Food Research 6 (Suppl. 2) : 9 - 17 (2022)
Journal homepage: https://www.myfoodresearch.com

FULL PAPER

The characterization of the bioactive compounds in fermented papaya pulp and leaves: providing new insights on the anti-ageing potential

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Article history:
Received: 28 August 2021
Received in revised form: 6 October 2021
Accepted: 9 October 2021
Available Online: 8 December 2022

Keywords:
Acetylcholinesterase (AChE),
Elastase,
Tyrosinase,
Flavonoid,
Organic acids

DOI:
https://doi.org/10.26656/fr.2017.6(S2).003

Abstract

Nowadays, fermented food and beverages derived from plants have become popular choices for consuming functional foods as they provide enormous advantages to human health. Fermented food and beverage might offer more therapeutic benefits than natural nutrition. Papaya contains high antioxidant, antitumor, and immune regulatory effects due to its valuable phytochemical properties. Despite all the advantages, papaya leaves were underutilised as they have an astringent and bitter taste. During this study, papaya pulp and leaves underwent fermentation to enhance their functional properties and improve their taste and flavour. Both papaya pulp and leaves were fermented using mixed cultures of *Komagataiebacter* sp. bacteria and *Dekkera* sp. yeast and incubated under a controlled fermentation process. The supernatant extracted from both fermented papaya pulp and leaves was collected and its inhibitory effect towards the elastase, tyrosinase and acetylcholinesterase was analysed. Elastase and tyrosinase were employed for anti-ageing as increasing in dermal enzymatic activities will cause skin disorders. Meanwhile, acetylcholinesterase will affect the cholinergic crisis in the brain. Thus, inhibiting these enzymes is important for preventing hyperpigmentation, skin ageing and improving cognitive function. In comparison to non-fermented papaya pulp and leaves, the fermented papaya extracts demonstrated significant differences ($P<0.05$) in the inhibition effect against elastase, tyrosinase and acetylcholinesterase with an inhibitory value above 80%. Most of the flavonoids obtained in fermented papaya pulp and leaves were higher than non-fermented pulp and leaves. Protocatechuic acid was the highest compound in fermented papaya pulp (1.22±0.11 µg/mL), while vitexin (113.20±1.18 µg/mL), rutin (10.56±0.52 µg/mL), and ellagic acid (8.76±0.40 µg/mL) highly contained in fermented papaya leaves. The highest organic acids obtained from fermented papaya pulp and leaves acetic acid (13495.42±308.99 µg/mL and 18572.48±78.45 µg/mL), followed by quinic acid (2148.71±63.31 µg/mL and 984.40±2.29 µg/mL) and citric acid (802.57±17.68 µg/mL and 151.83±6.50 µg/mL), respectively. The findings described the potential of fermented papaya pulp and leaves extracts as potential bio-ingredients to slow down ageing development.

1. Introduction

According to the World Health Organization (WHO), ageing can be defined as an accumulation of a wide variety of degenerative damage throughout time. Ageing will result in the emergence of several complex health conditions due to declining biological functions and adaptation to metabolic stress. The majority of anti-ageing studies are to develop therapies that can delay ageing-related diseases (Shetty *et al.*, 2018). Most researchers are intrigued by studying plant materials as a natural product to slow down and extend the life span of the ageing process. Similar interest is further enhanced by studying anti-ageing properties in fermented food and beverages.

In Malaysia, *Carica papaya* is one of the most commercially cultivated for fruit consumption production and generates revenue for trading activities, where Malaysia exported 24,470 tons of papaya in 2017 (Abu Dardak, 2019). Papaya became popular because the plant is fast-growing and available throughout the year,

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

produce a high yield with high nutritional value and taste delicious. The ripe papaya fruit is rich in vitamins A, C, and calcium source. Besides that, it contains proteolytic enzymes called papain and chymopapain that can help to treat several infectious diseases caused by microorganisms (Vij and Prashar, 2015). Application of fermentation process to papaya pulp and leaves has added several advantages: to prolong the papaya pulp shelf life and reduce papaya leaves' bitterness. Besides, fermented papaya pulp and leaves became more nutritious and provide a myriad of health benefits. Papaya leaves contain high phytochemical properties used as a folk medicine for many years. Studies have demonstrated that fermented papaya has anti-ageing (Logozzi *et al.,* 2020), anti-diabetic (Somanah *et al.,* 2018), anti-inflammatory, immunomodulatory, antimicrobial (Nafiu *et al.,* 2019), anti-viral (Sharma *et al.,* 2019) properties.

Nowadays, researchers are interested in effective strategies for delaying the ageing process through the consumption of fermented extracts. Several claims on anti-ageing related benefits associated with fermented foods have been made, as reported by Das *et al.* (2020). The health-promoting properties of fermented food have been attributed to microbial metabolic activities that occurred during the fermentation process. For example, fermented papaya with *Enterococcus faecalis* and *Aspergillus oryzae* has been successfully reported to restore age-related reductions in cell cytolytic activity of the patients due to the benefits of polyphenol metabolites released during the fermentation (Fujita *et al*., 2017). Furthermore, a study conducted by Logozzi *et al*. (2020) has shown the beneficial effects on the redox balance and anti-ageing in mice supplemented with the FPP® (yeast fermented papaya). Daily FPP® consumption has shown to be extremely effective in increasing the body's antioxidant reaction and improving the molecular signatures of ageing. The FPP also showed a consistent biological and gene-regulatory improvement in the biochemical or epigenomic skin (Bertuccelli *et al.,* 2016). In this study, the fermented papaya pulp and papaya leaves with a mixed culture of bacteria and yeast to produce functional extracts were studied. Elastase and tyrosinase are dermal enzymes that play an important role in skin ageing as their activations cause a decrease in elastin and induce excessive melanin production, resulting a loss in skin strength, elasticity and hyperpigmentation (Mostafa *et al*., 2021). Meanwhile, acetylcholinesterase inhibition will affect the cholinergic crisis in the brain. One of the most promising approaches for treating this disease is to use acetylcholinesterase inhibitors to reduce the breakdown of acetylcholine levels in the brain (Ingkaninan *et al*., 2003). Thus, in this study, the inhibitory activities of these enzymes on fermented papaya pulp and leaves will be evaluated as it shows an indicator for preventing skin ageing, hyperpigmentation, and improving cognitive function. This study aimed to determine the inhibitory effect of elastase, tyrosinase, acetylcholinesterase, profiling of flavonoids and organic acids to reveal their functional properties.

