Effect of roasting coffee on caffeine conten

ASSIGN BUSTER

Introduction:

Coffee has been a popular drink since 20th century, not only because of its different colors and flavors due to different degrees of roasting, but also the effect on which it can keep you to stay awake and to finish your job. It is commonly known that the reason for coffee to possess this effect is because of the caffeine inside the coffee beans. In general, there are four main types of roasting methods in the industry, namely, Light Roast, Medium Roast, Full Roast and Double Roast.

There are a variety of uses on caffeine nowadays. By interfering with adenosine in brain and body, it moderates new transmission of signals in CNS, Central Nervous System, and therefore keeps people awake[1]. Due to its short half-life in human body, around 4-10 hours on average[2], it can be used to increase the effect of analgesic for pain control[3] Also it has effect on delaying the muscle fatigue. However, caffeine can cause negative effect on human body, for example, increase the heart rate, breathing rate and makes people feel more alert and energetic, which is also the reason why International Olympic Committee forbid high caffeine consumption. Moreover, it is a mildly addictive drug, so some people cannot control the consumption of caffeine under overdose, which could lead to caffeinism.[4] Also, according to the a study held by Montreal University, if pregnant woman consume more than 1. 5 cup of coffee, the chance of miscarriage is doubled; if consume more than four, the chance of miscarriage is tripled.[5]

Therefore, the choice of caffeine consumption from coffee becomes critical due to its harmful effect on human body. It is advised that, if necessary, 100-300 mg per day of caffeine consumption is acceptable. However, people are

usually lack of knowledge about the caffeine content in the coffee and have no idea what amount exactly is in the coffee they are drinking. Thus, they often use their senses to “ identify” the caffeine content in coffee relative to each other. However, there are a lot of myths about this method. For example, those darker coffee has more caffeine than lighter coffee due to its darkness or that the caffeine is destroyed during roasting in higher temperature, so the caffeine content in lighter coffee is higher than that of darker coffee. To prevent over-dosing of caffeine from coffee, it is vital for people to have the general idea of comparing the caffeine content in different coffee when buying a them.

In this essay, the caffeine contents in coffee beans roasted in different degrees are examined by experiment through the extraction of caffeine by chemical means. The result would be interpreted by focusing on the roasting process, as coffee beans experience greatest change in physical or chemical change during roasting. Although there are a lot of sub-degrees of the main four roasting degrees, only one sub-degree from each degree would be selected as representative.

Research Question:

Does different roasting degree affect the caffeine content in coffee beans?

Background Information:

Original Coffee beans:

The original coffee beans are green in color. They contain non-volatile alkaloids, proteins and amino acid, carbohydrates, lipids, non-volatile chlorogenic acid, and volatile[6] compounds. Among the non-volatile

alkaloids, caffeine is the most abundant. It contains 1-2. 5% w/w [7] of green coffee been.

Caffeine:

Caffeine is a white, crystalline odorless and bitter tasting solid.[8] It exists in the plant of coffee beans as a natural pesticide. Its formal name is trimethylxanthine, or in systematic naming – 1, 3, 7-trimethylxanthine or 3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione.[9] The chemical formula of caffeine is C8H10N4O2. It is a polar[10] organic compound that contains carbon, nitrogen, hydrogen and oxygen. Its density in solid form is 1. 23 g/cm3. Due to the presence of nitrogen in the compound, caffeine is base in nature. Since it is a polar molecule, it is soluble to water, especially in hot water. The solubility of caffeine in water is 22 at 25, 180 at 80, and 670 at 100.[11] Under pressure of standard ambient temperature and pressure[12], the melting point of caffeine is 238[13] and it can also sublime at 178in nearly vacuum[14], which will be explained by phase diagram next.

Phase Diagram:

Three general phases of matter are solid, liquid and gas. Change of phase does not only depend on the temperature, but also the surrounding pressure acting on the chemicals. For example, the boiling point of water is 100, under standard ambient temperature and pressure. However, if the is placed in a lower atmospheric pressure, the boiling point of water is decreased since the vapor pressure is already greater than the atmospheric pressure, so water molecules can escape from the water surface in a lower required energy. Therefore, each compound can undergo phase change to the three phases in different combination of temperature and pressure. Figure 2 is a

phase diagram of . The lines dividing the diagram into sections represent that under certain temperature and pressure, would undergo change of phase, which is equilibrium between the two phases that the line is dividing. For example, the red line, if A < 1, then the corresponding melting point increases, while the corresponding boiling point, A', decreases.

