Original Research Article

**Effect of Post-harvest Chemical Dipping Treatments on Quality Parameters of Banana (*Musa* spp.) cv. Poovan (AAB)**

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ABSTRACT

**Aims:** To assess the efficacy of shelf life and quality of banana cv. Poovan.
**Study Design:** Completely Randomized Design (CRD) with six treatments and three replications.
**Place and Duration of Study:** Fruit Science Laboratory, SRM College of Agricultural Sciences, Baburayanpettai from 2023 to 2025.
**Methodology:** Post-harvest chemical dipping treatments using kojic acid (0.5% and 1%) and calcium lactate (1%) were applied individually and in combination. The observations to be recorded TSS, Titratable acidity, firmness, Total sugars, Reducing Sugars, Non-reducing sugars, fruit weight, PLW, and shelf life. The statistical analysis used by OPSTAT and significant differences at p = 0.05.
**Results:** Treatment T₅ (1% kojic acid + 1% calcium lactate) shows the best performing traits, such as highest TSS, lowest acidity, good firmness, and shelf life.
**Conclusion:** The above results indicate the potential of this treatment for commercial use in extending the banana shelf life and minimizing post-harvest losses. So, this technology can be recommended for highly perishable fruit commodities. (Add values of best treatment)

***Keywords:*** *Kojic acid, calcium lactate, postharvest dipping, banana*

1. INTRODUCTION

Bananas (*Musa* spp.), belonging to the family Musaceae, represent a globally significant fruit crop, predominantly cultivated in tropical and subtropical regions (FAO, 2020). The bananas were first domesticated in Southeast Asia around 8000 BCE, and the crop subsequently dispersed to Africa, Europe, and the Americas. It serves as a staple food and primary income source for millions of smallholder farmers. Bananas contribute substantially to the economies of agriculturally dependent nations worldwide (FAO, 2020). Furthermore, the fruit offers significant nutritional benefits, being particularly rich in essential minerals such as potassium (K), which plays a critical role in regulating muscle contraction (Anyasi *et al*., 2018). Various kinds provide macro- and micronutrients, including calcium, phosphorus, sodium, magnesium, zinc, iron, manganese, boron, and copper (Qamar and Shaikh, 2018). Q

Asia was the largest producer of bananas, followed by Africa, South America, Central America, and other regions. Asia supplied 68,041.17 tonnes of bananas, Africa 22,941.96 tonnes, South America 19,421.82 tonnes, Central America 10,188.53 tonnes, the Caribbean 2,017 tonnes, Oceania 1,696.17 tonnes, Europe 668.17 tonnes, and North America 2.78 tonnes (FAOSTAT, 2021). India has the leading global production share, about 29%. The countries of India, China, the Philippines, Brazil, and Ecuador provide about 60% of worldwide banana production (Nayak and Nahar, 2018). The green stages of bananas show a significant concentration of starch and a comparatively smaller amount of sugar. The sugar content markedly rises, whereas the starch content declines throughout the full-ripening process (Evans *et al*., 2020). Starch gets converted into sucrose through the reaction of sucrose phosphate synthase, while acid hydrolysis converts starch into non-reducing sugars (Netshiheni *et al*., 2019).

The Poovan banana, belonging to the AAB genomic group, is particularly valued for its desirable taste, texture, and nutritional content, making it a commercially important cultivar in South and Southeast Asia (Uma *et al*., 2005). The Poovan variety displayed the lowest at 5.0%. The difference in moisture content might be due to how well the final products stick together, which is affected by large molecules like cellulose, lignin, and hemicellulose (Mydhili *et al*., 2022).

Despite its economic and nutritional value, banana production is hindered by high post-harvest losses, which can range from 25% to 50% during storage, transport, and marketing (Kader, 2005; Dadzie and Orchard, 1997). The main cause of these losses is the fruit's extreme perishability, particularly under tropical ambient circumstances when ripening causes quick physiological and biochemical changes. As climacteric fruits ripen, they start to breathe more and produce ethylene, which causes them to become softer, lose color, dry out, and become more prone to rotting from microbes.

