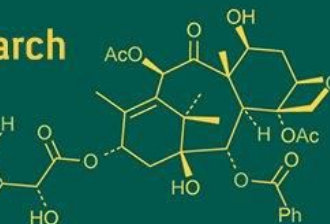


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(4): 222-226
www.biochemjournal.com
Received: 12-02-2026
Accepted: 15-03-2026

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Varietal screening of dietary fiber and pectin yield in five *Psidium guajava* L. cultivars

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4Sc.8089>

Abstract

Guava (*Psidium guajava* L.) is composed of significant amounts of both dietary fiber and bioactive compounds. However, how the genotype of these species in guava fruit affects the distribution of these structural carbohydrates has not been established to date. Therefore, this research aimed to assess five of the most popular commercial cultivars of guava in the Western Agro Climate Zone of Tamil Nadu, India (Taiwan Pink, Taiwan Red, L 46, L 49, and CO Local) for both their fiber profiles and pectin values from their fruit; a randomized complete block design (RCBD) with 4 replications was performed. The insoluble dietary fibers (IDF) and soluble dietary fibers (SDF) were quantified from the fruits of all cultivars utilizing the AOAC enzymatic-gravimetric methods. Pectin from the fruit was quantified with the calcium pectate method. All of the measured parameters for the cultivars exhibited significant statistical differences ($p < 0.05$) among each of the cultivars. Concentrations of IDF (5.10%), SDF (1.18%), and pectin (1.25%) were highest for cultivar L 49; whereas IDF (4.42%), SDF (0.75%), and pectin (0.94%) were lowest for cultivar Taiwan Pink. Overall, cultivar L 49 was identified as the highest producing cultivar in terms of IDF and pectin production, which provides rationale for the utilization of cultivar L 49 as an ingredient in the production of functional foods and nutraceuticals at a commercial level.

Keywords: Guava cultivars, dietary fiber, pectin content, genotype variation, functional foods

1. Introduction

Guava (*Psidium guajava* L.) is a leading tropical fruit in the family Myrtaceae and is grown in areas that grow tropical and subtropical crops. Although the fruit is commonly eaten as an excellent source of vitamin C and highly nutritious [Thomas A, *et al.* 2017]^[1], little research has been done looking at how guava is also used as a potential major source for industry of dietary fiber and pectin [Pawar V, *et al.* 2023]^[2].

One of the most significant components of a balanced diet is the amount of dietary fibre, as it helps regulate blood sugar and lipid levels, thus reducing the risk of developing metabolic diseases. [Kahleova H, *et al.* 2023]^[3] Multiple observational epidemiologic studies have found an association between high dietary fibre consumption and lower incidence of Type 2 diabetes, coronary heart disease, and bowel cancer. This could be due to the production of short-chain fatty acids in the gut as a result of fermenting dietary fibre by colonic bacteria and modifying the gut microbiome Dahl WJ *et al.* 2015^[12]. There have been only a small number of systematic studies on the characteristics of guava dietary fibre fractions related to regional genetic variations [Verma D, 2024]^[4]. The majority of the work done on guavas has focused on improving general quality characteristics of guavas.

Interest in clean, plant-based materials is increasing worldwide, and this has led some people to re-evaluate the way by-products associated with agriculture can be reused. Traditionally, industrial pectin production has used organic waste from citrus fruits and apple juice pomace; however, these sources are not always available and tend to have high prices Zhang X *et al.* 2018, Dahl WJ *et al.* 2015^[11, 12]. The guava grown in Tamil Nadu's Western Agro-Climatic Zone will be available throughout the year, thus representing an alternative, year-round source of low-cost materials for supplementing the traditional pectin supply chain.

Guava is a fruit that has a cell wall made up of primarily three components: cellulose, hemicellulose, and pectin.

