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Resistant starch formation in unripe plantain flour under two different drying conditions

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Abstract. Unripe plantain is a potential commodity for producing flour with low digestibility due to its dietary fiber and resistant starch content, thus more beneficial for health such as for blood glucose control and blood lipid profile improvement. The present study aimed to characterize the physicochemical and resistant starch content of unripe plantain flour produced under two different drying conditions namely: one-step drying and two-step drying technique. The unripe plantain flour control (unmodified) was produced by drying under one temperature for a fixed duration (60ºC for 6 hours), whereas the modified one was produced using a two-step drying process: first, at 40ºC for 5 hours, followed by 80ºC for 4 hours. The flour was characterized for its resistant starch content, proximate composition, colour and pasting properties. The results showed that modified unripe plantain flour contained higher resistant starch (6.98 ± 0.21%) as compared to the control one (5.21 ± 0.49%, $p<0.05$). Whereas starch content was 60.14 ± 0.19% and 55.76 ± 0.18% for the modified and the control flour respectively. The two-step drying condition also increased the pasting temperature of the modified unripe plantain flour by nearly 2ºC (84.37ºC) compared to the control flour (82.65ºC). Thus, two-step drying condition increased the resistant starch content and altered the pasting properties of unripe plantain flour.

1 Introduction

Unripe plantain contains 39.05% dietary fiber and resistant starch of nearly 54% of its total starch [1]. The high composition of indigestible carbohydrates in unripe plantain, makes it suitable for developing healthier food products with a lower glycemic index. Indonesia's banana production, including plantains, reached 9.69 million tons in 2023[2]. On the other hand, the climacteric nature of the fruit leads to a short post-harvest shelf life, especially under tropical ambient temperature [3]. Storage under modified atmosphere or controlled humidity is used to extend shelf-life without the use of chemical preservatives [3,4]. Besides

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storage modification, processing into flour can be an alternative to extend shelf life. However, this requires the plantain to be processed before reaching a certain stage of maturity. Unripe plantain, due to its high starch content has the potential to be processed into flour. Drying increases the shelf life of flour, by removing moisture from the fruit [5] thereby reducing spoilage.

Drying also plays a significant role in promoting the formation of resistant starch in unripe plantain flour. Tribess et al. [1] reported a combination of drying temperature at 55ºC and air velocity between 1 and 1.4 m/s using a tunnel dryer produced green banana flour with a high resistant starch content of approximately 58% dry basis. Another study [6] found that drying green banana using a spouted bed dryer at 80ºC and air velocity of 50 m/h produced flour with 42% resistant starch. However, when the temperature was increased to 90ºC, the resistant starch content decreased to 38%. A combination of an ultrasound and vacuum drying at 60ºC was reported to decrease resistant starch content [7]. Falodun et al. [5] found that cabinet drying technique produced higher resistant starch compared to freeze drying and sun drying in banana flour. This suggests that drying conditions (temperature; duration; air velocity) and techniques significantly influence resistant starch formation in green banana and potentially in plantain as well.

The present study aimed to compare resistant starch content and other physcochemical characteristics of unripe plantain flour subjected to one-step drying at 60ºC for 6 h and two-step drying at 40ºC for 5 h followed by 80ºC for 4 h.

2 Material and method

2.1 Material

Unripe plantains at the first stage of maturity [8–10] namely "Pisang Candi" a variety of "Pisang Tanduk" (horn banana) were obtained from a farmer in Malang Regency, East Java, Indonesia (**Figure 1**). Citric acid was used to prevent browning during flour preparation. Chemicals for analysis were ethanol, KOH, acetone, aquadest, K_2SO_4, H_2SO_4, NaOH, $Na_2S_2O_3$, HCl, Kjeldahl tablet as catalist, H_3BO_3 and hexane. Enzymes for resistant starch analysis were pepsin and pancreatic α-amilase.

The key equipments used in the present study were as follows: cabinet dryer (local), blender (Philips Indonesia, NL), glassware, electrical oven (Memmert, Germany), analytical balance, soxhlet aparatus, color reader (CR-10, Konika Minolta Sensing, Japan) and Rapid Visco Analyzer (Techmaster, Perkinelmer, USA).

