CHAPTER 2

LITERATURE REVIEW

Barley seed

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops and is used worldwide. It was one of the first crops domesticated for human consumption and can be grown under a large number of environmental conditions. Barley is used as a major animal-feed crop, as a popular seasoning, for malting and in health food.

The mature barley grain consists of two distinct parts: the embryo and the endosperm, which provides a store of carbohydrates (mainly starch) and protein to support the initial growth of the germinating embryo. The endosperm comprises the starchy endosperm, which is a non-living storage tissue, surrounded by a living non-starch cell layer called aleurone. The grain is protected by an outer husk (Figure 2.1)

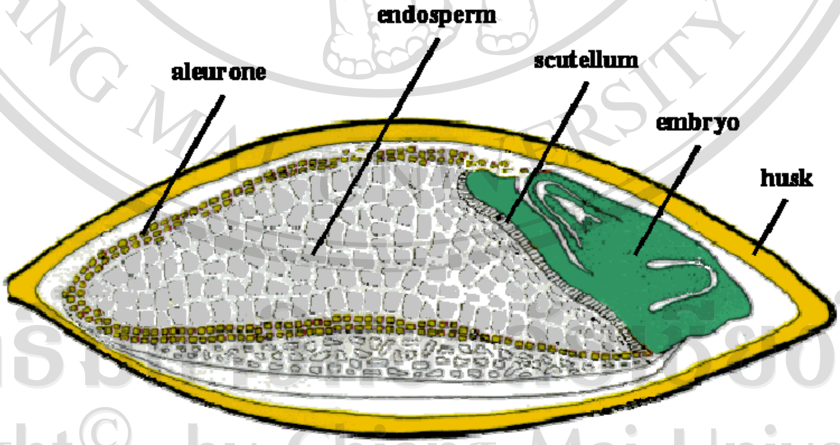

Figure 2.1 Structure of barley kernel (Online available on: www.crc.dk/flab/the.htm).

In tropical countries like Thailand, the planting of barley faces problems, because low-temperature crops such as this are not normally grown in these areas (due to low production levels). To brew beer in Thailand, barley has to be imported from temperate regions such as Europe (Cook, 1962). Thailand has to pay high prices for these imports, from companies who are not able to eliminate the fungi before exporting the grain. The development of fungi on malting barley occurs during the pre- and post-harvesting stages, and the steeping and germination steps in the malting process are favorable to further fungal growth. During the three stages (pre-harvest, post-harvest and malting), contamination probably degrades the seeds and affects the quality of the product, not only for the brewing process, but it also affects the consumer as several pathogens can be produced (Schwarz *et al.,* 1995; Kamil, 2007). The occurrence of mycotoxins in barley is of great concern worldwide, because their presence in processed feed and food appears almost unavoidable. Thus, disease free seeds from pathogenic fungi provide a front line of defense against the development and spread of plant diseases, which are responsible for large crop losses worldwide.

Seed-borne pathogen

Seeds play an important role in the transmission of pathogens and development of plant diseases. A seed-borne pathogen present externally or internally, or associated with the seed as contaminant, may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, as well as seedling damage which results in the development of diseases during the latter stages of plant growth, by systemic or local infection (Neergaard, 1977; Bateman and Kwasna, 1999; Dawson and Bateman, 2001; Khanzada *et al.,* 2002).

Barley seeds are known to attract by many seed-borne fungi (Hassan, 1999; Medina *et al.,* 2006). These infections can reduce grain yield, seed vigor, germination rates and more importantly, the pathogen can produce several mycotoxins (Kamil, 2007). These mycotoxins may pose a health hazard, as they can contaminate manufactured products such as beer, thereby entering the human food chain. The most important toxigenic fungi in stored or processed plant products are the species *Alternaria, Aspergillus, Fusarium* and *Penicillium* (Mateo *et al.,* 2004).