2. Materials and methods

2.1 Plant material and fermentation preparation

The papaya fruit at the maturity stage 5 (yellow with a trace of green) from 'Sekaki' variety was bought from a local fruit market and the leaves were harvested from Exotic Star, a papaya farm (Pahang, Malaysia). The papaya fruits were washed before the peel was removed using a fruit peeler. The papaya pulp was cut into small pieces and oven-dried at 50°C for 24 hrs. The pulp was kept vacuum-packed at 4°C for further usage. The papaya leaves were washed thoroughly to remove dirt and dried in a ventilated oven at 50°C for 24 hrs. The dried leaves were milled at 0.5 mm of particle size (Retsch ZM200, Germany) and kept in a vacuum-packed at 4°C for storage. The papaya pulp and leaves were undergone a submerged fermentation (5 L Erlenmeyer Flask) with selected microorganisms from the Collection of Functional Food Cultures (CFFC) MARDI. The papaya pulp and leaves were cultured with a mixed culture of bacteria and yeast (*Komagataiebacter* sp. and *Dekkera* sp.) at 30°C for eight days in order to achieve the concentration of acetic acid at 1.5%.

2.2 Evaluation of anti-ageing properties of fermented papaya pulp and leaves

2.2.1 Evaluation of elastase inhibition of fermented papaya pulp and leaves

A slight modification method of Liyanaarachchi *et al.* (2018) using porcine pancreatic elastase (PPE) was performed in elastase inhibitory activity. A 50 µL of the fermented papaya extract was added with 50 µL elastase dissolved in 0.2 mM Tris HCI buffer, pH 8. The mixture was then thoroughly mixed and pre-treated at 25°C for 10 mins before N-Succinyl-Ala-Ala-Ala-p-Nitroanilide (AAAPVN) solution was added and incubated at 25°C for another 30 mins in dark condition. The absorbance was measured at 410 nm. This study used oleanolic acid as a standard for a comparison purpose. The inhibitory enzyme activities were expressed in the percentage of inhibition (%). All the inhibitory enzyme activities were represented in the percentage of inhibition (%) as described in the equation below:

$$\%\ \text{Elastase Inhibtion Activity} = \frac{[\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})]}{\text{Abs}_{\text{control}}} \times 100$$

https://doi.org/10.26656/fr.2017.6(S2).003

Whereby $Abs_{control}$ is the absorbance reading of the sample without fermented papaya extract, Abs_{sample} is the absorbance reading of sample with fermented papaya extract and Abs_{blank} is the absorbance reading of Tris-HCI buffer

2.2.2 Evaluation of tyrosinase inhibition of fermented papaya pulp and leaves

The inhibition of tyrosinase activity of fermented papaya pulp and leaves was measured with minor modification (Suganya *et al.,* 2015). Tyrosinase and L-DOPA were used as enzymes and substrates, respectively. Approximately 40 µL of 5 mM L-DOPA was mixed with 100 µL of 0.1 M, pH 6.8 phosphate buffer in a 96-well microtiter plate. The mixture was thoroughly mixed and incubated at 37°C for 10 mins. Approximately 120 µL of fermented papaya extract and 40 µL of tyrosine (62 U/mL in 0.1 M, pH 6.8 phosphate buffer) were added and incubated at 37°C for 10 mins before the absorbance was measured at 475 nm. The kojic acid was used as a positive control in this experiment. All the inhibitory enzyme activities were expressed in the percentage of inhibition (%).

$$\%\ \text{Tyrosinase Inhibtion Activity} = \frac{[Abs_{control} - (Abs_{sample} - Abs_{blank})]}{Abs_{control}} \times 100$$

Whereby $Abs_{control}$ is the absorbance reading of sample without fermented papaya extract, Abs_{sample} is the absorbance reading of sample with fermented papaya extract and Abs_{blank} is the absorbance reading of phosphate buffer.

2.2.3 Evaluation of acetylcholinesterase (AChE) inhibition of papaya pulp and leaves

Sample (100 µL) was added into 20 µL acetylcholinesterase dissolved in 0.1 M phosphate buffer, pH 7.4. The mixture was thoroughly vortexed before 120 µL Ellman reagent was added. After 2 mins of reaction, 30 µL 25 mM acetylthiocholine iodide (ATCI) solution was added and incubated at 25°C for 30 mins as described by Ellman *et al.* (1961) with some modification as stated by Machado *et al.* (2015). The absorbance was measured immediately at 412 nm. The acetylcholinesterase activity was assayed in triplicate and using Tacrine as a standard. All the inhibitory enzyme activities were expressed in the percentage of inhibition (%) as in the equation below:

$$\%\ \text{AChE Inhibtion Activity} = \frac{[Abs_{control} - (Abs_{sample} - Abs_{blank})]}{Abs_{control}} \times 100$$

Whereby $Abs_{control}$ is the absorbance reading of sample without fermented papaya extract, Abs_{sample} is the absorbance reading of sample with fermented papaya extract and Abs_{blank} is the absorbance reading of phosphate buffer.

