

Pineapple juice drink: potential functional drink for reducing inflammation in athletes

*Wan Nur Zahidah W.Z., Mohd Nazrul Hisyam D., Syahida M., Hadijah H. and Norhida Arnieza M.

Food Science and Technology Research Center, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

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Abstract

Bromelain is well known as an anti-inflammatory compound derived from pineapple fruit and stem extract. This study investigated bromelain content in raw pineapple with different maturity indexes and also in pineapple juice drinks. Pineapple juice drink was produced by mixing fresh juice with other ingredients, homogenized and pasteurized. Bromelain content in raw and pineapple juice drinks was analyzed by using HPLC. The total soluble solids and pH values were also determined in the pineapple juice drink. This study also investigated the potential of pineapple juice drink as an anti-inflammatory drink using carrageen-induced paw edema in rat model. Bromelain content in raw pineapple showed that pineapple with index 4 contained significantly high ($p < 0.05$) bromelain compared to the others. The bromelain content in 200 mL of product is 106.33 ± 0.14 mg/ml and significantly ($p < 0.05$) reduced by 50% after 12 months of storage. The drink has a pH of 3.54 and 15°Brix. This study also revealed that bromelain in doses of 6.6 mL/kg showed 15% inhibition of paw edema at the end of three hrs. These studies suggested that bromelain does have anti-inflammatory properties which benefited athletes, especially for recovery purposes.

1. Introduction

Pineapple with the scientific name *Ananas Comosus (L) Merr (Bromeliace)* is one of the most promising demands of fruit in the local and export markets in Malaysia and out of 100 % pineapple produced, 70 % of it consumed as fresh fruit. Pineapple belongs to the bromeliad family, which consists of 50 genera and about 2,500 known species. In Malaysia, the popular varieties of pineapple include MD2, Moris, Jospine, and N36. Most of them are planted in Johor, where it ranked first in terms of production followed by Sarawak, Sabah, Kedah, Selangor, Negeri Sembilan, Pahang and Terengganu (Nurul Hidayah and Fazleen, 2019). The MD2 variety has been identified as a key crop under the National Key Economic Area (NKEA) of the Economic Transformation Program (ETP). Compared to other varieties, MD2 is better in several qualities and has great potential in the commercial market. They have uniform bright colour, and sweeter taste, are four times higher in vitamin C content, high in bioactive compounds, antioxidant activity, lower fibre, lower acidity, thinner skin, and smaller fruits at an average of 1.5 kg each (Thalip *et al.*, 2015).

Pineapple juice is a famous product in the market due to its delicious taste, pleasant aroma, flavour, and numerous functional properties. It is considered a functional drink due to its health-promoting properties such as anti-inflammatory, anti-ageing, and many other healing properties (Nouman *et al.*, 2016). Bromelain is well known as an anti-inflammatory compound derived from pineapple fruit and stem extract. The anti-inflammatory response is believed to have a great benefit during the recovery stage of athletes after performing strenuous physical activity on a regular basis (Scheffer and Latini, 2020).

Excessive pain after exercise and the body not healing as quickly as you would like can be a major problem for athletes. It can be a major barrier to subsequent training. Bromelain is a functional enzyme that has been used and proven to be a natural medicine for controlling inflammation. Bromelain had great potential in therapeutic value including an anti-inflammatory effect due to its biochemical and pharmacological properties. The main ingredient in crude bromelain is a proteolytic enzyme termed glycoprotein. Other than that, is an insoluble material,

*Corresponding author.

Email: zahidah@mardi.gov.my

such as minerals, coloured pigments, protease inhibitors, organic acids and organic solvents. Eight proteolytically active components have been isolated from bromelain (Vidhya *et al.*, 2016). Proteinases are considered to be the most active fraction, which comprises 2% of the total proteins. It was demonstrated that the majority of the physiological activity of bromelain may not be due to a single proteolytic fraction, and it is likely that the beneficial effects of bromelain are due to multiple factors (Vidhya *et al.*, 2016). Bromelain has not only been used to treat various health problems but it is also known as a nutritional supplement. Bromelain is absorbed into the human intestines and remains biologically active with a half-life of about 6 to 9 hrs. The highest concentration of bromelain was identified in the blood one hour after administration (Bhattacharyya, 2008). Therefore, in this study, the newly formulated pineapple juice drink from MD2 pineapple and other natural substances was developed based on the recommendation of taking bromelain to aid in the recovery of athletes.

