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Improving cell viability with protective agent, carrier support and stability agent of *Candida tropicalis* TISTR 5922 starter powder

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Abstract

A pure culture of *Candida tropicalis* TISTR 5922 was used as a starter culture in Ka - nom Tuay - fu production which is a Thai traditional food. The objective of this research was to improve the viability of *C. tropicalis* TISTR 5922 starter powder by 15% glucose (w/v) with a different protective agent (milk powder, skim milk powder and sweet whey powder) at 5, 10 and 15% (w/v)), carrier supports (rice flour, corn flour, wheat flour and cassava flour), which can enhance the survival by stability agents (gelatin, Arabic gum and maltodextrin at 1, 2 and 3% (w/v)), and drying at 40°C. The results showed that 15% glucose with 10% skim milk powder for protective agents, rice flour for carrier support and 3% Arabic gum as for stability agent were well processed. It also showed high cell viability of 8.45 log CFU/g. The starter powder had the moisture content and water activity at 7.15±0.48% and 0.40±0.04, respectively. The results indicated that good physical properties of starter powder were obtained.

1. Introduction

Yeast is eukaryotic microorganisms and play an important role during food fermentation (Rai *et al*., 2017) such as bread sourdough beer and vinegar. *Candida tropicalis* TISTR 5922 is one type of *Candida* genus yeast.

Candida tropicalis TISTR 5922 starter culture is the main ingredient for producing Ka - nom Tuay - fu. It is isolated from Loog - pang Khaomak as a traditional starter culture in Thailand (Maneesri *et al*., 2017). Starter cultures are used to ensure a fast and safe method to ferment food products with high and constant product quality (Foerst and Santivarangkna, 2015). Many factors can affect starter cultures and determine their high quality such as the growth conditions (Palmfeldt and Hahn - Ha gerdal, 2000), protective agents (De Valdez *et al*., 1983), the initial cell concentration and drying technologies (Lee *et al*., 2016).

Protective agents are one of the important factors that have a significant role in the conservation of viability. The best protective agent should be easily or fast dried, as well as stable and easy to rehydrate. Basholli - Salihu *et al*. (2014) studied skim milk and different sugars as protective agents to decrease the damaged cells caused by the drying method at low temperatures. Skim milk has a positive effect in terms of its ability to provide a coating layer that protects cells during the freezing and drying process (Abadias *et al*., 2001). Interestingly, several natural polymers have been reported to offer protection during drying and enhance the stability of starter culture which can be achieved by adding many protective compounds. Among carbohydrates, polysaccharides have a higher molecular weight and they include moltodextrin, gelatin and Arabic gum (Bustos and Bórquez, 2013; Yonekura *et al*., 2014; Nunes *et al*., 2018). Moreover, carriers support or carriers are usually directly mixed with the cell pellets or solution cells obtained after centrifugation. It can decrease water activity and moisture content of the final drying (Fu and Chen, 2011). The different water activity levels could affect the stress tolerance and change their behavior during storage.

The drying method is an important final process to reach a higher survival rate. Nowadays, usually, those spray and freeze - drying methods used for dehydration are considered a high - cost method. According to Santivarangkna *et al*. (2007), air drying is a cost - effective method which can lower the cost and attain positive results and comparatively less cell damage, as well as provide easier moisture control in the starter compared with other drying methods.

Aside from the benefits of *C. tropicalis* TISTR 5922

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the starter powder possesses, it also has disadvantages such as lesser cell viability, short shelf life and difficulty to use. Thus, this study aimed to evaluate the improved process of *C. tropicalis* TISTR 5922 starter powder by 15% glucose (w/v) with different protective agents (milk powder, skim milk powder and whey powder), carrier support (rice flour, corn flour, wheat flour and cassava flour), while enhancing its survival by the use of stability agent (gelatin, Arabic gum and maltodextrin), and in certain drying condition.

