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# Macromolecular Interactions During Gelatinisation and Retrogradation in Starch-Whey Systems as Studied by Rapid Visco-Analyser

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## Abstract

Gelatinisation and retrogradation of starch-whey mixtures were studied in water (pH 7) using the Rapid Visco-Analyser (RVA). The starch:whey ratios ranged from 0:100 – 100:0. Wheat starch, and whey protein concentrate (about 80% solids basis) and isolate (about 96% solids basis) were used. Mixtures with whey isolates were generally more viscous than those with whey concentrates, and this was attributed to fewer non-protein milk components in the former. Whey protein concentrates and isolates reduced the peak, trough and final viscosities of the mixtures, but the breakdown and setback ratios of the mixtures were increased. The gelatinisation temperature increased with whey substitutions indicating that whey protein delayed starch gelatinisation. The temperature of fastest viscosity development decreased as the amount of whey was increased. Whey protein isolate generally exercised a lesser effect than the concentrate. At between 40 - 50% whey substitutions, the dominant phase changed from starch to protein irrespective of the source of the whey protein. An additive law poorly defined selected RVA parameters. Both macromolecules interacted to define the viscosity of the mixture, and an exponential model predicted the viscosity better than the additive law. The results obtained in this study are discussed to assist the understanding of extrusion processing of starch-whey systems as models for whey-fortified snack and ready-to-eat foods.

**KEYWORDS:** high-protein low-carbohydrate foods, RVA, snack foods, ready-to-eat (RTE)

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## 1.0 INTRODUCTION

Starch is a base material of many products, and its rheology is very important in its processing. Starch rheological properties depend on concentration, type of starch [regular, root, waxy, and modified], test conditions [rate of heating and cooling, temperature, shear, time, and sample history], and additives [sugars, lipids, protein, emulsifiers, gums, salts, and pH modifiers] (Abu-Jdayil *et al.*, 2004; Chang *et al.*, 2004; Chen and Ramaswamy, 1999; Sopade *et al.*, 2004a). Upon heating in water, above 55°C for most starches, gelatinisation occurs with changes to the viscosity of the aqueous dispersion, and crystallinity and organisation of the granules. When gelatinised dispersions are cooled, retrogradation occurs where the starch molecules are re-associated into an ordered structure and form a gel (Olkku and Rha, 1973; Lai and Kokini, 1991; Roos, 1992). Gelatinisation and retrogradation phenomena are well studied, and both confer various properties on starch-containing materials. Whey proteins are known for their properties that relate to solubility, foaming, emulsion formation and gelling and present health benefits (Belobrajdic *et al.*, 2004; McIntosh *et al.*, 1998). The two main types are whey protein concentrate (WPC) with a protein content from 25-89%, and whey protein isolate (WPI) with a protein content of >90% protein on dry basis. Whey protein forms viscoelastic gels upon heating, and the thermal gelation involves an initial denaturation-unfolding step followed by aggregation into a protein network by hydrophobic and sulfhydryl-disulfide interactions (Mulvihill and Kinsella, 1987). WPC is generally of a higher lactose content than WPI, and the high concentration of lactose in WPC plays an important role in flavour, aroma and browning reactions. Bazinet *et al.* (2004) observed that relatively more studies have been conducted on the rheological properties of WPC than WPI despite both being extensively used in foods.

Both whey protein and starch can form unique mixed gels upon heating, and a synergic effect can occur because the two components are structurally compatible. Indeed, proteins contain many hydrophilic groups such as -OH, -NH<sub>2</sub>, -COOH and -SH in the alkyl side chains, which are capable of forming links with starch. For example, Goel *et al.* (1999) showed that the addition of casein to starch increased pseudoplasticity, as compared to starch alone, and postulated that casein and its hydrolysates interacted with amylose and outer branches of amylopectin through non-covalent hydrogen bonding. Yang *et al.* (2004) investigated the rheological properties of wheat starch-WPI cooled gels (> 55% solids). Although complex modulus decreased in the presence of WPI (weakened gel structure), it increased as WPI was increased, and this was related to increased enhancement of hydrophobic interactions. In studies of heated mixed WPI and regular or cross-linked waxy maize starch dispersions at pH 7, Shim and Mulvaney (2001) and Ravindra *et al.* (2004) identified three phase-separated

