Changes In Pectin Integrity During Mango Fruit Ripening and Thermal Processing

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Abstract

Pectin gives structural strength to the primary cell wall and plays a key role in fruit and vegetable texture changes during ripening and thermal processing. Understanding the degree and design of methoxylation is an important attribute in functional properties of pectin. Two mango fruit varieties, Apple and Sabine at the unripe, intermediate and ripe stages were obtained from Makueni County, Kenya and their Alcohol insoluble pectin (AIR) extracted and segmented into water soluble pectin (WSP), Chelator soluble pectin (CSP), Sodium soluble pectin (SSP) and residue (cellullose and hemicellulose) fractions (HSP). AIR recovery rate for both thermally treated and untreated samples recorded a yield of ⩾95%. WSP yield (%) increased with ripening and thermal processing while SSP and CSP decreased significantly ($p \leqslant 0.05$) with ripening and thermal processing. The HSP yield for Apple and Sabine at the unripe stage recorded yields of ⩾3.84%. Galacturonic acid increased significantly ($p \leqslant 0.05$) from unripe (16.16%) to ripe (28.72%) stage in WSP fraction, while it decreased significantly ($p \leqslant 0.05$) in SSP with ripening in the two varieties. For neutral sugars, arabinose occurred most abundantly while mannose was the least and both were significantly different ($p \leqslant 0.05$) across the maturity stages of the two varieties. The overall degree of esterification for the two mango varieties was ⩾50%, thus on processing, products such as jam and other preserves, external pectin must be added to form stable firm gels.

***Keywords*: Alcohol Insoluble Pectin, Methoxylation, Pectin, Esterification, Neutral Sugars, Fruit Ripening**

Introduction

In plants, the primary cell wall occupies 90% and is comprised of polysaccharides and structural proteins (Yashoda *et al*., 2006). During ripening, the fruits show ultrastructural and biochemical changes of the polysaccharides these include increased solubility, depolymerization, de-esterification and loss of neutral sugars mainly in pectins and hemicelluloses; to a lesser extent acids and inorganic ions (Yashoda *et al*., 2006). These changes are both enzymatic and non-enzymatically instigated (Ali *et al*., 2004; Karakurt, 2007; Yashoda *et al*., 2006, 2007).

Pectins form about 30-35% of the primary cell wall (Yashoda *et al*., 2006, 2007). Pectins, rich in galacturonic acid (GalA) are responsible for structural strength of the primary cell wall. During ripening, processing and storage, structural changes occur mainly in pectin and to a small extent, in cellulosic and hemicellulosic materials. This leads to modification of the functional properties of products from fruits and vegetable (Houben *et al.,* 2011). Fruit firmness is associated with the stage of maturity where it decreases with ripening due to changes in cell wall compounds; mainly pectin as a result of their differences in solubility. Alcohol Insoluble Residue (AIR) can be segmented into various phases. Water soluble pectin (WSP) which consists of polymers loosely attached to the cell via non-covalent and non-ionic bonds. The Chelator-soluble pectin (CSP) comprises mostly of ionic pectin. Sodium-soluble pectin (SSP) is mostly comprised of covalent ester bonds (Sila *et al*., 2006b). Difference in firmness and rate of softening during ripening is mainly influenced by different amount of starch (non-structuctral carbohydrate), pectin, cellulose and hemicellulose.

Mature mango and banana fruits are found to have higher amounts of starch (20-25%) than fruits like guava, papaya and star fruit which have very little or no detectable amounts (Ali *et al*., 2004). Pectin degrading enzymes like polygalacturonase are responsible for ripening and subsequent softening of fruits like tomatoes, bananas and guava. Cellulase enzymes have been associated with ripening and softening of pears and avocado. β-endo-mannanase and α-manannosidase enzymes are associated with textural softening in banana, capsicum and papaya, (Tharanathan *et al*., 2007). However, ripening and softening of fruits is a complex physical and biochemical process still under study and all these enzymes are responsible for textural changes in all the fruits at different levels and rates. Pectin is the cell wall component which is highly involved in textural change during ripening and thermal processing. This study investigated its content and distribution (Galacturonic acid) in AIR, WSP, CSP and SSP among selected mango varieties of *Apple* and *Sabine* at three stages of maturity as well its role in thermal and textural processes.

