Access this Article online
Quick Response Code:

Website:
jecajournal.com

Original Article

Palm Kernel Oil as an Alternative Clearing Agent in Histological Tissue Processing

[1]Nto N.J., [1]Ukwa C.J., [1,2]Oria R., [1]Oviosun A., [1]Katchy A.U., [1]Anyanwu G.E., [3]Nto-Ezimah U.A.

ABSTRACT

Xylene is the most commonly used clearing agent because of its easy accessibility. However, xylene poses significant health hazards and environmental risks due to its toxic and volatile nature. There is a need for a non-toxic, environmentally friendly, and cost-effective substitute for conventional agents. Palm Kernel Oil (PKO) is rich in saturated fatty acids, chemically stable, hydrophobic, and capable of tissue penetration. This study aims to determine the effect of PKO as a substitute for xylene as a clearing agent in histology. Liver and kidney were harvested from ten adult wistar rats. The tissues were divided equally into two groups; group A was cleared in xylene while group B was cleared in PKO. All tissues were subjected to the same histological processing except at the clearing stage. The two groups were evaluated for the following parameters; post-clearing tissue shrinkage, ease during sectioning, cellular architecture, staining intensity and uniformity, nuclear staining, and cytoplasmic contrast and clarity. The data was analysed on SPSS software version 23 and the means of both groups were compared using independent t-test tool. The results of the study reveal that the post-clearing tissue shrinkage, ease during sectioning, cellular architecture, staining intensity and uniformity, nuclear staining and cytoplasmic contrast and clarity in the PKO-cleared tissues were comparable to the Xylene-cleared tissues. In conclusion, the results of the study suggest that palm kernel oil can be used as an alternative clearing agent in histological preparations without losing the quality of histological details.

Keywords:
Palm kernel Oil, Clearing agent, Xylene, Histological tissue processing, PKO.

INTRODUCTION

Tissue processing is a critical methodology employed to prepare tissue specimens for microscopic examination. This multi-step technique involves fixation, dehydration, clearing, infiltration/impregnation, embedding, sectioning, mounting, and staining (Culling, 2013; Suvarna *et al.,* 2018). One pivotal step is the clearing process, where clearing agents are used to remove dehydrating agents, ensuring optimal tissue transparency. The choice of clearing agent significantly impacts the quality of subsequent steps, such as infiltration and sectioning (Culling, 1974; Swamy *et al.,* 2015; Suvarna *et al.,* 2018).

The efficacy of the clearing process is vital to avoid adverse effects on tissue sections (Culling, 1974; Suvarna *et al.,* 2018). Inadequate removal of dehydrating agents can compromise tissue refractive index, leading to subsequent challenges and poorly cut tissue sections. Clearing agents should remove dehydrating agents, enhance tissue transparency, facilitate staining, and ensure cellular components' easy identification (Culling, 1974; Suvarna *et al.,* 2018). Also, an ideal clearing agent should have a higher refractive index than the tissue, and be non-inflammable, non-toxic, and biodegradable (Radhika *et al.,* 2016).

While various clearing agents, including aromatic hydrocarbon solvents (xylene), essential oils (coconut oil) (Chandraker *et al.,* 2018), and cooking oil (palm oil) (Ravindran *et al.,* 2018), have been explored, xylene remains the most effective yet poses significant health and safety risks. Inhaling xylene vapor can result in symptoms such as headache, dizziness, nausea, and vomiting. Long-term exposure may lead to irritability, insomnia, tremors, impaired concentration, and acute neurotoxicity, among other health issues (Ramamoorthy *et al.,* 2016; Kandyala *et al.,* 2010). Efforts to find safer alternatives have yielded substitutes like carrot oil, olive oil, pine oil, rose oil (Swamy *et al.,* 2015), groundnut oil (Digala *et al.,* 2013), refined mineral oil (Premalatha *et al.,* 2013), and edible oils such as coconut and palm oil (Digala *et al.,* 2013; Swamy *et al.,* 2015; Ravindran *et al.,* 2018). However, challenges persist with commercially available substitutes being less effective, more expensive, and constituting health hazards comparable

[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Nigeria Enugu Campus, Enugu State; [2]Department of Anatomy, Faculty of Basic Medical Sciences, Cross River State University, Nigeria; [3]Department of Chemical Pathology, Faculty of Medical Sciences, University of Nigeria Enugu Campus, Enugu State.

