

## PONGAMIA OIL COMPOSITIONS, AND METHODS FOR PRODUCING AND USING THEREOF

## CROSS-REFERENCE(S) TO RELATED APPLICATION(S)

[0001] This application claims priority to and the benefit of U.S. Provisional Application No. 63/004,787, filed on April 3, 2020, the entire disclosure of which is incorporated herein by reference in its entirety.

## FIELD

[0002] The present disclosure relates generally to pongamia compositions, and more specifically, edible and non-bitter tasting pongamia oil, as well as methods for producing thereof, and methods for using thereof in food and beverage products.

## BACKGROUND

[0003] Growing concerns related to population growth, climate change and the viability of existing agricultural practices over the next several decades have led to a surge in the research and development of alternative food sources to ensure future global food security. Renewable plant-based sources have prompted significant interest as an environmentally friendly and sustainable means to alleviate pressure on the worldwide food supply.

[0004] *Millettia pinnata*, also known as *Pongamia pinnata* or *Pongamia glabra*, or more colloquially as pongamia or karanja, is tree that is common throughout Asia and may provide a major source for future plant-based food. Pongamia trees use a fraction of the land as required for soybean plants to produce the same amount of beans. Pongamia trees can grow on degraded soil, and allow for avoidance of deforestation issues created by soybeans. Pongamia also produces much larger amounts of protein and vegetable oil per acre than soybean. Pongamia oil, extracted from pongamia oilseeds, offers a potential renewable source of oil for use in foodstuffs comparable to soy. However, pongamia oilseeds also have other components that are known in the art to have disagreeable taste and odor, including karanjin and pongamol. It is desirable to minimize the amount of karanjin and pongamol in the oil for use as a viable food source.

[0005] The widespread use of pongamia-derived foodstuffs is currently prevented by the absence of methods for preparing pongamia compositions having low levels of karanjin and

pongamol while maintaining the high nutritional content (proteins, carbohydrates, etc.) intrinsic to the oilseeds. Existing methods for the removal of these undesirable components in pongamia seedcake and oil are insufficient and often require harsh, destructive conditions that reduce and degrade the nutrients to the point that the nutritional value of pongamia is severely impacted. The lack of methods for producing pongamia compositions having the critical balance of preserved nutritional content and sufficiently low levels of anti-nutrients have precluded the incorporation of pongamia-derived oil on a large enough scale to remain economically feasible.

[0006] Thus, what is desired in the art are commercially viable methods to obtain edible compositions from pongamia oilseeds that retain an optimal nutritional balance, while minimizing components such as karanjin and pongamol.

#### BRIEF SUMMARY

[0007] In some aspects, provided herein are pongamia oil compositions that are edible and non-bitter tasting. Such pongamia oil compositions can serve as useful ingredients in a variety of food and beverage products, and address the substantial unmet need in the industry for emerging plant-based products.

[0008] In certain aspects, provided are methods for producing pongamia oil compositions using solid-liquid separation. In some embodiments, the method comprises: combining crude pongamia oil with non-polar solvent to produce a crude mixture; eluting the crude mixture through silica gel with the non-polar solvent to separate at least a portion of the furanoflavonoids present in the crude mixture from the pongamia oil, and to produce a purified mixture that includes pongamia oil and the non-polar solvent; and removing at least a portion of the non-polar solvent from the purified mixture to produce a pongamia oil composition. In some variations, the non-polar solvent comprises alkane.

[0009] The crude pongamia oil contains karanjin and pongamol, and at least a portion of the karanjin and pongamol are adsorbed on the silica gel in the aforementioned method. The adsorbed components may be isolated. In certain embodiments, the method further comprises: eluting the silica gel with a polar solvent to isolate the karanjin and pongamol. In certain embodiments, the silica gel is eluted using a stepwise gradient with increasing proportions of

polar solvent in the non-polar solvent to isolate karanjin and pongamol separately. In some variations, the polar solvent comprises alkyl alkanoate.

[0010] In one aspect, provided is a pongamia oil composition produced according to any of the methods described herein. In other aspects, provided is a pongamia oil composition that is edible and non-bitter tasting.

[0011] In other aspects, provided are uses of the pongamia oil compositions in food or beverage products. In some variations, the pongamia oil compositions may be used as or in salad oil, frying oil, sauteeing oil, vinaigrettes, sauces, dressings, fats in meat mimetics, beverages, or blended margarines and other solid fat applications.

[0012] In other aspects, provided is an analytical method to measure the content of karanjin and pongamol that may be present in a pongamia oil sample. In some embodiments, the method comprises: combining pongamia oil with an extraction solvent to provide an extraction mixture; sonicating the extraction mixture; separating the sonicated mixture into an extracted pongamia composition and an extract that comprises karanjin or pongamol, or both; and measuring the concentration of karanjin or pongamol, or both, present in the extract. In some variations, the extraction solvent comprises alkyl ketone. In some embodiments, the measuring step involves determining the concentration of karanjin and/or pongamol by high performance liquid chromatography with an ultraviolet detector (*e.g.*, using a HPLC-DAD).

[0013] In one aspect, provided herein is a pongamia oil composition, having: less than or equal to about 1000 ppm of karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; less than or equal to about 1% by weight of unsaponifiable matter; a peroxide value of less than or equal to about 5 meq/kg; a p-anisidine value of less than or equal to about 10; and less than or equal to about 25 ppm of residual solvents. In some embodiments, the pongamia oil composition has less than or equal to about 150 ppm of karanjin as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; less than or equal to about 150 ppm of pongamol as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; less than or equal to about 1% by weight of unsaponifiable matter; a peroxide value

of less than or equal to about 5 meq/kg; a p-anisidine value of less than or equal to about 5; and less than or equal to about 25 ppm of residual solvents.

[0014] In another aspect, provided herein is a method for producing a pongamia oil composition, comprising: combining crude pongamia oil with non-polar solvent to produce a crude mixture, wherein the non-polar solvent comprises alkane, and wherein the crude pongamia oil comprises pongamia oil and furanoflavonoids; eluting the crude mixture through silica gel with the non-polar solvent to separate at least a portion of the furanoflavonoids from the pongamia oil, and to produce a purified mixture comprising pongamia oil and the non-polar solvent; and removing at least a portion of the non-polar solvent from the purified mixture to produce a pongamia oil composition, wherein the composition has less than or equal to about 1000 ppm of karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; less than or equal to about 1% by weight of unsaponifiable matter; a peroxide value of less than or equal to about 5 meq/kg; and a p-anisidine value of less than or equal to about 10.

[0015] In other aspects, provided herein are food or beverage products comprising pongamia oil compositions obtainable by the methods described herein. In some embodiments, the pongamia oil composition is light yellow as determined by the Lovibond Color - AOCS Scale; the composition comprises less than or equal to about 200 ppm karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition, and the composition has a neutral flavor. In other embodiments, the pongamia oil composition is yellow as determined by the Lovibond Color - AOCS Scale; the composition comprises less than or equal to about 150 ppm karanjin and less than or equal to about 150 ppm pongamol as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; and the composition has one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof.

#### DESCRIPTION OF THE FIGURES

[0016] The present application can be understood by reference to the following description taken in conjunction with the accompanying figures.

[0017] FIG. 1 depicts an exemplary analytical method to determine concentrations of karanjin and/or pongamol in a pongamia oil sample.

[0018] FIG. 2 depicts an exemplary process to purify a crude pongamia oil mixture to produce a pongamia oil composition that is edible and non-bitter tasting.

[0019] FIGS. 3A and 3B compare furanoflavonoids present in crude pongamia oil (FIG. 3A) versus those absent in an exemplary purified pongamia oil composition (FIG. 3B), as determined by HPLC.

#### DETAILED DESCRIPTION

[0020] The following description sets forth exemplary methods, parameters and the like. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure but is instead provided as a description of exemplary embodiments.

[0021] In some aspects, provided herein are pongamia oil compositions, and methods of producing such compositions. In some variations, the pongamia oil compositions pass human taste testing. In certain variations, the pongamia oil compositions are edible and non-bitter tasting. The methods provided to produce the edible pongamia oil removes or decreases the amount of furanoflavonoids present, including removing or decreasing the amount of karanjin and/or pongamol, which are typically considered inedible and potentially harmful to humans. Additionally, the pongamia oil compositions provided have various properties that make such compositions suitable for use in food and beverage products. For example, in certain variations, the pongamia oil compositions have low insoluble impurities, low soap content, high smoke point, low mono- and di-glycerides, low glycerol, fewer unidentified fatty acids, low total sterols, and light color (including, for example, low chlorophyll content).

#### Pongamia Oil Compositions

[0022] In some embodiments, the pongamia oil compositions provided herein are edible, non-bitter, and have an overall acceptable sensory profile in humans (*e.g.*, with respect to taste and smell).

*Unsaponifiable matter*

[0023] Unsaponifiable matter present in pongamia compositions generally include compounds other than the fatty acids. For example, unsaponifiable matter may include furanoflavonols, chlorophylls, tocopherols and sterols. In some embodiments, the pongamia oil compositions provided herein (including produced according to the methods herein) have a lower unsaponifiable matter content, as compared to the crude pongamia oil from which the compositions are obtained. In some embodiments, the pongamia oil compositions provided herein (including produced according to the methods herein) have a low unsaponifiable matter content. In some variations, the pongamia oil compositions provided herein (including produced according to the methods herein) have less than or equal to 5%, less than or equal to 4%, less than or equal to 3%, less than or equal to 2%, or less than or equal to 1% by weight of unsaponifiable matter in oil. In some variations, the pongamia oil compositions provided herein (including produced according to the methods herein) have at least 50%, at least 55%, at least 60%, at least 70%, at least 80%, or at least 90% by weight less unsaponifiable matter content as compared to the crude pongamia oil from which the composition was obtained. Any suitable methods known in the art to measure or determine the unsaponifiable matter content may be employed. In some variations, the unsaponifiable matter content is determined by AOCS Ca 6a-40.

[0024] As noted above, furanoflavonols are one type of unsaponifiable matter. Furanoflavonoids are a class of compounds that are typically present in pongamia oilseeds, and includes anti-nutritional compounds such as karanjin and pongamol. In some embodiments, provided are pongamia oil compositions having a low, negligible or non-detectable furanoflavonoids content. In some variations, the pongamia oil compositions have less than or equal to about 1000 ppm, less than or equal to about 750 ppm, less than or equal to about 500 ppm, less than or equal to about 300 ppm, less than or equal to about 250 ppm, or less than or equal to about 200 ppm of furanoflavonoids. In some variations, the pongamia oil compositions have less than or equal to 500 ppm, less than or equal to 450 ppm, less than or equal to 400 ppm, less than or equal to 350 ppm, less than or equal to 300 ppm, less than or equal to 250 ppm, less than or equal to 200 ppm, less than or equal to 150 ppm, less than or equal to 100 ppm, less than

or equal to 50 ppm, less than or equal to 40 ppm, less than or equal to 30 ppm, less than or equal to 20 ppm, or less than or equal to 10 ppm of furanoflavonoids.

[0025] In some embodiments, the pongamia oil compositions have less than or equal to 150 ppm of karanjin and/or pongamol. In some variations of the foregoing, the karanjin and pongamol concentrations are determined by the solvent extraction analytical methods described herein.

[0026] In some embodiments, the karanjin and pongamol contents of the pongamia oil composition are determined by HPLC analysis of an alkyl ketone extract obtained from the pongamia oil composition. In still other embodiments, the karanjin and pongamol contents of the pongamia oil composition are determined by HPLC analysis of an alkyl ketone extract obtained from the pongamia oil composition according to the analytical method described herein. In some embodiments, the alkyl ketone is acetone. In certain embodiments, the HPLC analysis of the alkyl ketone extract further comprises mass spectrometry detection or ultraviolet detection. In still certain other embodiments, the karanjin and pongamol contents of the pongamia oil composition are determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition according to the analytical method described herein.

