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IMPACT OF COMMINUTION ON ADSORPTION PROPERTIES OF GLUTEN-FREE WHEAT STARCH

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Abstract. The process of grinding of wheat grain endosperm leads to damage of the starch fraction. Starch damage in wheat flour changes its water absorption. Intact starch absorbs water in the amount of 70% of its mass, and damaged granules have up to 3-fold higher level of absorption, which is conditioned by the increased number of small crystalline structures and damage to many crystalline structures. These changes cause an increase of the volumetric water absorption capacity and it can be assumed that this will be reflected in the surface microstructure of starch particles that affect the adsorption of water vapour. The aim of the study was to evaluate the impact of comminution of gluten-free wheat starch on the adsorption properties of its granules. The study included the determination of adsorption isotherms of water vapour at 20°C with the static-desiccator method and the determination of adsorption parameters and microstructure of starch granule surface based on the GAB and Kelvin equations. The results confirmed the positive impact of mechanical processing on the capacity of surface water vapour adsorption by starch granules and this was indicated by a significantly diversified distribution of adsorption isotherms, surface adsorption parameters and parameters of microstructure of molecules on which adsorption was tested.

Keywords: wheat starch, adsorption isotherms, monolayer, specific adsorption surface, water activity, Guggenheim, Anderson and De Boer (GAB) equation

INTRODUCTION

The comminution of wheat grain endosperm results in damage to the starch fraction which consequently shows higher susceptibility to amylolytic enzymes, partially loses its crystalline structure and shows higher water adsorption capacity.

The damage of starch in wheat flour changes its water adsorption capacity. Intact starch adsorbs water up to 70% of its weight and damaged granules show a three-times higher level of adsorption as the number of small crystalline structures increases and numerous crystalline structures are disrupted (Jurga 2004). These changes stimulate the increase in water adsorption capacity and it is assumed that they may be reflected in the surface microstructure of starch molecules which conditions the process of water vapour adsorption.

Wheat starch consists of oval or lenticular granules with different sizes (Peterson *et al.* 2001), but some studies by Raeker *et al.* (1998) indicate that wheat starch granules show trimodal distribution. The size distribution of wheat starch granules may have an impact on the chemical composition of starch which consequently influences its utility (Darlington *et al.* 2000). Starch granules have a three-dimensional structure and amylopectin plays a major role in its formation. The branched fragments of amylopectin molecules are connected with hydrogen bonds and form dense crystalline areas called micelles. Between them there are spheres made of amylose. Native starch granules have both a crystalline and an amorphous structure (Achremowicz and Korus 2010).

During milling, individual starch granules are separated and many of them are also damaged. The degree of disruption depends on water diffusion into endosperm during conditioning of grain and on the technological conditions of the milling process (Jurga 2004, 2010).

The structure of starch also includes water molecules connected with hydrogen binding with hydroxyl groups of glucose residuals. Starch granules contain a minor amount of protein (app. 0.3%), lipids (0.4-0.8%) and minerals, which may influence the properties of starch (Raeker *et al.* 1998, Darlington *et al.* 2000, Gąsiorowski 2004). The properties of starch may change as a result of comminution, roasting, drying, extrusion and pelleting which are conditioned by thermal processes initiated during comminution, and of the temperature and period of storage (Dreher 1987).

Water content in starch depends on relative humidity in the environment. Starch granules are hygroscopic even at room temperature, though water adsorption is slow and limited. The phenomenon of water adsorption from the environment is based on reversible adsorption and is initially accompanied by a change in the size and shape of granules (Gąsiorowski 2004).

RESEARCH OBJECTIVE

The aim of the study was to evaluate the impact of comminution of gluten-free wheat starch on the physical condition of its granules determining the adsorption properties.

MATERIALS

The study was carried out on gluten-free wheat starch commercially manufactured by a factory of gluten-free products (Wytwórnia Artykułów Bezglutenowych “BEZGLUTEN” s.c., defined as N in the study – intact starch) and gluten-free wheat starch which was comminuted with a laboratory hammer mill type WŻ-1 (Zakład Badawczy Przemysłu Piekarskiego Sp. z o.o. w Bydgoszczy) for 60 s (defined as U in the study – damaged starch).

METHODS

The determination of water content was carried out with the routine reference method in accordance with PN-ISO 712:2002 for cereals and cereal products.

The water activity was evaluated in AquaLab (Series 3, TE model, Decagon Devices, Inc., Pullman, WA, USA) with an accuracy of ± 0.003 at 293.15 K.

