EuroDrying'2017 – 6th European Drying Conference
Liège, Belgium, 19-21 June 2017

MOISTURE SORPTION ISOTHERMS AND SHELF LIFE EVALUATION OF *PINHÃO* (*Araucaria angustifolia*) FLOUR

Angela Gava Barreto[1], Louise de Aguiar Sobral[2], André Fioravante Guerra[1], Regina Isabel Nogueira[3], Rossana Catie Bueno de Godoy[4] and Suely Pereira Freitas[5]

[1]Centro Federal de Educação Tecnológica Celso Suckow da Fonseca, Valença-RJ, Brazil
[3]Embrapa Agroindústria de Alimentos, Rio de Janeiro-RJ, Brazil
[2,5] Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro-RJ, Brazil
[4]Embrapa Florestas, Colombo-PR, Brazil

E-mail of the corresponding author: ***angelagava@gmail.com***

Abstract:

In this work moisture sorption isotherms and shelf life of pinhão flour were evaluated at 20 ±1 °C using static gravimetric methods in an aw range of 0.011 to 1.0. *Pinhão* endosperm, after convective drying at 40 ºC, 50 ºC and 60 °C, was milled to a finely ground powder (dp<1mm) and packed in metallized PET, vacuum sealed and stored in a low atmospheric relative humidity at 25 °C. The sorption data, fitted by GAB model, regardeless of drying temperature showed the characteristics of type III isotherm. Futhermore, the shel life of packged flour carried out during four months was proved the pinhão flour stability.

Keywords: shelf life, GAB model, water activity

Introduction

Araucaria angustifolia (Parana pine) is a traditional tree of the *Aracariaceae* family, a native conifer which grows mainly in the south of Brazil. It has been of cultural and economic importance apart from its ecological role in Araucaria forests. These seeds have an endosperm, denominated *pinhão* that is traditionally used in a regional cuisine in the many craft foods preparation in which taste is much appreciates for sensory characteristics. The availability of this material is quite limited by the low level of industrialization [1] and the development of new products from *pinhão* is one of the main challenges of the productive chain of this material.

The relation between a food raw material and its moisture content, called sorption isotherms, is of great value in predicting the dehydration parameters, the packaging selection among other applications [2]. Furthermore, moisture contents below certain levels inhibit the growth of microorganisms and the lowest water activity at which microorganisms are able to grow depends somewhat on the nature of medium, but approximate minimum limits may be defined [3].

Pinhão is constituted by an aqueous phase (water, sugars and salts) and scattered solids phases (carbohydrate, protein and insoluble polysaccharides and presented physical characteristics defined for these major components. In the present work the effect of the drying temperature in the sorption isotherms and shelf life of *pinhão* endosperm flour was evaluated. The three parameter GAB model was applied for fitting the experimental data at equilibrium moisture. This model takes into account the modified properties of the sorbate in the multilayer region as compared with the monolayer one and was recommended by the European Project Groups Cost 90 [4]. In this work, chemical composition, sorption isotherms and shelf life of *pinhão* flour were evaluated.

Material and method

Material

Pinhão seeds were collected in the producing regions of the Paraná State, by Embrapa Forestry, according to the authorization number 30147-1/2014 of the Environment Ministry. The seeds were peeled according to the procedure recommended by Cornejo et al. [5] and presenting irregular cuts of 8 mm average thickness.

Chemical Analysis

The moisture content, ashes, total proteins and total lipids were quantified, respectively, by gravimetric method in a drying oven for 90 minutes at 100 ° C, by incineration at 550 ° C, using Kjeldahl method [6] and according standard method recommended by AOCS (*Am 5-04)* [7]. The total carbohydrate was calculated by difference and total fiber content was determined applying enzymatic-gravimetric method [6]. All experimental data were carried out in duplicate.

