

Effect of freezing rates and freeze-thaw cycles on the texture, microstructure and pectic substances of mango

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<u>Abstract</u>

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Introduction

Freezing is a well-known preservation method widely used in the food industry. The freezing process combines the favorable effects of low temperatures with the conversion of water into ice. The waterice transition has the advantage of fixing the tissue structure and separating the water fraction in the form of ice crystals so that water is not available as either a solvent, as a reactive component (Delgado and Sun, 2001) or for microbial growth. However, this preservation technique may result in textural changes leading to food softening. The quality of frozen-thawed fruit can depend on many factors including the optimal freezing process and a proper distribution chain.

To elaborate, the freezing rate has a direct effect on the final product texture. The size and location of ice crystals formed during the freezing process may damage cell membranes and breakdown the physical structure of the fruit. The cause of undesirable physicochemical modifications during freezing can be due to both the water and solute crystallization process (Delgado and Sun, 2001). It is well-known that the crystallization of ice has two steps: the formation of nuclei and the later growth of the nuclei to a specific crystal size. The final ice crystal size is known to be a function of the rates of nucleation and crystal growth, and also of the final temperature

The objective of this study was to investigate the effect of freezing rates and freeze-thaw cycles on the texture, microstructure and pectic substances (i.e. alcohol insoluble solids (AIS), total pectin (TP), water soluble pectin (WSP) and ammonium oxalate soluble pectin (ASP)) of mango. With repeated freeze-thaw cycles, the WSP, ASP, and drip loss demonstrated a tendency to increase whereas firmness decreased. Although freezing reduced the firmness, fast frozen samples had the highest firmness values. A microstructure analysis showed that the cell walls of the frozen samples were destroyed by ice formation leading to cell shrinkage and collapse. The degradation of the cell walls was more obvious for slow frozen samples than for the fast and medium frozen samples. This study shows that freezing rates and repeated freezing and thawing have an obvious impact on the quality of soft fruits such as the ripe mango.

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(Martino *et al.*, 1998). Slow freezing (SF) generally leads to large ice crystals being formed exclusively in the extracellular areas that can damage cell structure and have an effect on the thaw behavior as well as on the sensory properties and nutritional value of foodstuffs. High freezing (HF) rates are known to produce small crystals evenly distributed throughout the tissue. Therefore, a fast freezing rate may be preferable since it can reduce damage to the fruit cell membranes.

However, the quality of the frozen product not only depends on a proper freezing rate, but also on the number of freeze-thaw cycles. It is often the case that temperature changes can occur in frozen products during transport and storage. Such fluctuations can include (i) food pack abuse in the outermost corner of a pallet of frozen food kept at room temperature, (ii) pack defrosting in a display cabinet, and (iii) abuse during transport of frozen food products to the home after being purchased by the customer.

Freeze-thaw methods have been widely used with starch-based foods such as rice starch gels (Charoenrein *et al.*, 2011) and Chinese water chestnut starch gels (Lan *et al.*, 2008) but the effects of freezethaw cycles on fruits have never been reported. In this research, freeze-thaw cycles were used to examine the conditions under which frozen mangoes are stored, distributed and consumer handled. Mangoes were chosen for this research because the effects of

freezing on mangoes have never been studied while that of other fruits such as apple (Chassagne-Berces et al., 2009), raspberry and blackberry (Sousa et al., 2007) and strawberry (Van Buggenhout et al., 2006) have been reported. Moreover, mangoes have a relatively short harvest season and storage life. The mango, Magifera Indica L. is well known for its excellent flavour and is usually referred to as the king of fruit (Sivakumar et al., 2011). It is a dicotyledonous plant belonging to the order sapindales in the family Anacardiaceae which is a popular and economically important fruit, widely cultivated in the tropics and subtropics. The 'Nam Dok Mai' mango is considered to be one of the best mango cultivars in Thailand. The popularity of this fruit in the international market is due to its excellent flavour, attractive fragrance, beautiful colour and beneficial nutritional properties. In addition, mangoes are a good source of ascorbic acid, carotenoids, phenolic compounds, and other dietary antioxidants (bioactive compounds) (Talcott et al., 2005). In Thailand, 'Nam Dok Mai' mangoes are only available for harvest from the beginning of March until the end of May. In addition, the storage life of mangoes is limited to 2-3 days at ambient temperature, or 2-3 weeks at 10-15 °C (Yahia, 1998). Therefore, preservation of the fruit to extend its shelf life could help solve the supply and demand problems related to these issues.

