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Exploring the Potential of *Nyonya Kuih* Residues as the Substrate for Yeast Cell Protein Production

(Meneroka Potensi Sisa Kuih Nyonya sebagai Substrat Penghasilan Protein Sel Yis)

PEI LING TANG* & WEI HERNG WONG

Department of Bioscience, Faculty of Applied Sciences, Tunku Abdul Rahman University of Management and Technology, 53300 Setapak, Kuala Lumpur, Malaysia

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ABSTRACT

Nyonya kuih, a popular Malaysian traditional cuisine that have short shelf life due to their high moisture and fat contents. The unsold *kuihs* usually ended up as food residue. Thus, *kuih talam* (KT) and *kuih lapis* (KL), two varieties of *Nyonya kuih* were chosen as the investigative substrates to explore the recycling potential of this food residue. Initially, the effect of fat content on the enzymatic hydrolysability of the residues to release fermentable sugar was investigated. The efficiency of lipase-pretreatment (LP), polyvinylpyrrolidone-post-treatment (PVP) and their combination in lipid removal was determined. Then, the potential of the resulting glucose-rich hydrolysates for *Saccharomyces cerevisiae* yeast cell biomass production was assessed, with molasses as the positive control. Results indicated that combined LP and PVP (LP-PVP) significantly (p<0.05) reduced crude fat content, while increasing the total sugar content of KT and KL hydrolysates by approximately 3.4 and 26.3 times, respectively. Yeast cell biomass produced from KT and KL hydrolysates were increased by 23% and 39%, respectively after LP-PVP treatment. Despite the yeast cells' yield (by dry cell weight) from molasses fermentation being 19% and 11% higher than those from KT and KL hydrolysates, respectively, KT and KL hydrolysates were proven superior due to the significantly higher (p<0.05) crude protein content in yeast cell produced from both hydrolysates. Further study is needed to enhance yeast cell yield from the fermentation of KT and KL hydrolysates, thereby improving the overall protein yield from the fermentation process. This study provides new insight into a novel food residue recycle strategy, promoting sustainable food production aligned with SDG 12.

Keywords: Amylase hydrolysis; lipase-pretreatment; *Nyonya kuih*; polyvinylpyrrolidone-post-treatment; yeast cell protein

ABSTRAK

Kuih Nyonya, sejenis hidangan tradisi Malaysia yang terkenal, namun mempunyai jangka simpanan yang pendek kerana kandungan air dan lemaknya yang tinggi. Kebiasaannya, kuih yang tidak terjual dibuang sebagai sisa makanan. Oleh itu, sisa kuih talam (KT) dan kuih lapis (KL) telah dipilih sebagai substrat dalam kajian ini untuk menerokai potensi kitar semula sisa makanan ini. Pada mulanya, kesan kandungan lemak terhadap prestasi hidrolisis berenzim untuk membebaskan gula boleh difermentasikan dikaji. Tambahan, keberkesanan pra-rawatan lipase (LP), pasca-rawatan polivinilpirolidon (PVP) dan gabungannya terhadap kecekapan penyingkiran lemak juga ditentukan. Kemudiannya, potensi kegunaan hidrolisat gula yang terhasil untuk penghasilan biojisim sel yis *Saccharomyces cerevisiae* dinilai, dengan menggunakan molas sebagai kawalan positif. Keputusan kajian menunjukkan bahawa gabungan LP dan PVP mengurangkan kandungan lemak kasar dalam hidrolisat dengan signigikan (p<0.05), sementara kandungan jumlah gula dalam hidrolisat KT dan KL meningkat masing-masing sebanyak 3.4 dan 26.3 kali. Biojisim sel yis yang terhasil daripada hidrolisat KT dan KL masing-masing meningkat sebanyak 23 % dan 39% selepas rawatan gabungan LP-PVP. Walaupun hasil sel yis (berdasarkan berat kering sel) daripada penapaian molas adalah 19% dan 11% lebih tinggi daripada penapaian hidrolisat KT dan KL, hidrolisate KT dan KL masih terbukti lebih unggul disebabkan kandungan protein kasar sel yis yang terhasil adalah lebih tinggi secara signifikan (p<0.05) berbanding dengan yang dihasilkan melalui penapaian molas. Kajian lanjut diperlukan untuk meningkatkan hasil sel yis daripada penapaian hidrolisat KT and KL, seterusnya meningkatkan hasil protein keseluruhan daripada proses penapaian ini. Maka, kajian awal ini dijangka akan memberi pandangan baharu mengenai strategi pengitaran semula sisa makanan yang baharu, seterusnya menggalakkan perkembangan sistem pengeluran makanan mampan yang sejajar dengan Matlamat Pembangunan Mampan 12 (SDG12).