networks depending on the composition of the blend; a WPI continuous network weakened by dispersed phase of starch granules when WPI fraction > 60% (WPI-dominated), two continuous phases (20% < WPI < 75%), and a starch continuous phase weakened by WPI aggregates when WPI < 20% (starch-dominated). While Champenois *et al.* (1998) found the storage and loss moduli of gluten-starch dispersions (6 – 30%) to increase with an increase in starch content, Shim and Mulvaney measured the complex modulus of 100% WPI to be greater than that of 100% starch (0% WPI), while at 5% or 15% total solids, the modulus exhibited a quadratic relationship with WPI content. The relationship between the modulus and WPI content appears to be affected by the solids content of the mixture because at 30% solids, Shim and Mulvaney obtained a consistent increase in the modulus as the WPI content was increased.

While the need for more studies on the rheology of WPI has been emphasised (Coughlan *et al.*, 2004), many studies on whey-starch mixtures, including those referred to above, concentrated on cooled gels, and information on changes to starch gelatinisation and retrogradation during cooking and cooling of whey-starch systems is limited. Goel *et al.* used the Brabender amylograph to study viscosity development in starch-casein systems at 5% solids. They obtained an increase in the viscosity of the dispersions with an increase in the casein content. Also, gelatinisation temperature decreased with the casein content, but Shim and Mulvaney did not obtain any substantial change in the gelatinisation temperature as WPI was increased. The conflicting results on the development of viscosity (or rheological parameters) in whey-starch or protein-starch systems emphasise the need for more studies in this area in order to understand the rheology of such systems, understand their processing (extrusion) and maximise their benefits. Therefore, the objectives of our extensive studies were to investigate gelatinisation and retrogradation in whey-starch mixtures using WPC and WPI, and to examine the nature of macromolecular interactions in such mixtures with techniques such as rapid visco-analyser, differential scanning calorimetry, rheometry, microscopy, and water absorption. However, the present paper discusses results from the rapid visco-analyser at pH of about 7 (distilled water).

## **2.0 EXPERIMENTAL**

### **2.1 Materials**

Whey protein concentrate (WPC) and whey protein isolate (WPI) were obtained from Total Foodtec Marketing Pty. Ltd., Darra QLD 4076 while wheat starch was purchased from Penford Australia Ltd., Lane Cove, NSW 2066. From the

manufacturer's data, WPC contains 77.0 % protein, 9.6% lactose, 5.2% fat, 5.5% moisture, 2.7% ash, and pH of 6.25, while WPI (ALACAN™ 894) contains 90.5 % protein, 5.2 % moisture, 0.2 % fat, 1 % total carbohydrate, 3.1 % ash, and pH of 6.80.

## 2.2 Preparation of powder samples

WPC or WPI was formulated in different mass ratios with starch (Table 1), and mixed on gently rotating rollers for at least three days before testing. The moisture content of each mixture was measured in a vacuum oven using standard procedures (AOAC, 2002).

Table 1. Formulations and codes of the mixtures

Form- ulation	Starch (%, solids)	Whey protein (%, solids)		Code	Moisture (%) <sup>a</sup>
		Concentrate	Isolate		
1	100	0	0	ST100	12.2 ± 0.3
2	80	20	0	ST80WPC20	10.1 ± 0.5
3	60	40	0	ST60WPC40	9.8 ± 0.2
4	50	50	0	ST50WPC50	8.9 ± 0.1
5	40	60	0	ST40WPC60	7.4 ± 0.2
6	20	80	0	ST20WPC80	7.1 ± 0.0
7	0	100	0	WPC100	5.1 ± 0.2
8	80	0	20	ST80WPI20	10.7 ± 0.1
9	60	0	40	ST60WPI40	8.4 ± 0.6
10	50	0	50	ST50WPI50	9.4 ± 0.5
11	40	0	60	ST40WPI60	8.0 ± 0.3
12	20	0	80	ST20WPI80	6.9 ± 0.0
13	0	0	100	WPI100	5.1 ± 0.2