[0027] In some variations, the pongamia oil compositions have less than or equal to 500 ppm, less than or equal to 400 ppm, less than or equal to 300 ppm, less than or equal to 250 ppm, less than or equal to 200 ppm, less than or equal to 150 ppm, less than or equal to 140 ppm, less than or equal to 130 ppm, less than or equal to 120 ppm, less than or equal to 110 ppm, less than or equal to 100 ppm, less than or equal to 90 ppm, less than or equal to 80 ppm, less than or equal to 70 ppm, less than or equal to 60 ppm, less than or equal to 50 ppm, less than or equal to 40 ppm, less than or equal to 30 ppm, less than or equal to 20 ppm, or less than or equal to 10 ppm of karanjin.

[0028] In some variations, the pongamia oil compositions have less than or equal to 500 ppm, less than or equal to 400 ppm, less than or equal to 300 ppm, less than or equal to 250 ppm, less than or equal to 200 ppm, less than or equal to 150 ppm, less than or equal to 140 ppm, less than or equal to 130 ppm, less than or equal to 120 ppm, less than or equal to 110 ppm, less than or equal to 100 ppm, less than or equal to 90 ppm, less than or equal to 80 ppm, less than or

equal to 70 ppm, less than or equal to 60 ppm, less than or equal to 50 ppm, less than or equal to 40 ppm, less than or equal to 30 ppm, less than or equal to 20 ppm, or less than or equal to 10 ppm of pongamol.

[0029] In other variations, the pongamia oil compositions may be characterized in terms of their combined karanjin and pongamol concentrations. For example, in some variations, the pongamia oil compositions have less than or equal to about 1000 ppm, less than or equal to about 750 ppm, less than or equal to about 500 ppm, less than or equal to about 300 ppm, less than or equal to about 250 ppm, or less than or equal to about 200 ppm karanjin and pongamol combined. In certain variations, the pongamia oil compositions have less than or equal to 200 ppm, less than or equal to 150 ppm, less than or equal to 140 ppm, less than or equal to 130 ppm, less than or equal to 120 ppm, less than or equal to 110 ppm, less than or equal to 100 ppm, less than or equal to 90 ppm, less than or equal to 80 ppm, less than or equal to 70 ppm, less than or equal to 60 ppm, less than or equal to 50 ppm, less than or equal to 40 ppm, less than or equal to 30 ppm, less than or equal to 20 ppm, or less than or equal to 10 ppm of karanjin and pongamol.

[0030] In still other variations, the pongamia oil compositions have a ratio of karanjin to pongamol of greater than or equal to about 1. In other variations, the pongamia oil compositions have a ratio of karanjin to pongamol of less than or equal to about 1.

[0031] In one variation, the pongamia oil compositions has a non-detectable amount of karanjin and/or pongamol, based on the solvent extraction analytical methods described herein.

[0032] In other variations, the pongamia oil compositions produced according to the methods described herein (*e.g.*, obtained from crude pongamia oil) have less than 100 times, less than 500 times, or less than 1000 times the amount of karanjin as compared to the crude pongamia oil from which the compositions were obtained. In some embodiments, the pongamia oil compositions produced according to the methods described herein (*e.g.*, obtained from crude pongamia oil) have less than 100 times, less than 150 times, or less than 200 times the amount of pongamol as compared to the crude pongamia oil from which the compositions were obtained.

*Fatty Acids*

[0033] In some embodiments, the pongamia oil compositions described herein have lower amounts of mono- and di-glycerides, low glycerol, and/or fewer unidentified fatty acids as compared to the crude pongamia oil from which the pongamia oil compositions are obtained (*e.g.*, according to the methods described herein). The pongamia oil compositions have certain fatty acid profiles.

[0034] In some embodiments, the amount of total identified fatty acids in the pongamia composition is at least 90%; or between 80% and 99%, or between 85% and 95%.

[0035] The pongamia oil compositions have a combination of various monounsaturated, polyunsaturated and/or saturated fatty acids. In some variations, the pongamia compositions have a greater monounsaturated fatty acid content than polyunsaturated fatty acids. In some variations, the pongamia compositions have a greater saturated fatty acid content than polyunsaturated fatty acids. In some variations, the pongamia compositions have a greater monounsaturated fatty acid content than saturated fatty acids.

[0036] In certain embodiments, the pongamia compositions have a low *trans* fatty acids content, or a lower *trans* fatty acids content as compared to the crude pongamia oil from which the pongamia oil compositions are obtained (*e.g.*, according to the methods described herein). In some variations, the amount of *trans* fatty acids in the pongamia compositions is less than or equal to 5%, less than or equal to 1%, less than or equal to 0.5%, or less than or equal to 0.25%.

[0037] In some embodiments, the methods provided herein do not change the healthful fatty acid profile, except in a positive way (for example, increasing oleic acid content on a % weight basis). This is generally in contrast to other methods known in the art that methods can radically change the fatty acid profile in an adverse way (for example, lower yield, less healthful or functional balance of fatty acids). In certain embodiments, the pongamia oil compositions comprise Omega 6 fatty acids, or Omega 9 fatty acids, or any combination thereof. In certain embodiments, the pongamia oil compositions comprise Omega 3 fatty acids, Omega 6 fatty acids, Omega 7 fatty acids, or Omega 9 fatty acids, or any combination thereof. In some variations, the amount of Omega 9 fatty acids is greater than Omega 6 fatty acids. In certain

variations, the amount of Omega 6 fatty acids and Omega 9 fatty acids combined is greater than the amount of Omega 3 fatty acids and Omega 7 fatty acids combined. In some variations, the amount of Omega 6 fatty acids and Omega 9 fatty acids combined is at least 50%, or at least 60%; or between 15% and 80%, or between 20% and 75%. In certain variations, the amount of Omega 3 fatty acids and/or Omega 7 fatty acids is less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%.

[0038] In certain embodiments, the pongamia oil compositions comprise myristic acid, palmitic acid, palmitoleic acid, margaric acid, heptadecenoic acid, stearic acid, vaccenic acid, oleic acid, linoleic acid, arachidic acid, gondoic acid, eicosadienoic acid, behenic acid, erucic acid, or lignoceric acid, or any isomers thereof, or any combination of the foregoing.

[0039] In some variations, the pongamia oil compositions comprise oleic acid. In one variation, the amount of oleic acid in the pongamia oil compositions is at least 40%, or at least 50%; or between 30% and 70%, between 30% and 60%, or between 45% and 55%.

[0040] In certain variations, the pongamia oil compositions comprise linoleic acid, or isomers thereof. In one variation, the amount of linoleic acid, or isomers thereof, in the pongamia oil compositions is at least 15%; or between 10% and 20%. In certain variations, the pongamia oil compositions comprise linolenic acid, or isomers thereof. In certain variations, the linolenic acid is alpha linolenic acid. In one variation, the amount of alpha linolenic acid in the pongamia oil compositions is between 1% and 5%.

[0041] In certain variations, the pongamia oil compositions comprise palmitic acid. In one variation, the amount of palmitic acid in the pongamia oil compositions is at least 5%; or between 5% and 10%.

[0042] In certain variations, the pongamia oil compositions comprise stearic acid. In one variation, the amount of stearic acid in the pongamia oil compositions is at least 5%; or between 5% and 10%.

[0043] In certain variations, the pongamia oil compositions comprise behenic acid. In one variation, the amount of behenic acid in the pongamia oil compositions is between 1% and 10%, or between 1% and 5%.

[0044] In certain variations, the pongamia oil compositions comprise arachidic acid, gondoic acid, or lignoceric acid, or any combination thereof. In one variation, the amount of arachidic acid, gondoic acid, or lignoceric acid in the pongamia oil compositions is independently between 1% and 5%.

[0045] In certain variations, the pongamia oil compositions comprise erucic acid. In one variation, the amount of erucic acid is at least 0.06%.

[0046] Any suitable methods or techniques known in the art may be used to measure fatty acid content in the compositions herein. For example, in some variations, the test method used is AOAC 996.06.

#### *Tocopherols*

[0047] In some embodiments, the pongamia oil compositions comprise tocopherol. In some variations, the tocopherol is alpha-tocopherol, beta-tocopherol, delta-tocopherol, gamma-tocopherol, or any combination thereof. In certain embodiments, the pongamia oil compositions have a total tocopherol content of at least 250 ppm, at least 300 ppm, at least 350 ppm, at least 400 ppm, or at least 450 ppm; or between 200 ppm and 500 ppm.

[0048] In some variations, the alpha-tocopherol content is the highest of the four aforementioned tocopherols. In certain variations, the content of alpha-tocopherol and gamma-tocopherol combined is greater than the content of beta-tocopherol and delta-tocopherol combined.

[0049] In one embodiment, the pongamia oil compositions have an alpha-tocopherol content of at least 200 ppm, at least 250 ppm or at least 300 ppm; or between 200 ppm and 500 ppm, between 200 ppm and 400 ppm, between 200 ppm and 350 ppm, or between 200 ppm and 300 ppm.

[0050] In another embodiment, the pongamia oil compositions have a gamma-tocopherol content of at least 100 ppm or at least 150 ppm; or between 100 and 200 ppm.

[0051] Any suitable methods or techniques known in the art may be used to measure tocopherol content in the compositions herein. For example, in some variations, the test method used is AOAC 971.30 with HPLC.

#### *Sterols*

[0052] In some embodiments, the pongamia oil compositions comprise sterols. In some variations, the pongamia oil compositions described herein have lower amounts of sterols as compared to the crude pongamia oil from which the pongamia oil compositions are obtained (*e.g.*, according to the methods described herein).

[0053] In some variations, the sterol is 24-methylene-cholesterol, beta-sitosterol, brassicasterol, campestanol, cholesterol, clerosterol, delta-5,23-stigmastadienol, delta-5,24-stigmastadienol, delta-5-avenasterol, delta-7-avenasterol, delta-7-campesterol, delta-7-stigmastenol, sitostanol, or stigmasterol, or any combination thereof. In certain embodiments, the pongamia oil compositions have a total sterol content of less than or equal to 2500 ppm, less than or equal to 2000 ppm, less than or equal to 1500 ppm, less than or equal to 1000 ppm, less than or equal to 750 ppm, less than or equal to 500 ppm, or less than or equal to 100 ppm.

[0054] In some variations, the pongamia oil compositions further comprise beta-sitosterol. In certain variations of the foregoing, the pongamia oil compositions further comprise campestanol, stigmasterol, or delta-5-avenasterol, or any combination thereof. In yet other variations of the foregoing, the pongamia oil compositions further comprise clerosterol, delta-5,24-stigmastadienol, or sitostanol, or any combination thereof.

[0055] Any suitable methods or techniques known in the art may be used to measure sterol content in the compositions herein. For example, in some variations, the test method used is COI/T.20/Doc No.10.

#### *Residual Solvent*

[0056] The methods for producing pongamia oil compositions as provided herein may result in the presence of residual solvent content in the pongamia oil compositions. Low levels of residual solvent in such pongamia oil compositions may be desirable as the presence of residual

solvent can may influence the sensory profile of the pongamia oil composition. In some variations, the pongamia oil compositions produced the methods herein may be subjected to processing techniques to remove residual solvent from, or to de-solventize, the pongamia oil composition in order to achieve the residual solvent levels as described herein.

[0057] In some embodiments, the pongamia oil composition comprises residual solvent. In some variations, the residual solvent comprises non-polar solvent used in the purification method described herein. In some variations, the alkane is a C1-20 alkane, or a C-10 alkane. In certain variations, the alkane is linear. In other variations, the alkane is branched. In yet other variations, a mixture of alkanes may be used. In one variation, the non-polar solvent comprises hexane. In still other embodiments, the pongamia oil composition comprises residual solvent, wherein the residual solvent comprises hexane. In yet other embodiments, the pongamia oil composition comprises residual solvent, wherein the residual solvent is hexane.

