

Development of an Infusion for LDL Cholesterol Based on Avocado Leaves (*Persea americana*), Guava Leaves (*Psidium guajava*) and Cinnamon

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ABSTRACT

An infusion based on avocado leaves, guava leaves and cinnamon were developed as a potential alternative for the treatment of LDL cholesterol. Avocado and guava leaves were washed, dehydrated and ground. Optimal dehydration length for avocado leaves was determined using weight loss curves, from which it was decided to treat leaves for 60 minutes at 40°C. Similarly, optimal dehydration conditions for guava leaves were achieved in a period of 80 minutes at 45°C. Three infusion formulations were tested. All formulations contained 1.5 g of guava leaves and 6 g of cinnamon, alongside 5, 10 and 15 g of dehydrated avocado leaves, respectively. A sensory analysis was carried out in which raters ranked the infusions in terms of taste and aroma, Basker's preference test was conducted and purchase intentions were gaged. Tasters preferred the formulation containing the lowest amount of dehydrated avocado leaves. 91.67% of tasters indicated that they would buy this infusion due to its possible functional properties.

Keywords: Infusion, avocado leaves, guava leaves, cinnamon, LDL cholesterol, sensory analysis.

INTRODUCTION

High levels of cholesterol in the blood have been related to an increased risk of cardiovascular disease in individuals aged above 40 years [1]. This increase is known as hypercholesterolemia, which is one of the most common metabolic diseases and results from high LDL (low-density lipoprotein cholesterol) cholesterol levels [2]. A high level of LDL cholesterol is a risk factor for the development of atherosclerosis, which can cause cardiovascular problems such as myocardial infarction and stroke [3]. This has led to the measurement of LDL cholesterol being considered as an indicator of great importance. Specifically, values <70 mg/dL in the blood indicate that an individual has a low risk of cardiovascular disease. In contrast, individuals with LDL values ≥190 mg/dL are classified as high risk, with this risk having to be controlled through medication [4]. Unfortunately, many of the drugs used to treat this condition can lead to a

higher incidence of diabetes mellitus and myopathy [5]. An example of this can be seen in the case of statins. Despite being the most used treatment for cholesterol, several investigations have revealed that they may promote the calcification of the cardiovascular disease coronary atheroma [6]. Likewise, acute memory loss could be related to the use of drugs to control cholesterol, due to the modification of the lipid composition of the brain [7]. The biological compounds of various foods have aroused great interest due to their possible effects on diseases such as hypercholesterolemia, diabetes, oxidative stress and hypertension [8]. One such food is avocado, whose pulp is commonly consumed and has a high composition of monounsaturated fatty acids. However, the peel, seed and leaves of this fruit have several compounds with functional properties [9]. In rats inoculated with carbon tetrachloride (known to contribute to fatty liver), 100-200 mg of an aqueous extract taken from the avocado leaf was found to significantly reduce total cholesterol and triacylglycerol levels, suggesting that this extract helps prevent hyperlipidemia and has a hepatoprotective effect in rats [10]. The antihypertensive effects of an aqueous extract taken from avocado leaves and *Cymbopogon citrates* has been studied in rats fed with honey as a source of ethanol and sucrose. This extract led to a lowering of glucose, total cholesterol, LDL cholesterol and triglyceride levels, revealing it to be a potential regulator of blood pressure [11]. Several investigations have reported that quercetin-3-glucoside and quercetin-3-rhamnoside are the main functional compounds present in avocado leaf [12]. These flavonoids improve endothelial dysfunction and reduce blood pressure, possibly by increasing the bioavailability of nitric oxide in the blood (potent vasodilator) [13].

Similarly, guava leaves have been shown to have great benefits in the treatment of cholesterol. These leaves have a considerable content of tannins, phenols, triterpenes and saponins. However, the most important bioactive compound that they have been found to contain is quercetin. This flavonoid has antioxidant, anticancer, anti-inflammatory and cardioprotective properties [14]. An ethanolic extract of guava leaves with isolated quercetin has been found to regulate lipid metabolism in rats previously inoculated with carbon tetrachloride. This study also found this extract to reduce levels of free fatty acids, phospholipids, LDL cholesterol and triglycerides. In addition, normal HDL cholesterol levels were restored [15]. Further, the effects of 400 mg/kg of weight of an aqueous extract of guava leaves has been evaluated in mice induced with diabetes via an injection of streptozotocin. At the end of the investigation, a decrease in the activity of hormone-sensitive lipase was demonstrated, alongside reduced levels of LDL cholesterol, triglycerides and total cholesterol. The authors of this study concluded that this extract improved the lipid profile of the mice, in addition to showing significant antidiabetic activity [16]. Finally, the effects of including guava leaf powder in the diet of Wistar mice subjected to a diet high in carbohydrates and fats over an 8-week period has been evaluated. This study concluded that the powder under study helped to prevent obesity, improved glucose intolerance, and decreased inflammation and oxidative stress in the liver of these animals [17].

