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Research Article

Enrichment of Doogh with Olive Leaf Extract and Investigation of Its Physicochemical, Microbial, and Sensory Properties during Storage at Room Temperature and Refrigerator

Laya Kalavari, Negin Nasiri, Fatemeh Ahmadian*, and Hamed Kioumarsi

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Abstract
Doogh is a popular Iranian drink with high nutritional value and in recent years has attracted the attention of many consumers. Nowadays, the problems caused by malnutrition and lack of useful compounds with high nutritional value and increasing shelf life are essential in the food industry. This research was performed in Shirin Cheshmeh Dairy Production Company and all experiments were performed in the Food Control Laboratory of the Food and Drug Administration, Guilan University of Medical Sciences. In this study, olive leaf extract was added to doogh at five levels of zero, 0.2, 0.5, 1, and 2% and stored for 45 days, its physicochemical, microbial, and sensory properties at two refrigerators at 2 °C and room temperature 25 °C were evaluated. Sampling was analyzed on days 0, 15, 30, and 45 days in 3 replications with Duncan's statistical analysis. The effect of using different treatments and storage time on most characteristics showed a significant difference. This study showed that the effect of olive leaf extract on the pH, mold, and yeast of the treatments is decreasing and the percentage of acidity and serum volume is increasing. According to the hedonic method, the acceptability of treatment 2 on the 15th day of maintenance was higher. In general, according to the results, it can be said that the production of useful products with plant compounds can promote an effective step to produce and design useful drinks in the consumer market and food industry factories.

Keywords: acidity, doogh, olive leaf extract, useful product

1. INTRODUCTION

Animal production is very important in Asia [1]-[4]. Doogh is a traditional Iranian fermented drink that is prepared by mixing yogurt with water and some salt. It is an acidic dairy drink mainly consumed in plain type or with aromatic compounds [5]. It is the result of lactic fermentation of milk, the dry matter of which is standardized by diluting yogurt (after fermentation) or milk (before fermentation). Similar products in Turkey, Afghanistan, and Azerbaijan, are consumed more than in other countries, including the Middle East and Central Asia [6][7]. Nowadays, due to increasing public awareness about the harmful consumption of carbonated beverages, desires to use natural drinks such as doogh are rising [8]. Though it is produced on large industrial scales, more data is needed to introduce its practical properties. The flow properties of doogh are determined for several purposes such as quality control, understanding the structure, process engineering applications, and correlations with sensory evaluation. The final properties of the doogh such as stability, texture, and appearance are directly dependent on its structure [5].

Healthy food production is the main priority of food technology and dairy products which plays an important role in the household food basket. Traditional dairy products take a large part in the market and sometimes are causing the transmission of various diseases to humans, particularly in the cheese market. The basic ingredients in milk fermented dairy products are similar to those in milk; As a result, these products are an excellent source of calcium, phosphorus, B vitamins such as thiamine (B1), riboflavin (B2), pantothenic acid (B5), cobalamin (B12) and magnesium and zinc, but the fermentation process changes the chemical structure. In addition to the nutritional benefits mentioned above, it contains beneficial bacteria that have many effects on the health of the gastrointestinal tract, so If used regularly, the beneficial bacteria Streptococcus thermophilus and Lactobacillus bulgaris in it will be placed in the

Table 1. pH changes in storage at refrigerator (4 °C) and room temperature (25 °C)

Samples (Concentration)	Day zero	Storage at refrigerator (4 °C)			Storage at room temperature (25 °C)		
		Day 15	Day 30	Day 45	Day 15	Day 30	Day 45
Control group	4.10 ± 0.01^{Aa}	4.04 ± 0.01^{Ba}	4.04 ± 0.01^{Ba}	4.01 ± 0.01^{Ba}	4.01 ± 0.01^{Ba}	3.90 ± 0.01^{Ca}	3.82 ± 0.02^{Da}
Sample 1 (0.2%)	4.08 ± 0.03^{Aa}	4.03 ± 0.02^{Ba}	4.02 ± 0.01^{Ba}	4.00 ± 0.01^{Ba}	4.00 ± 0.01^{Ba}	3.87 ± 0.02^{Da}	3.83 ± 0.01^{Da}
Sample 2 (0.5%)	4.07 ± 0.01^{Aa}	4.02 ± 0.02^{Ba}	4.01 ± 0.01^{Ba}	3.98 ± 0.01^{Ca}	3.98 ± 0.02^{Ca}	3.88 ± 0.01^{Da}	3.84 ± 0.02^{Da}
Sample 3 (1.0%)	4.04 ± 0.03^{Bb}	4.01 ± 0.01^{Ba}	3.95 ± 0.01^{Cb}	3.93 ± 0.01^{Cb}	3.95 ± 0.02^{Cb}	3.86 ± 0.01^{Da}	3.84 ± 0.01^{Da}
Sample 4 (2.0%)	4.00 ± 0.02^{Bb}	3.96 ± 0.01^{Cb}	3.94 ± 0.01^{Cb}	3.91 ± 0.01^{Cb}	3.94 ± 0.02^{Cb}	3.85 ± 0.02^{Db}	3.82 ± 0.01^{Da}

