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Extended Fermentation and Physical Scarification to Break Dormancy in Aren (*Arenga pinnata*) Seeds

Duryat*, Paksi Arenda Ayatullah Dewantara, Trio Santoso, Wahyu Hidayat

Department of Forestry, Faculty of Agriculture, University of Lampung, Bandar Lampung 35145, Indonesia

Corresponding Author Email: duryat@fp.unila.ac.id

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ABSTRACT

Aren seeds experience physical dormancy due to the thickness, hardness, impermeable seed coat, and the existence of potassium oxalate crystals. In addition to physical dormancy, palm seeds also experience physiological dormancy due to an imbalance in stimulating and inhibiting hormones. The study's objective was to obtain the effectiveness of fermentation and deoperculation methods, both as single and combination treatments, in breaking the dormancy of aren seeds. A factorial completely randomized design (CRD) with two treatments was tested: duration of fermentation and deoperculation treatment. There are four levels of fermentation duration: 0 weeks (F0); two weeks (F1); four weeks (F2); and six weeks (F3); as well as two levels of deoperculation: without deoperculation (D0) and with deoperculation (D1). Data analysis included homogeneity, variance analysis, and the least significant difference test (LSD). All tests were carried out at the 5% level of significance. The result showed that 4-week fermentation treatment combined with deopercolation, and 6-week fermentation treatment with or without deopercolation, were significantly proven to increase the percentage of germination and germination value, accelerate the seeds germination, and not reduce the seed viability of aren. Those three treatments have equal value in all observed parameters of seed germination.

1. INTRODUCTION

The aren palm (*Arenga pinnata*), a multi-purpose species prevalent mainly in tropical Asia, has demonstrated its utility across various sectors, including food, bioindustry, bioenergy, biofuel, and nano cellulose production [1-3]. The palm's sap, a key product, holds high economic value, with a single plant capable of yielding 20-30 litres per day [4]. Palm fiber of aren (*ijuk*) contains cellulose with high tensile strength and durability [5]. Aren fruit (*kolang-kaling*) contains antioxidants, flavonoids, alkaloids, and quinones, which have been empirically used to treat osteoarthritis [6]. Besides having the potential to produce various economic goods, aren is superior in soil and water conservation functions. The shallow and widening roots of aren help prevent soil erosion [7]. Furthermore, the dense palm leaves and the layer of ijuk covering the stems reduce splash erosion due to direct rainwater hitting the soil surface effectively [8].

The high economic potential of aren has made the plant popular and cultivated by farmers. The popularity of aren is supported by fluctuations in the prices of palm oil and rubber as the primary plantation commodities in Indonesia. Due to the relatively stable prices, many oil palm farmers have planted aren between the oil palm plants to increase the economic value of the land [9]. However, the high public interest in cultivating aren is still experiencing problems with the scarcity of high-quality seedlings. One of the significant obstacles to germinating aren is the nature of seed dormancy, which is hard to break. Therefore, it becomes an obstacle to providing adequate quality and quantity aren seedlings.

Aren seeds are characterized by a hard, impermeable seed coat, which restricts water and oxygen penetration, especially when the seed moisture content drops below approximately 14% during the final ripening stage [10, 11]. The high lignin content, coupled with potassium oxalate crystals, exacerbates the seed coat's impermeability, leading to physical dormancy [12]. Furthermore, the seeds experience physiological dormancy due to the imbalance of germination-stimulating and inhibiting compounds and the heightened calcium oxalate content in mature fruit [13]. Without pretreatment, aren seeds typically germinate 5-6 months post-sowing, and in nature, this process extends to a year [14]. Consequently, effective dormancy-breaking techniques are crucial for ensuring the availability of high-quality aren seedlings in sufficient quantities.