The process of bananas getting ripe includes enzymes such as polygalacturonase, pectin methyl esterase, and polyphenol oxidase (PPO), which help soften the fruit and turn it brown (Kanellis *et al*., 1991; Singh *et al*., 2016). These activities shorten the shelf life of bananas to only a few days at room temperature; thus, coming up with good ways to keep them fresh once they are picked is quite important (Yahia, 2011).

Among various post-harvest approaches, chemical dipping treatments have proven effective in preserving fruit quality and extending the shelf life of the fruits. They offer advantages in terms of ease of use, scalability, and affordability for both large and small-scale producers (Kader, 2002). Two compounds of particular interest are kojic acid and calcium lactate, which have demonstrated beneficial effects in food preservation.

Kojic acid, which comes from fungi like *Aspergillus oryzae* and *Aspergillus flavus*, is recognized for its ability to fight oxidation, kill germs, and bind to metals (Cabanes *et al*., 1994). It has been found to inhibit the activity of polyphenol oxidase and lessen browning in different fruits by binding to copper ions that are important for PPO activity. In addition to its browning inhibition, kojic acid also exhibits antifungal and antibacterial effects, which can suppress microbial spoilage during storage (Madhavi & Salunkhe, 1995).

Calcium ions help pectic substances in the middle lamella to strengthen cell walls, slow down softening, and make membranes less permeable. Studies have demonstrated that calcium treatments lower respiration rates, delay ethylene production, and enhance the mechanical resistance of fruits such as apples, strawberries, and bananas (Bangerth, 1979; Serrano *et al*., 2004). For example, bananas treated with 3% calcium lactate showed improved firmness and lower decay incidence during storage (Rai *et al*., 2011).

Using kojic acid and calcium lactate together might enhance the benefits of each treatment and reduce their drawbacks. While kojic acid primarily prevents oxidative damage and microbial growth, calcium lactate provides structural support and retards senescence. The integration of these compounds could lead to improved outcomes in terms of firmness retention, color preservation, and microbial control. This study aimed to identify the treatment combinations that give the best results to preserve fruit quality, reduce post-harvest losses, and extend the storage life.

2. material and methods

The experiment was conducted in the Fruit Science Laboratory at SRMCAS from 2023 to 2025. The experiment was laid out in the Completely Randomized Design (CRD) with six treatments and three replications. The treatment details are as follows:

TABLE 1. **Treatment details**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Chemical** | **Percentage** |
| T1 | Kojic acid | 0.5% |
| T2 | Kojic acid | 1% |
| T3 | Calcium lactate | 1% |
| T4 | Kojic acid + Calcium lactate | 0.5% + 1% |
| T5 | Kojic acid + Calcium lactate | 1% + 1% |
| T6 | Control | Water |

**Observations recorded**

The observations such as TSS (ºBrix), titratable acidity, firmness (N), Total Sugar (%), Reducing Sugar (%), Non-Reducing Sugar (%), fruit weight, physiological loss weight, and shelf life were recorded at green and ripe stage (Mention days after treatment).

**Fruit weight**

A digital electronic weighing balance was used to determine the hand's weight, which was then converted to kilograms (kg). A digital electronic weighing balance was used to determine the finger's weight, which was then reported in grams (g).

**TSS**

A digital refractometer was used to find out how much total soluble solids in the pulp. It was expressed as °Brix.

**Firmness**

The firmness of selected fruits was quantified using a penetrometer and reported in Newtons (N).

**Acidity (Like wise make subsection of each observations)**

Titratable acidity was estimated using the A.O.A.C. (2012) method, which requires collecting five grams of the fruit sample and diluting it to a final volume of 30 ml with distilled water. The liquid sample was filtered, and then 5 ml of the clear liquid was mixed with two to three drops of phenolphthalein indicator and slowly mixed with 0.1 N sodium hydroxide until it turned a light pink color. Titratable acidity was expressed as a percentage.

The Anthrone method, as described by Hedge and Hofreiter (1962), was utilized to estimate total sugar. Accurately measure 100 mg of the sample into a boiling tube. Hydrolyze using 5 ml of 2.5 N HCl in a boiling water bath for 3 hours. Cool and neutralize using solid sodium carbonate until effervescence has stopped. Prepare a final volume of 100 ml and subject it to centrifugation. Prepare aliquots of 0.5 ml and 1 ml for analysis. Prepare standards ranging from 0 to 1 ml using distilled water to achieve a final volume of 1 ml. Heat 4 ml of Anthrone reagent in a boiling water bath for 8 minutes, subsequently cool, and measure absorbance at 630 nm. The total sugar was expressed as a percentage.

