The Effect of Moisture Content in The Production of Agarwood Tea

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***Abstract*—Tea is a beverage that consume by people the most, aside water. Agarwood tea has so many benefits such as the antioxidant activities, anti-hypertensive and anti-inflammatory. A few benefits have been proven by a study on the Agarwood tea. The process of making Agarwood tea is the same as the process of making green tea from the leaves of *Camellia sinensis*. In this study, the equipment used for the drying process was Vacuum Far-Infrared Radiation (VFIR) dryer and the leaves used were *Aquilaria Subintegra* species. Based on the previous research, the optimum parameters of the VFIR dyer were set at 50˚C and 0.6 bar for 120 minutes for drying to study the chemical constituents in the Agarwood leaves. However, since previous study just used constant drying period, thus in this research, the first experiment conducted used the same parameters but only varying the time in order to find the optimum drying period. As for the result, 120 minutes is the optimum drying period after studying the moisture content removal analysis, colony count analysis and antioxidant activity. Another experiment conducted to study on the how moisture content of different storage conditions affects the lifespan of Agarwood tea. The two storage conditions used were the kitchen and air-conditioning room. The results of the samples were obtained every 7 days which repeated for 28 days by studying the moisture content removal analysis, colony count analysis and antioxidant activity. This study shows that the Agarwood tea stored in the air-conditioning room was actually increase its lifespan as long the tea was kept in a sealed packaging.**

***Keywords*— Agarwood tea, Moisture content, Aquilaria subintegra, Storage condition, Lifespan**

I. INTRODUCTION

There are many types of tea that have been commercialized in the market such as green tea, black tea, and Oolong tea. Black tea, Oolong tea and green tea come from the same leaves of *Camellia Sinensis* but undergo different level of fermentation [1]. Green tea does not undergo fermentation process while black tea and Oolong tea undergo the fermentation process. The difference between Oolong tea and black tea is Oolong tea is partially fermented while black tea is fully fermented [2]. As stated by Chung S. Yang, tea contains polyphenol that recognized with its antioxidant activities since it has the ability to scavenge the reactive oxygen [3].

The reason Agarwood tea has been chosen in this research because herbal tea can be made from the Agarwood leaves which provide a lot of benefits. Besides, if bacteria or wounds attack the *Aquilaria*, it can produce Agarwood resins [4]. There is also a specific study on the effects of *Aquilaria Subintegra* towards the Alzheimer Disease (AD). The study proves that the extract of *Aquilaria subintegra* stem may be a potential treatment for AD because of the valium independency and it is more effective compared to Berberine. Valium is a type of drugs that treat mental order diseases while Berberine is one of the drugs that inhibit Acetylcholinesterase (ACHe) [5].

Generally, tea gives high benefits towards human's health especially the green tea such as cancer prevention, anti-inflammatory and anti-oxidative [6]. Instead there is a researcher already did some research on the Agarwood tea to prove the benefits of the Agarwood tea. One of the benefits is ethanolic extract in the Agarwood tea displayed good in vitro antioxidant activity. Fine particles of Agarwood tea leaves provide the highest antioxidant activity [7].

There is a study stated that tea should contain only 3% to 5% of moisture content and once it has been packed, the tea should contain 5% to 6% of moisture [8]. Hence, the higher the moisture content removal, the better the tea production. Besides, commonly there is no microbiological problems in dried foods [9]. However, some of the dried foods may facing the microbiological problems because they may be contaminated due to the moisture content of surrounding or process of handling the products [10].

Basically, at home, people will store tea products in the shelf at the kitchen. In the market, the commercial tea products are place on the shelf in the air-conditioning space. Different storage conditions may affect the tea products in terms of their shelf life and chemical constituents. Kitchen condition obviously hotter than air-conditioning room. Besides, condensation may occur in air-conditioning room due to the contact between hot air and cold air [10]. So, it needs to be proven which storage condition that suitable for the products in order to preserve the chemical constituents and extend the shelf life.

Currently there is no scientific study on the effect of moisture content towards the chemical constituents and lifespan of Agarwood tea production. The leaves of *Aquilaria subintegra* contain many types of chemical constituents that give benefits related to chemical properties such as caffeine and antioxidants. Thus, further study need to be conducted to investigate the effect of moisture content on antioxidants activity, lifespan and colony count in Agarwood (*Aquilaria subintegra*) tea production.