Several dormancy-breaking techniques have been tested on aren seeds, both physically, chemically, and by administering growth-stimulating hormones. However, until now, an effective method has not been found. Scraping the seed coat using sandpaper; immersion in hot water with an initial temperature of 60°C; immersion in 3% H_2SO_4 solution; immersion in 3% KNO_3 solution; and immersion in 3% HCl still resulted in a low percentage of germinated aren seeds, i.e., 16.8%, 14.4%, 15.2%, 16.0%, and 14.4%, respectively [15]. The treatment of germination media using sand mixed with rice husk and combined with the 4-sided cutting of seeds near the operculum, round shape thinking right at the operculum; scraping the operculum using sandpaper; only resulted in the percentage of germination respectively 53.25%; 48.55%; and 46.25% [16]. Soaking seeds in warm water with an initial

temperature of 25℃ and 50℃ for 24 hours did not increase the germination percentage, germination speed, and germination power of aren seeds. On the other hand, soaking the seeds in warm water with an initial temperature of 75℃ for the same duration decreased the germination percentage and the germination rate of aren [17]. The scraping treatment combined with immersion in 1% NaOCl solution, GA3 solution 100 ppm, and H_2SO_4 75% resulted in the percentage of germination of aren seeds of 48%, 40%, and 0%, respectively [18]. In addition, using natural materials for soaking the seeds has not been able to break the dormancy of aren seeds. Soaking in 1% acetic acid solution, 100% aren sap, and 100% coconut water could not increase the germination percentage and accelerate the germination rate of aren [19].

In nature, germination of aren seeds is accelerated thanks to the help of the civet (*Paradoxurus hermaphrodites*) digestion. The civet consumes the ripe fruit, digests it, and secretes the seeds in its feces. Generally, Seeds in civet feces have a higher percentage of germination than those harvested directly from plants [20]. It is thought to be related to mechanical processes, enzymatic and microorganism activity in the civet digestive organs, which positively impact aren seed germination. In general, the microorganisms present in the feces are *Nitrosococcus* spp., *Pseudomonas striata, Nitrosomonas* spp., *Pseudomonas fluorescens* spp., *Streptomyces* spp., and *Trichoderma* spp. [21]. Cattle feces also contain hormones such as creatin, indole acetic acid, and auxin which could stimulate root growth [22].

The study was designed to adopt the processes in the civet digestion to break the aren seeds' dormancy by utilizing the activities of microorganisms and enzymatic processes found in ruminant feces. Until now, no scientific report has been related to the dormancy breaking of aren seeds through fruit fermentation utilizing microorganisms in cow dung. Through the process, it is expected that fungi and bacteria will be able to grow and degrade lignin, which is the main compound of aren seed coat. *Pseudomonas* and *actinomycetes* are gram-positive bacteria that effectively degrade lignin [23]. *Pseudomonas* is a bacterium that can be found in feces. The type of feces selected for the study was the cow dung (*Bos javanicus indicus*) of the Bragus variety, fed the natural food of weeds. Cow dung was chosen for several reasons, including being easy to prepare, having a soft texture to mix easily with aren fruit, and having a high water content to speed up the fermentation process. In addition to these reasons, cow dung is reported to contain many microorganisms that help digest grass which contains 15%-25% lignin [24]. Lignin is the compound responsible for the impermeable feature of aren seed coat. Bacterial enzymes generally catalyze the separation of various bonds in lignin, oxidation, demethylation, and cleavage of aromatic rings [23]. Cow dung contains *Lactobacillus* sp. and *Actinomycetes* sp. [25]. Those microorganisms are thought to produce phytohormones which play a role in spurring plant growth. These hormones include auxin, gibberellins, and cytokinins [15], which the embryos of aren can utilize to stimulate germination.

Preliminary treatment through fermentation by utilizing microorganism activity in cow dung has the potential to break the dormancy of aren seeds. This dormancy-breaking method is expected to be more effective when combined with scraping treatment (deoperculation) right on the operculum of the aren seeds after fermentation. Scratches on the operculum due to deoperculation will allow the imbibition process, an essential stage in seed germination. It is responsible for enzyme activation, breaking down starch into sugars, and transporting nutrients to the developing embryo [26]. Breaking physical dormancy through the destruction or release of water-inhibiting structures will signal seeds to start the germination process immediately [27]. The study's objective was to obtain the effectiveness of the fermentation and deoperculation methods, both as single and combination treatments, in breaking the dormancy of aren seeds.

2. METHOD

The research was conducted in the Faculty of Agriculture, University of Lampung greenhouse, from January to April 2021. The study was designed in a factorial, completely randomized design (CRD) with two treatments being tested: duration of fermentation and deoperculation. There were four levels of fermentation duration, namely 0 weeks (F0), two weeks (F1), four weeks (F2), and six weeks (F3), as well as two levels of deoperculation, namely without deoperculation (D0) and with deoperculation (D1).