gastrointestinal tract and will have positive effects [9][10]. Medicinal plants are endemic heritages of global importance Due to the different chemical compounds that plants produce, they have many applications in the food, medical, perfumery, cosmetic, and health industries. In the 21st century, due to the rapid population growth and the need to develop the food industry at the same time, methods must be used to be not only safe and high-quality products, but also economically and commercially appropriate and cost-effective [9][11][12].

Doogh is made by mixing yogurt, water, salt, and other additives, including peppermint essential oil. The use of sweet Doug has been common in the United States since 2002. The American people call this dairy product our yogurt. The doogh is a dairy drink that can replace soda in the food basket. In addition to nutritional benefits, doogh contains beneficial bacteria that have many effects on the health of the gastrointestinal tract, so its continuous consumption can prevent the growth of harmful organisms [13].

Throughout the history of civilization, the olive plant has been an important source of nutrition and medicine. Fermentation of food products is one of the oldest biotechnologies in production that produces food products with desirable properties such as long life and good organic properties [10] [14][15]. In recent years, the consumption of fermented beverages, due to their effects on health has increased. Olive leaf is effective in treating diabetes and lowering blood pressure and strengthening the immune system. This plant is also involved in lowering blood cholesterol and uric acid. Olive leaf extract strengthens the body's antioxidant defense systems such as catalase and superoxide dismutase. Polyphenols are massively bioactive molecules in olive leaves. In particular, aleuropine along with tyrosol and flavonoids are the main constituents. These compounds have antimicrobial and antioxidant activity in olive leaf extract and can make olive leaf extract suitable for use in the food industry and as a natural preservative [15]-[17]. Various results were obtained regarding the effects of plant extracts containing polyphenol compounds on the growth of semicircular organisms. Plant extracts have been shown to inhibit the growth of food-related pathogens and microorganisms responsible for food spoilage [9][17][18]. The purpose of this study, according to previous studies in which the effect of olive extract has been investigated separately or in combination, is to evaluate the enrichment of doogh with olive leaf extract and investigation of its physicochemical, microbial, and sensory properties during storage at room temperature and refrigerators.

2. MATERIALS AND METHODS

This study was performed in 2018 in Shirin Cheshmeh Dairy Production Company located in Fooman Industrial City in Guilan Province. Shirin Cheshme Gilan company with the brand name "Tenha" located in "Sardarjangal" industrial area, started operating in 2004, production equipment includes pasteurizer, homogenizer, separator, steel tanks, UF (ultrafiltration) equipment, etc. All experiments were performed in the Food Control Laboratory of the Food and Drug Administration, Guilan University of Medical Sciences. Hydroalcoholic extract of olive leaves was purchased from Adonis Goldaroo Company. First, raw milk with 2% of fat was pasteurized at 75 °C

for 20 seconds and then poured into a yogurt preparation tank. The milk temperature is decreased to 43 ° C, then 3% of the yogurt starter is added and stirred for 15 minutes, and finally, the incubation is continued for 8 hours to reach the desired acidity. Then, water, salt, and peppermint were added to stir for 15 minutes. The doogh is heated at 85 ° C for 15 seconds and then quickly cooled to 4 ° C and packaged in bottles. Concentrations of 0.2, 0.5, 1, and 2 mg of olive leaf extract were added to doogh and the treatments were stored in two refrigerators at 2°C and room temperature 25 °. Chemical, microbial and sensory evaluations were performed. The acidity of doogh was measured by titration method, pH was measured using a pH-Meter (Metrohm, Germany), and stability of doogh was also measured. Doogh microbial tests included the mold and yeast test and general enumeration of microorganisms. In determining the amount of a sensory evaluation of doogh through the sensory test (taste) which is done by five-point hedonic method (from very bad: 1 to very good: 5) in terms of color, odor, taste by a team of 15 trained evaluators (Standard No. 695, 1387). In this study, all physicochemical, microbial, and sensory properties of doogh containing different concentrations of olive leaf extract were performed in a completely randomized design with three replications. To compare the means, Duncan's multi -range test method was used at the level of 5% error probability, and in the end, all graphs were drawn with Excel 2007.