Nam Dok Mai mangoes are often soft when ripe. Textural softening in fruit during ripening is presumably related to the depolymerization and solubilization of pectic substances in the middle lamella of the cell wall (Ketsa et al., 1999). Pectic substances are the major cell component found in the primary cell wall and in the intercellular layers known as the middle lamella, and help cells to connect to one another. A previous study reported that pectic compounds are responsible for the mechanical strength of the primary cell wall of fruits and vegetables and the adhesion between cells. Changes in apple texture, such as softening, were found to be closely related to the chemical changes in the pectic substances and the middle lamella (Lapsley et al., 1992). However, the study of changes in pectic properties due to freezing is sparse. This study aimed to investigate the effects of freezing rates and freezethaw cycles on the texture, microstructure and pectic substances of the mango cv. 'Nam Dok Mai' fruit.

Materials and Methods

Mangoes (*Mangifera indica* L. cv. Nam Dok Mai) were purchased from an orchard in Chachoengsao (Thailand) during the fruiting season from March to

May of 2011. The fruits were carefully picked and selected for uniformity in size (350-400 g each) and maturity based on skin and flesh colour, texture, and total soluble solids. The total soluble solids, measured using a hand refractometer, were found to lie in the range of 18-20°Brix. Approximately twenty percent from both the stem and the blossom end of the fruit were discarded, as those areas are known to be highly diverse in terms of fruit properties. Therefore only the central parts of the fruits were used to minimize variations within the samples. The samples were washed, peeled, and cut into cubes of 1.5 cm in length. The samples were then packed 8 pieces per bag in nylon pouches (NY/LLDPE, 70 μ m, 100 x 150 mm) and sealed.

Freezing and freeze-thaw cycles

Samples were frozen at -80 (FF) and -40°C (MF) in a cryogenic freezer (Minibatch 1000L, Bangkok Industrial Gas Co., Bangkok, Thailand) and -20°C (SF) in a chest freezer (Sanyo refrigerator, model SF-C1497, Sanyo (Thailand) Co., Ltd., Bangkok, Thailand). The temperature at the cubic core was monitored with thermocouples placed in the cubes centre. Freezing was terminated once the thermal centre of the samples reached -20°C and the samples were then stored at -18°C. These measurements ensured that optimal freezing temperatures within the samples were reached and made it possible to estimate the freezing rates (FR) as the average of the ratio between the temperature gap (ΔT) and the freezing time (tf). Freezing rates (in °C/min) were determined according to the following equation:

$$FR = \frac{T_i - T_f}{ff}$$
(1)

where T_i is the initial temperature (20°C), T_f is the final temperature (-20°C) and tf is the time to reach final temperature from initial temperature (Olivera and Salvadori, 2009). The three tested protocols used fast (2.91°C/min), medium (1.69°C/min) and slow (0.05°C/min) freezing rates. The first freezing cycle consisted of a freezing stage at -18°C for 7 days followed by thawing at 4°C. This freeze-thaw cycle was repeated up to three times.

Frozen mango pieces were thawed in a lowtemperature incubator at 4°C until the thermal centre of the samples reached 0°C. The physical and chemical properties of the samples were then measured after 1, 2 and 3 freeze-thaw cycles. Fresh samples were used as reference samples for each experiment. The experiments were each repeated twice.

Pectic substances

The cell wall material was prepared by homogenizing 35 g of the fresh and freeze-thaw samples separately in 150 ml of 95% ethanol, filtering the samples (Whatman no.1), and then washing with 70% ethanol and acetone. The alcohol-insoluble solid residue (AIS) was dried for 24 h. at 40°C and weighed. The AIS was then analyzed for total pectin (TP), water soluble pectin (WSP) and ammonium oxalate soluble pectin (ASP) using the method of Taboada *et al.* (2010) by extracting three times for each fraction.

