CHAPTER 3.0 WATER ADSORPTION ISOTHERMS AND FUNGAL DEVELOPMENT ON STARCH-BASED FOOD STORED AT DIFFERENT LEVELS OF WATER ACTIVITY

3.1 INTRODUCTION

All foods contain water, and it is commonly observed that the foods most likely to show rapid deterioration due to biological and chemical changes are usually those with high water content. The concept of A_w introduced by Scott (1957), clearly quantitated the relationship between moisture in foodstuffs and the ability of microorganisms to grow on them. Hence, it is now widely accepted that A_w is the most useful expression of the water availability for microbial growth (Scott, 1957; Lacey and Magan, 1991) and enzyme activity (Acker, 1963).

The A_w levels corresponding to a range of moisture contents of foods are plotted to provide a water sorption isotherm. This isotherm is useful, not only in showing at what moisture contents certain desirable or undesirable levels of A_w are achieved, but also in indicating what significance small changes in moisture content will have in terms of A_w. It is also a useful guide to the storage life of foods held at moderate temperatures and preserved only by reduced A_w. Knowledge of changes in A_w during storage can explain further the evolution of the fungal flora (Sorger-Domenigg, *et al.*, 1955).

Food are very complex systems in which water sorption involves several different components, such as proteins, carbohydrates and lipids which can interact in more complex way than can be described by the simple relationship proposed by Labuza (1968). The effect of composition of a food on the shape of the isotherm has not been studied very much. Different foods with the same A_w may have very different moisture contents. For example, protein and starches adsorb much more water at low activities than do fatty materials or crystalline substances like sugars (Labuza, 1968). Pixton and Warburton (1971) further showed that oilseeds have a higher A_w at a given moisture content than starchy cereal seeds.

Water adsorption isotherms of cereals and its products have been studied by many workers, for example, of rice by Karon and Adams (1949), Rangaswamy (1973), Hunt and Pixton (1974), Gough and King (1980) and Charistian (1980) while of wheatflour, by Bailey, (1929), Fairbrother (1929), Anderson (1937), Anker *et al.* (1942), Morey *et al.* (1947), Bushuk and Winkler (1957) and Pratap *et al.* (1982). However, no published information is available on the isotherms of glutinous rice, glutinous riceflour and riceflour.

Spoilage of cereals for food use is more commonly due to discolouration, loss of milling quality, or visible mould growth. The first evidence relating A_w and storage life of foodstuffs was by Snow *et al.* (1944). It has long been recognized that water activity is only one of the environmental factors with which fungi must contend. Their growth and survival will be influenced by concomitantly by temperature, hydrogen ion concentration, oxygen and carbon dioxide concentrations, and the presence of preservatives. When any of these factors is suboptimal, the inhibitory effect of reduced A_w tends to be enhanced.

The present study is primarily concerned with the development of fungi on starch-based food stored within a range of water activity. The adsorption isotherm of starch-based food are included to obtain the maximum moisture content of each food to be maintained in order to prolong the shelf-life of each food.

3.2 MATERIALS AND METHODS

3.2.1 Sampling and subsampling of starch-based food

The starch-based food used were ordinary rice, glutinous rice, riceflour, glutinous riceflour, wheatflour and cornflour. Samples were of standard commercial grade purchased at random from retail outlets around Klang Valley. The flour samples consisted of various brands available in the market. Equal amount of samples of ordinary rice, glutinous rice, riceflour, glutinous riceflour, wheatflour and cornflour were bulked and mixed separately in a closed sterile polyethylene bags. Subsequently small proportions from different parts of the bulk samples were taken out using sterile spatula and mixed again in another closed sterile polyethylene bag. A suitable sized sub-samples from each composite samples of starch-based food were taken for subsequent experiments.

3.2.2 Determination of the initial moisture content of starch-based food

The moisture content (m.c.) of the composite samples of each starch-based food were determined by drying a weighed ground sample of food in a mechanically ventilated oven at 80 °C for 3 days. The samples were weighed until constant weight was achieved. The percentage moisture content was derived on a dry weight basis using the formula:

$$\text{m.c.}(\%) = \frac{\text{water loss from the sample (g)} \times 100}{\text{dry weight of the sample (g)}}$$

3.2.3 Determination of the water adsorption isotherm of starch-based food

The water adsorption isotherms were determined gravimetrically by exposing food samples to atmospheres of known equilibrium relative humidities ranging from 10% - 98%.

