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(54) Titre : PROCEDE AMELIORE D'EXTRACTION DE PROCYANIDINES DE CACAO
(54) Title: AN IMPROVED METHOD FOR EXTRACTING COCOA PROCYANIDINS

(57) Abrégé/Abstract:
A cocoa extract which is rich in procyanidin oligomers is made by extracting defatted cocoa beans with solvents or aqueous solvents which solubilize the procyanidins. The yield of procyanidins in an extract varies with the cocoa beans used, the solvent used, the pH of the solvent used and the extraction temperature. Extraction parameters can be optimized to increase procyanidin yield and to allow for the preferential extraction of higher or lower molecular weight oligomers. A preferred extraction method is a counter-current solvent extraction process in which the cocoa extract is recycled.

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(54) Title: AN IMPROVED METHOD FOR EXTRACTING COCOA PROCYANIDINS

(57) Abstract: A cocoa extract which is rich in procyanidin oligomers is made by extracting defatted cocoa beans with solvents or aqueous solvents which solubilize the procyanidins. The yield of procyanidins in an extract varies with the cocoa beans used, the solvent used, the pH of the solvent used and the extraction temperature. Extraction parameters can be optimized to increase procyanidin yield and to allow for the preferential extraction of higher or lower molecular weight oligomers. A preferred extraction method is a counter-current solvent extraction process in which the cocoa extract is recycled.

AN IMPROVED METHOD FOR EXTRACTING COCOA PROCYANIDINS

BACKGROUND OF THE INVENTION

Field Of The Invention

This invention is directed to improved methods for the extraction of cocoa polyphenols including catechin, epicatechin and cocoa procyanidin oligomers from cocoa solids.

It is known that regular consumption of dietary polyphenols, commonly found in a variety of fruits and vegetables, is beneficial. Red wine, green tea and cocoa have all been identified as being rich in polyphenols, and the regular consumption of red wine and green tea have both been shown to be inversely associated with heart disease deaths in industrialized countries.

Description of Related Art

It is well-known that the polyphenols of cocoa contribute significantly to the development of flavor in the fermented and roasted cocoa bean. Astringent and bitter flavors in cocoa have been traditionally associated with the presence of xanthine alkaloids and polyphenols in the cocoa beans. For this reason, various methods have been developed over the years to extract the cocoa polyphenols to verify their presence, to quantify their amounts, and to identify them. The cocoa polyphenols are primarily cocoa procyanidins. However, no extraction method has thus far been optimized to yield extracts high in cocoa procyanidins.

It is now known that the cocoa procyanidin oligomers show a clear relationship between structure and function, meaning that individual oligomers, or fractions containing several oligomers of a similar size, show specific biological functions which are not affected by other oligomers. Thus, it is important to ensure that the extraction procedure utilized not only results in the highest possible solubilization of the cocoa polyphenols, but that it is effective at extracting all the cocoa polyphenol oligomers present in the bean.

The extraction of cocoa beans using water or an organic solvent, or a mixture of water and an organic solvent, has been used to remove the xanthine alkaloids (predominantly caffeine and theobromine) and other soluble constituents of the cocoa bean which impart a bitter, disagreeable flavor. Included among these bitter-tasting soluble constituents are the procyanidins.

US Patent No. 1,750,795 (issued to Defren in 1926), discloses a process for removing the "greater part of the soluble bitter constituents of the beans" by soaking the beans in water at 60°C and then discarding the water and roasting the beans.

Hot water treatments have been used to remove the xanthines in order to provide stimulant-free cocoa beans. See US Patent No. 4,407,834 "Detheobromination of Cocoa" (issued to Chiovini et al., June 28, 1983) and US Patent No. 4,755,391 "Removal of Methylxanthines from Cacao Materials" (issued to Chiovini et al, July 5, 1988).

Cocoa extracts have been prepared by extracting cocoa solids, prepared from fermented, conventionally roasted cocoa beans or cocoa nibs, with water and/or alcohols. Osakabe et al. used ethanol, either absolute or aqueous at greater than 40% v/v with deionized water at ambient temperature to prepare a crude polyphenol extract. See JP 94-64717 "Food or Beverage Product for Preventing Gastric Ulcers" (October 4, 1995).

Zieglader et al. used methanol to extract the polyphenols from cocoa beans at ambient temperature. See "Antioxidative Effects of Cocoa" (Rev. Choc. Confect. Bak, 8:3-6, 1983) which discloses the preparation of a methanol extract. The extract contained "monomer tannin precursors (catechins, anthocyanidins and their soluble condensates)". It is used as an additive for oil to preserve it from oxidation. Griffiths et al. used methanol extracts, again obtained at ambient temperatures, to obtain polyphenol extracts from ripe cocoa nibs, which were used to study the characterization of plant polyphenols in cocoa and other plants. See "A Comparative Study of the Seed Polyphenols of the Genus *Theobroma*", (Biochemical J. 74: 362-365, 1960). Rigaud et al. made an extract from lyophilized cocoa beans and grape seeds, and noted that the use of methanol as a solvent precluded the presence of the higher oligomers. See "Normal-Phase High-Performance Liquid Chromatographic Separation of Procyanidins from Cacao Beans and Grape Seeds" (J. Chromatography 654:255-60, 1993). Jalal & Collin prepared extracts from different parts of the cocoa plant in order to analyze the polyphenols present in each part of the plant. The extraction was carried out using 70% cold methanol, followed by ethyl acetate. See "Polyphenols of Mature Plant, Seedling and Tissue Cultures of *Theobroma Cacao*" (Phytochemistry, 16:1377-1380, 1977).

Acetone/water has also been used for cocoa bean extractions. Clapperton et al. report the extraction of defatted cocoa powder made from fermented cocoa beans using cold 70% acetone. See "Polyphenols and Cocoa Flavor, Groupe Polyphenols," (XVIth Intern. Conf., Lisbon, Portugal, July 13-16, 1992). Rigaud combines a first extraction with ethanol with a second extraction using a 60% acetone/water mixture. See "Normal-Phase High-Performance Liquid Chromatographic Separation of Procyanidins from Cacao Beans and Grape Seeds" (J. Chromatography, 654:255-60, 1993).

Traditionally, cocoa beans are treated and processed in such a way as to minimize the bitter taste which the polyphenols impart to the beans, and this results in a reduction of the polyphenol content of the bean. The two ways in which the polyphenol content is significantly reduced are fermentation of cocoa beans in their husks and roasting the fermented cocoa beans in order to crack their husks and aid the de-hulling process. Zieglader at al. report a loss of antioxidant activity in extracts from cocoa beans which are fermented compared to extracts from unfermented beans, and correlate this reduction in antioxidant potential with a reduced amount of polyphenols in the extracts of fermented beans. All the previously discussed extraction methods were carried out using fermented beans, and in many cases the beans were also roasted. Therefore, the procyanidin yields are much lower than those found in extracts from unfermented beans.

It has been a common practice to follow an initial solvent extraction step with an ethyl acetate extraction step. See Forsyth & Roberts, "Cacao Polyphenolic Substances: 3. The Structure of Cacao Leucocyanidin 1" Biochem. J. 74, 374-378, 1960; Thompson et al., "Plant Procyanidins. Part I. Introduction; the Isolation, Structure and Distribution in Nature of Plant Procyanidins" J. Chem. Soc. Perkin I, Vol. 11, 1387-99, 1972; Jalal & Collins, "Polyphenols of Mature Plant, Seedling and Tissue Cultures of *Theobroma Cacao*", Phytochemistry, 16:1377-1380, 1977, Porter et al. "Flavans and Proanthocyanidins" Chapter Two *in* "The Flavonoids", Ed., J.B. Harborne, Chapman and Hall Ltd., London, 1988; US Patent 5,554,645 (issued October 3, 1994 to Romanczyk et al). This has the effect of producing an extract which contains the monomers and lower oligomers and none, or very little, of the higher oligomers. See Lea A. "The Phenolics of Ciders: Oligomeric and Polymeric Procyanidins", J. Sci. Fd Agric. 29, 471-477, 1978.

Whether or not the cocoa beans are defatted prior to extraction has an impact upon the yield of polyphenols extracted. If the beans are not defatted, the fat in the beans interferes with the solubilizing activity of the solvent, and the polyphenol yields are considerably reduced (Lazarus et al., "Flavonoids and Other Polyphenols" in Methods in Enzymology series. Edited by Lester Packer, Academic Press, New York, May 2001).

Cocoa extracts have been prepared from cocoa solids prepared from unfermented or fermented sun dried cocoa beans. The beans were ground, defatted and extracted. A 70% acetone/30% deionized water mixture was used, followed by a 70% methanol extraction and two chloroform extractions. Then follows an extraction with ethyl acetate, the addition of water, and the removal of the ethyl acetate. Alternatively, the beans were extracted with 70% acetone. In both cases the extractions were carried out at room temperature and the aqueous extracts were

freeze-dried. See U.S. 5,554,645 (issued October 3, 1994 to L. Romanczyk et al.) and WO 98/09533 published March 12, 1998.

Furthermore, cocoa extracts can be prepared from partially defatted cocoa solids that are prepared from cocoa beans which have not been roasted. The resulting cocoa solids, whether prepared from fermented, underfermented, or unfermented cocoa nibs, have a higher cocoa polyphenol content, i.e., cocoa procyanidin content, than cocoa solids prepared from conventionally roasted cocoa beans or cocoa nibs. See U.S. Patent No. 6, 015, 913 (issued January 18, 2000 to K.S. Kealey et al.).

Defatted, freeze-dried, unfermented cocoa beans contain about 2% xanthine alkaloids, and traditionally processed beans contain larger amounts. Theobromine is a degradation product of caffeine, and both are well-known stimulants of the nervous system. Excessive xanthine alkaloid intake is not thought to be beneficial. Hence, in some cases, it may be desirable to prepare cocoa extracts free of the xanthine alkaloids.

Thus, there is a need for an improved process to extract cocoa procyanidins from cocoa solids, and, in some cases, to decaffeinate and detheobrominate the cocoa extracts.

BRIEF SUMMARY OF THE INVENTION

The present invention provides an improved method for extracting cocoa polyphenols including catechin, epicatechin, and cocoa procyanidin oligomers from partially or fully defatted cocoa solids prepared from non-roasted cocoa beans. The improved process comprises an extraction, at atmospheric pressure or under pressure, with an acidified solvent capable of solubilizing the polyphenols or acidified aqueous solutions containing the solvent. Preferably, an acid is added to the solvent or aqueous-solvent solution in an amount sufficient to lower the pH from about 6.5 to about 2 to about 4. An edible or physiologically acceptable acid is preferred.

The cocoa solids are prepared from unfermented, underfermented, or fermented cocoa beans. The organic solvent may be selected from the group consisting of lower alkyl alcohols, lower alkyl ketones, and lower alkyl acetates. Suitable solvents include methanol, ethanol, isopropanol, acetone, methyl acetate, and ethyl acetate. The aqueous-solvent solution preferably contains only up to about 50% water by volume; however, it may contain more water, but with most aqueous solutions the extraction efficiency will be lower. Preferred aqueous-organic solvents include about 50 to about 70% isopropanol, about 80% to about 100% acetone and about 80% to about 95% ethanol. The use of ethanol alone preferentially extracts cocoa procyanidin oligomers higher than the heptamers. The mixture of about 80% isopropanol and about 20% water preferentially extracts monomers and oligomers up to and including hexamers

The present invention also provides a method of extracting cocoa polyphenols including catechin, epicatechin, and cocoa procyanidin oligomers from partially defatted or fully defatted cocoa solids prepared from cocoa beans that have not been roasted. The method comprises the step of extracting, at atmospheric pressure or under pressure, the cocoa solids with a non-acidified organic solvent capable of solubilizing the cocoa polyphenols. Preferably, the cocoa beans are unfermented or underfermented cocoa beans; however, fermented cocoa beans can be used but there will be fewer higher oligomers in the extract.

The underfermented cocoa beans typically have a fermentation factor of 275 or less. The preferred underfermented cocoa beans include slaty, purple, or purple brown cocoa beans, or mixtures of slaty and purple cocoa beans, purple and brown cocoa beans, or slaty, purple and brown cocoa beans. The unfermented or underfermented cocoa beans contain at least about 1% up to about 15-17% by weight of total cocoa procyanidins per gram of defatted cocoa solids, typically about 4-7%.

The solvent may be selected from the group consisting of a lower alkyl alcohol, a lower alkyl ketone, and a lower alkyl acetate. Suitable solvents are methanol, ethanol, isopropanol, acetone, methyl acetate, and ethyl acetate. The aqueous solvent solution can contain up to about

50% water by volume; however, more water can be present but the total amount of cocoa polyphenols extracted will not be as great. Preferred solvents include 50-60% acetone and 100% ethanol. The extraction can be carried out at a temperature from 0°C up to the boiling point of the solvent or aqueous solvent solution used at the extraction pressure used. When the solvent is methanol, the extraction is preferably carried out at room temperature. When the solvent is ethanol, the extraction is preferably carried out at about 20°C to about 50°C. When the solvent is isopropanol, the extraction is preferably carried out at about 70°C. When the solvent is an aqueous-acetone solution, the extraction is preferably carried out at about 50°C up to the boiling point of the solution.

In the above processes, when an acidified solvent or aqueous solvent solution is used, a lower extraction temperature is preferred. When a non-acidified solvent or aqueous solvent solution is used, a higher extraction temperature can be used.

