Effectiveness of Sodium Hypochlorite, Peroxyacetic Acid and Peroxycitric Acid in Reducing Microorganisms on the Surface of Fresh Whole Litchi Fruit and Its Arils

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ABSTRACT

The effectiveness of three sanitizers, sodium hypochlorite (NaOCl), peroxyacetic acid (PAA) and peroxycitric acid (PCA) in decreasing the total number of bacteria and yeast-molds on the peel of whole litchi fruit and its arils of three cultivars, cv. Honghuay, Gimjeng and Jugkapat were studied. First, the optimal concentration and treatment time of PAA and PCA were determined for whole litchi fruit (concentrations: 75, 100, 150 or 200 mg/L; treatment times: 1, 3 or 5 min) and for the arils (concentrations: 50 or 75 mg/L; treatment times: 1 or 3 min). Treatments were compared with undipped and dipped controls in tap water. The best treatments of PAA and PCA for sanitizing three cultivars of whole litchi fruit were 100 mg/L for 5 min and 200 mg/L for 3 min, respectively. For the arils, the best treatments of PAA and PCA for three cultivars were 50 mg/L for 1 min and 50 mg/L for 3 min, respectively. The effectiveness of PAA and PCA were then compared with NaOCl at a commercial recommendation levels (concentrations: 200 and 50 mg/L; treatment times: 3 min). The results showed that PAA was the most efficient in reducing microorganisms on whole litchi fruit and arils when compared with NaOCl and PCA. Therefore, PAA could be a potential alternative to NaOCl or chlorine as sanitizer for whole litchi fruit and its arils.

Key words: Sodium hypochlorite, Peroxyacetic acid, Peroxycitric acid, Litchi

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a subtropical Asian fruit with a natural red color, sweet acidic taste and aroma. The fruit has a high commercial value in the international market. The major factors reducing the storage life and marketability of fruit are microbial decay and browning of outer covering pericarp within 2-3 days after harvest at 20°C (Holcroft and Mitcham, 1996 ; Jieng, 2003). Thus, litchi

fruits are rejected by the market even though the edible arils (white, translucent, firm and juicy tissue covered by the pericarp) still remain in excellent condition. Arils or flesh of such litchi fruit can be preserved by minimal processing which provides fresh-like fruit with simplicity in use and convenience (Shah and Nart, 2008).

In minimal processing, washing of outer pericarp can reduce the overall potential for microbial food safety hazards because most microbial contamination is on the surface of the fruit. Final washing of arils after peeling helps remove some of the cellular fluids that could serve as a nutrient for microbial growth (USFDA, 2006). Chlorine is normally used for the disinfection of whole and fresh-cut fruit. Dipping in chlorine water containing 50-200 mg/L of free chlorine (recommended concentrations) is commonly used (Soliva-Fortuny and Martín-Belloso, 2003). Moreover, it is known that the reaction of chlorine with natural organic matter results in the formation of carcinogenic halogenated by-products (DBP), like trihalomethanes (THMs) and haloacetic acids (HAAs) (Artes et al., 2009). Use of chlorine is also associated with the production of high amounts of waste water with very high levels of biological oxygen demand (BOD) (Olmez and Kretzchmar, 2008). Due to the above described problems, alternative sanitizing agents to replace chlorine have gained much interest in recent years.

Peroxides such as peracetic acid (PAA) for sanitizing fruit and vegetables as an alternative to chlorine has been used on apples (Wisniewsky, 2000), lettuce (Beuchat et al., 2004 ; Kim et al., 2006), stone fruit (Mari et al., 2004) and whole mango fruit and flesh (Narciso and Plotto, 2005). Moreover, percitric acid (PCA) is one of the organic peroxides (Ferdousi et al., 2007, 2008). However, effectiveness of PCA in reducing microbial populations on fresh fruit and vegetable has not been reported. The objective of this study was to 1) determine the most effective concentration and treatment time of PAA and PCA in controlling microorganisms on whole litchi fruit and the arils of different cultivars and 2) using the most effective concentration and treatment time of the two sanitizers and compare its effectiveness with that of NaOCl of whole fruit and arils of different cultivars.