Address for Correspondence:
Nto-Ezimah U.A.
Department of Chemical Pathology, Faculty of Medical Sciences, University of Nigeria Enugu Campus, Enugu State, Nigeria.
uloaku.ntoezimah@unn.edu.ng

How to cite this article: Nto N.J., Ukwa C.J., Oria R., Oviosun A., Katchy A.U., Anyanwu G.E., Nto-Ezimah U.A. Palm Kernel Oil as an Alternative Clearing Agent in Histological Tissue Processing. *J Exp Clin Anat* 2022; 19(Suppl 2):6-10.

has to or exceeding xylene (Udonkang *et al.,* 2013).

Palm kernel oil (PKO) is a plant-based oil extracted from the seed of the oil palm fruit, *Elaeis guineensis* (Poku *et al.,* 2002; Zhang *et al.,* 2019). It is rich in saturated fatty acids, chemically stable, hydrophobic, and capable of tissue penetration (Aladetuyi, *et al.,* 2014). It has a non-volatile and non-toxic nature, and is widely available in many tropical and developing regions, and may serve as an alternative. With its unique fatty acid composition, which consists of lauric and myristic acids, and the potential to react with alcohols (Nainggolan & Sinaga, 2021), PKO offers characteristics suitable for clearing. PKO contains fatty acids; saturated fatty acids and unsaturated fatty acids (Nainggolan & Sinaga, 2021). The Saturated fatty acids include lauric acid (49.39%) and myristic acid (15.35%). The unsaturated fatty acids include Oleic acid (15.35%) and linoleic acid (3.10%), and also a small percentage of unidentified minor fatty acids (0.07%) present in PKO (Aladetuyi *et al.,* 2014). PKO has these fatty acids in the form of esters. An ester has the hydrogen atom (H) of at least one acidic hydroxyl group (−OH) of that acid is replaced by an organyl group (−R), it can react with alcohols and is soluble in paraffin wax (McNaught & Wilkinson, 1997). Hence, PKO can be considered as an alternate clearing agent.

This study evaluated the effectiveness of PKO as a substitute for xylene in histological tissue processing, considering its fatty acid profile, biodegradability, and potential application as an alternate clearing agent. The assessment criteria aimed to capture the impact of clearing agents on tissue sections, considering shrinkage, sectioning ease, cellular architecture, staining quality, and nuclear and cytoplasmic characteristics as described in Sermadi *et al.* (2014) and Ravindran *et al.,* (2018), will allow for the evaluation of the impact of PKO as a clearing agent in comparison to xylene in histological tissue processing.

MATERIALS AND METHODS

Reagents and Materials

PKO was purchased from a local vendor at Karu market, Abuja. The purchased PKO was was extracted mechanically by the locals. The mechanical extraction of PKO described in Alamu et *al.,* (2007) involves harvesting the ripe oil palm fruits and the removal of the outer fleshy part (used to extract palm oil). This is followed by cracking of the nuts inside the fruit to remove the kernels (seeds). The kernels were sun dried to remove moisture content and increase yield. The dried kernels were fed into a screw press to extract oil. The extracted oil was filtered to remove solid particles and impurities. Xylene used for this study was procured from a reputable supplier at Main market Enugu

Experimental Animals and Tissue Collection

Ten adult Albino male Wistar rats purchased from the Department of Veterinary Anatomy, University of Nigeria Nsukka were used for this study. The animals were sacrificed via cervical dislocation, and the liver and kidneys of each animal were harvested and divided into two halves.

Tissue Processing and Clearing Procedures

Each half was randomized into one of the two experimental groups (groups A and B) and fixed in 10% formal saline for 48 hours. Each tissue sample was progressively dehydrated using increasing concentrations of ethanol, from 70%, 80%, and 90% 30 minutes each and then in 100% in three changes, 30 minutes each, to remove water. Group A tissue specimen was cleared in xylene in two changes, 30 minutes each. Group B was cleared in PKO for 12 hours overnight. PKO appears more viscous (Aladetuyi et al., 2014) compared to xylene and may require more time to allow for the infiltration of tissues, hence it was allowed overnight. After clearing, the tissue was transferred to molten paraffin wax. Xylene and PKO were gradually replaced by the wax, which infiltrates the tissue, providing support and allowing for thin sectioning. The tissues were then sectioned and stained using the routine Haematoxylin and Eosin staining method.