Kata kunci: Hidrolisis amilase; Kuih Nyonya; pasca-rawatan polivinilpirolidon; pra-rawatan lipase; protein sel yis

INTRODUCTION

Nyonya kuih, one of the popular varieties of Chinese *Peranakan* desserts that emerged from the cultural borrowing and innovation through the contact with local ingredients and non-Chinese principles of food preparation. It is innovated by combining the Chinese cooking techniques with Malaysian ingredients and Indonesian spices and flavours, such as tamarind, ginger and lemongrass. In the local market, diverse types of *kuihs* such as *angku kuih*, *kuih dodol*, *kuih bangkit*, *kuih koci*, *kuih seri muka*, *kuih kosui*, *huat kuih, kuih talam*, and *kuih lapis* can be found in both Chinese and Malay food stalls (Ng & Karim 2016) (Supplementary 1). However, these desserts have a short shelf life of approximately 1 to 3 days due to high moisture and fat contents. Consumer demand for freshness restricts the use of chemical preservatives in these perishable products. Furthermore, re-processing of these products is almost impossible due to the abrupt change of texture (Seow et al. 1995). Therefore, discarding the unsold *kuihs* is the common practice among the *Nyonya kuih* manufacturers. This unwise practice not only generates substantial amounts of food residues in the industry, but it also contributes to resource depletion, financial loss and environment pollution (Mulya et al. 2022). Therefore, the recycling strategy of this food residue is utterly needed. In this preliminary study, steamed coconut and pandan layered rice cake (*kuih talam*) and steamed layered rice cake (*kuih lapis*) (Supplementary 1) were selected as the model substrate of investigation. To the best of our knowledge, this is the first exploratory research reported on the recycle potential of *Nyonya kuih* residue.

According to the current Malaysia Food Composition Database (https://myfcd.moh.gov.my/myfcdcurrent/), both *kuih talam* and *kuih lapis* are categorized into the group of rice and rice flour-based product, which are high in starch and sugar. Therefore, carbohydrate was the main targeted component for the conversion process explored in this study. Among the carbohydrate sources, glucose is the most preferred simple sugar by *Saccharomyces cerevisea*. Transporter proteins on the yeast cell membrane have a greater affinity for glucose (Struvf et al. 2017). Hence, the recovery of simple sugar from the *kuih* residue was targeted. Compared to acidolysis, enzymatic hydrolysis is preferred because it is green and safe in nature. Conventional acidolysis not only requires long reaction time, but also low in yield, high energy consumption and generates wastewater which potentially led to environmental pollution (Liang et al. 2024). Amylase, encompassing of endoamylases, exoamylase and debranching amylases, is the commonly utilized enzyme for starch hydrolysis. During the starch liquefaction process, a mixture of oligo-, tri-, di- and monosaccharides were released, subject to the reaction conditions (Mendonca, Reis & Barbosa-Tessmann 2023).

However, previous studies have reported that lipid significantly impact the performance of enzymatic hydrolysis of starch and the growth of *S. cerevisiae* (Barnett & Entian 2005; Crowe, Seligman & Copeland 2000; Parapouli et al. 2020). Hence, it is hypothesized that fat content in the *Nyonya kuih* could impede efficient sugar recovery through enzymatic hydrolysis of starch and fermentability of the produced hydrolysate by *S. cerevisiae*. In addition to carbohydrate, fat content of *Nyonya kuih* is anticipated to be high due to the inclusion of coconut milk in the recipe. Coconut milk is a fat-rich oil-in-water emulsion (with approximately 1.8% fat) extracted from coconut flesh (Zhu et al. 2014). Total fat content of *Nyonya kuih* varies based on the proportion of coconut milk and oil in the individual recipe. Therefore, lipid removal was expected to be a crucial step to maximize the efficiency of the bioconversion process.

Lipid can be extracted using either a chemical approach with solvent (such as hexane, acetone, chloroform, ethyl acetate, ethanol, butanol, and methanol) or an enzymatic approach employing lipases (Saini et al. 2021; Singh & Jana 2023). Up to recently, lipases have diverse applications in detergent, cosmetic, pharmaceutical, food processing, wastewater treatment industries (Chandra, Singh & Arora 2020; Singh & Jana 2023). The effectiveness of lipase pretreatment as an upstream process to enhance enzymatic reaction was reported in the studies by Song et al. (2016) and Yao et al. (2020). Therefore, lipase-pretreatment was investigated in this study.

Nevertheless, Wang, Yang and Kuo (2017) proposed the use of polyvinylpyrrolidone (PVP) – modified cotton as an effective filter for oil removal. The PVP-modified cotton exhibited superhydropholicity and superoleophobicity, making it an extraordinary material for efficiently separating oil residues from both surfactant-free and stabilized oil-in-water emulsions, with excellent antifouling properties for prolonged use. PVP is non-toxic, water-soluble, biocompatible, temperature-resistant, and pH-stable (Teodorescu & Bercea 2015). Given the demonstrated strengths and proven advantages of PVP in fat separation, the impact of post-treating glucose-rich hydrolysates produced from *kuih* residue using PVP-modified cotton was also investigated.