<sup>a</sup>Values are means ± standard deviations

## 2.3 Pasting measurements

The whey-starch mixtures were mixed with distilled water (pH 7) in order to obtain a 10% solution based on solids content. The RVA-4 (Newport Scientific, Warriewood NSW 2102) was used to obtain the pasting behaviours of the mixtures by modifying the procedures of Zhou *et al.* (2003) and Onwulata *et al.* (2004). Each sample was held at 50°C and stirred at 1000 rev min<sup>-1</sup> for 60 s followed by constant stirring at 320 rev min<sup>-1</sup>, heating from 50°C to 95°C in 4.5 min (equivalent to 10°C min<sup>-1</sup>), holding at 95°C for 5 min, cooling to 50°C in 6

min (equivalent to  $7.5^{\circ}\text{C min}^{-1}$ ) and holding at  $50^{\circ}\text{C}$  for 5 min. The total time of each test was 21.5 min, and Table 2 shows the direct and derived parameters from the pasting curve that were used in this study. In addition, the first derivative of viscosity with respect to temperature,  $d\mu/dT$ , during the heating period was obtained in order to estimate the temperature ( $d\mu/dT = 0$ ) of fastest change in viscosity.

Table 2. Direct and derived parameters

Direct parameters		Derived parameters	
Initial viscosity	$\mu_I$	Breakdown (BD)	$\mu_P - \mu_{IC}$
Temperature of onset of the rise in viscosity	$T_R$	Setback (SB)	$ \mu_{FC} - \mu_P $
Peak viscosity (PVIS)	$\mu_P$	Total setback (SBt)	$\mu_{FC} - \mu_{IC}$
Holding strength or initial cooling viscosity	$\mu_{IC}$	Breakdown ratio (BDR)	$\mu_{IC} / \mu_P$
Trough viscosity (TRVIS)	$\mu_T$	Setback ratio (SBR)	$\mu_{FC} / \mu_P$
Final cooling viscosity (FVISAC)	$\mu_{FC}$	Total setback ratio (TSBR)	$\mu_{FC} / \mu_{IC}$
Final viscosity after 5 min at $50^{\circ}\text{C}$ (FVIS)	$\mu_F$	Relative breakdown (RBD)	BD / SBt

The initial rise temperature provides an indication of the minimum temperature required to gelatinize a given sample, which can have implications for the stability of other components in a formula, and also indicate energy costs. The peak viscosity occurs at the equilibrium point between swelling and polymer leaching which causes an increase in viscosity, and rupture and polymer alignment, which causes it to decrease. It indicates also the water-binding capacity of the starch or mixture. It is often correlated with final product quality and, also provides an indication of the viscous load likely to be encountered by a mixing cooker. Final viscosity is the most commonly used parameter to define sample quality, as it indicates the ability of the material to form a viscous paste or gel after cooking and cooling. The breakdown represents the resistance of the material to heat and shear (Lim and Narsimhan, 2006). The setback and total setback indicate retrogradation or re-ordering of the starch molecules as well as being associated with syneresis or weeping. Final viscosity is correlated with the texture of the product, and Fitzgerald *et al.* (2003) observed that breakdown and setback have been correlated to the firmness, while stickiness has been correlated with the final viscosity.

### 3.0 RESULTS AND DISCUSSION

Figure 1 shows the pasting curves obtained for some of the whey-starch mixtures. Champenois *et al.* observed that the typical time-temperature combinations as in this study are sufficient for the effects of heating and cooling to occur. The curves generally exhibit the same trend, and both whey proteins, WPC and WPI, decreased the viscosity (peak, trough and final) with WPC generally exercising a greater effect than WPI. The stronger effect of WPC could be due to its higher content of lactose and fat. Proteins are generally known to reduce the viscosity of starch, and the presence of sugars dilutes starch viscosity also (Sopade and Filibus, 1995; Abubakar *et al.*, 1997). However, the patterns displayed in Fig. 1 appear to be contrary to those observed by Goel *et al.*, Yang *et al.*, Shim and Mulvaney, and Ravindra *et al.*, who measured an improvement of gel strength when protein content (casein or WPI) in starch-based products was increased. Also, Tárrega *et al.* (2005) reported an increase in the viscosity of starch dispersions when milk was substituted for water at the same starch concentration. The solids in milk will add to the overall viscosity, and if the solids content of the milk had been compensated for, a different trend could have been observed. However, the effects of WPC and WPI on starch gelatinisation and retrogradation are more clearly discussed using the direct and derived parameters (Table 2) from the viscograms.