Evaluation Parameters

The assessment of tissue sections involved the following parameters:

Post-Clearing Tissue Shrinkage: Measurement of the length and breadth of each tissue was taken before and after the clearing step Two-dimensional measurements were recorded, and scoring was based on a method adapted from Sermadi *et al.* (2014). Scores were assigned as follows: PKO cleared tissues inferior to xylene (score 0), similar to xylene (score 1), and superior to xylene (score 2).

Ease during Sectioning: Difficulty levels during tissue sectioning were noted, considering ribbon formation, microtome knife passage, and the presence of artifacts. Scoring method, adapted from Sermadi *et al.* (2014), included scores of 0 for PKO cleared tissues inferior to xylene, 1 for similar ease, and 2 for superior ease during sectioning.

Cellular Architecture: Assessment of nuclear-cytoplasmic contrast and cellular details based on a scoring method from Sermadi *et al.* (2014). Scores: 1 for distinct architecture and good nuclear-cytoplasmic contrast, 0 for indistinct/blurred contrast.

Staining Intensity and Uniformity: Direct microscopic observation for staining intensity and uniformity. Scoring method, as in Ravindran *et al.* (2018) and adapted from Sermadi *et al.* (2014): Poor (score = 0), Satisfactory (score = 1), Good (score = 2).

Nuclear Staining: Evaluation of nuclear stain uptake and clarity. Scoring: Poor (score = 0), Satisfactory (score = 1), Good (score = 2), following the method of Sermadi *et al.* (2014).

Cytoplasm Contrast and Clarity: Assessment of nuclear-cytoplasmic contrast and cytoplasmic staining. Scoring method: Poor (score = 0), Satisfactory (score = 1), Good (score = 2), adapted from Ravindran *et al.* (2018) and *Sermadi et al.* (2014).

All evaluations were conducted through direct microscopic observation by two independent scientist. Scores were assigned based on the visual analysis criteria mentioned above.

Statistical Analysis

Data was analysed using Statistical Package for Social Sciences (SPSS) Version 23 (IBM Computers USA). Student t-test was used to compare differences between the two experimental groups, employing appropriate tests for each parameter. The significance level was set at $p < 0.05$.

RESULTS

HISTOLOGICAL OBSERVATIONS OF THE TISSUES

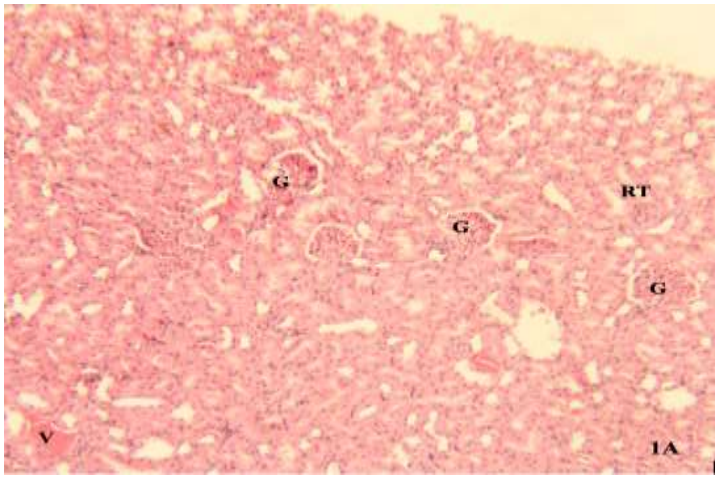

Figure 1A: — Photomicrograph Kidney sections of Xylene-treated specimen. Showing normal cytoarchitecture. The renal cortex appears well-preserved with visible glomeruli (g) and renal tubules (RT). The nuclear-cytoplasmic contrast is clear. H & E (X100)

