EDITORIAL BOARD OF THE JOURNAL OF AGRICULTURE AND DEVELOPMENT

EDITORIAL SECRETARIAT

Contact information:
Nong Lam University
Room 404, Thien Ly Building
Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam
Tel: (84-28)37245670
Email: jad@hcmuaf.edu.vn

The Journal of Agriculture and Development
Volume 23 - Issue 3 (2024)
pISSN 2615-9503
eISSN 2615-949X

CONTENT

Identification of sanchi samples based on DNA barcodes

Dong T. Pham, & Phong V. Nguyen*
Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: April 10, 2023
Revised: August 21, 2023
Accepted: September 20, 2023

Keywords

DNA barcodes
ITS1
MatK
Phylogenetic tree
RbcL
TrnH-psbA

***Corresponding author**

Nguyen Vu Phong
Email:
nvphong@hcmuaf.edu.vn

ABSTRACT

For centuries, sanchi has been used in traditional medicine in East Asian countries to promote health and fitness. To identify and distinguish sanchi from adulterants, this study was conducted to determine the sequence of four DNA barcodes. The PCR products of the four barcodes were 330 bp (*rbcL*), 822 bp (*matK*), 484 bp (*trnH-psbA*), and 438 bp (ITS1). Genetic relationship analysis showed that the four DNA barcode regions had high similarity with other *Panax* species. Three barcodes, *matK*, *trnH-psbA*, and ITS1, confirmed that the studied samples belonged to *Panax notoginseng*, distinguished from *Panax pseudoginseng*, and can be used to identify *Panax notoginseng*.

Cited as: Pham, D. T., & Nguyen, P. V. (2024). Identification of sanchi samples based on DNA barcodes. *The Journal of Agriculture and Development* 23(3), 1-10.

1. Introduction

Sanchi (*Panax notoginseng* (Burkill) F. H. Chen) is a precious medicinal herb belonging to the Araliaceae family. It contains valuable compounds such as saponins, polyacetylenes, and essential amino acids that can inhibit and destroy tumor cells, exhibit antibacterial activity, and promote human health. *Panax notoginseng* is a well-known and widely used herbal medication in Asian nations. Compared with other species of the genus *Panax*, it has unique chemical components and medicinal value. Thanks to its potential therapeutic benefits not only for blood illnesses but also for other types of chronic human ailments, *Panax notoginseng* has attracted a lot of attention and interest (Xu et al., 2018). The morphological similarities between *Panax notoginseng* and *Panax pseudoginseng* have led to confusion in their nomenclature. More importantly, sanchi rhizomes can be confused with other medicinal rhizomes (Huynh et al.,

2021). Therefore, an effective authentication approach for medicinal plants, including sanchi, is essential for developing the herbal medicine industry and for protection from adulterants. Traditionally, authentication of herbs relies on morphological and histological inspections. In many cases, such as in the authentication of different *Panax* species, this approach is far from reliable (Ngan et al., 1999). Recently, molecular biology techniques have been applied to identify and analyze genetic diversity through molecular taxonomy. DNA barcoding, introduced in the mid-1990s, has been utilized to classify species within the Panax genus. Barcodes used located in the nuclear genome such as the internal trancribed spacer (ITS) region, 18S-rRNA; in mitochondria such as nad1 or chloroplast genome such as *matK*, *psbA-trnH*, *psbK-I*, *pspM-trnD*, *rps16*, *trnC-trnD*. The ITS and *psbA-trnH* regions showed more single-nucleotide polymorphisms and could be used for species identification and taxonomy of the *Panax* genus (Zuo et al., 2011). In 2017, Trang et al. used four chloroplast DNA regions, including *matK*, *rbcL*, and *rpoB*, and one nuclear DNA region ITS for authentic *Panax vietnamensis*. The results showed that *matK* and *rpoB* were suitable for identification at the species and subspecies levels. Although not all scientists have agreed upon universal plant DNA barcodes, most applications use the standard markers, *rbcL*, *matK*, *trnH-psbA*, and ITS (Yu et al., 2021). In this study, four DNA barcodes were used to identify and distinguish sanchi from potential substitutes or adulterants.

2. Materials and Methods

2.1. Sample collection

Sanchi plantlets were collected from Lam Dong province and identified based on the basis of their morphology.

2.2. DNA extraction

Total DNA from leaf samples was extracted according to the protocol described by Aboul-Maaty & Oraby (2019). 100 mg of chopped leaf samples were pureed with buffer (3% CTAB (w/v), 1.4 M NaCl, 0.8 M Tris-HCl pH 8.0, 0.5 M EDTA pH 8.0; 0.3% 2-β-mercaptoethanol). The mixture was incubated for 60 min at 65°C and denatured with chloroform: isoamyl alcohol (C: I, 24: 1). Centrifuge 13,000 rpm for 15 min at room temperature, collect the supernatant and add 0.5 V 6M NaCl, 0.1 V potassium acetate. DNA was precipitated with isopropanol for 60 min at -4°C. The precipitate was centrifuged, and the DNA was washed with cooled 70% ethanol, collect and store the DNA at -20°C. After extraction, the DNA quality was examined by electrophoresis on 1% agarose gel, and a spectrometer (Nanodrop).

2.3. PCR and DNA sequencing

The composition of PCR to amplify four DNA barcodes (rbcL, matK, trnH-psbA, and ITS1) is as follows: 8.6 L Mastermix (Thermo Scientific), 50 ng DNA, 0.2 M of each primer (rbcL-F: GAC AAC TGT GTG GAC CGA TG, rbcL-R: CCA CCG CGA AGA CAT TCA TA) (Kress & Erickson, 2007); (1R_KIM-f: ACC CAG TCC ATC TGG AAA TCT TGG TTC, 3F_KIM-r: CGT ACA GTA CTT TTG TGT TTA CGA G) (Kim, unpublished); (trnHf: CGC GCA TGG TGG ATT CAC AAT CC, psbA3'f: GTT ATG CAT GAA CGT AAT GCT C) (Kress et al., 2005); (ITS-p5: CCT TAT CAY TTA GAG GAA GGAG, ITS-p4: CCG CTT AKT GAT ATG CTT AAA) (Cheng et al., 2016), and PCR water for a final volume of 20 L. PCR reactions were carried out under the following conditions: initial denaturation at 94°C for 5 min; 35 cycles of 30 s at 94°C, 30 s at Ta°C, 60 s at 72°C, and finally 7 min at 72°C to complete the reaction.

The PCR products were visualized by 1% gel electrophoresis using a 1 kb ladder (Bioline, UK) to estimate the amplified length. The correct PCR products were purified and bidirectionally sequenced by Sanger methods at the 1st BASE (Malaysia).

2.4. Phylogenetic analysis

The nucleotide sequences of both DNA strands were verified and edited to obtain a consensus sequence using the BioEdit Sequence Alignment Editor and Chromas 2.6.6. The assembled sequences were aligned to the available sequences in the NCBI GenBank database to obtain accession numbers and used for subsequent analysis. The phylogenetic tree was constructed using Molecular Evolution Genetic Analysis (MEGA X) with the maximum likelihood algorithm from DNA sequences with a bootstrap value set at 1,000 replicates (Kumar et al., 2018).

3. Results and Discussion

3.1. Morphology

The rhizome is radish-like in shape, with fibrous strands around it. The stem was upright, unbranched, and smooth with a spongy core. Compound leaves consisted of verticillate leaves arranged around the top of the stem. The leaflets were serrated; the leaf blade was elliptical, thin, biconvex, and green; the tip had a pointed tail; and the base was wedge-shaped, narrow, and long. Scattered trichomes were observed in the veins of both leaf surfaces (Figure 1).

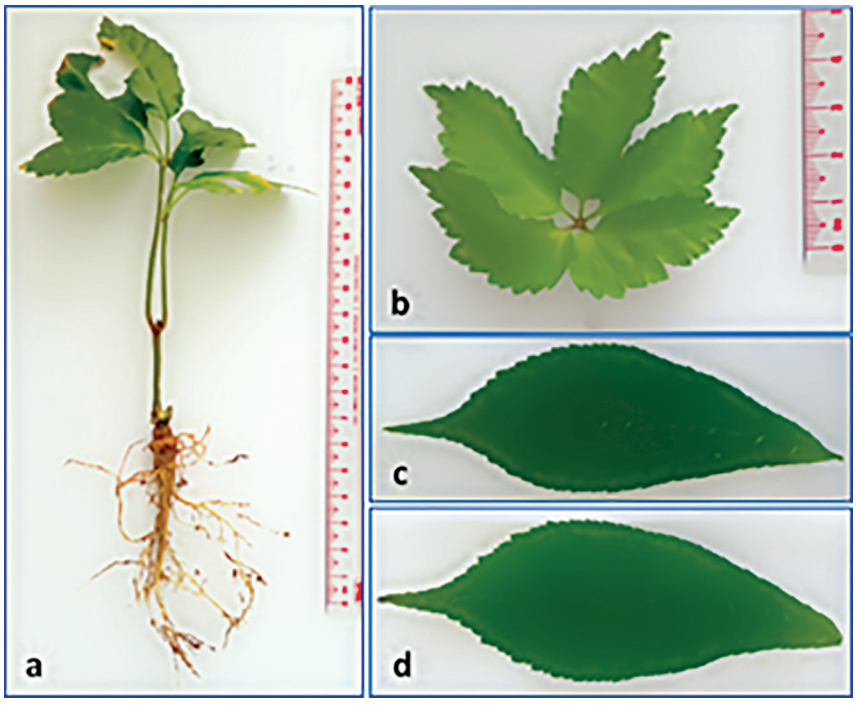

Figure 1. Sanchi sample. *a) Sanchi six months old; b) composite leaf; c-d) adaxial and abaxial sides.*

3.2. Amplify four regions of the DNA barcodes

The total DNA of the analyzed samples was extracted, quantified, and examined for purity. The results showed that the samples were purified with an OD 260/280 ratio of 1.89 - 1.97 and a concentration in the range of 48.8 ng/L - 380.1 ng/L. The finished product was clear and intact, and confirming its suitability for use in the PCR reaction (electrophoresis results are not shown). The results of the amplification of four DNA barcodes showed that the approximate product size was 400 bp for rbcL, 900 bp for matK, 500 bp for trnH - psbA, and in ITS1, which was close to the expected sequence. The PCR products were obtained, purified, and sequenced in two directions.

3.3. Nucleotide sequence analysis

Sequencing results were successful for all gene regions. The sizes of the products were 330 bp (*rbcL*), 822 bp (*matK*), 484 bp (*trnH-psbA*), and 438 bp (ITS1). BLAST results from NCBI showed 100% coverage and 99.7% to 100% similarity between the studied sequences and the corresponding *rbcL* gene sequences of *Panax* species; in the *matK* gene region were 99% and 93.79 - 93.30%, respectively; in the *trnH-psbA* gene region, 99 - 100% and 95.93 - 99.59%, respectively; and in the ITS1 gene region were 100% and 96.81% - 99.55%, respectively.

The studied *rbcL* region indicated a high similarity, reaching 100% similarity to sequences of all *Panax* species. The *rbcL* region is considered a universal highly conserved exhibiting and slow rate of evolution; therefore, it would only be used for classification at the genus level. The *matK* and *trnH-psbA* regions were highly similar to those of *P. notoginseng* (93.79% and 99.59%, respectively). The alignment results showed that the ITS1 sequence had the highest similarity (99.55%) to *P. notoginseng*, whereas *P. zingiberensis* showed the greatest difference (96.81%).

According to the rectification results, the research sample's *trnH - psbA* gene region contained a high similarity nucleotide to *P. notoginseng* (KP036468.1, South Korea), 3 different nucleotide positions and 5 gaps compared to *Panax* sp. 'sinensis', *P. quinquefolius*, *P. ginseng*, 7 different nucleotide positions from *P. wangianus*, *P. major*, *P. pseudoginseng* var. *elegantior*, *P. japonicus* var. *bipinnatifidus* and 9 - 10 nucleotide positions in *P. zingiberensis*, *P. vietnamensis* var. *fuscidiscus*, *P. vietnamensis*, *P. vietnamensis* var. *langbianesis* (Table 1).

In the ITS1 region, the research sample had a high nucleotide similarity to *P. notoginseng* (KP036468.1, South Korea) in the *trnH - psbA* region. There were nine nucleotide positions different from *P. wangianus*, *P. quinquefolius*, *P. japonicus* var. *japonicus*, *P.* sp 'sinensis, *P. ginseng*, *P. pseudoginseng* var. *bipinnatifidus*, *P. vietnamensis* and 10 - 12 positions different from *P. zingiberensis*, *P. japonicus*, *P. japonicus* var. *bipinnatifidus*, *P. variabilis*, *P. major*, respectively (Table 2). The diversity of nucleotides in the group of sanchi studied in Vietnam with other *Panax* species can be observed.

Table 1. Variable sites in *trnH - psbA* sequences from different *Panax* species

Species, Accession number / Nucleotide positions	140	147	188	232	235	250	271	272	276	280	281	289	389	405
Research sample	A	C	C	T	A	T	T	T	T	T	T	A	C	C
P. notoginseng KP036468.1														
P. wangianus MK408934.1		T		C		G	A	A				G	A	
P. major MW654097.1		T					A	A	A	A	A	G		
P. pseudoginseng var. *elegantior* NC062082.1		T					A	A	A	A	A	G		
P. japonicus var. *bipinnatifidus* OL543605.1		T					A	A	A	A	A	G		
P. zingiberensis MK408969.1		T		C		G	A	A	A	A	A	G		
P. vietnamensis var. *fuscidiscus* MT798587.1		T		C		G	A	A	A	A	A	G		
P. vietnamensis MF377623.1		T		C		G	A	A	A	A	A	G		T
P. vietnamensis var. *langbianensis* MT798584.1		T		C		G	A	A	A	A	A	G		T
Panax sp. 'sinensis' MK408967.1		T	A									G		
P. quinquefolius MK408953.1	T				G							G		
P. ginseng OL543607.1	T				G							G		

Table 2. Variable sites in internal transcribed spacer 1 sequences from different *Panax* species

Species, Accession number / Nucleotide positions	48	69	76	79	91	102	104	130	136	138	150	151	201	209	210	214	349
Studied sample	C	A	A	C	T	T	G	C		G	C	C	T	C	A	G	T
P. notoginseng MK408810.1	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
P. wangianus MK408801.1	T	C	C		C	C	A			A			C				C
P. quinquefolius MK408799.1	T	C			C	C	A			A		T	C				C
P. japonicus var. *japonicus* MH345184.1	T	C			C	C	A			A		T	C				C
P. sp. '*sinensis*' MK408796.1	T	C	C		C	C	A			A		T	C				C
P. ginseng KM207668.1	T	C			C	C	A			A	A	T	C				C
P. pseudoginseng var. *bipinnatifidus* AY271912.1	T	T		T	C	C	A					T	C			A	C
P. zingiberensis MH345201.1	T	C	C		C	C	A	Y	Y	R		T	C				C
P. vietnamensis KT380922.1	T	C			C	C	A					T	C		G		C
P. japonicus KJ740652.1		C	C		C	C	A			A		T	C		G		C
P. japonicus var. *bipinnatifidus* MZ149947.1	T	C	C		C	C	A			A		T	C				C
P. variabilis AY233330.1	T	C	C		C	C	A			A			C	T			C
P. major MH345179.1	T	C	C		C	C	A			A		T	C				C
P. japonicus var. *angustifolius* MH345153.1	T	C	C	T	C	C	A			A			C				C

3.4. Phylogenetic relationship

Phylogenetic trees of the studied samples and available sequences of *Panax* in GenBank were constructed using the MEGA X software with the Maximum Likelihood algorithm, and the bootstrap coefficient was set to 1.000. Results presented in Figures 2 - 5 showed that all species belonged to the same genus, *Panax*, and formed a distinct evolutionary clade. Additionally, they were found to be closely related to each other, with varying levels of support from bootstrap analysis. The clustering scores ranged from 64% for the *rbcL* gene region (Figure 2), 64% to 87% for the *matK* gene region (Figure 3), 52% to 96% for *trnH-psbA* (Figure 4), and 29% to 99% for ITS1 (Figure 5). Particularly for the *matK* gene region, there was clear branching of the research sequence group with 14 sequences of *Panax* species in GenBank (Figure 3). The phylogenetic tree also showed that the studied sequence group formed a separate clade with *P. notoginseng* compared to the *Panax* species in two gene regions: *trnH-psbA* and ITS1. These results show that the *trnH-psbA* and ITS1 DNA barcodes can be used to identify *Panax notoginseng*.

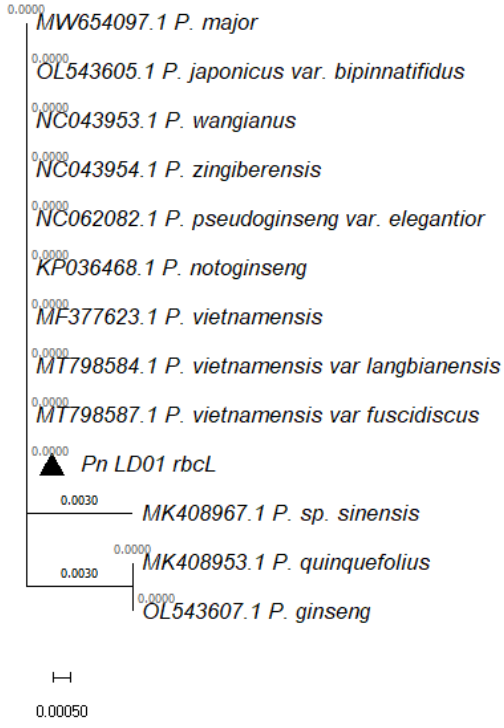

Figure 2. Phylogenetic relationship based on the *rbcL* sequence of the studied sample (Pn-LD01 *rbcL*) and 12 reference sequences with the Maximum Likelihood algorithm.

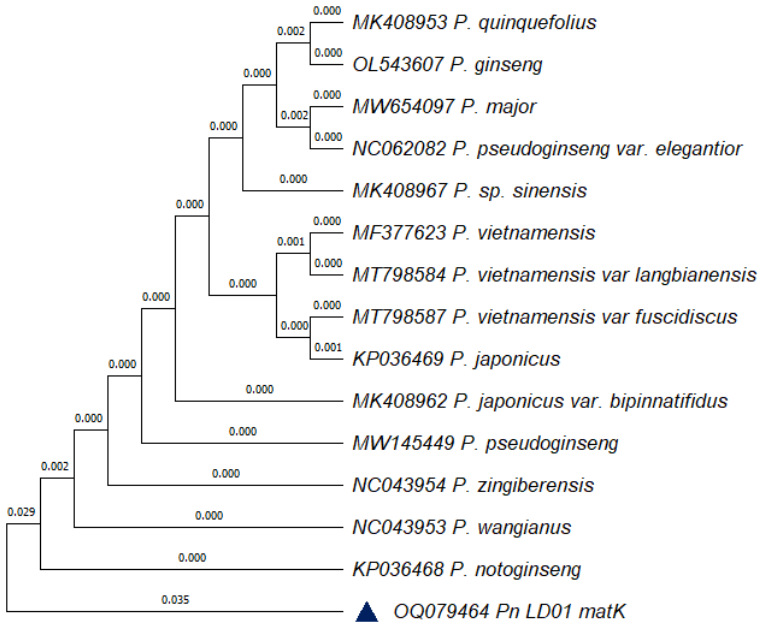

Figure 3. Phylogenetic relationship based on the *matK* sequence of the studied sample (Pn-LD01 *matK*) and 14 reference sequences with the Maximum Likelihood algorithm.

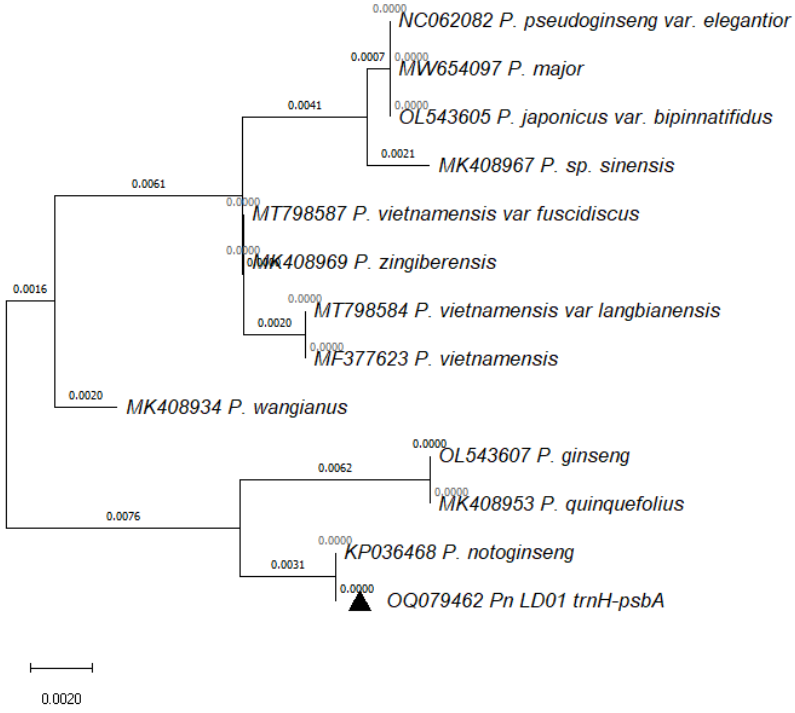

Figure 4. Phylogenetic relationship based on the *trnH-psbA* sequence of the studied sample (Pn-LD01 *trnH-psbA*) and 12 reference sequences with the Maximum Likelihood algorithm.

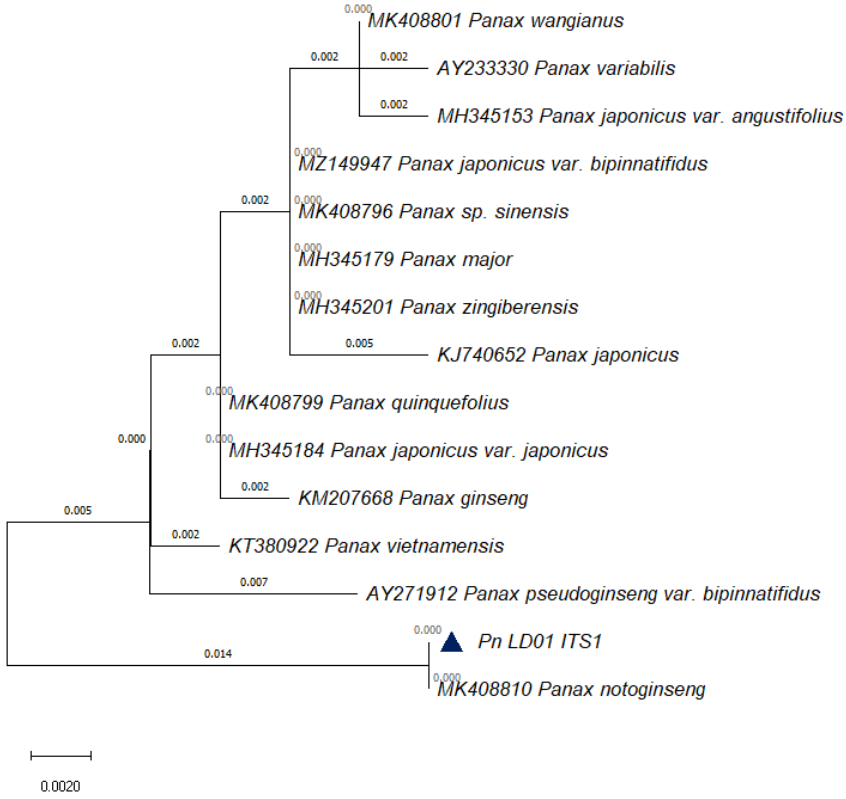

Figure 5. Phylogenetic relationship based on the internal transcribed spacer (ITS) 1 sequence of the studied sample (Pn-LD01 ITS1) and 14 reference sequences with the maximum likelihood algorithm.

The results proved the effectiveness of DNA barcodes in assessing the genetic relationships and species identification of *Panax*. The *trnH-psbA* and ITS1 sequences have almost identical nucleotide sequences in terms of the number of distinct nucleotides. However, compared with the *trnH-psbA* gene sequence, ITS1 displayed a larger differential nucleotide diversity. The *matK* region showed the greatest nucleotide difference. The four DNA barcodes displayed varying levels of differentiation, ranked from high to low as follows: *matK* > ITS1 > *trnH-psbA* > *rbcL*. This finding is consistent with the CBOL report on DNA barcoding in plants in 2009. These results provide a valuable database for further studies on this species.

4. Conclusions

The study successfully established a PCR procedure and sequenced four DNA barcode regions of the sanchi samples. The *matK, trnH-psbA*, and ITS1 sequences confirmed that the sanchi sample under study belonged to *Panax notoginseng*, distinguishing it from *Panax pseudoginseng* and other *Panax* species. These findings could be applied in the identification and discrimination of sanchi from adulterants.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

The authors gratefully acknowledge the support and encouragement of the Management Board of Nong Lam University (NLU). This research was funded by NLU under Grant No. CS-CB21-CNSH-01.

References

Aboul-Maaty, N. A. F., & Oraby, H. A. S. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre* 43(1), 1-10. https://doi.org/10.1186/s42269-019-0066-1.

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources* 16(1), 138-149. https://doi.org/10.1111/1755-0998.12438.

Huynh T. N., N. P. M., & Ho T. V. (2021). Design PCR primer for distinguishing between *Panax stipuleanatus* and *Panax notoginseng*. *Journal of Science, Technology, and Food* 21(2), 73-79.

Kress, W. J., & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS One* 2(6), e508. https://doi.org/10.1371/journal.pone.0000508.

Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of The National Academy of Sciences* 102(23), 8369-8374. https://doi.org/10.1073/pnas.0503123102.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6), 1547-1549. https://doi.org/10.1093/molbev/msy096.

Ngan, F., Shaw, P., But, P., & Wang, J. (1999). Molecular authentication of *Panax* species. *Phytochemistry* 50(5), 787-791. https://doi.org/10.1016/S0031-9422(98)00606-2.

Trang, N. T., Mai, N. T., Zhuravlev, Y. N., & Sciences. (2017). Application of DNA barcoding to authentic *Panax vietnamensis*. *American Scientific Research Journal for Engineering, Technology, and Science* 29(1), 60-67.

Xu, Y., Tan, H. Y., Li, S., Wang, N., & Feng, Y. (2018). *Panax notoginseng* for inflammation-related chronic diseases: a review on the modulations of multiple pathways. *The American Journal of Chinese Medicine* 46(05), 971-996. https://doi.org/10.1142/S0192415X18500519.

Yu, J., Wu, X., Liu, C., Newmaster, S., Ragupathy, S., & Kress, W. J. (2021). Progress in the use of DNA barcodes in the identification and classification of medicinal plants. *Ecotoxicology and Environmental Safety* 208, 111691. https://doi.org/10.1016/j.ecoenv.2020.111691.

Zuo, Y., Chen, Z., Kondo, K., Funamoto, T., Wen, J., & Zhou, S. (2011). DNA barcoding of *Panax* species. *Planta Medica* 77(2), 182-187. https://doi.org/10.1055/s-0030-1250166.

Investigation of small-scale farming status of Tire track eel (*Mastacembelus favus*) in the Mekong Delta, Vietnam

Nhi H. Y. Nguyen[1*], Hang T. T. Nguyen, Hanh T. B. Nguyen[2], Nguyen, Van T. T. Phan, Thang D. Tran, Man M. V. Ho, & Lan T. Trinh

[1]Department of Aquaculture, An Giang University, Vietnam National University Ho Chi Minh City, Vietnam

[2]Experimental and Practical Area, An Giang University, Vietnam National University Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: May 04, 2023

Revised: October 06, 2023

Accepted: October 12, 2023

Keywords

Feed and feeding

Mastacembelus favus

Mekong delta

Small-scale farming

Tire track eel

***Corresponding author**

Nguyen Huu Yen Nhi

Email:
nhynhi@agu.edu.vn

ABSTRACT

Tire track eel (*Mastacembelus favus*) is an economically important freshwater fish in the Mekong Delta. However, there is a lack of academic information about the current farming status, feed, and feeding related to this species in the region. Therefore, a field survey of small scale farming of tire track eel was conducted in An Giang, Dong Thap, Hau Giang and Kien Giang provinces of Vietnam. The objective of the survey was to determine the current farming practices information on households, especially the feed and feeding status of tire track eel in order to improve and develop a suitable feed for this fish in the future. The results showed that most of the small-scale farmers cultured fish in earthen ponds with or without plastic liners. Tire track eels were fed with feeds of other species and typically fed 3 - 5 times/d during the fingerling stage, and twice per day during the grow-out period. In terms of feed ingredients, crude protein contents in the feeds were 39.5 - 45.5% while crude lipid contents were 7.7 - 12.7%. Feed conversion ratios of tire track eels were 2 - 5. The farming periods of fish were 11.8 - 14 months when the fish reached the harvesting sizes of 318.2 - 421.4 g/fish, with the survival rates of 40 - 80%. It took 2.5 months to cultivate the fingerling stage (around 3 g/fish) from the fry with the survival rate of 55.7%. It was reported that this species was raised with a simple technique and got less illness compared to other fish species. However, the main reason of fish deaths during farming period related to management factors such as poor water quality due to decomposition of uneaten feed in water or lacks of dissolved oxygen due to high stocking density or power supply failures.

Cited as: Nguyen, N. H. Y., Nguyen, H. T. T., Nguyen, H. T. B., Phan, V. T. T., Tran, T. D., Ho, M. M. V., & Trinh, L. T. (2024). Investigation of small-scale farming status of Tire track eel (*Mastacembelus favus*) in the Mekong Delta, Vietnam. *The Journal of Agriculture and Development* 23(3), 11-22.

1. Introduction

Tire track eel (*Mastacembelusfavus*) is a native fish species that live mainly in freshwater but is also found in brackish water with low salinity (Pethiyagoda, 1991; Sokheng et al., 1999; Mongabay, 2020). In the wild, they are very widely distributed, at different habitats, from upstream to downstream, swampy areas, estuaries, or in riverbeds with fine or coarse sandy bottoms and places with thick vegetation (Ahmad et al., 2018; Jamaluddin et al., 2019; Jamaluddin et al., 2021). This is a hidden species, so they usually concentrate mainly on the bottom of flowing water bodies (canals, lakes) in the summer months or in flooded areas in the rainy season. They often bury themselves in the gravel bottom during the day and are active at night (Rainboth, 1996). Pethiyagoda (1991) reported that the maximum length of the tire track eel was 90 cm and the weight of 500 g. This fish has a slow growth rate. In the wild, one-year old tire track eels range from 150 - 250 g/fish in weight, 18 - 25 cm in length; while these parameters of two-year old fish fluctuated between 450 - 500 g/fish and 35 - 40 cm in weight and length, respectively. The natural spawning season of this fish in the Mekong river is the rainy season, from April to August every year, but mainly in June and July (Rainboth, 1996; Riede, 2004; Trieu, 2010). Tire track eel is an important food fish that is considered to be of high quality, contributing to local people's livelihoods and well-being in the Mekong Delta.