The present invention also provides a method for selectively extracting low molecular weight cocoa procyanidin oligomers from partially or fully defatted cocoa solids. The solvent used is a solvent which preferentially extracts the lower oligomers, e.g., methyl acetate or ethyl acetate. Preferably, the cocoa beans are unfermented or underfermented cocoa beans; however, fermented cocoa beans can be used. The extraction is preferably carried out at about 20°C to about 50°C. The resulting cocoa extracts consist essentially of monomers, dimers, and trimers.

The present invention also provides a method for selectively extracting higher molecular weight cocoa procyanidin oligomers from partially defatted or fully defatted cocoa solids prepared from cocoa beans that have not been roasted. The method comprises the steps of (a) extracting the cocoa solids with ethyl acetate; (b) recovering the extracted cocoa solids; (c) extracting the recovered cocoa solids with a solvent which is a good solvent for the higher oligomer, e.g., acetone, ethanol, and mixtures thereof with up to 50% water; (d) separating the cocoa solids from the cocoa extract; and (e) optionally drying the cocoa extract. The extraction is preferably carried out at about 20°C to about 50°C. Preferably the cocoa beans are unfermented or underfermented cocoa beans; however, fermented cocoa beans can be used. The cocoa extracts consist essentially of at least tetramers and higher oligomers.

The present invention further provides a continuous method for extracting, at atmospheric pressure or under pressure, cocoa polyphenols including catechin, epicatechin, and cocoa procyanidin oligomers from partially defatted or fully defatted cocoa solids using a solvent suitable for solubilizing cocoa procyanidins or aqueous solutions of the solvent. The process comprises the steps of (a) contacting a series of cells filled with the cocoa solids containing varying amounts of cocoa polyphenols with the solvent flowing in a counter-current direction,

and (b) recycling the cocoa extract. The liquid cocoa extract can be dried. Preferred solvents include lower alkyl alcohols, lower alkyl ketones, and lower alkyl acetates. Suitable lower alkyl alcohols are methanol, ethanol, and isopropanol. Ethanol is preferred. A suitable lower alkyl ketone is acetone. Suitable lower alkyl acetates are methyl acetate and ethyl acetate. Preferably, the cocoa extract is recycled until substantially no more cocoa polyphenols including epicatechin, catechin, and/or cocoa procyanidins are extracted from the cocoa solids.

The cocoa extracts are useful as liquid extracts, referred to as "tinctures" when the solvent is an alcohol, as an aqueous solution when the solvent is removed, or as a water-soluble dry powder after removal of the solvent or solvent and water. When a solvent is used, the cocoa extract is dried by flashing off the solvent. When an aqueous-solvent solution is used, the cocoa extract is dried by flashing off the solvent before drying the aqueous extract. The aqueous extracts can be dried by freeze drying, spray drying, flash drying, or drum drying.

The present invention provides a method for recovering cocoa procyanidins and theobromine from partially defatted or fully defatted cocoa solids prepared from cocoa beans. The process comprises the steps of (a) extracting the cocoa solids with ethanol at about 50°C to about 70°C, (b) chilling the ethanol extract at a temperature and for a time sufficient to precipitate out the theobromine, (c) separating the precipitated out theobromine, and (d) optionally removing the ethanol from the extract to recover the dried cocoa procyanidins. Preferably, the beans are unfermented or underfermented cocoa beans that have not been roasted; however, fermented cocoa beans and fermented roasted can be used.

The present invention also provides a method of minimizing the amount of theobromine in the cocoa procyanidins extracted from partially defatted or fully defatted cocoa solids. The method comprises the steps of (a) extracting the cocoa solids with isopropanol to produce an alcoholic extract containing cocoa procyanidins and theobromine, (b) chilling the alcoholic extract at a temperature and for a time sufficient to precipitate out the theobromine, and (c) separating the theobromine from the alcoholic extract.

In the above processes, the chilling is carried out for up to about 12 hours at 0° to about 5°C and the separating step is carried out by filtering the extract, centrifuging the extract, or by an adsorbing the theobromine on a suitable absorbent.

In one particular embodiment there is provided a process for selectively extracting low molecular weight cocoa procyanidin oligomers from partially or fully defatted cocoa solids, which process comprises the step of extracting the cocoa solids with a solvent which is methyl acetate or ethyl acetate.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1(a) is a graph showing the effect of temperatures of 0°, 20°, 50°, and 70°C on the oligomer recovery of monomer, pentamer, and decamer using 100% ethanol as the extraction solvent.

FIG.1(b) is a graph showing the effect of temperatures of 0°, 20°, 50°, and 70°C on the % oligomeric recovery of monomer, pentamer, and decamer using 100% isopropanol as the extraction solvent.

FIG. 1(c) is a graph showing the effect of temperatures of 0°, 20°, 50°, and 70°C on the % oligomeric recovery monomer, pentamer, and decamer using 100% methanol as the extraction solvent.

FIG. 2 is a graph showing that acetone and ethanol are most effective in extracting the higher oligomers. The recovery is reported as % yield of standard acetone:water:acetic acid (70:29.5:0.5v/v).

FIG. 3 is a graph showing the effects of fermentation and cocoa bean processing on the amount of monomer and procyanidin oligomers extracted using an acetone:water:acetic acid as the extraction solvent. The results are reported as % of yield from unfermented cocoa beans.

FIG. 4 is a graph showing the effect of fermentation and cocoa bean processing on the amount of monomer and procyanidin oligomer extracted using boiling water as the extraction solvent. The results are reported as % of yield from unfermented cocoa beans.

FIG. 5. is a schematic diagram of the process of extracting polyphenols from cocoa beans.

FIG. 6 is a schematic diagram showing the process of detheobrominating a cocoa extract. The theobromine-laden solvent vapor flows through the 3-way valve to the chilled plates on the left. The flow is then switched to the right side. The solvent is then purged from the left side. The left door of the chiller is opened and the solid crude theobromine is scraped from the chiller plates or washed off with a solvent and recovered in liquid form. The same steps are repeated on the right side. The solvent is recovered.

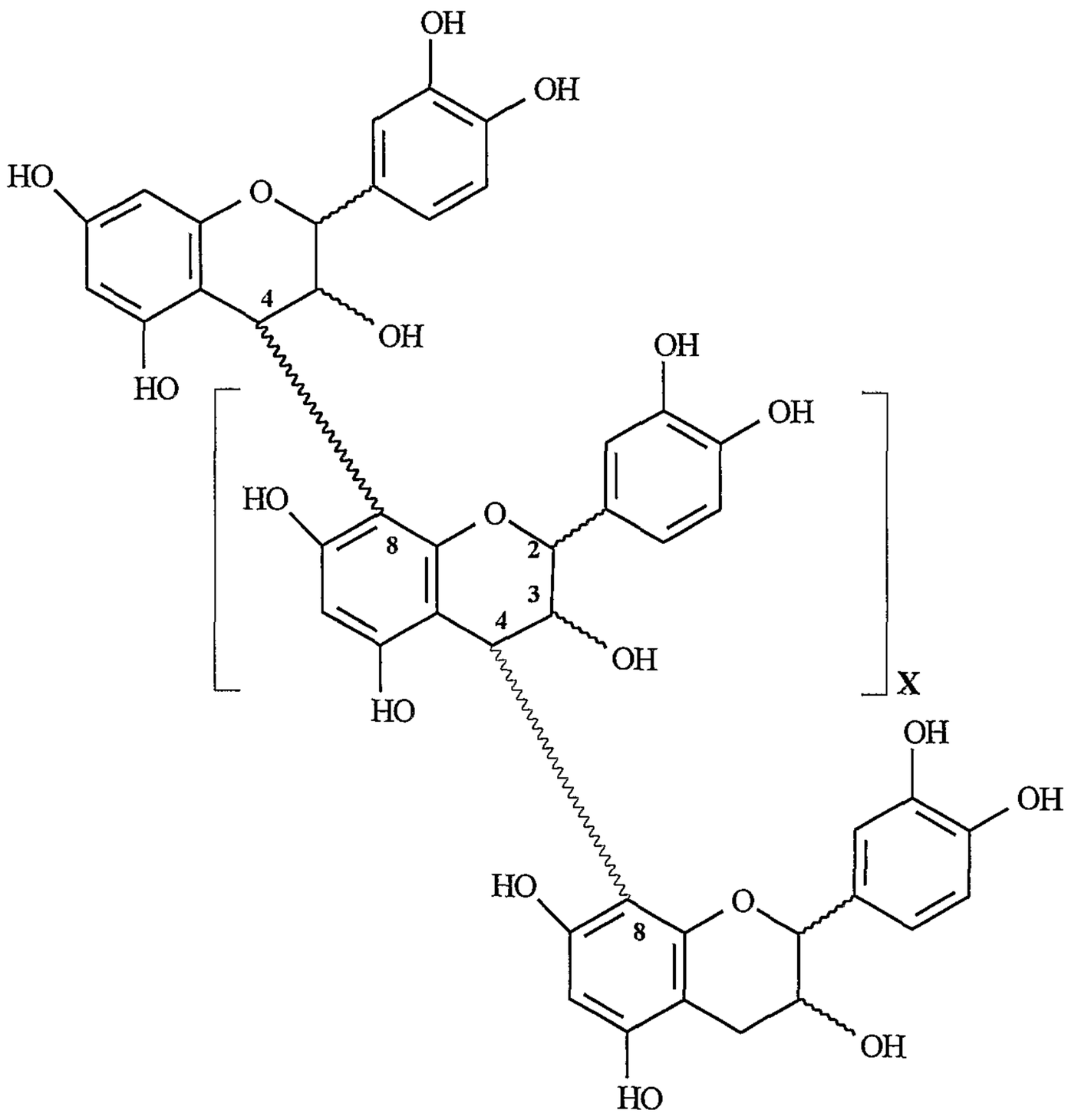
FIG. 7 is a graph comparing the oligomeric profiles of cocoa solids an extract prepared by extracting at 50°C cocoa solids prepared from unfermented cocoa beans with ethyl acetate with an extract prepared by then extracting the ethyl acetate-extracted, dried cocoa solids with

acetone:water:acetic acid (70:29.5:0.5). The % recovery is compared to an acetone:water:acetic acid (70:29.5:0.5) extract of cocoa solids from unfermented cocoa beans.

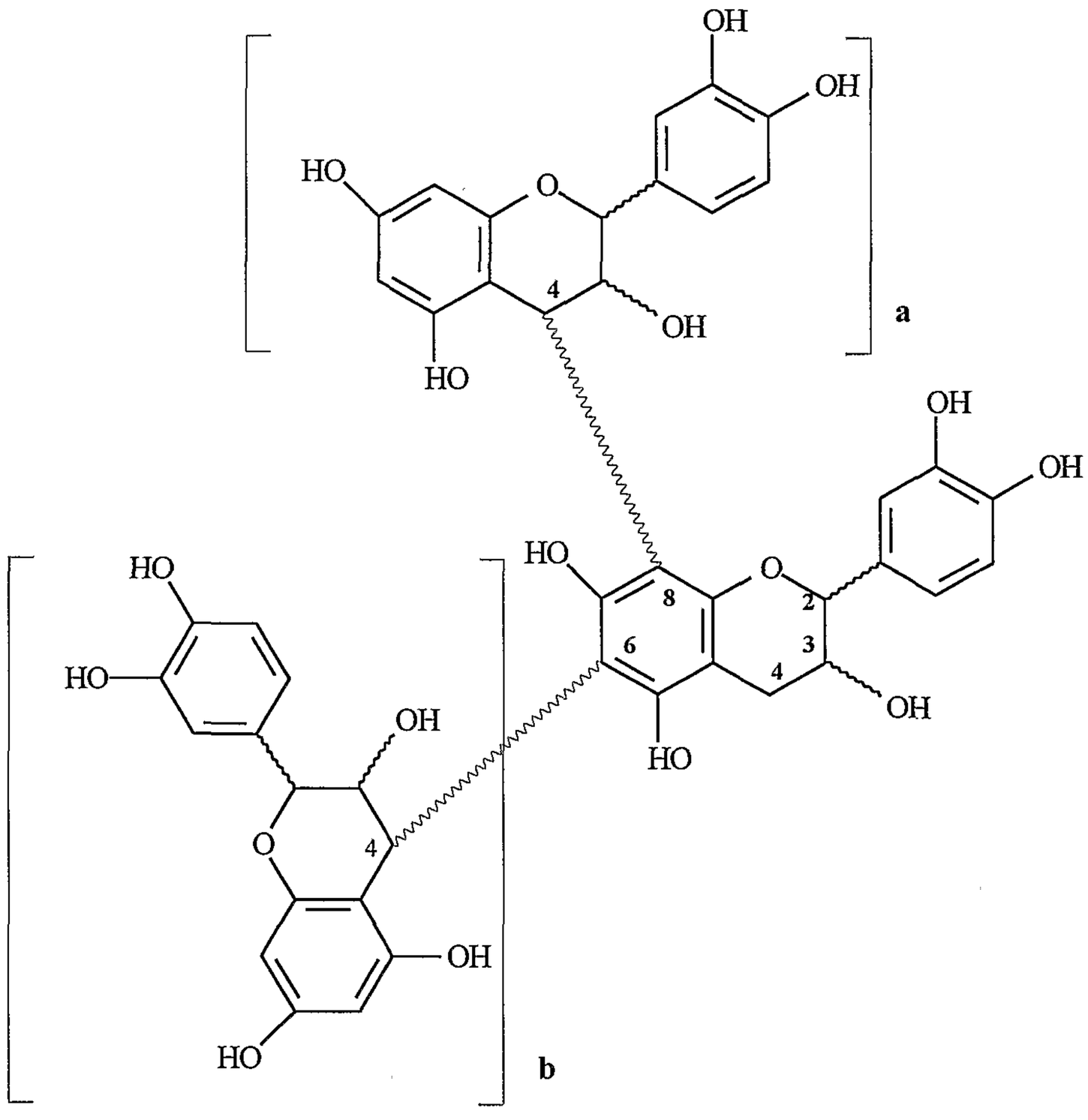
FIG. 8 is a comparison of the monomer and oligomer yields from the extractions of cocoa solids prepared from fermented and underfermented cocoa beans using 80% ethanol:20%water as the solvent.