Materials and Methods

Apple and *Sabine* mango varieties at mature green stage were selected for this study because they exhibited striking difference ($p \leq 0.05$) in their quality characteristics (Okoth E. M et al., 2013). *Apple* showed the most superior qualities, while *Sabine* the most inferior. They were harvested at Makueni County and transported to JKUAT workshop. The fruits were washed with detergent, dried with clean hand towel and left overnight to acclimatize to remove the field heat. They ripened at 25ºC±2 and relative humidity of 65-70% for 6-10 days.

Sample Preparation

The flesh was homogenized and analyzed at three stages of maturity (unripe, intermediate and ripe). The pasteurized ripe samples were prepared by heating the homogenized ripe pulp to 80°C and holding it for 5 minutes. They were cooled to room temperature and refrigerated at 4-10ºC for further analysis. The sterilized samples were prepared by canning in a retort at 121°C for 10 minutes. They were removed, cooled to room temperature and refrigerated at 4-10 ºC for analysis.

Isolation Of Pectin from The Mango Cell Wall

Cell wall material of *Apple* and *Sabine* mango varieties were isolated as Alcohol Insoluble Residue (AIR) (McFeeters and Armstrong, 1984). About 10g of the mango pulp was weighed, frozen under liquid nitrogen and homogenized twice in a blender/mixer for 20 seconds at 7500rpm. The mixture was then homogenized three times for six seconds in 64ml of 95% ethanol. The suspension was filtered and the residue was homogenized again in 32ml of acetone and stirred for 10 minutes in a cold room and filtered. The residue was placed in a large Petri dish and dried overnight at 40ºC. The AIR was ground in a mortar and pestle, weighed and stored in a desiccator over phosphorous pentoxide (P_2O_5) until analysis.

Fractionation of alcohol-insoluble residue (AIR)

The cell wall polysaccharides present in AIR were fractionated into various pectin fractions which included water-soluble pectin fraction (WSP), a chelator–soluble pectin fraction (CSP), sodium carbonate-soluble pectin fraction (SSP) and hemicellulose soluble pectin (HSP).

Water Soluble Pectin Fraction

This was done according to Sila *et al*., (2006). Approximately 0.25g AIR was incubated in 45mL of boiling water (100ºC) while stirring for 5minutes. The suspension was cooled in cold running tap water and filtered. The filtrate was adjusted to 50mL with water, frozen in liquid nitrogen and stored at -40ºC.

Chelator Soluble Pectin Fraction

The residue obtained from 3.3.1 was re-suspended in 45mL of 0.05M cyclohexane-trans-1, 2-diamine tetra-acetic acid (CDTA) in 0.1M potassium acetate at pH 6.5 for 6hrs. It was stirred for 15 minutes at room temperature, then in a shaking water bath at 28ºC. The suspension was filtered using filter paper and the volume adjusted to 50mLwith CDTA solution. The chelate soluble pectin sample was frozen in liquid nitrogen and stored at -40ºC.

Sodium Carbonate Soluble Pectin Fraction

The residue from 3.3.2 was re-incubated in 45mL 0.05M Na_2CO_3 containing 0.02M $NaBH_4$ for 16 hours with constant stirring. It was subsequently incubated for 6hours at 28ºC in a shaking water bath. The mixture was carefully filtered, and the volume adjusted to 50mL with a solution of Na_2CO_3 and $NaBH_4$, thus obtaining sodium carbonate-soluble pectin fraction, frozen in liquid nitrogen and stored at -40ºC. All the fractionation was carried out in triplicate.

Hemicellulose Soluble Pectin Fraction

This was done by the fast one-step acid hydrolysis method and compared with values determined by conventional NREL method (Sluiter et al., 2008b). In this method, 0.3g biomass (dry weight) that was hydrolyzed in 88 mL of 4.2% sulphuric acid at 121℃ for 1 hour. Then the liquid was drawn, centrifuged, and stored at 4℃ until analysis for sugar content.

Determination Of the Degree of Methyl Esterification (DE)

Degree of methyl esterification (DM) was determined as the ratio of methanol to GalA content. To quantify the amount of methanol, saponification of the ester bonds in the pectin with sodium hydroxide was done according to Ng and Waldron (1997). The amount of methanol released was determined spectrophotometrically according to Klavons and Bennett (1996).