Drying

Pinhão endosperms were dehydrated in a convective dryer [8] at 40 °C, 50 °C and 60 ºC and ground in a hammer mill (sieve 1 mm) resulting in a small size particles flours: F1, F2 e F3, respectively. The flours were packaged in metallized PET, vacuum sealed and stored in a low relative humidity at 25 °C to provide the safe samples for analysis.

Sorptions Isotherms

The isotherms were determined by the gravimetric method using saturated solutions of the salts LiCl, $MgCl_2$, K_2CO_3, NaCl, $BaCl_2$ and pure H_2O at 20 ºC whose water activities ranging from 0.093 to 1.0. The flour samples were placed in the desiccators containing the saturated solutions and remained in these environments until reaching equilibrium. The process was followed by weighing the samples every 24 hours. The equilibrium moisture values (x) were calculated on a dry basis. The GAB model (eq. 1) was used to fit the water sorption isotherms data [9] applying STATISTICA 12.0 [10].

$$X_{eq} = \frac{X_m.C.ka_w}{(1-ka_w).(1-ka_w+Cka_w)} \quad \text{.....[1]}$$

where:
X_m is the moisture content in the molecular monolayer (kg water.kg^{-1} dry solids)
C and k are the GAB parameters

Microbiological analysis

Pinhão flour samples (F1, F2 and F3) were microbiologically analyzed according to the Normative Instruction 62/2003 [11] and compared to the standards of RDC 12/2001 [12]. For this purpose, the flours are analyzed to total and fecal coliforms (NMP/g); *Bacillus cereus*, *Clostridium* sulphite reducer, positive and negative coagulase staphylococci, total count of mesophilic bacteria, yeast and molds and *Salmonella* sp.

Shelf life

Five packages of *pinhão* flour (F1) were maintained at 20 ± 1 °C and 35 ± 1 °C for 120 days and samples were collected at 0, 30, 60, 90 and 120 days, 25 g of flour was placed into 225 ml of sterile peptone water (0.1 w/v) and successive dilution was carried out up to 10^{-6} (w/v). Mesophilic total bacteria in Plate Count Agar (Himedia), yeast and molds in Potato Dextrose (Oxoid) agar acidified to pH 3.5 with sterile tartaric acid (Vetec) was analyzed by poor plate. The plates were incubated at 35 °C (Plate Count Agar) and at 30 °C (Potato Dextrose agar) for 48 h and 5 days, respectively. Plates containing between 25-250 and 15-150 cfu were selected to evaluate the results ($cfu.mL^{-1}$), using the software "Standard plate counting" [10].

***Bacillus cereus* (INCQS 3)**

To study the kinetics of microbial growth was chose *Bacillus cereus* because it is the sporogenous pathogenic microorganism most likely to be present in the flour.

Bacillus cereus strain was activated by three successive transfers in Brain Heart Infusion broth (Himedia). The growth of the last activation was centrifuged and washed twice with buffer solution at pH 7.2. After, the inoculum was adjusted to ca 10^5 $cfu.mL^{-1}$. Exactly 10 g of flour sample (F1) was inoculated with 0.1 mL of the inoculum ready to get final concentration of *ca* 10^3 $cfu.g^{-1}$. The samples were incubated at 30 °C and analyzed at 0, 30, 60 and 90 days by spread plate on Bacillus cereus agar (Himedia) according to Mossel until dilution 10^{-3}. Plates were incubated at 30 °C for 24 to 48 hours [13]. The results were calculated and expressed using the "*Bacillus cereus* enumeration" software [10].

Results

Proximate composition

The chemical composition of pinhão flour were: moisture, 4.95%; starch, 80.99%; protein, 5.98%; lipids, 1.56; crude fiber, 4.77 % and ashes, 1.75%. This results showed that starch and protein are the major components in this raw-seed, respectively, 91,2% and 6.74% in dry basis.

Sorption isotherms

In the Figure 1, the predicted and experimental values of the equilibrium moisture content were ploted as a function of water activity (a_w) at 20 °C. Futhermore, the GAB parameters X_m (monolayer moisture), *C* and k (constant to correct the properties of multilayer molecules with respect to the bulk liquid) are compared for F1, F2 and F3 (Table 1).