The estimation of reducing sugar was conducted using the Dinitro Salicylic Acid (DNS) method as described by Miller (1972). Transfer 0.5 to 3 ml of alcohol-free extract into test tubes, then dilute each tube to a total volume of 3 ml with water. Incorporate 3 ml of the DNS reagent and ensure thorough mixing. Boil for 5 minutes in a boiling water bath. Upon the development of color, introduce 1 ml of 40% Rochelle salt solution while maintaining heat, and stir the mixture. Before measuring the absorbance at 510 nm, cool the tubes using running tap water. The reduced sugar was expressed as a percentage.

By deducting reducing sugars from total sugars, the non-reducing sugars percentage was calculated as follows.

Non-Reducing Sugar (%): Total sugars (%) - Reducing sugars (%).

Physiological loss in weight (PLW) has been defined as the percentage difference between its initial weight and the weight observed at the time of assessment.

PLW (%) = (Initial Weight − Final Weight/Initial Weight​) ×100

 The shelf life of a fruit was determined by its appearance and marketability. Fruits that attain the edible ripe stage (without spoiling) have reached the final stage of their shelf life. It was represented as several days.

**Statistical analysis**

3. results and discussion

**Table 2: Effect of Postharvest treatments on TSS (ºBrix), Titratable Acidity (%), and Firmness (N) in Banana cv. Poovan (AAB)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **TSS (°Brix)** | **Titratable acidity (%)** | **Firmness (N)** |
| **Green****Stage** | **Ripe****Stage** | **Green stage** | **Ripe stage** | **Green stage** | **Ripe** **stage** |
| T1: Kojic Acid-0.5% | 16.4 | 20.3 | 0.45 | 0.31 | 32.1 | 19.3 |
| T2: Kojic Acid-1% | 16.7 | 20.9 | 0.43 | 0.28 | 37.5 | 24.7 |
| T3: Calcium Lactate - 1% | 17.2 | 21.7 | 0.42 | 0.27 | 33.4 | 23.2 |
| T4: Kojic Acid-0.5% + Calcium Lactate-1%  | 17.5 | 22.4 | 0.41 | 0.25 | 38.4 | 28.1 |
| T5: Kojic Acid-1% + Calcium Lactate-1%  | 17.9 | 23.5 | 0.38 | 0.22 | 39.4 | 21.3 |
| T6: Control | 16.2 | 19.6 | 0.49 | 0.35 | 30.8 | 16.8 |
| **SE (d)** | **0.038** | **0.287** | **0.019** | **0.027** | **0.961** | **0.493** |
| **CD (0.05)** | **0.085** | **0.632** | **0.042** | **0.060** | **2.117** | **1.087** |

**Table 3: Effect of Postharvest Treatments on Total Sugar (%), Reducing Sugar (%), and Non-Reducing Sugar (%) in Banana cv. Poovan (AAB)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Total Sugar (%)** | **Reducing Sugar (%)** | **Non-Reducing Sugar (%)** |
| **Green****Stage** | **Ripe****Stage** | **Green stage** | **Ripe stage** | **Green stage** | **Ripe** **stage** |
| T1: Kojic Acid-0.5% | 8.6 | 16.5 | 3.3 | 6.3 | 5.3 | 10.2 |
| T2: Kojic Acid-1% | 8.9 | 16.8 | 3.5 | 6.5 | 5.4 | 10.3 |
| T3: Calcium Lactate - 1% | 9.5 | 17.2 | 3.6 | 7.1 | 5.9 | 10.1 |
| T4: Kojic Acid-0.5% + Calcium Lactate-1%  | 9.2 | 18.4 | 3.6 | 8.2 | 5.6 | 10.2 |
| T5: Kojic Acid-1%+ Calcium Lactate-1%  | 9.8 | 16.2 | 4.5 | 6.1 | 5.3 | 10.1 |
| T6: Control | 8.2 | 22.5 | 5.1 | 12.1 | 3.1 | 10.4 |
| **SE (d)** | **0.211** | **0.287** | **0.098** | **0.137** | **0.102** | **0.210** |
| **CD (0.05)** | **0.464** | **0.632** | **0.216** | **0.303** | **0.224** | **N/A** |