2. Materials and methods

2.1 Preparation of Candida tropicalis TISTR 5922

Lyphilized stains of *C. tropicalis* TISTR 5922 were collected at the Thailand Institute of Scientific and Technological Research (TISTR). Subculture *C. tropicalis* TISTR 5922 in 300 mL of YPD broth. To obtain the logarithmic phase, the culture conditions were processed at 30°C, 110 rpm and 12 hrs. Cells were harvested by centrifugation at 9,000×*g* for 10 mins at 4° C. The supernatant was decanted and the cell paste was suspended in 35 mL distilled water. This process was repeated twice in order to collect the cells for subsequent mixing with protective agents (Maneesri and Masniyom, 2012).

2.2 Protective agents

Three types of milk (i.e., milk powder, whey powder and skim milk powder) with three concentrations (5, 10 and 15%) were mixed with 15% glucose as a protective agent and pasteurized at 80°C for 1 min. 15% glucose was used as a control. Protective agents were filled to cell paste in a centrifuge tube of 35 mL and then mixed together with vortex.

2.3 Carrier supports

Four types of flour (rice flour, corn flour, cassava flour and wheat flour) were used as carrier supports. They were purchased from a local supplier at the local market. All carrier supports were sterilized at 121°C for 15 mins. The cell solution was obtained from the cell paste and the protective agent (5 mL) was transferred to 5 g sterilized flour in a petri dish and mixed homogeneously.

2.4 Stability agents

Three stability agents i.e., gelatin, Arabic gum and maltodextrin at 1, 2 and 3% were mixed with the protective agent and pasteurized at 80°C for 1 min.

2.5 Starter powder properties

The viability of microorganisms before and after air drying was determined by 10 - fold serial dilutions and plate assays on YPD agar. Plates were incubated at 30°C for 24 hrs. Colonies with 30 - 300 per plate were selected for counting, and subsequently, the mean values of the three plates were reported.

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

2.6 Statistical analysis

The data were analyzed based on the ANOVA statistical analysis 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.

3. Results

3.1 Effects of protective agent and concentration on cell viability of Candida tropicalis TISTR 5922 starter powder

In this study, *C. tropicalis* TISTR 5922 was coated with 15% glucose with a dairy protective agent including milk powder, whey powder and skim milk powder at a concentration rate of 5, 10 and 15 (w/v). 15% glucose was a controlled sample. The results showed 15% glucose with all dairy protective agents had higher cell viability than the control. The cell viability range was between 7.79 - 8.92 log CFU/g (Figure 1). The 15% glucose with all concentrations of sweetened whey powder (5, 10 and 15) was found to possess fewer cells. In addition, 15% glucose with 15% dairy protective agent showed the effect on cell survival was as low as 7.79 - 7.98 log CFU/g. The 15% glucose used with dairy protective agent concentrations of 5 and 10 were similar in cell viability efficacy within the range of 8.09 - 8.92 log CFU/g (Figure 1). The best results showed 15% glucose with 5 and 10% skim milk powder (8.92 and

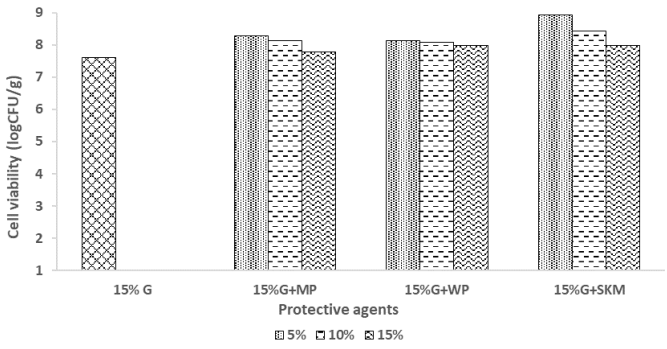

Figure 1. Cell viability of *C. tropicalis* TISTR 5922 with 15% glucose cell protector (15% G control), 15% glucose with milk powder (15% G+MP), whey powder (15% G+WP) and skim milk powder (15% G+SKM) at 5, 10 and 15% concentrations, drying at 40°C for 16 hrs.