2.2 Method

2.2.1 Preparation of unripe plantain flour

Unripe plantains were sorted and washed to remove dirt, after which the skins were completely peeled. The peeled plantains were sliced to a thickness of approximately 2 ± 0.2 mm using a mechanical slicer. To prevent enzymatic browning, the slices were soaked in a 0.5% (w/v) of citric acid solution at a plantain-to-citric acid solution ratio of 1:2, ensuring complete submersion for 15 minutes.

After draining, the slices were subjected to two different drying conditions, using a cabinet dryer:

- The one-step drying condition: sliced plantains were placed on a tray and dried at 60ºC for 6 hours

- The two-step drying condition: sliced plantains were placed on a tray and dried at 40ºC for 5 hours, followed by drying at 80ºC for 4 hours.

The dried plantain chips were milled into flour and passed through a 100-mesh sifter to obtain a fine powder. The resulting flour was stored in airtight containers at room temperature until further analysis.

2.2.2 Determination of flour color

Flour color was determined using a color reader, measuring lightness (L*), redness (a*) and yellowness (b*) [11].

2.2.3 Determination of proximate composition

The determination of moisture, ash, fat, protein and crude fiber content of the samples were carried out using the AOAC method [12]. Carbohydrate content was evaluated by difference.

2.2.4 Determination of resistant starch content

The resistant starch content was determined following a method described by Goni et al. [13] with modification. A 100 mg portion of dried sample was placed in a test tube, and 10 ml of KCl-HCl buffer (pH 1.5) was added. The desired pH was adjusted using either HCl 2 M or NaOH 0.5 M to create optimal condition for pepsin activity. Subsequently, 2 ml of pepsin solution (prepared by dissolving 1g pepsin in10 ml KCl-HCl buffer) was added to the mixture.

The enzyme-substrate mixture was incubated at 40ºC for 60 minutes, then cooled to room temperature. The pH was then adjusted to 6.9 using 0.5 M NaOH. Once the desired pH was reached, 1 ml of alpha-amylase was added, and the mixture was incubated at 37ºC for 16 hours in a waterbath with continuous stirring.

After incubation, the mixture was centrifuged at 3000 rpm for 15 minutes. The supernatant, which contained hidrolized starch, was discarded. The pellet was washed with 10 ml of distilled water and centrifuged again under the same conditions; the supernatant was discarded. The final residue representing resistant starch, was dried, weighed and quantified.

2.2.5 Observation of pasting properties

Pasting properties of the unripe plantain flour was determined using a Rapid Visco Analyzer (RVA). A 3 g sample was weighed and placed into an alumunium tube containing 25 mL of distilled water (aquadest). The suspension was initially heated to 50ºC and held for 1 minute, followed by a gradual increase to 95ºC, where it was maintained for 10 minutes. The mixture was then cooled back to 50ºC and held for 2 minutes. During the test, the following parameters were recorded: pasting temperature, peak viscosity, breakdown viscosity, setback viscosity, final viscosity and peak time [14].

2.3 Data analysis

Data were analysed using an independent sample t-test to compare samples from two different treatments. A statistically significant difference between the two independent means was determined at $p < 0.05$.

3 Results and discussion

3.1 Physicochemical properties of fresh unripe plantain

Fresh unripe plantains were obtained at the first stage of maturity, as indicated by their fully green color, in accordance with several studies [8–10]. The high moisture content observed was typical of fresh fruits. The phsyicochemical properties of the fresh unripe plantains are presented in **Table 1**.

Fig. 1. Unripe "Pisang Candi" plantain

Table 1. Physicochemical properties of fresh unripe plantain

Parameters	Values
Moisture (%)	62.33 ± 1.07
Starch (%)	26.64 ± 0.42
Texture:	
Hardness (N)	131.16 ± 5.25
Colour:	
Lightness (L*)	52.04 ± 2.13
Redness (a*)	-17.21 ± 0.68
Yellowness (b*)	39.60 ± 0.45

- Values are means ± SD's
- Moisture and starch content were analyzed in the plantain flesh
- Hardness, lightness, redness, yellowness was measured on the plantain skin

At the first stage of maturity, the fruit skin appeared fully green, as indicated by a negative redness (a*) value. Changes in external skin colour during ripening often reflects corresponding changes in flesh colour, as reported by previous studies [9,15].