[0058] In some variations, the pongamia oil composition has less than or equal to about 100 ppm, less than or equal to about 75 ppm, less than or equal to about 50 ppm, or less than or equal to about 25 ppm of residual solvent. In certain embodiments, the residual solvent comprises hexane. In certain variations, the pongamia oil composition has less than or equal to about 100 ppm, less than or equal to about 75 ppm, less than or equal to about 50 ppm, or less than or equal to about 25 ppm of residual hexane. Any suitable methods or techniques known in the art may be used to measure residual solvent content in the compositions herein. In some variations, the residual solvent is determined by AOCS Cg 4-94.

#### *Peroxide and p-Anisidine Values*

[0059] In some variations, the pongamia oil compositions may be further characterized by the level of oxidation products present in the oil. When exposed to oxygen and/or heat, fats and oils may undergo oxidation reactions, which cause the oils to develop an undesirable rancid flavor. As detailed above, the methods of the present disclosure for producing pongamia oil compositions provides means to remove or decrease the amount of furanoflavonoids and other unsaponifiable matter present. Existing methods for the removal of these components often utilize harsh conditions, such as highly caustic reagents and extreme temperatures (*e.g.*, reflux).

[0060] In contrast, the methods provided herein employ gentler temperature and solvent conditions to treat crude pongamia oil to remove furanoflavonoids and other unsaponifiable matter. As a result, the pongamia oil compositions obtained herein exhibit low furanoflavonoid content and low unsaponifiable matter content as well as minimal oxidation.

[0061] The extent of oxidation can be characterized by the presence and concentration of primary oxidation products that may form during initial oxidation and the secondary oxidation products that may form during the breakdown of the primary oxidation products with more extensive oxidation. The degree of primary oxidation may be assessed by measuring the peroxide value (in milliequivalents/kg), which is an index used to quantify the amount of hydroperoxides present in the oil. The extent of secondary oxidation may be assessed by measuring the p-anisidine value. Both the peroxide value and p-anisidine value are taken together to provide a complete representation of oxidation in the oil.

[0062] In some variations, the pongamia oil composition has a peroxide value of less than or equal to about 5 meq/kg, less than or equal to about 4 meq/kg, less than or equal to about 3 meq/kg, less than or equal to about 2 meq/kg, or less than or equal to about 1 meq/kg. In certain variations, the pongamia oil composition has a peroxide value of less than or equal to about 5 meq/kg. Any suitable methods or techniques known in the art may be used to measure peroxide value in the compositions herein. In some variations, the peroxide value is determined by AOCS test method AOCS Cd 8-53.

[0063] In other variations, the pongamia oil composition has a p-anisidine value of less than or equal to about 15, less than or equal to about 12, less than or equal to about 10, less than or equal to about 7, less than or equal to about 5, less than or equal to about 4, less than or equal to about 3, or less than or equal to about 2. In certain variations, the pongamia oil composition has a p-anisidine value of less than or equal to about 10. In certain other variations, the pongamia oil composition has a p-anisidine value of less than or equal to about 5. Any suitable methods or techniques known in the art may be used to measure p-anisidine in the compositions herein. In some variations, the p-anisidine value is determined by AOCS test method AOCS Cd 18-90.

#### *Thermal and Physical Properties*

[0064] The pongamia oil compositions provided herein may be further characterized by their thermal and physical properties. The array of applications and uses that are available to different fats and oil is largely determined by the thermal and physical behavior of the fats or oils under certain temperature conditions for the specific use. The thermal and physical behavior of the fats and oils are, in turn, largely influenced by the fatty acid profile of the fats and oils. As described above, the methods provided herein for producing pongamia oil compositions having reduced concentrations of karanjin, pongamol, and other unsaponifiable matter are contrasted by other methods in the art, which may affect the fatty acid content and profile of the resulting oil in an adverse way (for example, lower yield, less healthful or functional balance of fatty acids).

[0065] The thermal and physical properties of the pongamia oil compositions provided herein reflect the non-destructive methods for removing furanoflavonoids and other unsaponifiable matter used to obtain the compositions.

[0066] In some variations, the pongamia oil compositions of the present disclosure may be characterized by their physical state at a given temperature, or their temperature-dependent behavior, such as a melting profile. In some variations, the pongamia oil compositions are liquid at a temperature of greater than or equal to about 10°C. In some variations, the pongamia oil composition is liquid at room temperature. In other variations, the pongamia oil compositions are semi-solid at a temperature of about 0-10°C. In some variations, the melting profile is determined by differential scanning calorimetry (DSC).

[0067] In still other embodiments, the pongamia oil compositions of the present disclosure may be characterized by their solid-fat content at a given temperature. For example, in some embodiments, the composition has a solid fat content of between about 1% and about 10% at a temperature of about 0°C, about 2°C, about 5°C, or about 10°C. In certain variations, the composition has a solid fat content of between about 1% and about 10% at a temperature of about 5°C. Any suitable methods or techniques known in the art may be used to measure solid fat content in the compositions herein. In some variations, the solid-fat content is determined by AOCS test method AOCS-Cd 16b-93.

[0068] In other embodiments, the pongamia oil composition may be characterized by its dropping point. The dropping point is the upper temperature at which a fat or grease can retain

semi-solid structure. Above the dropping point, the fat or grease converts to a liquid state. In some embodiments, the pongamia oil composition has a dropping point of less than or equal to about 20°C, less than or equal to about 15°C or less than or equal to about 10°C. In certain embodiments, the pongamia oil composition has a dropping point of less than or equal to about 10°C. Any suitable methods or techniques known in the art may be used to measure dropping point in the compositions herein. In some variations, the dropping point is determined by AOCS test method AOCS Cc 18-80.

[0069] In some embodiments, the pongamia oil composition may be characterized by its flash point. The flash point is the lowest temperature at which the vapors of a substance may ignite, when in the presence of an ignition source. In some embodiments, the pongamia oil composition has a flash point of at least about 200°C, at least about 220°C or at least about 240°C. Any suitable methods or techniques known in the art may be used to measure flash point in the compositions herein. In some variations, the flash point is determined by AOCS test method AOCS Cc 9b-55.

[0070] In some embodiments, the pongamia oil composition may be characterized by its smoke point. The smoke point of an oil is the temperature at which an oil begins to generate continuous, visible smoke under defined conditions. Oils having higher smoke points may find enhanced utility in food-related applications, such as in pan frying or sautéing, deep frying or baking, where high temperatures are common. In still other embodiments, the pongamia oil composition has a smoke point of at least about 180°C, at least about 190°C, at least about 195°C, at least about 200°C, or at least about 210°C. In still other embodiments, the pongamia oil composition has a higher smoke point than the crude pongamia oil from which it is obtained. Any suitable methods or techniques known in the art may be used to measure smoke point in the compositions herein. In some variations, the smoke point is determined by AOCS test method AOCS Cc 9a-48.

[0071] In other variations, the pongamia oil compositions provided herein may be characterized by their viscosities. The viscosity of a liquid, such as an oil, is a measure of the liquid's resistance to flow and/or deform. In some embodiments, the pongamia oil composition has viscosity of at least about 30 centipoise, at least about 40 centipoise, or at least about 50

centipoise as determined at about 25°C. In other embodiments, the pongamia oil composition has viscosity of less than or equal to 600 centipoise, less than or equal to 500 centipoise, less than or equal to 250 centipoise, less than or equal to 100 centipoise, less than or equal to 90 centipoise, less than or equal to 80 centipoise, less than or equal to about 70 centipoise, or less than or equal to about 60 centipoise as determined at about 25°C. In certain embodiments, the pongamia oil composition has a viscosity of between about 30 centipoise and about 600 centipoise at about 25°C. In still other embodiments, the pongamia oil composition has a lower viscosity than the crude pongamia oil from which it is obtained as measured at the same temperature.

#### *Other Properties*

[0072] In some embodiments, the pongamia oil compositions have one or more of the following properties selected from:

- (i) a free fatty acid content of less than or equal to about 1%;
- (ii) less than or equal to about 0.1% of insoluble impurities;
- (iii) less than or equal to about 25 ppm of phosphorus;
- (iv) less than or equal to about 0.1 ppm of chlorophyll;
- (v) less than or equal to about 25 ppm of residual solvents;
- (vi) a moisture content of less than or equal to about 1%;
- (vii) less than or equal to about 1% glycerol;
- (viii) less than or equal to about 1% monoglycerides;
- (ix) less than or equal to about 5% diglycerides; and
- (x) at least about 90% triglycerides.

[0073] In some embodiments, the free fatty acid content is determined by AOCS test method AOCS Ca 5a-40. In some embodiments, the insoluble impurities content is determined by AOCS test method AOCS Ca 3a-46. In some embodiments, the phosphorus content is determined by

AOCS Ca 20-99, mod. In some embodiments, the chlorophyll content is determined by AOCS Ch 4-91. In some embodiments, the moisture content is determined by AOCS Ca 2b-38. In some embodiments, the glycerol content is determined by AOCS Cd 11c-93. In some embodiments, the monoglyceride content is determined by AOCS Cd 11c-93. In some embodiments, the diglyceride content is determined by AOCS Cd 11c-93. In some embodiments, the triglyceride content is determined by AOCS Cd 11c-93.

[0074] In certain embodiments, the pongamia oil compositions have a lower unsaponifiable matter content as compared to the crude pongamia oil from which the composition was obtained (*e.g.*, according to the methods described herein).

[0075] In addition to their compositional content, the pongamia oil compositions of the present disclosure may also be described in terms of their physical properties including but not limited to color and/or turbidity.

[0076] In certain embodiments, the pongamia oil compositions provided herein (*e.g.*, produced according to the methods described herein) have a lighter color as compared to the crude pongamia oil from which the compositions are obtained. In certain variations, the final color of the pongamia oil composition is lighter than the initial color of the crude pongamia oil. In one variation, the crude pongamia oil is red and/or brown (*e.g.*, including red, brown, reddish brown or brownish red); and the pongamia oil compositions obtained thereof (*e.g.*, according to the methods described herein) is yellow and/or white (*e.g.*, including yellow, light yellow, white, or off white). In certain variations, color of the same is determined using the Lovibond Color - AOCS Scale. In certain embodiments, the color is determined using the Lovibond Color-AOCS scale using a 1-inch or 5.25-inch cell path. Thus, in one variation, the crude pongamia oil has a Lovibond color of 1.5R, 70Y; and the pongamia oil compositions obtained thereof (*e.g.*, according to the methods described herein) has a Lovibond color of 0.5R, 18Y, according to the Lovibond Color - AOCS Scale (AOCS method Cc 13b-45) using a 5.25-inch cell path.

[0077] In some variations, the pongamia oil compositions have a Lovibond color, wherein the Y-value is less than 25, as determined by the Lovibond Color - AOCS Scale (AOCS method Cc 13b-45) using a 1-inch cell path. In certain variations wherein the Y-value is less than 25, as determined by the Lovibond Color - AOCS Scale (AOCS method Cc 13b-45) using a 1-inch cell

path, the pongamia oil composition is light yellow. In other embodiments, have a Lovibond color, wherein the Y-value is greater than or equal to 25, as determined by the Lovibond Color - AOCS Scale (AOCS method Cc 13b-45) using a 1-inch cell path. In certain other variations wherein the Y-value is greater than or equal to 25, as determined by the Lovibond Color - AOCS Scale (AOCS method Cc 13b-45) using a 1-inch cell path, the pongamia oil composition is yellow.

[0078] In addition to the color of the pongamia oil compositions, the pongamia oil compositions may be characterized by their haziness or turbidity by methods known in the art. In still other variations, the pongamia oil compositions provided in the present disclosure have reduced turbidity as compared to the crude pongamia oil from which the compositions are obtained.