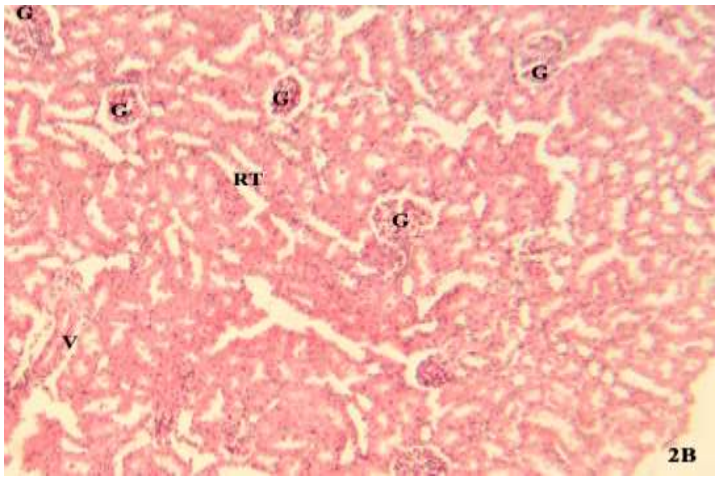

Figure 2B: Photomicrograph of Kidney sections of PKO treated specimen. Showing normal cytoarchitecture. The renal cortex appears well-preserved with visible glomeruli (g) and renal tubules (RT). The nuclear-cytoplasmic contrast is clear. H & E (X100)

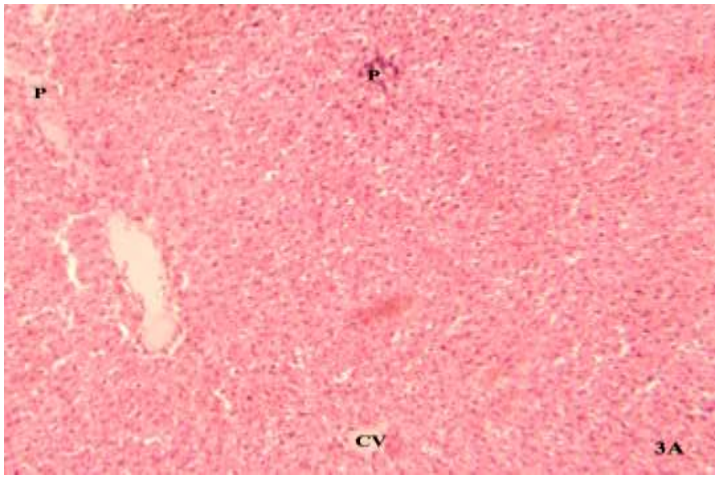

Figure 3A: Photomicrograph of Liver sections of Xylene-treated specimen. Showing normal cytoarchitecture. Shows a classic radial arrangement of hepatocytes (liver cells) around the central vein (CV). Also visible is the portal triad (P). H & E (X100)

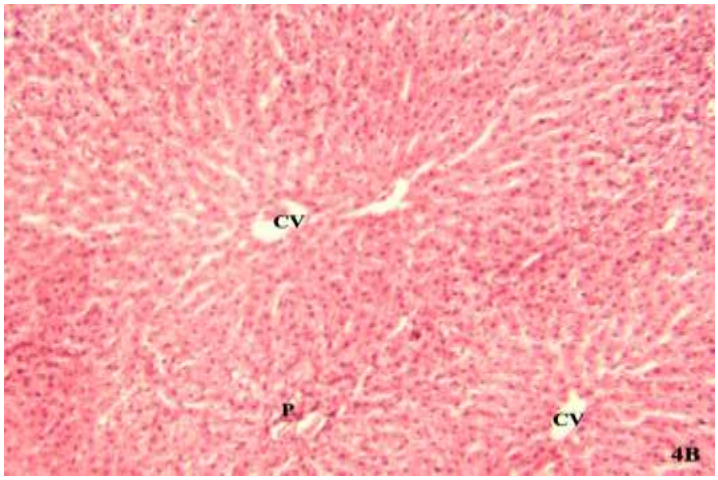

Figure 4B: Photomicrograph of Liver sections of PKO treated specimen. Showing normal liver cytoarchitecture. Shows a classic radial arrangement of hepatocytes (liver cells) around the central vein (CV). Also visible is the portal triad (P). H & E (X100)

The result on table 1 shows that there was no significant ($p>0.05$, student t-test) difference in Post-Clearing Tissue Shrinkage, Ease During Microtomy, Cellular Architecture, Staining Intensity and Uniformity, Nuclear Staining, and Cytoplasmic Contrast and Clarity in PKO-cleared tissues were compared to xylene-cleared tissues.

