EXTRACTION, OPTIMIZATION, AND SCREENING OF SELECTED AGRICULTURAL WASTES FOR PECTIN AND POTENTIAL PECTINASE PRODUCTION

ABSTRACT

Pectinases are a group of enzymes that degrade pectin into simpler units; as pectins are the substrates being degraded by pectinases. The enzymes have several industrial uses such as in the extraction and clarification of fruit juices. In this study, pectin was extracted from three agricultural wastes, namely: plantain (*Musa paradesiaca*) peels, orange (*Citrus sinensis*) peels, and the African Star Apple (*Chrysophyllum albidum*) locally called agbalumo peels. The extracted conditions: pH and boiling duration were optimized, and the extracted pectins were employed as substrates for assessing *Aspergillus niger* pectinase production potential. The results showed that agbalumo (*Chrysophyllum albidum*) peel had a higher yield ($p<0.05$) of pectin production compared to plantain (*Musa paradisiaca*), and orange (*Citrus sinensis*) peels. Relatively highly acidic medium (pH 2 to 3) and boiling durations of 45 to 60 minutes were optimal ($p<0.05$) for the extraction of pectin from the three agricultural wastes. The zone of hydrolysis of pectin using *Aspergillus niger* as pectinase source and modified Czapek media showed that pectin extracted from agbalumo (*Chrysophyllum albidum*) peel to be the best substrate with the largest zone of hydrolysis. The study concluded that the investigated fruit wastes have good prospects for the local production of pectin and the pectin could be employed as substrate during pectinase production.

Keywords: Agricultural wastes, Hydrolysis, Pectin extraction, Optimization, Pectinase.

INTRODUCTION

Citrus fruits used in industrial processing produces more than 10 million tons of waste annually (Zema et al. 2018). Fruits and vegetables are particularly important for nutrition because they include components such as pectins that help to control or promote digestion, function as laxatives or diuretics, and phenolic compounds to help maintain the pH of the intestines (Ibeawuchi et al. 2015).

Fruit's primary constituent, pectin, causes a gel to develop when heated and combined with sugar. Pectin is typically described as water-soluble pectinic acids with varied methyl ester concentrations that, under the right conditions, can form gels alongside sugar and acid. Pectin is a frequent cooking component because of its excellent gelling ability. Pectin is made up of linearly linked - α (1, 4) linked D-galacturonic acid units. The neutral sugar rhamnogalacturonan, which is also present in pectin molecules, is what splits and kinks the galacturonic acid chain (Narasimman & Sethuraman, 2016; Thakur et al. 1997). Plant cell walls contain protopectin, which is used to make pectin (Mohnen, 2008). Sources of pectin include fruits like citrus, and *Thaumatococcus daniellii* (Ametefe et al. 2022). Also, apple production generates waste like pomace, seeds, and peels, of which pectin is a constituent (Kumar et al. 2020). These various by-products mainly consists of monosaccharaides and disaccharides which are non-soluble carbohydrates (Zacharof, 2017).

Pectin is a complex mixture of polysaccharides found in virtually all primary cell walls of plant cells. It is especially prevalent in the non-woody sections of most terrestrial plants. Pectin can be found in both the intermediary lamella between plant cells and the primary cell walls, where it aids in the bonding of the cells. Pectin amounts, structures, and chemical composition vary

between plants, over time within a plant, and in various regions of a single plant (Srivastava & Malviya, 2011).

Pectin serves as a soluble dietary fiber. Hence, its consumption has been demonstrated to lower blood cholesterol levels. Pectin consumption makes the gastrointestinal tract viscous and reduces the absorption of cholesterol from the diet. Microorganisms are crucial in the breakdown of pectin, especially in the large intestine and colon, and they aid in the release of short-chain fatty acids that have positive effects on human health (Wakerly et al. 1996). Due to its beneficial effects on health, thickening, gelling, and emulsification capabilities, this biomolecule has been employed extensively in the food and pharmaceutical sectors for decades (Li et al. 2021).

The pectin enzymes (pectinase) have been implicated in the breakdown of pectin during the ripening process in plants, which causes the fruits to soften. This occurs as a result of the middle lamella, which is mostly made of pectin, breaking down from the enzyme action, leading to cell separation (Srivastava & Malviya, 2011).

Industry has utilized pectinases to break down the cell walls of plants. These enzymes now hold a 25 % market share of the world's food and beverage enzymes and are acknowledged as eco-friendly biocatalysts (Amin et al. 2019).

When it comes to factors like temperature, extraction duration, pH, among others, there is reported difference in the yield of pectin, the substrate for pectinase production (Munarin et al. 2012). It is for the above, that this study investigates the peels of plantain (*Musa paradisiaca*), agbalumo (*Chrysophyllum albidum*), and orange (*Citrus sinensis*) for pectin production and explore its potential as substrate for pectinase.

MATERIALS AND METHODS

SAMPLE COLLECTION

The samples, plantain (*Musa paradisiaca*), agbalumo (*Chrysophyllum albidum),* and orange (*Citrus sinensis*) peels were sourced from Obada, Ipetumodu, Osun State, and peeled.

PECTIN EXTRACTION

The fruit peels were obtained and sun-dried for about 5-7 days until they were completely dried. The dried peels were cut into small pieces to reduce surface area and weighed separately using an electronic weighing scale. The peels were placed in a beaker of distilled water at pH 2.7 and boiled at 100 °C for about 60 min.

After boiling, the sample was filtered using a Muslin cloth and left overnight. The residue (plant peels) was discarded and the filtrate was measured (Ametefe et al. 2022).

Each of the filtrates obtained was measured, transferred into beakers, and placed in the oven at 70 °C for three days to further concentrate by evaporation. The reduced samples were weighed. Percent pectin, calculated using the formula, weight of pectin concentrate (g)/ weight of Peel (g) x 100 [14].