Ten grams from each composite sample of starch-based food were placed in glass petri dishes and stored in the equilibrium relative humidity chamber described in Appendix B. To ensure moisture absorption in each case, the samples were first dried to less than 3 % moisture content. This drying was done at room temperature using activated silica gel. It took about 2 - 4 days for this drying. They were then irradiated with ultraviolet light for 30 mins according to Leiras and Iglesias, (1991) in order to prevent or delay microbial spoilage of samples at high relative humidity. Preliminary experiments showed that equilibration of the samples ranges from about 5 - 14 days before visible fungal growth. Equilibrium moisture content of the samples were determined as above, on a dry weight basis. The experiment was repeated to obtain triplicate data.

3.2.4 Fungal development in starch-based food stored at various levels of water activity

The time taken for fungal development were determined by storing 25g composite samples to atmospheres of known relative humidities ranging from 65% - 98% in the equilibrium relative humidity chamber described in Appendix B.

The samples were examined periodically under a binocular microscope (x 40 magnification) for the appearance of fungi. The number of days before visible fungal growth was noted for each starch-based food stored for a period of 6 months at the various water activity. The experiment was repeated three times.

3.3 RESULTS

3.3.1 The initial moisture content of starch-based food

Table 3.1 shows the initial moisture content (% dry basis) of each composite samples of starch-based food investigated. Glutinous rice grains has a higher average moisture content (10.14%) than ordinary rice grains (9.30%). Among the flours, riceflour has the highest average moisture content (6.76%) followed by wheatflour (6.67%) and cornflour (6.39%). Glutinous riceflour has the lowest average moisture content (5.75%).

Table 3.1 The initial moisture content of the composite samples of each starch-based food (% dry basis)

Number of replicates	Ordinary Rice	Glutinous rice	Riceflour	Glutinous riceflour	Wheatflour	Cornflour
1	9.54	10.00	6.91	5.94	6.41	6.44
2	9.51	10.06	7.04	5.65	6.68	6.91
3	9.27	10.12	6.91	5.78	6.56	6.81
4	9.15	10.75	6.22	5.28	6.76	6.02
5	9.01	10.00	6.80	5.96	6.63	6.00
6	9.34	9.93	6.65	5.90	6.98	6.18
Average	9.30	10.14	6.76	5.75	6.67	6.39
S.E.*	0.20	0.30	0.29	0.26	0.19	0.40

* standard error

3.3.2 Water adsorption isotherm of starch-based food

The equilibrium moisture content values for six starch-based food at 25 °C are given in Appendix D(3.1) and summarized in Table 3.2. A comparison of the equilibrium moisture content data reveals that glutinous rice grains has a higher sorptive capacity than ordinary rice grains while among the flours, wheatflour has the highest sorptive capacity followed by glutinous riceflour, riceflour and cornflour in that order.

The adsorption isotherm curves given by Fig. 3.1 - 3.6, characterizes the equilibrium state between the moisture content of starch-based food and the water vapour pressure of the surrounding atmosphere, the equilibrium being reached by gaseous exchange. On each graphs, **region A** corresponds to the adsorption of a monomolecular film of water, **region B**, to adsorption of additional layers over this monolayer; and **region C** to condensation of water in the pores of the material followed by dissolution of the soluble material present for each starch-based food. From Figs. 3.1 and 3.2, by extrapolation, the initial average moisture content of ordinary rice when sampled from retail outlets corresponds to a A_w of 0.25 while glutinous rice corresponds to a A_w of 0.36. Also, from Figs. 3.3 - 3.6, riceflour, glutinous riceflour, wheatflour and cornflour corresponded to a A_w of 0.29, 0.30, 0.31 and 0.30 respectively, when sampled from retail outlets.

