

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Date Palm (*Phoenix dactylifera* L.)

Date palms are a type of flowering plant in the *Arecaceae* (Palmae) family that produce dates, which are a type of sweet fruit that can be consumed. Dates are known botanically as *Phoenix dactylifera* L (Idowu *et al.*, 2020). There are many references related to date palms in the Islamic holy book, the Qur'an and prophetic Sunnah. Date palm is mentioned as the ultimate superfood for the preservation of good health. This is clearly stated in the verses of Surah An-Nahl: "And from the fruits of the palm trees and grapevines, you take intoxicant and good provision. Indeed, in that is a sign for a people who reason." (Al-Quran 16:66). Besides, a hadith narrated by Aisha reported that the prophet SAW as saying: "The 'Ajwa' dates of 'Al-Aliya' contain healing effects and are a remedy when taken first thing in the early morning." (Sahih Muslim, 23/5083).

There are four botanical stages of maturity after the cultivation of dates which are known by their Arabic names throughout the world as Kimri (unripe), Khalaal (full-size, crunchy), Rutab (ripe, soft), and Tamr (ripe, sun-dried) (Chandrasekaran & Bahkali, 2013). At the Kimri stage, the dates fruits remain green, hard, and have a bitter taste. The fruits rapidly grow in size, weight, and sugar content; this is also the time when acid activity is at its peak, and moisture content can reach up to 85%. The next six weeks comprise the Khalaal stage which the fruits remain hard and unripe, but their color changes to yellow, orange or red.

At the Khalaal stage, the fruits slowly gain weight. However, the sucrose content increases, moisture content goes down, and tannins will start to precipitate with the drawback of astringency.

The tips of the fruit start to turn brownish at the Rutab stage. This third phase is characterized by a decrease in weight due to moisture loss, and a partial reversion of sucrose content to reducing sugar. The color and taste of the dates are indicated as half-ripe with browning of the skin and softening of the tissues. The moisture content goes down to about 35% and the dates at this stage are harvested and sold as fresh fruit. At the final stage, the dates turn into Tamr only when they are left to continue to ripen on the palm trees for another two to three weeks until the color turns from brown to black-brown, altogether with climatic conditions permitting. The Tamr stage is characterized by a moisture content at which the dates are self-preserving, with the upper limit at around 24-25%, which is a lower moisture content than Rutab (Biglari *et al.*, 2008).

Cultivation of date palms in Malaysia has not yet been widely explored and is only available in small-scale farms which privately owned by the locals. Being an agricultural country, planting date palms can provide diversification and commercialization of fruit tree plantations (Mohd *et al.*, 2007). According to Kamarubahrin *et al.* (2018), date palm is still an extremely rare plant in Malaysia, added with limited research has been conducted on date palm due to the inadequacy of expertise either in date palm research and practice. A study suggests that technical feasibility should be conducted at the micro level to determine the most suitable location for date palm growth in Malaysia. By cultivating local date palm

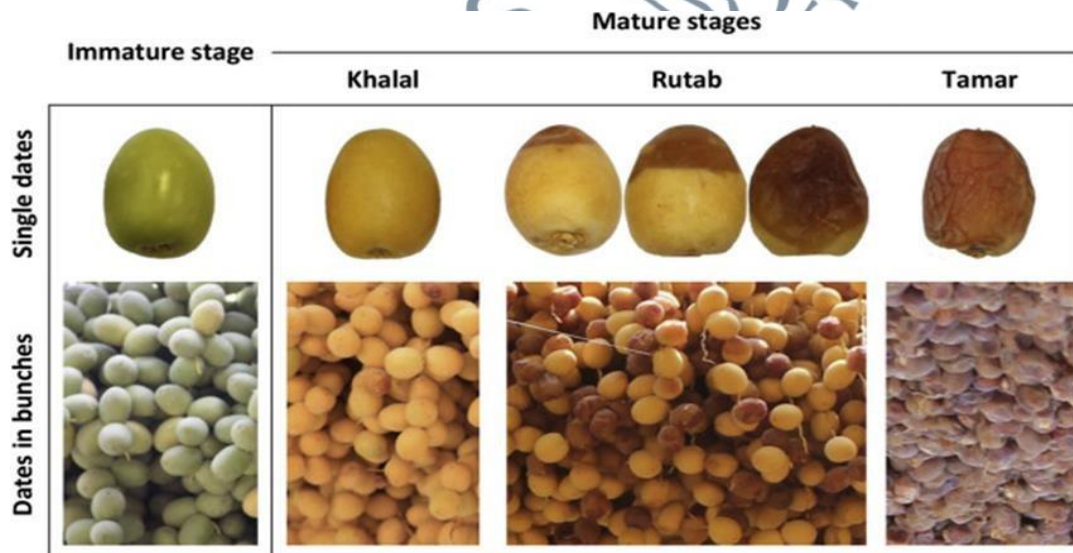
plantations in Malaysia, the dependency on date fruit importation can be reduced and their prices can be lowered (Kamarubahrin & Haris, 2020).

Date palm is a tree that commonly grows to a height between 10m to 15m and lives up to an age of over 100 years (Jahromi *et al.*, 2007). The date palm is one of the three economically important crops of the palm family. Ashraf and Hamidi-Esfahani (2011) reported that the date fruit is mostly cultivated in semi-arid regions of the world. In addition, the production and cultivation of date palms have long been the most important agricultural activity in arid regions of the Arabian Peninsula, North Africa, and the Middle East, as one of the oldest fruit crops grown. The ability of these crops to survive the arid condition makes them the most suitable crops to be used in improving public health. In fact, the date palm has been successfully introduced outside of its origins such as in Australia, Asia, Mexico, United States, India and Pakistan, to be new production areas for date palm cultivation (Chao & Krueger 2007). It is believed that date palm is one of the oldest fruit crops and the first trees to be cultivated for at least 5000 years (Zohary & Hopf, 2000).

There are many types of dates found worldwide, commonly known are, Ajwa, Khalas, Ruthana, Segae, Sukkary, Sefri, Hilali, Khodry and Munifi (Rahmani *et al.*, 2014). Since more people look for alternative treatments that are efficient and secure for curative purposes, dates have been documented as part of the human diet and considered natural remedies. It is known in Arabic as al-nakhl, which etymologically means “good, nourishing and nice smelling” (Zainan Nazri *et al.*, 2016). Dates are cheap to produce and preserve and are also very rich in nutrition. Date fruits have wide scope and potential for consumption as a staple

food and can be the main income source because of their nutritional and economic value (Khan & Khan, 2016).

The current scientific findings explore the various potential beneficial properties of the date palm to human health. Dates contain a high percentage of carbohydrates, 15 salts and minerals, a protein with 23 different amino acids, fat comprising 14 types of fatty acids, six vitamins and a high percentage of dietary fibre (Al Shahib & Marshall, 2003). Meanwhile, Hong *et al.* (2006) found that date fruits contain 6.5-11.5% total dietary fibers, about 2% proteins, 2% ash, 1% fat, and a rich source of phenolic antioxidants. Date fruits have a large potential to be specifically developed in the market as healthy and value-added food products. Hence nutritional values of dates fruits can be utilized to make the fruit an economically viable primary agricultural product (Ghnimi *et al.*, 2017).



**Figure 2.1:** Four major stages of maturity in date palm (Sources:

<https://www.sciencedirect.com/science/article/pii/S2352340919308698>)



**Figure 2.2:** Date palm plantation in Malaysia (Source: Date Palm Agro Bhd)

### 2.1.1 Nutritional Quality of Date Palm

#### a) Sugar

Dates are rich in carbohydrates as the major components, mainly the sugars, which are sucrose, glucose, and fructose, which constitute about 78%. As the dates contain almost half the number of sugars in the form of fructose, which is twice as sweet as glucose, this characteristic plays an important role in the flavour and desirability of the dates. The sugars in dates are easily digested and can immediately be transported to the blood after consumption and later metabolized quickly to release energy for assisting various cell activities in the human body (Ali *et al.*, 2012). Liu *et al.* (2000) reported that date flesh containing glucose and fructose is readily absorbed during the digestion process and can lead to a rapid boost of blood sugar.

The amount of carbohydrates in dates varies depending on the variety and level of ripeness, with the Tamr (completely ripe) stage having the maximum

concentration. The dry date fruit has a large amount of sucrose, while the soft date fruit is primarily made up of inverted sugars (fructose and glucose). Thus, classification of the date fruits can be done based on their type of sugar contents which are inverted sugar types, mixed sugar types, and cane sugar types. Firstly, the inverted sugar types of dates, such as Barhi and Saidy contain mainly glucose and fructose. Secondly, Khadrawy, Halawy, Zahidi, and Sayer are examples of dates with a high proportion of mixed sugar varieties. Lastly, Deglet Nour and Deglet Beidha date varieties are dominated by cane sugar containing sucrose as the main sugar (Ghnimi *et al.*, 2017).

#### **b) Dietary Fiber**

Dietary fibre is defined as plant foods that contain polysaccharides and lignin that are indigestible by enzymes in the human gastrointestinal tract. High value dietary fiber can be found abundantly in date fruits (Ghnimi *et al.*, 2017). Consumption of 100g of dates can provide approximately 50-100% of the recommended amount of dietary fiber needed by the human body. Date fruits have been reported to contain 6.5% - 11.5% total dietary fibers, which is up to 90% of insoluble dietary fiber and 10% of soluble dietary fiber (Al-Shwyeh, 2019). Hong *et al.* (2006) mentioned that date fruits contain 84 – 94% of insoluble and 6 – 16% of soluble dietary fiber, to the total dietary fiber in date fruits.

Both insoluble and soluble dietary fiber types have essential roles for the human body. The intestinal absorption of anti-nutritional factors such as cholesterol can be slowed down due to the formation of a viscous gel in the intestine, which is

contributed by the presence of soluble dietary fibers. Meanwhile, insoluble fibers allow bulking and aid fermentation and generation of short chain fatty acids in the intestine (Ötles & Ozgoz, 2014). In addition, Shafiei *et al.* (2010) identified that dates palm flesh contains water-insoluble fibers consisted of 49.9% lignin and 20.9% polysaccharides. They reported that date fruits fibers have high antioxidant and antimicrobial activities due to associated lignin and tannins contents.

### c) **Amino Acid**

Dates have been described to be a good source of essential amino acids and variations in amino acid contents are found in different date varieties (Al-Farsi *et al.*, 2005). A normal level of amino acids can be provided by diet as they cannot be formed in the human body. Histidine and arginine are examples of amino acids that play crucial importance in relation to the proper physiological functioning of the human body (Al-Aswad, 1971). Assirey (2015) reported that although the concentration of protein in date varieties studied was low (1.72 – 4.73%), dates contain essential amino acids to be considered an important nutritional source. It was discovered that the amino acid composition of date extracts contained low quantities of threonine, isoleucine, serine, tyrosine, methionine, phenylalanine, and lysine and high concentrations of leucine, proline, valine, glycine, histidine, and arginine.