Texture measurement indicated that the hardness of the fruit was attributed to its unripe condition. As the stage of maturity increased, fruit hardness decreased significantly. During ripening, plantain lose firmness and undergo softening due to several post-harvest physiological processes. Three key mechanisms have been identified to link with loss of firmness during ripening. First, starch degradation into glucose. Second, solubilization of pectic substances leading to the breakdown of cell wall integrity and a reduction of the middle lamella, thereby weakening tissue cohesion. Third, water migration from the skin to the flesh driven by increased osmotic activity [15]. These processes colectively alter the mechanical properties of the fruit, making texture a reliable indicator of ripening progression.

3.2 Chemical composition of unripe plantain flour

3.2.1 Proximate composition

The unripe plantains were processed into flour by means of two different drying conditions namely one-step drying and two-step drying conditions. The proximate composition of the unripe plantain flour is presented in **Table 2**.

Table 2. Proximate composition of unripe plantain flour

Composition	Modified unripe plantain flour (two-step drying)	Control unripe plantain flour (one-step drying)
Moisture (%)	7.19 ± 0.01^{a}	8.64 ± 0.048^{b}
Ash (%)	1.09 ± 0.03 ^{a}	1.71 ± 0.03 ^{b}
Fat (%)	0.76 ± 0.03 ^{a}	0.83 ± 0.02 ^{a}
Protein (%)	4.04 ± 0.04 ^{a}	3.79 ± 0.02 ^{b}
Carbohydrate by difference (%)	86.93 ± 0.02 ^{a}	85.03 ± 0.03 ^{b}

- Values are means ± SD's
- Different letters indicated significantly different at p value < 0.05 using independent sample t-test

The unripe plantain flour control exhibited slightly higher moisture content compared to the modified flour. The two-step drying allowed gradual water evaporation during the initial phase at 40ºC for 5 hours, minimizing the risk of surface case hardening that typically inhibit water migration. The subsequent drying at 80ºC for 4 hours evectively completed the dehydration process.

In contrast, the one-step drying employed a constant temperature at 60ºC from the beginning, which may have promoted case hardening before sufficient moisture had been removed from the inner layers, leading to the higher residual moisture content. Gulati and Datta [16] reported that case hardening is particularly intensified under conditions of higher drying temperature, increased air velocity in the drying chamber and reduced relative humidity in the surrounding air.

The dry matter content of unripe plantain flour was significantly influenced by the drying conditions, except for fat content, which remains similar across both treatments. Flour produced by one-step drying exhibited higher ash content, while other components including protein and carbohydrate were higher in flour produced by two-step drying. Generally, dry matter increases as moisture content decreases. In addition, the higher protein content in the modified unripe plantain flour may be attributed to the higher temperature that applied in the second drying phase (80ºC). Higher temperature stimulates interaction between protein and starch which leads to an increased surface-bound protein on starch granule [17].

3.2.2 Starch and resistant starch

Starch and resistant starch content of the unripe plantain flour are presented in **Table 3**. Both total starch and resistant starch contents were higher in unripe plantain flour produced by two-step drying. Since starch constitutes a major portion of the carbohydrate component, this result aligns with the carbohydate-by-difference values (Table 2). Resistant starch was also higher in unripe plantain flour produced by two-step drying. The use of cabinet dryer was informed by the findings of Falodun et al. [5], who reported higher resistant starch content in banana flour processed using this technique compared to sun drying and freeze drying. However, Ahmed et al.[18], observed contrasting results, suggesting that freeze drying was more effective than hot air drying in preserving resistant starch in green banana flour. The underlying mechanism remains unclear, but one hypothesis is that after rigidity is maintained during freezing, structural disorganization of starch granules may occur during water removal in drying, promoting retrogradation and resistant starch formation.

Table 3. Starch and resistant starch content of unripe plantain flour

Composition	Modified unripe plantain flour (two-step drying)	Control unripe plantain flour (one-step drying)
Starch (%)	60.14 ± 0.19^{a}	55.75 ± 0.18^{b}
Resistant starch (%)	6.98 ± 0.21^{a}	5.21 ± 0.50^{b}

- Values are means ± SD's
- Different letters indicated significantly different at p value < 0.05 using independent sample t-test
- % resistant starch was presented as per-100 g flour, not as per-100 g starch

A slower air-drying method over 10 days produced a higher resistant starch content (44%) in yam flour compared to hot air drying at 60ºC for 48 hours with a wind speed of 0.15 m/s in an oven dryer, which yielded 34% [19]. According to Correia and Beirăo-da-Costa [20], drying at 60ºC activates endogenous α-amylase, whose optimal activity occurs at 55-60ºC. This enzymatic activity promotes partial degradation of starch granule, thereby reducing resistant starch formation. However, prolonged drying in this temperature, may also facilitate the reasscociation of amylose and amylopectin chains, leading to the formation of retrograded resistant starch. This dual effect highlights the complexity of starch transformation during thermal processing.