The total soluble solids content increased significantly from the green to the ripe stage across all treatments. Treatment T5 - (Kojic Acid 1% + Calcium Lactate 1%) had the most sugar at 23.5 °Brix in fully ripe fruits, followed by T4 with 22.4 °Brix, and the control (T6) had the least at 19.6 °Brix (Table 2). This signifies increased sugar accumulation resulting from delayed ripening and improved metabolic management. The increase is because delayed ripening allows more time for enzymes to turn starch into sugars, similar to what happens in mangoes, where using calcium and antioxidants made them sweeter and better for selling (Khedr, 2022).

 Titratable acidity decreased from the green stage to the ripe stage across all treatments. T5 - (Kojic Acid 1% + Calcium Lactate 1%) showed the lowest acidity at the ripe stage, observed at 0.22%, showing effective acid metabolism during the ripening process. The control fruits (T6) were showing a higher acidity level of 0.35% (Table 2). This indicates that the fruits in the study were efficient at using acids during ripening, similar to mangoes treated with calcium lactate and kojic acid, where the reduction in acidity was associated with increased sweetness and extended shelf life.

 The low firmness gradually declines among all treatments from the green to ripening stages, which coincides with the activity of softening enzymes. The treatment T4 (0.5% kojic acid + 1% calcium lactate) showed the highest firmness (28.1 N) in ripe fruit, followed by other treatments T5 and T2 (Table 2). The high firmness is attributed to calcium ions, which strengthen the cellular framework by forming calcium pectate complexes that prevent cell wall degradation. Calcium lactate considerably maintained firmness during cold storage, unlike the mechanisms observed in mangoes (Khedr, 2022; Luna-Guzman and Barrett, 2000). According to Rodrigues *et al*. (2010), the firmness declines rapidly after ripening, even under cold storage conditions, and the calcium lactate proves helpful for retaining firmness.

Luna-Guzman and Barret (2000) noticed that calcium also increases cell wall turgor pressure and stabilizes the membrane turgidity.

 All treatments demonstrated a significant increase in total sugars at the ripening stage. The control (T6) showed a high total sugar content of 22.5%, probably resulting from accelerated starch conversion in untreated fruits. Treated fruits, especially T4 (18.4%) and T5 (16.2%) (Table 2), exhibited moderate sugar concentrations, indicating a more regulated ripening process that may enhance shelf life. Similar findings were observed in the banana cv. poovan, where the combination of calcium lactate1 % and kojic acid 1 % significantly enhanced the fruit quality and shelf life of banana. (Sandeep Saran *et al*, 2024).

 Similar results have been noticed, both reducing sugar and total sugar in T4 (8.2%) and the control (12.1%). Approximately 10% of non-reducing sugars were present in all treatments (Table 3). The rapid breakdown of starch is associated with an increase in higher levels of reducing sugar.

 The firmness is regulated in sugar levels, and low acid degradation in T5 indicates longer shelf life and less physiological weight loss (PLW). (Khedr, 2022; Barzegar *et al*., 2018) It concluded that in mango by using 2% calcium lactate gradually decreases the physiological loss weight (PLW) because it helps to enhance the cell wall membrane. The combination of treatment T5 - (Kojic Acid 1% + Calcium Lactate 1%) stopped polyphenol oxidase (PPO), which reduced the spoilage and browning, while calcium lactate helped to keep the structure and freshness of fruits (Cabanes *et al*., 1994; Khedr, 2022). This showed that kojic acid and calcium decreased the rate of deterioration and prolonged the shelf life and quality of the fruits.

 The treatment T5 - (Kojic Acid 1% + Calcium Lactate 1%) has a long shelf life, while the treatment (T6-control) has a shorter shelf life. Shah *et al*. (2017) observed by kojic acid in litchi fruit before storing it can slow down the browning of the pericarp. It also prevents browning by reducing the acidity and attaching to copper ions in the enzyme, which stops inhibiting the activity and enhances the shelf and provides better quality.