MATERIALS AND METHODS

Fruit

Litchi (*Litchi chinensis* Sonn.) fruit cv. Honghuay, Gimjeng and Jugkapat, at the fully-red color and commercially-harvesting stage were purchased from 3 different retailers in Chiang Mai Province, Thailand, during June-August, 2008. Fruit were then kept at 4±1°C overnight and selected for uniformity of size, shape, color and lack of physical damage and injury caused by insects, prior to use in the following two experiments.

Experiment 1. Determining the most effective concentration and treatment time of PAA and PCA for sanitizing whole litchi fruit and arils.

For whole litchi fruit, the experiment was designed as 4x3 factorial in Completely Randomized Design (CRD) with 3 replicates, 4 levels of concentration and 3 levels of treatment time. Five fruit per replication were dipped in 75, 100, 150 or 200 mg/L of PAA or PCA for 1, 3 or 5 min. After draining, fruit were analyzed for total bacteria (BAM, 2001) and yeast-molds (AOAC, 2000). Treatments were compared with undipped and dipped controls in tap water. Litchi fruit from three different locations were used.

For arils, the experiment was designed as 2x2 factorial in CRD with 3 replicates, 2 levels of concentration and 2 levels of treatment time. Five fruit per replication were washed with the best treatment of sanitizer for whole fruit. The seed was removed with sanitized sharp-point knife prior peeling. Then, five arils per replicate were dipped in 50 or 75 mg/L of PAA or PCA for 1 or 3 min. After draining, arils were evaluated for total bacteria (BAM, 2001) and yeast-molds (AOAC, 2000). Treatments were compared with undipped and dipped controls in tap water. Litchi fruit from three different locations were used.

Experiment 2. Comparison of PAA and PCA to NaOCl for sanitizing whole litchi fruit and arils.

PAA and PCA were compared against NaOCl in reducing total bacteria and yeast-mold populations on whole litchi fruit and arils of different cultivars using CRD with 3 replicates.

For whole litchi fruit, five fruit per replication were dipped in NaOCl at a commercially recommended level (concentration 200 mg/L for 3 min) or the best treatment of PAA and PCA obtained from Experiment 1. After draining, fruit were evaluated for total bacteria (BAM, 2001), and yeast-molds (AOAC, 2000).

For arils, five fruit per replication were washed with the best treatment of sanitizer for whole fruit. The seed was removed with sanitized sharp-point knife prior peeling. Then, five arils per replicate were dipped in NaOCl at a commercial recommendation level (concentration 50 mg/L for 3 min) or the best treatment of PAA and PCA obtained from Experiment 1. After draining, fruit were analyzed for total bacteria (BAM, 2001) and yeast-molds (AOAC, 2000).

Preparation of sanitizers

Chlorinated water (concentration 200 and 50 mg/L) was prepared with Clorox® USA (5.7% chlorine), adjusted to pH 6.5 with 50% citric acid. Five concentration levels 50, 75, 100, 150 and 200 mg/L of PAA at pH 2.55-3.54 were prepared from PAA solution (PAA 5%; Thaiperoxide Co., Ltd, Thailand). Five concentration levels of PCA 50, 75, 100, 150 and 200 mg/L at pH 2.30-4.42 were prepared from PCA solution (PCA 5%; Thaiperoxide Co., Ltd, Thailand).

Determination of microbial population

For whole litchi fruit, for each treatment, five fruit were transferred to a sterilized bag containing 50 ml of 0.1% phosphate buffer pH 7.2. Five fruit and phosphate buffer in bags were firmly hand-rubbed for 2 min. Samples were serially-diluted by a factor of ten in phosphate buffer. The undiluted mixture and serially-diluted mixture (0.1 ml in duplicate) were spread on plate count agar and potato dextrose agar for total bacteria and yeast-molds count, respectively. Then, plate count agar and potato dextrose agar were incubated at 35°C for 48 hr and 25°C for 48 hr, respectively. Values are reported as log CFU/fruit.