Single cell protein (SCP) is a nutrient dense source of high-quality protein with approximately 50 – 80% of protein content on a dry basis. SCP is obtained from the dried cells of microorganisms. Besides protein, SCP also contains other nutrients such as essential carbohydrates, vitamins, and minerals which originate from the biomass of the microorganisms. SCP can be obtained from different microbial sources such as microalgae, fungi, and bacteria. Among these sources, bacterial SCP is the most welcome due to its fast-growing capability under varying conditions and well-balanced essential amino acids profile (Malav et al. 2017). However, compared to bacteria, yeast cells offer

additional advantages, such as a larger size that facilitates easy cells separation in the downstream process, as well as an abundance of vitamin Bs and nuclei acids (Gervasi et al. 2017). Furthermore, Khan et al. (2022) suggested that producing SCP using food residue, such as banana peel, citrus peel, carrot pomace, and potato peel, is a highly promising approach in waste management. Hence, *S. cerevisiae* was selected as the model microorganism in this study to assess the potential of *Nyonya kuih* residue as a substrate for yeast cell protein production.

In general, this study was conducted to determine the effect of lipase-pretreatment, PVP-post-treatment, and their combination on the crude fat and sugar contents of the hydrolysates produced from *kuih talam* (KT) and *kuih lapis* (KL) via amylase hydrolysis. Then, the effects of crude fat and total sugar contents in the glucose-rich hydrolysates of KT and KL on the production of *S. cerevisiae* cell biomass were determined. Lastly, the yield and total crude protein content of the yeast cell biomass produced from the KT and KL hydrolysates were compared. This pioneer study explored the recycling potential of *Nyonya kuih* residue in the production of alternative microbial protein. This study is expected to provide new insight into a novel strategy in *Nyonya kuih* residue bioconversion.

MATERIALS AND METHODS

MATERIALS

The KT and KL residues were provided by Usaha Maju Kini Sdn. Bhd. (Seri Kembangan, Selangor). The residues were the leftover unsold *kuihs* collected from the restaurants and F & B outlets after 24 h of delivery. Baker's yeast (*Saccharomyces cerevisiae*) used in this study was the commercial instant dry yeast sold under the brand name of Mauripan (AB Mauri Malaysia, Balakong, Selangor, Malaysia). Molasses used as the positive control for yeast fermentation was organic certified with the brand name of MH Food (Matahari Sdn. Bhd., Selangor, Malaysia). All other chemicals used include polyvinylpyrrolidone (average molecular weight of 10 000), sulfuric acid, sodium hydroxide, hydrochloric acid, absolute ethanol, phenol, sodium dihydrogen phosphate, and disodium hydrogen phosphate (Merck, Germany) were analytical grade. Lipase (200 U/g) from *Aspergillus niger* and α-amylase (30 U/mg) from *Aspergillus oryzae* (Sigma, U. S. A.) were used.

SUBSTRATE PREPARATION

Initially, the collected KT and KL residues were separately ground using a kitchen blender (Model PB-3203L, Pensonic, Malaysia) into homogenous paste (Supplementary 2). Next, distilled water was added to the paste at a ratio of 1:1 (by weight), then homogenized using a high-speed homogenizer at a speed of 10 000 rpm for 3 min. The suspension was centrifuged at 8000 × g for 15 min at ambient temperature. Oil layer was observed on the top of the water layer. Then, the supernatant was discarded and distilled water was added (at 1:1 ratio). The homogenization and centrifugation processes were repeated for 3 cycles to remove the excess oil from the KT and KL. The KT and KL obtained were labelled as UKT and UKL, respectively.

The effects of lipase pretreatment (LP) and polyvinylpyrrolidone-post-treatment (PVP) on the enzymatic hydrolysablility of the *kuih* residue and the fermentability of the resulting hydrolysate were investigated through a 2 × 2 full factorial design, using UKT and UKL, respectively. The effects of the presence and absence of each factor were assessed.

LIPASE-PRETREATMENT (LP)

Lipase-pretreatment was carried out according to the method by Omar et al. (2016) with slight modification. Briefly, 20% w/v of the *kuih* paste obtained was suspended in 0.2 M phosphate buffer (pH 7), then 1.5% w/w of lipase was added. The pretreatment was conducted at 45 °C for 1 h in a water bath. Next, the suspension was centrifuged at 8000 × g for 15 min to recover the lipase-pretreated *kuih* paste. The lipase-pretreated KT and KL were labelled as LKT and LKL, respectively.

ENZYME HYDROLYSIS USING AMYLASE ENZYME

To produce glucose-rich hydrolysate, the UKT, UKL, LKT, and LKL at 20% w/v, respectively, were suspended in the 0.2 M phosphate buffer (pH 6), then 1.5% w/w of amylase was added. The hydrolysis was carried out at 55 °C for 1 h in a water bath (Gui et al. 2021). The hydrolysate was recovered through centrifugation (8000 × g, 15 min). All hydrolysates were boiled for 5 min to terminate the enzyme activity. Total crude fat and sugar contents of the hydrolysates were determined according to protocol described in the previous section.