**Fig. 1. Impact of post-harvest treatments on A. Fruit weight, B. Physiological loss weight, and C. Shelf life in Banana cv. Poovan**

**Result and Discussion**

**4. Conclusion**

The findings of the study showed that the combination treatment T5 (1% kojic acid + 1% calcium lactate) significantly enhanced total soluble solids (TSS), reduced acidity, and improved firmness and shelf life of Poovan bananas. These results indicate the potential of this treatment for commercial use in extending banana shelf life and minimizing post-harvest losses. This technology can be recommended for highly perishable fruit commodities.

**References**

Anyasi, T. A., Jideani, A. I. O., & Mchau, G. R. A. (2018). Phenolics and essential mineral profile of organic acid pretreated unripe banana flour. *Food Research International*, 104, 100–109. [https://doi.org/10.1016/j.foodres.2017.09.063](https://doi.org/10.1016/j.foodres.2017.09.063%22%20%5Ct%20%22_new)

A.O.A.C. (2012). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th ed. Washington, D.C.

Bai, R. K., Baldwin, E. A., & Hagenmaier, R. D. (2004). Use of edible coatings to preserve the quality of fresh-cut fruits. *Journal of Food Science*, 69(9), FQE60–FQE66. [https://doi.org/10.1111/j.1365-2621.2004.tb09952.x](https://doi.org/10.1111/j.1365-2621.2004.tb09952.x%22%20%5Ct%20%22_new)

Bangerth, F. (1979). Calcium-related physiological disorders of plants. *Annual Review of Phytopathology*, 17(1), 97–122. [https://doi.org/10.1146/annurev.py.17.090179.000525](https://doi.org/10.1146/annurev.py.17.090179.000525%22%20%5Ct%20%22_new)

Cabanes, J., Chazarra, S., Garcia-Carmona, F., & Lozano, J. A. (1994). Inhibition of melanogenesis by kojic acid. *Journal of Investigative Dermatology*, 102(4), 685–690. [https://doi.org/10.1111/1523-1747.ep12371756](https://doi.org/10.1111/1523-1747.ep12371756%22%20%5Ct%20%22_new)

Dadzie, B. K., & Orchard, J. E. (1997). *Routine post-harvest screening of banana/plantain hybrids: Criteria and methods*. INIBAP Technical Guidelines 2.

FAOSTAT. (2021). FAOSTAT online database. Retrieved January 2021, from [http://faostat.fao.org/](http://faostat.fao.org/%22%20%5Ct%20%22_new)

Ferguson, I. B. (1984). Calcium in plant senescence and fruit ripening. *Plant, Cell & Environment*, 7(6), 477–489. [https://doi.org/10.1111/1365-3040.ep11589615](https://doi.org/10.1111/1365-3040.ep11589615%22%20%5Ct%20%22_new)

Friedman, M. (1996). Food browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry*, 44(3), 631–653. [https://doi.org/10.1021/jf950389r](https://doi.org/10.1021/jf950389r%22%20%5Ct%20%22_new)

Glenn, G. M., & Poovaiah, B. W. (1990). Calcium-mediated postharvest changes in fruits and vegetables. *HortScience*, 25(8), 864–867. [https://doi.org/10.21273/HORTSCI.25.8.864](https://doi.org/10.21273/HORTSCI.25.8.864%22%20%5Ct%20%22_new)

Hedge, J. E., & Hofreiter, B. T. (1962). In: Whistler, R.L. & BeMiller, J.N. (Eds.), *Carbohydrate Chemistry*, Vol. 17, Academic Press, New York.

Kader, A. A. (2002). *Postharvest technology of horticultural crops* (3rd ed.). UCANR Publications.

Kanellis, A. K., Solomos, T., & Roubelakis-Angelakis, K. A. (1991). Cell wall degradation during fruit ripening. In *Plant Physiology* (pp. 264–290). Springer.