The adsorption isotherms were determined with the static-desiccator method. This method was based on the evaluation of humidity balance between a tested sample and the atmosphere with a given relative humidity regulated by saturated salt solutions. The scope of the study included water activity from 0.07 to 0.98. The temperature was 293.15 K (20^{o}C). The balance of configuration settled in 30 days after the samples were placed in a desiccator. Thymol was placed in the desiccators with water activity >0.7 in order to protect samples against the growth of microorganisms. Individual starch samples of approx. 1 g ± 0.1 mg, designated for the determination of adsorption isotherms in the first stage of the experiment, were placed in 35mm diameter weighing vessels in order to uniformly cover the whole surface of a vessel. In the next step, the vessels were placed in a desiccator containing P_2O_5 as a drying compound, at room temperature for 3 weeks in order to minimise humidity (~2%) in the tested samples. Following a 3-week period, the samples were weighed and placed in the desiccators with a saturated solution. The equilibrium water content was calculated and adsorption isotherms were drawn with the use of MS Excel based on the initial mass of product (determined after 3-week incubation in the desiccator with P_2O_5) and the changes of water content. The measurements of water activity 30 days after placing the samples in the desiccators were carried out with AquaLab.

The diversification of adsorption isotherms across the entire a_w range was statistically analysed with a Student’s t-test for differences between the means of matched pairs. The differences were regarded as statistically significant at a significance level not exceeding P = 0.001 (Sobczyk 2004, Łomnicki 2006).

The following GAB equation:

$$v = \frac{v_m CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \tag{1}$$

where: a_w – water activity (–);

v – equilibrium water content (g H_2O (100 g d.m.)$^{-1}$);

v_m – water content in the monolayer (g H_2O (100 g d.m.$^{-1}$);

C – Guggenheim energy constant;

K – constant correcting properties of multilayer molecules with relation to the liquid phase (Paderewski 1999, Figura and Teixeira 2007).

was determined based on the empirical data. The identification was carried out on the basis of non-linear regression with a Monte Carlo algorithm. Such an approach prevents inhibition of the estimation process by a local minimum. Minimisation of residual sum of squares was adopted as the target function, which is commonly used in statistical analysis (Ocieczek and Kostek 2009). The calculations were performed using MS Excel 2003. The errors of determined equation parameters were detected with the SolverAid macro command.

The adsorbent specific surface was calculated based on the following equation:

$$a_{sp} = \omega \frac{v_m}{M} N \tag{2}$$

where: a_{sp} – sorption specific surface (m^2 (g d.m.)$^{-1}$);

N – Avogadro number ($6.023 \cdot 10^{23}$ molecules mol^{-1});

M – water molecular weight (18 g mol^{-1});

ω – water setting surface ($1.05 \cdot 10^{-19}$ m^2 molecule^{-1}) (Paderewski 1999).

The size and volume of capillaries of the material tested were determined in the capillary condensation area by the Kelvin equation, assuming their cylindrical shape:

$$\ln a_w = -\frac{2\sigma V}{r_k RT} \tag{3}$$

where: σ – liquid surface tension at temp. T (N m^{-1});

r_k – capillary radius (nm);

R – gas constant (kJ (mol K)$^{-1}$)

T – temperature of process (K);

V – molar volume of adsorbate (m^3 mol^{-1}) (Paderewski 1999, Figura and Teixeira 2007).

DISCUSSION

The water content in intact starch (N) was 12.969 g (100 g)$^{-1}$ of dry matter and in damaged starch (U) it was 13.791 g (100 g)$^{-1}$ of dry matter. The water activity in both tested products was similar and amounted to 0.415 and 0.429, respectively. The results show that comminution intensifies water adsorption from the environment, which confirms the reports on strong hygroscopic properties of starch and the possibility of their increase due to disruption of starch granules during comminution.

The structure of native starch granules has been extensively studied for many years with numerous microscopic and scattering techniques, including optical and electron microscopy, light, X radiation and neutron scattering. The distribution and dynamic state of water in grain starch is much less researched and understood. This topic is important since water plays a key role in determining the response of starch granules to technological processes such as heating, microwaves, high pressure or chemical processing. The dynamic state of adsorbed water (up to 20%) on the surface of starch granules has been investigated with different methods. It has been found that the dynamic state of water adsorbed on the surface does not depend on the microstructure of granules and is determined by the interactions on the molecular level between water and starch. The role of granule microstructure in determining relaxation of water molecules with its high content when all sub-granules are saturated with water has not yet been investigated (Tang *et al.* 2000).

The impact of comminution on adsorption properties was evaluated based on a comparison of adsorption isotherms of water vapour drawn at 20°C (Fig. 1).