POLYVINYLPYRROLIDONE-POST-TREATMENT (PVP)

To investigate the efficiency of PVP-post-treatment in oil removal, the hydrolysates of UKT, UKL, LKT and LKL were filtered through the PVP-treated cotton. The PVP-treated cotton was prepared according to procedures described by Wang, Yang and Kuo (2017). Briefly, the degreased cotton was soaked in 1% w/v PVP solution for 30 min at room temperature, then dried at 85 °C and followed with curing at 150 °C for 5 min. The PVP-treated cotton was washed and dried at 85 °C for 3 cycles. The PVP-treated cotton was compressed on the Buchner funnel to completely cover up all holes. The hydrolysate was poured over the compressed PVP-treated cotton for

vacuum filtration. The PVP-post-treated UKT, UKL, LKT, and LKL hydrolysates were labelled as UKTP, UKLP, LKTP and LKLP, respectively. Total crude fat and sugar contents of the hydrolysates were determined according to protocol described in the previous section.

Saccharomyces cerevisiae FERMENTATION

All hydrolysates (UKT, UKL, LKT, LKL, UKTP, UKLP, LKTP, and LKLP) were sterilized at 121 °C for 15 min before inoculated with 4% w/v *S. cerevisiae* instant dry yeast. Molasses (diluted to total sugar content at 9.2 ± 0.1%) was served as positive control for the fermentation experiment. The fermentation progressed at 37 °C for 24 h in an incubator (modified Khan et al. 2022). After fermentation, the yeast cell biomass was separated from the hydrolysate through filtration using a piece of Whatman No. 4 filter paper. The separated cell biomass was rinse with distilled water before proceeding to the determination of dry cell weight. The dry cell weight of the yeast biomass was determined by drying the cell biomass in an oven at 105 °C overnight (16 h). The yield of cell biomass was determined by subtracting the dry cell weight of the inoculated yeast cells before fermentation from the final dry cell weight of the total yeast cells after 24 h fermentation. Total crude protein content of the yeast cell was determined using Kjeldahl method (AOAC International 2000). Total sugar content in the hydrolysate after 24 h fermentation was quantified according to protocol described in the previous section. Total sugar consumption by the yeast during fermentation was calculated by subtracting total sugar content in the hydrolysate after fermentation from the total sugar content in the hydrolysate before fermentation.

CHEMICAL ANALYSIS

Total sugar content of the hydrolysates was determined using phenol-sulfuric acid method according to protocol described by Zhang et al. (2020). Briefly, 2 mL of sample or glucose standard (20 – 100 ppm) was mixed with 0.8 mL of 5% w/v phenol solution and 5 mL concentrated sulfuric acid. The mixture was heated in boiling water for 20 min, then cool down to room temperature in an ice bath for 2 min. The absorbance of the mixture was measured at a wavelength of 490 nm using a spectrophotometer (Genesys, Thermo Scientific, U. S. A.). Calibration curve of absorbance versus glucose concentration with a regression equation $y = 0.009x + 0.104$ ($r^2 = 0.995$) was plotted. Concentration of the total sugar was determined by interpolating the glucose calibration curve.

Total crude fat content of the hydrolysate was determined through solvent extraction method. Briefly, the hydrolysate was mixed with petroleum ether at 1:1 ratio in a separating funnel, then shaken vigorously. The solvent was standing undisturbed until clear separation of 2 layers. The upper layer was collected. The extraction process was repeated for 3 times and the solvents (upper layer) collected were combined. The solvent was transferred into a pre-dried round flask and dried to dryness using a rotary evaporator (IKA, Germany). The flask containing oil residue was dried in the oven at 50 °C until constant weight. Weight gain of the round flask indicates total crude fat content.

STATISTICAL ANALYSIS

All experiments were performed in triplicates and the data was presented in mean ± standard deviation. Statistical difference between the means was determined through one-way ANOVA and post-hoc Tukey's multiple comparison using Minitab Statistical Software version 21.4 (Minitab, LLC, Pennsylvania, U. S. A.). The difference with p-value below 0.05 at 95% confidence level was considered significant.