[0079] Any suitable methods known in the art to measure or determine the properties above may be employed.

#### *Sensory Characteristic Profile*

[0080] As detailed above, the pongamia oil compositions of the present disclosure, for which the furanoflavonoid and other unsaponifiable matter content has been reduced, are edible, non-bitter, and have an overall acceptable sensory profile in humans (*e.g.*, with respect to taste and smell).

[0081] In still other variations, the pongamia oil compositions of the present disclosure may be characterized by the presence or absence of one or more sensory attributes including but not limited to pongamia flavor/notes, nuttiness, butteriness, grassiness, smoothness, sweetness, oiliness, astringency, sharpness, bitterness, and sourness. In some variations, the pongamia oil compositions have one or more sensory attributes selected from the group consisting of: pongamia flavor/notes, nuttiness, butteriness, grassiness, smoothness, sweetness, oiliness, astringency, sharpness, bitterness, and sourness, and any combinations thereof.

[0082] In some variations, the pongamia oil compositions may be characterized by the presence of one or more sensory characteristics selected from the group consisting of pongamia flavor/notes, nuttiness, butteriness, grassiness, smoothness, sweetness, and oiliness.

[0083] In still other variations, the pongamia oil compositions may be characterized by the absence of one or more sensory characteristics selected from the group consisting of astringency, sharpness, bitterness, and sourness.

[0084] In yet other variations, the pongamia oil compositions may be characterized by the mildness of sensory attributes. For example, in some variations, the pongamia oil compositions may be characterized as having a non-bitter taste, neutral flavor, blandness, clean flavor, or absence of aftertaste, or any combinations thereof.

[0085] In one aspect, provided herein is a pongamia oil composition having:

- (i) less than or equal to about 1000 ppm of karanjin and pongamol combined, e.g., as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition;
- (ii) less than or equal to about 1% by weight of unsaponifiable matter, e.g., as determined by AOCS Ca 6a-40;
- (ii) a peroxide value of less than or equal to about 5 meq/kg, e.g., as determined by AOCS Cd 8-53;
- (iv) a p-anisidine value of less than or equal to about 10, e.g., as determined by AOCS Cd 18-90;
- (v) less than or equal to about 25 ppm of residual solvent, e.g., as determined by AOCS Cg 4-94;
- (vi) at least 40% oleic acid present out of the total fatty acids, e.g., as determined by AOAC 996.06;
- (vii) light yellow or yellow color, e.g., as determined by the Lovibond Color - AOCS Scale;

(viii) a neutral flavor, or one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof; or

or any combinations of (i)-(viii) thereof.

[0086] In some embodiments of the present aspect, the pongamia oil composition has less than or equal to about 1000 ppm of karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; less than or equal to about 1% by weight of unsaponifiable matter, e.g., as determined by AOCS Ca 6a-40; a peroxide value of less than or equal to about 5 meq/kg, e.g., as determined by AOCS Cd 8-53; a p-anisidine value of less than or equal to about 10, e.g., as determined by AOCS Cd 18-90; and less than or equal to about 25 ppm of residual solvent, e.g., as determined by AOCS Cg 4-94.

[0087] In other embodiments of the present aspect, the pongamia oil composition has less than or equal to about 1000 ppm of karanjin and pongamol combined, e.g., as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; less than or equal to about 1% by weight of unsaponifiable matter, e.g., as determined by AOCS Ca 6a-40; a peroxide value of less than or equal to about 5 meq/kg, e.g., as determined by AOCS Cd 8-53; a p-anisidine value of less than or equal to about 10, e.g., as determined by AOCS Cd 18-90; and at least 40% oleic acid present out of the total fatty acids, e.g., as determined by AOAC 996.06.

[0088] In still other embodiments, the pongamia oil composition has:

- (i) less than or equal to about 1000 ppm of karanjin and pongamol combined, e.g., as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition;
- (ii) less than or equal to about 1% by weight of unsaponifiable matter, e.g., as determined by AOCS Ca 6a-40;
- (ii) a peroxide value of less than or equal to about 5 meq/kg, e.g., as determined by AOCS Cd 8-53;
- (iv) a p-anisidine value of less than or equal to about 10, e.g., as determined by AOCS Cd 18-90;
- (v) less than or equal to about 25 ppm of residual solvent, e.g., as determined by AOCS Cg 4-94;
- (vi) at least 40% oleic acid present out of the total fatty acids, e.g., as determined by AOAC 996.06;
- (vii) light yellow or yellow color, e.g., as determined by the Lovibond Color - AOCS Scale; and
- (viii) a neutral flavor, or one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof.

**[0089]** In some embodiments, the pongamia oil composition has a light yellow color as determined by the Lovibond Color - AOCS Scale and a neutral flavor. In other embodiments, the pongamia oil composition has a yellow color as determined by the Lovibond Color - AOCS Scale and one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof. In some embodiments, which may be combined with any of the foregoing embodiments, the pongamia oil composition is light yellow as determined by the Lovibond Color - AOCS Scale and the composition has less than or equal to about 200 ppm of karanjin and pongamol combined as

determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition.

[0090] In other embodiments, the pongamia oil composition:

- (i) is liquid at room temperature;
- (ii) has a viscosity of between about 30 centipoise and 600 centipoise, e.g., as determined at 25°C;
- (iii) has a solid fat content of between about 1% and about 10% at a temperature of about 5°C, e.g., as determined by AOCS-Cd 16b-93;
- (iv) has a smoke point of at least about 195 °C, e.g., as determined by AOCS Cc 9a-48; or
- (v) has a flash point of at least about 200°C, e.g., as determined by AOCS Cc 9b-55;

or any combinations of (i)-(v) thereof.

#### **Methods of Analyzing Pongamia Oil Compositions**

[0091] The high concentrations of karanjin and pongamol present in the oil and seedcakes obtained from pongamia oilseeds have generally prevented the use of the oil and seedcake in food products due to the lack of edibility due to adverse taste and smell, as well as potential toxicity. These compounds can render the oil and seedcake inedible and potentially harmful to humans and animals. Prior attempts to develop edible pongamia compositions have been unsuccessful in part due to the fact that consistent acceptable maximum thresholds for karanjin concentrations and other anti-nutrients for consumption have not yet been established. Moreover, existing methods for analyzing pongamia compositions have been inaccurate and unreliable such that assessing karanjin concentrations in pongamia compositions, let alone the further determining maximum acceptable karanjin concentrations, is a formidable endeavor. Thus, there remains a need for more accurate methods for determining the levels of karanjin and other anti-nutritional compounds present in pongamia compositions.

[0092] The present disclosure addresses this need by providing methods of analyzing pongamia oil compositions, namely methods of determining concentrations of karanjin and other chemical compounds intrinsic to pongamia oilseeds, with greater accuracy and precision than existing methods. Thus, in some aspects, provided herein are methods for analyzing the karanjin and/or pongamol concentrations in pongamia oil using a solvent extraction analytical method.

[0093] With reference to FIG. 1, provided is exemplary process 100 to analyze a pongamia oil sample. In step 102, an extraction mixture is provided by combining the sample with an extraction solvent. In some embodiments, the extraction solvent comprises alkyl ketone. In certain variations, the extraction solvent comprises methyl ketone. In one variation, the extraction solvent comprises acetone.

[0094] With reference again to FIG. 1, in steps 104 and 106, the extraction mixture is sonicated and then separated into an extracted pongamia composition (*e.g.*, oil) and an extract that has karanjin and/or pongamol.

[0095] In step 108, the concentration of karanjin and/or pongamol present in the extract is then measured. In some variations, the concentration of karanjin and/or pongamol is determined by high performance liquid chromatography with an ultraviolet detector (UV). In one variation, the ultraviolet detector is a diode array detector (*i.e.*, HPLC-DAD is employed).

[0096] In some aspects, provided is an analytical method, comprising: combining pongamia oil with an extraction solvent to provide an extraction mixture, wherein the extraction solvent comprises alkyl ketone, and wherein the pongamia oil comprises karanjin or pongamol, or both; sonicating the extraction mixture to produce a sonicated mixture; separating the sonicated mixture into an extracted pongamia composition and an alkyl ketone extract, wherein the extract comprises karanjin or pongamol, or both; and measuring the concentration of karanjin or pongamol, or both, present in the extract. In one variation, the alkyl ketone is acetone. In some embodiments of the foregoing, the measuring step comprises determining the concentration of karanjin or pongamol, or both, by high performance liquid chromatography with an ultraviolet detector. In one variation, the ultraviolet detector is a diode array detector.

[0097] In certain aspects, the analytical methods provided herein to detect the concentration of karanjin and pongamol are an improvement over analytical methods generally known in the art, including for example methods that involve the use of HPLC with detection by mass spectrometry (MS) and methods that were generally directed to analyzing a pongamia meal sample. The analytical methods provided herein allow for accurate determination of a pongamia oil sample, by using a particular sample preparation and HPLC with detection by UV (*e.g.*, HPLC-DAD) as opposed to HPLC with detection by mass spectrometry (*e.g.*, HPLC-MS-MS).

#### Methods of Producing Pongamia Oil Compositions

[0098] In some aspects, provided herein are methods of obtaining an edible pongamia oil from a crude pongamia oil is obtained from plant material derived from a pongamia tree or plant (also known as “*Cytisus pinnatus*”, “*Dalbergia arborea*”, “*Derris indica*”, “*Galedupa pungum*”, “*karanj*”, “*Millettia pinnata*”, “*pongam*”, “*pongamia*”, “*Pongamia glabra*”, “*Pterocarpus flavus*”, “*Pongamia pinnata*”, and “*Robinia mitis*”, “*Indian beech*”, and “*mempari*”). In some variations, the crude pongamia oil is obtained from pongamia oilseeds.

[0099] With reference to FIG. 2, exemplary purification process 200 includes step 202, in which crude pongamia oil is combined with non-polar solvent to produce a crude mixture. The resulting crude pongamia oil comprises pongamia oil and furanoflavonoids, such as karanjin and/or pongamol. In step 204, the crude mixture is eluted through silica gel with additional non-polar solvent to separate at least a portion of the karanjin and pongamol from the pongamia oil, and to produce a purified mixture comprising pongamia oil and the non-polar solvent. In step 206, at least a portion of the non-polar solvent is removed from the purified mixture to produce a pongamia oil composition that is edible and non-bitter tasting.

#### *Solvents*

[0100] In some embodiments, the non-polar solvent used in the purification method described herein comprises alkane. In some variations, the alkane is a C1-20 alkane, or a C-10 alkane. In certain variations, the alkane is linear. In other variations, the alkane is branched. In yet other variations, a mixture of alkanes may be used. In one variation, the non-polar solvent comprises hexane.

[0101] In some variations, the crude pongamia oil is combined with the non-polar solvent in a ratio of solvent-to-oil between 1:1 and 3:1 (w/v), between 1:1 and 2.5:1 (w/v), or between 1:1 and 2:1 (w/v).

*Silica gel*

[0102] In some variations, the silica gel has (i) an average particle size (*e.g.*, based on particle diameter) between 5  $\mu\text{m}$  to 1000  $\mu\text{m}$ ; or (ii) an average mesh particle size ranges between 18 units and 2,500 units; or (iii) an average porosity range between 30  $\text{\AA}$  to 300  $\text{\AA}$ , or any combination of (i)-(iii).

*Crude Pongamia Oil*

[0103] In some embodiments, the crude pongamia oil comprises pongamia oil, karanjin, pongamol, other furanoflavonoids, and other unsaponifiable matter.

[0104] In certain embodiments, the crude pongamia oil has at least 500 ppm, at least 10,000, or at least 30,000 ppm; or between 10,000 ppm and 30,000 ppm of unsaponifiable matter.