Khedr, E. H. (2022). Improving the quality of ‘Keitt’ mango using pre-harvest calcium lactate treatment and post-harvest applications of kojic and ascorbic acids. *Journal of Horticultural Science and Biotechnology*, 97(6), 757–772. [https://doi.org/10.1080/14620316.2022.2058427](https://doi.org/10.1080/14620316.2022.2058427%22%20%5Ct%20%22_new)

Lee, J., Durst, R. W., & Wrolstad, R. E. (2007). Determination of total phenolics using Folin–Ciocalteu reagent. *Journal of AOAC International*, 90(4), 1217–1225.

Lohani, S., Trivedi, P. K., & Nath, P. (2004). Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: Effect of 1-MCP. *Postharvest Biology and Technology*, 34(2), 121–129. [https://doi.org/10.1016/j.postharvbio.2004.04.006](https://doi.org/10.1016/j.postharvbio.2004.04.006%22%20%5Ct%20%22_new)

Luna-Guzman, I., & Barrett, D. M. (2000). Comparison of calcium chloride and calcium lactate effectiveness in preserving quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, 19(1), 61–72.

Madhavi, D. L., & Salunkhe, D. K. (1995). Toxicological aspects of food antioxidants. In Madhavi, D. L., Deshpande, S. S., & Salunkhe, D. K. (Eds.), *Food Antioxidants* (pp. 267–359). Marcel Dekker.

Miller, G. L. (1972). Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, 31(3), 426–428. [https://doi.org/10.1021/ac60147a030](https://doi.org/10.1021/ac60147a030%22%20%5Ct%20%22_new)

Mydhili, M., Pugalendhi, L., Indu Rani, C., Auxcilia, J., & Uma, D. (2022). Proximate composition of different varieties of banana pseudo-stem powder for nutritional and biochemical properties. *Biological Forum*, 14(3), 331–334.

Nayak, A. K., & Nahar, S. (2018). Growth, instability, and export performance of banana in India: An economic analysis. *Agriculture Industry*, 74(10), 25–33.

Prabha, T. N., & Bhagyalakshmi, N. (1998). Carbohydrate metabolism and respiration in sliced banana fruit during browning. *Journal of Plant Physiology*, 153(1–2), 125–130. [https://doi.org/10.1016/S0176-1617(98)80134-3](https://doi.org/10.1016/S0176-1617%2898%2980134-3%22%20%5Ct%20%22_new)

Rodrigues, J. D. P., Queiroz, M. A. Á., de Lucena, A. R., Costa, F. S., de Carvalho, D. T., da Costa, M. M., & Rodrigues, R. T. D. S. (2019). Inclusion of discarded banana in sugarcane silage decreases dry matter losses and improves its nutritional value. *Revista Colombiana de Ciencias Pecuarias*, *32*(1), 50-57.

Qamar, S., & Shaikh, A. (2018). Therapeutic potentials and compositional changes of valuable compounds from banana—A review. *Trends in Food Science & Technology*, 79, 1–9. [https://doi.org/10.1016/j.tifs.2018.06.016](https://doi.org/10.1016/j.tifs.2018.06.016%22%20%5Ct%20%22_new)

Rai, D. R., Sagar, V. R., & Kumar, R. (2011). Effect of calcium lactate and packaging on shelf life of bananas. *Indian Journal of Horticulture*, 68(2), 226–230.

Sadasivam, S., & Manickam, A. (1992). *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Limited, New Delhi, pp. 5–6.Serrano, M., Martínez-Romero, D., Guillén, F., & Valero, D. (2004). Effect of calcium dip and heat treatment on quality of table grapes. *Postharvest Biology and Technology*, 34(3), 209–217. [https://doi.org/10.1016/j.postharvbio.2004.05.003](https://doi.org/10.1016/j.postharvbio.2004.05.003%22%20%5Ct%20%22_new)

Singh, R., Sharma, R. R., & Singh, D. (2016). Post-harvest physiological and biochemical changes in banana. *International Journal of Agriculture and Food Science*, 6(2), 66–72.

Uma, S., Selvarajan, R., Sathiamoorthy, S., & Durai, P. (2005). Banana and plantain: An overview with reference to India. *Journal of Horticultural Sciences*, 1(1), 1–13.

Yahia, E. M. (2011). *Postharvest biology and technology of tropical and subtropical fruits*, Vol. 1. Woodhead Publishing.

A.O.A.C. (2012). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th ed. Washington, D.C.