The F-values and GAB model coefficients estimated by nonlinear regression, presented values above 690 and 0.999, respectively, indicating that GAB model fitted well the isotherms data. Regarding *pinhão* endosperm dried at 40 ºC, the GAB models parameters X_m and k are similar to reported data by CLADERA-OLIVEIRA et al [14] respectively, 0.0604 and 0.8768. The *X*m in the present work was found about 0.069 ± 0.001 and all k values was less than 1 (0.838 to 0.840) indicating that lower sorption data was predicted by GAB model as compared to ones fitted by two parameters BET models [15].

Table 1. Coefficients of GAB model for *pinhão* flours fitted by nonlinear regression.

Flour	X_m	C	k	R^2	F-value
F1 (40°C)	0.070	1.076	0.838	0.999	690
F2 (50°C	0.068	1.076	0.838	0.999	690
F3 (60°C	0.069	1.076	0.840	0.999	690

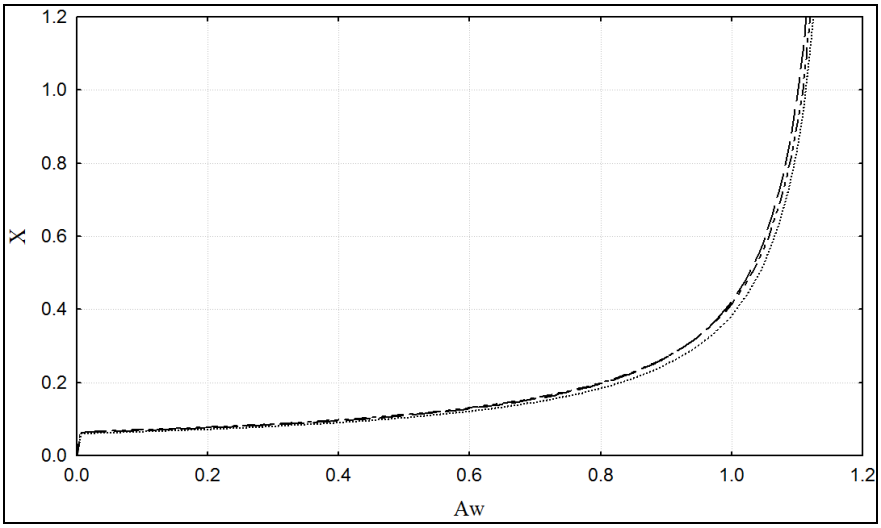

Fig.1. Sorption isotherms of the *pinhão* flour, F1 (———), F2 (·····) and F3 (——), fitted by three parameter GAB model.

Regarding the sorption isotherms of pinhão flour (Figure 1), at low water activity the plasticizing effect is very small and the mobility of the amorphous regions is restricted. However, as the water activity increases, the sorbed moisture promote a subsequent swelling of the flour and the degree of crystallinity decreases. These results are similar to reported data from starch powders by Al-Muhtaseb *et al*. [16]. This occour probably due to the proximate composition of *pinhão* in which the major component was starch (76.8% in dry basis).

Microbiological

The microbiological results from *pinhão* flour are shown in the Table 2.

Table 2 – Microbiological analysis

Microorganism	F1	F2	F3
Coliforms at 35°C - NMP.g^{-1}	<3.0	<3.0	<3.0
Coliforms at 45°C - NMP.g^{-1}	**<3.0**	**<3.0**	**<3.0**
Positive coagulase staphylococci - cfu.g^{-1}	<1.0 x 10^2	<1.0 x 10^2	<1.0 x 10^2
Negative coagulase staphylococci - cfu.g^{-1}	<1.0 x 10^2	<1.0 x 10^2	<1.0 x 10^2
***Salmonella* sp.25g^{-1}**	**Ausência**	**Ausência**	**Ausência**
Clostridium sulfite reducer - cfu.g^{-1}	<1.0 x 10^1	<1.0 x 10^1	<1.0 x 10^1
***Bacillus cereus* - cfu.g^{-1}**	**<1.0 x 10^2**	**<1.0 x 10^2**	**<1.0 x 10^2**
total count of mesophilic bacteria - cfu.g^{-1}	2.4 x 10^5	3.6 x 10^4	4.4 x 10^3
Yeast and Molds - cfu.g^{-1}	4.7 x 10^5	5.2 x 10^4	1.2 x 10^4