Pectin acts as a stabilizing structure for the plant cell wall by providing both gel-like and support characteristics. The location of pectin is found in the middle lamellae of the plant cell wall (middle lamella-the plant cell walls located between two adjacent plant cells), thus the reason for classifying pectin as part of the cell wall. Pectins are made up of many polymers (polysaccharides) that are derived mainly from the monosaccharide (simple sugar) galacturonic acid. Because of their unique structure, pectins have a very high degree of flexibility, making them ideal for use in food, pharmaceuticals and cosmetics Drira S, *et al.* 2022 [14]. The first published work describing the nutritional value of guava fruit was by Thomas *et al.*, [1] with most recent studies addressing guava fruit biomass as a lignocellulosic source. This study aims to quantify and compare the yield of insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and pectin from five genotypes of guava (Taiwan Pink, Taiwan Red, L 46, L 49, CO Local) to determine their potential best candidates for nutraceuticals or the pectin industry.

2. Materials and Methods

2.1 Experimental Site and Climatic Context

This experiment was conducted by the School of Agricultural Sciences at the Karunya Institute of Technology and Sciences in Coimbatore, India, where the mean daily temperature was between 24-34 °C and relative humidity was between 60-75% relative humidity. Under these semi-arid climatic conditions, fiber biosynthesis occurs as an adaptive mechanism to decrease transpiration stress for the tropical Myrtaceae (Arévalo-Marín E, *et al.* 2024). [7] In addition, high temperature and low moisture stress has been demonstrated to induce the expression of cellulose synthase and pectin methyltransferase genes and increase the total amount of structure carbohydrate in tropical woody species.

2.2 Experimental Design and Sample Preparation

Five different types of cultivars, including CO Local (Coimbatore Local), L46 (Lucknow 46), L49 (Lucknow 49), Taiwan Pink and Taiwan Red, were subject to a randomized complete block design (RCBD) experiment with four replications, and four replications of each cultivar were randomly assigned in each block. The fruits of these five different cultivars were shade dried and then ground into a fine powder for analysis. Fully matured fruit of each of the five different cultivars will be processed as soon as they are harvested and will be used to determine the amount of pectin present in each individual cultivar.

2.3 Extraction Procedures and Biochemical Analysis

2.3.1 Dietary Fiber Determination in Guava Fruit

Guava mesocarp has been analyzed for both insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) by AOAC 991.43 enzymatic-gravimetric method. Typically the fruit samples are oven-dried at 60 °C until reaching a constant weight and then ground to pass through a 0.5 mm mesh screen to ensure that the polysaccharide matrix is easily accessible by enzymes. AOAC 2019 [5]

Enzymatic digestion of guava mesocarp to isolate the various fiber fractions is accomplished sequentially in 3 stages using the following enzymes:

1. **Heat-stable alpha-amylase (EC 3.2.1.1):** The guava fruit mesocarp was initially treated with heat-stable alpha-amylase at 95 °C (pH 6.0) to gelatinize and hydrolyze the starch present in the mesocarp because the starch is typically very high in slightly immature (or mature) guava. The cleavage of the α -1, 4-glycosidic linkages by the enzyme converts the starch into soluble maltodextrins thus eliminating the potential for gravimetric interference due to the insoluble portion of the starch.
2. **Protease (EC 3.4.21.x):** The digestion was carried out at 60 °C (pH 7.5) to hydrolyze the proteins with in the guava mesocarp. This step is important because proteins usually form protein-polysaccharide complexes in the guava mesocarp and if these complexes are not hydrolyzed then the mass of the fiber will be overestimated.
3. **Amyloglucosidase (EC 3.2.1.3):** The digestion was continued with amyloglucosidase to convert the remaining portion of dextrins resulting from the enzymatic digestion back to their constituent glucose units to ensure that all digestible carbohydrates were completely removed from the sample.

2.3.1.1 Dietary Fibres Fractionation and Calculation

- After the sample has gone through final digestion, the solution is filtered. The residue on the filter represents the insoluble dietary fibre (IDF) and generally consists of cellulose, hemicellulose and lignin and is then washed, dried and weighed.
- The filtrate obtained from the first stage will have four volumes of 95% ethanol added to the solution to precipitate any soluble polysaccharides (i.e. pectins and gums). The precipitate will be filtered, dried and weighed to obtain the amount of soluble dietary fibre (SDF).
- The totals of the two fractions will then yield TDF (total dietary fibre) which is determined by adding IDF and SDF together.

