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The effects of frozen storage on the Physico-chemical characteristics of bread prepared using yeast isolated from different plant sources

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This study was conducted to determine the effects of frozen storage on the physico-chemical characteristics of bread fermented by yeast isolated from different sources pineapple (moris), papaya (sekaki), bamboo shoot (buluh minyak), palm sap and commercial yeast. The leavening ability of yeast in bread dough after 7 days stored at freezing temperature (-18ºC) was determined. The results showed that frozen storage of bread dough at -18C decreased the specific volume for all samples. However, yeast isolated from bamboo shoot produced higher ($p<0.05$) specific volume of bread than the control. On the other hand, the percentage of weight loss of bread showed an increasing trend after storage. Bread prepared using yeast from palm sap showed highest weight loss compare to commercial baker yeast. Frozen storage causes increased in the percentage of the crust moisture content and produce softer crust texture with the exception of bread prepared using yeast isolated from palm sap. On contrary, the moisture content of bread crumb showed a decreased after freezing and produced harder crumb texture. Bread containing yeast isolated from bamboo shoots had the softest breads crumb after freezing. The result suggested that Yeast from bamboo shoot produce best physico-chemical properties of bread after frozen storage. Thus, bamboo shoot yeast has the potential to be used as leavening agent for bread making.

Keywords: Frozen storage, physico-chemical properties, yeast, bread

INTRODUCTION

Bread is one of the staple foods in the world. Bread is one of the most popular bakery product because of its nutritional, sensory quality and texture (Patel et al., 2005). In Russia, bread gives 30% of calories and protein to their daily intake of food (Samsonov and Petrasov, 1993). In Ghana, bread is the main staple food for the country people (Ellis et al., 1997). Bread is a common food in United States. Currently, there is demand for different types of bread (Wang et al., 2002). Bread are made from different ingredient but the basic ingredient for bread are wheat flour, water, sugar, salt and yeast leavening agent. Bread can help to balance our diet because it contains starch and complex carbohydrate (Altamirano-Fortoul and Rosell, 2010). Bread is also being fortified to increase the nutrient content such as vitamin and mineral to meet the market needs.

Conventional baking technology produced bread with shorter shelf life at where the bread lost freshness, bread staling and organoleptic changes (Bárcenas et al., 2003). Hence, technologies were invented to prolong the bread shelf life. Frozen dough storage is one of the technologies which are able to produce almost the same quality with the bread baked by conventional method (Bárcenas and Rosell, 2007). This technology is able to produce fresh bread at any time, save labour and utilities cost (Indrani et al., 2002). Frozen dough is used widely in the bakery industry as it permits the baker to reduce night work and reduce logistic constraints. In the past, the in-house bake-off store needed to start from fresh raw dough in order to produce fresh bread in the store which demanded a lot of time and energy. Freezing technology is increasingly being implemented for the preservation of the dough and has been an important for the bakery industry as it is easier for the companies to produce “freshly baked” bread in stores, restaurants and others. Moreover, using frozen dough facilitates the centralization of dough production and extends the distribution area. According to Yu Sasano (2012), frozen dough baking technology has recently been used because it improves the labor conditions in the bakery industry and enables consumers to purchase fresh bread. Freezing of the dough offers main advantage which is reduction of losses caused by aging of products and harmonization of production with market demands. It is also important to ensure the frozen dough is stored in the suitable temperature to prolong the shelf life and to get the better freshness of the finished bakery products.

Bread quality baked from frozen dough is very much depending on the ingredient used especially yeast. Yeast, a leavening agent, produces carbon dioxide which contributes to the texture and loaf volume. Yeast strain, yeast cell activity, carbohydrate content and number of yeast cell would affect the carbon dioxide formation (Teunissen et al., 2002). Production cost for yeast is depending on the source for yeast to ferment. Yeast cost will be reduced if the cost of raw material is cheap and easy to find (Ejiofor et al., 1996). Malaysia which is rich with tropical fruits can be yeast source beside apple and grape. No work has been reported to look at the potential of indigenous *S. cerevisiae* as leavening agent bread making from local sources (Noroul Asyikeen et al., 2013). According to Noroul Asyikeen et al., (2013), yeasts that currently used by bakery industries in Malaysian are mostly imported from foreign countries such as Australia (Mauripan®), France (Saf-instant®), Canada (Fermipan®) and Turkey (Gold Pakmaya®). Thus, yeast from local fruits source were isolated to study the potential of those yeast to be used as leavening agent.

The objective of this study was to determine the effect of frozen storage towards the physicochemical properties of dough and white bread using yeast isolated from different source. The leavening and fermentation ability of yeast was also studied.