2.3 Determination of flavonoids of fermented papaya pulp and leaves using Ultra Performance Liquid Chromatography

The flavonoids content of the fermented papaya extracts was separated using Ultra Performance Liquid Chromatography (UPLC) system (Waters, USA) using Kinetex C18 100 A column (100 mm × 2.1 mm; 1.7 µm). The oven temperature was controlled at 40°C with the flow rate fixed at 0.4 mL/min. The absorbance was read at different UV spectrum of 280, 330 and 360 nm for particular flavonoids determination. The gradient elution consists of two types of mobile phase: mobile phase A (water: acetic acid, 97:3) and mobile phase B (methanol). The gradient elution mode was programmed as follow: 0–1 min, maintained at 100 % A; 1–10 mins, from 100 to 40 % A; 10–12 mins, from 40 to 100 % A and then maintained at 100 % A for another 2 mins. Quantification was performed using calibration curves obtained by injecting known amounts of targeted flavonoids as external standards with known retention time under a specific UV spectrum.

2.4 Determination of organic acids profiling of fermented papaya pulp and leaves using HPLC

Profiling of organic acids from fermented and non-fermented papaya pulp and leaves was performed with High Performance Liquid Chromatography (HPLC), Alliance Separation Module (Waters, 2695), equipped with a diode array (PDA) detector (Waters, 2996) according to a method previously described by Koh *et al.* (2019). The organic acids quantification was determined from a calibration curve obtained by the organic acids standard. All analysis was done in triplicate.

2.5 Statistical analysis

The data collected were analysed using statistical analysis software SPSS Version 18, SPSS Inc. The differences between different extracts were represented as mean value±standard deviation ($n = 3$) and compared using one-way analysis of variance (ANOVA) followed by Duncan's test at a significance level of $p < 0.05$.

3. Results and discussion

3.1 Evaluation of anti-ageing properties of fermented papaya pulp and leaves

In this study, fermented papaya pulp and leaves extracts were produced from mixed cultures of acetic acid bacteria and yeast (*Komagataiebacter* sp. and yeast, *Dekkera* sp.). Inhibition of elastase, tyrosinase, acetylcholinesterase activities, phenolic compounds and organic acids profiling were carried out on both fermented papaya pulp and leaves extracts. Non-

https://doi.org/10.26656/fr.2017.6(S2).003

fermented papaya pulp and leaves extracts served as a sample treatment in all the experiments.

3.1.1 Evaluation of elastase inhibition of fermented papaya pulp and leaves

Natural ageing caused by skin elasticity changes over time is called intrinsic skin ageing. In contrast, extrinsic skin ageing is developed through solar radiation exposure (Thring *et al.,* 2009). Elastin is an extracellular protein matrix that articulately holds skin elasticity, while elastase is an enzyme that breaks down peptide bonds in elastin, causing skin sagging (Tsuji *et al.,* 2007). Therefore, the inhibition of elastase activity can lead to skin elasticity improvement. As depicted in Figure 1, both fermented extracts from papaya pulp and leaves significantly exhibited elastase activity at 84.62% and 86.16%, respectively higher than the oleanolic acid standard of 69.27 at the $p<0.05$. However, non-fermented papaya leaves inhibited the elastase activity at a lower percentage of 12.45%, while non-fermented papaya pulp did not inhibit elastase enzyme. This finding was in accordance with Zofia *et al.* (2020) study, where the fermented green tea showed the ability to slightly inhibit more elastase enzymes compared to non-fermented green coffee extract at all three different concentrations (100, 500 and 1000 µg/mL).

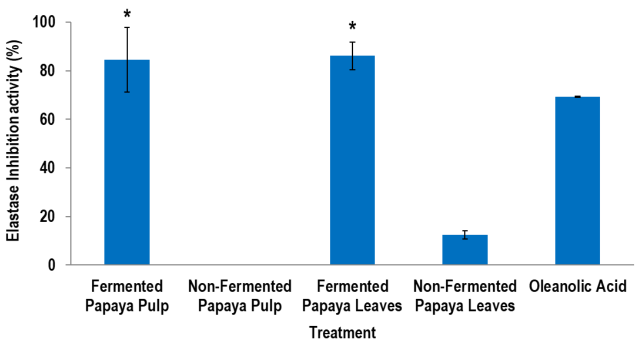

Figure 1. Elastase inhibition activity of fermented and non-fermented papaya pulp and leaves. Data were reported as mean±standard deviation of triplicate analyses. Bars with asterisk notation are significantly different between fermented and non-fermented extracts ($p<0.05$).

3.1.2 Evaluation of tyrosinase inhibition of fermented papaya pulp and leaves

Tyrosinase enzyme plays a vital role in the melanin biosynthesis process. Therefore, tyrosinase inhibition is pertinent in skin alteration protection. Similar finding with elastase inhibition activity, both fermented papaya pulp and leaves extracts showed good tyrosinase inhibition activity with the percentage inhibition of 84.25% and 82.97%, respectively (Figure 2), comparative effective with kojic acid, a well-known anti-pigmentation agent, which was observed to have percentage tyrosinase inhibition activity of 85.95%. The consistent finding in high tyrosinase inhibition effect in this study aligned with Chen *et al.* (2013) reported that, fermented *Rhodiola rosea* and *Lonicera japonica* showed a pronounced inhibitory effect compared to their ethanolic extract. Non-fermented papaya leaves was found not to have tyrosinase inhibition activity, while non-fermented papaya pulp was observed to have low inhibitory capacity at 34.43%.