The prepared AIS (0.05 g) was dispersed at room temperature in 100 ml of distilled water by stirring for 30 min, being centrifuged (7000 g) and the supernatant comprised of the water-soluble pectin (WSP) was removed. The residue was subsequently extracted with 100 ml 1% ammonium oxalate (pH 4.5) at room temperature and centrifuged as above. The supernatant containing the ammonium oxalatesoluble pectin (ASP) was then collected for analysis. The galacturonic acid content in each of the pectin fraction was spectrophotometrically determined by the alkaline m-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) using D-galacturonic acid monohydrate (Sigma-Aldrich, St. Louis, USA) as the standard.

Microstructure

The microstructure of fresh and freeze-thaw mango was determined by the method adapted from Sirijariyawat *et al.* (2012). A small section from the inner part of the mango cubes was excised with a razor blade. The thickness of the sections was approximately 1 mm. The microstructure of the samples was observed using a confocal laser scanning microscope (Axio Imager MI, Carl Zeiss PTe Ltd., Gottingen, Germany). The cell walls of the samples were stained with a Calcofluor white stain (Sigma-Aldrich, Buchs, Switzerland). The maximum emission wavelength of 500-520 nm and detecting wavelengths at approximately 488 nm were used. Images were taken using a 10x objective length.

Drip loss

Drip loss was determined by a method adapted from Lowithun and Charoenrein (2009). Frozen samples were laid over absorbent paper, placed into a zip lock plastic bags, and closed to eliminate evaporation during thawing. Then the samples were thawed at 4°C. Drip loss was evaluated by periodically weighing the absorbent paper until a constant value was reached. Our preliminary results indicated that an appropriate thawing time was 5 h. Measurements were done in triplicate, and the results were calculated as drip loss on a dry basis, according to the following equation:

$$DL(\%) = \frac{W_t - W_o}{W_s \times TS} \times 100$$
(2)

where W_o is the weight of absorbent paper before thawing, W_t is the weight of absorbent paper after thawing, W_s is the weight of the sample, and TS is the % dry matter of fruit after thawing.

Texture

The texture of both the fresh and freeze-thawed samples were determined using a compression test Texture Analyzer (TA-XT.plus, Stable Micro Systems, Surrey, UK) with a cylinder probe (P/36R) and 50% strain. The maximum force exerted during compression was recorded as the firmness of the samples. Twelve pieces of mango were used for each sample measurement.

Sensory evaluation

A sensory evaluation of the fresh and freezethawed mangoes was conducted by 7 trained panelists. This evaluation compared the texture of the reference samples using a scale from 1 to 5 which represented the firmness intensity of the fresh and freeze-thawed mangoes with 1 for a very low firmness intensity and 5 for very high firmness intensity values.

Statistical analysis

The experimental design used a completely randomized design. Analysis of variance (ANOVA) and Duncan's new multiple range test were used to determine statistically significant differences ($p \le 0.05$) of the mean values.

Results and Discussion

Pectic substances

The alcohol-insoluble solids (AIS) which form the principal constituents of cell walls are composed of salts, proteins, starch and different non-starch polysaccharides such as pectin, hemicelluloses and cellulose (Reinders and Their, 1998). The AIS values of fresh and freeze-thawed mangoes are shown in Figure 1(a). The AIS of the fresh and frozen samples stored for 1 to 3 freeze-thaw cycles were not significantly different (p>0.05). The AIS content decreased as the fruit ripened which is known to be due to the conversion of starch to soluble sugars and also to the conversion of fibre to alcohol soluble solids (El-Zoghbi, 1994). The freezing process had no effect on the component changes in the AIS. A



Figure 1. Effect of freezing rates and freeze-thaw cycles on alcohol-insoluble solid (a) and total pectin content (b) of fresh and frozen mangoes (FF – fast freeze rate, MF – medium freeze rate, and SF – slow freeze rate) ^a Means within the freezing rates with the same letter are not significantly different (p > 0.05)

^A Means within the freeze-thaw cycles with the same letter are not significantly different (p > 0.05)

similar result was found for TP as is shown in Figure 1(b) and the TP values did not change significantly during freezing (p>0.05). Changes in the structure of pectin polymers might have occurred during freezing and thawing in all treatments, but the total amount of TP did not change. Similarly, Galetto *et al.* (2010) observed that the total pectin content did not show any significant differences between fresh and frozen strawberries.

