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Effect of citric acid from *Citrus aurantiifolia* as a coagulant on natural rubber properties

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Abstract The results revealed that the ash content and nitrogen content of the natural rubber coagulated with 8% (w/v) citric acid were the lowest compared to natural rubber coagulated with 10% (w/v) and 12% (w/v) citric acid, and they were not significantly different from natural rubber coagulated with the standard coagulant, 2% (v/v) formic acid. The plasticity retention index of natural rubber coagulated with citric acid, regardless of the concentration, did not differ significantly from that of natural rubber coagulated with the standard coagulant. No significant differences were observed in dirt content and volatile matter among natural rubber coagulated with any of the tested coagulants, suggesting that the various coagulants had no effect on dirt content and volatile matter. The 8% (w/v) citric acid derived from *Citrus aurantiifolia* proves to be a comparable alternative coagulant for natural rubber, as it is equally effective, matching the performance of the standard coagulant.

Keywords: Ash content, *Hevea brasiliensis,* Latex coagulant, Nitrogen content, Plasticity retention index

Introduction

Natural rubber (cis-1,4-polyisoprene) is an essential material used in the manufacture of different products such as gloves, shoe soles, conveyor belts, automobile tires, and more. It is derived from the latex sap of the rubber tree (*Hevea brasiliensis*), commonly referred to as natural rubber latex (NRL) (Greve, 2000), and has been a significant export product for many countries, especially those in tropical areas (Orsuwan *et al*., 2023). Rubber tree is a major industrial crop in the Philippines (Philippine Statistics Authority, 2023), and the country is recognized as one of the rubber-producing nations, frequently ranks among the top ten global exporters of raw or semi-processed rubber (Daly *et al*., 2017).

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The Philippines exports natural rubber in primary forms, including raw cup lumps and semi-processed rubber products (Department of Trade and Industry, 2018). Raw cup lump is the unprocessed NRL that coagulates after being left in the tapper's cup after tapping (Daly *et al*., 2017; Orsuwan *et al*., 2023), a process known as natural coagulation, which is a type of coagulation technique associated with the activities of bacteria and coagulation enzymes present in NRL, initiating a sequence of biochemical reactions (Altman, 1947; Ng *et al*., 2022). Technically Specified Rubber (TSR), also locally designated as Standard Philippine Rubber (SPR), is one of the semi-processed rubbers that the Philippines has been exporting (Daly *et al*., 2017). The starting material for producing TSR is NRL, which is coagulated using a specific coagulant to form a coagulum. It then undergoes a series of processes, including granulation, drying, and baling. Alternatively, cup lumps also serve as the starting material, undergoing the same series of processes (DOST-PCAARRD, 2014; Daly *et al*., 2017; Orsuwan *et al*., 2023). The specific application of TSR depends on its rubber grade, which is determined by its properties such as ash content, dirt content, nitrogen content, plasticity retention index (PRI), and volatile matter (DOST-PCAARRD, 2014; Daly *et al*., 2017; Ng *et al*., 2022).

The quality of TSR, defined with a technical specification of its properties, is influenced by the quality of the starting material (Wisunthorn *et al*., 2015; Ng *et al*., 2022), with coagulation being a critical post-harvest activity that significantly affects the quality of this starting material (Chambon *et al*., 2017; Nepacina *et al*., 2019; Ng *et al*., 2022). Natural coagulation, the common technique practiced by farmers, is less efficient due to its slower coagulation process. Furthermore, cup lumps are of low quality and can only be used in manufacturing after undergoing several purification steps (Ferreira *et al*., 2005; Prasertsit *et al*., 2011). As a result, natural coagulation has gradually been replaced by coagulation using acids in most of the rubber-producing regions (Ng *et al*., 2022). Formic acid is the preferred coagulant for natural rubber production, known for its remarkable effectiveness in consistently delivering high-quality rubber products (Hietala *et al*., 2016; Oktriyedi *et al*., 2021). Nevertheless, due to the highly toxic nature of formic acid, specifically its corrosive effect on the skin, farmers and rubber workers are particularly vulnerable to accidents involving formic acid (Hietala *et al*., 2016).