[0105] In certain embodiments, the crude pongamia oil has at least 500 ppm, at least 10,000, or at least 30,000 ppm; or between 10,000 ppm and 30,000 ppm of furanoflavonoids. In certain embodiments, the crude pongamia oil has at least 10,000 ppm of karanjin and/or a pongamol. In some variations of the foregoing, the karanjin and pongamol concentrations are determined by the solvent extraction analytical methods described herein.

[0106] In some variations, the crude pongamia oil has at least 500 ppm, at least 10,000, or at least 30,000 ppm; or between 10,000 ppm and 30,000 ppm of karanjin. In other variations, the crude pongamia oil has at least 500 ppm, at least 10,000, or at least 30,000 ppm; or between 10,000 ppm and 30,000 ppm of pongamol. In other variations, the crude pongamia oil has at least 500 ppm, at least 10,000, or at least 30,000 ppm; or between 10,000 ppm and 30,000 ppm of karanjin and pongamol combined.

[0107] The crude pongamia oil used for the methods described herein may be produced from various methods and techniques known in the art or obtained from any commercially available sources. In some variations, the crude pongamia oil is obtained by mechanically separating

pongamia oilseeds. In one variation, the crude pongamia oil is obtained by cold-pressing pongamia oilseeds.

[0108] Optionally, the pongamia oilseeds may be dehulled to obtain the crude pongamia oil. Thus, in some variations, the crude pongamia oil is obtained by dehulling pongamia oilseeds to produce dehulled oilseeds; and mechanically separating the dehulled oilseeds to produce the crude pongamia oil and a seedcake that is at least partially deoiled. In other variations, the crude pongamia oil is obtained by heating pongamia oilseeds at a temperature between 25°C and 200°C for a suitable time to provide treated oilseeds; dehulling the treated oilseeds to produce dehulled oilseeds; and mechanically separating the dehulled oilseeds to produce the crude pongamia oil and a seedcake that is at least partially deoiled.

[0109] Dehulling typically involves passing pongamia beans through a dehuller to loosen the hulls and the bean, and separating the two fractions. Any suitable techniques known in the art may be employed to achieve dulling and hull separation. For example, in some variations, dehulling is performed by passing the pongamia beans through an impact type dehuller and loosening the hulls from beans. Other types of dehulling equipment such as abrasive/brushing type may be used for this purpose. Separation of the beans from the hulls can be performed by, for example, a gravity table or an aspirator.

[0110] The beans are then mechanically pressed (*e.g.*, cold-pressed), which typically may be performed using an expeller to remove free oil and produce reduced fat (*e.g.*, 10-14% fat) pongamia meal. Cold-pressing can be performed using any suitable techniques known in the art. For example, cold-pressing can be performed using various pieces of equipment, such as a Farmet FL-200 expeller press. In some variations, pressing can include passing the dehulled beans through the apparatus to produce free oil and reduced fat meal. The partially defatted mechanically pressed beans can remove approximately 60-75% of the original pongamia oil content.

### **Food and Beverage Products**

[0111] In certain aspects, provided are also food and beverage products incorporating or produced using the pongamia oil compositions herein. Such pongamia oil compositions may be

used as salad oil, frying oil, sauteeing oil, vinaigrettes, sauces, dressings, fats in meat mimetics, beverages, or blended margarines and other solid fat applications.

[0112] The pongamia oil compositions as provided herein have a number of favorable compositional properties, including low concentrations of karanjin, pongamol, and unsaponifiable matter, low peroxide values, low p-anisidine values, low residual solvent content, and high oleic acid content, that make the pongamia oil compositions suited for use in food applications. In addition to these compositional attributes, the pongamia oil compositions of the present disclosure also possess various organoleptic and functional properties that can be selected for various applications in which fats and/or oils are desired.

[0113] In some embodiments, provided herein are food and beverage products comprising a pongamia oil composition, wherein the pongamia oil composition has a light yellow color, e.g., as determined by the Lovibond Color - AOCS Scale, and a neutral flavor. In other embodiments, the pongamia oil composition has a yellow color, e.g., as determined by the Lovibond Color - AOCS Scale, and one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof. In some embodiments, which may be combined with any of the foregoing embodiments, the pongamia oil composition is light yellow, e.g., as determined by the Lovibond Color - AOCS Scale and the composition has less than or equal to about 200 ppm of karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition.

[0114] In other embodiments, the food or beverage product comprises a pongamia oil composition, wherein the pongamia oil composition:

- (i) has light yellow or yellow color as determined by the Lovibond Color - AOCS Scale; and
- (ii) has a neutral flavor, or one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof.
- (iii) is liquid at room temperature;
- (iv) has a viscosity of between about 30 centipoise and 600 centipoise as determined at 25°C;

(v) has a solid fat content of between about 1% and about 10% at a temperature of about 5°C as determined by AOCS-Cd 16b-93;

(vi) has a smoke point of at least about 195 °C as determined by AOCS Cc 9a-48; or

(vii) has a flash point of at least about 200°C as determined by AOCS Cc 9b-55;

or any combinations of (i)-(vii) thereof.

[0115] The food and beverages products can include various other components other than the pongamia oil compositions described herein. For example, the food and beverage products may include, for example, water, other fats and oils, sweeteners (such as sugar), salt, thickeners (such as pectin and other hydro colloids), anti-foaming agents, natural and artificial flavorings, preservatives, and coloring agents.

[0116] In one variation, the food product is pongamia oil mayonnaise. In another variation, the food product is pongamia oil margarine and spread. In yet another variation, the food product is pongamia oil salad dressing.

[0117] In another aspect, provided is a method of preparing food and/or beverages products. Such methods may include one or more of mixing/blending, pasteurizing and/or sterilizing, and packaging.

#### ENUMERATED EMBODIMENTS

[0118] The following enumerated embodiments are representative of some aspects of the invention.

1. An analytical method, comprising:

combining pongamia oil with an extraction solvent to provide an extraction mixture, wherein the extraction solvent comprises alkyl ketone, and wherein the pongamia oil comprises karanjin or pongamol, or both;

sonicating the extraction mixture to produce a sonicated mixture;

separating the sonicated mixture into an extracted pongamia composition and an alkyl ketone extract, wherein the extract comprises karanjin or pongamol, or both; and

measuring the concentration of karanjin or pongamol, or both, present in the extract.

2. The method of embodiment 1, wherein the alkyl ketone is acetone.
3. The method of embodiment 1 or 2, wherein the measuring step comprises determining the concentration of karanjin or pongamol, or both, by high performance liquid chromatography with an ultraviolet detector.
4. The method of embodiment 3, wherein the ultraviolet detector is a diode array detector.
5. A method for producing a pongamia oil composition, comprising:

combining crude pongamia oil with non-polar solvent to produce a crude mixture, wherein the non-polar solvent comprises alkane, and wherein the crude pongamia oil comprises pongamia oil and furanoflavonoids;

eluting the crude mixture through silica gel with the non-polar solvent to separate at least a portion of the furanoflavonoids from the pongamia oil, and to produce a purified mixture comprising pongamia oil and the non-polar solvent; and

removing at least a portion of the non-polar solvent from the purified mixture to produce a pongamia oil composition, wherein the composition is edible and non-bitter tasting.
6. The method of embodiment 5, wherein the composition has a lower furanoflavonoids content than the crude pongamia oil.
7. The method of embodiment 5 or 6, wherein the composition has less than or equal to 150 ppm of furanoflavonoids.
8. The method of any one of embodiments 5 to 7, wherein the furanoflavonoids comprise karanjin, or pongamol, or both.

9. The method of any one of embodiments 5 to 8, wherein the crude pongamia oil has an initial color, and the pongamia oil composition produced has a final color, wherein the final color of the pongamia oil composition is lighter than the initial color of the crude pongamia oil.
10. The method of embodiment 9, wherein the initial color is red and/or brown, and the final color is yellow.
11. The method of any one of embodiments 5 to 10, wherein the crude pongamia oil is mechanically separated pongamia oil.
12. The method of any one of embodiments 5 to 10, further comprising:  
  
dehulling pongamia oilseeds to produce dehulled oilseeds; and  
  
mechanically separating the dehulled oilseeds to produce the crude pongamia oil and a seedcake that is at least partially deoiled.
13. The method of any one of embodiments 5 to 10, further comprising:  
  
heating pongamia oilseeds at a temperature between 25°C and 200°C for a suitable time to provide treated oilseeds;  
  
dehulling the treated oilseeds to produce dehulled oilseeds; and  
  
mechanically separating the dehulled oilseeds to produce the crude pongamia oil and a seedcake that is at least partially deoiled.
14. The method of any one of embodiments 5 to 13, wherein the non-polar solvent comprises hexane.
15. The method of any one of embodiments 5 to 14, wherein the concentration of karanjin or pongamol, or both, is determined by the method of any one of embodiments 1 to 4.
16. The method of any one of embodiments 5 to 15, wherein the crude pongamia oil is combined with the non-polar solvent in a ratio between 1:1 and 3:1 (w/v).

17. The method of any one of embodiments 5 to 16, wherein the karanjin and pongamol are adsorbed on the silica gel, and the method further comprises:

eluting the silica gel with a polar solvent to isolate the karanjin and pongamol, wherein the polar solvent comprises alkyl alkanoate.
18. The method of any one of embodiments 5 to 16, wherein the karanjin and pongamol are adsorbed on the silica gel, and the method further comprises:

eluting the silica gel using a stepwise gradient with increasing proportions of polar solvent in the non-polar solvent to isolate karanjin and pongamol separately, wherein the polar solvent comprises alkyl alkanoate.
19. The method of embodiment 17 or 18, wherein the polar solvent comprises ethyl acetate.
20. A pongamia oil composition produced according to the method of any one of the preceding embodiments.
21. A pongamia oil composition having less than 150 ppm furanoflavonoids, wherein the composition is edible and non-bitter tasting.
22. The composition of embodiment 21, wherein the furanoflavonoids comprise karanjin, or pongamol, or both.
23. The composition of embodiment 22, wherein the composition has less than or equal to 150 ppm of karanjin or pongamol, or both.
24. The composition of embodiment 23, wherein the concentration of karanjin or pongamol, or both, is determined by the method of any one of embodiments 1 to 4.
25. The composition of any one of embodiments **Error! Reference source not found.** to **Error! Reference source not found.**, wherein the composition is obtained from a crude pongamia oil, and the composition has a color that is lighter than the color of the crude pongamia oil.
26. The composition of embodiment 25, wherein the color of the composition is yellow.