#### d) Mineral

Calcium, potassium, and phosphorus—three minerals that are crucial for human bodies—were present in date fruits at the recommended amounts, according to studies. For the date types Begamjangi, Haleeni, Dashtri, and Peshnah, the average mineral concentrations (calcium, phosphorus, potassium, sodium, and magnesium) ranged from 156.7 to 93.7 mg/100 g. Several date fruit varieties, soil types, and environmental factors affect the mineral content, particularly magnesium and sodium (Aslam *et al.*, 2019). Dates contain the appropriate amounts of calcium, potassium, and phosphorus, which are crucial for the metabolism of human cells (Sawaya *et al.*, 1983).

Based on a study by Assirey (2015), the results suggested that date palm flesh is an important nutritional source of high quantity minerals, with potassium as the predominant mineral. The potassium concentration was the highest which is 289.6 – 512mg/100 g dry matter, followed by calcium, magnesium, phosphorus, and sodium with the lowest concentration. It was discovered that Ajwa dates contained the most minerals when compared to other cultivars, including Mabroom, Shalaby, and Safawy. Magnesium and calcium are necessary for the growth of strong bones and the metabolism of energy, and dates with high potassium and low sodium contents make them ideal for consumption by those with hypertension (Ali *et al.*, 2012).

**e) Vitamin**

There are at least six vitamins which are thiamine, riboflavin, niacin, ascorbic acid (vitamin C), pyridoxin, and vitamin A have been reported to be present in date fruit at visible concentrations (Al-Shahib & Marshall, 2003). According to Ali *et al.* (2012), higher concentrations of vitamins are present in fresh dates compared to dry dates because the vitamin content is lost during the drying process. However, dried dates can be regarded as a moderate source of riboflavin, pyridoxine, niacin, and folic acid, meanwhile thiamine, ascorbic acid and vitamin A are present in relatively low concentrations in the dried dates. A nutritional analysis of date fruits reported that dates represented small amounts of vitamin C (0.7 – 0.9 mg%) and vitamin A (0.7 – 1.2 mg%) (Parvin *et al.*, 2015).

**f) Antioxidant**

The dates fruit has multiple medicinal advantages in the management of disease, as indicated by a review of preclinical tests. Among 28 fruits commonly consumed in China, date fruits were reported to have the second highest antioxidant activity (Guo *et al.*, 2003). Antioxidants are chemicals or materials that can neutralize and inhibit the actions of free radicals and consequently prevent them from causing harm (Rahmani *et al.*, 2014). Date fruits are a good source of natural antioxidants with antimicrobial properties that provides protection against several bacterial pathogens due to the presence of active phenolic compounds (Al-Shwyeh, 2019). The consumption of one hundred grams of dates is estimated to have

between 250 and 450 mg of phenolic substances (Ghnimi *et al.*, 2017). (Ghnimi *et al.*, 2017). The wide range of phenolic compounds present in date fruits, including p-coumaric, procyanidins, sinapic acids, flavonoids and ferulic are responsible for triggering antioxidant activity (Mansouri *et al.*, 2005).

### 2.1.2 Potential Health Benefits of Date Fruits

#### a) Prevention and Control of Diabetes Mellitus

Diabetes mellitus is a metabolic condition characterized by chronic hyperglycemia caused by abnormalities in insulin production, insulin action, or both. Various kinds of diabetes mellitus, such as type 1, type 2, gestational diabetes, and others, are compared based on diagnostic criteria, aetiology, and genetics (Kharroubi & Darwish, 2015). Carbohydrates are classified based on their glycemic responses, which were referred to as the glycemic index (GI) and are a widely accepted measure of the effect of carbohydrate foods on human health (Jenkins *et al.*, 2002). A high GI diet can cause hyperglycemia and hyperinsulinemia, which can increase the risk of developing chronic diseases. Meanwhile, low GI diets have been shown to improve blood glucose control, lower blood lipid levels, and reduce insulin demand, which is all thought to be important factors in the management or prevention of a number of chronic diseases (Brand-Miller, 2003).

A study conducted by Alkaabi *et al.* (2011) reported that in healthy people, the mean range of GIs for five different types of dates in the Tamr stage—Fara'd, Lulu, Bo ma'an, Dabbas, and Khalas—was 46 to 55; in type 2 diabetic patients, it was 43 to 53. According to the findings, the tested dates would have low GIs in

healthy participants, while diabetic patients who consume dates do not experience substantial postprandial glucose excursions. Dates are therefore categorized as low GI foods due to their high fructose and dietary fibre content. In addition, Tahraoui *et al.* (2007) mentioned that in Middle Eastern, dates had been used as a traditional medicine to treat diabetes.

A recent study found that consumption of fruit high in fructose was linked to an increase in plasma antioxidant capacity and plasma urate levels (Lotito & Frei, 2007). So, it's probable that eating fructose-rich date fruits will temporarily raise the plasma urate levels, improving the antioxidant capacity of the blood. The increase in plasma antioxidant capacities would thus attenuate the deleterious effects of reducing sugars on the cellular macromolecules, such as glycation of proteins and lipid oxidation.

Date sugars also show characteristics of being phenol-rich, powerful antioxidants, and strong inhibitors of alpha-glycosidase and alpha-amylase. Date sugar is directly related to the total phenolics present and inhibitory activity against DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical formation. Alpha-glucosidase inhibitors are commonly used to regulate blood glucose levels in type II diabetes by inhibiting membrane-bound intestinal alpha-glucosidases, which hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose in the small intestine. Alpha-Amylase is also considered a potential drug target for the development of anti-diabetic drugs. Salivary and pancreatic alpha-amylase inhibitors block the hydrolysis of complex starches to oligosaccharides. Because of the reduced rate of carbohydrate digestion caused by the inhibition of these enzyme systems, less glucose is absorbed into the circulatory system (Ranilla *et al.*, 2008).

Regular consumption of date fruit which is rich in minerals involved in glucose metabolism, can be a potential preventive and treatment strategy for diabetes (Vayalil, 2012). The important minerals include magnesium (Mg), zinc (Zn), chromium (Cr), manganese (Mn), copper (Cu), and selenium (Se). Mg is important for insulin release, glycolysis, oxidative energy metabolism, and energy-dependent transport mechanisms. The common mineral Zn plays a crucial role in the production, secretion, and signaling of insulin. Whereas Mn and Cu, which are proven to alter glucose homeostasis when deficient, also aid in improving insulin action. Se is a crucial mineral that has been linked to actions that mimic insulin (Stapleton, 2001). It promotes glucose absorption and controls the pentose phosphate pathway, gluconeogenesis, glycolysis, and fatty acid production. Hence, regular consumption of date fruit would maintain these minerals at physiological levels in the body, which could prevent the onset of diabetes in healthy people as well as regulate it in people who are deficient in them.

**b) Cardiovascular Disease**

Cardiovascular diseases (CVDs) remain the leading cause of disease burden for almost all countries (Ruan *et al.*, 2018). High blood pressure is correlated with the strongest evidence for causation among the risk factors for CVD, with a high prevalence of exposure (Fuchs & Whelton, 2020). The causes of essential hypertension remain unknown, however, there is a clear positive correlation between blood pressure and the risk of CVD, including stroke, myocardial infarction and heart failure (Carretero & Oparil, 2000). Date fruit is reported to have a potential protective effect on various conditions that lead to hypertension based

on its nutritional composition. Besides that, both eastern Africa and the Middle East have traditionally utilized date fruit as a meal and medicine to treat hypertension (Vayalil, 2012). Dates may significantly slow the onset and progression of cardiovascular disease by lowering hypertension, high cholesterol, and lipoprotein oxidation, improving serum antioxidant status, and minimizing the damaging effects of oxidative stress and probably inflammation on the vascular system (Vayalil, 2012).

Date fruit is known as an excellent dietary source of potassium with very low sodium content. Hence, frequent date consumption would provide the necessary potassium levels to maintain the body's electrolyte balance and could prevent the onset and progression of hypertension. Mg and Ca are other major dietary minerals at a very high concentration in date fruit that can provide protection from hypertension. Magnesium acts as a natural calcium channel blocker, which may reduce blood pressure. Magnesium is also a cofactor for the 6-desaturase enzyme, which is the rate-limiting step in the conversion of linoleic acid, a precursor to prostaglandin, a molecule that relaxes blood vessels and inhibits platelets. When calcium is combined with other ions like sodium, potassium, and magnesium, an ionic balance to the vascular membrane will happen and causing vasodilation and lowering blood pressure (Al-Dashti *et al.*, 2021).

#### **c) Pregnancy**

Date fruit appears to be a good dietary choice as part of a well-balanced diet for pregnant women, containing a lot of good nutritional value and potential health benefits. Zainan Nazri *et al.* (2016) mentioned that date fruit could contribute significantly to a healthy pregnancy in women due to its properties that can prevent

anaemia, reduce stress, control blood pressure, regulate blood sugar levels, help to restore depleted calcium, expel toxins, increase strength and for immune resistance.

In terms of cervical dilation, spontaneous labour, lower use of oxytocin, shorter apparent latent phase of the first stage of labour and fewer C-sections, women who consumed six dates per day for four weeks prior to the estimated date of delivery had significantly better childbirth than women who consumed none, according to Al-Kuran *et al.* (2011). In the last month of pregnancy, the uterine muscles are strengthened by stimulants found in dates. This is important for uterine dilation during labour and for reducing bleeding after delivery. Dates are considered by dieticians as the best food for pregnant women and women who are breast-feeding their infants since they also contain substances that reduce depression in mothers and increase nutrients in breast milk that children need for their health (Marwat *et al.*, 2009).

A recent non-randomised clinical trial was conducted among postpartum women to compare the effect of date fruit consumption and oxytocin immediately after placenta delivery. As a result, women who consumed 50 grams of oral dates had significantly less bleeding than those who were given 10 units of intramuscular oxytocin (Zainan Nazri *et al.*, 2016). Due to the presence of compounds in date fruit that imitated the action of oxytocin, date fruit consumption after giving birth reduces the amount of bleeding compared to intramuscular oxytocin in the first hour following placental delivery. Besides, the oxytocin-like effect in date palms also can increase the sensitivity of the uterus and stimulates uterine contractions. Thus, dates can be considered a good food supplement alternative in normal delivery (Khadem *et al.*, 2007). Accordingly, dates fruit consumption during late pregnancy

has been shown to positively affect the outcome of labour and delivery and is considered a safe supplement for mother and child.

## **2.2 Goat Milk**

Dairy goat and sheep farming have become a vital economic activity in many countries in Middle East, Europe, Oceania and Asia. It has a beneficial effect on both health and physiological function by providing nutrition to children and elderly people. The nutritional and socioeconomic well-being of developing and underdeveloped countries are strongly influenced by goat milk (Yangilar, 2013). Goat milk has been produced in Malaysia for some time. As goat milk has a significant ideal market, dairy goat farming has developed dramatically in this country, with the majority of dairy goats imported from several countries (Devendra *et al.*, 2012).