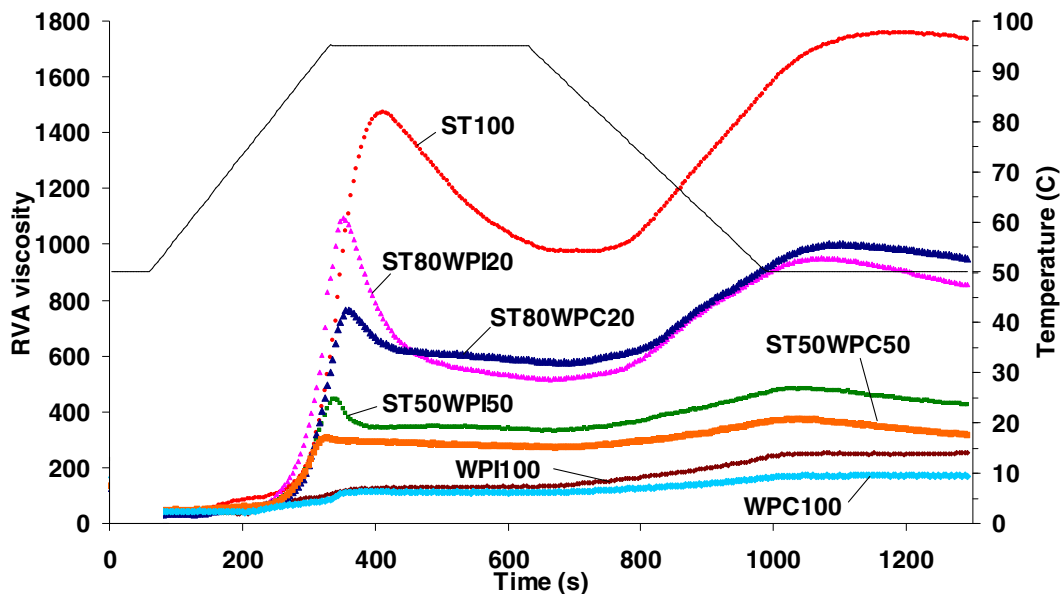


Figure 1. Typical pasting curves for the starch-whey mixtures

Figures 2 - 4 show the relationships between RVA parameters and whey protein (WPC/WPI) content. The higher the whey content, the lower are the peak, final and trough viscosities as well as the setback total, relative breakdown and the temperature of fastest rise in viscosity. It can be observed that with these parameters, the reduction in value is relatively steep at low concentrations of the whey, and above about 60% whey concentration, the viscosity is not materially different from that of the whey. This possibly confirms the change from a starch-dominated network to a whey-dominated one through a transition (40 – 60% whey) region as implied in earlier studies (Shim and Mulvaney; Ravindra *et al.*). Generally, starch forms a more viscous gel than proteins, and the whey proteins could have acted as a filler when the starch concentration was high. The observation with the viscosity of the starch-whey gel has processing implications as it implies that melt viscosity during extrusion cooking would be reduced when whey concentration is increased. This will affect expansion, specific mechanical energy and molecular fragmentation as well as physicochemical properties of starch-whey extrudates (Chuang and Yeh, 2004; Ding *et al.*, 2005). It should be stressed that expansion (diametral and longitudinal) during extrusion cooking is dependent on the viscoelastic property of the melt, and RVA results only reveals the viscous properties. Studies are in progress in our laboratory to investigate the viscoelastic property of the present starch-whey formulations. However, studies on mixtures of starch with other proteins such as gluten have been conducted, and Champenois *et al.* reported that both elastic and viscous moduli of starch-gluten mixtures reduced as the gluten content was increased.

