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Analysis of complexes between lipids and wheat starch

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Abstract

Starch–lipid complexes were formed by mixing wheat starch and various fatty acids and monopalmitin in a rapid visco analyser (RVA). The inclusion of the lipids in the starch pastes decreased the holding viscosity, whereas the final viscosity was increased. The changes in RVA parameters were correlated with the loss of iodine-binding capacity of the starch pastes, which was consistent with the formation of starch–lipid complexes. Mixtures of wheat starch with caprylic acid (C8:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:$1^{\Delta 9}$), linoleic acid (C18:$2^{\Delta 9,11}$), linolenic acid (C18:$3^{\Delta 9,11,13}$), and monopalmitin showed that maximal complex formation occurred at a different concentration for each lipid, which was related to the water solubility and critical micellar concentration of the lipid. Above a certain concentration, some of the lipids tended to self associate in preference to forming starch–lipid complexes.

Keywords: Wheat starch; Fatty acids; Starch–lipid complexes; Rapid visco-analyzer

1. Introduction

Starch and lipids are major food ingredients that have important functional interactions in food systems. Unbranched $\alpha(1 \rightarrow 4)$ glucan chains form helices with a hydrophobic interior, which can interact with a range of small non-polar molecules and the hydrophobic domains of amphilic molecules such as fatty acids, monoglycerides, and surfactants (Thomas & Atwell, 1999). Inclusion complexes form mainly with amylose and can modify the properties and functionality of starch, for example reducing solubility in water and retarding retrogradation and enzymic hydrolysis (Crowe, Seligman, & Copeland, 2000; Eliasson & Krog, 1985; Guraya, Kadan, & Champagne, 1997; Holm et al., 1983; Szczodrak & Pomeranz, 1992). There are also reports that linear outer branches of amylopectin may interact with surfactants (Biliaderis & Vaughan, 1987; Gudmundsson & Eliasson, 1990). Since these changes in the functionality of starch are of interest to the food industry and for human nutrition, starch–lipid complexes have been studied extensively using various methods. For example, crystallinity has been examined by X-ray diffraction (XRD), and differential scanning calorimetry has been used to analyze melting-transition characteristics and stability of the complexes (Bhatnagar & Hanna, 1994; Biliaderis & Seneviratne, 1990; Eliasson & Krog, 1985; Godet, Buleon, Tran, & Colonna, 1993; Karkalas & Raphaelides, 1986; Kowblansky, 1985; Nebesny, Rosicka, & Tkaczk, 2005; Tufvesson, Wahlgren, & Eliasson, 2003). Equilibrium dialysis has been used to measure the binding of lipids to starch in aqueous dispersions (Hahn & Hood, 1987). The effect of lipids on viscosity of starch pastes has been monitored by viscometry (Karkalas & Raphaelides, 1986; Kaur & Singh, 2000; Ozcan & Jackson, 2002), and the rapid visco analyser (RVA) has been used to show that fatty acids and monoglycerides increase the final viscosity of starch pastes (Deffenbaugh, Lincoln, & Walker, 1990; Liang, King, & Shih, 2002; Ravi, Sai Manohar, & Haridas Rao, 1999; Zhang & Hamaker, 2003, 2004).

Although starch–lipid complexes have been analyzed by many different methods, relatively few studies have been

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concerned with the influence of the concentration of lipid on complex formation with starch. Complex formation with different types of lipids has usually been studied using fixed lipid concentrations and the optimum lipid concentration has not been considered. Hence, there are inconsistencies between results obtained in studies in which different concentrations of lipids have been used to form complexes (for example, Eliasson & Krog, 1985; Fanta, Shogren, & Salch, 1999; Hahn & Hood, 1987; Hoover & Hadziyev, 1981; Kowblansky, 1985).

