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(Original Research)

Inhibitory Effects of Processed Bignay [*Antidesma bunius* (L.) Spreng.] Fruit Pulp Against Carbohydrate - Digesting Enzymes Related to Type 2 Diabetes

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Abstract

Background: *Antidesma bunius*, locally called bignay in the Philippines, is an indigenous fruit traditionally used as an antidiabetic agent. To promote its utilization, various processes can be applied to extend its shelf life and produce functional ingredients. **Methods:** In this study, freeze dried, oven dried, spray dried, and concentrated bignay were evaluated and compared with acarbose for their *in vitro* inhibitory activities against α-amylase, α-glucosidase, and sucrase as well as its potential to inhibit glucose movement across a membrane using spectrophotometric methods. **Results:** The inhibitory activities of processed bignay against α-amylase, α-glucosidase, and sucrase were lower than acarbose and showed concentration dependence. Among the processed bignay, the freeze dried showed the maximum inhibitory activity towards α-amylase (53.47 ± 0.34%), α-glucosidase (40.59 ± 1.01%), and sucrase (44.22 ± 1.82%) at 500 µg/mL. Moreover, the freeze dried bignay had the highest glucose movement inhibition across a dialysis membrane at 500 µg/mL and at different time intervals. **Conclusions:** The inhibition of the key enzymes for carbohydrate hydrolysis and glucose movement across a membrane were best exhibited by the freeze dried bignay, followed by oven dried bignay, concentrated bignay, and least by the spray dried bignay.

Keywords: *Antidesma bunius*, Enzyme inhibition, Glucose movement inhibition, Type 2 diabetes mellitus

1. Introduction

Type 2 diabetes mellitus (DM) is considered as one of the fastest growing chronic metabolic disorders caused by the lack of insulin production, insulin action, or both resulting in an increased blood glucose level or hyperglycemia [1]. Postprandial hyperglycemia should be controlled during the early stages of type 2 DM as it results in other health complications such as heart and blood vessel disease, kidney disease, retinopathy, and peripheral neuropathy [2]. Although the pathogenesis of type 2 DM is already well studied, its overall treatment still remains a challenge. As a result, there is a continuous effort to manage type 2 DM leading to recent advances both in drug and non-drug treatments [3].

Managing type 2 DM has two main considerations, first is the prevention of carbohydrate breakdown into glucose and second is the regulation of glucose diffusion through the intestinal membrane to the blood stream [4]. The

three major enzymes in carbohydrate hydrolysis are the α-amylase, α-glucosidase, and sucrase [5-6]. The α-amylase hydrolyzes carbohydrates to smaller oligosaccharides which are further broken into glucose by the α-glucosidase for absorption in the brush border membrane of the small intestine [7]. In addition, sucrase is also present in the small intestines' brush border membrane and hydrolyzes sucrose, releasing fructose and glucose [8]. Inhibiting these three enzymes can prevent postprandial hyperglycemia by reducing the blood glucose level [9]. Acarbose, miglitol, and voglibose are the most common prescription drugs in the market that can inhibit these carbohydrate-digesting enzymes, but these are reported to be costly and have several negative gastrointestinal effects such as recurrent stomach pain, flatulence, and diarrhea [1,6,10]. Thus, plants and their natural products have been explored as alternatives to regulate diabetes due to their pharmacological properties, minimal toxicity, and lesser side effects [1]. Different plant polyphenolic compounds have been reported to have antidiabetic properties [11] by inhibiting the different digestive enzymes involved in lipid and carbohydrate hydrolysis resulting in blood glucose control [12]. However, only around 10% of these secondary metabolites have been characterized and investigated for their antidiabetic activity.

Antidesma bunius, locally called as bignay in the Philippines, has been traditionally used as medicine for a widespread diseases such as indigestion, cough, hypertension, and diabetes [13]. Several studies report that its leaves, bark, and fruits have α-amylase and α-glucosidase inhibitory activities due to the presence of different phenolic compounds with antioxidant activities [9,14-16]. However, these reported studies were done on fresh samples and none yet on dried or processed bignay. Since the fruits of bignay is seasonal, it is usually stored by conventional freezing which may cause negative biochemical changes such as loss of antioxidant properties [17]. As an alternative, different drying and concentrating processes can be applied to extend its shelf-life and convert it to functional ingredients. Carbonera *et al.* (2023) [18] reported that freeze drying and oven drying bignay fruits can improve the polyphenolic content and antioxidant activity, all of which can contribute to its antidiabetic potential. Thus, it is important to analyze for the possible inhibitory effects of bignay fruit pulp against the carbohydrate-digesting enzymes linked to type 2 diabetes as well as its ability to inhibit glucose movement across a membrane to determine if the antidiabetic potential still remains after processing.

The main purpose of this study was to determine the inhibitory potential of bignay fruits which underwent freeze drying, oven drying, spray drying, and juice concentrating against enzymes related to carbohydrate digestion such as α-amylase, α-glucosidase, and sucrase. In addition, the ability of the processed bignay to prevent glucose movement across a membrane was also investigated. The combination of both these effects will support and establish the intent to convert bignay fruit into a functional ingredient with antidiabetic potential.

2. Materials and Methods

2.1 Chemicals and raw materials

The enzymes α-amylase, α-glucosidase, and sucrase (Sigma-Aldrich, USA) and standard drug acarbose (Thermo Fisher Scientific, USA) used for the inhibitory assays were all analytical grade. In addition, the other chemicals used were also analytical grade from Sigma-Aldrich (USA), Fluka™ (Switzerland), Ajax Finechem (New Zealand), Loba Chemie (India), J.T. Baker (China), and RCI Labscan (Thailand).