Tire track eel is a new culture fish species with high economic value, so many households in the Mekong Delta have been stocking it in form of small-scale farming. In the developing world, a small-scale or smallholder farm is a family-owned enterprise operating on up to 10 ha, or 24 acres, with most smallholder farmers cultivating less than 2 ha, or 5 acres, of land (Knight, 2022). The author also stated that small-scale farmers usually face challenges (e.g., related to their farm sizes, remote and rural locations), which hinder their ability to grow a prosperous business, while many lack the ability to access to credit, formal markets and high quality inputs like fingerling, farming equipment or medicine to maintain their animal healthy. In addition, there is a lack of academic information about the current farming status as well as the feed and feeding information of farming this species, thus this study has been conducted to provide some of this species and its farming information. The objectives of the study were to determine information on feed use, management skills, and techniques in raising tire track eels, as well as to examine advantages and disadvantages encountered in this farming. These will be basic useful information for farmers, local governments and researchers to do further research and improve the current system, and to develop suitable feeds for culturing this species with a more sustainability in the future.

2. Materials and Methods

2.1. Study sites

The study was carried out in An Giang, Dong Thap, Hau Giang, and Kien Giang provinces (Figure 1). These provinces were selected because they are the main Tire track eel farming areas in the Mekong Delta, Vietnam. Besides, these provinces have the specific agro-ecological characteristics of each location which form interlacing waterways and irrigation networks, providing a favourable environment for the agriculture and fishery sectors. Dong Thap is the upstream location of the Tien river and represents a wetland, with alkaline soil and agriculture with high production of fish. Hau Giang and An Giang are both located along the Hau river and produce large quantities of rice and fish. Kien Giang is one of the coastal provinces.

It has U Minh Thuong forest, one of the region contains peat swamp areas in the Mekong river basin which are important source of freshwater and fish for local consumption (Tran, 2016). The selected provinces are located in the lower basin of the Mekong Delta which distributes both branches of the Mekong river (Hau river and Tien river) and has both inland and coastal provinces of the Mekong Delta.

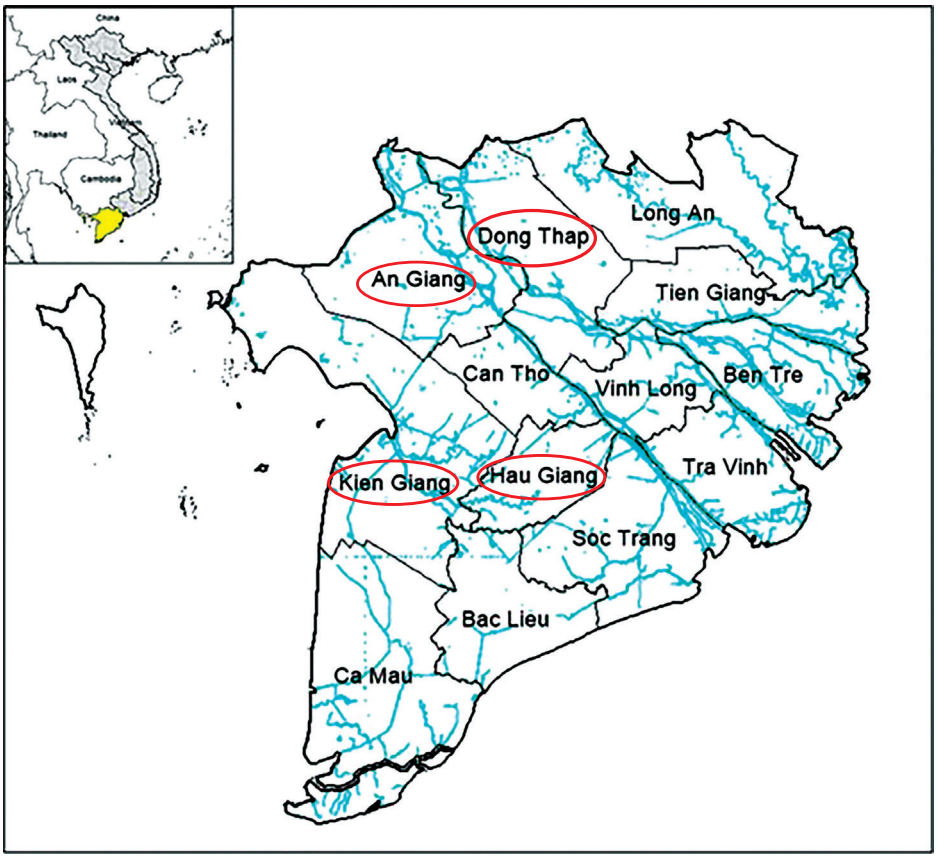

Figure 1. The provinces where the survey was conducted (Red circles).

2.2. Survey methods

Secondary data collection: annual reports and statistics on aquaculture as well as farming techniques of Tire track eel were collected from Department of Fisheries, Agricultural Extension Center, Statistical Department and Departments of Agriculture and Rural Development at the four surveying provinces. In addition, information related to the field of study is also collected from relevant websites. The secondary data collection was conducted before primary data collection.

The survey was carried out from April to November 2021. In total, ninety tire track eels farmers including 17 farmers in An Giang, 29 farmers in Dong Thap, 31 farmers Hau Giang and 13 farmers in Kien Giang were interviewed. The primary information was collected by observations, informal discussion and direct

interviews with farmers who were raising Tire track eel in these provinces, using a prepared questionnaire. The collected data included information on farming households, feed use; managements skills and techniques of raising Tire track eel; farming models; advantages and disadvantages encountered in the current farming models. Additionally, the feed used in the farming households were also sampled (5 samples/province) and analysed for nutritional compositions to evaluate the current status of feed use on farms.

2.3. Feed sampling and nutritional analysis

During the investigation, the feed samples (5 samples/province) were randomly collected from fish farms for nutritional analysis. The collected feed samples (between 200 g and 300 g) were analysed for moisture, protein, fat, carbohydrates (NFE), ash, fiber, energy, amino acids. Dry matters were determined by drying in an oven at 105°C until constant weights. Ash contents were determined by incineration of the sample at 550°C for 4 h. Crude proteins were calculated as 6.25 × % N analysed by the Kjeldahl method, ether extract (EE) were measured using the Soxhlet method and crude fibre (CF) contents were analysed using standard methods (AOAC, 2000). Amino acid contents were determined by high-performance liquid chromatography (HPLC) according to (Vázquez-Ortiz et al., 1995). Nitrogen-free extracts (NFE) were calculated according to methods described in AOAC (2000) as: (NFE = 100 - (%protein + %lipid + %fibre + %ash)). Gross energy contents (kcal/kg) were calculated according to (NRC, 1993), using values of 5.64, 9.44 and 4.11 kcal/g whole body for protein, lipid and carbohydrate, respectively.

2.4. Statistical analysis

The data were coded and analysed for descriptive statistics and analysis of variance using Microsoft Excel and Minitab (Version 16.0). Statistical analysis was conducted at 95% confident level ($P = 0.05$).

3. Results and Discussion

3.1. General information of farmers raising tire track eels

The interviewed tire track eel farmers were predominantly male and most of them owned the farms (Table 1). Most of them were over 30 years old with predominant from 30 - 50 years old. Males were responsibile for doing farm works.

Table 1. Educational backgrounds, sources of technological skills and other occupations of small scale fish farmers involved in the interview (Figures in table indicate % of the interviewees for each province) (n = 90)

Components	Descriptions	Provinces			
		An Giang	Dong Thap	Hau Giang	Kien Giang
Gender	Male	94.1	100	83.9	69.2
	Female	5.9	0.0	16.1	30.8
Year old	Under 30	11.8	6.9	0.0	0.0
	30 - 50	41.2	51.7	77.4	69.2
	Over 50	47.1	41.4	22.6	30.8
Role in farm	Owner	88.2	93.1	74.2	84.6
	Spouse of owners	0.0	0.0	19.4	0.0
	Child of owners	5.9	3.4	3.2	0.0
	Hired labor/technician	5.9	3.4	3.2	15.4
Education levels	Primary school (grades 1 - 5)	5.88	0	9.68	7.69
	Secondary school (grades 6 - 9)	52.9	51.7	71.0	69.2
	High school (grades 10 - 12)	0.00	20.7	9.68	7.69
	College, University	41.2	27.6	9.68	15.4
Technical skills learnt from	Own experiences	5.88	3.45	0.00	0.00
	Neighbors	88.2	75.9	38.7	61.5
	Workshops/training/school	0.00	0.00	9.68	0.00
	Media	5.88	13.8	45.2	7.69
	Neighbors & media	0.00	0.00	0.00	30.8
	Neighbors & training	0.00	6.90	6.45	0.00
Training (general)	Yes	11.8	6.9	74.2	76.9
	No	88.2	93.1	25.8	23.1
Living of the interviewees	Living in the farm	94.1	89.7	100	100
	Living in another place	5.9	10.3	0.0	0.0

In general, the educational levels of the interviewed farmers were relatively low with 5.56% and 61.1% at primary and secondary schools, respectively. The interviewees holding college or university degrees accounted for 22.2% and they lived mainly in An Giang and Dong Thap (Table 1). Most of the interviewed farmers directly lived in their farms to manage all farming activities.

Tire track eel is a new cultured species which was raised in farms recently after the artificial propagation of this species was succeeded (Loan, 2010; Trung, 2010), so most farmers did not have their own farming experiences. They learned farming techniques from each other, from their neighbors, from the mass media (e.g. YouTube, journals, newspapers, and so on), from Department of Agriculture and Rural development, agricultural extension station, production cooperative group, especially in An Giang and Dong Thap provinces.

3.2. Characteristics of the fish farms

Table 2. Type of fish farms in four different provinces in Vietnam (Figures in table indicate % of the interviewees for each province)

Descriptions	Provinces			
	An Giang	Dong Thap	Hau Giang	Kien Giang
Fingerling	17.7	13.8	61.3	0.00
Marketable sizes	64.7	72.4	32.3	69.2
Both fingerling and marketable sizes	11.76	3.45	3.23	0.00
Integrated with other species	5.88	10.34	3.23	30.8

Most of the interviewed farms raised marketable fish, especially in Dong Thap province with 72.4% farms (Table 2). Beside, some of farms raised the tire track eel in combination with other species such as freshwater prawn (*Macrobrachium rosenbergii*), marble goby (*Oxyeleotris marmoratus*), redtail botia (*Botia modesta*), siamese giant carp (*Catlocarpio siamensis*), and carp (*Cyclocheilichhthys enoplos*). Hau Giang was the province that has more fingerling farms involved in the interviews than other provinces.

Table 3. Types of raising facilities used in small-scale tire track eel farms in the surveyed provinces (Figures in table indicate % of all farmers within each province)

Descriptions	Provinces			
	An Giang	Dong Thap	Hau Giang	Kien Giang
Earthen ponds	64.7	86.2	9.68	76.9
Pond lined with PVC	11.8	10.3	19.4	0.00
Hapas in ponds	17.7	0.00	3.23	7.69
Hapas in rivers	5.88	0.00	0.00	0.00
Tanks lined with PVC	0.00	0.00	61.3	15.4
Cement tanks	0.00	3.45	3.23	0.00

Most of the farmers in An Giang, Dong Thap and Hau Giang raised marketable fish in earthen ponds while the farmers in Kien Giang reared fingerlings in tanks lined with PVC (Table 3). Some of the farms also used cement tanks to raise fingerlings.

Table 4. Farming areas (m^2) and stocking densities (fish/m^2) of hapas, tanks and ponds at different stages in small-scale farms in An Giang, Dong Thap, Hau Giang, and Kien Giang provinces

Fish period /Types of farming	Provinces							
	An Giang		Dong Thap		Hau Giang		Kien Giang	
	Area (m^2)	Stocking density (fish/m^2)	Area (m^2)	Stocking density (fish/m^2)	Area (m^2)	Stocking density (fish/m^2)	Area (m^2)	Stocking density (fish/m^2)
Fingerlings								
Earthen ponds	450	100	500	350	-	-	-	-
Cement tanks	20	200	-	-	200	225	-	-
Hapa-in- pond-systems	48	416	-	-	-	-	-	-
Ponds lined with PVC	-	-	-	-	400	200	-	-
Tanks lined with PVC	-	-	118	500	201	257	-	-
Fingerlings and marketable sizes								
Earthen ponds	8000	3	3000	2	-	-	-	-
Ponds lined with PVC	800	31	-	-	1500	25	-	-
Marketable sizes								
Earthen ponds	7543	3.21	2600	5.52	1067	7.83	4063	2
Ponds lined with PVC	-	-	200	15	575	28.75	100	30
Tanks lined with PVC	-	-	-	-	43	31.3	-	-
Hapas in river	80	13	-	-	-	-	-	-
Hapa-in- pond-systems	1525	6.5	-	-	-	-	200	5
Cement tanks	-	-	50	20	42	5.7	-	-
Marketable sizes with other species								
Pond lined with PVC	6500	7	-	-	-	-	-	-
Hapa-in- pond-systems	-	-	-	-	100	10	-	-
Earthen ponds	-	-	2667	11.3	2500	3	5000	1.95

Fingerlings of tire track eels were raised in small areas (20 - 500 m^2) with stocking densities from 100 - 400 fry/m^2 (Table 4). The marketable size tire track eels were cultured in several kinds of facilities such as earthen ponds, ponds lined with PVC, tanks lined with PVC, hapas in river or in ponds with their areas ranged from 100 - 8000 m^2. The stocking densities of marketable fish ranged between 2 - 31 fish/m^2. The stocking density was high in ponds with aeration.

Table 5. General information of the small-scale farming of marketable tire track eels in An Giang, Dong Thap, Hau Giang, and Kien Giang provinces

Descriptions	Provinces				SEM	*P*
	An Giang	Dong Thap	Hau Giang	Kien Giang		
Sizes of fingerlings (g/head)	4.2^{b}	2.7^{b}	3.2^{b}	22.1^{a}	1.09	0.001
Price of fingerlings (USD/head)	0.28^{b}	0.24^{b}	0.26^{b}	0.44^{a}	0.0	0.001
Culture periods (months)	12.8	13.6	11.8	14.0	2.1	0.335
Harvest sizes (g/head)	421^{a}	367^{ab}	318^{b}	394^{ab}	21.1	0.018
Selling price of fish (USD/kg)	11.8^{a}	8.91^{b}	12.1^{a}	11.7^{a}	0.4	0.001
Survival rates (%)	70^{a}	40^{b}	80^{a}	70^{a}	6.0	0.001

Values are given as LS means.

Means with different superscript letters within rows are significantly different (P < 0.05).

The tire track eels in marketable farms were stocked at the sizes of 2.7 - 22.1 g/head (Table 5). The sizes of fingerlings were similar in all surveyed provinces except Kien Giang. The price of fingerlings was 0.24 - 0.44 USD/head. The price of fingerlings in Kien Giang province was the highest among the surveyed provinces. The main reason for the hishest price could be that the farmers in Kien Giang province often stocked bigger size fingerlings compared to those of other provinces.

It often took 12 - 14 months to grow tire track eels to marketable sizes and was similar in all provinces, although Kien Giang province had larger fingerling and longer culturing time but harvesting sizes are almost similar to that of the other provinces. It can be effected by ecological environment conditions in Kien Giang, is one of the coastal provinces, not located directly on the main braches of Mekong river which can effect to the culture environment.

The harvesting sizes ranged from 318 - 421 g/head with the highest size in An Giang and lowest size in Hau Giang. Dong Thap sold the fish with the lowest price among the four provinces, that may be related with an abundance of wild fish for consuming in this area.

The survival rates of tire track eels ranged from 40 - 80%, in which the survival rates of fish in Dong Thap was the lowest. The average survival rate and rearing period from fry to fingerlings were 55.7% and 2.5 months, respectively. The average selling price of fingerling was approximately USD 0.23/head (around 3 g). The fingerling price of this fish is always higher than other fish species in the Mekong Delta.

3.3. Feeds and feeding

Tire track eels were typically fed twice per day (8:00 in the morning and 18:00 in the afternoon) during the grow out period, and 3 - 5 times per day during the fingerling stages. Feeds used in Tire track eel farming were mainly commercial pellets produced typically for other fish species such as snakehead fish, shrimp, barramundi, etc. Feeds were added with 40% water to soften for about an hour. Then vitamin C, probiotics, binder were added and shaped into balls with the average weights of 100 g. Besides, some farms also added earthworms, golden apple snails into the feed balls. This feed balls were put into feeding trays with feeding ratios of 3 - 10% the fresh fish body weights. This feeding strategy would raise environmental concerns. It can cause a negative affect on the farming system because of its difficulty in manage the feed. The feed balls were in a moist form, and feed ingredients would easily dissolve into the water, affecting the farming process. This practice needs improvement to make the fish farming more sustainable.

Results from chemical composition analysis show that the feeds used in different provinces significantly varied in their contents (Table 6). Because the farmers used various feed types, brands, and also added with different ingredients. The crude protein and crude lipid contents in feeds were 39.5 - 45.5% and 7.7 - 12.7%, respectively (Table 6). Feed conversion rate for grow-out periods of tire track eels ranged from 2 to 5.

Table 6. Chemical compositions of different compound feeds for tire track eels in An Giang, Dong Thap, Hau Giang, and Kien Giang provinces (5 samples/province)

Chemical compositions (%)	Provinces							
	An Giang		Dong Thap		Hau Giang		Kien Giang	
	Median	Range	Median	Range	Median	Range	Median	Range
Dry matters	91.2	89.7 - 91.6	90.1	89.3 - 92.1	92.6	92.1 - 93.1	90.3	89.8 - 91.1
Crude proteins	42.4	40.1 - 44.9	40.1	39.5 - 42.2	44.2	42.1 - 45.5	42.7	40.1 - 45.2
Lipids	10.2	7.7 - 10.5	10.3	8.0 - 11.8	11.8	9.6 - 12.1	11.1	9.1 - 12.7
Ashes	14.2	11.6 - 15.1	14.1	12.3 - 14.7	13.0	12.1 - 15.4	15.2	13.9 - 15.3
Crude fibres	3.7	3.1 - 4.6	4.2	3.1 - 5.6	3.3	2.8 - 4.4	2.3	2.0 - 3.9
Amino acids (g/kg)								
Arginin	56.1	44.4 - 60.1	46.7	42.6 - 59.7	62.5	43.6 - 63.7	60.4	44.2 - 62.1
Alanine	24.6	20.3 - 24.7	21.8	20.2 - 24.9	24.5	20.1 - 25.2	21.0	20.4 - 24.4
Isoleucine	17.6	16.9 - 21.5	20.1	17.8 - 10.6	22.1	19.4 - 23.1	20.6	17.2 - 20.8
Leucine	34.8	30.2 - 35.8	34.4	30.8 - 35.9	32.8	31.4 - 34.7	31.3	30.9 - 35.2
Lysine	21.7	20.6 - 25.8	23.4	20.9 - 31.0	29.6	20.7 - 30.5	26.7	20.4 - 28.1
Methionine	7.7	6.6 - 8.2	8.3	5.1 - 9.7	7.2	6.2 - 8.3	8.1	6.2 - 8.2
Phenillalanin	21.2	18.8 - 24.1	22.4	19.7 - 23.5	20.9	19.7 - 24.3	19.9	17.9 - 22.8
Threonine	14.8	14.2 - 19.4	19.9	14.5 - 20.1	16.2	15.3 - 18.9	13.7	13.1 - 18.9
Tryptophan	5.2	5.0 - 5.9	5.9	5.1 - 6.1	5.6	5.2 - 6.1	5.6	5.0 - 6.2
Valin	25.5	19.3 - 26.2	24.2	19.3 - 25.7	23.6	19.0 - 25.1	23.6	19.8 - 23.9
Cystin	24.2	10.1 - 25.9	18.3	12.6 - 19.5	23.5	12.6 - 14.2	16.0	12.0 - 25.9
Tyrosin	10.7	10.2 - 16.5	13.6	12.1 - 15.4	13.6	10.2 - 15.4	15.2	10.5 - 16.5

The chemical compositions of feed from this research showed that the farmers used high protein feeds to raise Tire track eel (from 39.5 - 45.5%) and made the feeds with a high cost. This concern needs to be solved by producing feeds for a more sustainable farming of this species in the future.

3.4. Managements the fish ponds

Many materials such as PVC tubes, chicken nets, water hyacinths, bamboo branches and nylon ropes were used as habitats for tire track eels in the pond system.

Sludge accumulated in the fish ponds was not removed during throughout each crop. Instead, the farmers used commercial probiotic products containing Bacilllus to treat the pond bottom environment for maintaining good water quality.

The water management process in Tire track eel ponds were done as the normal farms in Mekong Delta (Phan et al., 2009) that the water in the culture systems was exchanged at infrequent intervals ranging from daily to once a week. The rate of exchange at any one time ranged from 10 to 30% replenishment to ensure that all water parameters were always within the appropriate ranges for the normal growth of fish.

3.5. Advantages and challenges in farming tire track eels

The survey results show that the farmers were easy to sell their fish production. Culturing Tire track eel can give them high profit because they have high price and high market demand. In addition, the farmers reported that this species highly tolerated to the culture environment, as so obtaining high survival rate, well adapted to local natural conditions, and then the fish grows well; it can be integrated with other species to increase economic efficiency on the same farming area.

Besides, the farmers' opinion indicated that Tire track eel was one of the species for simple farming techniques, and it got less disease infection compared to other fish species raised in these provinces, such as pangasius, tilapia, snakehead fish, gourami, climbing perch, shrimp, etc. Fish only encountered a few diseases (e.g., skin, parasitic, intestinal diseases, fungus in gills, etc.), especially at small-size stages when the weather changes (e.g. rain, monsoon, etc.) or feed changes. However, the main reason of the fish deaths during farming period was mainly due to management factors such as poor water quality caused by decompositions of uneaten feed, low dissolved oxygen at high stocking density or power supply failures.

Infact, the moist feeds used in this farming would cause some water quality problems. This practice requires improvement in the future for the sustainable development of tire track eel farming.

4. Conclusions

Most of the small-scale farmers of tire track eel (*Mastacembelus favus*) were carried out in earthen pond covered with or without PVC liners, which developed and operated by self-learned experience and from neighbours' knowledge.

Fish were typically fed twice per day during the grow-out periods, and 3 - 5 times per day during the fingerling stages.

High nutrient feeds were used, specifically the crude protein and crude lipid contents in feeds were 39.5 - 45.5% and 7.7 - 12.7%, respectively. It was not a good practice while farmers used commercial pellets for other fish species such as snack head fish, shrimp, barramundi, etc to feed the tire track eel. Additionally, the current feeding strategy, which was the dry pellets were soaked with water to make large moist feed balls before feeding the fish, which needs improvement

because it would cause poor water quality.

Feed conversion rates for culturing growing-out tire track eels ranged from 2 to 5.

Commercial farming periods of tire track eels were from 11.8 - 14 months. The harvest sizes were from 318.2 - 421.4 g/head and the survival rates were from 40 - 80%.

The fingerlings were cultured around 2.5 months from the fry with a survival rate of 55.7% and were sold for USD 0.23/head (around 3 g/head).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was funded by Vietnam National University Ho Chi Minh City (VNU-HCM) under grant number B2021-16-01.

References

Ahmad, A. B., Zaini, N., Nayan, N., Fahmi-Ahmad, M., Rizal, S. A., & Yusuf, Y. (2018). Freshwater fish diversity of Sungai Setiu, Terengganu, Peninsular Malaysia. *Malayan Nature Journal* 70(4), 499-507.

AOAC (The Association of Official Analytical Chemists). (2000). *Official methods of analysis of AOAC international* (17th ed). Maryland, USA: The Association of Official Analytical Chemists.

Jamaluddin, J. A. F., So, N., Tam, B. M., Ahmad, A., Grudpan, C., Md Sah, A. S. R., & Mohd Nor, S. A. (2021). Genetic diversity of the tire track eel *Mastacembelus favus* in Southeast Asia inferred from microsatellite markers. *Ichthyological Research* 68, 529-540. https://doi.org/10.1007/s10228-021-00807-4.

Jamaluddin, J. A. F., So, N., Tam, B. M., Ahmad, A., Grudpan, C., Page, L. M., Khaironizam, Md. Z., & Mohd Nor, S. A. (2019). Genetic variation, demographic history and phylogeography of tire track eel, *Mastacembelus favus* (Synbranchiformes: Mastacembelidae) in Southeast Asia. *Hydrobiologia* 838(1), 163-182. https://doi.org/10.1007/s10750-019-03987-3.

Knight, A. (2022). What is a smallholder farmer? *Heifer International.* Food and Waste, Retrieved December 15, 2022, from https://www.heifer.org/blog/what-is-a-smallholder-farmer.html.

Loan, P. P. (2010). *Developing an artificial seed production process for Tire track eels (Mastacembelus favus) fish in An Giang* (research report). An Giang Department of Science and Technology, An Giang, Vietnam.

Mongabay. (2020). *Mastacembelidae* (Spiny eel) family. *Tropical fish*. Retrieved from April 12, 2020, from https://fish.mongabay.com/mastacembelidae.htm.

NRC (National Research Council). (1993). *Nutrient requirements of fish*. Washington, USA: National Academy Press.

Pethiyagoda, R. (1991). *Freshwater fishes of Sri Lanka*. Colombo, Sri Lanka: Wildlife Heritage Trust of Sri Lanka.

Phan, L. T., Bui, T. M., Nguyen, T. T., Gooley, G. J., Ingram, B. A., Nguyen, H. V., & De Silva, S. S. (2009). Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. *Aquaculture* 296(3-4), 227-236.

Rainboth, W. J. (1996). *Fishes of the cambodian mekong*. Wisconsin, USA: Food & Agriculture Organization of the United Nations.

Riede, K. (2004). *Global register of migratory species: from global to regional scales: final report of the R&D-Projekt 808 05 081*. Bonn, Germany: Federal Agency for Nature Conservation.

Sokheng, C., Chhea, C. K., Viravong, S., Bouakhamvongsa, K., Suntornratana, U.,

Yoorong, Nguyen, T. T., Tran, B. Q., Poulsen, A. F., & Jorgensen, J. V. (1999). Fish migrations and spawning habits in the Mekong mainstream: a survey using local knowledge (basin-wide). Assessment of Mekong fisheries: Fish migrations and spawning and the impact of water management project (AMFC). *AMFP Report* 2, 99.

Tran, T. (2016). *U Minh peat swamp forest: Mekong river basin (Vietnam)*. New York, USA: Springer. https://doi.org/10.1007/978-94-007-6173-5_174-4.

Trieu, N. V. (2010). Study on biological characteristics of Mastacembelus armatus. *Can Tho University Journal of Science* 2010(15b), 70-80.

Trung, N. T. (2010). *Artificial propagation of tire track eel (Mastacembelus favus) (*research report*)*. Can Tho Department of Science and Technology, Can Tho, Vietnam.

Vázquez-Ortiz, F., Caire, G., Higuera-Ciapara, I., & Hernández, G. (1995). High performance liquid chromatographic determination of free amino acids in shrimp. *Journal of Liquid Chromatography and Related Technologies* 18(10), 2059-2068.

Snail composition and its cercariae in rice field of Hoc Mon district, Ho Chi Minh City, Vietnam

Thien C. Pham*, & Quyen T. Duong

Department of Biology, Ho Chi Minh City University of Education, Vietnam

ARTICLE INFO

Research Paper

Received: May 17, 2023

Revised: October 05, 2023

Accepted: November 20, 2023

Keywords

Cercariae

Hoc mon

Rice field

Snails

***Corresponding author**

Pham Cu Thien

Email:
thienpc@hcmue.edu.vn

ABSTRACT

The research on snail composition and their cercariae by morphological analysis method in Xuan Thoi Thuong rice field of Hoc Mon district, Ho Chi Minh City, Vietnam was carried out in the wet season of August 2022 and the dry season of February 2023. A total of 993 snails were collected and 11 snail species belonging to 9 genera and 5 families were classified. There were 9 snail species collected in the wet season and 7 snail species found in the dry season. *Lymnaea viridis* and *Bithynia siamensis* were infected with trematode (cercariae stage) with the combined prevalence of 14.3% and 4.1%, respectively. The other nine snail species had cercariae free. Two cercariae morphotypes were discovered from snails including *Xiphidio cercariae* and *Echinostome cercariae*. More research on snails and their cercariae in other waterbodies should be done in Hoc Mon district and other places to identify the snail diversity and sources of trematodes affecting fish culture and human health.

Cited as: Pham, T. C., & Duong, T. Q. (2024).. Snail composition and its cercariae in rice field of Hoc Mon district, Ho Chi Minh City, Vietnam. *The Journal of Agriculture and Development* 23(3), 23-31.

1. Introduction

Hoc Mon is a suburb district of Ho Chi Minh City where rice cultivation still developed well with the total rice field area of 1,583.80 ha (PCHCMC, 2020). Pham et al. (2019) found that silver barb from fishing in Thay Cai - An Ha canal in Hoc Mon district was infected with metacercariae of *Haplorchis pumilio* with the prevalence of 80.0%. The interview's information from the farmers was that all silver barbs were mainly in rice fields where snails were available and just followed the water stream to get into the canal. The source of metacercariae in silver barb was questioned whether cercariae existed in snails in rice field and infected or not.

The study on trematode is necessary because foodborne trematodiasis is an emerging public health problem (Keiser & Utzinger, 2005). Little is known about the clinical importance of infections with minute intestinal flukes, but heavy infections can cause serious gastrointestinal symptoms (Nawa et al., 2005). Infections have significant human-health impact and cause substantial clinical or subclinical disease for small liver flukes (Dorny et al., 2009). The life cycles of intestinal flukes and liver flukes are similar (Yu & Xu, 2005). The heterophyid digeneans have a three-host life cycle involving snails as the first intermediate host, fish as the second intermediate host, and fish-eating animals and humans as the definitive host (Elsheikha & Elshazly, 2008).

In the natural condition, cercariae are released from snails then swim freely in the water and usually attract to the second intermediate hosts like fish, crabs, aquatic plants, etc (Thai, 2016). In a shedding experiment, cercariae emerge from infected snails during day and night, but more commonly in the morning and before noon. They are usually found in the lower half of a container with water (Saad, 1994). Cercariae remain active for 12 - 24 h and most die after 72 h. For cercariae of small liver flukes and intestinal flukes, they are free in the water, attract to fish and penetrate the fish's tissue within 5 - 10 min (Long-Qi et al., 2005).

The snail intermediate hosts for the heterophyid trematode species are primarily species of the Thiaridae and Bithynidae (Madsen & Hung, 2014). *Melanoides tuberculata*, *Thiara* and *Terabia granifera* are the first intermediate hosts of heterophyids (Waikagul & Radomyos, 2005). *Melanoides tuberculata* is the host of *Haplorchis pumilio* (Khalifa et al. 1977; Wang et al., 2002; Dechruksa et al., 2007) and *Thiara granifera* was found commonly infected with *H. pumilio* in Taiwan (Wang et al., 2002).