Each element has their own phase diagram to illustrate the transition of phases under different combination of temperature and pressure. Figure 3 is a phase diagram illustrating the phase transitions of caffeine, which is different from . This is critical in later interpretation of the result in the experiment. It is because roasting process involves temperature and pressure change.

Roasting process:

The main purpose of roasting coffee beans is to remove toxins, enhance the taste and concentrate the odor wanted. Since green coffee beans are hard, little smell and contain a lot of compounds that are bitter in taste. Therefore, by roasting, it can result in both physical and chemical change in the green coffee beans. Since caffeine is the main factor that is concerned in this essay, other chemical change will not be discussed. Although there are a lot different roasting processes in the industry, such as fluidized bed roasting, fast roasting or horizontal rotating drum, the basic process of roasting is similar. During roasting, there are 10% to 20% lost in weight from the green coffee beans. Here is a summary of roasting process of coffee beans:

1. Heating of Green Coffee Beans from 3-5 minutes (about 25 to 100)

The green coffee beans are heated so that the water in the original coffee beans evaporates at a very fast rate. As this point, the green coffee beans turn from green to yellow due to caramelization of sugar in the coffee beans.

1. Heating of Yellow Coffee Beans from 5th-9th minutes (about 170to 200)

The yellow coffee beans are further heated and start to turn to brown in color as more sugar being caramelized. Carbon dioxide and water are forced to escape out of the coffee beans due to the high pressure inside, as the temperature is very high, which also causes the expansion in size of coffee beans. The aroma also starts to give out at this stage.

1. First crack from 10th-11th minutes (about 210)

A first sound of crack gives out as the coffee beans expand to almost double in size from green coffee beans in high temperature. Familiar aroma is given out and the coffee beans turn golden brown at this stage. After this stage, according to different degrees of roasting required, the coffee beans will undergo different temperatures and time periods for further heating. During the process, the sugar is caramelized further and coffee oil is released.

Light roast:

To acquire light roasted coffee beans, the beans from stage 3 are to be roasted about one more minute in about 215 before the second crack.

Medium roast:

For further half to one minute from light roasted in about 230, a second crack occurs and it means medium roast is finished.

Full roast:

If continued for half to one minute from medium roasted in about 240, full roasted coffee beans are collected.

Double roast:

This is the most common final step that coffee beans would be roasted, which is acquired from continuing the roasting from full roasted coffee beans for about half a minute more in about 245. The sugar in the coffee beans at this stage is started to burn and degraded.

The above heating process can be achieved by either roasting the coffee beans on a hot plate, which is a traditional roasting method in industry or in home roasting, or passing the coffee beans by high temperature steam.

Method:

Variables:

Independent: Degree of roasted coffee beans

Dependent: Caffeine extracted from the coffee beans (g)

Controlled: Mass of roasted coffee beans (g)

Brand of coffee beans (Starbucks)

Apparatus: The following apparatus are used in this experiment.

Chemicals: The following chemicals are used in this experiment.

Procedures:

1. Extracting chemicals compounds out of coffee beans
2. Measure 100mL of distilled water by graduated cylinder

3. Pour the water into a 200mL beaker.
4. Place the beaker on a hot plate and heat the hot plate to 100.
5. Place a weighing boat on an electronic scale and tare it.
6. Measure 3g of coffee beans, in form of powder, on the electronic scale and record it.
7. Add the 3g of coffee powder into the boiling water.
8. Use glass rod to stir the solution during heating.
9. After the water boils, which means it reaches 100, set the temperature to 80 and allow the solution to stand for 20 minutes to extract as much caffeine as possible.

Explanation of Stage 1 procedure:

Since caffeine is a polar molecule, it dissolves in water, especially in hot water. It is because increase in temperature leads to increase in solubility of solids, as less energy is required directly from the molecule to break the water molecules apart from each other. Use of distilled water instead of tap water is to prevent any interruption from foreign ions exist, such as decreasing the solubility of caffeine. The water volume to coffee powder mass ratio has to be large to ensure that there is enough water to dissolve caffeine. Hotplate is preferred as it is easier to manipulate the temperature. The decrease in temperature from boiling point is to prevent any further evaporation of water, which may decrease the amount of caffeine extracted from coffee beans powder. Also, the coffee beans have to be in powder form in order to increase the surface area to dissolve completely in the solution.