Standard from RDC n° 12/2001 [12].

Maximum standards, specified by [12] for coliforms at 45 °C (NMP.g^{-1}), *Bacillus cereus* and *Salmonella* sp. are 1.0 x 10^2; 1.0 x 10^3 and absence, respectively. As the standards of Brazilian legislation guarantee the microbiological safety of foods, it is assumed that flours can be eating without microbiological risk to consumers.

The counts of total mesophilic aerobic bacteria and yeast and molds were between 4 and 5 logs of microbial growth. According to Ennadir *et al.* [17] counts of mesophilic total bacteria in flours in the order of 4 logs are of satisfactory microbiological quality. Aydin *et al.* [18] studied the level of contamination by mesophilic bacteria in wheat flour. Most samples contained between 3 and 4 logs of bacterial growth, but samples of 7 logs of growth were found.

Shelf life

A gradual reduction in about 1 log of the total mesophilic bacteria and molds counts was observed when the samples were processed at 40, 50 and 60 °C. These processing temperatures do not have an effect on microbial spores, so even the contamination being the majority of sporulated microbial genera, the presence of vegetative cells is not ruled out. The Figure 2 shows that the flours were microbiological safety [12] according the commercial conditions recommended from *pinhão* flours at 20 ± 1 °C

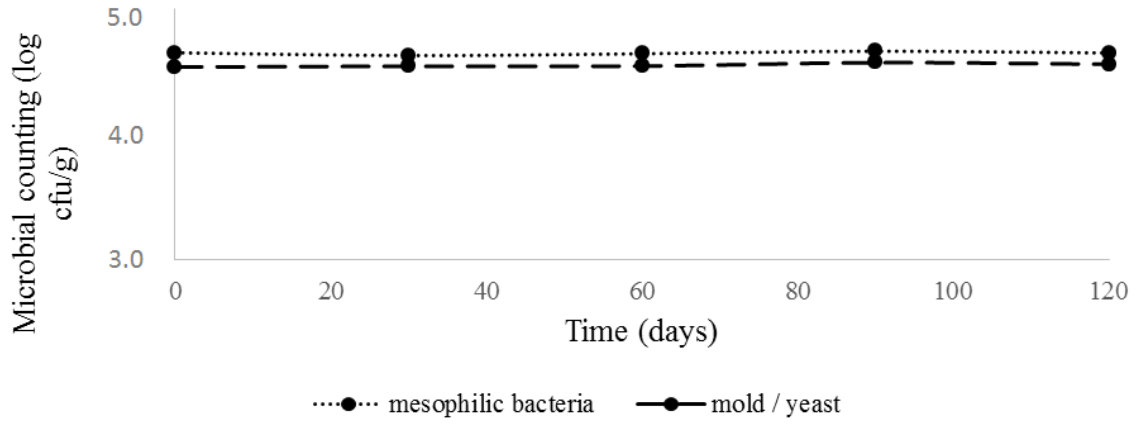

Figure 2. Growth (log cfu.g^{-1}) of total mesophilic bacteria and mold/yeast at room temperature (20 ± 1 °C).

There was no growth of total mesophilic bacteria and yeast and molds at the selected temperature. The first contaminant population remained constant throughout the 120 days of incubation. In the counting plates of mesophilic bacteria it was possible to see colonies with spreading behavior, typical of sporulated bacilli. A morphotinorial Gram test confirming the presence of this microorganism. The low water activity of the sample impairs microbial growth. The low water activity of the product impacts on microbial growth and when present, sporulated forms of microorganisms may remain in the product during storage.

