1). The highest viability of *C. tropicalis* starter culture was achieved under drying operated at 40ºC. The total count of cell from culture was decreased only 1 log CFU/g at 40ºC for 12 hr and kept stable until 24 hr. Whileas, drying at 50ºC for 4 hr found that the cell count of cell culture was slightly decreased 3 log CFU/g and no cell viability. The viability of *L. plantarum* starter culture was decreased 1.95 log CFU/g with drying at 40ºC for 16 hr. The temperature drying at 50ºC and 60ºC were obtained the cell viability of *L. plantarum* starter culture similar to *C. tropicalis.* The moisture content of *C. tropicalis* and *L. plantarum* starter culture after drying at 40ºC for 16 hr were 10.4%, respectively. The results showed that each temperatures were effect difference in cell viability and the moisture content. The water activity was related to the moisture content and the moisture content is an important parameter for cell viability. For the samples dried at 40ºC the water activity was 0.4. The high cell viability, sufficient moisture content and water activity were important in the stability of dried starter cultures. In our results, drying at 40ºC for 16 hr could suggested as optimum for cell starter culture and chosen to carry out further experiment. In addition, rice flour was used as carrier material might protect the cell of *C. tropicalis* and *L. plantarum* during longer drying process. This conditions were chosen as the most effective and economical method. Meuser *et al.* (1995) noted about the microbial activity of dried sourdough on the first at 40ºC and reduce to 30ºC that used as starters for sourdough fermentation. According to Hongpattarakere and Uraipan (2015), resistant starch from unripe saba banana was effectively protected *Lactobacillus plantarum* CIF17AN2 during drying process. Moreover, the survival of microorganisms have many factors, such strains (Bauer *et al*., 2012), temperature of drying (Fu and Chen, 2011) and protective agents (Hubalek, 2003).

Effect of protective agents

The effects of different protective agents and their concentrations on cell viability of *C. tropicalis* and *L. plantarum* starter cultures during drying process were investigated. Results are shown in Figure 2. The viability of *C. tropicalis* was increasing corresponding to the increase in concentrations of glucose and sucrose from 5% to 15%. The 10% and 15% glucose concentrations were effectively preserve the viability of *C. tropicalis* from 6.21 logCFU to 6.78 and 7.12 logCFU, respectively. The cell survival of *C. tropicalis* was 95 and 99%, respectively (Figure 3). When using 10% and 15% sucrose of protective agent showed the cell survival 95 and 94%, respectively. Whereas, the viability of *L. plantarum* starter culture with 15% glucose observed increase the viability from 6.02 logCFU/g to 7.03

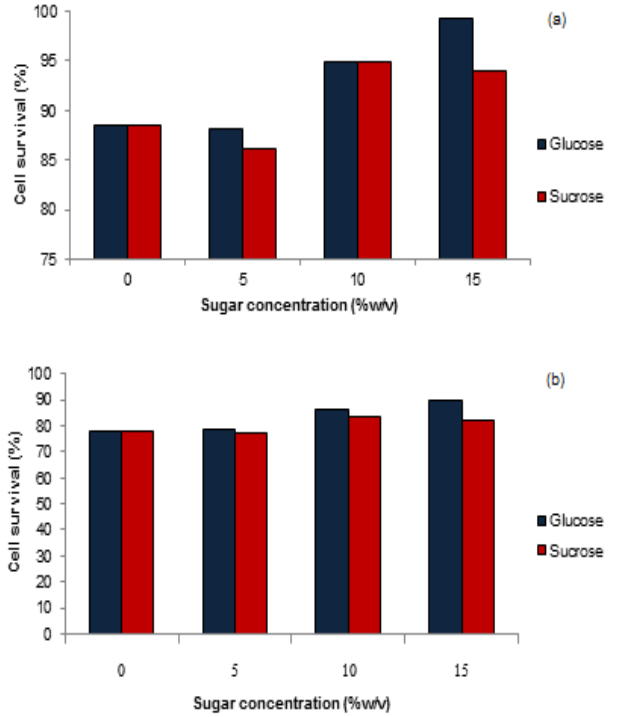

Figure 3. Cell survival of *C. tropicalis* (a) and *L. plantarum* (b) starter with a protectant agent as glucose and sucrose during drying at 40 ºC for 16 hr

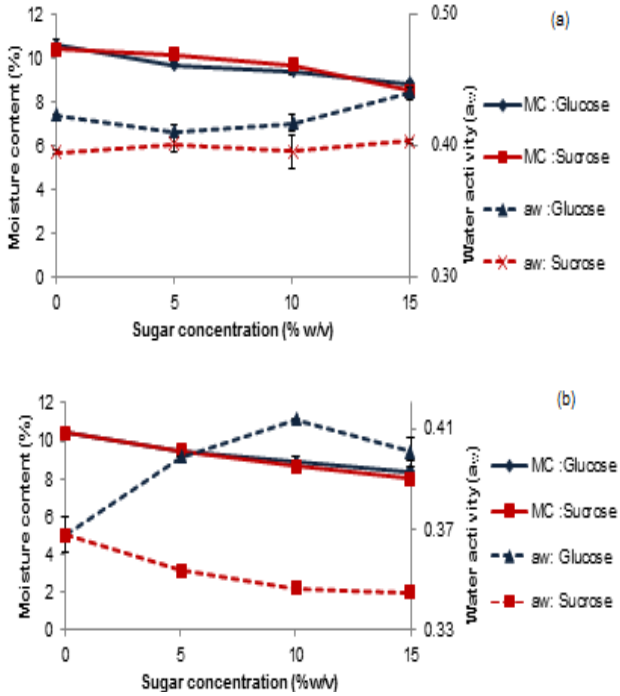

Figure 4. Moisture content and water activity (aw) of *C. tropicalis* (a) and *L. plantarum* (b) starter with a protectant agent as glucose and sucrose after drying at 40 ºC for 16 hr

logCFU/g and the cell survival was 90%. The viability of the cell was low when using 10% glucose. Glucose and sucose at 5% were no increasing in cell viability. Therefore, glucose at 15% concentration showed the highest cell survival up to 90%. As the result, it could be concluded that glucose greatly enhanced the