OPTIMIZATION PROCESS FOR MAXIMUM PECTIN PRODUCTION:

EFFECT OF PH ON PECTIN PRODUCTION

The effect of pH pectin production was measured at different pH values ranging from 2 to 5. The pH was adjusted using 1M HCl (hydrochloric acid) and 1M NaOH (sodium hydroxide).

EFFECT OF BOILING DURATION ON PECTIN PRODUCTION

The peels were separately boiled at the optimum pH (obtained previously in the above optimized experiment) for 15 to 60 minutes. After which the content was filtered using a Muslin cloth for each of the peels. The plantain (*Musa paradisiaca*), agbalumo (*Chrysophyllum albidum*), and orange (*Citrus sinensis*) peel pectin extracts were collected separately in a beaker.

MICROORGANISMS AND SCREENING FOR PECTINASE PRODUCTION

The *Aspergillus niger* in Ametefe et al. (2021) was used for the determination of the zone of hydrolysis for pectinase activity using the modified Czapek medium (substituting pectin extract for cellulose) (Chinedu et al. 2010).

DATA ANALYSIS

The data obtained were analyzed using the Microsoft Excel version 2016 (Washington, United States of America -USA). The charts drawn, and analysis of variance (ANOVA) and t-test obtained with a 5 % probability value (p-value), were performed using the same software.

RESULTS AND DISCUSSION

The results showed that agbalumo (*Chrysophyllum albidum*) peel produced highest pectin but not significantly ($p<0.05$) more than orange (*Citrus sinensis*) peel; however, the pectin in the agbalumo (*Chrysophyllum albidum*) peel was significantly ($p<0.05$) more than plantain (*Musa paradisiaca*) peel (Figure 1).

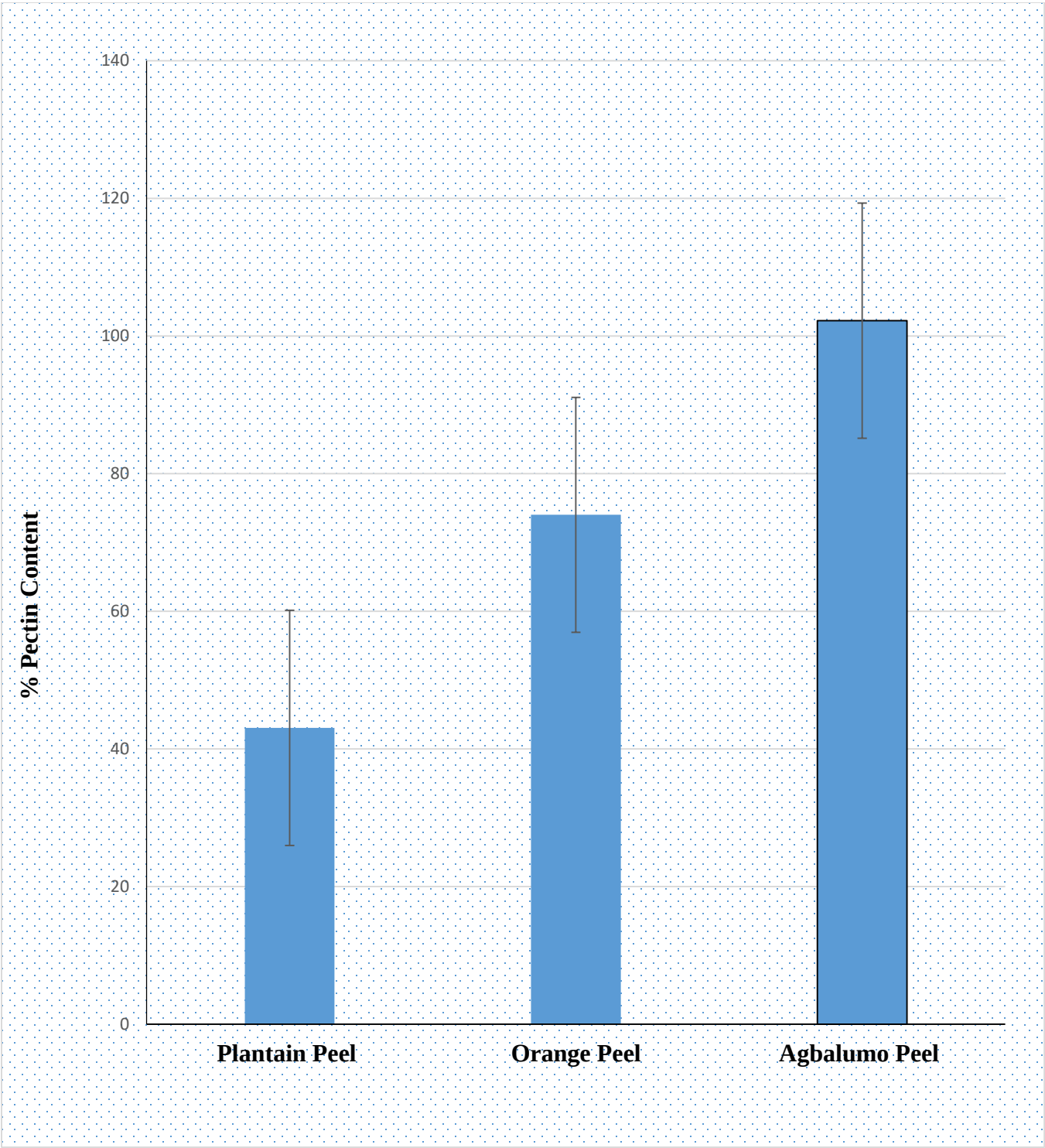

Figure 1: Yield of pectin from agrowastes

Bars represent Mean ± Standard Error

The significant ($p<0.05$) increase in pectin content of agbalumo (*Chrysophyllum albidum*) peel signified that it had the most pectin; however, the yield obtained in this study is lower than the as reported for the flavedo of *Citrrus sinensis* peel (Ametefe et al. 2022).