Arils from five fruit per replicate were cut with sterilized stainless steel scissors and a 10 g sample was weighed for analysis. The samples were transferred to a sterilized bag containing 90 ml of 0.1% phosphate buffer pH 7.2 and samples were macerated by stomacher (IVL Masticator 400, Spain) for 30 sec. The homogenized samples were serially-diluted by a factor of ten in phosphate buffer. The undiluted mixture and serially diluted mixture (0.1 ml in duplicate) were spread on plate count agar and potato dextrose agar for total bacteria and yeast-molds count, respectively. Then, plate count agar and potato dextrose agar were incubated at 35°C for 48 hr and 25°C for 48 hr, respectively. Values are reported as log CFU/g.

Statistical analysis

All experiments were replicated three times. Triplicate samples were analyzed and diluted samples were plated in duplicate (total n=18). Data were analyzed using SPSS program V.13 for analysis of variance. Duncanís multiple range test was used for comparison of means to determine differences in microbial counts for treatments.

RESULTS AND DISCUSSION

Effectiveness of PAA on three cultivars of whole litchi fruit

The number of total bacteria and yeast-molds initially on the surface of whole litchi fruit were 6.35-6.77 log CFU/fruit and 6.13-6.47 log CFU/fruit, respectively. Tap water treatment did not cause a significant change in total bacteria and yeast-molds count when compared with undipped control, the microbial reduction was less than 0.5 log CFU/fruit (data not shown). For treatment time of 1 or 3 min of whole fruit, 200 mg/L PAA achieved the highest reductions on total bacteria by 1.84 and 1.80 log CFU/fruit in Honghuay, 1.95 and 2.40 log CFU/fruit in Gimjeng and 1.56 and 2.23 log CFU/fruit in Jugkapat, respectively, and also yeast-molds by 1.84 and 1.83 log CFU/fruit in Honghuay, 2.32 and 2.63 log CFU/fruit in Gimjeng and 1.57 and 2.29 log CFU/fruit in Jugkapat, respectively (Table 1). The 5 min treatment time was more effective than the 1 or 3 min in reducing the total bacteria and yeast-mold populations, and no differences were noted among the concentration treatments with all three litchi cultivars. Therefore, the lowest concentration at 5 min, i.e., 100 mg/L PAA for 5 min was used for sanitation treatment in subsequent experiments.

Table 1. Log reductions of total bacteria and yeast-molds from whole litchi fruit treated with PAA at different concentrations and treatment times.

Treatment times (min)	Conc. (mg/L)	Log reductions (log CFU/fruit)					
		Honghuay		Gimjeng		Jugkapat	
		TBC	Y&M	TBC	Y&M	TBC	Y&M
1	75	0.30 h	0.29 f	0.14 g	1.28 h	0.77 g	0.76 g
1	100	1.34 f	1.35 d	1.59 e	1.75 f	1.05 f	1.03 f
1	150	1.43 ef	1.41 cd	1.92 c	2.17 d	1.08 f	1.08 f
1	200	1.84 b	1.84 b	1.95 c	2.32 c	1.56 e	1.57 e
3	75	0.51 g	0.58 e	1.48 f	1.55 g	1.11 f	1.04 f
3	100	1.55 de	1.53 cd	1.97 c	1.96 e	2.00 d	2.01 d
3	150	1.64 cd	1.63 bc	2.15 b	2.39 c	2.15 cd	2.17 cd
3	200	1.80 bc	1.83 b	2.40 a	2.63 ab	2.23 bc	2.29 bc
5	75	1.59 de	1.62 bcd	1.76 d	1.70 f	1.40 e	1.46 e
5	100	2.22 a	2.41 a	2.40 a	2.70 a	2.51 a	2.54 a
5	150	2.25 a	2.49 a	2.41 a	2.56 b	2.44 ab	2.50 ab
5	200	2.21 a	2.42 a	2.39 a	2.71 a	2.53 a	2.61 a

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means ± SD of n = 18.
Populations of TBC on undipped control were 6.51, 6.35 and 6.77 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively.
Populations of Y&M on undipped control were 6.20, 6.13 and 6.47 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively.
Values in each column with distinct letters represent the significantly different results ($p < 0.05$).