Bui et al. (2010) stated that there were 10 snail species in rice fields in Nam Dinh province, and most of snails in the families of Bithyniidae, Stenothyridae and Planorbidae dominated in rice fields. Nguyen et al. (2014) carried out research in An Hoa rice field of Tuy An district, Phu Yen province and found 9 snail species and *Melanoides tuberculata*, *Sermyla tornatella* and *Bithynia* sp. were found infected. In rice fields of Binh Khanh and Ly Nhon communes, Can Gio district of Ho Chi Minh city, Nguyen & Pham (2022) collected 8 snail species and discovered cercariae from *Bithinya* sp. and *Melanoides tuberculata* including xiphidio cercariae, furcocercous cercariae and pleurolophocercous cercariae. If snails in rice field of Hoc Mon district have pleurolophocercous cercariae, one of the questions for metacercariae in silver barb in this district can be answered. Therefore, the research on cercariae from snails in rice fields in Hoc Mon district needed to be implemented.

2. Material and Methods

2.1. Study areas

Xuan Thoi Thuong rice field was chosen to carry out the research because it had the largest

area of rice field in Hoc Mon district of Ho Chi Minh City (PCHCMC, 2020).

2.2. Sampling of snails

Two cross-sectional studies on snails were carried out in August 2022 (the wet season) and in February 2023 (the dry season). Snail sampling was done by using hand nets and hands with gloves to collect snails in the standard cell of 0.4 m wide x 10 m long x 0.1 m deep along the bank of rice field. Rice fields were in the rice-seedling stage in August 2022 and already harvested in February 2023. Fifteen samples per season were collected in each rice field with 500 m between the two sampling sites. The collected snails were washed and transferred to cloth bags and transported to the laboratory to analyze. Snails were classified into species following the keys of Dang et al. (1980).

2.3. Examination of snails for cercariae

The shedding method (Frandsen & Christensen, 1984; Bui et al., 2010) was used to examined for trematode infection (cercariae stage). Each snail was kept separately in 200 mL beakers and left for 24 h for shedding. Cercariae were checked twice per day at 8:00 AM and 14:00 PM in two days. Cercariae was recognized following the systematic key references (Frandsen & Christensen, 1984; Schell, 1985).

2.4. Data analysis

Microsoft Excel 2010 and SPSS (Statistical Package for Social Sciences version 20; SPSS Inc., Chicago, Illinois) was used for data entry and data analysis. The Chi-squared test was used to compare the difference of prevalence between the two seasons. A value of $P < 0.05$ was considered significant.

3. Results and Discussion

3.1. Snail composition and distribution in rice fields

Eleven snail species belonging to 9 genera and 5 families were collected and classified (Table 1). Nine snail species were found in the wet season and 7 snail species were caught in the dry season. This agreed with Brockelman et al. (1986) that snail populations are typically more abundant in the rainy season which provides good conditions for the development of snails. In addition, increasing rain in the wet season leads to an increase of snail populations in the area (Khamboonruang et al., 1997).

Total snail species in this study were much higher than what Bui et al. (2010) collected in rice field in Nam Dinh province with 10 snail species, or Nguyen et al. (2014) found in An Hoa rice field of Tuy An district, Phu Yen province with 9 snail species. Moreover, it was much higher than the finding from Nguyen & Pham (2022) with 8 snail species in two rice fields in Can Gio district, also belonging to Ho Chi Minh City, but Can Gio was located in brackish water site and Hoc Mon was in freshwater area; therefore, snails in Hoc Mon must be more abundant. Generally, it can be commented that total snail species in rice fields were not the same, but not very different among the North (Nam Dinh province), the Central (Phu Yen province) and the South of Vietnam (Ho Chi Minh City). Therefore, further research on snails in rice fields should continue doing to confirm the similarity of number of snail species in rice fields in Vietnam.

Table 1. Snail composition in rice field of Hoc Mon district, Ho Chi Minh City, Vietnam

Family	Genus	Snail species	Number of samples in the wet season	Number of samples in the dry season
Ampullariidae	*Pomacea*	*Pomacea canaliculata* (Lamarck, 1828)	240	197
Bithyniidae	*Bithynia*	*Bithynia siamensis* (Lea, 1856)	111	10
Viviparidae	*Angulyagra*	*Angulyagra polyzonata* (Frauenfeld, 1862)	1	0
	Cipangopaludina	*Cipangopaludina chinensis* (Gray, 1834)	9	0
		Cipangopaludina japonica (Martens, 1861)	154	0
	Filopaludina	*Filopaludina sumatrensis* (Dunker, 1852)	11	101
		Filopaludina martensi martensi (Frauenfeld, 1865)	21	4
	Sinotoia	*Sinotoia aeruginosa* (Reeve, 1863)	3	0
	Hippeutis	*Hippeutis umbilicalis* (Benson, 1836)	0	1
Physidae	*Physa*	*Physa acuta* (Draparnaud, 1805)	36	59
Lymnaeidae	*Lymnaea*	*Lymnaea viridis* (Quoy & Gaimard, 1832)	0	35

A total of 993 samples of snails were collected in August 2022 (the wet season) with 586 snails and in February 2023 (the dry season) with 407 snails. The snail species had high numbers including *Pomacea canaliculata* (44.0%), *Cipangopaludina japonica* (15.5%), *Bithynia siamensis* (12.2%), *Filopaludina sumatrensis* (11.3%), *Physa acuta* (9.6%), *Lymnaea viridis* (3.5%) and *Filopaludina martensi martensi* (2.5%). The other four snail species had low percentage including *Cipangopaludina chinensis* (0.9%), *Sinotoia aeruginosa* (0.3%), *Angulyagra polyzonata* (0.1%) and *Hippeutis umbilicalis* (0.1%) (Table 2).

Although Hoc Mon and Can Gio were districts of Ho Chi Minh City, snail species and snail composition were almost different. In rice fields of Can Gio district, the common snail species were *Sermyla tornatella* (47.6%), *Pomacea canaliculate* (27.2%) and *Melanoides tuberculate* (14.0%) (Nguyen & Pham, 2022) while the high percentage of three snail species in Hoc Mon district were *Pomacea canaliculata* (44.0%), *Cipangopaludina japonica* (15.5%) and *Bithynia siamensis* (12.2%). Nguyen et al. (2014) also found the three highest snail species

in rice fields of Phu Yen province were Bithynia sp., Pomacea sp. and Tarebia grannifera. It can be concluded that snail species and composition in rice fields in different areas were different from each other.

Table 2. Percentage contribution of each snail species in rice field of Hoc Mon district, Ho Chi Minh City, Vietnam

Snail species	Wet season (August 2022)		Dry season (February 2023)		Total	
	N	(%)	N	(%)	N	%
Angulyagra polyzonata (Frauenfeld, 1862)	1	0.2	0	0	1	0.1
Bithynia siamensis (Lea, 1856)	111	18.9	10	2.5	121	12.2
Cipangopaludina chinensis (Gray, 1834)	9	1.5	0	0	9	0.9
Cipangopaludina japonica (Martens, 1861)	154	26.3	0	0	154	15.5
Filopaludina sumatrensis (Dunker, 1852)	11	1.9	101	24.8	112	11.3
Filopaludina martensi martensi (Frauenfeld, 1865)	21	3.6	4	1.0	25	2.5
Hippeutis umbilicalis (Benson, 1836)	0	0	1	0.2	1	0.1
Lymnaea viridis (Quoy & Gaimard, 1832)	0	0	35	8,6	35	3.5
Sinotoia aeruginosa (Reeve, 1863)	3	0.5	0	0	3	0.3
Pomacea canaliculata (Lamarck, 1828)	240	41.0	197	48.4	437	44.0
Physa acuta (Draparnaud, 1805)	36	6.1	59	14.5	95	9.6
Total	586	100	407	100	993	100

3.2. Cercariae morphotypes infected in snails

The prevalence of cercariae infection in the dry season (14.3%) was significantly higher than in the wet season (4.5%) ($P < 0.05$). For the overall prevalence in two seasons for each snail species, *Lymnaea viridis*, the host of *Fasciolidae* (Thai, 2016), had the highest prevalence of 14.3% (N = 5/35) and *Bithynia siamensis*, a potential host for both Heterophyidae and Opisthorchiidae (Madsen et al., 2015), had the prevalence of 4.1% (N = 5/121). The other nine snail species had cercariae free (Table 3).

Lymnaea sp. was found infected with cercariae, but the snail species had the highest prevalence was *Melanoides tuberculata* in Nguyen et al. (2014). *Lymnaea* sp. was not found in Can Gio rice field in the research by Nguyen & Pham (2022) and in Cu Chi rice field in the study by Pham & Duong (2023). Thirty five *Lymnaea viridis* which were collected in Hoc Mon rice field in this research with the trematode prevalence of 14.3% were the new finding in the rice field of Ho Chi Minh City area. *Bithynia siamensis* was the second infected snails with the prevalence of 4.1%. This finding agreed with Nguyen & Pham (2022) that *Bithynia* in rice fields got the top prevalence. It was also like what Bui et al. (2010) and Nguyen et al. (2014) reported that *Bithynia* in canals and ponds had the high prevalence. For

the cercariae infection, the prevalence in the dry season was significantly higher than in the wet season ($P < 0.05$). This finding is similar to what Nguyen et al. (2014) that infection by trematode larvae in snails was high in the dry season and low in the wet season because of the temperature. The finding in this study also agreed with the publish by Nkwengulila & Kigadye (2005) that the prevalence of cercariae fluctuated by seasons, it was high in the dry season and decreased in the wet season. However, the result in this research was completely different from what Nguyen & Pham (2022) that the prevalence in the wet season was much higher than in the dry season. Although two districts belonged to Ho Chi Minh City but the result in Hoc Mon in this research was different from the finding from Can Gio; therefore, the different natural conditions might affect the distribution of snails and the trematode prevalence in snails when comparing between seasons.

Table 3. Trematode prevalence in snails in rice field of Hoc Mon district, Ho Chi Minh City, Vietnam

Snail species	Wet season (August 2022)		Dry season (February 2023)		Total	
	Infected snails/ Collected snails	Prevalence (%)	Infected snails/ Collected snails	Prevalence (%)	Infected snails/ Collected snails	Prevalence (%)
Bithynia siamensis	5/111	4.5	0/10	0	5/121	4.1
Lymnaea viridis	0	0	5/35	14.3	5/35	14.3

No *Pleurolophocercous cercariae* was found in snails from the research rice field. Xiphidiocercariae was more common and discovered from *Lymnaea viridis* and *Bithynia siamensis*. *Echinostome cercariae* was got in only one *Bithynia siamensis*. It was obvious that *Bithynia siamensis* had 2 different cercariae morphotypes whereas *Lymnaea viridis* had only one cercariae morphotype (Table 4). *Xiphidio cercariae* seemed to have more frequent occurrence than the others as it was recovered from 9 of 10 snails. This is similar to Nkwengulila & Kigadye (2005) that *Xiphidio cercariae* was the most prevalent in ponds and stream. It was also like what Pham & Tran (2021) that *Xiphidio cercariae* appeared the most in ponds of giant gourami culture in ponds.

Silver barb from rice field in Hoc Mon and released into Thay Cai - An Ha canal in Hoc Mon district was infected with metacercariae of *Haplorchis pumilio* (Pham et al., 2019), the species belongs to Heterophyidae (Chai et al., 2005). Pham & Nguyen (2005) stated that *Pleurolophocercous cercariae* was the cercariae of Heterophyidae; however, no *Pleurolophocercous cercariae* was found in two morphotypes of cercariae in this research in August 2022 and February 2023. Therefore, the infected source of metacercariae in silver barb has not been identified yet. More research on cercariae in snails in other rice fields of Hoc Mon in the different months should be done to check whether they exist to find the answer for one of the reasons why silver barb was infected.

Table 4. Cercariae morphotypes in infected snails in rice field of Hoc Mon district, Ho Chi Minh City, Vietnam

Cercariae	Wet season (August 2022)		Dry season (February 2023)	
	Bithynia siamensis	*Lymnaea viridis*	*Bithynia siamensis*	*Lymnaea viridis*
Xiphidio cercariae	4	0	0	5
Echinostome cercariae	1	0	0	0

4. Conclusions

Eleven snail species belonging to 9 genera and 5 families were identified. Lymnaea viridis and Bithynia siamensis were infected with trematode (cercariae stage) with the overall prevalence of 14.3% and 4.1%, respectively. The other nine snail species had cercariae free. Two cercariae morphotypes were recovered from snails including Xiphidio cercaria and Echinostome cercariae. Further research on this subject in different months and in other water bodies should be done in Hoc Mon district and other places to identify the sources of trematodes infected to fish in cultured ponds.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research is gratefully acknowledged for the financial support by Ministry of Education and Training in Vietnam (grant number: B2022-SPS-07).

References

Brockelman, W. Y., Upatham, E. S., Viyanant, V., Ardsungnoen, S., & Chantanawat, R. (1986). Field studies on the transmission of the human liver fluke, Opisthorchis viverrini, in northeast thailand: population changes of the snail intermediate host. *International Journal for Parasitology* 16(5), 545-552. https://doi.org/10.1016/0020-7519(86)90091-3.

Bui, T. D., Madsen, H., & Dang, T. T. (2010). Distribution of freshwater snails in family-based VAC ponds and associated waterbodies with special reference to intermediate hosts of fish-borne zoonotic trematodes in Nam Dinh Province, Vietnam. *Acta Tropica* 116(1), 15-23. http://dx.doi.org/10.1016/j.actatropica.2010.04.016.

Chai, J. Y., Murrell, K. D., & Lymbery, A. J. (2005). Fish-borne parasitic zoonoses: Status and issues. *International Journal for Parasitology* 35(11-12), 1233-1254. https://doi.org/10.1016/j.ijpara.2005.07.013.

Dang, N. T., Thai, T. B., & Pham, V. M. (1980). *Key of identification invertebrate in the North of Vietnam*. Ha Noi, Vietnam: Science and Technics Publishing House.

Dechruksa, W., Krailas, D., Ukong, S., Inkapatanakul, W., & Koonchornboon, T., (2007). Trematode infections of the freshwater snail family Thiaridae in the Khek river, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 38(6), 1016-1028.

Dorny, P., Praet, N., Deckers, N., & Gabriel, S. (2009). Emerging food-borne parasites. *Veterinary Parasitology* 163(3), 196-206. https://doi.org/10.1016/j.vetpar.2009.05.026.

Elsheikha, H. M., & Elshazly, A. M. (2008). Preliminary

observations on infection of brackish and freshwater fish by heterophyid encysted metacercariae in Egypt. *Parasitology Research* 103(4), 971-977. https://doi.org/10.1007/s00436-008-1043-z.

Frandsen, F., & Christensen, N. Ø. (1984). An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. *Acta Tropica* 41(2), 181-202.

Keiser, J., & Utzinger, J. (2005). Emerging foodborne trematodiasis. *Emerging Infectious Diseases* 11(10), 1507-1514. https://doi.org/10.3201/eid1110.050614.

Khalifa, R., El-Naffar, M. K., & Arafa, M. S., (1977). Studies on heterophyid cercariae from Assiut province, Egypt. I. Notes on the life cycle of *Haplorchis pumilio* (Looss, 1896) with a discussion on previously described species. *Acta Parasitologica Polonica* 25(1/10), 25-38.

Khamboonruang, C., Keawivichit, R., Wongworapat, K., Suwanrangsi, S., Hongpromyart, M., Sukhawat, K., Tonguthai, K., & Lima dos Santos, C. A. M. (1997). Application of hazard analysis critical control point (HACCP) as a possible control measure for *Opisthorchis viverrini* infection in cultured carp (*Puntius gonionotus*). *Southeast Asian Journal of Tropical Medicine and Public Health* 28, 65-72.

Long-Qi, X., Sen-Hai, Y., & Ying-Dan, C. (2005). *Clonorchiasis sinensis* in China. *Food-borne Helminthiasis in Asia* 1, 1-26.

Madsen, H., Bui, T. D., Dang, T. T., Nguyen, V. K., Dalsgaard, A., & Phan, T. V. (2015). The role of rice fields, fish ponds and water canals for transmission of fish-borne zoonotic trematodes in aquaculture ponds in Nam Dinh province, Vietnam. *Parasites and Vectors* 8(625), 1-11.

Madsen, H., & Hung, N. M. (2014). An overview of freshwater snails in Asia with main focus on Vietnam. *Acta Tropica* 140, 105-117. https://doi.org/10.1016/j.actatropica.2014.08.005.

Nawa, Y., Hatz, C., & Blum, J. (2005). Sushi delights and parasites: the risk of fishborne and foodborne parasitic zoonoses in Asia. *Clinical Infectious Diseases* 41(9), 1297-1303. https://doi.org/10.1086/496920.

Nkwengulila, G., & Kigadye, E. S. P. (2005). Occurrence of digenean larvae in freshwater snails in the Ruvu Basin, Tanzania. *Tanzania Journal of Science* 31(2), 23-30. https://doi.org/10.4314/tjs.v31i2.18417.

Nguyen, T. D., & Pham, C. T. (2022). The composition and trematode infections in snails in small canals and rice fields in Binh Khanh and Ly Nhon communes, Can Gio district, Ho Chi Minh City. *Scientific Proceedings for Master and Doctoral Students of Ho Chi Minh City University of Education in The School Year of 2022-2023* (30-42). Ho Chi Minh City, Vietnam: Ho Chi Minh City University of Education.

Nguyen, P. B. N., Nguyen, C. L., Vo, T. D., & Ngo, A. T. (2014). Infection of trematode larvae (cercariae) in freshwater snails in two communes, An My and An Hoa, Tuy An district, Phu Yen province. *Journal of Science-Fisheries Technology* 1.

PCHCMC (People's Committee of Ho Chi Minh City). (2020). Decision No. 2493/QD-UBND dated on July 10, 2020. Land use plan in 2020 of Hoc Mon district, Ho Chi Minh City. Retrieved May 12, 2022, from https://luatvietnam.vn/dat-dai/quyet-dinh-2493-qd-ubnd-ke-hoach-su-dung-dat-nam-2020-huyen-hoc-mon-tphcm-196296-d2.html.

Pham, C. T., & Duong, T. Q. (2023). The composition of snail species and cercariae in snails in rice field of Cu Chi district, Ho Chi Minh City, Vietnam. *Ho Chi Minh City University of Education Journal of Science* 20(5), 808-817.

Pham, C. T., Pham, T. T. D., Nguyen, T. H., Huynh, H., Pham, N. A. T., & Tong, X. T. (2019). Prevalence of trematode in wild fish from fishing in Ho Chi Minh City. *Ho Chi Minh City University of Education Journal of Science* 16(3), 152-161. https://doi.org/10.54607/hcmue.js.16.3.2467(2019).

Pham, C. T., & Tran, T. T. T. (2021). Freshwater snail diversity in grow-out ponds in giant gourami regarding intermediate hosts of trematode larvae (cercariae stage) in Tien Giang province. *Ho Chi Minh City University of Education Journal of Science* 18(9), 1649-1659.

Saad, A. I. (1994). The life cycle of *Centrocestus unequiorchalis* N. sp. (Heterophyidae: Centrocestiinae). *Journal of Islamic Academy of Sciences* 7(3), 193-198.

Schell, S. C. (1985). *Handbook of trematodes of North America, North of Mexico*. Moscow, Russia: Idaho Research Foundation.

Thai, T. B., (2016). *Invertebrates*. Ha Noi, Vietnam: Educational Publishing House.

Waikagul, J., & Radomyos, P. (2005). Intestinal trematode infections in Thailand. *Asian Parasitology* 1, 103-111.

Wang, J. J., Chung, L. Y., Lee, J. D., Chang, E. E., Chen, E. R., Chao, D., & Yen, C. M. (2002). Haplorchis infections in intermediate hosts from a clonorchiasis endemic area in Meinung, Taiwan, Republic of China. *Journal of Helminthology* 76(2), 185-188. https://doi.org/10.1079/JOH2002114.

Yu, S. H., & Xu, L. Q. (2005). Intestinal trematode infections in China. *Asian Parasitology* 1, 61-78.

Evaluating the growth performance of all male sex reversal and mixed ser tilapia *(Oreochromis niloticus)* cultured in earthen ponds in Binh Phuo province, Vietnam

Loi N. Nguyen[1*], Dang H. Nguyen[1], Nam B. Nguyen[1], Nhung T. H. Nguyen[2], & Hien T. Nguyen[2]

[1]Faculty of Fisheries, Nong Lam University, Ho Chi Minh City, Vietnam

[2]Agricultural Service Center of Binh Phuoc Province, Binh Phuoc, Vietnam

ARTICLE INFO

Research Paper

Received: October 30, 2023
Revised: November 16, 2023
Accepted: November 24, 2023

Keywords

Growth performance

Mixed sex

Nile tilapia

Reversed sex

***Corresponding author**

Nguyen Ngoc Loi

Email:
17116076@st.hcmuaf.edu.vn

ABSTRACT

The study was conducted to evaluate the growth performance, survival rate and yield of all male sex reversed and mixed sex Nile tilapia (Oreochromis niloticus) cultured in earthen ponds for 180 days. The reversed sex and mixed sex fingerlings (mean weight 7.43 ± 0.35 g) was randomly stocked in six earthen ponds (1,000 m^2/pond). The stocking density maintained was 5 fish/m^2. The stocked fish were fed a commercial pellet feed containing 35% crude protein. The feeding rate was adjusted according the size of fish, 5% at the beginning to 3% at harvest. Water environment parameters including dissolved oxygen (DO) (4.3 ± 0.8 mg/L), temperature (30 ± 0.7°C), NH3 (0.18 ± 0.2 mg/L), and pH (6.9 ± 0.5) were always within the appropriate range for the normal growth and development of tilapia. The growth rate of sex reversed tilapia was significantly higher ($P < 0.05$) than that of mixed tilapia throughout the experiment. The harvest weight and length of reversed sex tilapia were 410.5 ± 5.15 g and 25.48 ± 0.48 cm, respectively, and were significantly higher than that of mixed sex tilapia ($P < 0.05$). The survival rates and feed conversion ratio (FCR) of mono and mixed sex were 90.1% and 89.9%, respectively, but no significant differences were observed ($P > 0.05$). The relationship of fish lengths and weights expressed by power function revealed that the slope of the length weight regression lines was normal for reversed sex (2.72) and mixed sex Tilapia (2.93) with the high correlation coefficient (> 0.9). The findings of this study demonstrate that reversed sex tilapia has better growth performance compared to mixed sex tilapia. Therefore, reversed sex fingerlings should be used in commercial farming to increase tilapia production.

Cited as: Nguyen, L. N., Nguyen, D. H., Nguyen, N. B., Nguyen, N. T. H, & Nguyen, H. T. (2024). Evaluating the growth performance of all male sex reversal and mixed sex tilapia *(Oreochromis niloticus)* cultured in earthen ponds in Binh Phuoc province, Vietnam. *The Journal of Agriculture and Development* 23(3), 32-39.

1. Introduction

Nile tilapia, Oreochromis niloticus is one of the most important freshwater fish species in world aquaculture accounting for the second highest production in the world after carp (Admassu, 1996; Coward & Bromage, 1998). It is widely cultured in many tropical and subtropical countries around the world (Boyd & Tucker, 1998). Tilapia belongs to the Cichlidae family and was introduced from Africa (Gómez et al., 2015) and provides important economic and social benefits to rural communities (Jiménez-Badillo, 2006). Its fast growth rate, high tolerance to adverse environmental conditions, use of a variety of available feeds, ease of reproduction, disease resistance and consumer acceptance have made it be a commonly selected species for farming (Wohlfarth et al., 1983; Bahnasawy, 2009). However, one of the major constraints of tilapia farming with mixed-sex population is inherent high reproductive capacity resulting from early maturity, highly developed parental care, and multiple spawning cycles. Under favorable conditions they continue to reproduce, offspring will compete for food sources with the initially stocked fingerlings, resulting in stunted growth and unmarketable fish (Lévêque, 2002; Babiker & Ibrahim, 2006). Therefore, the need for monosex tilapia for intensive farming of this fish is an urgent requirement. Currently in the world, there are many all-male tilapia production techniques being used based on the treatment of male sex hormones in fry at a stage where they have not yet had sexual differentiation. Direct masculinization of tilapia using hormones is the most common method to produce unisexual males. Therefore, the objective of this study was to compare and evaluate the growth and productivity of unisex tilapia and mixed tilapia.

2. Material and Methods

2.1. Origin of experimental fish

Two groups of fish in the experiment including mixed sex (MS) and reversed sex (HR) were produced at provincial breeding center for freshwater aquaculture of Binh Phuoc province, Vietnam. The fry less than 14 days old were collected from the same brood stock, in which 50% of the fry were reversed sex by immersed in 17α - methyltestosterone for 2 h, while the remaining 50% were not treated with hormones. Fry of two groups were reared separately in the hapas (4 m x 4 m x 1 m) set in a pond until they reached experimental size.

2.2. Grow-out performance analysis

Fingerlings (7.1 ± 0.2 g; 6.5 ± 0.2 cm) of mixed sex and reversed sex were randomly sampled from the nursery hapas and stocked into six earthen ponds (1000 m^2), having three replicates of each with same stocking densities of 5 fish/m^2 at the experimental farm for aquaculture, Faculty of Fisheries, Nong Lam University. The fishes were fed twice a day on a commercial floating pelleted feed (Brand Tilapia feed, Cargill), with approximately 35% of crude protein, at a feeding rate of 3 - 5% of body weight daily, adjusted biweekly based on sample mean weight. Throughout the grow-out period (180 days), all water parameters (temperature, pH and dissolved oxygen) were closely monitored once a week. Growth parameters such as average weight gain (AWG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), survival rate were calculated as follows:

AWG (g/fish) = average final weight (g) – average initial weight (g)

DWG (g/day) = (Average final weight (g) – Average initial weight (g))/experimental period (day)

SGR (%/day) = (ln final weight (g) – ln initial weight (g)) X 100/experimental period (day)
FCR = feed intake (g)/ weight gain (g)

Survival rate (%) = final number of fish × 100/ initial number of fish

2.3. Statistical analysis

The data were expressed in terms of mean ± standard deviation. All growth parameters were statistically analyzed using SPSS version 16.0 in which data were subjected to one-way ANOVA and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means at 5% level of significance.

3. Results and Discussion

During the experiment, the physical and chemical parameters of ponds water were ranged within the appropriate range for normal growth and development of Nile tilapia (Table 1). Water temperature is one of the most important environmental factors affecting fish physiological responses of growth and feed utilization. The water temperature in the experiment was ranged from 29 - 31°C, but there were not statistical differences among experimental ponds. El-sayed & Kawanna (2008) stated that the optimal temperature for growth and reproduction was between 22°C to 32°C. The ideal DO level for tilapia farming is 4 - 5 mg/L. Dissolved oxygen in this study fluctuated between 4.3 ± 0.8 mg/L (mixed sex) and 4.4 ± 0.9 mg/L (reversed sex). There was not statistical difference in the dissolved oxygen concentration experimental ponds. The pH values of ponds were varied from 6.5 to 8.0 throughout experiment. The water mean values of total ammonia nitrogen concentrations of pond water were 0.17 ± 0.15 mg/L (mixed sex) and 0.19 ± 0.12 mg/L (reversed sex), but there was not statistical different among ponds.

Table 1. Physico-chemical parameters of pond water during the experimental period

Parameter	Mixed sex	Reversed sex
Water temperature (°C)	29.9 ± 0.7	30.0 ± 0.6
pH	6.7 ± 0.5	6.9 ± 0.5
Dissolved oxygen (mg/L)	4.3 ± 0.8	4.4 ± 0.9
Ammonia (mg/L)	0.17 ± 0.15	0.19 ± 0.12

All data are presented as mean ± SD.

Experimental results showed that although the initial weight was the same, the growth of reversed sex tilapia was significantly higher than that of mixed sex tilapia in all growth measurement times ($P < 0.05$). The results also indicated that the average harvest weight of reversed sex fish was 410.5 ± 5.15 g, while the weight of mixed sex fish was 315.9 ± 4.09 g (Table 2). Many studies have also found that male reversed sex fish have higher growth rates and harvest weights than mixed sex fish (Little et al., 2003; Chakraborty et al., 2011). Dagne et al. (2013) noted that male mono-sex tilapia showed significantly higher growth rate (weight, length, DWG, SGR) than mixed-sex group ($P < 0.05$).

Table 2. The weight gain (g) and length (cm) of mono sex and mixed sex tilapia at different time interval

Growing day	Weight (g)		Length (cm)	
	Reversed sex	Mixed sex	Reversed sex	Mixed sex
1	7.02 ± 0.21[a]	7.15 ± 0.19[a]	6.38 ± 0.16[a]	6.57 ± 0.18[a]
30	35.10 ± 0.99[a]	28.60 ± 1.76[b]	9.49 ± 0.23[a]	8.87 ± 0.25[b]
60	66.00 ± 1.94[a]	44.30 ± 1.42[b]	13.53 ± 0.39[a]	12.08 ± 0.34[b]
90	133.4 ± 2.41[a]	75.1 ± 1.54[b]	19.01 ± 0.39[a]	16.71 ± 0.26[b]
120	238.3 ± 2.31[a]	147.6 ± 1.43[b]	21.02 ± 0.28[a]	17.92 ± 0.36[b]
150	287.3 ± 8.19[a]	218.2 ± 5.98[b]	23.96 ± 0.45[a]	18.64 ± 0.35[b]
180	410.5 ± 5.15[a]	315.9 ± 4.09[b]	25.48 ± 0.48[s]	20.85 ± 0.38[b]

Values (mean ± standard deviation of data for triplicate groups) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey test, P < 0.05).

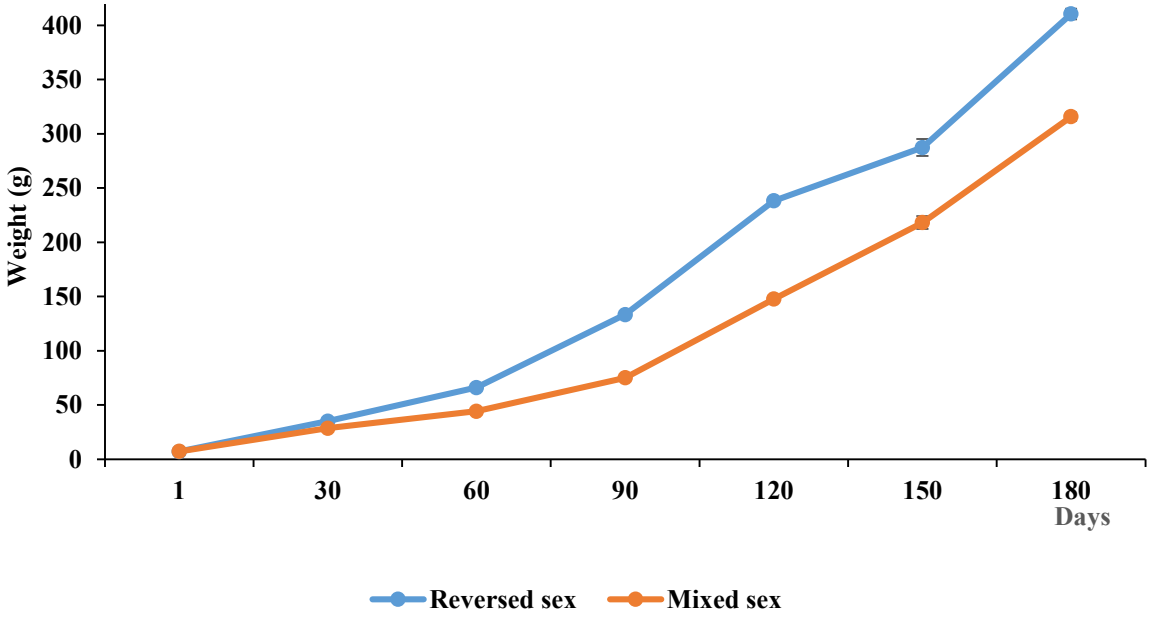

Figure 1. Growth trend in weight of reversed and mixed-sex tilapia throughout the experimental period.