The peak viscosity has been related to the water-holding ability (WHA) of the gel, and the measured reduction in the peak viscosity with an increase in whey suggests poor WHA in starch-whey gels when compared to starch gels. Sopade and Okonmah (1993) reported that proteins enhance water absorption capacity more than carbohydrates, and the whey proteins used in this study have been shown to have a higher sorptive ability than the wheat starch with the isolate absorbing more than the concentrate (Sopade *et al.*, 2007). However, Lupano and Gonzáles (1999) measured a reduction in WHA of starch-whey mixtures as whey protein concentrate was increased. While this is surprising in view of the availability of hydrophilic groups in both starch and protein, it possibly implies the modification of the hydrophilic sites by a form of macromolecular interactions in starch-whey systems. Perhaps, this interaction stabilised the gel against shearing (low breakdown) and modified or weakened the molecular sites that enhance retrogradation or re-ordering of starch molecules (total setback). Incidentally, Lim and Narsimhan attributed shear-thinning (breakdown) and setback in starch-soy protein gels to the formation of intermolecular hydrophobic interactions between starch and protein and/or



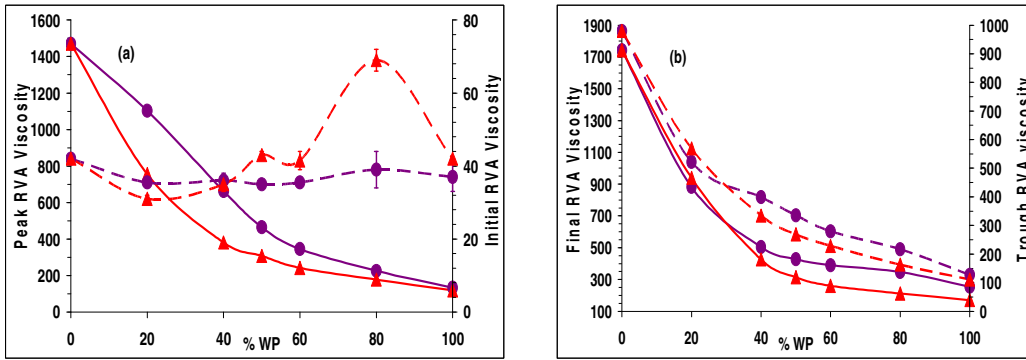


Figure 2 (a): Peak (— left hand axis) and initial RVA viscosity (— — right hand axis) for different concentrations of WPC (▲) or WPI (●)  
 (b): Final (— left hand axis) and trough RVA viscosity (— — right hand axis) for different concentrations of WPC (▲) or WPI (●)

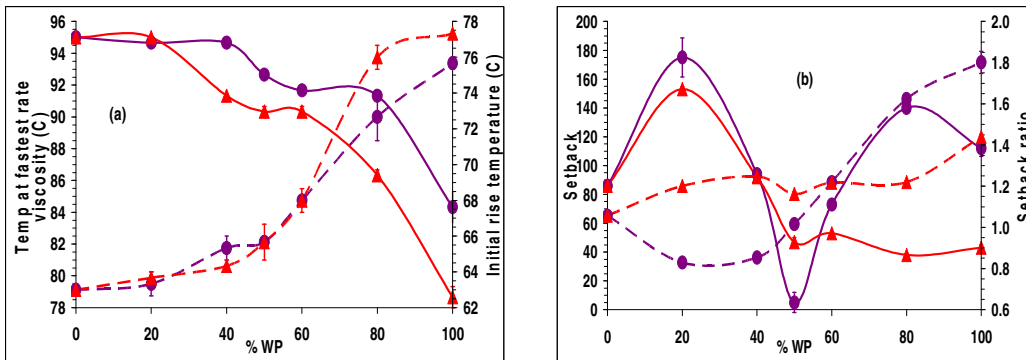


Figure 3 (a): Temperature at the fastest rate of change of viscosity (— left hand axis) and initial rise in temperature (— — right hand axis) for different concentrations of WPC (▲) or WPI (●)  
 (b): Setback (— left hand axis) and setback ratio (— — right hand axis) for different concentrations of WPC (▲) or WPI (●)