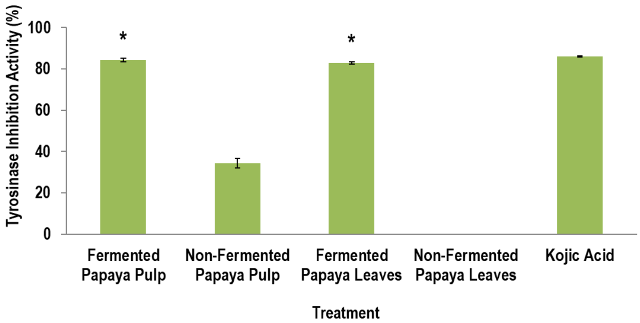

Figure 2. Tyrosinase inhibition activity of fermented and non-fermented papaya pulp and leaves. Data were reported as mean±standard deviation of triplicate analyses. Bars with asterisk notation are significantly different between fermented and non-fermented extracts ($p<0.05$).

3.1.3 Evaluation of acetylcholinesterase inhibition of fermented papaya pulp and leaves

Higher inhibitory capacity against acetylcholinesterase enzyme was observed in both fermented papaya pulp and leaves extracts at 92.50% and 92.33% respectively when compared to the tacrine standard at 76.80%, as shown in Figure 3. Meanwhile, the significantly lower inhibition ($p<0.05$) against acetylcholinesterase activity was obtained in both non-fermented papaya pulp and leaves at 27.97% and 46.55%, respectively. A similar finding was reported by Chupeerach *et al.* (2021), the fermented tea leaves exhibited a significant inhibitory effect on acetylcholinesterase at 90.19% compared to non-fermented and steamed tea leaves at only 59.13 and

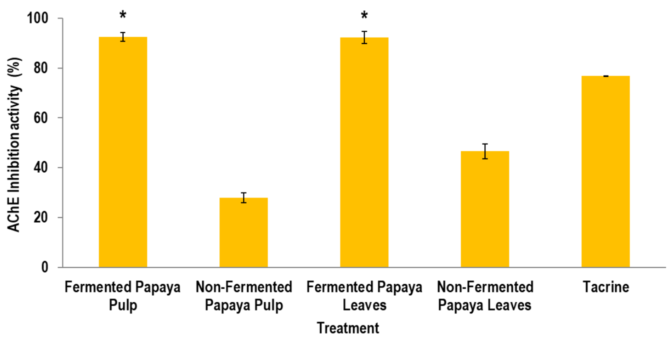

Figure 3. Acetylcholinesterase inhibition activity of fermented and non-fermented papaya pulp and leaves. Data were reported as mean±standard deviation of triplicate analyses. Bars with asterisk notation are significantly different between fermented and non-fermented extracts ($p<0.05$).

https://doi.org/10.26656/fr.2017.6(S2).003

61.75%. The cholinergic hypothesis suggested that neurotransmitter loss in Alzheimer's disease is due to the excessive cholinesterase enzymes (acetylcholinesterase and butyrylcholinesterase) secretion (Hardy and Selkoe, 2002). Profound interest in the relationship of fermented food and extracts in the neuroprotective effect that improves memory function has been recorded by several studies (Kim *et al.,* 2016). The extract from yeast-fermented papaya has significantly enhanced short and long-term memory impairment, implying that it may improve the ageing effects reported by Imao *et al.* (2001) where their PS-501 significantly inhibited the scopolamine and induced a decrease in spontaneous alternation performance and passive avoidance learning significantly.

3.2 Determination of flavonoids of fermented papaya pulp and leaves

Flavonoids are phenolic acids discovered in plants that have anti-ageing properties (Zhang *et al.*, 2020). During the fermentation, microorganisms may obtrude their wall breaking effect on the plant material, resulting in the rapid dissolution of flavonoids substance. Enzymes secretion by microorganisms throughout the fermentation process can also help convert flavonoids into the corresponding metabolites (Erel, 2004; Hole *et al.,* 2012). A large number of flavonoids were identified in both fermented papaya pulp and leaves extracts such as gallic acid, protocatechuic acid, hydroxybenzoic acid, 2,5 dihydroxybenzoic acid, ferulic acid, syringic acid, benzoic acid, salicylic acid, ellagic acid, caffeic acid, chlorogenic acid, vitexin, rutin, quercetin, luteolin and apigenin (Table 1). Papaya leaves is rich in phytochemicals that benefits human health. Therefore, flavonoids content were higher in papaya leaves than their pulp. Vitexin was found to be the highest flavonoids in fermented papaya leaves extract at 113.20±1.18 µg/mL, followed by rutin (10.56±0.52 µg/mL), ellagic acid (8.76±0.40 µg/mL), catechin (7.34±0.42 µg/mL) and protocatechuic acid (1.50±0.04 µg/mL). In fermented pulp extracts, the highest flavonoids obtained were protocatechuic acid (1.22±0.11 µg/mL), gallic acid (0.78±0.08 µg/mL), 2,5 dihydroxybenzoic acid (0.45±0.04 µg/mL), rutin (0.28±0.02 µg/mL) and chlorogenic acid (0.24±0.02 µg/mL). Findings by Samieri *et al.* (2014) have reported that flavonoids also importantly maintain better cognitive and mental health by decreasing oxidative stress and inflammation. Flavonoids content can be related to our significant findings on strong acetylcholinesterase inhibitory in both fermented extracts. Additionally, the review reported by Lima *et al.* (2018) briefly discussed the neuroprotective mechanisms of vitexin. Another study by Parihar and Brewer (2010) also reported the effect of vitexin in contributing to neural degeneration reduction, thus helping in cognition improvement. According to Choi *et al*. (2016), rutin improved skin elasticity and reduced length and wrinkles. The existence of vitexin and rutin can also be observed in fermented tea leaves (Gaggia *et al*., 2019). Moreover, ellagic acid found in fermented papaya leaves enables the skin to stretch and remain firm. In addition, ellagic acid has skin whitening properties and reduced ultraviolet-induced skin pigmentation by inhibiting tyrosinase activity through binding to copper at its active site (Sajna *et al*., 2015). Catechin, the natural antioxidant, help to prevent damage caused by oxidation and protects the skin against

