# CHARACTERISTICS OF CARBON DIOXIDE GAS ADSORPTION BY GRAIN AND ITS COMPONENTS.

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## INTRODUCTION

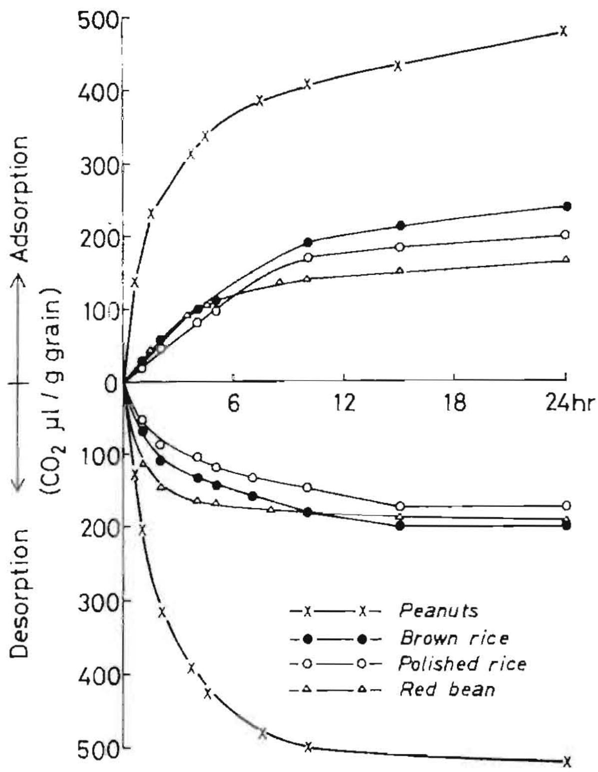
During experiments of grain storage in a $CO_2$ atmosphere, grains in flexible laminated plastic film bags originally containing $CO_2$ gas were found to be tightly packed as if packed in vacuo (Mitsuda et al., 1971). This led to the discovery of an interesting adsorption phenomenon of $CO_2$ gas by grains at a low moisture content and to the development of a technique for skin-packaging, the Carbon dioxide Exchange Method (CEM) (Mitsuda et al., 1972a). Various experiments have been carried out to examine the mechanism of this adsorption phenomenon (Mitsuda et al., 1972b, 1973, 1975, 1977). The purpose of this paper is to summarize the experimental results on the characteristics of $CO_2$ gas adsorption by grain and its components for the practical use of $CO_2$ gas in artificial controlled atmosphere storage of grains.

## CHARACTERISTICS OF CARBON DIOXIDE GAS ADSORPTION BY GRAIN

Equilibrium. Figure 1 shows the time course for $CO_2$ gas adsorption and desorption by grains at 25$^{o}$C. The velocity of adsorption varies with the species of grain. Fifty to sixty percent of the maximum adsorption takes place within the first 6 hours in the case of rice and red beans and in about 4 hours for peanuts seeds. The graph also shows that equilibrium is obtained after about 24 hours. Desorption curves show that the $CO_2$ gas adsorption by grains is almost completely reversed when the grain is allowed to stand in air. The velocity of desorption is larger than that of adsorption. The long time needed to reach adsorption equilibrium and the variation of the rate with the species of grain suggested that diffusion of $CO_2$ gas into the grain kernel is important in this phenomenon.

Temperature dependence. The amount of $CO_2$ gas adsorbed by grains varies depending on temperature, increasing at a lower temperature. The velocity of adsorption is not affected as much by temperature.

Moisture dependence. The maximum adsorption of $CO_2$ gas was measured at moisture contents of rice ranging from 0 to 16.3% to investigate the relationship between the moisture content and the amount of $CO_2$ gas adsorbed by rice grain. The $CO_2$ gas adsorption was found to depend on the moisture content of rice as shown in Fig. 2. Adsorption increases almost linearly in brown rice but decreases almost linearly in paddy rice as the moisture content of the grains increases. Hull and