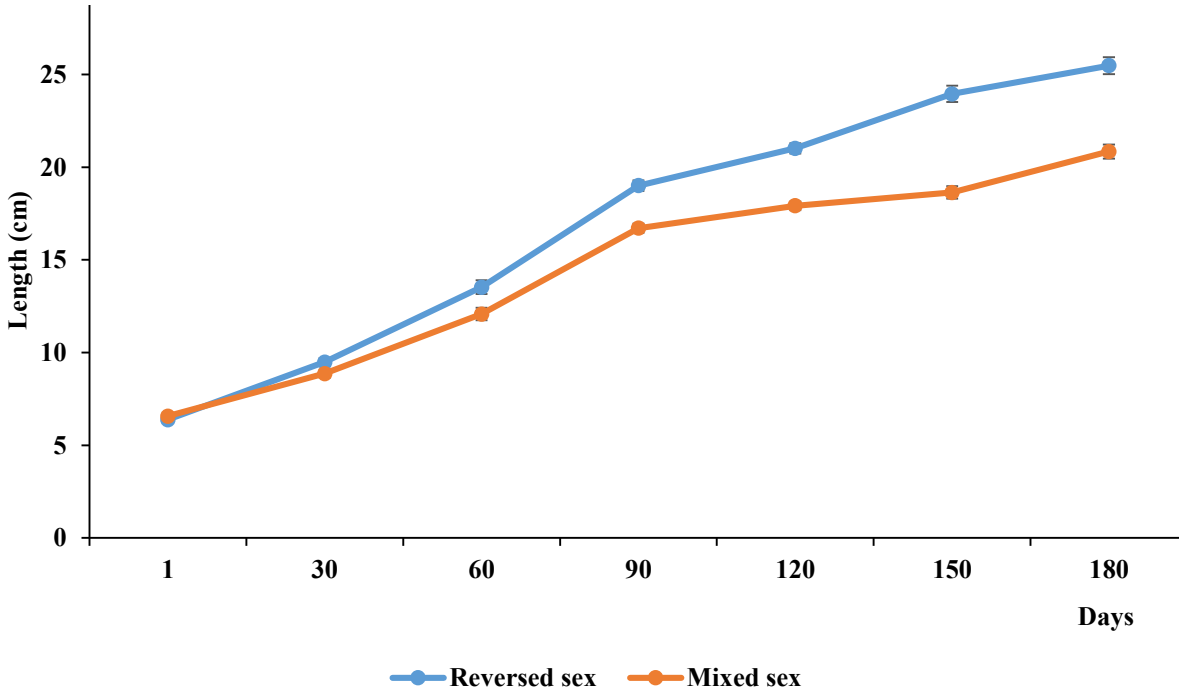

Figure 2. Growth trend in length of reversed and mixed-sex tilapia throughout the experimental period.

The average weight gain, daily weight and specific growth rate of reversed sex tilapia were 403.18 g/day, 2.08 g/day and 2.26%, respectively, which were significant higher than those of mixed sex tilapia ($P < 0.05$; Table 3). The higher growth rate of treated fish may be due to the anabolic effect of hormones to induce sex reversal in farmed tilapia (Jo et al., 1995). Mair et al. (1995) and Dan & Little (2000) reported that the anabolic effect of hormones showed an increase in growth rate of tilapia. Other studies have also shown that the higher average weight may be due to improved feed conversion efficiency of Oreochromis niloticus fry (Chakraborty & Samir, 2009; Dagne et al., 2013).

The FCR values of mixed sex group was higher than that of reversed sex group but the difference was not statistically significant difference. The FCR in this study was similar to the results of Islam et al. (2015) and Toguyeni et al. (1997) who reported that reversed sex male had a better feed conversion ratio than mixed sex tilapia.

The survival rate of reversed sex was slightly higher (88.4%) compared to mixed sex tilapia (86.2%) but the difference was not significantly different (Table 3). The survival rate of tilapia in this study was higher than the survival rate of tilapia in study of Nahiduzzaman & Awal (2023) varied between 73 and 78%, but lower than that in the study of Sultana et al. (1997) who recorded 95.75% and 81.25% for GIFT and existing Nile tilapia species, respectively. Several studies have demonstrated that 17 α-methyltestosterone has no negative impact on the survival of hormone-treated all male tilapia (Cruz & Mair, 1994).

Table 3. Data of stocking and harvest parameters of reversed-sex male and mixed-sex of Nile tilapia cultured in earthen ponds

Growth parameters	Mixed sex	Reversed-sex male
Initial body weight (g)	7.15 ± 0.19[a]	7.02 ± 0.21[a]
Final body weight (g)	315.9 ± 4.09[b]	410.5 ± 5.15[a]
Initial total length (cm)	6.38 ± 0.16[a]	6.57 ± 0.18[b]
Final body weight (g)	20.85 ± 0.38[a]	25.48 ± 0.48[b]
Average weight gain (g/fish)	308.70 ± 3.80[a]	403.18 ± 4.93[b]
Daily weight gain (g/day)	1.60 ± 0.02[a]	2.08 ± 0.03[b]
Specific growth rate (%/day)	2.10± 0.02[a]	2.26 ± 0.02[b]
Feed conversion ratio	1.54 ± 0.12[a]	1.42 ± 0.07[a]
Survival (%)	86.2 ± 1.8[a]	88.4 ± 2.6[a]

Values (mean ± standard deviation of data for triplicate groups) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey test, P < 0.05).

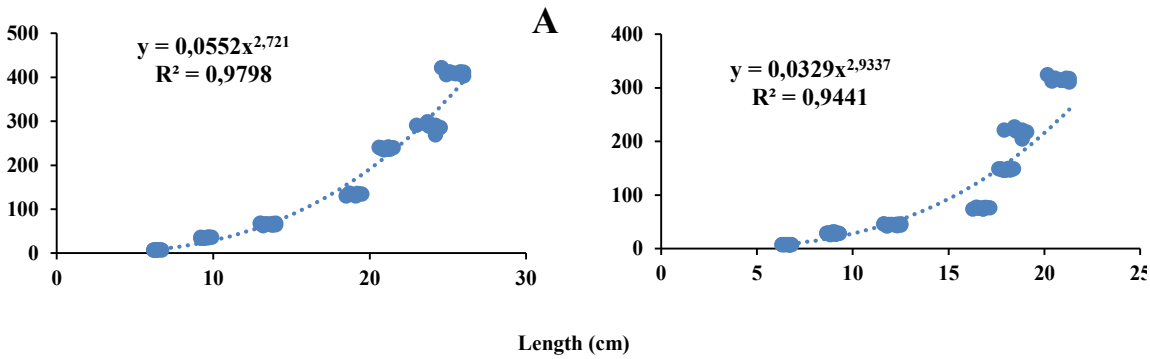

Figure 3. Length-weight relationships for (A) reversed sex male tilapia and (B) mixed sex population tilapia.

The relationship between length and fish weight expressed as an exponential function shows that the slope of the length-weight regression line is normal for reversed sex (2.72) and mixed sex tilapia (2.93) with high correlation coefficient (> 0.9; Figures 1, 2 & 3). Hopkins (1992) suggests that the slope of the length-weighted regression line when applying an exponential function should be between 2.5 and 3.5 with a high correlation coefficient (> 0.9) for farmed fish species.

4. Conclusions

The present findings indicated that reversed-sex tilapia had better growth rate than mixed-sex tilapia and had a low FCR, indicating that the sex-reversed tilapia is more viable for pond aquaculture compared to the mixed-sex tilapia.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

This study was made with financial support Nong Lam University (Ref. no. CS-SV22-TS-03) and from Department of Science and Technology, Binh Phuoc province.

References

Admassu, D. (1996). The breeding season of tilapia, *Oreochromis niloticus* L. in Lake Awassa (Ethiopian rift valley). *Hydrobiologia* 337(1), 77-83.

Babiker, M., & Ibrahim, H. (2006). Studies on the biology of reproduction in the cichlid Tilapia nilotica (L.): Gonadal maturation and fecundity. *Journal of Fish Biology* 14(5), 437-448.

Bahnasawy, M. (2009). Effect of dietary protein levels on growth performance and body composition of monosex nile tilapia, *Oreochromis niloticus* L. reared in fertilized tanks. *Pakistan Journal of Nutrition* 8(5), 674-678.

Boyd, C. E., & Tucker, C. S. (1998). *Pond aquaculture water quality management* (1st ed.) New York, USA: Springer.

Chakraborty, S., & Samir, B. (2009). Comparative growth performance of mixed-sex and monosex nile tilapia population in freshwater cage culture system under indian perspective. *International Journal of Biology* 2(1), 44-50.

Chakraborty, S. B., Mazumdar, D., Chatterji, U., & Banerjee, S. (2011). Growth of mixed-sex and monosex nile tilapia in different culture systems. *Turkish Journal of Fisheries and Aquatic Sciences* 11(1), 131-138.

Coward, K., & Bromage, N. (1998). Histological classification and the dynamics of ovarian recrudescence in Tilapia *zillii. Journal of Fish Biology* 53(2), 285-302.

Cruz, E. M. V., & Mair, G. C. (1994). Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). *Aquaculture* 122 (2-3), 237-248.

Dagne, A., Degefu, F., & Lakew, A. (2013). Comparative growth performance of mono-sex and mixed-sex nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. *International Journal of Aquaculture* 3(7), 30-34.

Dan, N. C., & Little, D. C. (2000). The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia *(Oreochromis niloticus)* in northern Vietnam. *Aquaculture* 184 (3-4), 221-231.

El-sayed, A., & Kawanna, M. (2008). Optimum water temperature boosts the growth performance of Nile tilapia *(Oreochromis niloticus)* fry reared in a recycling system. *Aquaculture Research* 39(6), 670-672.

Gómez-Márquez, J. L., Peña-Mendoza, B., Alejo-Plata, M. C., & Guzmán-Santiago, J. L. (2015). Culture mixed-sex and monosex of tilapia in ponds in Mexico city. *Agricultural Sciences* 6(2), 187-194.

Hopkins, K. D. (1992). Reporting fish growth: A review of the basics 1. *Journal of The World Aquaculture Society* 23(3), 173-179.

Islam, M. Z., Sarder, M. R. I., & Akhand, R. I. (2015). Growth performance of genetically male tilapia derived from YY male, sex reversed male tilapia and mixed sex tilapia of Oreochromis niloticus in earthen pond aquaculture system in Bangladesh. *International Journal of Fisheries and Aquatic Studies* 2(3), 186-191.

Jiménez-Badillo, L. (2006). Age-growth models for tilapia *Oreochromis aureus* (Perciformes, Cichlidae) of the Infiernillo reservoir, Mexico and reproductive behaviour. *Revista de Biología Tropical* 54(2), 577-588.

Jo, J. Y., Smitherman, R. O., & Tave, D. (1995). Effects of six levels of dietary 17α-methyltestosterone on sex-reversal and growth of *Oreochromis aureus* (Steindachner) and *Oreochromis niloticus* (Linnaeus). *Journal of Aquaculture* 8(2), 77-83.

Lévêque, C. (2002). Out of Africa: the success story of tilapias. *Environmental Biology of Fishes* 64(4), 461-464.

Little, D. C., Bhujel, R. C., & Pham, T. A. (2003). Advanced nursing of mixed-sex and mono-sex tilapia *(Oreochromis niloticus)* fry, and its impact on subsequent growth in fertilized ponds. *Aquaculture* 221(1), 265-276.

Mair, G. C., Abucay, J. S., Beardmore, J. A., & Skibinski, D. O. F. (1995). Growth performance trials of genetically male tilapia (G17α-MT) Derived from YY-Males in *Oreochromis niloticus* L.: on station compositions with mixed sex and sex reversed male populations. *Aquaculture* 137(1-4), 313-332.

Nahiduzzaman, M., & Awal, M. R. (2023). Determination of suitable species combination of two small indigenous fish species (sis) pabda *(Ompok pabda)* and gulsha *(Mystus cavasius)* with rohu *(Labeo rohita)* in farmers pond. *Research in Agriculture, Livestock and Fisheries* 10(2), 219-228.

Sultana, R., Kohinoor, A. H. M., Islam, M. S., Mazid, M. A., & Hussain, M. G. (1997). Comparative studies on growth of fry of GIFT and existing strain of Nile tilapia (*Oreochromis niloticus* L.). *Bangladesh Journal of Fisheries Research* 1(1), 25-30.

Toguyeni, A., Fauconneau, B., Boujard, T., Fostier, A., Kuhn, E. R., Mol, K. A., & Baroiller, J. F. (1997). Feeding behaviour and food utilisation in Tilapia, *Oreochromis niloticus*: Effect of sex ratio and relationship with the endocrine status. *Physiology and Behavior* 62(2), 273-279.

Wohlfarth, G. W., & Hulata, G. I. (1983). *Applied genetics of tilapias. ICLARM studies and reviews 6*. Manila, Philippines: International Center for Living Aquatic Resources Management.

Bacterial species causing subclinical mastitis in dairy cows: rapid identification and antimicrobial susceptibility testing

Son H. Ly, Tuan T. Pham, Han M. N. On, Bao D. Truong, & Thuong T. Nguyen*

Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: January 17, 2024
Revised: February 05, 2024
Accepted: February 19, 2024

Keywords

Antimicrobial resistance
Chromogenic media
Dairy cow
Subclinical mastitis

***Corresponding author**

Nguyen Thi Thuong
Email:
thuong.nguyenthi@hcmuaf.edu.vn

ABSTRACT

This study aimed to determine subclinical mastitis (SCM) caused by bacterial species, using chromogenic culture media and to assess the antimicrobial resistance rate in the isolated bacteria. From March to December 2023, 143 milk samples were collected from 71 Holstein Friesian cows with SCM across seven dairy farms in Ho Chi Minh City and Binh Duong province. Milk samples were incubated in triplicate chromogenic culture media to identify SCM caused by microorganisms. Our study revealed that 39.2% (56/143) of the samples had the growth of a single morphology, 26.6% (38/143) exhibited growth of two distinct morphologies, 9.0% (13/143) were found to be contaminated, and 25.2% (36/143) showed no growth. The isolated *Streptococcus* species were *Strep. agalactiae* 34.3% (49/143), *Strep. uberis* 22.4% (32/143), and *Enterococcus* spp. 1.4% (2/143). Besides, *S. epidermidis* 20.3% (29/143), *S. saprophyticus* 14.7% (21/143), and *S. aureus* 4.2% (6/143) were frequently isolated among *Staphylococcus species*. For gram-negative bacteria causing SCM, *E. coli* 2.8% (4/143), *Klebsiell*a spp. 1.4% (2/143), and *Pseudomonas* spp. 4.2% (6/143) were the most isolated. Regarding antimicrobial susceptibility testing, the resistance rate of each bacterial species to each antibiotic tested differed for *Staphylococcus*, *Streptococcus*, and gram-negative bacteria. *Staphylococcus aureus* was not resistant to gentamycin, florfenicol, and marbofloxacin. The resistance rate of *S. epidermidis* to gentamycin, florfenicol, trimethoprim-sulfadiazine, and amoxicillin-clavulanic acid varied from 10.3% to 17.2%. Marbofloxacin and trimethoprim-sulfadiazine were excellent choices in treating SCM caused by *S. saprophyticus* because of their low resistance rate (10.3 - 13.3%). *Streptococcus uberis* was sensitive to the combined antibiotic amoxicillin-clavulanic acid. The resistance rate of *Strep. agalactiae* to this combined antibiotic (amoxicillin-clavuclanic acid) was the lowest (10%). *Pseudomonas* spp. was resistant to the tested antibiotics. Our study suggests that identifying bacterial species and conducting antimicrobial susceptibility tests play a crucial role in improving the treatment effectiveness for bovine SCM.

Cited as: Ly, S. H., Pham, T. T., On, H. M. N., Truong, B. D., & Nguyen, T. T. (2024). Bacterial species causing subclinical mastitis in dairy cows: rapid identification and antimicrobial susceptibility testing. *The Journal of Agriculture and Development* 23(3), 40-52.

1. Introduction

Bovine mastitis is the inflammation of one or more of the four udders, primarily caused by a bacterial infection (Ruegg, 2011). Subclinical mastitis (SCM), one of the types of mastitis, results in decreased milk quality and quantity and increased veterinary costs (Bar et al., 2008; Hagnestam-Nielsen & Ostergaard, 2009). If left uncontrolled or untreated, it can progress to clinical mastitis, leading to more serious economic losses and compromising animal welfare. Previous studies have indicated that approximately 90% of microorganisms isolated from infected milk were environmental or infectious bacteria, such as *Staphylococcus aureus*, Negative Coagulase *Staphylococcus* (NCS), *Streptococcus agalactiae*, *Streptococcus uberis*, *Escherichia coli*, *Klebsiella* spp. (Bar et al., 2008; Hagnestam-Nielsen & Ostergaard, 2009). Each bacterial species has a different pathogenic mechanism (Côté-Gravel & Malouin, 2019). Therefore, identifying the causative agents of SCM plays an essential role in treatment efficacy. Previous studies showed that mastitis caused by *E. coli* and *Staphylococcus aureus* was not recommended of local intramammary antibiotic therapy except in severe cases (Pinzón-Sánchez et al., 2011; Suojala et al., 2013). Currently, the traditional methods for diagnosing SCM-causing bacteria rely on bacterial culture and biochemical tests. However, these methods have limitations, such as the requirement for aseptic sample collection and the time delay between sample sending and receiving results (Adkins & Middleton, 2018). Consequently, routine diagnosis of the microorganisms causing SCM is not performed on most dairy farms, leading to antimicrobial treatment without prior knowledge (Ly et al., 2022). This approach can contribute to the development of antibiotic resistance. CHROMagar is a type of chromogenic culture media developed to identify microbial pathogens based on the colors exhibited by microbial colonies. Compared to traditional methods, this chromogenic media helps rapid identification, reducing the biochemical tests to determine bacterial species. Indeed, the efficacy of CHROMagar for the rapidly identifying bacteria isolated from mastitis cows in Brazil has been published (Granja et al., 2021). However, studies on the use of CHROMagar to determine the SCM-causing agents in Vietnamese dairy farms are still limited. Thus, our study aimed to identify SCM caused by bacterial species using the chromogenic media, CHROMagar, and to assess the antimicrobial resistance rate of these isolated agents.

2. Materials and Methods

2.1. Time and locations

Our study was conducted from March 2023 to December 2023. Milk samples were collected from seven farms in Binh Chanh district and Cu Chi district of Ho Chi Minh city, and Tan Uyen city in Binh Duong province. The selected farms operated on an industrial scale, with the number of cows ranging from 200 to 2,000. Cows were fed using a total mixed ration and milked with a milking system. Bacterial identification and antimicrobial susceptibility testing were conducted at the Veterinary Hospital of Nong Lam University following the guidelines provided by NMC (2017).

2.2. Milk sample collection

In the present study, 143 milk samples were collected from 71 Holstein Friesian cows with subclinical mastitis. The California Mastitis Test (CMT) was used to detect subclinical mastitis udder. Initially, the teats were immersed in a teat disinfectant solution and then dried with paper towels. After discarding the initial three milk

streams, 2 mL from each udder was collected into the shallow cups on the paddle and mixed with 2 mL of CMT solution (DeLaval CMT®, DeLaval company, France). Milk samples were considered subclinical mastitis when the CMT results were more significant than 1+ (Kandeel et al., 2018). The subclinical mastitis milk samples were collected aseptically, with the teat ends disinfected using 70% iodized alcohol, and the milk was then collected in a sterile tube 15 mL (Aptaca®, Aptaca company, Italy) following the guidelines provided by NMC (2017). The samples were stored at 4°C and sent to the Veterinary Hospital of Nong Lam University for diagnosis.

2.3. Microbial identification by chromogenic culture media

Three chromogenic culture media (CHROMagar company, France) were utilized to identify subclinical mastitis-causing pathogens. The mechanism involves the chromogenic substrate being hydrolysed to release a coloured product that remains highly localized on microbial colonies. This allows clear differentiation of microbes producing the target enzyme from those that do not (Perry, 2017). The diagnostic performance (accuracy, sensitivity, and specificity) of chromogenic culture media in identifying microorganisms isolated from cows with clinical and subclinical mastitis has been demonstrated (Granja et al., 2021). The first medium specifically targeted *Staphylococcus* (CHROMagarTM *Staphylococcus*), the second medium selectively identified *Streptococcus* (CHROagarTM *Streptococcus*), and the third medium was designed to isolate gram-negative bacteria and yeasts (CHROMagarTM GramNeg). These culture media were plated in 90 x 15 mm tri-plate petri dishes. The milk samples were inoculated into the culture media using sterile swabs and then placed in an incubator at 37°C for 24 h under aerobic conditions. After the incubation period, the growth of colonies and microbiological identification were visually evaluated based on the colony-staining characteristics, according to the manufacturer's instructions: (1) pink = *Staphylococcus aureus*, (2) colorless to pinkish = *S. epidermidis*, (3) turquoise blue = *S. sarprophyticus* for *Staphylococcus plates*; (4) blue = *Streptococcus agalactiae*, (5) metallic blue = *Streptococcus uberis*, (6) mauve = *Enterococcus* spp. for *Streptococcus plates*; (7) mauve = *E. coli*, (8) colorless = *Pseudomonas* spp. for gram-negative plates (Figure 1).

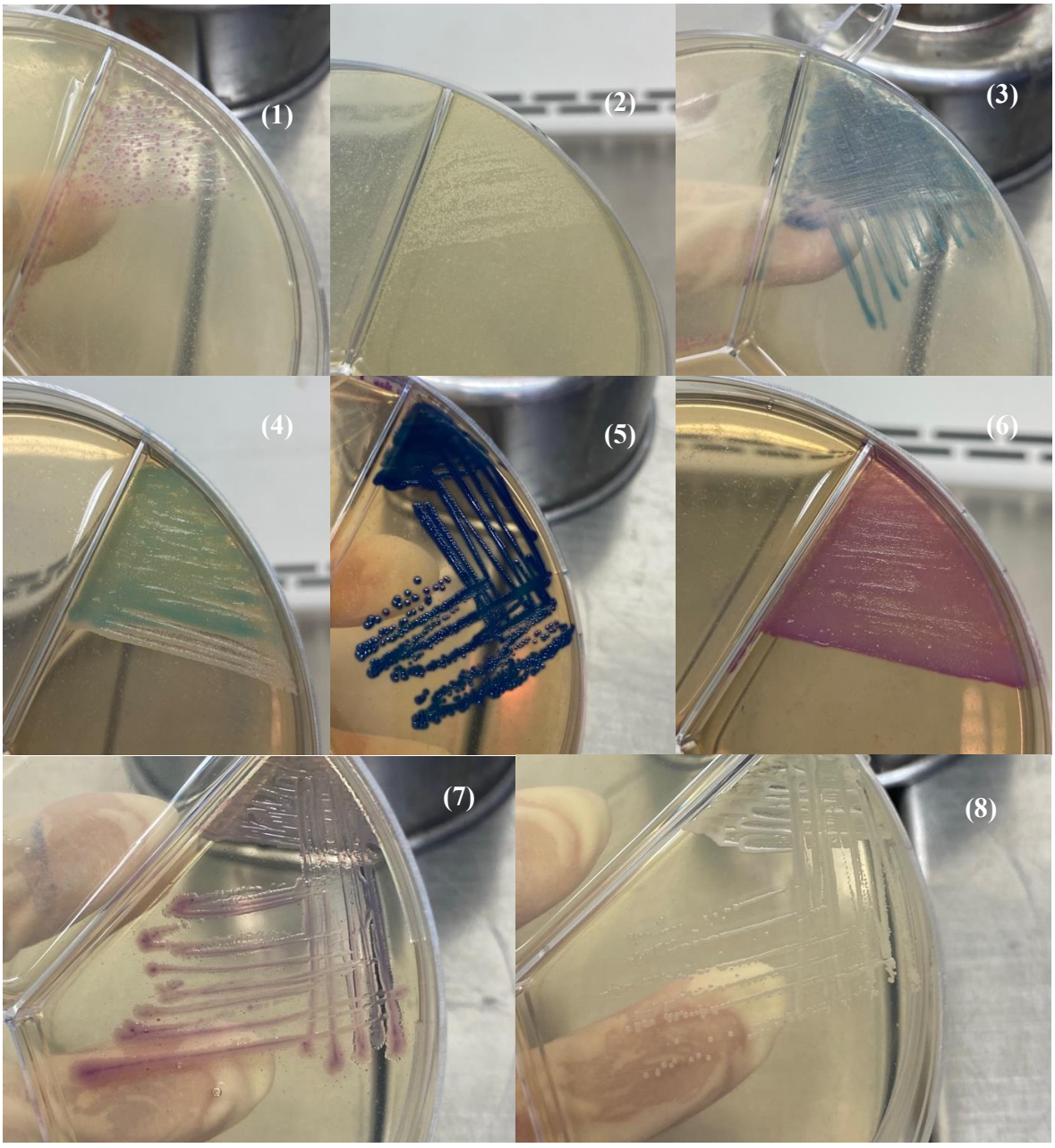

Figure 1. Colonies of subclinical mastitis-causing microorganisms inoculated in triplicate of chromogenic culture media: (1) *Staphylococcus aureus*, (2) *Staphylococcus epidermidis*, (3) *Staphylococcus sarprophyticus*, (4) *Streptococcus agalactiae*, (5) *Streptococcus uberis*, (6) *Enterococcus* spp.; (7) *Escherichia coli*, (8) *Pseudomonas* spp.

2.4. Antimicrobial susceptibility testing

The Kirby-Bauer disk diffusion method was applied to evaluate the antimicrobial susceptibility testing. In short, from 5 to 7 separate colonies of one species were picked and suspended in a 5 mL saline solution, resulting in 0.5 McFarland. The suspension was used for flooding the Mueller Hinton agar plates (MHA, Merck company, Germany), and the redundant solution was discarded. Streptococcus was examined on MHA plates supplemented with 5% sheep blood. The antimicrobial agents were selected according to their occurrence in commercially available products for mastitis treatment. Antibiotic discs (Oxoid company, United Kingdom) used including cetiofur (30 µg), amoxicillin (20 µg) + clavulanic acid (10 µg), ampicillin (10 µg), oxacillin (1 µg), cephalexin (30 µg), cefotaxim (30 µg), penicillin (10 UI), erythromycin (15 µg), trimethroprim (1.25 µg) + sulfadiazine (23.75 µg), tetracylin (30 µg), kanamycin (30 µg), gentamycin (10 µg), florfenicol (30 µg), marbofloxacin (5 µg), enrofloxacin (5 µg), clindamycin (2 µg), cefoperazone (30 µg). After 24 h incubation at 37oC, plates were read by measuring the inhibition zone diameters. Inhibition zone diameters were first evaluated by clinical breakpoints and provided by CLSI (2018) to determine resistant strains (Table 1).

2.5. Statistical analysis

Data were expressed as the parameters estimates and 95% confidence intervals using R version 4.2.3 (https://cran.r-project.org/). Confidence intervals (CI) were calculated based on the standard error obtained from a bnomial distibution following the formulas:

Standard Error (SE) $= \sqrt{\frac{p(1-p)}{n}}$ and CI 95% = estimate ± 1.96 x SE.

3. Results

3.1. Microbial identification by chromogenic culture media

A total of 143 subclinical mastitis samples were assessed. Among these, 39.2% (56/143) had growth of a single morphology, 26.6% (38/143) exhibited growth of two distinct morphologies, 9.0% (13/143) were found to be contaminated, and 25.2% (36/143) showed no microbial growth. Regarding the subclinical mastitis-causing agents, *Streptococcus* accounted for the majority at 62.9% (90/143). Within this group, *Strep. agalactiae* 34.3% (49/143) and *Strep. uberis* 22.4% (32/143) were the most frequently isolated strains. *Staphylococcus* was also isolated in 44.7% (64/143) of the samples. Specifically, negative coagulase *Staphylococcus*, such as *S. epidermidis* and *S. saprophyticus*, represented 35% of this group. Among the gram-negative bacteria, *Pseudomonas* spp., *E. coli*, and *Klebsiella* spp. were isolated in proportions of 4.2%, 2.8%, and 1.4%, respectively (Table 2).

3.2. Antimicrobial susceptibility testing

The percentage of resistant strains per species is provided in Table 3. The antibiotic resistance rate differed for each bacterial species within the *Staphylococcus* group on the tested antibiotics. *S. aureus* was utterly resistant to ampicillin, oxacillin, cephalexin, and cefotaxim. Conversely, gentamycin, florfenicol, and marbofloxacin were effective against *S. aureus in vivo*. The resistance rate of *S. epidermidis* varied widely from 10.3% to 93.1%. *S. epidermidis* resisted ampicillin, cephalexin, cefotaxim, and oxacillin, while this bacterial species was sensitive to gentamycin, florfenicol, and trimethoprim sulfadiazine. Similarly, *S. saprophyticus* was resistant to cefotaxime, cephalexin, ampicillin, and penicillin, with only marbofloxacin and

Table 1. Available clinical breakpoints for the pathogen in combination with the tested antimicrobials for a specific disk content (in µg)

Antibiotic	Disk content (µg)	*S. aureus*	*S. epidermidis*	*S. saprophyticus*	*Strep. uberis*	*Strep. agalactiae*	*Enterococcus* spp.	*E. coli*	*Pseudonomas spp.*
	RIS (mm)	RIS (mm)	RIS (mm)	RIS (mm)	RIS (mm)	RIS (mm)	RIS (mm)	RIS (mm)	RIS (mm)
Cetiofur	30	17 - 21	17 - 21	17 - 21	17 - 21	17 - 21	13 - 17	17 - 21	14 - 18
Amox/clav*	20/10	19 - 20	19 - 20	19 - 20	13 - 18	13 - 18	13 - 18	13 - 18	NA
Amoxicillin	10	28 - 29	28 - 29	28 - 29	18 - 26	18 - 26	16 - 17	13 - 17	NA
Oxacillin	1	17 - 18	17 - 18	17 - 18	≥ 20	≥ 20	NA	NA	NA
Cephalexin	30	22	22	22	≥ 24	≥ 24	NA	NA	NA
Cefotaxim	30	22	22	22	NA	NA	NA	22 - 26	NA
Penicillin	10 UI	28 - 29	28 - 29	28 - 29	≥ 24	≥ 24	14 - 15	NA	NA
Erythromycin	15	13 - 23	13 - 23	13 - 23	15 - 21	15 - 21	13 - 23	NA	NA
Tri-sufa**	1.25/23.75	10 - 16	10 - 16	10 - 16	10 - 16	10 - 16	NA	10 - 16	NA
Tetracylin	30	14 - 19	14 - 19	14 - 19	14 - 19	14 - 19	14-19	14 - 19	NA
Kanamycin	30	13 - 18	13 - 18	13 - 18	NA	NA	NA	13 - 18	NA
Gentamycin	10	12 - 15	12 - 15	12 - 15	NA	NA	NA	12 - 16	12 - 16
Florfenicol	30	12 - 18	12 - 18	12 - 18	18 - 22	18 - 22	NA	12 - 18	NA
Marbofloxacin	5	14 - 20	14 - 20	14 - 20	14 - 20	14 - 20	NA	14 - 20	20 - 25
Enrofloxacin	5	16 - 23	16 - 23	16 - 23	16 - 23	16 - 23	NA	16 - 23	16 - 23
Clindamycin	2	14 - 21	14 - 21	14 - 21	14 - 21	14 - 21	NA	NA	NA
Cefoperazone	30	17 - 23	17-23	17 - 23	≥ 18	≥ 18	NA	17 - 23	NA

R: resistant, I: intermediate, S: sensitive, NA: not available.