Bignay fruits [*Antidesma bunius* (L.) Spreng cv. 'common'] were collected from the Institute of Food Science and Technology, University of the Philippines Los Baños (UPLB) in the month of August 2020. The harvested fruits were sorted and only the dark purple to almost black mature fruits were used. Approximately 20 kg of the mature fruits were washed and fed through a de-pulper machine (Kiya Seisakusho, Ltd., Toyo Japan) to obtain the pulp and separate the seeds and peel. The collected pulp slurry was portioned at 2 kg each in polyethylene bags and kept in the freezer (Fujidenzo, Philippines) at -20 °C.

2.2 Processing of bignay

Freeze drying, oven drying, spray drying, and juice concentration were the processing methods used for this study. Freeze drying and oven drying were selected based on prior studies reporting their ability to enhance phenolic content

and antioxidant activity, which are linked to enzyme inhibition potential. Spray drying and juice concentrating were also included as these are commonly used in industry for scalability, despite known challenges such as thermal degradation of bioactive compounds

The portioned bignay pulp slurries were dried using different methods following the parameters based on previous studies [18-21] with minor modifications to achieve a moisture content of not more than 10%. For the freeze dried bignay (FDB), the slurry was placed in the freeze dryer (Gecar Machine Solutions Inc., Philippines) for 31 h with a heater setting of 35 °C with a chilling temperature of -30 °C [18-19]. For the oven dried bignay (ODB), a pack of the thawed slurry was placed in an industrial convection oven (Memmert GmbH + Co. KG, Germany) for 31 h set at 50 °C [20]. For the spray dried bignay (SDB), the slurry was prepared according to Carbonera *et al.* (2023) [18] and then loaded to the spray dryer (MachineLab Technology Inc., Philippines) with an inlet temperature of 180 °C and outlet temperature of 103 °C [21]. For the bignay concentrate (BC), the procedure of Carbonera *et al.* (2023) [18] was also followed to obtain a concentrate with a °Brix value within 25-60 which is the recommended total soluble solids for fruit concentrates [22].

The FDB, ODB, and SDB were placed in sealable dark colored containers then inside a desiccator at room temperature. On the other hand, the BC was placed in clean and sterile PET bottles then kept in refrigerated temperature (0–4 °C).

2.3 Extract preparation

Sample extracts of FDB, ODB, and SDB were obtained using the methods of Sayah *et al.* (2017) [23] with minor revisions. For each dried sample, 50 g was weighed, combined with 500 mL 80% methanol, and mixed using a mechanical shaker for 24 hr at room temperature. The extracts were filtered and the collected filtrate was concentrated using a rotary evaporator at 40 °C. On the other hand, the BC was filtered and did not undergo any extraction step. Appropriate dilution for each sample extract were prepared prior to each analysis.

2.4 α-amylase inhibition assay

The α-amylase inhibition assay was carried out following Shettar *et al.* (2017) [24]. Different concentrations of each sample and the standard drug acarbose at 100, 200, 300, 400, and 500 µg /mL were prepared. A volume of 0.5 mL for each sample was added with 0.5 mL α-amylase solution (0.5 mg/mL) in 0.02 M sodium phosphate buffer (pH 6.9). The mixture was allowed to stand for 10 min at room temperature and added with 0.5 mL 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9). The solution was mixed and allowed to stand for another 10 min at room temperature then added with 1 mL of dinitrosalicylic acid (DNS) to stop the reaction. The solution was placed in a 100 °C water bath for 5 min, cooled to 30 °C, added with 10 mL of deionized water, then mixed. The absorbance for each solution was read at 540 nM. A blank (buffer only) and control (buffer with amylase solution) were also prepared and absorbances were determined using the same wavelength. The α-amylase inhibitory activity was computed as:

$$\%\ \alpha - amylase\ inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

2.5 α-glucosidase inhibition assay

The α-glucosidase inhibition assay was done following Ma and Liu (2020) [25] and Bhatia *et al.* (2019) [1] with modifications using different concentrations of each sample and acarbose at 100, 200, 300, 400, and 500 µg/mL. The sample solutions, acarbose, α-glucosidase (0.1 U/mL), and *p*-NPG (1 mM) were prepared with 0.1 M phosphate buffer (pH 6.8). For each sample, 1 mL was added with 0.2 mL α-glucosidase solution and 2.5 mL 0.1 M phosphate buffer (pH 6.8) then mixed. The solution incubated at 37 °C for 20 min. Then, 0.4 mL 1mM *p*-NPG was added and the solution was incubated for another 30 min at 37 °C. The reaction was stopped with the addition of 1 mL 0.1 N Na_2CO_3. Then the absorbance determination was done at 405 nM. The absorbances of the blank (buffer only) and control (buffer instead of extract) solutions were also

obtained. The α-glucosidase inhibitory activity was calculated as:

$$\%\ \alpha - glucosidase\ inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

2.6 Sucrase inhibition assay

The sucrase inhibition assay was conducted following the procedure of Antora *et al.* (2018) [6] using different concentrations of each sample and acarbose at 100, 200, 300, 400, and 500 µg /mL. The samples, acarbose, sucrase (4.8 U), and sucrose (60 mM) were prepared using phosphate buffer saline (pH 7.4). In a test tube, 0.25 mL of each sample was added with 0.25 mL sucrase solution, mixed, and allowed to stand for 10 min at 37 °C. The mixture was added with sucrose solution, incubated for 30 min at 37°C, added with 1 mL DNS, and boiled for 5 min. It was then added with 1 mL distilled water, mixed, and the absorbance was read at 540 nM. The absorbances of the blank (buffer only) and control (buffer instead of extract) solutions were also read. The % inhibition was determined using the equation:

$$\%\ sucrase\ inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

2.7 Inhibitory concentration 50% (IC_{50}) value calculation

The concentration of each sample extract which can inhibit 50% of the enzyme activity or IC_{50} was computed including the standard drug acarbose for comparison. For each of the sample extract, a standard dose response curve was plotted using the different concentrations and the IC_{50} was calculated using the software Graph Pad Prism version 8.1.