The analysis results for the fruit will all be reported on an approximately dry weight basis which will enable comparison of different cultivars with regard to their initial moisture content.

2.3.2 Pectin Yield Determination

Using the calcium pectate method, each cultivar's mesocarp was used to extract pectin. Each cultivar's mesocarp was hydrolyzed with dilute HCl at 90 °C for 60 minutes to solubilize its protopectin as free pectic acid and make it soluble. The clarified filtrate was neutralized before treatment with CaCl_2 and the resulting precipitate (calcium pectate) was filtered, air-dried at 60 °C until constant weight was attained, and expressed on a fresh-weight basis as percent calcium pectate. (Gupta P *et al.* 2022) [10].

3. Results

3.1 Varietal Variation in Fiber Fractions

Statistical analysis showed a wide range of results for the five different varieties of *Psidium guajava* L., meaning that there is a lot of variation at the genetic level for the way structural carbohydrates are divided. The IDF, SDF, and TDF values for all five cultivars are found in Table 1.

L 49 had the highest values of IDF (5.10%), SDF (1.18%), and TDF (6.28%) respectively. The second highest cultivar was CO Local with IDF of 4.78%, SDF of 1.05%, and TDF of 5.83%. The third highest cultivar (6) was L 46 with IDF 4.70%, SDF 0.97%, and TDF 5.67%. Taiwan Red was fourth overall with IDF 4.62%, SDF 0.88%, and TDF 5.50%. Finally, Taiwan Pink had the lowest overall for every type of fraction (IDF 4.42%, SDF 0.75%, TDF 5.17%). The standard error of the difference (SE(d)) for TDF was ± 0.0088 while the critical difference $P = 0.05$ was 0.0191. This means that the differences between all pairs of cultivars were statistically significant. As well, the coefficient of variation (CV) for TDF was 0.21% which indicates good quality control throughout experimentation.

Table 1: Mean Performance and Statistical Constants for Dietary Fiber Fractions in Guava Cultivars.

Variety	IDF (%)	SDF (%)	TDF (%)
L 49	5.10 ^a	1.18 ^a	6.28 ^a
CO Local	4.78 ^b	1.05 ^b	5.83 ^b
L 46	4.70 ^c	0.97 ^c	5.67 ^c
Taiwan Red	4.62 ^d	0.88 ^d	5.50 ^d
Taiwan Pink	4.42 ^e	0.75 ^e	5.17 ^e
SE(d) $\pm$	0.0056	0.0032	0.0088
CD ($P = 0.05$)	0.0122	0.0069	0.0191
CV (%)	0.1673	0.4625	0.2115

IDF: Insoluble Dietary Fiber; SDF: Soluble Dietary Fiber; TDF: Total Dietary Fiber. Means within a column followed by different superscript letters are significantly different ($P = 0.05$, Duncan's Multiple Range Test).

3.2 Pectin Yield

There were significant differences ($p < 0.05$) between the amount of pectin produced by the different cultivars ($F = 261.73$; $CV = 1.34\%$; Table 2). The highest pectin yield was returned by L 49 (1.25%), followed by CO Local (1.18%), L 46 (1.12%), and Taiwan Red (1.06%). Taiwan Pink had the lowest yield at 0.94%. The standard deviation of difference (SED) was equal to 0.0105 while the critical difference (CD) for the significance level of $P = 0.05$ was equal to 0.0229. These findings indicate that all cultivars differ statistically significantly from one another. In addition, the low coefficient of variation (CV) suggests that the calculation of calcium pectate was done very precisely as it relates to different samples tested at the same time.

Table 2: Mean Performance and Statistical Constants for Pectin Yield in Guava Cultivars.