2. Materials and methods

2.1 Raw materials

MD2 pineapple variety was obtained from Ayer Hitam Plantation Johor. The collected samples were sorted into 3 different maturity indexes 4, 5 and 6. They were harvested in the morning according to the visual peel colour set up by Federal Agricultural Marketing Authority (FAMA) whereby the requirements for pineapple are referred: maturity 4; with 25% of fruits becoming yellowish in colour, maturity 5; with 50% of fruits are yellowish, while for maturity 6; almost 75% of fruits are yellowish and the pulp is squishy. The fruits were cleaned and peeled. Then the pulp was crushed using an industrial juicer (Boochii, Denmark) before being filtered using High-Speed Bean Grinding and Separating Machine (Santos, France).

2.2 Pineapple juice preparation and product development

Figure 1 shows the step of producing a pineapple juice drink. Pineapple juice drink was produced by mixing MD2 fresh juice extract with other ingredients (lime juice, kelulut honey, stabilizer and preservatives), homogenized and pasteurized at 80°C for 10 mins. Grade C MD2 pineapple is recommended in this process since it is more economical for production, while grades A and B are more expensive and are being used for export purposes. The pasteurized juices were filled into the amber glass bottle, capped properly and stored separately at room temperature (27°C) and chill temperature (4°C) for further analysis.

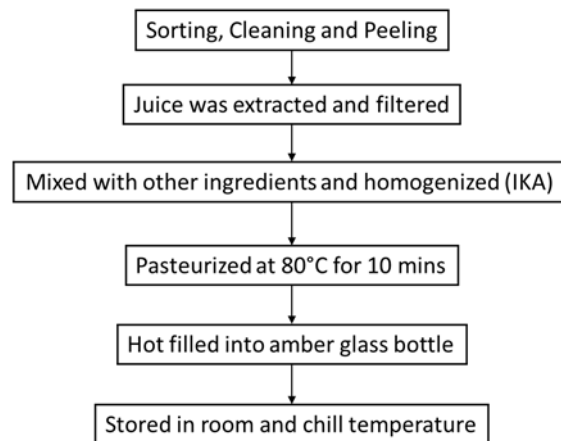


Figure 1. Processing of pineapple juice drinks

2.3 Determination of bromelain content

Bromelain content in raw pineapple and pineapple juice drinks was analyzed using a Waters HPLC system (Milford, MA, USA) consisting of a pump and system controller (Model 2695) and photo-diode array detector (Model 966). Bromelain separation was done by a reversed-phase column (4.6 × 150 mm, 4 μm; Phenomenex, Torrance, CA, USA). The mobile phase comprised 2% acetic acid: 30% acetonitrile: 68% distilled water in an isocratic program. The mobile phase was delivered at a rate of 0.7 mL/min, and samples (20 μL) were introduced into the column using an autosampler. The detection was monitored at 321 nm and data were integrated by Empower 2 software (Waters) (Milford, MA, USA).

2.4 Total soluble solid content and pH

The total soluble solid content (Brix) in the pineapple juice drink was determined using a refractometric method, measured with an Abbe refractometer (Atago, Japan). The refractometer was standardised with distilled water at 20°C. Two drops of juice at 20°C then dropped on the lens (sensitive surface) of the refractometer and measured (AOAC, 2004). The pH value was determined using AOAC, (2004) procedure. Pineapple juice drink was poured into a beaker and the pH probe of the pH meter (Metrohm, Herisau, Switzerland) was inserted into it after the pH meter has been standardised using buffer 4 and 7 solutions at 25°C.

2.5 Anti-Inflammatory Effect using carrageenan-induced paw edema

Anti-inflammatory activities were determined by carrageenan-induced edema test in the hind paws of rats according to Shankar *et al.* (2012) with some modification. Animals were fasted for 24 hrs before starting the experiment with free access to water. Rats were allocated randomly into 3 groups (n = 6); (a)

control negative (distilled water) (b) control positive (Ibuprofen 20 mg/kg BW) and (c) pineapple juice (6.6 mL/kg). Approximately 1 ml of a 1% suspension of Carrageenan (Sigma Aldrich, Darmstadt, Germany) in normal saline was freshly prepared before the experiment and was injected into the plantar side of the right hind paws of the rats. Treatments were given to the rats 1 hr before the carrageenan injection. The paw thickness was measured before the experiment begin (0hr) and every 1 hr interval until 3 hrs.