MATERIALS AND METHODS

Preparation of raw material

Baker yeast, commercial yeast was purchased from CS Brothers Sdn. Bhd. (Selangor, Malaysia). Pure yeast was isolated from pineapple Moris, bamboo shoot, buluh minyak, papaya Sekaki and palm sap which were obtained from previous study (Abdul Ghani et al., 2011). Glucose was obtained from Sigma Chemical Company, yeast peptone dextrose agar (YPD agar, peptone, yeast extract and agar media were obtained from Oxoid Ltd. High protein wheat flour, sugar, salt were purchased from bakery shop in Kajang.

Media preparation

Yeast peptone dextrose (YPD) agar

YPD agar powder (65g) was mixed with 1L distilled water. The solution was heated with hot plate and stirred with magnetic stirrer for 5 minutes. Next, the solution was autoclaved at 121℃ for 15 minutes. Sterile agar was poured into the petri dish and let cold under the UV lamina flow.

Yeast peptone dextrose (YPD) broth

Yeast extract (10 g), peptone (20 g), and glucose (20 g) were mixed with 1L distilled water. The solution was heated and stirred with magnetic stirrer for 5 minutes. The solution was poured into the universal bottle and autoclaved at 121°C for 15 minutes. Broth was stored in cold room for further use.

Single colony preparation

Pure yeast was cultured in petri dish. YPD agar was inoculated with 1 loop of pure yeast culture from agar with pure yeast culture. A single colony was obtained by using 4-way streak.

Growth profile of yeast

Growth profile of yeast was used to determine log phase of the yeast. The yeast in the log phase which was the most active would be used for yeast pellet preparation. Yeast pellet was used for bread making. A pure colony was inoculated into 10ml YPD broth and vortexed. First broth containing pure yeast was pipetted (1ml) and diluted with sterile distilled water (9 ml). Serial dilution was made up to 10^{-6} for first day at 0 hour and every 6 hours. From 36-hours onward, the serial dilution from 10^{-1} to 10^{-6} was done every 24 hours. On the 24-hours to 192-hours, the second YPD broth was used. The colony on YPD agar was enumerated after 48 hours of incubation. Colony forming unit per ml (N) was calculated with the following formula:

$N= C/v (n_1+0.1n_2) d$

Where C = Total colony in the petri dish,
v = Volume of sample
(0.1ml), d = Dilution factor,

n_1 = Total of petri dish at the first dilution and n_2 = Total of petri dish at the second dilution.

Yeast pellet preparation

From the growth profile, yeast from log phase was obtained. Yeast pellet preparation was based on Gül et al. (2005) method with modification. Broth containing yeast pellet was centrifuged at 10 000 rpm for 10 minutes at 4ºC. The pellet of yeast cells was washed with cold sterile distilled water after which resuspended in 10 mL sterile distilled water.

Dough and bread preparation

Preparation of dough and bread was modified from Yi and Kerr (2009) method. Dough containing high protein flour (100%), salt (2%), water (60%) and sugar (5%) was prepared and inoculated with yeast isolate (2.2×10^7 cfu mL^{-1}). Bakers' yeast (pure culture of *S. cerevisiae*) was used as positive control to ferment the dough. The dough was divided into small pieces and kept under -18±1°C for 7 days. After 7 days of storage, the dough was defrosted at 8°C for 180 minutes. Dough was fermented at 36°C for 90 minutes with relative humidity at 85%. After that, dough was baked for 15 minutes at 180°C. The bread was left to cool for 1 hour before further analysis. Preparation of bread without freezing was the same with preparation of freeze bread except after dough mixing, the dough was fermented and baked without freezing.

Physico-chemical analysis

Specific volume:
The bread volume was determined by the modification of AACC method 10-05, mustard seed displacement method. Specific volume of the bread was determined by dividing the bread volume.

Weight loss:
The weight loss of dough and bread (%) was determined by getting the difference of dough weight with bread loaf weight.

Moisture content:
Moisture content of bread crust and crumb was determined using AACC standard method 44-15A and preparation of sample was based on Mandala et al. (2007) method. Bread crust and crumb was cut and ground into fine powder. Bread powder (5.0 g) was put into a dried nickel plate. The sample was dried overnight for 24 hours at 105°C. The dried sample was cooled in a desiccator containing silica gel before weighing.

Texture Analysis:
The texture profile analysis was carried out to evaluate the crust and crumb texture using a texture analyzer (Shidmazu Twin-Column Texture Analyzer). Puncture test was used to evaluate the crust texture whereas the compression test was used to evaluate crumb texture. For puncture test which based on Altamrino-Fortoul and Rosell (2010) method, sample was placed in the middle of the Texture Analyzer. The probe head (diameter 4mm) was adjusted to reach the sample surface and poked into the sample for 10mm at the speed of 40 mm/min. The second and third poking was done 1cm away from the first poke. The maximum force in Newton unit (N) was recorded. The compression test was carried out based on Giannou and Tzia (2007) method. Sample was placed in the center of the texture analyzer. The probe head (diameter 45mm) was adjusted to the sample surface and was compressed for 15 mm at the speed of 3 mm/s. The maximum force used for compression was recorded in Newton (N).