The determined isotherms were sigmoidal in shape, which was typical of the physical adsorption of porous bodies accompanied by the formation of multi-molecule water layers on the surface of analysed granules (Roman-Gutierrez *et al.* 2002, Hébrard *et al.* 2003, Erbas *et al.* 2005, Limousin *et al.* 2007, Ocieczek 2007). The isotherms were continuous across the whole range of water activity, indicating that during adsorption of water no change of structure occurred due to a change in the organisation of the solid body matrix. The adsorption of water from the environment was particularly evident above 0.70 a_w; this phenomenon may be explained as an effect of capillary condensation. It should be emphasised that starch subjected to additional comminution (U) in the zone of capillary condensation (high humidity in the environment) adsorbed much more water than intact starch (N). This statement may confirm the thesis presented by Tang *et al.* (2000) indicating the lack of significant impact of starch granule microstructure on the dynamic state of water adsorbed on the surface, and simultaneously point at the need to carry out further research into the impact of granule microstructure on the state of water molecules when it is present at high contents.

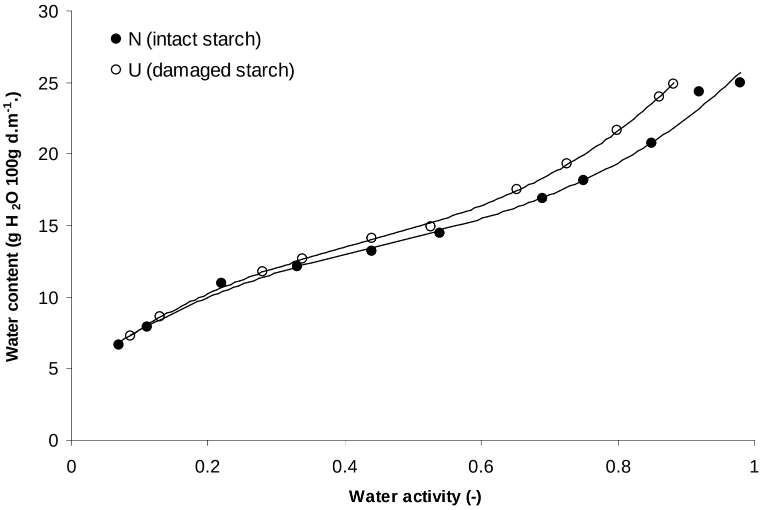

Fig. 1. Adsorption isotherms of water vapour by the investigated starch samples determined at 20°C

The damaged starch showed higher adsorption capacity, indicated by higher location of the isotherm. The adsorption capacity of starch is determined by distribution of polar groups, availability of these groups to water, degree of matrix crystallisation, relative strength of water-water and water-macromolecule interaction, as well as relative humidity in the environment (Elizalde *et al.* 1996). In order to statistically verify the null hypothesis, assuming the lack of differences in the distribution of both adsorption isotherms, Student's t-test was performed between the means for matched pairs. The calculated value for the *t* statistic was 4.593 and was higher than the critical value of the *t* test at significance level 0.001 and for 10 degrees of freedom. The null hypothesis should therefore be rejected and it should be stated that the isotherm for comminuted starch (U) is located significantly higher than the isotherm for intact starch (N).

The comparison of the parameters of adsorption isotherms based on the GAB model was the next component of evaluation (Tab. 1).

The choice of equation was influenced by the recommendation of European Project Group COST 90 indicating the usefulness of the GAB model in determination of water adsorption by food products (Timmermann *et al.* 2001, Timmermann

2003). The parameters of the GAB equation (v_m, C, K) and the values of water activity corresponding to monolayer coverage as well as the errors in the estimated parameters were determined with the SolverAid macro command.

Table 1. Parameters of the GAB equation for tested starch samples and a_w corresponding to v_m

Parameter of GAB equation	Product	
	C (intact starch)	U (damaged starch)
v_m – water content in the monolayer (g H_2O (100 g d.m.$^{-1}$)	10.36 ± 0.44	10.52 ± 0.27
K – constant correcting properties of multilayer molecules with relation to the liquid phase	0.611 ± 0.020	0.662 ± 0.013
C – Guggenheim energy constant	38.14 ± 8.69	33.16 ± 4.56
a_w – water activity (-)	0.228	0.224

The capacity of the monolayer is regarded as an indicator of the availability of polar sites for vapours. v_m capacity is determined by the quantity of individual compounds rich in polar sites and their physical state which, in turn, conditions the amount of water which is strongly bound to the food matrix (Mathlouthi 2001). The determined capacities of the monolayer ranged from 10.36 ± 0.44 to 10.52 ± 0.27 g H_2O per 100 g of product dry matter, which corresponded to the values measured by Karel (1975) for different food products. It was found that higher (v_m) was characteristic of damaged starch (U), which might indicate that comminution had an impact on the adsorption parameters of starch (Roman-Gutierrez *et al.* 2002, Erbas *et al.* 2005). The water activities corresponding to (v_m) were on a similar level.

