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# AIMS Agriculture and Food

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# Engineering properties of sorghum bioguma-variety for designing appropriate thresher and chopper machine

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## ABSTRACT

*Sorghum is a versatile plant with various parts that can be utilized. However, information on the physical and mechanical properties of the sorghum plant is crucial for designing agricultural machinery for primary handling processes such as threshing and chopping. This study aimed to determine the technical characteristics of sorghum plants (Bioguma variety) including the physical and mechanical properties of the stems, leaves, panicles and seeds to design a configuration system concept for threshing and chopping machines capable of processing sorghum plants with high moisture content immediately after harvesting. The study used a descriptive method and included samples of sorghum plants randomly taken from fields in Majalengka and Bogor, Indonesia. The physical and mechanical properties were measured using several replications, ranging from 3 to 30 depending on the parameter. The sorghum plants were harvested at at ages 80, 90 and 108 days after transplanting (DAT). It was found that the moisture content of sorghum stem and seeds decreased with the increase of plant ages where stem ranged between 84.18–79.81 %wb and seeds ranged between 51.7–29.4 %wb. The result revealed that planting ages influenced its properties including stem properties and seed properties. Longer DAT increased the stem hardness from 290.64 ± 29.41 to 350.00 ± 0.81 N and seed hardness from 8.2 ± 1.7 to 44.9 ± 5.4 N but decreased the tensile force of seed form panicles from 16.7 + 3.2 to 6.0 ± 0.8 N. The data on stem strength and seed hardness provide important considerations for the development of several equipment for sorghum processing. The findings of this study can serve as a basis for designing effective and efficient threshing and chopping machines for sorghum plants at high moisture content.*

***Keywords:*** *chopper machine; sorghum plants; technical characteristics; thresher design*

## 1. Introduction

Sorghum (Sorghum bicolor L.) is a cereal grain plant that is a member of the grass family (Poaceae). It is an important food crop and source of income for millions of people in the semi-arid tropics of Africa and Asia [1]. Sorghum is the fifth most important cereal crop globally after rice, wheat, barley and maize and is the staple food of around 500 million people [2,3]. Directly chopped sorghum leaves can be used for ruminant feed in the form of silage [4]. However, a study notes that although sorghum has potential as a source of food and industrial products, its utilization is constrained by several factors including the lack of efficient post-harvest processing technologies [5], especially sorghum with different varieties. Another study highlights the need for research to develop improved post-harvest management practices for sorghum and other crops to reduce losses and enhance food security [1,6,7].

According to Ndukwu and Ejirika [8], a crop's physical characteristics and in most cases its cultivar have the greatest impact on an agro-processing machine's ability to adapt. As also stated by Li et al. [9] and Ndukwu et al. [10], the physical properties of crops are part of the contact mechanics in postharvest processing and a major determinant in intelligent harvesting. Information about the physical and mechanical properties of the sorghum plant is needed for the primary handling process of the sorghum plant such as threshing the seeds and chopping the stems directly from harvest in the field so that all parts can be utilized. This is equivalent to the opinion of [11] which states that the physical characteristics of sorghum seeds are needed to design various types of agricultural machinery such as threshers and choppers. For immediate post-harvest processes with high water content (20-30 %wb) such as threshing of sorghum panicles and chopping of sorghum stems the physical and mechanical characteristics of the sorghum plant are needed. It is required for the hold-on type of threshing of sorghum panicles/seeds because the sorghum plant stems are clamped during threshing, thus, it is necessary to consider relevant information on seed moisture content, the tensile strength of seeds from panicles, panicle hardness, panicle-seed dimensions, seed-specific gravity, seed-to-panicle, plant ratio and terminal velocity. For the enumeration, information on the physical and mechanical properties of the stems and in the form of a pile of stems is needed including water content, hardness, specific gravity, dimensions, weight and cutting force of the sorghum stalks. According to Gely et al. [12] a thorough understanding of the physical properties of sorghum grains is helpful to improve the technology associated with operations and equipment related to post-harvest processes such as cleaning, sorting, transport, ventilation, drying and storage.

Several studies have reported some physical and mechanical properties of sorghum seeds [11–13] as affected by moisture content. However, these studies use different varieties and are not based on harvesting age. In addition, the research also only focused on sorghum seeds and without observing the sorghum plant as a whole. Thus, there is a gap in research regarding the engineering properties of the Bioguma variety which is necessary for the development of appropriate sorghum processing machines capable of directly processing seeds and stems at high moisture content and reducing processing costs such as panicle drying and seed milling. This research aims to fill this gap by obtaining technical characteristics of the sorghum plant (seeds, panicles and stems) which can serve as the basis for designing a configuration system concept for threshing and chopping machines that are capable of handling various sorghum seed varieties and plant conditions with seed moisture content ranging from 20–30% and chopped stems with moisture content of 76–90%. The findings of this research will be advantageous for the design and development of efficient sorghum processing machines. This research aimed to provide technical characteristics of the entire sorghum plant under different ages including the physical and mechanical properties of its stems, leaves, panicles and seeds to develop a configuration system concept for threshing and chopping machines. The study focuses specifically on the engineering properties of the Bioguma variety of sorghum plants, especially from Indonesia region.

## 2. Materials and methods

### 2.1. Sorghum raw materials

The material consists of sorghum Bioguma varieties grown on the fields of Majalengka and Garut farmers with latosol soil conditions and dry land. Bioguma sorghum varieties were harvested at the age of 80, 90 and 108 days after transplanting (DAT).

### 2.2. Physical properties measurements of sorghum stalks and seeds

The physical characteristics of the sorghum plant consisted of the stem, panicle, and seed components.

The parameters measured include plant height or length, dimension (diameter of main branch, diameter of panicle and seeds dimension), mass (plants, panicles and seeds) and bulk density (plant and seed piles). The method used in measuring the physical characteristics of the sorghum plant was a direct measurement with a measuring instrument namely a ruler, caliper and tape measure. The measurement of seed dimension is shown in Figure 1 based on [14–17]. The height or length of the plant was measured from the base of the main stem which was cut using a machete at a height of 10–20 cm from the ground to the top of the leaf. Figure 2 shows the measurement of stem diameter measured from the base of the main stem on the first internode after being cut at the lowest internode because the height or length of this plant is used to design conveyors for threshing and chopping machines. Bulk density was determined by weighing a sample of the material in the form of a pile of stems and then measuring the dimensions of the pile of stems (width and thickness).

The mass of stems and stem piles was measured by direct weighting per plant and plant pile. Bulk density was known to take into account the frictional pressure on sorghum plants calculated from the sample mass and plant volume [18] as in Equation (1).

$$\rho = \frac{m}{v} \quad (1)$$

where ρ is bulk density (g/cm3), m is sample weight (g) and v is volume occupied by sample (cm3).

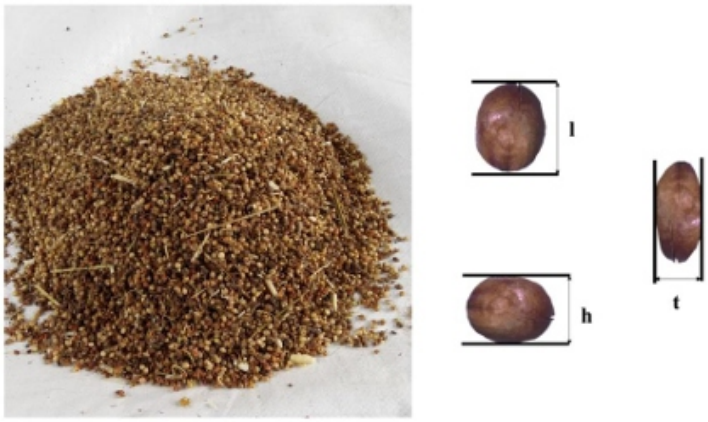


**Figure 1.** Dimensions of sorghum seeds (h: height; l: length; t: thickness).

**Figure 2.** Measurement of the diameter of the main stem and panicle stem.

## 2.3. Mechanical properties measurements of sorghum stalks and seeds

The mechanical characteristics observed were the tensile strength of the seeds from the panicle, the angle of seed dropping, terminal velocity, stem cutting force, stem hardness and plant friction coefficient. Measuring the mechanical characteristics of a sorghum plant is necessary to design a threshing machine and chopping machine as well as a conveyor system so that it becomes an appropriate technology that can help shorten the processing time of sorghum plants into poultry and ruminant feed.

2.3.1. Tensile strength of panicles

Tensile strength was measured by pulling the sorghum seeds from the panicles so that deformation occurred (Figure 3), namely, the sorghum seeds broke off from the panicle litter. The data obtained was in the form of changes in length and load which are then displayed as a stress-strain graph. The measuring tool used to test the tensile strength of sorghum seeds from panicles is a tensile strength tester (strain gauge) model ELK-500 (Yueqing Elecall Electric Co., Ltd., China) with an accuracy of 0.01 kg with maximum capacity of 50 kg and vernier callipers (Mitutoyo America Corp., USA) with an accuracy of 0.02 mm.To calculate the maximum tensile stress *(max)*the following formula was used [19] as in Equation (2).

$$\sigma_{max} = \frac{F_t}{A} \tag{2}$$

$$A = \frac{1}{4}\pi \times d^2 \tag{3}$$

where $\sigma_{max}$ is maximum tensile stress (N/m$^2$), $F_t$ is tensile force (N), $A$ is cross-sectional area of sorghum stem (m$^2$) and $d$ is diameter of sorghum stem (m).

The change in stress divided by the change in strain is called the modulus of elasticity. The modulus value is calculated as the linear slope of the stress-strain curve based on the regression method.

**Figure 3.** Sorghum seeds are pulled from the litter of the small stalks of the panicles.

2.3.2. Bulk angle of repose

The pouring angle was formed between the flat surface and the sloping side of the outpouring when several sorghum seeds were poured rapidly and flowed by gravity over the flat surface (Figure 4). The measurement of the pouring angle was carried out to find out how much the slope was formed when the sorghum seeds came out of the hole where whole seeds were removed from the hold-on type threshing machine. The pouring angle can be calculated using Equation 4 [20,21].

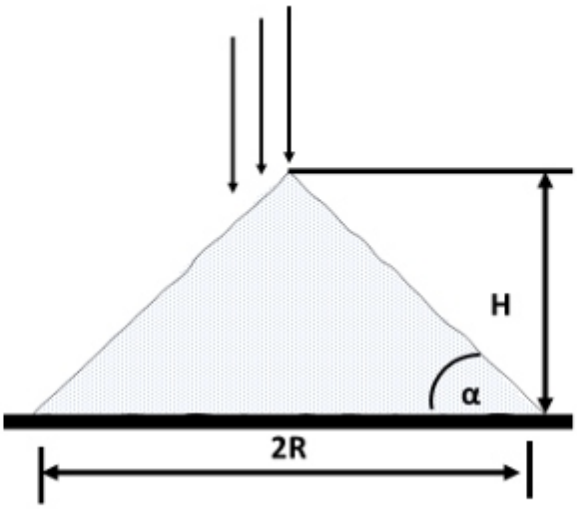


**Figure 4.** Measurement of Sorghum grain angle of repose.

$$\alpha = arc\tan\frac{H}{R} \quad (4)$$

where $\alpha$ is angle of repose (°), $H$ is height of sorghum seeds (cm) and $R$ is radius of sorghum grain demand base circle (cm).

2.3.3. Terminal velocity

Terminal velocity is the velocity at which an object changes until one condition where the drag is equal to the gravitational force [22]. In this case, terminal velocity of sorghum seed was measured by using an adjustable fan as shown in Figure 5.

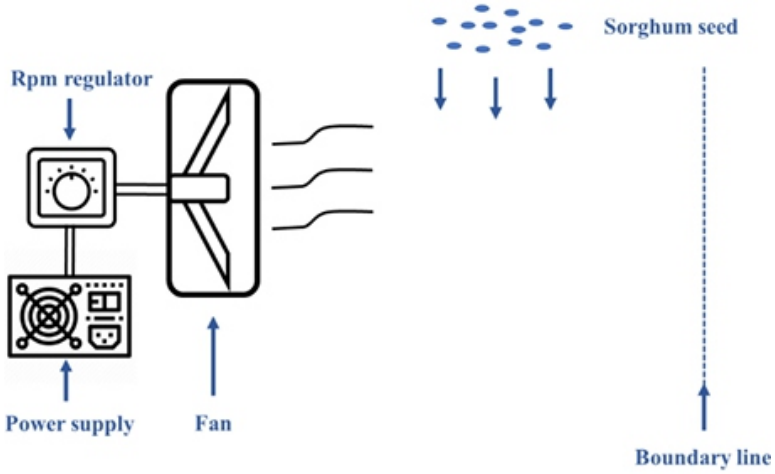


**Figure 5.** Method for terminal velocity measurement.

2.3.4. Hardness and tensile strength of stems and seeds

Stem hardness was measured by piercing or pressing the sorghum stems and seeds so that deformation occurred, namely the sorghum seeds or stems were damaged. The data obtained is in the form of changes in length and changes in load which are then displayed in the form of a stress-strain graph. The measuring instrument used in testing the hardness of sorghum stalks is the TA-XT Plus Texture Analyzer (Stable Micro Systems, UK) (Figure 6) with a loadcell capacity of 50 kg with a speed of 0.2 mm/s.

For stem tensile strength measurement, the samples of sorghum stem with a diameter of 0.62–1.91 mm and a length of 100 mm were prepared. The measurement of the diameter of the sample was carried out for the purposes of calculating the maximum tensile stress of the sorghum plant stems. To calculate the maximum tensile stress of sorghum stems ($\sigma s_{max}$) the Equation 5 was used [19].

$$\sigma s_{max} = \frac{F_t}{A} \tag{5}$$

where $\sigma s_{max}$ is maximum tensile stress of stem (N/m$^2$), $F_t$ is tensile force (N) and $A$ is cross-sectional area of sorghum stalks (m$^2$).

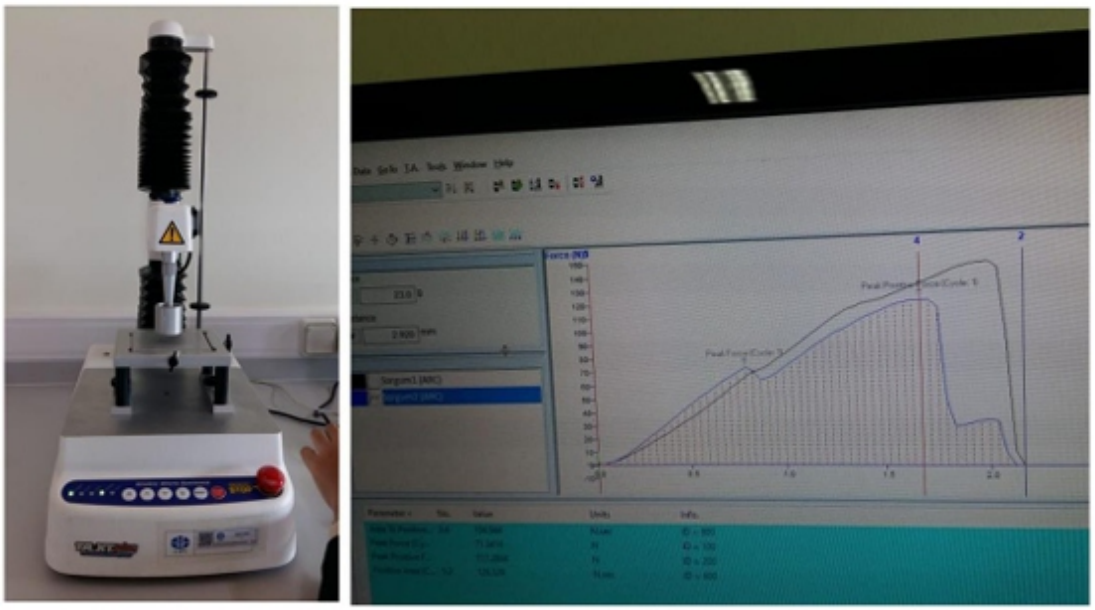

**Figure 6.** Texture analyzer used for measuring hardness of stems.

The change in stress divided by the change in strain is called the modulus of elasticity. The modulus value is calculated as the slope of the linear part of the stress-strain curve based on the regression method.

2.3.5. Cutting force

The cutting force is obtained by using the cutting force method. The ratio of the cutting resistance to the cross-sectional area of the cut sorghum stem is needed to calculate the specific cutting resistance. The cutting force acts when the strain gauge installed with a digital knife is pulled vertically downwards (Figure 7) and the cutting load will be read. The value reads in force units (N). The cutting resistance is calculated using the formula in Equation 6.

**Figure 7.** Cutting force measurement.

$$F_{BN} = \frac{F_{L1} \times L_F}{L_{TP} + R_b} \quad (6)$$

where $F_{BN}$ is stalk cutting resistance (N), $F_{L1}$ is force measured on the arm (N), $L_{TP}$ is length of cutting resistance arm (m), $R_b$ is radius of sorghum stem (m) and $L_F$ is length of force arm (m).

2.3.6. Friction coefficient

The coefficient of friction between the plants and the steel plate was measured by placing a vertical load on the sorghum plant and then pulling the load horizontally with a measuring scale while the sorghum plant was in a static state. The load used is a steel plate weighing 3 kg with dimensions of 25 × 10 × 5 cm. The plant friction coefficient is obtained from the relationship between the normal force and the friction force according to Equation 7.

$$F_g = \mu \times N \quad (7)$$

where $F_g$ is friction force (N), $\mu$ is friction coefficient and $N$ is normal force (N).

## 3. Results and discussion

3.1. Sorghum plant physical characteristics

Determining the sorghum plant's physical properties is necessary for designing a hold-on thresher and chopping machine. The sorghum plants studied from the Bioguma variety included plant dimensions and plant piles (p × l × t), plant/pile weight, plant/pile density and seed to plant/panicle ratio on the condition of sorghum plants after harvest directly from the field and then processed into feed. Plant density is needed for a conveyor design for carrying plant piles to a hold-on thresher with a clamping system on sorghum plant panicles. The physical properties of the seeds and plants of the sorghum Bioguma variety can be seen in Table 1.