DETAILED DESCRIPTION OF THE INVENTION

Cocoa polyphenols including cocoa procyanidins can be obtained from several *Theobroma cacao* genotypes by the procedures discussed hereinafter. The monomers include (+)catechin, (-)-epicatechin and their respective epimers (i.e., (-)-catechin and (+)-epicatechin). The procyanidin oligomers which have been identified in cocoa extracts include the dimers through the octadecamers. The oligomers may be linear or branched. The linear 4→8 oligomers have the structure:

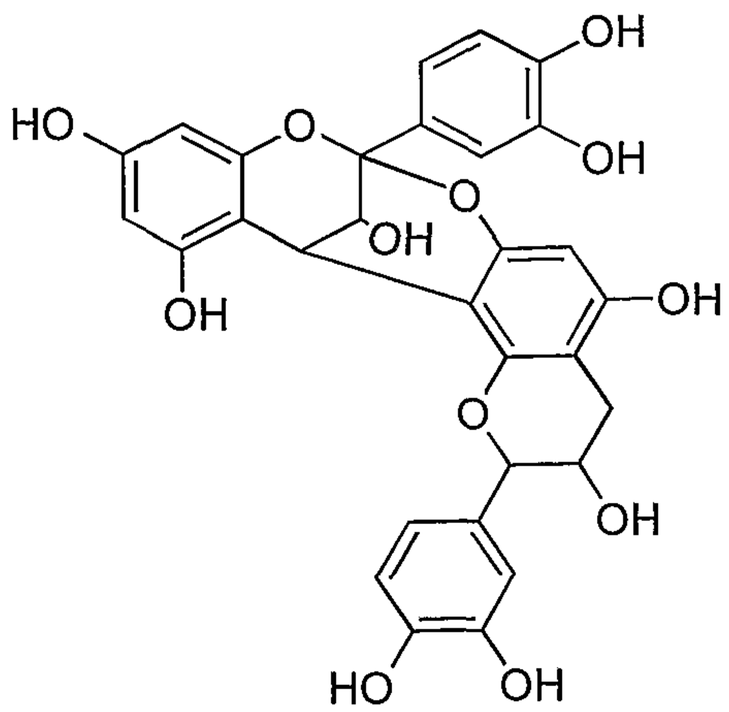


where x is an integer from 0-16. The branched oligomers have the structure:



where a and b are independently integers from 0-15.

Structural variations to procyanidin oligomers may also occur with the formation of a second interflavonoid bond by carbon-oxygen oxidative coupling to form A-type oligomers, as shown below (Porter et al. "Flavans and Proanthocyanidins" Chapter Two *in* "The Flavonoids", Ed., J.B. Harborne, Chapman and Hall Ltd., London, 1988; Porter In Methods in Plant Biochemistry, Vol. I. Plant Phenolics. Dey and Harborne, Eds; Academic Press: San Diego, Ca, 1989). Due to the complexity of this conversion, A-type proanthocyanidins are not as frequently encountered as the single-bonded oligomers:



Cocoa beans can be divided into four categories based on their color: predominately brown (fully fermented), purple/brown, and purple (underfermented), and slaty (unfermented). Preferably, the cocoa solids used for the maximum extraction of the cocoa procyanidins are prepared from unfermented or underfermented cocoa beans (i.e., slaty cocoa beans, purple cocoa beans, mixtures of slaty and purple cocoa beans, mixtures of purple and brown cocoa beans, or mixture of slaty, purple, and brown cocoa beans). More preferably, the cocoa beans are slaty and/or purple. Unfermented cocoa beans have a higher cocoa polyphenol content than fermented cocoa beans (Kim and Keeney J. Food Sci. 49 1090, 1984; Porter et al., "Cacao Procyanidins: Major Flavonoids and Identification of Some Minor Metabolites" Phytochemistry, Vol. 30, No. 5, 1657-1663, 1991).

The cocoa polyphenol content of cocoa beans or blends thereof is higher when the beans have a fermentation factor of 275 or less. The "fermentation factor" is determined using a grading system for characterizing the fermentation of the cocoa beans. For example, slaty beans are designated as 1, purple beans as 2, purple/brown beans as 3, and brown beans as 4. The percentage of beans falling within each category is multiplied by the weighted number. Thus, the "fermentation factor" for a sample of 100% brown beans would be 100 x 4 or 400, whereas for a 100% sample of purple beans it would be 100 x 2 or 200. A sample of 50% slaty beans and 50% purple beans would have a fermentation factor of 150 [(50 x 1) + (50 x 2)].

Preferably, the unfermented or underfermented cocoa beans will contain at least 1% by weight of total cocoa procyanidins based on the weight of the nonfat cocoa solids, preferably 3-7% by weight, more preferably 7-12% by weight, and most preferably 13-17% by weight.

A method for preparing a cocoa mass suitable for extraction is described in U.S. Patent Number 5,554,645 (issued September 10, 1996 to Romanczyk et al.). Harvested cocoa pods are opened and the beans with pulp are removed for freeze-drying. The pulp is manually removed from the freeze-dried mass and the beans are subjected to the following manipulations. The

freeze-dried cocoa beans are first manually dehulled and ground to a fine powdery mass with a TEKMAR Mill. The resultant mass is then defatted overnight by Soxhlet extraction using, for example, redistilled hexane as the solvent. Residual solvent is removed from the defatted mass by vacuum at ambient temperature. Alternatively, the cocoa beans are sun dried under conditions which retard fermentation and the beans are mechanically dried and then dehulled.

Partially defatted or nonfat cocoa solids having a higher cocoa polyphenol content, i.e., a high cocoa procyanidin content, can be obtained by processing the cocoa bean or cocoa nib without a roasting step and then milling the beans to chocolate liquor and recovering partially defatted cocoa solids, or by screw pressing the roasted beans or nibs to partially defatted cocoa solids without the milling step. Even higher levels of procyanidins can be achieved if underfermented cocoa beans are used in this process. This method conserves the cocoa polyphenols because it omits the traditional roasting step. The method consists essentially of the steps of: (a) heating the cocoa beans to an internal bean temperature just sufficient to reduce the moisture content to about 3% by weight and loosen the cocoa shell, typically using a infra red heating apparatus for about 3 to 4 minutes; (b) winnowing the cocoa nibs from the cocoa shells; (c) screw pressing the cocoa nibs; and (d) recovering the cocoa butter and partially defatted cocoa solids which contain cocoa polyphenols including cocoa procyanidins. Optionally, the cocoa beans are cleaned prior to the heating step, e.g., in an air fluidized bed density separator. Preferably, the cocoa beans are heated to an internal bean temperature of about 100°C to about 110°C, more preferably less than about 105°C. The winnowing can be carried out in an air fluidized bed density separator. The above process of heating the cocoa beans to reduce the moisture content and loosen the cocoa shell is disclosed in U.S. Patent No. 6,015,913 (issued January 18, 2000 to Kealey et al.).

The internal bean temperature (IBT) can be measured by filling an insulated container such as a thermos bottle with beans (approximately 80 – 100 beans). In order to maintain the temperature of the beans during transfer from the heating apparatus to the thermos, the insulated container is then appropriately sealed in order to maintain the temperature of the sample therein. A thermometer is inserted into the bean filled insulated container and the temperature of the thermometer is equilibrated with respect to the beans in the thermos. The temperature reading is the IBT temperature of the beans. IBT can also be considered the equilibrium mass temperature of the beans.

In the context of this application, the following definitions apply. As used herein, "higher oligomers" include the procyanidin hexamers to the decamers or higher and "lower oligomers" refer to procyanidins from the dimers up to and including the pentamers. As used herein, a

"cocoa extract" is an extract containing an unspecified mixture of compounds which are extracted from ground cocoa beans, preferably dehulled beans, by slurrying defatted, cocoa solids with a solvent or an aqueous solvent solution and removing the cocoa solids to recover a liquid extract. The liquid extract can be dried by conventional methods known to those skilled in the art.

The extraction process comprises the steps of slurrying the partially defatted or nonfat cocoa solids with an appropriate solvent or aqueous solvent to solubilize the cocoa polyphenols, separating the extracted cocoa solids from the solvent phase, for example by centrifuging, and optionally removing the solvent(s). Preferably, the extraction process is a continuous process.

The solvent used can be solvent, preferably a food grade solvent, which will solubilize the epicatechin and catechin monomers and cocoa procyanidin oligomers present in the cocoa solids. Preferably, the solvent is chosen from the group, consisting of lower alcohols such as methanol, ethanol, and isopropanol, acetates such as methyl or ethyl acetate, and acetone. The solvents can be used as aqueous solutions or undiluted. Surprisingly, preferable aqueous dilutions vary with the solvent used.

Acidification of the solvent or aqueous solvent solution to a pH of about 2 to about 4 can either improve or reduce the efficiency of the procyanidin extraction depending upon the solvent used in the extraction procedure. The presence or absence of 0.5% acetic acid in the solvent or aqueous solvent solution had a variable effect on the extraction efficacy depending on the solvent being used and the solvent concentration. The acid used in the extraction can be any non-mineral acid. Preferred acids are food grade or physiologically acceptable acids such as formic acid, citric acid, phosphoric acid and acetic acid. Acids are not preferred if the extraction is carried out at a higher temperature.

The cocoa extracts can be detheobrominated and decaffeinated and these methylxanthines can be recovered and purified. The preferred solvent differs according to whether or not the theobromine is to be discarded or recovered. If the theobromine is to be discarded, the best extraction solvent is isopropanol at a low temperature. However, if the theobromine is to be recovered for purification, the best solvent is ethanol and the extraction should be carried out at between 50-70°C. The optimum conditions for removal of the methylxanthines must be balanced against the optimum conditions for procyanidin extraction to provide an effective simple process for the production of a theobromine-free cocoa polyphenol extract.

Alternatively, the methylxanthines can be removed from the extract using gel permeation chromatography, as described in US Patent 5,554,645 (issued to Romanczyk et al. on September 10,1996). Briefly, the partial purification process is carried out using liquid chromatography on

Sephadex™ LH 20 (28 x 2.5 cm). Separations are aided by a step gradient into deionized water. The initial gradient composition started with 15% methanol in deionized water, which was followed step-wise every 30 minutes with 25% methanol in deionized water, 35% methanol in deionized water, 70% methanol in deionized water, and finally 100% methanol. The effluent following the elution of caffeine and theobromine was collected as a single fraction which represents a xanthine alkaloid free sub-fraction of the original extract.

The methylxanthines can also be removed from the cocoa extract by adsorbing them onto a solid adsorbent, after which the extract, substantially free of caffeine and theobromine, is washed through the adsorbent. Various solid adsorbents can be used in the process, such as polymeric resins and activated carbon. Preferably, the adsorbent is substantially neutral in water. An example of a neutral resin adsorbent is semi-calcined resin XE-340 (manufactured by Rohm & Haas). Neutral activated carbon can be obtained either by acid washing of thermally activated carbon followed by rinsing with water to neutrality or by neutralization of acid-activated carbon with an aqueous alkali followed by rinsing with water to neutrality.

The solvent extraction of the defatted cocoa solids can be carried out using either a batch process or a continuous process, preferably a counter-current process. In the batch process a large solvent to solids ratio is required to extract the cocoa polyphenols from the cocoa solids. Additionally, several consecutive extractions are usually required to extract all of the available cocoa polyphenols including the procyanidins. The preferred method of extraction is a continuous counter-current extraction. In this process the efficiency of extraction and the cost-effectiveness of the process is optimized, allowing it to be scaled up for manufacturing purposes. Use of a continuous counter-current flow of solvent through the cocoa solids allows the solvent to solids ratio to be much lower since the solvent can be continuously recycled. Additionally, due to the continuous flow of the solvent through the cocoa solids, it is not necessary to perform dual solvent extractions or to wash the solids with an aqueous solvent to ensure that all the procyanidins are extracted from the cocoa solids.

As will be shown hereafter, the efficacy of the procyanidin extraction varies significantly depending upon which solvent is used. Furthermore, it has been surprisingly found that while some solvents will preferentially extract the higher oligomers, other solvents will preferentially extract the monomers and lower oligomers. The type of solvent used, whether or not the solvent is an aqueous solution, the temperature at which the extraction is carried out, and other factors can affect the selective extraction of high or low molecular weight procyanidin oligomers. By sequentially carrying out the extraction of the same cocoa powder with two separate solvents, one of which extracts monomers and the lower oligomers and one of which extracts the higher

oligomers, it is possible to obtain separate procyanidin fractions during the same extraction procedure without having to subject the extract to complicated separation methods to separate the higher and lower oligomers. Thus, a further embodiment of the invention is a process by which oligomers can be selectively extracted from cocoa solids in a large-scale, single step process which avoids the costly and complex process of chromatographically separating the oligomers.

Test Procedures

Control Extract

In the examples which follow all solvent extractions were compared to control extracts which were prepared by extracting unfermented, freeze-dried, defatted, cocoa beans prepared using the procedures described in Part A of Example 1. The solvent used was a mixture of 70% acetone, 29.5% water, and 0.5% acetic acid. All aqueous solutions are expressed as % (v/v). Ten grams of defatted cocoa mass were slurried with 100 mL of this solvent mixture and sonicated for 5-10 min. at 50°C. The slurry was centrifuged for 15 min. at 4° C at 3000 x G and the supernatant was passed through glass wool. The yields of crude procyanidins ranged from 15-20%.