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Table 1. Moisture content and water activity of *C. tropicalis* TISTR 5922 starter powder with different protective agents and concentrations, drying at 40°C for 16 hrs.

Protective agents	Concentration (%)	Moisture content (%)	Water activity
15% G (control)		6.61±0.60^{bc}	0.38±0.03^{b}
15%G + MP	5	6.64±0.52^{c}	0.42±0.07^{ab}
	10	6.48±0.68^{bc}	0.40±0.03^{ab}
	15	6.40±0.45^{c}	0.40±0.02^{ab}
15%G + WP	5	7.17±0.95^{ab}	0.48±0.07^{ab}
	10	7.34±0.70^{a}	0.48±0.07^{a}
	15	7.10±0.97^{ab}	0.45±0.05^{ab}
15%G + SKM	5	6.30±0.50^{c}	0.38±0.02^{b}
	10	6.58±0.32^{bc}	0.38±0.01^{b}
	15	6.31±0.26^{c}	0.37±0.01^{b}

Values are presented as mean±SD of triplicates. Values with different superscripts within the same column are statistically significantly different (P<0.05). 15%G: 15% glucose, MP: milk powder, WP: whey powder, SKM: skim milk powder.

8.45 log CFU/g, respectively).

The moisture content of starter powder after drying using 15% glucose with the dairy protective agent ranged between 6.30±0.50 - 7.34±0.70% (Table 1). The 15% glucose with all concentrations of sweetened whey powder was higher in terms of moisture than in the other experiments. However, the starter powder after drying using 15% glucose with all concentrations of skim milk powder had the lowest moisture content (range of 6.30±0.50 - 7.58±0.32). The water activity is associated with the moisture content in the same direction. The water activity was ranged from 0.37±0.01 - 0.48±0.07. The effect of water activity, with 15% glucose in all concentrations of skim milk powder was lowest 0.37±0.01 - 0.38±0.02. From the results of the experiment, 15% glucose with 10% skim milk powder was selected for further studies or investigations.

3.2 Effect of carrier supports on cell viability of Candida tropicalis TISTR 5922 starter powder

From the experiment, four types of carrier supports were used namely, rice flour, corn flour, wheat flour and cassava flour. Cell solution coated with 15% glucose and 10% skim milk powder) and 5 mL per 5 g of carrier supports was stirred and mixed together. It was dried by hot air - drying method at 40°C for 16 hrs. Cell viability was not significantly different in the range of 8.12 - 8.29 log CFU/g. The moisture content of starter culture with rice flour, corn flour and cassava flour was found to be insignificantly different (6.48±0.56%, 6.73±0.25% and 6.80±0.63%) from the water activity (0.42±0.02, 0.43±0.04 and 0.49±0.14), respectively (Table 2). In addition, wheat flour showed the moisture content and water activity of the starter culture were higher, 12.16±3.54% and 0.72±0.10, respectively. From the results of the study, rice flour was selected as the carrier support. Because cell viability was not significantly different, moisture content and water activity are within good dry yeast criteria and this explained why Rice flour is the main ingredient of Ka - nom Tuay - fu.

3.3 Effect of stability agents and concentration on cell viability of Candida tropicalis TISTR 5922 starter powder

In this study, protective agents (15% glucose and 10% skim milk powder) were mixed with stability agents including gelatin, Arabic gum and maltodextrin at concentrations of 1, 2 and 3 (w/v) to improve the drying efficiency. The result showed all stability agents and concentrations had increased in cell viability after drying than the control. Cell viability was ranging between 8.00 - 8.66 log CFU/g, while the control showed a cell viability of 7.88 log CFU/g (Table 3). Gelatin and maltodextrin were similar in the 7.93 - 8.27 log CFU/g. Effects of Arabic gum showed the highest cell viability at all stability agents. In particular, 3% Arabic gum had shown the highest cell viability 8.66 log CFU/g (Figure 2).

Table 2. Cell viability, moisture content and water activity of *C. tropicalis* TISTR 5922 starter powder with different carrier supports.