The GAB equation is based on the BET theory and therefore it is assumed that the heat of multilayer adsorption of molecules from the first to the ninth layer differs from the heat of condensation (Anderson 1946). It has also been shown (van den Berg 1981, 1984, 1985, Jong *et al.* 1996) that this deviation describes the value of the K parameter in the GAB equation. When K equals 1, multilayer water has the properties of water in a liquid state. The value of K makes it possible to determine whether the GAB equation correctly maps a specific set of empirical data. Lewicki (1997) showed that one of the conditions for the correct application of the GAB equation to describe an experiment is K-value located in the range from 0.24 to 1. This condition was fulfilled and, therefore, the parameters of the GAB equation can be regarded as an appropriate description of the real situation. Comminuted starch had a higher K value. It may be assumed that the degree of damage to starch granules has an impact on the energy state of configuration, which consequently leads to an increase in the difference between the heat of multilayer adsorption and the heat of condensation. It should be presumed that the process mechanism does not change, whereas the energy state of water molecules forming a multilayer configuration becomes diversified.

According to Lewicki (1997), the second parameter indicating the usefulness of the GAB model, which determines the gain calculation error monolayer at level lower than 15.5%, is the value of the constant C greater than 5.67. This condition was also fulfilled. In addition, the value of the C parameter describes the nature of the adsorption phenomenon since it reflects the difference between enthalpy of desorption from the monolayer and enthalpy of evaporation from liquid adsorbent. The value of enthalpy of physical adsorption is approximately 20 $\mathrm{kJ \cdot mol^{-1}}$ and does not influence the identity of physically adsorbed molecules. During chemical adsorption, the molecules form bonds with the surface. The enthalpy of chemisorption is much higher than the physical adsorption and usually amounts to approximately 200 $\mathrm{kJ\ mol^{-1}}$ (Atkins 2003). However, Pérez-Alonso *et al.* (2006) suggested that the value of the empirical C parameter obtained from calculations may lack a physical sense.

This paper also discusses the structural characteristics of tested starch with the Kelvin equation (Tab. 2). This equation allows a transformation of equilibrium relative pressures into corresponding radiuses of capillaries. In order to determine the capacity of the capillaries and to determine their distribution as a function of radius the mercury porosimetry method or designation of the type II adsorption isotherm can be used. The method of mercury porosimetry can only determine the capacity of capillaries with radii larger than 7.5 nm. However, the adsorption method allows to specify the radius of capillaries in the range of 1.5-100 nm (Paderewski 1999).

Table 2. Structural characteristics of the starch samples

Product	Sorption specific surface ($\mathrm{m^2\ g^{-1}}$ d.m.)	Total capacity of capillaries ($\mathrm{mm^3\ 100\ g^{-1}}$ d.m.)	Size of capillaries at a_w=0.75 (nm)
N (intact starch)	364.1	88.16	3.987
U (damaged starch)	369.7	90.61	4.356

Native starch granules are different in shape and size (from 0.1 to 100 µm) depending on its origin (Capouchová *et al.* 2003, Wilpiszewska and Spychaj 2006, Dai *et al.* 2008, Berski 2010). A single starch granule is a partially crystalline, complex layered structure (Wilpiszewska and Spychaj 2006). Starch granules consist of amorphous and semi-crystalline alternating layers with a thickness of 100 to 400 nm (Angellier-Coussy *et al.* 2009). These layers are called “growth rings”.

Layers of relatively structured granules are examined by X-ray techniques using SAXS and WAXS and XRD (Angellier-Coussy *et al.* 2009). The crystallinity of starch is the result of the presence of hydrogen bonds between the hydroxyl groups of the polysaccharide chains. The degree of crystallinity (15-45%) of native starch affects primarily the amylopectin fraction (Wilpiszewska and Spychaj 2006). This fraction forms a thin, lamellar domain (approximately 4.5 nm in thickness), which consist of about 10 double helices. Double helices are packed densely, and are characterised by a high degree of regularity, such as occurs in the crystal (Maningat and Seib 2010). Very little is known about the amorphous parts of the “growth rings”. The α-1,6-branch points in amylopectin are clustered between crystalline layers. The branched region of amylopectin with the amylose molecules constitute the amorphous layer of starch granules. The amorphous layer of a fully hydrated native starch granule is accessible to water-soluble reagents with a cut-off molecular weight of $\approx$ 1,000 da (Brown and French 1977).