**Amoxicillin + clavulanic acid.*

***Trimethroprim sulfadiazine.*

Table 2. Distribution of subclinical mastitis-causing agents identified by CHROMagar

Variable	Number of samples	%	CI 95%
Total samples	143	100	
No growth	36	25.2	18.1 - 32.3
Colonies with one morphology	56	39.2	31.1 - 47.2
Colonies with two morphology	38	26.6	19.3 - 33.8
Contamination	13	9.0	4.3 - 13.7
Staphylococcus	64	44.7	36.5 - 52.8
Staphylococcus aureus	6	4.2	0.9 - 7.5
Staphylococcus epidermidis	29	20.3	13.6 - 26.7
Staphylococcus saprophyticus	21	14.7	9.0 - 20.5
Other *Staphylococcus*	8	5.6	1.8 - 9.3
Streptococcus	90	62.9	54.9 - 70.8
Streptococcus uberis	32	22.4	15.6 - 29.2
Streptococcus agalactiae	49	34.3	26.5 - 42.1
Enterococcus spp.	2	1.4	0.5 - 3.0
Other *Streptococcus*	7	4.9	1.3 - 8.4
Gram-negative bacteria	12	8.4	3.8 - 12.9
Escherichia coli	4	2.8	0.9 - 5.5
Klebsiella spp.	2	1.4	0.5 - 3.0
Pseudomonas spp.	6	4.2	0.9 - 7.5

CI 95%: confidence interval 95%.

Table 3. Antimicrobial resistance of isolated pathogens from bovine subclinical mastitis

Antibiotic tested	Disk content (µg)	*S. aureus* (n = 5)	*S. epidermidis* (n = 29)	*S. saprophyticus* (n = 15)	*Strep. uberis* (n = 22)	*Strep. agalactiae* (n = 30)	*Enterococcus* spp. (n = 2)	*E. coli* (n = 1)	*Pseudonomas* spp. (n = 5)
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Cetiofur	30	3 (60)	12 (41.4)	4 (26.7)	8 (36.3)	10 (33.3)	0	0	5 (100)
Amox/clav*	20/10	3 (60)	5 (17.2)	6 (40.0)	0	3 (10.0)	0	0	NA
Ampicillin	10	5 (100)	25 (86.2)	12 (80.0)	9 (40.9)	10 (43.3)	0	1 (100)	NA
Oxacillin	1	5 (100)	18 (62.0)	10 (66.7)	21 (95.4)	28 (93.3)	NA	NA	NA
Cephalexin	30	5 (100)	25 (82.7)	14 (93.3)	18 (81.8)	26 (86.7)	NA	NA	NA
Cefotaxim	30	5 (100)	27 (93.1)	15 (100)	NA	NA	NA	1 (100)	NA
Penicillin	10 UI	4 (80)	23 (79.3)	12 (80.0)	15 (68.1)	23 (76.6)	0	NA	NA
Erythromycin	15	3 (60)	13 (44.8)	9 (60.0)	19 (86.3)	24 (80.0)	2 (100)	NA	NA
Tri-sufa**	1.25/23.75	2 (40	5 (17.2)	3 (20.0)	20 (90.9)	23 (76.6)	2 (100)	0	NA
Tetracylin	30	1 (20)	16 (55.2)	8 (53.3)	15 (68.1)	24 (80.0)	2 (100)	0	NA
Kanamycin	30	1 (20)	11 (37.9)	10 (66.7)	NA	NA	2 (100)	0	NA
Gentamycin	10	0	3 (10.3)	3 (20.0)	10 (45.4)	19 (63.3)	NA	0	0
Florfenicol	30	0	3 (10.3)	5 (33.3)	6 (27.2)	12 (40.0)	NA	0	NA
Marbofloxacin	5	0	8 (27.6)	2 (13.3)	NA	NA	NA	NA	5 (100)
Enrofloxacin	5	2 (40)	10 (34.4)	5 (33.3)	8 (36.3)	14 (46.6)	NA	NA	2 (40)
Clindamycin	2	3 (60)	9 (31.0)	5 (33.3)	10 (45.4)	17 (56.6)	NA	NA	NA
Cefoperazone	30	3 (60)	6 (20.7)	5 (33.3)	8 (36.3)	9 (30.0)	NA	NA	NA

**Amoxicillin + clavulanic acid, ** Trimethroprim sulfadiazine, NA: not available.*

ceftiofur remaining effective against this species. For *Streptococcus*, the antibiotic resistance rate also varied among bacterial species. *S. uberis* resisted oxacillin, trimethoprim-sulfadiazine, cephalexin, erythromycin, penicillin, and tetracycline. Conversely, this strain showed sensitivity to amoxicillin/clavulanic acid. *S. agalactiae* was resistant to oxacillin, cephalexin, erythromycin, tetracycline, and penicillin, while the most effective antibiotic was ampicillin. *Enterococcus* spp. exhibited complete resistance to erythromycin, trimethoprim. In contrast, amoxicillin/clavulanic acidsulfadiazine, tetracycline, kanamycin, and ampicillin remained sensitive to this bacterial species. The number of isolated gram-negative bacteria was not substantial. Initially, *Pseudomonas* spp. showed intensely extreme resistance to ceftiofur and marbofloxacin, while gentamicin was effective against this bacterium.

4. Discussion

The present study used a triplate containing chromogenic culture media to rapidly identify microorganisms in subclinical mastitic milk samples. Of 143 samples, it observed a prevalence of positive samples was 65.8%. This result aligns with the findings of Granja et al. (2021), who also used chromogenic culture media to isolate agents-causing subclinical mastitis in Brazil. Among these positive samples, it found that the microorganisms with the highest prevalence were *Strep. agalactiae*, *Strep. uberis*, and negative coagulase *Staphylococcus*. The results of this study were like those described for the distribution of microorganisms causing subclinical mastitis in dairy cows in Southern Vietnam (Östensson et al., 2013). The results of contamination (9.0%) were higher than those described by previous studies from 0.6 - 2.9% (Cameron et al., 2013; Ganda et al., 2016; Granja et al., 2021). This differentiation could be associated with practical skill, although the milk sample protocol was followed by the NMC (2017). In this study, 25.2% of the samples had no microbial growth. Previous studies showed that the percentage of subclinical mastitis milk samples isolated without colonies varied between 28.6% and 31.3% (McCarron et al., 2009; Ganda et al., 2016; Granja et al., 2021). It could be due to a low bacterial concentration to be detected by the culture method, or belong to other bacteria species, such as *Mycoplasma* (Fox, 2012). Regarding the *Staphylococcus* group isolated from SCM samples, the chromogenic culture media differentiated contagious pathogens (*S. aureus*, 4.2%) and environmental pathogens (NCS). Similarly, the differentiation capacity among *Streptococcus* group isolated in the chromogenic media. In this case, rapid identification results could be used to separate cows with contagious transmission (*Strep. agalactiae*) and environmental transmission (*Strep. uberis*). One of the limitations of using the CHROMagar *Streptococcus* is the lack of differentiation of *Strep. agalactiae* and *Strep. dysgalactiae* because they have similar colony color. In our study, gram-negative bacteria constituted an insignificant proportion of subclinical mastitis cases. This finding was identical to those reported by Ashraf and Imran (2018). Indeed, gram-negative bacteria, such as *E. coli*, are often the cause of severe clinical mastitis. Thus, the assessment of the effectiveness of chromogenic media in SCM-caused by gram-negative bacteria was limited. It is necessary to conduct another research on milk samples from cows with clinical mastitis to further evaluate the efficacy of CHROMagar in identifying gram-negative bacterial species.

For the *Staphylococcus* group, the results showed that the resistance rate of each bacterial species to each antibiotic tested was different.

This finding suggested that it was essential to identify bacterial species causing SCM could help to select more sensitive antibiotics, thereby increasing treatment effectiveness. For example, ceftiofur, which has been widely used on dairy farms, had resistance rates to *S. saprophyticus* (26.7%), *S. epidermidis* (41.8%), and *S. aureus* (60%). In our study, *S. aureus* was highly resistant to the β-lactam antibiotic group, while this bacterial species was sensitive to gentamycin, florfenicol, and marbofloxacin. Our findings were like those of previous studies (Erskine et al., 2002; Roesch et al., 2006; Botrel et al., 2010). The antimicrobial resistance rate of *S. aureus* from bovine mastitis for gentamycin in the United States, Chile, Europe, and Iran varied between 0 and 6.8% (Oliver & Murinda, 2012). For *S. epidermidis*, we found that antibiotics with low resistance rates for this bacterium, including gentamycin, florfenicol, and trimethoprim-sulfadiazine, varied from 10.3 % to 17.2%. The previous studies from Botrel et al. (2010), Kalmus et al. (2011), and Persson et al. (2011) were also similar. In the current study, marbofloxacin was the best choice in the treatment of subclinical mastitis caused by *S. saprophyticus* because of the lowest resistance rate (13.3%), followed by trimethoprim sulfadiazine (20%) and ceftiofur (26.7%). Cetiofur and trimethoprim sulfadiazine were found also sensitive to this species in the research of Suriyasathaporn (2010), with a resistance rateo of 16.7%. In a systemic review, Peter et al. (2012) indicated that *S. saprophyticus* was sensitive to cetiofur and trimethoprim sulfadiazine, with a resistance rate of 0% and 17%, respectively.

Like the *Staphylococcus* group, the results of the present study indicated that different bacterial species within the *Streptococcus* group had different rates of resistance to various antibiotics. Florfenicol, enrofloxacin, gentamycin, clindamycin, and tetracycline were effective against *Strep. uberis*, while *Strep. agalactiae* was sensitive to others such as trimethoprim sulfadiazine, erythromycin, cefoperazone. Those findings confirmed the importance of identifying bacterial species in selecting antibiotic treatment. In this study, the resistance rate of the combined antibiotic amoxicillin-clavulanic acid to *Strep. uberis* and *Strep. agalactiae* was the lowest, 0% and 10% respectively. Similar results were described by Petrovski et al. (2015) that all isolated *Strep. uberis* strains were susceptible to the amoxicillin-clavulanic acid in the United States and New Zealand. Bacteria *Enterococcus* spp. recorded to be sensitive to some beta-lactam antibiotics commonly used to treat mastitis, including ceftiofur, amoxicillin-clavulanic, amoxicillin-clavulanic acid, and ampicillin. Ampicillin resistance rates were also reported by Ebrahimi et al. (2007) and Nam et al. (2009). However, a limitation encountered in our study was the small number of antibiotic samples for *Enterococcus* spp. Similarly, although the number of isolated gram-negative bacteria was insignificant, our results showed that *Pseudomonas* spp. resisted tested antibiotics. of the report ed by Rajala-Schultz et al. (2004), which mentioned that resistant ability. Another limitation of our study was that *Klebsiella* spp. did not grow on Mueller Hinton agar plates for the antimicrobial susceptibility testing.

5. Conclusions

The chromogenic media, CHROMagar, are an option for rapid species-level identification of bacteria causing subclinical mastitis without biochemical tests in Vietnam livestock conditions. Isolated colonies from this chromogenic media can be utilized in antimicrobial susceptibility testing. Most isolated bacterial pathogens were resistant to β-lactam antibiotics. Additionally,

varying levels of antibiotic resistance were observed across different bacterial species. Thus, identifying the bacterial species and conducting antimicrobial susceptibility tests play a crucial role in improving the effectiveness of treatment for bovine subclinical mastitis.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors gratefully acknowledge financial support from Nong Lam University, Ho Chi Minh City, Vietnam, project ID CS-CB23-CNTY-04.

References

Adkins, P. R. F., & Middleton, J. R. (2018). Methods for diagnosing mastitis. *Veterinary Clinical North America Food Animal Practice* 34(3), 479-491. https://doi.org/10.1016/j.cvfa.2018.07.003.

Ashraf, A., & Imran, M. (2018). Diagnosis of bovine mastitis: from laboratory to farm. *Tropical Animal Health Production* 50, 1193-1202. https://doi.org/10.1007/s11250-018-1629-0.

Bar, D., Tauer, L. W., Bennett, G., González, R. N., Hertl, J. A., Schukken, Y. H., Schulte, H. F., Welcome, F. L., & Gröhn, Y. T. (2008). The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. *Journal of Dairy Science* 91(6), 2205-2214. https://doi.org/10.3168/jds.2007-0573.

Botrel, M. A., Haenni, M., Morignat, E., Sulpice, P., Madec, J. Y., & Calavas, D. (2010). Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. *Foodborne Pathology Disease* 7(5), 479-487. https://doi.org/10.1089/fpd.2009.0425.

Cameron, M., Keefe, G. P., Roy, J. P., Dohoo, I. R., MacDonald, K. A., & McKenna, S. L. (2013). Evaluation of a 3M petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. *Preventive Veterinary Medicine* 111(1-2), 1-9. https://doi.org/10.1016/j.prevetmed.2013.03.006.

CLSI (Clinical and Laboratory Standard Institue). (2018). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals* (4th ed.). Pennsylvania, USA: Clinical and Laboratory Standard Institue.

Côté-Gravel, J., & Malouin, F. (2019). Symposium review: Features of *Staphylococcus aureus* mastitis pathogenesis that guide vaccine development strategies. *Journal of Dairy Science* 102(5), 4727-4740. https://doi.org/10.3168/jds.2018-15272.

Ebrahimi, A., Kheirabadi, K. H. P., & Nikookhah, F. (2007). Antimicrobial susceptibility of environmental bovine mastitis pathogens in west central Iran. *Pakistan Journal Biology Sciences* 10(17), 3014-3016. https://doi.org/10.3923/pjbs.2007.3014.3016.

Erskine, R. J., Walker, R. D., Bolin, C. A., Bartlett, P. C., & White, D. G. (2002). Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. *Journal of Dairy Science* 85(5), 1111-1118. https://doi.org/10.3168/jds.S0022-0302(02)74172-6.

Fox, L. K. (2012). Mycoplasma mastitis: causes, transmission, and control. Veterinary Clinics of North America: *Food Animal Practice* 28(2), 225-237. https://doi.org/10.1016/j.cvfa.2012.03.007.

Ganda, E. K., Bisinotto, R. S., Decter, D. H., & Bicalho, R. C. (2016). Evaluation of an on-farm culture system (accumast) for fast identification of milk pathogens associated with clinical mastitis in dairy cows. *PloS One* 11(5), e0155314. https://doi.org/10.1371/journal.pone.0155314.

Granja, B. M., Fidelis, C. E., Garcia, B. L. N., & dos Santos, M. V. (2021). Evaluation of chromogenic culture media for rapid identification of microorganisms isolated from cows with clinical and subclinical mastitis. *Journal of Dairy Science* 104(8), 9115-9129. https://doi.org/10.3168/jds.2020-19513.

Hagnestam-Nielsen, C., & Ostergaard, S. (2009). Economic impact of clinical mastitis in a dairy herd assessed by stochastic simulation using different methods to model yield losses. *Animal* 3(2), 315-328. https://doi.org/10.1017/S1751731108003352.

Kalmus, P., Aasmäe, B., Kärssin, A., Orro, T., & Kask, K. (2011). Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. *Acta Veterinaria Scandinavica* 53(1), 1-7. https://doi.org/10.1186/1751-0147-53-4.

Kandeel, S. A., Megahed, A. A., Arnaout, F. K., & Constable, P. D. (2018). Evaluation and comparison of 2 on-farm tests for estimating somatic cell count in quarter milk samples from lactating dairy cattle. *Journal of Veterinary Internal Medicine* 32(1), 506-515. https://doi.org/10.1111/jvim.14888.

Ly, S. H., Nguyen, N. Q., & Nguyen, D. T. (2022). Characterization of clinical mastitis in dairy cows in industrial farms. *Journal of Veterinary Science and Technology* 29(6), 70-74.

McCarron, J. L., Keefe, G. P., McKenna, S. L. B., Dohoo, I. R., & Poole, D. E. (2009). Laboratory evaluation of 3M Petrifilms and university of Minnesota Bi-plates as potential on-farm tests for clinical mastitis. *Journal of Dairy Science* 92(5), 2297-2305. https://doi.org/10.3168/jds.2008-1661.

Nam, H. M., Lim, S. K., Kang, H. M., Kim, J. M., Moon, J. S., Jang, K. C., Joo, Y. S., Kang, M. I., & Jung, S. C. (2009). Antimicrobial resistance of streptococci isolated from mastitic bovine milk samples in Korea. *Journal of Veterinary Diagnosis Investigation* 21(5), 698-701. https://doi.org/10.1177/104063870902100517.

NMC (National Mastitis Council). (2017). *Laboratory handbook on bovine mastitis* (3rd ed.). Minnesota, USA: National Mastitis Council.

Oliver, S. P., & Murinda, S. E. (2012). Antimicrobial resistance of mastitis pathogens. *Veterinary Clinics of North America: Food Animal Practice* 28(2), 165-185. https://doi.org/10.1016/j.cvfa.2012.03.005.

Östensson, K., Lam, V., Sjögren, N., & Wredle, E. (2013). Prevalence of subclinical mastitis and isolated udder pathogens in dairy cows in Southern Vietnam. *Tropical Animal Health Production* 45, 979-986. https://doi.org/10.1007/s11250-012-0320-0.

Perry, J. D. (2017). A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. *Clinical Microbiology Reviews* 30(2), 449-479. https://doi.org/10.1128/CMR.00097-16.

Persson, Y., Nyman, A. K. J., & Grönlund-Andersson, U. (2011). Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Veterinaria Scandivanica* 53(1), 1-8. https://doi.org/10.1186/1751-0147-53-36.

Petrovski, K. R., Grinberg, A., Williamson, N. B., Abdalla, M. E., Lopez-Villalobos, N., Parkinson, T. J., Tucker, I. G., & Rapnicki, P. (2015). Susceptibility to antimicrobials of mastitis-causing *Staphylococcus aureus*, *Streptococcus uberis* and *Str. dysgalactiae* from New Zealand and the USA as assessed by the disk diffusion test. *Australian Veterinary Journal* 93(7), 227-233. https://doi.org/10.1111/avj.12340.

Pinzón-Sánchez, C., Cabrera, V. E., & Ruegg, P. L. (2011). Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. *Journal of Dairy Science* 94(4), 1873-1892. https://doi.org/10.3168/jds.2010-3930.

Rajala-Schultz, P. J., Smith, K. L., Hogan, J. S., & Love, B. C. (2004). Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. *Veterinary Microbiology* 102(1-2), 33-42. https://doi.org/10.1016/j.vetmic.2004.04.010.

Roesch, M., Perreten, V., Doherr, M. G., Schaeren, W., Schällibaum, M., & Blum, J. W. (2006). Comparison of antibiotic resistance of udder pathogens in dairy cows kept on organic and on conventional farms. *Journal of Dairy Science* 89(3), 989-997. https://doi.org/10.3168/jds.S0022-0302(06)72164-6.

Ruegg, P. L. (2011). Managing mastitis and producing quality milk. *Dairy Production Medicine* 207-232. https://doi.org/10.1002/9780470960554.ch18.

Suojala, L., Kaartinen, L., & Pyörälä, S. (2013). Treatment for bovine *Escherichia coli* mastitis - an evidence-based approach. *Journal of Veterinary Pharmacology and Therapeutics* 36(6), 521-531. https://doi.org/10.1111/jvp.12057.

Suriyasathaporn, W. (2010). Milk quality and antimicrobial resistance against mastitis pathogens after changing from a conventional to an experimentally organic dairy farm. *Asian-Australasian Journal of Animal Sciences* 23(5), 659-664.

Inventorying and proposing solutions for street tree management in Thu Duc city, Ho Chi Minh City

Tien T. M. Duong*, Thuy T. Vuong, The M. T. Ngo, & Thanh T. Nguyen
Faculty of Environment and Natural Resources, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: January 04, 2023
Revised: June 21, 2023
Accepted: July 17, 2023

Keywords
Street trees
Thu Duc city
Tree inventory
Trees in Ho Chi Minh city
Urban trees management

***Corresponding author**

Duong Thi My Tien
Email:
duongmytien@hcmuaf.edu.vn

ABSTRACT

Tree inventory plays an essential role in the urban landscape master plan. It serves as the foundation for data acquisition that supports the planning strategy and decisions relating to the community's interests, particularly in recently established cities like Thu Duc city. This research was carried out from October 2021 to December 2022 in this city and aimed to inventory to gather qualified information for managing the city's street trees. The study used an inventory form of street trees including criteria such as name, family, diameter at breast height (DBH), total height, and indicators of vitality and distance from trees to infrastructure. By this form, 287 streets were surveyed. The total number of investigated trees was 31.023 trees belonging to 65 species and 24 flora families, of which the species with a high percentage (from 10% or more) were *Dipterocarpus alatus*, *Hopea odorata*, *Peltophorum pterocarpum*, and *Lagerstroemia speciosa*. The most numerous plant family was Fabaceae. These trees typically had a height of less than 10 m, with a DBH of less than 20 cm. Many streets did not guarantee the distance between trees and infrastructure, particularly the distance to the electrical systems. The survey also showed that the majority of trees were healthy and grow healthily. From the current situation of Thu Duc city street trees, three solutions must be prioritized in order to improve street trees in this city.

Cited as: Duong, T. T. M., Vuong, T. T., Ngo, T. M. T., & Nguyen, T. T. (2024). Inventorying and proposing solutions for street tree management in Thu Duc city, Ho Chi Minh City. *The Journal of Agriculture and Development* 23(3), 53-66.

I. Introduction

Since the nineteenth century, street trees have become an important component of the urban landscape, playing many ecological and socio-economic roles (Caneva et al., 2020). They are an integral part of urban ecosystems, improving environmental quality by providing ecological benefits (Dover, 2015). Street trees significantly reduce air pollution by removing pollutants such as SO_2, NO_x, CO, and O_3 through their leaf stomata and by capturing dust on their leaf surfaces (Smith, 1984). Additionally, street trees contribute to reducing the urban heat island effect and have a clear role in improving the microclimate (Chang et al., 2007). "Greening the city" is a crucial issue on the agendas of major cities around the world, and trees are an important aspect of it (Braverman, 2008). Urban tree planting initiatives are being actively promoted as a planning tool for urban areas to adapt to and mitigate climate change, enhance urban sustainability, and improve health and well-being (Salmond et al., 2016).

From a landscape architecture perspective, street trees are one of the most important components of urban green space, playing an important role in street aesthetics (Li et al., 2011). The first impression of a city often comes from its streetscape (Jacobs, 1993). Over the centuries, street trees have remained enduring features of urban landscapes (Merse et al., 2008). However, data on species diversity of street trees, the criteria for species selection, and regulations on tree planting and maintenance are still limited (Caneva et al., 2020).

However, data on the species diversity of street trees, the criteria for species selection, and regulations on tree planting and maintenance are still limited (Caneva et al., 2020). For street trees, the design and selection of species reinforce the identity and distinctiveness of a city. Species diversity contributes to improving the aesthetics and health of trees in urban areas (Ware, 1994; Jim, 1999; Thaiutsa et al., 2008). Besides suitability to local climatic conditions, factors such as size and aesthetic value should be considered when selecting species (Caneva et al., 2020). In urban environments, trees are adversely affected by atmospheric pollutants, poor drainage, harsh soil conditions, mechanical impact, and limited growth space due to high and low ambient temperatures (Ware, 1994; Jim, 1999; Thaiutsa et al., 2008). Design and species selection depend on urban-specific characteristics such as land use (commercial land, residential land, etc.), street specifications (size, width, length), and the location of trees (sidewalks, medians) (Li et al., 2011). Successful street tree planting can only be achieved if multiple criteria are met (Pauleit, 2003).

Cities around the world have issued regulations for the selection of species, technical parameters to be ensured when planting and maintaining street trees, planting location, and the distance between trees and road edges, intersections, fire hydrants, and manholes. Some examples include the Street Tree Manual issued by SDOT in 2013, the Design Standards for Street Trees published by TDPRLC (2007), the Street Tree Planting Standards published by NYC Parks (2016), the Regulation of Street Trees: Maintenance Of Street Trees And Sidewalks issued by TFM (2022), and Street Trees promulgated by City of Tulare, California (CTCCO, 2008). In Vietnam, standards and specifications for street trees are stipulated by MOC (2005) of the Ministry of Construction on guiding the management of urban green trees, GOV (2010) of the government on urban green tree management, the national standard (MOST, 2012) on planning green trees for public use in

urban areas - design standards; GOV (2005) detailing and guiding the implementation of a number of articles of the Electricity Law on safety protection of high-voltage grid works.

In Ho Chi Minh City, regulations governing the selection of plant species are outlined in PCHCMC (2013) by the People's Committee, which provides a list of encouraged and restricted trees and prohibits certain types of planting. Thu Duc city was recently established in 2021 according to the Resolution SCNA (2020) of the National Assembly Standing Committee, and is currently focused on improving the quality of its "green-clean-beautiful" street landscape. However, there is currently no inventory of street trees in the city. This study aims to assess the current situation of street trees in Thu Duc city in relation to government regulations, specifically in terms of species composition and compliance with specifications. The findings of this study will contribute to the development of a database for the management and research of urban trees.

2. Materials and Methods

This research utilized a standard urban tree inventory form from the urban forestry discipline, which included various technical parameters of the road (such as length, road width, sidewalk width, and the presence or absence of a separator), plant species information (including botanical name, flora family, total height, diameter at breast height - DBH, and planting location with other components like intersections, fire hydrants, manholes, and electricity), and vitality. Certain pieces of information were selected for this inventory, such as tree height and DBH, for specific reasons. Total tree height is one of the most important tree attributes in forest inventory (Jurjević et al., 2020). This indicator is usually used to calculate individual trees, it directly affects the calculation of other attributes (Wang et al., 2019). The DBH is a widely-used forestry management measurement that has been utilized since the 19th century to estimate wood volume, identify trees, and measure tree growth (Magarik, 2020) without cutting down the trees (Chaudhuri, 2016).

The gathered information was evaluated and compared to the guidelines on species composition, height, and regulation on location and planting distance specified in the government's legal documents (listed and discussed in section 3.1).

The data was collected via field survey methods like observing, recording information, taking photos, and collecting samples according to Klein and Klein's method (1970) for unidentified species. Tape measures and clinometers were also used to collect street width, total tree height, DBH, and other parameters.

To identify the species' botanical names, samples, photos, and descriptions of trees were compared morphologically with species in some books like "An Illustrated Flora of Vietnam" by Pham (2000) and "500 Useful Plants in Landscape Design" by Dinh (2021). This study was also updated with the latest scientific name according to the website http://www.worldfloraonline.org/. The collected data were synthesized in Microsoft Excel 2015 and then analyzed, evaluated, and compared to (MOC, 2005) and (PCHCMC, 2013).

3. Results and Discussion

3.1. Inventory of street trees

Streets in the investigation areas

The inventory was conducted on 287 streets in 3 old districts of Thu Duc city (district 2, district 9 and Thu Duc district). There are only 24 streets

with separator in width from 1 - 8 m. These are streets that are already available or qualified for tree planting. The width of the streets ranges from 3 m to 60 - 70 m (Mai Chi Tho street, Pham Van Dong street and Hanoi highway). The sidewalk width is mostly 3 - 5 m.

Number of trees and number of species

The study investigated 31,023 trees belonging to 65 different species, as shown in Table 1. Some species, including Dipterocarpus alatus, Hopea odorata, Peltophorum pterocarpum, and Lagerstroemia speciosa, accounts for 10% or more of the total trees. Additionally, some species constituts 3 - 7% of the total trees, such as Mimusops elengi, Khaya senegalensis, Pterocarpus macrocarpus, Pterocarpus indicus, Delonix regia, Cassia fistula, and Tamarindus indica. On the other hand, some species, mostly fruit trees like Syzygium samarangense, Dimocarpus longan, Chrysophyllum cainito, and Cocos nucifera, are available in small number. Despite the variety of street tree species in Thu Duc city, there are also several species on the list of trees banned from planting and restricted from planting under Decision 52/2013/QD-UBND of the People's Committee. For example, Ficus racemosa roots damage the infrastructure, and ripe figs falling on the street can lead to unhygienic conditions. Terminalia captappa, which has 648 trees, is vulnerable to pests and diseases. Alstonia scholaris has easily broken branches and smelly flowers that cause discomfort to the citizens. Khaya senegalensis, with its protruding roots on the ground, can damage sidewalks and the street surface and pose a danger to pedestrians. Additionally, fruit trees like Dimocarpus longan, Mangifera indica, Cocos nucifera, and Chrysophyllum cainito, when ripe, can attract children to climb dangerously or fall, leading to hazardous conditions for people. Acacia auriculiformis has branches that pose a risk to pedestrians, and Hura crepitans is forbidden to plant because of its toxic seeds and latex. Therefore, it is essential to take timely action to eliminate these restricted and banned species from the streets to ensure aesthetic appeal and safety for citizens.