As a comparison to cow milk, goat milk fat contains much more short-, medium-, and polyunsaturated fatty acids (Devendra, 2012). Goat milk has higher concentrations of monounsaturated, polyunsaturated, and medium-chain triglycerides than cow milk, all of which are recognized to be good for human health, especially for cardiovascular conditions (Haenlein, 2004). The diverse dietary nutritional contributions made by goat milk are particularly important for resource-poor farming families that are constantly on the edge of subsistence and vulnerability. The majority of milk is marketed informally in rural parts of the country (Devendra, 2012). Bhattarai (2012) mentioned that goats are highly contributed in supplying milk and milk products, which has a significant role in

rural economy sector and health purposes. In addition, the acceptance of goat milk and its products is excellent among consumers. Thus, more efforts in research and development are needed to explore the maximum benefits of goats, particularly their meat, milk, and milk product that have potential medicinal value for human health.

### **2.2.1 Nutritional Quality of Goat Milk**

Goat milk is very famous as a natural food resource that has excellent nutritional properties. Goat milk contains an average of 12.2% total solids, consisting of 4.1% lactose, 3.8% fat, 3.5% protein, and 0.8% ash. These reported data indicate that it has more fat, ash, and protein and less lactose than cow milk but provides a similar level of calories (70 kcal/100mL) for human nutrition as cow or human milk does (Park, 2006). Goat milk is responsible for better digestibility as compared to cow milk because its curd tension is below than cow milk (Bihaqi & Jalal, 2010). Coni *et al.* (1999) reported that goat milk differs from cow or human milk in terms of having better digestibility, alkalinity, buffering capacity, and certain therapeutic values in medicine and human nutrition. The advantage of goat milk consumption should be popularized due to its high nutritional value and this consequently can enhance the manufacturing and utilization of goat milk (Kumar *et al.*, 2012).

#### **a) Carbohydrate**

Goat milk has a basic composition with many similarities to cow milk, such as carbohydrates, lipids, protein, minerals and vitamins (Getaneh *et al.*, 2016). Kunz *et al.* (2000) reported that the major carbohydrate in goat milk is lactose, with a content slightly lower than in cow milk. Glucose and galactose present in the mammary gland synthesized lactose, where the milk protein  $\alpha$ -lactalbumin plays an essential role. Lactose is important because it aids intestinal absorption of calcium, magnesium, and phosphorus and the utilization of vitamin D (Lad *et al.*, 2017). Goat milk also contains small amounts of oligosaccharides, glycopeptides, glycoproteins, and nucleotides. The prebiotic and anti-infective properties of milk oligosaccharides are beneficial to human nutrition when consumed (Lara-Villoslada *et al.*, 2004). The oligosaccharide structures found in goat milk are most similar to those found in human milk. Thus, goat milk also can provide a significant complementary effect in infant nutrition (Lad *et al.*, 2017).

#### **b) Protein and Amino Acid**

A soluble phase made up of whey proteins and an unstable micellar phase made up of casein are the two different phases of milk proteins. When comparing goat milk to cow milk, there are almost comparable amounts of the k-casein fractions, lower levels of the alpha s-casein, and higher amounts of the beta-casein fractions. Hence, beta-casein is the main protein found in goat milk (Diaz-Castro *et al.*, 2010). Also, compared to cow milk, goat milk proteins are thought to be easier to digest and their amino acids are absorbed more effectively. When acidified, caprine milk produces a curd that is softer and more friable, which may be due to

the milk having a lower alpha s1-casein level. Goat milk curds that are smaller and more friable will be degraded by stomach proteases more quickly, which will improve their digestibility (Mestawet *et al.*, 2014). Turkmen (2017) mentioned that goat milk has a better buffering capacity than cow milk due to its relatively higher content of protein, phosphate content, and non-protein N, which would be helpful for treating stomach ulcers. Additionally, goat milk contains greater concentrations of key essential amino acids, including cysteine, tyrosine and lysine, and the branched-chain AAs, isoleucine and valine which is important for human nutrition (Greppi *et al.*, 2008).

#### **c) Mineral and Vitamin**

The vitamin and mineral content of goat milk is almost similar to that cow milk. Krstanovic *et al.* (2010) reported that goat milk has a higher content of Calcium, Potassium, Copper, Chloride, Phosphorus, Zinc, and Selenium than cow milk. Goat milk is a good source of vitamins such as vitamin D, vitamin E, thiamine, niacin and riboflavin. Goat milk, however has a low level of folate (Park, 2006). The consumption of goat milk needs to be supplemented with folic acid because goat milk contains less than 10% of the amount of folic acid contained in cow milk (Getaneh *et al.*, 2016). As with cow milk, goat milk is an excellent source of calcium and phosphorus to human nutrition. Both calcium and phosphorus of goat milk can be absorbed by human infants, thus providing a great excess of such minerals to the energy of young children due to the higher content of most of the minerals (Turck, 2013).

Vitamin A content in goat milk is similar to human milk but higher than in cow milk because goats convert all  $\beta$ -carotene from their foods into vitamin A in the milk (Conesa *et al.*, 2008). Immune responses such as cell-mediated defense and antibody production require vitamin A for both innate and adaptive immune functions (Lad *et al.*, 2017). Besides, goat milk also contains greater amounts of vitamin C than in cow milk, and this vitamin is well-known as a water-soluble antioxidant.

#### **d) Lipid**

The nutritional benefits of goat milk over cow milk are not due to variations in the minerals or proteins but from the lipids, especially the fatty acids within the lipids (Haenlein, 2004). The observation on fat globules of goat milk found a similarity to that of cow milk in lipids composition and properties, but goat milk lacks agglutinin. Goat milk fat contains much more butyric, caproic, caprylic, capric, lauric, myristic, palmitic and linoleic fatty acids than cow milk fat (Jenness, 1980; Mollica *et al.*, 2021). In addition, goat milk has higher values of free lipids than that of cow milk (Cerbulis *et al.*, 1982; Mollica *et al.*, 2021 ).

Lad *et al.* (2017) mentioned that medium chain triglycerides (MCTs) are able to supply energy without being deposited in the fatty tissue of the body. The high amounts of short and medium chain fatty acids (MCT) in goat milk fat contribute significantly to human nutrition. Firstly, it may be more rapidly digested than cow milk fat because lipase attacks ester linkages of short or MCT more easily than those of longer chains. Secondly, these fatty acids have positive effects on

cholesterol metabolism, including hypocholesterolaemia action on tissues and blood via inhibition of cholesterol deposition and gallstone cholesterol dissolution (Alferez *et al.*, 2001).

## **2.2.2 Potential Health Benefits of Goat Milk**

### **a) Allergy and Anti-Inflammatory Effects**

The definition of allergy is an abnormal tissue reaction following exposure to foreign antigen (McCullough, 2003). Protein is the most common antigen. The most frequent protein sensitivity occurs in infants, with approximately 2-6 % incidence (Lara-Villoslada *et al.*, 2004). According to the researcher, alpha s-1 casein is often the cause of cow milk intolerance. Goat milk is less allergic because the level of the alpha s-1 casein goat milk is 89 % lower than that of cow milk. Goat milk has been proven to show improvements in colic, minor digestive disorders, asthma and eczema over cow milk and individuals with cow milk sensitivities (McCullough, 2003). Lara-Villoslada *et al.* (2004) reported a study about allergenicity of goat milk and cow milk in mice. The results demonstrated that goat milk provides immunological benefits in reducing specific markers involved in the allergic response (Lara-Villoslada *et al.*, 2004).

Albenzio *et al.* (2012) in their studies proved goat milk exhibited anti-allergy benefits upon drinking when a similar trial was taken among children with cow milk protein allergies. In addition to the lack of inflammatory effects with goat milk, those who drank goat milk had higher levels of the anti-inflammatory cytokine IL-10. IL-10, which inhibits the production of pro-inflammatory cytokines

like TNF-, is hypothesized to aid the immunological suppression and thereby limiting reactivity to antigens in cow milk (Albenzio *et al.*, 2012). Besides, goat milk contributes to almost all biological reactions and utilizes antioxidant and anti-inflammatory effects on the human body. Due to the smaller size of the fat globules in goat milk, which is only one-ninth of the size of cow milk fat globules, goat milk does not cause irritation effect in the gut (Lad *et al.*, 2017). Shea *et al.* (2004) supported that probiotics and prebiotics contents in goat milk help to maintain a healthy intestinal microflora which is important for protection against the pathogenic infection allergy that can cause negative effects, including many diseases.

## **b) Malabsorption Syndrome**

Physiological digestion and absorption of nutrients within the gastrointestinal tract is a complex process with the possibility of disturbances in gastrointestinal motility and related diseases that may lead to malabsorption. Milder motility disturbances can contribute to various symptoms, such as diarrhoea, constipation, and abdominal pain. Meanwhile, severe motility disturbances can induce impairment of nutrient absorption (Keller & Layer, 2014). According to Lad *et al.* (2017), goat milk contains medium chain triglycerides (MCTs) that may be used to treat the absorption disorders such as diarrhoea, coeliac disease, liver disease, steatorrhoea (fat indigestion) and digestion problems due to gastrectomy.

In an animal study, Alferez *et al.* (2001) examined the effect of goat and cow milk fat on the digestive processes connected to lipid metabolism. They discovered that rats given a diet high in medium-chain triglycerides from goat's milk digested fat more efficiently than those given a diet based on cow's milk. Goat milk consumption decreased cholesterol levels while maintaining normal levels of triglycerides and high-density lipoprotein. This is due to the presence of higher level medium chain triglyceride (MCT) (36% in goat milk vs 21% in cow milk), which decreases the synthesis of endogenous cholesterol. Consuming goat milk increases the body's absorption of iron and copper, according to Lad *et al.* (2017). Several research demonstrated that goat milk assimilates easily and increases the bioavailability of nutrients (Lad *et al.*, 2017).

**c) Nutritional Nourishing**

Approximately 35% of the daily calcium needed in a glass can be provided by consuming goat milk. In addition, a glass of goat milk provides up to 20% of the daily needs of riboflavin. Furthermore, goat milk also contains high levels of potassium and vitamin B12. According to the researchers, goat milk can be considered a functional and nutraceutical health drink. Goat milk can be a useful source of nutrients for people who have a cow milk allergy or intolerance, especially those who suffer from anaemia, osteoporosis, or malabsorption (Lad *et al.*, 2017). According to Zago and Oteiza (2001), goat milk improves Zn bioavailability, which is a well-known mineral with antioxidant capacity. Regular consumption of goat milk may lead to a positive effect on genomic stability, even during the Fe-overloading feeding routine, which could be due to the high bioavailability of Mg and Zn (Diaz-Castro *et al.*, 2009), altogether with goat milk's better quality of fat content (Alferez *et al.*, 2001).