Separation And Quantification Of The Procyanidin Oligomers

In the examples which follow, the analytical methods described below were used to separate and quantify, by degree of polymerization, the monomers and procyanidin oligomers in the cocoa extracts. The method, described below for identifying procyanidin oligomers in the cocoa extract is based upon work reported in Hammerstone, J. F. et al, "Identification of Procyanidins in Cocoa (*Theobroma cacao*) and Chocolate Using High-Performance Liquid Chromatography/Mass Spectrometry", *J. Ag. Food Chem.*; 1999; 47 (10) 490-496. The analytical methods described below were used in a qualitative study of a broad range of food and beverage samples reported to contain various types of proanthocyanidins, as reported in Lazarus, S. A., et al. "High-performance Liquid Chromatography/Mass Spectrometry Analysis of Proanthocyanidins in Foods and Beverages", *J. Ag. Food Chem.*; 1999; 47 (9); 3693-3701. The method of Lazarus et al. (1999) reported analysis using fluorescence detection because of higher selectivity and sensitivity.

The samples were analyzed using the analytical method reported in Adamson, G.E. et al., "HPLC Method for the Quantification of Procyanidins in Cocoa and Chocolate Samples and Correlation to Total Antioxidant Capacity", *J. Ag. Food Chem.*; 1999; 47 (10) 4184-4188. Samples were then compared directly to the control extract to accurately determine the levels of

monomers and procyanidin oligomers in comparison to the monomers and procyanidin oligomers in the control extract.

The total amount of procyanidin monomers and oligomers in an extract can also be determined using the method reported in Adamson et al. However, instead of comparing sample levels with those of the control extract, the samples are compared with composite standard stock solutions of each of the monomers and oligomers, thus allowing the absolute amount of each procyanidin present in the extract to be calculated. Composite standard stock solutions and calibration curves were generated for the monomers and procyanidin oligomers by the method reported in Adamson at al.

EXAMPLES

Example 1

This example describes the preparation of cocoa powders containing varying amounts of cocoa polyphenols.

Part A – Preparation From Freeze-Dried Cocoa Beans

Methods for preparing a defatted cocoa mass are described in U.S. Patent Number 5,554,645 (issued to Romanczyk September 10, 1996). Unless otherwise stated, the cocoa beans used were unfermented Sulawesi cocoa beans. They were processed in the following manner. Harvested cocoa pods were opened and the beans with pulp were removed for freeze-drying. The pulp was manually removed from the freeze-dried mass and the beans were subjected to the following manipulations. The freeze-dried cocoa beans were first manually dehulled and ground to a fine powdery mass with a TEKMAR™ Mill. The resultant mass was then defatted overnight by Soxhlet extraction using redistilled hexane as the solvent. Residual solvent was removed from the defatted mass by vacuum at ambient temperature.

Part B – Preparation From Roasted Cocoa Beans

The cocoa powder was prepared using West African cocoa beans by a conventional method which comprises the steps of roasting the cocoa beans to an internal bean temperature of 95°C to 160°C, winnowing the cocoa nibs from the roasted cocoa beans, milling the roasted cocoa nibs into chocolate liquor, pressing the liquor to extract the cocoa butter, and recovering the cocoa butter and partially defatted cocoa solids. The cocoa solids can be further defatted using hexane as a solvent, as previously described in Part A.

Part C – Preparation from Non-Roasted Cocoa Beans

Partially defatted cocoa solids having a high cocoa polyphenol content, i.e., a high cocoa procyanidin content, were obtained by processing sun-dried, unfermented Sulawesi cocoa beans to cocoa solids without a bean or nib roasting step and, if desired, without the step of milling the beans to chocolate liquor, i.e., by screw pressing the beans to provide cocoa butter and partially defatted cocoa solids. The cocoa polyphenols were conserved because the traditional roasting step was omitted. The method includes the steps of: (a) heating the cocoa beans to an internal bean temperature just sufficient to reduce the moisture content to about 3% by weight and loosen the cocoa shells; (b) winnowing the cocoa nibs from the cocoa shells; (c) screw pressing the cocoa nibs; and (d) recovering the cocoa butter and partially defatted cocoa solids which contain cocoa polyphenols including cocoa procyanidins. Typically, the heating was carried out in an infra red heating apparatus for about 3 to 4 minutes. Optionally, the cocoa beans were cleaned prior to the heating step, e.g., in an air fluidized bed density separator. Preferably, the cocoa beans were heated to an internal bean temperature of about 100°C to about 110°C, more preferably less than about 105°C. The winnowing can be carried out in an air fluidized bed density separator. The above process of heating the cocoa beans to reduce the moisture content and loosen the cocoa shell is disclosed in U.S. Patent Number 6,015,913 (issued January 18th, 2000).

Example 2

In this example, the effectiveness of various solvents and aqueous solvents at 50°C was studied. The solvents used for the extractions were varied, as well as the pH of the solvents. The extracts were compared in order to assess the efficiency of the different solvents.

Extracts were prepared using cocoa solids prepared from unfermented, freeze-dried, defatted, cocoa beans as described in Part A of Example 1. Ten grams of the defatted cocoa mass were slurried with 100 mL of solvent for 5-10 min. at 50°C. For the acidified solvents 0.5% acetic acid was added to the solvents and aqueous solvents. The solvents used were ethanol, methanol, isopropanol, and acetone. The solvent to water percentage was varied. The slurry was centrifuged for 5 min. at ambient temperature at 3000 x G and the supernatant was passed through a 0.45 micron filter. The amounts of procyanidins extracted and the oligomeric profiles of the cocoa extracts were determined using the analytical methods described above. The quantity of each oligomer detected was expressed as a percentage of the amount of the same oligomer extracted using the control extract described above.

Isopropanol

The isopropanol results are shown in Table 1 (acidified) and 2 (non-acidified).

Table 1 – Acidified Isopropanol

% solvent	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	94.92	91.17	90.37	90.53	84.23	78.98	69.86	60.42	43.83	35.79
60	97.26	93.03	91.17	89.46	80.58	72.75	59.97	50.09	35.11	26.49
70	94.96	90.52	86.12	78.97	64.32	50.80	35.59	25.53	14.56	8.60
80	94.70	86.16	73.96	57.96	38.61	24.02	12.71	6.66	0.00	0.00
100	58.45	50.89	50.12	49.49	46.67	44.33	40.38	38.49	34.85	29.12

Table 2 – Non-Acidified Isopropanol

%	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	93.25	88.96	86.61	84.58	82.82	75.01	66.54	60.77	50.05	18.18
60	92.59	87.90	84.57	80.67	79.46	63.73	55.08	48.00	36.44	9.09
70	93.32	90.43	83.09	73.31	65.00	45.45	30.11	24.13	14.15	1.52
80	40.52	35.71	25.75	20.38	17.77	14.16	11.93	11.73	8.88	1.61
100	42.14	38.95	37.56	36.24	35.81	32.10	32.05	30.75	28.22	22.14

There was a pronounced decrease in procyanidin extraction with increase in oligomeric size for aqueous isopropanol at all dilutions. The 80% isopropanol and 20% water solution was the least effective. The 50%, 60% and 70% isopropanol solutions were the most effective for all oligomers apart from the decamers, for which 100% isopropanol was the most effective. It should be noted that for the monomer and lower oligomers (up to the pentamer) the 50-70% isopropanol solutions were more than twice as effective as the 80% and 100% isopropanol.

The results show that the acid addition resulted in an overall increase in oligomeric extraction at all aqueous dilutions (50%, 40%, 30%, and 20% water). However, the effect of adding acid to the 80% isopropanol doubled the amount of lower oligomers extracted, while leaving the amount of higher oligomers extracted largely unaltered.

Methanol

The methanol results are shown in Table 3 (acidified) and Table 4 (non-acidified).

Table 3 – Acidified Methanol

%	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	86.98	85.48	80.24	74.98	72.26	58.27	55.21	50.00	39.88	0.00
60	91.60	89.66	85.22	81.26	77.00	66.19	56.50	48.93	31.90	0.00
70	92.46	95.23	95.57	97.15	90.90	84.33	72.67	60.82	41.82	28.95
80	88.53	94.10	92.86	92.43	85.80	78.57	66.00	54.12	36.36	21.05
100	92.40	90.73	90.76	92.06	92.82	86.94	80.34	77.63	61.13	43.86

Table 4 – Non-Acidified Methanol

%	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	79.50	74.32	69.48	63.73	56.62	46.92	35.86	28.09	17.02	8.33
60	81.04	76.33	72.66	67.65	62.15	52.56	39.80	32.58	20.21	12.50
70	87.07	82.60	76.19	39.18	60.85	51.18	42.18	36.71	35.48	18.75
80	89.49	84.94	77.82	70.78	62.09	52.35	42.18	34.18	29.03	12.50
100	84.94	76.41	77.73	78.06	76.62	76.41	69.74	71.91	62.77	66.67

The data shows that when 70% and 80% methanol was used, the extractions of lower oligomers was best when 100% methanol was the extraction of the higher oligomers was greater. For oligomers higher than the pentamer, the lower concentrations of alcohol were less effective with increasing oligomeric size.

The addition of acid resulted in an increase in oligomeric yield. The 100% methanol, however, was unaffected by the presence of acid.

Acetone

The acetone results Table 5 (acidified) and Table 6 (non-acidified).

Table 5 – Acidified Acetone

%	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	95.93	93.96	93.97	95.69	99.64	107.73	114.39	112.33	126.81	121.05
60	95.28	94.81	94.84	95.78	97.65	100.78	104.29	103.62	111.35	111.40
80	97.14	95.21	95.56	94.08	92.57	91.95	94.11	76.32	82.86	66.91
100	77.30	73.43	74.55	77.56	76.03	74.05	63.03	54.02	13.04	13.16

Table 6 – Non-Acidified Acetone

%	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	89.36	84.69	86.20	85.61	86.67	94.77	94.17	108.94	108.21	140.59
60	98.18	115.95	75.54	98.67	100.16	102.95	102.95	105.39	100.94	131.95
80	103.54	103.34	102.89	100.64	94.97	93.21	80.23	80.56	68.06	24.42
100	72.07	68.20	69.57	73.20	73.88	78.36	71.70	68.66	0.00	0.00

For the lower acetone concentrations (50 and 60%) the extraction efficiency increased with oligomeric size. For the 80% and 100% acetone the extraction efficiency decreased with oligomeric size.

The ethanol results are shown in Table 7 (acidified) & Table 8 (non-acidified).

Table 7 – Acidified Ethanol

%	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	86.02	83.17	80.77	79.85	77.00	69.94	66.73	52.51	37.80	19.09
60	88.12	84.02	81.00	77.88	75.92	68.33	61.83	44.00	32.21	15.76
70	87.97	82.66	78.61	74.24	68.21	58.39	48.77	32.99	18.87	3.33
80	91.49	82.90	75.51	67.29	57.72	45.53	36.21	24.08	13.34	0.00
100	81.48	75.53	75.15	75.76	76.69	76.10	78.53	71.12	68.92	63.03

Table 8

%	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
50	84.43	82.32	79.33	78.40	76.55	67.21	53.33	39.88	29.07	3.33
60	87.99	86.04	82.58	81.69	78.17	66.74	51.11	36.90	23.26	6.67
70	87.96	83.40	78.00	74.01	67.99	55.58	41.11	28.57	17.44	6.67
80	91.85	86.62	83.11	81.00	71.02	63.13	53.08	43.75	37.36	18.33
100	89.44	81.87	83.24	87.94	85.76	90.00	95.29	97.75	102.91	109.86

The results show that the ethanol was better than the aqueous ethanol and that the acidified ethanol was better than the non-acidified ethanol. The data also shows that when 70% and 80% ethanol was used, the extraction of lower oligomers was best when 100% ethanol was used, the extraction of higher oligomers was greater. For oligomers higher than the pentamer, the lower concentrations of alcohol were less effective with increasing oligomeric size.

Thus, the choice of whether to use 100% of solvent or an aqueous-solvent mixture will depend on the oligomeric profile of the procyanidins desired in the cocoa extract.

Example 3

Experiments were carried out to determine the effect of temperature on the extraction of cocoa procyanidins using methanol, ethanol, and isopropanol. The extractions were carried out as described above except that they temperature was 0°C, 20°C, 50°C and 70°C except for methanol which has a boiling point of 64°C. The results are shown in Tables 9-11.

Table 9 – 100% Isopropanol

°C	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
0	2.44	1.94	1.71	1.47	1.12	0.88	0.68	0.46	0.00	0.00
20	5.85	4.97	4.54	4.11	3.55	3.06	2.63	2.39	0.93	1.03
50	38.21	36.43	36.04	35.27	33.57	31.62	30.56	30.44	25.27	25.89
70	72.76	69.92	68.99	65.72	62.41	59.30	57.32	60.45	54.34	61.33

Table 10 – 100% Methanol

°C	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
0	91.22	89.43	85.98	83.10	79.01	76.36	69.78	68.47	58.15	37.72
20	92.20	95.87	93.65	92.94	88.56	91.54	87.13	91.54	84.38	87.35
50	90.47	92.41	91.91	90.22	88.33	87.46	86.85	88.67	82.52	80.69

Table 11 – 100% Ethanol

°C	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
0	67.66	68.17	67.84	67.49	63.93	62.28	58.98	53.72	49.69	46.06
20	73.27	72.88	73.03	72.90	70.61	69.83	64.53	63.43	59.68	53.85
50	89.12	88.22	89.39	91.22	88.84	86.35	86.26	86.87	73.57	80.90
70	90.52	88.21	89.17	91.32	88.97	87.93	87.06	87.06	82.82	79.31

When 100% isopropanol was used, the extraction efficiency for all of the procyanidin oligomers increased dramatically as the extraction temperature was increased (see Figure 1b). When 100% ethanol was used, the extraction efficiency for all of the oligomers increased up to 50°C (see Figure 1A). When 100% methanol was used, the effect of temperature varied with oligomeric size. For example, the yield of the monomers was unaffected by temperature, the yield of the dimers to the heptamers increased as the temperature was increased from 0°C to 20°C; and yield of the higher oligomers (octamers to decamers) increased substantially when the temperature was increased from 0°C to 20°C, but above 20°C, the yield decreased (see Figure 1c).