Assumption in Stage 1 procedure:

1. The coffee beans are originated from the same coffee beans farm.

2. No other procedure apart from roasting and grinding is done.
3. The coffee beans are roasted in the same method.
4. The degree of roasting is exactly same as listed on the package.

Separate caffeine from other chemicals in the solution

1. Use filter paper and filter funnel to filter out large remaining solids.
2. After the solution in stage 1 is cooled down, measure 2g of sodium carbonate by electronic scale and add it to the solution.
3. Use glass rod to stir the solution and allows sodium carbonate to dissolve in the solution.
4. Use a pipette of 25 mL to transfer 25 mL of dichloromethane to the solution in order to obtain accurate measurement and stir it with a glass rod for a few minutes.
5. Pour the solution into a separating funnel and cover it.[20]
6. Use a stand to hold the separating funnel and allow the solution in it to separate into two immiscible layers.

Precaution of Stage 2:

1. Since dichloromethane is a volatile organic solvent, it is toxic when inhaled. Thus, starting from step 3 in Stage 2 and onward, all steps in the experiment have to be carried in the fume board.
2. Make sure the opening of the separating funnel is closed before pouring the solution.

Explanation of Stage 2 procedure:

Apart from caffeine, the solution from stage 1 also contains different compounds extracted from the coffee beans, which are soluble in water too. The most abundant amount among the other compounds is tannin, which

usually exists in plants products. Therefore, the addition of sodium carbonate is to remove those compounds other than caffeine by allowing those compounds to be more water-soluble and hence, allow them to dissolve in water instead of other solvents.

The addition of dichloromethane is the most important procedure. It is a good organic solvent to dissolve caffeine. Although caffeine is a polar molecule and has dipole moment of 3. 64D[22], it dissolves more readily in less polar dichloromethane, which has dipole moment[23] of 1. 14D[24], while H2O 1. 84D[25]. It is because caffeine is a large molecule, so small charges are distributed over a large distance, which makes the molecule less polar than water even though is has larger value of dipole moment than water. Therefore, it dissolves more readily in dichloromethane than in water.

Since the polarity of dichloromethane is far less than water and is almost non-polar, it is not miscible with water, and due to its molar mass, 84. 93g/mol, which is heavier than water molecule, 18. 02g/mol, it sinks in the separating funnel.

Assumptions in Stage 2 procedure:

1. All caffeine in coffee beans dissolves in the boiling water from stage 1
2. All caffeine is dissolved in dichloromethane from water and no other compounds.

Completion of caffeine extract (In fume board)

1. Label a beaker for the number of trial of a particular kind of coffee beans.
2. Weigh the mass of a beaker by electronic balance and record it.

3. Place the measured beaker under the separating funnel and open the funnel to run the lower dichloromethane down to the beaker.
4. Cover can be opened a little in order to add atmospheric pressure inside the funnel, so that solution can run down smoother.
5. Close the cover of the funnel when reaching the separating line and allow the solution to run slowly.
6. Close the funnel when the line of separation is reached at the tip of the funnel.
7. Place the beaker with collected dichloromethane on a hot plate.[26]
8. Turn the temperature of hot plate to 100.
9. Wait until all solutions are evaporated and the beaker is dried and white crystals remain.
10. Allow the beaker to cool down then measure the mass again on the electronic scale and record it. The difference between the mass of beaker in step 1 and step 9 in Stage 3 is the mass of caffeine extracted from the coffee beans.

Explanation for Stage 3 procedure:

Each beaker has different mass, so every individual mass must be weighed before usage. Step 1 must be done if trials are being done simultaneously. If some extra solutions from the upper part of the separating funnel are obtained, the entire procedure has to be started again. Since the boiling point of dichloromethane is 40, 100can make sure that any water and dichloromethane are evaporated while 100is still far lower than the point of sublimation of caffeine in vacuum.

Assumption in Stage 3 procedure:

1. All dichloromethane is collected in the beaker.
2. Only caffeine remains in the beaker after evaporation of other solution.