2.8 Glucose diffusion inhibition assay

This inhibition assay proceeded following the methods of Roy and Mahalingam [4] with minor modifications using different concentrations of each sample and acarbose at 100, 200, 300, 400, and 500 µg/mL. Strips of 12000 MW dialysis membrane (Sigma Aldrich, USA) were prepared following the protocol provided by the manufacturer. The prepared dialysis membrane was soaked in distilled water and kept in the refrigerator at 4 °C prior to the analysis.

In a test tube, 2 mL of each sample and the acarbose solution were added with 2 mL of 0.15 M sodium chloride with 0.22 mM glucose solution, mixed, and then transferred to the prepared dialysis membrane with one end sealed. The other end of the dialysis membrane strips was also sealed and placed in 100 mL beakers containing 40 mL 0.15 M sodium chloride solution, and 10 mL distilled water. A magnetic stirrer was added at the bottom of the beaker and then placed in a stirring plate set at 200 rpm. Sample aliquot of 1 mL was taken from each beaker every 30 min for 3 hr. The collected sample aliquots were tested for glucose concentration using phenol sulfuric acid method using the protocols of Tamboli *et al.* in 2020 [26]. The relative inhibition of glucose

$$\%\ Inhibition\ of\ glucose\ movement = \frac{(glucose\ conc._{initial} - glucose\ conc._{final})}{glucose\ conc._{initial}} \times 100$$

movement (%) was computed as:

2.9 Statistical analysis

The assays were done in three trials for each concentration of all the samples as well as the acarbose and the calculated data were presented as mean ± standard deviation. The results were tested for homogeneity with Levene's test and evaluated by one-way analysis of variance (ANOVA) using the Minitab® version 18.1 (Minitab, Inc., USA). Tukey's Honestly Significant Difference (HSD) test ($p \leq 0.05$) then followed to determine the significant differences among the means.

3. Results

3.1 α-amylase, α-glucosidase, and sucrase inhibitory activities

The α-amylase inhibitory activity of the different processed bignay in comparison with the standard drug acarbose is presented in Figure 1. Results showed that all processed bignay inhibited α-amylase activity in a concentration-dependent manner (100–500 µg/mL) wherein a higher concentration resulted in higher inhibitory activity. For each concentration, the α-amylase

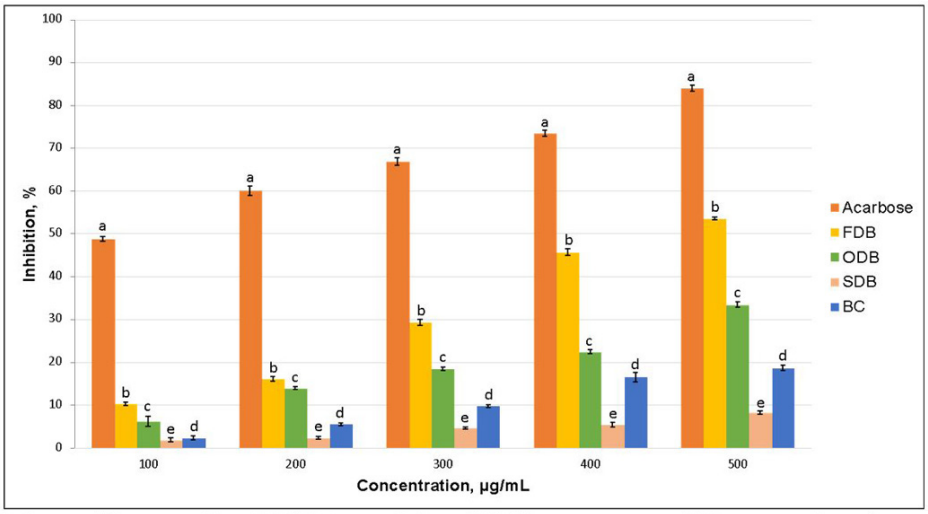

Figure 1. α-amylase inhibitory activity of different processed bignay and acarbose at different concentrations. Processed bignay samples: freeze dried bignay (FDB), oven dried bignay (ODB), spray dried bignay (SDB), and bignay concentrate (BC). Means keyed with different letters within each concentration indicate significant differences at $p < 0.05$.

inhibitory activity of all processed bignay were significantly different from each other and were all lower than the standard drug acarbose. Among the processed bignay at the 500 µg /mL concentration, FDB has the highest α-amylase inhibitory activity (53.47 ± 0.34%) followed by the ODB (33.42 ± 0.62%), BC (18.61 ± 1.04%), and lastly the SDB (8.19 ± 0.48%).