Turning attention to cinnamon, this is an additive used to improve the smell, flavor and color of food. This plant is of great importance due to its polyphenol and glutathione content, which ensures anti-inflammatory and anticancer properties, as well as potentially playing a protective role against heart disease through the reduction of LDL cholesterol and the increase of HDL cholesterol [18] [19]. The effect of a cinnamon extract on glucose and lipid levels has been evaluated in Sprague-Dawley diabetic rats. Cinnamon was found to lower blood glucose, lead

to craving and improve lipid parameters [20]. The aim of the present research was to develop an infusion made from avocado leaves, guava leaves and cinnamon as a potential alternative for the treatment of LDL cholesterol.

MATERIALS AND METHODS

Plant Material

The avocado leaves, guava leaves and cinnamon used in the present experiment were purchased from a supermarket. Avocado and guava leaves were classified according to size, appearance and the absence of defects. The leaves were then washed and dried at room temperature. In the same way, the cinnamon sticks were classified according to size, appearance and the absence of defects.

Dehydration of Avocado and Guava Leaves

The washed and dried avocado and guava leaves were placed in trays within a Rational brand dehydrator. In the case of guava leaves, a hot air dehydration process was used with the temperature being set at 45°C. This temperature was selected based on the results obtained in previous studies [15]. With regards to avocado leaves, the same dehydration process was used, however, the temperature was set at 40°C, in accordance with that established by previous investigations [10]. During this treatment, avocado and guava leaves were weighed every 20 minutes until a constant weight was reached.

Formulation of the Infusion

Dehydrated avocado and guava leaves were ground in a Bosch ErgoMixx mill. The same procedure was carried out with the cinnamon sticks. Three formulations were made. All formulations contained the same quantity of dehydrated guava leaves, specifically, 1.5 g, in accordance with outcomes produced by previously conducted research [17]. Likewise, the amount of cinnamon added to the sample was held constant at 120 mg/kg in all three infusions, in accordance with outcomes reported by previously conducted research [20].

In the case of avocado leaves, three different amounts were compared. Quantities were selected based on prior research considering effects on cholesterol control [10] and in order to enable an examination of different sensory aspects (pleasant taste and aroma for the consumer). The amounts used were 100 mg/kg, 200 mg/kg and 300 mg/kg.

Amounts of avocado leaf and cinnamon were calculated in consideration of a reference body weight of 50 kg.

Sensory Analysis

Sensory analysis was carried out via two types of tests. The first was a ranking test and the second was Basker's preference test. In order to carry out these tests, 60 tasters were required. All tasters were given three mugs (formulation 1, 2 and 3) containing 15 ml of the infusion made from avocado, guava and cinnamon leaves. All samples were coded using a four-digit code.

In the ranking test, tasters were asked to place the tasted samples in descending order based on their flavor and aroma attributes. In the case of Basker's test of the "critical value of the difference between the sum of categories", tasters were requested to evaluate samples using a pre-established scale indicating preference, where 1 pertained to the most preferred sample

and 3 the least preferred sample. Raters also reported whether they would be willing to purchase the infusion from which the sample had been drawn.

Packaging

The three formulations were placed in Kraft paper sachets coated with food grade resin. Subsequently, a logo and name for the avocado, guava and cinnamon leaf infusion were conceived. In this process, suggestions were made that adequately described the type of product and its benefits, with the best ideas being put forwards for further assessment. The aim of this process was to achieve the best possible introduction of the product onto the market.

RESULTS

Dehydration of Avocado Leaves

Avocado leaves decreased in weight during dehydration. At the beginning of the treatment, the plant material had a weight of 250 g, following 60 minutes of dehydration, avocado leaves weighed 238.70 g. From this point onwards, weight loss appeared to stabilize as this parameter showed no further variation following another 80 minutes of treatment.