Galacturonic Acid Determination

This was done using Ahmed and Labavitch, (1978) method. Approximately 10mg of AIR, WSP, CSP SSP, HSP and the residue were hydrolyzed with 8 ml of concentrated sulphuric acid and stirred continuously for 1 hour. The uronic acid was quantified by a spectrophotometer at 520 nm at 25°C according to Blumenkrantz and Asboe-Hansen (1973). All the hydrolysates were performed in duplicate and three colorimetric analyses performed for each.

Neutral Sugar Determination

Approximately 1 mL of dialyzed and lyophilized Alcohol insoluble Residue (AIR) and the pectin fractions were hydrolyzed with 1 ml of 4 M trifluoroacetic acid for 1.5 hours at 110°C under a reflux condenser. The samples were cooled and trifluoroacetic acid evaporated. The samples were then diluted with de-mineralized water to 0.1% w/v. Quantification of the neutral sugars was done with a high-performance liquid chromatography (HPLC); fitted with 6mm thermo Fisher refractive index detector, (column NH2P amino, 5µm, 250 x 4.60 mm; eluted with acetonitrile/water 73:27 (v/v) as a mobile phase at a flow rate of 1 ml/min. Commercial neutral sugar standards were used to identify and quantify specific neutral sugars present in the samples.

Results and Discussion

Yield (%) Of the Pectin Fractions

Pectin is key in biochemical processes such as ripening and thermal processing. Percent yield of the pectin fractions of untreated and thermally treated *Apple* and *Sabine* mango varieties at the three stages were determined and results presented in Table 1.

Table 1 Percentage Yield Pectin In WSP, CSP, SSP, HSP And Residue of Treated And Untreated Mango Fruit Samples

SAMPLE	WSP%	CSP%	SSP%	HSP%	RESIDUE%
Apple unripe	15.75±1.2h	56.61±2.0a	20.48±1.2c	3.84±0.5a	3.32±0.02d
Apple intermediate	21.11±1.5g	52.80±1.8b	18.60±1.0ef	2.99±0.2abc	4.33±0.05cd
Apple ripe	35.42±1.2e	43.36±1.5c	17.23±1.0fg	1.45±0.02cde	2.54±0.05ab
Apple pasteurized	43.33±2.0c	30.73±1.5f	18.81±1.8ef	0.33±0.01e	6.80±0.03abc
Apple sterilized	41.15±1.2d	29.33±1.2f	19.15±1.0de	2.79±0.1abcd	7.58±0.05cd
Sabine unripe	23.16±1.0g	44.18±1.2c	26.81±1.2a	3.85±0.1a	2.00±0.05a
Sabine intermediate	32.69±1.5f	39.67±1.3d	24.11±1.2b	2.85±0.1abc	0.68±0.05ab
Sabine ripe	35.24±1.5e	36.85±1.2e	22.16±1.0bc	1.22±0.1de	4.53±0.02bcd
Sabine pasteurized	49.03±2.0b	15.93±1.0h	22.23±1.0b	2.21±0.1bcd	10.6±0.02bcd
Sabine sterilization	59.18±2.1a	18.50±1.2g	16.44±1.0g	2.19±0.2bcd	3.69±0.02ab
LSD (p≤0.05)	1.849	1.91	1.724	1.436	0.163

Mean (±SD) sharing similar superscript letters in a column are not significantly different at p≤0.05; WSP: water soluble pectin; CSP: chelator soluble pectin; SSP: sodium soluble pectin; HSP: hemicellulose soluble pectin; (n=3).

Yield determination is important in the quantification of the recovered pectin. There was % yield increase in WSP with ripening and thermal processing, while CSP, SSP and hemicelluloses fractions decreased. Similar results were obtained from sweet cherries (Rodríguez *et al*., 2005). The recovery of AIR for both thermally treated and untreated pectin fraction samples was considerably high (≥95.68%). The concentration of galacturonic acid was higher in EDTA-soluble fraction, indicating that the pectin complex in the cell wall of mango pulp is ionic in nature. These results were inconsistent with those obtained on analyzing Alphonso mango fruit, (Yashoda *et al*., 2006) and other selected tropical fruits (mango, guava, dates and strawberry).