For a general comparison of solvent effectiveness see Figure 2. For each solvent, the dilution which gave the best overall procyanidin yield was selected. As can be seen, the differences in the yields of the monomers and oligomers up to the hexamers did not vary greatly between the solvents. However, for the higher oligomers it became clear that some solvents were preferentially extracting the lower oligomers, while other solvents were preferentially extracting the higher oligomers. For example, isopropanol was an extremely effective solvent for the monomer and lower oligomers, but its efficacy dropped off dramatically for the higher oligomers. Conversely, ethanol was the least effective solvent for extracting the monomer and lower oligomers, but was highly effective for extracting the higher oligomers.

Example 4

In this example the effect of fermentation on the procyanidin yield was studied. The differences in the procyanidin recovery levels from the following groups of cocoa beans was studied: 1) unfermented cocoa beans which were freeze-dried and ground according to the method of Part A of Example 1; 2) fermented cocoa beans which were processed to cocoa solids using the same method as that used for the unfermented cocoa beans.. For most bean varieties the amount of time required for normal fermentation to occur varies according to the size of the mass of cocoa pods and the frequency with which the pods are turned. It takes about three days for beans to be lightly fermented, between five and seven days for full fermentation to take place, and beans will be over-fermented after eight days. The third group of cocoa beans were not fermented and were not roasted. The cocoa beans were removed from the pod, sun-dried, and processed according to the method disclosed in the U.S. Patent No. 6.015,913 (to Kealey et al.). The hulls were removed by heating the cocoa beans only for a time and at temperature sufficient to reduce the moisture content and loosen the cocoa shells so that the shells could be separated from the nibs in the winnowing step. The beans were subsequently reduced to a powder by the method of Example 1. The cocoa procyanidins were extracted using the "control" extraction method where the solvent was a mixture of 70% acetone, 29.5% water and 0.5% acetic acid. The amount of each of the procyanidin monomers and oligomers present in each extract was determined using the analytical procedure previously described.

As shown in Figure 3, extracts from fermented cocoa beans contained between 75 to 100% less cocoa procyanidins than the extract prepared from unfermented cocoa beans. The relative loss of procyanidins increased with oligomeric size. Heating the beans to remove the shells (as in the '913 process) also resulted in extract with reduced cocoa procyanidin levels. The cocoa extract from the heated cocoa beans contained levels of procyanidin oligomers which ranged between 10-50% of those found in the cocoa extract from the unfermented, manually dehulled cocoa beans.

The results show that the fermentation and subsequent processing of the cocoa beans have a more dramatic effect on the procyanidin content of the extract than the choice of solvent(s).

Example 5

The experiment of Example 4 was repeated using the same groups of beans, but altering the method of extraction by using boiling water as the solvent. As can be seen in Figure 4, the use of boiling water as the solvent drastically reduced the overall yield of cocoa procyanidins. The loss increased with oligomeric size such that the higher oligomers were not extracted using hot water.

Example 6

This example is a comparative example which repeats the extraction process described in a group of Japanese patent applications and patents owned by a Meiji Seika Kaisha Ltd. where hot aqueous ethanol is the preferred solvent. See JP 9206026, published August 12, 1997; JP 7274894, published October 24, 1995; JP 9224606, published September 2, 1997. In these publications, it is not disclosed if the beans were fermented and roasted. Presumably, the cocoa beans were subjected to a "traditional" of fermentation followed by roasting and winnowing of the cocoa beans to recover the dehulled beans. As shown above, fermentation and roasting deplete the available supplies of procyanidins in the cocoa beans.

Experiments were carried out to compare the amount of procyanidins and the oligomeric profile of the extract obtained when an extraction was carried out using (a) fermented, (b) underfermented, and (c) unfermented, non-roasted cocoa beans processed according to the procedure of U.S. Patent No. 6,015,913 (Kealey et al.). Cocoa solids were prepared from the three groups of beans by the methods described in Part A of Example 1. Two extracts were made from each group. The first extract was prepared at 50°C using the standard solvent mixture of acetone/water/acetic acid (70%/29.5%/0.5%). The second extract was prepared at 80°C using a mixture of 80% ethanol and 20% water as the solvent. The amount of each oligomer present in the aqueous ethanol extract was reported as a percentage of the amount oligomer present in the standard acetone/water/acetic acid extract.

The results are shown in Table 12.

Table 12

Cocoa Powder	mono-mer	dimer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
unfer-mented beans	93.27	105.99	99.58	95.26	81.02	64.44	46.03	30	18.8	12.5
fermented beans	64	56.41	50	36.84	33.33	33.33	0	0	0	0

The results show that the yield of procyanidins was dramatically reduced when the extraction was carried out using fermented beans. Furthermore, there were virtually no higher oligomers in the extract from the fermented beans. The use of 100% ethanol as the solvent resulted in extremely high yields of the nonamers and decamers (103% for the nonamer and 110% for the decamer compared to the 100% for the standard solvent). The extraction with 80% ethanol gave much lower yields (37% for the nonamer, 18% for the decamer) compared to 100% for the standard solvent.

Example 7

Extraction Of Cocoa Procyanidins From Cocoa Powder Using A Counter-Current Extraction Method.

In a continuous counter-current extraction process, an organic solvent or an aqueous organic solvent passes through an extraction system comprising a plurality of cells containing partially defatted cocoa solids. The organic solvent or aqueous-organic solvent mixture enters the extraction system at a cell containing the most extracted batch of cocoa solids, passes through progressively fresher batches of cocoa solids contained in successive cells, and is finally drawn off from the cell containing the freshest batch of cocoa solids. Thus, the solvent and the solids to be extracted are moving through the extraction system counter-current to one another. The temperature of the solvent mixture entering the cell containing the most extracted cocoa solids may be from about 20°C to about 100°C, preferably from about 50°C to about 95°C at atmospheric pressure, or higher if the extraction takes place under pressure. The solvent should be in the liquid phase, as opposed to the gaseous phase, therefore the reaction temperature must either be below the boiling point of the solvent or the reaction must be carried out in a pressurized vessel. Since experimental data generated in the above batch processes suggests that the extraction process is more effective at higher temperatures, it may be preferable to carry out the counter-current extraction at a higher pressure. The number of cells and cycle time are chosen to give the maximum yields of extracted procyanidins from the cocoa solids. The procyanidin content of the extract is concentrated by recycling the extract in the continuous counter-current system. In such a system the extract passes continuously through the cells in series. Periodically, the cell containing the most extracted cocoa solids is removed from the system and one containing fresh cocoa solids is added. The optimum number of cells in the series and the length of each cycle will vary with the particle size of the cocoa solids and extraction parameters such as temperature and pressure. Methods of determining the optimum conditions are well known to those skilled in the art. See "Unit Operations of Chemical Engineering" 3rd Edition, Chapter 7, September 2000, Eds. McCabe, W., and Smith J., McGraw Hill.

Example 8

Selective Extraction of Procyanidin Oligomers According to Their Size

This example demonstrates how extracts containing predominantly high molecular weight cocoa procyanidin oligomers or predominantly low molecular weight cocoa procyanidin oligomers can be obtained by the selective use of solvents which preferentially solubilize certain procyanidin oligomers.

One gram of cocoa powder, obtained from unfermented cocoa beans by the method described in Example 1 was extracted three times with 100% ethyl acetate at 50°C. The extracts were combined and concentrated by rotary evaporation under vacuum until dry. The dry extract was reconstituted with 10 mls of the standard acetone/water/acetic acid (70%/29.5%/0.5%) solvent, purified, and analyzed for oligomeric content as described previously. The ethyl acetate-extracted cocoa solids were then dried in a vacuum oven overnight to remove any residual solvent. The dried solids were extracted with the standard acetone/water/acetic acid solvent (70%/29.5%/0.5%) at 50°C, purified, and then analyzed for oligomeric content as described previously. The oligomeric content of both extracts was determined as a percentage of the oligomeric content of an extract prepared from unfermented cocoa beans using the standard acetone/water /acetic acid solvent (70%/29.5%/0.5%) at 50°C. The results are shown in Table 13.

Table 13

	mono-mer	di-mer	tri-mer	tetra-mer	penta-mer	hexa-mer	hepta-mer	octa-mer	nona-mer	deca-mer
Acetone/water/ acetic acid extract from unfermented beans	100	100	100	100	100	100	100	100	100	100
Ethyl acetate extract from unfermented beans	47.77	34.94	25.77	17.63	9.98	5.28	3.43	0	0	0
Acetone/water/ acetic acid extract of ethyl acetate-extracted cocoa solids	66.47	72.81	78.5	83.45	89.05	92.96	96.08	97.87	100	100

As can be seen in Table 13 and Figure 7, the use of ethyl acetate as the solvent resulted in the extraction of only the monomer and lower oligomers, leaving all the higher oligomers in the

extracted cocoa solids. The higher oligomers were then efficiently extracted using the standard acetone/water/acetic acid solvent (70%/29.5%/.05%) or 100% ethanol.

Example 7

Detheobromination Of The Cocoa Extract

In these experiments, various solvents were used at different temperatures to extract cocoa procyanidins from cocoa solids. The extracts were then analyzed to quantify the amount of caffeine and theobromine present in the cocoa extracts. The solvents used were isopropanol, ethanol and methanol. The extractions were carried out at 0°, 20°, 50° and 70°C using the method described in Example 1.

The results are shown in Table 14.

Table 14

Solvents/Temp.	Theobromine mg/g/	Caffeine mg/g
isopropanol @ 0 C	0	0
isopropanol @ 20 C	Trace	Trace
isopropanol @ 50 C	1.7	Trace
isopropanol @ 70 C	3.5	0.15
methanol @ 0 C	1.17	Trace
methanol @ 20 C	1.56	0.16
methanol @ 50 C	1.88	0.19
ethanol @ 0 C	1.16	Trace
ethanol @ 20 C	3.58	0.16
ethanol @ 50 C	4.45	0.18
ethanol @ 70 C	3.73	0.16

The yields of theobromine were the greatest when ethanol was used as the solvent and the least when isopropanol was used as the solvent. The yields increased with increasing temperature. The results show that theobromine is most soluble in ethanol and least soluble in isopropanol, and that solubility increases with temperature.

To achieve minimum theobromine extraction, and therefore facilitate its removal from the extract, the optimum extraction will be with isopropanol carried out at the lowest temperature.

Thus, results in the extraction of the procyanidins. The theobromine will then be precipitated out of the extract by chilling the extract overnight at about 0°-4°C and recovered by filtering the extract.

To recover the maximum amount of theobromine from cocoa beans, the ideal extraction will be with ethanol at between 50°-70°C. Subsequent to the extraction, the ethanol extract will be chilled to between 0-5°C, which should result in the precipitation of the theobromine. The theobromine is separated by filtration, by centrifugation in a contiguous centrifuge, or by adsorption onto a neutral adsorbent followed by elution.

Preferably, the theobromine is collected as a crude solid by passing the theobromine-laden solvent vapor through a system of chilled plates within closed cabinets (as shown schematically in Figure 6). The solvent is then purged from the system and recovered for further use. Meanwhile, the crude theobromine is scraped off the chilled plates, and dissolved in an aqueous solution. Purified, pharmaceutical grade theobromine is obtained by recrystallizing the theobromine, drying the crystals, and redissolving them in de-ionized double-distilled water.

Having thus described in detail the preferred embodiments of the present inventions, it is to be understood that the present inventions defined by the appended claims are not to be limited by the particular details set forth in the above descriptions as many apparent variations thereof are possible without departing from the spirit or scope of the present inventions.

CLAIMS

1. A process for selectively extracting low molecular weight cocoa procyanidin oligomers from partially or fully defatted cocoa solids, which process comprises the step of extracting the cocoa solids with a solvent which is methyl acetate or ethyl acetate.

2. A process according to claim 1, wherein the solvent is acidified to a pH of about 2 to about 4 with an edible acid or a physiologically acceptable acid.

3. A process according to claim 1 or 2, wherein the extraction is carried out at a temperature of from 0°C up to the boiling point of the solvent used.

4. A process according to any one of claims 1 to 3, wherein the cocoa beans are unfermented or underfermented cocoa beans.

5. A process according to claim 4, wherein the underfermented cocoa beans have a fermentation factor of 275 or less.

6. A process according to claim 4, wherein the cocoa beans are slaty cocoa beans, purple cocoa beans, or purple brown cocoa beans, or mixtures of slaty cocoa beans and purple cocoa beans, purple cocoa beans and brown cocoa beans, or slaty cocoa beans, purple cocoa beans, and brown cocoa beans.