Weight loss in avocado leaves is related to a decrease in water content, with this being characteristic of dehydration treatments. Figure 1 shows the weight lost by avocado leaves during the dehydration process.

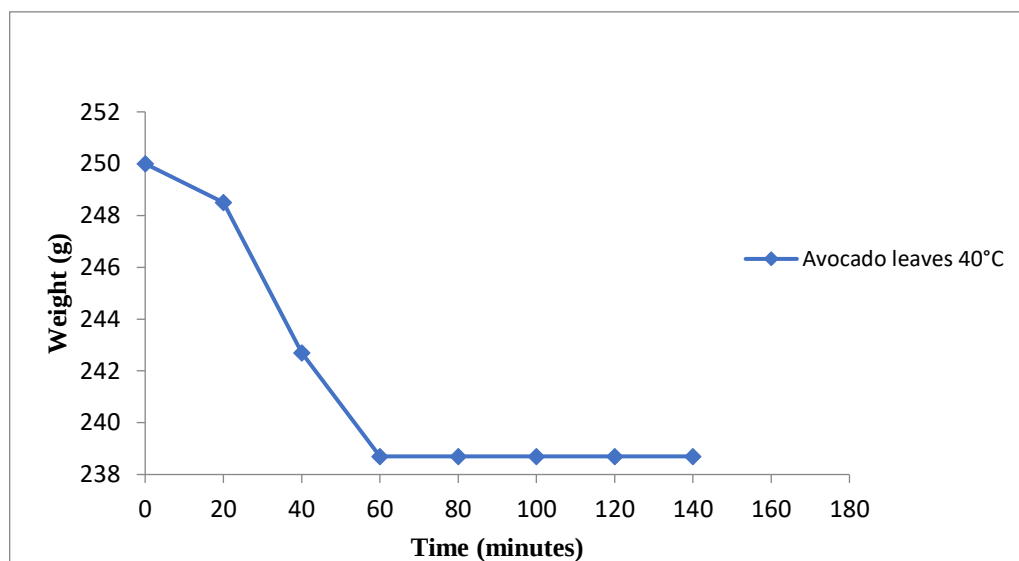


Fig. 1. Weight Loss Curve Pertaining to Avocado Leaves

Dehydration of Guava Leaves

In the same way, guava leaves showed a similar behavior during dehydration. At the beginning of the study, material comprising guava leaves weighed 180 g. After 80 minutes of treatment, the weight of matter from guava leaves reduced to 167.90 g. From this point onwards, weight loss stabilized as no further weight changes were recorded.