Galacturonic Acid Content of Untreated Mango Samples

Galacturonic Acid content of AIR and the pectin fractions were evaluated by measuring them colorimetrically. The results obtained were as presented in Table 2.

Table 2 Galacturonic Acid Content of Alcohol Insoluble Pectin (AIR) And The Pectin Fractions (Mg/G) In Apple And Sabine Varieties At Three Stages Of Ripeness

SAMPLE	WSP	CSP	SSP	HSP	RESIDUE	AIR
AVU	16.16±0.01d	58.09±0.09a	21.02±0.01b	3.94±0.04ab	0.15±0.01^{c}	99.36±0.04^{ab}
AVI	20.86±0.03c	52.18±0.02b	18.38±0.01c	2.96±0.01abc	0.17±0.01^{c}	94.55±0.02^{b}
AVR	22.78±0.01^{c}	28.72±0.01^{d}	9.91±0.01^{d}	1.09±0.02^{c}	0.30±0.01^{c}	62.80±0.04^{d}
SVU	21.55±0.1^{c}	47.25±0.04^{c}	26.53±0.02^{a}	4.12±0.02^{a}	0.50±0.01^{b}	99.95±0.01^{a}

SVI	24.65±0.01^{b}	21.46±0.01^{e}	19.40±0.03^{bc}	3.10±0.02^{abc}	0.29±0.01^{c}	68.90±0.06^{c}
SVR	28.72±0.02^{a}	17.02±0.03^{f}	11.50±0.01^{d}	2.07±0.01^{bc}	0.69±0.01^{a}	60.00±0.01^{f}
LSD:p≤0.05	1.926	1.820	1.933	1.700	0.178	2.255

Mean (±SD) sharing similar superscript letters in a column are not significantly different at p≤0.05 AVU: *Apple* variety Unripe; AVI: *Apple* variety Intermediate; AVR: *Apple* variety Ripe; SVU: *Sabine* variety Unripe; SVI: *Sabine* variety Intermediate SVR: *Sabine* variety ripe.

Galacturonic acid is the main cell wall sugar in pectin. Its content is used to estimate the contribution of pectin in each polysaccharide fraction to the entire pectin population embodied by the AIR (Azad *et al.*, 2014). The results revealed that different mango varieties have different contents of pectin in different fraction; WSP, CSP, SSP and residue in their cell wall at different stages of ripeness. With progressive ripening and texture softening, a decreasing trend in % pectin in CSP, SSP HSP and AIR were observed in both varieties. The decrease rate in pectin (%) from unripe to ripe in *Apple* samples was significantly different from that of *Sabine* at p≤0.05. They had; 51.79% and 63.98% (CSP); 52.85% and 56.65% (SSP); 72.33% and 49.76% (HSP) respectively. The pectin in WSP and the residue fraction in the two varieties showed a significantly different increasing trend at p≤0.05. The increase was 29.06% (*Apple*) and 24.96% (*Sabine*) respectively. Pectin increase in WSP may have been due to the shift of galacturonan from the CDTA- and CO_2-soluble fractions to the water-soluble fraction as observed in Carabora (Yadao *et al*., 2012). *Apple* and *Sabine* recorded a significant difference in residue fraction of 50% and 27.53%, respectively during ripening.

The ionically bound cell wall pectin fraction (CSP) in *Apple* variety was most abundant (58.09, 52.18 and 28.72 mg GalA/g of AIR) at the three stages of ripeness respectively. In *Sabine* variety, pectin in CSP was dominant at the unripe stage (47.25mg GalA/g of AIR); whereas pectin in WSP was dominant at the intermediate (24.65mg GalA/g of AIR) and ripe stage (28.72mg GalA/g of AIR), respectively. The decrease in AIR with ripening shows that the large alcohol–insoluble polymers are degraded to shorter alcohol-soluble polymers during ripening (Prasanna, *et al*., 2003; Yashoda *et al*., 2006). Since the SSP (protopectin) is structurally bound to the cell wall, (Ketsa *et al*., 1998), the fruit softening during ripening resulted from disintegration of the cell wall caused by degradation of the protopectin.