II. METHODOLOGY

A. Materials

Aquilaria subintegra leaves collection

The fresh mature leaves of *Aquilaria subintegra* were collected at Jalan Kebun Shah Alam. The leaves were rinsed and wiped using the laboratory tissue to remove all the dirt and residue. After that, the leaves were kept in the vacuum seal bag and stored in the refrigerator.

B. Experimental Procedure

i. ***Drying process***

The 15 clean leaves chosen were dried using VFIR at 50˚C and 0.6 bar by varying the drying period. The drying period started at 60 minutes. After the drying process, the dried leaves were put in the vacuum seal bag and stored in a glass desiccator containing silica gel. The dried leaves were grounded using the dry mill for about 1 minute until they became small particles as the commercial tea powder in the market. The grounded leaves were weighed about 2g using the weighing balance and transferred into tea bag. The tea bag stored in the plastic container. Those steps were repeated with the drying period at 90 minutes, 120 minutes, 150 minutes and 180 minutes.

As for the dried sample used in the lifespan analysis, 15 clean leaves chosen were dried using VFIR at 50˚C and 0.6 bar for 120 minutes as the optimum drying period. The dried leaves then were grounded using the dry mill about 1 minute until they became tea powder. The tea powder was weighed about 2g using the weighing balance and transferred into the tea bag. Steps repeated until 20 tea bags of the samples have been prepared. Ten tea bags placed in the plastic container and divided to two storage conditions respectively. Those two storage conditions were the kitchen and the air-conditioning room.

ii. *Moisture content removal*

The analysis of moisture content removal was conducted using the Sartorius Infrared Moisture Analyzer MA35. The equipment was set at 100˚C for 5 minutes for the samples from the tea bags that have been weighed around 2g initially. The analysis of moisture content removal ran for each sample that has been dried at 60 minutes, 90 minutes, 120 minutes, 150 minutes and 180 minutes. The data has been recorded for the determination of optimum drying period.

The parameters of the equipment have been fixed for the samples used in lifespan analysis. A tea bag containing 2g of tea powder from each storage condition has been taken for moisture content removal analysis. The data of moisture content removal of each sample has been recorded. The steps repeated every 7 days for 28 days.

iii. *Colony count*

The nutrient agar was prepared with some modifications. 20g of nutrient powder was added in 1L distilled water. Nutrient powder need to be mixed well in the distilled water. The solution then sterilized using the autoclave at 120˚C for 15 minutes. The solution was left for a while to cool it down then transferred into petri dish. One petri dish contained about 20ml of the solution and was left in the biological safety cabinet allowed it to solidify. The nutrient agar stored in the refrigerator [11].

The 2g of tea powder in a tea bag from the sample that has been dried for 60 minutes was swabbed using the cotton bud and spread on the nutrient agar. The result was observed on the next two days using the Stuart Colony Counter. The data has been recorded and steps were repeated using the sample from different drying period at 90 minutes, 120 minutes, 150 minutes and 180 minutes.

For the lifespan analysis, one tea bag of 2g tea powder was taken from each storage condition in first 7 days. The tea powder from each tea bag was swabbed using the cotton bud and spread on the nutrient agar. The result also has been observed on the next two days using the Stuart Colony Counter. The data has been recorded and steps were repeated in next 7 days for 28 days.

iv. *DPPH (2,2-diphenyl-1-picrylhydrazy) radical scavenging activity*

About 7.9mg of 0.2mM DPPH powder was weighed and mixed well with 100ml of 95% methanol. The DPPH solution was transferred into Erlenmeyer flask and has been covered with aluminium foil to avoid light from penetrate into the bottle. The DPPH solution was stored in the refrigerator.

200ml ultra-pure water has been boiled in the beaker at 100˚C. After it has reached 100˚C, one tea bag containing 2g of tea powder from the sample that dried for 60 minutes was immersed in the 200ml boiled ultra-pure water. It has been done for the process of tea extraction. The tea solution then transferred into conical flask and has been filtered using the filter paper. 3ml of the filtered tea solution has been transferred into the small bottle sample to mix with the DPPH solution.

Concisely, 3ml of DPPH solution was taken and reacted with 3ml of the tea extraction that has been prepared in the small bottle sample respectively. After that, the solution was placed in a dark space for 30 minutes at room temperature for incubation. The result of absorbance (A) was measured using the spectrophotometer at 517nm single wavelength. The steps were repeated for the tea sample from each drying period at 90 minutes, 120 minutes, 150 minutes and 180 minutes.

