Enhancement of the pasting properties of teff and maize starches through wet-heat processing with added stearic acid

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List of abbreviations:
CI: Complexation Index
CLSM: confocal laser scanning microscopy
FITC: Fluorescein-isothiocyanate
rpm: rate per minute
RVA: Rapid Visco Analyser
SEM: Scanning electron microscopy

ABSTRACT

This study determined the effects of stearic acid on the functional properties of teff starch, a compound granule starch in comparison to maize, a simple type granule starch. Stearic acid was incorporated into teff and maize starches and pasted (held for 5 or 120 min at 91°C) with an RVA (Rapid Visco Analyser). Teff starch with added stearic acid (0.25 and 1.5% starch basis) did not produce a pasting peak viscosity within short holding time (5 min) compared to maize starch. The paste viscosity of both teff and maize starches with stearic acid increased to about three times with long pasting (120 min). This increase in paste viscosity occurred earlier for teff starch than maize starch. Teff starch with stearic acid was more viscous and was non-gelling. Confocal laser scanning microscopy showed that stearic acid did not diffuse in teff starch granules, but seemed to coat them. However, stearic acid diffused inside maize starch granule through channels. This microstructural difference may explain the different pasting behavior. The early high paste viscosity and non gelling properties of the teff starch modified with stearic acid could have promising applications in foods for example better mouthfeel with lower starch concentration.

Key words: teff, maize, starch, pasting, amylose–lipid complex, confocal laser scanning microscopy

1. Introduction

Starch is often chemically modified to improve its functionality, for example better resistance to shear, extreme pH and reduced retrogradation (Singh et al., 2007). The chemicals used for modification, for example propylene oxide, acetic anhydride and octenyl succinic anhydrides are synthetically derived. These chemicals may leave residues in the final product, which can raise some health concerns amongst consumers (Lui, 2005). Thus, there is need for more natural alternatives to modify starch properties. Food grade/or naturally occurring alternatives, for example fatty acids from hydrolysis from triacylglycerols, can be used. Fatty acids, for example stearic acid, can reduce starch peak viscosity, increase time to peak viscosity and increase final viscosity for rice (Zhou et al., 2007) during a pasting cycle of 30 minutes or less. Similar findings have been found for wheat starch (Tang and Copeland, 2007). The addition of fatty acid has been also found to result in increase or decrease in final viscosity during pasting, depending on type of fatty acids (Tang and Copeland, 2007). These effects may be attributed to the formation of amylose-fatty acid complexes (Kaur and Singh, 2000) and formation of micelles between fatty acid molecules when the fatty acid is in excess (Tang and Copeland, 2007). Amylose-lipid complexes have also been suggested to play a role in the formation of a second viscosity peak, which occurs when maize starch is pasted for a long period, i.e. more than 30 min holding at high temperatures during pasting (Nelles et al., 2000).

The starch of teff (*Eragrostis tef*) has been subjected to limited research, but teff starch has been shown to have some unusual and potentially useful properties. The starch granules are small (2-6 μm), do not seem to have pores and are from compound granules (Bultosa et al., 2002). Teff starch also has lower peak, setback viscosities than maize starch and shows some resistance to breakdown during pasting (Bultosa et al., 2002). The effect of fatty acid on pasting properties of teff starch has not been reported.

This study therefore determined the effects of adding stearic acid on the pasting properties of teff starch, with maize starch used as reference.

2. Experimental

2.1 Materials

Witkop, a white teff variety, was obtained from PANNAR (Kroonstad, South Africa). This variety the most cultivated in South Africa. Stearic Acid (Grade I, approx. 99% capillary GC grade) was obtained from Sigma-Aldrich (Product Code S4751-25G). A commercial maize starch, Amyral (from a white maize cultivar), was kindly donated by Tongaat Hulett®, Edenvale, South Africa, and was used as reference. The cultivar is commonly used for starch extraction in South Africa. All other chemicals were of analytical grade.

2.2 Starch extraction

Teff grain was milled in a laboratory hammer mill to pass through an 800 µm opening screen. The flour was then defatted three times with hexane (1 part flour: 3 parts hexane) for 1 hour at 25°C. Teff starch extraction was conducted by the method described by Bultosa et al. (2002). In brief, flour (100 g) was suspended in 500 ml distilled water for an hour. The slurry was wet-milled in a Retsch Mill ZM 200 (Haan, Germany) with a 250 µm opening screen, followed by sieving (to remove the fibrous components) and centrifuged (to remove the insoluble protein). Starch samples were then freeze-dried. The protein (Nx6.25), ash, crude fat were 1.5, 0.5, and 0.15% (dry basis) respectively. The amylose content was about 30% starch basis (similar to maize starch). The reported chemical analyses values for teff starch were similar to the commercial maize starch.

2.3 Incorporation of stearic acid into starch

Stearic acid was added to the teff and maize starches in the concentrations 0, (control) 0.25, 0.5, 1.5, 2.0, 3.0 and 4.0% (dry weight basis of the starch). The stearic acid was first dissolved in absolute ethanol. The starch was added to this solution and covered with Parafilm and aluminium foil and placed in a shaking water bath at 50°C for 30 min. The ethanol was then evaporated off in a forced draught oven at 40°C.

2.4 Starch pasting properties

Pasting was conducted using a Rapid Visco Analyser (RVA Model 3D) (Newport Scientific, Warriewood, Australia). Starch (3 g; 14% moisture) was suspended in distilled water and the weight adjusted to 28 g. For the short pasting condition, an initial stirring speed of 960 rpm at 50°C for 30 second was used and then stirring was at 160 rpm for the entire period thereafter. The temperature was increased at a rate of 5.5°C/min to 91°C and held at this temperature for 5 min. The pastes were then cooled to 50°C at a rate of 5.5°C/min. The extended pasting condition was also investigated, which differed from the short pasting cycle by using a holding time of 120 min at 91°C.