**Table 1.** Average physical properties of sorghum stems and seeds based on DAT and water content.

| No | Physical parameters | Plant age (moisture content of stems, seeds (%wb)) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 80 DAT (84.18%, 51.7%) | | 90 DAT (80.64%, 30.7%) | | 108 DAT (79.81%, 29.4%) | |
| | | Range | Average | Range | Average | Range | Average |
| 1 | Pile width (cm) | 54–26 | 38.00 ± 10.64 | 52–26 | 38 ± 10.64 | 53–26 | 38.00 ± 10.64 |
| 2 | Pile height (cm) | 16–11 | 14 ± 0.76 | 15–10 | 13.5 ± 0.76 | 14–12 | 13.00 ± 0.76 |
| 3 | Pile weight (kg) | 8.1–3.9 | 6.61 ± 1.27 | 7.6–3.7 | 6.11 ± 1.27 | 7.1–3.9 | 5.61 ± 1.27 |
| 4 | Plant height (cm) | 244–128 | 183.36 ± 30.69 | 260–182 | 210 ± 26.72 | 266–202 | 222 ± 10.08 |
| 5 | Stem diameter (cm) | 1.5–0.8 | 1.09 ± 0.24 | 1.9–1.1 | 1.07 ± 2.2 | 1.8–1.0 | 1.77 ± 2.1 |
| 6 | Seed size: | | | | | | |
| | *l* (mm) | 4.98–3.95 | 4.81 ± 0.15 | 4.78–3.98 | 4.71 ± 0.14 | 4.28–3.95 | 4.18 ± 0.13 |
| | *w* (mm) | 4.53–5.88 | 4.15 ± 0.66 | 4.43–5.88 | 4.18 ± 0.68 | 4.23–5.88 | 4.15 ± 0.64 |
| | *t* (mm) | 2.85–2.49 | 2.68 ± 0.10 | 2.80–2.49 | 2.66 ± 0.10 | 2.75–2.49 | 2.62 ± 0.10 |
| 7 | Weight: | | | | | | |
| | Plant (g) | 289–94 | 166.09 ± 73.19 | 691–154 | 317.73 ± 153.07 | 700–400 | 560.00 ± 15.07 |
| | Panicle (g) | 54–15 | 34.27 ± 14.38 | 178–28 | 74.09 ± 46.07 | 236.07–55.35 | 138.62 ± 63.08 |
| | Seeds in one panicle (g) | 46–12 | 27.91 ± 11.59 | 157–24 | 63.27 ± 39.81 | 210.72–47.60 | 121.43 ± 51.56 |
| 8 | Seed–panicle ratio (%) | 0.91–0.51 | 0.83 ± 0.11 | 0.93–0.73 | 0.85 ± 0.05 | 0.89–0.85 | 0.87 ± 0.01 |
| 9 | 1000 grain weight (g) | 38–30 | 32.0+0.2 | 39–30 | 36.0+0.2 | 49–36 | 44.0+0.5 |
| 10 | Seed diameter (cm) | 0.51–0.35 | 0.40 ± 0.04 | 0.50–0.38 | 0.42 ± 0.04 | 0.50–0.39 | 0.45 ± 0.05 |
| 11 | Litter diameter (cm) | 3.3–3.0 | 3.10 ± 0.09 | 3.9–3.1 | 3.27 ± 2.0 | 3.8–3.0 | 4.57 ± 2.4 |
| 12 | Small panicle stalk diameter (cm) | 1.5–0.8 | 1.09 ± 0.24 | 1.9–1.1 | 1.07 ± 2.2 | 1.8–1.0 | 1.77 ± 2.1 |
| 13 | Stem diameter (cm) | 5.8–5.1 | 5.4 ± 0.19 | 6.1–5.1 | 5.8 ± 0.18 | 5.7–5.0 | 5.6 ± 0.17 |
| 14 | Seed–plant Ratio (%) | 22–10 | 17.0 ± 2.37 | 20–10 | 16.0 ± 2.17 | 17–9 | 12.0 ± 1.86 |
| 15 | Seed density (kg/m$^3$) | 78–60 | 64.0 ± 2.37 | 77–55 | 62.0 ± 2.17 | 76–54 | 61.79 ± 1.86 |
| 16 | Stem stack density(kg/m$^3$) | 56–40 | 48.70 ± 2.37 | 52–45 | 41.2 ± 2.17 | 58–42 | 49.7 ± 1.86 |

The physical characteristics of the Bioguma sorghum include dimensions and shape. The shape of the Bioguma sorghum at 80 DAT, 90 DAT and 108 DAT was round. The sorghum seeds' shape and the seeds' dimensions are needed as a basis for designing thresher and output outlet dimensions. Meanwhile, the ratio of both seed-to-panicle and seed-to-plant ratios is needed to determine the threshing machine's capacity and the conveyor's capacity to carry the sorghum plants to the thresher. The density of the stem stack of Bioguma sorghum has an average value of 41.2 ± 2.17 until 49.7 ± 1.86 kg/m3. The density of plant piles is used to design the carrying conveyor capacity for the threshing machine and the conveyor capacity for the chopping machine. The density of the plant pile also determines the capacity of the hold-on type thresher and the capacity of the cross-flow type thresher. The ratio of seeds to sorghum plants is significant in designing the capacity of a sorghum threshing machine combined with a sorghum stalk chopper under conditions of high moisture content of seeds (20–30%) and stems (70–90%). According to [23], the grain ratio is needed to determine the threshing machine's capacity and the conveyor carrying the sorghum plants to the thresher.

In this study, it was found that the Bioguma variety had a mass of 1000 seeds ranging from 32–44 grams. This value is greater than the study by Gely et al. [12] which use cultivar SDK DK51 where the value ranges from 26–29 grams. This was due to the water content in this study being higher (above 29%wb) while in Gely et al. [12], the water content was below 21%wb.

3.2. Sorghum plant mechanical characteristics

Parameters of the seeds' mechanical characteristics include the seeds' pulling force from the panicle, the angle of seed dropping and the terminal velocity. A previous study reported that litter and small panicles are needed to design threshing machine designs such as topical by calculating the power requirements, the need for separatory sieve mechanisms and blowers and the total power requirement for threshing machines [24]. The mechanical properties of stems including panicle hardness, stem hardness and stem cutting force are needed to design the components of a sorghum stalk chopping machine including knife hardness, number of blades, chopped length, driving force requirements and others. The average mechanical properties of sorghum stems and seeds are presented in Table 2.

**Table 2.** Mechanical properties of sorghum stems and seeds of Bioguma varieties based on DAT and moisture content.

| No | Physical parameters | Plant ages (moisture content of stems, seeds (%wb)) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 80 DAT (84.18 %, 51.7%) | | 90 DAT (80.64%, 30.7%) | | 108 DAT (79.81%, 29.4%) | |
| | | Range | Average | Range | Average | Range | Average |
| 1 | Tensile force of seeds from panicles (N) | 26.2–13.8 | 16.7+3.2 | 16.5–12.0 | 13.8 ± 1.4 | 7.83–5.07 | 6.0 ± 0.8 |
| 2 | Stem hardness(N) | 344–229 | 290.64 ± 29.41 | 380–260 | 315.91 ± 34.85 | 440–280 | 350.00 ± 0.81 |
| 3 | Seed hardness (N) | 10.5–5.8 | 8.2 ± 1.7 | 13.8–5.3 | 9.76 ± 2.4 | 54–30 | 44.9 ± 5.4 |
| 4 | Terminal velocity of litter (m/s) | 2.9–2.1 | 2.53 ± 0.23 | 2.7–2.0 | 2.43 ± 0.25 | 2.6–1.9 | 2.23 ± 0.26 |
| 5 | Terminal velocity of small panicle stalk (m/s) | 2.7–1.8 | 2.33 ± 0.25 | 2.6–1.8 | 2.33 ± 0.26 | 2.5–1.8 | 2.23 ± 0.28 |
| 6 | Terminal velocity of chopped rod (m/s) | 2.9–2.1 | 2.45 ± 0.34 | 2.9–2.1 | 2.45 ± 0.34 | 2.9–2.1 | 2.45 ± 0.34 |
| 7 | Terminal velocity of chopped leaves (m/s) | 2.9–2.1 | 2.34 ± 0.32 | 2.9–2.1 | 2.45 ± 0.34 | 2.9–2.1 | 2.45 ± 0.34 |
| 8 | Terminal velocity of seeds (m/s) | 14.2–11.3 | 12.2 ± 0.89 | 14.2–11.4 | 12.3 ± 0.49 | 13.2–11.3 | 12.1 ± 0.59 |
| 9 | Coefficient of friction (N) | 0.93–0.81 | 0.86 ± 0.06 | 0.87–0.75 | 0.80 ± 0.05 | 0.83–0.71 | 0.76 ± 0.03 |
| 10 | Bar cutting force (N) | 334–220 | 290.64 ± 28.32 | 360–260 | 315.91 ± 24.85 | 423.4–223.7 | 354.00 ± 0.71 |
| 11 | Tensile strength stems (GPa) | 3.29–0.64 | 1.67 ± 0.97 | 0.44–0.26 | 0.33 ± 0.06 | 0.44–0.26 | 0.33 ± 0.06 |
| 12 | Angle of repose (°) | 35.5–25.1 | 34.3+1.67 | 34.2–23.8 | 33.0 ± 3.72 | 33.70–22.10 | 32.34 ± 0.15 |

3.3. Tensile force of seeds from panicles

The tensile force of the sorghum seeds from the panicle of sorghum causing deformation occurs, namely the sorghum seeds break or cracks occur in the range of the maximum tensile force value of 26.2 N at a seed moisture content of 51.7%, 16.5 N at a moisture content seed 30.7% and 7.85 N at a seed moisture content of 29.4%. The results of this study indicate that the tensile strength of the seeds decreases with decreasing moisture content. This research is not in line with research by [25] on patchouli plants that showed the higher the water content, the lower the tensile strength of the patchouli stems. Likewise, it is also not in line with research on bamboo plants which shows that the lower the water content of bamboo, the higher the tensile strength of bamboo [26]. This is because the seeds are attached to the leaf litter on the tip of the small panicle stalk and not on the panicle stalk. So, the withdrawal process occurs on the leaf litter and if the leaf litter dries out the sorghum seeds will fall off more easily. Likewise, this is because when an object experiences a pull, the object experiences an internal force and an external force in the process of its attraction. The external force is the force that changes the initial state of the object while the internal force is the force that comes from within the object. The results of the tensile force of

sorghum seeds from panicles based on different planting ages are presented in Figure 8.

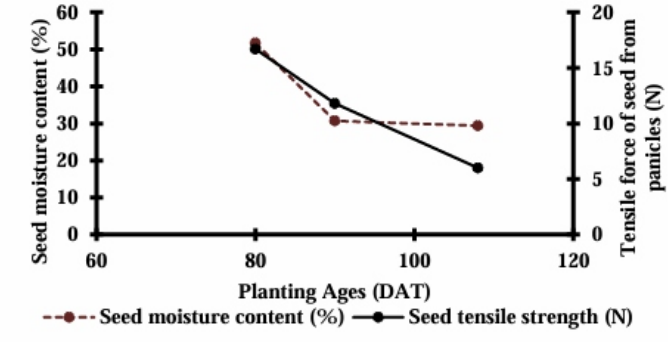


**Figure 8.** Tensile force test of seed from panicles at various seed moisture contents and planting ages.

3.4. Hardness of sorghum stems and seeds

Stem and seed hardness is the property of resistance to breaking due to the applied compressive force. Hardness is the ability to withstand pressure that causes the stems/seeds to break. The hardness of the samples was shown on the intact stems and seeds. The Bioguma variety has a hardness of around 8.2–44.9 N. This value was similar to that of the Seredo variety but lower than the Serena and Karimtama varieties [11]. The stem hardness of the Bioguma variety ranges from 290–350 N which is still below the hardness range of sugarcane stems around 775 N [27]. The hardness of sorghum stems and seeds will be higher with lower stem moisture content and seed moisture content. The effect of DAT on moisture content and sorghum grain hardness is presented in Figure 9 and the effect of DAT on stem moisture content and stem hardness is presented in Figure 10.

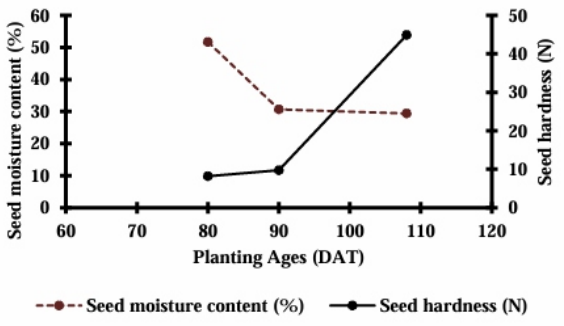


**Figure 9.** Influence of DAT on moisture content and sorghum grain hardness.

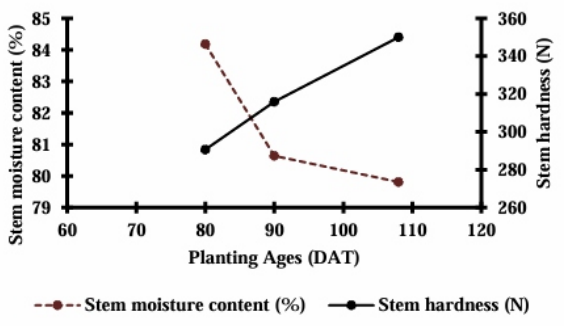


**Figure 10.** Influence of DAT on stem moisture content and stem hardness.

3.5. Angle of repose and coefficient of friction

The pouring angle is needed for the design of the slope of the outlet for threshing beans. The sorghum grain pouring angle was carried out three times with an average pouring angle of 34.3 ± 1.67° at a condition of 51.7% seed moisture content, an average pouring angle of 33.0 ± 3.72° at a seed moisture content of 30.7% and an average pouring angle of 32.34 ± 0.15° at 29.4% seed moisture content. This indicates that an increase in the bulk angle can occur with an increase in water content. In accordance with research conducted by [28] that the corn bulk angle increased significantly from 49° to 58° in the range of 4.73% to 22% moisture content. [29] recommended using galvanized iron for the manufacture of grain seed vessels with a tilt angle of 40°. The characteristic coefficient of friction based on the calculation of Equation 6 above an average of 0.76 at a moisture content of 29.4% is necessary for the design of the rod carrying conveyor to the chopping machine.

3.6. Terminal velocity

The terminal velocity of leaf litter, seeds and small panicle stalks which are part of the sorghum panicle is used as the basis for designing the separation of impurities from threshed seeds in a hold-on type sorghum threshing machine. The measurement results showed that the terminal velocity of the litter was around 2.9–2.10 m/s, small panicle stalks around 2.6–1.80 m/s, leaf chopping around 2.9–2.10 m/s, stem chopping around 2.9–2.10 m/s and seeds around 14.1–11.2 m/s. This can be the basis for determining the blower speed above 2.9 m/s and below 14 m/s so that litter and other debris are blown away but not the sorghum seeds. The results of the terminal velocities of sorghum seeds and other impurities are in line with several studies on the separation of impurities in grains, stalks and leaves that have been carried out by several researchers aimed at improving the quality and quality of the product. Gorial and O'callaghan [30] performed particle separation using horizontal airflow. Five different samples were used in this experiment wheat germ mix, different grain mix (soybean, pea, Adzuki bean, mung bean, sorghum, millet and oilseed rape), wheat germ mix, oilseed rape mix and soybean/grass seed mix. Sacks with a width of 20 cm each are attached to the dispensing hole. Air flow is blown with a speed of 8 m/s and 11 m/s on a continuous channel with a length of 4 m using a blower with a 2 kW motor power. In order to eliminate the effects of turbulence and eddy, there are two fine screens placed on the feeder line which is close to the discharge section. The results showed that the effectiveness of separating grain was influenced by air velocity, grain size and density. The air velocity of 11 m/s causes the material to float along the channel and most of the husks are accommodated at the farthest outflow. However, the low air velocity (8 m/s) causes most of the mixture to be contained in the three sacks near the feed, the low air velocity combined with the high feed rate results in poor separation. At an airspeed of 11 m/s, 1.1% of the soybean seeds are found in the grass seeds and 0.85% of the grass seeds are found in the soybeans. However, when the air velocity decreased to 8 m/s half as much soybean was found in the grass seeds and 3.3% of the grass seeds remained among the soybeans. At a continuous channel length of 160 cm and an air speed of 11 m/s, it allows the wheat to be separated by 95%.

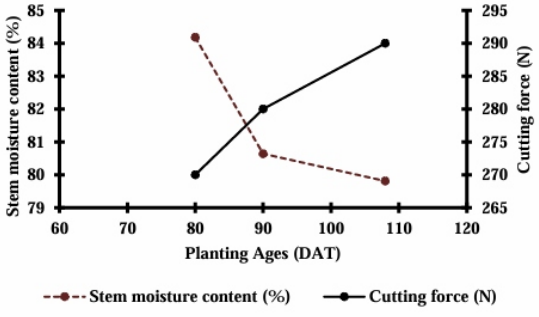


**Figure 11.** Stem cutting force at various seed moisture contents.

3.7. Sorghum stem cutting force

The design concept of the sorghum stalk chopping machine is based on the stem cutting force test. This parameter exhibited an overall increase as the number of DAT increases. At 80 DAT, the force required to sever the stem is 270.00 N which rises to 280.00 N at 90 DAT and 290.00 N at 108 DAT. This trend indicates that as the plant matures the stems become tougher and require greater force to cut. It suggests a possible accumulation of structural components or changes in stem anatomy leading to increased resistance to cutting forces. The graph of the cutting force at various moisture contents is shown in Figure 11.

## 4. Conclusions and recommendation

This research examined the physical and mechanical properties of sorghum stems and seeds which are relevant to design a hold-on thresher and sorghum chopping machine. The tensile force of seed from panicles decreased significantly from 80 to 108 DAT with average values of 16.7, 11.8 and 6.0 N respectively. In addition, seed hardness increased over time from 8.2 to 44.9 N while stem hardness also increased ranging from 290.64–350 N. Furthermore, cutting force exhibits an overall increase from 270 to 290 N as DAT increased. This trend indicates that as the plant matures the stems become tougher and require greater force to cut. These parameters are relevant for the development of a thresher machine for sorghum because they affect the machine's ability to efficiently separate the grain from the rest of the plant material. The decrease in tensile force from 80 to 108 DAT may make it easier for the thresher to separate the grain from the plant material. The increase in seed hardness over time suggests that the thresher machine can use greater force without damaging the seed to effectively separate them from the rest of the plant material. This may require adjustments to the machine's design or operating parameters to ensure that it can efficiently separate the grain without damaging the seeds. The machine will need to be designed to effectively separate the grain from the rest of the plant material while minimizing damage to the stems and ensuring efficient separation of the harder seeds. Future research can explore the engineering properties of other sorghum varieties. Different varieties may exhibit variations in physical and mechanical characteristics which can influence the design and optimization of threshing and chopping machines. Comparing multiple varieties will provide a broader understanding of sorghum plants and facilitate the development of machinery suitable for diverse sorghum crops. While this study lays the foundation for designing appropriate machinery, future research should conduct long-term field trials to evaluate the performance and durability of the developed threshing and chopping machines in real-world scenarios. These trials should consider factors such as maintenance requirements, reliability and adaptability to varying field conditions. Feedback from farmers, industry stakeholders and end-users can further refine and improve the machinery.

## Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

## Conflict of interest

The authors declare no conflict of interest.

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# Mealybug vectors: A review of their transmission of plant viruses and their management strategies

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## ABSTRACT

*Mealybugs cause mechanical damage and diseases to plants. Through their feeding activities, they reduce the yield, quality and productivity of crops. This review discusses mealybug vectors of plant viruses, the economic losses they cause, mealybug species and their hosts. Among the numerous mealybug species, Planococcus species are the most effective vector of plant viruses, transmitting many Ampeloviruses. Diverse methods for the control and regulation of mealybugs are also discussed. Physical, cultural and biological control methods are labor-intensive but environmentally friendly compared to chemical methods. However, chlorpyrifos are one the active ingredients of insecticides effective against several mealybug species. Using plant products as neem oil as a biocontrol method has been effective, similar to other insecticides. Notwithstanding, the biological method of controlling mealybugs is effectively slow but safe and highly recommended. The Anagyrus species have the highest success rate amongst other natural parasites of mealybugs. Also, farm sanitation and pruning as cultural methods help reduce mealybug populations.*

***Keywords:*** *Mealybugs; Planococcus; Pseudococcus longispinus; Dysmicoccus brevipes; Closteroviridae; Ampeloviruses*

## 1. Introduction

The world's population is growing so fast that at the end of 2021 it was 7.9 billion people [1]. This is a rapid growth rate compared to ten years ago (6.194 billion) [2]. With this growth rate, it is estimated that the world population will be around 9,782,061,758 in 2051 [3].

This exponential growth trend has an adverse impact on food security. To meet the dietary requirements for this population growth, food production needs to be scaled up. Among the various food sources, plants are the largest food source for humans. A total of 50–90% of the human diet is of plant origin [4]. However, these contributions of plant products to the human diet are likely to diminish due to several factors. The decrease in agricultural land use size, plant diseases and other biotic factors threaten plant productivity. Among these factors, plant pathogenic diseases are the major problem, where 16% of annual plant yield losses are due to plant pathogenic diseases [5].

Plant pathogens contributes a troubling decline in global food security and crop production [6]. Plant pathogens are transmitted through a variety of vectors. Insects and nematodes [7] play a role in pathogen transmission. Insects, the most popular and effective of all the vectors, pose a concern for plants, animals and human health [8].

