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Amino acid profile and protein quality of starter cultures fermented African Yam bean (*Sphenostylis sternocarp*) seed condiment

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ABSTRACT

African yam bean seeds condiment is an unpopular nutritive local food seasoning compared to Iru and is mostly produced by natural fermentation. Production with a defined starter culture will give a novel functional condiment with enhanced nutritive values, especially amino acids, to prevent hidden hunger, shorten the fermentation time, and improve product uniformity. The effect of different monoculture bacterial starters and natural fermentation on the amino acids profile of condiments produced from African yam bean seeds was investigated. The result revealed a total of 18 amino acids with glutamic and aspartic acids as the most abundant amino acids in all the samples portions, with a range of values of 9.79-11.44 g/100 g protein and 9.20 -10.67 g/100 g protein respectively. The inoculated samples were rich in all the amino acids quality than un-inoculated. The total amino acid content of the samples was significantly different ($p \leq 0.05$). *Bacillus amyloliquefacien* starter fermented sample had the highest total amino acid value of 95.80 g/100 g protein while *Bacillus subtilis* fermented and un-inoculated seed portion samples had a total amino acid value of 86.81 g/100g and 74.04 g/100 g protein respectively. The essential amino acids (EAA_{10}) were significantly different ($p \leq 0.05$) and were in the range of 35.75-46.32 g/100 g protein with leucine, arginine, lysine, valine, isoleucine and phenylalanine the most abundant in inoculated and un-inoculated samples portions and were more concentrated in inoculated samples. Non-essential amino acids were slightly higher (38.29 - 49.47 g/100 g protein) than the total essential amino acids. The total acidic amino acids varied from 18.99 – 22.11 g/100 g protein and were significantly higher than the basic amino acids (12.88 – 15.95 g/100 g protein). The amino acid content of the *Bacillus amyloliquefacien* starter fermented sample was better enhanced than the sample fermented with *Bacillus subtilis* starter. The study concluded that controlled fermented condiments produced with *B. amyloliquefacien* culture boosted the amino acids value and profile.

Introduction

Contains a high amount of essential amino acids. Protein malnutrition is one of the acute results of deficiency in dietary protein in children foods. Leguminous plant seeds are fermented to produce food condiments, which are used as a flavouring agent for soups and sauces. African yam bean seed *(Sphenostylis sternocarpa)* is an annual crop that belongs to the leguminous family and sub-family of

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papilonacea sp. (Ihekoronye and Ngoddy, 1985). It is an underutilized leguminous crop grown mainly in West African countries like Nigeria, Cameroon, Cote d'Ivoire, Ghana, and Togo (Ihekoronye and Ngoddy, 1985). It is a vigorous, herbaceous, climbing vine reaching 1.5 m-2 m in height with trifoliate leaves with beautifully coloured flowers, which are about 2.5 cm long. The glabrous linear flat seed pod has raised margins and contains 20 to30 seeds, showing considerable variations in size and colour. The seed colour varies from white to various shades of cream, black, dark brown, grey and speckled grey. Some cultivars of African yam bean produce edible tubers, which may be up to 0.5 kg in weight and about 5 to 7 cm long, under the soil. African yam bean seed is a promising raw material for condiment production. Its nutritional composition is the same as of most edible legumes, such as soybean, locust bean seed and bambara nut seeds. Its nutritional composition has been reported to contain about 21.10% protein, 5.70% crude fibre, 74.10% carbohydrate, 3.20% ash, 8.5% moisture and 8.25% fat (Nnayelugo et al., 1995; Jeff-Agboola, 2007). Research studies on AYB seed have focused mainly on the utilization of the unfermented seeds as part of composite flour in confectionery industries (Azeke et al., 2005; Idowu, 2015; Inyang and Eyo, 2015; Okoye, 2017; Iwe et al., 2016; Yusufu et al., 2016). Food fermentation is an age-long practice, targeted at increasing the bioavailability of certain nutrients in food, such as amino acids and vitamins, as well as a reduction in the availability of antinutrients. Increased food digestibility and diversification, enhanced flavours and aromas are some of the important benefits associated with food fermentation. (McGovern et al., 2004; Fowomola and Akindahunsi, 2008; Jeyaram et al., 2009).

