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# Adsorption of milk proteins onto rice starch granules

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ABSTRACT

The adsorption of sodium caseinate (NaCAS) and whey protein isolate (WPI) to both normal and waxy rice starch granules was investigated using SDS-PAGE. This showed that the proteins present in NaCAS and WPI do adsorb to normal and waxy rice starch granules and that in the case of NaCAS, for both normal and waxy rice starch, $\alpha_s$-casein adsorbed preferentially and in higher amounts than β-casein. In the case of WPI, although the amount of adsorbed β-LG is higher than that of α-LAC, no preferential adsorption is observed. Confocal laser scanning microscopy, with specific dyes, was also used in an attempt to locate the milk proteins adsorbed to the starch granules. The adsorption isotherms of these two milk protein ingredients to rice starch granules, were modelled using the BET equation which is usually used to model multilayer adsorption, and a simple equation is proposed to take into account possible absorption of the proteins into the core of the starch granules. Good agreement is found between these models and the experimental results. Possible mechanisms involved in the interaction between milk proteins and starch granules are discussed.



## 1. Introduction

Milk proteins and starch co-exist in several food formulations such as yoghurts, processed cheese and custards. Despite their industrial importance, only a few fundamental studies have been dedicated to the investigation of the interactions between these two food ingredients. Recently, it was shown that the addition of sodium caseinate (NaCAS) and whey protein isolate (WPI) into waxy and normal rice starch dispersions did affect their pasting behaviour; that is their viscosity when the temperature was increased gradually from ambient temperature to 95 °C (Noisuwan, Bronlund, Wilkinson, & Hemar, 2008). In fact, it was found that the addition of very small amounts (0.1 wt%) of NaCAS did result in the shift of the temperature at which the viscosity reaches a maximum to higher temperature values. This led to the suggestion, in view of the small amount of the protein involved, that interaction between the milk proteins and the starch granule could occur through hydrophobic interactions leading to the adsorption of the proteins to the starch granule interface.

NaCAS and WPI are known to be very good emulsifiers, and their adsorption to oil–water, air–water and solid–water interfaces are well documented. In brief, milk proteins are large complex amphipathic macromolecules having a combination of ionic, polar and non-polar regions (Dickinson, 1999). Although, several studies have shown that other proteins do adsorb onto starch granules (Dahle, 1971; Dahle et al., 1975; Eliasson and Tjerneld, 1990; Lundh et al., 1988; Ryan & Brewer, 2005a, 2005b; Wannerberger, Wahlgren, & Eliasson, 1996) that this adsorption reduces the ability of the starch granules to absorb water and swell, to the best of our knowledge, the adsorption of milk proteins to starch granules was not previously reported. However, the adsorption of BSA, low molecular weight wheat protein fraction (WP1), and high molecular weight wheat protein fraction (WP2) onto wheat, maize and potato starch granules was previously studied, and the adsorption behaviour was found to be low for BSA and WP1 but higher for WP2, and that the adsorption behaviour depended also on the type of starch (Eliasson & Tjerneld, 1990). In fact, removal of the native wheat starch granule surface protein and resulted in a decrease in the binding of added proteins, suggesting that native granule proteins might mediate the binding of exogenous protein (Ryan & Brewer, 2006).

Despite the recent increase in awareness of the interactions between exogenous proteins and starch granules (Ryan & Brewer, 2005a, 2005b), the underlying mechanism of these interactions is still not fully understood. The objective of this paper was to establish whether caseins and whey proteins, the major proteins in milk, do adsorb to the surfaces of rice starch granules and to identify the mechanisms of the interactions involved. To do so, we quantify the amount of the adsorbed proteins by adapting an SDS-PAGE method, which was proven to quantify accurately the adsorption

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**Table 1**
Chemical composition, including carbohydrates (Carb) and lactose (Lact) of dairy ingredients and rice starches. NaCAS, sodium caseinate; WPI, whey protein isolate.

| | Lact. | Carb. | Protein | Fat | Ash | Moisture |
|---|---|---|---|---|---|---|
| NaCAS | 0.1 | – | 93.0 | 0.7 | 3.6 | 2.6 |
| WPI | 0.6 | – | 93.2 | 0.3 | 2.1 | 3.8 |
| Normal rice starch | – | 87.60 | 0.42 | 0.58 | 0.17 | 11.20 |
| Waxy rice starch | – | 87.92 | 0.30 | 0.11 | 0.17 | 11.50 |

of milk protein in emulsions. In an attempt to confirm the presence of these milk proteins at the surface of the starch granule, confocal laser scanning microscopy with specific protein dyes was implemented.

## 2. Materials and methods

### 2.1. Materials

Normal and waxy rice starch were generously donated by National Starch and Chemical Co., Bangkok, Thailand. Sodium caseinate (NACAS) and whey protein isolate (WPI) were obtained from Fonterra Co-operative Group Ltd, New Zealand. The chemical compositions of the starch and milk protein ingredients are reported in Table 1.

All chemicals used were of analytical grade and were purchased from either Sigma Chemical Co. (St Louis, MO, USA) or BDH Chemicals (BDH Ltd, Poole, England).

### 2.2. Sample preparation

A 10% (w/w) stock solution of NaCAS or WPI was made by mixing the protein powder in MilliQ water using a magnetic stirrer at room temperature for at least 2 h. This stock solution was kept overnight at 4 °C to ensure complete hydration. The stock solution was centrifuged at 2000 × *g* for 10 min to remove any undissolved protein aggregates, and different protein solutions of different concentrations were made by mixing the appropriate amount of the stock solution and MilliQ water. The milk protein/rice starch mixtures were made by mixing, at room temperature, 3 g of rice starch and 30 g of the milk protein solutions with a magnetic stirrer.