Table 1. Number of trees and species investigated in the streets of Thu Duc city

No.	Names	Family	Quantity	Percentage (%)
1	*Dracontomelon duperreanum* Pierre	Anacardiaceae	7	0.02
2	*Mangifera indica* L.	Anacardiaceae	32	0.10
3	*Monoon longifolium* (Sonn.) B.Xue & R.M.K.Saunders	Annonaceae	119	0.38
4	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	32	0.10
5	*Holarrhena pubescens* Wall. & G.Don Wall. ex G.Don	Apocynaceae	1	0.00
6	*Kopsia arborea* Blume	Apocynaceae	9	0.03
7	*Plumeria rubra* L.	Apocynaceae	9	0.03
8	*Plumeria obtusa* L.	Apocynacesae	289	0.93
9	*Araucaria columnaris* Hook.	Araucariaceae	4	0.01
10	*Adonidia merrillii* (Becc.) Becc.	Arecaceae	329	1.06
11	*Archontophoenix alexandrae* (F.Muell.) H.Wendl. & Drude	Arecaceae	3	0.01
12	*Cocos nucifera* L.	Arecaceae	1	0.00
13	*Elaeis guineensis* Jacq.	Arecaceae	143	0.46
14	*Roystonea regia* O.F.Cook	Arecaceae	103	0.33
15	*Spathodea campanulata* P. Beauv.	Bignoniaceae	17	0.05
16	*Tabebuia rosea* (Bertol.) Bertero ex A.DC.	Bignoniaceae	585	1.89
17	*Tecoma stans* (L.) Kunth	Bignoniaceae	5	0.02
18	*Bucida molineti* (M.Gomez) Alwan & Stace	Combretaceae	420	1.35
19	*Terminalia captappa* L.	Combretaceae	352	1.13
20	*Terminalia chebula* Retz.	Combretaceae	180	0.58
21	*Dipterocarpus alatus* Roxb. & G.Don	Dipterocarpaceae	4,513	14.55
22	*Hopea odorata* Roxb	Dipterocarpaceae	4,279	13.79
23	*Diospyros decandra* Lour	Ebenaceae	2	0.01
24	*Hura crepitans* L.	Euphorbiaceae	3	0.01
25	*Acacia auriculiformis* A.Cunn. ex Benth.	Fabaceae	4	0.01
26	*Acacia mangium* Willd.	Fabaceae	1	0.00
27	*Afzelia xylocarpa* (Kurz) Craib	Fabaceae	42	0.14
28	*Bauhinia purpurea* L.	Fabaceae	509	1.64
29	*Bauhinia variegata* L.	Fabaceae	9	0.03
30	*Cassia fistula* L.	Fabaceae	981	3.16
31	*Dalbergia oliveri* Gamble ex Prain	Fabaceae	38	0.12
32	*Delonix regia* (Bojer ex Hook.) Raf.	Fabaceae	932	3.00

Table 1. Number of trees and species investigated in the streets of Thu Duc city (cont.)

33	*Erythrina fusca* Lour.	Fabaceae	26	0.08
34	*Erythrophleum fordii* Oliv.	Fabaceae	7	0.02
35	*Peltophorum pterocarpum* (DC.) Backer ex K.Heyne	Fabaceae	3,586	11.56
36	*Pterocarpus indicus* Willd.	Fabaceae	1,662	5.36
37	*Pterocarpus macrocarpus* Kurz	Fabaceae	970	3.13
38	*Samanea saman* (Jacq.) Merr.	Fabaceae	421	1.36
39	*Tamarindus indica* L.	Fabaceae	1,047	3.37
40	*Tectona grandis* L.f.	Lamiaceae	508	1.64
41	*Cinnamomum camphora* (L.) J.Presl	Lauraceae	78	0.25
42	*Barringtonia acutangular* (L.) Gaertn.	Lecythidaceae	507	1.63
43	*Couroupita guianensis* Aubl.	Lecythidaceae	24	0.08
44	*Lagerstroemia calyculata* Kurz	Lythraceae	7	0.02
45	*Lagerstroemia reginae* Roxb.	Lythraceae	490	1.58
46	*Lagerstroemia speciosa* (L.) Pers.	Lythraceae	3,952	12.74
47	*Michelia alba* DC.	Magnoliaceae	8	0.03
48	*Ceiba pentandra* (L.) Gaertn.	Malvaceae	10	0.03
49	*Azadirachta indica* A.Juss.	Meliaceae	3	0.01
50	*Chukrasia tabularis* A.Juss	Meliaceae	572	1.84
51	*Khaya senegalensis* A.Juss.	Meliaceae	1,002	3.23
52	*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg	Moraceae	149	0.48
53	*Artocarpus heterophyllus* Lam.	Moraceae	8	0.03
54	*Ficus benjamina* L.	Moraceae	29	0.09
55	*Ficus microcarpa* L.f.	Moraceae	76	0.24
56	*Ficus racemosa* L.	Moraceae	18	0.06
57	*Ficus religiosa* L.	Moraceae	15	0.05
58	*Ficus rumphii* Blume	Moraceae	8	0.03
59	*Muntingia calabura* L.	Muntingiaceae	7	0.02
60	*Syzygium cumini* (L.) Skeels	Myrtacea	1	0.00
61	*Syzygium samarangense* (Blume) Merr & L.M.Perry	Myrtaceae	2	0.01
62	*Averrhoa carambola* L.	Oxalidaceae	6	0.02
63	*Dimocarpus longan* Lour.	Sapindaceae	1	0.00
64	*Chrysophyllum cainito* L.	Sapotaceae	2	0.01
65	*Mimusops elengi* L.	Sapotaceae	1,868	6.02
			31,023	100.00

Number of families

65 species of trees investigated belong to 24 flora families, in which Fabaceae accounted for the majority in both the number of species (15 species, 23%) and the number of trees (10,340 trees, nearly 35%). This can be explained by the fact that Fabaceae is a family with many beautiful flowering trees that can be grown in the street. In addition, Fabaceae is the third largest family of flowering flora after Asteraceae and Orchidaceae (Judd et al., 2002), Fabaceae is also a large plant family in Vietnam with about 600 species (Nguyen, 2003). Some other families also are considered noteworthy such as Moraceae (7 species), Apocynaceae, and Arecaceae (5 species of each), the remaining families account for 1 - 3 species of each (Figure 1). The family Dipterocarpaceae had only 2 species but in very large number (7,265 trees, ranked second after Fabaceae). Two other families, Lythraceae and Meliaceae, had only 3 species per family, but the number of trees was also considerable (4,117 trees and 1,577 trees).

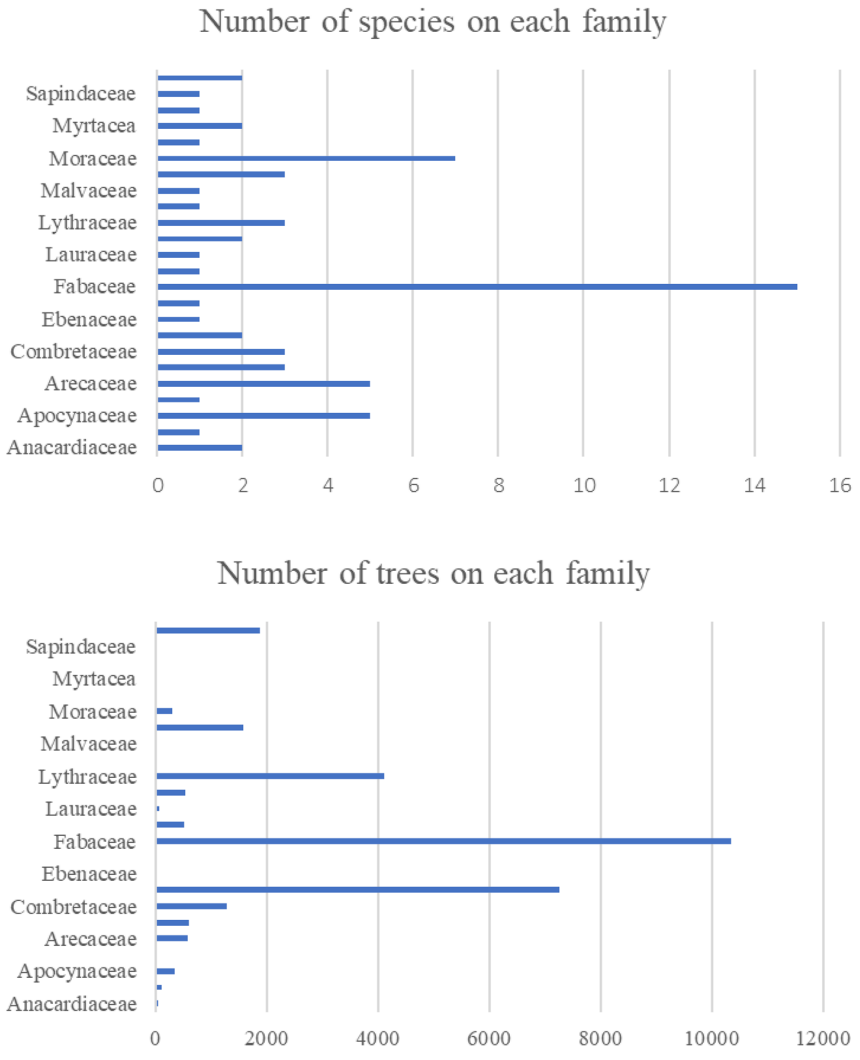

Figure 1. Tree families.

Total height of trees

The inventory showed that most of the street shade trees in Thu Duc city had a total height of less than 10 m (74%). According to the classification of (MOC, 2005), there are type 1 trees. The proportion of type 2 ones (total height from 10 m to 15 m) accounts for 24% and type 3 ones (trees with a total height greater than 15 m) accounts for only about 2% (Figure 2). According to Table 1, 42 species (accounting for 65%) at maturity are type 3. There are some reasons for this situation, such as recently planted trees, trees height is limited because of entangled with power lines or to limit falls, broken branches due to weather. Regarding 587 trees on type 3, the majority is Dipterocarpus alatus Roxb. & G. Doni and Hopea odorata Roxb with the number of 167 and 249 plants, respectively.

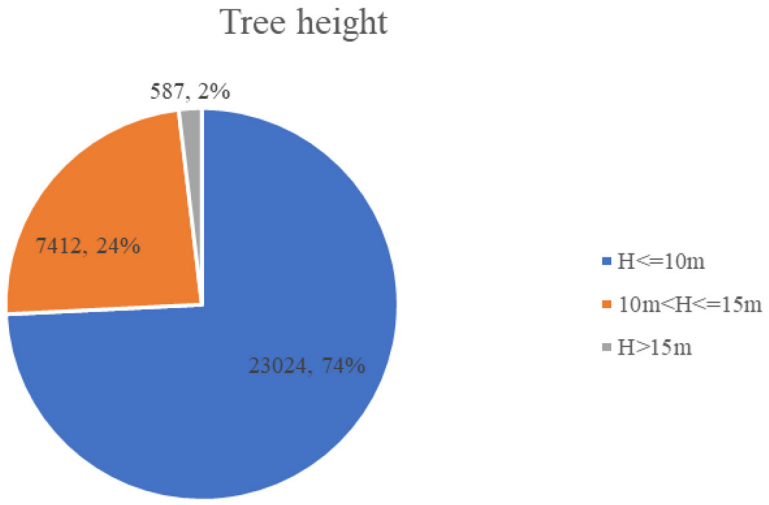

Figure 2. Tree height class according to Ministry of Construction (2005).

Diameter at breast height (DBH)

In this inventory, 56% of street trees in Thu Duc city had a DBH of less than 20 cm, 43% of trees had DBH between 20 and 50 cm, and only 1% (corresponding to 264 trees) was found to have DBH greater than 50 cm (Figure 3) in which the main species were Samanea saman, Khaya senegalensis and Delonix regia. These were species that grow and develop quite rapidly, easily reaching bigger diameters than other common street tree species. Species with a large number of trees such as Dipterocarpus alatus, Hopea odorata, Peltophorum pterocarpum, and Lagerstroemia speciosa were mostly at DBH mainly distributed in less than 20 cm class and 20 cm 50 cm class.

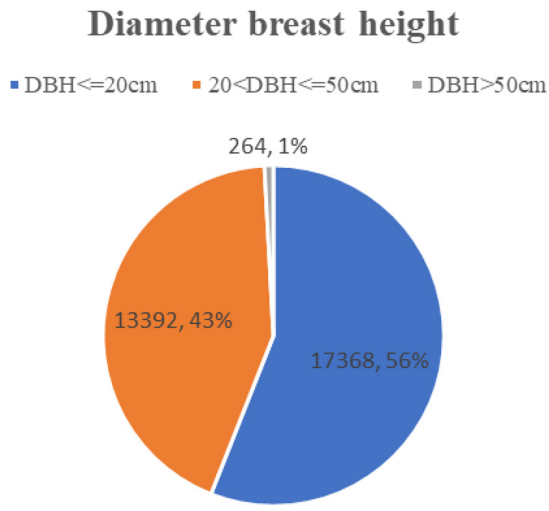

Figure 3. Diameter at breast height class according to Ministry of Construction (2005).

Distance of trees to infrastructure

As can be seen in the Table 2, there are 17/287 streets with street trees that have not to meet the stipulation of Circular No. 20/2009/TT-BXD of the Ministry of Construction amending and supplementing MOC (2005) the distance from trees to the manhole is from 1 m to 2 m, but the ones are on Nguyen Hoang street (district 2) and some streets such as Dinh Phong Phu, Man Thien, 3rd Avenue, No. 10, No. 13, etc (district 9) with tree distance to the manhole less than 1 m.

According to the above regulation, there are 30/287 streets with trees planted illegally, which is less than 1 m from light such as Thong Nhat, Hiep Binh, Ngo Chi Quoc streets (Thu Duc district), Nguyen Van Huong, Xuan Thuy, Nguyen Hoang, Vu Tong Phan, etc (district 2) and some streets in district 9 such as Dinh Phong Phu, Duong Dinh Hoi, etc. Compared with the regulations on the distance between street trees and intersections, it was found that there are 18/287 streets with tree planting distances and intersections less than 5 - 8 m, namely streets B, D, Tam Chau (Thu Duc district) and streets 442, No. 10, No. 14 (district 9), etc.

Regarding the distance from the trees to the fire hydrant, there are 11/287 streets with a distance from the tree to the fire hydrant less than 2 - 3 m, such as No. 71-TML, No. 103-TML, No. 57-CL, etc (district 2) and No. 12, B (Thu Duc district). Trees planted along the electricity must ensure safety according to the provisions of GOV (2005) detailing and guiding the implementation of the Electricity Law on safety protection of high-voltage. However, the study found up to 104/287 streets which have trees that did not meet this regulation (Figure 4). This is an issue that Thu Duc needs to pay attention to improve the safety of urban people and ensure a good development environment for street trees.

Table 2. Distance from street trees to infrastructure

Distance to infrastructure	G	P
Distance of trees to manholes	270	17
Distance of trees to street lights	257	30
Distance of trees to intersections	269	18
Distance of trees to fire hydrants	276	11
Distance of trees to electricity	183	104

Figure 4. The unsafety of trees and electricity at Chu Manh Trinh and Cong Ly streets.

Tree vitality

Healthy trees provide a variety of advantages for the urban environment (Wargo, 2002). Nevertheless, several environmental pressures might have an impact on urban trees' vitality like pests and biotic diseases (Dobbertin, 2005; Percival, 2005). Street trees in Thu Duc city are evaluated in terms of vitality according to the following 4 classes. There are Class A - Good (trees without decay or rots at the trunk and full foliage/crown, no pests, and diseases); Class B - Fair (full foliage/crown, show signs of pests and diseases but are not significant to growth, unbalanced canopy, percentage of parasites and pet or diseases, rot less than 10%); Class C - Moderate (significantly less vitality, yellow/decay leaves, damaged by pests and diseases, poorly crown, percentage of parasitic and pet or diseases from 10 to 30%); and Class D - Poor, need to replace other trees (dead or nearly dead). As shown in Figure 5, 80% of street trees in Thu Duc city are classified A, and 19% ones in class B. However, there had 1% (432 trees) in classes C and D. These are trees that need to be monitored closely or replaced immediately.

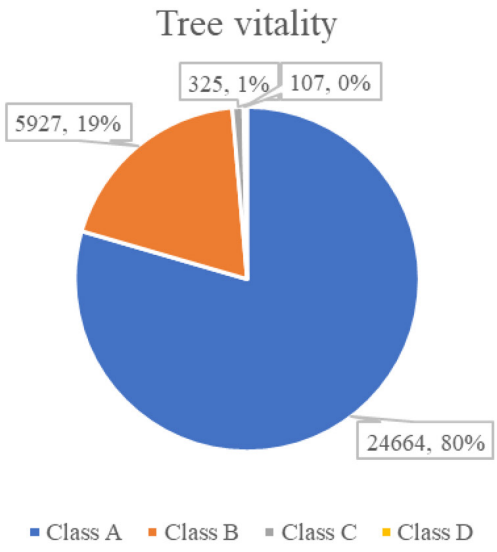

Figure 5. Tree vitality class.

3.2. Solutions for street tree management in Thu Duc city

To improve the street trees in Thu Duc city, the research proposes three main solutions based on legal documents, which are issued by the government related to urban trees and the current tree situation.

Firstly, it is important to choose accurate and suitable species. As shown in Table 1, many species do not comply with street tree regulations and must be replaced to ensure a beautiful and safe streetscape. Future street planning must also follow this solution. Suitable species that can be considered include *Chukrasia tabularis* A.Juss., *Diospyros martabanica* C.B.Clarke, *Anisoptera costata* Korth., *Terminalia chebula* Retz., *Dipterocarpus alatus* Roxb. ex G.Don, *Hopea odorata* Roxb, *Pterocarpus macrocarpus* Kurz, *Swietenia macrophylla* King, *Cinnamomum camphora* (L.) J.Presl, and *Dalbergia oliveri* Gamble ex Prain, among others.

Secondly, it is necessary to renovate existing street trees. This involves replacing dead or nearly dead trees, monitoring and closely caring for them, removing trees that do not comply with regulations, ensuring proper distance from infrastructure, and trimming to ensure safety and aesthetics.

Thirdly, in planning and designing trees for new streets, it is important to follow general regulations and also choose a different form of tree planting for each street depending on its length to create a unique landscape aesthetic and promote environmental efficiency.

4. Conclusions

The study surveyed 287 streets in Thu Duc city, counting 31,023 trees of 65 species and 24 flora families. The most prevalent family was Fabaceae, and the most abundant species were *Dipterocarpus alatus*, *Hopea odorata*, *Peltophorum pterocarpum*, and *Lagerstroemia speciosa*. However, some trees are currently

prohibited or restricted from being planted and should be replaced as soon as possible. The trees on the streets are generally less than 10 meters tall and have a diameter at breast height of less than 20 cm. Unfortunately, many trees are not properly distanced from infrastructure, including electricity/power line, presenting safety hazards. Despite this, most trees exhibit good vitality and growth. To improve the street trees in Thu Duc city, three solutions must be prioritized: selecting appropriate species, providing timely maintenance, care, and replacement of trees, and enhancing the planning and design of new streets.

Conflict of interest

The authors declare that they have no conflict of interest in this article.

Acknowledgements

This research was financially supported by the Nong Lam University research funding (the research code: CS-CB22-MTTN-11).

References

Braverman, I. (2008). Everybody loves trees: Policing American cities through street trees. *Duke Environmental Law and Policy Forum* 19, 81-118.

Caneva, G., Bartoli, F., Zappitelli, I., & Savo, V. (2020). Street trees in italian cities: Story, biodiversity and integration within the urban environment. *Rendiconti Lincei Scienze Fisiche e Naturali* 31(2), 411-417. https://doi.org/10.1007/s12210-020-00907-9.

Chang, C. R., Li, M. H., & Chang, S. D. (2007). A preliminary study on the local cool-island intensity of Taipei city parks. *Landscape and Urban Planning* 80(4), 386-395. https://doi.org/10.1016/j.landurbplan.2006.09.005.

Chaudhuri, B. R., & Pandey, N. K. (2016). *Forest mensuration*. West Bengal, India: Development Circle, Directorate of Forests Government of West Bengal.

CTCCO (City of Tulare, California Code of Ordinances). (2008). *Street trees*. Ohio, USA: American Legal Publishing.

Dinh, D. Q. (2021). *500 useful plants in landscape design*. Ho Chi Minh City, Vietnam: Ho Chi Minh City Science and Technics Publishing House.

Dobbertin, M. (2005). Tree growth as indicator of tree vitality and of tree reaction to environmental stress: A review. *European Journal of Forest Research* 124, 319-333. https://doi.org/10.1007/s10342-005-0085-3.

Dover, J. W. (2015). *Green Infrastructure: Incorporating plants and enhancing biodiversity in buildings and urban environments* (1st ed). London, UK: Taylor & Francis. https://doi.org/10.4324/9780203121993.

GOV (Government of the Socialist Republic of Viet Nam). (2010). Decree No. 64/2010/ND-CP dated on June 11, 2010. On management of urban green trees. Retrieved June 26, 2022, from https://thuvienphapluat.vn/van-ban/Xay-dung-Do-thi/Nghi-dinh-64-2010-ND-CP-quan-ly-cay-xanh-do-thi-107156.aspx.

GOV (Government of the Socialist Republic of Viet Nam). (2005). Decree No. 106/2005/ND-CP dated on August 17, 2005. Detailing and guiding the implementation of a number of articles of the electricity law on protection of safety of high-voltage power grid works. Retrieved May 20, 2022, from https://thuvienphapluat.vn/van-ban/Xay-dung-Do-thi/Nghi-dinh-106-2005-ND-CP-bao-ve-an-toan-cong-trinh-luoi-dien-cao-ap-huong-dan-luat-dien-luc-3032.aspx.

Jacobs, J. (1992). *The death and life of great American cities*. New York, USA: Vintage Books. Retrieved August, 24, 2022, from http://www.petkovstudio.com/bg/wp-content/uploads/2017/03/The-

Death-and-Life-of-Great-American-Cities_Jane-Jacobs-Complete-book.pdf.

Jim, C. Y. (1999). A planning strategy to augment the diversity and biomass of roadside trees in urban Hong Kong. *Landscape and Urban Planning* 44(1), 13-32. https://doi.org/10.1016/S0169-2046(98)00113-3.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F., & Donoghue, M. J. (2002). *Plant systematics: A phylogenetic approach* (2nd ed). Oxford, UK: Sinauer Associates.

Jurjević, L., Liang, X., Gašparović, M., & Balenović, I. (2020). Is field-measured tree height as reliable as believed - Part II, A comparison study of tree height estimates from conventional field measurement and low-cost close-range remote sensing in a deciduous forest. *ISPRS Journal of Photogrammetry and Remote Sensing* 169, 227-241. https://doi.org/10.1016/j.isprsjprs.2020.09.014.

Klein, R. M., & Klein, D. T. (1970). *Methods of plant research*. Ha Noi, Vietnam: Science and Technics Publishing House.

Li, Y. Y., Wang, X. R., & Huang, C. L. (2011). Key street tree species selection in urban areas. *African Journal of Agricultural Research* 6(15), 3539-3550. https://doi.org/10.5897/AJAR11.461.

Magarik, Y. A., Roman, L. A., & Henning, J. G. (2020). How should we measure the DBH of multi-stemmed urban trees? *Urban Forestry and Urban Greening* 47. https://doi.org/10.1016/j.ufug.2019.126481

Merse, C. L., Buckley, G. L., & Boone, C. G. (2008). Street trees and urban renewal: A Baltimore case study. *Geographical Bulletin - Gamma Theta Upsilon* 50(2), 65-81.

MOC (Ministry of Construction). (2005). Circular No. 20/2005/TT-BXD dated on December 20, 2005. On guidelines for urban forestry. Retrieved October 18, 2022, from https://thuvienphapluat.vn/van-ban/Xay-dung-Do-thi/Thong-tu-20-2005-TT-BXD-huong-dan-quan-ly-cay-xanh-do-thi-7249.aspx.

MOST (Ministry of Science and Technology) (2012). National standard TCVN 9257:2012 promulgated by the Ministry of Science and Technology on the planning of trees for public usage in urban areas - design standards. Retrieved November 4, 2022, from https://vanbanphapluat.co/tcvn-9257-2012-quy-hoach-cay-xanh-su-dung-cong-cong-tieu-chuan-thiet-ke.

NYC Parks (City of New York Parks and Recreation). (2016). *Street tree planting standards for New York city*. New York, USA: City of New York Parks and Recreation.

Nguyen, B. T. (2003). *Legumes (Fabaceae) in the list of plant species of Vietnam*. Ha Noi, Vietnam: Agricultural Publishing House.

Pauleit, S. (2003). Urban street tree plantings: identifying the key requirements. *Proceedings of The Institution of Civil Engineers - Municipal Engineer* 156(1), 43-56. https://doi.org/10.1680/muen.2003.156.1.43.

PCHCMC (People's Committee of Ho Chi Minh City). (2013). Decision No. 52/2013/QD-UBND dated on November 25, 2013. Promulgating the list of trees prohibited from growing on the streets in Ho Chi Minh City. Retrieved October 20, 2022, from https://thuvienphapluat.vn/van-ban/Xay-dung-Do-thi/Quyet-dinh-52-2013-QD-UBND-Danh-muc-cay-cam-trong-tren-duong-Ho-Chi-Minh-214358.aspx.

Percival, G. C. (2005). The use of chlorophyll fluorescence to identify chemical and environmental stress in leaf tissue of three oak (Quercus) species. *Arboriculture and Urban Forestry* 31(5), 215-227. https://doi.org/10.48044/jauf.2005.028.

Pham, H. H. (2000). *An illustrated flora of Vietnam*. Ho Chi Minh City, Vietnam: Youth Publishing House.

Salmond, J. A., Tadaki, M., Vardoulakis, S., Arbuthnott, K., Coutts, A., Demuzere, M.,

& Wheeler, B. W. (2016). Health and climate related ecosystem services provided by street trees in the urban environment. *Environmental Health*, 15(36) 95-111. https://doi.org/10.1186/s12940-016-0103-6.

SCNA (Standing Committee of National Assembly). (2020). Resolution No. 1111/NQ-UBTVQH14 dated on December 9, 2020. On arrangement of commune-level and district-level administrative divisions and establishment of Thu Duc city affiliated to Ho Chi Minh City. Retrieved July 18, 2022, from https://luatvietnam.vn/hanh-chinh/nghi-quyet-1111-nq-ubtvqh14-thanh-lap-thanh-pho-thu-duc-tphcm-195853-d1.html.

SDOT (The Seattle Department of Transportation). (2013). *Street tree manual*. Washington, USA: Retrieved September 24, 2022, from https://www.seattle.gov/documents/Departments/SDOT/Trees/StreetTreeManuaWEB.pdf.

Smith, W. H. (1984). *Pollutant uptake by plants*. New York, USA: John Wiley & Sons.

TDPRLC (The Department of Parks and Recreation of Lincoln City). (2007). *City of design standards*. Nebraska, USA: The Department of Parks and Recreation of Lincoln City.

TFM (The Fremont Municipal). (2022). *Regulation of street trees*. California, USA: The Fremont Municipal.

Thaiutsa, B., Puangchit, L., Kjelgren, R., & Arunpraparut, W. (2008). Urban green space, street tree and heritage large tree assessment in Bangkok, Thailand. Urban Forestry and Urban Greening 7(3), 219-229. https://doi.org/10.1016/j.ufug.2008.03.002.

Wang, Y, Pyörälä, J., Liang, X., Lehtomäki, M., Kukko, A., Yu, X., Kaartinen, H., & Hyyppä, J. (2019). In situ biomass estimation at tree and plot levels: What did data record and what did algorithms derive from terrestrial and aerial point clouds in boreal forest. *Remote Sensing of Environment* 232. https://doi.org/10.1016/j.rse.2019.111309.

Ware, G. H. (1994). Ecological bases for selecting urban trees. *Journal of Arboriculture* 20(2), 98-103. https://doi.org/10.48044/jauf.1994.018.

Wargo, P. M., Minocha, R., Wong, B. L., Long, R. P., Horsley, S. B., & Hall, T. J (2002). Measuring changes in stress and vitality indicators in limed sugar maple on the Allegheny plateau in north-central Pennsylvania. *Canadian Journal of Forest Research* 32, 629-641. https://doi.org/10.1139/x02-008.

Comparison of the physicochemical properties and biological compounds of acerola fruit varieties grown in Vietnam through the various maturation stages

Diep T. N. Duong[1*], Nhi X. Ngo[1], & Binh Q. Hoang[2]

[1]Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City, Vietnam.

[2]Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City, Vietnam.

ARTICLE INFO

Research Paper

Received: April 17, 2023

Revised: September 14, 2023

Accepted: September 20, 2023

Keywords

Acerola fruit

Antioxidant activity

Maturation stages

Physicochemical properties

Variety

***Corresponding author**

Duong Thi Ngoc Diep

Email:
duongngocdiep@hcmuaf.edu.vn

ABSTRACT

The objective of the present study was to find the changes in physicochemical properties, bioactive compounds, and antioxidant activity of acerola fruits under different cultivars (i.e., Brazilian acerola (*Malpighia emarginata* D.C) and sour acerola (*Malpighia glabra* L.)) and maturation stages (unripe, half-ripe, and ripe). For any species, the study found an increase in total soluble solid and a* value, whereas there was a decrease in the content of bioactive compounds (i.e., polyphenols, flavonoids, vitamin C), total acidity, and antioxidant activity, which followed the maturation development of fruits. Briefly, the unripe acerola fruits (Brazillan cultivar) were an excellent source of vitamin C (32.97 mg/g) and phenolic content (25.62 mg GAE/g).

Cited as: Duong, D. T. N., Ngo, N. X., & Hoang, B. Q. (2024). Comparison of the physicochemical properties and biological compounds of acerola fruit varieties grown in Vietnam through the various maturation stages. *The Journal of Agriculture and Development* 23(3), 67-78.

1. Introduction

The tropical fruit like acerola is not only high in ascorbic acid but also a source of phenolic compounds and flavonoids. Hence, this fruit is being used as a nutritional dietary supplement for enhancing immune response, antioxidant capability, and dietary requirements. (Hanamura et al., 2005). Besides being a functional material good for human health, acerola could be used as an ingredient for making various delicious food and beverage products such as nectar, jam, powder, fermented drinks, and ice cream (Hoang et al., 2022). Acerola fruits are extensively grown in some regions of Vietnam's Mekong Delta. The yield of this fruit is noticeably high in Tien Giang province.

There are two major acerola fruit cultivars in Vietnam: Malpighia glabra L. (Vietnamese called it sour acerola fruit) and Malpighia emarginata D.C. (Vietnamese called it Brazilian acerola fruit). The raw material characteristics are one of the most important factors influencing the final product's quality. The previous studies about pomegranate (Al-Maiman & Ahmad, 2002), mango (Barbosa-Gamez et al., 2017), and mulberry (Mahmood et al., 2012) revealed the phytochemical compositions, bioactive compounds, and bio-functions of fruit modified during the maturation period. On the other hand, these attributes were affected by the plant cultivar (Ribeiro & Freitas, 2020). However, there has not been any available information about the impact of both factors on the physicochemical properties of acerola fruit varieties grown in Vietnam.

2. Material and Methods

2.1. Materials

The two varieties of acerola, including sour acerola fruits (SAF) and Brazilian acerola fruits (BAF), were purchased from cooperative gardeners in Go Cong town, Tien Giang province, during the summer of 2022. The fruits were not damaged, moldy, or rotten. Acerola was collected, washed, and drained: After pretreatment, both materials were divided into 3 different maturity levels, such as unripe, half-ripe, and ripe, based on their visual appearance (Tables 1 & 2). All samples were packed into plastic bags and stored in a freezer until used for analysis. The sampling was carried out in 3 replications, where each iteration was performed with 200 g (approximately 45 fruits).