The daily consumption pattern of the greater portion of the Nigerian population goes with the intake of various fermented food products, which include condiments from leguminous seeds. Fermented leguminous seed products abound in many parts of the world and include Nigeria "dawadawa" or "iru" from African locust bean (Parkia biglobosa), ogiri from melon (*Citrullus vulgaris*) (Adedeji et al., 2017), ugba from African oil bean (*Pentaclethra macrophylla*) (Ahaotu et al., 2013; Olasupo et al., 2016), dawadawa-like condiment from Bambara groundnut (*Vigna subterranean L Verdc.*) (Akanni et al., 2018), soy-dawadawa from Soya bean (*Glycine max (L.) Merr*.) (Ezeokoli et al., 2016); Maari from Baobab seeds (*Adansonia digitata*) (Parkouda et al., 2015). These condiments form an important part of everyday local delicacies and provide an affordable and cheap source of plant proteins and calories for many low-income families. Condiments are characterized by the breakdown of amino acids, resulting in overtones of ammonia and its production is primarily done at the local household level, leading to non-uniformity in the final products, as well as attendant safety issues across the different producers. It is important to standardize the production of fermented food condiments from leguminous seeds by selecting appropriate microorganisms to be used as a starter culture for the reliable and reproducible fermentation process in order to increase the acceptability and to encourage the consumption of a cheap source of protein.

Well-defined molecular-typed starter cultures, mainly from *Bacillus* species for controlled fermentation of this product, will not only improve food safety and product consistency in terms of taste, quality, shortened production time, and improved marketability (Odunfa 1986), but will also enhance the bioavailability of amino acids content. Despite the high nutritive value of the African yam bean seed, there is still a dearth of information on the effect of starter cultures on the fermentation of African yam bean seeds for condiment production. This work is targeted at evaluating amino acids composition and protein quality of monoculture fermented condiments produced from African yam bean seeds under controlled conditions.

Materials and methods

Source of raw materials

African yam bean seeds *(Sphenostylis sternocarpa)*, used for this experiment, was purchased from the Isi gate market in Umuahia, Abia state. The seeds were characterized as varieties TSS-5 and TSS-45 at IITA, Ibadan. The seeds were collected in polythene bags, aerated for 24 hours and stored in the refrigerator (6± 2 °C) until use.

Bacterial culture characterization

Starter cultures (*B. amyloliquefacien, B. subtilis and B. siamensis*) used in this study were previously obtained from natural fermented AYB seeds. The isolates were maintained on a Tryptone soy agar (TSA; Oxoid CM131, Basingstoke, UK) slant in the refrigerator until use. These organisms were molecularly characterized with the techniques of sequencing of 16S rRNA gene, obtained from amplification of intergenic transcribed spacer gene region of 16S-23S rDNA (ITS-PCR), together with repetitive sequence-based PCR (rep-PCR). The sequence was subjected to a blasting program on the NCBI site for isolate identity.

Preparation and purification of starter inoculum

Preparation of starter culture was done according to the modified method of Omodara and Aderibigbe (2013). Agar slant stored pure culture of starter culture strain was inoculated on Tryptone Soy Agar (TSA; Oxoid CM131, Basingstoke, UK) plate and incubated overnight at 30 ± 2 °C. The *Bacillus* strains were sub-cultured in 50 ml of Tryptone Soy Broth (TSB; Oxoid CM129, Basingstoke, UK) in 100 ml conical flask and incubated at 30 ± 2 °C for 24 hours with an incubator shaker. The turbid cultures were centrifuged with a refrigerated centrifuge (Centurion, Scientific Ltd, UK) for 10 mins at the speed of 10,000 rpm. Cell pellets were harvested and re-suspended in 5 ml of 0.9% sterile normal saline (containing 9 g l^{-1} NaCl; pH 7.0). The number of viable cells in the resuspension was determined with a spectrophotometer (T60UV, PG Instruments Ltd, UK) at a wavelength of 540 nm and diluted to 0.5 McFarland standard to contain 1.5x 10^8 cells per ml. About 0.05 g broth/ g seed concentration of starter inoculum was used to inoculate the sterile AYB seeds.