The main fungi infecting the heads and seeds of barley in the field belong to the genus *Fusarium.* The species' infecting the barley heads include: *F. graminearum, F. poae, F. avenaceum, F. sporotrichoides* (Salas *et al.,* 1997), *F. culmorum, F. moniliforme and F.nivale* (Dubin *et al.*, 1997). These species are also involved in the formation of head blights that have been found worldwide (Gilchrist and Dubin, 2002). Furthermore, the fungus can produce several mycotoxins, including deoxynivalenol (DON) and zearalenone (ZON) found in contaminated feed, which can reduce growth rates, while zearalenone can cause reproductive problems. Barley infections of *F. graminearum* can cause problems for the malting and brewing industries, due to the phenomenon known as 'gushing' or 'excessive foaming'. Many malting companies reject barley for the malting process, if it is found to contain detectable levels of DON (Turkington *et al.*, 2002)

Apart from *Fusarium* spp., the species *Alternaria, Cladosporium* and *Dreschlera* can infect the grains especially on the embryo side before harvest, causing black points. All seed infecting fungi reduce the quality of the seed, and can be a main cause of spoilage. The fungi not only reduce the quality of the grains, but also the toxins produced by some of the species can cause health problems for livestock and humans alike (Barry *et al.,* 2002).

Seeds infected by such species are also more vulnerable to storage fungi such as *Penicillium* spp. and *Aspergillus* spp. In general *Aspergillus* species can be adapted to conditions without free water and can grow at a lower humidity of R.H.70% (Dube, 1990), whereas *Penicillium* species are abundant mainly in grains with a high moisture content stored at lower temperatures. Similar to *Penicillium* spp., species' of *Rhizopus*, *Mucor* and *Nigrospora* can also invade high moisture content grains before or during storage (Sauer *et al.,* 1992).

Control of seed-borne fungi

At present, one of most common practices used to reduce seed fungal infection is chemical seed treatment (Platz *et al.,* 2001). However, the extensive use of seed treatment fungicides has been found to cause risks in terms of pollution of the environment, not only in terms of leaving a residue on the seed, but also in terms of the potential negative influence on the health of people working with and handling them on a regular basis. Another issue is the development of pathogen resistance to commonly used chemical compounds.

One possible alternative is to eliminate seed-borne pathogens using a thermal process. Various thermotherapy methods such as hot water, hot and dry air, solar energy and hot vapour can be used for seed pathogen control. Hot water treatment was first used in practice for the sanitization of seeds from seed-borne diseases 50 to 60 years ago. However, conventional hot water treatment is laborious and is also very energy intensive. Several study reports show that using hot water treatment may cause reduced seed germeability. One positive effect of treating with hot, dry air, obtained occasionally, is a reduction in the number of seed-borne pathogens with little or no lowering of germinability, but this is often at the expense of using long exposures and high temperatures. A recent innovative approach to eradicate seed-borne pathogen has been the use of Radio Frequency (RF). The thermal energy from RF is transported into the material by an alternating electromagnetic field, such that heat can be generated within the product rapidly. Research shows that RF heat treatment is effective for the decontamination of food materials and many agricultural products (Cwiklinski and Hörsten, 2001; Clear *et al.*, 2002; Geveke *et al.,* 2002; Forsberg, 2004).

Radio frequency (RF)

Radio frequency is a band within the electromagnetic spectrum which covers a region between 1 to 300 MHz, as shown in Figure 2.2. Microwaves, which are rather similar to RF waves in their heating behavior, are of a higher frequency range; between 300 MHz and 300 GHz (Ryynänen, 1995). Both RF and microwaves are considered non-ionizing radiation, because they have insufficient energy (less than 10 eV) to ionize biologically important atoms. Since these waves lie in the radar range and can interfere with communication systems, only certain frequency bands are permitted for domestic, industrial, scientific and medical applications. These frequencies are 13.56 (central wavelength 22 m), 27.12 (central wavelength 11 m), 40.68 (central wavelength 7.3 m) (RF), 915 (central wavelength 32.8 m) and 2450 MHz (central wavelength 12.2 cm), as well as 5.8 (central wavelength 5.2 cm) and