27. The composition of any one of embodiments 21 to 26, further comprising a non-polar solvent, wherein the non-polar solvent comprises alkane.
28. The composition of embodiment 27, wherein the non-polar solvent is present at a concentration of less than 25 ppm.
29. The composition of any one of embodiments 21 to 28, wherein the composition comprises fatty acids.
30. The composition of embodiment 29, wherein the fatty acids are monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, trans fatty acids, Omega 3 fatty acids, Omega 6 fatty acids, Omega 7 fatty acids, or Omega 9 fatty acids, or any combination thereof.
31. The composition of any one of embodiments 21 to 28, wherein the composition comprises oleic acid, linoleic acid, palmitic acid, stearic acid, behenic acid, alpha linolenic acid, lignoceric acid, arachidic acid, gondoic acid, oleic acid, vaccenic acid, palmitoleic acid, eicosadienoic acid, linoleic acid, margaric acid, gondoic acid, erucic acid, palmitoleic acid, heptadecenoic acid, or myristic acid, or any isomers thereof, or any combination of the foregoing.
32. The composition of any one of embodiments 21 to 31, wherein the composition has:
  - (i) a free fatty acid content of less than or equal to 1%;
  - (ii) less than or equal to 0.1% of insoluble impurities;
  - (iii) less than or equal to 25 ppm of phosphorus;
  - (iv) less than or equal to 0.1 ppm of chlorophyll;
  - (v) less than or equal to 25 ppm of residual solvents;
  - (vi) a moisture content of less than or equal to 1%;
  - (vii) less than or equal to 1% glycerol;

- (viii) less than or equal to 1% monoglycerides;
  - (ix) less than or equal to 5% diglycerides; or
  - (x) at least 90% triglycerides,
- or any combination of (i)-(x).
- 33. The composition of any one of embodiments 21 to 32, wherein the composition has a lower unsaponifiable matter content as compared to the crude pongamia oil from which the composition was obtained.
  - 34. The composition of any one of embodiments 21 to 33, wherein the composition further comprises tocopherols.
  - 35. The composition of embodiment 34, wherein the tocopherols comprise alpha-tocopherol, beta-tocopherol, delta-tocopherol, or gamma-tocopherol, or any combination thereof.
  - 36. The composition of embodiment 34 or 35, wherein the composition has at least 400 ppm of tocopherols.
  - 37. The composition of any one of embodiments 34 to 36, wherein the composition has at least 200 ppm of alpha-tocopherol.
  - 38. The composition of any one of embodiments 21 to 37, wherein the composition further comprises sterols.
  - 39. The composition of embodiment 38, wherein the sterols comprise beta-sitosterol, campesterol, cholesterol, clerosterol, delta-5,24-stigmastadienol, delta-5-avenasterol, sitostanol, or stigmasterol, or any combination thereof.
  - 40. The composition of embodiment 38 or 39, wherein the composition has less than 2500 ppm of sterols.
  - 41. Use of the pongamia oil composition of any one of embodiments 21 to 40 as salad oil, frying oil, sauteeing oil, vinaigrettes, sauces, dressings, fats in vegan meat mimetics,

beverages, or blended margarines and other solid fat applications, or any combination thereof.

42. A food or beverage product, comprising the pongamia oil composition of any one of embodiments 21 to 40.
43. The product of embodiment 42, wherein the product is salad oil, frying oil, sauteeing oil, vinaigrettes, sauces, dressings, fats in meat mimetics, beverages, or blended margarines and other solid fat applications.
44. A pongamia oil composition, having:
  - less than or equal to about 1000 ppm of karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition;
  - less than or equal to about 1% by weight of unsaponifiable matter as determined by AOCS Ca 6a-40;
  - a peroxide value of less than or equal to about 5 meq/kg as determined by AOCS Cd 8-53;
  - a p-anisidine value of less than or equal to about 10 as determined by AOCS Cd 18-90;
  - and
  - less than or equal to about 25 ppm of residual solvents as determined by AOCS Cg 4-94.
45. The composition of embodiment 44, having:
  - less than or equal to about 150 ppm of karanjin as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition;
  - less than or equal to about 150 ppm of pongamol as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition;

less than or equal to about 1% by weight of unsaponifiable matter as determined by AOCS Ca 6a-40;

a peroxide value of less than or equal to about 5 meq/kg as determined by AOCS Cd 8-53;

a p-anisidine value of less than or equal to about 5 as determined by AOCS Cd 18-90;  
and

less than or equal to about 25 ppm of residual solvents as determined by AOCS Cg 4-94.

46. The composition of embodiment 44 or 45, wherein the residual solvents comprise a non-polar solvent.
47. The composition of any one of embodiments 44 to 46, wherein the residual solvents comprise a non-polar solvent and wherein the non-polar solvent comprises alkane.
48. The composition of any one of embodiments 44 to 47, wherein the composition is liquid at room temperature.
49. The composition of any one of embodiments 44 to 48, wherein the composition has a viscosity of between about 30 centipoise and 600 centipoise as determined at 25°C.
50. The composition of any one of embodiments 44 to 49, wherein the composition has a solid fat content of between about 1% and about 10% at a temperature of about 5°C as determined by AOCS-Cd 16b-93.
51. The composition of any one of embodiments 44 to 50, wherein the composition has a smoke point of at least about 195 °C as determined by AOCS Cc 9a-48.
52. The composition of any one of embodiments 44 to 51, wherein the composition has at least 400 ppm of tocopherols as determined by AOAC 971.30 with HPLC.
53. The composition of any one of embodiments 44 to 52, wherein the composition has less than 2500 ppm of sterols as determined by COI/T.20/Doc No.10.

54. The composition of any one of embodiments 44 to 53 wherein the composition comprises oleic acid, linoleic acid, palmitic acid, stearic acid, behenic acid, alpha linolenic acid, lignoceric acid, arachidic acid, gondoic acid, oleic acid, vaccenic acid, palmitoleic acid, eicosadienoic acid, linoleic acid, margaric acid, gondoic acid, erucic acid, palmitoleic acid, heptadecenoic acid, or myristic acid, or any isomers thereof, or any combination of the foregoing, as determined by AOAC 996.06.
55. The composition of embodiment 54, wherein the composition comprises at least 40% oleic acid as determined by AOAC 996.06.
56. The composition of any one of embodiments 44 to 55, wherein the color of the composition is yellow or light yellow as determined by the Lovibond Color - AOCS Scale using a 1-inch cell path, wherein:
- when the composition is yellow, the composition has a Lovibond Color Y-value of greater than or equal to 25; and
- when the composition is light yellow, the composition has a Lovibond Color Y-value of less than 25.
57. The composition of any one of embodiments 44 to 56, wherein the composition has one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof.
58. The composition of any one of embodiments 44 to 57, wherein the composition has a ratio of karanjin to pongamol of greater than about 1.
59. The composition of any one of embodiments 44 to 56, wherein the color of the composition is light yellow and the composition has a Lovibond Color Y-value of less than 25 as determined by the Lovibond Color - AOCS Scale using a 1-inch cell path.
60. The composition of any one of embodiments 44 to 56 and 59, wherein the color of the composition is light yellow, and wherein the composition comprises less than or equal to about 200 ppm karanjin and pongamol combined.

61. The composition of any one of embodiments 44 to 56, 59 and 60, wherein the composition has a neutral flavor.
62. The composition of any one of embodiments 44 to 56 and 59 to 61, wherein the composition has a ratio of karanjin to pongamol of less than or equal to 1.
63. The composition of any one of embodiments 44 to 62, wherein the composition has:
  - (i) a free fatty acid content of less than or equal to about 1% as determined by AOCS Ca 5a-40;
  - (ii) less than or equal to about 0.1% of insoluble impurities as determined by AOCS Ca 3a-46;
  - (iii) less than or equal to about 25 ppm of phosphorus as determined by AOCS Ca 20-99, mod.;
  - (iv) less than or equal to about 0.1 ppm of chlorophyll as determined by AOCS Ch 4-91;
  - (v) a moisture content of less than or equal to about 1% as determined by AOCS Ca 2b-38;
  - (vi) less than or equal to about 1% glycerol as determined by AOCS Cd 11c-93;
  - (vii) less than or equal to about 2% monoglycerides as determined by AOCS Cd 11c-93;
  - (viii) less than or equal to about 5% diglycerides as determined by AOCS Cd 11c-93; and
  - (ix) at least about 90% triglycerides as determined by AOCS Cd 11c-93,
  - (x) or any combination of (i)-(ix).
64. An analytical method, comprising:

combining pongamia oil with an extraction solvent to provide an extraction mixture, wherein the extraction solvent comprises alkyl ketone, and wherein the pongamia oil comprises karanjin or pongamol, or both;

sonicating the extraction mixture to produce a sonicated mixture;

separating the sonicated mixture into an extracted pongamia composition and an alkyl ketone extract, wherein the extract comprises karanjin or pongamol, or both; and

measuring the concentration of karanjin or pongamol, or both, present in the extract.

65. The method of embodiment 64, wherein the alkyl ketone is acetone.
66. The method of embodiment 64 or 65, wherein the measuring step comprises determining the concentration of karanjin or pongamol, or both, by high performance liquid chromatography with an ultraviolet detector.
67. The method of embodiment 68, wherein the ultraviolet detector is a diode array detector.
68. A method for producing a pongamia oil composition, comprising:

combining crude pongamia oil with non-polar solvent to produce a crude mixture, wherein the non-polar solvent comprises alkane, and wherein the crude pongamia oil comprises pongamia oil and furanoflavonoids;

eluting the crude mixture through silica gel with the non-polar solvent to separate at least a portion of the furanoflavonoids from the pongamia oil, and to produce a purified mixture comprising pongamia oil and the non-polar solvent; and

removing at least a portion of the non-polar solvent from the purified mixture to produce a pongamia oil composition, wherein the composition has less than or equal to about 1000 ppm of karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition; less than or equal to about 1% by weight of unsaponifiable matter as determined by AOCS Ca 6a-40; a peroxide value of less than or equal to about 5 meq/kg as determined by AOCS Cd 8-53;

and a p-anisidine value of less than or equal to about 10 as determined by AOCS Cd 18-90.

69. The method of embodiment 68, wherein the crude pongamia oil has an initial color, and the pongamia oil composition produced has a final color, wherein the final color of the pongamia oil composition is lighter than the initial color of the crude pongamia oil.
70. The method of embodiment 69, wherein the initial color is red and/or brown, and the final color is yellow or light yellow.
71. The method of any one of embodiments 68 to 70, wherein the crude pongamia oil is mechanically separated pongamia oil.
72. The method of any one of embodiments 68 to 71, further comprising:  
  
dehulling pongamia oilseeds to produce dehulled oilseeds; and  
  
mechanically separating the dehulled oilseeds to produce the crude pongamia oil and a seedcake that is at least partially deoiled.
73. The method of any one of embodiments 68 to 72, further comprising:  
  
heating pongamia oilseeds at a temperature between 25°C and 200°C for a suitable time to provide treated oilseeds;  
  
dehulling the treated oilseeds to produce dehulled oilseeds; and  
  
mechanically separating the dehulled oilseeds to produce the crude pongamia oil and a seedcake that is at least partially deoiled.
74. The method of any one of embodiments 68 to 73, wherein the non-polar solvent comprises hexane.
75. The method of any one of embodiments 68 to 74, wherein the concentration of karanjin or pongamol, or both, is determined by the method of any one of embodiments 21 to 24.

76. The method of any one of embodiments 68 to 75, wherein the crude pongamia oil is combined with the non-polar solvent in a ratio between 1:1 and 3:1 (w/v).
77. The method of any one of embodiments 68 to 76, wherein the karanjin and pongamol are adsorbed on the silica gel, and the method further comprises:  
  
eluting the silica gel with a polar solvent to isolate the karanjin and pongamol, wherein the polar solvent comprises alkyl alkanoate.
78. The method of any one of embodiments 68 to 77, wherein the karanjin and pongamol are adsorbed on the silica gel, and the method further comprises:  
  
eluting the silica gel using a stepwise gradient with increasing proportions of polar solvent in the non-polar solvent to isolate karanjin and pongamol separately, wherein the polar solvent comprises alkyl alkanoate.
79. The method of embodiment 77 or 78, wherein the polar solvent comprises ethyl acetate.
80. A pongamia oil composition produced according to the method of any one of the embodiments 68 to 79.
81. Use of the pongamia oil composition of any one of embodiments 44 to 63 and 80 as salad oil, frying oil, sauteeing oil, vinaigrettes, sauces, dressings, fats in meat mimetics, beverages, or blended margarines and other solid fat applications, or any combination thereof.
82. A food or beverage product, comprising the pongamia oil composition of any one of embodiments 44 to 63 and 80.
83. The product of embodiment 82, wherein the composition is light yellow; the composition comprises less than or equal to about 200 ppm karanjin and pongamol combined, and the composition has a neutral flavor.
84. The product of embodiment 82, wherein the pongamia oil composition is yellow; the composition comprises less than or equal to about 150 ppm karanjin and less than or

equal to about 150 ppm pongamol; and the composition has one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof.