Variety	Pectin (%)
L 49	1.25 ^a
CO Local	1.18 ^b
L 46	1.12 ^c
Taiwan Red	1.06 ^d
Taiwan Pink	0.94 ^e
SE(d) $\pm$	0.0105
CD ($P = 0.05$)	0.0229
F-Value	261.73
CV (%)	1.34

Values followed by different superscript letters within the column differ significantly ($P = 0.05$). Pectin expressed as percent calcium pectate on a fresh-weight basis.

4. Discussion

4.1 Genetic Regulation of Fiber Architecture

The significant inter-cultivar variation in IDF, SDF, and TDF (5.17-6.28%) confirms that structural carbohydrate accumulation in *P. guajava* L. is under strong genotypic control, consistent with observations in other tropical Myrtaceae (Meena RL *et al.* 2023, Sharma R, *et al.* 2020^[6, 8]). This genetic variability modifies the partitioning of photosynthetically fixed carbon toward specific cell wall polymers irrespective of shared environmental conditions in the Western Agro-Climatic Zone of Tamil Nadu (Arévalo-Marín E, *et al.* 2024, Sharma R, *et al.* 2020).^[7, 8]

4.2 Insoluble Dietary Fiber: Structural Adaptation

The predominant fruit fraction of IDF was the most important structural component of the cell wall. IDF was observed to be superior in L49 (5.10%) compared with Taiwan Pink (4.42%), indicating that the secondary cell wall of L49 had greater thickening and more deposition of lignocellulose due to adaptation to high irradiance and transpiration stress in tropical *Myrtaceae* (Arévalo-Marín E, *et al.* 2024, Rodríguez L *et al.* 2018)^(17, 9). Higher IDF levels are likely to reflect increased levels of cellulose and hemicellulose cross-linkage within the fruit tissue, providing greater mechanical strength and stability of fruit tissue due to turgor pressure within the cells of the fruit (Rodríguez L *et al.* 2018)^[9]. A cell wall comprises cellulose microfibrils embedded within a hemicellulose matrix; the amount of hemicellulose deposited within the cell wall of a fruit has been directly correlated with the higher gravimetric value of IDF found using enzymatic-gravimetric methods (Tan L, *et al.* 2018)^[16].

4.3 Soluble Dietary Fiber and Pectin: Biochemical Significance of L 49

There is a greater amount of soluble dietary fiber (1.18%) and pectin (1.25%) in L 49 than in Taiwan Pink (soluble dietary fiber 0.75%, pectin 0.94%), which can be attributed to an accumulation of protopectin in the middle lamella of the cell walls and a more controlled enzymatic conversion of protopectin to soluble pectin throughout the fruit maturation process^[10]. The process of making pectin begins with the methylesterification (the addition of methanol) of the polygalacturonic acid chains in the Golgi apparatus followed by release into the apoplast where the enzyme pectin methylesterase (PME) regulates how much of a chain has been methylesterified and thus how much calcium can cross-link the pectin network (Willats WGT *et al.* 2021)^[17]. In the assay of the calcium pectate, the fraction of the pectin that is measured is actually calcium pectate, which is described as the insoluble calcium pectic acid salt and is the only chemical form of pectin that is precipitated by a calcium chloride (CaCl_2) treatment (Gupta P *et al.* 2022)^[10]. Therefore, the 1.25% found for L 49 indicates a higher concentration of calcium pectate than in Taiwan Pink, which suggests the cell wall architecture of the cells of L 49 provides greater amounts of low-methoxyl pectin, a form of pectin that is highly sought after for use in industrial extraction. Both strength of gel and rate of setting are affected by the degree of methylesterification; therefore, low-methoxyl pectins, which have been precipitated as calcium pectate, are particularly desirable for sugar-free and calorie-reduced food formulations Fraeye I, *et al.* 2018^[18]