3. RESULTS AND DISCUSSIONS

3.1. PH Measurement Results

By measuring the pH of different samples, it was found that the highest pH belonged to the control sample on day zero and the lowest pH belonged to the sample stored for 45 days at room temperature. By adding olive leaf extract, the pH of the samples decreases so that the more the amount of extract, the more the pH decreases, but this increase is not significant up to 0.5% concentration, but in sample 3, with increasing olive leaf extract concentration to 1 and 2%, the control pH decreases significantly. Also, with increasing shelf life, both in the refrigerator and at room temperature, the pH decreases with increasing storage time. In the 15-day for the sample stored in the refrigerator, we see a significant decrease in pH, but in 30-day and 45-day the changes are not significant. We also see a decrease in pH in the samples stored at room temperature, but this decrease has a greater slope compared to the samples stored in the refrigerator so a significant decrease is observed in the 15-day samples and the measurements of the 30-day samples. The decrease in pH is also significant and at the end of 45 days, we see the greatest decrease in pH in samples stored at room temperature. In 30-day and 45-day samples stored at room temperature, pH does not decrease significantly with the increasing concentration of the extract.

3.2. Stability Measurement Results (Phase Separation Rate)

Stability measurement was performed to determine the degree of phase separation by measuring the volume of isolated serum in milliliters, the results of which are shown in Table 3. The results show that the lowest isolated serum or the highest stability is related to the 15-day control sample stored at refrigerator temperature and the highest isolated serum, which indicates the lowest stability, is related to the 45-day controlled control sample at room temperature. With

Table 2. Changes in the acidity of the samples according to the percentage of lactic acid.

Samples (Concentration)	Day zero	Storage at refrigerator (4 °C)			Storage at room temperature (25 °C)		
		Day 15	Day 30	Day 45	Day 15	Day 30	Day 45
Control group	0.46 ± 0.05^{Aa}	0.49 ± 0.05^{Aa}	0.50 ± 0.03^{Aa}	0.51 ± 0.09^{Ba}	0.52 ± 0.04^{Ba}	0.35 ± 0.02^{Ca}	0.58 ± 0.01^{Da}
Sample 1 (0.2%)	0.50 ± 0.09^{Ab}	0.51 ± 0.04^{Aa}	0.52 ± 0.04^{Ba}	0.52 ± 0.04^{Ba}	0.56 ± 0.02^{Ba}	0.57 ± 0.01^{Da}	0.58 ± 0.01^{Da}
Sample 2 (0.5%)	0.51 ± 0.04^{Ab}	0.52 ± 0.01^{Ba}	0.54 ± 0.04^{Ba}	0.57 ± 0.05^{Cb}	0.56 ± 0.04^{Ca}	0.57 ± 0.01^{Db}	0.59 ± 0.04^{Da}
Sample 3 (1.0%)	0.52 ± 0.03^{Bb}	0.54 ± 0.01^{Bb}	0.55 ± 0.02^{Cb}	0.57 ± 0.04^{Cb}	0.56 ± 0.05^{Cb}	0.58 ± 0.01^{Db}	0.59 ± 0.05^{Da}
Sample 4 (2.0%)	0.53 ± 0.09^{Bb}	0.56 ± 0.01^{Cb}	0.56 ± 0.04^{Cb}	0.59 ± 0.05^{Cb}	0.58 ± 0.04^{Cb}	0.58 ± 0.02^{Db}	0.59 ± 0.04^{Da}

Table 3. Changes in the volume of isolated serum.