85. The product of any one of embodiments 82 to 84, wherein the product is salad oil, frying oil, sauteeing oil, vinaigrettes, sauces, dressings, fats in meat mimetics, beverages, or blended margarines and other solid fat applications.
86. A pongamia oil composition, having:
- (i) less than or equal to about 1000 ppm of karanjin and pongamol combined as determined by HPLC-DAD analysis of an acetone extract obtained from the pongamia oil composition;
  - (ii) less than or equal to about 1% by weight of unsaponifiable matter as determined by AOCS Ca 6a-40;
  - (iii) a peroxide value of less than or equal to about 5 meq/kg as determined by AOCS Cd 8-53;
  - (iv) a p-anisidine value of less than or equal to about 10 as determined by AOCS Cd 18-90; and
  - (v) less than or equal to about 25 ppm of residual solvent as determined by AOCS Cg 4-94;
  - (vi) at least 40% oleic acid present out of the total fatty acids as determined by AOAC 996.06;
  - (vii) light yellow or yellow color;
  - (viii) a neutral flavor, or one or more sensory attributes selected from the group consisting of: nuttiness, butteriness, grassiness, smoothness, and sweetness, and any combinations thereof;
  - (ix) any combinations of (i)-(viii) thereof.

## EXAMPLES

[0119] The presently disclosed subject matter will be better understood by reference to the following Examples, which are provided as exemplary of the invention, and not by way of limitation.

### Example 1

#### Production of Pongamia Oil

[0120] This example demonstrates the production of edible (*e.g.*, non-bitter) pongamia oil by liquid:solid adsorption chromatography of crude (*e.g.*, bitter) pongamia oil. The crude pongamia oil undergoes adsorptive purification using silica gel. Karanjin, pongamol, and potentially other anti-nutritional factors and/or bitterness compounds are chromatographically removed from mechanically separated pongamia oil to give a purified oil product.

#### Column Preparation (Wet Pack Method)

[0121] The column used was fritted, 1 L in volume with a spherical reservoir with another approximately 1 L capacity on the top. The silica used had a 40-63  $\mu\text{m}$  particle size and 60 Å pore size.

[0122] The silica (500-550 g, ~1 L volume) was combined with n-hexane (1000-1100 mL) in a flask and swirled to form a silica slurry. A large funnel was used to transfer silica into the flask. The stopcock at the column outlet was opened just enough to allow for 1 drip/sec flow rate. After allowing the silica slurry to reach room temperature, the silica slurry was poured into the column. Additional hexane was added when the slurry became too thick to pour. Once the column was fully packed, the stopcock at the column outlet was closed, allowing 1-2 cm of solvent to remain above the column head. Glass wool was added on top of the silica slurry bed.

#### Crude Mixture Preparation

[0123] Approximately 1100 mL (1000-1100 g) of crude pongamia oil was combined with 500 mL of n-hexane in a 2 flask with a glass stopper. Initially, two layers were observed to form.

The top layer had milky-yellowish appearance indicating an emulsion. The two layers were mixed, forming a homogenous solution.

#### Chromatography Procedure

[0124] 750 mL of the crude mixture prepared above was poured onto the glass wool at the top of the column bed. The stopcock at the column outlet was opened to allow for a flow rate of 2 drips/second (approximately 7 mL/min), while the top of the column was capped with aluminum foil to minimize evaporation. Colorless hexane was eluted and collected first, followed by a yellow fraction. Remaining crude mixture was added, and colorless hexane and yellow fractions were eluted and collected until the column head nearly emptied. 1 L hexane was added to the column, and colorless hexane and yellow fractions were eluted and collected. Inner walls of the spherical reservoir and the glass wool were rinsed with aliquots of hexane, followed by elution and collection. Another 1 L hexane was added to the column, and eluted and collected. Finally, colorless hexane and yellow fractions collected were combined, with the exception of those collected during the rinses, which were found to contain additional minor peaks (but no pongamol or karanjin).

#### Elution of Polar Compounds

[0125] 1 L of 100% ethyl acetate was added to the column to flush out all silica-bound polar compounds, including karanjin and pongamol, at a flow rate of 1 drip/second. The eluent was collected into a flask. Second, another 1 L of ethyl acetate was added to the column, and the eluent was collected into another flask. The fractions were stored at room temperature.

[0126] In other variations, alternative methods for eluting the polar compounds may be employed. For example, in other experiments performed, 1 L each of 5%, 10%, 20% and 30% ethyl acetate in hexane was prepared for a stepwise gradient elution. Next, 1 L of 5% ethyl acetate in hexane was added to the column, and the eluent was collected as a yellow fraction and a nearly colorless fraction. Then, 1 L each of 10%, 20%, 30% ethyl acetate in hexane, followed by 500 mL of 100% ethyl acetate were added to the column, and corresponding eluting fractions were collected. The flow rate was increased after the yellow fraction eluted with hexane. The

flow rate was adjusted to allow for a fast drip (approximately 10 mL/min). Finally, solvents of each fraction were evaporated.

[0127] Table 1 below compares the chemical compositions of crude pongamia oil (“crude oil”) with the pongamia oil purified in accordance with the chromatography procedure set forth in this example (“purified oil”). The methods used to determine the components measured are set forth in Table 1, where AOCS refers to the American Oil Chemists’ Society and their test methods are publicly available.

**Table 1.** Chemical composition<sup>1, 2</sup>

| Component (unit of measure)                       | Crude oil      | Purified oil | Test method           |
|---------------------------------------------------|----------------|--------------|-----------------------|
| Free Fatty Acids (FFA)                            | 1.3            | 0.13         | AOCS Ca 5a-40         |
| Peroxide Value (meq/kg)                           | 1.1            | 4.55         | AOCS Cd 8-53          |
| p-Anisidine Value                                 | ND             | 4.7          | AOCS Cd 18-90         |
| Neutral Oil (%)                                   | 98.75          | 99.91        | AOCS Ca 9f-57         |
| Iodine value (calculated from FA profile)         | 78.4           | 84.6         | AOCS Cd 1c-85         |
| Insoluble impurities (%)                          | 0.02           | <0.01        | AOCS Ca 3a-46         |
| Soap Content (g/kg)                               | 4564.5         | <0.1         | AOCS CC17-95, CC15-60 |
| OSI (Oxidative Stability Index; hours)            | 7.83           | 9.6          | AOCS Cd 12b-92:1997   |
| Phosphorus (oil specific) (ppm)                   | 2.6            | 22.5         | AOCS Ca 20-99, mod.   |
| Chlorophyll (ppm)                                 | 0.8            | <0.1         | AOCS Ch 4-91          |
| Residual solvents (ppm)                           | NA             | <0.50        | AOCS Cg 4-94          |
| Moisture (%)                                      | 0.05           | 0.03         | AOCS Ca 2b-38         |
| Lovibond Color - AOCS Scale (5.25-inch cell path) | 1.5R, 70Y      | 0.5R, 18.0Y  | AOCS Cc 13b-45        |
| Smoke point (°F)                                  | 326            | 414          | AOCS Cc 9a-48         |
| Glycerol %                                        | 1              | <1           | AOCS Cd 11c-93        |
| Monoglycerides %                                  | 9              | <1           | AOCS Cd 11c-93        |
| Diglycerides %                                    | 2.5            | 1.0          | AOCS Cd 11c-93        |
| Triglycerides %                                   | 85.6           | 95.9         | AOCS Cd 11c-93        |
| Hexane residual (ppm)                             | Not applicable | 2.3          | AOCS Cg 4-94          |

<sup>1</sup> Data represents the average of multiple experiments.

<sup>2</sup> ND: non-detectable

[0128] Table 2 below compares the amount of fatty acid classes in the crude oil with the purified oil. The methods used to determine the components measured are set forth in Table 2, where AOAC refers to the Association of Official Analytical Chemists and their test methods are publicly available.

**Table 2.** Fatty acid classes (% of total)

| Class                             | Crude oil | Purified oil | Test method |
|-----------------------------------|-----------|--------------|-------------|
| Total Identified Fatty Acids      | 84.72     | 92.69        | AOAC 996.06 |
| Total Monounsaturated Fatty Acids | 46.59     | 52.09        | AOAC 996.06 |
| Total Polyunsaturated Fatty Acids | 18.3      | 18.44        | AOAC 996.06 |
| Total Saturated Fatty Acids       | 19.62     | 21.93        | AOAC 996.06 |
| Total Trans Fatty Acids           | 0.22      | 0.24         | AOAC 996.06 |
| Total Omega 3 Fatty Acids         | 2.41      | 1.99         | AOAC 996.06 |
| Total Omega 6 Fatty Acids         | 15.8      | 16.37        | AOAC 996.06 |
| Total Omega 7 Fatty Acids         | 0.58      | 0.65         | AOAC 996.06 |
| Total Omega 9 Fatty Acids         | 46.57     | 51.12        | AOAC 996.06 |

[0129] Table 3 below shows the amount of fatty acid compositions in the crude oil with the purified oil. The methods used to determine the components measured are set forth in Table 3.

**Table 3.** Fatty acid composition (% of total)<sup>1</sup>

| Fatty acid | Common name                      | Crude oil | Purified oil | Test method |
|------------|----------------------------------|-----------|--------------|-------------|
| 14:0       | Myristic acid                    | 0.03      | 0.03         | AOAC 996.06 |
| 16:0       | Palmitic Acid                    | 8.26      | 8.84         | AOAC 996.06 |
| 16:1c9     | Palmitoleic Acid                 | <0.04     | <0.04        | AOAC 996.06 |
| Σ16:1      | Total Palmitoleic Acid + isomers | 0.09      | 0.15         | AOAC 996.06 |
| 17:0       | Margaric Acid                    | 0.08      | 0.09         | AOAC 996.06 |
| 17:1c9     | Heptadecenoic Acid               | 0.04      | 0.04         | AOAC 996.06 |
| 18:0       | Stearic Acid                     | 6.08      | 6.84         | AOAC 996.06 |
| 18:1c11    | Vaccenic acid                    | 0.55      | 0.61         | AOAC 996.06 |
| 18:1c9     | Oleic Acid                       | 44.87     | 49.98        | AOAC 996.06 |
| Σ18:1      | Total Oleic Acid + isomers       | 45.62     | 50.87        | AOAC 996.06 |
| 18:2n6     | Linoleic Acid                    | 15.64     | 16.23        | AOAC 996.06 |
| Σ18:2      | Linoleic Acid + isomers          | 15.76     | 16.35        | AOAC 996.06 |
| 18:3n3     | Alpha Linolenic Acid             | 2.18      | 1.99         | AOAC 996.06 |
| Σ18:3      | Total Linolenic Acid + isomers   | 2.18      | 1.99         | AOAC 996.06 |
| 20:0       | Arachidic Acid                   | 1.15      | 1.33         | AOAC 996.06 |
| 20:1c11    | Gondoic Acid                     | 0.90      | 1.05         | AOAC 996.06 |
| Σ20:1      | Total Gondoic Acid + isomers     | 0.95      | 1.12         | AOAC 996.06 |
| 20:2n6     | Eicosadienoic Acid               | 0.15      | 0.14         | AOAC 996.06 |
| 22:0       | Behenic Acid                     | 2.87      | 3.42         | AOAC 996.06 |
| 22:1c13    | Erucic Acid                      | 0.06      | 0.07         | AOAC 996.06 |
| Σ22:1      | Total Erucic Acid + isomers      | 0.06      | 0.08         | AOAC 996.06 |
| 24:0       | Lignoceric Acid                  | 1.13      | 1.36         | AOAC 996.06 |

<sup>1</sup> The following fatty acids have less than 0.02% of total fatty acids: C4:0, 6:0, 8:0, 10:0, 11:0, 12:0, 14:0, 14:1c9, 15:0, 15:1, 16:2, 16:3, 16:4, 18:3n6, 18:4n3, 20:3n3, 20:3n6, 20:4n6, 20:5n3, 22:2n6, 22:3n3, 22:4n6, 22:5n3, 22:5n6, 22:6n3, 24:1n9.