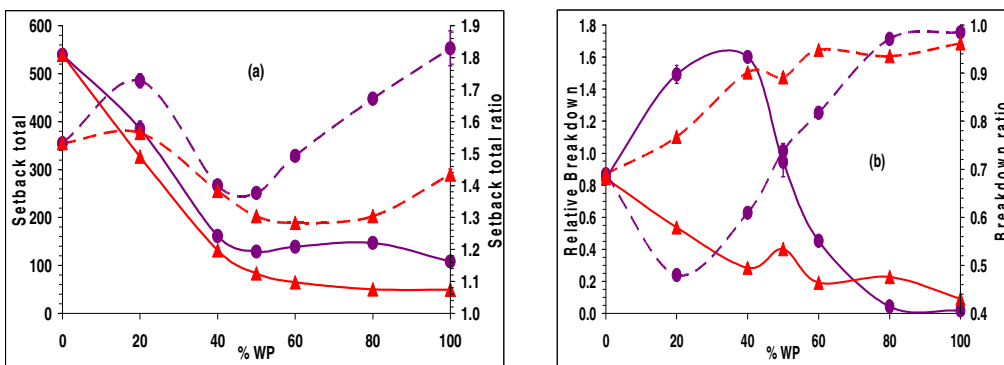


Figure 4 (a): Setback total (— left hand axis) and setback total ratio (— — right hand axis) for different concentrations of WPC (▲) or WPI (●)  
 (b): Relative breakdown (— left hand axis) and breakdown ratio (— — right hand axis) for different concentrations of WPC (▲) or WPI (●)

soyproteins themselves through the formation of reversible hydrogen bonds and thermally irreversible hydrophobic and/or covalent bonds. Although useful for following cooking and cooling in food, RVA data are limited on mechanistic information on starch gelatinisation and retrogradation, and supportive techniques are required to further probe these transformations.

The onset of gelatinisation reveals information about hydration and swelling of starch granules, and as shown in Figure 3a, an increase in whey protein increased the temperature of initial rise of viscosity (gelatinisation temperature). This shows that whey solids delayed the onset of gelatinisation, and whey protein concentrate exercised more effect than the isolate. Lupano and Gonzáles attributed the delay in gelatinisation temperature to the proteins, lactose and calcium in whey solids, and with more lactose in the concentrate than the isolate, the observed effects are not unexpected. While our results agree with those of Champenois *et al.* in starch-gluten systems, they contradict the observations of Shim and Mulvaney that indicate an increase in the temperature followed by a decrease as whey protein isolate was increased. Although differences in molecular conformation between gluten and whey proteins might be responsible for this, Lim and Narsimhan, using soy proteins, obtained a trend that agrees with the present findings, which contradict those of Goel *et al.* who used casein. It is well established that food components or additives (sugars, salt, hydrocolloids) affect gelatinisation temperature, and mechanisms have been proposed to explain this (Sopade *et al.*, 2004b). Starch usually gelatinises at a temperature that is lower than that required for protein denaturation, and this is evident in Figure 3a. This implies that gelatinisation is initiated before protein denaturation, and changes to granule swelling and rupturing as well as amylose exudation for gel formation by the presence of whey protein, will influence initiation of gelatinisation. Because excess water was used, Champenois *et al.* did not associate the delay in gelatinisation in the presence of gluten to limited available water or a delay in the diffusion of water to the starch granules. These authors hypothesised that the presence of gluten hinders granule-granule interactions as the gluten becomes embedded between the starch granules thereby delaying the formation of a network by the granules. Unfortunately, these authors did not include microstructural evidence for their proposed model of starch-gluten paste network, but scanning electron microscopy of Shim and Mulvaney lends credence to the model despite the latter authors measuring a contrary trend with the gelatinisation temperature. The exact mechanisms have not been established, and it seems that a simultaneous microscopy and rheometry (e.g. in Rheoscope<sup>TM</sup>) during heating and cooling of starch-whey dispersions will contribute to the understanding.

It is worth noting that after the delay, gelatinisation proceeded faster as shown by the temperature of fastest rate of increase in viscosity decreasing as

the whey content increased (Figure 3a). The temperature at which peak viscosity was obtained also decreased. In other words, upon overcoming the gelatinisation (swelling and rupturing) threshold, the exudation of amylose from the starch granules to form a gel network proceeded faster on increasing the amount of whey solids in the dispersion. In a study of starch-sugar systems, Perry and Donald (2002) observed that gelatinisation proceeded as in excess water after the initial delay as a result of sugars in the water. However, using differential scanning calorimetry (DSC), Sopade *et al.* (2004b) measured a broadening of the gelatinisation transition in the presence of sugars to suggest that even after surmounting the gelatinisation threshold, the process did not proceed as in excess water. A major deduction from gelatinisation in starch-whey systems is that both macromolecules interact in defining the RVA viscogram and its associated parameters.

