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Evaluation of malt produced from different cultivars of indigenous *Oryza glaberrima* (L.)

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Abstract
Brewing of malts and beer using cereals other than barley is a well embraced trend. Research evidence suggested that use of glutten-free brown rice malt against milled rice cultivars in brewing could enhance the sensory profile and provided added health advantage for people with celiac disease. This study investigates the proximate composition and wort quality of malt produced from four varieties of indigenous brown 'Ofada' rice. The germinative energy of all the cultivars studied increases with changes in temperature from 24h < 48h < 72h with 20 – 93% and were below least ideal level of 96 percent for commercial malting brewing as reported in literatures. Oryza glaberrima type A had the highest malting loss (33.3 ± 0.14%) while Oryza glaberrima type C had the lowest (24.1 ± 0.0%). Moisture content ranges from 11.0 – 11.85% and 11.87 – 12.15% for unmalted and malted samples, respectively. There is trend in the variation of crude fat content of the grains studied only with high content in malted cultivars. The lowest crude protein content of 8.35 ± 0.07% was recorded in Oryza glaberrima type A and highest observed in Oryza glaberrima type C (9.05 ± 0.07%) with no significant different (p < 0.05). The highest range of carbohydrate content was recorded in unmalted rice (80.23 – 82.47%) compared lower range in malted samples (75.45 – 75.95%) with no significant difference. The result of this pilot study reveals the contingency for using 'Ofada' rice as potential brewing malts.

Keywords: *Brewing, Mashing, Proximate composition, Malting, Germinative energy*

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I. Introduction

Approximately fifty percent of people worldwide accept rice as their major staple food (Mayer et al., 2014). According to United States Department of Agriculture, Foreign Agricultural Services report, about five hundred million tons of milled rice was produced globally as at 2019 and 2020 market year. The leading country was China with 146.7 million metric tons (about 30 % of production) followed by India with 118.9 million metric tons (24 %), Indonesia and Bangladesh were at par (about 7% of production) and Thailand with 4 % production rate (*World Rice Production & Trade in Brief*, n.d.). Rice grain of the family Gramineae is grouped into two species based on stages of milling; Oryza *sativa* L. which is the white rice and *Oryza glaberrima* L., the brown rice (Mayer et al., 2014). The white rice is milled while the brown rice is unmilled and it is mainly grown in Africa (Mohd. Esa et al., 2013). Local brown rice known as 'Ofada rice' is a common staple food among Egba indigenes of Ogun State, Nigeria. It is an important ingredient for poultry feedstuff formulation (Asyifah et al., 2012). The high level of zinc, calcium , and carbohydrate contents of local brown rice has been reported (Yankah et al., 2020). Reports have shown that brown rice is high in dietary fibre, tocopherol, niacin and lysine content compared with white rice with minimal composition (Mohd. Esa et al., 2013). Research evidence suggested that consumption of glutten-free diets is one of the remedy for the treatment and control of celiac disease and rice have been confirmed to be a glutten-free cereal that is readily available to people (Ceccaroni et al., 2019). Production of beer from malted barley have been extensively studied and documented (Ceccaroni et al., 2019; Zhao et al., 2008). However, production of beer from standard malts such as wheat or barley has proved scientifically burdensome and previous studies have shown that prospective malts can be obtained from cereals including millet, maize , rice and sorghum (Ceccaroni et al., 2019). Also, barley importation to Nigeria is attributable to high cost of production and sales of malted drinks (Ceccaroni et al., 2019). Owing to the little knowledge we have, little report so far on the exploration of indigenous brown 'Ofada' rice as an ingredient for malting and brewing had been documented. This study aims at investigating the malting potentials of four different cultivars of brown rice indigenous to South West, Nigeria.

II. Methodology

2.1 Rice Samples: Four varieties of brown 'ofada' rice, *Oryza glaberrima* L. including *short grain, medium grain, light brown* and *long brown* rice , cultivated in South West, Nigeria were examined. The samples were labeled *Oryza glaberrima* (L.) A, *Oryza glaberrima* (L.) B, *Oryza glaberrima* (L.) C and *Oryza glaberrima* (L.) D respectively.