Pectin content in WSF, CSP, and SSP are the major polysaccharides which undergo depolymerization during ripening, thus contributing to tissues softening and textural – organoleptic changes (Yashoda *et al*., 2006) among other processes. The results are in agreement with those reported for other climacteric fruits like, Carambola and sweet cherries (Lazan *et al*., 1999), respectively. An increase in pectin with increased ripeness was recorded of rasberryfruits (Vincente, et al., 2014) Lemon pomace fruits showed an overall decrease in pectin from premature to mature, then an increase from mature to ripe stage (Azad *et al.*, 2014). We concluded that pectin content varies in different fractions, species, variety and stage of ripeness.

Degree Of Methyl Esterification Of AIR, Water and Chelator Fraction

The DE of AIR, WSP and CSP in *Apple* and *Sabine* mango varieties at the three stages of maturity were analyzed and results presented in Table 3.

Table 3 Degree Of Methyl Esterification (DE) Of AIR, WSP And CSP In Apple And Sabine Mango Varieties

SAMPLE	AIR	WSP	CSP
Apple Unripe	64.80±0.22a	61.87±0.22b	77.62±2.76b
Apple Intermediate	**46.81±0.60c**	**60.56±0.60B**	**46.53±0.13e**
Apple Ripe	43.83±0.96d	30.38±0.08e	42.49±0.13f
Sabine Unripe	**56.12±1.17b**	**77.30±0.17a**	**85.17±4.52a**
Sabine Intermediate	19.36±0.26e	54.38±0.01c	67.80±3.78c
Sabine Ripe	**14.07±0.23F**	**49.45±0.28d**	**63.16±3.32d**
LSD (p≤0.05)	1.881	1.838	1.779

Mean (±SD) sharing similar superscript letters in a column are not significantly different at p≤0.05; AIR: Alcohol insoluble residue; WSP: water soluble pectin; CSP: chelator soluble pectin; (n=3).

The degree of methyl esterification gives a measure of the methyl esters removed from pectin during ripening process which brings about the softening of the plant tissues. Degree of esterification (DE) depends on species, type of tissue and stage of ripeness (Azad *et al*., 2014). The results reveal that the unripe samples had the highest DE which decreased with ripening in the two mango varieties. CSP had the highest DE for both varieties, *Apple* (77.62%) and *Sabine* (85.17%) at the unripe stage of ripeness respectively. The decrease in *Apple* variety was 32.36% (AIR), 50.60% (WSP) and 45.26% (CSP), while that of *Sabine* variety was 69.49% (AIR), 36.03% (WSP) and 25.84% CSP) respectively. This shows that variety and stage of ripeness has influence on the DE. Based on DE pectin classification, ≥ 50% DE pectin is high methoxy pectin, while DE of pectin ≤50% is low methoxy pectin (Azad *et al*., 2014; Sayeeb & Singh, 2014). The results reveal that WSP and CSP fractions exhibited high methoxy pectin at the three stages of ripeness while AIR exhibited high methoxy pectin at the unripe stage only. A similar decrease was recorded on several fruits as they ripened, this included peach fruit from 74.40% (Unripe) to 46.80% (fully ripe), (Lurie *et al*., 2003); Lemon pomace fruits from 79.51 to 33.59%, (Azad *et al.*, 2014); Citrus maxima from 76.30 to 33%, (Sotanaphun *et al*., 2012) and on dragon fruit from 52% to 31% (Ismail *et al*., 2012). Low DE could be as a result of the conversion of protopectin into pectin which leads to high sugar level and a softer fruit as it matures and ripens (Azad *et al*., 2014).

The type of pectin determines the mechanism for gel formation (ability and rate of gelling). Low methoxy pectin forms gel with the addition of low quantities of sugar or without addition of sugar in a divalent cation like Ca^{2+}. They are also not as sensitive to pH as high methoxy pectin. A higher degree of methyl-esterification results in rapid setting of a gel (Sayeeb and Sigh, 2014). The high methoxy pectin requires heating in sugar solutions of concentrations more than 55% and pH of 3.5 for a good gel to form (Woo *et al*., 2010; Ranajit *et al*., 2013).

Changes In Overall Neutral Sugar Composition in *Apple* and *Sabine* Mango Fruit Cell Wall During Ripening

Neutral sugars, arabinose, mannose, xylose, galactose, glucose and rhamnose were analyzed at different stages of ripeness and results presented in Table 4.