Table 1. Experimental design-matrix encoding the independent variables

Maturity	Brazilian acerola (*Malpighia emarginata* D.C).		Sour acerola (*Malpighia glabra* L.)	
Unripe				
Half-ripe				
Ripe				

Table 2. Organoleptic properties of acerola fruit at the different maturity indexes and varieties

Maturity	Brazilian acerola (*Malpighia emarginata* D.C).	Sour acerola (*Malpighia glabra* L.)
Unripe	Bold greenness	Bright greenness
	Hard	Hard
	Sour	Quite Sour
	Uncharacteristic aroma	Uncharacteristic aroma
Half-ripe	Orange yellow	Orange yellow
	Semi-soft	Semi-soft
	Sour and a little sweet	Little sour and a little sweet
	Uncharacteristic aroma	Uncharacteristic aroma
Ripe	Redness	Redness
	Juicy	Juicy
	Sweet and little sour	Sweet and little sour
	A quite characteristic aroma	Strong aroma

2.2. Chemical

The chemicals used for this assay were Na_2CO_3 ≥ 99,5%, $AlCl_3$, CH_3COONa, NaOH, Methanol 100%, Iodine, Starch, (Xilong, China); Gallic acid 99%, Quercetin (95%), Ascorbic acid, Folin - Ciocalteu 99.5% (Sigma Aldrich, USA); 2.2-diphenyl-1-picrylhydrazyl (DPPH) and 2.2'-azisbonis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS) (TCI, Japan).

2.3. Analytical method

2.3.1. Moisture content

The samples were weighed and dried in an oven (Memmert UM200, Atmosafe, Germany) at 105^{0}C until the weight remained constant. The samples were cooled in a desiccator untill cool and weighed. Moisture content was measured according to the formula:

$$MC = \frac{m1 - m2}{m1} \times 100\%$$

Where: MC is moisture content (%); m1 is the sample weight before drying (g); m2 is the sample weight after drying (g).

2.3.2. Ascorbic acid measurement

The procedure was carried out according to Pathy (2018). Weigh 10 g of the crushed sample dissolved in distilled water and makeup to 100 mL. Add 2 drops of 0.2% HCl to stabilize the sample for 10 min. Take 10 mL of the diluted sample, add 2 drops of the saturated starch solution, and titrate with the 0.01 N iodine solution. Ascorbic acid content was calculated according to the formula:

$$X\ (mg/g) = \frac{V \times V_1\ x\ 0.88}{V_2\ x\ m\ x}$$

Where: V: mL of 0.01 N I_2 that used for titration, V_1: volume of sample solution used in the experiment, V_2: volume of sample solution taken to determine vitamin C content, 0.88: The mg of vitamin C corresponds to 1 mL of 0.01 N I_2 solution, m: sample mass (g).

2.3.3. Total phenolic content (TPC)

The antioxidant components in acerola fruits were extracted with methanol using a modified version of the Xu et al. (2008) method. One g of the crushed acerola fruit was weighed into a 50 mL falcon tube. The extraction procedure was performed with 9 mL of 80% methanol for 30 min at room temperature 29 - 31°C, in a dark condition. Next, the sample was filtered through a filter paper to collect the solution.

The TPC measurement is referenced according to Lim et al. (2007). In a test tube, 0.3 mL diluted acerola fruit solution was vortexed with 1.5 mL Folin-Ciocalteu 10%. Samples were allowed to stand for 5 min in a darkened area. The mixture was then mixed thoroughly with 1.2 mL of 7.5% Na_2CO_3 and left for 30 min. The samples were tested for optical density (OD) by using a UV-Visible Spectrophotometer (V730, Germany) at a wavelength of 765 nm. The standard curve was carried out using gallic acid and TPC was performed as mg gallic acid equivalent (GAE)/g.

$$TPC = \frac{(y - b)\ x\ V\ x\ df}{a\ x\ m\ x\ 1000}$$

Where y: OD value of sample. a and b are the coefficient in the standard curve. V: volume of extracted solution (mL). DF: dilution factor. m: sample mass (g). 1/1000: the coefficient converts from µg/g to mg/g.

2.3.4. Total flavonoid content (TFC)

The TFC measurement is referenced according to Do et al. (2014). The aliquot of 2 mL of extract was mixed well with 0.1 mL of 10% AlCl3 solution and 0.1 mL of 0.1 mM CH3COOK solution. After that, the samples were kept at room temperature for 30 min in dark condition. The total flavonoid content was measured by using a spectrophotometer (UV-Visible Spectrophotometer, V730, Germany) at a wavelength of 415 nm. The standard curve was carried out using quercetin solution and TFC was performed as mg quercetin equivalent (QE)/g

$$TFC = \frac{(y - b)\ x\ V\ x\ df}{a\ x\ m\ x\ 1000}$$

Where y: OD value of sample. a and b are the coefficient in the standard curve. V: volume of extracted solution (mL). DF: dilution factor. m: sample mass (g). 1/1000: the coefficient converts from µg/g to mg/g.

2.3.5. DPPH assay

The DPPH reagent was used for antioxidant activity determination (Phuong et al., 2020) The aliquot of 0.2 mL of extract was added to 4 mL of 0.1 mM DPPH solution. After vortexing, the samples were left for 30 min in dark condition. The antioxidant activity was determined by using a spectrophotometer (UV-Visible Spectrophotometer, V730, Germany) at a wavelength of 517 nm. The standard curve was prepared using solutions of ascorbic acid (AA) and antioxidant activity was expressed as mg AAE (ascorbic acid equivalent)/g.

$$DPPH = \frac{(y - b)\ x\ V\ x\ df}{a\ x\ m\ x \times 1000}$$

Where y: OD value of sample. a and b are the coefficient in the standard curve. V: volume of extracted solution (mL). DF: dilution factor. m: sample mass (g). 1/1000: the coefficient converts from µg/g to mg/g.

2.3.6. ABTS assay

The ABTS assay was used for antioxidant activity determination (Phuong et al., 2020). The aliquot of 80 µL of the extract was added to 3.2 mL of ABTS working solution. After shaking well, the samples were left for 5 min in a dark condition. The antioxidant activity was determined by using a spectrophotometer (UV-Visible Spectrophotometer, V730, Germany) at a wavelength of 734 nm. The standard curve was prepared using solutions of ascorbic acid (AA) and antioxidant activity was expressed as mg AAE/g.

$$ABTS = \frac{(y - b)\ x\ V\ x\ df}{a\ x\ m\ x \times 1000}$$

Where y: OD value of sample. a and b are the coefficient in the standard curve. V: volume of extracted solution (mL). DF: dilution factor. m: sample mass (g). 1/1000: the coefficient converts from µg/g to mg/g.

2.3.7. Color measurement

The color parameters were performed and expressed in the color space CIE L*a*b* by using a handheld chroma meter (Konica Minolta, CR-400, Japan). Where, L* indicated the bright from darkness (zero value) to lightness (positive value), a* indicated the color from greenness (-a*) to redness (+a*), and b* indicated the color from blueness (-b*) to yellowness (+b*).

2.3.8. Physicochemical properties

The total soluble solid (TSS) was determined by a hand-held refractometer (ATAGO 0.0 ~ 33.0%, Japan). The titration procedure with 0.1 N NaOH and 1% phenolphthalein as an indicator was used to determine the total acidity (TA). The fruit size such as weight and length were evaluated by using an electronic balance (FX-1200i, A&D, Japan) and digital caliper (150 mm, Miyutoyo, Japan), respectively. The pH was measured using a pH meter (Hanna, HI2210-02, Romania).

2.3.9. Statistical analysis

The methods such as One way-ANOVA and least significant difference (LSD) which were applied by using software JMP version 13 with P = 0.05.

3. Results and Discussion

3.1. Physicochemical properties parameter

Regarding morphological characteristics, the shape of the acerola fruit was different under the various cultivars (Table 1). For example, the Brazilian acerola had a long, round shape with three lobes (zones) and a surface with sharp notches, while the sour acerola had a round, smooth, glossy surface with three lobes (zones). Besides, the current study found a development of the fruit size during maturation progression (Table 3). Herein, the diameter expanded gradually from 1.64 to 1.87 mm (BAF) and from 1.64 to 1.85 mm (SAF), while the weight of fruit increased continuously from 5.08 to 5.95 g (BAF) and from 4.40 to 4.63 g (SAF). However, the statistical analysis showed no different significance among the samples at $P > 0.05$ for both attributes.

On the other hand, the current study obtained different statistical significance for color properties among fruits under the various maturation stages. Table 3 displayed an a* value that increased dramatically from -16.74 to 44.34 (BAF) and from -13.14 to 47.72 (SAF), where a* indicated the red tones at a positive value and the green tones at a negative value. Moreover,

Table 3 also performed the highest value of b* at half-ripe fruit; herein, b* indicated the yellow tones at a positive value and the blue tones at a negative value. Another study about the acerola (Malpighia punicifolia L) also recorded that b* had the peak value (12.13) at an immature stage, while a* (25.97) was achieved at the mature stage (Vendramini & Trugo, 2000).

The report of several authors also obtained the external appearance color of acerola fruit transferred from green to red (Batista-Silva et al., 2018; Ribeiro & Freitas, 2020). The reason could come from the change in pigment compounds such as β-carotene, cryptoxanthin, lutein, and violaxanthin (Mezadri et al., 2005). The research on acerola fruit planted in Brazil (Lima et al., 2005) and *Maclura tricuspidata* (Kim et al., 2019) pointed out that the enhancement of carotenoid concentration in fruit pulp during the ripening period was considerable. In addition, the increase in the action of the chlorophyllase enzyme caused chlorophyll degradation (Nassur et al., 2015).

Generally, TSS, total acidity, and pH of both acerola fruit cultivars changed during ripening. (Table 3). Herein, the TSS increased slightly from 7.10% to 7.93% (BAF), and from 8.00% to 8.30% (SAF); due to the action of an enzyme promoted during the maturation process, which leads to the hydrolysis of starch and polysaccharides into simple sugar (Kulkarni & Aradhya, 2005). Whereas TA dropped slightly from 1.63% to 1.02% (BAF), and from 0.91 to 0.76% (SAF). Organic acid can be used as a material for fruit's respiration during maturation, leading to a reduction in the acidity of fruit (Mini, 2017). In comparison with the various studies, the behavior of soluble solids and acidity in fruit during ripening showed variation among the different fruits. For example, TSS of mango (Nassur et al., 2015; Barbosa-Gamez et al., 2017) and *Bunchosia glandulifera* fruit (Blank et al., 2018) amplified during the growth of fruit, whereas TSS seems unchanged for pomegranate (Al-Maiman & Ahmad, 2002). TA of mango (Nassur et al., 2015; Barbosa-Gamez et al., 2017) and pomegranate fruits (Al-Maiman & Ahmad, 2002) decreased during ripeness, while TA increased for *Bunchosia glandulifera* fruit (Blank et al., 2018). The pH value of BAF lower than SAF for any maturation index. The pH changed from 3.13 to 3.09 (BAF), and from 3.43 to 3.38 (SAF) from unripe to ripe.

Table 3. Effect of maturity indexes and acerola fruit varieties on the physicochemical properties of fruits

Attributes	Varieties					
	Brazilian acerola (*Malpighia emarginata* D.C)			Sour acerola (*Malpighia glabra* L.)		
	Unripe	Half-ripe	Ripe	Unripe	Half-ripe	Ripe
TSS (%)	$7.10^{d} \pm 0.08$	$7.60^{c} \pm 0.14$	$7.93^{b} \pm 0.05$	$8.00^{b} \pm 0.08$	$8.10^{b} \pm 0.08$	$8.30^{a} \pm 0.08$
TA (%)	$1.63^{a} \pm 0.04$	$1.21^{b} \pm 0.03$	$1.02^{c} \pm 0.02$	$0.91^{d} \pm 0.15$	$0.86^{d} \pm 0.11$	$0.76^{e} \pm 0.28$
pH	$3.13^{c} \pm 0.02$	$3.08^{d} \pm 0.01$	$3.09^{d} \pm 0.02$	$3.36^{b} \pm 0.01$	$3.43^{a} \pm 0.02$	$3.38^{b} \pm 0.00$
Diameter (mm)	$1.64^{a} \pm 0.32$	$1.80^{a} \pm 0.23$	$1.87^{a} \pm 0.32$	$1.64^{a} \pm 0.25$	$1.70^{a} \pm 0.17$	$1.85^{a} \pm 0.26$
Weight (g)	$5.08^{b} \pm 0.31$	$5.89^{a} \pm 0.15$	$5.95^{a} \pm 0.08$	$4.40^{c} \pm 0.24$	$4.63^{c} \pm 0.13$	$4.74^{b} \pm 0.05$
L*	$44.94^{cd} \pm 1.80$	$60.82^{b} \pm 3.65$	$40.8^{d} \pm 2.66$	$68.18^{a} \pm 1.04$	$65.42^{ab} \pm 2.19$	$47.92^{c} \pm 2.98$
a*	$-16.74^{f} \pm 0.41$	$27.14^{c} \pm 0.59$	$44.34^{b} \pm 0.64$	$-13.14^{e} \pm 0.55$	$11.27^{d} \pm 1.37$	$47.72^{a} \pm 1.34$
b*	$34.00^{b} \pm 0.82$	$44.86^{a} \pm 1.26$	$34.17^{b} \pm 3.84$	$42.66^{a} \pm 2.13$	$44.06^{a} \pm 0.72$	$40.59^{a} \pm 1.96$
Moisture content (%)	$93.92^{f} \pm 0.02$	$94.22^{e} \pm 0.06$	$94.64^{d} \pm 0.14$	$92.27^{c} \pm 0.02$	$91.40^{b} \pm 0.04$	$91.08^{a} \pm 0.04$

All data are the mean ± SD of three replicates. The data within a row followed by the same superscript letter (a, b, c) are not statistically significant difference. TSS: total soluble solid; TA: total acidity.

3.2. Bioactive compounds and antioxidant activity

As fruit maturity progressed, this study observed the reduction of bioactive compounds such as vitamin C, polyphenols, and flavonoids in acerola fruit. There were significant differences ($P < 0.05$) in these attributes among varieties, wherein the unripe fruit had the highest value, and the ripe fruit had the lowest value (Table 4). For instance, the vitamin C content of the unripe fruit of BAF was the highest at 32.97 mg/g, which was significantly different compared to the ripe fruits (17.89 mg/g). Similarly, unripe fruit of SAF had a vitamin C content (18.95 mg/g) greater than ripe fruit (13.23 mg/g). Similarly, the significantly highest TPC was 25.62 mg/g for the unripe fruit of the Brazilian variety, while the ripe fruit of the sour variety had the lowest value (12.97 mg/g). The degradation of polyphenols may be affected by the actions of some enzymatic browning (e.g., polyphenol oxidase) during the development of fruit. Another reason is that phenolic compounds may be used as substrates for the biosynthesis of different compounds (Kulkarni & Aradhya, 2005). Likewise, the deterioration of TFC during ripening also obtained from 0.092 to 0.063 mg/g (BAF) and from 0.064 to 0.062 (SAF). Moreover, Table 4 showed the content of vitamin C, TPC, and TFC in BAF was 2 times higher than that in SAF within a ripeness index. As a result of this phenomenon, the antioxidant activity of BAF was stronger compared to that of SAF. The antioxidant activity of the unripe fruit of BAF was 0.19 mg/g (DPPH assay) and 26.17 mg/g (ABTS assay), while its value reached 0.15 mg/g (DPPH assay) and 15.05 mg/g (ABTS assay) for SAF.

The loss of TPC and vitamin C as well as antioxidant activity in acerola fruit samples from unripe to fully ripe stages is consistent

with various fruits such as *Maclura tricuspidata* (Kim et al., 2019), mango (Barbosa-Gamez et al., 2017), and pomegranate (Al-Maiman & Ahmad, 2002). However, the different studies obtained an increase in TFC and TPC in mulberry pulp (Mahmood et al., 2012), and *Cudrania tricuspidata* (Shin et al., 2015), *Bunchosia glandulifera* (Blank et al., 2018) during ripeness progression. It could be concluded that there is no general pattern for the variation of biological compounds and antioxidant activity of fruit during the ripening period. The change in these properties belongs to the horticultural condition and genotype type (Mahmood et al., 2012; Barbosa-Gamez et al., 2017).

The vitamin C content and TPC of acerola fruit samples in the current study are higher than in previous studies. For acerola fruit (*Malpighia emarginata* DC) samples planted in Brazil, the vitamin C content of fresh pulp from immature to mature ranged from 2424 to 957 mg/100 g (de-Assis et al., 2001), 1900 - 970 mg/100 g (Righetto et al., 2005). The other studies on acerola fruits (*Malpighia glabra* L.) verified their antioxidant activity of 959.1 mg/100 g (DPPH assay), and 1198.9 mg/100 g (ABTS assay), total phenolics of 1055.9 mg/100 g (Kuskosk et al., 2005). These data are different compared to our results due to the different weather conditions, farming techniques, and locations.

Table 4 also displayed the phenolic content in acerola fruit is higher than that of apples (1.97 mg/100 g), blackberries (3.01 mg/100 g), oranges (0.75 mg/100 g), and pomegranates (1.33 mg/100 g), soursop (54.8 mg/100 g), pineapple (38.1 mg/100 g), sweetsop (81.7 mg/100 g), jackfruit (29.0 mg/100 g) (Ruiz-Torralba et al., 2018; Almeida et al., 2011). Likewise, the vitamin C content in acerola fruit is higher than kiwi (0.92 mg/100 g), orange (0.59 mg/100 g), lime (0.29 mg/100 g), apple (0.04 mg/100 g), soursop (3.3 mg/100 g), pineapple (13.0 mg/100 g), sweetsop (29.6 mg/100 g), jackfruit (1.2 mg/100 g) (Almeida et al., 2011; Mieszczakowska-Frąc et al., 2021).

Table 4. Effect of maturity indexes and varieties on the antioxidant compounds in acerola fruits

Characteristic	Varieties					
	Brazilian acerola (*Malpighia emarginata* D.C)			Sour acerola (*Malpighia glabra* L.)		
	Unripe	Half-ripe	Ripe	Unripe	Half-ripe	Ripe
Vitamin C (mg/g)	$32.97^{a} \pm 0.04$	$21.82^{b} \pm 0.12$	$17.89^{d} \pm 0.3$	$18.95^{c} \pm 0.29$	$17.34^{d} \pm 0.37$	$13.23^{e} \pm 0.32$
TPC (mg GAE/g)	$25.62^{a} \pm 0.3$	$18.57^{b} \pm 0.19$	$16.31^{c} \pm 0.43$	$15.65^{c} \pm 0.34$	$13.68^{d} \pm 0.46$	$12.97^{d} \pm 0.29$
TFC (mg QE/g)	$0.092^{a} \pm 0.008$	$0.063^{b} \pm 0.002$	$0.057^{bc} \pm 0.001$	$0.064^{b} \pm 0.001$	$0.065^{b} \pm 0.001$	$0.062^{b} \pm 0.002$
DPPH (mg AAE/g)	$0.19^{a} \pm 0.01$	$0.16^{b} \pm 0.01$	$0.13^{c} \pm 0.01$	$0.15^{d} \pm 0.01$	$0.11^{e} \pm 0.01$	$0.06^{f} \pm 0.01$
ABTS (mg AAE /g)	$26.17^{a} \pm 1.06$	$17.72^{b} \pm 0.77$	$14.95^{c} \pm 0.45$	$15.05^{c} \pm 0.25$	$9.59^{d} \pm 0.24$	$6.71^{e} \pm 0.45$

All data are the mean ± SD of three replicates. All attributes are present under mg/g fresh weight. The data within a row followed by the same superscript letter (a, b, c) are not statistically significant difference. TPC: total phenolic content; TFC: total flavonoid content; DPPH: 2.2-diphenyl-1-picrylhydrazyl; ABTS: 2.2'-azisbonis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium.

3.3. Correlation among color properties, bioactive compound, and antioxidant

For Brazilian cultivars, Table 5 provided that TSS had a positive correlation with diameter (r = 0.99), weight (r = 0.94), and moisture content (r = 0.98); in contrast, it had a negative correlation with pH (r = -0.99) and TA (r = -0.86). Table 5 also revealed the positive correlation between vitamin C and polyphenols (r = 0.99), vitamin C and TFC (r = 0.99), and polyphenols and flavonoids (r = 0.99). On the other hand, Table 5 displayed a significant correlation between the bioactive compounds and antioxidant activity. All coefficient correlations had a positive value (i.e., r > 0.83), which means the decrease in bioactive compounds leads to a decline in antioxidant activity. Vitamin C was the major compound that contributed to the antioxidant activity of acerola fruit, followed by polyphenols and other compounds (Mezadri et al., 2008). Table 5 also stated a negative correlation between the L*, a*, and b* with bioactive compounds or antioxidant activity for any cultivar. Most of the coefficient correlation values range from -0.99 to -0.93 (a*) and are lower than 0.5 (L* and b*). Regardless of sour cultivar, Table 6 demonstrated a positive correlation between bioactive compounds and antioxidant activity with r value from 0.83 to 0.99.

Table 5. Pearson's correlation coefficient among the color properties, bioactive compounds, and antioxidant activity in Brazilian acerola

	TSS	TA	Diameter	Weight	pH	Moisture content	L*	a*	b*	TPC	TFC	Vit C	DPPH	ABTS
TSS	1.00													
TA	-0.99	1.00												
Diameter	0.99	-0.99	1.00											
Weight	0.94	-0.97	0.96	1.00										
pH	-0.86	0.90	-0.90	-0.98	1.00									
Moisture content	0.98	-0.95	0.96	0.85	-0.73	1.00								
L*	-0.08	-0.02	0.00	0.27	-0.44	-0.29	1.00							
a*	0.99	-1.00	0.99	0.98	-0.92	0.94	0.05	1.00						
b*	0.13	-0.23	0.21	0.46	-0.62	-0.08	0.98	0.26	1.00					
TPC	-0.96	0.98	-0.98	-0.99	0.96	-0.88	-0.19	-0.99	-0.39	1.00				
TFC	-0.94	0.97	-0.97	-0.99	0.98	-0.85	-0.25	-0.98	-0.45	0.99	1.00			
Vit C	-0.98	0.99	-0.99	-0.99	0.95	-0.91	-0.13	-0.99	-0.33	0.99	0.99	1.00		
DPPH	-0.97	0.94	-0.94	-0.82	0.70	-0.99	0.33	-0.93	0.13	0.86	0.83	0.89	1.00	
ABTS	-0.99	0.99	-0.99	-0.98	0.92	-0.94	-0.06	-0.99	-0.27	0.99	0.98	0.99	0.92	1.00

TSS: total soluble solid; TA: total acidity; TPC: total phenolic content; TFC: total flavonoid content; DPPH: 2.2-diphenyl-1-picrylhydrazyl; ABTS: 2.2'-azisbonis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium.

Table 6. Pearson's correlation coefficient among the color properties, bioactive compounds, and antioxidant activity in sour acerola

	TSS	TA	Diameter	Weight	pH	Moisture content	L*	a*	b*	TPC	TFC	Vit C	DPPH	ABTS
TSS	1.00													
TA	-0.99	1.00												
Diameter	1.00	-0.99	1.00											
Weight	0.92	-0.94	0.88	1.00										
pH	0.11	-0.16	0.02	0.48	1.00									
Moisture content	-0.90	0.92	-0.85	-1.00	-0.54	1.00								
L*	-0.98	0.97	-0.99	-0.83	0.10	0.79	1.00							
a*	1.00	-0.99	0.99	0.95	0.19	-0.93	-0.96	1.00						
b*	-0.79	0.82	-0.73	-0.97	-0.69	0.98	0.65	-0.84	1.00					
TPC	-0.89	0.91	-0.84	-0.99	-0.56	0.99	0.77	-0.92	0.99	1.00				
TFC	-0.95	0.96	-0.91	-0.99	-0.43	0.99	0.86	-0.97	0.95	0.99	1.00			
Vit C	-0.99	0.99	-0.97	-0.97	-0.24	0.95	0.94	-0.99	0.87	0.94	0.98	1.00		
DPPH	-0.99	0.99	-0.97	-0.98	-0.28	0.96	0.93	-0.99	0.88	0.95	0.99	0.99	1.00	
ABTS	-0.92	0.94	-0.88	-0.99	-0.48	0.99	0.83	-0.95	0.97	0.99	0.99	0.97	0.98	1.00

TSS: total soluble solid; TA: total acidity; TPC: total phenolic content; TFC: total flavonoid content; DPPH: 2.2-diphenyl-1-picrylhydrazyl ; ABTS: 2.2'-azisbonis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium.

4. Conclusions

The total acidity, a* value, bioactive compounds, and antioxidant activity of the Brazilian acerola varieties were higher than the sour acerola variety. The content of these parameters decreased gradually according to the fruit's maturation development, excess a* value. The unripe Brazilian acerola fruit had the highest bioactive compounds and antioxidant activity. This sample can be used as material for the extraction of bioactive compounds or as a functional ingredient to improve the antioxidant activity of foodstuffs.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Nong Lam University was supported for this study through project CS-CB21-HHTP-08.

References

Al-Maiman, S. A., & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. *Food Chemistry* 76(4), 437-441. https://doi.org/10.1016/S0308-8146(01)00301-6.

Almeida, M. M. B., de Sousa, P. H. M., Arriaga, Â. M. C., do Prado, G. M., de Carvalho Magalhães, C. E., Maia, G. A., & de Lemos, T. L. G. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International* 44(7), 2155-2159. https://doi.org/ 10.1016/j.foodres.2011.03.051.

Barbosa-Gamez, I., Caballero-Montoya, K. P., Ledesma, N., Sayago-Ayerdi, S. G., García Magaña, M. D. L., Bishop von Wettberg, E. J., & Montalvo-González, E. (2017). Changes in the nutritional quality of five Mangifera species harvested at two maturity stages. *Journal of The Science of Food and Agriculture* 97(14), 4987-4994. https://doi.org/10.1002/jsfa.8377.

Batista-Silva, W., Nascimento, V. L., Medeiros, D. B., Nunes-Nesi, A., Ribeiro, D. M., Zsögön, A., & Araújo, W. L. (2018). Modifications in organic acid profiles during fruit development and ripening: correlation or causation? *Frontiers in Plant Science* 9, 1-20. https://doi.org/10.3389/fpls.2018.01689.

Blank, D. E., Justen, D., Fraga, S., Peixoto, C. R., & de Moura, N. F. (2018). Chemical composition and antioxidant activity of *Bunchosia glandulifera* fruit at different ripening stages. *Food and Nutrition Sciences* 9(10), 1147-1159. https://doi.org/ 10.4236/fns.2018.910083.

de Assis, S. A., Lima, D. C., & de Faria Oliveira, O. M. (2001). Activity of pectinmethylesterase, pectin content and vitamin C in acerola fruit at various stages of fruit development. *Food Chemistry* 74(2), 133-137. https://doi.org/ 10.1016/S0308-8146(01)00104-2.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis* 22(3), 296-302. https://doi.org/10.1016/j.jfda.2013.11.001.

Hanamura, T., Hagiwara, T., & Kawagishi, H. (2005). Structural and functional characterization of polyphenols isolated from acerola (*Malpighia emarginata* DC.) fruit. *Bioscience, Biotechnology, and Biochemistry* 69(2), 280-286. https://doi.org/10.1271/bbb.69.280.

Hoang, Q. B., Pham, N. T., Le, T. T., & Duong, T. N. D. (2022). Bioactive compounds and strategy processing for acerola: A review. *Can Tho University Journal of Innovation and Sustainable Development* 14(2), 46-60. https://doi.org/10.22144/ctu.jen.2022.011.

Kim, D. W., Lee, W. J., Asmelash Gebru, Y., Choi, H. S., Yeo, S. H., Jeong, Y. J., Kim, S., Kim, Y. H., & Kim, M. K. (2019). Comparison of bioactive compounds and antioxidant activities of *Maclura tricuspidata* fruit extracts at different maturity stages. *Molecules* 24(3), 567. https://doi.org/10.3390/molecules24030567.

Kulkarni, A. P., & Aradhya, S. M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry* 93(2), 319-324. https://doi.org/10.1016/j.foodchem.2004.09.029.

Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry* 103(3), 1003-1008. https://doi.org/10.1016/j.foodchem.2006.08.038.

Lima, V. L., Mélo, E. A., Maciel, M. I. S., Prazeres, F. G., Musser, R. S., & Lima, D. E. (2005). Total phenolic and carotenoid contents in acerola genotypes harvested at three ripening stages. *Food Chemistry* 90(4), 565-568. https://doi.org/10.1016/j.foodchem.2004.04.014.

Mahmood, T., Anwar, F., Abbas, M., & Saari, N. (2012). Effect of maturity on phenolics (phenolic acids and flavonoids) profile of strawberry cultivars and mulberry species from Pakistan. *International Journal of Molecular Sciences* 13(4), 4591-4607. https://doi.org/ 10.3390/ijms13044591.

Mezadri, T., Pérez-Gálvez, A., & Hornero-Méndez, D. (2005). Carotenoid pigments in acerola fruits (*Malpighia emarginata* DC.) and derived products. *European Food Research and Technology* 220, 63-69. https://doi.org/ 10.1007/s00217-004-1042-y.

Mezadri, T., Villaño, D., Fernández-Pachón, M. S., García-Parrilla, M. C., & Troncoso, A.

M. (2008). Antioxidant compounds and antioxidant activity in acerola (*Malpighia emarginata* DC.) fruits and derivatives. Journal of Food Composition and Analysis 21(4), 282-290. https://doi.org/10.1016/j.jfca.2008.02.002.

Mieszczakowska-Frąc, M., Celejewska, K., & Płocharski, W. (2021). Impact of innovative technologies on the content of vitamin C and its bioavailability from processed fruit and vegetable products. *Antioxidants* 10(1), 54. https://doi.org/ 10.3390/antiox10010054.

Mini, M. (2017). Influence of harvesting stage and storage temperature on nutritional quality of tomato (*Lycopersicon esculentum* Mill) cv. PKM-1. *International Journal of Biochemistry Research and Review* 16(4), 1-8. https://doi.org/10.9734/IJBCRR/2017/31858.

Nassur, R. D. C. M. R., González-Moscoso, S., Crisosto, G. M., Lima, L. C. D. O., Vilas Boas, E. V. D. B., & Crisosto, C. H. (2015). Describing quality and sensory attributes of 3 mango (*Mangifera indica* L.) cultivars at 3 ripeness stages based on firmness. *Journal of Food Science* 80(9), 2055-2063. https://doi.org/10.1111/1750-3841.12989.

Pathy, K. (2018). Process for preparation of vitamin C and method for determination of vitamin C in tablets. *Surgery and Case Studies: Open Access Journal* 1(3), 1-14. https://doi.org/10.32474/SCSOAJ.2018.01.000114.

Phuong, N. N. M., Le, T. T., Dang, M. Q., Van Camp, J., & Raes, K. (2020). Selection of extraction conditions of phenolic compounds from rambutan (*Nephelium lappaceum* L.) peel. *Food and Bioproducts Processing* 122, 222-229. https://doi.org/10.1016/j.fbp.2020.05.008.