Figure 1. (a) Physiologically ripe aren fruit, (b) Cow dung of Bragus variety which is fed with weeds, (c) Aren fruit fermentation, and (d) Deoperculated aren seed

The research implementation begins with the preparation of aren fruit. The selected palm fruit is physiologically ripe with brownish yellow colour and soft flesh texture Figure 1(a). Furthermore, the fruit is selected to acquire a uniform size

(diameter 4-5 cm) and defect-free. The next step was the cow dung preparation (Figure 1(b)). The selected cow was the type of Bragus variety that was fed with weeds. The cow dung used was the fresh dung taken on the same day when fermentation began. Therefore, the dung still contained much energy for the microorganisms to grow.

The fermentation begins by digging a hole in the ground, measuring 60 cm × 60 cm with a depth of 50 cm. Before being put into the fermentation pit, the aren fruit was stirred with cow dung with a volume ratio of 1:1. Fifty liters of fruit and cow dung mixture was then put into each fermentation pit Figure 1(c). The mixture was then covered with soil and watered regularly once a day in the morning. After getting fermentation treatment, the seeds were extracted by separating them from the remains fruit and cow dung flesh. The seeds were then selected to obtain those uniform sizes and defects-free. After being fermented, deoperculation of seeds was then conducted by scraping the seed on the operculum until the color turned whitish brown Figure 1(d). Scraping was done using sandpaper with a particle size of 190-256 micrometers (FEPA No P 80).

Germination of seeds was carried out in fine sand media (passed a 2.0 mm sieve), which was placed in a germination tub. Germination was performed by immersing the aren seeds to a depth of 3 cm with a distance between seeds of 3 cm × 3 cm. The germination media was watered every morning using a hand sprayer to keep the media moist.

The data collected in the study were the number of germinated seeds from the first day to the 60th day after sowing and the number of seeds that still had the potential to germinate (vigor) on the last day of observation. The parameters observed in the study were the percentage of germination (%G), average germination time (AGT), germination value (GV), and seed viability (SV) [28].

$$\%GT = \frac{A}{B} \times 100\% \quad (1)$$

where, A is the number of seeds germinated, and B is the number of seeds sown.

$$AGT = \frac{(N1 \times H1) + (N2 \times H2) + \ldots (Ni \times Hi)}{(N1 + N2 + \ldots Ni)} \quad (2)$$

where, N is the number of germinated seeds on the day i, and H is the number of days in the seed germination process.

$$GV = PV \times ADG \quad (3)$$

where, PV is the peak value, and ADG is the average daily germination value.

$$SV = \frac{\Sigma GS + \Sigma VNG}{\Sigma \text{ sown seed}} \times 100\% \quad (4)$$

where, SV is seed viability, GS is the germinated seed, and VNG is the viable, not germinated seed.

Data were analyzed through three stages of testing, namely the homogeneity test using the Bartlet Test, analysis of variance, and test of significant difference between treatment mean using the least significant difference test (LSD). All tests were carried out at the 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Effectiveness of fermentation and deoperculation methods and combination of the two treatments to break dormancy of aren seed

Bartlet test results show that count X^2 (5.219) is smaller than table X^2 (14.067), indicating that the data is categorized as homogeneous. The results of the ANOVA test showed that there was at least one treatment of fermentation duration as a single treatment which had a significant effect on the percentage of germination, germination value, and average germination time. However, fermentation as a single treatment did not significantly affect seed germination. Slightly different, deoperculation as the single treatment significantly affected the germination and germination value percentage. However, it had no significant effect on the average germination time and seed viability of aren seeds. The combined treatment of fermentation and deoperculation showed a very significant effect on all observed parameters. The results of the ANOVA test on the effectiveness of the fermentation and deoperculation treatments to break aren seeds dormancy are presented in Table 1.