In the Philippines, formic acid is the standard coagulant (PNS/BAFS 286:2019). This preference aims to ensure that the coagulum produced by predominantly smallholder rubber growers is consistently high in quality. Processors must maintain consistency in the quality of their semi-processed rubber products, which is why the coagulum they purchase from these smallholders should also be consistently of high quality. This enables them to command competitive prices in the global market (DOST-PCAARRD, 2014; Department of Trade and Industry, 2018). However, due to logistical challenges and issues with formic acid supply, non-standard acids like vinegar-derived acetic acid and automobile battery-derived sulfuric acid have been extensively used, resulting in the sale of "non-standard coagulum" labelled as formic acid-coagulated. This fraudulent behaviour is prompted by the higher price of formic acid-coagulated coagulum as opposed to non-formic acid-coagulated coagulum (Nepacina *et al*., 2019). Additionally, the study by Othman and Chan (1980) revealed that rubbers coagulated with sulfuric acid, as measured by aged tensile properties, exhibit distinctly inferior aging behaviour in the tread mix and tend to have a 15% higher ash content compared to rubbers coagulated with formic acid. Although acetic acid has been shown to have a less negative impact on the properties of natural rubber than sulfuric acid (Ng *et al.*, 2022), it is a weaker acid and less efficient than formic acid, resulting in much higher consumption of acids (Hietala *et al*., 2016; Oktriyedi *et al*., 2021). In fact, the concentration of acetic acid used in the processing of rubber at the village level was 50% (v/v) (Castillo *et al.*, 2014). As a result, rubber processors continue to receive low prices for semi-processed rubber due to concerns about contaminated output and poor quality. Consequently, this is one of the challenges undermining the competitiveness of the Philippine rubber industry (Daly *et al*., 2017). Therefore, it is essential to find a nontoxic and accessible coagulant to improve the quality of the semi-processed natural rubber produced.

Citric acid, 2-hydroxypropane-1,2,3-tricarboxylic acid, has been utilized in various industrial applications owing to its remarkable physicochemical properties and non-toxic nature (Kirimura *et al*., 2011) and is naturally concentrated in citrus fruits like lemons and limes (Penniston *et al*., 2008). In the study of Chukwu *et al*. (2010), citric acid from lemon was used as a coagulant for natural rubber. The results revealed that natural rubber coagulated with citric acid exhibited the highest PRI values when compared to those coagulated with acetic acid and formic acid. Nevertheless, it is important to note that the property assessed in this study was solely PRI.

Thus, the objective of the study was to assess the effect of citric acid extracted from *Citrus aurantiifolia* (Christm.) Swingle, an available and lesser-utilized citrus variety locally known as *dayap*, as a coagulant on the properties of natural rubber, including ash content, dirt content, nitrogen content, PRI, and volatile matter.

Materials and methods

The study was conducted at Bibs Ramos Rubber Farm in Santa Maria, Laguna, Philippines, from October 17, 2023, to October 18, 2023. The experiment was laid out in a completely randomized design (CRD) with three replicates for each treatment. There were four treatments: three treatments with citric acid at concentrations of 8% (w/v), 10% (w/v), and 12% (w/v), and one treatment with 2% (v/v) formic acid as the control. The use of 2% (v/v) formic acid aligns with the recommended concentration specified in PNS/BAFS 286:2019, and this same concentration was employed in the studies conducted by Baimark and Niamsa (2009) as well as by Triawan *et al.* (2022).

Materials

Fresh NRL was collected from rubber trees (*Hevea brasiliensis*) commercially grown at Bibs Ramos Rubber Farm in Santa Maria, Laguna, Philippines. Its specific gravity was 0.9535. The citric acid was extracted from *Citrus aurantiifolia* (Christm.) Swingle, and the formic acid (AR grade) was obtained from Scharlau and used without further purification. Distilled water was used in preparing the treatments and during the dilution of the NRL to ensure the absence of impurities and contaminants that could affect the accuracy and consistency of the experiment.

Methods

Processing of NRL into TSR was done following the procedure of the Philippine National Standard (PNS) for Good Agricultural Practices (GAP) for Natural Rubber developed by the Bureau of Agriculture and Fisheries Standards (BAFS) or the PNS/BAFS 286:2019.

Clean collecting cups were used to collect 20,400 mL of fresh NRL before sunrise. Tapping trees before sunrise maximizes NRL dripping by taking advantage of the high turgor pressure in NRL vessels. The NRL was then bulked into a container to ensure uniformity and strained to remove impurities. Subsequently, the bulked NRL was diluted with an equal amount of distilled water. The diluted NRL was divided into 15 individual pans, each serving as an experimental unit.

The coagulation experiments were carried out by randomly assigning each of the four treatments three times within this set of 15 experimental units. Each time a treatment was added to the diluted NRL, it was stirred for 10 seconds until homogeneous, and left until coagulation was complete. The volume of each treatment added to the diluted NRL followed a 1:10 ratio, based on the rubber processing practices described in the study by Castillo *et al.* (2014). Each of the resulting slabs (coagulum) was then pressed into sheets, with the excess serum squeezed out. The formed sheets were shredded before drying in an oven at 120℃ for four hours. The dried rubber was then cooled and tested for its physical properties.