Table 4 Changes in Neutral Sugar Composition In Apple And Sabine Mango Fruit Cell Wall During Ripening

		Sugar composition (mg/g)					
Variety	**Ripeness stage**	**Arabinose**	**Mannose**	**Xylose**	**Galactose**	**Glucose**	**Rhamnose**
Apple	Unripe	9.15±1.2a	0.82±0.8e	2.38±1.2c	5.62±0.2a	3.55±0.2b	1.30±0.8c
	Intermediate	6.25±2.5b	1.52±1.2b	2.86±0.5b	3.45±0.2c	4.75±0.5a	1.90±1.2b
	Ripe	3.92±1.8e	1.85±1.0a	3.17±0.2a	2.52±0.2f	2.85±0.2c	2.54±1.2a
Sabine	Unripe	5.84±1.5c	0.40±0.1f	0.12±0.1f	4.23±0.2b	1.30±0.2cd	0.45±0.5d
	Intermediate	5.50±1.0d	1.35±0.2d	1.05±0.4e	3.15±0.02d	2.65±0.1c	1.42±1.2c
	Ripe	3.25±0.8f	1.53±0.4b	2.06±0.2d	2.84±0.02e	2.10±0.1d	1.85±0.8b
LSD	($p \leq 0.05$)	0.197	0.017	0.139	0.133	0.151	0.178

Mean (±SD) sharing similar superscript letters in a column are not significantly different at $p \leq 0.05$.

Arabinose was most abundant in the two mango varieties at the three stages of maturity followed by galactose and glucose respectively. The results compare well with those obtained by Azad *et al*., (2014) on lemon pomace fruit. Mannose exhibited the least occurrence of these sugars. Arabinose, xylose and galactose decreased with ripeness while mannose and rhamnose contents increased. Glucose in *Apple* and *Sabine* varieties increased by 33.80% and 103.84% from unripe to intermediate stages, then decreased in the ripe stage by 50.94% and 20.75%, respectively. All the neutral sugar proportions in *Apple* mango variety were higher than those observed in *Sabine* variety at the three stages of ripeness. This suggested variability among mango variety in cell wall sugar composition and its modification with ripening. This compares well with those observed on analyzing Kent and Tommy Atkins varieties (Brito *et al*., 2012). Kent had higher neutral sugar contents at all stages of ripening than Tommy Atkins.

A decrease in all the six neutral sugars with ripening was reported on Sapodilla fruits (Morais *et al*., 2008); while an increase of the six sugars was reported on lemon pomace fruits (Azad *et al*., 2014); A decrease in rhamnose, arabinose, galactose and glucose was reported on Tommy Atkins, while xylose and mannose increased (Carrillo-López *et al*., 2016). According to the reports on raspberry fruits, arabinose, xylose and mannose increased with ripening, while rhamnose, fucose and galactose decreased. This reveals that, not only did the neutral sugars vary in content and stage of ripeness, but also across different fruit species.

Conclusion

The study revealed that different mango varieties have different pectin contents in different fraction, WSP, CSP SSP and hemicellulose in their cell wall at different stages of maturity. They were also found to have different neutral sugar proportions. With progressive ripening and texture softening, a decreasing trend in percentage pectin in CSP, SSP and HSP was observed in the two varieties. The ionically bound cell wall pectin fraction (CSP) in *Apple* variety was the most abundant at the three stages of ripeness. The decrease in AIR with ripening shows that the large alcohol–insoluble polymers are degraded to shorter alcohol-soluble polymers during ripening.

There was a percentage yield increase in WSP and residue (cellulose) fractions with ripening and thermal processing, while yield in CSP, SSP and hemicelluloses fractions decreased. The amount of galacturonic acid was higher in EDTA-soluble fraction, indicating that the pectic complex in the cell wall of mango pulp is essentially ionic in nature. There was a decrease in the degree of methyl-esterification (DE) of pectin with ripening in the two mango varieties, where the variety and stage of ripeness had influence on the DE. The mango fruits analyzed had HMP in their WSP and CSP fraction, while AIR had HMP in its unripe stage. The pectin content in the two mango varieties studied was not sufficient to form a stable gel in preserves and jam hence the authors recommend addition of external or commercial pectin when processing these products. Further studies on ethylene production at the various stages of ripeness would be relevant to add more information on the changes occurring during ripening.

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Conflict of Interest Statement

The authors wish to state that there is no conflict of interest among the authors.

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