As for the lifespan analysis, one tea bag containing 2g of tea powder was taken from each storage condition. They were immersed in the 200ml boiling water at 100˚C respectively. The tea solution then transferred into conical flask and has been filtered using the filter paper. 3ml from the filtered tea solution has been transferred into the small bottle sample to mix with the DPPH solution.

The procedure of the reaction of 3ml of DPPH solution with 3ml of filtered tea solution is the same as mentioned earlier. The data has been recorded for further analysis. Besides, the steps were repeated every 7 days for 28 days. The blank used in this analysis was the mixture of 3ml of distilled water and 3ml of tea extraction which gave the value of 0.994. The percentage of DPPH scavenging activity using the following equation [12]:

$$\text{Free radical scavenging activity (\%)} = \frac{A\,(\text{blank}) - A\,(\text{extract})}{A\,(\text{blank})} \times 100$$

III. RESULTS AND DISCUSSION

A. The suitable drying period determination of Agarwood tea

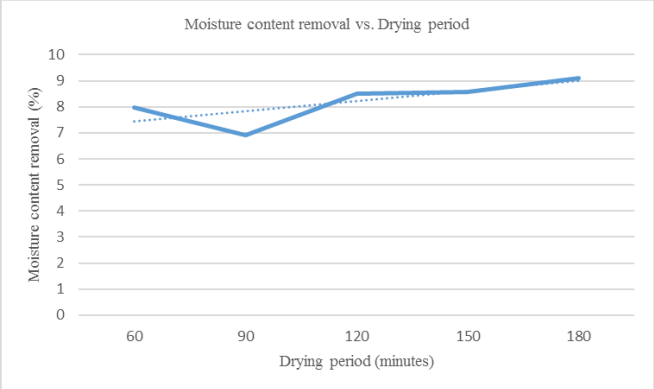

Figure 1: Percentage of moisture content removal of Agarwood tea against the drying period.

Figure 1 shows the moisture content removal of 2g grounded Agarwood tea sample for each drying period. Based on the trendline of the graph, as the drying period increases, the moisture content removal increases. This has been proven that more moisture content has been removed when longer time taken for drying [13]. For this analysis, obviously the leaves that were dried for 180 minutes have the highest moisture content removal. This is actually the best condition for the tea production because it can prevent the growth of microorganisms. It is rare for isolation of pathogenic organisms in most dried foods, but it does not mean that it is impossible for the microorganisms to inhibit [9]. However, it is good when the moisture content removal is high in the food product because low probability for microorganisms to grow.

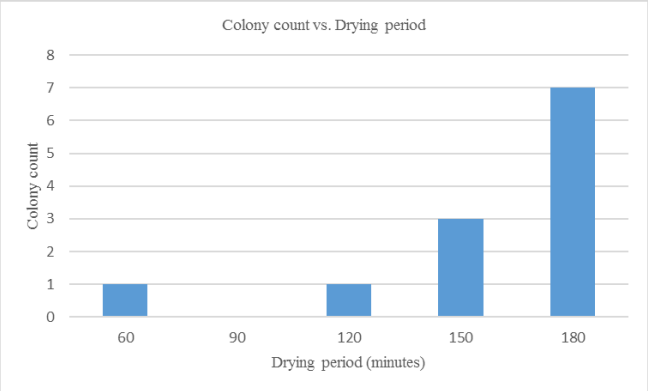

Figure 2: The number of colony count observed on Agarwood tea sample after 2 days for each drying period.

Figure 2 shows the observation of the number of colony count on the 2g of grounded Agarwood tea sample for each drying period after 2 days. Based on the observation above, the number of colony count started to increase on the sample at 120 minutes drying time and the highest number of colony count is 7 which has been observed on the sample at 180 minutes. The best sample was the sample that has been dried at 90 minutes because no microorganisms observed on the sample.

Logically, the higher the moisture content removal, the lower the number of microorganisms. This is because microorganisms actively grow in the moisture. However, in this analysis, microorganisms appear on the sample that has been dried at 120 minutes, 150 minutes and 180 minutes which already remove a lot of moisture. One of the reasons that can prove this situation to occur is poor handling [10]. Before the experiment started, workplace and hands (with gloves) need to be sprayed by the methanol to ensure everything was cleaned. Mouth also need to be covered to avoid the saliva from affecting the sample and the nutrient agar. Thus, further study need to be conducted carefully and triplicated the data.

Besides, the other reason may be spore forming bacteria on the dried products which is commonly happen in life. *Enterobacteriaceae* is one of the microorganisms can survive in long period of time in the dried foods. Mycotoxins also may often contain in the dried foods during mould growth on raw materials when they are still moist. The colder parts of a packaged product also may allow sufficient amount of water vapor to migrate which can permit spore germination of the more zerophilic moulds [9].