The IDF:SDF ratio of L 49 (about 4.3:1) appears to be beneficial for fulfilling glycemic management and lipid regulation functions because they produce unique physiological activities when combined (Kahleova H, *et al.* 2023) [3]; IDF will also promote accelerating transit through intestines, thereby decreasing time with potentially harmful substances from a carcinogen standpoint to come in contact with the intestinal epithelial cells (i.e., colon); while on the other hand, the fermentation of SDF will yield propionate and butyrate which will suppress cholesterol production in liver cells (liver), and feed (i.e., provide nutrients for) colon cells (i.e., colorectal cancer prevention) 9 Zhang X *et al.* 2018 Flint HJ, *et al.* 2017) [11, 19]. The amount of pectin present in L 49 (1.25%) matches up with published amounts of tropical fruit pectin between 0.88-1.79% which places guava fruit biomass as a viable, year-round option for pectin production versus using seasonal crops such as either citrus or apple pomace to produce pectin.

4.4 CO Local: Resilience and Regional Valorization

The local-origin DF occurred second (ranging from 1.05% for source-dried samples to 4.78% for final DFs) across all parameters being measured (IDF = 4.78%, SDF = 1.05%, and pectin content = 1.18%). Uniquely suited to grow locally, azimuthal adaptation provides an ideal opportunity for sustainable production of functional foods in situations in which limitations on transportation preclude either

developing an improved variety (such as L 49) or having any source of water available for crop irrigation. The Comparative Structural Carbohydrate Profile of the [CO local] DFs is dramatically superior to that of either hybrid tallow or Taiwan Pink and Taiwan Red, and thus suggests the use of this genotype as an integrated approach to fruit-derived biorefining operations.

4.5 Industrial and Zero-Waste Implications

The findings of the study showed that L 49 and CO Local varieties produce high levels of both IDF and pectin indicating that these two varieties could produce materials for use as a biorefinery where both fruit biomass could be processed to extract both fiber and a gelling agent from fruit (Gupta P *et al.* 2022) [10]. Additionally, since guava fruits are available year-round in the Western Agro-Climatic Zone of the Tamil Nadu region and the coefficient of variation in extraction assays of the fruits were low CV (1.34), this should enable a high degree of reproducibility when implementing a large scale commercially viable biorefinery operation resulting in low per unit extraction costs (Gupta P *et al.* 2022) [10]. Also, as the global market for functional food ingredients is expected to increase significantly by 2030, there is potential for commercial development of regionally grown clean label ingredients such as fiber & pectin produced from under-utilised tropical crops like guava (Grand View Research 2023) [19].

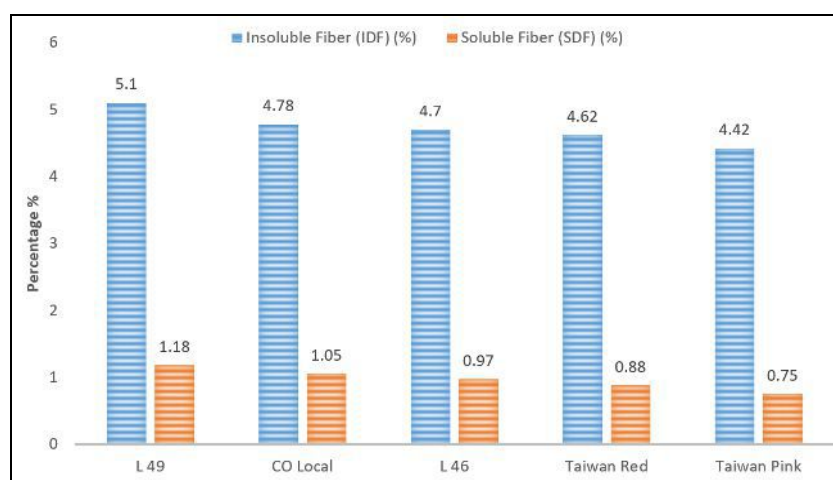


Fig 1: Comparative analysis of Insoluble Dietary Fiber (IDF) and Soluble Dietary Fiber (SDF) percentages in five guava cultivars (CO Local (Coimbatore Local), L46 (Lucknow 46), L49 (Lucknow 49), Taiwan Pink and Taiwan Red)