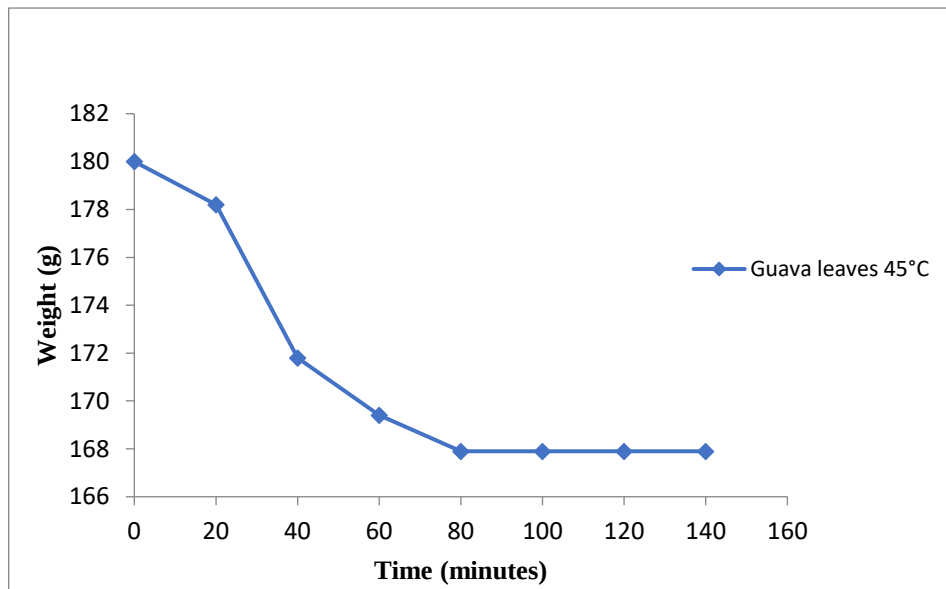


Fig. 2. Weight Loss Curve Pertaining to Guava Leaves

Formulation of the Infusion

As mentioned above, quantities of the ingredients making up the three infusions were calculated based on a reference individual with a weight of 50 kg. In this way, the 100 mg/kg of avocado required for formulation 1 was equivalent to 5g, whilst the 200 mg/kg of avocado leaves required for formulation 2 corresponded to 10 g. Likewise, in the case of formulation 3, the 300 mg/kg of avocado leaves required was equivalent to 15 g. In the case of cinnamon, 120 mg/kg was equivalent to 6 g. Table 1 presents the quantities of avocado leaf, guava leaf and cinnamon used for each of the three formulations.

Table 1. Infusion formulations

Formulation 1			Formulation 2			Formulation 3		
Avocado leaf (g)	Guava leaf (g)	Cinnamon (g)	Avocado leaf (g)	Guava leaf (g)	Cinnamon (g)	Avocado leaf (g)	Guava leaf (g)	Cinnamon (g)
5	1.5	6	10	1.5	6	15	1.5	6

Taste Ranking Test

Figure 3 shows outcomes of the taste ranking test. Outcomes of this test confirmed that tasters were able to differentiate between the different flavor intensities of the three formulations as determined by the different amounts of avocado leaf used. This conclusion is borne out by the fact that 61.67% of raters considered formulation 3 to have a better flavor than formulations 1 and 2.

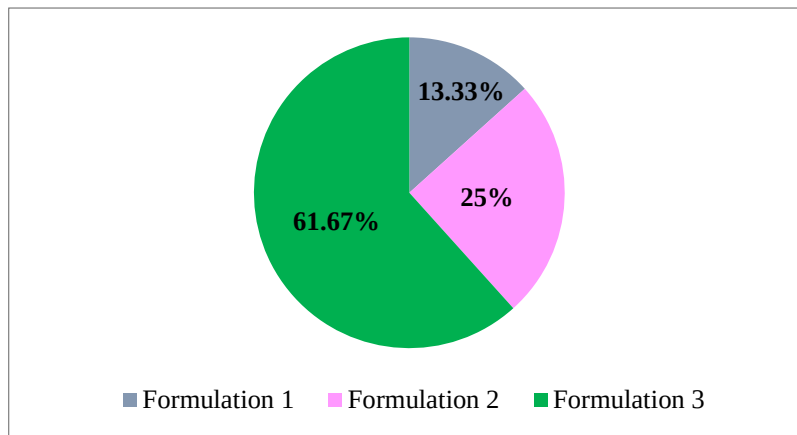


Fig.3. Taste ranking test

Aroma Ranking Test

With regards to the aroma ranking test, similar patterns to those revealed in the taste test were evidenced. Figure 4 shows that 80% of tasters perceived formulation 3 to be characterized by a greater aroma intensity.

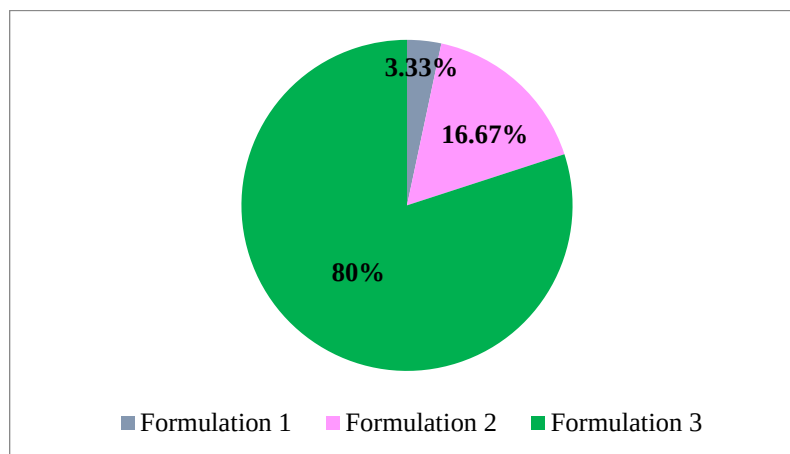


Fig.4. Aroma ranking test

Basker's Preference Test

Table 2 presents outcomes of the Basker preference test. Gathered data reveals that tasters preferred formulation 2, as evidenced by the lower value, specifically, 92, assigned to it by the rank sum test. Likewise, comparison values pertaining to formulation 2 (44 and 40) are both higher than the critical value of the Basker test (25.7). This provides further support that this formulation is the most preferred.