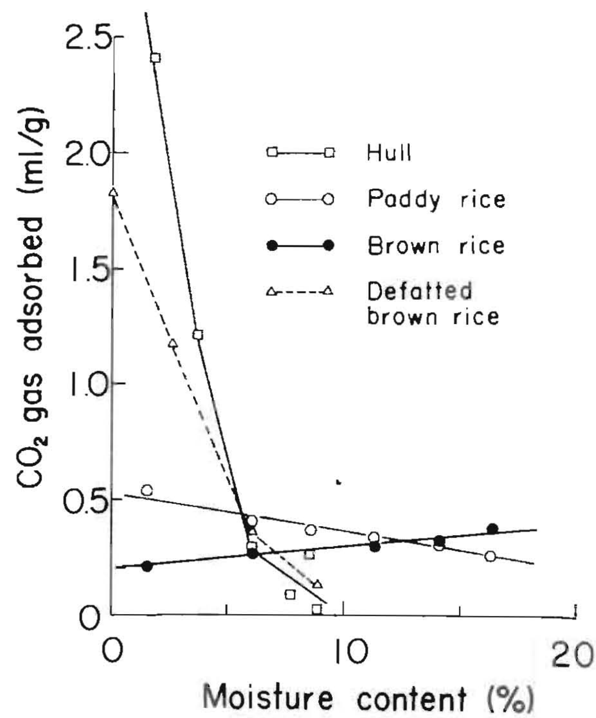




Fig. 1 (left). Time course of $CO_2$ gas adsorption and desorption by grains (at $25^{o}C$). One gram of grains was put into a vessel of a Warburg' s manometer and was shaken in an atmosphere of $CO_2$ gas at a constant temperature bath adjusted to $25^{o}C$. The amount of $CO_2$ gas adsorbed was measured with the lapse of time. To study desorption one gram of grains was placed in an atmosphere of $CO_2$ gas for more than 48 hrs to adsorb the gas until fully saturated. This grain was transferred quickly into the vessel and then shaken in air.

Fig. 2 (right). Relationship between the moisture content and $CO_2$ gas adsorbed by rice grain. Defatted brown rice was prepared as follows: Grains were dehydrated and defatted by a gradual ethanol-aceton or ethanol-hexane schedule. Samples were then heated at $105^{o}C$ for 24 hr to remove the solvent remained in the kernel. Grain samples with various moisture contents were prepared by equilibrating them for 3 to 14 days in desiccators with saturated salt solution. The amount of $CO_2$ gas adsorbed by the grains was measured in the same way as described in Fig. 1.

defatted brown rice adsorb remarkable amounts of $CO_2$ gas at the lower moisture content. The maximum solubility level of $CO_2$ gas in water contained in grains, calculated by Bunsen's absorption coefficients, is found to be lower than one-half of the amount of $CO_2$ gas adsorbed. This suggests that the $CO_2$ gas adsorption phenomenon in grains is not merely caused by dissolution of gas into the water.

Kinds of grains. Many kinds of cereal grains and pulses showed a similar adsorption phenomenon. Table 1 shows the adsorption amount of $CO_2$ gas by each of these grains at 20°C for 3 hr. Wheat, corn, peanuts, soybeans, red beans, sesame seeds, and their flours show the same adsorption phenomenon as rice, but the velocity of adsorption and the maximum amount of $CO_2$ gas adsorbed vary with the kinds of grains. Peanuts, soybeans, and sesame seeds are good adsorbents, and wheat, rice and flours seem to be relatively poor ones. Compared with conventional adsorbents, such as silica gel and activated charcoal, these grains are all poor adsorbents.

TABLE 1.
The amount of $CO_2$ gas adsorbed by various grains.

| Kinds of grains | Adsorption amount at 20°C for 3 hr | Kinds of grains | Adsorption amount at 20°C for 3 hr |
|---|---|---|---|
| Paddy rice | 86 ml/kg | Peanuts | 560 ml/kg |
| Brown rice | 90 | Soybean flour | 440 |
| Polished rice | 70 | Soybean flour | 216 |
| Rice flour | 60 | Red beans | 64 |
| Wheat | 75 | Coffee beans | 123 |
| Wheat flour | 60 | Sesame seeds | 230 |
| Corn | 170 | Black tea | 115 |

Grain components. As shown in Table 2, an inverse relationship was obtained between fat content of brown rice and the amount of $CO_2$ gas adsorbed. Carbohydrates such as sugars, cellulose and starch in purified forms failed to adsorb the $CO_2$ gas. A clear relationship was not observed between the protein content of grains and the amount of $CO_2$ gas adsorbed, although many kinds of pulses of high protein content were found to be good adsorbents.