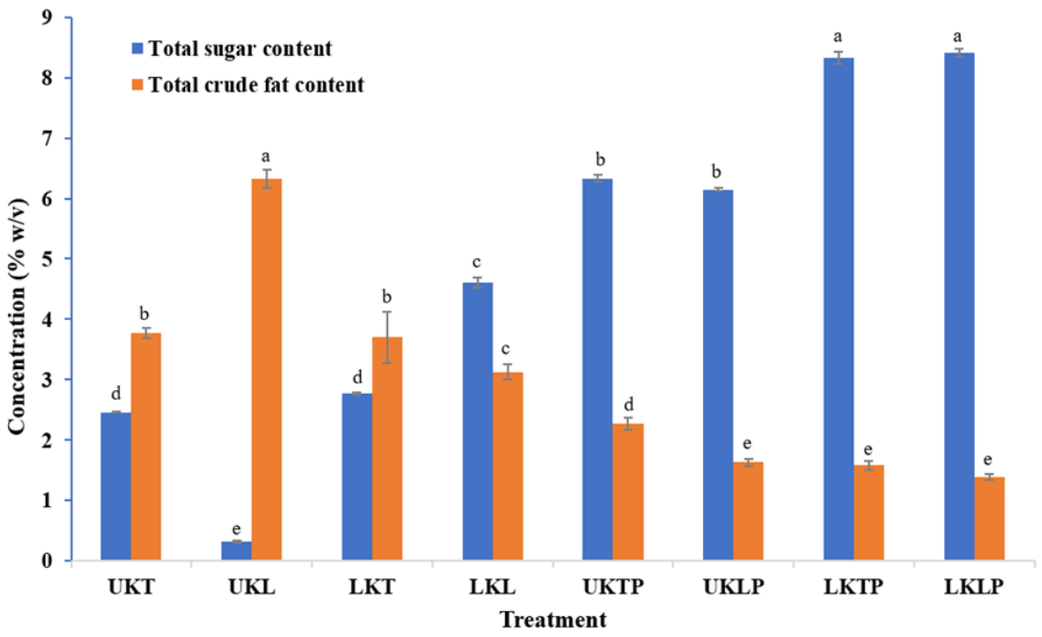
RESULTS AND DISCUSSION

EFFECTS OF LP, PVP AND THEIR COMBINED TREATMENT (LP-PVP) ON THE ENZYMATIC HYDROLYSABILITY OF *KUIH* RESIDUE

Figure 1 shows the effects of LP, PVP and their combined treatment (LP-PVP) on the total sugar and crude fat contents of hydrolysates produced from KT and KL. Based on the results, hydrolysate of UKL contained the highest crude fat content at 6.33 ± 0.15%, which is about 2.6% higher than UKT (3.77 ± 0.08%). However, LP did not exert a significant effect on the total crude fat content of LKT hydrolysate, but it significantly reduced total crude fat content of LKL hydrolysate. Perhaps, the efficacy of LP in fat removal is notably influenced by the total fat content of substrate. Total fat content in KT (3.77 ± 0.08%) was about 40% lower than that of KL (6.33 ± 0.15%). Total crude fat content of LKL hydrolysate (3.13 ± 0.12%) was 3.2% lower than UKL hydrolysate. There was no significant difference ($p>0.05$) between total crude fat content of UKT and LKT. Among the hydrolysates, total crude fat content of UKLP, LKTP, and LKLP were the lowest (in the range of 1.39 – 1.63%). Total crude fat content of these hydrolysates was not significantly different ($p>0.05$). This result proposes that fat content of KL can be effectively removed through PVP without the need of LP. Even though total crude fat content of UKTP was not the lowest, it was significantly lower ($p<0.05$) than UKT and LKT. This finding suggests that PVP was more efficient and promising than LP in fat removal.

Up to presently, the strength of lipase enzyme in wastewater bioremediation has been widely reported. Lipase enzyme has extensive industrial applications due to its robustness under different environmental conditions and ability to hydrolyse triacylglycerol without the need of cofactor (Nimkande & Bafana 2022). However, the hydrolytic efficiency of lipase is influenced by fatty acids composition and structure of lipid in the oil. The hydrolytic efficiency dropped over time due to the changes of substrate availability and accumulation of by-products (Matias et al. 2023). Unlike LP, PVP physically removed oil residues from the reaction environment by forming complexes. Study by Wang, Yang and Kuo (2017) reported that PVP-modified cotton could efficiently separate both surfactant-free and stabilized oil-in-water emulsion. The study showed that PVP possesses both superhydrophilic and superoleophilic properties, which makes it an excellent material for efficient separation of water rich immiscible oil droplets. Besides, numerous studies also reported the improved filtration efficiency and antifouling characteristic of filter membrane incorporated with PVP (Kapoor et al. 2024; Shalbafan, Esmaeilzadeh & Vekili-Nexhaad 2020; Zhao et al. 2023). In addition, the excellent performance of PVP in oil and dye removal were also reported in the study by Teodorescu and Bercea (2015). The previous studies explain the reason why PVP was superior to LP in fat removal in this study.

According to Figure 1, total sugar content of UKL hydrolysate (0.32 ± 0.01%) was 7.7-fold lower than UKT hydrolysate (2.46 ± 0.01%), and it was the lowest among the samples. Additionally, the results also indicate that LP did not significantly increase the total sugar content of LKT hydrolysate, as there was no significant difference (p>0.05) observed when compared to UKT hydrolysate. However, LP significantly increased the total sugar content of LKL hydrolysate by 14.4 folds, reaching 4.61 ± 0.08%. In addition, PVP was also proven to substantially increase total sugar content of UKTP, UKLP, LKTP, and LKLP. PVP increased the total sugar content of UKTP and UKLP by 2.6 and 19.2 folds, respectively, compared with UKT and UKL. However, total sugar content of UKTP and UKLP were no significant differences (p>0.05). Among the hydrolysates, LKTP and LKLP contain the highest total sugar content (in the range of 8.33 - 8.41%), which is about



a – e: Different alphabets indicate there is significant difference (p<0.05) between the mean of the same chemical component

UKT indicates KT without undergoing any pre- and post-treatment, UKL indicates KL without undergoing any pre- and post-treatment, LKT indicates LP-treated KT, LKL indicates LP-treated KL, UKTP indicates KT underwent PVP without LP, UKLP indicates KL underwent PVP without LP, LKTP indicates KT underwent both LP and PVP treatments, and LKLP indicates KL underwent both LP and PVP treatments

FIGURE 1. Total sugar and crude fat contents of different glucose-rich hydrolysates produced from KT and KL (n=3)

2% higher than UKTP and UKLP. This finding shows that LP-PVP was the best method to maximize the recovery of sugars from the *kuih* residue. Besides, the result of Pearson correlation analysis suggested that total sugar content of the hydrolysate was negatively correlated with its total crude fat content. The correlation relationship between total sugar and crude fat was very strong with r = - 0.947 at $p<0.01$. This finding proposes that crude fat content negatively impacted performance of amylase catalysis, hence affect sugar released from the starchy *kuih* residue.