Wheat starch is a major source of carbohydrates in the human diet. The formation of starch–lipid complexes has been shown to affect gelatinisation and pasting properties of wheat starch (Mira, Eliasson, & Persson, 2005; Morrison, 1988; Richardson, Langton, Bark, & Hermansson, 2003). In this study, we have used the RVA as a simple and expedient method to mix wheat starch and lipids to form and analyze complexes, and relate effects to functionality. An advantage of this approach is that the lipids can be mixed directly with starch and not, as in many other studies, dissolved in a solvent, which may also influence the behaviour of the starch. The effect of various lipids on starch paste viscosities was compared with the reduction in iodine-binding capacity to relate changes in viscoelastic properties to the formation of starch–lipid complexes.

2. Materials and methods

Wheat starch was obtained from Penford Australia Pty Ltd (Lane Cove, NSW, Australia) and was used in experiments as supplied. According to the supplier's specifications, the moisture content was 9.9% (w/w), lipid content was 0.25–0.30% of dry matter, and the proportion of amylose was 25.5%. Free fatty acid acidity was estimated titrimetrically using Method 02-02A of the American Association of Cereal Chemists to be equivalent to 3.5 mg of KOH per 100 g of starch (0.016% palmitic acid equivalents). Starch damage was estimated to be 4% using the Megazyme Kit (AACC Method 76-31).

Caprylic acid (C8:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1Δ^9), linoleic acid (C18:2$\Delta^{9,11}$), α-linolenic acid (C18:3$\Delta^{9,11,13}$), monopalmitin (MP), and tripalmitin (TP) were from Sigma–Aldrich (St. Louis, MO, USA).

2.1. Rapid viscosity analysis

The viscoelastic properties of wheat starch were examined using a Rapid Visco Analyser-4 (Newport Scientific, Australia) according to Standard Method 1 (STD1) provided by the instrument manufacturer. Lipids were weighed accurately into a test canister and 25 ml of distilled water added, followed by 2.5 ± 0.01 g of starch (10% moisture). The mixture was agitated by raising and lowering the plastic paddle through the canister 10 times before inserting the canister into the instrument. The starch suspension was stirred at 960 rpm for 10 s before the shear input was decreased and held constant at 160 rpm during the subsequent heating and cooling cycles. The suspension was heated from 50 to 95 °C in 3 min and 42 s, held at 95 °C for 2 min and 30 s before cooling to 50 °C over 3 min and 48 s. Reference traces were recorded for each set of tests using samples that contained only starch. Final viscosity (FV) was measured as indicated in Fig. 1, and changes in final viscosity due to the addition of lipid (ΔFV) were calculated as follows:

$$\Delta FV = (FV_{Starch\text{–}lipid} - FV_{Starch\text{-}only})/FV_{Starch\text{-}only} \times 100.$$

2.2. Complexing index

The complexing index (CI) of starch was measured by the method of Gilbert and Spragg (1964) with modifications as follows. Starch paste (5.0 g) was removed from the RVA canister immediately after the completion of the profile and mixed with 25 ml of distilled water at 50 °C in a 50 ml capped tube. The tube was vortexed for 2 min, and 100 μl of the resulting dispersion was mixed with 15 ml of distilled water, followed by the addition of 2 ml of iodine solution (2.0% KI and 1.3% of I_2 in distilled water). The absorbance at 690 nm was measured. Pastes that contained only starch were used as a reference. To avoid starch retrogradation the tests were performed within 60 min. Complexing index was calculated as follows:

$$CI = (Abs_{Reference} - Abs_{Starch\text{–}lipid})/Abs_{Reference} \times 100.$$

2.3. X-ray diffraction analysis

Starch pastes obtained from the RVA were stored at 4 °C for 24 h, freeze-dried, and ground into a powder using a small domestic coffee grinder. The moisture con-