Table 1. Profile of flavonoids content in fermented and non-fermented papaya pulp and leaves

Bioactive Component	Fermented Papaya Pulp (µg/mL)	Non-fermented Papaya Pulp (µg/mL)	Fermented Papaya Leaves (µg/mL)	Non-fermented Papaya Leaves (µg/mL)
Gallic acid	0.78±0.08	7.13±0.10	0.45±0.05	0.83±0.08
Protocatechuic acid	1.22±0.11	1.32±0.03	1.50±0.04	0.72±0.19
Hydroxybenzoic acid	0.29±0.03	0.00±0.00	0.80±0.06	0.91±0.04
2,5 Dihydroxybenzoic acid	0.45±0.04	0.36±0.02	0.81±0.03	0.22±0.02
Ferulic acid	0.00±0.00	0.00±0.00	0.25±0.02	0.26±0.02
Syringic acid	0.04±0.00	0.00±0.00	1.24±0.24	1.06±0.03
Benzoic acid	0.00±0.00	0.00±0.00	1.03±0.08	1.04±0.19
Salicyclic acid	0.00±0.00	0.00±0.00	1.26±0.19	2.02±0.24
Ellagic acid	0.07±0.01	0.00±0.00	8.76±0.40	4.01±0.6
Caffeic acid	0.02±0.00	0.02±0.00	0.32±0.05	2.48±0.09
Chlorogenic acid	0.24±0.02	0.00±0.00	2.34±0.04	1.02±0.04
Vitexin	0.00±0.00	0.00±0.00	113.20±1.18	93.11±4.35
Rutin	0.28±0.02	0.05±0.01	10.56±0.52	10.83±2.01
Quercetin	0.04±0.01	0.04±0.01	0.00±0.00	0.00±0.00
Luteolin	0.05±0.01	0.03±0.01	0.00±0.00	0.00±0.00
Apigenin	0.17±0.01	0.05±0.01	0.00±0.00	0.00±0.00
Catechin	0.16±0.02	0.10±0.02	7.34±0.42	1.18±0.10
Epigallocatechin	0.07±0.01	0.36±0.01	0.60±0.12	0.87±0.16

https://doi.org/10.26656/fr.2017.6(S2).003

ultraviolet radiation. In contrast to this study, catechin decreased in most fermented tea leaves due to enzyme mechanisms excreted by yeast and bacteria in the kombucha culture (Jayabalan *et al*., 2007; Gaggia *et al*., 2019). Furthermore, protocatechuic acid was shown to have potential in the anti-wrinkle effect. It contains anti-ageing properties based on both *in vitro* assays and *in vivo* skin testing in humans, as published by Shin *et al.* (2020). These results supported the significant inhibition of elastase, tyrosinase and acetylcholinesterase enzymes beneficial to ageing by both papaya pulp and leaves extracts as discussed earlier.

3.3 Determination of organic acids profiling of fermented papaya pulp and leaves

In the fermentation process, multiple organic acids are produced through microorganisms and biochemical reactions. According to Petsiou *et al.* (2014), organic acids also contributed to the fermented fruit's organoleptic qualities, altered the fermented papaya extract aroma, taste and become more pleasant. In this study, organic acids showed a high yield in both fermented products compared to non-fermented papaya with the domination of acetic acid. This phenomenon may occur due to acetic acid bacteria (*Komagataiebacter* sp.) used in the fermentation process, which helps convert the intermediate substance into acetic acid. Both fermented papaya pulp and leaves extracts shared similar organic acids profile content, as shown in Table 2. The highest organic acids obtained from fermented papaya pulp and leaves were acetic acid (13495.42±308.99 µg/mL and 18572.48±78.45 µg/mL, respectively), followed by quinic acid (2148.71±63.31 and 984.40±2.29 µg/mL), citric acid (802.57±17.68 and 151.83±6.50 µg/mL), malic acid (249.40±4.17 and 123.62±1.25 µg/mL). Most of the organic acids produced are slightly higher in fermented papaya pulp compared to fermented papaya leaves except for oxalic acid (43.67±1.58 and 69.97±1.88 µg/mL) and kojic acid content (4.81±0.27 and 20.32±0.32 µg/mL). This result was consistent with other studies where most fermented extracts showed an increment in acetic acid concentration and produced complex compositions of organic acids (Jayabalan *et al*., 2007; Kong *et al.,* 2018; Anal, 2019; Gaggia *et al.,* 2019). According to Leal *et al*. (2018), acetic acid is responsible for the acidic smell and taste of fermented extracts. In *Caenohabditis elegans* model systems studied by Zhang *et al*. (2012), quinic acid is active in enhancing DNA repair, immunity, and neuroprotective effects in neuron cells. This substance may support fermented papaya's significant anti-ageing activities associated with neuronal cell death in neurodegenerative diseases. In addition, Shirazi-thyme, cinnamon and cardamom flavoured kombucha studied by Shahbazi *et al*. (2018) also produced a notable malic acid compound at 312.00±32.67, 276.00±23.13 and 98.00±11.27 mg/L, respectively. Findings by Yamamoto *et al*. (2006) and Tang and Yang (2018) indicated that malic acid increases skin renewal rate and has more diverse biological functions in skin ageing. Moreover, organic acids exert some health benefits like antioxidants and antimicrobial effects (Budak *et al.,* 2014).