### 2.3. Determination of starch granule size, density and specific area

A Malvern MasterSizer 2000 (Malvern Instruments Ltd, Malvern, UK) was used to determine the average particle size of the starch granules using the general purpose (spherical) analysis mode. Because the starch granules are relatively large, and not spherical, starch granule particle size was obtained using the Fraunhofer approximation of light scattering theory. Result showed that both normal and waxy starch granules could be represented by a bi-modal size distribution, with the first distribution ranging from 0.5 to 2 μm and the second distribution ranging from 2 to 15 μm approximately (Fig. 1). The average Sauter-mean radius $R_{32}$ calculated from the particle size distributions was found to be equal to 5.60 and 5.79 μm, for normal and waxy rice starch granules respectively. Note that bi-modal size distributions have been previously reported for rice starch granule dispersions (Cardoso, Samios, & Silveira, 2006; Chen, Lii, & Lu, 2004; Zhong et al., 2009) including for the same rice starch granules that were used in this study (Zuo, Knoerzer, Mawson, Kentish, & Ashokkumar, 2009).

The absolute density ($\rho$) of rice starch was determined using a modification of the xylene displacement method (Schoch & Leach, 1964). The measurements yielded values of 1.5151 g/cm$^3$ and 1.5023 g/cm$^3$ for normal and waxy rice starch respectively,

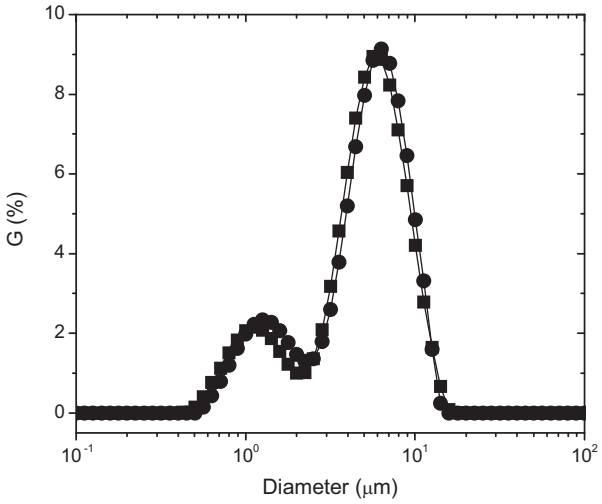


**Fig. 1.** Particle size distribution of normal (■) and waxy (●) rice starch granules.

which are in very good agreement with that previously reported in literature (Juliano, 1984). Using the values of the density and those of $R_{32}$ a specific surface area of 0.484 m$^2$/g for normal rice starch and 0.491 m$^2$/g waxy rice starches are obtained.

### 2.4. Determination of the amount of milk protein adsorbed onto the rice starch granules

The method developed by Hunt and Dalgleish (1994) was adapted to determine the amount of protein and the proportions of the individual proteins that were adsorbed onto the rice starch granules. The proportions of the individual proteins in milk protein ingredients were also determined by this method, which is based on the quantification of the milk proteins by sodium dodecyl sulphate polyacrylamide gels electrophoresis (SDS-PAGE).

The milk protein/starch mixture sample for SDS-PAGE experiment were prepared as follow: approximately 30 g of the rice starch/milk protein ingredients mixtures were weighed (to 4 decimal places) directly into 50 ml polypropylene centrifuge tubes (catalogue number 430829, Corning Co., Corning, New York, USA) and centrifuged at 1000 × *g* for 10 min in a Heraeus Megafuge 1.0 (Heraeus, Hanau, Germany). The supernatant was removed and the pellet was resuspended in MilliQ water and centrifuged to remove any non-adsorbed milk proteins. The procedure was repeated six times. Then the final pellet was resuspended in enough MilliQ water to produce a 40% (w/w) starch slurry. The adsorbed milk proteins were desorbed from the rice starch granule with a 2% SDS in buffer (0.5 M Tris, 0.009% bromophenol blue, pH 6.8). The 40% starch slurry was weighed out (~0.6 g) into 1.5 ml Eppendorf vials, and 500 mg of SDS sample buffer was added. The vial was mildly shaken using a Vortex shaker for 30 mins, then centrifuged at 14,000 rpm for 5 min at 20 °C using an Eppendorf centrifuge (5417R; Eppendorf AG, Hamburg, Germany). A 20 μl aliquot of the supernatant was loaded onto the sodium dodecyl sulphate-polyacrylamide electrophoresis gel (SDS-PAGE). All the SDS-PAGE measurements were performed at least in duplicate.

Quantification of the protein was performed by scanning the SDS-PAGE gels on a Molecular Dynamics Scanner (Molecular Dynamics, Sunnyvale, CA) and by determining the intensity volume of proteins in each band of the samples using ImageQuant 5.0 software. Standards were prepared and loaded in exactly the same way as the samples. In each SDS-PAGE gel, the standard protein solution was run in conjunction with samples to indicate the position of known proteins on the gel. An example of a scanned SDS-PAGE gel, obtained for normal rice–NaCAS mixtures is shown

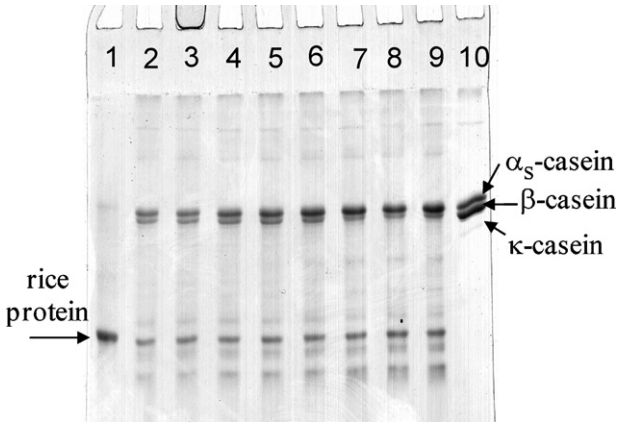


**Fig. 2.** SDS-PAGE patterns of adsorbed α-casein and β-casein obtained from normal rice as function of NaCAS concentration. Lane 1: starch in MilliQ water, lane 2: starch in 0.25% NaCAS, lane 3: starch in 0.50% NaCAS, lane 4: starch in 0.75% NaCAS, lane 5: starch in 1% NaCAS, lane 6: starch in 2.5% NaCAS, lane 7: starch in 5% NaCAS, lane 8: starch in 7.5% NaCAS, lane 9: starch in 10% NaCAS, lane 10: 0.0075% NaCAS.

in Fig. 2. The amount of protein in each band for each milk protein ingredient was determined from the respective protein concentration/volume band densitometry standard curve. All the standard curves were linear in the dilution range used for the experiments.