Finally, repeat the entire procedure to obtain 5 trials result for each kind of roasted coffee beans. Therefore, there will be 20 results obtained.

After all procedures are done, make sure all the apparatus which have contact with dichloromethane are placed inside the fume board and all solutions are poured into a big beaker. Label the beaker as “ toxic organic waste” in order to allow the technician to dispose or treat them correctly.

Raw data:

Observation:

1. White precipitates remained.
2. The precipitates are odorless.
3. White precipitates stick on the bottom of the beaker.

Conclusion and Analysis:

From the calculations and Diagram 1, it can be seen that the percentages of caffeine existing in each coffee beans roasted in different degree are very closed. Therefore, the answer to the research question based on the result should be: the caffeine content in a coffee bean is not affected during the roasting process. The result is reliable since all percentages lie between 3. 2% and 4. 0%, which are between the greatest value of lower limits and the lowest value in the upper limits of all percentages. Moreover, the trend shows neither increasing nor decreasing as the degree of roasting increases. Thus, there should not be any relationship between the degree of roasting

and the caffeine content in the corresponding coffee beans. I will now interpret the results with regarding the background knowledge.

Referring back to the roasting process, it is clear that no chemical is added during the roasting process. However, the roasting process involves high temperature on direct heating with metal pans or using high temperature steam as alternative. One myth about the roasting process is that people claim that some caffeine is destroyed or evaporated during roasting under high temperature, so lighter coffee beans contain more caffeine than darker coffee beans. However, most of the roasting temperatures are under the melting point of caffeine, which is, indeed, not enough energy to destroy intra-molecular bonds. Although the temperature used for double roasted coffee beans are a little higher than the melting point of caffeine, the temperature only stands for about half a minute, which is not enough for the internal part of coffee beans to rise to melting point of caffeine. Neither, therefore, destroy nor evaporation of caffeine is unlikely to occur during the roasting process.

Moreover, some myths say that due to the fact that the point of sublimation of caffeine is 178 and the temperature for full roast and double roast is already beyond that temperature, which exceeds 200, thus, the caffeine content in darker coffee beans is again described as less caffeine content that lighter coffee beans. It seems to be reasonable at first, but apart from temperature, we also consider the pressure in roasting process, since phase change depends on temperature and pressure as mentioned before in this essay. Considering the method of heating green coffee beans directly, in order to keep the temperature over 200, it is necessary that the system of

the roasting process is under a closed condition, so that less energy is lost to the surrounding. According to the Ideal Gas Law, PV = nRT, where P is the pressure, V is the volume, n is the number of moles of gas molecules, R is the gas constant and T is temperature. As the system is closed, the volume, amount of gas and R, indeed, are constant. Therefore, if the temperature increases, the pressure also increases in the system. Same condition is in the method of using high temperature steam. Although it is commonly known that gas is the highest phase and the temperature cannot increase anymore, it is possible to create a steam with higher temperature than its boiling point, which is in a closed system. So if 200 steam is to be used, the roasting system has to be closed. Applying the same principle as before, the pressure also increases with the increase in temperature.

Refer to figure 3, the phase diagram of caffeine. As the pressure increases, the blue line, the melting point of caffeine increases, the red line. Since the atmospheric pressure is increased to against the vapor pressure of caffeine, so more energy needed to break the forces between caffeine molecules in solid, which is an exothermic reaction[27]. Also, from the diagram, the line representing the sublimation point, purple rectangle, is only under relatively low pressure and temperature, as 178under vacuum condition, which is not the case in roasting coffee beans. Thus, it is incorrect to say that caffeine sublimes during roasting process.

To conclude, high temperature and pressure in roasting process are the reasons that the caffeine content is not affected regardless the degree of roasting that the coffee beans have achieved. Therefore, even different degree of roasted coffee beans is used to brew coffee, the caffeine

consumption is the same no matter which type of coffee you prefer. Indeed, decaffeinated coffee is not taken into account.

Limitations and Evaluations:

Random errors: Random error arises since caffeine may not be all dissolved in dichloromethane since it still has a little solubility in room temperature water. Furthermore, the caffeine extracted by dichloromethane may contain impurities, which affect the mass of caffeine recorded. Also, the measurement using graduated cylinder causes uncertainties. Moreover, the use of other equipments such as electronic balance, which causes 0. 01g on the mass measured.