4. Conclusion

This study highlights the potential of fermented papaya pulp and leaves in the inhibition of elastase, tyrosinase, acetylcholinesterase activity and rich with flavonoids and organic acids, which can be associated with slowing down ageing development. Moreover, the fermentation process provides alternative methods to enhance the functional properties, taste and favour of the extracts. The information gathered in this study may provide new insights on the exploration of the health benefits of fermented papaya products.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank the Ministry of Agriculture and Food Industry (MAFI) for the financial support under the RMK-11(P21003004050001) grant.

Table 2. Profile of organic acids content in fermented and non-fermented papaya pulp and leaves

Organic acids	Fermented Papaya Pulp (µg/mL)	Non-fermented Papaya Pulp (µg/mL)	Fermented Papaya Leaves (µg/mL)	Non-fermented Papaya Leaves (µg/mL)
Acetic Acid	13495.42±308.99	0.00±0.00	18572.48±78.45	0.00±0.00
Citric Acid	802.57±17.68	726.37±6.14	151.83±6.50	459.21±5.38
Succinic Acid	0.00±0.00	399.65±19.51	0.00±0.00	0.00±0.00
Oxalic Acid	43.67±1.58	180.56±2.33	69.97±1.88	249.49±4.09
Malic Acid	249.40±4.17	55.59±1.72	123.62±1.25	346.00±3.12
Kojic Acid	4.81±0.27	2.57±0.19	20.32±0.32	7.18±0.30
Quinic Acid	2148.71±63.31	84.38±2.01	984.40±2.29	81.10±3.56

https://doi.org/10.26656/fr.2017.6(S2).003

References

Abu Dardak, R. (2019). Trends in production, trade, consumption of tropical fruit in Malaysia. Food and Fertilizer Technology Center for the Asian and Pacific Region, No. 1381. Retrieved from FFTC Agricultural Policy Platform (FFTC-AP) website: https://ap.fftc.org.tw/article/1381#:~:text=The%20trend%20of%20Malaysia%27s%20export,Malaysian%20consumers%20indicates%20declining%20trends.

Anal, A.K. (2019). Quality Ingredients and Safety Concerns for Traditional Fermented Foods and Beverages from Asia: A Review. *Fermentation*, 5(1), 8. https://doi.org/10.3390/fermentation5010008

Bertuccelli, G., Zerbinati, N., Marcellino, M., Kumar, N.S.N., He, F., Tsepakolenko, V., Cervi, J., Lorenzetti, A. and Marotta, F. (2016). Effect of a quality-controlled fermented nutraceutical on skin ageing markers: An antioxidant-control, double-blind study. *Experimental and Therapeutic Medicine*, 11(3), 909-916. https://doi.org/10.3892/etm.2016.3011

Budak, N.H., Aykin, E., Seydim, A.C., Greene, A.K. and Guzel-Seydim, Z.B. (2014). Functional properties of vinegar. *Journal of Food Science*, 79(5), 757–764. https://doi.org/10.1111/1750-3841.12434

Chen, Y.S., Liou, H.C. and Chan, C.F. (2013). Tyrosinase inhibitory effect and antioxidative activities of fermented and ethanol extracts of *Rhodiola rosea* and *Lonicera japonica*. *The Scientific World Journal*, 2013, 612739. https://doi.org/10.1155/2013/612739

Choi, S.J., Lee, S.N., Kim, K., Joo, D.H., Shin, S., Lee, J., Lee, H.K., Kim, J., Kwon, S.B., Kim, M.J., Ahn, K.J., An, I.S., An, S. and Cha, H.J. (2016). Biological effects of rutin on skin aging. *International Journal of Molecular Medicine*, 38(1), 357-363. https://doi.org/10.3892/ijmm.2016.2604

Chupeerach, C., Aursalung, A., Watcharachaisoponsiri, T., Whanmek, K., Thiyajai, P., Yosphan, K., Sritalahareuthai, V., Sahasakul, Y., Santivarangkna, C. and Suttisansanee, U. (2021). The effect of steaming and fermentation on nutritive values, antioxidant activities and inhibitory properties of tea leaves. *Foods*, 10(1), 117. https://doi.org/10.3390/foods10010117

Das, G., Paramithiotis, S., Sivamaruthi, B.S., Wijaya, C.H., Suharta, S., Sanlier, N., Shin, H.S. and Patra, J.K. (2020). Traditional fermented foods with anti-aging effect: A concentric review. *Food Research International*, 134, 109269. https://doi.org/10.1016/j.foodres.2020.109269

Ellman, G.L., Courtney, K.D., Andres Jr. V. and Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7(2), 88-90. https://doi.org/10.1016/0006-2952(61)90145-9

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*, 37(4), 277-285. https://doi.org/10.1016/j.clinbiochem.2003.11.015

Fujita, Y., Tsuno, H. and Nakayama, J. (2017). Fermented papaya preparation restores age-related functions in peripheral blood mononuclear cell cytolytic activity in tube-fed patients. *PLoS ONE*, 12 (1), e0169240. https://doi.org/10.1371/journal.pone.0169240