In the present study, the higher resistant starch content in unripe plantain flour produced by two-step drying may be attributed to the initial low-temperature phase (40ºC), which likely prevented the activation of endogenous α-amylase. The subsequent high-temperature phase (80ºC) may have inactivated any residual enzyme activity, creating more favorable conditions for resistant starch formation. Nonetheless, the structural quality of the resistant starch produced under these conditions remains to be fully characterized.

The two-step drying, which begins at a lower temperature (40ºC), may help preserve the native structure of starch granule, rendering them less accesible to enzyme hydrolysis [20]. Consequently, the resistant starch produced under these conditions may be classified as type-2 resistant starch (RS2), which is characterized by ungelatinized structure [21]. RS2 is thermally sensitive and can be lost during processing or cooking due to starch gelatinization.

On the other hand, retrograded resistant starch (RS3) begins upon gel cooling [21]. The mechanism by which increased drying temperature promotes resistant starch formation remains unclear. Liu et al. [22] reported that the higher the temperature, the higher the resistant starch content in potato flour, suggesting that higher temperature may facilitate molecular rearrangement conducive to RS formation. However, the thermal sensitivity of resistant starch varies across botanical sources according to Correia and Beirão-da-Costa [20], indicating that starch composition and granule architecture play critical role in thermal behaviour.

3.3 Physical properties of unripe plantain flour

3.3.1 Colour

The preparation of unripe plantain flour included a soaking step in a 0.5% citric acid solution, aimed in perventing enzymatic browning. This treatment was particularly important due to the high polyphenol content in the plantains, which can trigger browning reactions through polyphenol oxidase activity [15]. Citric acid acts as an acidulant, lowering pH and inhibiting enzyme activity thereby preventing discoloration. The visual appearance of the resulting flour is presented in **Figure 2** and the colour space values are shown in **Table 4**.

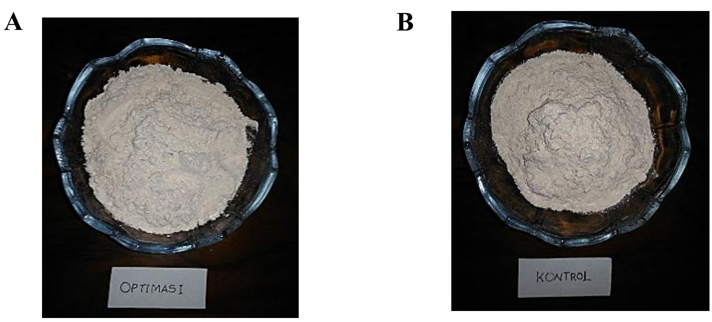

Fig. 2. Unripe plantain flour produced by two-step drying (A) and one-step drying (B)

The flour produced from unripe plantains appeared off-white or broken white, regardless of whether it was treated by one-step or two-step drying. However, the lightness (L*) value was slightly but significantly lower in the flour produced by two-step drying. This reduction in lightness may be attributed to prolonged heat exposure (a total of 9 hours) which can promote the degradation of heat-sensitive natural pigments or phenolic compounds [15]. Pico et al. [23] reported that drying can lead to cellular collapse due to dehydration, resulting in decompartmentalization and the release of phenolic compounds and polyphenol oxidase (PPO) enzymes. Although soaking in citric acid solution can inhibit enzymatic browning, residual phenolic compounds may still be present and released during drying. Their interaction with PPO enzymes produced trace of yellow or brown colour, subtly affecting the final colour of the flour.