### 2.5. Confocal laser scanning microscopy (CSLM)

To ensure that only the milk protein was stained, the Alexa Fluor 488 protein labelling Kit A-10235 (Molecular Probes, Eugene, OR, USA) was used. This reactive dye (Alexa Fluor 488 carboxylic acid, succinimidyl ester, dilithium salt, $M_W$ ~643) has a succunimidyl ester moiety, which can react efficiently with primary amines in the proteins to form stable and highly fluorescent dye-protein conjugates. The milk proteins (2 mg/ml) were mixed with the dye and stirred for 1 h, then eluted through a Bio-rad BioGel P-30 fine size exclusion resin column. This allows the separation of the proteins from the excess dye. Starch granule dispersions were added to the stained protein solutions to give a concentration of 1% starch granule in the stained protein solution. The starch granules and stained protein mixtures were then gently mixed with the starch granules for 30 min using a magnetic stirrer, and then left overnight at 4 °C. Note that the final concentration of the milk protein to rice granules is 0.2 g/g. The next day the mixtures were centrifuged at 1000 × *g* for 10 min and the supernatant discarded, in order to remove any excess of the labelled proteins or dye from the mixture. The pellet was rewashed by resuspending in Milli-Q water and further centrifuged at 1000 × *g* for 10 min to remove any non-adsorbed labelled proteins or unincorporated dye. This washing procedure was repeated 3 times. An aliquot of the sample was transferred into a glass slide with a cavity and a coverslip was placed on it and sealed with nail polish to prevent water evaporation. The CSLM observations were carried out on a Leica TCS 4D confocal microscope (Leica Lasertechnik GnbH, Heidelberg, Germany) with a 100 mm oil immersion objective lens equipped with an air-cooled Ar/Kr laser, for which the absorption and fluorescence emission maxima were 494 and 519 nm, respectively.

## 3. Results

Because NaCAS and WPI are known to behave differently at interfaces, the results of their adsorption measurements to starch granules are reported separately. Comparison of their adsorption into starch granule behaviour will be reported in Section 4.

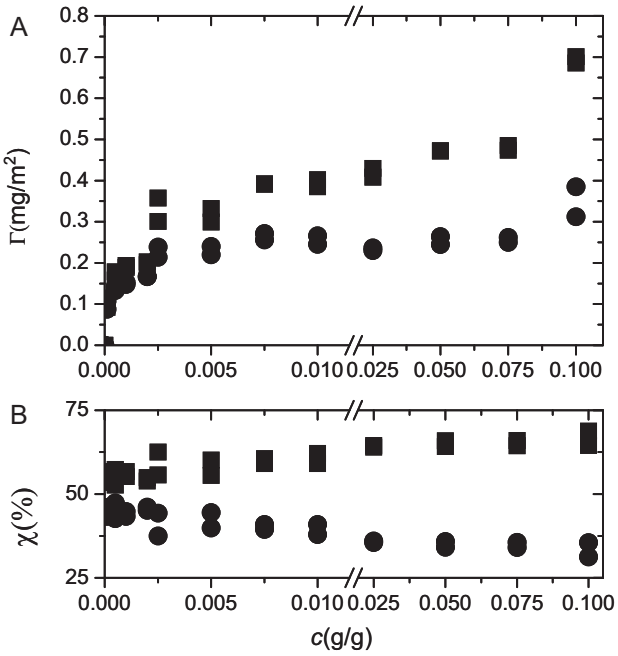


**Fig. 3.** Adsorption isotherms of NaCAS on normal rice starch granules ($\Gamma$) and the proportion of the individual proteins ($\chi$) as a function of added protein concentration *c*. NaCAS proteins are: $\alpha_s$-casein (■) and β-casein (●).

### 3.1. Adsorption of NaCAS to rice starch granules

SDS-PAGE measurements were carried out in order to establish whether milk proteins are adsorbed to rice starch granules. The amount of protein present in the mixture of 10% rice starch containing milk protein, after six washings, was measured for different mixing times ranging from 15 min to 24 h. It was found that the measured amount reached a constant value after 30 min (result not shown). Thus, all the SDS-PAGE measurements were performed on starch–milk protein mixtures, mixed at room temperature for 1 h. Fig. 3A shows the amount (mg) of $\alpha_s$-casein and β-casein present in the NaCAS/normal rice starch mixture as a function of the initial amount of NaCAS added. The proportion of these two proteins as a function of the added NaCAS to the 10 wt% normal starch dispersion is reported in Fig. 3B. κ-Casein, which is also present in very small amounts in NaCAS, was not detected by the SDS-PAGE method. At low NaCAS concentrations, the amount of adsorbed $\alpha_s$-casein gradually increased with the increase in NaCAS to reach a plateau value of 0.35 mg/m$^2$ at 0.25% added NaCAS. At concentrations greater than 7.5% NaCAS, the amount of adsorbed protein suddenly increased again to reach a value of 0.7 mg/m$^2$ at the maximum (10 wt%) NaCAS concentration used. A similar behaviour is observed for β-casein, where the amount of adsorbed protein increased to reach a plateau value of 0.25 mg/m$^2$ at 0.25% NaCAS. At NaCAS concentrations higher than 5%, the amount of adsorbed β-casein also increased to reach a maximum value of 0.3 mg/m$^2$ at 10 wt% added NaCAS.