**d) Cardiovascular Disease**

Monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and medium chain triglycerides (MCT) contents in goat milk are better than that of cow milk, which is beneficial for cardiovascular conditions. At the same time, Haenlein (2004) also mentioned that goat milk has a lower level of cholesterol than cow milk. Goat milk has a balanced fatty acid profile, which may contribute to the prevention of atherosclerosis, heart attacks, strokes, and other heart complications.

Goat milk also has a high content of potassium which can reduce the blood pressure level. Goat milk is a good choice for preventing cardiac disorders because its fat can reduce total cholesterol levels. Alférez *et al.* (2019) concluded in their paper that the consumption of fermented goat milk improves haematological status and promotes beneficial metabolic response, which may reduce the severity of cardiovascular risk factors during anaemia recovery and iron overload to reduce the inflammatory response, macrophages activation, and atherosclerosis development.

## **2.3 Metabolism of Iron**

### **2.3.1 Iron Bioavailability**

The amount of ingested iron absorbed and used by the body for physiological functions and storage is known as iron bioavailability. Dietary iron has two main forms, namely heme and non-heme. Heme iron comes from haemoglobin and myoglobin in animal source foods, whereas non-heme iron is found in both plant and animal sources. Heme iron is better absorbed than non-heme iron. Approximately 40 % of iron from animal sources is heme iron, whereas plant-source iron is exclusively non-heme iron. In adults with adequate iron stores, approximately 25 % of heme iron is absorbed (Monsen *et al.*, 1978). Non-heme iron often absorbs significantly less efficiently than heme iron. The amount of absorption of all non-heme dietary iron that enters the common iron pool in the digestive system varies depending on the balance between absorption inhibitors and enhancers as well as the individual's iron status.

**a) Factors Influencing Iron Bioavailability**

Enhancers and inhibitors in the diet significantly impact the bioavailability of dietary iron. Depending on the ratio of enhancers to inhibitors in the meal, the iron absorbed might range from 1% to 40%. (WHO, 2001). To increase the bioavailability of iron in normal diets, meal plans might be changed to favour enhancers, reduce inhibitors or both. It is recognized that various variables affect how well iron is absorbed from food. Heme iron absorption is more moderately influenced by the iron status of the host, but absorption of non-heme iron is strongly influenced by iron status and also affected by dietary factors. Animal tissue (meat, fish, and poultry), calcium salts, phytates, ascorbic acid, and polyphenols ((beans, coffee and tea) are dietary components that affect the absorption of non-heme iron. In addition, the amount of non-heme iron consumed also affects absorption capacity, with higher intakes resulting in a lower percentage of absorption. Iron absorption may also be influenced by other host factors such as hepcidin, inflammation or infection, and even genetic factors. Ascorbic acid and animal tissue are two dietary components that improve iron absorption, whereas phytic acid, polyphenols, and calcium salts inhibit it.

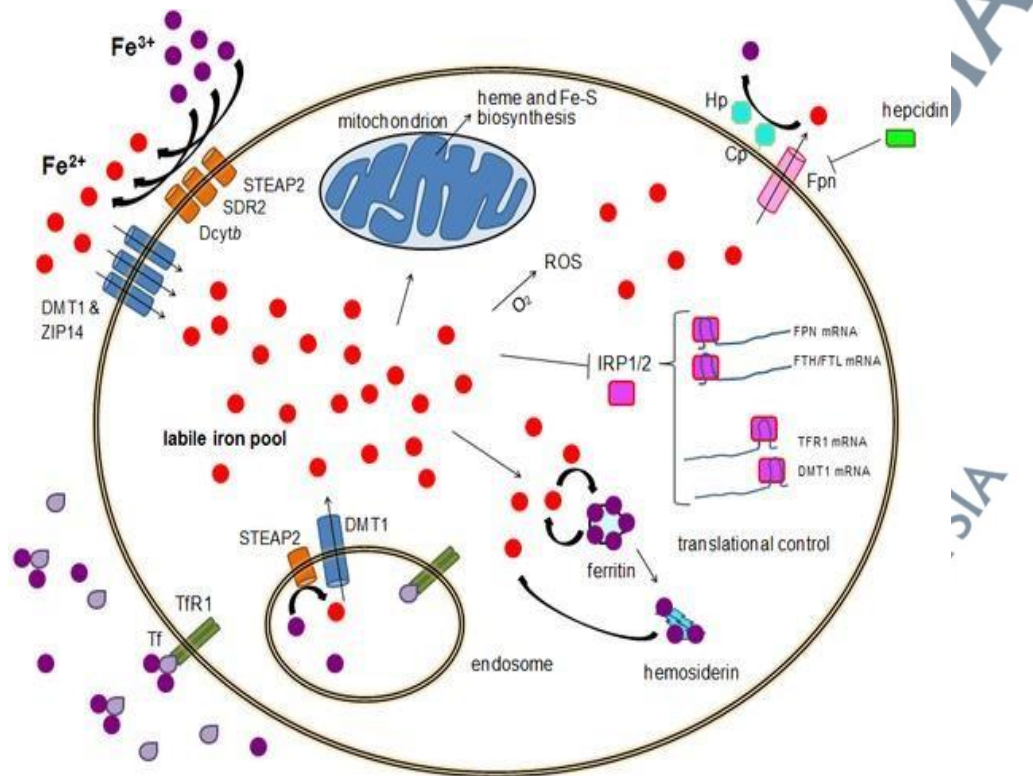
## **b) Method in Estimating Iron Bioavailability**

The study of iron bioavailability in humans can be imitated by using Caco-2 cell line. Caco-2 cells are human epithelial cell line that behaves like intestinal cells derived from a human colonic adenocarcinoma. It is well established as an *in vitro* model in many research of iron absorption. This method is usually combined with a gastrointestinal model, a solubility test, or a dialysability test. For example, after the food sample has been digested, ferritin production in a monolayer of Caco-2 cells can be used to measure iron intake. These combined techniques have the benefit for allowing the researcher to study iron absorption and transport mechanisms. Wood and Tamura (2001) mentioned that many researchers are convinced that human absorption studies are the benchmark for evaluating human iron bioavailability. However, the studies of iron bioavailability in humans are usually high-cost as they may require tracer iron. There is an absence and limitation in published work that can prove the direct relation between estimates of relative iron bioavailability determined in the Caco-2 cell system with human iron absorption studies. However, Au and Reddy (2002) published a study that showed a significant correlation ( $r = 0.92$ ) between iron uptake in Caco-2 cells from various food digests and human iron absorption. It is necessary to conduct more validation studies, especially regarding iron supplements, even though Beard (2007) believed that an *in vitro* approach using Caco-2 cells is promising in predicting iron bioavailability in human studies. In addition, the *in vitro* methods for evaluating iron bioavailability could be a rapid and low-cost alternative.

Yun *et al.* (2004) demonstrated gastric and intestinal digestion and absorption using *in-vitro* digestion coupled with a Caco-2 cell culture model to replicate meal intake in human studies, such as egg meals with different ascorbic acid concentrations

and wheat rolls with various tannic acid concentrations. This study is conducted to determine how iron bioavailability using cell culture can be used to assess human iron bioavailability. The Caco-2 cells formed a ferritin substance that serves as a marker for cell iron uptake and is subsequently used as a functional indicator of iron bioavailability in both absorption and utilization of iron in the tested materials. This method found a strong significant correlation between iron uptake by Caco-2 cells model from the meals and humans. However, there is almost no possibility of having a concrete measure of iron bioavailability because iron is measured in the tested material and the percent of absorbable iron cannot be calculated from the ferritin concentration measured in Caco-2 cell lysate. On the other hand, this model can also be utilized to study the interactions between iron and enhancers or inhibitors. For example, a study was conducted to determine the effect of fructose on iron uptake in Caco-2 cell by adding fructose 1,6-biphosphate (F17BP) into iron and resulted to an increase in ferritin formation in Caco-2 cells (Rodriguez-Ramiro *et al.*, 2019). It has been shown that cabbage has the highest relative bioavailability of iron, followed by broccoli, pepper, kale, and spinach. It is possible that the complex formation of the fructose contained in cabbage explains the higher iron bioavailability in that vegetable (Rodriguez-Ramiro *et al.*, 2019).

### 2.3.2 Iron Absorption



**Figure 2.3:** Diagram showing a generalized view of cellular iron homeostasis in humans. (Source: [https://en.wikipedia.org/wiki/Human\\_iron\\_metabolism](https://en.wikipedia.org/wiki/Human_iron_metabolism))

Heme and non-heme iron make up dietary iron. The origin of heme iron is exclusively from animal sources, mainly from the breakdown of haemoglobin and myoglobin (Dunn *et al.*, 2007), with approximately 40 % of iron from animal sources is heme iron (Du *et al.*, 2000). On the other hand, non-heme iron comes from both plant and animal sources.

Heme and non-heme iron are absorbed through different pathways, with the absorption process mostly taking place in the duodenum. Iron from non-heme sources is in the ferric form and must first be converted into ferrous iron before absorption, which is facilitated by the ferric reductase duodenal cytochrome b (DCYTB) (Hentze

*et al.*, 2004). Then, ferrous iron enters the enterocytes through the apical membrane. The uptake of non-heme iron is mediated by divalent metal transporter I (DMT1), also known as divalent cation transporter I (DCT1). Heme iron is different from non-heme because it is already in the ferrous form. It is transported across the apical membrane by a mechanism that is still not well known among researchers. It was suggested that heme carrier protein (HCP1) assists in transporting heme iron. However, HCP1 has been shown to transport mainly folate (Qiu *et al.*, 2006). Steinbicker and Muckenthaler (2013) explained that heme enters the enterocyte as an intact iron protoporphyrin complex. The iron is released from heme by the hemoxygenase 1 (HO-1) once its is inside the enterocyte. Then, both sources of iron enter a common labile iron pool (Dunn *et al.*, 2007).

The iron is either stored as ferritin or exported out of the enterocyte by ferroportin (FPN1) (Steinbicker and Muckenthaler, 2013). The excess iron that mostly stored as ferritin is lost through exfoliation of the cell. Approximately 10 % of the iron stored in ferritin is later exported out of the enterocyte (Lynch, 2000). FPN1 is expressed in the basolateral membrane of the enterocytes and in macrophages, liver, placenta and spleen (Lieu *et al.*, 2001). Hepcidin is a peptide hormone that controls the surface expression of FPN1, which also regulates its function by triggering its internalization and degradation following iron replete conditions (Steinbicker and Muckenthaler, 2013). The ferrous iron exported by FPN1 is taken up and transported by the transport protein transferrin and converted to ferric iron due to ferroxidase activity. The ferroxidase activity in the enterocytes is provided by hephaestin, a homologue of ceruloplasmin. Meanwhile, the ferroxidase activity in non-intestinal cells is provided by ceruloplasmin. Steinbicker and Muckenthaler (2013) also explained that the mRNA expression for

DCYTB, DMT1, HO-1 and FPN1 is controlled by hypoxia inducible factor 2 (HIF-2), while posttranscriptional of DMT1 and FPN1 is regulated by iron regulatory proteins (IRPs) in response to intracellular iron levels.