Table 3.2 Equilibrium moisture content of starch-based food at different water activities at 25 °C (Average of three replicates)

A_w	Equilibrium moisture content (% dry basis)					
	Ordinary rice	Glutinous rice	Riceflour	Glutinous riceflour	Wheatflour	Cornflour
0.98	21.93	25.57	20.87	23.63	23.11	20.69
0.95	20.73	24.02	19.48	21.19	22.66	19.43
0.85	16.43	16.92	15.28	15.42	16.11	15.60
0.75	14.13	14.21	11.18	11.83	12.44	12.70
0.65	13.01	12.87	9.56	10.61	10.72	10.52
0.50	11.79	11.38	8.18	8.53	8.59	8.80
0.35	10.34	9.93	7.09	6.42	7.11	6.75
0.20	8.62	8.00	5.79	4.47	5.32	5.43
0.10	7.11	6.36	4.22	3.68	4.08	3.85

Fig. 3.1 Water adsorption isotherm of ordinary rice at 25°C.

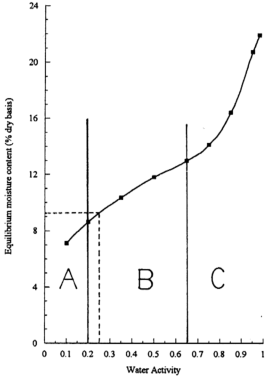


Fig. 3.2 Water adsorption of glutinous rice at 25°C.

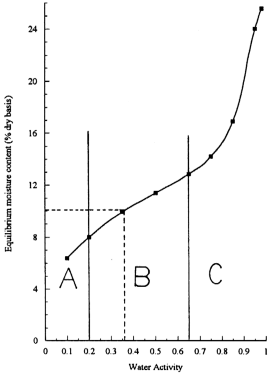


Fig. 3.3 Water adsorption isotherm of riceflour at 25°C.

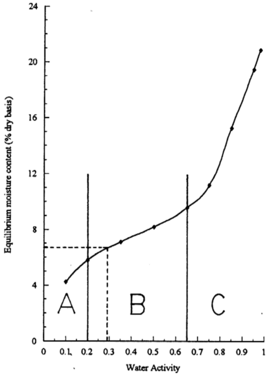


Fig. 3.4 Water adsorption of glutinous riceflour at 25°C.

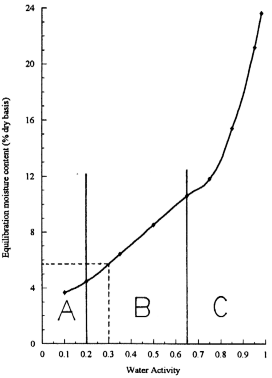


Fig. 3.5 Water adsorption isotherm of wheatflour at 25 °C

The moisture content of wheatflour was obtained by weighing after equilibration with the vapour pressure of the surrounding atmosphere. Taking water activity of 0.65 as the safe storage level, the critical moisture content of wheatflour is 10.72%. The average initial moisture content of wheatflour is 6.67% corresponding to a water activity of 0.31.

Fig. 3.6 Water adsorption isotherm of cornflour at 25 °C

The moisture content of cornflour was obtained by weighing after equilibration with the vapour pressure of the surrounding atmosphere. Taking water activity of 0.65 as the safe storage level, the critical moisture content of cornflour is 10.52%. The average initial moisture content of cornflour is 6.39% corresponding to a water activity of 0.30.

Fig. 3.5 Water adsorption isotherm of wheatflour at 25°C.

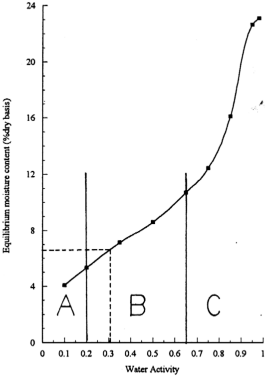
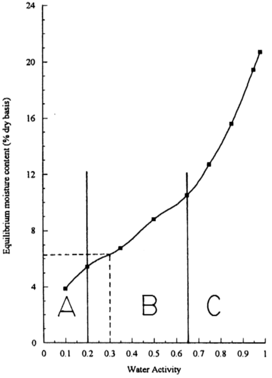


Fig. 3.6 Water adsorption isotherm of cornflour at 25°C.



3.3.3 Time taken for the development of fungi in starch-based food stored at various levels of water activity at 25 °C

The number of days before visible fungal development on six starch-based food are give in Appendix D(3.2) and summarized in Table 3.3. The data indicated a shelf-life of about 20 days for ordinary rice and glutinous rice grains, about one month for riceflour and wheatflour, three months for glutinous riceflour whilst cornflour can be stored for more than five months at a A_w of 0.75.