In addition, the α-glucosidase inhibitory activity of the processed bignay and the standard drug acarbose at different concentrations are summarized in Figure 2. Results also showed that all processed bignay inhibited α-glucosidase activity in a concentration-dependent manner (100–500 µg/mL) and at a higher concentration, the higher the inhibition. The inhibitory activity of α-glucosidase for all processed bignay showed significant differences with each other at each concentration and were also lower than acarbose. At 500 µg/mL, FDB also has the highest inhibitory activity (40.59 ± 1.01%) among the processed bignay against α-glucosidase followed by the ODB (28.63 ± 0.81%), the BC (18.95 ± 1.45%), while the SDB has the lowest inhibitory activity (13.98 ± 1.01%).

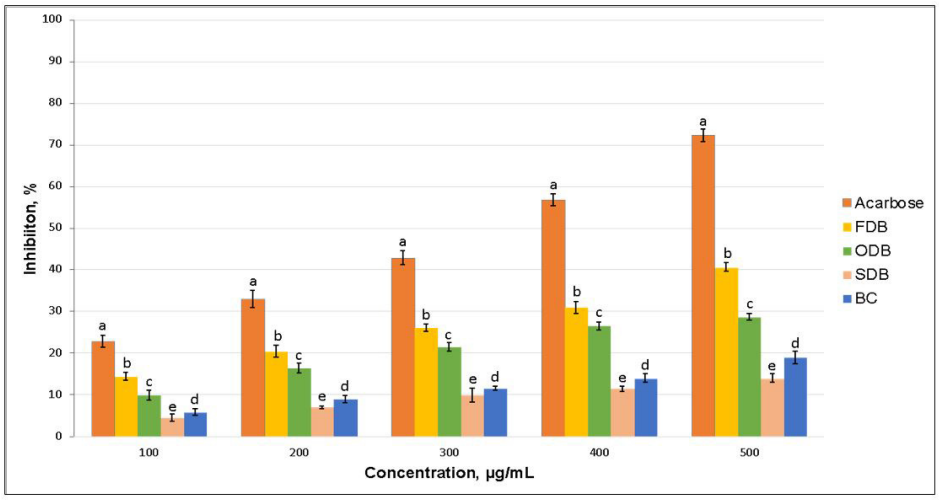

Figure 2. α-glucosidase inhibitory activity of different processed bignay and acarbose at different concentrations. Processed bignay samples: freeze dried bignay (FDB), oven dried bignay (ODB), spray dried bignay (SDB), and bignay concentrate (BC). Means keyed with different letters within each concentration indicate significant differences at $p < 0.05$.

Likewise, the processed bignay have also shown concentration-dependent (100–500 µg/mL) inhibitory effect on sucrase (Figure 3). The inhibitory effect of all processed bignay at the different concentrations were also lower than acarbose. Comparing the processed bignay at the highest concentration, FDB showed the highest inhibitory effect on sucrase (44.22 ± 1.82%), followed by the ODB (31.99 ± 1.42%), the BC (20.70 ± 1.01%), and lastly by the SDB (13.98 ± 0.84%).

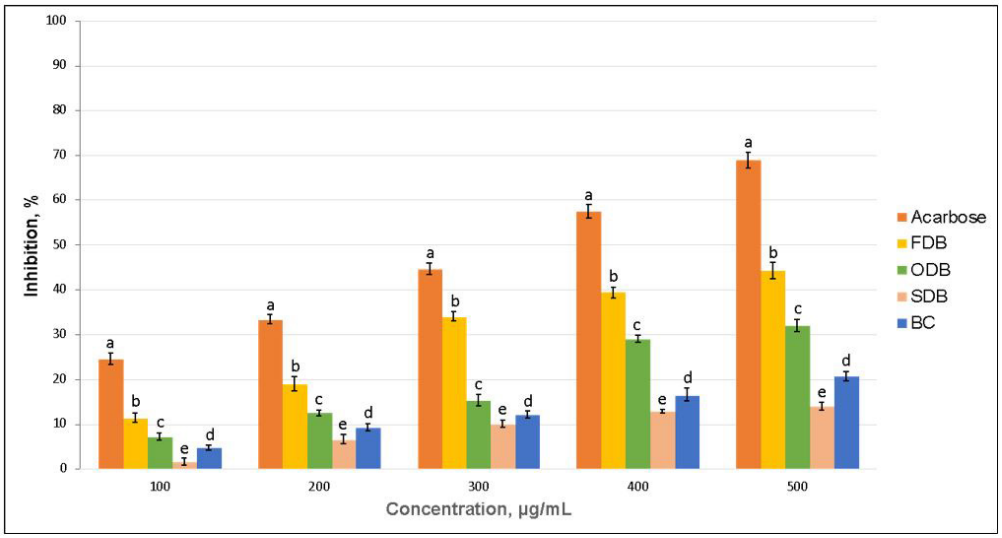

Figure 3. Sucrase inhibitory activity of different processed bignay and acarbose at different concentrations. Processed bignay samples: freeze dried bignay (FDB), oven dried bignay (ODB), spray dried bignay (SDB), and bignay concentrate (BC). Means keyed with different letters within each concentration indicate significant differences at $p < 0.05$.

The IC_{50} values of the different processed bignay and acarbose showed significant differences within the three tested carbohydrate-digesting enzymes as presented in Table 1. The IC_{50} values of acarbose for α-amylase (100.68 ± 7.97 µg/mL), α-glucosidase (336.61 ± 1.90 µg/mL), and sucrase (337.06 ± 3.44 µg/mL) indicated strong inhibition as compared to all processed bignay. Among the processed bignay, FDB is the most potent inhibitor against α-amylase, α-glucosidase, and sucrase having IC_{50} values of 464.30 ± 1.90 µg/mL, 675.93 ± 35.71 µg/mL, and 538.09 ±18.91 µg/mL, respectively, while the lowest was the SDB for all three tested enzymes. The substantial differences in the IC_{50} values of acarbose and the processed bignay is mainly because acarbose is a

Table 1. The IC_{50} values for α-amylase, α-glucosidase, and sucrase of processed bignay and acarbose.