TABLE 2.
Relationship between the fat content of brown rice and the amount of $CO_2$ gas adsorbed.

| | | Fat content[a)] | $CO_2$ gas adsorbed[b)] |
|---|---|---|---|
| Raw brown rice | | 2.67 % | 128 µl/g |
| Defatted brown rice with: | Ethanol | 2.51 | 129 |
| | Ether | 2.16 | 185 |
| | Chloroform-methanol (2:1) | 1.35 | 568 |
| | Ethanol-acetone schedule | 0.20 | 1758 |

a) Determined by Soxhlet methods with ether.
b) Determined by the Warburg's manometry at 25°C.
All figures are shown on a dry basis.

Porosity. The porosity of the grain kernel was proved to influence the $CO_2$ gas adsorption phenomenon. A high pressure mercury penetration porosimeter was used for the determination of pore-volume and pore-size distribution of rice kernel in the range of 42 - 300 Å. A relatively larger size of pores was found in paddy compared with those of brown and polished rice. Defatting and/or heat-drying treatment to rice kernel, however, produced abundant pores in their intergranular spaces of starch. Pore volume between the range of 42 Å and 12 μ in diameter was estimated to be 264, 134, 73.7, 31.2 and 21.3 μl/g (on dry basis) for hull, defatted brown rice, paddy, brown and polished rice respectively.

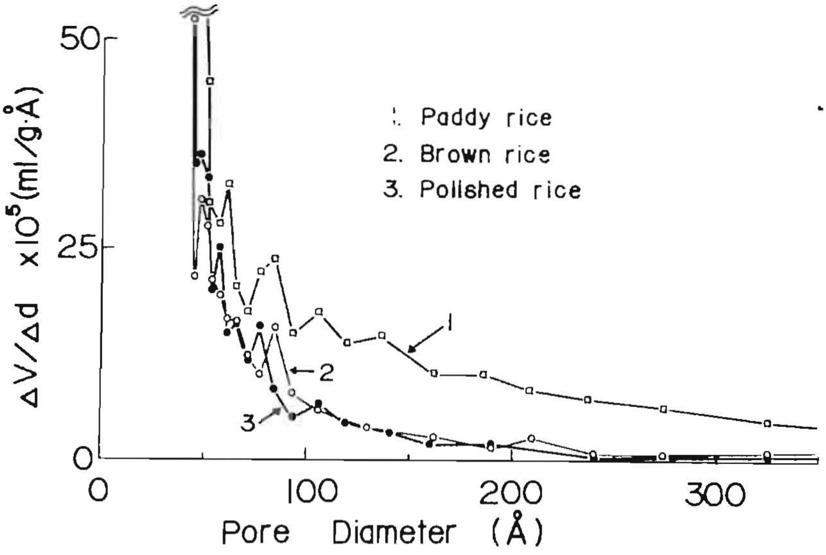



Fig. 3. Pore-size distribution of rice grain.

TABLE 3.
Comparison of pore-volumes of rice grain and hull.

| | Hull | Paddy | Brown | Polished |
|---|---|---|---|---|
| Total pore volume (42 Å - 12 μ) (μl/g) | 264 | 73.7 | 31.2 | 21.3 |
| Micro-pore volume (42 Å - 354 Å) (μl/g) | 136 | 38.1 | 15.4 | 11.0 |

Sorption isoterm. According to the classic sorption isoterm of Freundlich, a linear relation is obtained when the logarithms of pressure (P) and sorption volume (V) are plotted. This relation may be written:
$\log V = \log k + n \log P$, where k and n are constants.