Colour Analysis:
The colour of the crust and crumb were determined by using colorimeter (Minolta Colorimeter Model CR 200). L*(lightness), a*(redness) and b*(yellowness) was used to determine the color of the bread. Image analysis

was carried out to evaluate the air hole of the bread. Digital camera (Lumix DMC-FH3) was used to capture the image of bread air hole and cell size of bread. A scale was set to compare and the image was taken at well illuminated site to capture consistent image.

Statistical analyses

Data was analysed using SAS software. Analysis of Variance (ANOVA) and Duncan Multiple Range test (DMRT) was used to compare the significance difference among the sample and treatment at significance level of p<0.05.

RESULTS AND DISCUSSION

Growth profile of isolated yeast

Growth profile of yeast was constructed to determine the log phase of the yeast. Log phase is the most active phase for yeast during the growth phase. The growth phase starts from lag phase, log phase, stationary phase and lastly death phase. According to Ejiofor et al., (1996), growth rate should be controlled in order to obtained yeast which can give favorable quality towards the bread. Thus, at the end of the log phase and near beginning of stationary phase, yeast pellet was extracted as active division of cell was still occurring. Based on Figure 1, five types of the yeast showed similar growth pattern with different CFU/ml. Yeast isolated from bamboo shoot, palm sap and pineapple had higher CFU/ml then the commercial yeast. Yeast from bamboo shoot had the highest CFU/ml, while yeast from papaya had the lowest CFU/ml and showed the earliest (at the fourth hour) to reach log phase. The log phase that started early means that the yeast quickly adapts to the growth state.

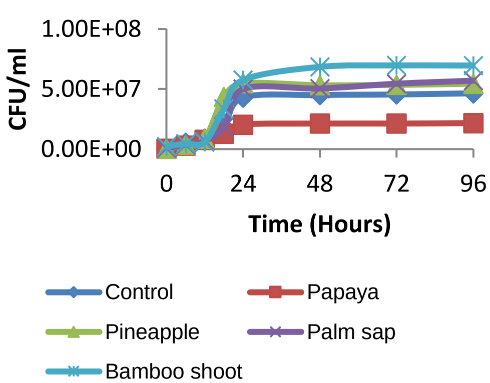

Figure 1; Growth profile for different yeast isolated from different local sources

In the context of baking, the growth rate that is faster in the log phase is a good characteristic for the yeast, as it reflex to the quantity of yeast produce in this phase is higher and the possibility of more fermentation activity occurs to leaven the dough is better compare to other yeast. It can be concluded that the most active phase that yeast can growth occur around 18 – 24 hours. Therefore the cultivation of starter culture for bread dough preparation was between 18 10 24 hours of incubation. Besides that according to Charoenchai et al., (1998) the temperature, pH and concentration of carbohydrate also would affect the growth rate of yeast.

Physico-Chemical Analysis

Specific volume

Bread specific volume is the most important physical characteristics for bread as it affect the texture of the bread. Bread with higher specific volume will have a softer texture. The properties of bread dough fermented with five different yeast isolated from various plant sources are showed in Figure 2. All data showed the specific volume of bread baked from dough without freezing was higher than the specific volume of bread baked from stored frozen dough (7 days).

Among this samples, the specific volume of bread dough fermented by yeast isolated from bamboo shoot showed highest volume for both before and after freezing with the significant different p<0.05. Bread baked using yeast isolated from bamboo shoot also had larger diameter and size. Yeast from papaya produced lowest specific bread volume in both bread baked from fresh dough and stored frozen dough. According to Havet et al., (2000), freezing would reduce the bread volume at least 20% and also causes the yeast to die as much as 9%.

Yeast from bamboo shoot was the most resistant to freezing temperature as the reduction of specific volume of bread was only 4.24%. However, yeast from papaya had the highest reduction of specific volume of bread which was 14.61% as yeast from papaya was not resistant to freezing temperature. Yeast was unable to produce sufficient carbon dioxide to give rise to the bread volume under temperature stress. Thus, the specific volume of bread baked from frozen dough decreased.