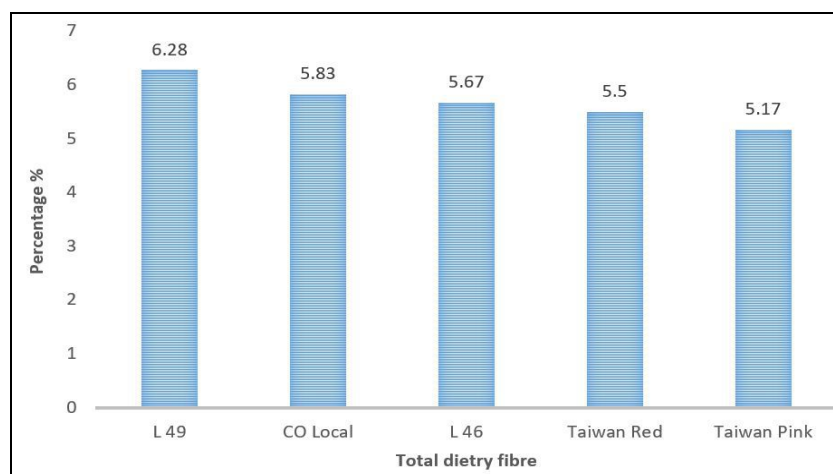


Fig 2: Total Dietary Fiber (TDF) content across five guava cultivars.(CO Local (Coimbatore Local), L46 (Lucknow 46), L49 (Lucknow 49), Taiwan Pink and Taiwan Red)

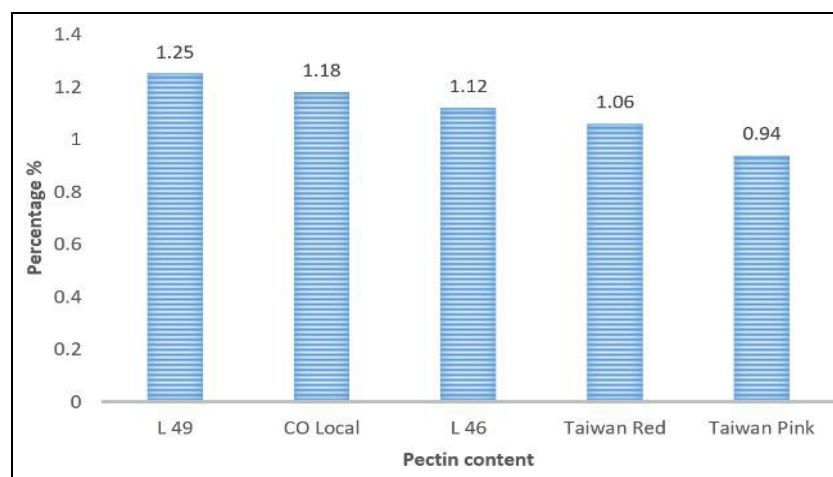


Fig 3: Comparison of Pectin yield (expressed as percent calcium pectate) among five guava cultivars. (CO Local (Coimbatore Local), L46 (Lucknow 46), L49 (Lucknow 49), Taiwan Pink and Taiwan Red)

5. Conclusion

The results of this research shows that the structural carbohydrates found in *Psidium guajava* L. are affected by the genotype of the plant. Of the five cultivars that were tested, L 49 had the greatest quantities of IDF (5.10%), SDF (1.18%), and pectin (1.25%) compared to all of the other cultivars; therefore, L 49 is the best candidate for industrial extraction of dietary fiber and pectin in the Western Agro-Climatic Zone of Tamil Nadu. The second best cultivar, CO Local, is also a good choice for commercial production since it has similar physical qualities to L 49 but can be grown in a wider area. The horticulture industries have the potential to implement sustainable production methods that produce a "zero-waste" industry by focusing on these two superior cultivars to access the ever-growing demand for functional foods using both fruit biomass.

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