2.2 Malting Procedure:

2.2.1Steeping and germination: The modified depicted method of Mayer et al. (2014) was adopted. Approximately 2000 g of each rice sample was weighed and made cleared by hand picking to remove solid contaminants such as stones, crop debris and the sorts. It was afterwards rinsed in clean water and the floating grains were skimmed off. The grains were steeped in tap water at room temperature (27 ± 2 ℃) to evenly hydrate the cereal starchy endosperm and raise the moisture content of the grains. The steeped water was changed every six hours to avoid microbial contamination. Steeping continued for 36 h. and thereafter, aqueous layer was removed and samples ready for germination. The steeped samples were all made to germinate by spreading the grain samples separately on foil paper at room temperature (27 ± 2 ℃) for four days. Distilled water was sprinkled on the grains every six hours and grains turned simultaneously to aid even germination. The germinative energy and capacity were determined during the course of germination (Mayer et al., 2014).

***2.2.2Kilning*:** On the sixth day of germination, growth was terminated and kilning was performed by placing the germinated grains in hot air oven at (48 ± 2 ℃) for 24 h (Mayer et al., 2014).

***2.2.3De-culming*:** The culms (shoots and rootlets) were all removed by rubbing the malted grains between the palms (Adebowale *et al.,* 2010).

***2.2.4Milling*:** The kilned and dried grains were milled using a blender into fine powder and stored dry in an airtight container (Adebowale *et al.,* 2010).

2.3Determination of the malting parameters of the grains

***2.3.1Germinative energy*:** This was determined by germinating 100 grains of each of the working grains. The germinated grains were counted at 24 h, 48 h and 72 h and expressed in percentage as the Germinative energy (Mayer et al., 2014)**.** equation (1)

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$$\%\ \textbf{Germinative energy} = \left(\frac{\textbf{Number of viable grains}}{\textbf{Total number of grains}}\right) * \textbf{100} \quad \textbf{equation(1)}$$

***2.3.2Germinative capacity*:** After 72 h, grains that showed no evidence of germination were placed between moist filter paper and allowed to germinate for another 48 hours while grains that showed sign of germination were counted and added to the germinated mass after 72 h. The new percentage was then estimated as germinative capacity according to (Omah & Okafor, 2015) equation (2).

$$\text{Germinative Capacity} = \%\ \text{Germinative energy} - \%\ \text{Dormancy} \quad \text{equation (2)}$$

***2.3.3Malting loss*:** The malting loss was determined as depicted by Badau et al.(2007). It is given as differences between the weights of hundred unmalted grains against malted grains, expressed in percentage.

***2.3.4Malting yield*:** This was determined as the ratio of weight of hundred malted grains to weight of hundred raw or unmalted grains expressed in percentage (Mayer et al., 2014).

***2.3.5Malt Storage*:** The malted grain was stored cool and dry in sealed containers to prevent absorption of moisture, spoilage and mainly to arrest the decline in enzyme levels.

2.4 Proximate analysis of the malted and unmalted rice samples: The rice samples were evaluated for proximate composition by AOAC (2016) standard procedures.

***2.4.1Moisture content*:** This was estimated by placing 10 g of each sample in a pre-weighed crucible, carbonized in the oven for 5 – 8 h at 105 ºC. The samples were cooled in a desiccator and weighed using an electronic analytical balance. Carbonization was repeated until a steady weight is gained. The percentage moisture loss was estimated thus:

$$(\%)\text{Moisture} = \left(\frac{\{X2 - X3\}}{\{X2 - X1\}}\right) \times 100 \quad \text{equation (3)}$$

Where: X_1 = mass of empty crucible; X_2= mass of crucible + sample before drying
X_3= mass of crucible + sample after drying to constant mass.