The effect of pH on the pectin production from plantain (*Musa paradisiaca*), orange (*Citrus sinensis*), and the agbalumo (*Chrysophyllum albidum*) peel was investigated at pH 2.0-5.0. It was observed that the three agricultural wastes produced highest pectin at highly acidic pH (pH 2 and 3). Beyond pH 3, a decline in pectin yield was observed for the three pectin sources (Figures 2, 3, and 4). However, agbalumo (*Chrysophyllum albidum*) peels showed the highest pectin extract of 3.72 %, while orange (*Citrus sinensis*) and plantain (*Musa paradisiaca*) peels resulted in 2.52 % and 1.85 % respectively. I think there's a need to site one or two researcher who have done something similar and the research for the high yeild at acidic pH. Parhaps because of dissolution ability of acid.

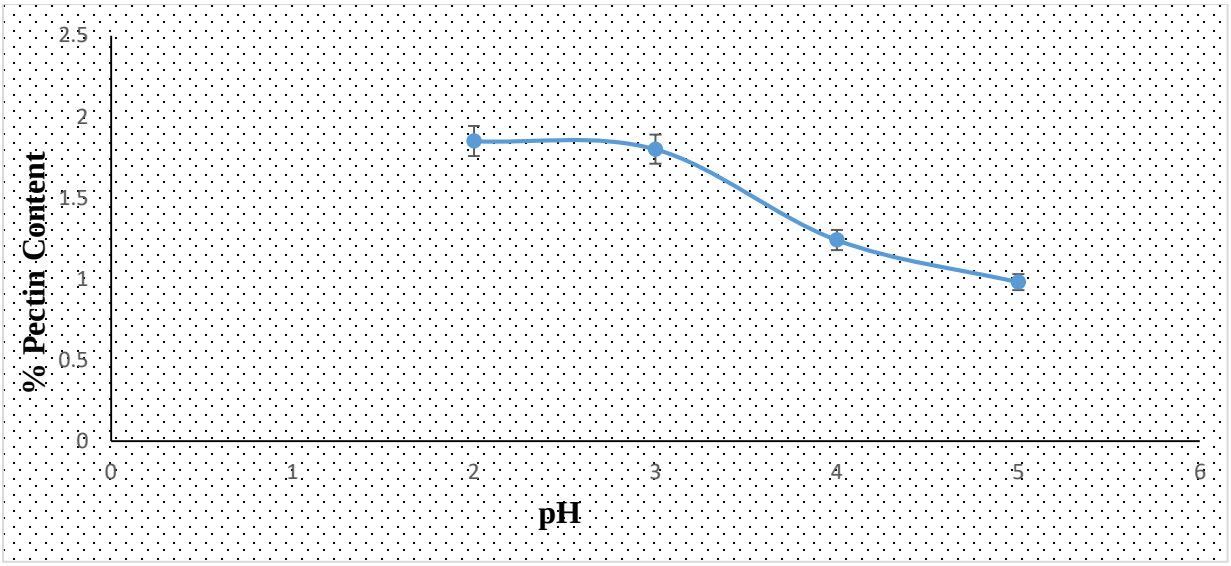

Figure 2: Effect of pH on extraction of plantain (*Musa paradisiaca*) pectin

Bars represent Mean ± Standard Error

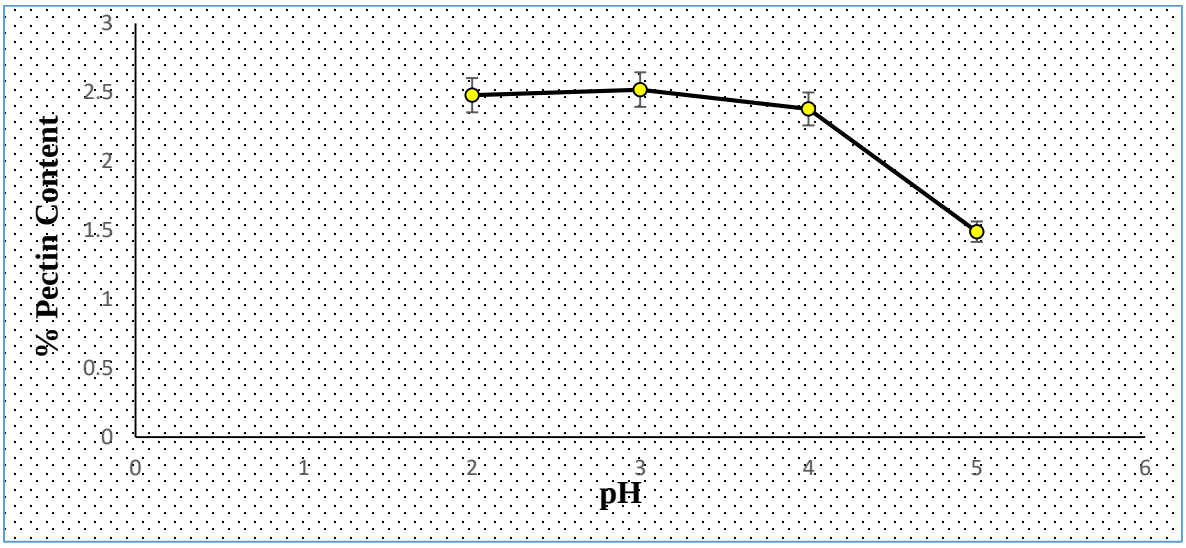

Figure 3: Effect of pH on extraction of orange (*Citrus sinensis*) pectin

Bars represent Mean ± Standard Error