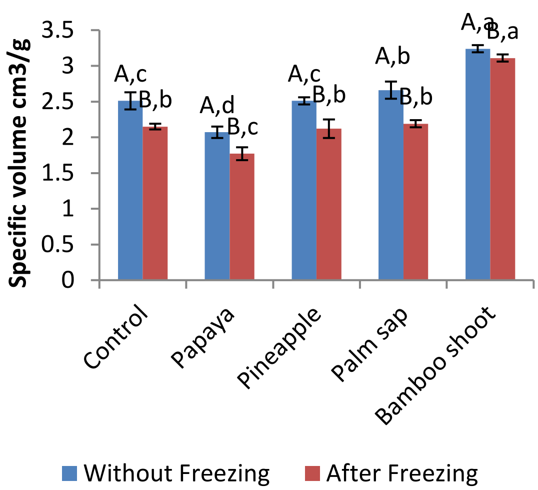

Figure 2; Mean value for specific volume (ml/g) for non-frozen and frozen bread (n=2).

A-D* Mean values with the same letter (upper case) are not significantly different (p<0.05) for dough baked with or without freezing process.

a-b* Mean values with the same letter (lower case) are not significantly different (p<0.05) between the bread baked from non-frozen dough and frozen dough.

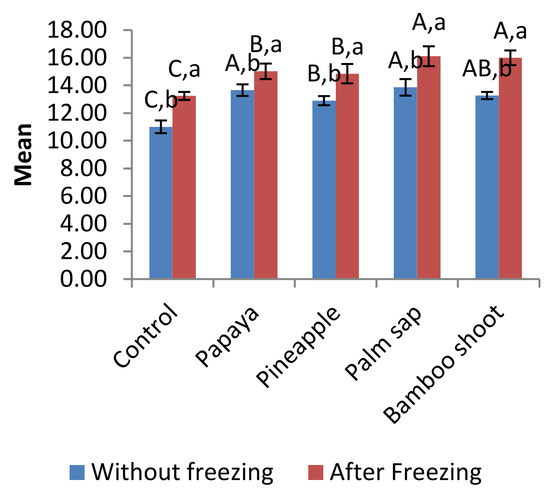

Figure 3; Mean value of weight loss of bread (%) with or without (n=2).

A-C* Mean values with the same letter (upper case) are not significantly different (p<0.05) for dough baked after freezing and without freezing.

a-b* Mean values with the same letter (lower case) are not significantly different (p<0.05) between the bread baked from non-frozen dough and frozen dough.

Weight loss of bread crust and crumb

Weight loss of dough during baking is correlated with the loss of moisture content. In the figure 3, it shows that the weight loss increased for the bread baked after frozen storage. Control bread baked from dough without freezing had the lowest weight loss, 11.01±0.46%, compared to 4 others yeast used in bread making. Weight loss for the control bread baked after frozen storage was 13.24±0.29%. The highest weight loss of bread without freezing and frozen dough was bread baked using yeast from palm sap. The weight loss was 16.11±0.72% for bread baked from frozen dough using yeast (palm sap). Freezing temperature caused cell damage in yeast and denatured the enzyme from yeast (Serra et al., 2005). Thus, frozen dough had less viable yeast to form a good gluten network to hold water and caused the loss of moisture in the bread and weight loss of the bread.

Weight loss of dough was in the range 12-15% (Schnitzer, 2004) and 10-13% (Lai and Lin, 2006). Ice crystal was formed during freezing. Small ice crystal would combine to form large ice crystal which would destroy the gluten network in the dough (Xu et al., 2009).

Once the gluten network was interrupted, the originally bound water would be drawn out from the dough during defrosting and caused the reduction of weight. Fermentation rate of bread baked using yeast from bamboo shoot was high. High concentration of carbon dioxide was trapped in the dough before baking contributing weight to the bread. Beside bread made from palm sap yeast, the weight loss of bread made from bamboo shoot yeast was as high as 16±0.53% because carbon dioxide was eliminated during baking. During baking, the air cell, which carbon dioxide tight to, expanded and transformed the dough into elastic bread. The transformation caused weight loss in bread (Keetels et al., 1996).

Moisture content of bread crust and crumb

Moisture content of bread crust affects the bread texture. When the dough is baked, the water from the surface of the dough will evaporate causing the water content on the crust become lower compare to the crumb. The water distribution between the crust and crumb contributes to the organoleptic perception to the final product (Vanin et al., 2009). The crispy texture on bread crust is due to low moisture content and water activity (Stokes and Donald, 2000).