Table 4. Colour of unripe plantain flour

Colour	Modified unripe plantain flour (two-step drying)	Control unripe plantain flour (one-step drying)
Lightness (L*)	82.87 ± 0.06 [a]	84.43 ± 0.06 [b]
Redness (a*)	0.90 ± 0.10 [a]	0.63 ± 0.06 [b]
Yellowness (b*)	12.67 ± 0.06 [a]	11.67 ± 0.06 [b]

- Values are means ± SD's
- Different letters indicated significantly different at p value < 0.05 using independent sample t-test

In line with the observed reduction in lightness, both redness and yelowness values were elevated in the modified flour compared to the control. The thermal stability of phenolic compunds varies significantly, cathecins are susceptible to thermal degradation while flavanols particularly quercetins are quite heat stable [23]. The interplay between pigment breakdown and retention during drying likely contributes to the final appearance of the flour.

3.3.2 Pasting properies

Pasting properties are among the important functional characteristics of starch, reflecting its behaviour during heating and hydration. When an aqueous suspension of starch is heated above a critical temperature, granule swell irreversibly, and both amylose and amylopectin leach out into the aqueous phase resulting in increased viscosity [24]. In the present study, different drying conditions were shown to produce unripe plantain flour with distinct chemical compositions including variations in resistant starch content. These compositional differences modified the pasting properties of the flour as ilustrated in **Figure 3**.

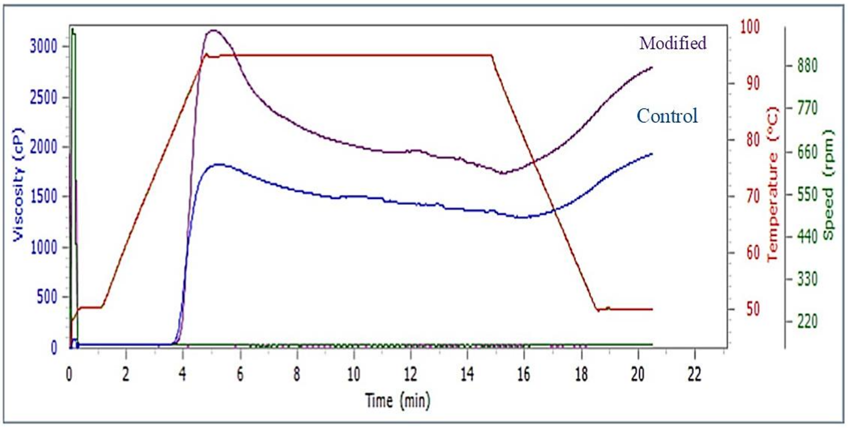

Fig. 3. Rapid visco analyser pattern of unripe plantain flour

The flour produced under both drying conditions exhibited similar pasting pattern during Rapid Visco Analyser testing. In both cases, the starch reached peak viscosity followed by major breakdown and a broad plateau, characteristics of a type-A viscosity profile as described by Tsakama et al.[25]. The pasting properties of the unripe plantain flour were further detailed in **Table 5**.

The heating phase during RVA testing induced swelling of starch granules, followed by gelatinization, which was evident from the peak viscosity. Notably, plantain flour produced by two-step drying exhibited higher peak viscosity than that produced by one-step drying. In the two-step drying, the initial low-temperature phase (40ºC) effectively reduced moisture while preserving the structural integrity of starch granules. As a result, during the pasting test, these intact granules underwent more complete gelatinization, leading to higher viscosity.

Table 5. Pasting properties of unripe plantain flour

Pasting properties	Modified unripe plantain flour (two-step drying)	Control unripe plantain flour (one-step drying)
Peak viscosity (cP)	3113.67 [a]	2001.67 [b]
Breakdown viscosity (cP)	1124.00 [a]	629.33 [b]
Setback (cP)	908.67 [a]	743.67 [b]
Final viscosity (cP)	2817.00 [a]	2111.33 [b]
Peak time (minutes)	5.14 [a]	5.33 [b]
Pasting temperature (ºC)	84.37 [a]	82.65 [b]

- Different letters indicated significantly different at p value < 0.05 using independent sample t-test

Peak viscosity indicates the ability of starch granules to absorb water and swell during heating [26]. This property is influenced by starch granule size, amylose-to-amylopectins ratio and chain length distribution of amylopectins [27]. Given that both starches were originated from the same botanical source, the drying condition may have altered the molecular arrangement of amylose and amylopectins within the starch granules. Higher peak viscosity has been associated with weaker molecular interactions among starch granules, making them more susceptible to disintegration under thermal and mechanical treatment [24]. In the present study, unripe plantain flour produced by two-step drying exhibited a higher peak viscosity than that produced by one-step drying. This property was accompanied by a shorter time to reach peak viscosity (peak time), suggesting an inverse relationship between peak viscosity and peak "pasting" time. Such trend has also been reported in previous studies [24,25].