Two orders of insects (Hemipterans and Thysanopteras) have the most devastating effect on crop yield [9]. Among the various orders of insects, Hemipteran [10], Coleopteras [11], Thysanoptera (generally thrips) [12–15], Lepidoptera [16] and Diptera [17,18] are known vectors of plant viruses, fungi and

bacteria. Close to one-fourth of all plant viruses require insect vectors for effective transmission [9]. Coleopterans are effective transmitters of Bromoviruses, Carmoviruses, Comoviruses, Machlomoviruses, Sobemoviruses and Tymoviruses [19]. Hemipterans (aphids, whiteflies and leafhoppers), transmit most plant viruses and bacteria. They are infamous for the transmission of more than one pathogen. Furthermore, their brisk reproductive cycles and diverse plant hosts give them an advantage in plant virus transmission [20,21]. Although thrips, aphids and whiteflies account for over 50% of plant virus transmission [22,23], the role of mealybugs in plant viruses transmission is noteworthy. Of the Homoptera insect order, mealybugs are one of the major vectors of plant viruses [24]. With various biotic problems of plants, viral diseases are one of the biggest constraints to plant health [25]. Transmission of plant viruses is dependent on the mealybug life stage, temperature and suitable host. However, information about the viruses transmitted by mealybugs is not comprehensive as that of aphids, whiteflies and thrips. This article provides a comprehensive review of the different types of plant viruses transmitted by different species of mealybugs, and various management strategies to reduce and control the devastating effects of mealybugs.

## 2. Morphology of mealybugs

Mealybugs (Pseudococcidae) are destructive insect pests of crop plants. Mealybugs are either monophagous [26] or polyphagous. Mealybugs perfectly homogenize with their host plant, thanks to the wax produced by the host plant, which covers them and offers them camouflage. It is estimated that 149 mealybug species feed on plants with their piercing and sucking feeding behavior. The Planococcus species are the most common and destructive [27], causing severe mechanical damages to crop plants. Despite having a diverse feeding host, woody and herbaceousplants are most preferred. They pierce and suck the plant sap, which causes sooty mold from releasing sap materials, reduces the plant chlorophyll content and thus affect photosynthesis [28–30]. During feeding, viral particles (especially those retained in the stylet and foregut) are released through their stylet [31]. The stylets are withdrawn into the body after and when not feeding [32].

Mealybugs are approximately 5mm long [33], with adult females 3-5mm and males average 3mm long [34,35]. Adult females retain some nymph-like features attributable to incomplete metamorphosis and are wingless. Similarly, male adults also undergo incomplete metamorphosis, but they are much smaller than the females and possess wings that aid them in moving to female mealybugs for mating [26,33,36].

### 2.1. Life cycle of mealybugs

The lifecycles of mealybugs differ according to their sex and species [37]. Male and female mealybugs have the same life cycle from the egg stage to the 2nd instar stage. In males, the prepupa stage is the next stage after the 2nd instar stage, then follows the Pupa, and finally to the adult male stage. However, unlike the male, the female mealybug has a 3rd instar stage that ends at the adult stage [38], as observed in Figure 1a and b below.

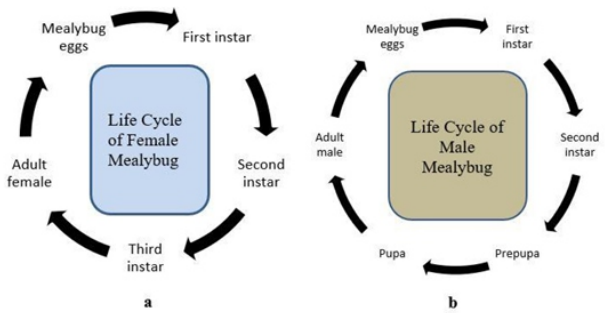


**Figure 1.** Illustration of female and male mealybug lifecycles (adapted and altered from [37,38]).

As observed from Figure 1a and b, male and female mealybugs have similar life cycles. However, certain mealybug species may require additional stages. The male pineapple mealybug (Dysmicoccus brevipes Cockerell), longtailed mealybug (Pseudococcus longispinus) were confirmed to have a third Instar stage between the second instar and prepupa stage [37,39]. Also, the female pineapple mealybug had a crawler stage instead of the first instar stage. Daane et al. (2008) also reported a similar life cycle of Planococcusficus to that of Dysmicoccus brevipes.

2.2. Hosts of mealybugs

Different species of mealybugs are found on plants in greenhouses, nurseries, plants and landscapes. Over the world, approximately 246 of several plant families serve as hosts for almost 5000 species of mealybugs [41]. Poaceae are the most popular host plants (585 species) for mealybugs, with Cyperaceae having the least number of host plants (75 species), and [42–44] emphasized that mealybugs feed on nearly 149 plant species, through the sucking of plant sap which causes leaves to distort and fall.. Destruction and pathogen transmission by mealybugs have been reported on guava, citrus, pomegranate, grapes, sugarcane, banana, black pepper, pineapple plantain, stone fruit, berries, yam, cassava, cashew, papaya, pawpaw and cocoa [31,42,45–50].

Weeds such as Amaranthus vividus, Bidens Pilosa, Sonchus oleraceus, Chenopodus ambrosoides, Commelina sp., Cucumis anguria, Momordica charantia, Cyperus rotundus, Chamaesyce hirta, Croton sonderianus, Jatropha urens, Mimosa pudica, Piptadenia moniliformis, Serra macranthera, Herissanthia crispa, Sida cordifolia, Sida galheirensis, Sida rhombifolia, Sidastrum micranthum, Sidastrum sp., Digitaria horizontalis, Talinum paniculatum, Watheria douradinha, Malva sylvestris L., Redroot pigweed, Amaranthus retriflexus L., Crimson clover, Trifolium incarnatum L., Toadflax, Linaria sp. and Chorizococcus rostellum also serve as host plants for mealybugs [51,52]. Various degrees of mealybug infestation have been reported in Kenya [53], Nigeria [54], Ghana [48,50,55,56], Indonesia [57], New Zealand [58], India [57,59–61] and Israel [26]. In Turkey, 25 weed species from 14 plant families were found to host 5 mealybug species [51].

2.3. Some reported economic losses due to mealybugs

Several studies have confirmed the economic losses caused by the destructive effects of mealybugs and the virus diseases they transmit. According to Asare Bediako et al. [50], the mealybug wilt of pineapple (MWP) causes approximately US $248/ha in losses to pineapple fruit yield in Ghana, while causing 30–50 % of fruit yield loss in Hawai in the United States, depending on the age of the plant. Three cassava mealybugs (Phenacoccus manihoti, Planococcus herreni and Planococcus spp.) have been reported to cause cassava yield reduction in Sub-Saharan Africa. Phenacoccus manihoti is estimated to cause an 80% reduction in cassava yield [62]. Similarly, the Hibiscus mealybug (Maconellicoccus hirsutus) is indicated to cause a US $75 million loss annually in the United States of America [26]. Prabhakar et al.[30] reported various economic losses in several countries due to cotton mealybug (Phenacoccus solenopsis),Papaya mealybug (Paracoccus marginatus) and several mealybugs on cotton plant yields. Previous studies show that green coloration occurs in situations where both the male and female mealybug are found (especially between Dysmicoccus brevipes and Dysmicoccus neobrevipes) but are absent in a situation where only one parent was found (either male or the female) [63]. This green colouration affects the quality and market value of the produce. It is worth noting that the direct effect of viruses transmitted by mealy bugs is difficult to estimate since other factors in combination with the virus diseases cause economic damages to the crops. In a by Franco et al. [64], Planoccocus citri and other species of mealybug cause economic losses in citrus orchards in the Mediteranean regions.

**Table 1.** A summary of selected plant virus mealybug vectors, their common names and host plant information.

| Species of Mealybugs | Common Names | Host Plants | References |
|---|---|---|---|
| *Pseudococcus longispinus* | Longtailed mealybug | Citrus, grapes, nursery stock, indoor ornamentals, citrus, taro, avocado, guava, eggplant. | [34,35,65] |
| *Pseudococcus maritimus* | Grape mealybug | Grapes, Pears, Pomegranate other fruit trees, apricots | [34,35,66] |
| *Planococcus citri (cryptus)* | Citrus mealybug | Citrus, landscape shrubs | [34,35,51] |
| *Planococcus ficus* | Vine mealybug | Grapes, fruits, ornamental plants | [34,35,40,67] |
| *Rastrococcus iceryoides* and *R. invadens* | Mango Mealybug | Mango and Citrus | [35,68] |
| *Dysmicoccus brevipes* | Pineapple mealybug | Pineapple, avocado, banana, celery, citrus, clover, cocoa, coconut, coffee, custard apple, figs, ginger, guava, maize, mango, oil palm, orchids, groundnut, peppers, plantain, potato and sugarcane. | [26,35,69,70] |
| *Planococcus kenyae* | Kenya mealybug | Coffee, yam, pigeon pea, passion fruit, sugarcane and sweet potato | [27,71] |
| *Saccharicoccus sacchari* | Sugarcane mealybug | sorghum, rice and some grasses, sugarcane | [26,72] |
| *Ferrisia virgata* | Striped mealybug | Common on most crops | [26,34] |
| *Ferrisia gilli* | Gill's mealybug | Pistachios | [73] |
| *Heliococcus bohemicus* | Bohemian mealybug | Grapevine | [74] |
| *Phenacoccus aceris* | Apple mealybug | Grapevine, apple | [74] |
| *Planococcus solani* Ferris *Phenococcus solenopsis* Tinsley | Solanum mealybug | Solanaceous crops | [34,35] |
| *Maconellicoccus hirsutus* | Pink hibiscus mealybug | Hibiscus | [35,75] |
| *Paracoccus marginatus* | Papaya mealybug | Papaya, Solanaceous crops, cotton, pomegranate, pea, sweet potato. | [30,53,76] |
| *Nipaecoccus viridis* | Spherical mealybug | Cotton | [77] |
| *Planococcus kraunhiae* | Japanese mealybug | Broad bean | [26,78] |
| *Planococcus minor* | Passionvine mealybug | Vine | [79] |
| *Planococcus njalensis* | Cocoa mealybug | Cocoa | [54] |
| *Pseudococcus viburni* | Tuber mealybug | Donkey lettuce, Whitestem filaree, Tubular flower, Spanish needle, Hairy fleabane, grapes, persimmon | [80–82] |

## 3. Mealybug vectors of plant viruses

Compared to aphids and whiteflies, mealybugs are transmitters of a few genera of plant viruses. Due to their less mobile nature, they are less effective in transmitting plant viruses than aphids, leafhoppers and other insect vectors. In addition, the sex and age of mealybugs affect virus transmission rates. For example, old female mealybugs are less efficient in transmitting plant viruses [83]. Also, the life stages of the nymph affect their transmission rate of viruses (adults are more effective than nymphs) [19].

Mealybugs transmit viruses of the genus Ampelovirus [31,63], and some Closteroviruses [84], of the Closteroviridae family. Mealybugs also transmit badnavirus [47,85] of the Caulimoviridae family. Closteroviridae generally consists of four genera- Closterovirus, Ampelovirus and Crinivirus, Velarivirus [86,87]. Some studies have also confirmed the transmission of vitiviruses by some mealybug species [83]. These viruses trigger leaf discoloration, deformation, mottling and leaf yellowing.

### 3.1. Ampeloviruses transmitted by mealybugs

Based on the organization of the positive-strand RNA genomes, Ampeloviruses can be subdivided into different groups [87]. Ampeloviruses have a non-enveloped capsid, 1400–2200 nm long virion, 13.0–18.5 kb segmented genome [86] and filamentous shape. The genome of Ampelo-like air potato virus 1 (AiPoV1) is estimated to be around 13,398 nucleotides [88]. Mealybugs are the main vectors of Ampeloviruses. In a semi-persistent manner, mealybugs transmit Ampelovirus and other viruses in the Ampelovirus genus in a semi-persistent mode (GLRaV 3) [31,89]. In semi-persistent (foregutborne) virus transmission, viruses are spread from the stylet of the insect up to the foregut. The virus does not spread beyond the foregut of the insect vector. Within 20 minute-period, the mealybug picks up the virus and infects the host [90]. The virus does not reproduce and multiply in the vector, and retention of the virus in the host spans from hours to days [9]. Studies indicate that semi-persistent viruses influence the feeding behavior of their host [91]. The injuries caused by their stylets during feeding, triggers plant defense response [62]. During feeding, the saliva of some mealybug species, especially Maconellicoccus hirsutus, causes harmful effects to plants [92]. Ampeloviruses cause vascular diseases with obscure symptoms. However, studies show that Ampelovirus, when combined with other viruses, causes mixed infection in plants [88]. Most Ampeloviruses are transmitted by Dysmicoccus brevipes (Pseudococcus brevipes), Dysmicoccus neobrevipes [63] and Pseudococcus longispinus.

From Table 1, Planoccocus mealybug species are more active in transmitting plant Ampeloviruses. Planococcus ficus is a regular transmitter of five strains of Grapevine leafroll associated viruses [93]. These can cause mixed infections since the mealybugs are vectors of numerous viruses [88] as observed in Table 2. According to a study by Sether et al. [94], Pineapple mealybugassociated wilt viruses, when associated with pineapple's Mealybug wilt virus, resulted in 100% yield loss. Also, mealybugs acquire and transmit viruses with or without association with other viruses. For example, mealybugs (Dysmicoccus brevipes and Dysmicoccus neobrevipes) were found to transmit Pineapple Mealybug-associated virus-3 (PmaV-3) without the transmission of PmaV-1 [94], although they are vectors of these two viruses [95]. It is worth noting that some other insect species do actively transmit Ampeloviruses. For example, Parthenolecanium corni (Coccidae) was reported to transmit GLRaV-3 [96].

**Table 2.** A summary of some ampeloviruses and their mealybug vectors.

| Virus species | Mealybug vectors | Hosts | References |
|---|---|---|---|
| Air potato ampelovirus (AiPoV 1) | *Planococcus* spp. | Air potato | [88] |
| Blackberry Vein banding associated virus | *Planococcus* spp. | Blackberry | [97,98] |
| Grapevine leafroll-associated virus 1 | *Planococcus ficus, Pseudococcus longispinus, Phenacoccus aceris, Heliococcus bohemicus* | Grapevine | [19,74,99] |
| Grapevine Leafroll - associated virus 3 | *Planococcus ficus, Pseudococcus. longispinus, Ferrisia gilli, Phenacoccus aceris, Pseudococcus calceolariae, Heliococcus bohemicus,Pseudococcus maritimus* | Grapevine | [19,74,96,99–102] |
| Grapevine leafroll-associated virus 4 | *Planococcus ficus, Pseudococcus longispinus, Phenacoccus aceris* | Grapevine | [19,101,103] |
| Grapevine leafroll associated virus 13 | *Planococcus ficus, Pseudococcus longispinus* | Grapevine | [19,95] |
| Pineapple mealybug associated viruses 1 and 3 | *Dysmicoccus brevipes,Dysmicoccus neobrevipes* | Pineapple | [19,104] |
| Pineapple mealybug associated virus 2 | *Dysmicoccus brevipes, Dysmicoccus neobrevipes* | Pineapple | [19,50,63,98] |
| Pistachio ampelovirus | *Planococcus ficus* | Pistachio | [105,106] |
| Fig leaf mottle associated viruses 1 and 2 | *Ceroplastes* spp. | Fig | [107,108] |
| Manihot esculenta virus 1 | *Phenacoccus manihoti, Phenacoccus herreni* | Cassava | [62] |

3.2. Closteroviruses transmitted by mealybugs

The genus Closterovirus belongs to the family Closteroviridae. Closteroviruses have two huge gene modules: one for genome replication, and the other for genome packaging and transport within the cells. The genome of Closterovirus is linear, positive RNA, with a maximum size of 19.3 kb [109]. In comparison to Ampeloviruses, fewer Closteroviruses are transmitted by mealybug vector species. For example, the Little cherry virus 2 belonging to the closterovirus genera is transmitted by Phenacoccus aceris [83,110].

3.3. Badnavirus transmitted by mealybugs

The genus Badnavirus belongs to the Caulimoviridae family. Viruses found in Caulimoviridae have semicircular double-stranded DNA. They have a genome length range of 7.2–9.2 kbp. Eight divisions (Badnavirus, Caulimovirus, Cavemovirus, Petuvirus ,Rosadnavirus, Solendovirus, Soymovirus and Tungrovirus) are members of the Caulimoviridae family based on host range, insect vector and the basis of genome organization [111]. Badnaviruses affect monocots and dicots. Most Badnaviruses are horizontally transmitted through mealybugs and aphids [111,112]. Fewer or no symptom is associated with Badnavirus infections [113]. The effectiveness of their transmission is dependent on the species of mealybugs. Badnavirus often have more than one species of the same vector as transmitters (Table 3). For example, 14 established vectors of Cocoa Swollen Shoot Virus [114], of which Planoccoides njalensis, Planococcus citri, Ferrissia virgata are potent transmitters of the Cocoa Swollen Shoot Virus [115]. Mealybugs usually feed on the flowers and pods. Like Ampelovirus, Badnaviruses are transmitted by mealybugs in a semi-persistent manner [115].

**Table 3.** A summary of badnaviruses transmitted by mealybug vectors.

| Virus Species | Mealybug vectors | Hosts | Reference |
|---|---|---|---|
| Cocoa Swollen Shoot Virus | *Planoccoides njalensis, Planococcus citri, Ferrissia virgata* | Cacao | [114,115] |
| Banana Streak Virus | *Planoccocus citri Risso,Saccharicoccus sacchari,Dysmicoccus brevipes,Ferrisia virgata* | Banana | [69,83] |
| Citrus Yellow Mosaic Badnavirus | *Planococcus citri* | Citrus | [33] |
| Sugarcane bacilliform virus | *Saccharicoccus sacchari* | Sugarcane | (Sastry, 2013) |
| Piper yellow mottle virus | *Ferrisia virgata,Planococcus citri,Pseudococcus elisae,* | Black pepper | [19,83] |
| Sugarcane mild mosaic virus | *Saccharicoccus sacchari* | Sugarcane | [19] |
| Taro bacilliform badnavirus | *Pseudococcus solomonensis* | Taro | [83] |
| Schefflera ringspot virus | *Planococcus citri* | Schefflera | [83] |
| Dioscorea bacilliform RT virus | *Planococcus spp* | Yam | [116] |

3.4. Vitiviruses transmitted by mealybugs

Vitiviruses belong to the family Flexiviridae and they are flexuous, filamentous, 12–13 in diameter [117] and 725–825 nm in length [118]. They are monopartite, positive sense and singlestranded. Vitiviruses were initially considered Trichoviruses, but the differences in their genome organizations provided a basis for their differentiation [119]. Their virions contain RNA genome in a tail-like structure facilitating their transmission to plants by their insect vector [117].

**Table 4.** A summary of vitiviruses and mealybug vectors.

| Plant virus | Mealybug vector | Hosts | References |
|---|---|---|---|
| Grapevine virus A (Kober Stem Grooving) | *Pseudococcus spp,Planococcus ficus* | Grapevine | [31,82,83] |
| Grapevine virus B (Corky bark disease) | *Planococcus ficus* | Grapevine | [31,82,83] |
| Grapevine Virus D | *Phenacoccus spp* | Grapevine | [83] |
| Grapevine Virus E | *Heliococcus spp* | Grapevine | [31,83] |

Vitiviruses are transmitted by mealybugs and other insect genera (Pseudococcus, Planococcus, Phenacoccus, Heliococcus, Neopulvinaria, Parthenolecanium, Cavariella and Ovatus)(Table 4) in a semipersistent manner [83].

## 4. Management strategies for mealybugs

Recent technological advances have influenced the methods and dynamics of controlling and managing mealybugs. Feeding behaviors determine the control strategy. The common management strategies are physical, chemical, cultural and biological. Environmental conditions such as temperature, humidity and others are considered when designing pest management strategies.

**Table 5.** A summary of mealybug species and physical control.

| Mealybug Species | Physical Method | Key findings | Reference |
|---|---|---|---|
| *Dysmicoccus brevipes, Dysmicoccus neobrevipes* | Ant barriers | Red ants were controlled causing the decrease in pink pineapple mealybug transportation | [120] |
| *Planoccocus njalensis* | Crop barriers, Barrier cropping | Farms with barrier crops had low mealybug infestation cases in comparison to those with none | [115,121] |
| *Drosica mangiferae* | Crop rotation | Adequate control of mango mealybug | [122] |
| *Planococcus ficus* | 51–53 °C hot water treatment of grape cuttings | Eradication of more than half of *Planococcus ficus* population | [123] |
| *Planococcus ficus* | Ultralow oxygen treatment | Complete eradication of all life stages of *Planococcus ficus* | [82] |
| *Dysmicoccus brevipes, Dysmicoccus neobrevipes* | 50 °C-30 minutes hot water treatment of pineapple propagules | Most of the mealybug population were destroyed | [124] |
| *Planococcus citri* Rossi, *Pseudococcus odematti* Miller and Williams | Hot water immersion of propagules | 90–95% of the mealybug population were eliminated | [123] |

### 4.1. Physical methods

The physical (mechanical) pest control method involves using hurdle to reduce the contact between the pest and the crop. Physical control eliminates the pest or triggers behavioral or feeding changes in the pest [125].