Each sample was tested for five physical properties, including, ash content (PNS ISO 247:2015) Method A, dirt content (PNS ISO 249:2015), nitrogen content (PNS ISO 1656:2007), PRI (PNS ISO 2930:2015), and volatile matter (PNS ISO 248-1:2015), at the Regional Standards and Testing Laboratories of the Department of Science and Technology – Region IX in Zamboanga City, Philippines.

Statistical analysis

The data were analysed using the Analysis of Variance (ANOVA) function in Statistical Tool for Agricultural Research (STAR) 2.0.1 software, developed by the International Rice Research Institute (IRRI). This analysis aimed to determine whether there were statistically significant differences among the means of the four treatments for properties such as ash content, dirt content, nitrogen content, PRI, and volatile matter. Additionally, mean comparisons were performed and interpreted using the Least Significant Difference (LSD) test at a 0.05 level of significance.

Results

Ash content

The ash content of natural rubber coagulated with 8% (w/v) citric acid was 0.23% (by mass), the lowest compared to that of natural rubber coagulated with other citric acid concentrations, and not significantly different from that of natural rubber coagulated with 2% (v/v) formic acid (Table 1). Additionally, as the concentration of citric acid increased from 8% (w/v) to 12% (w/v), the ash content also increased significantly ($p = 0.0038$), from 0.23% to 0.33% by mass.

Table 1. Effect of different coagulants on the ash content of natural rubber

Coagulant	Concentration	Ash content, % (by mass) [1]
Formic acid	2% (v/v)	0.20^{c}
Citric acid	8% (w/v)	0.23^{bc}
Citric acid	10% (w/v)	0.28^{ab}
Citric acid	12% (w/v)	0.33^{a}

[1]Different letters indicate statistically significant differences at p-value < 0.05

Dirt content

The dirt content of natural rubber coagulated with citric acid, regardless of the concentration, remained consistently at 0.02% by mass (Table 2). It was not found to be significantly different from that of natural rubber coagulated with 2% (v/v) formic acid at a 0.05 level of significance, suggesting that the various coagulants had no effect on the dirt content.

Table 2. Effect of different coagulants on the dirt content of natural rubber

Coagulant	Concentration	Dirt content, % (by mass) [1]
Formic acid	2% (v/v)	0.02[a]
Citric acid	8% (w/v)	0.02[a]
Citric acid	10% (w/v)	0.02[a]
Citric acid	12% (w/v)	0.02[a]

[1]Means with the same letter are not significantly different at p-value > 0.05

Nitrogen content

The nitrogen content of natural rubber coagulated with 8% (w/v) citric acid was 0.49% (by mass), the lowest among various citric acid concentrations, and not significantly different from that of natural rubber coagulated with 2% (v/v) formic acid (Table 3). Moreover, as the concentration of citric acid increased from 8% (w/v) to 12% (w/v), the nitrogen content also increased significantly ($p = 0.0299$), from 0.49% to 0.62% by mass.

Table 3. Effect of different coagulants on the nitrogen content of natural rubber

Coagulant	Concentration	Nitrogen content, % (by mass) [1]
Formic acid	2% (v/v)	0.50[b]
Citric acid	8% (w/v)	0.49[b]
Citric acid	10% (w/v)	0.54[ab]
Citric acid	12% (w/v)	0.62[a]

[1]Different letters indicate statistically significant differences at p-value < 0.05

Plasticity retention index (PRI)

The PRI of natural rubber coagulated with 10% (w/v) citric acid was 85.67, the highest among the three citric acid concentrations, and the lowest with 12% (w/v) citric acid. Additionally, these values were lower than those of natural rubber coagulated with 2% (v/v) formic acid (Table 4). However, the variation in PRI among natural rubber coagulated with different coagulants showed no statistical significance ($p = 0.2646$) at the 0.05 level of significance.

Table 4. Effect of different coagulants on PRI of natural rubber

Coagulant	Concentration	Plasticity Retention Index[1]
Formic acid	2% (v/v)	88.67[a]
Citric acid	8% (w/v)	85.33[a]
Citric acid	10% (w/v)	85.67[a]
Citric acid	12% (w/v)	82.33[a]

[1]Means with the same letter are not significantly different at p-value > 0.05

Volatile matter

The volatile matter of natural rubber coagulated with 8% (w/v) and 12% (w/v) citric acid was 0.22% by mass, which was the same as that of natural rubber coagulated with 2% (v/v) formic acid. In contrast, natural rubber coagulated with 10% (w/v) citric acid was the highest at 0.23% by mass (Table 5). However, the volatile matter content of the natural rubber coagulated with different coagulants, showed no significant differences ($p = 0.9085$). This suggests that the various coagulants had no significant effect on the volatile matter of natural rubber.