Sample	IC_{50} values (µg/mL)		
	α-amylase	α-glucosidase	Sucrase
Acarbose	100.68 ± 7.97^{e}	336.61 ± 1.90^{e}	337.06 ± 3.44^{e}
FDB	464.30 ± 1.90^{d}	675.93 ± 35.71^{d}	538.09 ±18.91^{d}
ODB	794.12 ± 15.72^{c}	919.54 ± 10.05^{c}	767.54 ± 33.92^{c}
SDB	3196.02 ± 367.86^{a}	2062.21 ± 311.54^{a}	1637.56 ± 178.68^{a}
BC	1206.72 ± 33.15^{b}	1542.77 ± 181.59^{b}	1255.03 ± 70.05^{b}

Value Means ± SD (n=3); IC_{50}: Half-maximal inhibitory concentration
FDB = Freeze-dried bignay; ODB = Oven dried bignay at 50 °C; SDB = Spray dried bignay; BC = Bignay concentrate.
Means keyed with different letters within each column indicate significant differences at $p < 0.05$.

pure standard drug while the extracts from the processed bignay were crude and not purified.

3.2 Glucose diffusion inhibitory activity

The relative inhibition of glucose movement across a membrane of the different processed bignay and acarbose at 500 µg/mL and different time intervals is summarized in Figure 4. It can be observed that there is a constant decrease in the percent inhibition of glucose movement over time for all processed bignay as well as the acarbose. In addition, the relative inhibition of glucose movement of the FDB and ODB were not significantly different with each other from 30 to 90 min while the rest of the processed bignay were significantly different from each other at each time interval and were all lower than the standard drug acarbose. Among the processed bignay, FDB and ODB showed substantial amounts of inhibition while the SDB showed poor inhibition.

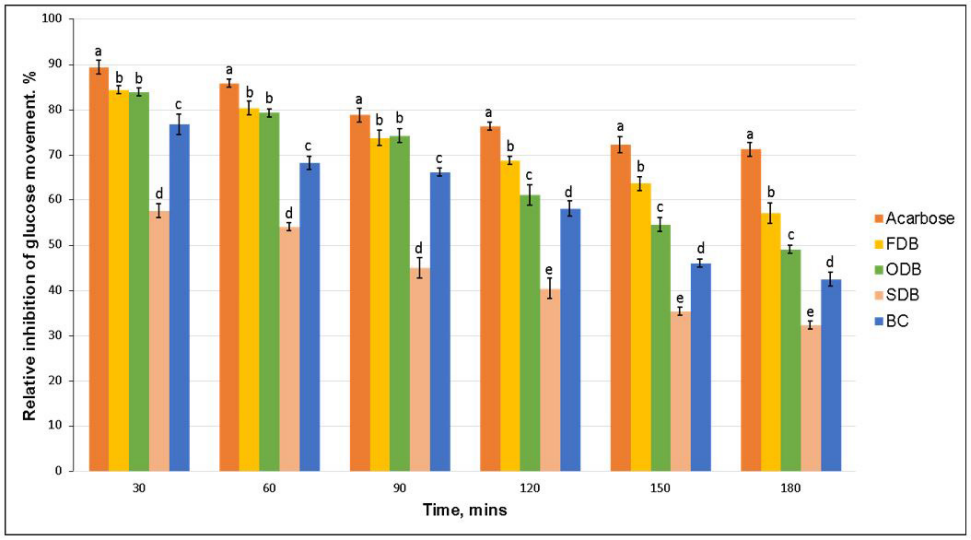

Figure 4. Glucose movement inhibition across a membrane of different processed bignay and acarbose at 500 µg/mL at different time intervals. Processed bignay samples: freeze dried bignay (FDB), oven dried bignay (ODB), spray dried bignay (SDB), and bignay concentrate (BC). Means keyed with different letters within each time indicate significant differences at $p < 0.05$.

4. Discussion

The inhibitory property of the processed bignay, specifically the FDB, against the enzymes responsible for carbohydrate digestion could be related to the phenolic compounds present. Several studies report that different phenolic compounds show inhibitory activities against α-amylase, α-glucosidase, and sucrase [6,23, 27-28]. Specifically, flavonoids, flavonols, and anthocyanins show inhibitory effect to α-amylase and α-glucosidase [1, 29] while eugenols, rosmarinic acids, luteolin, apigenin, glucosides, saponins were found to have sucrase inhibitory property [30-31].

Fresh bignay fruits are reported to have high total phenolics, total flavonoids, and total anthocyanins [19, 32] which have inhibitory effects towards α-amylase, sucrase [9] as well as α-glucosidase [10]. For processed bignay fruits, Carbonera et al. in 2023 [18] reported that freeze drying as well as oven drying significantly improved the total phenolic, total flavonoid, and total anthocyanin contents while spray drying and juice concentration have detrimental effects. In addition, the major phenolic compounds in dehydrated bignay are reported to be epicatechin, catechin, and gallic acid [18] and these have inhibitory effects towards α-amylase [32]. This study extends previous related studies by demonstrating that processing methods such as freeze drying can improve the antidiabetic activity of bignay fruit. But since phenolic compounds are heat sensitive due to their thermolabile molecular structure [33], the high temperature of spray drying (103–180 °C) and juice concentrating (80 °C) may have caused thermal degradation on the phenolic compounds, which in turn affected their inhibitory effects against α-amylase, α-glucosidase, and sucrase. Similar studies also reported that heat treatment at 100°C and higher

can decrease the activity of polyphenolic compounds to inhibit α-amylase [34] and α-glucosidase [35].