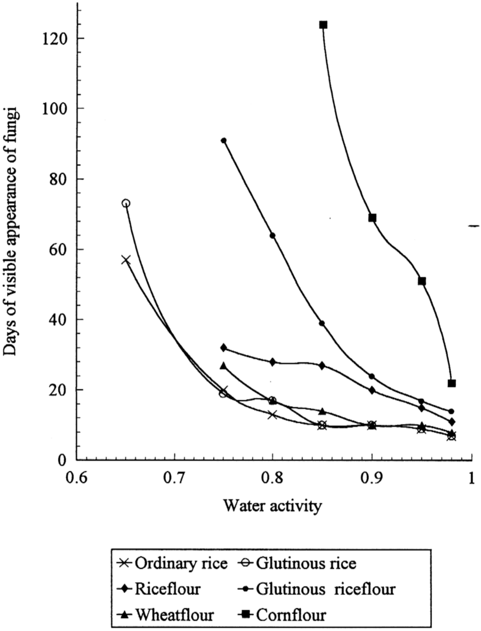
Table 3.3 Days before visible appearance of fungi on six starch-based food at 25 °C. (Average of three replicates)

A_w	Rice	Glutinous rice	Riceflour	Glutinous riceflour	Wheatflour	Cornflour
0.98	7 ± 2	7 ± 2	11 ± 3	14 ± 2	8 ± 1	22 ± 3
0.95	9 ± 1	9 ± 2	15 + 4	17 ± 4	10 ± 1	51 ± 2
0.90	10 ± 2	10 ± 3	20 ± 1	24 ± 3	10 ± 1	69 ± 2
0.85	10 ± 2	10 ± 0	27 ± 1	39 ± 2	14 ± 3	124 ± 4
0.80	13 ± 1	17 ± 1	28 ± 3	64 ± 4	17 ± 1	*
0.75	20 ± 2	19 ± 1	32 ± 1	91 ± 3	27 ± 2	*
0.65	57 ± 2	73 ± 1	*	*	*	*

* No fungal development at 6 months incubation.

Fig. 3.7 indicates that fungi developed within one month or less on ordinary rice, glutinous rice, riceflour and wheatflour when stored continuously at 25 °C under 0.75 - 0.98 A_w. At A_w of 0.65, fungal growth on rice and glutinous rice, while not entirely prevented, took place after a latent period of two months or more. No fungal growth occurred on riceflour, glutinous riceflour and wheatflour when stored for six months. On the other hand, fungal development did not occur on cornflour after six months storage at a A_w of 0.80 and below.

Fig. 3.7 Relation between water activity and the time before visible appearance of fungi on starch-based food at 25℃.



Starch-based food were stored at various water activity at 25°C and the number of days before visible fungal growth was recorded.

3.4 DISCUSSION

Cereals, before being consumed as food, goes through the processes of cultivation, harvesting, drying, preparation and marketing (including storage) all under natural conditions, and therefore, often involves microbiological contamination and infection. The water activity level in food is of practical importance as it may affect the onset and severity of mold spoilage particularly in low or intermediate moisture foods.

In this experiment, the starch-based food investigated displayed a type II isotherm in accordance with other studies of isotherms of cereals (e.g. Bushuk and Winkler, 1957; Pratap *et al.*, 1982). It is generally considered that fungal growth on cereals is not significant at A_w less than 0.62 - 0.70 (Pitt 1975; Gough and Bateman, 1977; Gough and King, 1980; Hill and Lacey 1983). Hence, the moisture content corresponding to this A_w range is of particular interest as the "critical moisture content" for safe storage. Thus, from the present study, taking A_w of 0.65 as the safe storage level, as extrapolated from the adsorption isotherms in Figs. 3.1 - 3.6, the critical moisture content (% dry basis) of ordinary rice grains at 25 °C is 13.01 %, glutinous rice grains is 12.87 %, riceflour is 9.56 %, glutinous riceflour is 10.61 %, wheatflour is 10.72 % and cornflour is 10.52 %. From Table 3.1, the initial average moisture contents (% dry basis) of the composite samples of each starch-based food viz. ordinary rice (9.30 %), glutinous rice (10.14 %), riceflour (6.76 %), glutinous riceflour (5.75 %), wheatflour (6.67 %) and cornflour (6.39 %) when sampled randomly at various retail outlets were found to be below the critical moisture content levels calculated from the isotherm graphs given above. As shown in Figs. 3.1 - 3.6, all these moisture contents corresponds to a water activities in either regions A or B whereby water is loosely or tightly bound and is unavailable for reaction as defined by Labuza (1968), Duckworth (1975) and Troller and Christian (1978). The critical moisture content can vary widely from one foodstuff to another and is estimated to be 14.5 % for sorghum (Christensen, 1973), 12.5 - 13.5 % for wheat and maize (Christensen, 1973), but as low as 8% for groundnuts (McDonald, 1968). This depends on the composition of the material which affects the function of the water-holding ability of the plant material. It must be acknowledged also that for grains, the relation of A_w to moisture contents may vary depending on the kinds of grains (Hunt and Pixton, 1974; Charistian, 1980) and the variety of grains (Gough and King, 1980). In general, the shape of the isotherm curve of a material is affected by the experimental procedure adopted i.e. the adsorption or desorption and the composition of the material.