Table 5. Effect of different coagulants on volatile matter of natural rubber

Coagulant	Concentration	Volatile Matter, % (by mass)[1]
Formic acid	2% (v/v)	0.22[a]
Citric acid	8% (w/v)	0.22[a]
Citric acid	10% (w/v)	0.23[a]
Citric acid	12% (w/v)	0.22[a]

[1]Means with the same letter are not significantly different at p-value > 0.05

Discussion

Ash content is one way to estimate the mineral matter content in natural rubber. According to Greve (2000), NRL typically contains approximately 0.5% inorganic salts. The ash content of the natural rubber coagulated with the different coagulants tested in this study was below the average ash content of natural rubber, which may be attributed to the dilution process. Dilution of NRL typically leads to a decrease in Dry Rubber Content (DRC) and, consequently, may result in lower ash content (Graham, 1969; Ng *et al*., 2022). Furthermore, the average ash content values in this study are similar to the values reported in the study of Othman and Chan (1980). Moreover, the ash content requirement for the highest TSR grade should not exceed 0.60% (DOST-PCAARRD, 2014), a threshold that is met by both natural rubbers coagulated with 2% (v/v) formic acid and by those coagulated with 8% (w/v), 10% (w/v), and 12% (w/v) citric acid.

Dirt content, encompassing fine sand and bark and representing the level of insoluble dirt, and can indicate poor quality when it is high. The observed dirt content of the natural rubber coagulated with the different coagulants fell below the specified maximum allowable requirement for the highest TSR grade, which is 0.05% (DOST-PCAARRD, 2014). This noteworthy outcome can be attributed to the uniform straining process employed, where all experimental units underwent filtration using the same size strainer to effectively remove impurities. A similar study by Baimark and Niamsa (2009) also yielded comparable observations and results, suggesting that the type of coagulant does not significantly impact the dirt content of natural rubber.

The nitrogen content serves as an indicator of non-rubber components in NRL. Nitrogen occurs in natural rubber primarily as proteins, and together with lipids, proteins, carbohydrates, and minerals, they collectively constitute approximately 5-6% of the components of NRL (Ng *et al*., 2022). The nitrogen content of natural rubber coagulated with 12% (w/v) citric acid surpassed the maximum allowable requirement of 0.6% for all grades of TSR, as per DOST-PCAARRD (2014). This suggests that the concentration of citric acid as a coagulant should be kept below 12% (w/v). Moreover, a parallel trend was noted in the study by Othman and Chan (1980), where nitrogen content increased as pH levels decreased; similarly, in this study, a corresponding increase in nitrogen content was found with increasing concentration of citric acid.

The PRI serves as a measure of raw natural rubber's resistance to oxidation (Baker, 1980), with a higher PRI value indicating greater resistance and, consequently, higher quality (Chukwu *et al*., 2010). Natural rubber coagulated with citric acid at 12% (w/v) produced the lowest value, as compared to citric acid at 8% (w/v) and 10% (w/v). This trend aligns with the findings of Othman and Chan (1980), where an increase in concentration led to a decrease in PRI. Moreover, these values have surpassed the minimum PRI requirement of 60 for the highest-grade TSR, as stipulated by DOST-PCAARRD (2014).

The dryness of rubber is indicated by its volatile matter, with a high value suggesting incomplete drying. Additionally, the source of rubber is another factor influencing volatile matter, where the presence of hydrophilic materials such as fatty acids, as well as ammoniated latices, latex concentrates, and skim latices, contributes to moisture retention, resulting in elevated volatile matter values (DOST-PCAARRD, 2014). The lack of significant differences in the volatile matter content among natural rubbers coagulated with different coagulants may be attributed to the fact that all samples underwent the same drying process and originated from the same source of NRL. Additionally, similar results were observed in the study by Baimark and Niamsa (2009), indicating that the type of coagulant does not affect the volatile matter of natural rubber. Furthermore, the volatile matter values fell within the requirements of DOST-PCAARRD (2014), remaining below the maximum threshold of 0.8% for volatile matter in all grades of TSR.

In summary, citric acid derived from *Citrus aurantiifolia*, an available and not extensively utilized citrus fruit in the Philippines, proves to be a comparable alternative coagulant for natural rubber when kept below 12% (w/v). Conclusively, the study suggests that an 8% (w/v) concentration of citric acid is sufficient to minimize consumption, as it proves equally effective, matching the performance of the standard coagulant (2% v/v formic acid) in crucial parameters such as ash content, dirt content, nitrogen content, plasticity retention index, and volatile matter for grading semi-processed natural rubber. Citric acid is safer than formic acid and more accessible to rubber farmers. Additionally, citrus fruits are a good intercrop for the rubber agroforestry system (DOST-PCAARRD, 2014). This is because they not only provide an additional source of citric acid but also, as pointed out by Chhiev and Jongrungrot (2021), intercropping rubber trees with upper canopies and plants such as citrus fruits with lower canopies helps prevent soil erosion.

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