7. A cocoa extract obtained by a process as claimed in any one of claims 1 to 6.

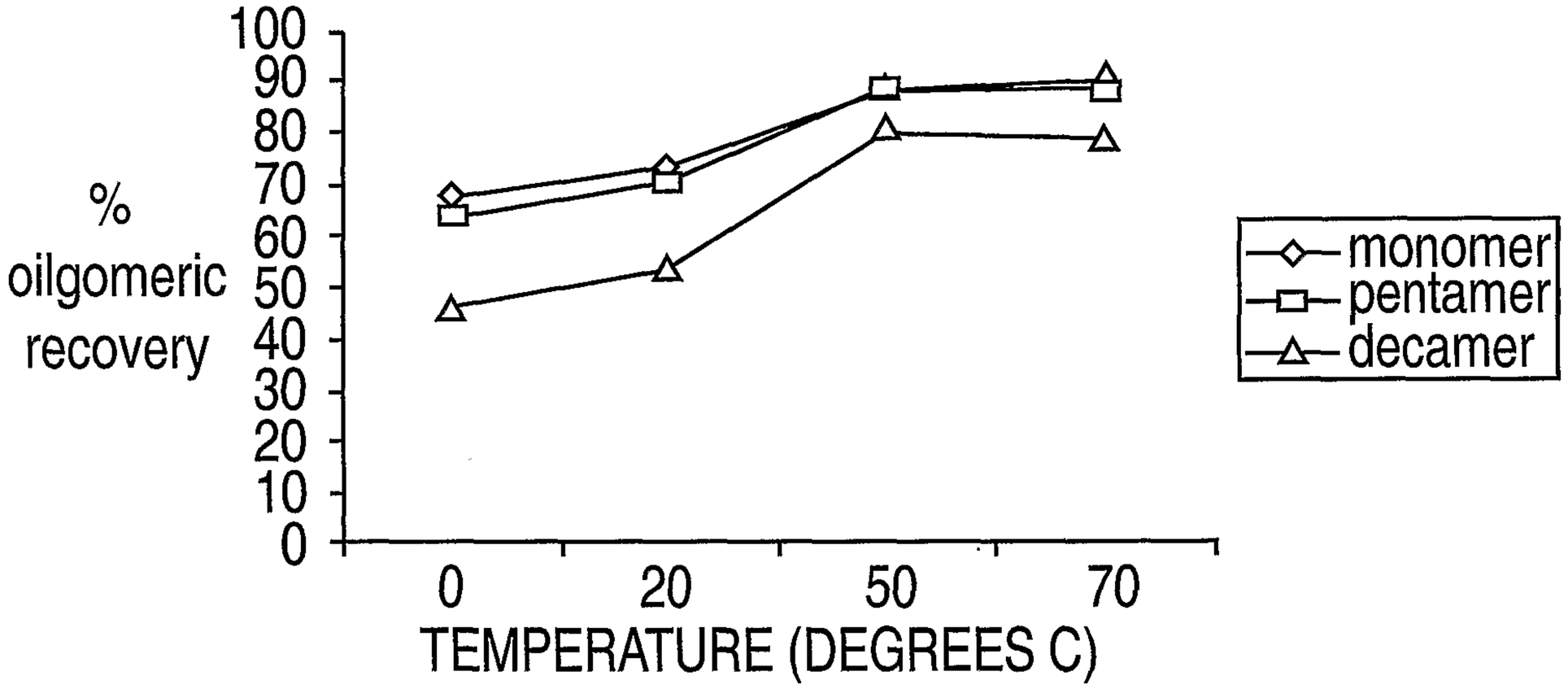

100
90
80
70
60
50
40
30
20
10
0
% oilgomeric recovery
0
20
50
70
TEMPERATURE (DEGREES C)
monomer
pentamer
decamer

FIG. 1A

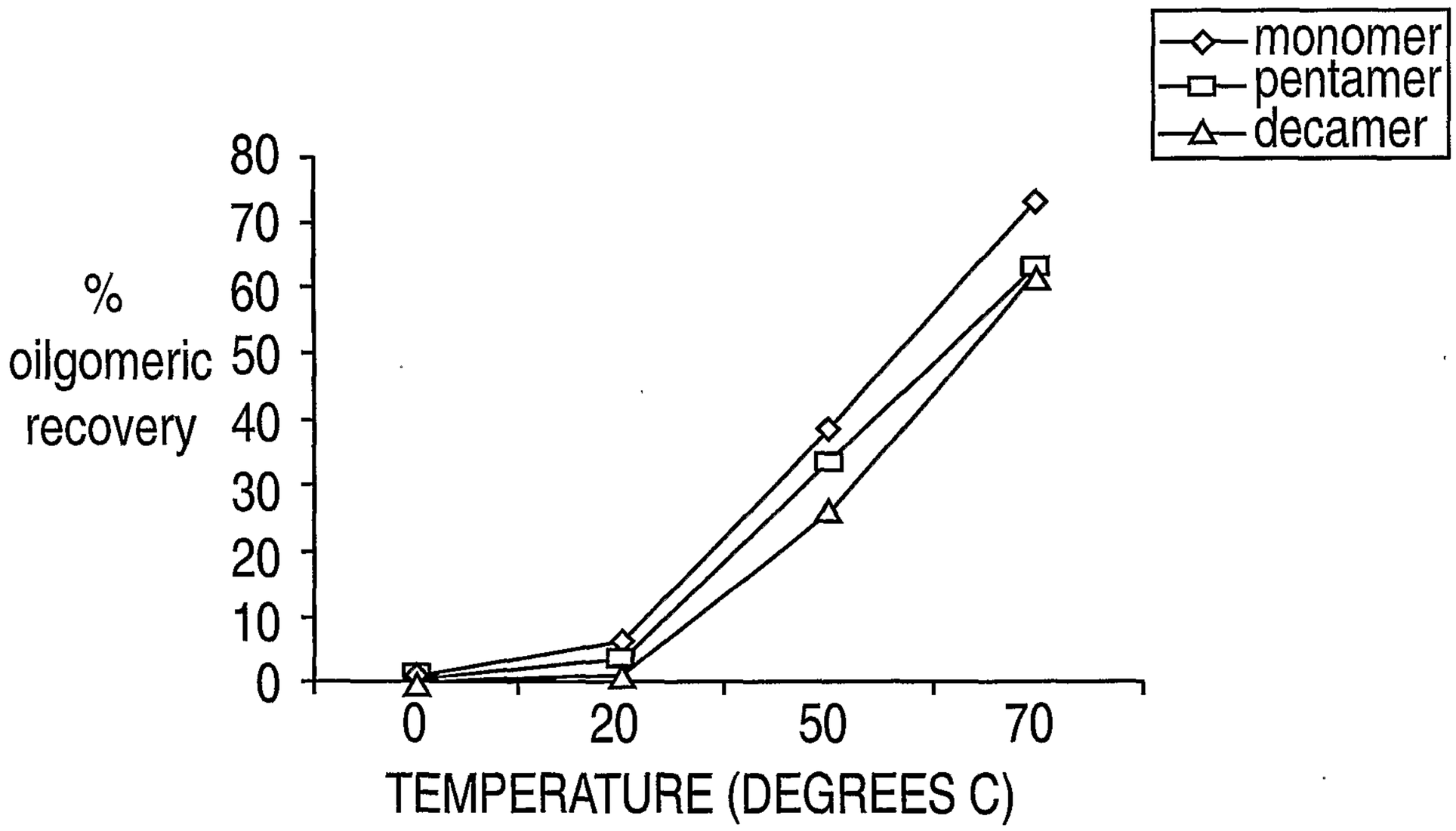

80
70
60
50
40
30
20
10
0
% oilgomeric recovery
0
20
50
70
TEMPERATURE (DEGREES C)
monomer
pentamer
decamer

FIG. 1B

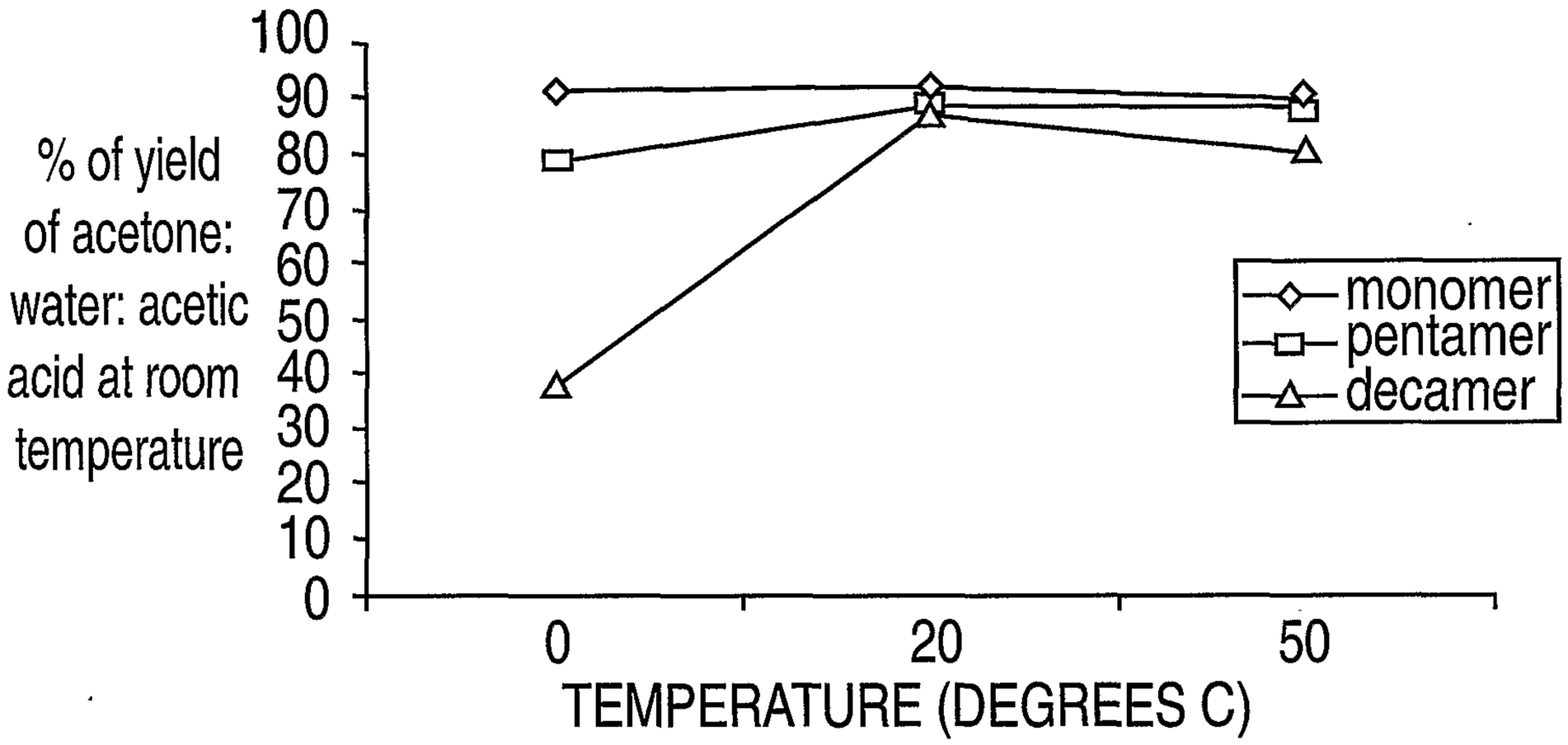

100
90
80
70
60
50
40
30
20
10
0
% of yield of acetone: water: acetic acid at room temperature
0
20
50
TEMPERATURE (DEGREES C)
monomer
pentamer
decamer