According to Moran (2021), the amylose helix - fatty acid complex forms when gelatinized starch exposed to fatty acids. The complexes are readily form at room temperature with any fatty acid and their resistance to amylase is contingent on the nature of the lipid in the complex. In the study by Crowe, Seligman and Copeland (2000), lauric acid, myristic acid, palmitic acid and oleic acid were reported to reduce the extent of amylose hydrolysis by about 50%. Furthermore, it was also reported the inhibition of starch hydrolysis by fatty acids occurred mainly in the amylose fraction and not in the amylopectin fraction. In the *kuih* residue which is rich in coconut milk that contains lauric acid and myristic acid as the dominant fatty acids (Nadeeshani et al. 2015), the fatty acids are expected to facilitate the formation of amylose – fatty acid complexes that are resistance to amylase catalysis, hence reduce the release of reducing sugar. During LP, lipase enzyme releases free fatty acids from the triacylglycerols, which subsequently might promote the formation of amylose – fatty acid complexes which resist hydrolysis. Therefore, based on results in Figure 1, integrating PVP with LP was observed to be more efficient in fat removal. Fatty acids released after LP were expected to be complexed with PVP during the post-treatment stage, hence minimizing the formation of amylose – fatty acid complexes, eventually increasing the hydrolytic efficiency of starch by amylase enzyme.

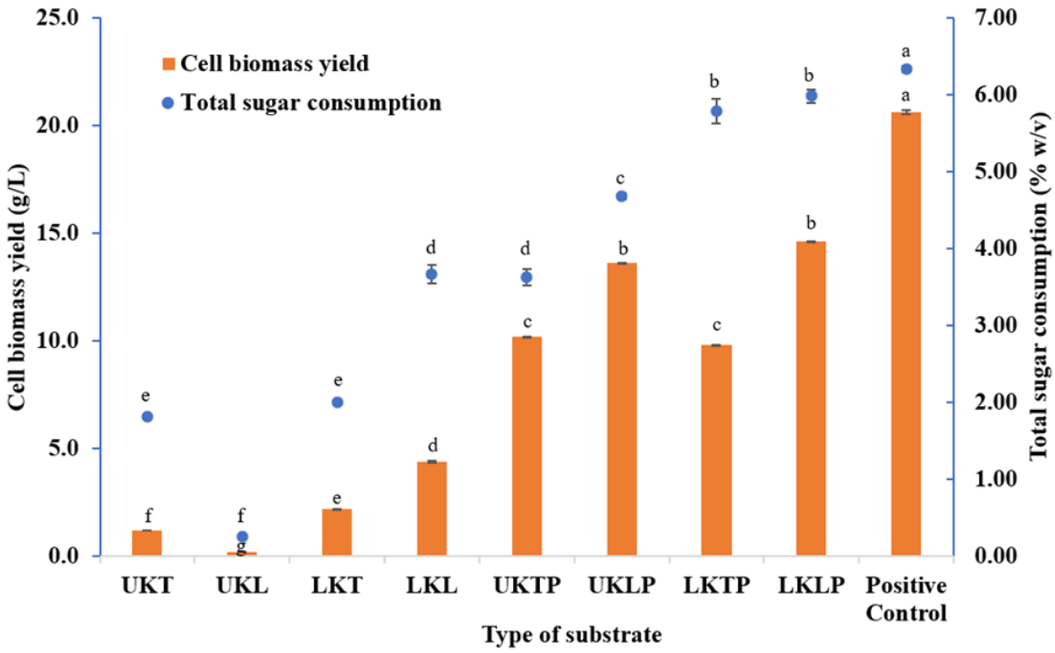
EFFECTS OF CRUDE FAT AND TOTAL SUGAR CONTENTS ON *Saccharomyces cerevisiae* FERMENTATION

Figure 2 presents the yield of yeast cells biomass and total sugar consumption by the yeast in 24 h fermentation in different hydrolysates of KT and KL. The result shows that total sugar consumption by the yeast in the molasses (positive control) was the highest (6.33 ± 0.04 %) with the highest cell biomass yield (20.6 ± 2.0 g/L). Among the hydrolysates, fermentation of UKLP and LKLP hydrolysates produced the highest yield of cell biomass (13.6 ± 0.6 g/L), followed by UKTP (10.2 ± 0.2 g/L) and LKTP (9.8 ± 0.6 g/L). The cell biomass yield of UKTP and LKTP was significantly lower ($p<0.05$) than that of UKLP and LKLP. Yeast cells yield from the hydrolysate of KL and KT was 30% and 50%, respectively, lower than the molasses. Besides, Table 1 shows the yeast cell yield on substrate (dry cell weight per glucose consumption) in different hydrolysates.