In Figure 4, it shows that moisture content of bread crust for bread baked without freezing using control yeast had no significance difference with dough using palm sap yeast. The mean moisture content of control bread was 18.19±0.27%. Bread baked from bamboo shoot yeast had the highest moisture content which was 20.16±0.59%. For stored frozen dough, the moisture content for control bread was 20.26±0.42%, which was also the lowest moisture content among other treatment. Bread with bamboo shoot yeast had the highest moisture content which was 22.05±0.85%. Bamboo shoot yeast was active yeast as it had the highest growth rate in the growth profile of yeast compared to other yeast. Thus, more water was entrapped in the dough for yeast metabolism for both non-frozen and frozen dough. Papaya yeast and control yeast were less active than bamboo shoot yeast, therefore the need for water was lesser for metabolism. Low moisture content in the crust was not favorable in the bread because the temperature of bread crust could increase above 100̊C which could induce Maillard reaction to form toxic component that was harmful to human health (Ahrne et al*.,* 2007). As a result, bamboo shoot yeast had the potential to be developed as commercial yeast to be used in bread for safer and healthier bread. There was a significance difference in moisture content of crust for bread baked using papaya yeast, bamboo shoot yeast and control yeast after frozen storage. There was a significance difference between bread baked from frozen dough and non-frozen dough. The moisture content (bread crust) of breads baked from frozen dough was higher than non-frozen dough which could be observed from figure 2.

According to Mandala et al., (2007), the moisture content changes were the highest in frozen dough. Freezing would cause the transfer of moisture in crumb to crust and to the surrounding. Moisture transfer occurred according to concentration gradient at which it transfers from higher moisture content to lower moisture content. Since the moisture in the crust was transferred to surrounding during frozen storage, the crumb which had higher moisture content would transfer the moisture to the crust. Thus, the crust would have higher moisture content after freezing.

The moisture content of bread plays an important role in the texture of bread, especially in terms of its softness. The optimum moisture content of bread will produce soft and longer shelf life.

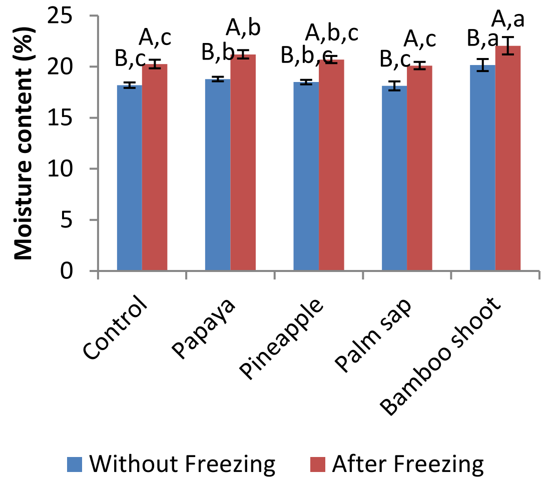

Figure 4; Mean value for bread crust moisture content (%) for non-frozen and frozen bread (n=2).

A-D* Mean values with the same letter (upper case) are not significantly different ($p<0.05$) for dough baked after freezing and without freezing.

a-b* Mean values with the same letter (lower case) are not significantly different ($p<0.05$) between the bread baked from non-frozen dough and frozen dough.

Higher moisture content will support the growth of microorganisms thus lowering the shelf life of the bread. Moisture content in bread crumb is also important in affecting the bread texture. According to figure 5, bread baked used bamboo shoot without freezing had the highest moisture content and showed a significant different with other bread crust samples with 41.6±0.49%. In contrast, bread baked using pineapple yeast had the lowest moisture content which was 38.96±0.22%.

For bread baked after freezing, moisture content in the control bread crust was 38.96±0.58% which was the lowest moisture content. The trend was similar for bread baked after frozen storage with bread baked from fresh dough. Overall the moisture content of bread crust and crumb fermented by bamboo shoot yeast had highest moisture content compare to the bread samples. Besides that, by products during fermentation of yeast may strengthen the gluten network and made it more capable to retain moisture content. During fermentation of yeast, yeast would produce enzyme, beta-glucans, some unknown soluble protein, amino acid containing sulfur under stress. The compound produced depending on the genera, species and strain of the yeast (Querol and Fleet, 2006).

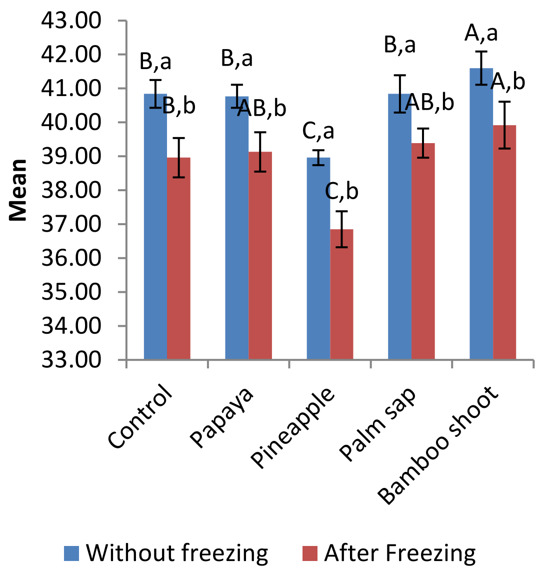

Figure 5; Mean value for bread crumb moisture content (%) for non-frozen and frozen bread (n=2).
A-C* Mean values with the same letter (upper case) are not significantly different ($p<0.05$) for dough baked after freezing and without freezing.
a-b* Mean values with the same letter (lower case) are not significantly different ($p<0.05$) between the bread baked from non-frozen dough and frozen dough.