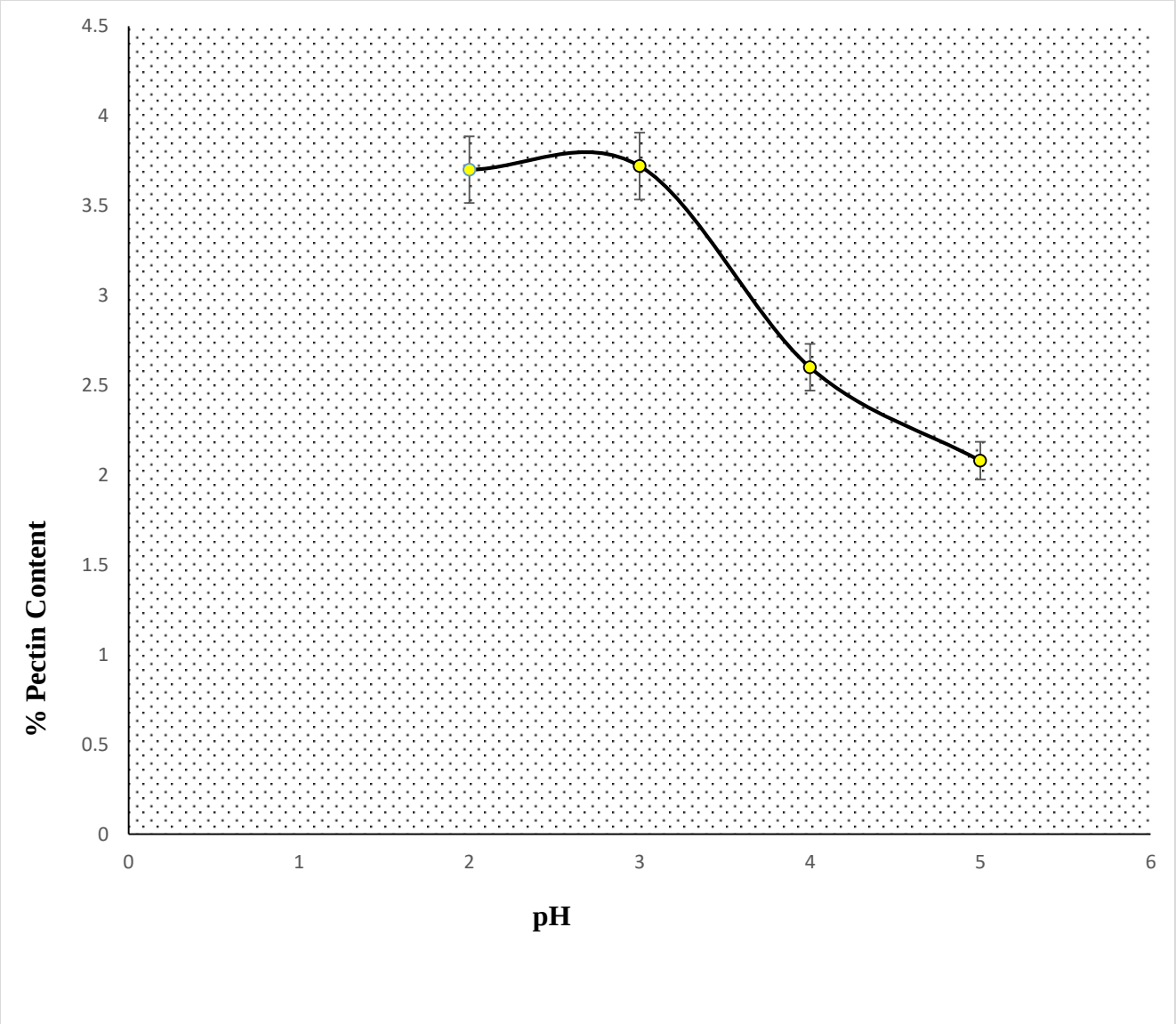

Figure 4: Effect of pH on extraction of agbalumo (*Chrysophyllum albidum*) pectin

Bars represent Mean ± Standard Error

According to studies, pectin molecules can be partially soluble in plant tissues without degradation using weakly acidic aqueous solvents, but some pectin fractions are not extractable in this way (Udonne et al. 2016). This is due to the probably stronger attachment of the pectins to other cell wall components. To extract these forms of pectin, acid hydrolysis was performed to enhance the pectin molecules' tendency to precipitate (Ametefe et al. 2022; Karim et al. 2014). In another related study, a pH of 1.5 was recorded for pectin extraction from sweet potatoes (Hamidon & Zaidel, 2017). Protopectin is reportedly hydrolyzed more quickly when there is a high concentration of hydrogen ions present in the solvent (at a low pH); further justifying the lower pH for better pectin extraction (Kertesz, 1951). However, the difference in pH values with the present study could be due to the differences in the source of the agricultural wastes used for the extraction of pectin.