The amounts and proportions of $\alpha_s$-casein and β-casein adsorbed onto waxy rice starch granules are reported in Fig. 4A and B respectively. The adsorption behaviour is similar to that observed in the case of normal rice starch, although the amounts of $\alpha_s$-casein and β-casein measured were smaller in the case of waxy rice starch. The amount of adsorbed $\alpha_s$-casein reached a plateau value of 0.3 mg/m$^2$ at 0.2% added NaCAS, then increased at concentrations higher than 2.5% to reach a maximum value of 0.4 mg/m$^2$ when 10 wt% NaCAS is added to the waxy rice starch dispersion (Fig. 4A). The amount of adsorbed β-casein also reached a plateau

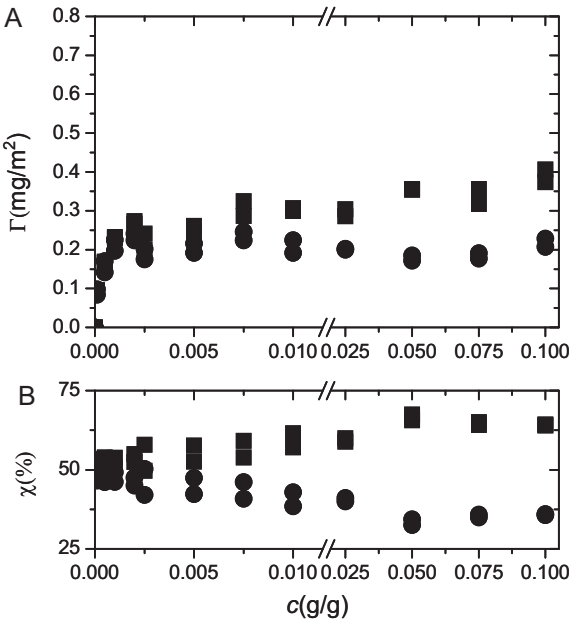


**Fig. 4.** Adsorption isotherms of NaCAS on waxy rice starch granules ($\Gamma$) and the proportion of the individual proteins ($\chi$) as a function of added protein concentration *c*. NaCAS proteins are: $\alpha_s$-casein (■) and β-casein (●).

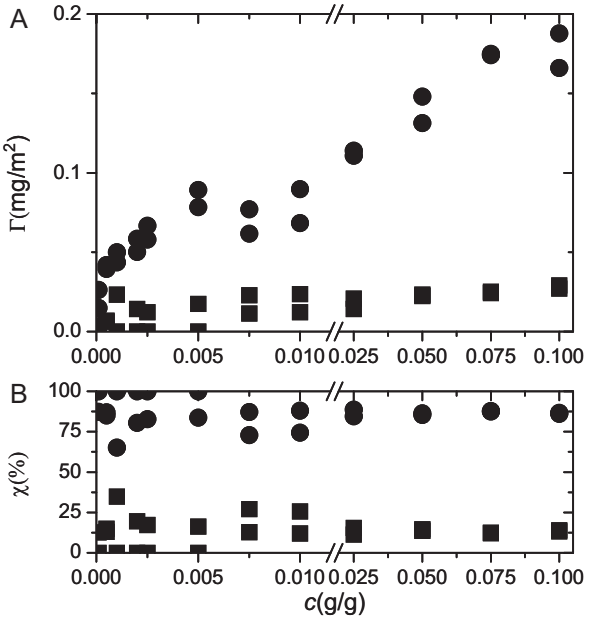


**Fig. 5.** Adsorption isotherms of WPI on normal rice starch granules ($\Gamma$) and the proportion of the individual proteins ($\chi$) as a function of added protein concentration *c*. WPI proteins are: α-LAC (■) and β-LG (●).

value of 0.2 mg/m$^2$ at 0.2% added NaCAS concentration. The amount of $\alpha_s$-casein that was adsorbed onto both normal and waxy rice starch granules was higher than the amount of β-casein for all concentrations of NaCAS, and the difference in the amounts adsorbed of the two proteins increased with the increase in NaCAS concentration (Figs. 3B and 4B). This indicates a preferential adsorption of $\alpha_s$-casein to β-casein to the rice starch granules.

### 3.2. Sorption of WPI to rice starch granules

The amounts of adsorbed α-lactalbumin (α-LAC) and β-lactoglobulin (β-LG) onto normal starch granules and waxy rice starch granules, when WPI is added, are shown in Figs. 5A and 6A respectively. The amount of adsorbed β-LG and α-LAC gradually increased with increases in WPI concentration. A pseudo-plateau is observed when WPI was added in the range of 0.25–2.5%, with a value of 0.1 and 0.02 mg/m$^2$ for β-LG, and α-LAC, respectively. The amount of adsorbed β-LG, and α-LAC increased when the amount of added WPI is increased from 5% to 10%, to reach a maximum value of 0.2 mg/m$^2$ and 0.03 mg/m$^2$ for β-LG and α-LAC at 10% added WPI, respectively (Fig. 5A). Similarly to normal rice starch, when WPI is added to waxy rice starch, the amount of adsorbed protein also increased with added WPI concentration (Fig. 6A). A pseudo-plateau is also observed, in the range of 0.25–2.5% added WPI, with a value of 0.1 and 0.02 mg/m$^2$ for β-LG, and α-LAC, respectively, as observed in the case of normal rice starch. A second increase in the amount of adsorbed β-LG, and α-LAC is observed when the amount of added WPI is increased from 5 to 10%, where a maximum of 0.15 and 0.05 mg/m$^2$ for β-LG, and α-LAC respectively at 10% added WPI.