#### **a) Inhibitors of Iron Absorption**

##### **i) Phytate**

Phytic acid is also known as an iron absorption inhibitor. It is abundant in grains, legumes, nuts, and oil seeds. The salt form of phytic acids such as magnesium, calcium, or potassium salt is referred to as phytate (Brown *et al.*, 2004). The mechanism of iron inhibition by phytate is due to its ability to bind to iron and subsequently form insoluble complexes that are unable to be absorbed at intestinal pH. (Minihane and Rimbach, 2002). The phytic acid molecule contains negative charge phosphate, which binds to metallic cations such as iron, calcium, and zinc, leaving them insoluble and decreasing bioavailability.

Higher inositol phosphates (myo-inositol hexaphosphate-IP6 and myoinositol hexaphosphate IP5) level are the most common cause of inhibitory effects of phytates on iron absorption. Sandberg *et al.* (1999) studied the effects of inositol phosphates with different number of phosphorus groups on iron absorption and he found that IP5 inhibited iron absorption but no effect with IP3 and IP4. The in vitro studies have also shown that even small quantities of 0.5  $\mu\text{mol}$  of IP5 and IP6 can significantly reduce iron solubility, meanwhile, IP3 and IP4 did not (Sandberg *et al.*, 1989). Hallberg *et al.* (1989) also mentioned that the effect of phytic acid on non-heme iron absorption is dose-dependent, similar with ascorbic acid.

## ii) Polyphenol

Polyphenols are a class of natural chemicals that have phenolic structural characteristics (Tsao, 2010). Based on their chemical structure, Manach *et al.* (2004) categorized them as phenolic acids, flavonoids, polyphenolic amides and other polyphenols. The health advantages obtained are determined by the bioavailability of polyphenols and the amount consumed. The antioxidant content of various polyphenols found in our food sources, such as edible plants, is the most prevalent dietary source of antioxidants. The properties are critical for enhancing human health, especially in the prevention of degenerative diseases such as cancer, neurological diseases, diabetes, cardiovascular disease, and osteoporosis (Scalbert *et al.*, 2005). Scalbert *et al.* (2005) also stated that fruits, vegetables, whole grains, tea, coffee, chocolate, red wine, legumes, and other foods possess a high concentration of polyphenols.

Many fruits and foods naturally contain a group of phytonutrients called flavonoids (Manach *et al.*, 2004). Flavanols may exist as simple monomeric (catechins), dimeric (theaflavins), or polymeric (procyanidins). Green tea, chocolate, red wine, and fruits like apricot, grapes, and berries are the main sources of catechins. However, theaflavin is a molecule found in black tea that is produced during green tea fermentation by the oxidation of monomeric flavanols (Manach *et al.*, 2004).

Polyphenols are also known to have anti-nutritional effects. Anti-nutritional factors can interact with nutrients, reducing their bioavailability. Polyphenols inhibit the absorption of iron that is released from meals by binding and forming complexes with iron which take place in the gastrointestinal tract (Schönfeldt *et al.*, 2016).

### iii) Calcium

Calcium is an important mineral for human nutrition that cannot be produced in the body and must be gained from diet. Milk, yogurt, and cheese are examples of dairy products that are common sources of calcium. The effect of calcium on iron absorption has raised concerns because children and women are at risk of iron insufficiency, although the same populations are also advised to increase their calcium consumption.

Calcium is unique among dietary nutrients; it is the only known dietary component that inhibits both heme and non-heme iron absorption. Regarding how calcium affects iron absorption, there is still some debate. According to Lynch (2000), the inhibitory effect of calcium on iron absorption is caused by the interaction of calcium and dietary components that alter iron bioavailability. Moreover, the interaction of calcium on luminal surface receptors during iron uptake may possibly be the cause of iron inhibition. Hallberg *et al.* (1991) postulated that the effect of calcium is at the mucosal level, in which both heme and non-heme iron undergo a common process.

## **b) Enhancers of Iron Absorption**

### **i) Ascorbic Acid**

Ascorbic acid is a water-soluble vitamin C present in food that has both enzymatic and non-enzymatic activities. It acts as a cofactor for several metabolic reactions in its enzymatic activities. Ascorbic acid, on the other hand, is a powerful antioxidant that aids in the scavenging of free radicals and reactive oxygen species (Lykkesfeldt *et al.*, 2014). The majority of vitamin C intake comes from vegetables and fruits, particularly citrus fruits. High quantities of vitamin C have been found in kiwi, mango guava and broccoli.

By converting iron from ferric to ferrous form, vitamin C is a common booster of non-heme iron absorption. As a result, iron is effectively transported across the enterocytes' apical membrane. Ascorbic acid also aids in iron chelation by forming a soluble compound across a wide pH range (Lynch and Cook, 1980). In addition, ascorbate, ascorbic acid salt, donates an electron, acting as a free radical scavenger and reducing iron oxidation states to ferrous ion form, which is the only bioavailable form for enterocyte cells (Smirnov, 2018). ferrous ion form is the only iron that can be absorbed through iron transporters of intestinal enterocyte cells (Gulec *et al.*, 2014).

Kuhn *et al.* (1968) found that adding ascorbic acid to corn and wheat diets increased iron absorption. Using data from a 1970 study by Callendar and Marney, they discovered that adding 100 ml of orange juice to an egg meal increased iron absorption from 3.7 to 10.4%. Ascorbic acid's effect on the absorption of non-heme iron has also been demonstrated to be dose-dependent (Cook and Monsen, 1977). Even when inhibitors are present, ascorbic acid has a remarkable ability to overcome their

inhibitory effects. In research by Hallberg *et al.* (1989), adding 50 or 100 mg of ascorbic acid led to a noticeably higher rate of iron absorption from meals containing 25 and 250 mg of phytate phosphorus as an inhibitor.

## **ii) Animal Tissue**

Animal foods are well acknowledged as a well-known diet that can aid in non-heme iron absorption. To increase the bioavailability of iron in food, more animal tissue is being added to meals (Reddy *et al.*, 2006). Bach *et al.* (2003) proved the influence of animal tissue on iron absorption by incorporating 50g of meat into a low bioavailability test meal, which increased iron bioavailability by more than 40%. The impact of adding meat (with or without ascorbic acid) to weaning gruel meals on the absorption of non-heme iron was examined by Hallberg *et al.* in 2003. They discovered that adding meat powder dramatically boosted gruel's ability to absorb iron by 85%.

The overall amount of iron is absorbed more effectively due to the heme component of animal tissue found in meat, fish, and poultry. Animal tissue improves non-heme iron absorption in vegetarian diets and numerous potential mechanisms by which meat improves non-heme iron absorption has been postulated. One of the proposed mechanisms is the stimulation of gastric juice secretion. They also observed that the test subjects' ability to absorb iron was increased by the addition of gastric juice. Another suggested mechanism is the discharge of digestion products of animal tissue. According to Hurrell *et al.* (2006), animal tissue releases partially digested products that can bind iron via histidine and cysteine residues. The binding mechanism causes iron to become soluble, then increase iron absorption. In this approach,

inhibitors such as phytates and polyphenols found in meals are unable to bond with iron, preventing iron inhibition.

### **iii) Fructose**

Fructose is a monosaccharide, a type of simple sugar that naturally exists in fruits, honey, and some vegetables. According to O'Dell (1993), fructose increases iron bioavailability via enhancing ferrous iron production. Christides and Sharp (2013) also discovered that fructose dramatically increases iron bioavailability in human intestinal epithelial cells by increasing ferrous iron production utilizing in vitro cell models of the gut and liver. The results reveal that when fructose is mixed with FeCl<sub>3</sub>, the ferrozine-chelatable ferrous iron levels increase by roughly 300%. This study also discovered that fructose increases the levels of iron-induced hepatic ferritin.

### **2.3.3 Iron Transport and Cellular Uptake**

Transferrin is a glycoprotein, which is a major iron transport protein synthesized mainly by the liver. It has two domains for binding iron molecules and binds iron with very high affinity at a pH of 7.4 (Lane and Richardson, 2014). The ferric iron is taken up by transferrin and transported to target cells. The holotransferrin (HTF) is an iron-transport protein sourced from plasma, which is taken up into the cell by transferrin receptor (TfR), a transmembrane protein consisting of two identical subunit. Each of the two subunits of the TfR binds to one transferrin molecule. There are two types of TfR, namely TfR1 and TfR2. TfR1 is expressed in all tissues and is synthesized in the endoplasmic reticulum. Meanwhile, TfR2 is expressed only in hepatocytes, duodenal

crypt cells and erythroid cells (Lane and Richardson, 2014). Besides, TfR2 binds to transferrin with a lower strength of binding than TfR1 due to a mutation in its gene linked to hemochromatosis (Papanikolaou and Pantopoulos, 2005). At the target cell surface, TfR1 binds to the holotransferrin and the complex is then endocytosed. The release of the iron is mediated by acidification of the endosome by ATPase proton pump. The iron is released at a pH of approximately 5.5. Divalent metal transporter 1 (DMT1) is the major iron transporter and plays a role in non-heme iron uptake in most types of cells (Yanatori *et al.*, 2019). DMT1 transports the iron across the membrane of the endosome into the cytoplasm (Chahine and Pakdaman, 1995). The iron can be utilized for cellular purposes, or it can be stored in the primary iron storage protein ferritin. However, iron is also stored in hemosiderin at higher concentrations. The apo-transferrin-TfR1 complex then returns to the cell surface to be able to bind new transferrin.

#### **2.3.4 Iron Storage**

The body stores 0-15 mg/kg of iron, largely as the iron storage protein ferritin, with a small percentage of around 5% as hemosiderin. About half of the body's ferritin is found in the liver, with the remaining present in muscles and the reticuloendothelial system. According to Cullis *et al.* (2018), ferritin is a soluble protein that has 24 subunits and a cavity that may store more than 4,000 iron atoms in the ferric form (Arosio *et al.*, 2009). Mammalian ferritin is a heteropolymer composed of Heavy (H) and Light (L) chain components. The H-ferritin protein has a molecular weight of 21 kDa, whereas the L-ferritin protein has a molecular weight of 19 kDa (Cullis *et al.*, 2018). Around 800 iron (Fe III) sites are filled when ferritin is 20% saturated in vivo

(Beard, 2001). Ferritin, with a high amount of H subunit, is found mostly in the heart, red blood cells, and the brain, and it has a ferroxidase site with high ferroxidase activity. The L-type chain, meanwhile, is primarily present in the liver and spleen, which have larger cavities for storing iron. It serves to accelerate the nucleation of iron and the turnover of the ferroxidase center (Hentze *et al.*, 2010). The chemical interaction of ferritin with ferrous iron oxidizes it to ferric iron for deposition into its cavity. In the reaction that takes place at the ferroxidation center, ferritin uses dioxygen as an oxidant during the oxidation process. The iron oxide substance is kept in the central cavity. Because this process uses both ferrous and oxygen, it is considered to prevent the production and formation of free radicals in cells and tissues (Romeu *et al.*, 2013). The body's metabolism then releases the iron that has been stored in ferritin when needed, primarily by proteolytic ferritin breakdown. In disease conditions such as iron deficiency, ferric iron in ferritin may be reduced to ferrous iron as an alternative approach that results in the release of iron (Arosio *et al.*, 2009).