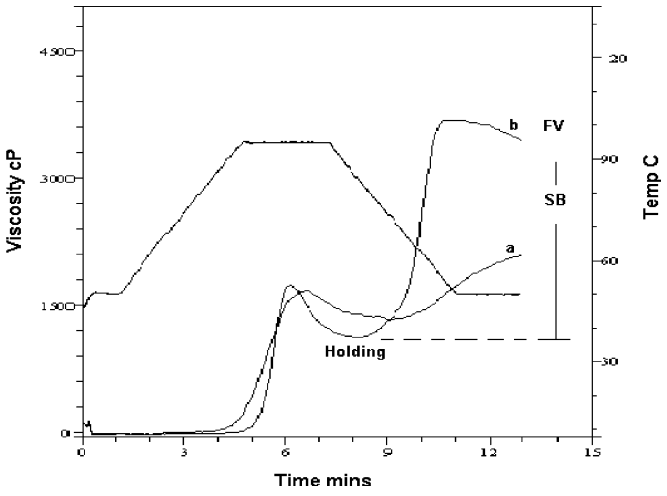

Fig. 1. RVA traces of starch and a starch–monopalmitin mixture. Pastes of starch (a) and starch mixed with MP (b) were prepared and analysed as described. The data are representative of triplicate experiments.

tent of the dried and ground pastes was 7–10%. Specimens were deposited in 1 mm-thick films on an aluminium sample holder and X-ray diffraction patterns obtained using a Shimadzu D6000 X-ray diffractometer equipped with a graphite monochromator. The X-ray source was CuK_α radiation (wavelength = 0.15405 nm), and operating conditions were 40 kV and 30 mA. Data were collected over the 2θ range from 2° to 36° at a scanning speed of 0.06 deg/min and a step size of 0.02°. Peaks in the traces were analysed using EVA software (version 3.00) for XRD.

3. Results and discussion

When wheat starch was heated to 95 °C with stirring in the RVA, there was a sharp increase in viscosity to a peak after about 6 min. Subsequently, the viscosity declined to a holding value, before increasing again to a final value as the mixture was held at 50 °C (Fig. 1). The difference between the final and holding viscosities is referred to as the set back. These phases of the RVA profile correspond to the starch granules initially absorbing water and swelling, followed by the disruption of the granule structure under the shear force and leaching of starch molecules, and finally the retrogradation of starch molecules into a gel and/or semi-crystalline aggregates held together by intermolecular interactions. The addition of 0.056 mmol of MP to 2.5 g of the starch (13.9 mmol of Glc equivalents) decreased the holding viscosity of the starch paste by about 20% and increased final viscosity by about 63% compared to starch-only pastes. Adding more MP to the starch did not result in any further significant changes in the RVA profile. The addition of MP to the wheat starch had little effect on the peak viscosity, and there was a small delay in the time at which the peak viscosity occurred (Fig. 1).

To determine whether the rheological changes observed were related to formation of starch–lipid complexes, the loss of iodine binding capacity of the starch, as measured by the CI, was compared with RVA parameters. Amylose helices occupied by lipid have reduced capacity to bind iodine, and will give a lower absorbance than starch alone

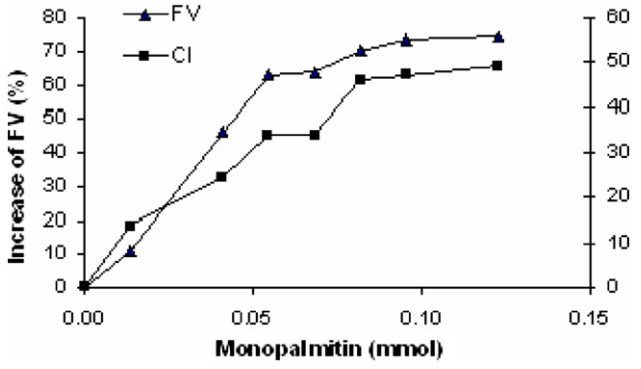

Fig. 2. Effect of adding MP to starch on final viscosity (FV) and complexing index (CI). Increasing amounts of MP were added to starch pastes, and CI and the increase in FV over the starch-only control were calculated as described. The data are representative of duplicate experiments.