Bacillus cereus

Bacillus cereus is a gram-positive, endemic, beta-hemolytic beta bacterium that lives in soil. Some strains are harmful to humans and cause food poisoning, while other strains may be beneficial, such as probiotics for animals. Therefore, the microorganisms from the raw material and/or processing can cause a reduction in the shelf life of food formulations from

the *pinhão* flour. This occurs due to the water activity increase in the bulk. In the Figure 3 was showed the growth kinetics of *Bacillus cereus* of this target microorganism.

There was no observed growth of *Bacillus cereus* in the flour samples during 90 days of incubation at 30 °C. In Figure 3 it can be seen that the number of inoculated microorganism remained constant throughout the entire period of incubation. These results confirm that the inherent barriers to flour processing guarantee that there is no development of pathogenic microorganisms. The safety of the food, in this case, is inherent to the initial contamination of the raw material and processing of pathogens. After packaging, the population of these microorganisms remains constant.

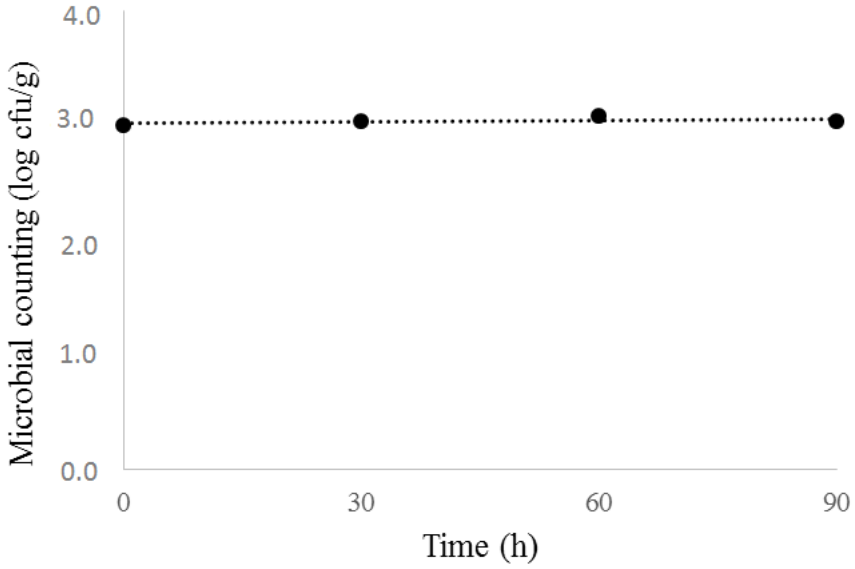

Figure 3. Growth of *Bacillus cereus* until 3 months.

Conclusion

GAB model was appropriate to predicted equilibrium moisture for *pinhão* flour in all range of water activity. All k estimated values indicating that lower sorption values were predicted by three parameters GAB equation as compared to ones fitted by two parameters BET models. Drying temperatures used to obtain *pinhão* endosperm flour did not change significantly the hygroscopic properties of this product. Furthermore, *pinhão* flours are safe for human consumption.

Acknowledgement

To the Centro Federal de Educação Tecnológica Celso Suckow da Fonseca - CEFET/RJ (Campus Valença), Embrapa Florestas, Embrapa Agroindústria de Alimentos and Universidade Federal do Rio de Janeiro - UFRJ/TPQB.

References

[1] BALBINOT, R.; GARZEL, J. C. L.; WEBER, K. S.; RIBEIRO, A. B. Tendências do consumo e preço de comercialização do pinhão (semente de Araucaria angustifólia (Bert.) O.kzte.) no Estado do Paraná. Ambiência. 4, 3, (2008), 463-472.

[2] IGLESIAS, H. A.; CHIRIFE, J. Handbook of food isotherms: water sorption parameters for food and food components. New York (1982), 347 p.