The proportions of β-LG and α-LAC that were adsorbed from WPI onto normal rice starch granules and waxy rice starch granules are shown in Figs. 5B and 6B respectively. For both types of rice starch granules, it was found that the ratio of adsorbed α-LAC to β-LG was not affected by the added WPI concentration, indicating that these two proteins showed no preferential adsorption to rice starch granules.

### 3.3. Confocal microscopy of protein/rice starch granule mixtures

CSLM images for normal and waxy rice starch granules after 24 h incubation with the incorporated Alexa Fluor™ 488 fluorescent dye are shown in Fig. 7A1 and B1, respectively. Although the majority of starch granules are mostly seen as dark particles, the core of some starch granules in both normal and waxy starches show a fluorescence background similar to that observed in the continuous phase made of water containing the fluorescent dye. This indicates that the dye can diffuse into the starch granules. The micrographs show also that, as indicated previously, the starch granules are not spherical but have a polyhedral morphology.

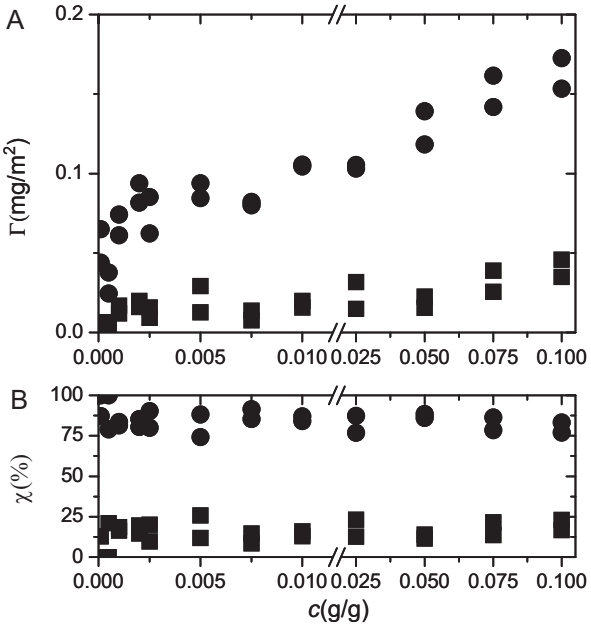


**Fig. 6.** Adsorption isotherms of WPI on waxy rice starch granules ($\Gamma$) and the proportion of the individual proteins ($\chi$) as a function of added protein concentration *c*. WPI proteins are: α-LAC (■) and β-LG (●).