DISCUSSION

This study assessed the viability of PKO as an alternative to xylene in tissue processing. Due to the inherent toxicity and hazardous nature of xylene, there is need for alternative substitutes that are non-toxic, less bio-hazardous, and economically viable for laboratory use. Several attempts have been made to replace xylene with substitutes like limonene, aliphatic hydrocarbons, and mineral oils (Kandyala *et al.,* 2010); however, these alternatives have proven less effective and more expensive than xylene. Exploring natural products as potential clearing agents,

Table 1: Descriptive Statistics and Comparison of the Mean Scores of the Six Parameters Assessed for the Xylene group and the PKO group

Parameter	Xylene Mean±Std Error	PKO Mean±Std Error	t-test for Equality of Means t	Df	p-value	Mean difference	Std. Error Difference
Post-Clearing Tissue Shrinkage	1.00±0.10	1.00±0.26	0.000	18	1.000	0.000	0.248
Ease During Microtomy	1.00±0.12	0.80±0.25	0.802	18	0.433	0.200	0.237
Cellular Architecture	0.60±0.16	0.60±0.16	0.000	18	1.000	0.000	0.231
Staining Intensity and Uniformity	1.00±0.21	1.20±0.25	0.612	18	0.548	-0.200	0.327
Nuclear Staining	1.60±0.16	1.40±0.16	0.866	18	0.398	0.200	0.231
Cytoplasmic Contrast and Clarity	1.50±0.17	1.60±0.16	-0.429	18	0.673	-0.100	0.233

studies have considered cedarwood oil, pine oil, rose oil, carrot oil, coconut oil, and bleached palm oil (Rahmawati *et al.,* 2020; Ravindran *et al.,* 2018; Swamy *et al.,* 2015; Indu *et al.,* 2014; Digala *et al.,* 2013).

In evaluating tissue shrinkage post-clearing, both xylene and PKO exhibited a slight, statistically non-significant tissue shrinkage. This is comparable to the Rahmawati *et al.,* (2020) work comparing different oils to xylene in clearing liver and kidney tissues. The comparable results suggest that PKO can induce similar effects as xylene in terms of tissue shrinkage.

The ease of tissue sectioning, encompassing ribbon formation, microtome knife passage, absence of artifacts, and tissue embedding, showed no statistically significant difference between xylene and PKO. This finding is consistent with study by Ravindran *et al.* (2018) using bleached palm oil, indicating no difference in ease during sectioning.

Evaluation of cellular architecture revealed no statistically significant difference between xylene and PKO-cleared tissue specimens. This outcome corresponds with Rahmawati *et al.,* (2020) findings, further supporting that PKO preserves cellular architecture similarly to xylene.

Assessment of staining intensity and uniformity demonstrated no statistically significant difference between xylene and PKO-cleared sections. This is in line with Rahmawati *et al.,* (2020) study, reinforcing the notion that PKO can achieve staining quality comparable to xylene.

Examining nuclear staining, no statistically significant difference was observed between xylene and PKO. This result aligns with Ravindran *et al.,* (2018) findings, suggesting that PKO can provide adequate nuclear staining similar to xylene.

Similarly, evaluation of cytoplasm contrast and clarity yielded no statistically significant difference between xylene and PKO-cleared sections, consistent with Rahmawati *et al.,* (2020) observations.

The cumulative findings of this study, indicating no significant differences between xylene and PKO in various histological parameters, suggest that PKO could serve as a substitute for xylene in routine histology lab procedures. Further exploration of PKO's potential in histopathological and immunohistochemical procedures is warranted. The application of PKO in medical diagnosis, scientific studies, autopsy, and forensic investigations appears promising based on this comparative study.

Conclusion: In conclusion, this comparative study sought to assess the viability of palm kernel oil as a substitute for xylene in tissue clearing. The results indicate no significant differences between palm kernel oil and xylene, suggesting that palm kernel oil can be employed as an alternative clearing agent in histological preparations without compromising the quality of histological details. This inference opens avenues for further exploration and potential utilization of palm kernel oil in various histological applications.

Funding: None

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no competing interests.

REFERENCES

Aladetuyi, A., Olatunji, G.A., Ogunniyi, D.S., Odetoye, T.E. (2014) Production and characterization of biodiesel using palm kernel oil, fresh and recovered from spent bleaching earth. Biofuel Research Journal 134-138.