Carrier supports	Cell viability (log CFU/g)	Moisture content (%)	Water activity
Rice flour	8.27±0.27	6.48±0.56^{b}	0.42±0.02^{b}
Corn flour	8.29±0.08	6.73±0.25^{b}	0.43±0.04^{b}
Wheat flour	8.12±0.17	12.16±3.54^{a}	0.72±0.10^{a}
Cassava flour	8.19±0.02	6.80±0.63^{b}	0.49±0.14^{b}

Values are presented as mean±SD of triplicates. Values with different superscripts within the same column are statistically significantly different (P<0.05).

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Table 3. Moisture content and water activity of *C. tropicalis* TISTR 5922 starter powder with different stability agents and concentration.

Stability agents	Concentration (%)	Moisture content (%)	Water activity
Control		5.30±0.16^{d}	0.33±0.01^{f}
Gelatin	1	6.49±0.71^{bc}	0.40±0.05^{cd}
	2	7.53±0.64^{a}	0.47±0.04^{a}
	3	7.09±0.49^{ab}	0.45±0.02^{ab}
Arabic gum	1	6.12±0.62^{c}	0.36±0.02^{e}
	2	6.10±0.65^{c}	0.36±0.01^{e}
	3	6.65±0.57^{bc}	0.38±0.04^{de}
Maltodextrin	1	7.05±0.51^{ab}	0.42±0.03^{bc}
	2	7.08±0.43^{ab}	0.41±0.03^{cd}
	3	7.16±0.64^{ab}	0.43±0.02^{bc}

Values are presented as mean±SD of triplicates. Values with different superscripts within the same column are statistically significantly different (P<0.05).

The moisture content of stability agents was in the range of 6.10±0.65 - 7.53±0.64%. There were statistically significant differences. The moisture content of Arabic gum was less than gelatin and maltodextrin with water activity in the range of 0.36±0.01 - 0.47±0.04. Table 3 shows all concentrations of Arabic gum had the lowest water activity in the range of 0.36±0.01 - 0.38±0.04. In this experimental study, 3% Arabic gum was selected as a stability agent for further investigations. This was due to the highest cell viability of 8.66 log CFU/g. The moisture content and water activity had been found to be in the range of 6.97±0.72% and 0.38±0.04%, respectively.

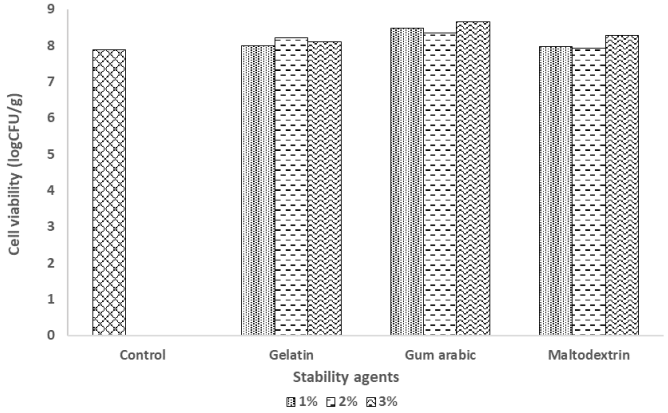

Figure 2. Cell viability of *C. tropicalis* TISTR 5922 starter powder with different stability agents and concentrations.

3.4 Effect of drying condition on cell viability of Candida tropicalis TISTR 5922 starter powder

The optimum temperature for *C. tropicalis* TISTR 5922 starter culture was investigated at 40, 50 and 60°C and the sampling process was carried out every 4 hrs for 24 hrs. The result found out that drying at 40°C was a low - temperature drying situation. The moisture content of the starter culture was slightly decreased and the drying process at 8 hrs. *C. tropicalis* TISTR 5922 was slightly increased from 0 hr, 7.68 log CFU/g to 8.16 log CFU/g. The maximum cell viability was increased at 16 h (8.45 log CFU/g) (Figure 3). While drying condition at 50°C was found to have less cell viability. Starter powder had cell viability during 0 to 24 hrs ranging from 7.77 - 6.55 log CFU/g. Meanwhile, drying conditions at 60°C had no cells at 4 hrs.