Systematic errors: The quantity of sample is too small, which causes unclear results obtained since large difference may be obtained if sample has larger quantity. If there is any difference of caffeine content, it would be more obvious if increase the amount of each coffee powder sample used. However, due to the fact that school laboratory do not have a large beaker at the time the experiment was performed, which required more than 200mL, only small amount of sample in each trial can be used in order to keep a large ratio between the volume of water and the mass of the coffee powder.

Moreover, the major systematic error is the separation of two layers in separating funnel. Since the running of dichloromethane into a beaker is manipulated by hands and through the observation. To ensure that all dichloromethane is collected, the closure of the separating funnel is a little delayed due to the bubbles in the separating line, which blurred the exact

separating level. Therefore, a little solution of the upper part, which contains the impurities from coffee beans, is added into the dichloromethane in the beaker. After the evaporation, since sodium carbonate is added before and it has high boiling point due to its ionic structure, its mass contributes to the mass weighed on the electronic balance.

Methods to resolve the major systematic error- Sublimation of caffeine:

In order to resolve the major systematic error, a further step can be done if equipment is allowed in school laboratory. Since caffeine sublimes well under vacuum at 178, as no air molecules against the vapor pressure of caffeine, the collected caffeine can be placed into a flask connecting to a aspirator, which keeps the condition in vacuum, and have a cold finger above it and a heat source below the flask. Caffeine can then sublime and precipitate on the cold finger. Thus, pure caffeine can be collected and weighed.

Further unresolved question and suggested investigation:

Although the caffeine content is not affected by roasting, a step backward can result in an unresolved question, which is whether green coffee beans grown from different places contain different level of caffeine inside? A suggested way to do so is that; buy different coffee beans of same roasted level from different locations around the world. This can be done by using internet shopping. After, extract caffeine from each example and compare the caffeine content in each coffee bean from different location.

Since there are more and more methods of roasting in the industry, such as fluidized bed roasting and fast roasting[29], it would be interesting to investigate whether these new methods of roasting can affect the caffeine

content in the coffee beans even they are claimed to be same degree of roasting after all.

Bibliography:

1. A, Nehlig; JL, Daval; G, Debry (1992). “ Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic, and psychostimulant effects”. Brain Res Rev
2. FP, Meyer; E, Canzler; H, Giers; H. Walther (1991). “ Time course of inhibition of caffeine elimination in response to the oral depot contraceptive agent Deposiston. Hormonal contraceptives and caffeine elimination”. Zentralbl Gynakol
3. Oxford A-Z of Medicinal Drugs Oxford Press.
4. Kent, Michael (1997). “ Oxford Food & Fitness (A Dictionary of diet and exercise)” Oxford University Press.
5. Anderson, Jean; Deslein, Barbara. “ The Nutrition Bible” William Marrow and Company, Inc.
6. LEUNG, T. M.; LEE, C. C. “ Inorganic Chemistry and Chemistry in Action” Fillans.
7. “ Library for Science”. .
8. “ About. com”
9. “ Drug bank”
10. “ Purdue University Online Writing Lab”
11. “ Look for chemicals”
12. “ Carleton College»
13. “ University of British Columbia”
14. “ Coffee-Makers-Café”

15. “ Coffee-Tea”
16. “ Sweet Marias”
17. “ Sonora Environmental Research Institute, Inc.”
18. “ The Scripps Research Institute”
19. “ City Collegiate”
20. “ New Mexico Tech”
21. « Ezine Article»
22. Beverage. cc
23. PubMed database

Appendix

Chemicals in green coffee beans

Non-volatile alkaloids:

Caffeine, theophylline, theobromine, paraxanthine, liberine, and methylliberine are present, while caffeine is the most abundant non-volatile alkaloid, which is about 1-2. 5% w/w of a green coffee bean. Also, caffeine acts as a natural insecticide for the plant. Moreover, caffeine’s half life is 5. 7 hours in a normal adult body.[31]

Proteins and amino acids

Proteins and amino acids make up 8-12% w/w of a green coffee bean.

Carbohydrates

Carbohydrates account for 50% w/w of a green coffee bean, mostly polysaccharides.

Lipids

Lipids, ester, long chained unsaturated fatty acids and amides are found in green coffee beans. The fatty acid is saturated during roasting process, which accounts for the coffee oil.