24.124 GHz (central wavelength 1.24 cm) (MW) (Piyasena *et al*., 2003; Orsat and Raghavan, 2005; Marra *et al.,* 2008).

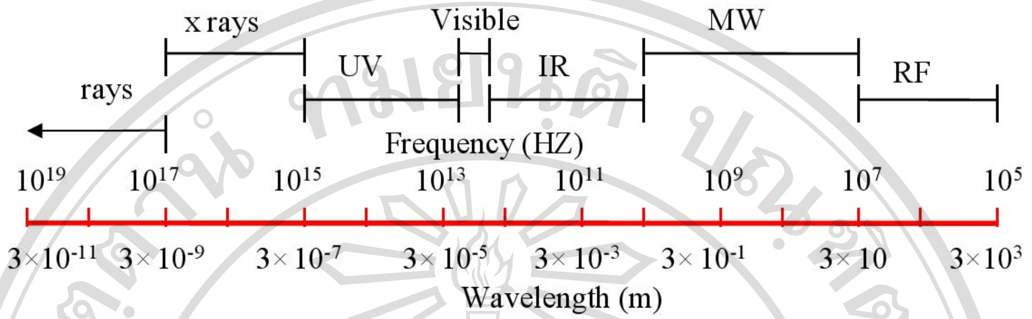

Figure 2.2 The electromagnetic spectrum (Marra *et al.,* 2008).

The difference between RF and Microwave is principally the technology. In RF, an electric field is developed between electrodes whereas in Microwave, heating occurs in a metal chamber with resonant electromagnetic standing wave modes, as in a microwave oven or waveguide. In the microwave frequency range, the dielectric heating mechanism dominates up to moderate temperatures. The water content of the materials is an important factor for microwave heating performance. For normal wet material the penetration depth from one side is approximately from 1 to 2 cm at 2450 MHz (Datta and Davidson. 2001). Because RF heating uses longer wavelengths than microwave heating, electromagnetic waves in the RF spectrum can penetrate deeper into the product so that there is no overheating or dominance of hot or cold spots; a common problem with microwave heating. RF heating also offers simple uniform field patterns as opposed to the complex non-uniform standing wave patterns in a microwave oven. RF heating is a very attractive processing method to provide safe and high quality products, because of the rapid and uniform heating patterns and the significant penetration depth. The use of RF heating can also result in reduced energy consumption; a great advantage over the traditional method of heating. (Zhao *et al.*, 2000; Barrett *et al*., 2005)

Mechanism of RF heating

RF heating is also known as high frequency dielectric heating. In an RF heating system, the RF generator creates an alternating electric field between two electrodes (Figure 2.3). The material to be heated is placed between the electrodes where the alternating energy causes polarization, and where the molecules in the material continuously reorient themselves to face opposite poles. When the electric field is alternating at certain radio frequencies, for example 27.12 MHz, the electric field alternates at 27,120,000 cycles per second (Piyasena *et al.,* 2003). The friction resulting from the rotational movement of the molecules and the space charge displacement causes the material to rapidly heat throughout its mass. The amount of heat generated in the product is determined by the frequency, the square of the applied voltage, the dimensions of the product and the dielectric loss factor of the material, which is essentially a measure of the ease with which the material can be heated by radio waves.