Samples (Concentration)	Storage at refrigerator (4 °C)			Storage at room temperature (25 °C)		
	Day 15	Day 30	Day 45	Day 15	Day 30	Day 45
Control group	31.0	32.0	34.0	20.0	26.0	30.0
Sample 1 (0.2%)	28.0	29.0	30.0	24.0	26.0	30.0
Sample 2 (0.5%)	26.0	27.0	27.0	26.0	26.0	30.0
Sample 3 (1.0%)	26.5	27.0	28.0	24.0	20.0	27.0
Sample 4 (2.0%)	23.0	24.0	25.0	28.0	26.0	23.0

increasing storage time in all samples, we see a decrease in stability and an increase in isolated serum. This increase in samples stored at room temperature is more than samples stored in the refrigerator and it is also observed that the addition of olive leaf extract changes the amount. The serum is isolated and stability is observed, but these changes are not in a definite order and are not recognized as significant.

3.2. Doogh Sensory Analysis Results

Sensory properties of control doogh containing olive leaf extract based on the five-point hedonic method are specified in Table 4. The taste and odor were very good, but the sample obtained 4 lower scores. The lowest taste and odor scores are related to the samples stored for 15 days at room temperature. With increasing the concentration of the extract from 0.2 to 0.5% and above, we see a change in taste and odor score in 15-day samples stored in the environment. The results show that the taste and odor scores related to the 15-day samples stored in the refrigerator are higher than the 15-day samples stored at room temperature.

3.3. Results of Acidity Measurement in Terms of Percentage of Lactic Acid

By measuring different samples, it was found that the lowest acidity was related to the control sample on day zero and the highest acidity was related to the 45-day sample stored at room temperature. With the addition of olive leaf extract, the acidity of the samples increases significantly compared to the control sample and with increasing the amount of extract, an increase in acidity is observed, but this increase is not significant among the samples containing the extract. This increase in acidity in samples stored at room temperature is greater than samples stored in the refrigerator.

3.4. Microbial Analysis Results

The results of the microbial test are presented in Table 4. During this test, it was found that all samples were negative for mold and yeast, coliform, total count, and Staphylococcus aureus coagulase on the day of manufacture. However, with increasing storage time on the 15th day, the samples stored in the refrigerator remained negative, but mold and yeast were observed in samples stored at room temperature, while the total, coliform, and Staphylococcus aureus count as negative as the samples stored in the refrigerator. The highest amount of mold and yeast in the samples kept at room temperature on the 15th day was related to the control sample and the lowest amount of mold and yeast was related to sample 1, which contains 0.2% of olive leaf extract. In the total count experiment of 30-day samples stored in the refrigerator, in samples No. 2 and 4, which contain 0.5 and 2% of olive leaf extract, respectively, 30 and 1700 cfu/g of the colony were observed, while other samples in Other microbial tests including mold and yeast, coliform and Staphylococcus aureus were negative. The 30-day samples stored at room temperature, like the 30-day refrigerated samples, were negative in coliform and Staphylococcus aureus tests, but molds and yeast were found in samples 1, 2, 4, and in control samples, 1 and 2 in the counting test. A total colony was observed. But other samples were negative. In total, the number of infected samples and the number of colonies in the samples stored at room temperature is more than the samples stored in the refrigerator. The total number of colonies in the samples containing olive leaf extract is less than the control sample. It is observed that in sample 3

stored at room temperature, the total count of microorganisms is negative. 45-day samples were stored at refrigerator temperature in all microbial tests, but 45-day samples were stored at room temperature, although negative in the measurement of coliform and Staphylococcus aureus, but have mold and yeast so in the control sample Mold and yeast were obtained at 4000cfu/g. However, in samples containing olive leaf extract, there is a significant decrease in mold and yeast with increasing extract, so even in samples 1 and sample 4, which contain the lowest and highest amount of extract, mold, and yeast become negative.

3.5. Discussion

As can be seen in Table 1, the pH values of the measured samples are different and this difference is significant in some cases. The pH of the control sample was highest on day 0 and decreased with increasing storage time and storage temperature. Abbase et al. [19] in a study that investigated the effect of guar gum on stability and qualitative properties of doogh found that, with increasing the storage time, the pH of doogh samples containing gum compared to the control sample has a significant decrease and the reason is the activity of lactic acid bacteria and lactic acid production.