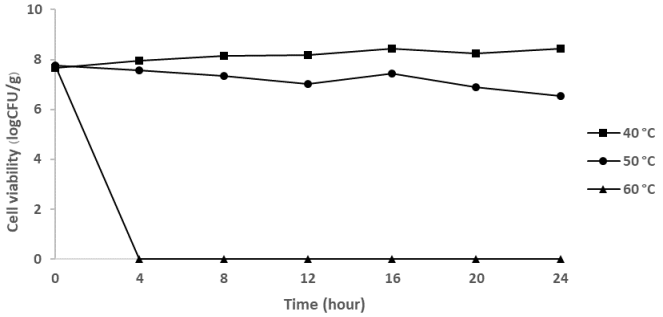

Figure 3. Cell viability of *C. tropicalis* TISTR 5922 starter powder with drying condition at 40, 50 and 60°C.

The effects of drying rate on moisture content and water activity during drying are shown in Figure 4. Initial moisture content was in the range of 45.64±0.64 - 45.87±0.33 with water activity at 0.99. Drying conditions at 40°C the drying rate showed an initial moisture content of 45.64±0.64%, and water activity of 0.99±0.005 that continuously decreased over 12 hrs. The moisture content was entering lower drying at 7.15±0.48% and water activity of 0.40±0.04. While drying at 50°C, the moisture content and water activity had decreased rapidly at 8 hrs from 45.82±0.61% and 0.99±0.004 to 5.85±0.65% and 0.35±0.10, respectively. Drying at 60°C had caused a faster evaporation process. The moisture content and water activity had decreased rapidly for 4 hrs from 45.87±0.33% and 0.99±0.006 to 3.32±0.32% and 0.20±0.04, respectively. From the results, the temperature of 40°C was found to be the optimum drying temperature for *C. tropicalis* TISTR 5922 starter powder.

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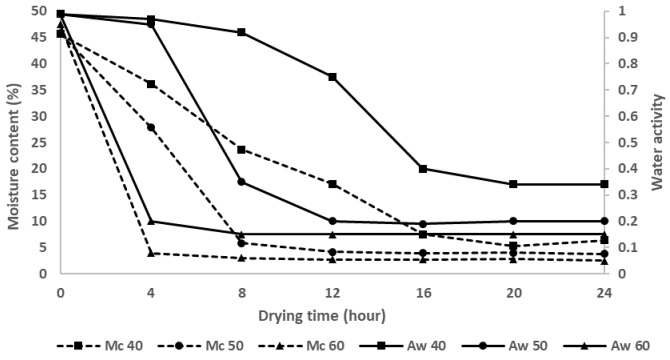

Figure 4. Moisture content and water activity of *C. tropicalis* TISTR 5922 starter powder with drying conditions at 40, 50 and 60°C.

4. Discussion

In the results of protective agents and concentrations, 15% glucose with skim milk powder gave the best results. This was due to the high protein content which was 34 - 37% (milk powder protein and sweet whey powder at 26.3% and 13%, respectively) (Glass and Hedrick, 1976; Chandan, 1997; Patel *et al.*, 2007; Soga *et al.*, 2015). Protein is a key stabilization factor for cell walls during drying (Lapsiri *et al*., 2013), thus reducing cell damage during drying (Chen *et al*., 2015). Concentrations of skim milk powder had effects on the cell survival of the *Lactobacillus brevis* ED25 during freezing and lyophilization (Gul *et al*., 2020). When the level of skim milk powder and sugar was increased, it could increase cell survival during the freeze - drying of *Lactobacillus reuteri* significantly (Schwab *et al*., 2007). In addition, skim milk powder can increase the viscosity of the solution. The formation of a viscous layer on the cell surface by milk proteins helps maintain the structure of cell morphology during drying (Carvalho *et al*., 2004). Skim milk powder contains phosphates and citrates that have buffer capacity and play a role in stabilizing the pH of the solution (Gul *et al*., 2020). According to Basholli - Salihu *et al*. (2014) skim milk powder with different levels of sugar content could reduce the damage during drying process because skim milk powder contains calcium ions (Ca^{2+}) that contribute to the cell wall coating and sugar had the ability to protect cell membranes to be stable during drying (Hubálek, 2003).