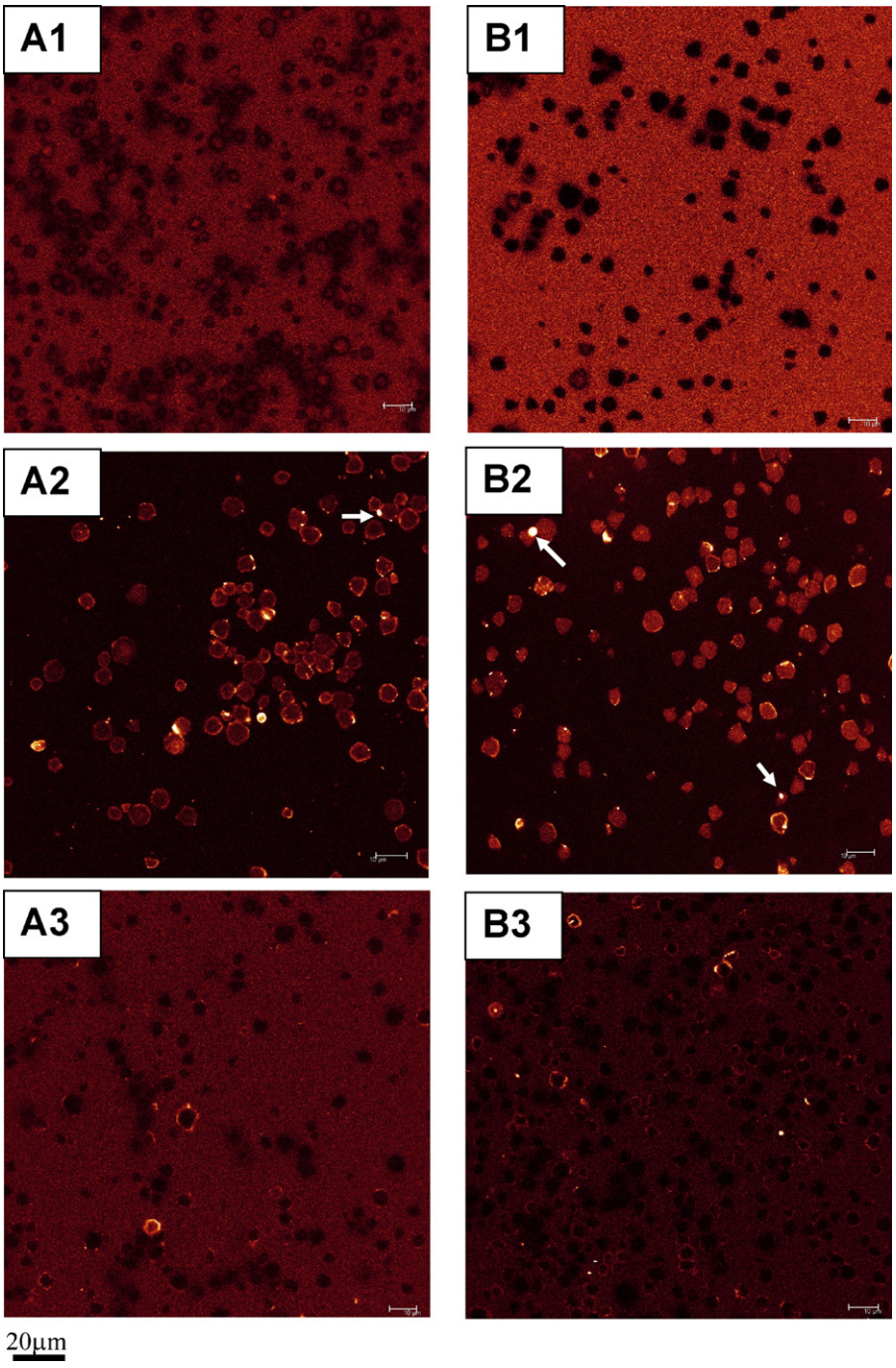


**Fig. 7.** Confocal micrographs of normal rice starch (A1, A2, and A3) and waxy rice starch (B1, B2, and B3) after incubation for 24 h at 4 °C with; incorporation of Alexa Fluor™ 488 dye (A1 and B1), labelled proteins from 0.01% NaCAS (A2 or B2), and labelled proteins from 0.01% WPI (A3 or B3), scale bar represents 10 μm. Arrows in A2 and B2 indicate intensely fluorescent particles.

The CSLM images of rice starch granules, after 24-h incubation with the labeled NaCAS proteins for normal and waxy rice starch are reported in Fig. 7A2 and B2. In the case of both normal and waxy rice starches, few individual granules showed brightly fluorescent regions on the granule surface or a ring around the granule's periphery. A similar behaviour could be observed in the case of labelled WPI proteins (Fig. 7A3 and B3), where some starch granules appear to be covered by a fluorescent layer. Note that the confocal micrographs also show intensely fluorescent particles (indicated by arrows in Fig. 7A2 and B2). Although, it is difficult to identify clearly these particles as they could be small starch granules or the result of the optical section intersecting only a small part of the starch granule. Alternatively, it cannot be overruled that these particles are made of milk protein; particularly NaCAS particles are known to self associate through hydrophobic interactions. However, the NaCAS aggregates have been reported to be smaller than 100 nm (Chu, Zhou, Wu, & Farrell, 1995; Nash, Pinder, Hemar, & Singh, 2002) while the aggregates seen in Fig. 7A2 and B2 are in the μm range. In addition, the protein solutions were of low concentration (2 mg/g) and were eluted through a size exclusion column during their staining, and the protein–starch mixtures being three times washed from any excess protein. Thus, it is expected that the successive washing would result in the removal of the non-adsorbed NaCAS aggregates from the continuous phase.

### 3.4. Modelling of the adsorption isotherms

At higher added concentration, the adsorption isotherms, for both starches and the two milk protein ingredients, showed clearly that there is a second increase in the amount of adsorbed proteins after the plateau is reached. This behaviour could be the result of the protein adsorbing at the granule interface as a multi-layer, when the amount of added proteins is in excess. Usually, similar adsorption isotherms are modelled using the Brunauer, Emmett, and Teller (BET) equation where the adsorbed surface protein concentration $\Gamma$ as a function of the protein concentration $c$ is given by (Brunauer, Emmett, & Teller, 1938):

$$\Gamma = \frac{A_{BS}\Gamma_{\max}c}{\left[(c_S - c)(1 + (A_{BS} - 1)(c/c_S)\right]} \quad (1)$$

where $A_{BS}$ is the affinity of the adsorbed molecules and is related to the enthalpy of adsorption, $\Gamma_{\max}$ is the monolayer coverage value of surface protein concentration and $c_s$ is the solute concentration in the subsurface area.

Another plausible explanation to the increase in the amount of adsorbed proteins is their diffusion into the starch granules. Starch granules are reported to present pits, channels and holes at the hilum of starch granules (Baldwin, Adler, Davies, & Melia, 1994). It was suggested that these pores and channels are large enough for water, reagents or enzymes to diffuse into the starch granule (Baldwin et al., 1994; Fannon, Hauber, & Bemiller, 1992). This is shown in the confocal micrographs (Fig. 7) where the core of some starch granules appears fluorescent (Fig. 7). In the case, where absorption to these cavities does occur, we propose to model the isotherm using the following simple equation:

$$\Gamma = \frac{A_{BS}\Gamma_{\max}c}{(1 + A_{BS}c)} + A_{DS}c \quad (2)$$

This equation is simply the addition of the absorption behaviour to the Langmuir adsorption behaviour. The absorption behaviour stipulates here that the amount of protein that diffuses freely into the starch granule is proportional to the added concentration through the constant $A_{DS}$. The constants in the Langmuir equation have the same definition as in Eq. (1).

Eqs. (1) and (2) are used to model the adsorption isotherms of NaCAS and WPI to normal and waxy rice starches and are reported in Fig. 8. Note that the adsorption isotherms reported in Fig. 8 represent the total amount of the protein adsorbed, calculated by summing the amounts of the individual proteins, $\alpha_s$-casein and β-casein for NaCAS, and α-LAC and β-LG for WPI. Using OriginPro8 software, fits to Eq. (1) (solid line) and to Eq. (2) (dashed lines) were carried out, and the results are reported in Fig. 8. NaCAS is known to form casein aggregates at high NaCAS concentration (Chu et al., 1995; Creamer & Berry, 1975), thus the high value obtained at 10% NaCAS addition (Fig. 8A) could be due to its adsorption onto starch granules in its aggregated form. To take into account this peculiar adsorption pattern, Eqs. (1) and (2) were also used to fit the same data in Fig. 8A with the last data points corresponding to 10% NaCAS addition excluded (dotted line in Fig. 8A). Values of the different parameters resulting for these different fits are reported in brackets in Table 2.