Glucose movement across the small intestines' brush border membrane plays an important role in the regulation of carbohydrate uptake where generally glucose is transferred by the sodium-dependent glucose transporter (SGLT1) and the facilitated-transporter glucose transporter (GLUT2) [36]. The processed bignay can decrease glucose movement because of the presence of polyphenols. The different phenolic compounds can suppress the absorption of glucose into the intestinal cells by inhibiting SGLT1 and GLUT2 [11, 37]. This is also in agreement with other studies on plants with high phenolic content and antioxidant activity such as *Caralluma europaea* [38], *Cleome viscora* [39], and *Phoenix roebelenii* leaves [4] which also demonstrated significant inhibitory effect on the movement of glucose through a dialysis membrane to an external solution at different time intervals. Furthermore, a study on the effect of spray drying on the blueberry juice polyphenolic compounds showed a 76-78% loss in the total phenolics and 57% loss on the anthocyanins [40]. Thus, the poor inhibition activity exhibited by the SDB is due to the high temperature of the spray drying process which resulted in the thermal degradation of the phenolic compounds in bignay.

While the *in vitro* assays in this study provide valuable insights into the inhibitory effects of processed bignay fruit on carbohydrate-digesting enzymes and glucose movement, they do not account for the complexities of physiological systems. Thus, further studies that include in vivo models to evaluate bioavailability, metabolism, and potential side effects in diabetic individuals should be considered.

5. Conclusions

Processed bignay displays potential antidiabetic activity by preventing the breakdown of complex carbohydrates through inhibition of the key carbohydrate-digesting enzymes as well as reducing the rate of glucose movement across a membrane. The inhibitory effect of the processed bignay is more towards α-amylase, followed by sucrase, and lastly towards α-glucosidase – all of which are concentration-dependent. Among the processed bignay, the FDB had the highest inhibitory activity against the three tested enzymes and on the inhibition of glucose movement. Therefore, it is recommended to use this drying process in converting bignay fruit into a functional ingredient. Since this study included only *in vitro* assays, further validation through in vivo animal models or clinical studies is recommended to confirm and validate the observed effects.

Availability of Data and Materials

All data are available in this study

Author Contributions

Conceptualization, Resources, Funding acquisition, and Writing – Review & Editing, A.F.A.C, L.M.A, M.A.C.E, S.M.D., R.C.M.L.A., and K.A.T.C.I.; Methodology, A.F.A.C. and K.A.T.C.I.; Investigation and Writing – Original Draft, A.F.A.C.; Supervision and Project administration, L.M.A, M.A.C.E, S.M.D., R.C.M.L.A., and K.A.T.C.I.

Ethics Approval and Consent to Participate

Not applicable

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Not applicable

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Conflict of Interest

The authors of this study declare no conflict of interest.

References

[1] Bhatia, A., Singh, B., Arora, R., & Arora, S. (2019). *In vitro* evaluation of the α-glucosidase inhibitory potential of methanolic extracts of

traditionally used antidiabetic plants. *BMC Complementary and Alternative Medicine, 19,* 74. https://doi.org/10.1186/s12906-019-2482-z.

[2] Elhefnawy, M.E., Ghadzi, S.M.S., & Noor Harun, S. (2022). Predictors associated with type 2 diabetes mellitus complications over time: A literature review. *Journal of Vascular Diseases,* 1(1), 013-023. https://doi.org/10.3390/jvd1010003.

[3] Galindo, R.J., Trujillo, J.M., Low Wang, C.C., & McCoy, R.G. (2023). Advances in the management of type 2 diabetes in adults. *BMJ Medicine, 2,* e000372. https://doi.org/10.1136/bmjmed-2022-000372.

[4] Roy, M. & Mahalingam, G. (2017). The *in-vitro* antidiabetic activity of *Phoenix roebelenii* leaf extract. *International Journal of Green Pharmacy,* 11(1), S128-S134. https://doi.org/10.22377/ijgp.v11i01.884.

[5] Gong, L., Feng, D., Wang, T., Ren, Y., Liu, Y., & Wang, J. (2020). Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. *Food Science & Nutrition,* 8(12), 6320-6337. https://doi.org/10.1002/fsn3.1987.

[6] Antora, R.A., Rabeta, M.S., & Norazatul Hanim, M.R. (2018). Evaluation of *in vitro* sucrase inhibitory and non-enzymatic glycation properties of *Ocimum tenuiflorum* L. leaves. *Food Research,* 2(4), 368-377. https://doi.org/10.26656/fr.2017.2(4).076.

[7] Li, X., Bai, Y., Jin, Z., & Svensson, B. (2022). Food-derived non-phenolic α-amylase and α-glucosidase inhibitors for controlling starch digestion rate and guiding diabetes-friendly recipes. *LWT, 153,* 112455. https://doi.org/10.1016/j.lwt.2021.112455.

[8] Li, W.T., Chuang, Y.H., & Hsieh, J.F. (2019). Characterization of maltase and sucrase inhibitory constituents from *Rhodiola crenulate. Foods, 8*(11), 540. https://doi.org/10.3390/foods8110540.