Table 1: Comparison of antioxidants activity of Agarwood tea with different drying time

Drying Period (minutes)	60	90	120	150	180
DPPH Radical Scavenging (%)	83.30 ±0.06	95.91 ±0.03	90.91 ±0.03	88.93 ±0.60	92.19 ±0.13

Based on the table above, the most effective DPPH is the tea sample that dried after 90 minutes since it has the highest value of DPPH radical scavenging [14]. Antioxidants play a big role in order to protect our body from the free radicals formation and slow down or prevent lipid peroxidation occurrence. Free radicals can develop various degenerative diseases diabetic, gastric ulcer and cancer [15].

However, the optimum drying period has been analyzed by comparing the analysis of moisture content removal, colony count and antioxidant activity. The optimum drying period for the *Aquilaria Subintegra* Agarwood tea has been decided at 120 minutes. This is because the moisture content removal is the second highest and almost similar to the sample that has been dried for 180 minutes. The reason drying period at 180 minutes was excluded because the colony count of the sample is the highest among the others which is not suitable in the Agarwood tea production. The microorganisms may affect the chemical constituents in the tea and indeed affect the lifespan too. People will not consume the contaminated products.

As for the colony count analysis, absolutely tea sample dried at 90 minutes is the good one because no microorganisms grew on it. But only one colony count observed in the tea sample that dried at 120 minutes. There must be a reason that inhibit the growth of microorganisms on the nutrient agar as mentioned earlier in the part of colony count analysis. As an example, poor handling might be the cause of the microorganisms to grow.

For the antioxidant activity, the tea sample that dried at 90 minutes was the highest but it cannot be chose because the colony count and moisture content removal respectively is not suitable as the optimum drying condition in overall. As can be seen, no microorganisms on the tea sample but the moisture content removal is the lowest. During the short term, maybe it was good to consume but the moisture inside the tea was still high which may inhibit the growth of microorganisms later on. Thus, in overall, 120 minutes is the optimum drying period for the Agarwood tea in order to provide the best production quality.

B. The analysis on the lifespan of Agarwood tea production

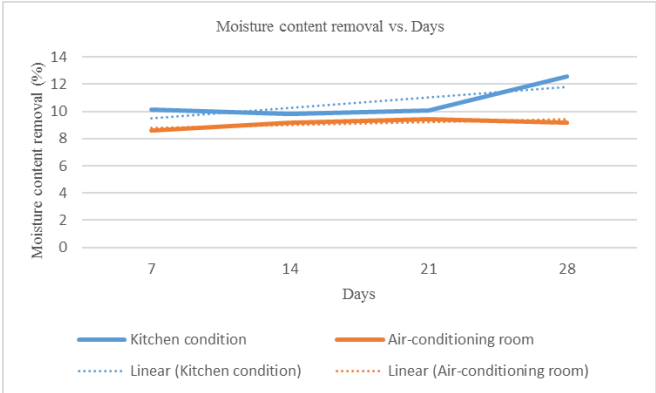

Figure 3: Comparison of moisture content removal of Agarwood tea stored in the kitchen and air-conditioning room for 28 days.

Figure 3 shows the study on the moisture content removal of the Agarwood tea production in two storage conditions. These two storage conditions are the common places for the tea storage in daily life. Kitchen represent a very common tea storage since every house has a kitchen while air-conditioning room represent the pantry in the office or the cabinet in the supermarket. That is the reason why the analysis has been conducted in these conditions. Based on the trendline in both graphs, as 7 days went by, the moisture content removal increases. However, the amount of moisture content removal of the Agarwood tea sample in the kitchen is higher than the air-conditioning room.

As stated in the graph, the amount of moisture content removal of the air-conditioning room sample do not exceed 9.50% while the amount of moisture content removal of kitchen sample are mostly more than 10%. Theoretically, the relative humidity is the ratio of water vapor pressure present to the saturation pressure at the temperature of the same air. Air can hold more moisture when the temperature increases but condensation may occur when this air contact with the cold surface. When the relative humidity is high, dry goods may suffer if they absorb the moisture from the air. Some problems might occur in the low humidity levels such as static electricity and increase the product water loss [10].

Refrigerators in most household use air-cooled condensers. The condenser place at the back of the refrigerator will release heat and dissipated to the room air [16]. Since the Agarwood tea sample in the kitchen was placed in the shelf above the refrigerator that has low humidity and high temperature, the sample was affected by those conditions which resulted in high moisture content removal compared to the air-conditioning room sample.