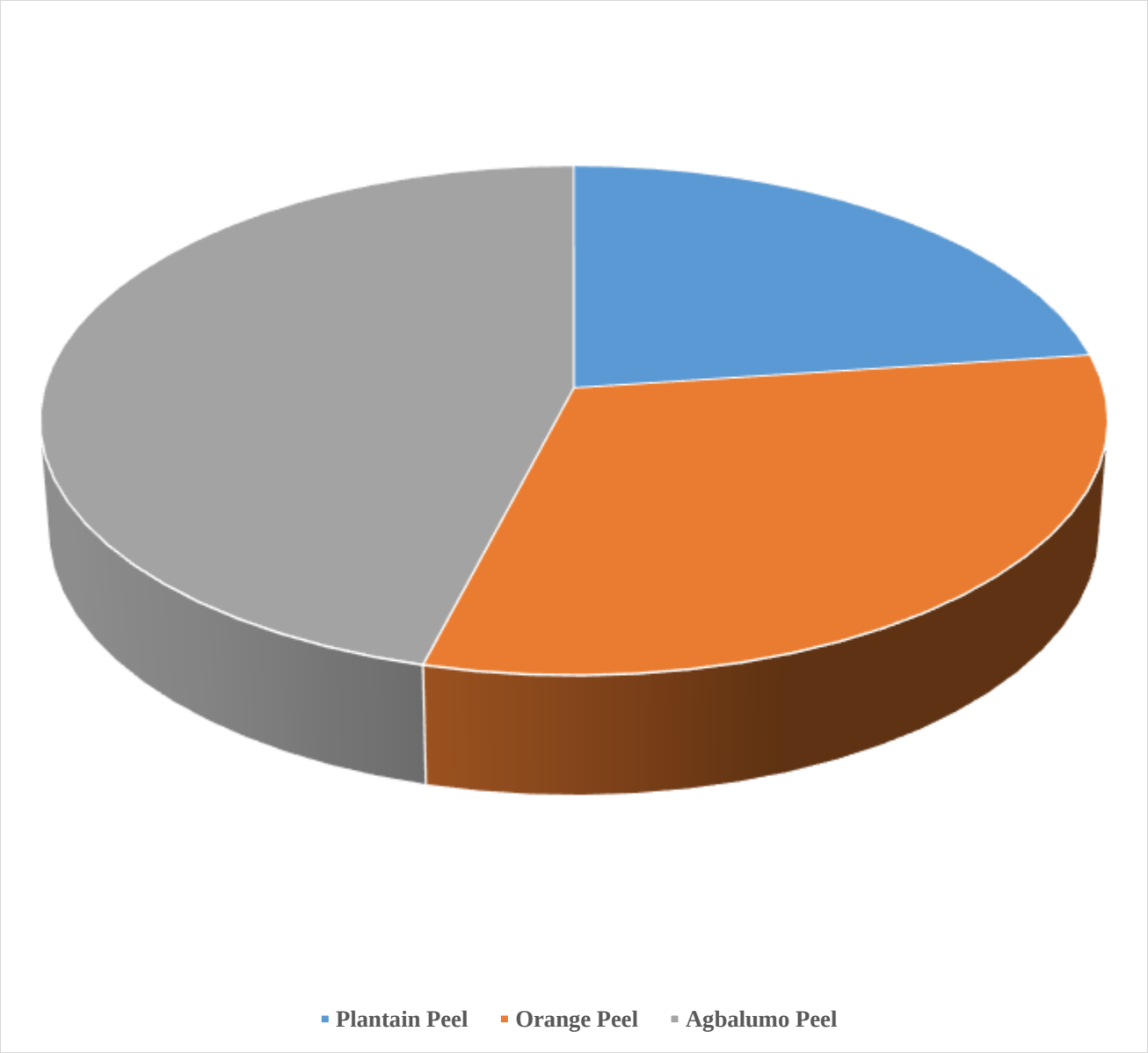

Figure 5: Optimal pH extraction for the three extracted pectins

Bars represent Mean ± Standard Error

The increase in agbalumo (*Chrysophyllum albidum*) pectin over orange (*Citrus sinensis*) peel could come from the fact that the albedo was more in the agbalumo (*Chrysophyllum albidum*) from physical examination compared to the more flavedo in the orange peels. So, the acidic medium favoured the breakdown of the cell walls in the pectin-rich albedo of agbalumo (*Chrysophyllum albidum*) compared to the more flavedo-rich orange (*Citrus sinensis*) and plantain (*Musa paradisiaca*) peels (Figure 5) (Ametefe et al. 2022).

The effect of the duration of boiling on the substrates was obtained at 100 °C. The optimization of the duration was from 15-60 minutes. The effect of temperature duration showed that higher durations of boiling increased the yield of pectin extraction to a peak of about 45 to 60 minutes (Figures 6, 7, and 8). Figure 9 showed that the optimum boiling duration obtained for the agricultural wastes indicated more capacity for the pectin extracted from orange (*Citrus sinensis*) peels than that from plantain (*Musa paradisiaca*) peels; with the agbalumo (*Chrysophyllum albidum*) peel being the highest.

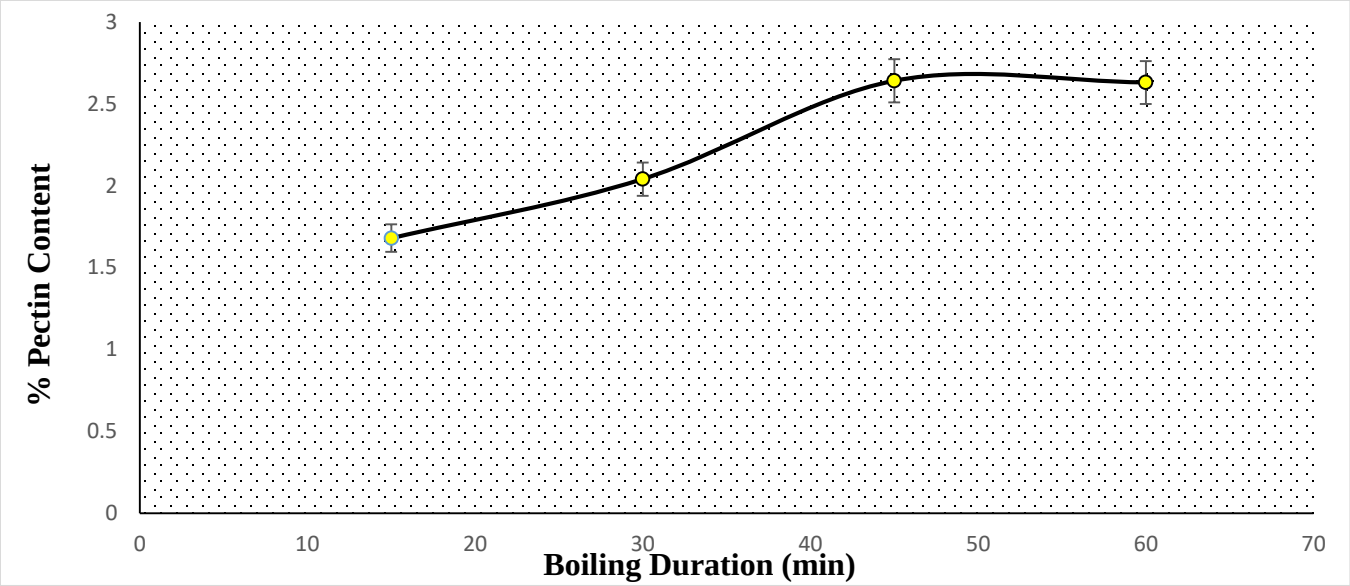

Figure 6: Effect of boiling durations on the extraction of orange (*Citrus sinensis*) pectin