High moisture content was needed in bakery products especially bread because it would delay the staling process in bread (Oreopoulou, 2006). Ideal and high moisture content could increase bread specific volume (Gallagher et al., 2003). Moisture content (bread crumb) for all bread baked after frozen storage had lower moisture content compared to bread baked from dough without freezing. This was due to the migration of water from crumb to the crust. Though that, the moisture content in bread crumb was higher than bread crust because bread crust hardening set a barrier for water to escape from bread crumb. Thus, the loss of moisture content in bread crumb was lower in bread crust.

Texture of bread

Puncture test was used to evaluate the texture of the bread crust. The puncture test correlated the failure force (newton) obtained from probing with the force during first bite to break some substances (Altamirano-Fortoul and Rosell, 2010). Table 1 showed that the minimum force used to probe the bread crust baked from different yeast under frozen and non-frozen storage. For the bread baked from frozen dough, pineapple yeast had the hardest bread crust, at which the force used to probe was 3.8±0.12N. Bamboo shoot yeast had the softest bread crust which only 1.88±0.07 N force was used to probe. There was a significance difference for the penetration force applied to bread for all yeast except palm sap yeast and bamboo yeast. For bread baked from frozen dough, bamboo shoot yeast also produced the softest bread crust which the force used was 1.34±0.11 N. This could correlate with the moisture content in the bread crust (bread baked from bamboo shoot). The moisture content in bread crust (bread baked from bamboo shoot) for both frozen and non-frozen storage was the highest. Moisture content would affect the texture of bread crust. The higher moisture content of bread crust, the texture of bread crust would be softer. Yeast from palm sap produced hardest bread which needed 3.11±0.38 N to probe the bread crust.

Table 1; Mean value for crust and crumb texture (N) for non-frozen and frozen bread (n=2).

Texture	Fresh (N)	Frozen (N)
Crust		
Control	$2.75±0.13^{C,a}$	$2.17±0.12^{C,b}$
Papaya	$3.40±0.25^{B,a}$	$2.54±0.20^{B,b}$
Pineapple	$3.80±0.12^{A,a}$	$2.37±0.24^{BC,b}$
Palm sap	$2.08±0.15^{D,b}$	$3.11±0.38^{A,a}$
Bamboo shoot	$1.88±0.07^{D,a}$	$1.34±0.11^{D,b}$
Crumb		
Control	$7.45±0.11^{A,b}$	$8.36±0.36^{B,a}$
Papaya	$7.21±0.21^{A,b}$	$9.02±0.84^{AB,a}$
Pineapple	$7.58±0.27^{A,b}$	$9.22±0.49^{A,a}$
Palm sap	$5.51±0.32^{B,b}$	$9.28±0.24^{A,a}$
Bamboo shoot	$3.68±0.35^{C,b}$	$4.52±0.21^{C,a}$

A-D* Mean values with the same letter (upper case) are not significantly different ($p<0.05$) for dough baked after freezing and without freezing.
a-b* Mean values with the same letter (lower case) are not significantly different ($p<0.05$) between the bread baked from non-frozen dough and frozen dough.

According to Altamirano-Fortoul and Rosell (2010), bread with thicker and harder crust would need higher force. Visual inspection on bread crust (bamboo shoot yeast) showed that the crust was thinner than bread crust (palm sap yeast). Thus, the bread crust (bamboo yeast) was softer and lower force was needed to penetrate.

There was significance difference between bread baked from frozen dough and non-frozen dough for different yeast used. Redistribution of moisture during frozen storage was also an important factor that affects the bread crust texture (Mandala et al., 2007). Redistribution of moisture content in the bread system would change the gluten network and might affect the bread crust texture. The force needed to penetrate into bread crust for bread baked from frozen dough was lower than the bread baked from fresh dough for all yeast except palm sap yeast. Moisture content of bread baked from frozen dough was higher than bread baked from non-frozen dough which correlated to the texture of bread crust. The migration of water from bread crumb to bread crust during freezing caused the bread crust contain higher moisture content after freezing. Palm sap yeast made a harder bread crust after freezing because it could not tolerate the freezing temperature.