[3] TAYLOR, A. A. Determination of moisture equilibria in dehydrated foods. Food Tecnology, 15, (1961), 536-540.

[4] SPIESS, W., WOLF, W. The results of the COST 90 project on water activity. R. Jowitt (Ed.), et al., Physical Properties of Foods, Applied Science Publisher, London (1983).
[5] CORNEJO, F. E. P., NOGUEIRA, R. I., DE CARVALHO, C. W. P, DE GODOY, R.C.B, OLIVEIRA, A. H., SANTOS, L. F. C., BARRETO, A. G., FREITAS, S. P. Descascamento e secagem de pinhão (Araucária angustifólia) para a obtenção de farinha. (Embrapa Agroindústria de Alimentos. Comunicado técnico, 206), 2014, 3p.
[6] AOAC - Association Of Official Analytical Chemists. Official Methods of Analysis of the AOAC International. 18 th ed. Washington, DC, 2005. AOAC (2005) rev. 2010.
[7] AMERICAN OIL CHEMIST´S SOCIETY - AOCS. Official methods and recommended practices of the American Oil Chemists' Society. Champaign 1200p. 2009.
[8] NOGUEIRA, R. I.; CORNEJO, F. E. P.; WILBERG, V. C. Manual para Construção de um Desidratador de Produtos Agroindustriais. Rio de Janeiro: Embrapa Agroindústria de Alimentos, 2015, (21), 24 p. (Documentos / Embrapa Agroindústria de Alimentos).
[9] VAN DEN BERG, C. Development of B.E.T like models for sorption of water of foods; theory and relevance. In D. Simatos and J. L. Multon (Eds), Properties of Water in Foods, (1985), 119-135
[10] Softwares: available in www.microbiologia-de-alimentos.com. Access in 15th march (2017).
[11] BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n o 62, de 26 de agosto de 2003. Oficializa os Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. Diário Oficial da União, Brasília, 26 de Agosto de 2003. Seção 1.
[12] BRASIL. Agência Nacional de Vigilância Sanitária. RDC n° 12 de 2 de janeiro de 2001. Aprova o Regulamento Técnico sobre padrões microbiológicos para alimentos. Diário Oficial da União, Brasília, 10 de Janeiro de 2001.
[13] MOSSEL, D. A. A.; KOOPMAN. M. J.; JONGERIUS, E. Enumeration of Bacillus cereus in foods. Applied Microbiology, 15, (1967), 650-653.
[14] CLADERA-OLIVEIRA, F. MARCZAK, L. D. F., NOREÑA, C. P. Z., PETTERMANN, A. C. Modeling water adsorption isothems of pinhão (araucaria angustifolia seeds) flour and thermodynamic analysis of the adsorption process. Journal of Food Process Engineering, 34, (2011), 826-843.
[15] CHIRIFE, J. E. O.; TIMMERMANN, H. A.; IGLESIAS; R. B. Some features of the parameter k of the GAB equation as applied to sorption isotherms of selected food materials. Journal of Food Engineering, 15, 1, (1992), 75-82.
[16] AL-MUHTASEB, A. H.; MCMINN, W. A. M.; MAGEE, T. R. A. Water sorption isotherms of starch powders. Part 1: mathematical description of experimental data. J. Food Engineering, 61, (2004), 297–307.
[17] ENNADIR J.; HASSIKOU R.; OHMANI, F.; HAMMAMOUCHI J.; BOUAZZA F.; QASMAOUI A.; MENNANE Z.; TOUHAMI, A. O.; CHAROF, R.; KHEDID K. Qualité microbiologique des farines de blé consommées au Maroc. Canadian Journal of Microbiology, 58, 2 (2012), 145-150.
[18] AYDIN, A; PAULSEN, P.; SMULDERS, J. M. The physico-chemical and microbiological properties of wheat flour in Thrace. Turk J. Agric For., 33, (2009), 445-454.