The results showed that yeast cells yield on substrate in UKTP and UKLP were the highest (0.28 – 0.29 g dry cell/g sugar) among the hydrolysates, which is only about 12 – 15% lower than positive control. This finding proposes that feeding substrate significantly impacted yeast growth during fermentation. Besides, the findings also recommend that KL could be a better substrate than KT for yeast cell production.

In addition, results in Figure 2 also show that oil removal exerted significant effects on the yeast cell production. According to Figure 2, yeast cells growth in the hydrolysates prepared from the untreated *kuih* residue (UKT and UKL) were the lowest. The yeast cell yields in UKT and UKL hydrolysates were 1.2 ± <0.0 g/L and 0.2 ± <0.0 g/L, respectively. When LP was employed, the yield of yeast cell biomass was increased to 2.2 ± 0.2 g/L and 4.4 ± 1.0 g/L, respectively, in LKT and LKL hydrolysates. The yeast cells production in the hydrolysate of KT and KL was further increased by 4.6 and 3.1 folds, respectively, when PVP and LP-PVP employed. Moreover, the results of Pearson correlation analysis unveil that the yeast cell yield was positively correlated (r = 0.849, $p<0.01$) with total sugar consumption by the yeast and negatively correlated with the total crude fat content of the hydrolysate (r = -0.845, $p<0.01$). In addition, referring to Table 1, yeast cell yield on substrate of UKTP and UKLP were observed higher than LKTP and LKLP. These findings suggest that PVP was a pivotal process step to remove oil residues in the hydrolysate, subsequently facilitating the yeast cells growth in the fermentation. This hypothesis is in accordance with the finding reported by Parapouli et al. (2020), whereby fat was reported to adversely impact the yeast cells growth during fermentation. Besides, Bruna-Garcia et al. (2023) also proved that complex lipid matrix caused oxidative damage and stress to yeast *Saccharomyces cerevisiae*.

Figure 3 shows total crude protein content of yeast cells biomass produced from different hydrolysates. The result shows that total crude protein of yeast cell biomass produced from LKLP was the highest (52.15 ± 0.46%), followed by LKTP (49.47 ± 0.61%), UKLP (47.19 ± 0.37%) and UKTP (44.92 ± 1.19%). Generally, single cell protein is recognised for being an excellent high protein source, within the range of 30 – 80%. Among the various sources of single cell protein, the protein content of yeast cell biomass constitutes 35 – 60% of the yeast dry weight (Ma et al. 2023; Salazar-Lopez et al. 2022). Hence, the yeast cell biomass produced from the hydrolysates has been proven to be a suitable and promising alternative protein source for the future.



a – g: Different alphabets indicate there is significant difference ($p<0.05$) between the mean of the same chemical component

UKT indicates KT without undergoing any pre- and post-treatment, UKL indicates KL without undergoing any pre- and post-treatment, LKT indicates LP-treated KT, LKL indicates LP-treated KL, UKTP indicates KT underwent PVP without LP, UKLP indicates KL underwent PVP without LP, LKTP indicates KT underwent both LP and PVP treatments, and LKLP indicates KL underwent both LP and PVP treatments

FIGURE 2. Effect of different feeding substrates on the total sugar consumption and yield of yeast cell biomass (n = 3)

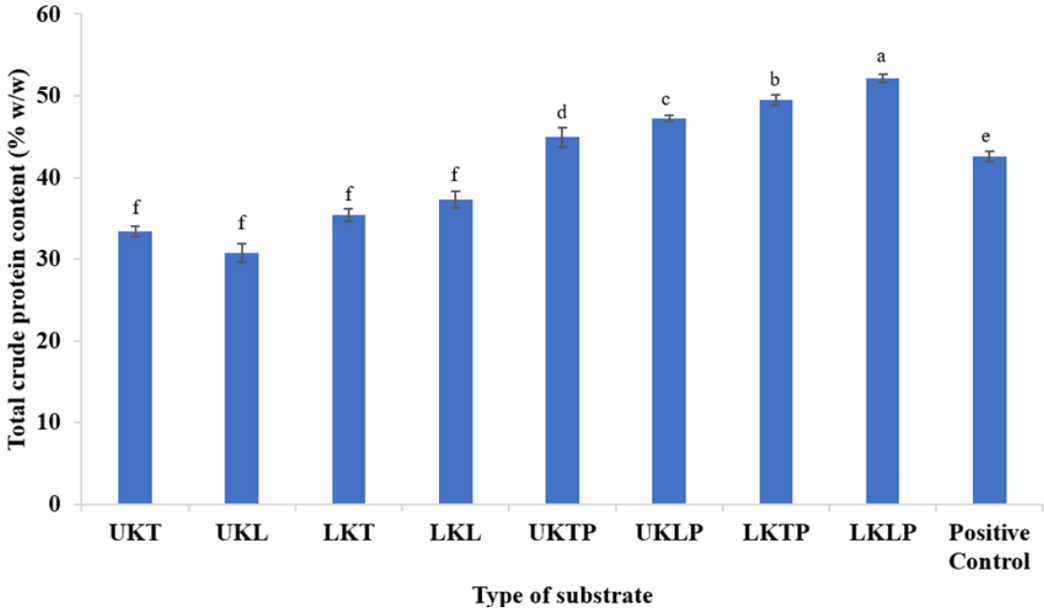
TABLE 1. Yield on substrate of yeast cells biomass in different hydrolysates