Preparation of starter fermented condiment from AYB seeds

The modified method of Jeff-Agboola (2007) was used in the preparation of AYB seeds for starter culture fermentation. The seeds were thoroughly sorted to eliminate any contaminants and subsequently washed. The cleaned seeds were soaked in tap water (30 °C) for 6 h, dehulled, boiled for two hours, drained and mashed into a paste. Approximately 100 g of the wet paste was added into a 250 ml conical flask, each in four sample portions. The samples were sterilized at 121 °C for 15 minutes with an autoclave. The sterile samples were allowed to cool down to room temperature and thereafter, the first and the second portion were inoculated with broth culture of each of the two bacterial starters, at a concentration of 0.005 g broth/g seed containing 1.5 x 10^8 CFU/ml. The third portion was left uninoculated. The samples were fermented in a fermenter (incubator) at 30 ± 2 °C for 120 hours.

Evaluation of amino acid profile from AYB seed condiment

The determination of amino acids of the samples was conducted according to the standard method of AOAC (2006), as described by Oluwashina et al. (2019), using Applied Biosystems PTH Amino Acid Analyzer (Model 120A, Thermo Fisher Scientific, USA). The sample was dried to steady known weight at 70 °C with a hot air oven (Model NYC-101) and defatted with Soxhlet apparatus using chloroform/methanol (ratio of 2:1) for 15 hours. The defatted sample was hydrolyzed with 7ml of 6M HCL solution in the absence of oxygen, sealed in a glass ampoule and heated for 22 hours at 105 ± 5 °C in an oven. The glass ampoule was cooled, opened and humins removed from its hydrolyzed sample by filtration. The filtrate was then evaporated to dryness using a rotary evaporator and the residue was re-dissolved with 5ml to acetate buffer (pH 2.0). About 60 microlitres of the treated sample was loaded into the cartridge of the analyzer and analysed for the different classes of amino acids present in it.

Results

Table 1 shows the distribution of amino acid composition of two mono starter fermented and un-inoculated samples. The total amino acid composition from this study ranged from 74.04 to 95.80 g/100 g protein. The un-inoculated sample had the less amount of the total amino acid composition of 74.04 g/100 g protein. The high value indicates that the African yam bean seed is rich in protein. Generally, controlled microbial fermentation of leguminous seeds/nuts increases the bioavailability of amino acids. Methionine increased from 1.10 g/100 g crude protein in the un-inoculated sample to 1.65 g/100 g crude protein in *B. amyloliquefacien* fermented sample. Across the individual amino acid components, microbial fermentation enhanced their availability. The result of the total amino acids profile indicated that glutamic acid, aspartic acid followed by leucine, arginine, tyrosine, phenylalanine, glycine and lysine were the predominant amino acids components found in *Bacillus amyloliquefacien* fermented sample. The amino acids profile of the *Bacillus subtilis* fermented sample followed a similar trend with glutamic acid, aspartic acid, leucine, arginine, phenylalanine, glycine and lysine being the most predominant amino acids recorded. The amino acids concentration of the sample fermented with *Bacillus amyloliquefacien* were slightly higher than those obtained from *B. subtilis* fermented sample. The results from these starter inoculated samples agrees closely with the reports of Ibrahim et al. (2018), Nazidi et al. (2018), Adeyeye, (2011), Olaofe and Akintayo (2000) and Aremu et al. (2006) on amino acids composition of legumes and nuts. Similarly, Duodu et al. (2018) also recorded a rise in amino acid components of starter fermented samples from groundnut and cottonseed. The higher abundance of the amino acids components in the inoculated samples could be attributed to the proteolytic activities of these organisms on the seed substrate matrix leading to the breakdown of protein structures into amino acids. The un-inoculated sample revealed a seemingly good concentration of amino acids composition. However, the un-inoculated sample had a lower concentration of amino acids and this indicated that the starter cultures influenced the production of amino acid in African yam bean seed condiment. The concentration of amino acid in the un-inoculated sample was marginally close to the reported values from findings of Ibrahim et al. (2018) on condiments from *Hibiscus sabdariffa* seeds.

Table 2 revealed the concentration of the essential and non-essential amino acids composition of inoculated and un-inoculated African yam bean seed condiments. The result shows that essential amino acids from each of the mono starter fermented samples are better improved and have higher concentration values compare to the un-inoculated sample.