Effectiveness of PAA as a sanitizer is based on the release of active oxygen, which oxidize sensitive sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites of the bacteria and yeast-molds (Kitis, 2004).

Effectiveness of PCA on three cultivars of whole litchi fruit

The number of total bacteria and yeast-molds initially on the surface of whole litchi fruit were 6.53-6.82 log CFU/fruit and 6.11-6.33 log CFU/fruit, respectively. Tap water treatment did not cause a significant change in total bacteria and yeast-molds count when compared with undipped control, the reduction was less than 0.5 log CFU/fruit (data not shown). Total bacteria and yeast-molds were reduced by less than 0.80 log CFU/fruit when whole litichi fruit of all three cultivars were dipped in 75, 100 or 150 mg/L PCA at all treatment times (Table 2). The microorganisms were reduced by more than 0.80 log CFU/fruit; approximately 1.03 log CFU/fruit, when treated with 200 mg/L PCA for 3 or 5 min. Therefore, the 3 min treatment with 200 mg/L PCA was used for sanitation treatment in subsequent experiments.

Effectiveness of three sanitizers on whole litchi fruit

The effectiveness of the three sanitizers used in this study were compared (Table 3). The highest reduction resulted from treatment of whole litchi fruit with 100 mg/L PAA for 5 min, achieving reductions of total bacteria and yeast-molds by 2.44 and 2.67 log CFU/fruit in Jugkapat, 2.32 and 2.57 log CFU/fruit in Gimjeng

Table 2. Log reductions of total bacteria and yeast-molds from whole litchi fruit treated with PCA at different concentrations and treatment times.

Treatment times (min)	Conc. (mg/L)	Log reductions (log CFU/fruit)					
		Honghuay		Gimjeng		Jugkapat	
		TBC	Y&M	TBC	Y&M	TBC	Y&M
1	75	0.07 e	0.09 e	0.19 h	0.25 g	0.44 f	0.24 e
1	100	0.49 d	0.54 d	0.43 f	0.52 ef	0.67 de	0.53 cd
1	150	0.59 cd	0.56 d	0.59 de	0.64 de	0.74 c	0.63 bc
1	200	0.67 bcd	0.68 bcd	0.69 bc	0.79 bc	0.84 b	0.65 bc
3	75	0.19 e	0.23 e	0.34 g	0.43 f	0.47 f	0.24 e
3	100	0.56 cd	0.61 cd	0.60 de	0.69 cd	0.71 cd	0.54 bc
3	150	0.81 abc	0.77 bc	0.62 cd	0.67 d	0.83 b	0.70 ab
3	200	1.03 a	1.06 a	0.96 a	0.98 a	0.98 a	0.82 a
5	75	0.52 d	0.56 d	0.51 ef	0.57 de	0.62 e	0.39 d
5	100	0.80 abc	0.80 bc	0.74 b	0.80 bc	0.76 c	0.63 bc
5	150	0.86 ab	0.87 b	0.74 b	0.81 b	0.98 a	0.83 a
5	200	1.03 a	1.07 a	1.02 a	1.03 a	1.01 a	0.85 a

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means ± SD of n = 18.
Populations of TBC on undipped control were 6.53, 6.37 and 6.82 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively.
Populations of Y&M on undipped control were 6.15, 6.11 and 6.33 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively.
Values in each column with distinct letters represent the significantly different results ($p \leq 0.05$).

and 2.25 and 2.45 log CFU/fruit in Honghuay, respectively, followed by treatment with 200 mg/L NaOCl for 3 min where reduction was less than 1.5 log CFU/fruit. Treatment with 200 mg/L PCA for 3 min was less effective, reductions did not exceed 1.3 log CFU/fruit (Table 3). These results agree with those of Narciso and Plotto (2005) who showed that 100 mg/L PAA was more effective than 200 mg/L NaOCl on the reduction of microbial populations in whole mango fruit.