Experimental results obtained with the adsorption of $CO_2$ by brown rice at various partial pressures of $CO_2$ were found to agree with the isoterm as shown in Fig. 4. An almost linear relation between log V and log $P_{CO_2}$ was obtained in the higher partial pressure of the $CO_2$ ($P_{CO_2}$) region but not in the lower one. The constant n was calculated as about 1.5.

$$V = \frac{(C_o - C_e)\, v}{100 - C_e}$$

V: volume of $CO_2$ gas adsorbed by brown rice (ml)
$C_o$: original $CO_2$ gas composition (%)
$C_e$: equilibrated $CO_2$ gas composition (%)
v: original mixed gas volume (ml)

$$\log V = \log k + n \log P_{CO_2}$$

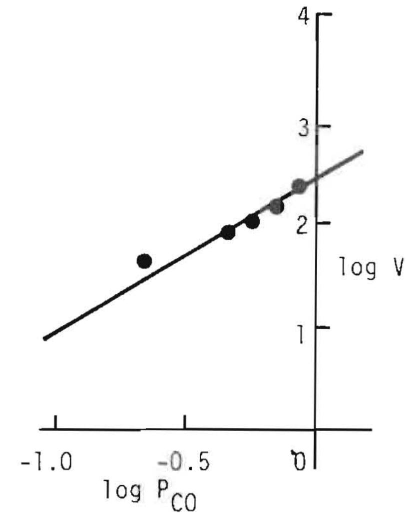



Fig. 4. Relation between the amount of $CO_2$ gas adsorbed and the partial pressure of $CO_2$ (at 25$^oC$). Brown rice was packed in plastic film bags under different compositions of $N_2$ and $CO_2$ gas. After the adsorption equilibrium was obtained at 25$^oC$ under atmospheric pressure, the composition of headspace gases was analyzed by gas chromatography and compared with the original composition. The amount of $CO_2$ gas adsorbed by brown rice was calculated using the equation shown in this figure.

Results obtained in these experiments can be summarized as follows:

(1) Solubility of $CO_2$ gas into the moisture and lipids of the grain is assumed to have a minor effect on the $CO_2$ adsorption phenomenon. No direct relationship is also presented between any special components of grain and the amount of $CO_2$ gas adsorbed.

(2) Diffusion of $CO_2$ gas into the grain is important in this phenomenon. This adsorption phenomenon is very similar to that observed in sorption of gases by charcoal and silica gel, which sorb gases into their many pores.

THE $CO_2$ GAS-PROTEIN INTERACTION IN A GAS-SOLID PHASE.

In the course of investigations to elucidate the mechanism involved in the $CO_2$ gas adsorption phenomenon by grains, various proteins in solid form were found to be capable of adsorbing $CO_2$ gas. The characteristics of $CO_2$ gas adsorption by highly purified protein has been investigated by Warburg manometry and the B.E.T. surface area analyzer.

Adsorption of $CO_2$ gas by proteins. Carbon dioxide gas was adsorbed by casein at more than 500 µl/g/24hr while ethylene and oxygen were less than 200 and 50 µl/g/24hr respectively. Approximate equilibrium was obtained after 24 hours. Helium, nitrogen, hydrogen and air were also examined but none of them was adsorbed considerably by casein under the same conditions in the case of $CO_2$ gas. The amount of $CO_2$ gas adsorbed by proteins is shown in Table 4. Various proteins were found to adsorb $CO_2$ gas when they were placed in the high partial pressure of the gas concerned. Casein, gelatin and raw silk were revealed to be the better adsorbents as compared with the others.

TABLE 4.
Adsorption of $CO_2$ gas by various proteins in dried state.

| Proteins | $CO_2$ gas adsorbed[a)] |
|---|---|
| Casein | 662 µl/g |
| Gelatin | 532 |
| Egg albumin | 267 |
| Glutenine | 231 |
| Hemoglobin | 182 |
| Zein | 150 |
| Soybean flour | 220 |
| Whole milk | 200 |
| Bleached *Chlorella* algae | 163 |
| Hydrocarbon-assimilating yeast | 115 |
| Gluten | 111 |
| Rice bran | 109 |
| Raw silk | 690 |
| Fibroin, from silk | 110 |
| Chinese white rabbit hair | 106 |

a) Determined by Warburg manometry at 25°C for 24 hr.
All figures are shown on a wet basis.

Moisture dependence. In Fig. 5, the amount of $CO_2$ gas adsorbed by casein and gelatin are plotted against their moisture content. The lower the moisture is, the greater the adsorption amount of $CO_2$ increases.

Temperature dependence. The amount of $CO_2$ gas adsorbed by zein varies depending on temperature, as shown in Fig. 6, increasing at a lower temperature. Other proteins also show a similar tendency as shown in Fig. 7. The temperature dependence of $CO_2$ adsorption by protein is close to that of physical adsorption. Although large amounts of $CO_2$ gas were adsorbed by histamin, tyramine, L-lysine and L-arginine, temperature dependence of the adsorption is somewhat different from that of protein, such as casein and gelatin.