2.6 Statistical analysis

The collected data were statistically analyzed using a one-way analysis of variance (ANOVA). The values obtained at $p < 0.05$ were considered significant. The SAS software suite was used to analyze the data.

3. Results and discussion

Table 1 shows the bromelain content in three different maturity indexes of MD2 pineapple. Results showed that index 4 was higher in bromelain content and was significantly ($p < 0.05$) different from other indexes. Table 2 shows the total soluble solids and pH in three different maturity indexes of MD2 pineapple. The results from Table 1 are in agreement with the finding from Pang *et al.* (2020) whereby unripe fruits had higher proteolytic activity than ripe fruits (higher index). Fruit bromelain is a proteolytic enzyme harbouring cysteine catalytic residue found abundantly in pineapple fruit. The expression of cysteine proteases is usually regulated during fruit ripening.

Table 1. Bromelain content in raw pineapple juice

Raw Pineapple juice index	Bromelain content unit (mg/100 mL)
4	259.9±0.5 ^a
5	127.4±0.3 ^b
6	123.1±0.6 ^b

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p < 0.05$).

Table 2. Total soluble solids, pH and colour analysis in raw pineapple juice

	Juice Index 4	Juice Index 5	Juice Index 6
°Brix	14.05±0.91 ^b	14.65±0.35 ^b	15.65±0.21 ^a
pH	3.92±0.06 ^a	4.01±0.04 ^a	3.99±0.03 ^a

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p < 0.05$).

A total soluble solid (TSS) is one of the most important quality factors for most fruits. A TSS value between 13.8 to 17.0% for pineapple indicates the highest quality during the harvesting stage (Kamol *et al.*,

2014). The TSS value increased as the maturity stages of fruits increased. From Table 2, there was no significant difference in pH values among the 3 ripening stages.

The bromelain content in pineapple juice drink during 12 months of storage in both room and chill temperature was shown in Table 3. The bromelain content in 200 mL of product is significantly reduced ($p < 0.05$) by 50% after 12 months of storage. Storage time and temperature do affect the degradation of bromelain contents. It has been proven that increasing temperature and storage time causes a general decrease in content for a bromelain compound according to Abdul Majid *et al.* (2008)

Table 3. Total bromelain content in pineapple juice drink during storage.

Storage period (month)	Storage conditions	Total bromelain content (mg/100 mL)
0	Room	106.33±0.14 ^c
	Chill	161.74±0.6 ^a
2	Room	105.19±0.2 ^c
	Chill	160.20±0.3 ^a
4	Room	91.53±0.3 ^d
	Chill	155.51±0.2 ^a
6	Room	84.0±0.2 ^d
	Chill	123.9±0.3 ^b
8	Room	61.14±0.9 ^c
	Chill	101.9±1.23 ^c
10	Room	42.36±1.15 ^f
	Chill	82.34±1.0 ^d
12	Room	41.17±1.06 ^f
	Chill	83.51±0.4 ^d

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p < 0.05$).

From a previous literature review, it appeared that the quality of pineapple juice is largely influenced by the technology used during processing (Nauman *et al.*, 2016). Little studies have been performed on the effect of storage time and pasteurization on the quality of pineapple juice parameters such as colour, sugar content, acidity, vitamin and bromelain content. Therefore, in this study, the evaluation of the effect of temperature and storage time on bromelain was carried out. The results indicated that bromelain in pineapple juice was found stable even after going through high-temperature treatment during the pasteurization process. This observation was in the agreement with the hypothesis by Poh and Abdul Majid (2011), where they found the highest temperature at which the maximum activity complexed bromelain exhibited was 85°C. High tolerance to heat denaturation under this high temperature could become an advantage for bromelain. It would signify that bromelain complexed with polyphenol would be more stable by retaining this enzymatic activity

under the condition employed for food and beverage processing, manufacturing for drugs and pharmaceuticals and other industrial application.