The liability of foodstuffs to fungal growth depends on several factors, of which the water activity and the length of storage are most important. In this study, at 25 °C, for short term storage (i.e. < 90 days), it is recommended that ordinary rice, glutinous rice, riceflour and wheatflour be stored at A_w of less than 0.65 which corresponded to their critical moisture content while glutinous riceflour can be maintained at a higher A_w of 0.75 with a moisture content of 11.86 % and cornflour at 0.85 A_w with a moisture content of 12.73 % (Table 3.3). A somewhat larger safety margin is advisable for long storage periods. Work by Barton-Wright and Tomkins, (1940) on the development of fungal growth on samples of flour, bran and middlings at different relative humidities indicate that in a period of 4-month storage, fungal growth was prevented on samples of flour exposed to 0.79 A_w and on bran at 0.75 A_w. Snow *et al.* (1944), recommended a water activity not exceeding 0.72 for 3 months storage and not above 0.65 when storage for two to three years was required. In tropical conditions however, a water activity of less than 0.7 is not low enough (Scott, 1957).

Differences were observed in the time taken for the appearance of fungi on the different starch-based food stored at the same water activity. This may be due to the concentration of the initial inoculum and the physiological properties of the different variety of fungal species occurring in the different samples might have affected the rate of development of moulding. Also, the type of starch-based food, particularly the availability of the soluble nutrients, might have affected the time of germination of fungal spores and the production of fungal mycelium. It has been shown by Snow *et al.* (1944) that fungi developed most rapidly on samples where a balance of nutrients was provided i.e. the starch and protein mixtures and also food-borne fungi grow more vigorously in media rich in carbohydrates (Pitt and Hocking, 1977).

It is also shown from this study that fungal growth occurred on rice and glutinous rice grains at A_w of 0.65 whereas their corresponding flours did not develop fungal growth at the same A_w even after a period of 6 months. This may be due to flours being a highly processed foodstuffs has a lower sorptive capacity than grains at low water activities (< 0.75 A_w) as indicated in Table 3.2. Also Scott (1957), states that fine structure or texture of foods may affect susceptibiliy to attack by affecting the chances of discontinuities in the aqueous phase, or the probability that the required assortment of molecules will occur together in the micro-environment of the cell.

The A_w of ordinary rice at 13.01 % moisture content and glutinous rice at 12.87 % moisture content is 0.65 whereas at A_w of 0.75 the moisture content of ordinary rice and glutinous rice is 14.13 % and 14.21 % respectively. Ordinary rice and glutinous rice at 0.75 A_w may spoil in about 20 days but at 0.65 A_w, it would be safe for about 2 months. Hence, an error in the moisture content of 1.12% for rice and 1.34% for glutinous rice is disastrous. Similarly, riceflour, glutinous riceflour and wheatflour at A_w of 0.65 and cornflour at A_w of 0.80 is safe for 6 months but at A_w of 0.75 riceflour and wheatflour will spoil at about 1 month and 3 months for glutinous riceflour. Cornflour on the other hand, will spoil at 4 months at 0.85 A_w. Hence, knowledge of water adsorption isotherm of foods will indicate the shelf-life of foods and the desirable moisture contents of each food at which it should be maintained.