Ribeiro, B. S., & de Freitas, S. T. (2020). Maturity stage at harvest and storage temperature to maintain postharvest quality of acerola fruit. *Scientia Horticulturae* 260, 108901. https://doi.org/10.1016/j.scienta.2019.108901.

Righetto, A. M., Netto, F. M., & Carraro, F. (2005). Chemical composition and antioxidant activity of juices from mature and immature acerola (*Malpighia emarginata* DC). *Food Science and Technology International* 11(4), 315-321. https://doi.org/10.1177/1082013205056785.

Ruiz-Torralba, A., Guerra-Hernández, E. J., & García-Villanova, B. (2018). Antioxidant capacity, polyphenol content and contribution to dietary intake of 52 fruits sold in Spain. *CyTA-Journal of Food* 16(1), 1131-1138. https://doi.org/10.1080/19476337.2018.1517828.

Shin, G. R., Lee, S., Lee, S., Do, S. G., Shin, E., & Lee, C. H. (2015). Maturity stage-specific metabolite profiling of *Cudrania tricuspidata* and its correlation with antioxidant activity. *Industrial Crops and Products* 70, 322-331. https://doi.org/10.1016/j.indcrop.2015.01.048.

Vendramini, A. L., & Trugo, L. C. (2000). Chemical composition of acerola fruit (*Malpighia punicifolia* L.) at three stages of maturity. *Food Chemistry* 71(2), 195-198. https://doi.org/10.1016/S0308-8146(00)00152-7.

Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y., & Shi, J. (2008). Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chemistry* 106(2), 545-551. https://doi.org/10.1016/j.foodchem.2007.06.046.

Influence of the supplementation of macadamia oil cake powder on nutritional and sensory qualities of bread

Chuyen V. Hoang*, & Duy L. A. Do
Faculty of Chemical and Food Technology, HCMC University of Technology and Education,
Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: March 21, 2024
Revised: April 25, 2024
Accepted: May 10, 2024

Keywords

Bread
Macadamia oil cake
Nutritional quality
Physical property
Sensory quality

***Corresponding author**

Hoang Van Chuyen
Email:
chuyenhv@hcmute.edu.vn

ABSTRACT

Although macadamia oil cake (MOC) is a by-product of macadamia oil processing, this material still has high nutritional value, making it a promising ingredient for food products. This study aimed to investigate effects of the MOC supplementation at different ratios on physical properties, nutritional composition and sensory quality of bread. The results show that the addition of MOC led to increases in protein, ash and fat content, while carbohydrate content was lower in the supplemented bread. The specific volume and springiness of the bread were significantly affected by the MOC supplementation while no significant change in hardness and spread ratio was observed ($P < 0.05$). For the sensory quality of fortified bread, the differences in color, flavor, texture and overall acceptability among 4 levels of MOC addition were insignificant, except for the taste score. Microbiological analyses also confirmed that the MOC supplemented bread product met microbial safety standards. The obtained results suggest that the bread sample with 20% MOC addition (9.35 g protein, 12.32 g fat, 46.13 g carbohydrate, 4.29 g dietary fiber and 332.77 kcal per 100 g) should be selected for developing high nutritional bread products.

Cited as: Hoang, C. V., & Do, D. L. A. (2024). Influence of the supplementation of macadamia oil cake powder on nutritional and sensory qualities of bread. *The Journal of Agriculture and Development* 23(3), 79-89.

1. Introduction

Macadamia nut is known as the Australia's only commercial food crop indigenous and the world's finest nuts due to its unique, delicate flavor, crunchy texture. Macadamia nuts are an abundant source of nutrition including unsaturated fats, plant protein, and vital minerals with and exceptional lipid content that varies from 69.1% to 78.4%. Macadamia nuts contain 77.4% MUFA per 100 g of fat, of which 58.5% is oleic acid (18:1ω9) and 18.7% is palmitoleic acid (16:1) (Munro & Garg, 2008).

The extraction of macadamia oil normally uses cold pressing method (Navarro & Rodrigues, 2016). Because of their high nutritional value and protein content (15% to 50%), the seed and nut cakes are commonly utilized as feedstuffs for poultry and cattle (Acheampong-Boateng et al., 2017). In addition to the potential nutritional value, the macadamia oil cake (MOC) can also produce typically attractive flavors so it is suggested to be used as an ingredient to improve sensory quality of food products (Sarkis et al., 2014).

Different types of oil cake and nuts are utilized to make food products. The addition of oil cakes into food products could be a great way to increase the consumption of oil industry by-products to solve malnourishment (Behera et al., 2013; Szydłowska-Czerniak et al., 2021). A research on bread fortified with walnut oil cake and walnut oil showed positive results, especially the high amount of antioxidants and health aspects. However, some definite disadvantages in that research were recorded, such as the increase of hardness, dark crumb, reduction in bread volume (Pycia et al., 2020). Another research on hemp cake bread also showed a decrease in volume and negative effects on structural and textural characteristics of bread crumb. Nevertheless, the addition of hemp cake in bread helps to improve nutritional quality, especially proteins and some macro-elements, micro-elements such as iron (Pojić et al., 2015).

Thus, this research focused on producing bread supplemented with different ratios of MOC to improve the nutritional and sensory qualities of the products.

2. Material and Methods

2.1. Materials

Macadamia oil cake (from the cold pressing process) was provided by Damaca Nguyen Phuong Company (Dak Lak, Vietnam). The oil cake was dried, ground and sieved to obtain powder with the size less than 0.25 mm and then stored at -18^{0}C until used.

The MOC was analyzed and confirmed to have a moisture content of 6.83%, crude protein content of 21.6%, crude fat content of 30.4%, ash content of 3.57%, and crude fiber content of 6.27%.

2.1. Preparation of bread supplemented with macadamia oil cake

Table 1. Composition of bread

Ingredients	Samples			
	S0	S15	S20	S25
Wheat flour (g)	200	170	160	150
Macadamia oil cake (g)	0	30	40	50
Sugar powder (g)	15	15	15	15
Butter (g)	25	25	25	25
Non-sugar fresh milk (g)	130	130	130	130
Yeast (g)	3	3	3	3
Condensed milk	30	30	30	30
Salt (g)	1	1	1	1

Procedure of bread making:

All the ingredients were put and mixed well in a mixing bowl until becoming consistent. After that, the mixture was rested for 15 min at room temperature for 4 resting cycles. The rested dough was then folded the outer edge to the middle about 10 - 12 times. The obtained dough was divided the dough into 6 equal parts, about 60 g each. The edge of the dough was folded in the middle until the surface was smooth before the rolling and shaping to form pillow bread. The shaped dough was placed in the mold with 3 parts on each mold. The mold was then covered with a towel to be prevented from drying out for incubating until the bread has doubled in size (about 30 min).

Before baking process, the oven was preheated to 160°C in 10 min and then the trays were put into the oven and bake at 160°C in 30 min. After baking, the bread samples were placed on a cooling rack for cooling to room temperature and kept zip-top bags with desiccant packs in a cool and dry location.

2.3. Evaluation of physical parameters

- Moisture content: Moisture content of samples was determined by using hot-air oven at 105° until reaching constant weight according to the Method by AACC (2000).

- Specific volume: Each bread was weighed and then measured for volume using a rapeseed displacement volume-meter. Specific volume (cm^3/g) was calculated as the ratio of the volume (cm^3) and the mass of the samples (g) following the AACC Method (AACC, 2000).

- Texture analysis: The texture characteristics of the bread were analyzed using a texture analyzer (CT3, Brookfield Ametek Inc., MA, USA). TA-AACC3 type probe with trigger load of 4.0 g and test speed of 1 mm/s was employed for texture profile analysis (TPA). The hardness, elasticity, cohesiveness, and stalling rate of the TPA curve were analyzed.

2.4. Color analysis

L*, a* and b* values of the bread were determined using the CR-400 colorimeter (Konica Minolta, USA). The color differences between fortified samples with the control were calculated based on following equation (Mokrzycki & Tatol, 2011).

$$\Delta E = \sqrt{(L^* + L^*_0)^2 + (a^* + a^*_0)^2 + (b^* + b^*_0)^2}$$

Where

L*, a*, b* are the color values of fortified samples;

L^*_0, a^*_0, b^*_0 are the color values of control sample.

2.5. Nutritional composition

- Crude protein was determined by Kjeldahl method according to AACC 46-10.01.

- Crude fat was determined by a Soxhlet extractor with petroleum ether as the solvent according to AACC 30-25.01.

- Total ash content was determined by heating in an oven at temperature of 600º in 6 h according to AACC 08-01.01 standard.

- Total carbohydrate was determined according to FAO (2019) using the following equation:

Total carbohydrate% = 100 - (Protein% + Fat% + Ash% + Moisture%) (1 g protein: 4 kcal energy; 1 g fat: 9 kcal energy; 1 g carbohydrate: 4 kcal energy)

- Energy value of samples was calculated based on method of FAO (2019) using the following equation:

Energy = (weight of Carbohydrate × 4) + (weight of Protein × 4) (weight of fat × 9)

- Other indexes including dietary fiber, mineral content and microbiological criteria were analyzed by Eurofins Hai Dang food testing laboratories (Ho Chi Minh City, Vietnam).

2.6. Sensory evaluation

The sensory test was conducted using 9-point hedonic scale ranging from (1, dislike very much to 9, like very much) (Ghoshal et al., 2020). The coded bread samples were served to 20 untrained panelists including 10 males and 10 females (their age was from 20 to 50 years old). The panelists were asked to score the samples for color, shape, texture, sweetness, flavor, mouth feel and overall acceptability.

2.7. Data analysis

All experiments were performed in triplicate, and the results were expressed as the mean ± standard deviation. Comparisons amongst values were based on the LSD tests. Differences were considered to be significantly different at $P < 0.05$.

3. Results and Discussion

3.1. Effect of MOC substitution on physical properties of MOC bread

The analysis of physical properties of MOC supplemented bread (Table 2) showed that the addition of MOC caused significant variation in the specific volume of the bread samples. This change may be due to the ability to absorb water of the dough which is impacted by the uneven kneading process among the samples thereby leading to the unstable expansion during baking (Plazzotta et al., 2018).

Table 2. Physical properties of macadamia oil cake-supplemented bread

Samples	Specific volume (cm^3/g)	Hardness cycle 1 (N)	Hardness cycle 2 (N)	Springiness (mm)
S0	2.33 ± 0.01^{c}	2003.83 ± 16.28^{a}	2005.08 ± 13.32^{a}	17.00 ± 2.37^{b}
S15	2.65 ± 0.02^{a}	1994.67 ± 12.21^{a}	1982.50 ± 12.13^{a}	11.31 ± 0.48^{c}
S20	2.49 ± 0.03^{b}	1987.00 ± 22.10^{a}	1995.67 ± 15.75^{a}	21.51 ± 2.42^{ab}
S25	2.67 ± 0.04^{a}	1990.00 ± 11.26^{x}	2007.00 ± 10.58^{a}	26.00 ± 1.42^{a}

Values are mean ± SD of three replicates. Means of the same row followed by superscript letters are significantly different ($P < 0.05$).

The hardness cycle 1 of S0 (2003.8 N) was the highest among the four samples, followed by S15 (1994.7 N). But when compressing the second time, the hardness cycle 2 of 3 samples S0, S20, S25 increased, the highest was S25 (2007N), only sample S15 decreased and gave the lowest value (1982.5N). Bread hardness is usually affected by airspace formation, gluten networks, other components present in the flour mixture and especially moisture content. In this study, when increasing the amount of MOC added, the moisture content decreased in sample S15 (29.65%), then increased in turn in samples S20 (31.12%), S25 (32.23%).

Regarding the springiness of MOC-fortified bread, it can be seen in Table 2 that the springiness decreased in sample S15 (11.3 mm), then gradually increased in samples S20 (21.5 mm), S25 (26.0 mm). Because the protein in the MOC is higher than the protein in the wheat flour (11% < 21.6%), it helps the gluten network to grow stronger, helping to increase the bread's flexibility, springiness, structure and shape. The reduced springiness in sample S15 can be attributed to the uneven mixing of the samples affecting the water absorption ability, causing the dough to not expand properly and having a low springiness value (Kumala & Sutrisno, 2020).

3.2. Effect of MOC substitution ratio on color of bread

The color analysis showed that when replacing wheat flour with MOC at the ratios of 15%, 20%, 25%, the L*, a*, b* values were significantly different to the control sample 0% (Table 3). Value of a* was directly proportional to the ratio of MOC addition and conversely the L* value and b* value were inversely proportional. As the amount of additional MOC is increased, L* and b* decreased while the value of a* increased.

Table 3. Color parameters of macadamia oil cake-added bread

Samples	L*	a*	b*	ΔE*
S0	59.71 ± 1.56^{a}	5.49 ± 1.77^{c}	28.57 ± 1.36^{a}	-
S15	51.04 ± 1.43^{b}	8.97 ± 2.47^{b}	23.96 ± 1.69^{b}	10.80 ± 2.04^{b}
S20	49.28 ± 1.00^{b}	12.28 ± 0.76^{a}	24.42 ± 0.77^{b}	13.31 ± 2.18^{b}
S25	44.62 ± 1.90^{c}	13.46 ± 0.49^{a}	19.18 ± 2.22^{c}	$19,71 \pm 2.71^{a}$

Values are mean ± SD of three replicates. Means of the same row followed by superscript letters are significantly different (P < 0.05).

The more MOC is added, the darker the color tends to be and the control is the brightest bread sample. For the value of a*, an uptrend can be seen in the Table 3, which means that the more MOC is added, the redder the sample will be. As a result, the surface color of S20 and S25 is significantly redder than that of S0 and S15, and they are not significantly different ($P > 0.05$). A significant difference in a* value at S0 and S15 was noted ($P > 0.05$). The value of b* decreases significantly. The higher the amount of MOC added to the bread dough, the lighter the yellow color of the surface will be. A significant difference was noted between M0 and all other 3 samples ($P < 0.05$).

Figure 1. Color of 4 different supplemented ratios of macadamia oil cake bread samples.

The ΔE* value of all three samples was greater than 5, indicating that the observer can see the color difference between the samples, the larger the ΔE* value, the greater the color difference. In addition to the analyzed color values, the obvious variation in color of the bread samples is also illustrated via the visual observation as shown in Figure 1.

3.3. Chemical composition of MOC added bread

The changes in contents of moisture, ash, protein, lipid, carbohydrate and energy value of bread added with different ratios of MOC are shown in the Table 4. The moisture content of the bread significantly increased with the high level supplementation of MOC, which were 31.12% for S20 and 32.23% for S25. In contrast, the moisture content of S15 (29.65%) was lower than that of the control (30.73%). The lower moisture content can prevent microbial spoilage and prolong the shelf life of the products (Demirkesen, 2016). Therefore, bread sample S15 has the longest shelf life, followed by reference sample (S0) due to its relatively low moisture content.

Table 4. Chemical composition of macadamia oil cake bread

Samples	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Energy value (kcal)
S0	30.73 ± 0.03b	0.90 ± 0.04^{b}	7.78 ± 0.13^{b}	8.79 ± 0.19^{c}	51.81 ± 0.34^{a}	317.44 ± 0.86^{c}
S15	29.65 ± 0.11c	0.97 ± 0.01^{b}	9.19 ± 0.11^{a}	11.90 ± 0.23^{b}	48.29 ± 0.44^{b}	336.99 ± 0.86^{a}
S20	31.12 ± 0.39b	1.08 ± 0.02^{a}	9.35 ± 0.12^{a}	12.32 ± 0.17^{b}	46.13 ± 0.53^{c}	332.77 ± 1.72^{b}
S25	32.23 ± 0.59a	1.17 ± 0.04^{a}	9.47 ± 0.23^{a}	10.3 ± 0.12^{a}	44.03 ± 0.90^{d}	331.88 ± 1.69^{b}

Values are mean ± SD of three replicates. Means of the same row followed by superscript letters are significantly different ($P < 0.05$).

The ash is generally the indication of mineral content in foods (Oguntoyinbo et al., 2021). In this experiment, the ash content in MOC bread increased with the addition of MOC, respectively, S0 (0.90%), S15 (0.97%), S20 (1.08%) and sample S25 (1.17%) had the highest value. This increase may be due to the effect of higher ash content in MOC (3.57%) than in wheat flour (0.8%). Macadamia nuts are also one of the nuts that can be considered as a source of essential and beneficial minerals for human health such as calcium, iron, magnesium (Munro & Garg, 2008).

Protein content in the bread samples ranged from 7.78 to 9.47% along with the increase in the MOC addition level. Because protein content in wheat flour is lower than in MOC (11% to 21.6%), the increase in protein content of the MOC added bread may be caused by the higher protein in the supplemented MOC. A previous study on bread supplemented with moringa seed powder also revealed similar results with the present research (Bolarinwa et al., 2019).

The amount of fat in the wheat flour used in this experiment is 3%, whereas the amount of fat in MOC is 30.4%. With an increase in MOC level, bread's fat content also greatly elevated. The sample with the highest fat content was S25, while the control sample with the least fat content was S0. The same outcome was discovered in (Bolarinwa et al., 2019) when they conduct a research on bread fortified with moringa seed powder. As a result of the change in nutritional composition, the energy value ranged from 317.44 to 331.88 kcal, which may be primarily attributable to MOC's fat content.

3.4. Effect of MOC on mineral content of MOC bread

It can be claimed that the mineral content of the sample will be lower the smaller the amount of ash contained in the flour sample (Bilge et al., 2016). S0 and S25, however, have ash contents of 0.9% and 1.08%, respectively (Table 5). The samples S0 (control sample) and S20 (the sample that received the most positive feedback) were selected to examine the mineral content present and explain the change of each element.

Table 5. Mineral content of macadamia oil cake bread

Samples	Ca (mg/kg)	Fe (mg/kg)	Mg (mg/kg)
S0	504	13.9	159s
S20	626	19.5	440

Overall, all minerals increased between the two samples of bread. In two samples of bread, calcium is the element that is most prevalent. Iron is the least dominant element, followed by magnesium. The calcium content in this study ranged from 504 to 626 mg/kg, increasing the calcium in S20 by 122 mg/kg. The amount of calcium the body needs each day is roughly 450 mg.

3.5. Sensory evaluation of MOC bread

Table 6. Sensory evaluation of macadamia oil cake-added bread samples

Samples	Color	Flavor	Taste	Texture	Overall acceptability
S0	6.33 ± 1.88^{a}	6.22 ± 1.56^{a}	6.33 ± 1.46^{ab}	6.89 ± 1.41^{a}	6.44 ± 1.82^{a}
S15	6.61 ± 1.94^{a}	6.22 ± 1.52^{a}	5.72 ± 1.93^{ab}	6.00 ± 1.61^{a}	5.72 ± 1.67
S20	6.33 ± 1.71^{a}	6.50 ± 1.58^{a}	6.83 ± 1.42^{a}	6.83 ± 1.62^{a}	6.22 ± 1.66^{a}
S25	6.83 ± 1.42^{a}	5.83 ± 2.04^{a}	5.33 ± 1.94^{b}	5.67 ± 1.94^{a}	5.44 ± 1.42^{a}

Values are mean ± SD of three replicates. Means of the same row followed by superscript letters are significantly different ($P < 0.05$).

The sensory scores of the bread samples are presented in Table 6. In terms of color, All samples were evaluated with "like slightly" and "like moderately" scores. S20 sample got the same score as the control sample, while S25 had the highest acceptance for its eye-catching golden brown color. The Maillard, caramelization, and dextrinization reactions cause the color changes, and the natural color of MOC contributes to some of the bread color variations (Demirkesen, 2016). The flavor score of sample S15 is comparable to the flavor of the control. The highest score belongs to the S20 sample, which shows that this is the sample most accepted by the panelists. The lowest score belongs to S25 below "like slightly" (5.83). In terms of taste, there are only 2 samples scored between "like slightly" and "like moderately" namely S0 and S20, the remaining 2 samples were between "neither like or dislike" and "like slightly". The S20 became the most popular sample with the highest score of 6.83 of all samples, which indicates that panelists liked MOC's creamy in moderation. The most preferred texture samples can be said to be S0 and S20 because the difference is not significant (6.89 and 6.83). This can be explained in structural measurements, both of which have moderate hardness and springiness.

Overall, panelists widely accepted the S20 and gave this sample a score of 6.22, slightly lower than the control sample (6.44). Therefore, S20 sample (20% MOC fortified) is the selected sample for publication. Furthermore, the nutritional parameters of 20% MOC fortified breads (S20) are optimal, with protein content 9.35%, fat content 12.32%, carbohydrate content 46.13%, dietary fiber content 4.29% and energy value 332.77 kcal.

3.6. Microbiological quality of MOC added bread (S20)

The microbiological parameters of 20% MOC fortified bread sample (S20) were analyzed at Eurofins Hai Dang food testing laboratories and shown in Table 7. The results indicate that the microbial quality of selected bread sample satisfies all the microbiological requirements according to TCVN 5909-1995.

Mold and yeast growth are the main sources of microbial spoilage in confectionery products, which are characterized by high solids content and low moisture level (Loureiro & Querol, 1999). Controlling microbial growth or outbreak in confectionery plants requires an understanding of microorganism nature (Kačániová, 2011).

Table 7. Microbiological parameters of macadamia oil cake-added bread (S20)

Microbial indexes	Unit	Results	TCVN 5909-1995
Clostridium perfringens	CFU/g	Not detected (LOD=10)	Satisfy
Coliforms	CFU/g	Not detected (LOD=10)	Satisfy
Escherichia coli	CFU/g	Not detected (LOD=10)	Satisfy
Aerobic plate count	CFU/g	Not detected (LOD=10)	Satisfy
Total spores of yeast and mold	CFU/g	Not detected (LOD=10)	Satisfy

Clostridium perfringens is a gram-positive, anaerobic spore-forming organism. Heat-resistant spores that can live at cooking temperatures are produced by *C. perfringens* (Lee, 2016). A heat labile enterotoxin produced by *C. perfringens*, which is cytotoxic and responsible for food poisoning, damages the membrane of epithelial cells and causes diarrhea (Andersson et al., 1995). Macadamia oil cake bread adheres to the TCVN 5909-1995 standard for *C. perfringens*, which forbids its presence in bread, so the MOC bread sample (S20) are safe based on microbial standards.

Coliforms are non-spore-forming gram-negative bacteria that can break down lactose into acids and gas within 48 h. The presence of coliforms, which are regarded hygienic indicators and are typically detected when heating is insufficient or secondary contamination results from heating (Tominaga & Ishii, 2020). Coliforms shall not exceed 10^2 bacteria/g in accordance with TCVN 5909-1995; consequently, MOC bread satisfies this requirement. The statistics indicated that the bread sample is safe for microbiological quality based on the microbial standards. *Escherichia coli* is a gram-negative, non-spore-forming bacteria, a sign of unhealthy conditions, which may include using water that is of poor quality (Al-Nasiry, 2020). As a result, the bread sample (S20) is free of *E. coli*, making it safe for microbiological quality. An indicator of the amount of microorganisms contained in a food product is the aerobic plate count (Maturin & Peeler, 2001). The maximum amount of aerobic plate count allowed by TCVN 5909-1995 is 5 x 10^3 bacteria/g. The S20 sample thus met the microbiological requirements.

Total yeast and mold spores: Products made of confectionery have a shorter shelf life because to mold and yeast growth. the potential for acidification caused by the mycotoxin formation on those items. Secondary metabolites known as mycotoxins are harmful for human intake (Oranusi et al., 2013). PọMolds that create mycotoxins must not be present in the product, although yeast is permitted with a concentration of no more than 102 bacteria/g. MOC is capable of adjusting to both yeast and mold parameters. In summary, the bread sample fortified with 20% macadamia oil (S20) is safe for microbiological quality.

4. Conclusions

This study revealed that the fortification of MOC significantly improved the nutritional quality and sensory quality of bread. The optimum ratio for MOC fortification was 20% for the bread. The bread produced from MOC were higher nutritional than basic bread in terms of ash, protein, fat and mineral content, especially magnesium content. The hardness of 20% MOC was moderate and this and the control sample were the most preferred samples

in terms of texture. All sensory criteria of the S20 bread samples were above 6 which fall between "Like slightly" and "Like moderately". Besides, the developed bread samples satisfied standard microbial safety requirements of TCVN 5909-1995. Based on the obtained results, it can be concluded that the MOC (a food processing waste) supplementation can help improving nutritional and sensory of bread. The promising results of this study suggest that MOC may also be used as a nutritional supplement ingredient for producing other bakery products instead of being regarded as a waste of food processing.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research is supported by Ho Chi Minh City University of Technology and Education (HCMUTE), Vietnam. The authors also acknowledge Damaca Nguyen Phuong Company for providing macadamia oil cake.

References

Acheampong-Boateng, O., Bakare, A. G., Nkosi, D. B., & Mbatha, K. R. (2017). Effects of different dietary inclusion levels of macadamia oil cake on growth performance and carcass characteristics in South African mutton merino lambs. *Tropical Animal Health and Production* 49(4), 733-738. https://doi.org/10.1007/s11250-017-1250-7.

Al-Nasiry, B. S. A. N. (2020). Detection of bacterial contamination in filled and dried biscuit products of young children. *Annals of Tropical Medicine and Health* 23(16), 231-602. https://doi.org/10.36295/asro.2020.231602.

Andersson, A., Ronner, U., & Granum, P. E. (1995). What problems does the food industry have with the spore-forming pathogens *Bacillus cereus* and clostridium perfringens? *International Journal of Food Microbiology* 28(2), 145-155. https://doi.org/10.1016/0168-1605(95)00053-4.

Behera, S., Indumathi, K., Mahadevamma, S., & Sudha, M. L. (2013). Oil cakes-a by-product of agriculture industry as a fortificant in bakery products. *International Journal of Food Sciences and Nutrition* 64(7), 806-814. https://doi.org/10.3109/09637486.2013.801405.

Bilge, G., Sezer, B., Eseller, K. E., Berberoglu, H., Koksel, H., & Boyaci, I. H. (2016). Ash analysis of flour sample by using laser-induced breakdown spectroscopy. *Spectrochimica Acta Part B: Atomic Spectroscopy* 124, 74-78. https://doi.org/10.1016/j.sab.2016.08.023.

Bolarinwa, I. F., Aruna, T. E., & Raji, A. O. (2019). Nutritive value and acceptability of bread fortified with moringa seed powder. *Journal of the Saudi Society of Agricultural Sciences* 18(2), 195-200. https://doi.org/10.1016/j.jssas.2017.05.002.

Demirkesen, I. (2016). Formulation of chestnut cookies and their rheological and quality characteristics. *Journal of Food Quality* 39(4), 264-273. https://doi.org/10.1111/jfq.12209.

FAO (Food and Agriculture Organization). (2019). Chapter 2: Methods of food analysis. Retrieved February 20, 2024, from https://www.fao.org/4/Y5022E/y5022e03.htm.

Ghoshal, G., & Kaushik, P. (2020). Development of soymeal fortified cookies to combat malnutrition. *Legume Science* 2(3), e43. https://doi.org/10.1002/leg3.43.

Kačániová, M., & Juhaniaková, L. (2011). Microorganisms in confectionery products. *Journal of Microbiology, Biotechnology and Food Sciences* 1(1), 57-69.

Kumala, T., Sutrisno, A., & Yunianta (2020). Glucomannan as an anti-staling agent to improve the texture value of whole wheat bread.

IOP Conference Series: Earth and Environmental Science 475. https://doi.org/10.1088/1755-1315/475/1/012030.

Lee, C. A., & Labbé, R. (2016). Distribution of enterotoxigenic clostridium perfringens spores in US retail spices. *Journal of Food Protection* 81(3), 394-399. https://doi.org/10.4315/0362-028X.JFP-17-352.

Loureiro, V., & Querol, A. (1999). The prevalence and control of spoilage yeasts in foods and beverages. *Trends in Food Science and Technology* 10(11), 356-365. https://doi.org/10.1016/S0924-2244(00)00021-2.

Maturin, L., & Peeler, J. T. (2001). *BAM Chapter 3: Aerobic plate count*. Maryland, USA: US Food and Drug Administration.

Mokrzycki, W., & Tatol, M. (2011). Colour difference delta E-A survey. *Machine Graphics and Vision* 20(4), 383-411.

Munro, I., & Garg, M. (2008). *Nutrient composition and health benefi cial effects of macadamia nuts*. In Alasalvar, C., & Shahidi, F. (Eds.). Tree Nuts: Composition, phytochemicals and health effects (1st ed., 263-272). Florida, USA: CRC Press.

Navarro, S. L. B., & Rodrigues, C. E. C. (2016). Macadamia oil extraction methods and uses for the defatted meal byproduct. *Trends in Food Science and Technology* 54, 148-154. https://doi.org/10.1016/j.tifs.2016.04.001.

Oguntoyinbo, O. O., Olumurewa, J. A. V., & Omoba, O. S. (2021). Physico-chemical and sensory properties of cookies produced from composite flours of wheat and banana peel flours. *Journal of Food Stability* 4(3), 1-21.

Oranusi, S., Braide, W., & Oguoma, O. I. (2013). Antifungal properties of lactic acid bacteria (LAB) isolated from ricinus communis, pentaclethra macrophylla and yoghurts. *Global Advanced Research Journal of Food Science and Technology* 21, 01-06.

Plazzotta, S., Sillani, S., & Manzocco, L. (2018). Exploitation of lettuce waste flour to increase bread functionality: effect on physical, nutritional, sensory properties and on consumer response. *International Journal of Food Science and Technology* 53(10), 2290-2297. https://doi.org/10.1111/ijfs.13820.

Pojić, M., Hadnađev, T. D., Hadnađev, M., Rakita, S., & Brlek, T. (2015). Bread supplementation with hemp seed cake: A by-product of hemp oil processing. *Journal of Food Quality* 38(6), 431-440. https://doi.org/10.1111/jfq.12159.

Pycia, K., Kapusta, I., & Jaworska, G. (2020). Walnut oil and oilcake affect selected the physicochemical and antioxidant properties of wheat bread enriched with them. *Journal of Food Processing and Preservation* 44(8), e14573. https://doi.org/10.1111/jfpp.14573.

Sarkis, J. R., Côrrea, A. P. F., Michel, I., Brandeli, A., Tessaro, I. C., & Marczak, L. D. F. (2014). Evaluation of the phenolic content and antioxidant activity of different seed and nut cakes from the edible oil industry. *Journal of the American Oil Chemists' Society* 91(10), 1773-1782. https://doi.org/10.1007/s11746-014-2514-2.

Szydłowska-Czerniak, A., Poliński, S., & Momot, M. (2021). Optimization of ingredients for biscuits enriched with rapeseed press cake-changes in their antioxidant and sensory properties. *Applied Sciences* 11(4), 1558. https://doi.org/10.3390/app11041558.

Tominaga, T., & Ishii, M. (2020). Chapter 11 - Detection of microorganisms with lateral flow test strips. *Methods in Microbiology* 47, 351-394. https://doi.org/10.1016/bs.mim.2019.11.007