Carrier support is very important in generating a stable powdered form and shape for improving the stability and quality of the final product. In this study, rice flour was selected as carrier support because rice flour is one of the ingredients for making Ka - nom Tuay - fu as well as having a good quality starter powder product. While wheat flour has the highest moisture content and water activity. because it contains starch and two types of proteins, glutamine and gliadin. This is called gluten and it can create a strong network for holding gas and moisture. In addition, wheat flour has the ability to absorb water up to 60 - 65% (Rattanapanon, 2010). Moreover, Lee *et al*. (2016) in contrary reported that wheat flour was not suitable for preparing starter products because they led to the formation of lumps after air - blast drying. Fessas and Schiraldi (2001) further described that wheat flour and cell solution happened as a result of the higher water activity and increased viscosity due to starch gelatinization and binding gluten network.

Arabic gum was found to completely prevent the dehydration of cells and stable cells during storage (Liu *et al*., 2017). Furthermore, the concentration of Arabic gum was found to increase survival rate efficiency. Proteins in the Arabic gum coating layer on the cell wall and fibers provide partial replacement of water molecules sites in the cells during drying. Similarly, 1 - 10% Arabic gum was used to dry probiotic micro - encapsulation powder by spray drying method. High Arabic gum concentration was found to increase the cell viability of *Lactobacillus acidophilus* (NCDC 016) (Arepally and Goswami, 2019) and also increase the thermal resistance during spray drying of *Lactobacillus paracasei* NFBC 338 (De Medeiros *et al*., 2014).

Temperature and time for drying were related to cell viability and water activity. This important parameter determines the stability and shelf life of starter powder (Tsaousi *et al*., 2008). The highest viability of *C. tropicalis* TISTR 5922 starter powder was obtained by drying at 40°C. This caused the water mass to evaporate slowly and the cell could grow higher surviving cell at 50 and 60°C. In addition, a protective agent is one of the factors contributing to cell proliferation. Skim milk powder contains nutrients that can be a source of food for yeast growth. At the temperature of 50 and 60°C, the macromolecular structure deteriorates. This leads to changes in the flow within the cell membrane and physical properties such as lipid peroxidation caused by dehydration of the cell membranes (Fu and Chen, 2011). Drying at 40°C for 16 hrs is suggested as an optimum condition for *C. tropicalis* TISTR 5922 starter powder. These conditions were a cost - effective method. According to Hongpattarakere and Uraipan (2015) *Lactobacillus plantarum* CFI17AN2 was dried by vacuum drying at 37°C for 12 hrs and using SABA powder as a support. The highest survival obtained was at 85.81%.

5. Conclusion

This study showed that the protective agent, carrier support and stability agent employed influence microbial cell viability. A 15% glucose plus 10% skim milk as a

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protective agent had enhanced cell viability of *C. tropicalis* TISTR 5922 starter powder. Rice flour, corn flour, cassava flour and wheat flour showed no difference significant in terms of cell viability. Wheat flour was not suitable for carrier support because it contains the highest moisture content and water activity. Therefore, rice flour was selected since it was one of the ingredients for Ka - nom Tuay - fu production. Because cell viability was 8.0±27.27 log CFU/g and not different to corn flour and cassava flour. 3% Arabic gum used is better in terms of stability agent and improved cell viability. Moreover, drying conditions at 40°C for 16 hrs can decrease cell damage due to thermal and stress cell. The optimal condition of the protective agent (15% glucose plus 10% skim milk), carrier support (rice flour), stability agent (3% Arabic gum) and drying condition (40°C for 16 hrs) were high in cell viability (8.45 log CFU/g), low water activity (0.40±0.04) and moisture content (7.15±0.48%).

Conflict of interest

The authors declare no conflict of interest.

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