### **2.3.5 Regulation of Iron Storage and Uptake**

#### **a) Ferritin and Transferrin Receptor (TfR)**

Regulation of iron absorption, transport and storage occurs mainly at the translational level but some control mechanisms exist at the transcriptional level. Homeostasis of iron is accomplished through the iron regulatory proteins (IRPs) and iron-responsive elements (IRE) signalling pathway (Zhou & Tan, 2017). IRPs bind to IREs in target mRNAs to regulate the expression of genes involved in the metabolism of iron (Zhang *et al.*, 2014). There are two types of IRPs, namely IRP1 and IRP2. Both

types of IRP are expressed in all tissues; however, IRP2 is expressed in lesser quantities in most tissues. The function of this protein depends on the intracellular iron concentration. The IREs are responsible as either enhancers or inhibitors of translation under different circumstances. They are located either at the 3' untranslated region (UTR) or the 5' UTR of the mRNA. The binding of IRP to the protein's IRE located at the 3' UTR leads to the stability of the mRNA resulting in translation and increased protein synthesis. Meanwhile, the location of IRE at the 5' UTR causes the binding of IRP to the IRE blocks mRNA translation (Hentze *et al.*, 2010). The IRE for ferritin is located at the 5' UTR but the IRE for transferrin receptor (TfR) is located at the 3' UTR. In a condition of iron deficiency, the IRP1 and IRP2 binds to the IRE for both ferritin and TfR, consequently decreasing iron storage and increasing iron intake. The ferritin translation is blocked and ferritin synthesis is prevented because the binding activity to the 5' UTR impedes ribosome binding and translation of the protein. The binding to the 3' UTR obscures the mRNA to endonuclease digestion, resulting in translation and increased protein synthesis. Therefore, there is increased stability of the TfR mRNA.

In contrast, the binding activity of IRPs to the IRE is reduced in the presence of iron. There is increased translation of the ferritin mRNA and increased ferritin synthesis, thus allowing iron storage and reduced iron uptake. TfR mRNA, on the other hand, is rendered unstable, affecting the translation and synthesis of the protein. Iron regulates the function of IRP1, which is also capable of functioning as an isoform of cytoplasmic aconitase. According to Gourley *et al.* (2003), there is an assembly of [4Fe-4S] clusters taking place in a condition in which iron is presented that reduces the affinity of IRP1 for the IRE. However, there is a disassembly of the [4Fe-4S] cluster in iron deficiency conditions. Thus, IRP1 has a strong affinity for the IRE, therefore, can easily bind IRE,

functioning as a DNA-binding protein. Unlike IRP 1, IRP2 does not have those [4Fe-4S] clusters and its regulation by iron occurs through degradation by proteasomes. In addition, ferritin is also a major predictor of iron absorption from meals. Hunt *et al.* (2010) reported that ferritin is entitled to at least 60 % of the variability in iron absorption from non-heme sources by affecting absorption by 10-15 folds. According to Armah *et al.* (2013), ferritin influences 35 % of the variability in non-heme iron absorption from whole diets. In contrast, Reddy *et al.* (2000) reported that ferritin accounted for 32 % of the variability in non-heme iron absorption. Ferritin is also said to be inversely related to iron absorption because as iron stored increases, the absorption of non-heme iron decreases and vice versa (Cook *et al.*, 1991).

#### **b) The Role of Hepcidin in Iron Metabolism**

Hepcidin is an antimicrobial hormonal peptide, considered the main regulator of iron absorption. It is important in iron homeostasis, playing a key role in iron absorption, recycling and mobilization. Hepcidin is a systemic iron regulatory hormone composed of 25-amino acids and is mainly produced by the liver (Zhang *et al.*, 2014). Hepcidin regulates iron homeostasis by controlling the iron export protein, ferroportin (FPN1) expression and function. FPN1 is a multi-domain transmembrane protein that acts as the main exporter of iron from the enterocyte, hepatocytes and macrophages. It is known that ferric iron in the diet must first be reduced to ferrous iron for non-heme iron absorption. Then, the divalent metal transporter 1 (DMT1) will be able to transport iron across the apical membrane into the enterocytes. Once inside the cell, FPN1 exports the part of newly absorbed iron across the basolateral membrane into circulating blood (Abboud and Haile, 2000). Hepcidin has been shown to regulate the

expression of iron transporter in response to iron deficiency and anaemia conditions. Hepcidin is able to adjust iron export from enterocytes into the blood by regulating the expression of FPN1 on the basolateral membrane of enterocytes (Ganz and Nemeth, 2011). According to Nemeth *et al.* (2004), hepcidin that is circulated can bind to FPN1 on the plasma membrane to reduce iron influx into the blood in a feedback manner and cause the impaired release of iron from macrophages. These results are due to the binding of hepcidin to FPN1 which causes FPN1 to be internalized, dephosphorylated and degraded.

Dysregulation of the hepcidin-FPN1 interaction is implicated in anaemia of inflammation and chronic disease, and also in the systemic iron overload disease such as hemochromatosis, because of the role of hepcidin in regulating iron homeostasis (Zhang *et al.*, 2014). Anaemia of inflammation is linked to increased hepcidin levels and characterized by a decrease in iron absorption in the intestines and macrophage iron release, including an increase in reticuloendothelial iron. During infection or inflammation, the concentration of hepcidin is increased. The cytokine IL-6 is mentioned to contribute to the stimulation of hepcidin production in inflammation (Andrews, 2004). According to Steinbicker and Muckenthaler (2013), hepcidin is an acute-phase protein and the increased production of hepcidin is mediated by lipopolysaccharides (LPS) and cytokines mainly interleukin- 6 (IL-6). LPS may mediate hepcidin production by inducing endoplasmic reticulum stress and activating C-AMP responsive element binding protein H (CREBH) (Kroot *et al.*, 2011). Hemochromatosis is a type of disorder mainly caused by insufficient production of hepcidin by the hepatocytes. It is characterized by inappropriately low levels of hepcidin and increased intestinal iron absorption and iron release from macrophages.

This condition leads to tissue iron accumulation and organ dysfunction mediated by iron due to an expanded circulating iron pool (Beutler, 2007).

### 2.3.6 Iron Utilization and Recycling

Mitochondria have been assigned crucial roles in the metabolism of iron because they assemble iron-sulfur (Fe-S) protein clusters, act as the site for heme synthesis, and participate in most of intracellular iron metabolism (MacKenzie *et al.*, 2008). Fe-S clusters are protein cofactors found in the mitochondria, nucleus and cytosol that participate in catalytic and regulatory processes in electron transport, tricarboxylic acid (TCA) cycle, amino acid biosynthesis and DNA replication and repair and the regulation of gene expression (Lill *et al.*, 2012). The efficiency of Fe-S cluster synthesis is important to assist the cell in regulating iron homeostasis and maintaining proper intracellular iron distribution. Additionally, the synthesis of heme occurs mainly in developing red blood cells in the marrow, with a small fraction, approximately 15 %, occurring in the liver. The synthesized heme is incorporated into haemoglobin and other hemoproteins. The biosynthesis of heme occurs in the following four steps. Firstly, the formation of the pyrrole; secondly, assembly of the tetrapyrrole, followed by modification of the tetrapyrrole sidechains; and the final step in the heme biosynthetic pathway is the formation of protoporphyrin IX and insertion of iron (Ajioka *et al.*, 2006). The insertion of iron into protoporphyrin IX is catalyzed by ferrochelatase (FECH). Ferrochelatase known as heme synthase is an Fe-sulfur protein located in the inner mitochondria membrane.

Macrophages are important in iron metabolism and recycling. Red pulp macrophages (RPMs) in the spleen, central nurse macrophages (CNMs) in bone marrow, and Kupffer cells (KCs) in the liver are the tissue macrophages responsible for iron recycling. After phagocytosis, they use heme oxygenase to catabolize the heme from senescent and damaged erythrocytes, releasing the iron. Macrophages retrieve iron for haemoglobin production. They are able to recycle 90-95% of bodily iron while also efficiently maintaining erythropoiesis. For example, haemoglobin production needs approximately 20 mg of iron per day, and iron recycling contributes significantly to daily iron demand (Steinbicker and Muckenthaler, 2013). The significant amount of iron gained through haemoglobin degradation is recycled by iron recycling macrophages, and also recycle other used-up cells into iron that can be used in hemoglobin synthesis, such as producing new erythrocytes (Kong *et al.*, 2008). Most of the iron homeostasis and trafficking is contributed by these specialized macrophage functions.

## 2.4 Function of Iron

Iron is essential for oxygen transport as well as the respiratory chain. Several of its functions are associated with its role in heme biosynthesis. Heme is made up of a ferrous iron that is inserted inside a protoporphyrin ring, which is made up of four pyrrole rings. It is an essential part of haemoglobin, which is necessary for the transportation of carbon dioxide and oxygen throughout the circulatory system. Haemoglobin, a tetrameric protein made up of four polypeptide chains (2 alpha and 2 beta globin chains), is found mostly in erythrocytes. It transports oxygen from the lungs to the tissues and carbon dioxide from the tissues back to the lungs. Heme is a cofactor for several enzymes, including catalases and peroxidases. It is also a component of cytochromes, which are electron transport proteins found mostly in mitochondria and perform significant functions in energy metabolism (Liu, 2014). Iron serves a variety of functions outside of cytochromes, such as participating in Fe-S clusters. Furthermore, Fe-S clusters are crucial components in the respiratory chain. Similar to cytochromes, Fe-S clusters play a role in electron transport. They also perform a number of additional biological functions, including activating and binding to substrates, controlling gene expression, controlling enzyme activity, and disulfide reduction (Johnson, 2005).

## 2.5 Prevalence of Iron Deficiency Anaemia

Iron deficiency anaemia (IDA) is a global health problem and the most frequent cause of anaemia in developed and developing countries, including Malaysia. According to the National Health and Morbidity Survey (NHMS) of 2015, the overall occurrence of anaemia was recorded at 24.6%. NHMS 2015 data also revealed a higher prevalence of anaemia among women in their reproductive years, standing at 34.5% (Awaluddin *et al.*, 2017). This statistic surpassed the global estimate of 29% provided by the World Health Organization (WHO, 2015). In response, the World Health Assembly aimed to decrease the prevalence of anemia among women of reproductive age by 50% by the year 2025 as part of its global nutrition objective (WHO, 2014). The study conducted by Loh and Khor (2010) stated that the overall prevalence of anaemia was 20.9% while IDA was at 10.3%, indicating that about 50% of anaemia cases were caused by iron deficiency.