[0130] Table 4 below shows tocopherol content in the crude oil with the purified oil. The methods used to determine the components measured are set forth in Table 4.

**Table 4.** Tocopherol content (ppm)

| Tocopherol        | Crude oil | Purified oil | Test method           |
|-------------------|-----------|--------------|-----------------------|
| Alpha-Tocopherol  | 319       | 294          | AOAC 971.30 with HPLC |
| Beta-Tocopherol   | 53.3      | 47.3         | AOAC 971.30 with HPLC |
| Delta-Tocopherol  | <46.6     | <46.6        | AOAC 971.30 with HPLC |
| Gamma-Tocopherol  | 173       | 141          | AOAC 971.30 with HPLC |
| Total Tocopherols | 545       | 482          | AOAC 971.30 with HPLC |

[0131] Table 5 below shows sterol contents in the crude oil with the purified oil. The methods used to determine the components measured are set forth in Table 5, where “COI/T.20/Doc No.10” is a publicly available test method put forth by the International Olive Council.

**Table 5.** Sterol content

| Sterol                                       | Crude oil | Purified oil | Test method        |
|----------------------------------------------|-----------|--------------|--------------------|
| 24-Methylene-cholesterol (% total sterols)   | <0.01     | <0.01        | COI/T.20/Doc No.10 |
| Apparent Beta-Sitosterol (% total sterols)   | 56.54     | 79.6         | COI/T.20/Doc No.10 |
| Beta-Sitosterol "real" (% total sterols)     | 51.58     | 67.78        | COI/T.20/Doc No.10 |
| Brassicasterol (% total sterols)             | <0.01     | <0.01        | COI/T.20/Doc No.10 |
| Campestanol (% total sterols)                | 0.98      | <0.01        | COI/T.20/Doc No.10 |
| Campesterol(% total sterols)                 | 6.12      | 7.47         | COI/T.20/Doc No.10 |
| Cholesterol (% total sterols)                | <0.01     | <0.01        | COI/T.20/Doc No.10 |
| Clerosterol (% total sterols)                | 0.56      | 1.13         | COI/T.20/Doc No.10 |
| Delta-5,23-stigmastadienol (% total sterols) | <0.01     | <0.01        | COI/T.20/Doc No.10 |
| Delta-5,24-stigmastadienol (% total sterols) | 0.62      | 1.1          | COI/T.20/Doc No.10 |
| Delta-5-avenasterol (% total sterols)        | 3.36      | 8.95         | COI/T.20/Doc No.10 |
| Delta-7-avenasterol (% total sterols)        | <0.01     | <0.01        | COI/T.20/Doc No.10 |
| delta-7-Campesterol (% total sterols)        | <0.01     | <0.01        | COI/T.20/Doc No.10 |
| Delta-7-stigmastenol (% total sterols)       | <0.01     | <0.01        | COI/T.20/Doc No.10 |
| Sitostaol (% total sterols)                  | 0.41      | 0.63         | COI/T.20/Doc No.10 |
| Stigmasterol (% total sterols)               | 36.36     | 12.93        | COI/T.20/Doc No.10 |
| Total sterol - (mg/kg fat)                   | 2630      | 1090         | COI/T.20/Doc No.10 |

[0132] Table 6 below compares the karanjin and pongamol content in the crude oil with the purified oil. The karanjin and pongamol contents were determined in accordance with the protocol described in Example 2 below.

**Table 6.** Karanjin and pongamol content (ppm)

| Pongamia-specific furanoflavonoids | Crude oil | Purified oil |
|------------------------------------|-----------|--------------|
| Karanjin                           | 11,662    | <10          |
| Pongamol                           | 2,288     | <10          |

[0133] Table 7 below compares the color of the crude oil with the purified oil. The method used to determine color are set forth in Table 7.

**Table 7.** Lovibond color description

| Oil type     | Lovibond color |               | Description of color | Test method                          |
|--------------|----------------|---------------|----------------------|--------------------------------------|
|              | Red (0-20)     | Yellow (0-70) |                      |                                      |
| Crude oil    | 1.5            | 70            | brownish red         | AOCS Cc 13b-45 (5.25-inch cell path) |
| Purified oil | 0.5            | 18            | yellow               | AOCS Cc 13b-45 (5.25-inch cell path) |

## Example 2

### Characterization of Pongamia Oil

[0134] This example provides the general protocol for characterizing pongamia oil. The protocol provided herein and set forth in FIG. 1 was used to characterize the crude oil and the purified oil as described in Example 1 above.

[0135] A pongamia oil sample was combined with acetone to prepare an extraction mixture. Then, the extraction mixture was sonicated to extract the liquid portion with karanjin and/or pongamol from the oil. This liquid portion was injected onto the HPLC column equilibrated with 40% acetonitrile for component analysis. Table 8 below summarizes HPLC-DAD settings used.

**Table 8.** Summary of basic HPLC-DAD settings

| Category            | Description                               |
|---------------------|-------------------------------------------|
| Column              | Agilent Poroshell C18, 4.6×100 mm, 2.6 µm |
| Column temperature  | 35 ± 0.5°C                                |
| Mobile phase A      | HPLC Grade Water                          |
| Mobile phase B      | Acetonitrile                              |
| Seal wash           | 90:10 Water:Acetonitrile                  |
| Needle wash         | 90:10 Acetonitrile:Water                  |
| Needle wash time    | 6 seconds (Flush Port)                    |
| Flow rate           | 0.800 mL/min                              |
| Injection volume    | 1.0 µL                                    |
| Signal A wavelength | 304 nm (bandwidth 4 nm)                   |
| Signal B wavelength | 350 nm (bandwidth 4 nm)                   |

[0136] Once the sample extract was loaded onto the C18 column equilibrated with 40% acetonitrile, the relative concentration of acetonitrile was, while maintaining the flow rate at 0.8 mL/min throughout, (i) linearly increased to 90% over 18 minutes, (ii) maintained at 90% for 4 minutes, (iii) linearly decreased to 40% over 1 minute, and (iv) maintained at 40% for 2 minutes, as summarized in Table 9 below.

**Table 9.** Elution program

| Time (min) | Flow volume (mL) | A (%) | B (%) | Flow rate (mL/min) |
|------------|------------------|-------|-------|--------------------|
| 0          | 0                | 60    | 40    | 0.8                |
| 18         | 14.4             | 10    | 90    | 0.8                |
| 22         | 17.6             | 10    | 90    | 0.8                |
| 23         | 18.4             | 60    | 40    | 0.8                |
| 25         | 20               | 60    | 40    | 0.8                |

[0137] Karanjin was observed to elute around 9.6 minutes, corresponding to the relative acetonitrile concentration of around 67%. Pongamol was observed to elute around 14.4 minutes, corresponding to the relative acetonitrile concentration of around 80%. Based on the spectral analysis of elution fractions corresponding to karanjin and pongamol, the ppm concentration of each component was determined.

[0138] Results using this method to characterize the karanjin and pongamol in the crude oil and purified oil of Example 1 above are provided in Table 6 above. FIGS. 3A and 3B also compare the removal of furanoflavonoids and other chemicals from edible oil in the crude oil and purified oil of Example 1 above.

### **Example 3**

#### **Thermal Properties of Purified Pongamia Oil**

[0139] The present example details evaluation of the thermal and temperature-dependent physical properties of purified pongamia oil.

[0140] Purified pongamia oil is obtained in accordance with the protocols described in Examples 1 or 2. Solid fat content (SFC) is measured using Nuclear Magnetic Resonance (NMR) following AOCS-Cd 16b-93. In addition, flash point, dropping point and smoke point are determined on the same sample in accordance with AOCS Cc 9b-55, AOCS Cc 18-80, and AOCS Cc 9a-48, respectively.

[0141] Melting (heating) and crystallization (cooling) profiles of pongamia oil are studied using differential scanning calorimetry (DSC).

### **Example 4**

#### **Pongamia Oil Sensory Evaluation**

[0142] The present example describes a protocol for assessing the organoleptic properties of pongamia oil compositions that may be obtained according the methods of Examples 1 and 2.

[0143] Internal pongamia oil tasting involves the participation of 6 individuals. Each participant is asked to assess each oil sample on the basis of color, turbidity, odor, taste, and overall acceptability. For taste and smell evaluation, the participants perform free choice profiling by blindly tasting each purified pongamia oil sample and assigning attributes that they believe to best describe the flavor of each oil sample. The free choice descriptors are aggregated for each sample.

**Example 5****Food Products**

[0144] This example provides various examples of food products that can be produced using the pongamia oil compositions obtained in accordance with the protocols set forth in Example 1 above. Table 10 provides an exemplary formula for pongamia oil mayonnaise. Table 11 provides an exemplary formula for pongamia oil margarine and spread. Table 12 provides an exemplary formula for pongamia oil salad dressing.

**Table 10.** Pongamia Oil Mayonnaise Formula

| <b>Ingredient</b> | <b>Wt%</b> |
|-------------------|------------|
| Pongamia oil      | 75         |
| Egg yolk          | 6          |
| Vinegar 5% (w/v)  | 10         |
| Salt              | 1.1        |
| Sugar             | 2.5        |
| Water             | 4.5        |
| Mustard           | 1.5        |
| Guar gum          | 0.4        |
| Potassium Sorbet  | 0.07       |
| Sodium benzoate   | 0.03       |

**Table 11.** Pongamia Oil Margarine and Spread Formulas

| <b>Ingredient</b>                                | <b>Wt% in Finished Product</b> |                |                |
|--------------------------------------------------|--------------------------------|----------------|----------------|
|                                                  | <b>80% Fat</b>                 | <b>60% Fat</b> | <b>40% Fat</b> |
| <b>Oil phase</b>                                 |                                |                |                |
| Liquid and fully hydrogenated pongamia oil blend | 79.884                         | 59.584         | 39.384         |
| Soybean lecithin                                 | 0.1                            | 0.1            | 0.1            |
| Soybean oil mono- and diglycerides (IV 5, max.)  | 0.2                            | 0.3            | —              |
| Soybean oil monoglyceride (IV 60)                | —                              | —              | 0.5            |
| Vitamin A palmitate-b-carotene blend             | 0.001                          | 0.001          | 0.001          |
| Oil-soluble flavor                               | 0.015                          | 0.015          | 0.015          |

| Aqueous phase        |      |         |           |
|----------------------|------|---------|-----------|
| Water                | 16.2 | 37.36   | 54.86     |
| Gelatin (250 bloom)  | —    | —       | 2.5       |
| Spray-dried whey     | 1.6  | 1       | 1         |
| Salt                 | 2    | 1.5     | 1.5       |
| Sodium benzoate      | 0.09 | —       | —         |
| Potassium sorbate    | —    | 0.13    | 0.13      |
| Lactic acid          | —    | to pH 5 | to pH 4.8 |
| Water-soluble flavor | 0.01 | 0.01    | 0.01      |

Table 12. Pongamia Oil Salad Dressing Formula

| Ingredient                   | Wt% |
|------------------------------|-----|
| Water                        | 15  |
| Apple vinegar                | 12  |
| Apple juice                  | 12  |
| Honey                        | 10  |
| Pongamia oil                 | 42  |
| Mustard powder               | 5   |
| Xanthan gum                  | 0.5 |
| Pregelatinized potato starch | 0.5 |
| Colourant E-150d             | 0.5 |
| Acidulant E-330              | 0.1 |
| Preservative E-202           | 0.1 |
| Salt                         | 1   |
| Sugar                        | 1.3 |

[0145] The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, “about x” includes and describes “x” *per se*. In some embodiments, the term “about” when used in association with a measurement, or used to modify a value, a unit, a constant, or a range of values, refers to variations of +/- 2% of the stated value or parameter.

[0146] Reference to “between” two values or parameters herein includes (and describes) embodiments that include those two values or parameters per se. For example, description referring to “between x and y” includes description of “x” and “y” per se.