Table 1. Analysis of variance in the effectiveness of the fermentation and deoperculation treatments to break aren seeds' dormancy

Source of Variety	Parameter			
	%G	AGT	GV	SV
Fermentation	**	*	**	ns
Deoperculation	ns	ns	**	ns
Interaction	**	**	**	**

Notes: ns=not significant, * =significant (significant level 5%), ** =very significant (p=1%)

As a single treatment, 4-week and 6-week fermentation treatments have increased the germination percentage of aren seeds. However, deoperculation as a single treatment did not affect germination percentage. On the other hand, fermentation combined with deoperculation has been shown to increase the percentage of germination. The best treatment to increase germination percentage was fermentation for four weeks combined with deoperculation and fermentation for six weeks with or without deoperculation. The fermentation and deoperculation, as a single or combination treatment, were proven to shorten the average germination time. All treatments given were equally effective in accelerating seed germination. The treatment of fermentation and deoperculation as a single treatment did not increase the germination value of the seed. However, the 4-week and 6-week fermentation treatment combined with deoperculation increased the germination value. The two combined treatments were equally effective in increasing the germination value of aren seed. Fermentation and deoperculation given either as a single or combination treatment did not increase the seed viability of aren seed. The results of the LSD test on the effectiveness of fermentation, deoperculation, and the combination of both treatments to break the dormancy of aren seeds are presented in Table 2.

Table 2. The LSD test on the effectiveness of fermentation, deoperculation, and the combination of both treatments to break the dormancy of aren seeds

Parameter	Deoperculation	Duration of Fermentation			
		P0	P1	P2	P3
%G	D0	0.74f	28.89def	40.00bcd	64.44ab
	D1	4.44ef	28.89cdef	75.56a	82.22a
AGT (day)	D0	60.00b	32.31a	33.78a	32.69a
	D1	29.33a	30.60a	30.18 a	33.88a
GV	D0	0.01g	0.21efg	0.58cdefg	1.06bcdefg
	D1	0.01fg	0.39defg	1.66abcd	2.27ab
SV (%)	D0	88.89ab	86.66ab	75.55b	77.77ab
	D1	88.88ab	86.66ab	84.44ab	97.78a

Notes: LSD value: % G=33.86, AGT=22.34, GV=1.30, and SV=20.13

3.2 Best method to break aren seed dormancy

Based on the LSD test results, it is known that 4-week and 6-week fermentation treatments with or without deoperculation were the best treatments to break aren seed dormancy. These treatments resulted in the same effectiveness for all observed parameters. However, the 6-week fermentation combined with deoperculation gave a better result for all parameters than the other two. A comparison of the results of fermentation and deoperculation treatment of all observed parameters is presented in Figure 2.

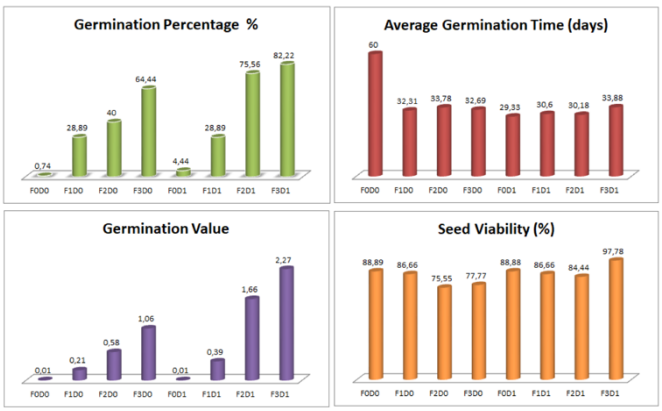

Figure 2. Chart of LSD test results on the effectiveness of fermentation, deoperculation, and combination of both treatments to break the dormancy of aren seeds

3.3 Discussion

The visual appearance of aren seed after fermentation treatment and deoperculation is shown in Figure 3. Deoperculation as a single treatment did not affect the germination percentage of aren seeds. It differs from the result tested by the study [29] deoperculation improved the germination percentage by 65% 90 days after the seeds were sown. In this study, deoperculation only resulted in a germination percentage of 4.4% 60 days after the seeds were sown. The low germination percentage of single deoperculation treatment indicated that the dormancy in aren seeds was not only a physical dormancy caused by the seed coat's hardness, thickness, and impermeable structure. Deoperculation creates gaps in the seed coat, making it easier for water to enter the embryo sac (*imbibition*) [30]. Seeds treated with deoperculation by scraping the seed coat had a higher imbibition rate than those not treated [31]. In physical dormancy, destroying or releasing water-inhibiting structures will signal seeds to start germination immediately [32]. Once water-inhibiting structures are open, seeds can absorb water quickly and germinate in various conditions. Imbibition is the initial stage of the germination process due to the protoplasm's softened seed coat and hydration [33]. The perfect imbibition process will increase secondary metabolic activity to maintain seed viability and germination [34].