Most physical methods share some similarities in their pest-elimination strategies. Despite their effectiveness, they are time-consuming and labor-intensive. Hand-picking of mealybugs, and off tree parts heavily infested by mealybugs control mealybugs [34,35,115]. Growing barrier crops and destroying wild mealybug host plants have reduced contact between the mealybug vector and the host plant. Ameyaw et al.[115] reported on using citrus and oil palm in cocoa farms as barrier crops since they are not appropriate hosts for the mealybug vectors of Cocoa Swollen shoot virus. These crops break the mealybug vectors cycle since they are unsuitable hosts.

Results from studies performed by Franco et al.(2004) on pheromone traps to control Planococcus citri and Pseudococcus cryptus male mealybugs indicated that male mealybugs of the Planococcus citri population was significantly reduced. Also, trapping and eliminating mealybugs have proven to be a

population regulator of mealybugs. Sticky plate traps help regulate some mealybugs species, especially Planococcus citri [26]. Similarly, the pheromone of some mealybug species can be manipulated to attract predators or natural enemies to them (as in the case of Anagyrus pseudococci in the control of Planococcus ficus) [26]. Also,the use of biological barriers,heat treatment amongst others are suitable in the control of mealybug species as seen in Table 5.

4.2. Cultural Methods

Like other pest control methods, cultural methods are diverse. They are environmentally friendly but labor-intensive. The cultural method involves a combination of practices that reduces the population and interrupt the infection cycle of pests. They include crop rotation, sanitation practices [34,35] and humidity control on the farm (Table 6).

**Table 6.** A summary of mealybug species and cultural controls.

| Mealybug species | Cultural method | Key findings | References |
|---|---|---|---|
| *Planococcus ficus* | resistant rootstocks (IAC 572,10-17A, RS-3) | Resistant rootstocks were more resistant to *Planococcus ficus* infested as compared to other rootstocks | [82,123,126] |
| *Planococcus ficus* | Low soil nitrogen content | Grape plants on low nitrogen level soil had low mealybug presence in comparison to other grape plants on soils with high nitrogen content | [82] |
| *Formicoccus njalensis, Planococcus citri* | Breeding resistant varieties | Mealybug infestation was less in comparison to non-resistant varieties | [121] |
| *Sachharicoccus sacchari* | Resistant varieties (Giza 96/74, Ph 8013) | Self-peeling varieties were less infected by the S*accharicoccus* mealybug as compared to other varieties | [127,128] |
| *Planococcus njalensis* | Roguing and pruning | Cocoa crops with pruned diseased parts had less mealybug infestation as compared to those not pruned | [121] |
| *Saccharicoccus sacchari* | Flood irrigation, burning of dry leaves in the field | Number of mealybug infestation per plant was reduced | [128] |
| *Saccharicoccus sacchari* | Low nitrogen fertilizer application, roguing, farm sanitation | Mealybug population was lower in farms where these practices were enforced | [128] |
| *Saccharicoccus sacchari* | Drip irrigation | Increased drip irrigation method significantly reduced S*accharicoccus sacchari* population | [128] |

Some crops have a genetic combination that helps them rejuvenate and regenerate after heavy mealybug feeding. AR23 (cassava genotype), an improved variety of cassava, was found to develop new leaves and rejuvenate into a healthy plant after severe damage was caused by the cassava mealybug [62]. Inter-Upper Amazon Hybrids of cocoa also have resistivity against heavy mealybug infestation [90]. However, there is innate resistance in some plants against some species of mealybugs.

For example, different citrus varieties are reported to show varying levels of susceptibility to the citrus mealybug [79]. This underlines mealybug species preferences for special kinds of plants over others.

In addition, regular pruning of trees in and around the farm is encouraged. Mealybugs develop and multiply rapidly in a warm and humid environment [83]. Pruning trees deprives mealybugs of the necessary moist conditions. Thus, it exposes them to harsh weather conditions, such as sunlight that will slow or stop their rapid growth and gradual extinction.

Sanitation practices on the farm should be considered. The destruction of old and new heavily infested plant propagules should be practiced. In addition, farm equipment should be sanitized to reduce the transport of mealybug eggs within the farm. The destruction of cocoa trees affected by the Cocoa swollen Shoot Virus reduced the spread of the plant virus to healthy cocoa plants [90].

Also, fertilizers and irrigation within the farm should be regulated. Studies have demonstrated a relationship between wet soils coupled with high nitrogen content and mealybug growth [92]. There is a significant multiplication of mealybugs in the farm if the soil has high water content with significantly higher nitrogen levels. Daane et al. [40] confirmed the increase in the Planococcus ficus population due to increased nitrogen fertilizer use. Soils with high moisture content and adequate nitrogen levels help regenerate new plant parts. The mealybug then has new and succulent plant parts to feed on, and reproduction is encouraged. Adversely, Rae et al. [72] observed an increase in the Saccharicoccus sacchari at 320mg/L of nitrogen, but their population declined at a relatively higher nitrogen concentration.

### 4.3. Biological control

Biological pest control methods use natural enemies to eliminate or reduce the population of pests. Biological control methods, although labor-intensive, are environmentally friendly. Recently, biological pest control methods have been gaining popularity. Several natural parasitoids of mealybugs have been enacted, but only a few have proven very effective. Aphelinidae and Platygasterida species have yielded appreciable results [26]. Natural enemies of mealybugs are numerous e.g. parasitic wasps, ladybird beetles, hoverflies, lacewings [35], etc. This wasp lays its eggs on the maturing mealybugs, killing these mealybugs and feeding on them. Gyranusoidea, Coccophagus, Leptomastix, Allotropa, Pseudaphycus and Acerophagus are reported to be parasitic wasps of mealybugs [26,129]. In Africa and South America, Apoanagyrus lopezi and Epidicarno lopezi are reported to be effective in regulating the Cassava mealybug (Phenaccocus manihoti) [35,62]. Gyranusoidea tebygi and Anagyrus mangicola are natural enemies of the mango mealybugs, Rastrococcus invadens and Rastrococcus iceryoides [34,35]. In addition, the population of citrus mealybug is reported to be reduced by natural parasites, such as Leptomastidea abnormis (Girault), Leptomastix dactylopii Howard, Chrysoplatycerus splendens Howard and Anagyrus pseudococci (Girault). However, parasitic fungus, such as Entomophthora fumosa and other natural parasites (brown lacewing, Sympherobius barberi (Banks) and green lacewing, Chrysopa lateralis Guérin, trash bugs, syrphid fly larvae and scale-eating caterpillars, Laetitia coccidivor, Cryptolaemus montrouzieri Mulsant, Decadiomus bahamicus (Casey) Scymnus flavifrons Melsheimer, Chilocorus stigma (Say) and Olla abdominalis var. plagiata (Say), are reportedly effective against some species of mealybug [130].

The mode of action by which these parasitoids and predators suppress and eliminate different species of mealybugs differs. For example, Epidicarnosis lopezi, a parasitoid of cassava mealybugs, lays eggs on the mealybug and their larvae feed on them [26]. Similarly, the mealybug predator Cryptolaemus montrouzieri, reported by Anjana and Joy [37], can feed on a maximum of 5000 mealybug eggs in various life stages. Additionally, Anagyrus kamali controls the pink mealybug population by piercing the adult mealybug and laying eggs in them. The eggs hatch and the contents of the mealybug are used to nourish itself until it attains adulthood [37].

Consideration should be given to other insects (especially ants) that may antagonize the success of this

biological control based on their relationship with mealybugs. The population of ants must be under control since they can mitigate the effectiveness of this method. Some ants have a mutualistic relationship with mealybugs [26,37,40], since they benefit from the honeydews made by mealybugs. Ants have an antagonistic relationship with the natural enemies of mealybugs. Also, ants play a role in the transportation and dispersion of several mealybug species [90], as several studies have demonstrated the transport and dispersal of mealybugs by ants [26,131].

The citrus mealybug (Planococcus citri) is reported to be effectively controlled by a range of parasites such as Eptomastidea abnormis (Girault), Leptomastix dactylopii Howard,Chrysoplatycerus splendens Howard and Anagyrus pseudococci (Girault) (Table 7).

**Table 7.** A summary of mealybug species and biological control agents.

| Mealybug species | Natural Predators | Key findings | References |
|---|---|---|---|
| *Planococcus citri* | *Leptomastix dactylopii* | L*eptomastix* was superior t*o* other natural enemies | [26,132] |
| *Phenacoccus manihoti* | *Apoanagyrus lopezi, Epidinocarsis lopezi,Apoanagyrus diversicornis* | Apoanagyrus species had maximum control of the cassava mealybug species in relation to other natural enemies | [133–135] |
| *Rastrococcus invadens* | *Gyranusoide tebygi, Anagyrus mangicola* | Effective control of *Rastrococcus invadens* | [35] |
| *Planococcus ficus* | *Anagyrus pseudococci, Nephus angustus, Nephus quadrivattus, Nephus ninaevatus, Nephus sp., Hyperaspis felixi, Sycmnus nubilis Mulsant, Cynodia lunata, Rhizobiellus sp., Hippodamia sp., Chrysopa sp.* | The Anagyrus species was more effective in controlling *Planoccocus ficus* mealybug | [26,136] |
| *Phenacoccus solenopsis* | *Oenopia* (Synharmonia) *conglobata*(L.), *Cheilomenes propingua* (Mulsant) *Chrysoperla carnea* (Stephens), *Chrysoperla mutata* (Mc Lachlan) (Neuroptera: Chrysopidae), *Sympherobius elegans* (Stephens); *Sympherobius fallax* (Navas), (Neuroptera: Hemerobiidae) | These parasitoids had higher parasitizing activity as compared to other predators | [137,138] |
| *Dysmicoccus brevipes* | *Heterorhabditis amazonensis* (NEPET 11 and IBCD.n40) | These two isolates reduced over 80% of t*he Dysmicoccus brevipes* population | [139] |
| *Dysmicoccus brevipes* | *Metarhizium anisopliae, Beauveria bassiana and Lecanicillium lecanii* | These fungi had maximum control of pink pineapple mealybug and other mealybugs | [140] |
| *Planococcoides njalensis* | *Acerophagus notativentis, Acerophagus pallidus, Aenasius abengoroui, Aenasius martini, Anagyrus aurantifrons, Anagyrus beneficiens, Arhopoides sp., Blepyrus saccharicola, Leptomastix bifasciatus, Leptomastix dactylopii, Platynapsis higginsi, Pseudaphycus sp., Scymnus sp., Tetracnemoidea sydneyensis, Tropidophryne melvillei* | These predators have higher success in t*he* control of *Planococcoides njalensis* | [141] |
| *Maconellicoccus hirsutus* | *Anagyrus kamali* | *Anagyrus kamali* fed on more than 78 % of *Maconellicoccus hirsutus* reducing their population | [142,143] |

The use of chemicals during biocontrol methods should be regulated. In addition, non-selective insecticides tend to kill or neutralize several beneficial insect pollinators.

4.4. Chemical control

In recent times, chemical control methods have generated public outcry due to the accumulation of chemical residues in plant and food products [144,145] and their negative effects on the environment [146,147]. Cocco et al. [82] reported on the harmful levels of imidacloprid and chlorpyrifos (active ingredients in the control of several mealybug species) in waterbodies in Spain.Similarly, Babar et al. [148] emphasized on the need to regulate the use of profenofos, carbosulfan and methidathion during the control of Drosicha mangiferae on citrus farms in Pakistan. Mansour et al. [149] proposed in their review paper that the use of spirotetramat in combination with other treatments will effectively help reduce the population of Planococcus ficus and Planococcus citri. Chemicals used in pest control include acaricides, insecticides, rodenticides, fungicides, larvicides. The use of insecticides in the control of mealybugs is not recommended because their outer covering, made up of wax, protect them against the insecticides [26]. With time, they develop resistance to these chemical insecticides. Phenacoccus solenopsis is reported to show a minimal reaction to insecticides that are lethal to other mealybug species [150]. Also, mealybugs hide underneath leaves and their large group makes it difficult for the chemicals to have maximum contact [150]. Their rapid reproduction cycle is also reported to contribute to their resistivity to insecticides [151].

Insecticides containing dinotefuran, imidacloprid, or pyrethroids [26,152], which are active ingredients that are effective against crawling mealybugs but have serious irritations on other beneficial insect pollinators. Daane et al. [40] confirmed the reduction in the population of Planococcus ficus when insecticides with chlorpyrifos active ingredients were applied.

Alcohol is effective in the control of mealybug. A previous study confirmed the association between alcohol application and mealybug mortality. A spray with a 70% concentration of isopropyl alcohol killed 70-80% of most mealybug species when applied against them [92].

Biopesticides where plant extracts are used to combat mealybugs infestation are also effective against mealybugs. Extracts from plants, such as neem, have proven effective against plant pathogens and pests [153–156]. According to Abul Monjur Khan et al. [157], 2% of neem oil effectively reduced 30% of the papaya mealybug when applied. Azadirachtin, a compound in neem trees, slows insect metamorphosis and reproduction. the Azadirachtin compound leads to a reduced growth rate and death of insects [158]. Neem Kernel water extracts are deadly to young cassava mealybugs [34,35]. 1–2% concentration of insecticidal soaps and vegetable oil as biopesticides have successfully controlled mealybugs [34,35]. Additives, such as oil, dissolve and break up the thick covering of the mealybug [26].

Insecticides that disrupt the nervous system of insects, like the organophosphates class of insecticides (chlorpyrifos, acephate, dichlorvos and diazinon), are recommended to control mealybugs (Table 8). When applied in the right amount, this class of insecticides has been proven to eliminate most species of mealybugs [26].

**Table 8.** A summary of some chemical controls on mealybug species.

| Mealybug species | Chemical Control | Key Findings | References |
|---|---|---|---|
| *Dysmicoccus brevipes* (Cockerell)/Pineapple mealybug | 50% Fenithrothion,50% Fenthion,40.8% Chlorpyrifos | After 21 days, the mixture of these chemicals resulted in higher mealybug mortality after the second dose than the other tested chemicals | [159] |
| *Dysmicoccus brevipes* | Omethoate,48mg of AI Phorate per plant | More than half of the *Dysmicoccus brevipes* population were eliminated | [160] |
| *Phenacoccus solenopsis* | Acephate, Chlorpyrifos | *Planococcus solenopsis* mealybug was reduced by 69% after Acephate and Chlorpyrifos as compared to other chemical treatments | [161] |
| *Phenacoccus solenopsis* | Brufozen | After 3 days, Brufozen decreased the mealybug population by 95% | [161] |
| *Phenacoccus manihoti* | Diazinon,Phosphamidon, Methidathion | Diazinon, Phosphamidon and Methidathion were 12.7,10.8 and 7.3% effective in controlling the cassava mealybug as compared to the control | [162] |
| *Pseudococcuscoccus njalensis* | (CR409) Bisdimethylamino-fluoro-phosphine oxide | CR409 was superior in the control of the cocoa mealybug | [163] |
| *Planococcus citri* | 0.075% Zethiol,0.075% Nogos 100 EC, Bisdimethylamino-fluoro-phosphine oxide (CR409) | 0.075% Zethiol and 0.075% Nogos 100EC completely eliminated *Pseudococcus citri*.CR409 had complete control over *Planococcus citri* | [164] |
| *Maconellicoccus hirsutus* | Spirotetramat, bifenthrin, flypyradifurone, fenpropathrin | In the nymph stage, the fecundity of mealybug was highly affected after day 6 | [165] |
| *Planococcus ficus* | Chlorpyrifos, Mevinphos | Chlorpyrifos, mevinphos had superior control as compared to other methods | [126] |

## 5. Conclusions

This paper reviewed the economic losses caused by mealybugs, mealybug-transmitted plant viruses, their mode of transmission, host plants of mealybugs and the control methods of mealybugs. The paper also highlighted someeconomic losses of mealybugs. In times of evolving plant viruses, the role of mealybugs cannot be underestimated.

Mealybugs are active in transmitting plant viruses' genera belonging to the Closteroviridae family. Of these genera, Ampeloviruses and Badnaviruses are actively transmitted by mealybug species. Due to various environmental pollution problems, chemicals should be reduced or replaced by other safe control methods. Therefore, the biological control method of environmentally friendly mealybugs should be encouraged. For example, the Anagyrus species are effective against several mealybug species as biological control methods. Additionally, the use of plant products with insecticidal properties (neem seeds, leaves) to control mealybugs should be well researched.

Breeding of more mealybug-resistant varieties of plants should be encouraged. Genes that allow crop plants to withstand the aggressive feeding of mealybugs must be well studied. The acquisition and use of more tolerant varieties will help small-scale farmers who cannot afford expensive control methods. Using one control method at a time makes the mealybug species build up resistance in a shorter time. In

effect, further studies should be conducted on using Integrated Pest Management (IPM) strategies in the management of mealybug species. IPM strategies are critical in controlling and managing mealybugs in the long term.

**Use of AI declaration**

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

**Conflict of interest**

The authors declare they have no conflict of interest.

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# The influence of hot-air mechanical drying on the sensory quality of specialty Colombian coffee

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## ABSTRACT

*The main aim of this study was to evaluate the impact of mechanical drying on the sensory quality of specialty coffee produced on three Colombian coffee farms. The technique involved a study of the coffee bean drying process parameters, such as temperature (35, 45 and 55 °C), airflow (100 m3/min·m2) and thickness (0.2 m) for mechanical drying, vs conventional drying in the open sun until 11% of moisture content was reached. For mechanical drying, the effective diffusion coefficient, electrical conductivity and drying kinetics were evaluated. A sensory test was performed for three storage periods (3, 6 and 9 months) using the Specialty Coffee Association (SCA) protocol. The results showed that the effective diffusion coefficient varied from 3.21 to 8.02 × 10−7 m2/s for mechanical drying and from 4.21 × 10−11 m2/s for drying in the open sun. The time drying time was established at 20.35 ± 0.06, 29.10 ± 0.09 and 71.52 ± 0.11 hours for mechanical drying at 55 °C, 45 °C and 35 °C respectively and 54.48 ± 11.37 hours for drying in the open sun system. The average moisture content at the end of all drying operations was 12.5%. Electrical conductivity rose from 11.71 to 16.86 µS/cm·g at drying temperatures ranging from 35 to 55 °C. The sensory test revealed that storage duration had no effect on the quality of the coffee drink when in touch with the drying process, with mechanical drying yielding higher sensory ratings. The coffee beans were dried at 55 °C, yielding coffee samples with SCA scores more than 85 points. In overall, it is determined that the convective mechanical drying method is a viable approach for the processing of specialty coffee beans since it allows for the retention of high-quality sensory qualities, allowing it to command higher market pricing.*

***Keywords:*** *specialty coffee; drying kinetics; diffusivity coefficient; sensorial quality*

## 1. Introduction

Coffee is one of the most popular beverages around the world and a rather relevant food commodity from an economic standpoint. In that sense, green beans are a largely produced and commercialized commodity worldwide, with an average global production of approximately million 60-kg bags [1]. Botanically, coffee belongs to the genus Coffea of the Rubiaceae family, with the commercially relevant species being C. arabica and C. canephora and it is only produced in tropical regions that have specific soil and climatological characteristics [2]. A complex system has been related to the coffee supply chain, which involves several agents such as agricultural inputs firms, farmers, commodity traders, food industries, retailers, coffee shops and the final consumer [3]. Likewise, coffee fruit processing includes steps such as harvesting, postharvest process (dry, semi-wet and wet processing), dehulling, size

grading, roasting, grinding, extraction and drying, the last step for industrial coffee factories.