2.4.2 Ash content: was determined by the method previously described (Unuofin *et al.*, 2017). About 2 g each of the samples was put in a crucible of known weight and heated at 550 °C using a muffle furnace until when the sample changes to white, or grayish-white. The ash was removed and weighed after cooling (AOAC 2006). Mathematically, ash content was measured as:

$$\% \text{ Ash } = \left(\frac{\{\text{Wy} - \text{Wz}\}}{\{\text{Wy} - \text{Wx}\}}\right) \times 100 \qquad \text{equation (4)}$$

***2.4.3 Crude fat content*:** Fat content was estimated by standard procedures of AOAC, (2006). Exactly 2 grams each of the samples were extracted with petroleum ether for 12 h. The sample was concentrated to about 50 % with a rotary evaporator and oven dried at 105 °C to a steady weight. The percentage of fat content was determined Kumari et al. (2017) according to equation (5).

$$\% \text{ Crude fat } = \left\{\frac{\text{W2} - \text{W1}}{\text{W3}}\right\} \text{x } 100 \qquad \text{equation (5)}$$

***2.4.4Crude protein*:** Crude protein content was estimated from the total organic nitrogen by the Macro-Kjeldhal method (Koyuncu et al., 2014). The total Nitrogen was determined using the conversion factor of 6.25. Approximately 2 g of the sample each was subjected to boiling in 10 ml of concentrated sulphuric acid with addition of selenium as catalyst. The process was performed in a fume cupboard until a clear ammonia digest is obtained. The distilled ammonia digest was obtained by mixing with 100 ml of distilled water and 10 ml of 45 % NaOH solution using a kjeldhal distillate apparatus. About 100 ml of 4 % boric acid solution and 3 drops of methyl red and bromocresol green were added until a green distillate solution was obtained. Exactly 50 ml of distillate was titrated against 0.02 N Sulphuric acid solutions until a deep red end point was observed. A reagent blank without the sample was titrated accordingly. Percentage nitrogen content was determined and percentage crude protein was estimated thus:

$$\% \text{ Crude protein } = \{\% \, Nitrogen\} \text{x } 100 \qquad \text{equation (6)}$$

***2.4.5Crude fibre content*:** Crude fibre was analyzed using the AOAC (2006) standard procedures. About 1g of the malted sample was soaked in 3 mL of H_2SO_4 and 17 ml of 1.25 % hot Sulphuric acid solution was added. The residue obtained after filtration was washed with hot water and 3 mL, 0.313 M Sodium hydroxide was added. Exactly 17.0 mL, 0.313 M Sodium hydroxide hot solution was also added. The mixture was placed in a shaker for 30 min and re-filtered gravitationally with No1Whatman filter paper. The residue was washed with 1 % HCl and boiling water simultaneously. The residue was then subjected to ethanol and ether wash and oven-dried at 100 °C to obtain constant weight. Fibre content was estimated thus: Abifarin *et al.* (2021).

$$\% \text{ Crude fibre } = \left\{\frac{\text{W2} - \text{W1}}{\text{W3}}\right\} \text{x } 100 \qquad \text{equation (7)}$$

***2.4.6Carbohydrate content*:** The carbohydrate content was calculated as:

$$\% \text{ Carbohydrate } = 100 - \{\text{A} + B + C + D + E2\} \qquad \text{equation (8)}$$

where A = Moisture, B = Protein, C = Fat, D = Ash, E_2 = Crude fibre

***2.4.7Preparation of caramel colour agent*:** The brown colour was performed by adding 100 g of sugar granules to 50 mls of distilled water and homogenized vigorously. The syrup obtained was heated in a water bath at 100 °C. Exactly 3mls of $(NH_4)_2SO_4$ was added to the boiling sample and stirred continuously until a steady brown colour was obtained. The sample was allowed to cool and kept for future use (Ceccaroni et al., 2019).

2.5Production of malt drink: This was performed according to the modified procedures depicted by Ceccaroni et al. (2019). About 500 L of water was added to brown rice grit and allowed to stand for 15 to 30 min. at temperature below 70 °C. The slurry obtained was allowed to stand for another 30 min. during which protein conversion to amino acid is achieved. After this, the aqueous layer was poured in a separate container and the concentrated layer was put in water bath until a jelly-like substance was achieved at temperature below 70 °C. The aqueous layer was later added after cooling and the mixture was allowed to stand for 30 min.to enable decomposition of beta-amylases. The temperature was adjusted to favour decomposition of alpha- amylases for another 30 minutes in the water bath and complete mashing process was achieved. The mashed sample was filtered using cheese cloth to separate the sweet wort from grain particles. About 60 g of sugar granules was

added to 1 L of the sweet wort and heated to boil for 1h. Exactly 0.5 g citric acid was added and the whole was repeated until a dark-brown coloured wort was achieved using the prepared caramel as standard. The malted sample was collected in a screw capped clean glass bottles (Ceccaroni et al., 2019; Mayer et al., 2014).