2.5 Confocal laser scanning microscopy

Approximately 10 mg of the starch, before pasting, was placed on a microscope slide. Fluorescent stains, Nile Red (1 g/L in ethanol) and fluorescein-isothiocyanate (FITC) (0.05 g/L in 50% ethanol) were used to stain stearic acid and starch, respectively. Emmambux and Stading (2007) used Nile Red for a non-polar substance, while Savary et al. (2007) used FITC to stain starch. The stains were mixed into the sample and left overnight in a dark cold room for 24 hr. The starches were then viewed with a Zeiss LSM 510 META Confocal Laser Scanning Microscope (Zeiss SMT, Jena, Germany). The excitation and emission spectra for Nile Red were 488 nm and 640-750 nm, respectively, and 488 nm and 486-539 nm for FITC.

265 Flow properties of starch pastes at varying shear rates

The flow properties of starch pastes (sampled at end of both pasting cycles) were measured using the Bob and Cup method with a Physica MCR 301 Rheometer (Anton Paar, Ostfildern, Germany). Measuring bob (diameter: 27 mm) was inserted into the cup (diameter: 28.9 mm) containing the starch paste. To prevent moisture loss, paraffin oil was added to the top of the starch paste and allowed to form a layer until the entire surface was covered. This was done to prevent moisture loss from the sample. The starch paste was then allowed to equilibrate at 60°C for 30 min. The starch pastes were measured at shear rates ranging between 0.1 and 800 s^{-1}. The data were fitted into a Power-Law Model as follows:

$$\tau = \kappa \dot{\gamma}^{n}$$

where τ as the shear stress (Pa), k as the consistency coefficient (Pa.s), $\dot{\gamma}$ as the shear rate (s^{-1}) and n as the flow behaviour index.

2.7 Complexation Index

Complexation Index (CI), the percentage complexation between stearic acid and starch (amylose), was determined as described by Guraya et al. (1997). This method determines the amount of iodine complexed with free amylose component of the stearic acid treatment relative to the control (untreated starches).

2.8 Starch paste clarity

Paste clarity was determined according to the method of Craig et al. (1989), as modified by Bultosa and Taylor (2004). The method is based on the principle that the whiteness of a starch paste is related to the association of starch chains that reflect light.

2.9 Starch gel texture

The texture of starch gels obtained after cooling the pastes at 25°C overnight, were analysed as described by Bultosa and Taylor (2004). Gel firmness was analysed using a TA-XT2 texture analyser (Stable Micro Systems, Godalming, England) with a P/20p cylinder probe (20 mm diameter). Gels were compressed 5 mm at a speed of 2 mm/s at 25°C.

2.10 Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant differences due the addition of stearic acid, for maize and teff starches. Means were then compared using Fischer's Least Significant Difference Test (LSD). The experiments were repeated four times.

3. Results and Discussion

3.1 Pasting properties

With stearic acid addition, teff starch showed some unusual pasting effects, when compared to maize starch (Fig. 1). With teff starch plus stearic acid, a peak viscosity was not reached within the first 15 min of pasting (5 minutes holding at 91°C). Nonetheless, it seems that the viscosity during pasting decreased with increase in stearic acid addition to teff starch. This suggests that stearic acid delayed the pasting of teff starch. With maize starch, stearic acid at increasing concentration significantly ($P<0.05$) decreased the peak and decreased breakdown viscosity (the difference between the peak and trough viscosities) when pasted for 5 minutes holding at 91°C (Fig. 1). The final viscosities (viscosity at the end of the pasting cycle) seemed to show a small decrease with low stearic concentrations (0.25-2.0%) and then an increase at high concentration (3-4% stearic acid). Peak time (time taken to reach peak viscosity) also increased with stearic acid concentration.

The non-occurrence of a pasting peak of teff starch with stearic acid addition is not a common phenomenon. Other authors, namely Raphaelides and Georgidis (2006) working on maize starch; Tang and Copeland (2007) working on wheat starch and Zhou et al. (2007) working with rice starch have reported a delay and lower pasting peak of the starches during pasting with fatty acids, but not the absence of the pasting peak. Richardson et al. (2003) proposed that the addition of a lipid compound to wheat starch would result in increased hydrophobicity of the granule. This in turn would result in a reduced ability of the granules to take up water as well as a reduced swelling of starch granules during pasting, as shown by Raphaelides and Georgiadis (2006) for maize starch pasted with stearic acid.

Teff starch with added stearic acid was pasted for a longer period (120 min holding at 91°C) to further understand why addition of stearic acid did not produce a pasting peak. Here only the fatty acid concentrations of 0.25 and 1.5% (w/w of starch) were used. Teff starch (control) showed two pasting peaks, one before 30 min and one about 90 minutes (Fig. 2). The addition of 0.25% stearic acid resulted in a pasting peak only at about 100 min, i.e. the viscosity increased from about 2.5 min until 105 min (Fig. 2). This viscosity was almost three times (3x increase) higher than pasted teff starch without stearic acid. A peak was not observed when teff starch was modified with 1.5% within the pasting time, but the viscosity kept on increasing from 2.5 to 130 min and this viscosity was higher than pasted teff starch with 0.25% and without stearic acid (Fig. 2). The pasting patterns were somewhat different for maize starch treated with stearic acid (Fig. 2). Maize starch (control and modified with 0.25% stearic acid) showed a second pasting peak after 30 minutes in addition to a first peak at about 10 mins. Maize starch modified with 1.5% stearic acid did not produce a second peak, but the viscosity kept on increasing from 60 to 130 mins. This increase was about three times the control (3x increase) at 120 mins. The viscosity of maize starch with added 1.5% stearic acid was the highest followed by maize starch added with 0.25% stearic and maize starch without stearic acid during pasting. Teff starch with added stearic acid had a higher rate of viscosity increase compared to maize starch. This can be seen with a 2x increase and a 2.7x at one hour pasting for teff with added 0.25 and 1.5% respectively, compared with no increase and a 1.4x for maize with added 0.25 and 1.5% respectively. This suggests less wet heat processing time required to modify/increase teff viscosity with stearic acid compared to maize starch.