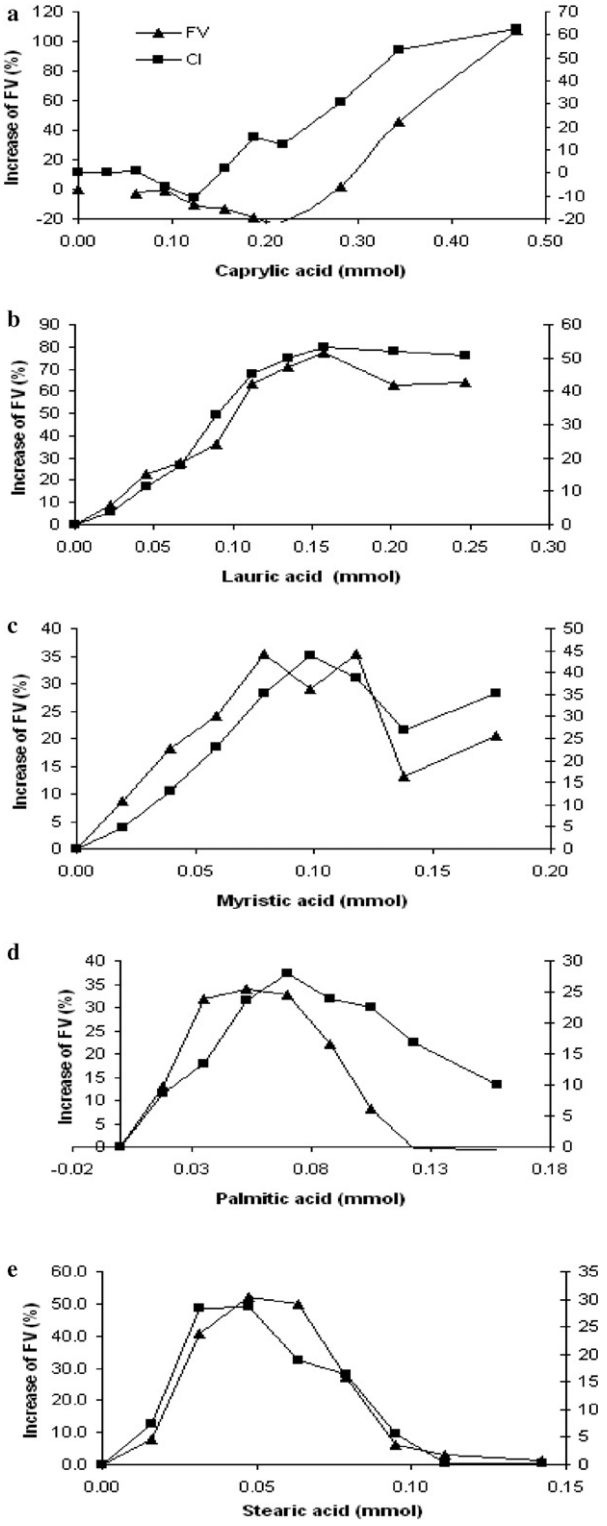

Fig. 3. Effect of adding saturated fatty acids to starch pastes on final viscosity and complexing index (CI). Starch was mixed with caprylic acid (a), lauric acid (b), myristic acid (c), palmitic acid (d), and stearic acid (e), and CI and the increase in FV over the starch-only control were calculated as described. The data are representative of duplicate experiments.

when mixed with iodine, that is the CI increases as the iodine binding capacity decreases.

The effect on the CI of adding increasing amounts of MP to the starch followed a similar trend to that observed for ΔFV (Fig. 2), consistent with the formation of complexes between the starch and MP in the RVA pastes. Plots of RVA parameters against CI showed a linear relationship between changes in ΔFV and CI ($r = 0.967$), and between setback and CI ($r = 0.970$). An inverse linear relationship was observed between CI and holding strength ($r = -0.954$), but there was no correlation between peak viscosity and CI.