Qualitatively, it can be clearly seen in Fig. 8 that both Eqs. (1) and (2) yield very similar results. A slight discrepancy could be seen at higher added proteins, where the fit obtained by Eq. (2) is linear, while the fit obtained by Eq. (1) is curved upward. In fact this is expected, and could be simply demonstrated by subtracting the Langmuir term from each equation and performing a Taylor series development to the second order. This results for small added protein concentrations, in $\Gamma \sim c$ for Eq. (1) and $\Gamma \sim c^2$ for Eq. (2) respectively.

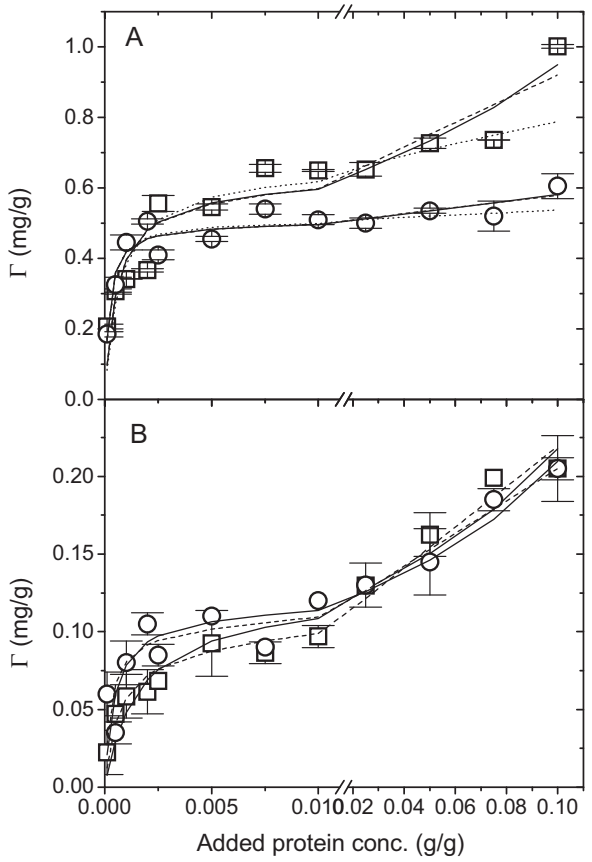


**Fig. 8.** Absorption isotherms of NaCAS (■) and WPI (●) to normal (A) and waxy (B) rice starches as a function of the concentration of added protein $c$. Lines are fits obtained using Eq. (1) – BET equation (solid line) and Eq. (2) – modified Langmuir equation (dashed line). Dotted line in A is a fit obtained using Eq. (2) to data with the highest NaCAS concentration (10%) omitted. Error bars are standard deviations.

## 4. Discussion

The measured adsorption isotherms, using SDS-PAGE, demonstrated clearly that there is adsorption of both NaCAS and WPI to both normal and waxy rice starches. However, the fitting exercise could not establish whether the different milk proteins were adsorbed as a multilayer to the granules or alternatively if the proteins were absorbed into the starch granule. Confocal microscopy observations could not establish clearly whether the respective constituent proteins of NaCAS or WPI had been just adsorbed at the surface of the granules or whether there was both adsorption and absorption by the two types of starch granules (Fig. 7). As mentioned in the introduction, to the best of our knowledge there are no published reports on the interactions between caseins or whey proteins with starch granules, thus the results obtained in this work will be discussed in the light of the adsorption of these milk proteins onto oil–water interfaces or solid interfaces, which are well documented.

In the case of the adsorption of NaCAS to normal and waxy rice starch granules respectively, the value for full monolayer coverage $\Gamma_{max}$ obtained was ~0.6 mg/m$^2$ and ~0.5 mg/m$^2$, and ~0.1 for the adsorption of WPI for both normal and waxy rice starch granules (Table 2). The low amount found in the case of the adsorption of WPI compared to NaCAS is due to the highly flexible structure of extended casein molecules, compared to the more rigid structure of globular whey proteins, as was previously suggested in the case of the adsorption of these proteins in oil–water emulsions (Euston & Hirst, 1999). In the case, of NaCAS the caseins exist as free casein molecules and self-assembled protein particles; both casein com-

**Table 2**
Constant values obtained from the fit to the adsorption isotherms using the BET equation and the modified Langmuir equation. Values in brackets are obtained from the fit to the isotherms of NaCAS–starch mixtures where the highest (10%) NaCAS concentration has been omitted.

| BET | Normal rice starch | | Waxy rice starch | |
|---|---|---|---|---|
| | NACAS | WPI | NACAS | WPI |
| BET | | | | |
| $A_{dS}$ | 512.09 ± 161.20 (770.97 ± 496.44) | 146.128 ± 39.50 | 3577.80 ± 1366.63 (8367.19 ± 13640.21) | 487.31 ± 206.34 |
| $\Gamma_{max}$ | 0.60 ± 0.04 (0.65 ± 0.05) | 0.12 ± 0.01 | 0.50 ± 0.02 (0.51 ± 0.28) | 0.11 ± 0.01 |
| $c_s$ | 0.27 ± 0.05 (0.54 ± 0.43) | 0.22 ± 0.03 | 0.68 ± 0.28 (1.72 ± 2.98) | 0.22 ± 0.031 |
| Modified Langmuir | | | | |
| $A_{dS}$ | 1978.49 ± 864.85 (1470.82 ± 533.02) | 1561.26 ± 0.02 | 5231.72 ± 1431.82 (4868.52 ± 1285.50) | 3647.70 ± 2135.22 |
| $\Gamma_{max}$ | 0.592 ± 0.05 (0.643 ± 0.06) | 0.092 ± 0.01 | 0.50 ± 0.02 (0.50625 ± 0.02052) | 0.10 ± 0.01 |
| $A_{bs}$ | 3.30459 ± 0.89751 (1.49097 ± 1.19754) | 1.28177 ± 0.11648 | 0.82 ± 0.39 (0.32 ± 0.54) | 1.04 ± 0.18 |