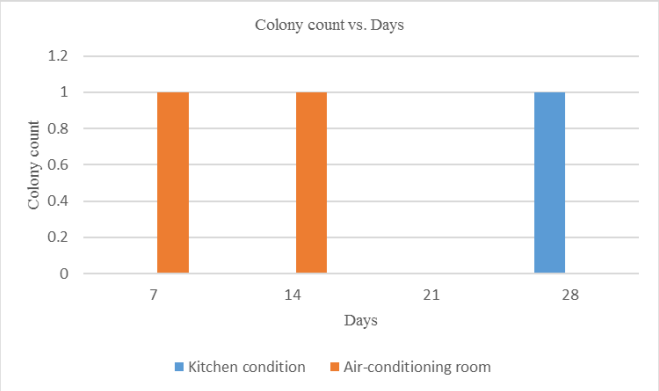

Figure 4: Comparison of colony count of Agarwood tea stored in the kitchen and air-conditioning room for 4 weeks.

Figure 4 above shows the comparison of colony count between the Agarwood tea stored in the kitchen and air-conditioning room. Generally, tea production either by using *Camellia Sinensis* or *Aquilaria Subintegra*, the leaves will undergo drying process (removal of moisture). Potential for spoilage of a food product can be reduced by using drying method because bacteria has low ability to grow without water and degrading enzymes [17].

As shown in the graph, there is no colony count from first 7 days up until 21 days for Agarwood tea stored in the kitchen but after 28 days, there is a colony count observed. The result obtained after 2 days of the agar was left in the incubator shaker. As for the Agarwood tea sample in the air-conditioning room, there is one colony count observed in first 7 days up to 14 days. However, starting from 21 days to 28 days, there is no colony count observed from the sample.

Based on the past research, raw tea or teabags were highly contaminated and microorganisms keep on increasing from time to time [18]. This prove the result obtained for the Agarwood tea sample placed in the kitchen which on week 4, the microorganisms started to grow on the sample. Hence, a very good handling needed to perform towards the sample or product in order to preserve it and lessen the contamination.

Foods might get affected from the surrounding. One of the effects is airborne contamination. Some of the sources of airborne contamination are people, raw materials and packaging. In air-conditioning room, when condensation occur, it may give rise to corrosion, microbial growth and other moisture related problems [10]. This condition resulted in the appearance of microorganisms on the Agarwood tea sample store in the air-conditioning room. Poor handling also may be the other reason for the microorganisms to grow on the nutrient agar.

Table 2: Comparison of antioxidants activity of Agarwood tea between the samples that stored in the kitchen and air-conditioning room

Days	7	14	21	28
DPPH Radical Scavenging (%) Kitchen	89.74 ± 0.06	87.93 ± 0.00	91.15 ± 0.06	87.79 ± 0.12
DPPH Radical Scavenging (%) Air-conditioning room	89.24 ± 0.12	89.17 ± 0.15	87.56 ± 0.07	88.20 ± 0.09

Based on the table above, the value of DPPH radical scavenging of the tea sample stored in the kitchen and air-conditioning room were both decreases. However, the value of the sample stored in the air-conditioning room decreases slowly and likely constant in 28 days. For the tea sample stored in the kitchen, the value of DPPH radical scavenging decreases rapidly after 14 days and the value were not consistent starting from 21 days until 28 days.

The phenolic compound and the antioxidant activity found in the *Aquilaria spp.* that known as herbs do give potential to human health and benefit to pharmaceutical industry [12]. Thus, since the value of DPPH radical scavenging of the tea sample in air-conditioning room decreases slower than the sample in the kitchen, the best storage for tea production is air-conditioning room. This is because high value of DPPH radical scavenging is the most effective and the condition preserved the antioxidant activity inside the product [14].

For the lifespan analysis, two different storage conditions have been provided in order to find out which storage condition is the optimum condition to maintain the lifespan of Agarwood tea without affecting a lot on the nutrients of the tea itself. Based on the trend line of moisture content removal analysis, the sample in the air-conditioning room remove low percentage of moisture content while the sample in the kitchen remove very high percentage of moisture content as days went by.

Condensation occur in the air-conditioning room which resulted in low moisture content removal for the Agarwood tea in that storage condition. Tea is preferable to be stored in cool condition and it is much better if it is store in the dark condition to increase the shelf life. However, since the samples were kept in the transparent plastic container in both storage conditions, thus the cool condition provide its best to the Agarwood tea.