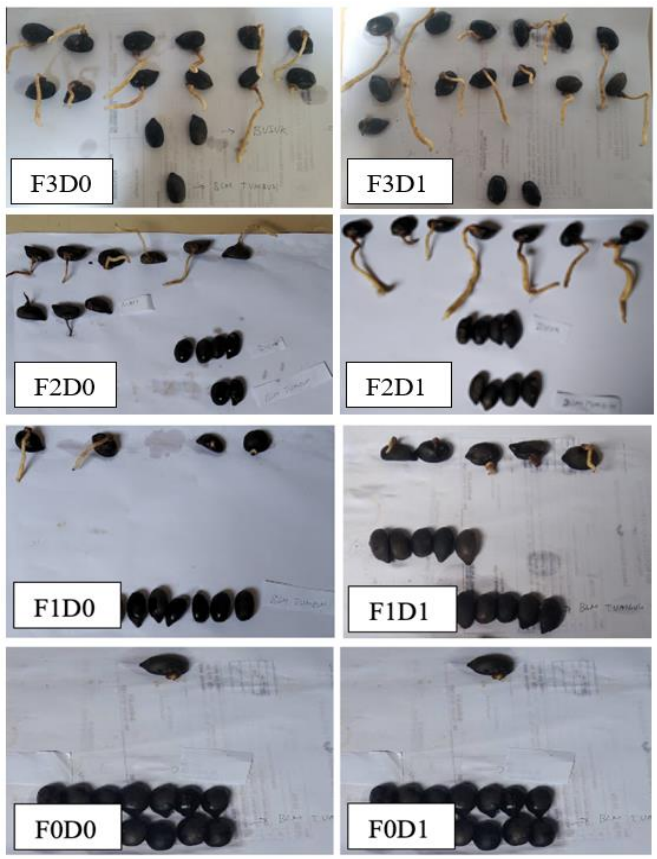

Figure 3. The treatment of fermentation duration, deoperculation, and a combination of both and their effect on aren seed germination

Apart from the thick, rigid, and impermeable seed coat, the dormancy of the aren seeds is also thought to be triggered by an imbalance of stimulant and inhibitor compounds that affect seed germination [12]. Seed dormancy can be caused by the impermeability of the seed coat to water and gas (oxygen), embryos that have not yet fully grown, mechanical resistance of the seed coat to embryo growth, growth regulators that have not yet been formed, or due to an imbalance between stimulant and inhibitors substances in the embryo [35]. Apart from the thickness of the seed coat, another thing that is thought to be

the cause of the aren seeds dormancy is the presence of calcium oxalate in the ripe fruit [36].

Crystals of calcium oxalate (CaOx) are plant ergastic objects produced from calcium (Ca) bonds with oxalic acid compounds ($C_2H_2O_4$) [37]. Calcium oxalate crystals are a waste product of cell metabolism that plants no longer use. The abundance of inactive oxalic acid in plants serves to help plants from excess calcium ions so that calcium oxalate crystals form [38]. Calcium oxalate is naturally used to protect plants against pests since this substance is irritating and causes a burning and itching sensation for most organisms [37]. Larvae of lepidopteran species (*Polytella clines*) and land snails (*Eremina desertorum*) avoid feeding on the tissue of negev desert lily (*Pancratium sickenbergeri*) since it contains calcium oxalate [39].

The aren seed coat is composed of lignified palisade cells. The lignin content in the seed coat increases as the seed gets riper, which is thought to play a role in increasing the impermeability of the seed [40]. The palisade cells of the seed coat are highly lignified and contain deposits of tannins and calcium oxalate crystals [41]. Calcium oxalate crystals are thought to increase the impermeability properties of the aren seed coat since this material is in the form of crystals and can crowd the cavities between the cells of the seed coat, making it even more impermeable [38]. Calcium oxalate is an insoluble compound in water but in acidic solutions. The lower the pH of the solution, the easier it is to convert water-insoluble calcium oxalate compounds into water-soluble oxalic acid compounds [38]. The HCl solution is one of the acids that can reduce calcium oxalate compounds in aren seeds [42]. A study proved that aren seeds treated with HCl with a concentration of 0.3% had the fastest germination time of 49 days, and the highest percentage of normal germination was 95.83% [43].