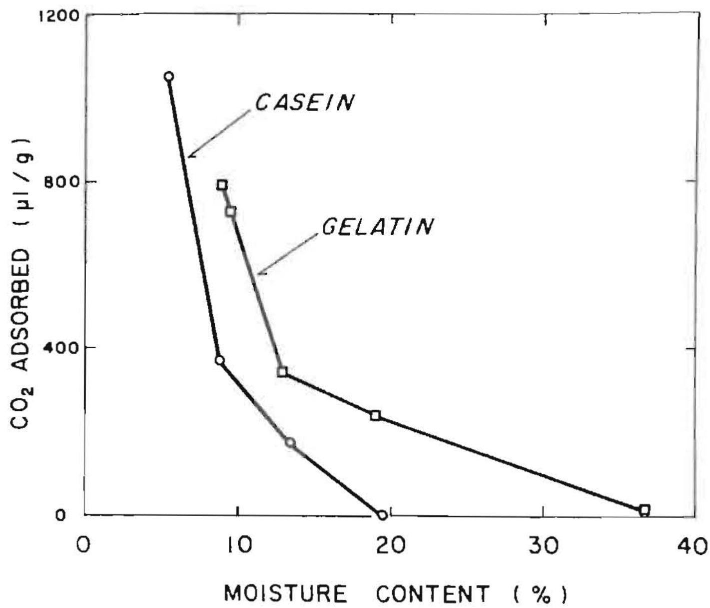
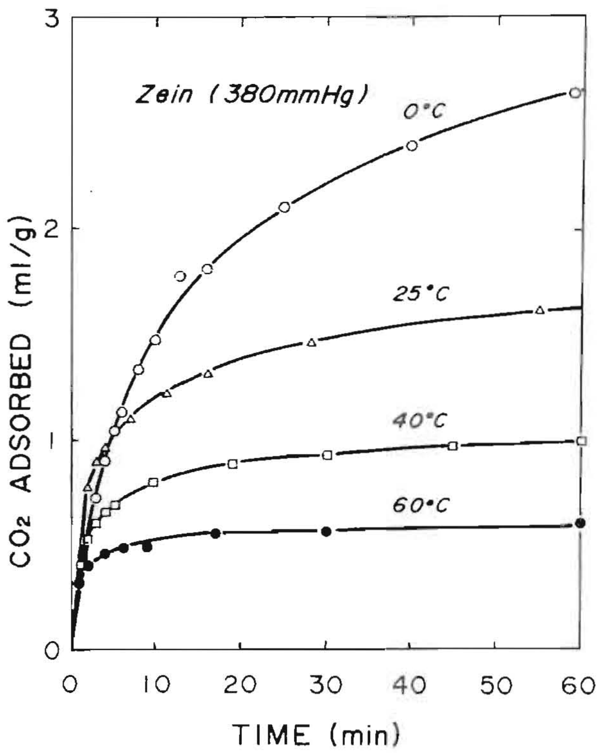



Fig. 5. Effect of moisture content on the amount of $CO_2$ gas adsorbed by casein and gelatin. Protein samples at various moisture contents were prepared by equilibrating them for 3 to 14 day in desiccators with saturated solutions of LiCl, $K_2CO_3$ , $Mg(NO_3)_2$ , NaCl and $KNO_3$ at relative humidities between 11 and 88%.

Reversibility of $CO_2$ gas adsorption by protein and others. Reversibility of the $CO_2$ gas adsorption by protein, protein hydrolyzates, amines and amino acids was examined. More than 90% of $CO_2$ gas which had been adsorbed by casein and gelatin desorbed when they were put into an atmosphere of low partial pressure of $CO_2$ but only a few percentage desorbed in the case of amino acid and histamine. Interaction between $CO_2$ gas and amines and amino acids seemed to be "chemical reaction" or "chemisorption" rather than "physical adsorption".