Softening and textural modifications of fruit samples have also been correlated to changes in pectin structure. Yashoda et al. (2006) reported that textural softening in fleshy fruit is primarily due to cell wall modification resulting in structural changes to pectic substances. The pectin dissolution and solubilization is related to textural softening in several fruits. Figure 2 shows the WSP of fresh and freezethawed mangoes. The fresh mangoes had the lowest WSP. After freezing and thawing, the WSP increased, possibly resulting from the cell wall pectins which were damaged due to the growth of ice crystals during freezing. Roy et al. (2001) reported that the freezing process resulted in substantial dissolution, depolymerization and apparent destruction of cell wall pectins leading to the presence of smaller sized polymers. The average molecular weight of pectins also decreased as the amount of WSP increased (Varanyanond et al., 1999). For freezing rates, the samples with the slowest freezing rates had the highest WSP for both one and two freeze-thaw cycles $(p \le 0.05)$. This occurred because slow freezing leads to large ice crystals being formed exclusively in the extracellular areas causing increased amounts of cell damage (Chassagne-Berces et al., 2009). Phothiset and Charoenrein (2014) reported that ice crystal formation cause cell wall damage, with large cell wall polymers being degraded to shorter cell wall



Figure 2. Effect of freezing rates and freeze-thaw cycles on water soluble pectin of fresh and frozen mangoes (FF – fast freeze rate, MF – medium freeze rate, and SF – slow freeze rate

^{a,b} Means within the freezing rates with the different letters are significantly different ($p \le 0.05$)

polymers and resulting in an increase in WSP. The larger ice crystal formed induced higher degree of cell wall damage and therefore caused higher WSP. However, for the third freeze-thaw cycle, samples for all freezing rates showed no significant difference in WSP (p>0.05). Ketsa et al. (1999) found that the WSP increased during mango ripening resulting in textural softening. For multiple freeze-thaw cycles, the WSP increased with an increasing number of freeze-thawed cycles, with the third cycle having the highest WSP ($p \le 0.05$). This increase in WSP may be due to degradation of pectin polymers in the middle lamella of cell walls by cell wall hydrolases during the thawing process (Chassagne-Berces et al., 2009). This well known phenomenon results in the solubilization of the insoluble pectic substances to a soluble pectic fraction (Ben-Arie et al., 1979) resulting in an increase in the WSP of mango fruits with a loss of firmness. This result supports the research of Barbier and Thibault (1982) who reported that the pectic substances and pectolytic enzymes from cherry fruits (Montmorency cv Prunus cerasus) affected the firmness of the fruit during brining, bruising and freezing.

In general, texture changes in most fruits are the result of cell wall degradation. The pectin fraction which is soluble in ammonium oxalate solution (ASP) was one of the most strongly implicated components in fruit softening. ASP exists as low-methoxyl pectins or pectic acids which can bind calcium to form a cross-linkage structure (Yu *et al.*, 1996). Ketsa *et al.* (1999) found that the ASP in chilled fruit appeared to have a major structural role in maintaining the firmness of the chilled fruit samples. Figure 3 shows that the ASP of freeze-thaw mangoes were significantly influenced by repeated freeze-thaw cycles ($p \le 0.05$) which caused the ASP to increase. The ASP of slow freeze



Figure 3. Effect of freezing rates and freeze-thaw cycles on ammonium oxalate soluble pectin of fresh and frozen mangoes (FF – fast freeze rate, MF – medium freeze rate, and SF – slow freeze rate)