The change in pasting viscosity of both teff and maize starches with addition of stearic acid suggested that there was starch-stearic acid interaction. The Iodine Complexation Index (CI) assay is an indication of amylose-lipid complexes (Guraya et al., 1997). At the end of the pasting cycle of 5 minutes holding at 91°C, both teff and maize starches showed a significant increase ($P< 0.05$) in CI (Table 1) with increase of stearic acid. This suggests there could have been some amylose-stearic acid interaction during the short pasting cycle. CI further increased with longer pasting (120 minutes holding at 91°C) and with increase addition of stearic acid from 0.25 to 1.5%. The CI data thus suggests that amylose-stearic interaction was involved in the increase pasting viscosity of maize and teff starches with added stearic acid. This agrees with the suggestions of Zhou et al. (2007) working on rice starch, and Tang and Copeland (2007) who also showed an increase in final viscosity with addition of stearic acid during pasting. A peak after 30 min pasting of maize starch has also been reported by Nelles et al. (2000) and amylose lipid complexes was suggested to play role in this phenomenon.

The non-occurrence of a peak viscosity for stearic acid added teff starch (Fig. 2) may be related to the differences in microstructure between teff and maize starch granules. Fig. 3a shows confocal laser scanning micrographs of teff and maize starches with and without stearic acid addition before pasting. The starch is stained green and the stearic acid is seen as red spots. Teff and maize starch granules without stearic acid fluoresced green with FITC and showed very little red fluorescence for teff and virtually no red fluorescence for maize. The addition of stearic acid (0.25%) showed more red bright coloured spots for both teff and maize starches. The red fluorescence appeared to be inside the granules of maize starch but outside for teff starch granules.

To confirm whether stearic acid was inside the maize starch granules and not on the surface, Z-slices micrographs at various depths into the granules were taken for teff and maize starch with added stearic acid. The bright red spot areas (stained as stearic acid) seen in the Z-slices for maize starch might have indicated that stearic acid had diffused into the maize starch granules and these spots seemed to be in channels (Fig. 3b). Fannon et al. (1992) found pores on the surface of maize starch granules (using Scanning Electron Microscopy (SEM)). These pores were found to be opening for channels to inside the granules (Huber and BeMiller, 1997). Stearic acid could have entered the maize starch granules through these pores during the complexing stage. The teff starch control and the stearic acid-treated sample showed some bright red areas/spots on the surfaces of the granules (Fig. 3a). The Z-slices indicate that there was no red fluorescence from inside the granules (Fig. 3b). Bultosa et al. (2002) did not find pores on the surface of teff starch granules using SEM. The presence of stearic acid around the surface of the teff starch granules indicates that stearic acid did not diffuse into the teff starch granules during the complexation stage. Thus, the presence of stearic acid outside the teff starch granules may be linked to the unusual pasting behaviour, i.e. large increase in viscosity without giving a first viscosity peak, probably as a result of coating the granule during pasting.

3.2 Flow properties of starch pastes at different shear rates

Starch pastes are Non-Newtonian, i.e. viscosity decreases with increasing shear rates. Therefore, it was important to determine the stability of the starch pastes (treated and non-treated samples) at different shear rates. For this reason, the flow properties of the starch pastes were measured at varying shear rates using a rheometer. The flow properties of both starch pastes and their treated counterparts followed the Power-Law Model (Table 2) with a fit of $r^2 \geq 0.97$ for all the treatments. Teff and maize starch pastes (control, treated and pasted for 5 min or 120 min holding at 91°C) displayed shear thinning behaviour because n was less than one. The addition of stearic acid reduced n, the Power-Law flow index, for both starches after the short and extended pasting cycles (Table 2). This indicates that the starch with stearic acid pastes were more shear thinning than starch pastes alone. Stearic acid is widely used in the pharmaceutical industry as a lubricating agent in tablets (Husseini and Pitt, 2008). Thus, stearic acid-starch paste may have allowed the starch molecules to align more easily in the direction of shear and caused the paste to shear thin more.

The Power-Law consistency coefficient, K, indicative of viscous properties as a function of shear rates were not significantly different between the treatments but were different between the starches during pasting for 5 minute holding at 91°C (Table 2). K was significantly higher ($P<0.05$) for both starches (maize and teff) after pasting for 120 min holding at 91°C compared to pasting for 5 minutes holding at 91°C. The addition of stearic acid led to a further increase in K for extended pasting, suggesting that the viscosity is always higher for starches added with stearic acid at different shear rates.

3.3 Clarity of starch pastes

In terms of paste clarity, both teff and maize starches pasted for 30 minutes had significantly ($P<0.05$) lower paste clarity with added stearic acid (Table 3). Paste clarity was lower when the starches were pasted for 120 min holding at 91°C as compared with

30 min at 91°C. According to Craig et al. (1989), ‘whiteness’ or opacity is brought about by the degree of light scattered by the association of starch chains after pasting. The reduced transmittance of starch modified with stearic acid suggests that stearic acid may interfere with the association of the starch polymers. In fact, amylose-lipid complexes have been found to interfere with the re-aligning of amylose chains during retrogradation (Gudmundsson, 1992 and 1994).

3.4 Gel texture of starches

The gels of both starches had reduced firmness with the 0.25% added stearic acid after pasting for 120 min holding at 91°C treatment (Table 4). However, teff starch gels remained firmer than maize starch gels. The findings about the modified starches agree with those of Raphaelides (1992) and Richardson et al. (2004), who found that the rigidity of starch gels was reduced with the addition of fatty acids and emulsifiers, respectively. Richardson et al. (2004) also found that potato amylose dispersions formed open structured gels with many pores with addition of low levels of an emulsifier (glycerol monostearate) to produce weaker gels.