Gaggia, F., Baffoni, L., Galiano, M., Nielsen, D.S., Jakobsen, R.R., Castro-Mejia, J.L., Bosi, S., Truzzi, F., Musumeci, F., Dinelli, G. and Gioia, D.D. (2019). Kombucha beverages from green, black and rooibos tea: A comparative study looking at microbiology, chemistry and antioxidant activity. *Nutrients*, 11(1), 1. https://doi.org/10.3390/nu11010001

Hardy, J. and Selkoe, D.J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science,* 297, 353–356. https://doi.org/10.1126/science.1072994

Hole, A.S., Rud, I., Grimmer, S., Sigl, S., Narvhus, J. and Sahlstrøm, S. (2012). Improved bioavailability of dietary phenolic acids in whole grain barley and oat groat following fermentation with probiotic *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, and *Lactobacillus reuteri*. *Journal of Agricultural and Food Chemistry,* 60(25), 6369–6375. https://doi.org/10.1021/jf300410h

Imao, K., Kameyama, T. and Ukai, M. (2001). PS-501, fermented papaya preparation, improves scopolamine-induced amnesia in mice. *Research Communications in Pharmacology and Toxicology*, 6(2), 197–204.

Ingkaninan, K., Temkitthawon, P., Chuenchom, K., Yuyaem, T. and Thongnoi, W. (2003). Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *Journal of Ethnopharmacology*, 89(2-3), 261-264. https://doi.org/10.1016/j.jep.2003.08.008

Jayabalan, R., Marimuthu, S. and Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chemistry*, 102(1), 392-398. https://doi.org/10.1016/j.foodchem.2006.05.032

Kim, B., Hong, V.M., Yang, J., Hyun, H., Im, J.J., Hwang, J., Yoon, S. and Kim, J.E. (2016). A review of fermented foods with beneficial effects on brain and cognitive function. *Preventive Nutrition and Food Science*, 21(4), 297-309. https://doi.org/10.3746/pnf.2016.21.4.297

Koh, S.P., Sharifudin, S.A., Abdullah, R.A., Hamid, N.S., Mirad, R. and Mustaffa, R. (2019). Antimicrobial efficacy of fermented mango leaves beverage towards selected foodborne pathogens. *Malaysian Journal of Microbiology,* 15 (4), 320– 326. https://doi.org/10.21161/mjm.191548

Kong, C.T., Ho, C.W., Ling, J.W.A., Lazim, A., Fazry, S. and Lim, S.J. (2018). Chemical Changes and Optimisation of Acetous Fermentation Time and Mother of Vinegar Concentration in the Production of Vinegar-like Fermented Papaya Beverage. *Sains Malaysiana*, 47(9), 2017–2026. https://doi.org/10.17576/jsm-2018-4709-09

Leal, J.M., Suarez, L.V., Jayabalan, R., Oros, J.H. and Escalante-Aburto, A. (2018). A review on health benefits of kombucha nutritional compounds and metabolites. *CyTA-Journal of Food*, 16(1), 390-399. https://doi.org/10.1080/19476337.2017.1410499

Lima, L.K.F., Pereira, S.K.S., Junior, R.S.S., Santos, F.P.S., Nascimento, A.S., Feitosa, C.M., Figueredo, J.S., Cavalcante, A.N., Araujo, E.C.C. and Rai, M. (2018). A brief review on the neuroprotective mechanisms of vitexin. *BioMed Research International*, 2018, 4785089. https://doi.org/10.1155/2018/4785089

Liyanaarachchi, G.D., Samarasekara, J.K.R.R., Mahanama, R. and Hemalal, P. (2018). Tyrosinase, elastase, hyaluronidase, inhibitory and antioxidant activity of Sri Lankan medicinal plants for novel cosmeceuticals. *Industrial Crops and Products*, 111, 597-606. https://doi.org/10.1016/j.indcrop.2017.11.019

Logozzi, M., Di Raimo, R., Mizzoni, D., Andreotti, M., Spada, M., Macchia, D. and Fais, S. (2020). Beneficial Effects of Fermented Papaya Preparation (FPP®) Supplementation on Redox Balance and Aging in a Mouse Model. *Antioxidants*, 9(2), 144. https://doi.org/10.3390/antiox9020144

Machado, L.P., Carvalho, L.R., Young, M.C.M., Cardoso-Lopes, E.M., Centeno, D.C., Zambotti-Villela, L., Colepicolo, P. and Yokoya, N.S. (2015). Evaluation of acetylcholinesterase inhibitory activity of Brazilian red macroalgae organic extracts. *Revista Brasileira de farmacognosia*, 25(6), 657-662. https://doi.org/10.1016/j.bjp.2015.09.003

Mostafa, E.S., Ahmed, M.D.A., Gad, S.S. and Mahmoud, E.N. (2021). A unique acylated flavonol glycoside from *Prunus persica* (L.) var. Florida Prince: a new solid lipid nanoparticle cosmeceutical formulation for skincare. *Antioxidants*, 10(3), 436. https://doi.org/10.3390/antiox10030436

Nafiu, A., Alli-Oluwafuyi, A.M., Haleemat, A., Olalekan, I.S. and Rahman, M.T. (2019). Papaya (*Carica papaya* L., Pawpaw) In: Nabavi, S. and Silva, A. (eds), Nonvitamin and Nonmineral nutritional Supplements, p. 335-359. Amsterdam: Elsevier Press. https://doi.org/10.1016/B978-0-12-812491-8.00048-5

Parihar, M.S. and Brewer, G.J. (2010). Amyloid-β as a modulator of synaptic plasticity. *Journal of Alzheimer's Disease*, 22(3), 741–763. https://doi.org/10.3233/JAD-2010-101020