^{a,b} Means within the freezing rates with the different letters are significantly different ($p \le 0.05$)

 $^{\rm A,B,C}$ Means within the freeze-thaw cycles with the different letters are significantly different (p $\leq 0.05)$

(SF) samples had higher ASP than those with other freezing rates after one freeze-thaw cycle ($p \le 0.05$). This result indicates that the slow freezing produced large ice crystals which are able to degrade pectin molecules more than smaller crystal formation. After the second freeze cycle, all freezing rates lead to significantly increased amounts of pectin damage (p>0.05). After the third cycle though freeze rate no longer had an effect and equal amounts of damage were done by all treatments. This may be because the cumulative amount of damage done by the ice crystals after the second and third cycles reached a maximum level which was only partially mitigated by the fast freezing rate after the second cycle. This suggests that the number of freeze-thaw cycles had more influence than freezing rates. The increase in ASP may also be due to ice crystals which affected the degradation of egg-boxes pectin which are known to release homogalacturonan with calcium ions when egg-box structures rupture. Ammonium oxalate ions which are chelating agents can also solubilize low methoxylated pectin fractions by chelating calcium ions (Taboada et al., 2010). As a result, the pectins were lost to interpolymer associations and were either released from the primary cell walls and middle lamella or remained loosely bound to the walls by relatively sensitive bonds. Consequently, wall strength and firmness of the tissues were degraded. (Roy et al., 2001)

Microstructure

Confocal laser scanning microscopy was used to visualize the cell wall of the mango tissues. Figure 4 shows images of the cell walls in fresh and frozenthawed mango tissues. For the fresh state, the cell wall is clearly visible and filled up the total cell volume.



Figure 4. Effect of freezing rates and freeze-thaw cycles on the microstructure of fresh and frozen mangoes. bar = $100 \ \mu m$

The cells are relatively spherical, tightly packed and uniformly distributed throughout the tissue (Figure 4a). For the first cycle, a minimal amount of cell degradation was observed after freezing at the fast freezing rate (-80°C). The cells were still round and similar to cells from fresh tissues (Figure 4b). The medium freeze samples showed a slightly flat cellular structure (Figure 4c) whereas the slow freezing rate induced large changes in the cellular structure and a decrease in the uniformity of the cells. Some intercellular spaces could be seen (Figure 4d) in all samples, but for two freeze-thaw cycles, intercellular spaces increased in size and number. The fast freeze samples presented apparently flat cells which were contracted and misshapen (Figure 4e). Samples frozen at medium freezing rates showed a tearing of the cell walls (Figure 4f). The slow freeze samples appeared to have more cell damage and the cells seemed to be collapsed with a reduction in cell to cell contact (Figure 4g). Moreover, for the third cycle, a high number of collapsed cells, irregular cells shape and tearing of the cell walls were found in the cell images (Figure 4h-j). The cells of these frozen samples were destroyed by ice crystals formation leading to cell shrinkage and gross cell damage.

The degradation of cell walls seemed to be higher for slower freezing rates (at -20°C) than for faster freezing rates after each cycle and this damage was probably caused by the osmotic imbalance due to ice crystal formation (Mazur, 1984). Freezing at the slow freezing rate (-20°C) lead to the growth of fewer ice crystals which consequently became

Freeze-Freezing rate Properties thaw Fresh FF MF SF cycle Drip loss 14.56+1.63°C 22.19±2.36^{bC} 31.15+2.72^{aB} 1 30.77±1.47^{cB} 41.90<u>+</u>3.86^{bB} 53.77+3.71ªA (%) 2 42.09+4.90^{bA} 57.63+3.85^{aA} 60.70+5.50^{aA} 3 Firmness 5.94<u>+</u>0.16 value (N) 2.30±0.14^{aA} 1.43 ± 0.07^{bA} 0.92±0.09^{cA} 1 1.07<u>+</u>0.10^{aB} 0.91<u>+</u>0.01^{aB} 0.69<u>+</u>0.04^{ыв} 2 3 0.72<u>+</u>0.02^{aC} 0.67<u>+</u>0.03^{aC} 0.65±0.03^{aB} Firmness 2.49<u>+</u>0.60 1 0.96±0.10^{aA} 0.84<u>+</u>0.01^{aA} 0.59±0.09bB score 0.45 ± 0.07^{aB} 2 0.44+0.02^{aB} 0.31+0.03^{aB}