In the other hand, compression test is used to evaluate the softness of bread. This test mimics how consumers choose bread by pressing the bread to determine the freshness and hardness of the bread. Hardness of the bread is the physical quality of bread as it gives the perception of freshness of the bread (Faridi and Faubion, 1990). Results from table 1 indicated that for control bread treated without freezing, had maximum force as 7.45±0.11 N. There was no significance difference for the hardness of the bread for all yeast except for bamboo shoot yeast and palm sap yeast. Bread baked from bamboo shoot yeast was the softest bread and bread baked from palm sap yeast had intermediate softness. Bamboo shoot yeast might have higher fermentation rate and then produced more carbon dioxide which increase the air hole in the bread and thus reduced the compressibility of bread. For bread without freezing, the bread baked palm sap yeast was a better leavening agent compared to papaya yeast, control yeast and pineapple yeast as the texture was softer. However, after freezing, bread baked from palm sap yeast was the hardest. The maximum force needed was 9.28±0.24 N. Palm sap yeast was unable to tolerate freezing temperature. Fermentation rate decreased due to the cell damage in yeast and less carbon dioxide was produced. The cell hole produced was lesser, smaller and uneven which contributed to the undesirable bread texture. There was a significance difference between the bread baked from frozen dough and fresh dough for all the yeast used. This indicated that bread baked after freezing was harder and need higher force of compression. During the cold storage, crystallization of ice occurred and the crystal size was big. The redistribution of water and ice crystal formation caused changes in gluten network and rearrangement of amylose and amylopectin. The changes occurred during starch gelatinization and retrogradation (Ribotta et al., 2003). Retrogradation and loss of moisture content was the mechanism that affected the hardness of bread crumb because starch is the main component in bread (Selumulyo and Zhou, 2007). However, in Martin et al., (1991) study, main factor of bread crumb hardness was due to the formation of hydrogen bonding between gluten and starch granule. The reduction of moisture content would encourage the formation of hydrogen bond with starch polymer or starch with protein which increased the hardness of the bread (Schiraldi and Fessas, 2001). Since the moisture content of bread decreased after freezing, the hardness of bread increased as stated in previous mentioned studies.

Colour of bread crust and crumb

Color of bread crust is an indicator of bread quality (Yi and Kerr, 2009). According to Giannou and Tzia (2007) bread crust should be golden brown. In table 2, L* value for control bread baked from fresh dough was 63.99±0.61 and showed no significant different for bread baked after freezing. The similar trend showed by bread fermented by pineapple and palm sap yeast. In contrast, the bread baked using papaya and bamboo shoot showed significant different for the L* value for frozen and non-frozen, which showed darker in colour after freezing.

According to Farvili et al., 1997, he categorized degree of brightness for the bread into three categories which were 50 was dark colored, 60 was optimum colored and 70 was bright colored. The L* values for the bread baked from different yeast for frozen and non-frozen dough were in the range of 60-70 except bread baked from pineapple yeast. Color of bread crust was affected by the activity of yeast during baking (Boboye and Dayo-Owoyemi, 2009). Metabolites from different yeast were different, thus giving different brightness towards the bread crust. Yeast helped in the formation of Maillard precursor which later on would turn into volatile component or give color to the bread crust (Zehentbauer and Grosch, 1998). Therefore, different yeast could provide different Maillard precursor with different quantity and thus gave different bread crust color. In table 2, it showed that the brightness of bread crust increased for bread baked from frozen dough. There was significance difference for brightness of bread crust between the bread baked from dough without freezing and frozen dough. Giannou and Tzia (2007) had stated that frozen storage of dough would increase the brightness of bread because of white spot formation on the bread crust. The a* values and b* values were shown in Table 2. Positive a* value indicates degree of redness and negative a* value indicates degree of greenness. The a* value for control bread baked from non-frozen dough was 11.19±0.59. Other breads using different yeast had lower a* value than the control bread. However, a* value for breads baked after frozen storage had higher a* value than control bread as the a* value for control bread was -0.56±0.08. After freezing, the redness decreased might be due to the formation of white spot on the crust. Positive b* value indicates the degree of yellowness and negative b* indicates the degree of blueness. Only yellowness was observed in all samples for bread baked from non-frozen dough and frozen dough. Yellowness of bread decreased for bread baked from frozen dough.

Besides bread crust color, bread crumb color is the key quality factor of bread (Cauvain and Young, 2006). Bread crumb should be creamy white and the color is attracting and even (Giannou and Tzia, 2007). Table 2 shown that most of the bread sample using different yeast gave even white colour of bread crumb. For bread baked from non-frozen dough, L* values for five types of dough were from 71.34 to 74.35. Cell structure would affect the brightness of bread crumb because of light diffraction from bread cell. Air hole size, quantity and distance might affect the degree of lightness of bread crumb. Different yeast formed different air hole size, quantity and distance in bread. Thus, different degree of lightness was observed. Bread baked from pineapple yeast formed smaller and evenly distributed air hole. L* value for the bread was 74.24±0.70. After freezing, the L* value decreased significantly at which the value differed from 66.56 to 68.93.