The *Bacillus amyloliquefacien* starter culture produced condiment sample has high values of leucine (8.78 g/100 g) and arginine (7.04 g/100 g). The sample also has threonine (3.77 g/100 g), valine (4.72 g/100 g), isoleucine (4.36 g/100 g), phenylalanine (5.76 g/100 g), histidine (3.34 g/100 g), lysine (5.55 g/100 g), methionine (1.65 g/100 g) and tryptophan (1.33 g/100 g) in appreciable levels. This result is in line with the observations by Nazidi et al. (2018) and Ibrahim et al. (2018). Amongst the two inoculated samples, the *Bacillus amyloliquefacien* starter culture produced condiment had the highest essential and non-essential amino acids concentrations.

Table 1. Amino acid composition in Bambara nut condiment

Amino acids	**Inoculated sample (g/100 g protein)**		**Un-inoculated sample**
	B. amyloliquefacein	***B. subtilis***	**(g/100 g protein)**
Leucine	8.78^{d}±0.01	7.79^{b}±0.00	7.04^{a}±0.00
lysine	5.55^{b}±0.01	5.56^{b}±0.00	4.65^{a}±0.00
Isoleucine	4.36^{c}±0.01	3.80^{a}±0.00	3.58^{a}±0.00
Phenylalanine	5.76^{c}±0.00	5.57^{b}±0.00	4.22^{a}±0.00
Tryptophan	1.33^{d}±0.01	1.23^{b}±0.00	1.00^{a}±0.00
Valine	4.72^{c}±0.01	4.19^{b}±0.00	3.10^{a}±0.00
Methionine	1.65^{c}±0.02	1.39^{b}±0.00	1.10^{a}±0.00
Proline	4.45^{c}±0.01	3.72^{a}±0.00	3.76^{b}±0.00
Arginine	7.04^{c}±0.00	6.95^{b}±0.00	6.14^{a}±0.00
Tyrosine	6.54^{c}±0.01	4.00^{b}±0.00	3.19^{a}±0.00
Histidine	3.34^{b}±0.01	3.44^{d}±0.00	2.09^{a}±0.00
Cystine	1.73^{c}±0.01	1.25^{b}±0.00	0.79^{a}±0.00
Alanine	4.74^{bc}±0.36	4.49^{ab}±0.00	4.07^{a}±0.00
Glutamic acid	11.44^{c}±0.02	10.82^{b}±0.00	9.79^{a}±0.00
Glycine	5.56^{b}±0.01	5.73^{d}±0.00	4.79^{a}±0.00
Threonine	3.77^{c}±0.01	3.48^{b}±0.00	2.83^{a}±0.00
Serine	4.32^{c}±0.01	3.35^{b}±0.00	2.70^{a}±0.00
Aspartic acid	10.67^{d}±0.01	10.05^{b}±0.00	9.20^{a}±0.00
Total Amino Acids	95.80^{c}±0.21	86.81^{b}±0.00	74.04^{a}±0.00
TEAA	46.32^{c}±0.06	43.40^{b}±0.00	35.75^{a}±0.00
TNEAA	49.47^{c}±0.27	43.41^{b}±0.00	38.29^{a}±0.00

Values with different superscripts along the same row are significantly different ($p \leq 0.05$).
TEAA= Total essential amino acids; TNEAA= Total non-essential amino acids

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Table 2. Essential and non-essential amino acid content of AYB seed mono starter fermented condiment