Breakdown viscosity reflects the degree of disintegration of starch granules and the stability of the paste under shear stress [24]. At breakdown, swollen granules disrupt further and amylose generally leaches into solution, contributing to changes in viscosity and gel structure [25]. Starches that exhibit higher peak viscosity often show higher breakdown viscosity, indicating susceptibility to shear stress and produce weak gels.

The higher setback viscosity observed in the modified plantain flour produced by two-step drying suggested a lower susceptibility to retrogradation upon cooling [24] which may translate to a reduced staling rate in products made from this flour [28]. However, contrasting findings have been reported by other researchers. For instance, one study [26] reported that lower setback values are associated with improved cold paste stability and reduced susceptibility to retrogradation, highlighting the complexity of interpreting setback viscosity across different starch system. Final viscosity which represents the cold paste viscosity, thus indicates the stability of cooked starch paste in the actual use [24,25,28].

The differences observed accross all pasting parameters between unripe plantain flour produced by two-step drying and one-step drying were consistent with trends reported for other starch-based flour. However, an anomaly was noted in the pasting temperature; flour produced by two-step drying exhibited higher pasting temperature despite showing higher values in all other viscosity-related parameters. Typically, pasting temperature is inversely correlated with peak viscosity, breakdown viscosity, setback viscosity and final viscosity as reported by previous studies [24,25,28]. This unexpected trend may be explained by differences in proximate composition, slightly higher protein, carbohydrate, starch and resistant starch may collectively increased the thermal energy required to initiate granule swelling and gelatinization, thereby elevating the pasting temperature.

Although unripe plantain flour produced by two-step drying exhibited a higher resistant starch content, its pasting properties suggest that the thermal stability of this resistant starch was relatively low. This indicates that most of the resistant starch present was likely type-2 (RS2), composed of intact starch granules that are susceptible to degradation upon heating. During cooking, these granules gelatinize and lose their resistance, which limits their functional contribution in thermally processed foods.

Conversely, the gel formed from cooked starch of this flour demonstrated low susceptibility to retrogradation, as shown by high setback viscosity. Retrogradation leads to the formation of resistant starch type-3 (RS 3) [25], which is thermally stable and formed through the reassociation of amylose and amylopectins upon cooling. RS3 is considered the most stable and functionally beneficial of RS in processed foods.

From nutritional and functional standpoint, unripe plantain flour produced by one-step drying may offer greater advantages in food application. Despite its lower initial resistant starch content, it exhibited more desirable pasting properties and a higher potential for RS2-to-RS3 conversion during cooking and cooling. Therefore, when evaluating resistant starch formation in unripe plantain flour, it is essential to consider not only its quantity but also its structural characteristics and behaviour under processing conditions, as these factors directly influence its contribution to texture, digestibility, and health benefits in final food products.

4 Conclusions

Unripe plantain flour produced by two-step drying exhibited lower moisture content and higher concentration of dry matter components including ash, protein, carbohydrates, starch and resistant starch but similar fat content compared to one-step drying. The appearance of the flour was less light than that produced by one-step drying.

Despite higher resistant starch in unripe plantain flour produced by two-step drying, the pasting properties suggested that this starch had low stability to heat and mechanical treatment upon cooking, and when cooling, the formation of more stable resistant starch (retrograded starch) was likely to be low.

The advantage of the two-step drying was that the condition facilitated the formation of RS2 in a higher quantity, but the disadvantage was that the condition did not promote the conversion of RS2 to RS3 during cooking and cooling, RS3 is more stable resistant starch towards higher temperature. Its content in flour and food products reduced starch digestibility or lowered glycemic response.

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Declaration

I acknowledge the use of Microsoft Copilot in helping me to review my writing at the final stage of preparing my assessment. I used the following prompt : "Suggest ways to improve clarity of this complex sentence" I reviewed the feedback generated by Microsoft Copilot critically and, based on this, revised the writing using my own words and expressions.