The 1.5% stearic acid treatment with pasting for 120 min holding at 91°C prevented gels from being formed. This non-gelling behaviour is also in agreement with Richardson et al. (2004) working with wheat starch and added emulsifier. These authors found that high levels of added emulsifier only resulted in the formation of a thick opaque paste, without gelling of wheat starch. Gel formation is largely dependent on starch retrogradation, i.e. the re-alignment of amylopectin molecules and formation of amylose double helices (network formation). Thus, the-non gelling suggests that amylose interacted with stearic acids may prevent interaction between starch during the recrystallisation process (Gudmundsson, 1994).

4. Conclusions

Teff starch with added stearic acid produces an earlier high paste viscosity compared to maize starch with added stearic acid. This high viscosity paste with added stearic acid was also non-gelling. The pasting pattern of teff starch with stearic acid during wet heat processing in the RVA shows no pasting peak and this may be related to the absence of channels of teff starch granules. This high viscosity and non gelling properties of teff starch modified with stearic acid can open new avenues in food application for example better mouthfeel with lower starch concentration.

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Fig. 1. Effect of stearic acid addition on the pasting properties of teff and maize starches held for 5 minutes at 91°C

Each concentration is a representative graph of 4 repetitions

T-0%, T-0.25%, T-0.5%, T-1.5%, T-2.0%, T-3.0%, T-4.0% are teff starches with 0, 0.25, 0.5, 1.5, 2.0, 3.0 and 4.0% stearic acid respectively

M-0%, M-0.25%, M-0.5%, M-1.5%, M-2.0%, M-3.0%, M-4.0% are Maize starches with 0, 0.25, 0.5, 1.5, 2.0, 3.0 and 4.0% stearic acid respectively

Fig. 2. Effect of stearic acid addition on the pasting properties of teff and maize starches held for 120 min at 91°C

Each concentration is a representative graph of 4 repetitions

T-0%, T-0.25%, T-1.5%, are teff starches with 0, 0.25, and 1.5, stearic acid respectively

M-0%, M-0.25% and M-1.5%are Maize starches with 0, 0.25 and 1.5 stearic acid respectively

Fig. 3. Confocal laser scanning micrographs of (a)starch granules before pasting and (b) their Z-slices

A: teff starches, B: teff starches with stearic acid, C: maize starches, D: maize starches with stearic acid

T-X. T-Y, T-Z are Z-slices of teff starch with stearic acid at depth of 0.87, 2.61 and 5.21 μm, respectively

M-X. M-Y, M-Z are Z-slices of maize starch with stearic acid at depth of 0.70, 3.51 and 7.70 µm, respectively

The spots (red) represent stearic acid

The scale bars for A, B, C and D, M-X, M-Y and MZ are 10 µm and T-X, T-Y and T-Z are 5 µm

Table 1

Complexation Indices of teff and maize starches after first peak during pasting for 5 min or after second peak during pasting for 120 min at 91°C

Stearic Acid (%)	**Complexation Index (CI)**			
	5 min holding at 91°C		120 min holding at 91°C	
	Teff	**Maize**	**Teff**	**Maize**
0	na	na	na	na
0.25	$13.3^{a} \pm 0.8$	$17.8^{a} \pm 1.9$	$24.3^{a} \pm 0.5$	$20.8^{a} \pm 1.1$
1.50	$42.3^{b} \pm 1.3$	$48.1^{b} \pm 0.5$	$52.3^{b} \pm 0.2$	$46.5^{b} \pm 1.6$

Values within the same columns with different letters are significantly different (P <0.01) and are the means of at least 3 repetitions.

*na = not applicable (0% added stearic acid taken as 0% complexation/blank

Table 2

Effect of stearic acid on the flow properties in terms of the Power- Law Model coefficients for teff and maize starches pasted for 5 or 120 min at 91°C.

	n	K (Pa.s)
5 min holding at 91°C		
Teff	$0.41^{c} \pm 0.01$	$15.6^{a} \pm 0.2$
Teff + 0.25% stearic acid	$0.39^{c} \pm 0.01$	$17.6^{a} \pm 1.7$
Maize	$0.35^{b} \pm 0.01$	$23.4^{b} \pm 0.2$
Maize + 0.25% stearic acid	$0.33^{a} \pm 0.01$	$24.4^{b} \pm 0.6$
120 min holding at 91°C		
Teff	$0.44^{d} \pm 0.01$	$29.5^{d} \pm 1.6$
Teff + 0.25% stearic acid	$0.40^{c} \pm 0.01$	$34.2^{e} \pm 1.2$
Maize	$0.40^{c} \pm 0.02$	$26.5^{c} \pm 0.2$
Maize + 0.25% stearic acid	$0.39^{c} \pm 0.01$	$30.4^{d} \pm 2.8$

Values within columns with different letters are significantly different ($p<0.01$) and the pastes for the short and extended cycles separately.

n is the flow index and K is the consistency coefficient from the Power law model

Table 3

Effect of stearic acid on the clarity of teff and maize starches pasted in boiling water for 30 or 90 min

Stearic acid (%)	Transmittance (%)			
	Teff		Maize	
	30 min	90 min	30 min	90 min
0	$38.1^{b} \pm 1.5$	$27.0^{c} \pm 0.7$	$43.2^{b} \pm 1.9$	$23.3^{b} \pm 1.6$
0.25	$39.4^{b} \pm 1.1$	$23.7^{b} \pm 1.0$	$40.4^{a} \pm 0.8$	$21.9^{b} \pm 0.7$
1.50	$34.3^{a} \pm 0.5$	$22.0^{a} \pm 0.6$	$39.3^{a} \pm 0.6$	$19.8^{a} \pm 0.6$