With caprylic acid (C8:0), complex formation did not occur at low fatty acid concentrations, as indicated by the lack of an effect on ΔFV and CI, but was evident when amounts of caprylic acid greater than 0.28 mmol were mixed with 2.5 g (13.9 mmol of Glc equivalents) of wheat starch (Fig. 3a). Increases in ΔFV and CI indicated that complex formation between wheat starch and lauric acid (C12:0), myristic (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) increased as the amounts of fatty acids increased (Fig. 3b–e). The ΔFV and CI with lauric acid increased to maximum values when about 0.1 mmol of the fatty acid was mixed with 2.5 g of starch, and these parameters did not change significantly as the amount of lauric acid in the mixtures was increased further (Fig. 3b). In contrast, both the ΔFV and CI for mixtures of starch and myristic and palmitic acids increased to maxima with about 0.1 and 0.05 mmol, respectively, of the fatty acid per 2.5 g of starch, and then decreased as the amount of fatty acid was increased beyond this amount (Fig. 3c and d). Similar results were observed with starch and stearic acid mixtures (Fig. 3e). Thus, with the long chain saturated fatty acids, increasing the concentration of lipid led firstly to an increase in amount of starch–lipid complexes, and then as the concentration of the fatty acids reached a threshold there was a decrease in the extent of complex formation (Fig. 3).

The extent of the setback reflects the nature of the aggregates formed as the starch retrogrades, with predominantly the amylose molecules interacting closely to form junction zones between the chains (Whistler & BeMiller, 1997). It is possible that amylose complexed with lipids, which has a single-helical "V" structure, produces gels with increased spacing between junction zones, giving aggregates that are less compact and hence have a higher viscosity.

X-ray diffraction patterns obtained with freeze-dried starch pastes formed by mixing 2.5 g of wheat starch and 0.05 mmol of stearic acid showed peaks at 2θ values of 7.4°, 12.7° and 19.8° (Fig. 4), which matched the V pattern observed for amylose–lipid complexes in other studies (Bhatnagar & Hanna, 1994; Godet, Bizot, & Buleon, 1995). The peaks corresponding to amylose–lipid complexes were substantially reduced when the amount of stearic acid added to the starch was increased to 0.14 mmol. Instead, peaks were observed at 2θ of 21.5° and 23.9°,