The diameter of a water molecule is about 0.2 nm, which allows it to freely penetrate structures such as starch granules, even within the semi-crystalline layer. The water molecule is a strongly polarized dipole and in structures comparable to it in terms of size it plays an active role. It affects the distribution of the particle electric charge and often becomes an integral part of the structure determining the mechanical stability of biological particles.

Examination of the surface microstructure of gluten-free wheat starch granules was based on the use of hydrophilic interactions taking place between the water molecule and the native, intact starch granules (N) and starch granules damaged during mechanical action (U).

The estimation of the surface microstructure of tested samples indicated that the specific surface area of adsorption of wheat starch expanded as a result of the comminution of samples. Taking into account the chemical integrity of the tested starch samples it may be assumed that the observed change in specific surface area of adsorption was only conditioned by the physical state of the starch granules determined by comminution. On the one hand, comminution leads to a decrease in the size of individual particles, which consequently results in the expansion of the total surface area of the new molecules. On the other hand, comminution disrupts the degree of arrangement of the starch matrix and induces the occurrence of amorphous zones capable of adsorption of water molecules. It should be emphasised that inter-distribution of adsorption isotherms indicates that comminution has a bigger impact on the susceptibility of starch to water at higher concentrations than at lower concentrations.

The determination of the radii of capillaries and the total capacity of capillaries filled after initiation of capillary condensation (a_w = 0.75) revealed that increased values of these parameters were characteristic of the starch sample comminuted in the laboratory hammer mill type WŻ-1 for 60 s. This indicates that the pores with larger radius condition a higher total capacity of capillaries in the product. Then again, higher porosity of material facilitates water diffusion and, in turn, increases adsorption in the range of high a_w values.

CONCLUSIONS

1. The adsorption isotherms for both tested starch samples (N and U) were sigmoidal, which is typical of products rich in starch. The shape of adsorption curves indicated multi-layer adsorption and a porous structure of the tested products.
2. The significantly higher adsorption capacity was characteristic for comminuted starch (U), especially in the range of full saturation of all sub-granules with water, with its high concentration in the environment.
3. The comminuted starch (U) showed higher total capacity of capillaries and the initiation of capillary condensation was accompanied by the filling of capillaries with larger radiuses.
4. It should be stated that the tested starch samples which were chemically homogeneous differed in adsorption characteristics as determined by their physical state.
5. It is assumed that the affinity of starch granules (damaged and intact) to water, analysed in the entire volume of the material, may show a higher diversity of water binding capacity than in the case of processes located only on the surface of molecules.

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WPŁYW ROZDRABNIANIA NA WŁAŚCIWOŚCI SORPCYJNE SKROBI PSZENNEJ BEZGLUTENOWEJ

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Streszczenie. Proces rozdrabniania bielma ziarna pszenicy prowadzi do powstawania uszkodzeń frakcji skrobiowej. Uszkodzenie skrobi w mące pszennej powoduje zmiany jej wodochłonności. Skrobia nieuszkodzona absorbuje wodę w ilości 70% swojej masy, a granule uszkodzone wykazują nawet 3-krotnie wyższy poziom absorpcji, co uwarunkowane jest zwiększeniem ilości drobnych struktur krystalicznych oraz uszkodzeniem wielu struktur krystalicznych. Zmiany te wpływają na wzrost zdolności pochłaniania objętościowego wody i zakładać można, że znajdą odzwierciedlenie w mikrostrukturze powierzchniowej cząstek skrobi warunkującej przebieg zjawiska adsorpcji pary wodnej. Celem badania była ocena wpływu rozdrabniania skrobi pszennej bezglutenowej na jej właściwości sorpcyjne. Badanie obejmowało wyznaczenie izoterm sorpcji w temperaturze 20°C metodą statyczno-eksykatorową, określenie parametrów procesu sorpcji i mikrostruktury powierzchni granul skrobiowych na podstawie równania GAB oraz równania Kelvina. Otrzymane wyniki potwierdziły dodatni wpływ mechanicznego oddziaływania na zdolność powierzchniowego pochłaniania pary wodnej przez granule skrobiowe, co wykazano poprzez zidentyfikowanie istotnie zróżnicowanego przebiegu izoterm sorpcji, parametrów charakteryzujących pochłanianie powierzchniowe oraz parametrów opisujących mikrostrukturę cząstek, na których sorpcję badano.

Słowa kluczowe: skrobia pszenna, izotermy sorpcji, monowarstwa, powierzchnia właściwa sorpcji, aktywność wody, równanie Guggenheima, Andersona i De Boera (GAB)