Effectiveness of PAA on three cultivars of litchi arils

The numbers of total bacteria and yeast-molds initially on the surface of arils were 3.63-3.93 log CFU/g and 3.39-3.59 log CFU/g, respectively. Treatment with PAA at two levels of concentration or two levels of treatment time were not significant ($p \leq 0.05$) in reducing the microorganism populations. Total bacteria and yeast-molds were reduced by 1.30 and 1.50 log CFU/g in Honghuay, 1.70 and 2.20 log CFU/g in Gimjeng and 1.70 and 1.70 log CFU/g in Jugkapat, respectively (Table 4). Therefore, 50 mg/L PAA for 1 min was the optimal treatment for the three cultivars of litchi arils.

In contrast, Kim (2006) reported that the 3 min treatment was more effective than the 1 min time in reducing the *Enterobacter sakazakii* population when shredded lettuce were treated with 40 or 80 mg/L PAA. The reason for difference is not known. No differences were found between the two concentration treatments.

Table 3. Populations of total bacteria and yeast-molds recovered from whole litchi fruit when treated with three types of sanitizers.

Micro organisms	Experiment units	Microbial populations (log CFU/fruit)		
		Honghuay	Gimjeng	Jugkapat
Total bacteria	100 mg/L PAA for 5 min	3.55 ± 0.12 c	4.07 ± 0.09 b	4.03 ± 0.06 c
	200 mg/L PCA for 3 min	4.87 ± 0.03 a	5.40 ± 0.15 a	5.67 ± 0.12 a
	200 mg/L NaOCl for 3 min	4.43 ± 0.24 b	5.49 ± 0.04 a	5.47 ± 0.07 b
Yeast & molds	100 mg/L PAA for 5 min	3.45 ± 0.16 c	3.79 ± 0.10 b	3.80 ± 0.08 c
	200 mg/L PCA for 3 min	5.09 ± 0.05 a	5.32 ± 0.07 a	5.56 ± 0.10 a
	200 mg/L NaOCl for 3 min	4.76 ± 0.12 b	5.29 ± 0.05 a	5.26 ± 0.15 b

Values are means ± SD of n = 18.
Populations of total bacteria on undipped control were 5.76, 6.39 and 6.47 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Populations of yeast and molds on undipped control were 5.90, 6.30 and 6.42 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Values in each column with distinct letters represent the significantly different results ($p \leq 0.05$).

Table 4. Log reductions of total bacteria and yeast-molds from arils treated with PAA at different concentrations and treatment times.

Treatment times (min)	Conc. (mg/L)	Log reductions (log CFU/g)					
		Honghuay		Gimjeng		Jugkapat	
		TBC	Y&M	TBC	Y&M	TBC	Y&M
1	50	1.36 ns	1.54 ns	1.73 ns	2.26 ns	1.70 ns	1.78 ab
1	75	1.33 ns	1.49 ns	1.71 ns	2.19 ns	1.70 ns	1.86 a
3	50	1.39 ns	1.55 ns	1.72 ns	2.23 ns	1.60 ns	1.66 bc
3	75	1.39 ns	1.50 ns	1.72 ns	2.18 ns	1.61 ns	1.60 c

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means + SD of n = 18.
Populations of TBC on undipped control were 3.63, 3.87 and 3.93 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Populations of Y&M on undipped control were 3.39, 3.80 and 3.59 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Values in each column with distinct letters represent the significantly different results ($p \leq 0.05$).

Effectiveness of PCA on three cultivars of litchi arils

The number of total bacteria and yeast-molds initially on the surface of arils were 3.60-3.96 log CFU/g and 3.30-3.73 log CFU/g, respectively. Tap water treatment did not cause a significant change in total bacteria and yeast-mold count when compared with undipped control (data not shown). Treatment with PCA at two levels of concentration were not significant ($p \leq 0.05$) on the microorganism populations (Table 5). However, dipping the arils in PCA at all concentrations for 3 min did reduce microorganism populations than the 1 min treatment. Total bacteria and yeast-molds in Honghuay, Gimjeng and Jugkapat cultivars were reduced by 0.97, 1.04, 1.27 log CFU/g and 1.06, 1.13, 1.24 log CFU/g, respectively (Table 5). Therefore, the best treatment of PCA for sanitizing three cultivars of litchi arils was 50 mg/L for 3 min.