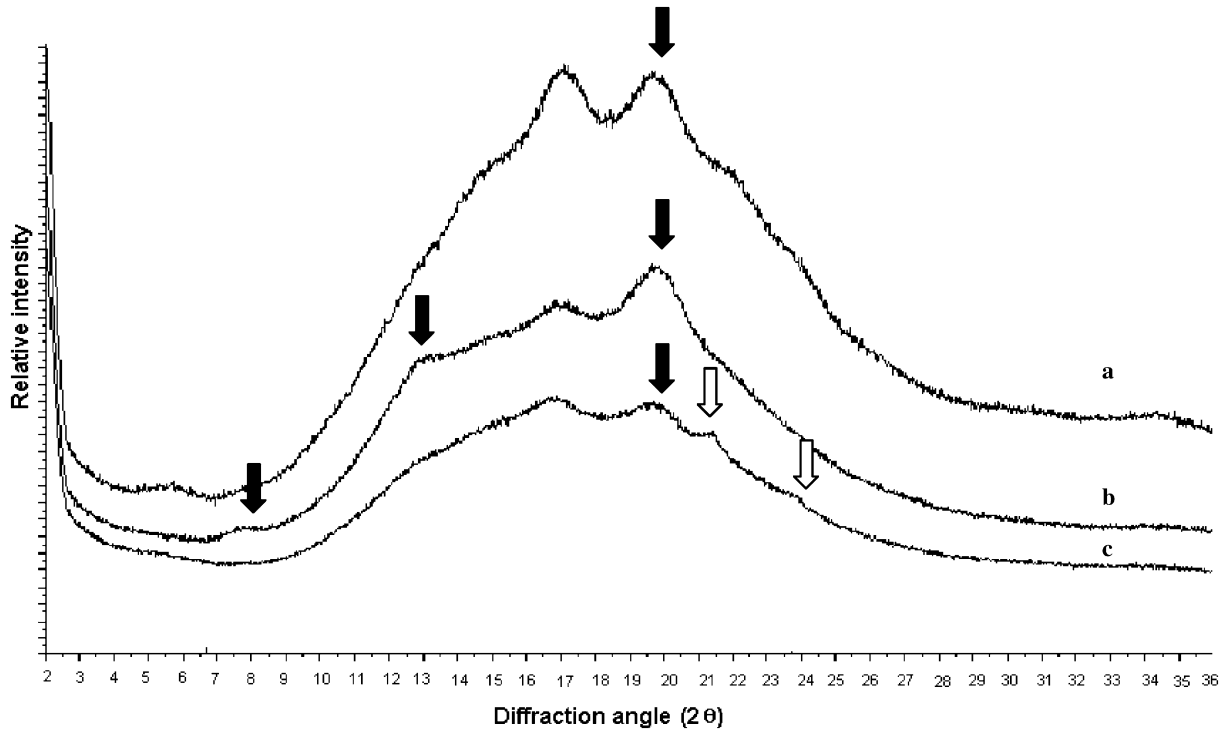

Fig. 4. X-ray diffraction patterns of (a), starch mixed with 0.05 mmol stearic acid (b) and starch mixed with 0.14 mmol stearic acid (c). Peaks identified as corresponding to amylose–lipid complexes and stearic acid micelles are designated by filled and open arrows, respectively. The data are representative of triplicate experiments.

which were identified as corresponding to the crystalline pattern of stearic acid aggregates (Fig. 4). The XRD patterns observed were consistent with the interpretation that above a certain concentration, lipids with low water solubility have a tendency to self-associate rather than form complexes with glucan helices. The formation of starch–lipid complexes in RVA pastes of rice starch was also observed by XRD (Liang et al., 2002). The greater water solubility of caprylic and lauric acids meant that starch–lipid complex formation with these fatty acids did not decrease at the high concentrations, as occurred with palmitic and stearic acids. Earlier studies have indicated that complexation with starch requires the monomer form of the lipid (Eliasson & Krog, 1985) and is influenced by the solubility of the lipid in water (Fanta et al., 1999; Hahn & Hood, 1987; Kowblansky, 1985).

The addition of oleic acid (C18: 1 Δ^9), linoleic acid (C18: 2 $\Delta^{9,11}$) and α-linolenic acid (C18: 3 $\Delta^{9,11,13}$) to starch pastes resulted in similar effects to those observed with saturated fatty acids (Fig. 5). Both ΔFV and CI increased to a maximum with the addition of increasing amounts of unsaturated fatty (Fig. 5). Complex formation decreased when the amount of oleic or linoleic acid added was greater than approximately 0.063 mmol per 2.5 g of starch. The decrease in complex formation at high concentrations of linolenic acid was less evident than with oleic and linoleic acids (Fig. 5), which may be related to the increased hydrophilicity of double bonds compared to saturated bonds (Hahn & Hood, 1987; Howard & Meylan, 1997). The addition of tripalmitin to wheat starch had no effect on RVA parameters or CI (results not shown), which is consistent with triglycerides not forming complexes with starch (Liang et al., 2002; Morrison, 1988).

4. Conclusion

The results of the present investigation have shown that there is an optimum concentration range for fatty acids to form complexes with starch. This may help to explain why