Effect of particle size on $CO_2$ adsorption by bacto-peptone and casein. Bacto-peptone, one of the protein hydrolyzates for a microbial culture medium, showed an interesting result on $CO_2$ adsorption. This was one of the samples which fairly adsorbed $CO_2$ gas when they were tested in larger particles above 32 mesh. Gas adsorption increased more than 20 times when the sample was ground. The less the peptone particle size, the more the amount of $CO_2$ gas adsorbed. This result suggested that the gas diffusion process in the particles was the rate-limiting factor in $CO_2$ gas adsorption by proteins. This was confirmed by the determination of a diffusion coefficient of $CO_2$ gas in casein particles of three different sizes. By applying the Boyd equation, the degree of the adsorption ($V_t/V_{eq}$) was found to be in proportion to a square root of time in the region below one-half of $V_t/V_{eq}$ as shown in Fig. 9. The diffusion coefficient obtained in these samples was around $10^{-9} cm^2/sec$. This value was in the proper range of the $CO_2$ gas diffusion coefficient in the solid, but lower in liquid and in gas ($10^{-5}$- 1 $cm^2/sec$).



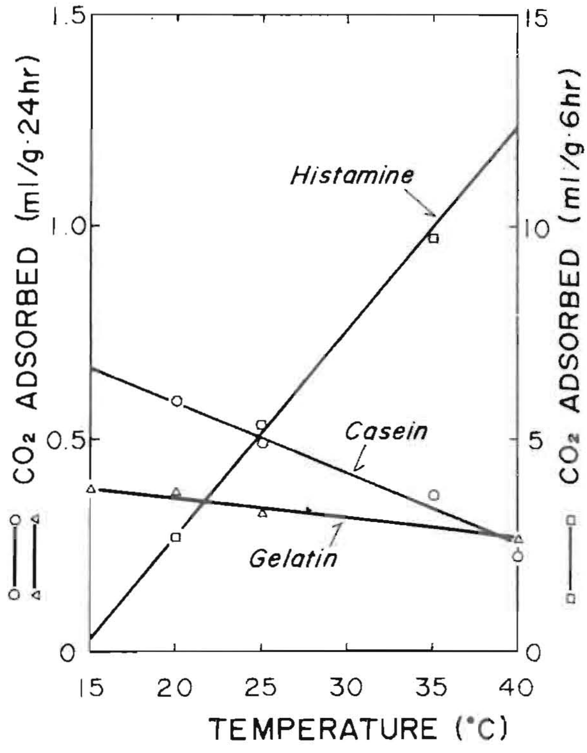




Fig. 6 (left). Effect of temperature on $CO_2$ gas adsorption by zein. The amount of $CO_2$ gas adsorbed was measured with the lapse of time by the B.E.T. surface area analyzer at one-half of atmospheric pressure.

Fig. 7 (right). Temperature dependence of $CO_2$ gas adsorption by casein, gelatin and histamine. The amount of $CO_2$ gas adsorbed was measured in the same way described in Fig. 1.

Adsorption isoterms. Fig. 10 indicates the adsorption isoterms for $N_2$ and $CO_2$ in casein determined by the B.E.T. method. A typical polymolecule adsorption curve (BET type) was obtained with $N_2$ gas adsorption in -196°C. The carbon dioxide gas adsorption curve, however, showed an adsorption curve of the Henry type in which the amount of gas adsorbed was in proportion to the pressure. Because the experimental pressure (76 cmHg) at 25°C is situated in the lower region of the saturated vapor pressure (4,830 cmHg) of $CO_2$ gas, experimental results can also be assumed to be in the early part of BET, Langumuir, Freundlich and other types of isoterms.

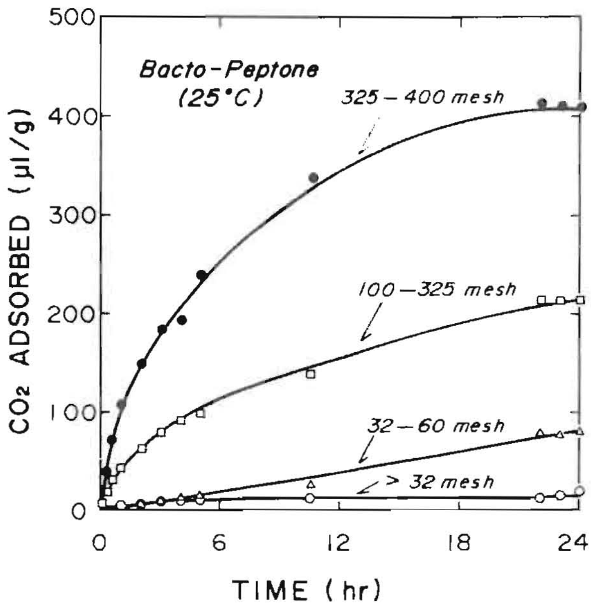



Fig. 8. Effect of particle size on $CO_2$ adsorption by bacto-peptone.