However, the effectiveness of PCA as a sanitizer for fruit and vegetable has not been reported. The results from this work showed that effectiveness of PCA was equivalent in the reduction of mesophilic bacteria on fresh-cut lettuce with dipping in 1% citric acid, which was reduced by 1.50 log CFU/g (Akbas and Olmez, 2007).

Table 5. Log reductions of total bacteria and yeast-molds from arils treated with PCA at different concentrations and treatment times.

Treatment times (min)	Conc. (mg/L)	Log reductions (log CFU/g)					
		Honghuay		Gimjeng		Jugkapat	
		TBC	Y&M	TBC	Y&M	TBC	Y&M
1	50	0.74 b	0.80 b	0.89 b	0.97 b	1.06 b	1.07 b
1	75	0.77 b	0.85 b	0.90 b	1.02 b	1.03 b	1.09 b
3	50	0.95 a	1.01 a	1.04 a	1.11 a	1.27 a	1.24 a
3	75	0.98 a	1.10 a	1.04 a	1.15 a	1.26 a	1.24 a

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means ± SD of n = 18.
Populations of TBC on undipped control were 3.60, 3.85 and 3.96 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Populations of Y&M on undipped control were 3.30, 3.66 and 3.73 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Values in each column with distinct letters represent the significantly different results ($p \leq 0.05$).

Effectiveness of three sanitizers on arils

The effectiveness of the three sanitizers used in this study were statistically different ($p \leq 0.05$). Treatment with 50 mg/L PAA for 1 min was the most effective among the treatments, which reduced the number of total bacteria by 1.58, 2.06, 1.91 log CFU/g and 1.73, 2.25,1.96 log CFU/g on yeast-molds in Honghuay, Gimjeng and Jugkapat, respectively (Table 6). PCA treatment with 50 mg/L for 3 min was the least effective in reducing microbial populations, followed by 50 mg/L NaOCl for 3 min and 50 mg/L PAA for 1 min. These results agree with those of Ruiz-Cruz et al., (2007) who showed that 40 mg/L PAA was more effective than 200 mg/L NaOCl on the reduction of *Salmonella* spp. in fresh-cut carrot under processed water condition (COD 35,000 mg/L).

CONCLUSION

Results from this work showed that 100 mg/L PAA for 5 min and 50 mg/L PAA for 1 min are an alternative to NaOCl treatment for sanitizing three cultivars of whole litchi fruit and its arils, respectively. PAA has the advantage of being more stable and can preserve its efficacy even in the presence of organic matter. It caused higher reduction of total bacteria and yeast-molds.

Table 6. Populations of total bacteria and yeast-molds recovered from arils when treated with three types of sanitizers.

Micro organisms	Experiment units	Microbial populations (log CFU/fruit)		
		Honghuay	Gimjeng	Jugkapat
Total bacteria	50 mg/L PAA for 1 min	2.23 ± 0.08 c	2.07 ± 0.12 c	2.49 ± 0.08 c
	50 mg/L PCA for 3 min	2.87 ± 0.19 a	3.11 ± 0.12 a	3.22 ± 0.07 a
	50 mg/L NaOCl for 3 min	2.55 ± 0.08 b	2.64 ± 0.26 b	2.69 ± 0.05 b
Yeast & molds	50 mg/L PAA for 1 min	2.02 ± 0.10 c	1.69 ± 0.10 c	2.10 ± 0.10 c
	50 mg/L PCA for 3 min	2.73 ± 0.13 a	2.82 ± 0.12 a	2.76 ± 0.11 b
	50 mg/L NaOCl for 3 min	2.33 ± 0.06 b	2.45 ± 0.11 b	2.82 ± 0.07 a

Values are means ± SD of n = 18.
Populations of total bacteria on undipped control were 3.81, 4.13 and 4.40 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Populations of yeast and molds on undipped control were 3.75, 3.94 and 4.06 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.
Values in each column with distinct letters represent the significantly different results ($p < 0.05$).

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