With current changes in the preferences of consumers, who are increasingly aware of the ethical and environmental implications, the production processes and the people behind their food, the specialty coffee market has become one of the products with the highest growth and interest worldwide. Specialty coffee is defined as a beverage with unique and distinct sensorial attributes. It is derived from green coffee beans obtained by selective harvesting of ripe fruits (handpicking), which are free of primary defects (stones, sticks, black and sour beans). Specialty coffee is processed by a controlled fermentation, followed by a traditional open sun drying process [4]. The fermentation process has been previously examined by different authors, who define it as the process with a major impact on volatile compounds, composition, quality and value of the final product. These factors allow the product to reach higher market prices due to superior qualification values, as defined by the SCA scale (>85) [4,5].

After the fermentation process, the coffee beans must be dried to avoid bacterial or mold activity, thus preventing over fermentation of coffee beans. The drying process aims to evaporate the water, or the volatile constituents present in the food material and to reduce water activity (aw) through a complex phenomenon that involves processes of heat and mass transfer [6,7]. Several authors have reported the use of drying as a method of processing agro-industrial products and by-products such as avocado [8], passion fruit [9] and coffee and coffee byproducts [6], among others. In the coffee industry, drying of green coffee beans is a critical step for the overall quality, since drying avoids damage and weight loss. Since green beans must be dried immediately due to the high moisture content derived from the washing and fermentation processes (>50%), coffee is considered a perishable product [10,11]. Overall, the drying process is associated with the country of the coffee's origin and can be performed by hot-air or open sun drying.

In Colombia, it is common that farmers apply open sun drying, which is carried out on flat ground, platforms or concrete terraces until the beans reach the desired water content (<12%). This method is used to reach the moisture content required by the Colombian standard. Open sun drying is a procedure that has not altered significantly since the beginning of coffee production in Colombia and it is unlikely to change in the future. This type of drying technique using solar energy, makes it an economical process that is advantageous mainly for small farmers. However, this process need at least 100 square meters of drying area, takes several days depending on the climatic conditions and the coffee beans need to be homogenized 3 times a day, Otherwise, it can be compromised the sensory characteristics of the final product [4,5,11,12]. Nowadays, for Colombian farmers, mechanical drying is a technology that is still unknown and viewed with suspicion and is frequently associated with high cost and low quality.

The mechanical drying of coffee is a technique that allows for a better utilization of the properties of coffee and is used to reduce the moisture content in coffee beans [13]. This technique involves the use of drying machines that apply heat and hot air to accelerate the process of water evaporation in the beans. Looking for to achieve a faster and more efficient drying process, [14] evaluated the influence of different drying techniques (direct sun exposure, cabinet sun drying, heat pump drying, hot air drying and freeze-drying) on the bioactive components, fatty acid composition, and volatile chemical profile of green robusta coffee beans. The authors reported that freeze-drying is an efficient way to preserve saturated and unsaturated fatty acids as well as organic acids as well as more than 62 volatile chemicals. According to the authors, the maximum concentration of volatiles was achieved with heat pump drying, while the highest quantity of volatiles was obtained with lyophilization. Finally, the drying techniques direct exposure to the sun were shown to have a tight association. However, the lyophilization and hot air-drying methods were notably different from the remainder of the drying process.

According to the above, the traditional method takes several days to process, requires large drying spaces and is an uncontrolled process based on the experience of the farmer. To the best of our

knowledge, this is the first time to work about the effect of mechanical drying with different conditions over the sensorial quality of specialty green coffee beans. The objective of the present work was to evaluate the effect of the mechanical drying process on the sensorial quality of specialty coffee produced in three different Colombian coffee farms and compare the results with samples obtained by traditional open sun drying technique. This work provides a processing alternative to farmers in the coffee industry, aiming to reduce drying process time and produce coffee beans that can be sold in the international market as a specialty coffee.

## 2. Materials and methods

### 2.1. Materials

150 kg of Castillo® variety coffee was collected by handpicking in a state of optimum maturity from trees planted on three different farms located at 1700 meters above sea level in Valle del Cauca, Colombia. The farms are La Esmeralda farm (4°17'00"N; 75°49'15"W), La Morelia 2 farm (4°16'39"N; 75°48'40"W) and Villa Laura farm (4°16'07"N; 75°5'01"W). The collected coffee was processed by the wet method and the fermentation process was controlled with the Fermaestro® method.

### 2.2. Fermaestro® method

The Fermaestro® method has proven to be an effective tool in accurately determining the washing point when natural fermentation is carried out using a device that helps to determine the optimal washing point [15,16]. In this regard, the Fermaestro® implement consists of a truncated cone of half a liter with holes in the base and walls, which is filled with freshly depulped coffee and placed in the mass of coffee that is fermenting; this way, the coffee inside the Fermaestro® follows the same fermentation process as the coffee in the tank [16].

### 2.3. Drying process of the coffee samples

After washing, two drying processes were applied to coffee samples until 11% w.b of moisture content was reached (Figure 1).


**Figure 1.** (a) Diagram of the equipment used for drying coffee with hot air and (b) open sun.

The weight change over time was measured with a gravimetric method for both drying techniques [17,18]. The air-hot drying process (mechanical drying) was carried out in a static layer silo with a maximum capacity of 15 kg of coffee samples. The mechanical drying process was performed using a silo dryer equipped with a heat source, fan and devices based on Arduino technology. Specifically, the equipment consisted of a fan coupled to an electrical resistance for air heating, which passed through a tunnel with a height of 40 cm. The electrical resistance consisted of a 6-inch tubular plate with a working range of 110 to 120 volts (Haceb, Colombia). The temperature of the drying air was set at 35, 45 and 55 ±

1 °C, with data collection performed by a data acquisition system every 60 minutes. The air velocity rate was set at 100 ± 0.1 m3/min·m2 and the maximum bed height of coffee was 0.20 m. The microcontroller of the Arduino mega board was programmed through a computer using serial communication via the RS-232 port available on the board. The firmware of the system was programmed to sense relative humidity using DHT11 sensors and to control the temperature using type K thermocouples. The signals from the sensors were sent to the computer software control and automation system, which consisted of a user interface (UI) developed using C# technology. The open sun drying process was carried out in a patio under direct exposure to the sun. The thickness of the coffee was 0.01 m, and the sample was mixed every 2 hours. At night, the coffee was packed to protect it from relative humidity and avoid re-moistening of the coffee.

After the coffee drying processes were completed, the parchment coffee was packed in GrainPro® Hermetic PouchTM bags (GrainPro, USA) and stored for three, six and nine months at 23 ± 2 °C and 75 ± 3 relative humidity inside a darkroom.

## 2.4. Modeling of coffee beans drying process

A dimensionless moisture ratio (MR) was calculated from the drying curves as shown in Equation 1, where Xt is the moisture content at any time t (g water/g dry basis), Xe is the moisture content at the equilibrium (g water/g dry basis) and X0 is the initial moisture content (g water/g dry basis).

$$MR = \frac{X_t - X_e}{X_0 - X_e} \tag{1}$$

values of $X_e$ are considered relatively small compared to $X_t$ or $X_0$ [6].

The effective diffusion coefficient ($D_{eff}$) was determined using Fick's second law for an infinite slab (open sun drying) and spherical geometry (mechanical drying), shown in equations 2 and 3, respectively [19,20]. Fick's law was used for one-dimensional transport with the assumptions that moisture migrates only by diffusion, negligible shrinkage occurs, and the diffusion coefficients and temperature are constant [21].

$$MR = \frac{8}{\pi^2}\sum_{i=1}^{\infty}\frac{1}{(2i-1)^2}e^{\left(\frac{-(2i-1)^2\,\pi^2\,D_{eff}\,t}{4L^2}\right)} \tag{2}$$

$$MR = \frac{6}{\pi^2}\sum_{i=1}^{\infty}\frac{1}{j^2}e^{\left|-j^2\,\pi^2\,D_{eff}\frac{t}{r^2}\right|} \tag{3}$$

However, for long drying times (MR < 0.6), only the first terms of equations 2 and 3 are relevant for the evaluation of MR and can be simplified as shown by equations 4 and 5, respectively.

$$MR = \frac{8}{\pi^2}e^{\left(\frac{-D_{eff}\times\pi^2\times t}{4L^2}\right)} \tag{4}$$

$$MR = \frac{6}{\pi^2}e^{\left|\pi^2\,D_{eff}\frac{t}{r^2}\right|} \tag{5}$$

Deff is the effective moisture diffusion coefficient ($m^2.s^{-1}$), t is the drying time (s), L is the half-thickness of the slice (m) and r the radius of the sphere (m). Different semi-theoretical methods were used to provide an understanding of the transport processes and to demonstrate a better fit to the experimental data. All the temperatures were modeled, in that sense 55 °C was selected in order to show graphically the behavior of the mechanical drying process. The semi-theoretical models are shown in Table 1.

**Table 1.** Semi-theoretical models to describe drying kinetics.

| No | Model | Equation | Reference |
|---|---|---|---|
| 1 | Page | $MR = \exp(-kt^n)$ | (Akoy, 2014) [22] |
| 2 | Henderson and Pabis | $MR = a \exp(-kt)$ | (Hashim, Daniel & Rahaman, 2014) [23] |
| 3 | Midilli et al. | $MR = a \exp(-kt) + bt$ | (Ayadi, Mabrouk, Zouari & Bellagi, 2014) [24] |
| 4 | Demir et al. | $MR = a \exp(-kt)^n + b$ | (Demir, Gunhan & Yagcioglu) [25] |

*2.5. Moisture and electrical conductivity*

The obtained coffee samples were tested for moisture according to the methodology described by the norma técnica colombiana NTC 2325/2005 [26]. The electrical conductivity was tested following the methodology described by [27] and a Hanna brand HI8733 portable conductivity meter was used (µS/cm·g).

*2.6. Sensorial quality*

Sensorial analysis of the coffee samples was carried out applying a methodology reported by [28,29]. Sensory evaluation was performed in different sessions involving a total of 15 expert panelists. The description of the sensory attributes and the score of the beverage was carried out according to the SCA protocol for specialty coffee. After carrying out the coffee roasting process according to SCA protocol, 50 grams of roasted coffee were ground, ensuring that 70–75% of the particles passed through a 20-mesh sieve (Retsch, Germany) and 5 cups of coffee were prepared with a ratio of (55 g coffee/1 L $H_2O$). Frag/aroma, flavor, aftertaste, acidity, body, uniformity, balance, clean cup, sweetness and overall quality were tested. The total score of each coffee sample was converted into an SCA point scale and the average of the panelists' scores was calculated.

*2.7. Experimental design and statistical analysis*

A 4 × 3 randomized factorial experimental design was performed with two independent variables: drying process temperature (55 °C, 45 °C, 35 °C and solar drying) and storage time (3, 6 and 9 months), with a block factor (3 farms). The responses that were measured included diffusivity coefficient ($D_{eff}$), moisture content, electrical conductivity and sensorial test. Data were expressed as mean ± SD of three replicates. The data and RMS were analyzed and performed using R software (R Development Core Team, 2004). An analysis of variance (ANOVA) was applied where the effects were considered significant when $p < 0.05$. The FactoMineR package in R language was used for the factorial analysis of mixed data (FAMD) to find the similarities between the quantitative and qualitative results in the analyzed variables [30,31].

## 3. Results and discussion

3.1. Effect of mechanical drying process conditions on effective diffusion coefficient analysis of green coffee beans

The influence of drying conditions (35 °C, 45 °C, 55 °C and open sun drying) on drying time, moisture content (MC), diffusivity coefficient (Deff) and electric conductivity (EC) of the coffee samples is

presented in Table 2.

According to the data shown in Table 2, the drying time required to get an MR = 0.1 (Equiation1) varied from 20.35 to 71.52 hours. Increasing the process temperature results in lower process time. The Diffusivity value (Deff), based on Fick's second law, presented significant differences ($p < 0.05$) for all drying processes. The Deff ranged from 3.21 to 8.02 × 10−7 m2/s for mechanical drying and values of R2 ranged between 0.83 to 0.96. On average, open sun drying showed diffusivity of 4.21 × 10−11 m2/s.

In general, the previous values are in accordance with those reported by [32], who related that overall, the diffusivity values for food matrices are between 10−11 and 10−8 m2/s. The values obtained for Deff from mechanical drying were lower than these values, indicating a faster water evaporation process in mechanical drying compared to sun drying. This is because mechanical drying is a controlled process, whereas open sun drying depends on climatic conditions (temperature and relative humidity). These conditions are not constant in tropical regions like Colombia, where the climate is characterized by rainy seasons, cloudiness and limited hours of sunlight. Likewise, the effective diffusivity values increased greatly with increasing drying temperature, as an elevated heating energy leads to an increase in the activity of water molecules, thus higher moisture diffusivities [22].

**Table 2.** Results for drying process (mechanical and solar drying) applied to specialty Colombian coffee samples.

| Drying process | Variable | | | |
|---|---|---|---|---|
| | Drying time (h) | MC (% db) | $D_{eff}$ ($m^2$/s) | EC (µS/cm·g) |
| 35 °C | 71.52 ± 0.11 $^{a}$ | 12.67 ± 0.03 $^{ab}$ | 3.21E-07 ± 4.96E-10 $^{a}$ | 11.71 ± 0.10 $^{a}$ |
| 45 °C | 29.10 ± 0.09 $^{b}$ | 12.59 ± 0.22 $^{ab}$ | 6.32E-07 ± 1.79E-09 $^{b}$ | 14.40 ± 0.09 $^{b}$ |
| 55 °C | 20.35 ± 0.06 $^{c}$ | 12.42 ± 0.02 $^{a}$ | 8.02E-07 ± 1.61E-08 $^{c}$ | 16.86 ± 0.13 $^{c}$ |
| Open sun drying | 58.48 ± 11.37 $^{d}$ | 12.79 ± 0.24 $^{b}$ | 4.21E-11 ± 5.37E-12 $^{d}$ | 11.87 ± 0.08 $^{d}$ |

Note: Values are expressed as the mean ± standard deviation. Means in same column with different superscript letters are significantly different ($p \leq 0.05$) by Fisher's LDS test.

Figure 2 shows the drying curves obtained using the operating conditions that produced the dehydrated product in 20 h (55 °C), 29 h (45 °C) and 72 h (35 °C).

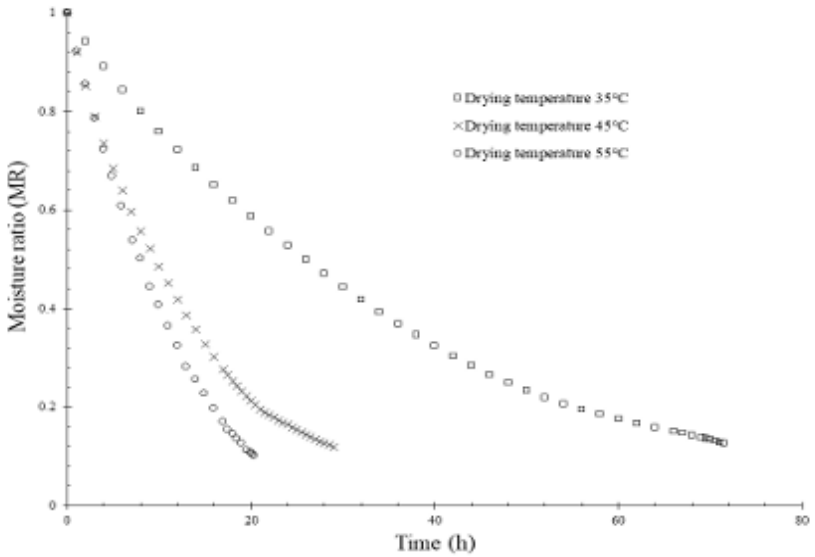


**Figure 2.** Convective drying curve obtained from the operating drying conditions.

Subsequently, an Arrhenius-type adjustment was made of the Deff values obtained as a function of the inverse of the temperature to establish the activation energy (Ea) of the process. On average, the Ea of the mechanical process was 900.6 J/mol. However, when the Ea was calculated for each farm, the following values were obtained: 892.29 J/mol (La Esmeralda farm), 886.30 J/mol (La Morelia 2 farm) and 922.55 J/mol (Villa Laura farm). The differences in the values could be explained by the geographical location of each farm, as that can have an influence on the behavior of the process.

The final moisture content was between 12.42 and 12.79 g/100 g d.b, in the sense that a moisture content of 10 to 11% (wet basis) was obtained to commercialize parchment coffee. The electric conductivity varied between 11.71 to 16.86 μS/cm/g. The drying temperature (T) significantly influences the electric conductivity ($p < 0.05$), with higher values of temperature correlating to an increase in the electric conductivity. This behavior indicates that the cell membrane of coffee beans is affected by the temperature, which favors the diffusivity process and hence the loss of water. Likewise, higher temperature is related to an increase in the enthalpy of the system, which increases the transfer of mass and energy, thus accelerating the migration of water [6,22]. The results found in this work are like those reported by [33] and lower than those reported by [27].

Table 3 shows values of the drying constants and drying coefficients of the selected models.

**Table 3.** Results for the drying kinetics described by semi-theoretical models.

| Model | Parameters | 35 °C | 45 °C | 55 °C |
|---|---|---|---|---|
| 1 | $R^2$ | 0.9926 | 0.9696 | 0.9927 |
| | k | 9.05E−05 | 5.94E−04 | 1.57E−04 |
| | n | 1.2531 | 1.1534 | 1.3926 |
| | Standard error | 0.0265 | 0.0481 | 0.0276 |
| 2 | $R^2$ | 0.9809 | 0.9645 | 0.9706 |
| | a | 1.0594 | 1.0348 | 1.0874 |
| | k | 6.54E−04 | 1.71E−03 | 2.12E−03 |
| | Standard error | 0.0419 | 0.0521 | 0.0556 |
| 3 | $R^2$ | 0.9991 | 0.9692 | 0.9994 |
| | a | 1.0008 | 1.0050 | 1.0142 |
| | b | 0.0000 | 0.0000 | −0.0002 |
| | k | 4.90E−04 | 1.49E−03 | 1.37E−03 |
| | Standard error | 0.0087 | 0.0491 | 0.0081 |
| 4 | $R^2$ | 0.9994 | 0.9710 | 0.9999 |
| | a | 1.1529 | 0.9809 | 1.2436 |
| | b | −0.1664 | −0.0192 | −0.2489 |
| | k | 4.42E−04 | 1.47E−03 | 1.28E−03 |
| | n | 1.0496 | 1.1800 | 1.1055 |
| | Standard error | 0.0072 | 0.0483 | 0.0033 |

From the Table, the drying constant (k) is a function of temperature, where an increase in drying temperature leads to an increase in the drying constant. In all cases, the R2 values for the models were greater than 0.95, indicating a good fit and varied between 0.9696 and 0.999. These values show that the tested drying models predict the drying process of coffee beans adequately. Figure 3 shows the plotting of the experimental data with the predicted values using Page, Henderson, Midilli and Demir models for coffee samples processed at 55 °C by mechanical drying.

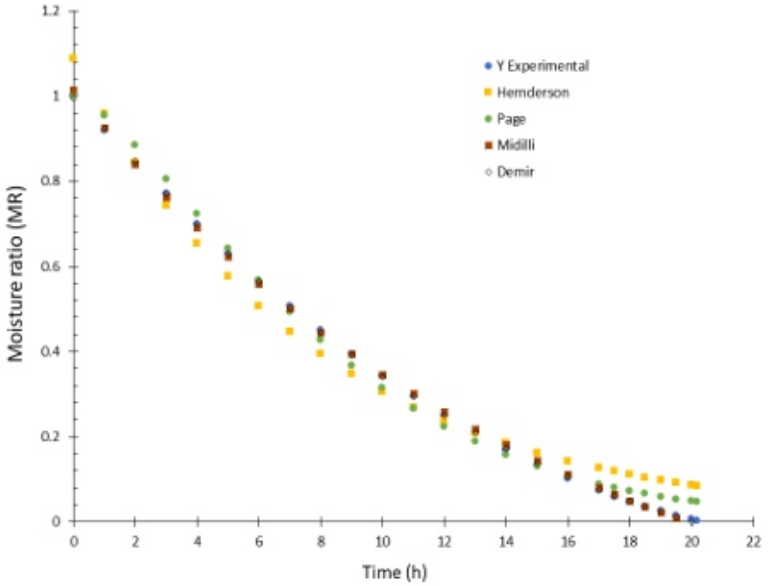


**Figure 3.** Predicted $MR_t$ versus Experimental MR by Page, Henderson, Midilli and Demir models at 55 °C.

The diagram shows that the observations are clustered along the linear regression line, which demonstrates the adequacy of the selected models in describing the drying characteristics of coffee beans.