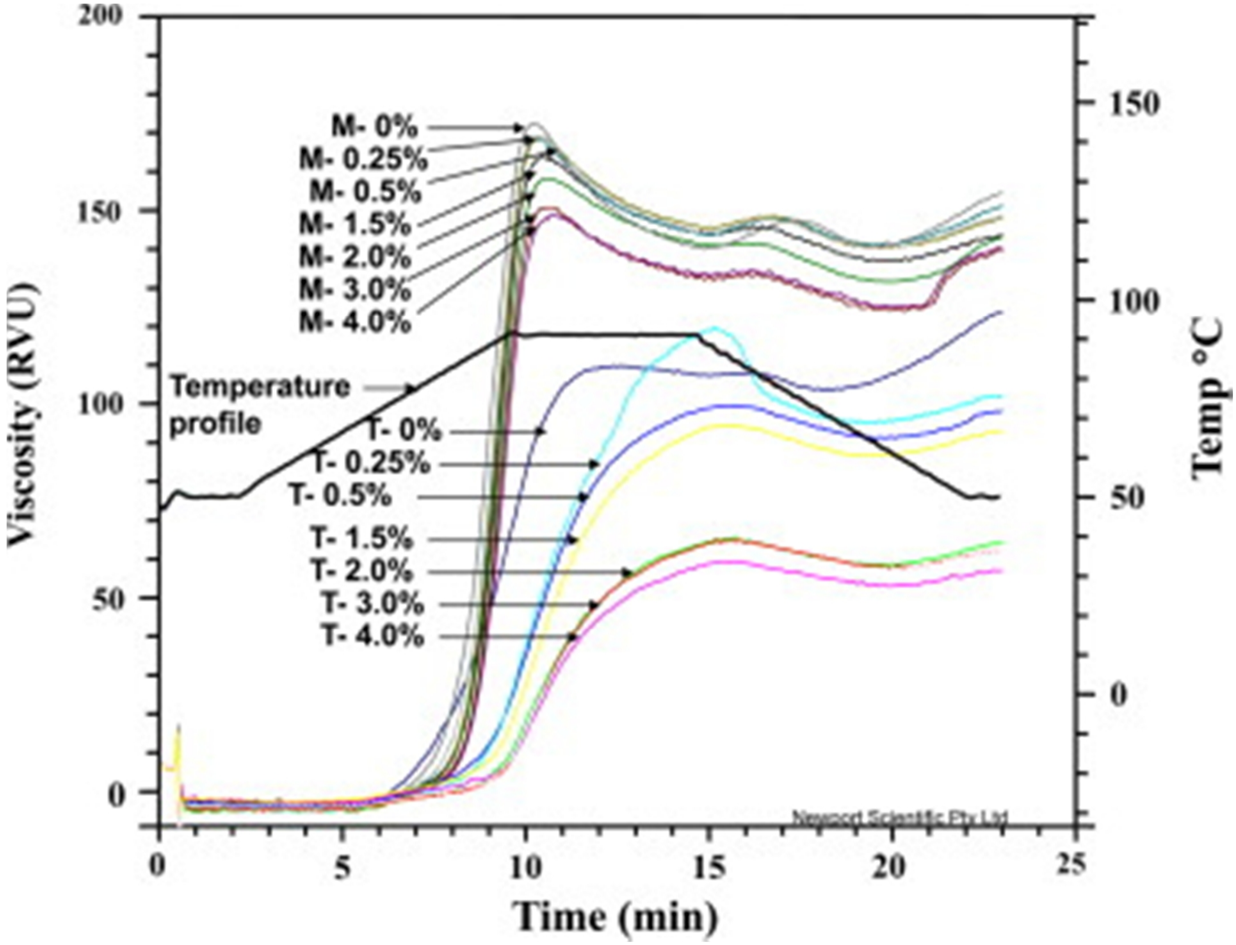
Values within the same columns with different letters are significantly different ($p < 0.05$)
Only 0.25% and 1.50% stearic acid used for extended pasting

Table 4

Texture of gels of teff and maize starches pasted for 120 min holding at 91°C in RVA

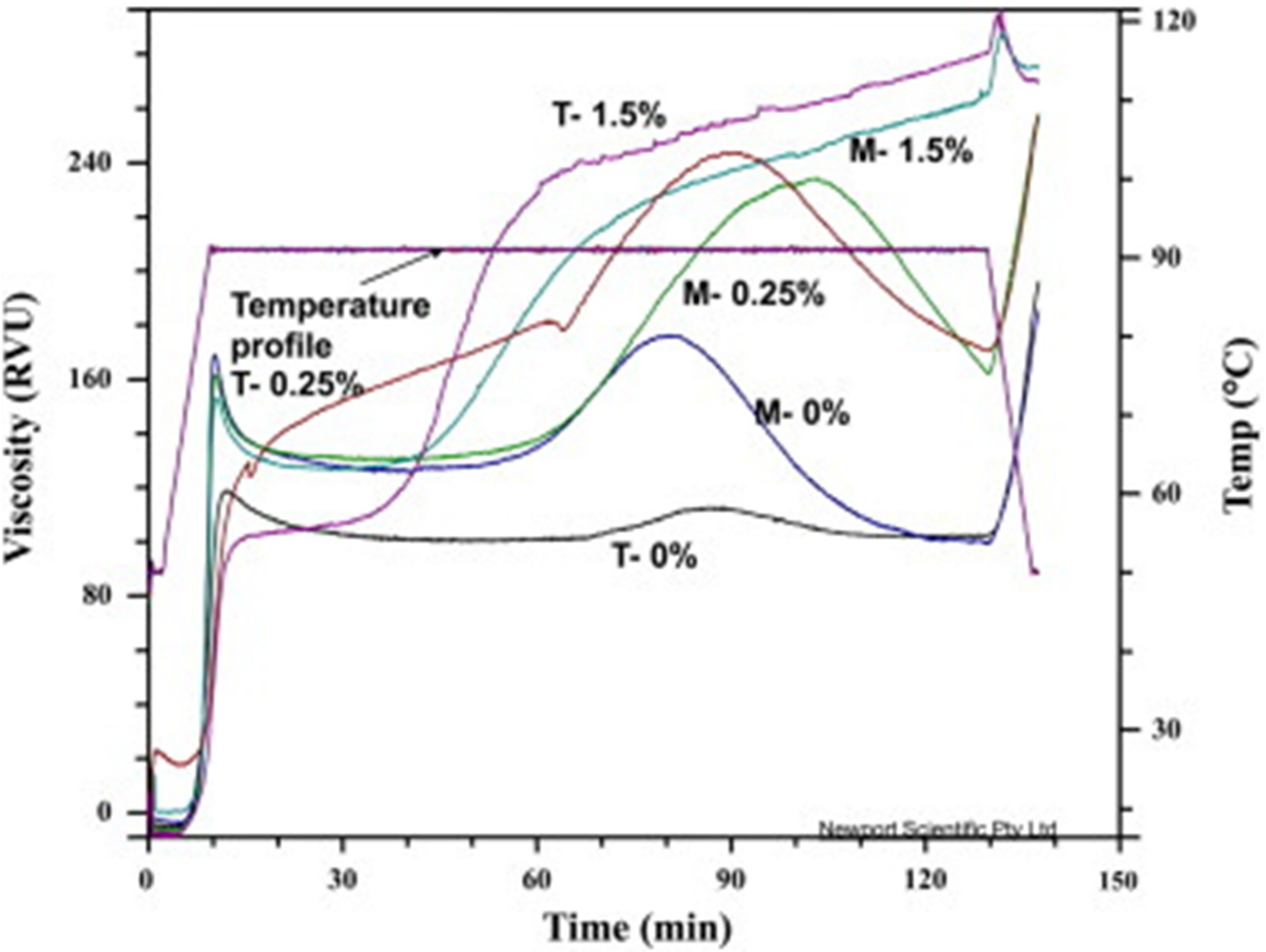
Stearic acid (%)	**Teff**		**Maize**	
	***Force (N)**	**Observations**	***Force (N)**	**Observations**
0	$4.2^{b} \pm 0.1$	Short; white	$3.8^{b} \pm 0.2$	Short; white
0.25	$3.4^{a} \pm 0.3$	Softer; white	$2.5^{a} \pm 0.3$	Softer; white
1.50	Did not gel but remained a very viscous paste			