Laghaei and Zomorodi, measured the effect of zedo, commercial stabilizer CHO, Arabic, and xanthan gum on the stability and qualitative properties doogh and according to the sensory evaluation, with increasing concentration of xanthan gum and commercial stabilizer CHO the score of flavor and with increasing concentration any of four types of stabilizers the score of consistency decreased [20]. Mirchooli Borazgh et al. [21] investigated the effect of temperature and packaging on the shelf life of non-carbonated doogh and reported that with increasing the storage temperature of doogh, the pH shows a significant decrease. Therefore, the results of the present study are consistent with the mentioned findings. Also, by adding the extract to the doogh on day 0 of storage, we see a significant decrease in pH in the samples containing the extract compared to the control sample and also a regular decrease in pH with increasing the extract from 0.2 to 2%. This can be due to the acidic pH of olive leaf extract. The low PpHh of olive leaf extract can also be due to the presence of caffeic acid in the extract. The mentioned is similar to the findings of the previous researchers [22].

The findings in Table 2 shows that with increasing storage time and temperature, the amount of acidity in terms of the percentage of lactic acid shows a significant increase so this increase in acidity increases significantly with increasing storage temperature both in refrigerated samples and samples stored in the environment, the acidity increases with increasing storage time, so this increase in acidity can be attributed to the activity of lactic acid-producing bacteria during storage. Mirchooli Borazgh et al [21], investigated the effect of temperature and packaging on the shelf life of non-carbonated doogh and reported that with increasing the storage temperature of the doogh, the acidity shows a significant increase which is in line with the findings of the current study.

As shown in Table 3, the results show that the lowest amount of isolated serum or the highest stability is related to the sample stored for 15 days in the refrigerator temperature and the highest amount of isolated serum is the lowest stability. It is related to the 45-day control sample stored at room temperature. With increasing storage time in all samples, we see a decrease in stability and an increase in isolated serum. This increase in samples stored at room temperature is more than samples stored in the refrigerator and is also observed.

With the addition of olive leaf extract, changes in the amount of isolated serum and stability are observed, but these changes did not have a specific order and were not recognized as significant. Therefore, it can be concluded that adding olive leaf extract to doogh in the present study had a significant effect on increasing or has not reduced the stability of the doogh. Mahmoudzadeh et al. [23] studied the effect of Thymus Kotschyanus essential oil on the physicochemical and sensory properties of doogh and reported that essential oil doesn't have significant changes in the Physico-chemical properties of doogh. Therefore, the above results are also consistent with the results of the present study. Sensory properties of control doogh and containing olive leaf extract based on the five-point hedonic method are specified in Table 4. The taste and odor were very good, but the sample got 4 lower scores. The reason for this score is probably

due to the high amount of extract and the creation of an astringent taste in the mouth. The lowest taste and odor scores are related to 15-day samples stored at room temperature. With increasing the concentration of the extract from 0.2 to 0.5% and above, we see a decrease in taste and odor scores in 15-day samples stored in the environment. The results show that the taste and odor scores related to the 15-day samples stored in the refrigerator are higher than the 15-day samples stored at room temperature. It seems that storage at room temperature will have an adverse effect on the sensory properties of doogh. The results of this study are consistent with the reports of Mirchooli Borazgh et al. [21] who studied the effect of temperature and packaging on the shelf life of non-carbonated buttermilk.

According to the results (Table 4) during this experiment, it was found that all samples on the day of manufacture were negative for mold and yeast, coliform, total count (Total Count), and Staphylococcus aureus coagulase, which indicates that Provides hygienic observance in the preparation and manufacture of tested samples. However, with increasing storage time on the 15th day, the samples stored in the refrigerator remained negative, but the samples stored at room temperature, mold, and yeast were observed, while the total, coliform and Staphylococcus aureus counts as negative as the samples stored in the refrigerator.

These results are consistent with the studies of Mirchooli Borazgh et al. [21] which investigated the effect of temperature and packaging on the shelf life of non-carbonated buttermilk. The highest amount of mold and yeast in the samples stored at room temperature on the 15th day is related to the control sample and the lowest amount of mold and yeast is related to sample 1, which contains 0.2% of olive leaf extract, which can be due to anti-inflammatory properties of olive leaf extract.

In the total count experiment of 30-day samples stored in the refrigerator, in samples No. 2 and 4, which contain 0.5 and 2% of olive leaf extract, respectively, 30 and 1700 cfu/g of the colony were observed, while other samples in other microbial tests including mold and yeast, coliform and Staphylococcus aureus were negative. The 30-day samples stored at room temperature, like the 30-day refrigerated samples, were negative in coliform and Staphylococcus aureus tests, but molds and yeast were found in samples 1, 2, 4, and in control samples, 1 and 2 in the counting test. The total number of colonies was observed but other samples were negative. In total, the number of infected samples and the number of colonies in the samples stored at room temperature is more than the samples stored in the refrigerator.