3.2. Sensory analysis of dried coffee beans

The scores obtained for fragrance/aroma, flavor, aftertaste, acidity, body, uniformity, balance, clean cup, sweetness and overall quality for samples coffee evaluated as presented in Table 4. The total score of each coffee sample was converted into an SCA point scale and all samples were given a score higher than eighty. Overall, the drying process presented a significant effect ($p < 0.05$) for all the coffee samples, while storage time did not present a significant effect ($p > 0.05$) over the sensory attributes evaluated.

The uniformity, clean cup, and sweetness of the beverages scored a value of 10 in all the samples, which indicates that the storage conditions and drying processes produced coffee beans with the minimum quality requirements for the specialty coffee market. On the other hand, the samples produced at 55 °C and for the entire storage time reached higher scores for fragrance/aroma, flavor, residual flavor, acidity, body and balance. The results obtained for global score (Table 4) indicate that coffee samples dried at 55 °C and 45 °C benefit the sensorial characteristics of coffee samples and reach the SCA requirement to be selected for the specialty coffee market. According to the results obtained in the sensorial test, it can be inferred that shorter drying time and higher temperature favors the sensory profile of the samples. These factors favor the concentration of important chemical compounds in the formation of flavor and aroma during the roasting process, as reported by additional authors [14,34,35].

**Table 4.** Sensory attributes evaluated in specialty coffee samples for the drying process.

| Sensory attributes | Drying process | | | |
|---|---|---|---|---|
| | 35 °C | 45 °C | 55 °C | Open sun drying |
| Fragrance/aroma | $8.03 \pm 0.19^{a}$ | $7.96 \pm 0.29^{ab}$ | $8.50 \pm 0.17^{c}$ | $7.83 \pm 0.14^{b}$ |
| Flavor | $7.78 \pm 0.12^{a}$ | $7.80 \pm 0.12^{ab}$ | $8.11 \pm 0.18^{b}$ | $7.63 \pm 0.25^{a}$ |
| Aftertaste | $7.50 \pm 0.21^{a}$ | $7.78 \pm 0.20^{b}$ | $7.94 \pm 0.18^{b}$ | $7.50 \pm 0.20^{a}$ |
| Acidity | $7.78 \pm 0.19^{ab}$ | $7.67 \pm 0.19^{a}$ | $7.89 \pm 0.13^{b}$ | $7.76 \pm 0.13^{ab}$ |
| Body | $7.52 \pm 0.18^{a}$ | $7.75 \pm 0.22^{b}$ | $7.97 \pm 0.15^{c}$ | $7.53 \pm 0.18^{a}$ |
| Uniformity | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ |
| Balance | $7.66 \pm 0.06^{a}$ | $7.79 \pm 0.14^{b}$ | $7.97 \pm 0.15^{c}$ | $7.62 \pm 0.14^{a}$ |
| Clean cup | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ |
| Sweetness | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ | $10 \pm 0^{a}$ |
| Overall | $7.80 \pm 0.14^{a}$ | $7.86 \pm 0.14^{a}$ | $8.08 \pm 0.36^{b}$ | $7.73 \pm 0.18^{a}$ |
| Total score SCA | $84.00 \pm 0.48^{ab}$ | $84.60 \pm 0.80^{b}$ | $86.50 \pm 1.00^{c}$ | $83.60 \pm 0.74^{a}$ |

Note: Values are expressed as the mean ± standard deviation. Means in same row with different superscript letters are significantly different ($p \leq 0.05$) by Fisher's LDS test.

For a better understanding of the effect of temperature the sensory profiles of the cup based on the 10 attributes during the storage time are shown in Figure 4.

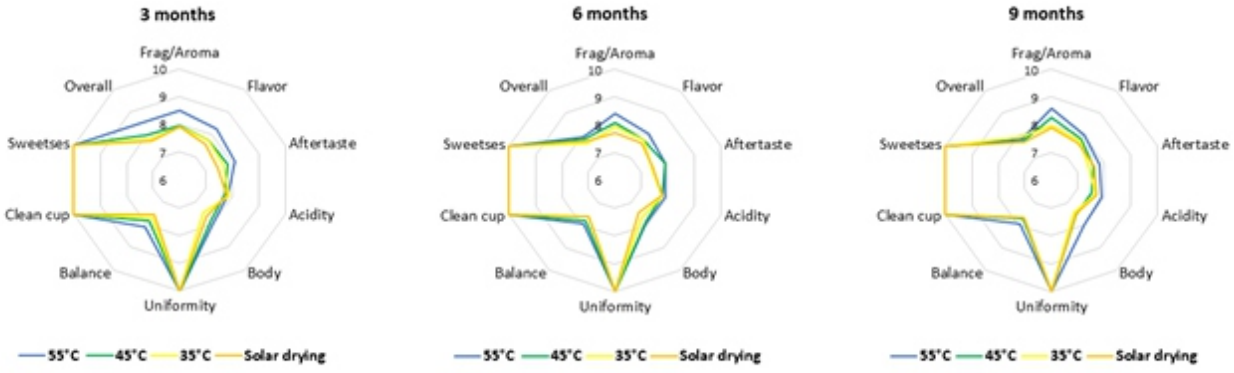


**Figure 4**. Sensory radar profiles of coffee samples according to SCA protocol for the different drying processes and storage time.

It is observed that the sensory profiles retain their tendency as time passes, while the coffee dried at 55 °C differs from the rest of the drying processes in the fragrance/aroma, flavor, residual flavor, body, balance and overall. These results show the importance of guaranteeing adequate storage conditions for coffee using packaging that protects the grain from moisture, oxygen and light. This can allow low impact on the chemical composition of the grain, leading to preserved sensory attributes over time. In general, higher cup scores are obtained in samples handled with mechanical drying procedures. Because this sort of technique eliminates or decreases the effects of exposure to light, air, humidity and environmental conditions as well as microbiological, enzymatic and oxidative processes, which standard drying samples are subjected to.

Figure 5 shows the factor analysis of mixed data (FAMD) for the quantitative variables (sensory attributes and drying time) evaluated during storage for all drying processes. FAMD was chosen as an

appropriate multivariate approach for explaining the link between sensory qualities and drying time in relation to drying procedures and storage duration. The first two primary dimensions (Dim1 and Dim2) explain 59.4% of the variation in the observed variables, where the drying processes and drying time are clearly separated from the sensory qualities. This form of study is used to describe how drying methods affect sensory, chemical and physical properties [35].

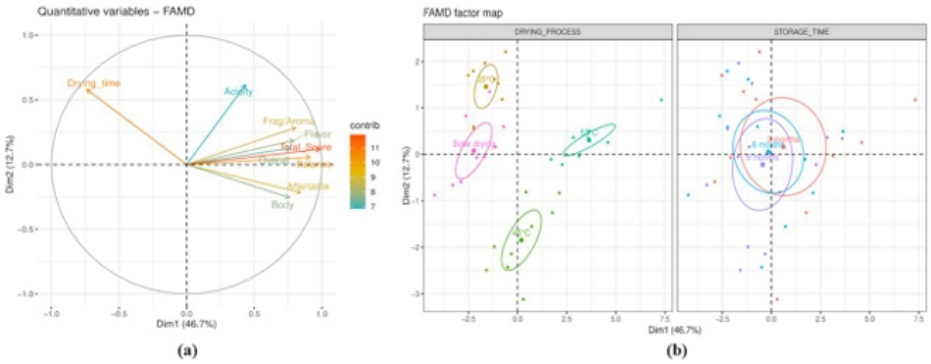


**Figure 5**. Factor analysis of mixed data (FAMD) for (a) quantitative variables and (b) map with drying process and storge time in months.

In Figure 5 (a) it is observed that there is a negative correlation between sensory attributes and drying time, indicating that drying processes with less time favor the sensory attributes evaluated in roasted coffee. This tendency could be due to longer drying times causing changes in the concentration of chemical components, which affect the sensory profile of the coffee drink [36]. The drying time effect may be related to different physicochemical and microbiological processes that occur inside and outside the coffee beans during drying. Water activity (aw) is an important attribute in coffee quality preservation and when it is slow dried, the aw is higher in the grains, enabling microbiological growth phenomena, oxidation processes, hydrolysis processes and enzymatic activity [37].

Figure 5 (b) shows the differences between the drying processes with the confidence ellipses and their centers of gravity. The drying procedures used on green coffee beans have an impact on the values obtained for sensory characteristics and evaluated variables. It is reasonable to believe that the drying procedures used have an effect on the amounts of chemical components in coffee beans. When performing the coffee roasting process, the concentration of these chemical components permits the development of scents and tastes, influencing the sensory profile of the coffee drink. According to current study, the drying technique utilized can ensure a higher or lower concentration of chemical components in the coffee beans after drying [14,35,38].

The opposite is observed for the storage time where all the ellipses are intercepted, indicating that the sensory attributes are preserved over time. The FAMD results confirm the ANOVA results in that storage time had no influence on the tested variables. This may be due to the fact that the packaging utilized helps the stability and conservation of the physicochemical qualities of the coffee. In this regard, the packaging used to keep parchment coffee must be very resistant to water vapor, oxygen and light.

## 4. Conclusions

In general, it can be concluded that the hot-air drying was a suitable technique for processing green coffee beans since the mechanical drying is a controlled process. This regulated environment yields a product with strong sensory qualities that has the potential to be commercialized in the specialty coffee market. The sensory quality of the coffee enhanced when the air temperature was elevated during mechanical drying. When compared to direct sun drying, a drying air temperature of 55°C led in greater

ratings for the characteristics fragrance/aroma, flavor, aftertaste, body and balance. The mechanical drying technology that we examined provides a value-added option for Colombian coffee farmers, allowing them to produce high-quality green coffee beans while also opening up new financial prospects. Finally, the greatest coffee cup score was obtained at a temperature of 55 °C, which can be attributed to a quicker drying period as compared to direct sun drying. In this context, technologies such as microwave drying, heat pump drying, and dehumidified air drying can achieve faster drying periods. These technologies have the potential to improve the coffee cup score.

**Use of AI tools declaration**

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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**Conflict of interest**

The authors declared that there is no conflict of interest.

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# Bioactive and nutritional compounds in fruits of pepper (Capsicum annuum L.) landraces conserved among indigenous communities from Mexico

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## ABSTRACT

*Farmers' varieties or landraces of chili are regularly heterogeneous, selected and preserved by small traditional farmers and highly demanded by regional consumers. The objective of this study was to evaluate the variation in the content of phenolic compounds, vitamin C, carotenoids, capsaicinoids and antioxidant activity in fruits of a population collection of the landraces Huacle and De Agua, which originated in Oaxaca, Mexico, and a commercial variety of Jalapeño (control). The collection was grown in greenhouse conditions under a random block design. At harvest, a sample of ripe fruits was obtained to evaluate the content of phenolic compounds, vitamin C and antioxidant activity by UV–visible spectrophotometry and the concentration of capsaicin and dihydrocapsaicin was measured by high-resolution liquid chromatography. Significant differences were observed between the Huacle and De Agua landraces and between these and Jalapeño. The studied fruits exhibit the following pattern for flavonoid and carotenoid contents: Huacle > De Agua > Jalapeño. The opposite pattern was observed for total polyphenol and vitamin C contents: Jalapeño > De Agua > Huacle. The general pattern for capsaicinoids in fruits was Jalapeño > De Agua > Huacle. Huacle and De Agua populations showed high variability in all compounds evaluated, with positive correlations with antioxidant activity. The capsaicin content in Huacle populations varied ranging from 7.4 to 26.2 mg 100 g−1 and De Agua ranged from 12.4 to 46.8 mg 100 g−1.*

***Keywords:*** *antioxidant activity; bioactive compounds; indigenous food systems; underutilized landraces; plant genetic resources*

## 1. Introduction

Current crop domestication is an ongoing dynamic process of coevolutionary nature involving plants and humans. It is influenced by anthropogenic disturbances, climate change, food transitions (e.g., diet changes) and market demands, among other factors, which Krug et al. [1] named as a new era of crop domestication in the interrelationship of genetic and phenotypic particularities of the crop species, including their wild relatives, agronomic and cultural practices (e.g., human selection, management) and ecogeographical factors. In this context, the chili pepper (Capsicum annuum L.) is an important crop, with a world production from 4.2 to 4.8 million tons [2]. This species continues in domestication and their perspectives or priorities for breeding have changed due to the new role for diet such as the addition of nutrients and bioactive compounds and facing climate changes, not only high yield [3]. In these cases, countries with high agrobiodiversity and centers of origin, domestication and diversification

of cultivated plants have certain advantages regarding access to a greater diversity of food products [4], e.g., the diversity of Capsicum annuum L. in Mexico. Perry and Flannery [5] showed different archaeobotanical evidence of the Mexican Mesoamerican origin of C. annuum from pre-Columbian times, and Kraft et al. [6] indicated that the presence of phytoliths, pollen and starch grains in vessels provides multiple lines of evidence of domestication in Mexico. In this sense, evolution under domestication continues, resulting in various lines of genetic differentiation that we now identify as autochthonous varieties, fruit morphotypes or landraces [7]. Therefore, human selection in C. annuum has a significant effect on the differentiation among regional variants or types [8,9] and, consequently, significant effects on fruit composition, with high variation between and within landraces or groups of native populations [10]. Notably, some of these landraces are unknown outside their area of distribution and usually commercialized in regional markets.

Fresh or dried chili fruits provide sugars, vitamins, minerals, organic acids, ascorbic acid, tocopherols, carotenoids and several phenolic compounds, all of them important in the family diet, community health and global gastronomy. The most well-known and studied bioactive compounds are capsaicinoids, flavonoids and carotenoids, which, in addition to their biological function as a defense mechanism against biotic and abiotic factors in plants, and when they are consumed have functional and antioxidant activities that counteract diseases associated with eating disorders and chronic degenerative diseases between consumers [11]. For example, flavonoids and capsaicinoids participate in preventing cancer, inflammation, diabetes, obesity, hypertension and gastric system problems and act as analgesics and antimicrobials [12,13]. In addition, the specific sensory characteristics of each type of pepper are related to variations in the content of sugars, organic acids, capsaicinoids, carotenoids, phenolic compounds, vitamins and volatile compounds, among other compounds [10,14–19]. This complex of compounds in the fruits is released during processing stages such as drying, boiling, or toasting and confers different functional nutraceutical and nutritional properties to processed foods [20–23]. Pungency and color are some of the parameters used to determine the quality and commercial value of chili fruits based on their types or morphological group (e.g., Jalapeño, nonpungent variants, Ancho, Habanero, etc.). In the placenta and endocarp of the fruit, 20 or more capsaicinoid compounds responsible for itching or pungency are biosynthesized [24], among which capsaicin [(E)-N(4hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide)] and 6,7-dihydrocapsaicin represent more than 90% of the total [25]. Carotenoids, such as β-carotene, β-cryptoxanthin, capsanthin, antheraxanthin, capsorubin and zeaxanthin, confer different shades of color to the fruit and accumulate in the endocarp and pericarp when the fruit ripens [26,27]. However, the composition of the fruit changes due to management during production, environmental factors, genetic traits and genetic-environmental interactions, but little is known about the traditional varieties in the centers of origin that continue under domestication and facing climate changes. In this study, variation in phenolic compound, carotenoid, vitamin C, and capsaicinoid contents and antioxidant activity were evaluated in fruits of a collection of populations of Capsicum annuum native from Oaxaca, Mexico, grouped in the Huacle and De Agua landraces and cultivated under greenhouse conditions.

## 2. Materials and methods

### 2.1. Sampling of landraces and cultivation

Ten populations of Huacle landrace were collected in different communities of the municipality of San Juan Bautista Cuicatlan and eleven populations of De Agua landrace from five municipalities of Valles Centrales, both from Oaxaca, Mexico, between November 2016 and April 2017. In this work we use the landrace term to group all populations with similar fruit morphology, using the local name, such as

referenced previously by Vera-Guzman et al. [10]. For experimental purposes, the variety Jalapeño (Hortaflor® seed) was included as control in the experiment, considering their commercial importance and most know by consumers in the international market [28] (Figure 1, Table 1).

In summary, 21 populations more one control were sown in polystyrene trays containing commercial substrate (peat moss, Sphagnum sp.) and later seedlings were transplanted in September 2017 under a random block design with three replicates in greenhouse conditions. Conventional management was provided for the plants, and fertilization was performed through the irrigation system using the commercial formulas Ultrasol® 15-30-15, 18-18-18 and 13-6-40 and with added calcium nitrate. For the control of pests and diseases, propamocarb (1 mL L−1 of water), imidacloprid (1 mL L−1 of water), cupric hydroxide (1 mL L−1 of water), diethyl-dithio-carbamate (1 g L−1 of water) and chlorothalonil (1 g L−1 of water), among other products, were used.

## 2.2. Sample preparation

At harvest, 250 g of mature fruits was randomly chosen from each experimental plot. The fruits were washed with distilled water and dried at room temperature until water residues were removed and then blended in a food processor (Nutribullet ®) to obtain a puree. Then, the sample was divided into three fractions. In the first fraction, total carotenoid and vitamin C contents were analyzed immediately to avoid degradation. The second fraction was stored at −20 °C until analysis for total polyphenol and flavonoid content and antioxidant activity. In the third fraction, whole fruits were dried in an oven at 45 °C until a constant weight was reached. The fruits were then processed and placed in amber plastic flasks for storage at 20 °C until analysis for capsaicinoid contents. Overall, the percent of moisture was determined gravimetrically by standard method 930.15 AOAC [29] and used to correct fresh weights to dry weights


**Figure 1.** Sample of mature fruits of the A) Huacle and B) De Agua landraces collected and cultivated in Oaxaca, Mexico and the commercial variety Jalapeño (C).