***2.5.1Assessment of malted sample*:** The quality of malted sample was performed in relation to density and pH of the samples using standards of Analytica Brewery Convention protocols (EBC, 2007).

2.6 Statistical Analysis

The results obtained was truly depicted as mean ± standard deviation and subjected to one way analysis of variance (ANOVA) where $P < 0.05$ was regarded as significant (Ceccaroni et al., 2019).

III. Results and Discussion

The germination parameters of the four brown rice cultivars studied are shown in Table 1 below. At 24 h the highest germinative energy recorded was from *O. glaberrima* B (43.0 ± 1.41^{a}) while *O. glaberrima* A had the lowest germinative capacity (20.0 ± 1.41^{d}). The germinative energy of all the cultivars studied increases with changes in temperature in the ranges 24 h < 48 h < 72 h. This against the value found in literature for milled rice which decreases in the range 21 h > 72 h > 120 h (Mayer et al., 2014). At 48 h, *O. glaberrima* B had the highest Germinative capacity (86.0 ± 1.41 %) with *O. glaberrima* type A having the lowest (56 ± 0.0 %) but at 72 h *O. glaberrima* C recorded the highest germinative energy (93 ± 1.41 %) while *O. glaberrima* D had the lowest (88 ± 0.0 %). However, the germinative capacities of all the cultivars studied at different temperature ranges were significantly different and in comparison with research conducted by (Ceccaroni et al., 2019). The germinative energy shows the amount of grains that will sprout under favourable and specified temperature level of about 20 ^{o}C. In this research, the sprouting temperature was at 24 ^{o}C which is most suitable for rice germination. However, the resulting germinative energy levels of the cultivars studied were below 96 percent which is the least ideal level for commercial malting brewing as reported in literatures. Hence, the tested samples can be sample trial for brewing malt (Mayer et al., 2014). The highest percentage dormancy obtained was from *Oryza glaberrima* D (12.0 ± 0.00 %*)* and the lowest were from *Oryza glaberrima* C (7.00 ± 1.414 %). *Oryza glaberrima* type A and *Oryza glaberrima* type B had 10.5 ± 0.71 % and 9 ± 1.41 % respectively. *Oryza glaberrima* type A had the highest malting loss (33.3 ± 0.14 %) while *Oryza glaberrima* type C had the lowest (24.1 ± 0.0 %). *Oryza glaberrima* type B and *Oryza glaberrima* type D had 75 ± 0.21 and 32.25 ± 0.07 respectively. The malting loss of the cultivar A and B are not significantly different, likewise that of C and D. *Oryza glaberrima* type C recorded the highest malting yield (80.3 ± 0.42 %) while *Oryza glaberrima* type A recorded the lowest (75 ± 2.83 %). *Oryza glaberrima* type B and *Oryza glaberrima* type D had 76 ± 0.28 % and 75.6 ± 0.28 % respectively. This is implicated in the malt yield content of all the samples which are relatively at the same level as this confirmed the report of Mayer et al., 2014.

***Proximate analysis of the unmalted and malted grains*:** The mean and standard deviation of the proximate analyses of unmalted and malted samples were shown in Table 2 and 3 respectively. The lowest crude protein content of 8.35 ± 0.07 % was recorded in *Oryza glaberrima* type A and highest observed in *Oryza glaberrima* type C was 9.05 ± 0.07 % with no significant ($p<0.05$) difference. This is also noticed in malted samples except with slight difference in range 8.35 - 8.95 %). This range (8.35 - 9.05 %) /(8.35 -- 8.95 %) are relatively higher than that of literature for paddy rice with low protein content (5.8 - 7.7 %) as documented (Mayer et al., 2014).The moisture content ranges from (11.0 -- 11.85 %) and (11.87 -- 12.15 %) for unmalted and malted samples respectively. These permitted the storage of the samples at low temperature as compared with that of (11.0 -- 14.1 %) reported for rice (Ceccaroni et al., 2019; Mayer et al., 2014). The foaming property, taste and fermentation of dietary yeast during brewing process have been implicated with relatively high protein content of brown rice studied and this confirmed their use in malting (Mayer et al., 2014).