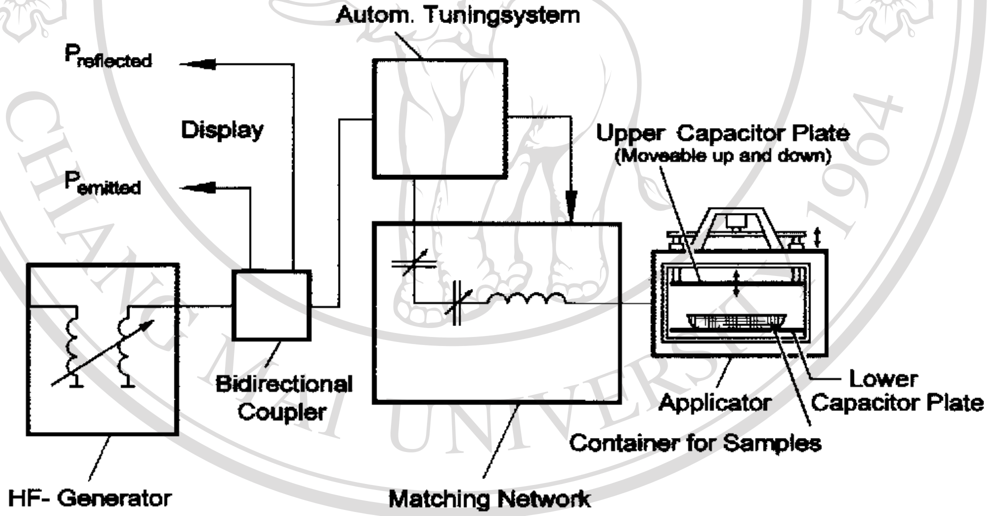

Figure 2.3 Schematic diagram of radio frequency application unit (Oberndorfer and Lücke 1999).

The polarization in an RF field is illustrated in Figure 2.4 When a material is placed in an alternating field at RF, its charged carriers are pulled towards opposite electrodes at the speed of the alternating electric potential. At the same time, its polar molecules are rotated with the alternating field in search of the proper alignment. The realignment of charge carriers and polar molecules with the alternating field results in the generation of heat within the material, caused by the polarization itself and by the friction between the moving parts (Yang, 2003; Lagunas-Solar *et al.,* 2006).

Alternating electric field

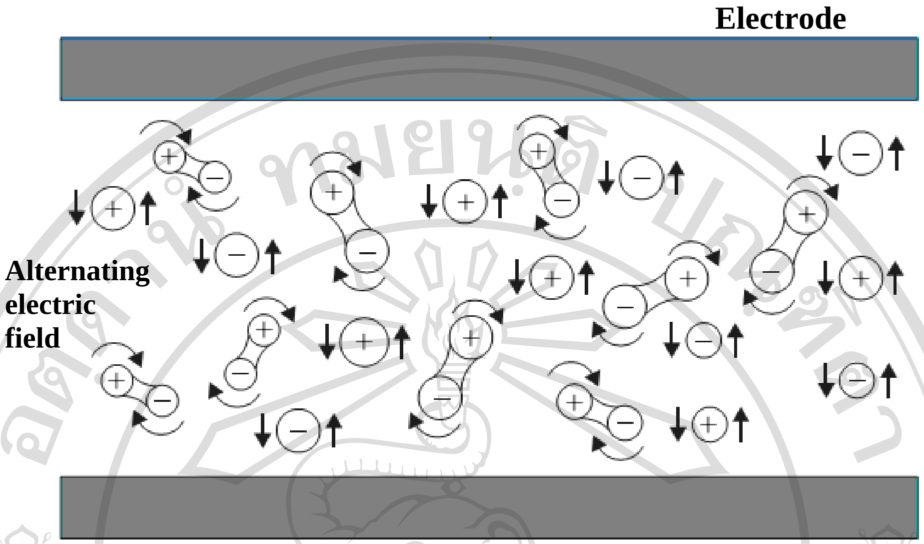

Figure 2.4 Space charge and dipolar polarization in an alternating electric field at radio frequencies (Orsat and Raghavan, 2005).

Factors influencing RF heating

Dielectric properties

The dielectric properties or permittivities of materials are important in understanding the behavior of these materials when exposed to electromagnetic fields in the process of RF. The dielectric properties that are related in RF heating are permeability, permittivity (capacitivity) and electrical conductivity of the heated material. The permittivity determines the dielectric constant; the dielectric and loss factors influence the RF heating.

The permittivity describes dielectric properties that influence the reflection of electromagnetic waves at interfaces, and the attenuation of the wave energy within materials. The complex relative permittivity ε* of a material can be expressed in the following complex form:

$$\varepsilon^* = \varepsilon' - j\varepsilon''$$

The real part ε' is referred to as the dielectric constant or ‘capacitivity’. The dielectric constant is a measure of the capacity of a material to store electric energy, that is, a measure of the induced dispersion in a material. It is a constant for a material at a given frequency, while the dielectric loss factor ε'', which is the imaginary part, influences energy absorption and attenution, and $j = \sqrt{-1}$ (Wang *et al.*, 2003). Both properties are highly correlated with frequency, moisture content, bulk density and temperature.