**Table 1.** List of the native populations of the Huacle and De Agua landraces and origin municipalities in Oaxaca, Mexico.

| Pop. ID | Landrace | Municipality (Oaxaca, Mexico) | Altitude (m) | Latitude N | Longitude W |
|---|---|---|---|---|---|
| CH-01 | Huacle | San Juan Bautista Cuicatlán | 649 | 17° 44' 41.60" | 96° 57' 37.44" |
| CH-02 | Huacle | San Juan Bautista Cuicatlán | 655 | 17° 48' 37.30" | 96° 57' 36.09" |
| CH-03 | Huacle | San Juan Bautista Cuicatlán | 625 | 17° 48' 09.87" | 96° 57' 48.99" |
| CH-04 | Huacle | San Juan Bautista Cuicatlán | 623 | 17° 48' 07.74" | 96° 57' 52.83" |
| CH-05 | Huacle | San Juan Bautista Cuicatlán | 620 | 17° 48' 08.87" | 96° 57' 50.79" |
| CH-06 | Huacle | San Juan Bautista Cuicatlán | 600 | 17° 47' 41.63" | 96° 57' 50.79" |
| CH-07 | Huacle | San Juan Bautista Cuicatlán | 605 | 17° 47' 36.19" | 96° 57' 36.22" |
| CH-08 | Huacle | San Juan Bautista Cuicatlán | 615 | 17° 48' 09.87" | 96° 57' 37.44" |
| CH-09 | Huacle | San Juan Bautista Cuicatlán | 622 | 17° 48' 08.87" | 96° 57' 47.99" |
| CH-10 | Huacle | San Juan Bautista Cuicatlán | 620 | 17° 48' 09.87" | 96° 57' 19.44" |
| CA-62 | De Agua | San Jerónimo Tlacochahuaya | 1582 | 17° 00' 25.93" | 96° 35' 24.51" |
| CA-25 | De Agua | San Bernardo Mixtepec | 1643 | 16° 49' 55.30" | 96° 53' 58.13" |
| CA-42 | De Agua | Ejutla de Crespo | 1495 | 16° 34' 09.10" | 96° 42' 10.91" |
| CA-36 | De Agua | Zimatlán de Álvarez | 1646 | 16° 51' 25.64" | 96° 48' 56.17" |
| CA-20 | De Agua | Santa Cruz Zenzontepec | 1040 | 97° 29' 43.05" | 16° 32' 00.32" |
| CA-53 | De Agua | Santa Cruz Zenzontepec | 1040 | 16° 30' 35.6" | 97° 24' 02.9" |
| CA-38 | De Agua | Ejutla de Crespo | 1483 | 16° 32' 47.01" | 96° 42' 28.65" |
| CA-40 | De Agua | Ejutla de Crespo | 1491 | 16° 34' 06.69" | 96° 42' 12.78" |
| CA-24 | De Agua | San Bernardo Mixtepec | 1697 | 16° 49' 35.21" | 96° 54' 04.51" |
| CA-33 | De Agua | San Bernardo Mixtepec | 1649 | 16° 39' 35.54" | 96° 53' 58.06" |
| CA-32 | De Agua | San Bernardo Mixtepec | 1649 | 96° 54' 08.92" | 16° 49' 30.47" |

2.3. Total carotenoids and vitamin C content analysis

Total carotenoids were measured using the method suggested by Vera-Guzmán et al. [10] with some modifications. The samples were ground using an extraction mix solution of ethanol, acetone and hexane at a ratio of 1:1:2 (v/v). The ground sample was placed in an ice bath and stirred for 20 min, distilled water was added and the mixture was allowed to rest at room temperature for 5 min but protected from light. An aliquot of the upper phase was taken to prepare a hexane-based dilution. The absorbance of the solution was measured with a UV/Vis spectrophotometer (Shimadzu UV 1800, Kyoto, Japan) at 446 nm. Total carotenoids in the sample were calculated using the measured absorbance and a calibration curve for a β-carotene standard (β-carotene with 97.0% purity; Fluka, Buchs SG, Switzerland) from 0.8 to 4.0 µg mL−1 (r2 = 0.999). Total carotene concentrations were expressed as milligrams of β-carotene per gram of dry weight (mg βC g−1 dw). The vitamin C content in fresh fruits was determined using the method described by Dürust et al. [30] with modifications. Samples were ground with oxalic acid (0.4%) at a ratio of 1:10 (w/v) and placed in a dark room for 20 min before centrifugation at 11500 rpm. Then, 1 mL of the supernatant was mixed with sodium acetate buffer and 2,6-dichlorophenol indophenol solution. The absorbance of the solution was measured at a wavelength of 520 nm, and vitamin C was calculated based on an adjusted calibration curve for a L-ascorbic acid standard (99% purity; Sigma, St Louis, Missouri, USA) from 1 to 5 µg mL−1 (r2 = 0.999). The estimated concentration of vitamin C was reported as milligrams of ascorbic acid per gram of dry weight (mg AA g−1 dw).

2.4. Total polyphenol and flavonoid contents and antioxidant activity

To measure total polyphenol and flavonoid contents and antioxidant activity, 3 g of sample underwent 60% ethanol extraction or 80% methanol extraction, respectively. Then, total polyphenol content was determined using the method described by Singleton and Rossi [31]. First, 2.4 mL of deionized water and 0.2 mL of Folin-Ciocalteu reagent were added to 0.4 mL of the diluted extract and the solution was allowed to rest for 5 min. Subsequently, 2 mL of 7% Na2CO3 was added and the solution was incubated for 1 h at room temperature (23 ± 3 °C). Finally, the absorbance readings were measured at 750 nm. The total polyphenol content was estimated using a calibration curve for gallic acid (40 to 160 µg mL−1, r2 = 0.995) and the values were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g−1 dw).

The flavonoid content was determined using the aluminum chloride colorimetric method [32]. A total of 0.5 mL of homogenate was mixed with 1.5 mL of 95% alcohol, 0.1 mL of 10% aluminum chloride hexahydrate (AlCL3), 0.1 mL of 1 M potassium acetate (CH3COOK) and 2.8 mL of deionized water. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm. Flavonoid content was calculated using a calibration curve for a quercetin standard (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one; 98% purity; Sigma, St Louis, Missouri, USA) from 10 to 70 µg mL−1 (r2 = 0.996). Data was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE g−1 dw).

Antioxidant activity was measured using the DPPH method (2,2-diephenyl-1-picrylhydrazyl) described by Brand-Williams et al. [33]. The DPPH radical was added to 100 µL of the methanol extract. Then, the solution was vortexed and allowed to stand for 30 min in the dark. Subsequent readings were performed in triplicate at 517 nm. Antioxidant activity was recorded based on a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman2-carboxylic acid) calibration curve (0.13 to 0.79 µmol mL−1, r2 = 0.993) and expressed in µmol Trolox equivalents per gram of dry weight (µmol TE g−1 dw).

Antioxidant activity was also determined by the FRAP method. The antioxidant capacity, expressed as iron reduction, was measured using the method described by Benzie and Strain [34]. A total of 3 mL of FRAP reagent (sodium acetate buffer, pH 3.6, 10 mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) and 10 mM FeCl3 6H2O) was added to 100 µL of the extract. This solution was incubated for 30 min at 37 °C, and the absorbance was read at 593 nm. The quantification of antioxidant activity was performed using a Trolox calibration curve (0.10 to 0.80 µmol mL−1, r2 = 0.999) and the results were expressed as µmol equivalents of Trolox per gram of dry weight (µmol TE g−1 dw).

2.5. Capsaicinoids analysis by high-performance liquid chromatography (HPLC) with diode-array detection (DAD)

Capsaicinoids were extracted from 5 g of the ground sample in 10 mL of acetonitrile and heated at 70 °C for 4 h with stirring every 30 min [35,36]. The suspended material was centrifuged at 11500 rpm for 15 min at 4 °C, and then the supernatant was transferred to a vial. The extract was filtered through acrodiscs (45 µm PTFE) and frozen at −20 °C until further analysis. Capsaicin (CAP) and dihydrocapsaicin (DIH) analyses were performed using a 1.00 mL min−1 flow rate on a Hypersil ODS column (length 250 mm, ID 4 mm and particle size 5 µm, Agilent, USA) in an HPLC (Model 1260 Infinity II; Agilent Technologies, CA USA) system with an acetonitrile/water (45:55%) mobile phase using a diode array detector in isocratic mode (280 nm wavelength). The analysis time was 20 min, and the injection volume was 20 µL. The retention times for capsaicin and dihydrocapsaicin were 11.5 and 17.1 min, respectively. Capsaicin and dihydrocapsaicin contents were estimated using an external standards calibration curve: capsaicin (8-methylN-vanillyl-trans-6-nonenamide of capsicum with 95% purity; Sigma, St. Louis, Missouri, USA) from 0.1 to 1 mg mL−1, (r2 = 0.996) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide of capsicum with 90% purity; Sigma, St. Louis, Missouri, USA) from 0.1 to 1 mg mL−1, (r2 = 0.996). Data was expressed as milligrams of capsaicin or dihydrocapsaicin per 100 g of dry weight (mg CAP or DIH 100 g−1 dw).

2.6. Statistical analysis

The databases were integrated from the evaluation of compounds in each experimental sample and subsequently subjected to analysis of variance to evaluate the differences between Huacle and De Agua landraces and between populations within each landrace, including the control (Jalapeño) or differences between and within landraces. The nesting effect of populations in landraces and the effect of laboratory replicates nested in greenhouse replicates were considered. Multiple comparisons of means were conducted using the Tukey test ($p < 0.05$). In addition, a simple Pearson correlation analysis between antioxidant activity and bioactive compounds was performed and a descriptive analysis of the main bioactive compounds and antioxidant activity by population was performed. Complementarily, a principal component analysis (PCA) was performed to describe the variation populations within Huacle and De Agua landraces and control. All statistical analyses were performed with the SAS statistical package [37].

## 3. Results

3.1. Phenolic compounds, vitamin C, carotenoids and antioxidant activity

The analysis of variance indicated significant differences ($p \leq 0.01$) between landraces (Huacle and De Agua) and between populations within landraces in total polyphenols, flavonoids, vitamin C and

carotenoid contents and antioxidant activity evaluated by DPPH and FRAP methods. Based on the magnitude of variance or square means for landrace and population effects, the variance due to the landraces effect was more than double and up to 19 times that of the variance due to populations within landraces for all the measured variables (Table 2), i.e., the differences between the Huacle and De Agua landraces were greater than those between the populations within each landrace.

**Table 2.** Significance of square means from the analysis of variance in phenolic compounds, vitamin C, carotenoids and antioxidant activity in chili fruits of the Huacle and De Agua landraces of *Capsicum annuum*.

| Sources of variation | Total polyphenols | Flavonoids | Vitamin C | Carotenoids | Antioxidant activity | |
|---|---|---|---|---|---|---|
| | | | | | DPPH | FRAP |
| Landraces (L) | 68.8** | 11.0** | 4.8** | 9.39** | 20.06** | 15.40** |
| Populations/L[1] | 24.2** | 2.4** | 0.3** | 0.49** | 3.6** | 3.56** |
| Rep. (R) | 149.5** | 0.67$^{ns}$ | 1.7** | 0.22$^{ns}$ | 5.60** | 5.86* |
| Lab. replicates/R[1] | 0.04$^{ns}$ | 0.06$^{ns}$ | <0.001$^{ns}$ | 0.06$^{ns}$ | 0.01$^{ns}$ | 0.007$^{ns}$ |
| Error | 7.1 | 0.34 | 0.06 | 0.11 | 0.56 | 0.90 |
| Coeff. var. (%) | 24 | 27.8 | 12.8 | 19.8 | 15.5 | 13.6 |

$^{ns}$ Not significant ($p > 0.05$); * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; [1] Indicate nesting of populations in landraces and nesting of laboratory replicates in repetitions of greenhouse cultivation, respectively.

The commercial variety Jalapeño used as the control differed significantly from the Huacle and De Agua landraces regarding total polyphenol, flavonoid and vitamin C contents and antioxidant activity evaluated by FRAP. The carotenoid contents in Jalapeño was similar to De Agua landrace. In antioxidant activity (DPPH method), Jalapeño and Huacle were similar. De Agua and Huacle showed equivalent total polyphenol contents and antioxidant capacity (FRAP method). Huacle had higher concentrations of flavonoids and carotenoids and antioxidant activity (DPPH) than did De Agua, but the trend for vitamin C was reversed. In this sense, the studied fruits exhibit the following pattern for flavonoid and carotenoid contents: Huacle > De Agua > Jalapeño. The opposite pattern was observed for total polyphenol and vitamin C contents: Jalapeño > De Agua > Huacle (Figure 2).

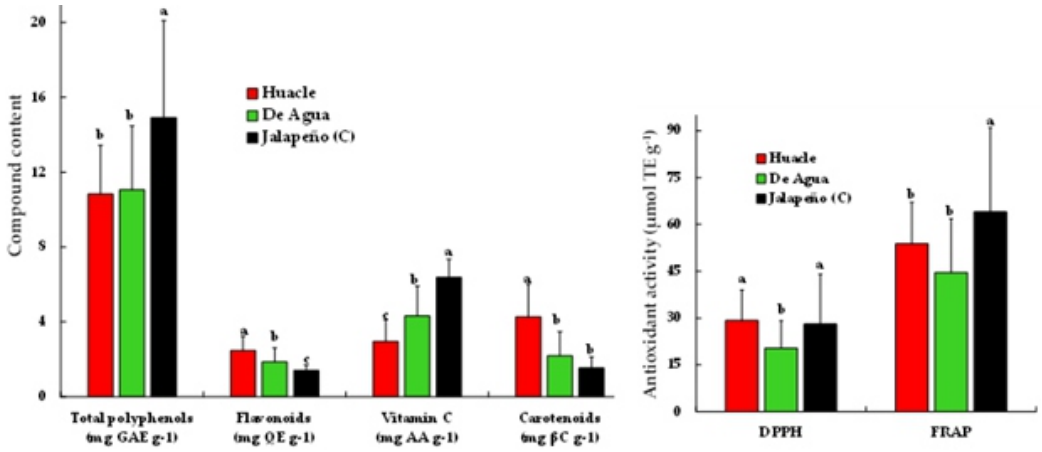


**Figure 2.** Differentiation between De Agua and Huacle chili landrace regarding total polyphenol, flavonoid, vitamin C, and carotenoid contents and antioxidant activity (DPPH and FRAP methods).

There was high variability between populations within each landrace. Among the Huacle populations, total polyphenols ranged from 7.9 to 13.8 mg of GAE g−1 and flavonoids ranged from 1.6 to 3.2 mg of QE g−1. For De Agua, total polyphenols ranged from 9.0 to 14.1 mg of GAE g−1 and flavonoids ranged

from 1.1 to 2.7 mg of QE g−1. In Huacle, carotenoids ranged from 2.5 to 5.6 mg C g−1 and in De Agua, carotenoids ranged from 1.4 to 3.7 mg C g−1. In this study, the Huacle populations exhibited slightly more variation than did De Agua populations. Among Huacle populations, vitamin C ranged from 1.8 to 4.3 mg AA g−1. Among De Agua populations, vitamin C ranged from 3.3 to 5.9 mg AA g−1. The variety of Jalapeño presented the highest total polyphenol and vitamin C contents. Regarding antioxidant activity, as evaluated by DPPH and FRAP, the populations of both landraces exhibited similar differential patterns (Table 3).

**Table 3.** Variation in phenolic compounds, vitamin C, carotenoids and antioxidant activity among populations of De Agua and Huacle landraces of chili pepper.

| Pops. | Total polyphenols | Flavonoids | Vitamin C | Carotenoids | Antioxidant activity (μmol TE $g^{-1}$) | |
|---|---|---|---|---|---|---|
| ID | (mg GAE $g^{-1}$) | (mg QE $g^{-1}$) | (mg AA $g^{-1}$) | (mg βC $g^{-1}$) | DPPH | FRAP |
| Huacle landrace: | | | | | | |
| CH1 | 7.9 ± 2.3 e[1] | 1.6 ± 0.3 efg | 1.8 ± 0.4 h | 2.5 ± 1.1 c-f | 16.9 ± 4.2 ef | 37.2 ± 12.5 c |
| CH2 | 10.5 ± 3.2 a-e | 1.7 ± 0.2 d-g | 2.9 ± 1.1 e-h | 3.6 ± 1.4 a-f | 35.5 ± 10.8 abc | 53.9 ± 7.2 abc |
| CH3 | 9.3 ± 1.0 cde | 2.3 ± 0.5 a-f | 2.2 ± 0.2 gh | 4.2 ± 2.0 a-d | 23.6 ± 3.4 c-f | 57.5 ± 5.3 abc |
| CH4 | 10.4 ± 2.6 a-e | 2.1 ± 0.4 c-g | 3.6 ± 1.4 d-h | 3.7 ± 1.0 a-e | 31.8 ± 2.0 a-d | 59.3 ±6.4 abc |
| CH5 | 11.8 ± 3.1 a-e | 2.9 ± 0.9 abc | 2.8 ± 0.6 e-h | 4.7 ± 1.3 a-c | 33.8 ± 10.8 abc | 63.3 ± 22.7 ab |
| CH6 | 12.5 ± 1.6 a-e | 3.2 ± 0.5 a | 4.3 ± 2.3 b-f | 5.1 ± 1.0 ab | 37.4 ± 10.0 ab | 64.6 ± 5.6 a |
| CH7 | 13.8 ± 2.3 abc | 3.1 ± 1.2 ab | 3.5 ± 0.5 e-h | 4.2 ± 0.6 a-d | 38.8 ± 8.1 a | 59.2 ± 7.8 abc |
| CH8 | 10.7 ± 1.3 a-e | 2.5 ± 0.3 a-e | 2.9 ± 0.5 e-h | 4.8 ± 1.0 ab | 27.0 ± 3.5 a-f | 55.1 ± 12.9 abc |
| CH9 | 10.2 ± 1.9 b-e | 2.5 ± 0.3 a-e | 2.5 ± 0.4 fgh | 4.2 ± 1.0 a-d | 20.2 ± 3.4 def | 44.0 ± 4.5 abc |
| CH10 | 11.0 ± 1.3 a-e | 2.7 ± 0.2 a-d | 3.0 ± 0.6 e-h | 5.6 ± 3.6 a | 27.1 ± 6.5 a-f | 43.8 ± 7.5 abc |
| De Agua landrace: | | | | | | |
| CCA20 | 14.1 ± 6.8 ab | 2.6 ± 1.3 a-d | 5.5 ± 0.6 abc | 3.4 ± 2.2 a-f | 25.8 ± 13.1 a-f | 59.2 ± 20.2 abc |
| CCA24 | 12.8 ± 3.8 a-d | 2.1 ± 0.3 b-g | 4.2 ± 1.3 b-f | 1.7 ± 0.2 e-f | 25.1 ±6.9 b-f | 45.4 ± 10.0 abc |
| CCA25 | 11.3 ± 0.6 a-e | 1.9 ± 0.3 c-g | 5.4 ± 2.1 a-d | 3.1 ± 1.8 b-f | 23.3 ± 3.5 c-f | 49.1 ± 12.5 abc |
| CCA32 | 10.3 ± 1.1 a-e | 1.3 ± 0.2 fg | 3.3 ± 0.3 e-h | 1.6 ± 0.4 ef | 15.9 ± 5.8 ef | 37.3 ± 10.5 c |
| CCA33 | 10.0 ± 2.4 b-e | 1.8 ± 0.3 d-g | 3.5 ± 0.8 e-h | 1.5 ± 0.4 ef | 13.9 ± 2.6 f | 40.0 ± 3.3 bc |
| CCA36 | 13.3 ± 4.9 a-d | 2.7 ± 1.1 a-d | 5.9 ± 3.0 ab | 3.7 ± 1.7 a-f | 28.4 ± 11.5 a-e | 63.4 ± 32.2 ab |
| CCA38 | 9.3 ± 2.5 cde | 1.3 ± 0.5 g | 3.4 ± 0.5 e-h | 1.5 ± 0.5 ef | 13.8 ± 5.7 f | 36.9 ± 12.2 c |
| CCA40 | 10.4 ± 1.1 a-e | 1.9 ± 0.5 c-g | 3.7 ± 0.5 c-g | 1.4 ± 0.4 def | 24.8 ± 9.8 b-f | 42.6 ± 18.9 abc |
| CCA42 | 10.5 ± 2.0 a-e | 1.9 ± 0.4 c-g | 4.1 ± 0.7 b-f | 2.3 ± 0.5 def | 19.3 ± 5.7 def | 40.6 ± 8.8 abc |
| CCA53 | 10.6 ± 2.4 a-e | 1.7 ± 0.4 d-g | 4.6 ± 2.0 a-e | 2.2 ± 0.8 def | 16.2 ± 6.5 ef | 39.7 ± 14.2 bc |
| CCA62 | 9.0 ± 1.8 de | 1.1 ± 0.1 g | 3.6 ± 0.4 c-h | 1.6 ± 0.5 ef | 17.4 ± 5.2 ef | 36.6 ± 8.8 c |
| Jalapeño type (control): | | | | | | |
| JAL | 14.9 ± 5.2 a | 1.4 ± 0.3 fg | 6.4 ± 1.0 a | 1.5 ± 0.6 e-f | 28.0 ± 16.0 a-e | 64.1 ± 27.0 ab |

[1] In columns, means with the same letter are not significantly different (Tukey's test, $p \leq 0.05$); GAE = gallic acid equivalents; QE = Quercetin Equivalents; AA = Ascorbic Acid; βC = β-Carotene; TE = Trolox Equivalents.

Pearson correlation analysis (r) was used to identify positive and significant correlations between total carotenoid, vitamin C, total polyphenol and flavonoid contents in relation to antioxidant activity evaluated by DPPH method ($0.22 < r < 0.63$; Student's t test, $p \leq 0.01$, n = 66 samples evaluated) and FRAP method ($0.39 < r < 0.60$; Student's t test, $p \leq 0.01$, n = 66); in the latter case, the correlation with vitamin C was not significant ($r = -0.04$, Student's t test, $p > 0.05$, n = 66). These correlations suggest that the concentration of phenolic compounds and vitamin C influence the ability to capture free radicals and singlet oxygen.