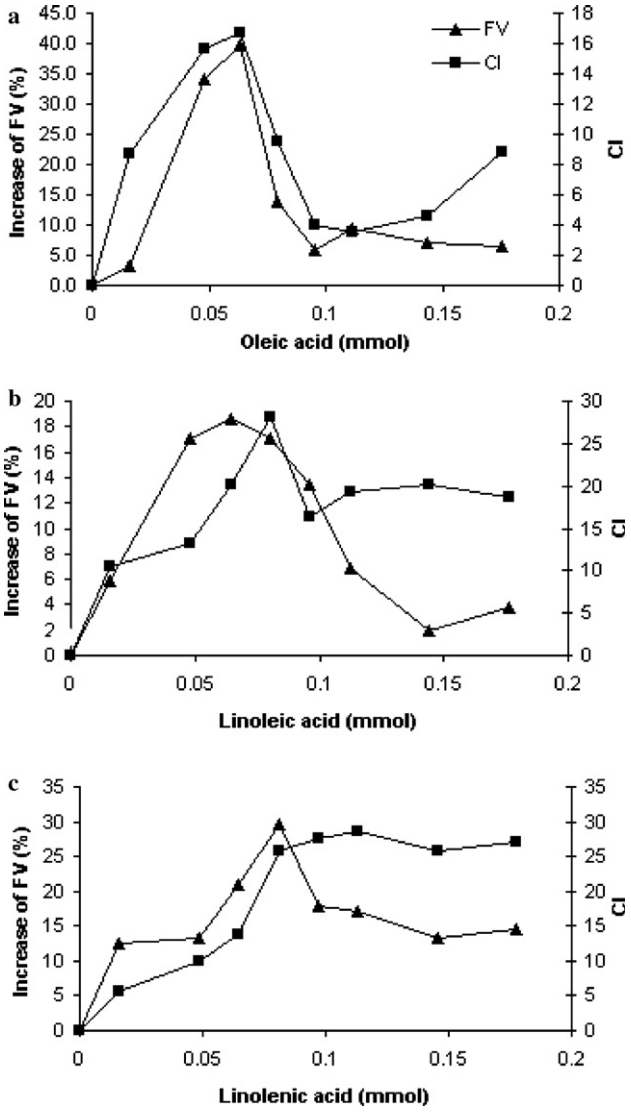

Fig. 5. Effect of adding unsaturated fatty acids to starch pastes on final viscosity and complexing index. Starch was mixed with oleic acid (a), linoleic acid (b) and, linolenic acid (c), and CI and the increase in FV over the starch-only control were calculated as described.

Table 1
Lipid concentration range for maximum complex formation

Lipid	Maximum CI	Amount of lipid for maximal starch–lipid complex formation per 2.5 g starch		Water solubility (%) at 20 °C[a]
		mg	mmol	
Caprylic acid	62.7	>60	>0.42	0.068
Lauric acid	53.2	>27	>0.13	0.0055
Myristic acid	44.0	18–27	0.08–0.12	0.0020
Palmitic acid	28.0	14–23	0.05–0.09	0.00072
Stearic acid	28.7	9–14	0.03–0.05	0.00029
Oleic acid	16.7	14–18	0.05–0.06	
Linoleic acid	28.2	18– 23	0.06–0.08	
α-Linolenic acid	28.7	>27	>0.10	
Monopalmitin	48.8	>27	>0.08	

Starch (2.5 g) and lipids were mixed and analysed in the RVA as represented in Figs. 2, 3, and 5. The maximum CI and concentration range of lipid that gave maximum CI and ΔFV are shown.

[a] Data from Singleton (1960).

little complex formation was observed when amylose was mixed with stearic acid in some studies, whereas in other studies amylose was found to complex strongly with stearic acid (Eliasson & Krog, 1985; Fanta et al., 1999; Hahn & Hood, 1987; Kowblansky, 1985). The increase in final viscosity of starch pastes in the presence of fatty acids and monopalmitin gave a quantitative measure of the extent of starch–lipid complex formation. In a starch–lipid–water system, the lipid molecules can interact with the solvent water, form complexes with amylose in starch, or self-associate into micellar structures. The critical micelle concentration (the concentration at which micellar aggregation first occurs) for fatty acids decreases as carbon chain length increases and water solubility decreases (Gurr & James, 1975; Mukerjee & Mysels, 1971). Formation of complexes of the lipids with amylose is therefore likely to be influenced by the solubility of the lipid in water and the critical micellar concentration. This means that the concentration range for maximum complex formation between starch and lipids will vary depending on the type of the lipid and its water solubility (Table 1).

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