A material with a high dielectric loss factor will absorb energy at a faster rate than a material with a lower loss factor. Sample values for the dielectric constant and dielectric loss can be found in Table 2.1. Materials that show no dielectric heating are those which contain no free ions and no unsymmetrical molecules (for example, polystyrene) or those with bound ions in a lattice structure (a low loss factor). Most readily heated materials contain unsymmetrical molecules such as polar molecules, conducting components and/or free ions. Water, which has one dipolar molecule in the absence of any other electric field, absorbs the energy of a RF electric field very easily.

Factors influencing dielectric properties

The dielectric properties of most materials depend on several factors. In hygroscopic materials, such as agricultural products, the amount of water in the materials is generally a dominant factor. The dielectric properties also depend on the frequency of the applied alternating electric field, the temperature of the materials, and on the density and structure of the materials. In granular or particulate materials, the bulk density of the air-particle mixture is another factor that influences their dielectric properties. Certainly, the dielectric properties of materials are dependent on their chemical composition and particularly on the permanent dipole moments associated with any molecules making up the materials of interest (Grant *et al.*, 1978).

In theory, when a mixture of materials such as grain and insects is exposed to an RF field, the insects absorb energy at a higher rate than the seeds, providing a selective or differential heating condition. The heating rate of each material then depends on the frequency and intensity of the field, and on the loss factor, specific heat and density of each material (Nelson, 1992a). If selective heating of pests in relation to the grain they infest were possible, dielectric heating would offer an advantage over conventional heating for stored-grain pest control. In a situation where the dielectric loss factor for the pests and the grain are different, the electric field intensities in the pests and the grain might also differ, offering a great potential for selective heating (Nelson, 1991). Therefore, it might be possible to use selective heating to control seed-borne pathogen in the seed.

Table 2.1 Dielectric constant and Dielectric loss factor of selected foods (Piyasena *et al.,* 2003).

Material	Dielectric Constant (ε') (F/m)	Dielectric Loss (ε'') (F/m)	Frequency MHz	Source
Polystyrene	2.35	0.001	10	Orfeuil, 1987
Water-based glue	5	0.25	10	Orfeuil, 1987
Water (25°C)	78	0.36	10	Orfeuil, 1987
Salt water	80	100	10	Orfeuil, 1987
Codfish (10°C)	95.2	1085	10	Bengtsson et al., 1963
Beef fat (−20,0°C)	3.5;12		2	Ede et al., 1951
Beef fat (−20,0,20°C)	3.0: 7;7		20	Ede et al., 1951
Dried cream milk (20°C)	2.4		2	Ede et al., 1951
	2.3		20	
Flour (3.2%MC, 20°C)	3.1		2	Ede et al., 1951
	2.7		20	
Sugar at 20°C	2.3		2	Ede et al., 1951
	2.3		20	
Salt at 20°C	2.6		2	Ede et al., 1951
	2.6		20	
100% Honey	40.29	1.92	10	Puranik et al., 1991
	30.48	13.80	100	
50% Honey	72.92	685.93	10	Puranik et al., 1991
	73.45	69.53	100	
Saturated sugar	54.08	2.55	10	Puranik et al., 1991
	42.35	8.13	100	
Yellow corn at 24°C (10%MC)	4.1	8.3	20	Nelson, 1979
Apple, 86.5% MC at 23°C	63.4	16.0	200	Nelson, 1994
Strawberry, 92.1%MC	75.1	36.7	200	Nelson, 1994
Potato, 79.3% MC	67.9	65.7	200	Nelson, 1994
Onion, red, 90.3%MC	71.1	42.2	200	Nelson, 1994
Orange, 87.5%MC	75.9	33.5	200	Nelson, 1994
Brown rice at 2°C (11.7%MC)	3.1	0.32	20	Nelson, 1992
Beef at 1.2 and 23.4°C, 73%MC	63.6; 75.4	115.3; 194.3	100	Tran and Stuchly, 1987
Beef liver, at 1.2 and 24.2°C, 65%MC	64.2; 70.3	166.4; 153.1	100	Tran and Stuchly, 1987
Chicken, at 1.2 and 25.0°C, 74%MC	59.9; 74.2	115.7; 147	100	Tran and Stuchly, 1987
Salmon at 1.2 and 25.0°C, 71%MC	60.7; 66.5	107.9; 144.7	100	Tran and Stuchly, 1987
Sausage emulsion	186	147.0	27	Houben et al., 1991
Cooked tortillas 0%, 0.2%, 0.5% of lime	35.95 74.03 58.15		10.2 KHz	Rodriguez et al., 1996