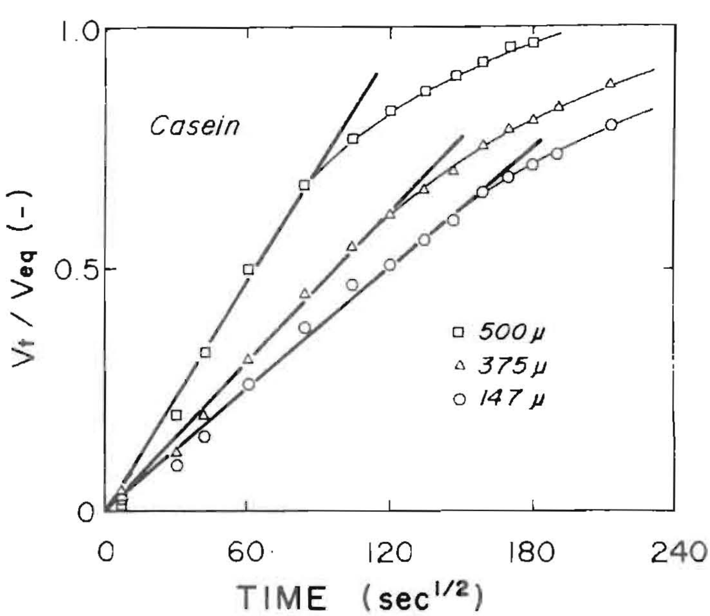



Fig. 9. Effect of particle size on $CO_2$ adsorption by casein.

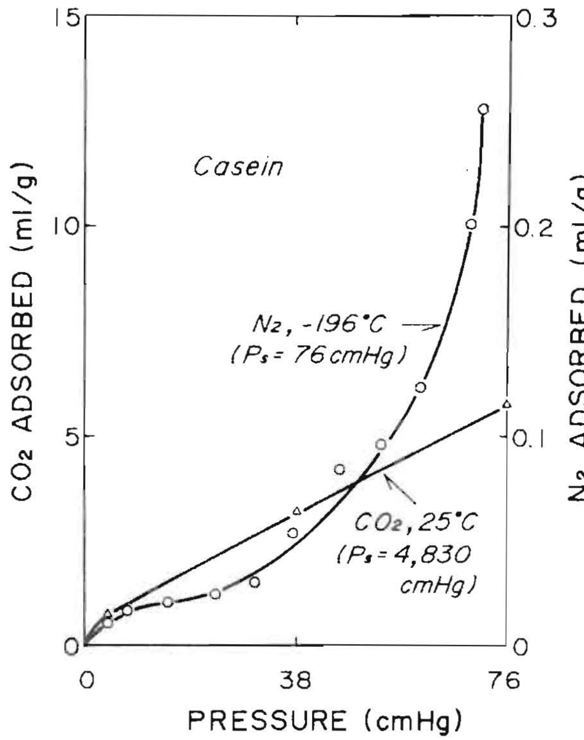



Fig. 10. Adsorption isoterms for $N_2$ and $CO_2$ in casein. The amount of gas adsorbed was measured by the B.E.T. surface analyzer in the condition described in this figure.

Particular functional groups involved in $CO_2$ gas adsorption by protein.