[9] Aksornchu, P., Chamnansilpa, N., Adisakwattana, S., Thilavech, T., Choosak, C., Marnpae, M., Makynen, K., Dahlan, W., & Ngamukote, S. (2020). Inhibitory effect of *Antidesma bunius* fruit extract on carbohydrate digestive enzymes activity and protein glycation *in vitro. Antioxidants, 10*, 32. https://doi.org/10.3390/antiox10010032.

[10] Quiming, N., Asis, J.L., Nicolas, M., Versoza, D., & Alvarez, M.R. (2016). *In vitro* α-glucosidase inhibition and antioxidant activities of partially purified *Antidesma bunius* fruit and *Gynura nepalensis* leaf extracts. *Journal of Applied Pharmaceutical Science, 6(*5), 97-101. https://doi.org/10.7324/JAPS.2016.60515.

[11] Golovinskaia, O. & Wang, C.K. (2023). The hypoglycemic potential of phenolics from functional foods and their mechanisms. *Food Science and Human Wellness, 12,* 986-1007. https://doi.org/10.1016/j.fshw.2022.10.020.

[12] Grussu, D., Stewart, D., & McDougall, G.J. (2011). Evaluation of the nutritional, phytochemical and antioxidant properties of the peels of some selected mango varieties. *Journal of Agricultural and Food Chemistry, 59*(6), 2324-2331. https://doi.org/10.12691/ajfst-5-5-2.

[13] Islam, S., Ahammed, S., Sukorno, F.I., Koly, S.F., Biswas, M., & Hossain, S. (2018). A review on phytochemical and pharmacological potentials of *Antidesma bunius*. *Journal of Analytical & Pharmaceutical Research, 7*(5), 602-604. https://doi.org/10.15406/japlr.2018.07.00289.

[14] Yusif, M., Pirman, Fitriani, U.N., Amri, I., & Juwita, A.I. (2021). Identification of polyphenols and α-amylase inhibitory activity of multi herbal formulation: Cocoa beans *(Theobroma cocoa),* buni *(Antidesma bunius L. Spreng)* and cinnamons *(Cinnamomum cassia). Journal of Physics: Conference Series,* 1783. https://doi.org/10.1088/1742-6596/1783/1/012004.

[15] Ratnadewi, A.A.I., Wahyudi, L.D., Rochman, J., Susilowati, Nugraha, A.S., & Siswoyo, T.A. (2020). Revealing anti-diabetic potency of medicinal plants of Meru Betiri National Park, Jember – Indonesia. *Arabian Journal of Chemistry,* 13(1), 1831-1836. https://doi.org/10.1016/j.arabjc.2018.01.017.

[16] Mauldina, M.G., Sauriasari, R., & Elya, B. (2017). α-Glucosidase inhibitory activity from ethyl acetate extract of *Antidesma bunius* (L.) Spreng stem bark containing triterpenoids. *Pharmacognosy Magazine,* 13(52), 590-594. https://doi.org/10.4103/pm.pm_25_17.

[17] Vaishali, H.P.S., Uttam, D., Sugandha, S., & Arpit, P. (2020). Effect of freezing systems and storage temperatures on overall quality of perishable food commodities. *The Pharma Innovation Journal, 9*(9), 114-122.

[18] Carbonera, A.F.A., Atienza, L.M., Estacio, M.A.C., Duque, S.M.M., Lizardo-Agustin, R.C.M., Flandez, L.E.L., & Castillo-Israel, K.A.T. (2023). Effects of various processing methods on the dietary fiber and antioxidant properties of Bignay *(Antidesma bunius* L. Spreng*)* fruit. *Food Chemistry Advances, 3,* 100561. https://doi.org/10.1016/j.focha.2023.100561.

[19] Castillo-Israel, K.A.T., Sartagoda, K.J.D., Ilano, M.C.R., Flandez, L.E.L., Compendio, M.C.M., & Morales, D.B. (2020). Antioxidant properties of Philippine Bignay *(Antidesma bunius* (Linn.) Spreng cv. 'Common') flesh and seeds as affected by fruit maturity and heat treatment. *Food Research, 4*(6), 1980-1987. https://doi.org/10.26656/fr.2017.4(6).215.

[20] Garau, M.C., Simall, S., Rosello´, C., & Femenia, A. (2007). Effect of air-drying temperature on physico-chemical properties of dietary fibre and antioxidant capacity of orange (*Citrus aurantium* v. Canoneta) by-products. *Food Chemistry, 104*, 1014-1024. https://doi.org/10.1016/j.foodchem.2007.01.009.

[21] Phisut, N. (2012). Spray drying technique of fruit juice powder: Some factors influencing the properties of product. *International Food Research Journal, 19*(4), 1297-1306.

[22] Kosters, P.S.R. & Waldron, K.W. (2009). Handbook of waste management and co-product recovery in food processing Volume 2. Woodhead Publishing. Cambridge, England.

[23] Sayah, K., Marmouzi, I., Mrabti, H.N., Cherrah, Y., & Faouzi, M.E. (2017). Antioxidant activity and inhibitory potential of *Cistus salviifolius* (L.) and *Cistus monspeliensis* (L.) aerial parts extracts against key enzymes linked to hyperglycemia. *Biomed Research International,* 2789482. https://doi.org/10.1155/2017/2789482.

[24] Shettar, A.K., Sateesh, M.K., Kaliwal, B.B., & Vedamurthy, A.B. (2017). *In vitro* antidiabetic activities and GC-MS phytochemical analysis of *Ximenia americana* extracts. *South African Journal of Botany, 111*, 202-211. https://doi.org/10.1016/j.sajb.2017.03.014.