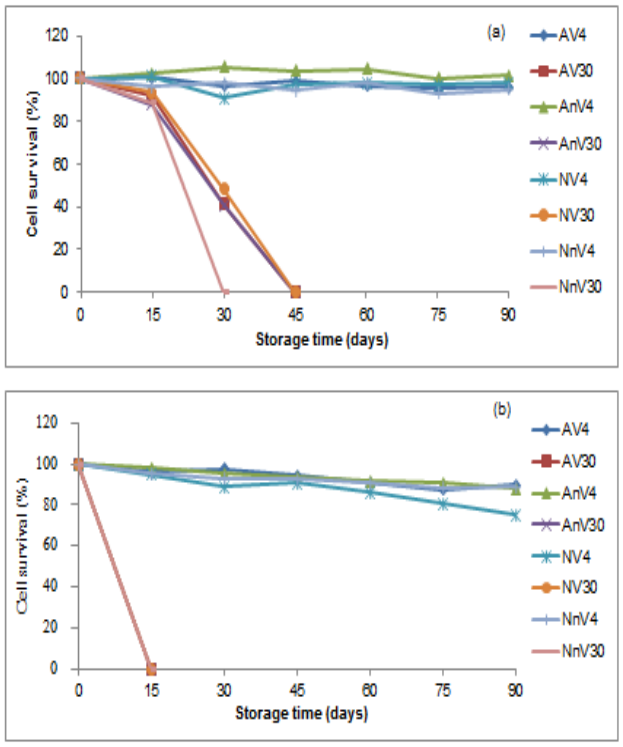

Figure 5. Cell survival of *C. tropicalis* (a) and *L. plantarum* (b) starter with a different package, vaccum or non- vaccum sealing and storage at 4ºC and room temperature at 30 ºC for 90 days. The complete description of the abbreviated is listed as aluminium foil (A),plastic bag (N), vaccum packing (V), non- vaccum sealing (nV) and temperature for storage (4, 30 ºC)

retention of viability of *C. tropicalis* and *L. plantarum* starter cultures during drying. The moisture content of *C. tropicalis* and *L. plantarum* starter culture after drying were slightly decreased. But the effect of glucose concentration was increased the water activity (Figure 4). At the highest survival obtained at 15% glucose concentration for *L. plantarum* starter culture, moisture content were 8.39% and water activity 0.4. Results of glucose as the protective agent and addition to the cell suspension before drying has been reported that sugars are used in preparation of dried starter cultures for fermented food. When drying to a slowly low moisture content and leads to a lower survival rate (Santivarangkna *et al*., 2007; Santivarangkna *et al*., 2008). Interestingly, dried starter cultures at low temperature drying is higher viability than freeze-dried starter. For freeze drying, the cell survival of *C. tropicalis* and *L. plantarum* are 73 and 92%, respectively (data not shown).

Survival of C. tropicalis *and* L. plantarum

Survival of *C. tropicalis* and *L. plantarum* after storage in each conditions at different package as aluminium foil (A), plastic bag (N), vacuum (V) or non vaccum (nV), temperatures (4, 30 ºC) by count cell viability. The slight viability loss of *C. tropicalis*

and *L.plantarum* starter powder were observed from the beginning to 90 days of storage (Figure 5).

At 4ºC storage, the starter powder product in an aluminium foil sachet and plastic bag were higher cell viability than that stored at the room temperature. Cell survival of *C. tropicalis* under storage under treatment AV4, AnV4, NV4 and NnV4 showed closely and higher than 95%. Cell survival of *L. plantarum* was the highest at 90% in AV4 and following to NnV4, AnV4 and NV4 as 89, 88 and 75%, respectively. At room temperature, a sharp decrease of viable cells were detected at both package within 45 days. Our result showed the temperature of storage was effective for protecting cells. It can be concluded that storage at low temperature is required to maintain viability of culture starter powder. The water activity and moisture content were slightly increase and not effective to survival of *C. tropicalis.* But the survival of *L. plantarum* cells were a few reduced during in 90 days.

Conclusion

Drying were affected on *C. tropicalis* and *L. plantarum* starter culture, with decreased viability of cell during the drying process. Cell can be damage due to thermal and dehydration stress. This study demonstrated the possibility of addition of glucose as protective agent were improved the survival depend on concentration. In optimization condition of drying can be use 40ºC for 16 hr. and the better results in cell viability, drying time, even comparable cost to preparation of *C. tropicalis* and *L. plantarum* starter culture for production of Ka-nom Tuay-fu. In this work, it was shown that the starter culture powder for Ka-nom Tuay-fu can be use easily and increase the consuming in the home. Therefore, to application for production of Ka-nom Tuay-fu and can use for future studies to development the product.

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