Bars represent Mean ± Standard Error

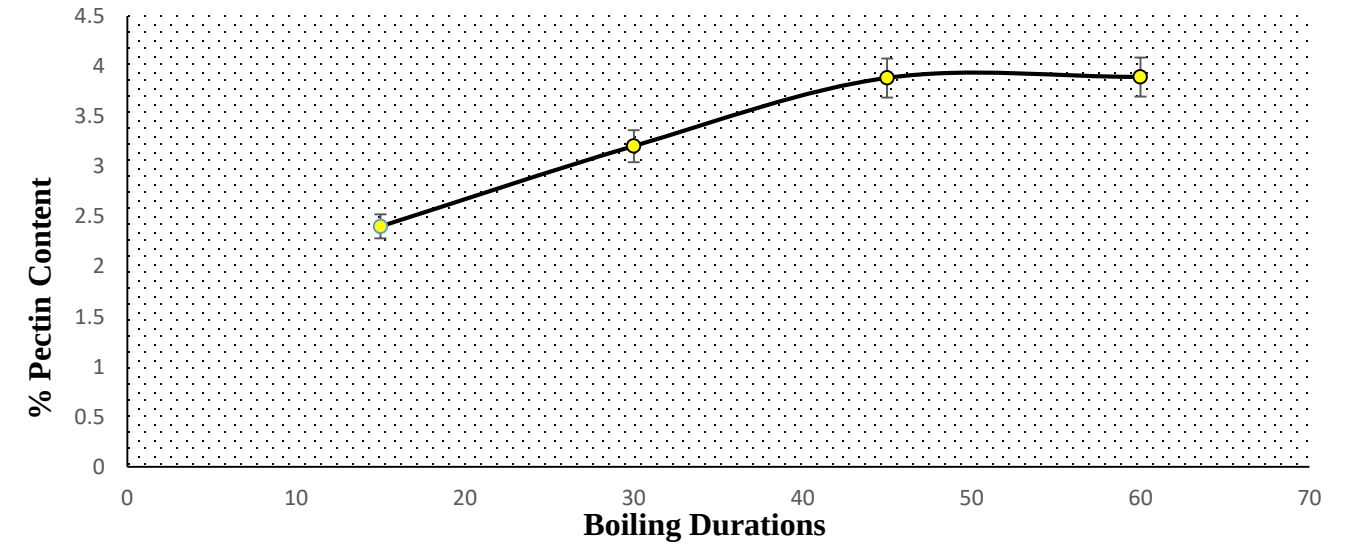

Figure 7: Effect of boiling durations on the extraction of agbalumo (*Chrysophyllum albidum*) pectin