plexes and aggregates (Creamer & Berry, 1975; Srinivasan, Singh, & Munro, 1999). The main caseins that adsorbed onto normal and waxy rice starches were $\alpha_s$-casein and β-casein, with $\alpha_s$-casein being preferentially adsorbed compared to β-casein (Figs. 3B and 4B). This result is in agreement with the results previously reported on the adsorption of these proteins to oil-water emulsions (Euston, Singh, Munro, & Dalgleish, 1995; Euston & Hirst, 1999). The ratio of adsorbed $\alpha_s$-casein to β-casein also slightly increased with increasing NaCAS concentration. Finally in the case of WPI, for both normal and waxy rice starches, the adsorbed amount of β-LG and α-LAC from WPI continuously increased with increases in WPI concentration. This continuous increase could be due to the ability of β-LG to adsorb as a multilayer to both hydrophobic and hydrophilic surfaces (Addesso & Lund, 1997; Elofsson, Paulsson, & Arnebrant, 1997; Hunt & Dalgleish, 1994).

Despite this qualitative agreement between the adsorption behaviour of milk protein at oil–water interface and their adsorption behaviour at the starch granule–water interface, the measured amounts of adsorbed proteins to oil–water interfaces are much higher than that measured in the case of normal and waxy rice starch. For instance, Hunt and Dalgleish (1994) reported values for the surface coverage values of about 3.20 mg/m$^2$ for both NaCAS and WPI, while values <1 mg/m$^2$ and <0.15 mg/m$^2$ were measured for NaCAS and WPI respectively on rice granules. These low values for the surface coverage are not surprising as starch granules are considered to be hydrophilic (Seguchi, 1986). In fact milk proteins have been found to adsorb to hydrophilic solid surfaces in smaller amounts compared to hydrophobic surfaces. For example β-LG is reported to be adsorbed between 0.1 to 0.4 mg/m$^2$ at hydrophilic surfaces and between 1.0 and 2.0 mg/m$^2$ at hydrophobic surfaces (Marsh, Jones, & Sferrazza, 2002).

Section 4 above, naively considers starch granules as having homogeneous surface active properties. In reality the surface of the starch granule is very complex, as it is known to contain proteins and lipids (see Table 1) and a proportion of these components are present at the starch granule surface. It is thus plausible that the adsorption of the milk proteins onto starch granules is mediated by these indigenous lipids and proteins as suggested by several authors (Barlow, Buttrose, Simmonds, & Vesk, 1973; Eliasson, Carlson, & Larsson, 1981; Seguchi, 2001). Since the amount of protein and fat is higher for normal rice starch than waxy rice starch (Table 1), this could explain the higher amount of protein adsorbed to normal rice starch when compared to that adsorbed to waxy rice starch. To investigate the influence of the starch surface lipids and proteins, we first used 2% sodium dodecyl sulfate (SDS) to displace these proteins and wash the starch granules as suggested by Debet and Gidley (2006). To these washed starch granules, milk proteins were added. Both CSLM observation and SDS-PAGE analysis showed that milk proteins were adsorbed to the starch granules. An example of the electrophoresis patterns of adsorbed $\alpha_s$-casein and β-casein from NaCAS or α-LAC and β-LG from WPI obtained from waxy rice starch granules with their native proteins and fat

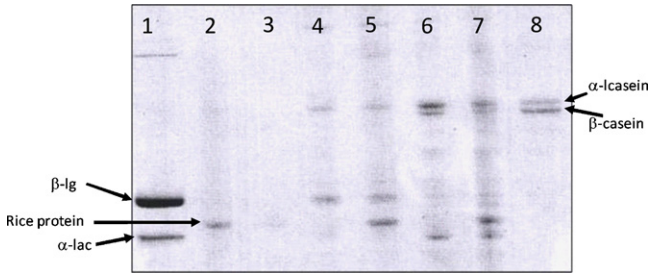


**Fig. 9.** Electrophoresis patterns of adsorbed $\alpha_s$-casein and β-casein from NaCAS or α-LAC and β-LG from WPI obtained from waxy rice with and without their native proteins and fat. Standard WPI (Lane 1); waxy rice starch without adsorbed milk proteins (Lane 2), SDS-washed waxy rice starch (Lane 3), SDS-washed rice starch with adsorbed whey protein (Lane 4), waxy rice starch with adsorbed whey protein (Lane 5), SDS-washed rice starch with adsorbed NaCAS (Lane 6), waxy rice starch with adsorbed NaCAS (Lane 7) and standard NaCAS (Lane 8).

either present or removed by SDS washing are shown in Fig. 9. For waxy rice starches without adsorbed milk proteins there was a surface protein (band in Lane 2). This band almost disappeared (Lane 3) when the starch was treated with SDS to remove the surface proteins and fat, indicating that most of the surface protein was removed by the SDS extraction. The addition of WPI proteins (Lanes 4 and 5) and NaCAS (Lanes 6 and 7) resulted in the adsorption of the milk proteins to the surfaces of the waxy rice granules that either had (Lanes 4 and 6) or were free of their native proteins and fat (Lanes 5 and 7). The same electrophoresis pattern was obtained in the case of normal rice starch granules (result not shown). Therefore, it is uncertain to what extent surface protein and fat mediate the binding of the added milk proteins.

Finally, although further investigations are required to fully elucidate the mechanisms by which milk proteins interact with starch granules, the findings of this study could at least explain indirectly why the addition of milk proteins in small amounts can affect drastically the pasting (gelatinisation) behaviour of rice starch granule dispersions. In other words, the presence of the milk proteins either at the interface of the granule or in the voids (pits, channels and holes) which are present at the hilum of starch granules would restrict the diffusion of water into the starch granule during pasting.

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