The influence of whey solids on the setback, setback ratio, relative breakdown, breakdown ratio and initial viscosity does not follow a simple trend as evidenced in Figures 2a, 3b, 4a, 4b. Previous studies (Aguilera and Rojas, 1997) have shown that the evolution of storage modulus in cassava starch-whey protein isolate gels does not follow an additive law;  $G'_{\text{mix}} = \lambda G'_{\text{starch}} + (1 - \lambda) G'_{\text{whey}}$ , where  $\lambda$  = fraction of starch in the mixture, and  $G'$  is the storage modulus of the mixture (<sub>mix</sub>), starch (<sub>starch</sub>) and whey (<sub>whey</sub>). Figure 5 confirms that the additive law is unsuitable for the selected viscosity-related RVA parameters of the present formulations. We are presently investigating various models to describe the RVA viscograms, from which we would mathematically describe the degree of interactions between the macromolecules. However, a non-linear regression analysis revealed that an exponential model (Eq 1) is suitable in describing the dependence of the viscosity parameters on starch content (Table 3).

$$\mu_{\text{mix}} = A \exp [B (\lambda \mu_{\text{starch}} + \{1 - \lambda\} \mu_{\text{whey}})]$$

or 
$$\mu_{\text{mix}} = A' \exp [B' \lambda] \quad (1)$$

where A, B, A', B' = constants,  $\mu$  = viscosity of the mixture (<sub>mix</sub>), 100% starch (<sub>starch</sub>) and 100% whey (<sub>whey</sub>). A, A' and B, B' are different for the concentrate and isolate with the former being substantially higher with the isolate than concentrate, while the reverse applies for the latter constants. As to be expected from their composition (protein, fat and lactose), the observation with the constants of the exponential equation confirms that the concentrate and isolate affected starch gelatinisation and retrogradation differently with the latter showing a reduced effect. Also, from the setback, starch-WPI gels are expected to have firmer or stronger texture.