Bars represent Mean ± Standard Error

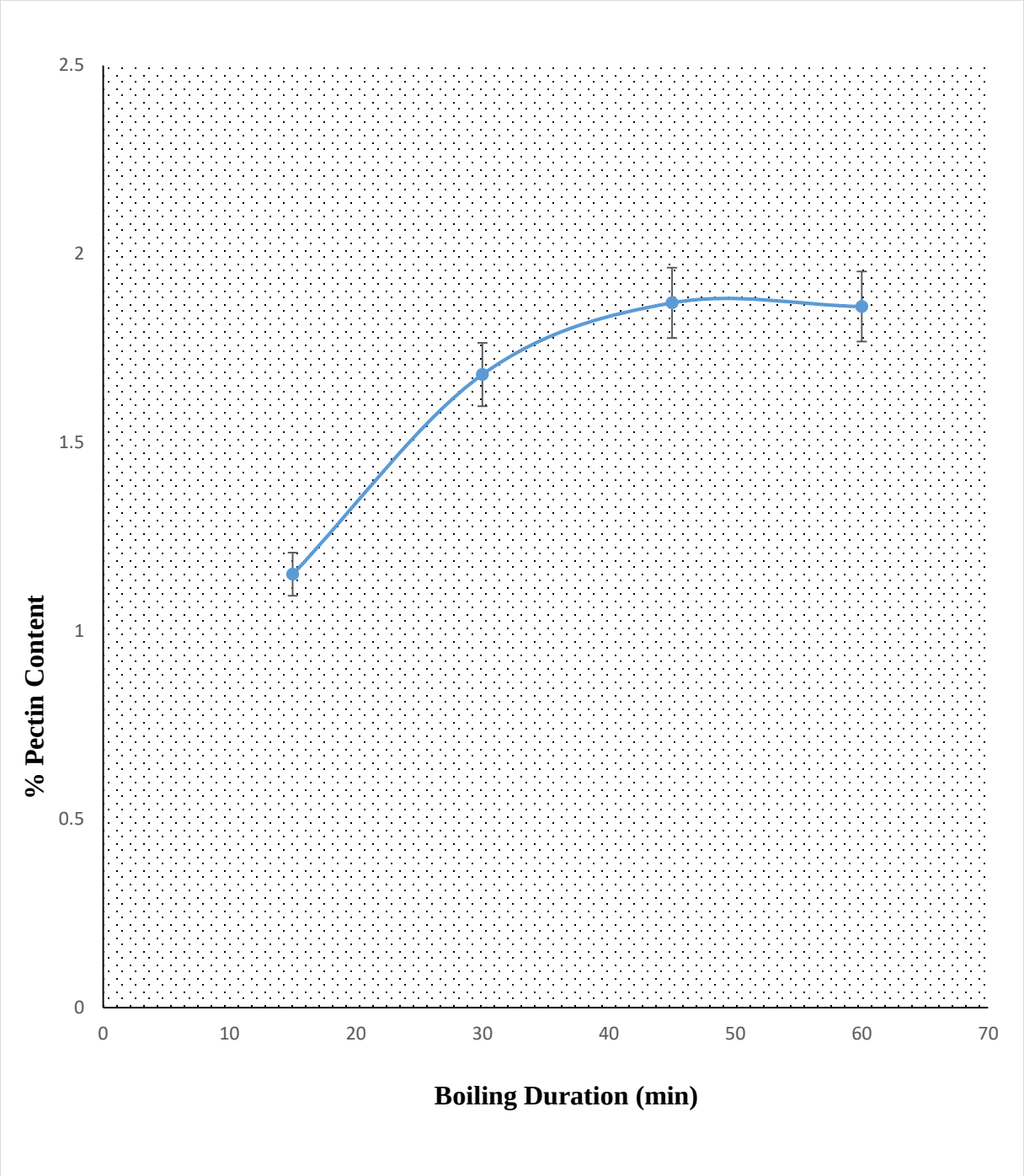

Figure 8: Effect of boiling duration on the extraction of plantain (*Musa paradisiaca*) pectin

Bars represent the Mean ± Standard deviation

A study by Hamidon et al. (2017) showed that higher durations at an average temperature of 90 ^{o}C led to the extraction of more pectin from sweet potatoes. However, in the same study, the yield decreased as the temperature was beyond 60 minutes. So, prolonged extraction time beyond 60 minutes might have induced the digestion of pectin thus making it difficult to be precipitated by ethanol which lowered the yield of pectin, as obtained in this study. Hence, the optimal contact duration between the solvent molecule and fruit peels significantly influenced the extraction times of pectin, a carbohydrate polymer (Karim et al. 2022; Nining et al. 2021).

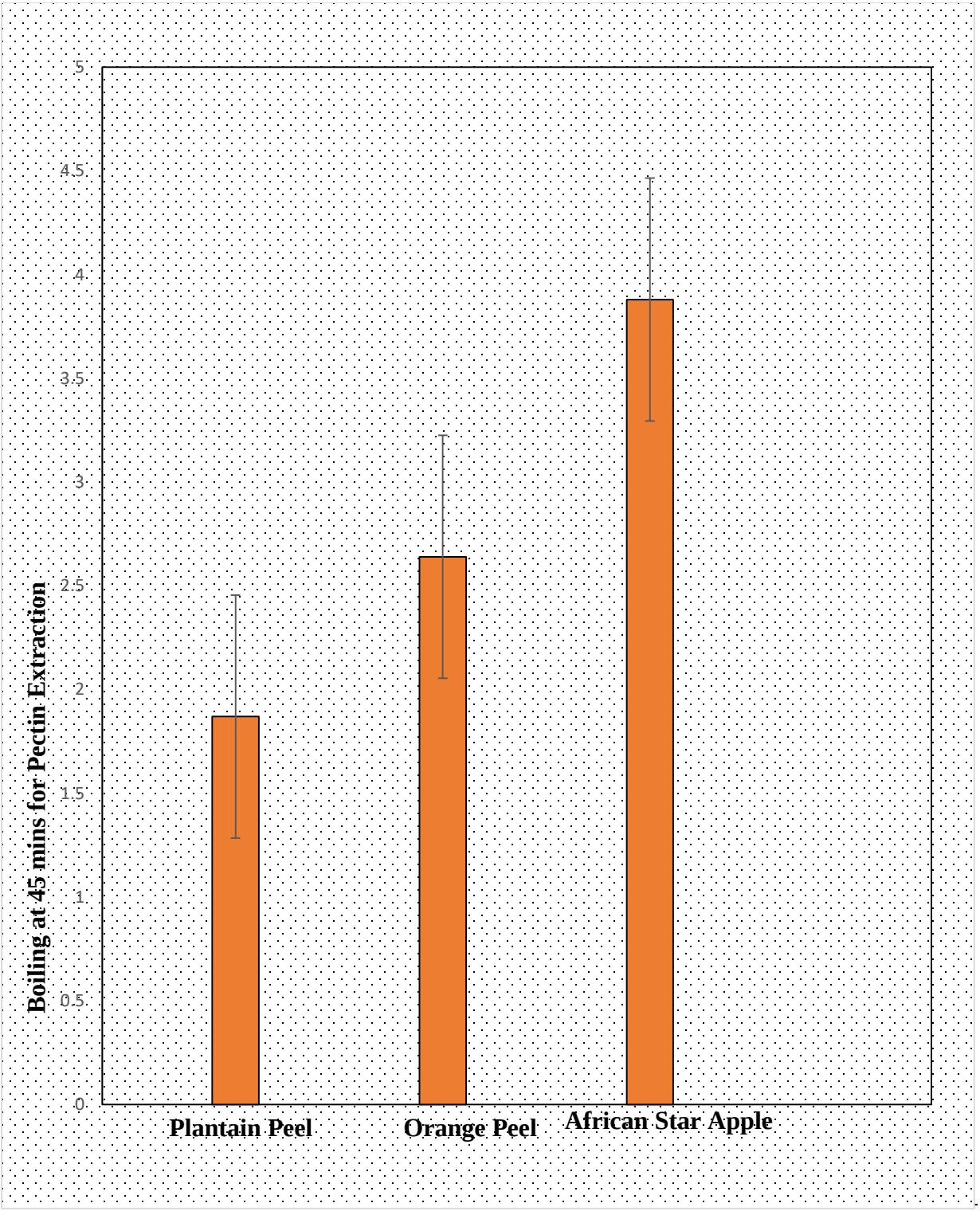

Figure 9: Effect of optimal boiling durations on pectin extraction

Bars represent Mean ± Standard Error

ZONE OF HYDROLYSIS FOR THE EXTRACTED PECTIN SOURCES

The use of *Aspergillus niger* for screening of pectinase showed that agbalumo (*Chrysophyllum albidum*) resulted in a higher zone of hydrolysis compared to orange (*Citrus sinensis*) peels, signifying that, agbalumo (*Chrysophyllum albidum*) has more potential for pectinase production compared to orange (*Citrus sinensis*) and plantain (*Musa paradisiaca*) peels (Figure 4.10).