Alamu, O.J., Waheed, M.A., Jekayinfa, S.O., (2007). Alkali-catalysed Laboratory Production and testing of biodiesel fuel from Nigeria Palm Kernel Oil. Agricultural Engineering International. CIGR J. 9, 1–11.

Chandraker R, Rathod VC, Chandraker NK, Pundir S, Dixit S, Desai V. (2018) Comparison between xylene and coconut oil in tissue processing. Mod Med Lab J. 2(1):96-99.

Culling, C. F. (1974). Handbook of Histopathological and Histochemical Techniques: Including Museum Techniques. 3rd ed. London: Butterworth-Heinemann.

Digala, P., Bollu, D., Karthicka, C., Vincy, S. J., Selvam, R., Kandaswamy, S. (2013) Alternative to reduce occupational hazards for paramedical staffs in histopathology department. J Pharm Biol Sci. 12(5):5-12.

Indu, S., Ramesh, V., Indu, P., Prashad, V., Premalatha, B., Ramadoss, K. (2014) Comparative efficacy of cedarwood oil and xylene in hematoxylin and eosin staining procedures: An experimental study. J Nat Sci Biol Med. 2014;5:284-7.

Kandyala, R., Raghavendra, S.P., Rajasekharan, S. T. (2010) Xylene: An overview of its health hazards and preventive measures. J Oral Maxillofac Pathol. 14(1):1-5.

McNaught, A. D., & Wilkinson, A. (1997). *IUPAC compendium of chemical terminology* (2nd ed.). Blackwell Scientific Publications.

Nainggolan, M, Sinaga, A. G. S. (2021). Characteristics of fatty acid composition and minor constituents of red palm olein and palm kernel oil combination. J Adv Pharm Technol Res. 12(1):22–6.

Poku, K. (2002). Origin of oil palm. In: Small-Scale Palm Oil Processing in Africa (FAO Agricultural Services Bulletin 148, p. 3). Food and Agriculture Organization. ISBN 92-5-104859-2.

Premalatha, B. R., Patil, S., Rao, R. P., Indu, M. (2013). Mineral oil—A biofriendly substitute for xylene in deparaffinization: A novel method. J Contemp Dent Pract. 14(2):281-6.

Radhika, R., Roma, Y., Amit, B. (2016). Biosafe substitutes to xylene: A review. Int J Inf Res Rev. 3(6):2529-32.

Rahmawati, S., Wulan, A. J., Utami, N., Danan Jaya, B. P. (2020). Preliminary study: the potency of vegetable cooking oil as an alternative clearing agent for histological preparations. In: International Conference on Agromedicine and Medical Sciences (ICAMS)

Ramamoorthy, A., Ravi, S., Jeddy, N., Thangavelu, R., Janardhanan, S. (2016). Natural alternatives for chemicals used in histopathology lab - A literature review. J Clin Diagn Res. 10(11):01-4.

Ravindran, R., Sruthi, A. K., Ameena, M., Harish, R. N. K. D. (2018). Bleached palm oil as a bio-friendly substitute for xylene: A comparative study. Oral Maxillofac Pathol J. 9(2):63-9.

Sermadi, W., Prabhu, S., Acharya, S., Javali, S. (2014) Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory. J Oral Maxillofac Pathol. 18(Suppl 1):S49-S53.

Suvarna, K. S., Layton, C., Bancroft, J. D. (2018) Bancroft's Theory and Practice of Histological Techniques E-Book Elsevier Health Sciences

Swamy, S. R., Nandan, S. R., Kulkarni, P. G., Rao, T. M., Palakurthy, P. (2015). Bio-friendly alternatives for xylene - Carrot oil, olive oil, pine oil, rose oil. J Clin Diagn Res. 9(11):ZC16-8.

Udonkang, M., Eluwa, M., Ekanem, T.B., Asuquo, O.R., Akpantah, A. O. (2014) Bleached palm oil as a substitute for xylene in histology. J Phys Chem Solids. 8(1):8-17.

Zhang, W., Zhao, F., Yang, T., Liu, S. (2019). Solid-state fermentation of palm kernels by Yarrowia lipolytica modulates the aroma of palm kernel oil. Sci Rep. 9(1):2538.