FIG. 1C

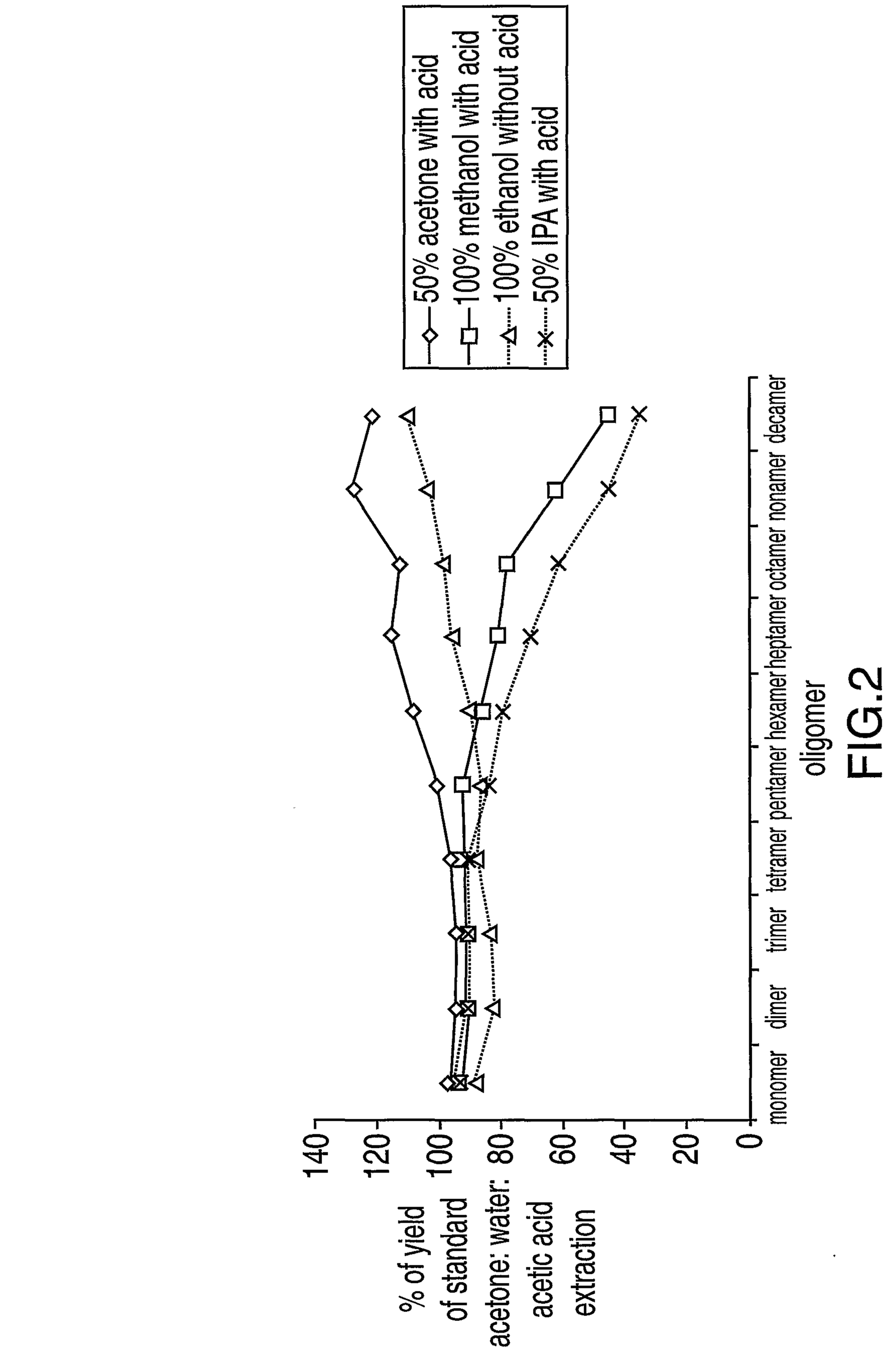

50% acetone with acid
100% methanol with acid
100% ethanol without acid
50% IPA with acid
% of yield of standard acetone: water: acetic acid extraction
140
120
100
80
60
40
20
0
monomer
dimer
trimer
tetramer
pentamer
hexamer
heptamer
octamer
nonamer
decamer
oligomer

FIG.2

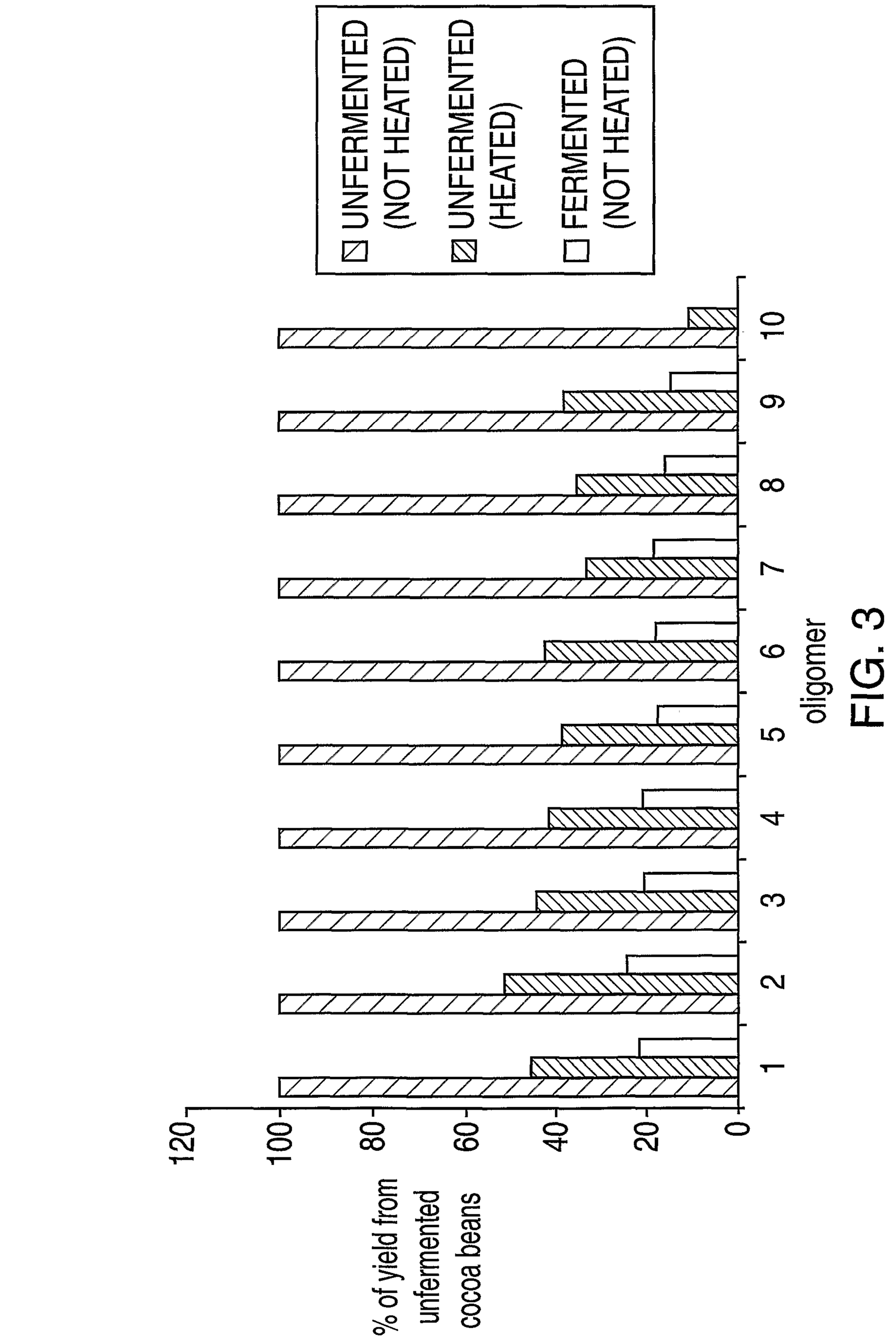

UNFERMENTED (NOT HEATED)
UNFERMENTED (HEATED)
FERMENTED (NOT HEATED)
oligomer
1
2
3
4
5
6
7
8
9
10
% of yield from unfermented cocoa beans
0
20
40
60
80
100
120

FIG. 3

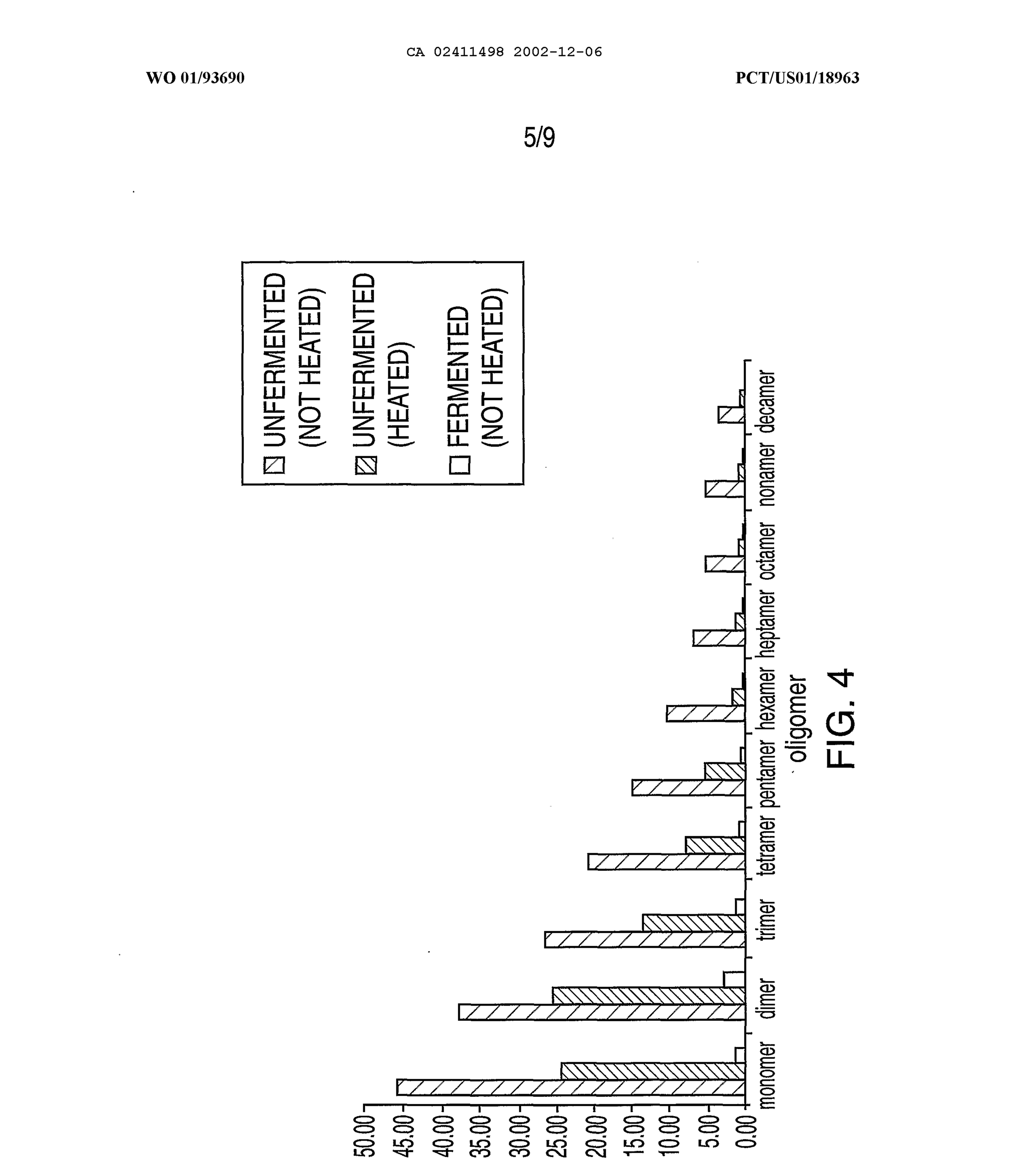

UNFERMENTED (NOT HEATED)
UNFERMENTED (HEATED)
FERMENTED (NOT HEATED)
FERMENTED (HEATED)
% of yield from acetone: water: acetic acid extraction
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
monomer
dimer
trimer
tetramer
pentamer
hexamer
heptamer
octamer
nonamer
decamer
oligomer

FIG. 4

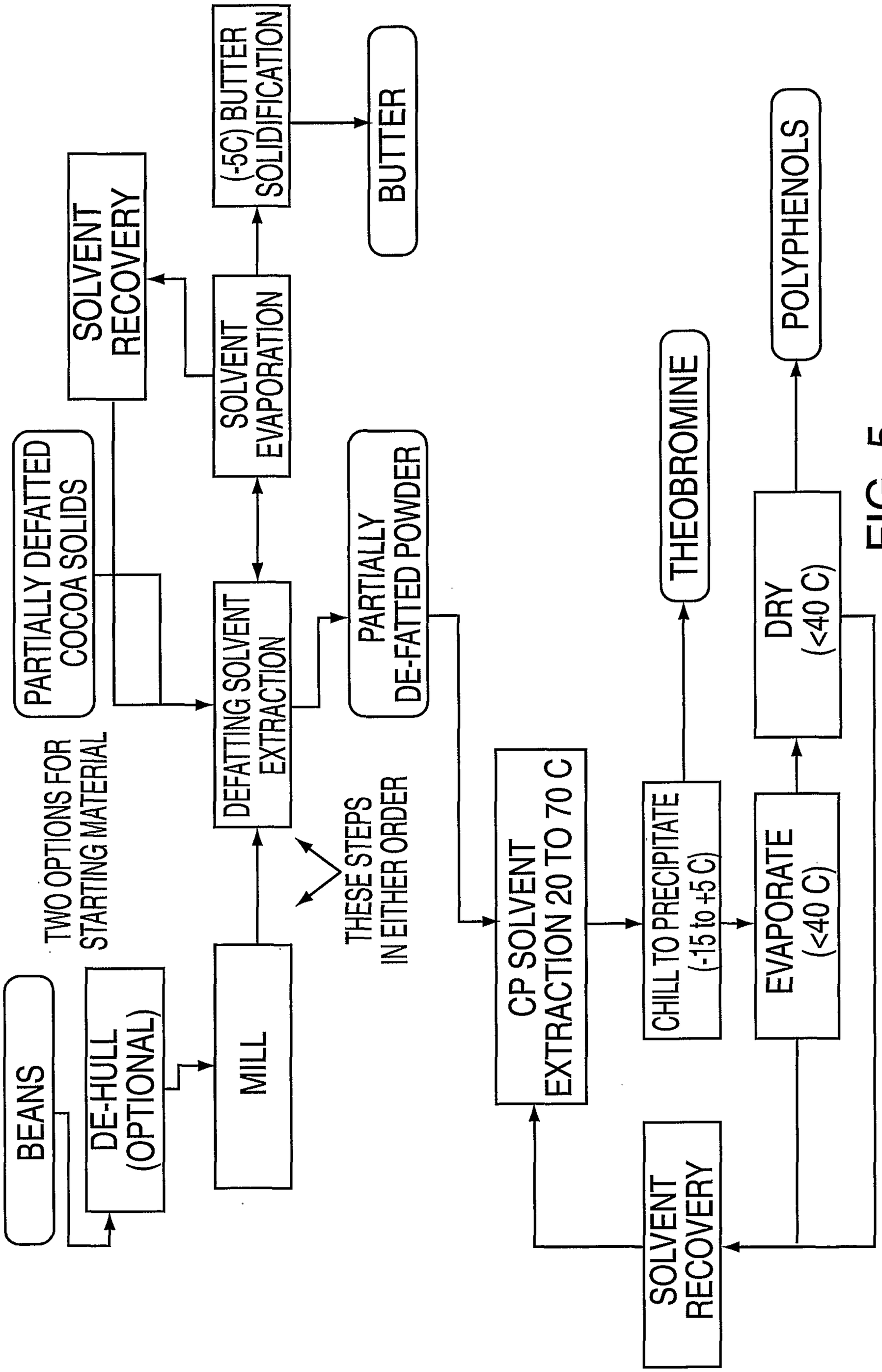

BEANS
DE-HULL (OPTIONAL)
MILL
TWO OPTIONS FOR STARTING MATERIAL
PARTIALLY DEFATTED COCOA SOLIDS
DEFATTING SOLVENT EXTRACTION
SOLVENT RECOVERY
SOLVENT EVAPORATION
PARTIALLY DE-FATTED POWDER
(-5C) BUTTER SOLIDIFICATION
BUTTER
THESE STEPS IN EITHER ORDER
THEOBROMINE
POLYPHENOLS
CP SOLVENT EXTRACTION 20 TO 70 C
CHILL TO PRECIPITATE (-15 to +5 C)
DRY (<40 C)
EVAPORATE (<40 C)
SOLVENT RECOVERY