As time goes by, tea will be contaminated. The microorganisms appeared starting after 28 days in the kitchen condition. On the other hand, the microorganisms for the air-conditioning room sample was observed on the first 7 days up to 14 days which suddenly turned out to be 0 after 21 days and 28 days. This may occur because of poor handling during the experimental work. Besides, hot storage condition and open space attract more microorganisms on the product. Thus, air-conditioning room provide the best condition for the lifespan in Agarwood tea production.

IV. Conclusion

In conclusion, the moisture content in the food and surrounding do affect the food itself either the nutrients content or the physical of the food. When the water activity of the food is marginal, the dried food product may become mouldy [9]. Even though the condition of air-conditioning room might affect the moisture content because of the process of condensation occur from the contact of hot air with cold air but as long as the tea was kept in the container, the moisture content can be maintained and prevent it from being contaminated. As stated by a postdoctoral from Department of Biology, Selena Ahmed, tea best stored in sealed packaging in dark and cool conditions in order to increase its shelf life [19]. Further analysis on the other chemical constituents need to be conducted and longer period of time needed to study the lifespan of the Agarwood tea.

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References

[1] Dalong Zhu, Y. J. (2013). Green Tea Consumption and Diabetes. In V. R. Preedy, Tea in Health and Disease Prevention (pp. 675-680). Elsevier Inc.

[2] Figueira, M. E. (2019). Tea Extracts. In A. S. Seyed Mohammad Nabavi, Nonvitamin and Nonmineral Nutritional Supplements (pp. 433-436). Elsevier Inc.

[3] Chung S. Yang, J. M. (2000). Effects of Tea Consumption on Nutrition and Health. The Journal of Nutrition, 2409-2412.

[4] Veronica Alexander Jok. Nurhaslina Che Radzi, K. H. (2015). A Review: Pharmacological Properties of Aquilaria spp. Advanced Materials Research, 193-197.

[5] Hirbod Bahrani, J. M. (2014). Isolation and Characterisation of Acetylcholinesterase Inhibitors from Aquilaria subintegrafor the

Treatment of Alzheimer's Disease (AD). Current Alzheimer Research, 1-9.
[6] Chacko, S. M. (2010). Beneficial effects of green tea: A literature review. Chinese Medicine.
[7] Adam, A. Z. (2017). Pharmacological properties of agarwood tea derived from Aquilaria (Thymelaeaceae) leaves: An emerging contemporary herbal drink. Journal of Herbal Medicine, 37-44.
[8] Evans, D. I. (n.d.). The Moisture Content of Made Tea. 54-58.
[9] D.A.A. Mossel, J. L. (1976). Micro-organisms in dried foods: their significance, limitation and enumeration. Journal of Food Technology, 205-220.
[10] K. Brown (Chairman), K. A. (2006). Guidelines on air handling in the food industry. Food Science and Technology, 331-336.
[11] Laboratories, H. (2018). Technical Data: Nutrient Agar. Mumbai: HiMedia Laboratories Pvt. Limited.
[12] Nik Noor Asma Nik Wil, N. A. (2014). In vitroantioxidant activity and phytochemical screening of Aquilaria malaccensis leaf extracts. Journal of Chemical and Pharmaceutical Research, 688-693.
[13] Berk, Z. (2018). Dehydration. In Z. Berk, Food Science and Technology (pp. 513-566). Elsevier.
[14] Moosa, S. (2010). Phytochemical and Anti Oxidant Screening of Aquilaria Malaccensis Leaves. Research and Development Seminar.
[15] A.W.N Huda, M. M. (2009). Antioxidant activity of Aquilaria malaccensis (thymelaeaceae) leaves. Phcog Res, 1: 270-3.
[16] Reny Varghese, N. R. (2014). Heat Recovery System in Domestic Refrigerator. International Journal for Research & Development in Technology, 2349-3585.
[17] Ruth MacDonald, C. R. (2017). Food Processing. In C. R. Ruth MacDonald, Understanding Food Systems (pp. 179-225). Elsevier Inc.
[18] Christine Wilson, M. M. (2004). Pathogen growth in herbal teas used in clinical settings: A possible source of nosocomial infection? AJIC Brief Report, 117-119.
[19] Ahmed, S. (26 May, 2011). TuftsNow. Retrieved from Does tea lose its health benefits if it's been stored a long time? And is it better to use loose tea or tea bags?: https://now.tufts.edu/articles/tea-health-benefits-storage-time