## 3.2. Variation in capsaicinoid contents

The analysis of variance of the capsaicinoid contents indicated significant differences ($p \leq 0.05, 0.01$) in capsaicin (CAP), dihydrocapsaicin (DIH), total capsaicinoid (CAP + DIH) contents and the CAP/DIH ratio between landraces and populations within landraces. The mean square or variance due to landraces was 5 to 10 times more than due to differences between populations within landraces (Table 4). This indicates that the differences between landraces were significantly greater than the differences between populations.

**Table 4.** Significance of square means from the analysis of variance in capsaicin and dihydrocapsaicin in fruits of Huacle and De Agua landraces of *Capsicum annuum*.

| Sources of variation | Capsaicin (CAP) | Dihydrocapsaicin (DIH) | CAP + DIH | CAP/DIH |
|---|---|---|---|---|
| Landraces (L) | 46.4** | 13.0** | 55.5** | 2.6** |
| Populations/L[1] | 4.7** | 2.5** | 6.9** | 0.3* |
| Replicates (R) | 7.5** | 7.9** | 14.1** | 2.2** |
| Lab. replicates/R[1] | $0.01^{ns}$ | $<0.04^{ns}$ | $0.05^{ns}$ | $0.08^{ns}$ |
| Error | 0.8 | 0.5 | 1.2 | 0.1 |
| Coeff. Var. (%) | 20.6 | 21.3 | 20.0 | 20.0 |

$^{ns}$ not significant ($p > 0.05$); * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; [1] Indicates nesting of populations in landraces and nesting of laboratory replicates in repetitions of greenhouse cultivation, respectively.

In the comparison of capsaicinoids between landraces and the control variety (Jalapeño), the capsaicin (CAP), dihydrocapsaicin (DIH) and total capsaicinoid (CAP + DIH) contents were higher in the Jalapeño control (Table 5). The CAP content was always higher than the DIH content, i.e., up to 2.1 times higher in De Agua and significantly different from that in Huacle (1.7 times). Significant differences were observed between landraces, with higher CAP and CAP + DIH contents in De Agua than in Huacle. DIH concentration was similar between the landraces. The general pattern for CAP and CAP + DIH content in fruits was Jalapeño > De Agua > Huacle and for DIH was Jalapeño > De Agua = Huacle; in all cases, the CAP content was always higher than the DIH content (Table 5). These patterns indicate significant differences between landraces with respect to the Jalapeño control.

**Table 5.** Differences in capsaicin and dihydrocapsaicin contents between Huacle and De Agua landraces and the commercial variety Jalapeño (control) of chili pepper.

| Landraces and control | Capsaicin (CAP, mg 100 $g^{-1}$) | Dihydrocapsaicin (DIH, mg 100 $g^{-1}$) | CAP + DIH (mg 100 $g^{-1}$) | CAP/DIH |
|---|---|---|---|---|
| Huacle | 14.1 ± 7.4 c[1] | 9.4 ± 5.8 b | 23.5 ± 12.6 c | 1.7 ± 0.6 b |
| De Agua | 27.0 ± 14.4 b | 13.1 ± 6.7 b | 40.0 ± 21.0 b | 2.1 ± 0.3 a |
| Jalapeño | 44.0 ± 10.6 a | 24.1 ± 6.4 a | 68.1 ± 16.9 a | 1.8 ± 0.1 ab |

[1] In columns, means with the same letter are not significantly different (Tukey's test, $p \leq 0.05$).

Between Huacle and De Agua populations, there was high variability in capsaicin (CAP), dihydrocapsaicin (DIH), total capsaicinoid (CAP + DIH) content and in the CAP/DIH ratio (Table 6). In Huacle, a similar pattern was observed between CAP and DIH. Populations with higher or lower CAP values also had high or low DIH values. The trend was similar in De Agua. However, the De Agua populations with higher CAP (32.8 to 46.8 mg 100 g−1) and DIH (21.2 mg 100 g−1) contents significantly surpassed Huacle populations with higher values of CAP (26.2 mg 100 g−1) and DIH (18.0 mg 100 g−1) contents. The populations with the highest total capsaicinoid content (> 40 mg 100 g−1) were Huacle CH1 and De Agua CCA24, CCA25, CCA33, CCA40 and CCA62. The content in Jalapeño was 68.2 mg 100 g−1. In eight De Agua populations, the CAP/DIH ratio was greater than double, unlike

in Huacle populations, among which only one population presented this pattern (Table 6).

**Table 6.** Variation in capsaicin, dihydrocapsaicin, total capsaicinoid (CAP + DIH) contents and CAP/DIH ratio in fruits of populations from the Huacle and De Agua landraces of *C. annuum*.

| Pop. ID | Capsaicin (CAP, mg 100 g$^{-1}$) | Dihydrocapsaicin (DIH, mg 100 g$^{-1}$) | CAP + DIH (mg 100 g$^{-1}$) | CAP/DIH |
|---|---|---|---|---|
| Huacle landrace: | | | | |
| CH1 | 26.2 ± 6.1 b-f[1] | 18.0 ± 5.9 abc | 44.3 ± 10.6 a-e | 1.5 ± 0.4 abc |
| CH2 | 15.3 ± 7.8 d-g | 11.5 ± 6.6 b-g | 26.8 ±14.3 c-g | 1.4 ± 0.1 bc |
| CH3 | 11.5 ± 10.4 efg | 11.5 ± 6.7 b-g | 27.0 ± 16.2 d-g | 1.2 ± 1.1 c |
| CH4 | 7.4 ± 3.2 g | 3.5 ± 1.0 g | 10.8 ± 3.6 g | 2.2 ± 0.9 a |
| CH5 | 15.5 ± 7.0 d-g | 8.4 ± 3.8 c-g | 23.9 ± 10.7 d-g | 1.8 ± 0.2 abc |
| CH6 | 14.5 ± 3.4 efg | 8.0 ± 0.8 d-g | 22.5 ± 3.2 d-g | 1.8 ± 0.5 abc |
| CH7 | 15.1 ± 6.6 d-g | 10.6 ± 7.1 c-g | 25.7 ± 13.6 d-g | 1.6 ± 0.3 abc |
| CH8 | 14.7 ± 1.9 d-g | 9.6 ± 2.5 c-g | 24.3 ± 4.0 d-g | 1.6 ± 0.4 abc |
| CH9 | 11.7 ± 5.2 efg | 7.9 ± 4.6 d-g | 19.6 ± 9.2 efg | 1.6 ± 0.5 abc |
| CH10 | 9.4 ± 3.0 fg | 5.0 ± 2.2 fg | 14.4 ± 5.1 fg | 1.9 ± 0.4 abc |
| De Agua landrace: | | | | |
| CCA20 | 21.1 ± 5.5 c-g[1] | 11.1 ± 3.2 c-g | 32.3 ± 8.6 b-g | 1.9 ± 0.2 abc |
| CCA24 | 32.8 ± 16.8 a-d | 16.7 ± 9.5 a-e | 49.5 ± 25.8 a-d | 2.2 ± 0.6 a |
| CCA25 | 37.5 ± 22.2 abc | 17.7 ± 9.6 a-d | 55.2 ± 31.7 ab | 2.1 ± 0.1 ab |
| CCA32 | 26.5 ± 6.3 b-f | 13.3 ± 4.9 b-f | 39.8 ± 11.2 b-f | 2.1 ± 0.4 ab |
| CCA33 | 28.3 ± 14.2 b-e | 13.9 ± 6.9 b-f | 42.2 ± 20.7 a-e | 2.1 ± 0.5 ab |
| CCA36 | 21.6 ± 5.1 c-g | 11.4 ± 3.3 b-g | 33.1 ± 8.5 b-g | 1.9 ± 0.1 abc |
| CCA38 | 14.9 ± 2.0 d-g | 7.4 ± 0.8 efg | 22.4 ± 2.7 efg | 1.9 ± 0.2 abc |
| CCA40 | 46.8 ± 8.6 a | 21.2 ± 3.3 ab | 68.0 ± 11.9 a | 2.2 ± 0.1 a |
| CCA42 | 12.4 ± 8.4 efg | 6.0 ± 4.5 fg | 18.4 ± 12.9 efg | 2.1 ± 0.3 ab |
| CCA53 | 17.6 ± 4.0 d-g | 8.5 ± 1.4 c-g | 26.0 ± 5.3 d-g | 2.1 ± 0.3 abc |
| CCA62 | 37.0 ± 9.9 abc | 16.5 ± 4.1 a-e | 53.5 ± 13.6 abc | 2.2 ± 0.3 a |
| Jalapeño type (control): | | | | |
| JAL | 44.1 ± 10.6 ab | 24.1 ± 6.4 a | 68.2 ± 16.9 a | 1.8 ± 0.1 abc |

[1] In columns, means with the same letter are not significantly different (Tukey's test, $p \leq 0.05$)

In the principal component analysis (PCA), the first two components explained 94.5% of the total variation in terms of fruit composition (Figure 3). The differences between populations of each landrace were associated with antioxidant activity evaluated by DPPH and FRAP methods, indirectly reflecting the ability to capture free radicals by bioactive compounds, and capsaicin and dihydrocapsaicin contents helped differentiate landraces and populations within each landrace. The populations of the Huacle landrace were associated with low concentrations of capsaicinoids and higher antioxidant activity. Conversely, the populations of the De Agua landrace were associated with higher capsaicinoids content and lower antioxidant activity, as shown in Figure 3.

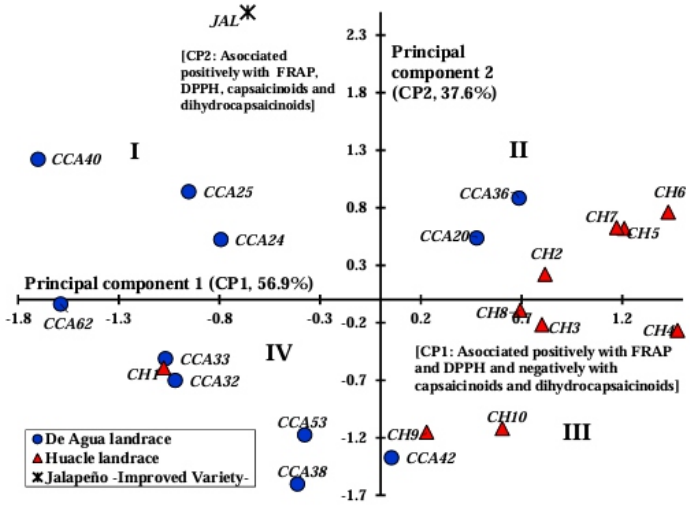


**Figure 3**. Scatterplot of pepper landrace populations as a function of the two principal components, on base phenolic compounds, vitamin C and capsaicinoid contents and antioxidant activity.

## 4. Discussion

The composition of chili fruit and its perception by consumers through aroma, flavor and texture determines, in part, the preferences and demands in regional, national and international markets and the nutritional-nutraceutical benefits for health. Consumers demand landraces of chili to recreate flavors is a medium that allows the conservation of these populations or traditional varieties by farmers and help researchers to differentiate plants and/or fruits between landraces and allow monitor the evolution process under domestication [7,11,17]. In this sense, Huacle and De Agua are names of pepper landraces cultivated, selected and preserved on-farm by traditional farmers in Oaxaca, Mexico but with demand local and regional of combinations of flavor and aroma. In this study, the Huacle and De Agua landraces with different fruit morphologies were significantly different regarding flavonoids and vitamin C content and antioxidant activity evaluated by DPPH, capsaicin (CAP) and total capsaicinoid (CAP + DIH) contents and CAP/DIH ratio, with substantial differences with respect to the control (Jalapeño, Tables 3 and 6). In this study, the capsaicinoids content was higher in Jalapeño than in both landraces, i.e., twice that in Huacle and 50 to 60% more than in De Agua (Table 5), indicating that the pungency of Huacle and De Agua is very low or almost null compared to the Jalapeño. In Oaxaca, Mexico, Huacle is frequently used dry, and De Agua is usually roasted. In contrast, Jalapeño is consumed fresh or in sauces. Composition analyses help to propose a chemotaxonomy of Capsicum annuum landraces based on the chemical structure of the secondary metabolites in fruit [38]. Hervert-Hernandez et al. [39] obtained similar results in the differential composition of fruits between five spicy varieties of C. annuum, and Martínez-Ispizua etal. [40] obtained similar patterns among landraces from Valencia, Spain.

The variation in total polyphenol contents (7.9 to 14.9 mg GAE g−1) between Huacle, De Agua and Jalapeño (control) was slightly higher than the values reported in different landraces and commercial varieties of C. annuum –2.47 to 8.331 mg GAE g−1– [10,40–42] but lower than those in wild populations of C. annuum var. glabriusculum (26.2 to 42.4 mg GAE g−1) and for hot pepper varieties (23.2 to 28.4 mg GAE g−1) [39,43]. Even when there are certain methodological differences in laboratory protocols, in each investigation, different germplasm sources or genetic sources are evaluated and fruit composition can differ due to genetic, environmental or agroecological effects, genetic-environmental interactions and management practices, in addition to the selection of diverse landraces by farmers [8,44].

Huacle and De Agua landraces and Jalapeño exhibited high variation in flavonoid contents (1.1 to 3.2 mg QE g−1), but the values were within the range for average flavonoid content reported by Ionică et al. [41] for five commercial varieties of chili (1.42 to 5.46 mg QE g−1) but, in certain cases, lower than the specific variation in quercetin equivalents for 14 commercial varieties of chili (0.2 to 7.9 mg QE g−1) [45]. However, the estimated variation in this study was slightly lower than those for wild chili based on catechin equivalents (3.53 to 4.14 mg EC g−1) [43]. This indicates that the native populations of Huacle or De Agua traditionally consumed by communities within Oaxaca, Mexico, have a total flavonoid content similar to commercial or traditional varieties from other regions and that, perhaps, the combination of flavonoids and other secondary metabolites confer flavors and specific aromas that are preferred by consumers [46,47].

In general, in different countries there are not breeding programs to improve landraces. So, based on this work, we suggest a breeding program of plant and fruit selections in order to maintain the characteristic fruit composition, which can promote their demand not only at regional/national level even international. The vitamin C content in fruits of Huacle and De Agua varied from 1.8 to 4.3 and from 3.4 to 5.9 mg AA g−1, respectively, and was 6.4 mg AA g−1 in Jalapeño, indicating significant differences where variation that slightly exceeded that reported by Martínez-Ispizua et al. [40] for 18 sweet or non spicy chili landraces (0.6 to 2.47 mg AA g−1). The vitamin C content in Huacle populations was within

the range reported by Vázquez-Flores et al. [43] for wild populations of chili (2.58 to 3.07 mg AA g−1) but lower than those reported for Jalapeño and De Agua. Ionică et al. [41] reported values of 0.17 to 1.60 mg AA g−1 in five commercial varieties, concentrations lower than those found in this study. In this context, the chili fruits evaluated herein are excellent sources of vitamin C. For example, the consumption of 100 g per day can meet the basic vitamin C consumption needs of an adult [11,48,49]. Besides, ascorbic acid is a nutritional-functional compound with antioxidant activity and is abundant in immature and mature chili [50–54].

The variation in carotenoid content in Huacle and De Agua landraces and Jalapeño was 1.5 to 5.6 mg βC g−1, values lower than the range reported by Vázquez-Flores et al. [43] for wild populations (5.7 to 6.03 mg βC g-1) but higher than the range reported by Vera-Guzmán et al. [10] for landraces from Oaxaca, Mexico, 0.034 to 1.329 mg βC g−1. However, the final carotenoid content in fruit depends on the ripening color, e.g., red, yellow or orange and brownish yellow. In the populations evaluated in this study, mature Huacle fruits were dark brownish yellow and those for De Agua and Jalapeño were red. Thus, certain populations of Huacle have a higher carotenoid content than do De Agua and Jalapeño (Figure 1).

The variation in capsaicin (CAP) and dihydrocapsaicin (DIH) contents in Huacle, De Agua and Jalapeño ranged from 7.4 to 46.8 mg 100 g−1 and from 3.5 to 24.1 mg 100 g−1, respectively. These values are within the range (0.49 to 222.9 mg 100 g−1 of CAP) reported by Ionică et al. [41] in fruits of commercial varieties, and by Paredes-Andrade et al. [55] in a germplasm of C. annuum from Central America (20 to 260 mg 100 g−1 of CAP) and also in the range reported by Cisneros-Pineda [25] (7.5 to 631.89 and 3.7 to 50.4 mg 100 g−1 of CAP and DIH, respectively) in fruits of landraces of C. annuum which differ from those reported by Vázquez-Flores et al. [43], 661 to 710 mg 100 g−1 of CAP and 149 to 212 mg 100 g−1 of DIH. These estimates for capsaicin and dihydrocapsaicin allow differentiating levels of pungency. For example, low values (<10 mg 100 g−1 of CAP or DIH) are considered sweet to slightly pungent, and high concentrations of CAP and/or DIH (> 10 mg 100 g−1 of CAP or DIH) are moderately to highly pungent [41]. Therefore, the Huacle and De Agua landraces vary from sweet to moderately pungent based on the values recorded. The De Agua populations with the highest CAP content (32.8 to 46.8 mg 100 g−1) were CCA24, CCA25, CCA40 and CCA62, and the CH4 and CH10 populations of Huacle (7.4 to 9.4 mg 100 g−1 of CAP and 3.5 to 5.0 mg 100 g−1 of DIH) can be used as sweet or non-pungent peppers in several gastronomic preparations. The antioxidant activity evaluated by DPPH and FRAP methods provided complementary information to differentiate populations within each landrace and allow to understand the complexity in the composition of the fruits evaluated. Antioxidant activity reflects the combined effect of total polyphenols, flavonoids, vitamin C, capsaicinoids, phenolic acids and other compounds on the reducing capacity or capture of free radicals [20,56]. The positive correlations between antioxidant activity and compounds are explained by their ability to donate hydrogen atoms from a hydroxyl group to the benzene ring and their redox properties, which increase their ability to adsorb and sequester free radicals [57,58]. In this sense, gallic acid, quercetin and capsaicinoids in isolated fractions of chili fruits have high DPPH antioxidant activity [59]. Capsaicin can sequester or eliminate DPPH radicals when the reaction site is located at the C7 position of the benzyl carbon [60]. In addition, capsaicin, dihydrocapsaicin and total polyphenols in chili peppers are associated with high antioxidant activity, as evaluated by the FRAP method [61].

All evaluated compounds were useful to find similitudes and differences between Huacle and De Agua landraces, and consequently to define distinctive traits from a phytochemical point of view to formulate on-farm conservation strategies and support a denomination of origin from a vegetal product. Currently, chili fruits are a source from vitamins, minerals and bioactive and antioxidant compounds, which contribute to improve the nutritional health of consumers and communities of small-scale farmers. For

agronomic purposes, the populations evaluated are a source of useful traits or genes for pepper breeding. For example, there are non-pungent populations with a common trait in many commercial varieties, but also it was evident that many populations have a combination of polyphenols, flavonoids, vitamin C, carotenoids and capsaicinoids more convenient in the international cuisine.

## 5. Conclusions

The results indicate significant differentiation between Huacle and De Agua regarding flavonoids, vitamin C, carotenoids, capsaicin and total capsaicinoids contents and antioxidant activity evaluated by DPPH. The Huacle and De Agua landraces differed from the Jalapeño control in total polyphenol, flavonoid and vitamin C contents, antioxidant activity as evaluated by FRAP, capsaicin (CAP), dihydrocapsaicin (DIH) and total capsaicinoid (CAP + DIH) contents. The differentiation between Huacle and De Agua landraces can contribute to increase their value added and a start point for a breeding program focused on fruit quality because the evaluation of fruit composition allowed the differentiation of populations with higher total polyphenol, flavonoid, vitamin C, carotenoid, capsaicin and dihydrocapsaicin contents (in Huacle, CH1, CH6 and CH7; in De Agua, CCA24, CCA25, CCA40 and CCA62). Populations with non-pungent fruits were identified within Huacle and De Agua landraces, which can contribute to the demand for this type of product. In addition, this variation can be exploited directly by consumers through potential nutritional-nutraceutical value. The results provide useful phytochemical composition information that can be used to formulate conservation strategies and promote diversity.

**Use of AI tools declaration**

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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**Conflict of interest**

The authors declare that they have no conflicts of interest to report regarding the present study.

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