The zone of hydrolysis around the colonies indicated the pectinolytic activity of fungi used in the study (Ametefe et al. 2021). Hence, the pectin extracted from agbalumo (*Chrysophyllum albidum*) peel had the highest effect in inducing the microorganism to secrete the corresponding enzyme for the hydrolysis of the extracted pectin substrate added in the Czapek medium (Ametefe et al. 2021; Sudeep et al. 2020).

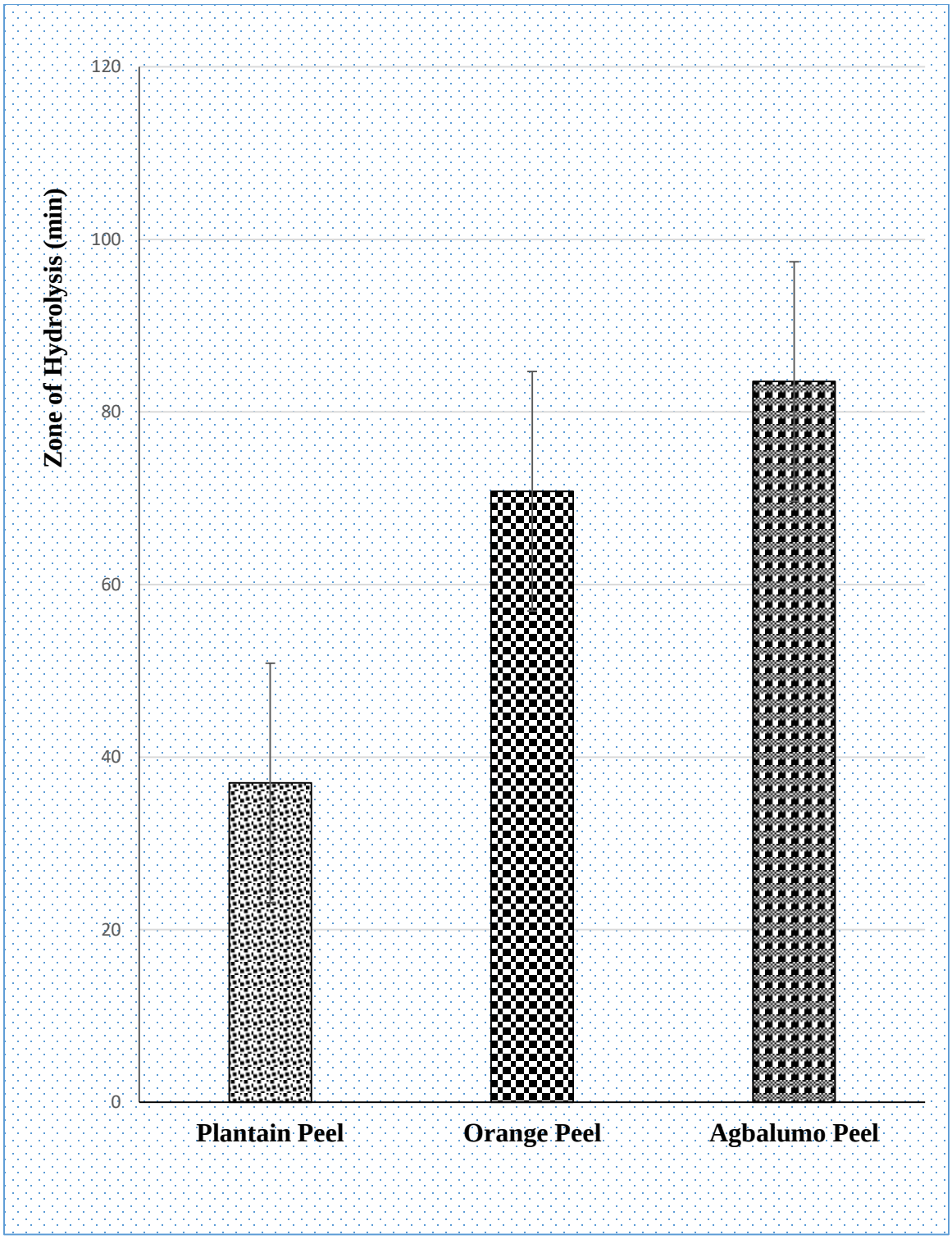

Figure 10: Effect of extracted agrowastes pectin on the potential for pectinase production

Bars represent Mean ± Standard Error

Upon flooding using potassium iodide solution for determination of the zone of hydrolysis, the maximum hydrolytic zone on the media measured in Roy et al. (2018) is in agreement with this study; showing that the larger the zone of pectin hydrolysis, the more potential for the substrate to be used for pectinase production.

CONCLUSION

It is evidenced from this study that agbalumo (*Chrysophyllum albidum*) peel has highest potential for pectin production compared to orange (*Citrus sinensis*) and plantain (*Musa paradisiaca*) peels. The highly acidic medium and the relatively longer durations of boiling in the range of 45 to 60 minutes demonstrated to be more suitable for pectin extraction from the three agricultural wastes. Pectin extracted from the agbalumo (*Chrysophyllum albidum*) peels and orange (*Citrus sinensis*) peels utilized in this study showed more potential for pectinase production. Hence, since the agro wastes used in this study are readily available in the western part of Nigeria, and our study has demonstrated that they contain a considerable amount of pectin suitable for pectinase production potential; this study therefore suggests that additional research be conducted to further optimize the agro wastes pectin production, perhaps by separating the outer layer of the peel from the inner part.

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COMPETING INTERESTS

The authors declare that they have no competing interests

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

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