Amino acids	Inoculated sample (g/100 g protein)		Un-inoculated sample (g/100 g protein)
	B. amyloliquefacein	***B. subtilis***	
Essential Amino acids			
Leucine	8.78^{d}±0.01	7.79^{b}±0.00	7.04^{a}±0.00
Arginine	7.04^{c}±0.00	6.95^{b}±0.00	6.14^{a}±0.00
Phenylalanine	5.76^{c}±0.00	5.57^{b}±0.00	4.22^{a}±0.00
lysine	5.55^{b}±0.01	5.56^{b}±0.00	4.65^{a}±0.00
Valine	4.72^{c}±0.01	4.19^{b}±0.00	3.10^{a}±0.00
Isoleucine	4.36^{c}±0.01	3.80^{a}±0.00	3.58^{a}±0.00
Threonine	3.77^{c}±0.01	3.48^{b}±0.00	2.83^{a}±0.00
Histidine	3.34^{b}±0.01	3.44^{d}±0.00	2.09^{a}±0.00
Methionine	1.65^{c}±0.02	1.39^{b}±0.00	1.10^{a}±0.00
Tryptophan	1.33^{d}±0.01	1.23^{b}±0.00	1.00^{a}±0.00
Total EAA	**46.32^{c}±0.06**	**43.40^{b}±0.00**	**35.75^{a}±0.00**
Non-Essential Amino acids			
Glutamic acid	11.44^{c}±0.02	10.82^{b}±0.00	9.79^{a}±0.00
Aspartic acid	10.67^{d}±0.01	10.05^{b}±0.00	9.20^{a}±0.00
Tyrosine	6.54^{c}±0.01	4.00^{b}±0.00	3.19a±0.00
Glycine	5.56^{b}±0.01	5.73^{d}±0.00	4.79^{a}±0.00
Alanine	4.74^{bc}±0.36	4.49^{ab}±0.00	4.07^{a}±0.00
Proline	4.45^{c}±0.01	3.72^{a}±0.00	3.76^{b}±0.00
Serine	4.32^{c}±0.01	3.35^{b}±0.00	2.70^{a}±0.00
Cystine	1.73^{c}±0.01	1.25^{b}±0.00	0.79^{a}±0.00
Total NEAA	**49.47^{c}±0.27**	**43.41^{b}±0.00**	**38.29^{a}±0.00**

Values with different superscripts along each row are significantly different ($p \leq 0.05$).
TEAA= Total essential amino acids; TNEAA= Total non-essential amino acids.

This result also reflects the release of amino acids by different microorganisms since some strains of *Bacillus* bacteria have been shown to possess the ability to hydrolyse proteins found in their medium to liberate the amino acids.

The total essential amino acids (TEAA) concentration of the un-inoculated sample was 35.75 g/100 g protein This value of total essential amino acids concentration increased to 46.32 g/100 g protein in samples inoculated with *B. amyloliquefacien* and to 43.40 g/100 g crude protein for the *B. subtilis* culture inoculated sample after 120 hours of fermentation. The TEAA concentrations from inoculated samples in this study were slightly closer to the protein of 47.9 g/100 g, 46.1% and 43.6% reported Oshodi et al. (1993); Aremu et al. (2006) and Ogbuagu et al. (2018) respectively but far higher than observed TEAA values of 22.80 g/100 g and 25.80 g/100 g protein for un-inoculated and inoculated Opagha seed samples respectively reported by Afolabi et al. (2019). The values of TEAA from this study imply that the condiment is a comparable rich source of essential amino acids.

Table 3 displayed the different categories of amino acids composition in the un-inoculated and inoculated samples. The total non-essential amino acids were slightly higher than TEAA and ranged from 38.29 g/100 g crude protein in the un-inoculated sample to 49.47 g/100 g crude protein in *B. amyloliquefacien* fermented sample. These values are comparable with the results of 47.5 g/100 g - 55.42 g/100 g reported by Ogbuagu et al. (2018) and 32.9 g/100 g - 33.4 g/100 g by Oluwashina et al. (2019) for both un-inoculated and inoculated samples. The microbial fermented samples had a total acidic amino acids composition of 20.87 -22.11 g/100 g protein while the un-inoculated samples recorded a total acidic amino acid value of 18.99 g/100 g protein. The total acidic amino acids in the samples from this study were noted to be higher than the total basic amino acids composition (12.88 to 15.95 g/100 g protein), this indicated that the samples protein was likely acidic. The Sulphur containing amino acids recommended WHO standard value of 5.8 g/100 g protein was observed to be greatly higher than the scores recorded from this study for both inoculated and un-inoculated samples (1.89 g/100 g protein to 3.38 g/100 g protein). Total aromatic amino acids (10.80 - 26.51 g/100 g protein and 8.41 g/100 g protein) for inoculated and un-inoculated samples respectively were fairly above the suggested reference standard of 6.8 g/100 g protein for infants. The result of the different categories of the investigated amino acids in the present study compares favourably with the report of Ogbuagu et al. (2018) on African oil bean seed condiments. The starter fermented samples had a higher concentration of all the categories of amino acids than the un-inoculated sample. The result indicated that the bacterial cultures used in the control enhanced the liberation of the amino acids through their proteolytic activities. The quality of food protein is measured by comparing the essential amino acids content with the World Health Organization (WHO) reference standard for ideal protein based on the amino acids needed for children aged 2- 5 years (FAO/WHO/UNU, 1991). Table 4 presented the comparison of the essential amino acid value of starter inoculated and un-inoculated African yam bean seed condiments. The result indicated that the score values from inoculated samples for leucine (7.79% - 8.98%), phenylalanine + tyrosine (9.57% - 12.30%), valine (4.19% - 4.72%) and isoleucine (3.80%- 4.36%) recorded higher values than their respective reference standards.