There is trend in variation of crude fat content of the samples studied only with high content in malted cultivars. The lowest crude fat was obtained for *Oryza glaberrima* unmalted type A (0.91 %) and malted type C (2.18 %) which were not significantly different with that of unmalted type C (0.97 %) and malted type D (2.41 %) respectively. The fibre contents of both the malted and malted cultivars followed similar trend with highest crude fibre content of 1.25 ± 0.35 % in *Oryza glaberrima* type A and the lowest crude fibre content of 1 ± 0.00 % found in *Oryza glaberrima* type C. The ash content of *Oryza glaberrima* type C recorded the lowest ash content of 1.11 ± 0.15 %. The mean ash content recorded for the other grains were *Oryza glaberrima* type A (1.35 ± 0.07 %), *Oryza glaberrima* type B (1.58 ± 0.14 %) and *Oryza glaberrima* type D (1.15 ± 0.21 %) for unmalted samples. *Oryza glaberrima* type C recorded the lowest Ash content of 1.10 ± 0.15 % in malted sample and this is significantly different from that of type B (1.58 ± 0.14 %) been the highest value (Yankah et al., 2020). The highest range of carbohydrate content was recorded in unmalted rice (80.23 -- 82.47 %) compared while malted samples recorded lower range of (75.45 -- 75.95 %) and were not significantly different.

According to Kumar et al., (2016), the carbohydrates content for millet, maize and brown rice were 67.5 %, 74.3 % and 76.2 %, respectively. This is in comparison with the samples tested. Reduction in the carbohydrate content of the malted samples could be attributable to complete saccharification process which is an index for evaluating the potential of brewing malt (Aboagye et al., 2020; Mayer et al., 2014; Yankah et al., 2020).

Wort Quality: The wort quality of the malted rice samples were evaluated for pH value and density. It was observed that the samples have relatively low pH value range of 4.8 -- 5.96; the malted sample A is more acidic (pH 4.8) than malted sample B (pH 5.96). Low pH of malted samples have been reported to be relative to improved saccharification process, though these values were not in correlation with the ideal pH 8.17 of wort extract following standard Analytica European Brewery Convention procedures for assessing the quality of brewery malt (Ceccaroni et al., 2019; Yankah et al., 2020).

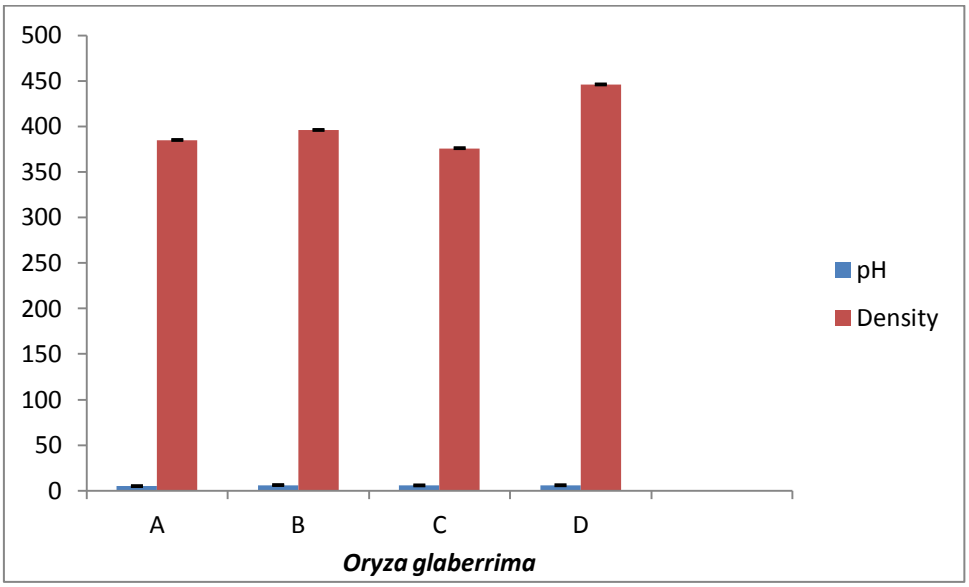

Fig.1: Mean value of wort quality of samples

Table 1: Malting Parameters of Selected Rice Grains

Values of the Means of Duplicate Determinations in the same column having same superscripts depicts not significantly different (P<0.05), Keys: % = Percentage, G.E = Germinative Energy, G.C= Germinative Capacity, D.M= Dormancy, M.L = Malt Loss; M.Y = Malt Yield