Fermentation of aren fruit has been proven effective in breaking the dormancy of seeds, both as a single treatment and combined with deoperculation. It is thought to occur due to the enzymatic activity of the fungi and bacteria during the fermentation process, which positively impacts the germination of aren seeds. In nature, bacteria and fungi are involved in the degradation and modification of lignin. Several microorganisms, including bacteria and fungi, can degrade lignin and recycle its carbon content [8]. Many bacterial and fungal strains can metabolize lignin compounds, and some mineralize and dissolve lignin polymers. Bacterial enzymes catalyze the separation of various bonds in lignin, oxidation, demethylation, and cleavage of aromatic rings [23]. Lignin degradation by bacteria can occur under aerobic and anaerobic conditions [44].

The progress during the fermentation process allows fungi and bacteria to degrade lignin, an essential compound of aren seed coat responsible for impermeability. *Pseudomonas* and *Actinomycetes* are gram-positive bacteria effective in degrading lignin [23]. *Pseudomonas* is a bacterium that is abundant in the feces.

Feces contain a large number of bacteria *Nitrosococcus* spp., *Pseudomonas striata*, *Nitrosomonas* spp., *Pseudomonas fluorescens* spp., *Streptomyces* spp., and *Trichoderma* spp. [21] *lactobacillus*, fungi, and yeasts such as *Saccharomyces* [45], as well as *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus, Bacillus subtilis, Enterococcus diacetylactis*, *Bifido*bacteria and yeast (*Saccharomyces cerevisiae*) in the lower part of cow intestine [46]. These results indicate that fungi and bacteria derived from cow digestion effectively degrade lignin. Cows consume grass that contains 15%-25% lignin [24]; therefore, fungi and bacteria isolated from the cows' digestion can degrade lignin. Bacteria isolated from habitats such as soil containing degraded lignin of plant material or from anaerobic ecosystems in sediments and in the rumen where lignocellulosic material is degraded are expected to metabolize lignin and its derivative compounds [23].

The effectiveness of the fermentation treatment on the parameters of aren germination is also thought to be supported by the presence of hormones found in cow feces. Cattle feces contain hormones such as creatin, indole acetic acid, and auxin, which can stimulate root growth [22]. Auxin (IAA) and Gibberellins (GA) are hormones that are very important in initiating germination. Auxin can break seed dormancy, stimulate germination, and increase crop quantity [47]. In addition, auxin can better stimulate the formation and growth of roots. Auxin helps accelerate the breaking of rubber seed dormancy [48]. In addition, it increases embryonic cell division and elongation after an overhaul of nutrition reserves in the endosperm so that seeds germinate more quickly [45]. While indole-3-acetic acid (IAA) has a role in cell enlargement, division of cambium cells, and stem development [49]. However, these hormones will only be effective after being able to enter the core of the seed. It was indicated by the fermentation treatment for six weeks, which resulted in the best germination, with or without deopercolation. With the long fermentation time, the enzymatic activity of fungi and bacteria has occurred to degrade lignocellulose and make the seed coat permeable. It allows hormones to enter the core of the seed along with water which then plays a role in stimulating seed germination [30].

4. CONCLUSION

The treatment of 4-week fermentation combined with deopercolation, and 6-week fermentation treatment with or without deopercolation, was significantly proven to increase the germination percentage and germination value, accelerate the seeds germination, and not reduce the seed viability of aren. Those three treatments have equal value in all observed parameters of seed germination. However, for practical reasons, a 6-week fermentation treatment without deopercolation would be more efficient and effective for large-scale nurseries. The limitation of this study is the maximum fermentation duration which was only six weeks. It is possible that if the duration were extended, it would increase germination, especially in the germination percentage. Therefore, it is essential to carry out further research, with the addition of the duration of fermentation. Feces from other ruminants, such as buffalo, goats, and sheep, can also be tested as a source of starter microorganisms in aren fermentation. It is essential to accommodate the aren nurseries where cows are not cultivated.

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