The values for lysine (5.55% - 5.56%), tryptophan (1.23% - 1.33%) and threonine (3.48% - 3.77%) were marginally above the WHO reference limits. However, the value for methionine + cysteine contents (3.38 g/100 g protein for *B. amyloliquefacien* and 2.64 g/100 g protein *B. subtilis*) of the starter inoculated sample are higher than the recommended reference standard while the score from the un-inoculated sample was much lower than the reference standard. The result also indicated that only leucine (7.04%), phenylalanine + tyrosine (7.41%) and isoleucine (3.58%) essential amino acids from the un-inoculated sample were above the WHO standard reference limit. While the other essential amino acids were below the set reference limits for the respective standard. The result from this study followed the trend of similar reports for *Rhynchophorus phoenicis* (Adobeze and Enemor 2020), and African Oil Bean (*Pentaclethra macrophylla*, bent ham) Seed (Ogbuagu et al., 2018).

Table 3. Amino acid groups in African yam bean seed mono starter fermented condiment

Groups of Amino acids	**Inoculated sample (g/100 g protein)**		**Un-inoculated sample (g/100 g protein)**
	B. amyloliquefacein	***B. subtilis***	
Essential amino acid	46.32	43.40	35.75
Non-essential amino acid	49.47	43.41	38.29
Acidic amino acid	22.11	20.87	18.99
Basic amino acid	15.93	15.95	12.88
Aromatic amino acid	26.51	10.80	8.41
Aliphatic amino acid	32.61	29.25	26.34
Hydroxylic amino acid	8.09	6.83	5.53
Sulphur-containing amino acid	3.38	2.64	1.89

Table 4. Comparison of the essential amino acid composition of inoculated and un-inoculated African yam bean seed condiment with FAO/WHO/UNU reference values

Amino acids	Inoculated sample (g/100 g protein)		Un-inoculated sample (g/100 g protein)	FAO/WHO/UNU (1991) Ref. Value
	B. amyloliquefacein	***B. subtilis***		
Leucine	8.78	7.79	7.04	6.6
Arginine	7.04	6.95	6.14	--
Phenylalanine + Tyrosine	12.30	9.57	7.41	6.3
lysine	5.55	5.56	4.65	5.8
Valine	4.72	4.19	3.10	3.5
Isoleucine	4.36	3.80	3.58	2.8
Threonine	3.77	3.48	2.83	3.4
Histidine	3.34	3.44	2.09	--
Methionine + Cystine	3.38	2.64	1.89	2.5
Tryptophan	1.33	1.23	1.00	--

Conclusions

This study has shown that condiments with excellent values of amino acid composition can be produced adequately under controlled conditions with *B. amyloliquefacien*. African yam bean seed condiment could be considered as an ideal food that provides a wide range of total essential amino acids, acidic amino acids, and aromatic amino acids. The findings, however, revealed that amino acids are readily produced at an appreciable level during starter-assisted fermentation than in naturally fermented condiments. Monostarter fermented African yam bean seed condiment can be used as a food supplement thus addressing the incidence of protein malnutrition in many local families.

Author Contributions: Pius Ifeanyi Okolie: Conceptualized the project, Performed the HPLC analysis, Project Administration, Data Collection, Formal Analysis, Writing - Original manuscript draft preparation, review, and editing. Adewale Obadina: Project conceptualization, Supervision, Review, and editing. Martins E. Itohan and Emilymary Chima Okolie: Analyzed the experimental data. All authors read and approved the final manuscript.

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