Values within the same columns with different letters are significantly different ($p < 0.01$)
*Force required to compress gel by 5 mm



200
150
100
50
0
Viscosity (RVU)
150
100
50
0
Temp °C
0
5
10
15
20
25
Time (min)
M- 0%
M- 0.25%
M- 0.5%
M- 1.5%
M- 2.0%
M- 3.0%
M- 4.0%
Temperature profile
T- 0%
T- 0.25%
T- 0.5%
T- 1.5%
T- 2.0%
T- 3.0%
T- 4.0%
Newport Scientific Pty Ltd

Figure 1



Viscosity (RVU)
0
80
160
240
Temp (°C)
30
60
90
120
Time (min)
0
30
60
90
120
150
T- 1.5%
M- 1.5%
M- 0.25%
Temperature profile
T- 0.25%
M- 0%
T- 0%
Newport Scientific Pty Ltd

Figure 2

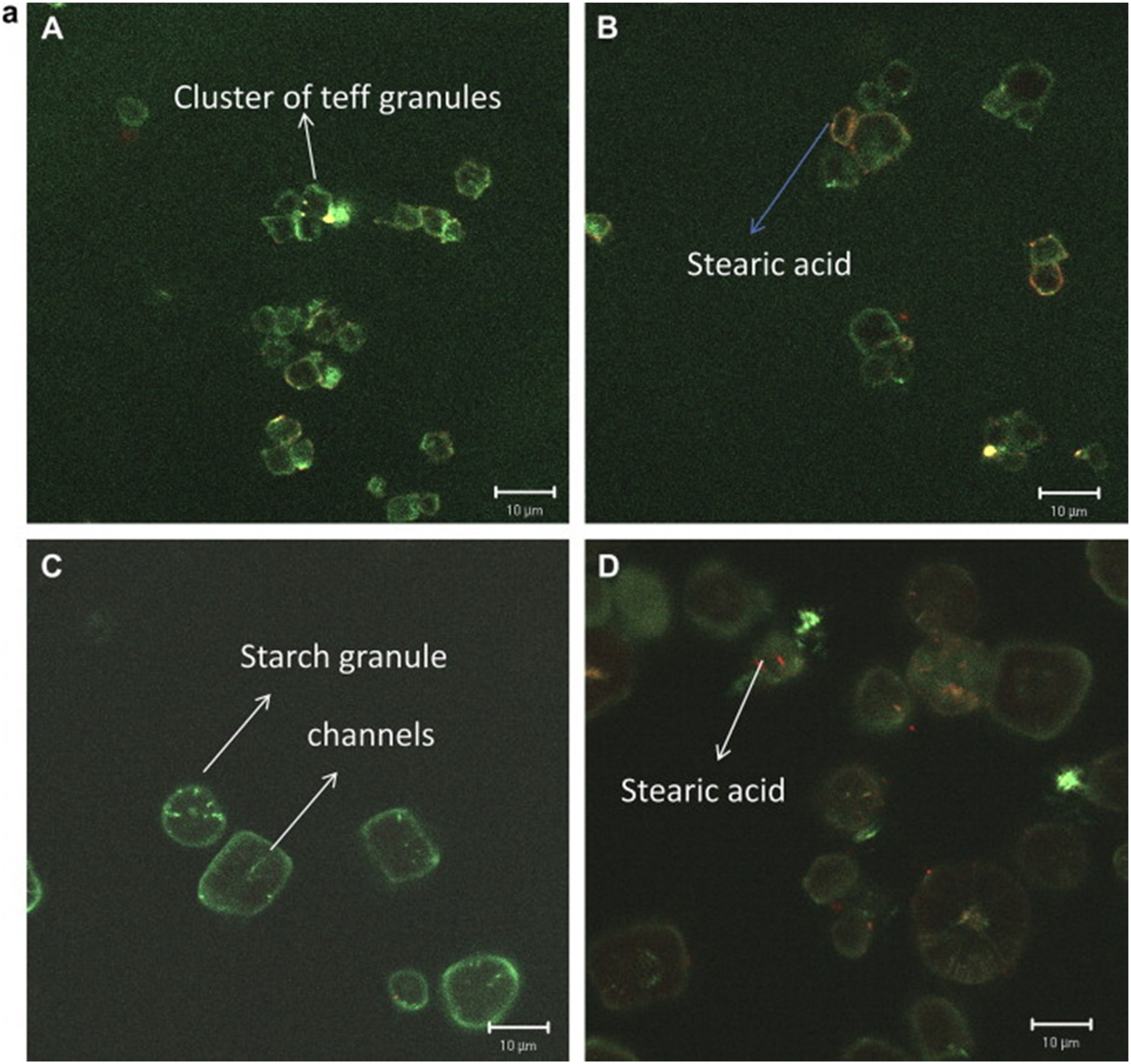
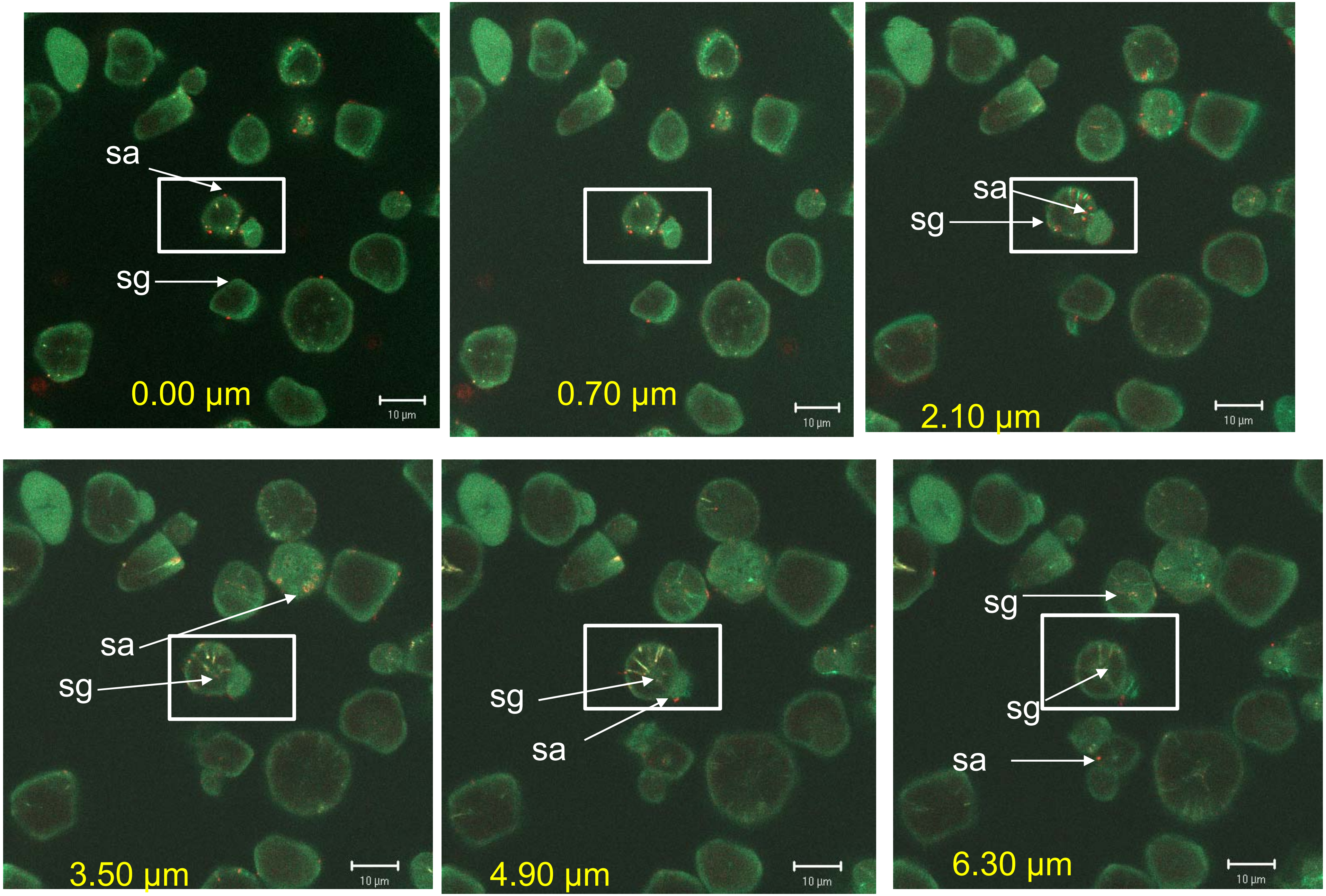

a
A
Cluster of teff granules
10 µm
B
Stearic acid
10 µm
C
Starch granule
channels
10 µm
D
Stearic acid
10 µm

Figure 3

Maize


sa
sg
0.00 µm
10 µm
0.70 µm
10 µm
sa
sg
2.10 µm
10 µm
sa
sg
3.50 µm
10 µm
sg
sa
4.90 µm
10 µm
sg
sg
sa
6.30 µm
10 µm