Haniff *et al.* (2007), in their multicentre cross-sectional study, found that the overall prevalence of anaemia in Kuala Lumpur was 35% (cut-off level at 11g/dL). The majority was in the mild type and the teenage group had a higher incidence, with more cases among Indians followed by Malays and Chinese being the least. A cross-sectional study conducted among 178 non-pregnant women aged between 18 to 35 years old at Universiti Sains Islam Malaysia discovered ninety-nine subjects (55.62%) were found to have Hb levels less than 12 g/dl. Sixty-five subjects (36.52%) were found to have ferritin levels of less than 30 ng/ml. The IDA prevalence in this study was higher than the previous report among non-pregnant females in Malaysia (Nurul, 2017). It has been reported that a moderately

high prevalence of anaemia is found among infants, young children and women of childbearing age. For example, among 490 preschool children in Kelantan, 38.9% were iron deficient (Siti Noor *et al.*, 2006).

Anaemia in pregnancy is one of the major public health problems in developing countries. More than half of the pregnant women in developing countries are estimated to be anaemic, which is 52% compared to 23% in industrialized countries. WHO reported that about 10.8 million in Africa, 9.7 million in Western Pacific and 24.8 million pregnant women in South East Asia are anaemic. In Kelantan, Hassan *et al.* (2005) reported that 21.2% of women attending the antenatal clinic had IDA. A larger study conducted in Kuala Lumpur found that 1066 of 73,048 pregnant women attending the antenatal clinic were anaemic and 35% were associated with IDA (Loh, 2010). Haniff *et al.* (2007) reported that the prevalence was greater among pregnant women in the third trimester, especially from the urban residential areas and the prevalence showed an increasing trend with ongoing pregnancy.

### **2.5.1 Causes of Iron Deficiency**

#### **a) Nutritional Factor**

The human body regularly gets iron from the foods consumed on a daily basis. If too little iron is consumed, over time human body can become iron deficient. Examples of iron-rich foods include red meat, eggs, leafy green vegetables and iron-fortified foods. For proper growth and development, infants and children need iron from their diets too. Nutritional iron deficiency (ID) occurs when physiological requirements cannot be fulfilled by iron absorption from diet supplies. Researchers also suggested a method of selective plant breeding and genetic engineering to improve dietary iron nutritional quality. Iron deficiency in the population can be controlled through targeted iron supplementation, iron fortification of foods, or both (Zimmermann & Hurrell, 2007). Insufficient bioavailable iron could be lost from the gastrointestinal tract and skin, in the urine, and through menstruation (Lynch, 2011).

#### **b) Blood Loss**

Red blood cells in the blood contain iron, thus, if a person loses blood, their iron level will drop. Due to their prolonged and substantial blood loss, women who have heavy menstrual cycles are more likely to develop iron deficiency anaemia. Iron deficiency anaemia can also be brought on by slow, ongoing blood loss within the body, such as caused by colorectal cancer, peptic ulcers, hiatal hernias, colon polyps, or other conditions. Aspirin is frequently used as an over-the-counter pain medication, and also can cause gastrointestinal bleeding. Johnson-Wimbley and

Graham (2011) state that patients with intestinal angiodysplasia, for example, who have gastrointestinal blood loss that exceeds their ability to absorb iron, may develop iron deficiency anaemia that is unresponsive to oral iron supplementation. The most common cause of iron-deficiency anaemia is continuous blood loss, particularly in older patients (Warner & Kamran, 2021).

**c) Inability to Absorb Iron**

In the human small intestine, iron from the diet is absorbed into the bloodstream. Iron deficiency anaemia can be caused by an intestinal problem, such as celiac disease, which impairs the intestine's capacity to absorb nutrients from the food that has been digested. The majority of ingested nutrients, including lipids, carbohydrate and proteins, as well as micronutrients like iron, vitamins, and minerals, as well as water and electrolytes, are absorbed in the small intestine. Surgery to bypass or remove a portion of the small intestine may impair a patient's capacity to absorb iron and other nutrients. Disorders of malabsorption lead to decreased iron absorption and develop iron deficiency anaemia (Saboore *et al.*, 2015). Malabsorption can be caused by premucosal (luminal), mucosal, and postmucosal (postabsorptive). Premucosal causes lead to maldigestion, meanwhile, mucosal and postmucosal conditions are related to actual malabsorption (Vaníèková *et al.*, 2012).

#### **d) Pregnancy**

Many pregnant women develop iron deficiency anaemia without iron supplements because their iron stores must serve both their own increased blood volume and as a source of haemoglobin for the developing foetus. Anaemia in pregnancy is more than 20% of the population worldwide, and Garzon *et al.* (2020) claim that this is a significant public health issue. Compared to a woman who is not pregnant, pregnant women require more iron. This is due to an exponential rise in the amount of iron required to raise plasma volume, produce more red blood cells, maintain fetal-placental unit growth, and compensate for iron lost during birth. During the start of pregnancy, roughly 40% of women exhibit low or absent iron stores, and up to 90% of women have iron reserves of less than 500 mg (Fisher and Nemeth, 2017).

### **2.6 Pathological Effect of Iron Deficiency Anaemia**

#### **a) Cognitive Development**

The most frequent nutrient deficiency in children between the ages of 6 and 24 months is iron insufficiency (ID). This is the period when the brain grows the most and numerous neurodevelopmental processes are at their peak. Since this is the period when the brain grows the most, getting enough iron in diet is essential at this point. Dopaminergic-peptidergic areas have a significant localization of iron (globus pallidus, substantia nigra, red nucleus, thalamus, caudate nucleus and nucleus accumbens). Iron insufficiency results in lower non-heme iron concentrations in the brain and decreases dopamine D2 receptors (Noor Fadzilah,

2013). The role of iron in the development of the hippocampal and the action of neurotransmitters, particularly in the dopaminergic system, is a reason for the impact of iron shortage on cognition.

Another proposed mechanism is connected to the high concentrations of iron found in the cerebellum and basal ganglia, which are crucial for motor function and play a role in myelin synthesis and maintenance (Lozoff, 2000). A deficiency of iron can affect cognitive function and is associated with neurophysiological impairments in infants, pre-schoolers and school-age children, adolescents and adults. The main cognitive impairments caused by iron shortage include those referring to intelligence, sensory perception, and attention span, as well as those related to emotions and behaviour, which are frequently directly linked to the presence of iron deficiency anaemia (Lobera, 2014).

Throughout the first two years of life, iron deficiency anaemia (IDA) is associated with poor psychomotor development and behavioural abnormalities, such as decreased levels of responsiveness to people and stimuli, impatience, and inhibition. Several studies show that effects were seen during infancy last for a very long time. According to reports, preschoolers who were previously anaemic are less energetic, more restrained, and shy than the corresponding controls (Oyarzun, 1993). Children have been found to have worse fine-hand motions and poorer academic achievement (Walter, 2003). Besides, a chronic ID group showed significantly lower scores on language, environmental sound perception, and motor measures when compared with infants with normal nutritional iron status at 6 months and 14–18 months (Beltran-Navarro, 2012).

## **b) Reduced Work Capacity and Productivity**

Poor working ability and reduced work tolerance are two common anaemia signs. Those with low iron levels were much less productive than employees with normal haemoglobin levels. According to studies examining the connection between haemoglobin concentration and work capacity, anaemic individuals have lower work tolerance than those with normal haemoglobin concentrations (Gardner *et al.*, 1977). The decreased work capacity in anaemic patients may be mostly explained by haemoglobin alterations rather than by changes in iron status (Edgerton *et al.*, 1981). A very helpful critical overview of the evidence, especially the laboratory findings on humans and animals, is provided by Haas and Brownlie (2001). The major conclusions are that IDA in humans results in decreased maximal physical working capacity and decreased endurance. The majority of laboratory investigations on both animals and humans show that IDA lowers aerobic work capacity.

Research also shows that IDA impairs aerobic capacity and endurance. Animal and human studies suggested a strong causal relationship between all levels of iron deficiency and voluntary physical activity. The connection between anaemia and work capacity may be caused by iron's involvement in energy production. The respiratory chain, which involves the transport of oxygen, and numerous enzymes, all contain iron. Hemoglobin, which contains iron and is a component of red blood cells, is essential for carrying oxygen from the lungs to the body's tissues. Another iron-containing protein called myoglobin, found in the cytoplasm of muscle cells, aids in the transport of oxygen to the mitochondria. Iron is necessary for the electron

transfer chain-related cytochromes in mitochondria and enzymes like dehydrogenases that oxidize substrates (Haas & Brownlie, 2001).

### c) **Adverse Pregnancy Outcome**

One of the main causes of anaemia in infants and young children is iron deficiency during pregnancy. The last trimester of pregnancy is when pregnant women are most at risk for anaemia and iron deficiency. This happens because iron transfer from mother to fetus is supported by an increase in maternal iron absorption during pregnancy which is regulated by the placenta (Harris, 1992; Starreveld, 1995). Because iron is used for the physiological expansion of the maternal red blood cell mass, serum ferritin typically decreases significantly between the 12th and 25th week of gestation. During 30 weeks of gestation, the foetus receives the majority of the iron, increasing the need for iron. There are more transferrin receptors in the placenta when the mother's iron status is low, which allows the placenta to absorb more iron. Excessive iron transport to the fetus may be prevented by the placental synthesis of ferritin. Poor dietary iron consumption during pregnancy increases the risk of iron shortage, especially in the third trimester when the foetus is dependent on the mother's iron stores.

In a recent study, Lin (2018) discovered that pregnant women with anaemia had higher rates of polyhydramnios, preterm birth, low birth weight ( < 2500 g), newborn problems, and NICU hospitalization. Affected mothers frequently experience breathing issues, dizziness, exhaustion, palpitations, and problem sleeping (Lee *et al.*, 2004). Moreover, they have a higher risk of bleeding, pre-eclampsia, and perinatal infections. Besides that, behavioural issues and postpartum cognitive impairment were reported (Milman, 2012; Murray-Kolb, 2013).

Intrauterine growth retardation, preterm, and low birth weight are examples of adverse perinatal outcomes that all have high mortality risks, especially in impoverished countries (Bhutta *et al.*, 2005). Iron deficiency also has been linked to abnormalities in the implantation and growth of the embryo in animals, particularly in the development of lungs, heart and brain (Noor Fadzilah, 2013).