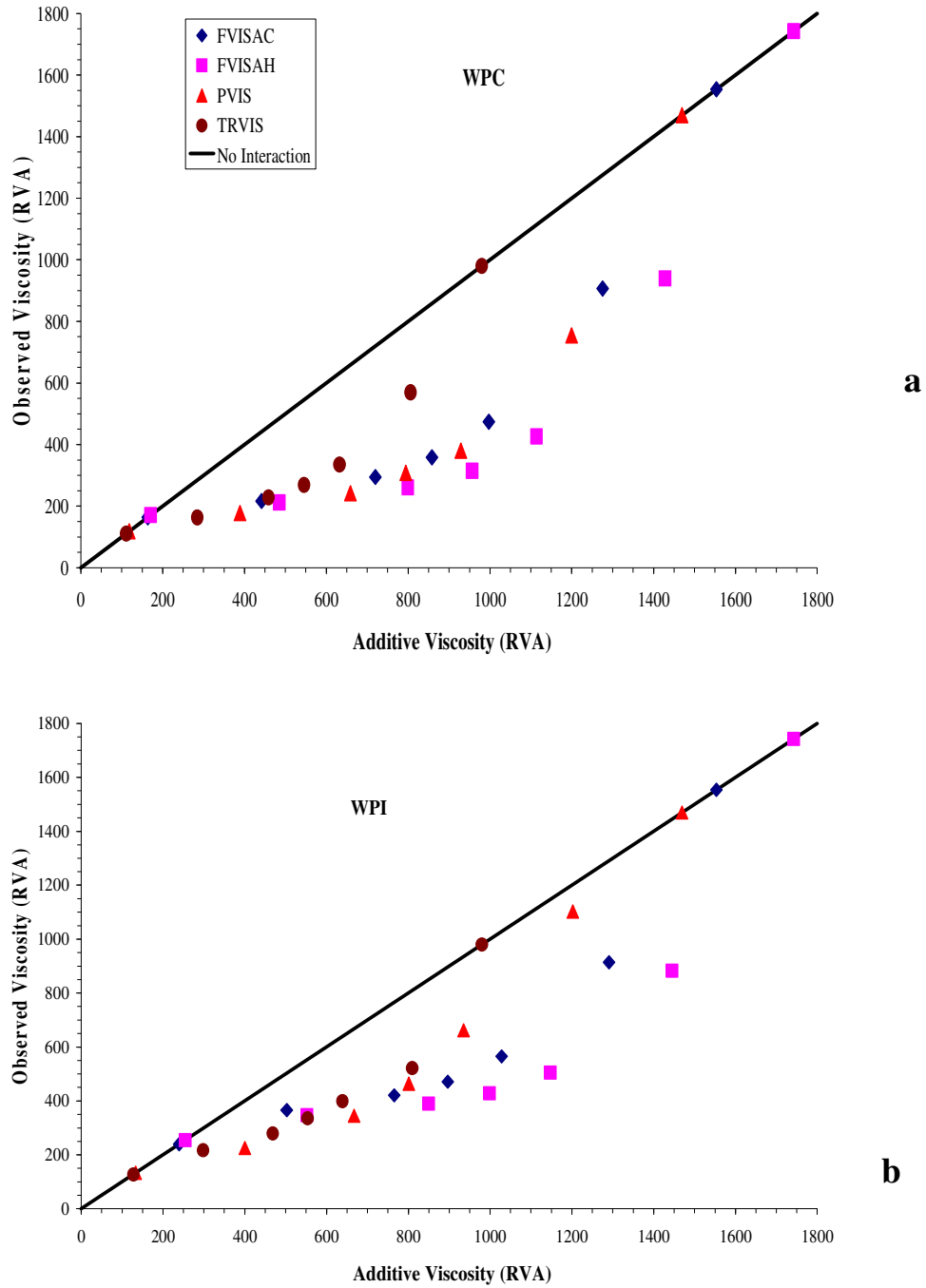


Figure 5. The relationship between observed and additive viscosities

Table 3: The parameters of the exponential model for the selected viscosity parameters

Parameter	Viscosity Parameter			
	After cooling to 50°C (FVISAC)	After holding at 50°C for 5 min. (FVISAH)	Peak (PVIS)	Trough (TRVIS)
<i>Whey protein concentrate (WPC)</i>				
A	71.3	51.4	49.9	58.9
A'	98.6	72.5	65.5	80.8
B x 10 <sup>-3</sup>	1.98	2.02	2.29	2.85
B'	2.75	3.18	3.10	2.48
r <sup>2</sup>	0.99	0.99	0.99	0.99
μ <sub>starch</sub> (RVA)	1553.5	1742.5	1469.5	980.0
μ <sub>whey</sub> (RVA)	163.5	170.5	118.5	111.0
<i>Whey protein isolate (WPI)</i>				
A	114.5	78.7	131.0	82.7
A'	170.3	123.0	163.7	113.3
B x 10 <sup>-3</sup>	1.66	1.75	1.67	2.48
B'	2.18	2.61	2.23	2.11
r <sup>2</sup>	0.98	0.96	0.98	0.97
μ <sub>starch</sub> (RVA)	239.5	254.5	133.0	127.5

#### 4.0 CONCLUSIONS

Whey protein concentrate and isolate decreased the viscosity of wheat starch when heated and cooled in water. At high starch concentrations, starch appeared to dominate the viscous network, which became whey protein-dominated at concentrations above 50% whey protein. The transition for the phase change probably occurred at whey concentration of 40 – 60%. Above 60% whey protein, the gelatinisation temperature was substantially increased by whey protein and the denaturation of whey protein became the determining process for a rise in the viscosity of the starch-whey gels. Whey proteins increased the stability of the gels, and with lower protein and higher lactose and fat contents, the concentrate exercised a stronger effect on starch gelatinisation and retrogradation in the mixtures. Although processing of starch-whey system is expected to be at a higher solids content than 10% used in the present studies, the trends obtained are

valuable in process design and optimisation. Although macromolecular interactions in the starch-whey systems are confirmed, other techniques (e.g. differential scanning calorimetry, rheometry, microscopy, X-ray diffraction, water absorption) are needed to probe the mechanisms. Also, pH of and salts in the dispersing medium will influence the characteristics of the gel in view of the amphoteric nature of proteins and their isoelectric points.

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