Petsiou, E.I., Mitrou, P.I., Raptis, S.A. and Dimitriadis, G.D. (2014). Effect and mechanisms of action of vinegar on glucose metabolism, lipid profile, and body weight. *Nutrition Review*, 72(10), 651–661. https://doi.org/10.1111/nure.12125

Sajna, K.V., Gottumukkala, L.D., Sukumaran, R.K. and Pandey, A. (2015). White Biotechnology in Cosmetics. In Pandey, A., Hofer, R, Taherzadeh, M., Nampoothiri, K.M. and Larroche, C. (eds.), Industrial Biorefineries and White Biotechnology, p. 607-652. India: Elsevier. https://doi.org/10.1016/B978-0-444-63453-5.00020-3

Samieri, C., Sun, Q., Townsend, M.K., Rimm, E.B. and Grodstein, F. (2014). Dietary flavonoid intake at midlife and healthy aging in women. *The American Journal of Clinical Nutrition*, 100(6), 1489-1497. https://doi.org/10.3945/ajcn.114.085605

Shahbazi, H., Cahruie, H.H., Golmakani, M.T., Eskandari, M.H. and Movahedi, M. (2018). Effect of medicinal plant type and concentration on physicochemical, antioxidant, antimicrobial and sensorial properties of kombucha. *Food Science and Nutrition*, 6(8), 2568-2577. https://doi.org/10.1002/fsn3.873

Sharma, N., Mishra, K.P., Chandra, S., Bhardwaj, V., Tanwar, H., Ganju, L., Kumar, B. and Singh, S.B. (2019). Evaluation of anti-dengue activity of *Carica papaya* aqueous leaf extract and its role in platelet augmentation. *Archives of Virology*, 164, 1095-1110. https://doi.org/10.1007/s00705-019-04179-z

Shetty, A.K., Kodali, M., Upadhya, R. and Madhu, L.N. (2018). Emerging anti-aging strategies – Scientific basis and efficacy. *Aging and Disease*, 9(6), 1165-1184. https://doi.org/10.14336/AD.2018.1026

Shin, S., Cho, S.H., Park, D. and Jung, E. (2020). Anti-skin aging properties of protocatechuic acid *in vitro* and in vivo. *Journal of Cosmetic Dermatology*, 19 (4), 977-984. https://doi.org/10.1111/jocd.13086

Somanah, J., Putteeraj, M., Aruoma, O.I. and Bahorun, T. (2018). Discovering the Health Promoting Potential of Fermented Papaya Preparation - Its Future Perspectives for the Dietary Management of Oxidative Stress During Diabetes. *Fermentation*, 4 (4), 83. https://doi.org/10.3390/fermentation4040083

Suganya, P., Karnan, J., Mallavarapu, G.R. and Murugan, R. (2015). Comparison of the chemical composition, tyrosinase inhibitory and anti-inflammatory activities of the essential oils of *Pogostemon plectranthodes* from India. *Industrial Crops and Products,* 69, 300-307. https://doi.org/10.1016/j.indcrop.2015.02.045

Tang, S.C. and Yang, J.H. (2018). Dual effects of alpha-hydroxy acids on the skin. *Molecules*, 23(4), 863. https://doi.org/10.3390/molecules23040863

Thring, T.S.A., Hili, P. and Naughton, D.P. (2009). Anti-collagenase, anti-elastase and antioxidant activities of extracts from 21 plants. *BioMed Central Complementary and Alternative Medicine*, 9, 27. https://doi.org/10.1186/1472-6882-9-27

Tsuji, N., Moriwaki, S., Suzuki, Y., Takema, Y. and Imokawa, G. (2007). The role of elastase secreted by fibroblasts in wrinkle formation: Implication through selective inhibition of elastase activity. *Photochemistry and Photobiology*, 74(2), 283-290. https://doi.org/10.1562/0031-8655(2001)0740283TROESB2.0.CO2

Vij, T. and Prashar, Y. (2015). A review on medicinal properties of *Carica papaya* Linn. *Asian Pacific Journal of Tropical Disease*, 5(1), 1-6. https://doi.org/10.1016/S2222-1808(14)60617-4

World Health Organization. (2015). World report on aging and health, p. 25. Geneva, Switzerland: WHO Press.

Yamamoto, Y. Uede, K., Yonei, N., Kishioka, A., Ohtani, T. and Furukawa, F. (2006). Effects of alpha -hydroxy acids on human skin of Japanese subjects: The rationale for chemical peeling. *The Journal of Dermatology*, 33(1), 16-22. https://doi.org/10.1111/j.1346-8138.2006.00003.x

Zhang, L., Zhang, J., Zhao, B. and Zhao-Wilson, X. (2012). Quinic acid could be a potential rejuvenating natural compound by improving survival of *Caenorhabditis elegans* under deleterious conditions. *Rejuvenation Research*, 15(6), 573-583. https://doi.org/10.1089/rej.2012.1342

Zhang, S., Cheng, M., Li, Z., Guan, S., Cai, B., Li, Q. and Rong, S. (2020). Composition and biological activity of rose and jujube kernel after fermentation with kombucha SCOBY. *Journal of Food Processing and Preservation*, 44(10), e14758. https://doi.org/10.1111/jfpp.14758

Zofia, N.L., Aleksandra, Z., Tomasz, B., Martyna, Z.D., Magdalena, Z., Zofia, H.B. and Tomasz, W. (2020). Effect of Fermentation Time on Antioxidant and Anti-Ageing Properties of Green Coffee Kombucha Ferments. *Molecules*, 25(22), 5394. https://doi.org/10.3390/molecules25225394

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