[25] Ma, Y. & Liu, B. (2020). Preparation and α-glucosidase inhibitory activity of gallic acid-dextran conjugate. *Natural Product Communications, 15*(8), 1-5. https://doi.org/10.1177/1934578X20941289.

[26] Tamboli, F.A., More, H.N., Bhandugare, S.S., Patil, A.S., Jadhav, N.R., & Killedar, S.G. (2020). Estimation of total carbohydrate content by phenol sulphuric acid method from *Eichhornia crassipes* (mart.) Solms. *Asian Journal of Research in Chemistry, 13*(5), 357-359. https://doi.org/10.5958/0974-4150.2020.00067.X.

[27] Ishnava, K., Kotadia, R., & Patel, S. (2015). Nutritional properties and chemical composition of *Corallocarpus epigaeus* (Arn.) Cl: As remedy to control diabetes mellitus. *Chiang Mai Journal of Science, 42*(4), 806-815.

[28] Wongsa, P., Chaiwarith, J., Voranitikul, J., Chaiwongkhajorn, J., Rattanapanone, N., & Lanberg, R. (2019). Identification of phenolic compounds in colored rice and their inhibitory potential against α-amylase. *Chiang Mai Journal of Science, 46*(4), 672-682.

[29] Moein, S., Moein, M., & Javid, H. (2022). Inhibition of α-amylase and α-glucosidase of anthocyanin isolated from *Berberis integerrima* Bunge fruits: A model of antidiabetic compounds. *Evidence-Based Complementary and Alternative Medicine,* 6529590. https://doi.org/10.1155/2022/6529590.

[30] Verma, S. (2016). Chemical constituents and pharmacological action of *Ocimum sanctum* (Indian holy basil-Tulsi). *The Journal of*

Phytopharmacology, 5(5), 205-207. https://doi.org/10.31254/phyto.2016.5507.

[31] Kaewnarin, K. & Rakariyatham, N. (2017). Inhibitory effects of phenolic compounds in *Ocimum sanctum* extract on the α-glucosidase activity and the formation of advanced glycation end-products. *Chiang Mai Journal of Science, 44*(1), 203-214.

[32] Muhammad, M., Zhu, Y., Wen, A., Liu, N., & Qin, L. (2022) Phenolic profile, alpha-amylase inhibitory activity, and *in vitro* glycemic index of adzuki beans. *Frontiers in Nutrition, 9*, 1063602. https://doi.org/10.3389/fnut.2022.1063602.

[33] Esparza, I., Cimminelli, M.J., Moler, J.A., Jim´enez-Moreno, N., & Ancín-Azpilicueta, C. (2020). Stability of phenolic compounds in grape stem extracts. *Antioxidants, 9*(8), 720. https://doi.org/10.3390/antiox9080720.

[34] Oboh, G., Akinyemi, A.J., & Ademiluyi, A.O. (2012). Inhibition of α-amylase and α-glucosidase activities by ethanolic extract of *Telfairia occidentalis* (fluted pumpkin) leaf. *Asian Pacific Journal of Tropical Biomedicine, 2*(9), 733-738. https://doi.org/10.1016/S2221-1691(12)60219-6.

[35] Hardoko, H., Sasmito, B.B., Puspitasari, Y.E., Okviani, Y., & Halim, Y. (2018). The effect of heating temperature on inhibitory activity of mangrove *Rhizophora mucronate* fruit extract toward α-glucosidase. *Asian Journal of Pharmaceutical and Clinical Research, 11*(7), 237. https://doi.org/10.22159/ajpcr.2018.v11i7.25175.

[36] Pico, J. & Martinez, M.M. (2019). Unraveling the inhibition of intestinal glucose transport by dietary phenolics: A review. *Current Pharmaceutical Design, 25*(32), 3418-3433. https://doi.org/ 10.2174/1381612825666191015154326.

[37] Müller, U., Stübl, F., Schwarzinger, B., Sandner, G., Iken, M., Himmelsbach, M., Schwarzinger, C., Ollinger, N., Stadlbauer, V., Höglinger, O., Kühne, T., Lanzerstorfer, P., & Weghuber, J. (2018). *In vitro* and *in vivo* inhibition of intestinal glucose transport by guava *(Psidium Guajava)* extracts. *Molecular Nutrition & Food Research, 62,* 1701012. https://doi.org/10.1002/mnfr.201701012.

[38] Ouassou, H., Bouhrim, M., Bencheikh, N., Addi, M., Hano, C., Mekhfi H, Ziyyat, A., Legssyer, A., Aziz, M., & Bnouham, M. (2021). *In vitro* antioxidant properties, glucose-diffusion effects, α-amylase inhibitory activity, and antidiabetogenic effects of C. *Europaea* extracts in experimental animals. *Antioxidants (Basel), 10*(11), 1747. https://doi.org/10.3390/antiox10111747.

[39] Suresh, Y., Rajasekar, G., Lavanya, T., Lakshminarsimhulu, B., Reddy, K.S., & Reddy, S.D. (2020). Antioxidant and antidiabetic properties of isolated fractions from methanolic extract derived from the whole plant of *Cleome viscosa* L. *Future Journal of Pharmaceutical Science, 6,* 103.

[40] Wilkowska, A., Ambroziak, W., Czyżowska, A., & Adamiec, J. (2016). Effect of microencapsulation by spray drying and freeze drying technique on the antioxidant properties of blueberry *(Vaccinium myrtillus)* juice polyphenolic compounds. *Polish Journal of Food and Nutrition Sciences, 66*(1),11-16. https://doi.org/10.1515/pjfns-2015.