The efficacy study of this product towards anti-inflammation showed that pineapple juice drink can reduce the swelling rate to normal as good as commercial drugs. Judging from the data in Figures 2 and 3, rats fed with bromelain drinks showed the greatest decrease in swelling as early as 4 hrs. The effect of bromelain in overcoming the problem of inflammation is very fast compared to the commercial drug used in this study which is Ibuprofen. After 24 hrs, all rat hind paws had returned to their original shape and the percentage of decrease was also almost the same. It should be emphasized that carcinogenic injections used to induce inflammation are temporary in nature and will recover after 24 hrs. Thus, in this study, the most important time in recording a rat's paw volume to determine response was at 0–4 hrs. The results are significant with the finding from Hale *et al.* (2010) where they reported that long-term dietary supplementation with fresh pineapple juice with active bromelain enzymes was safe and decreased inflammation severity and the incidence and multiplicity of inflammation-associated colonic neoplasia in a murine model of inflammatory bowel disease.

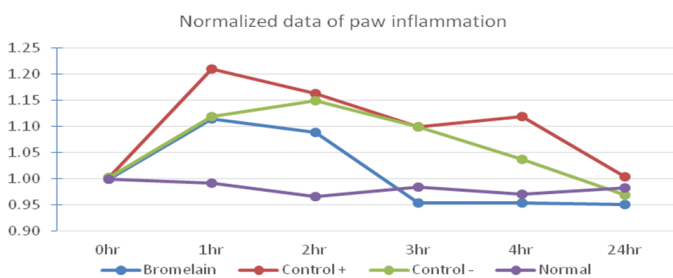


Figure 2. Normalized data on anti-inflammatory effect using carrageenan-induced paw edema

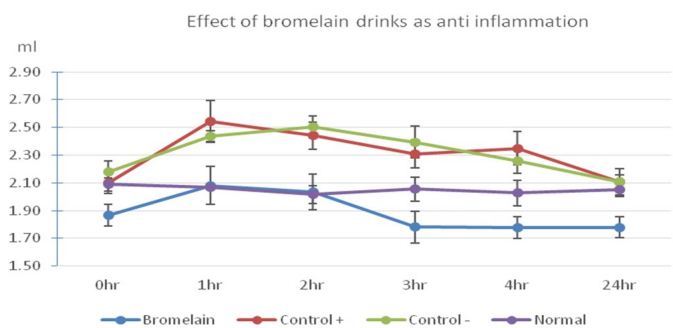


Figure 3. Anti-inflammatory effect using carrageenan-induced paw edema

Fresh pineapple juice is also known for having a healing power against acute tendon injuries. Studies by Aiyegbusi *et al.* (2011) compared the effects of commercial bromelain and fresh pineapple juice on tenocyte proliferation and the malondialdehyde (MDA) level in the early stage of healing in a crush injury to the

Achilles tendon of Sprague Dawley rats. They concluded that pineapple juice significantly lowered the MDA level compared with both the control and bromelain-treated groups. Majeed and Borole (2013) also evaluated the anti-inflammatory effect of pineapple juice in rheumatoid and osteoarthritic models in rats and concluded weak anti-inflammatory activity can be used as a dietary adjuvant to anti-inflammatory drugs. Vitamin C found in pineapple juice also helps as a great remedy for oral health and can reduce the risk of gingivitis and periodontal disease. It also helps the body to fight against the bacteria and the toxins that invade human gum tissues and help in repairing damaged tissues and in keeping the lymphatic system working healthy.

The studies by Agus (2005), mentioned that carrageenan-induced edema in the rat hind paw is most widely used for the screening of new anti-inflammatory agents. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenan-induced edema is mediated through the release of prostaglandin and slow reacting substances which peak at 3 hrs. In the carrageenan-induced paw edema, the bromelain-induced dose-dependent reduction of paw edema in the rat. Results from Agus (2005) showed that bromelain in doses of 20 and 40 mg/kg, produced significant ($p < 0.05$) inhibition of paw edema as compared to the control. These results indicate that bromelain possesses inhibition of prostaglandin release mediated analgesic and anti-inflammatory properties as same as found in this study.

4. Conclusion

The newly developed pineapple juice drink can contribute to the development of functional beverages for the local and international markets. Their anti-inflammatory effects can meet not only the athlete's preference but also active people.

Conflict of interest

The authors declare no conflict of interest.

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