In order to identify the functional groups which really contribute to $CO_2$ gas adsorption by proteins, ε-amino groups of lysine residues of egg albumin were chemically modified with trinitrobenzene sulfonic acid to various degrees. Experimental results showed that about 60% of the total amount of $CO_2$ gas adsorbed by solid egg albumin diminished by completely modification of the ε-amino groups of lysine residues. This directly provides evidence that the ε-amino group of lysine is capable of adsorbing the $CO_2$ gas in protein in the same manner as in free amino acid. As to the remaining 40% of the total amount of $CO_2$ gas adsorbed by egg albumin, the guanidinium group of arginine residues and α-amino group in protein were shown to take part in it by the following experiments. Correlation coefficients found between the amount of $CO_2$ and each of the variables, the lysine content, the arginine content and the total of lysine and arginine contents of protein and its hydrolyzates were 0.76, 0.54 and 0.85 respectively. Chemical modification of the guanidinium groups of arginine residues of lysozyme by 1,2-cyclohexanedione also showed the decrease of the amount of $CO_2$ gas adsorbed by lysozyme. The ability of $CO_2$ gas adsorption by α-amino group of amino acids and oligopeptides was found to be developed by the elongation of the peptide chain of glycine and other amino acids, by the removal of the α-carboxyl group of histidine and tyrosine to corresponding amines and by the esterification of α-carboxyl group of leucine with p-nitrophenol. These results clearly indicate that $CO_2$ binding sites in protein in the gas-solid phase system are ε-amino, α-amino and guanidinium groups. Table 5 gives the amounts of $CO_2$ gas adsorbed by a series of oligopeptides and collated with the $pK_a$ value for their α-amino groups reported in the literature.

TABLE 5.
Effect of peptide chain length on the $CO_2$ gas adsorption by amino group.

| | $pK_a$ values ($\alpha$-$NH_2$) | ($\varepsilon$-$NH_2$) | $CO_2/NH_2$ (mmoles/mole) |
|---|---|---|---|
| Gly | 9.78 | | 0.024 |
| Gly-Gly | 8.25 | | 0.111 |
| Gly-Gly-Gly | 8.09 | | 0.465 |
| Gly-Gly-Gly-Gly | - | | 1.47 |
| Polyglycine | 7.4 | | - |
| Lys | 9.18 | 10.79 | 3.97 |
| Lys-Gly | - | | 4.49 |
| Lys-Glu-Ala | - | | 6.50 |
| γ-Glu-Cys-Gly | 8.75 | | 1.03 |

Differences between the $CO_2$ gas adsorption by proteins and by amino acids and amines.

The reasons why similar functional groups of proteins and amino acids were able to show different reactivity to $CO_2$ gas, such temperature dependence, moisture dependence and reversibility were examined. Differences between the $CO_2$ gas adsorption by proteins and by amino acids and amines can be best explained with the following two different interactions between the amino group and $CO_2$:

1. Carbamate formation.

$$R\text{-}NH_2 + CO_2 \rightleftharpoons R\text{-}NHCOOH$$

2 Anionic reaction.

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \qquad < pH\ 7.0$$

$$OH^- + CO_2 \rightleftharpoons HCO_3^- \qquad > pH\ 7.0$$

$$R\text{-}NH_3^+ + HCO_3^- \rightleftharpoons (R\text{-}NH_3^+)(HCO_3^-)$$

The $pK_a$ values of amino groups in protein are lower than those in amino acids. In the solid state of protein, therefore, amino groups must be present in the unprotonized form ($R-NH_2$) and form carbamate salts with $CO_2$. This interaction is weak and reversible as is well known in the interaction of hemoglobin and $CO_2$ in blood. Characteristics of $CO_2$ gas adsorption by protein are assumed to be caused by the carbamate formation. On the other hand, amino groups in free amino acids and amines seem to be present in the protonized form ($R-NH_3^+$) even in a solid state for their higher $pK_a$ values. This amino group in protonized form may react with the bicarbonate ion which is formed from $CO_2$ and water via carbonic acid. An experiment on the $CO_2$ gas adsorption by the basic amino acids such as lysine and arginine in dried, and moistened form revealed that the amount of $CO_2$ gas adsorbed by these free amino acids increased remarkably with their increased moisture content, while an inverse relationship was observed in protein as shown in Fig. 5.

## CONCLUSION

The $CO_2$ gas adsorption phenomenon observed in grain storage in a $CO_2$ atmosphere is assumed to be caused by a mechanism combined with a diffusion process in the grain kernel and a carbamate formation of $CO_2$ gas with functional groups of protein which are exposed in the internal surface of the grain kernel. The reversible interaction of $CO_2$ gas with functional groups of protein is assumed to contribute to retaining grain qualities during the storage.

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