#### **d) Pica**

Patients with a rare illness known as pica experience cravings for unhealthy substances that pose serious health concerns. A pregnant woman was the first recorded case of pica in the sixth century AD (Coltman, 1969). Although the precise cause of pica is unknown, iron deficiency is recognized to be strongly linked to the condition (Kathula, 2008). Several hypotheses have been postulated to explain pica. It was initially described in iron-deficient animals and thought to be a response to nutritional deficiencies (Hyslops, 1977). Pica has been seen in men and women of all ages and ethnicities, but it is more common in lower socioeconomic levels and is most noticeable in people with developmental difficulties (Grotegut *et al.*, 2006). Patients in several reported cases of pica have admitted to consuming items such as ice cubes, clay, dried spaghetti, chalk, starch, paste, tomatoes, lemons, cigarette butts, hair, lead, and laundry starch. (Coltman, 1969; Hackworth and Williams, 2003; Kushner *et al.*, 2004; Grotegut *et al.*, 2006). Pica causes serious health problems that frequently call for medical attention. These patients are at risk for metabolic and electrolyte disorders, lead and mercury poisoning, hypokalemia, parasitic infections, tooth wear, intestinal blockage, and a variety of gastrointestinal issues. (Barker, 2005; Grotegut *et al.*, 2006; Khatula, 2008)

## 2.7 Prevention of Iron Deficiency Anaemia

Similar to other nutritional deficiencies, iron deficiency mainly results from poverty. It affects a significant percentage of persons in particularly vulnerable groups, even in wealthy countries. A wide range of sectors and organizations must contribute ideas and resources to provide prevention initiatives, particularly in the case of iron shortage. For example, the agriculture, health, commerce, industry, education, and communication sectors should be included in any strategy. Consecutively, these sectors need to collaborate with communities and non-governmental groups. Targeted initiatives should be made to minimize poverty, increase access to a variety of meals, enhance health care and sanitation, and encourage improved feeding and caring behaviours, particularly in rural areas. They are essential components of any health program to enhance nutritional health generally but are particularly crucial for the development of the status in the Malaysian population.

**a) Dietary Improvement through a Food-Based Approach**

The most ideal and long-lasting strategy for reducing micronutrient deficiency is an approach that is focused on nutrition. These strategies aim to enhance the dietary consumption of micronutrients. Hence, food-based methods should include strategies to increase the year-round availability of micronutrient-rich foods, guarantee that households, particularly those at risk, have access to these foods, and alter feeding habits. The ability of these food-based approaches to provide a variety of nutritional advantages is one of the greatest strengths. These advantages can therefore result in both immediate impact and long-term sustainability. Food production, preservation, processing, marketing, and preparation should appear first in actual food-based systems. Secondly, they should address feeding practices, such as intra-family food distribution and care for vulnerable groups.

In order to combat iron deficiency, initiatives should be made to increase access to and availability of foods high in iron. Organs and flesh of cattle, birds, fish, and poultry, as well as non-animal foods such as legumes and cruciferous vegetables, are some examples. The focus should be on meals that improve iron absorption or utilization. Examples include those of animal origin and non-animal foods - such as some fruits, vegetables, and tubers - that are good sources of vitamins A and C and folic acid. Finally, to boost the demand for and consumption of such foods, effective nutrition education may be required. This includes providing information on health and nutrition for both supply and demand aspects of programs.

Because there is limited information available about the amounts of phytates and iron-binding polyphenols in various diets, it is difficult to interpret

iron bioavailability. Such information is vitally required to promote healthy food choices among consumers. The age of the target group, seasonal availability, and other factors that affect food intake and meal patterns should all be considered when making recommendations. It should be highlighted that without additional data on meal composition and eating habits, food-frequency surveys are not a reliable source from which to determine a person's predicted iron status. The bioavailability of iron is influenced by the techniques used in food preparation and processing. All inositol phosphates reduce the ability of the body to absorb iron in proportion to the total number of phosphate groups; therefore, processing procedures that lower the number of phosphate groups improve the bioavailability of non-haem iron.

Building food-based approaches around the needs and activities of women can be especially effective. This is important because women play multiple responsibilities, including being the primary carers and providers of food. For example, encouraging home gardens and small animal husbandry, as well as advancing technologies for food preservation and home or community processing, can be particularly helpful in improving iron status. These approaches are strengthened by initiatives to increase women's income and by improved nutrition education. The major objective of dietary modification is to enhance and maintain the iron status of a population, involves behavioural changes that increase the selection of foods that are rich in iron and result in meal patterns that are favourable to increased bioavailability. Although sometimes difficult to achieve, such changes in dietary habits can bring about important sustainable improvements, not only in iron status but also in nutrition in general.

## **b) Iron Supplementation**

According to the authors of a study on the impact of ID on children's cognitive development, the effectiveness of short-term iron therapy in anaemic children under the age of two is debatable (Grantham-McGregor, 2001). Iron treatment appears to be more helpful for anaemic children older than two years of age. Regarding the behavioural issues linked to ID, it appears that early iron supplementation of infants with moderately low birth weight has no effect on cognitive skills at age 3.5 years but significantly reduces the prevalence of behavioural issues (Lobera, 2014). For other authors, iron seems to have a modest effect on linear growth in deficient populations. The effects of iron supplementation on immunological function, physical performance, thermoregulation, cognition, and restless leg syndrome have been observed (Lobera, 2014). These effects are independent of an increase in haemoglobin. Considering the cognitive functions, the point is whether improvement can be achieved through iron supplementation.

The advantages of iron supplementation in an urban population of nonanemic, iron-deficient adolescent girls were documented by Bruner *et al.* in 1996. The addition of iron supplements enhanced verbal learning and memory. Another study found that the decreased cognitive performance in healthy formerly iron-deficient children may persist even after iron therapy (Lobera, 2014). The scientists hypothesized that long-lasting changes in myelination and energy metabolism, perhaps especially in the hippocampus, might account for these long-term effects on an important aspect of human cognitive development.

Devaki *et al.* (2007) evaluated two groups of teenagers (nonanemic ID and iron-deficient anaemic) after 8 months of iron supplementation. Cognitive function

and academic performance significantly improved as a result of the supplementation. Ebenezer *et al.* (2013) reported that there is no significant impact on concentration levels and test scores in a shorter follow-up trial with 6 months of iron supplementation. When contrasting anaemic children with children who are iron-rich, it appears that anaemic youngsters develop their motor skills more quickly. In contrast to iron-sufficient children, children with ID appear to have a dose-response relationship between haemoglobin and cognitive function, which has not been observed in those without ID.

In a study by Christian *et al.* (2010), it was found that prenatal iron/folic acid supplementation was favourably associated with fine motor performance in offspring in a region where ID was common. Friel *et al.* (2018) administered an iron supplement to breastfed infants aged 1–6 months. Regarding the development impacts, that supplementation resulted in improved developmental indices and visual acuity at 13 months of age. After controlling for background factors, children with severe, chronic ID in infancy demonstrated poorer motor functioning, written expression, mathematics achievement, and some particular cognitive processes (spatial memory, selective recall, and tachistoscopic threshold), and this occurred 10 years after treatment.

Children between the ages of 16 and 24 months who had received iron in a timely manner performed better on neurodevelopmental tests, according to Matiasvili *et al.* (2012). There have been some reports of cognitive function gains in preschoolers following iron supplementation. According to Landim *et al.* (2016), feeding low-income preschoolers cookies supplemented with heme iron may boost their cognitive development. Moreover, after iron supplementation in children,

Pollitt (2001) observed improvement in cognitive brain function. Young children with IDA have been found to benefit from iron treatment in the short term in terms of their mental or psychomotor development, but the impact of longer-term treatment is yet unknown. In addition, supplementing with a combination of iron and folic acid tends to improve young teenage girls' cognitive abilities (Thompson *et al.*, 2013).

## **2.8 Beneficial Effect of Date Palm on Iron Deficiency Anaemia**

Numerous scientific studies reported that date fruit is a good source of many nutrients and are able to alleviate symptoms of IDA (Alfe'reza *et al.*, 2006; Abdel Rahman, 2006; Onuh *et al.*, 2012). The date palm has been shown to be rich in iron (Eldaim & Elnadi, 2014) and was reported can increase the haemoglobin level in rats (Onuh *et al.*, 2012; Zen *et al.*, 2013). Date palm also has been described as a major source of iron among fruits and berries (Sahari *et al.*, 2007). Date fruit has huge potential in maintaining human health due to its properties as energy-rich food and a source of a variety of beneficial minerals (Al Farsi *et al.*, 2005). It is also a significant source of dietary fibres, essential vitamins and minerals (Al Shahib & Marshall, 2003). Moderate concentrations of manganese, iron, phosphorus and calcium per 100 g of dates provide over 7% of the daily requirement (Al-Farsi & Lee, 2008). Consumption of date palm would contribute significantly to the daily requirement of many of these components, such as carbohydrates and minerals (Fe, Mg, Ca, Zn, P, Cu, Se, I, etc.) and provides reasonable amounts of vitamins such as niacin, B6, and folate (Vayalil, 2012). Therefore, daily consumption of dates can

be a good alternative for the treatment of deficiency-related diseases, particularly anaemia.

## **2.9 Beneficial Effect of Goat Milk on Iron Deficiency Anaemia**

The children who consumed goat milk gained more weight, height, skeletal mineralization, and blood serum contents of vitamin A, calcium, thiamin, riboflavin, niacin and haemoglobin as compared to those who consumed cow milk suggesting that goat milk supplies adequate amounts of vitamin A, thiamine, riboflavin and pantothenic acid (Díaz-Castro *et al.*, 2015). Park (2006) reported that goat milk is deficient in iron, vitamin B6, B12, C, D, and folic acid compared to cow milk. Alfereza *et al.*, (2006) demonstrated that milk and other dairy products obtained from cows, being rich in calcium, interfere with the absorption of Fe from the diet. However, recent studies have found that goat milk does not only increase Fe bioavailability in anemic rats but also minimizes the interference of Fe absorption by improving the metabolic and digestion of Ca and also minimizes the possible interactions of Fe with other minerals such as Ca, P and Mg, in comparison with cow milk-fed animals fed (López-Aliaga *et al.*, 2000).

## **2.10 Promotion of Alternative Medicine**

There is a lack of valid experimental data and scientific design of the efficacy of alternative medicine toward human health. These gaps arise mainly due to a lack of interest in therapeutic properties hidden in traditional herbs, foods and medicine among researchers and leading industries. However, several issues involving side effects of conventional treatment involving modern drugs have initiated efforts to find better treatments that involve the usage of more effective methods and alternative medicine. Therefore, modern researchers and scientists have come up with the idea of disease prevention through foods or plant-based and healthy lifestyles. The release of the WHO Traditional Medicine Strategy 2002–2005 also plays a major role in the significant advances in research regarding the effect of traditional and complementary medicine products on health (WHO, 2013). This highlight attracts many researchers in developing countries to get involved in this sector supported by the development of biotechnology sectors. Traditional and complementary medicine sector now plays a significant role in the economic development of a number of countries (WHO, 2013). Dates have gained popularity in recent years due to their multiple health benefits; as a result, numerous in vitro and animal studies, as well as the identification and quantification of several classes of phytochemicals, are being conducted worldwide (Vayalil, 2012). The high value of goat milk has also drawn attention to its use as a crucial component of human nutrition, according to Clark & Garca (2017).