Radio frequency heating applications

The application of RF is a thermal method to heat any biological material. It can be used for dehydration, defrosting, drying and decontamination (sterilization and pasteurization) (Von Hörsten and Cwiklinski, 1999). In common conventional systems, thermal energy is transported as heat to the material by convection and conduction from a hot medium to a cooler product, resulting in large temperature gradients, whereas RF heating involves the transfer of electromagnetic energy directly into the product, initiating volumetric heating due to frictional interaction between molecules (heat is generated within the product). For this reason, electromagnetic energy, with its rapid heating potential, may offer a competitive edge in agricultural and food applications.

Radio frequency and agricultural product

A seed is composed of various chemical components which will most likely have an effect on the special variability of its dielectric properties. So far, there has been no measurement made of the dielectric property of the various parts of a seed. Rather, the measurement of dielectric properties has been made on powdered grains or on bulk samples of grains. Thus, the values that are available are for the seed as a whole and not for the parts of a seed. If we take the example of wheat, the primary component of the seed is starch, which makes up the composition of the endosperm; the composition of the embryo is mostly protein and to a lesser extent, oil. It is likely that there are differences in the dielectric properties at various locations through a seed, especially between the embryo and the endosperm which have significant differences in composition. In light of the composition of both these parts, the assumption that can be made is that the embryo has a greater dielectric loss factor than the endosperm, so the seed will probably absorb a greater amount of energy at the embryo level. However, the seed is of a relatively small size and so selective heating due to the embryo's higher dielectric loss factor may be of little consequence, due to the rapid dispersion of the heat throughout the entire volume of the seed during the period of a treatment (Nelson, 1992b; Orsat, 1999).

Control seed-borne pathogen by RF

Heat disinfection of grain using high temperatures has the benefit of being very effective; it is rapid and there is no residue. However, it offers no medium- to long-term protection, and its use to disinfect grain has yet to be implemented. Many researches indicate that thermal electromagnetic treatments can effectively eliminate the contamination of seed. Cwiklinski and von Hörsten (1999; 2001) reported that the application of microwave and RF technology leads to a complete eradication of *Fusarium culmorum* (Smith) Saccardo in seed, while maintaining germination. They reported a complete eradication of the fungus on wheat seeds at temperatures of 70°C to 75°C, and treatment times of 150 to 180 seconds, when the initial seed moisture content was 15%, while using RF at 65°C with an initial seed moisture content of 16%, also completely eradicated *F. graminearum* and maintained germination to 85%. Vassanacharoen (2005) treated sesame seed with RF, and found the most effective level to be at 70ºC, with a seed moisture content of 10%; all fungi invasion decreased to 51%, whereas the seed germination percentage was 73%. Furthermore, corn with an initial moisture content of 10.5% and 14% was treated with RF temperatures of 65, 70, 75, 80 and 85°C for 10 min. The results show that an increase in temperature led to a decreased percentage of *F. semitectum* infection, and at 85°C and a 14% moisture content, the percentage of fungi infection was only 2% (Vassanacharoen *et al.*, 2006). Nelson *et al.* (2002) also studied the radio-frequency heating of alfalfa seeds, to reduce human pathogens. The RF exposures needed to provide the required reduction in pathogens, caused significant damage to seed germination. Reduced RF intensity treatments provided a moderate reduction in pathogens, while improving seed germination by reducing hard seed percentages. According to Janhang *et al.* (2005), using RF heat treatment at temperatures of 70, 75, 80 and 85°C for 180 seconds to control seed-borne *Trichoconis padwickii* in rice seeds (*Oryza sativa* L.) with an initial seed moisture content 10.4%, suggests that the best temperature to use is 75 °C. *Trichoconis padwickii* infestation dropped to 17.8%, while the percentage of seed viability was as high as 82%, and the moisture content decreased to 9.5%.