Table 2. Sum of Categories

	Formulation	1	2	3
Formulation	Rank sum test	136	92	132
1	136	0	44	4
2	92	-44	0	-40
3	132	-4	40	0

With regards to the purchase intention assessment, tasters stating that they would be willing to buy any one of the three formulations were considered to have positive purchase intentions. As can be seen in Figure 5, 91.67% of tasters reported that they would be interested in purchasing the infusion in any one of the three formulations, as they considered it to be a safer alternative, with fewer side effects, for the treatment of high cholesterol levels.

The 8.33% who responded that they would not be willing to buy this product stated that they preferred to treat high cholesterol levels with medication. However, they considered that the product presented excellent organoleptic properties.

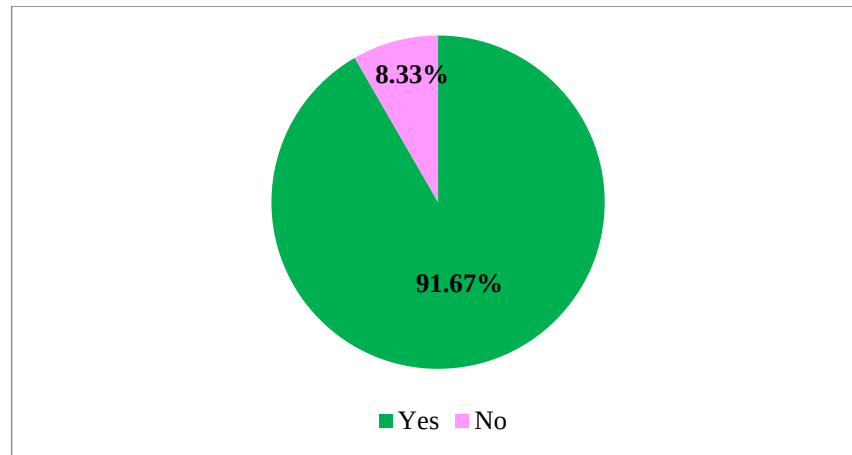


Fig. 5 Purchase intention test outcomes

Label Design

White and violet colors were used for the design of the label. The name selected for the infusion was Nativa Fit. The word 'native' refers to the use of ancestral medicinal plants, whilst the word 'fit' refers to the potential use of this product for the treatment of cholesterol. Figure 6 shows the final label.



Fig. 6. Native Fit Infusion Label

Final Product

Acceptability outcomes pertaining to the sensory analysis performed revealed that formulation 2 was perceived by tasters to be the best infusion option. It was, therefore, selected for elaboration of the final product. Thus, 10 g of dehydrated avocado leaves, 1.5 g of dehydrated

guava leaves and 6 g of cinnamon were placed in food grade filter paper bags. Packages of 10 units of these bags were made up and packaged in cardboard boxes. Finally, the label discussed above ("Nativa Fit") was attached to one end of the thread used to seal the bag.

CONCLUSIONS

Monitoring the weight lost by avocado leaves during dehydration enabled optimal conditions for this treatment to be established. Indeed, it was found that a treatment of 40°C for a period of 60 minutes avoided any damage from occurring to the plant material. In the same way, optimal dehydration conditions for guava leaves were observed to be 45° for 80 minutes. Further, avocado leaves have a sweet flavor which means that they played a role in improving the sensory characteristics of the examined infusion. This was shown through both the taste and aroma raking tests, in which this leaf was easily identified and received good acceptance ratings from tasters. Basker preference test outcomes revealed that tasters preferred formulation 2, which contained 10 g of avocado leaves. For this reason, this formulation was used to prepare the final product. Purchase intention assessment revealed that 91.67% of participating tasters were willing to buy the examined infusion due to its possible functional properties against high cholesterol levels.

The success of the infusion developed through the present study is dependent on a change in individuals' medicinal habits and more in-depth understanding of the different functional benefits of these plants.

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