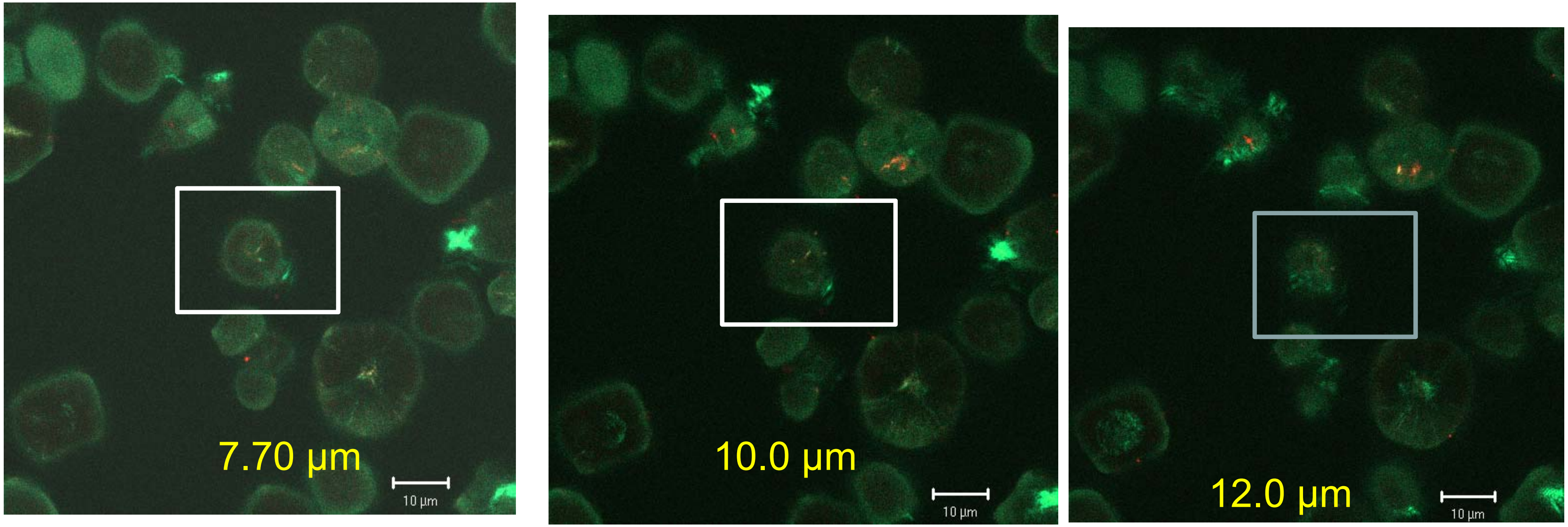


Figure X: Confocal laser scanning micrographs of maize starch granules before pasting
Z-slices at relative depth are shown in the figure
Follow the white rectangle to show the stearic acid stained as red

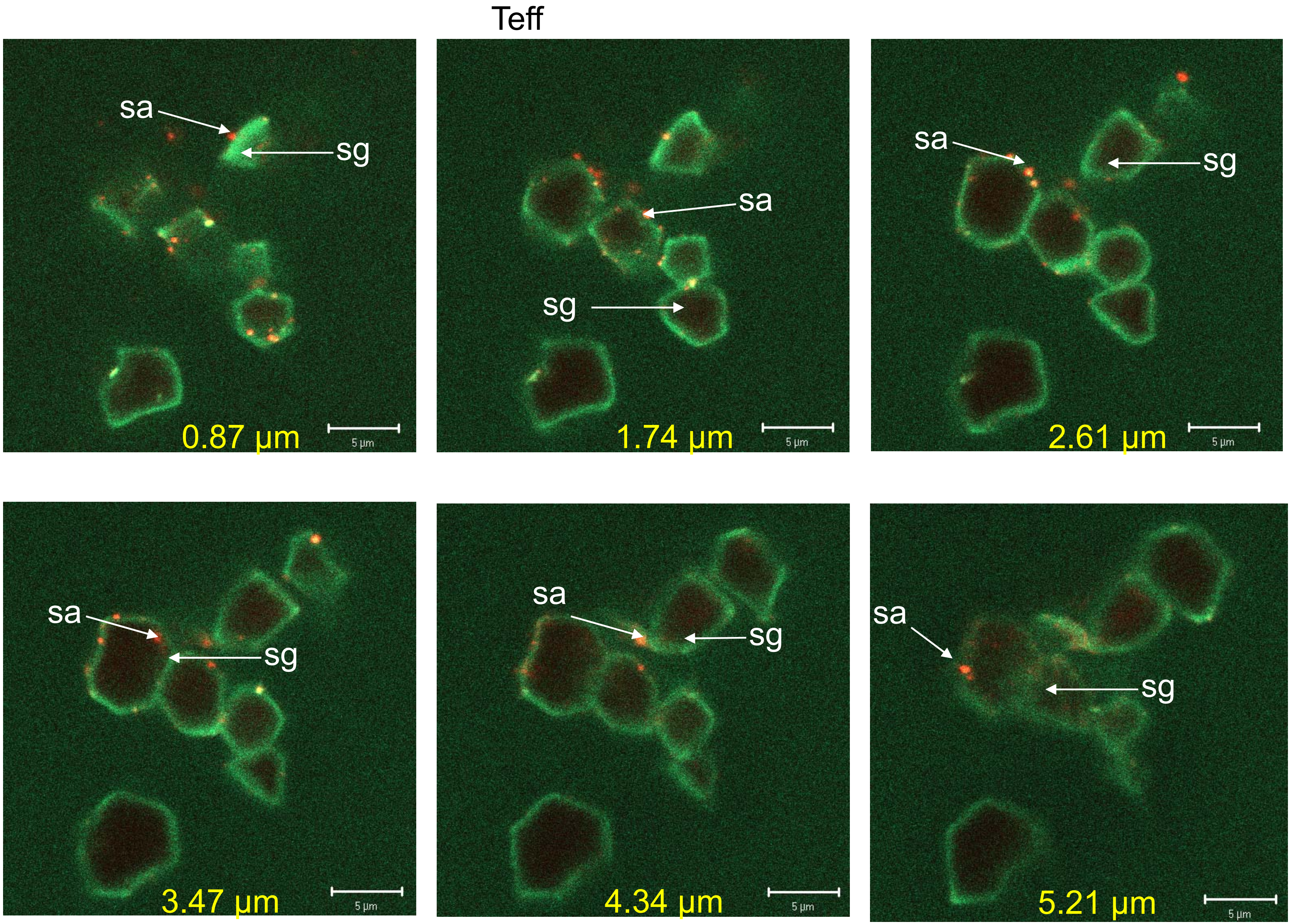


Fig. Y. Confocal laser scanning micrographs of teff starch granules before pasting
Z-slices at relative depth are shown in the figure