FIG. 5

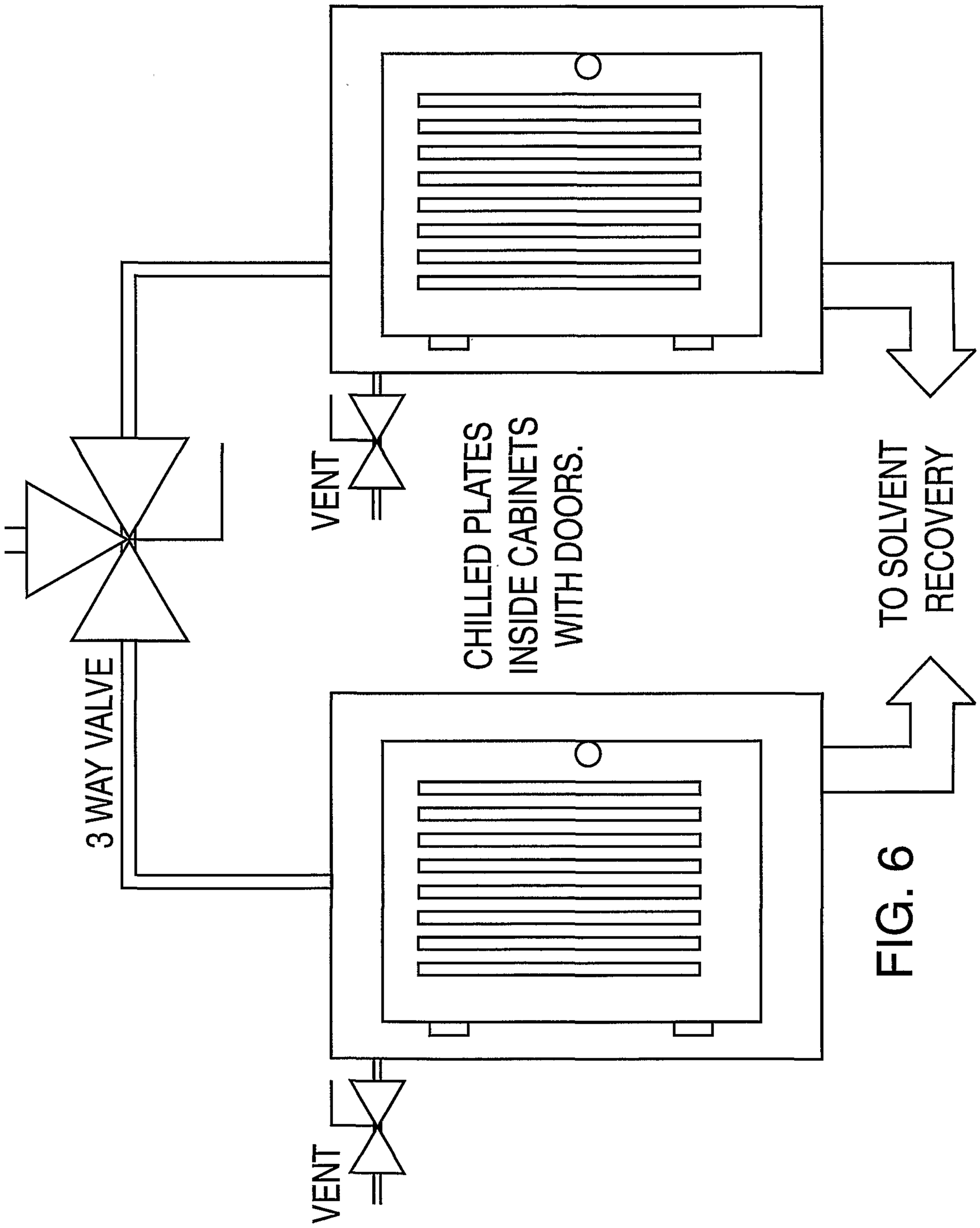

VENT
CHILLED PLATES
INSIDE CABINETS
WITH DOORS.
TO SOLVENT
RECOVERY
3 WAY VALVE
VENT

FIG. 6

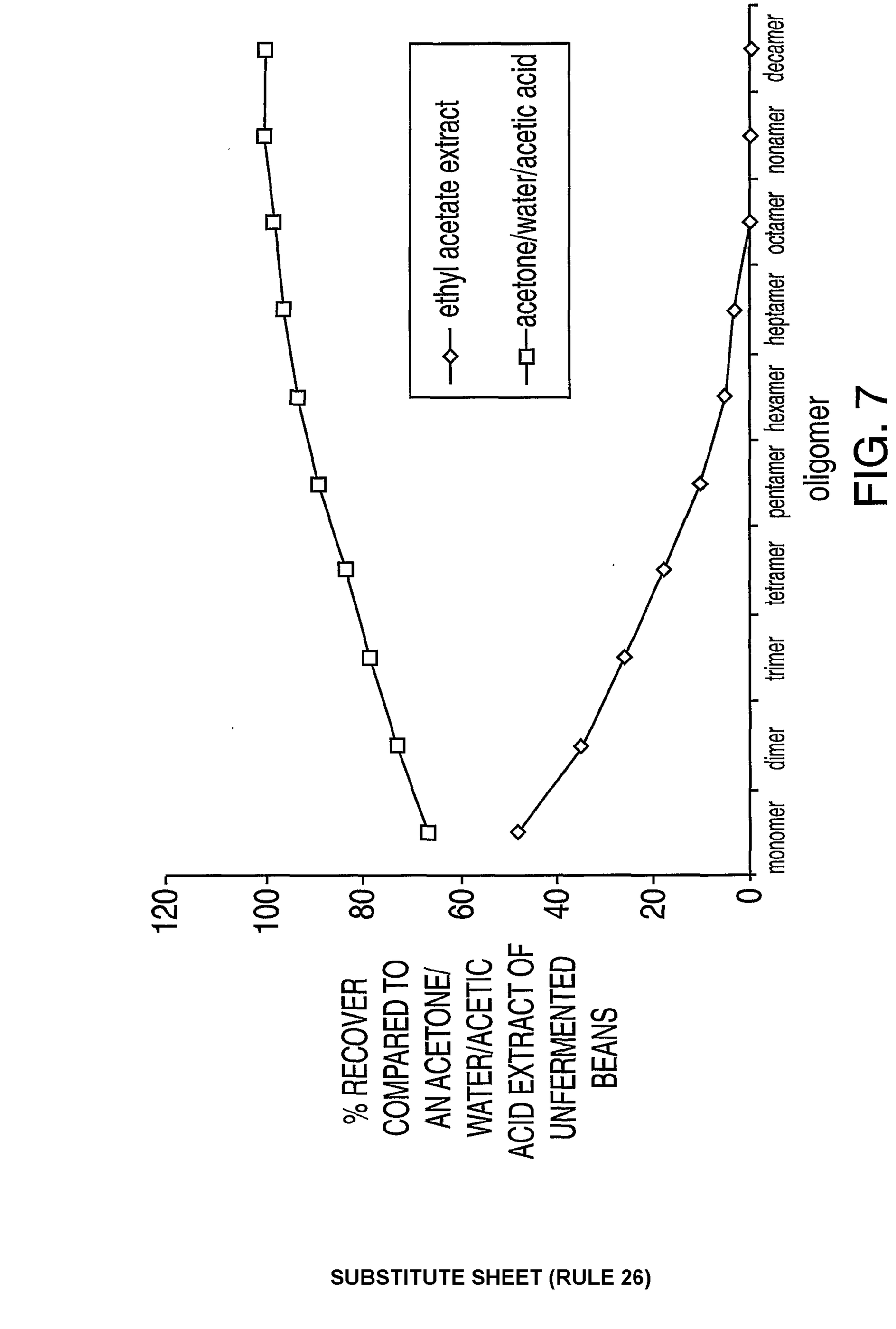

ethyl acetate extract
acetone/water/acetic acid
monomer
dimer
trimer
tetramer
pentamer
hexamer
heptamer
octamer
nonamer
decamer
oligomer
0
20
40
60
80
00
20
% RECOVER COMPARED TO AN ACETONE/ WATER/ACETIC ACID EXTRACT OF UNFERMENTED BEANS

FIG. 7

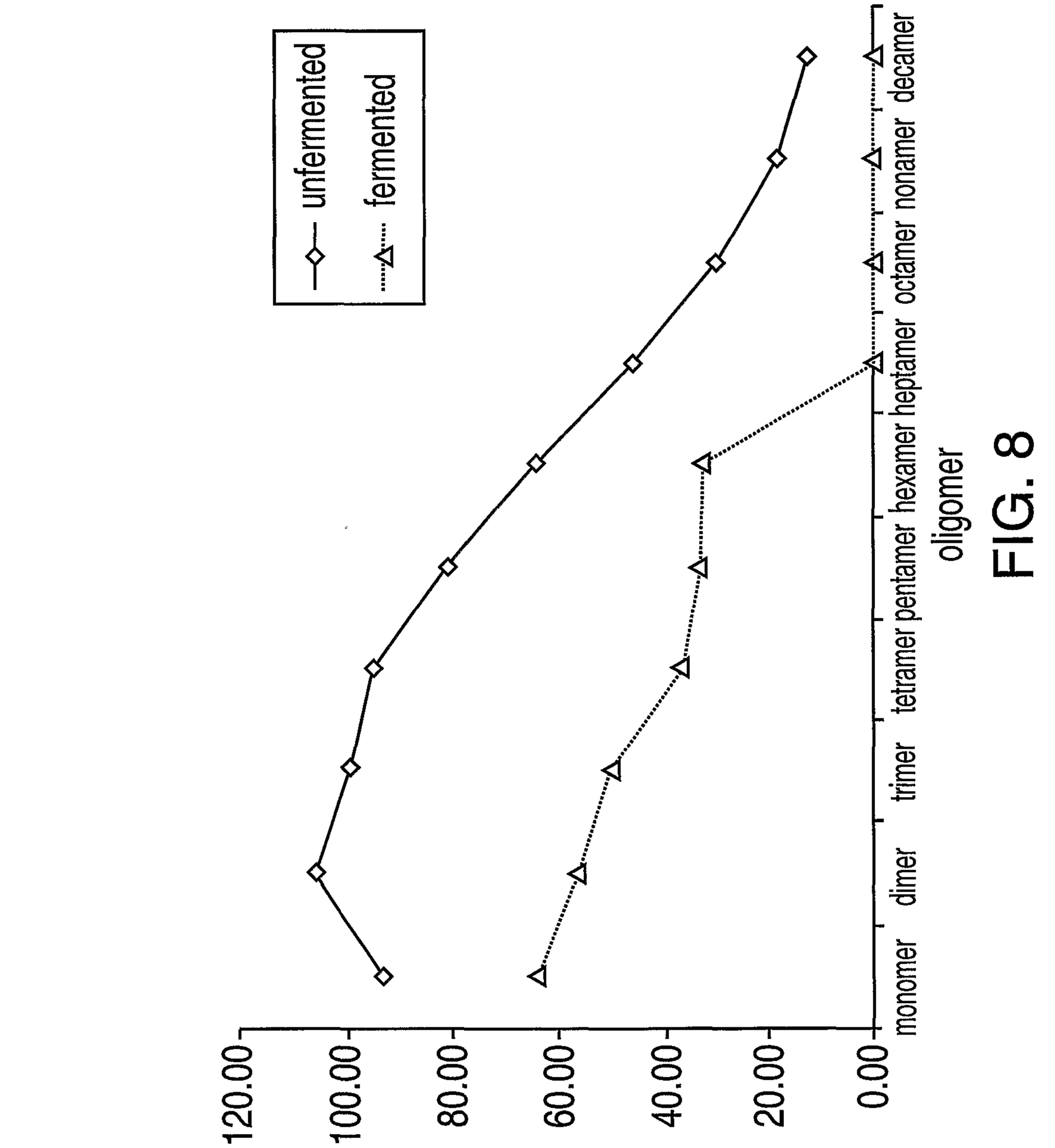

unfermented
fermented
% of oligomeric yield from a acetone/water acetic acid extraction
120.00
100.00
80.00
60.00
40.00
20.00
0.00
monomer
dimer
trimer
tetramer
pentamer
hexamer
heptamer
octamer
nonamer
decamer
oligomer

FIG. 8