Cereal grains	N	% G.E 24 h	% G.E 48 h	% G.E 72 h	% D.M	% G.C	% M.L	% M.Y
O. glaberrima A	2	20.0 ±1.41^{d}	56.0 ±0.00^{d}	89 ±0.71^{c}	10.5 ±7.07^{a}	79 ±1.41^{b}	33.30 ±0.141^{a}	75.0 ±2.828^{b}
O. glaberrima B	2	43.0 ±1.41^{a}	86.0±1.41^{a}	91 ±1.41^{b}	9.00±1.414^{b}	82± 2.828^{a}	30.75 ±.212^{a}	76.5 ±0.283^{b}
O. glaberrima C	2	35.0 ±1.41^{b}	70.0 ±0.00^{b}	93 ±1.41^{a}	7.00±1.414^{b}	86± 2.828^{a}	24.10 ±.000^{b}	80.3± 0.424^{a}
O. glaberrima D	2	25.0 ±0.0^{c}	57.0±1.41^{c}	88 ±0.0^{d}	12.0±0.00^{a}	76±0.00^{b}	32.25±.071^{b}	75.6 ±0.283^{a}

Table 2: Proximate analysis of unmalted cultivars

Un-malted cultivars	N	% Moisture	% Protein	% Fat	% Crude fibre	% Ash	% CHO
O. glaberrima A	2	11.85±0.07[a]	8.85 ±0 .07[a]	0.91±0 .14[b]	1.25± 0.35[a]	1.34±0 .07[a]	80.60±0 .07[a]
O. glaberrima B		11.00±0.00[c]	9.04 ±0 .16[a]	0.98±0.01[a]	1.01±0.014[b]	1.57±0 .60[a]	80.23±0 .28[a]
O. glaberrima C	2	11.45±0.07[b]	9.05 ±0 .07[a]	0.97±0.03[b]	1.00 ±0 .00[c]	1.10± 0.15[b]	81.21±0 .07[a]
O. glaberrima D	2	11.85± 0.07[a]	8.96 ±0 .06[a]	0.99± 0.13[a]	1.03 ±0 .02[b]	1.15 ± 0.21[b]	82.47 ±0 .01[a]

Values of the Means of Duplicate Determinations in the same column having same superscripts depicts not significantly different (P<0.05), KEYS: N = number of grains analyzed, % =Percentage, CHO = Carbohydrate

Table 3: Proximate analysis of malted cultivars

Malted rice	N	% Moisture	% Protein	% Fat	% Crude fibre	% Ash	% CHO
O. glaberrima A	2	12.010 .09[a]	8.35 ±0 .07[a]	2.7 ±0.14[a]	1.25±0.35[a]	1.35±0 .07[a]	75.95 ±0 .07[a]
O. glaberrima B	2	11.87±0 .00[b]	8.41 ±0 .16[a]	2.40± 0.01[b]	1.01±0.014[b]	1.58±0 .14[a]	75.6 ±0 .28[a]
O. glaberrima C	2	12.15±0 .02[a]	8.95 ±0 .07[a]	2.18± 0.03[b]	1.00 ±0.00[c]	1.11± 0.15[b]	75.45 ±0 .07[a]
O. glaberrima D	2	12.2 ±0.07[a]	8.46 ±0 .06[a]	2.41± 0.13[b]	1.03±0 .02[b]	1.15± 0.21b	76.49 ±0 .01[a]

Values of the Means of Duplicate Determinations in the same column having same superscripts depicts not significantly different (P<0.05), KEYS: n = number of grains analysed, %=Percentage, CHO = Carbohydrate

IV. Conclusion

To the best of our knowledge the production of malt from four different local cultivars of brown rice was investigated for the first time. The result of this pilot study reveals the contingency for using 'Ofada' rice as potential brewing malts. Suggested evidences in literatures showed possibility of enhancing wort quality, improved saccharification as well as germinating energy of the rice malts obtained from this research and brewing an all-rice beer of acceptable quality in further study.

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Conflicts of Interest
None

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