Substrate	Yield on substrate (g dry cell /g sugar)
Positive control	0.33 ± 0.03^{a}
UKT	$0.07 \pm <0.00^{f}$
UKL	$0.08 \pm <0.00^{f}$
LKT	$0.11 \pm <0.01^{e}$
LKL	0.12 ± 0.02^{e}
UKTP	$0.28 \pm <0.00^{b}$
UKLP	0.29 ± 0.01^{b}
LKTP	0.17 ± 0.01^{d}
LKLP	0.24 ± 0.01^{c}

a – f: Different alphabets indicate there is significant difference ($p<0.05$) between the means

Yield on substrate = Total cell biomass yield (g) / Total sugar consumption (g)

UKT indicates KT without undergoing any pre- and post-treatment, UKL indicates KL without undergoing any pre- and post-treatment, LKT indicates LP-treated KT, LKL indicates LP-treated KL, UKTP indicates KT underwent PVP without LP, UKLP indicates KL underwent PVP without LP, LKTP indicates KT underwent both LP and PVP treatments, and LKLP indicates KL underwent both LP and PVP treatments



a – f: Different alphabets indicate there is significant difference ($p<0.05$) between the mean of the same chemical component

UKT indicates KT without undergoing any pre- and post-treatment, UKL indicates KL without undergoing any pre- and post-treatment, LKT indicates LP-treated KT, LKL indicates LP-treated KL, UKTP indicates KT underwent PVP without LP, UKLP indicates KL underwent PVP without LP, LKTP indicates KT underwent both LP and PVP treatments, and LKLP indicates KL underwent both LP and PVP treatments

The total crude protein content of yeast cell protein should fall within the range of 35 – 60 % (Ma et al. 2023)

FIGURE 3. Effect of different feeding substrates on the total crude protein content of the yeast cell biomass (n = 3)

Additionally, yeast cell biomass produced from these four hydrolysates contained significantly higher ($p<0.05$) total crude protein content than the positive control (42.64 ± 0.62 %). In the study by Yilmaz, Berk and Gokmen (2024), Baker's yeast and Brewer's yeast secreted diverse amino acid derivatives under environmental stresses, including temperature, pH, alcohol, phenolic and osmotic pressure. Besides, Mekoue et al. (2019) proved that polyphenols in grapes must significantly impact metabolism of yeast during fermentation. In the Nyonya *kuih* making process, natural colorants, such as Pandan leaf extract, blue pea flower extract, and carrot extract had been added. Therefore, it is strongly believed that phytochemicals derived from the natural colorant used in the *kuih* making are the components that impact the yeast fermentation.

Even though the yeast cells yield and yield on substrate in the four hydrolysates were significantly lower than the positive control, the higher total crude protein content in the yeast cell biomass produced confers novelty to this preliminary study. Given its high total crude protein, Nyonya *kuih* residue is recommended as an economically feasible substrate for yeast cell protein production. However, further study is needed to enhance the yield and fermentation efficiency, thereby improving the success rate of technology transfer from laboratory to industry. To increase the success rate of process scale-up application, further investigation into the types of nutrients in the *kuih talam* and *kuih lapis* residues that contribute to higher crude protein content in yeast cell biomass need to be carried out. Besides, the experiment will also be extended to include more varieties of *Nyonya kuihs* so that the economic feasibility of the proposed bioconversion process can be thoroughly evaluated.

CONCLUSIONS

The study proposes a novel green approach to utilise the oily and starchy *Nyonya kuih* (*kuih talam* and *kuih lapis*) residue as the substrate for yeast cell protein production. In this study, the Baker's yeast *S. cerevisiae* was used as the model of investigation. The study proved that *kuih lapis* was a better substrate than *kuih talam* for producing cell biomass with a significantly higher crude protein content. Besides, the combined lipase-pretreatment and PVP-post-

treatment has also been proven to be the best technique to reduce crude fat content of the hydrolysate produced from the residue, subsequently turning the hydrolysate into a feasible fermentable substrate for yeast cell protein production. This study provides new insights into a novel food residue recycle approach to reduce food wastage in the food supply chain. The study was conducted to align with the call of SDG 12 (Responsible consumption and production) by the United Nations.

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*Corresponding author; email: tangpl@tarc.edu.my

SUPPLEMENTARY 1. (a) Variety of Nyonya desserts in Malaysia local market, (b) Kuih Talam, and (c) Kuih Lapis

a)

b)

c)

SUPPLEMENTARY 2. Smashed kuih waste for processing