Table 2;Mean value for crust and crumb colour (N) for dough baked after freezing and without freezing (n=2).

FRESH			
Colour	**L***	**a***	**b***
Crust			
Control	63.99±0.61^{B,b}	11.19±0.59^{A,a}	22.28±0.62^{A,a}
Papaya	63.70±1.72^{B,b}	11.13±1.10^{A,a}	20.77±0.73^{B,a}
Pineapple	71.92±0.75^{A,b}	9.04±0.59^{B,a}	20.64±0.66^{B,a}
Palm sap	63.32±0.74^{BC,b}	9.85±0.60^{B,a}	19.94±0.52^{BC,a}
Bamboo shoot	62.02±0.47^{C,b}	7.52±0.46^{C,a}	19.51±0.51^{C,a}
Crumb			
Control	72.76±0.58^{B,a}	-1.80±0.03^{D,b}	7.50±0.36^{A,b}
Papaya	73.31±0.27^{AB,a}	-1.33±0.05^{BC,b}	10.79±0.75^{C,b}
Pineapple	74.24±0.70^{A,a}	-1.40±0.14^{C,b}	10.28±0.35^{A,b}
Palm sap	74.35±0.84^{A,a}	-1.22±0.09^{B,a}	10.25±0.53^{A,a}
Bamboo shoot	71.34±0.78^{C,a}	-0.62±0.06^{A,b}	9.47±0.15^{B,b}
FROZEN			
Colour	**L***	**a***	**b***
Crust			
Control	71.33±0.23^{B,a}	-0.56±0.08^{E,c}	14.63±0.74^{C,b}
Papaya	68.48±0.82^{C,a}	5.36±0.32^{B,b}	17.98±0.54^{A,b}
Pineapple	76.89±0.51^{A,a}	1.10±0.12^{D,b}	17.94±0.75^{A,b}
Palm sap	68.24±0.97^{C,a}	4.36±0.35^{C,b}	15.94±1.04^{B,b}
Bamboo shoot	65.62±1.04^{D,a}	10.48±0.39^{A,a}	16.46±0.46^{B,b}
Crumb			
Control	66.56±1.25^{C,b}	-1.40±0.10^{C,a}	9.31±0.39^{C,a}
Papaya	68.58±1.00^{B,b}	-0.42±0.10^{B,a}	9.23±0.19^{C,b}
Pineapple	71.08±0.65^{A,b}	-0.31±0.07^{AB,a}	11.72±0.22^{A,a}
Palm sap	68.93±0.70^{B,b}	-1.46±0.06^{C,b}	8.37±0.43^{D,a}
Bamboo shoot	68.43±0.79^{B,b}	-0.23±0.04^{A,a}	10.05±0.20^{B,a}

A-E* Mean values with the same letter (upper case) are not significantly different ($p<0.05$) for dough baked after freezing and without freezing.
a-c* Mean values with the same letter (lower case) are not significantly different ($p<0.05$) between the dough baked after freezing and without freezing

Giannou and Tzia (2007) reported that the phenomena of decreasing brightness of bread crumb after frozen storage was known as darkening of bread crumb. Darkening of bread crumb occurred might be due to the reduction of moisture content and stimulated the browning reaction. For bread baked from non-frozen dough, a* value for all breads were negative at which only greenness was observed. Control bread showed intensity of greenness compared to other bread. There was significance difference between the control bread and other bread baked from different yeast source. Greenness of bread crumb increased after frozen storage except bread baked from bamboo shoot yeast. Bamboo shoot yeast bread had lowest degree of greenness among the five breads. The a* values differed from -0.23 to -1.46 for frozen storage bread. The b* values of all bread using different yeast and different treatment were positive values ranging from 9.47 to 10.79. For bread baked from non-frozen dough, control bread had the lowest b* value which was 7.50±0.36. There was significance difference for the bread baked from different yeast with control bread. For bread baked after freezing, b* value of bread baked from papaya yeast and palm sap yeast had decreased. The b* value for bread baked using commercial yeast, pineapple yeast and bamboo shoot yeast increased.

CONCLUSION

This study had proved that yeast from different source had different effect towards the physic-chemical properties for frozen storage bread and non-frozen storage bread. Yeast from bamboo shoot produce best physico-chemical properties of bread after frozen storage and have potential characteristic as a leavening agent of bread and relatively better than the commercial baker's yeast.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors involved in the design and performing the experiment as well as analysis of data.

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