Non-volatine chlorogenic acids

Chlorogenic acids are antioxidant. They are good for health, but 70% of them are destroyed during roasting process.

Volatile compounds

Volatile compounds are found in green coffee beans as aldehydes, short-chained fatty acids and nitrogen containing aromatic molecules. However, the aromatic molecules in green coffee beans are unpleasant, which is the reason that coffee beans are roasted in order to form pleasant odor of aromatic molecules.

Degree of roasting

It can be seen that although dipole moment of caffeine is very large, larger than water, the size of the molecule is very large compared to water and dichloromethane. Therefore, charges are spread widely, which makes the polarity of caffeine molecule less polar. Thus, caffeine dissolves more readily in dichloromethane than in water or sodium carbonate, which is ionic compound. Sodium carbonate is used to react and make some compound other than caffeine to be more soluble to water, such as tannin.[34]

1. Nehlig, A, Daval JL, Debry G (1992). “ Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic, and psychostimulant effects”. Brain Res Rev

2. Meyer, FP, Canzler E, Giers H, Walther H. (1991). “ Time course of inhibition of caffeine elimination in response to the oral depot contraceptive agent Deposiston. Hormonal contraceptives and caffeine elimination”. Zentralbl Gynakol
3. Oxford A-Z of Medicinal Drugs, Oxford Press.
4. Michael Kent (1997). “ Oxford Food & Fitness (A Dictionary of diet and exercise)”. Oxford University Press.
5. Jean Anderson, Barbara Deslein. “ The Nutrition Bible”. William Marrow and Company, Inc.
6. Volatile means tendency of vaporization.
7. w/w = weight/volume percentage solution
8. Library for Science: http://www. chromatography-online. org/directory/analt-235/page. html About. com: http://chemistry. about. com/od/moleculescompounds/a/caffeine. htm
9. Drug bank: http://www. drugbank. ca/cgi-bin/getCard. cgi? CARD= DB00201
10. Electrons are unevenly distributed.
11. Purdue University Online Writing Lab: http://employees. oneonta. edu/knauerbr/chem226/226expts/226_expt06_pro. pdf
12. 25and 1 atm
13. T. M. LEUNG, C. C. LEE, “ Inorganic Chemistry and Chemistry in Action”, Fillans.
14. Look for chemicals: http://www. lookchem. com/Caffeine/
15. Original from Carleton College: http://serc. carleton. edu/research_education/equilibria/other_diagrams. html Red line added as example.

16. Original from University of British Columbia: http://www. chem. ubc. ca/courseware/123/tutorials/exp10A/sublimation/
17. Coffee-Makers-Cafe: http://www. coffee-makers-cafe. com/coffee-roasting. html#roastHomeCoffee-Tea: http://www. coffee-tea. co. uk/commercial-roasting. php Sweet Marias: http://www. sweetmarias. com/roasting-VisualGuideV2. php
18. The method of extracting caffeine from coffee is improved by myself from originally design from Sonora Environmental Research Institute, Inc.: www. seriaz. org/downloads/4-caffiene. pdf
19. All coffee beans are from same brand- Starbucks.
20. Using separating funnel is more accurate than pouring the solution in another beaker by hand and using filter paper to remove the remaining unwanted solution from the original design.
21. Fig. 4 separating funnel’s diagram is from Jindal Medical & Scientific Instrument
22. D = Debye = unit of dipole moment. Look for chemical: http://www. lookchem. com/Caffeine/
23. Dipole moment is the vector sum of polarity.
24. The Scripps Research Institute: http://www. scripps. edu/chem/finn/Scipdfiles/dipolemoments. pdf
25. City Collegiate: http://www. citycollegiate. com/dipolemoment. htm
26. Calcium sulphate is not used as mentioned in the original procedure, since it cannot be removed after wards, which could affect the mass of caffeine collected.

27. Exothermic reaction means there is a net energy given out as heat.

28. New Mexico Tech: http://infohost. nmt. edu/~jaltig/Chem333LCaffeine. pdf

29. Ezine Articles: http://ezinearticles. com/? A-Look-at-the-Coffee-Roasting-Process&id= 1802022

30. Beverage. cc

31. PubMed database

32. “ Sweet Marias”

33. Rare case that the coffee beans are totally blackened.

34. Sonora Environmental Research Institute, Inc.