The total number of colonies in the samples containing olive leaf extract is less than the control sample so, in sample 3 stored at room temperature, the total count of microorganisms is negative. 45-day samples were stored at refrigerator temperature in all microbial tests, but 45-day samples stored at room temperature, although negative in the measurement of coliform and Staphylococcus aureus, but have mold and yeast so in the control sample Mold and yeast were obtained at 4000 cfu /

Table 4. Results of sensory analysis of samples.

Samples (Concentration)	Day zero		Day 15			
			Storage at refrigerator (4 °C)		Storage at room temperature (25 °C)	
	Taste	odor	Taste	odor	Taste	odor
Control group	5	5	4	3	3	2
Sample 1 (0.2%)	5	5	3.5	3	2.5	2
Sample 2 (0.5%)	5	5	4	3	2.5	1
Sample 3 (1.0%)	5	5	4	3	2.5	1
Sample 4 (2.0%)	4.5	4.5	4	3	2	1

Notes: 1-very week; 2-week; 3- average; 4-good; 5-very good

g, but in samples containing olive leaf extract, the increase in mold and yeast extract decreased significantly, so that even in samples 1 and sample 4, which contain the lowest and highest amount of extract, mold, and yeast become negative. The above cases show that although with increasing temperature and shelf life, the number of mold and yeast colonies and the total microbial count increase, but with the presence and increase of the extract concentration due to antimicrobial properties of olive leaf extract causes a significant decrease in mold colonies and yeast and the general population of microorganisms. These results are consistent with the observations of Rafiee et al. [24].

4. CONCLUSIONS

Increasing the nutritional value and shelf life of traditional dairy products has been considered much in recent years. The use of natural food additives with functional properties instead of chemical preservatives is more interested in the consumer and food industry. The quality and shelf life of yogurt are important topics in the dairy industry [8, 9, 25, 26]. According to the results and discussions, it can be concluded that adding olive leaf extract to doogh can reduce the pH and its storage at refrigerator temperature is recommended to help the minimum pH changes during storage, the acidity of doogh stored at refrigerator temperature is the lowest. It has changed in comparison with doogh stored at room temperature. It was also found that olive leaf extract has no effect on the stability of doogh, but storage conditions in the refrigerator increase the stability of doogh. According to our findings, the antimicrobial activity of olive leaf extract is confirmed in the samples during the storage time, especially with the improvement of the storage conditions. Also, no significant adverse effects were observed in the sensory characteristics of the samples with the addition of olive leaf extract. Therefore, the positive effect of olive leaf extract as a natural safe compound with different biological activities on enriched doogh was confirmed in the present study. However, additional research to increase the quality and acceptability of enriched products is needed.

AUTHOR INFORMATION

Corresponding Author

Fatemeh Ahmadian — The Food and Drug Administration, Guilan University of Medical Sciences, Rasht-43 (Iran);
orcid.org/0000-0001-9986-7701
Email: ahmadian.f94@gmail.com

Authors

Laya Kalavari — Department of Food Industry, University of MehrAeen, Bandar Anzali-38 (Iran);
orcid.org/0000-0002-0008-9709

Negin Nasiri — Department of Food Industry, University of MehrAeen, Bandar Anzali-38 (Iran);
orcid.org/0000-0001-7547-2617

Hamed Kioumarsi — Department of Animal Science Research, Gilan Agricultural and Natural Resources Research and Education Center, Rasht -43 (Iran);
orcid.org/0000-0002-0783-0934

Author Contributions

L. K. The work presented derives from her master's thesis project, and she conducted the research. N. N. She is the supervisor of the thesis (conceptualization, writing original draft preparation, funding acquisition, resources, methodology, investigation, data analysis). F. A. She is the supervisor of the thesis (conceptualization, writing original draft preparation, funding acquisition, resources, methodology, investigation, data analysis). H. K. He served as the Head of the Faculty of Agriculture, Mehraeen Higher Education Institution (original draft preparation, resources, methodology, review, and editing).

Conflicts of Interest

The author(s) declared no conflict of interest

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