Table 1. Effect of freezing rates and freeze-thaw cycles on the drip loss and texture of fresh and frozen mangoes

^{a,b,c} Means within the same row with the different letters are significantly different ($p \le 0.05$)

^{A,B,C} Means within the same column in each characteristic with the different letters are significantly different ($p \le 0.05$)

larger ice crystals. This also resulted in high osmodehydration which damaged the cell walls and degraded the cellular structure resulting in overall structure degradation and thus a decrease in firmness. In contrast, freezing at medium (-40°C) and fast (-80°C) freezing rates, produced a large number of small ice crystals which may have helped retain cell compartments and the cellular structure in the frozen state. The combined effect of turgor pressure decrease and cell wall alteration may be responsible for the tearing of tissues associated with fruit softening (Chassagne-Berces *et al.*, 2009).

Drip loss

The drip loss of the freeze-thawed mangoes after being subjected to various freezing rates and freeze-thaw cycles are shown in Table 1. These results show that the slow freeze samples had the highest drip loss. For the first cycle, the drip loss of the frozen mangoes was 31.15% for slow freezing whereas the fast freeze samples only lost14.56%. For the third freeze-thaw cycle, the percentage drip loss for the slow freeze samples was even higher reaching 60.70%. Fuchigami et al., (1995) found that the amount of drip increased as the freezing rate decreased in frozen carrots. This was explained by the fact that a slow freezing rate causes formation of large ice crystals, which can break the cell walls (Roy et al., 2001). In addition, the drip loss increased with repeating freeze-thaw cycles. This indicates that the mango structure collapsed during thawing resulting in loss of the cells water holding capacity leading to

drip loss (Van Buggenhout et al., 2006).

Texture analysis

The freezing rate determines the size of ice crystals that form, and this in turn determines the degree of structural damage, which produces a change in the texture of the tissue (Sousa et al., 2007). It is generally accepted that the texture quality is better preserved with fast than with slow freezing rates. In this study instrumental measurement and sensory evaluation were used to analyze the texture of fresh and freeze-thaw mangoes. The firmness values measured using the instrument for the fresh and freeze-thawed mangoes are shown in Table 1. After freezing and thawing, the firmness values of the samples decreased compared with the fresh mangoes (5.94 N). After the first freeze-thaw cycle, FF samples (2.30 N) had higher firmness value than MF (1.43 N)and SF (0.92 N) samples, respectively ($p \le 0.05$). In the second freeze-thaw cycle, the firmness values of the SF samples were significantly lower than those of the FF and MF samples ($p \le 0.05$). These results are in agreement with other works on the freezing rate. The effects of freezing rate on texture have been previously studied by several authors (Chassagne-Berces et al., 2009; Chassagne-Berces et al., 2010) all of which show lower textural damage with faster freezing rates. Fast freezing induces the formation of a large number of small ice crystals while slow freezing led to fewer ice crystals of larger size which degraded the cell structures of the mango (Chassagne-Berces et al., 2010). The firmness values of samples with the three freezing rates in the third freeze-thaw cycle were not significantly different (p>0.05). As the number of freeze-thaw cycles increased from the first to the third cycle, the firmness value of three different freezing rates decreased significantly (p \leq 0.05). This result is most likely due to the formation of larger ice crystals during the freezing process and the recrystallization during repeated freezing and thawing, both of which can cause damage to the structures of the cell walls (Fabloul *et al.*, 1996).

The firmness scores from the sensory evaluation of the fresh and freeze-thawed mangoes subsequent to one to two cycles are shown in Table 1. These results show that a significant decrease in firmness scores was observed for all freezing rates compared to fresh mangoes. FF and MF samples had higher firmness scores than the SF samples after one freezethaw cycle ($p \le 0.05$). For the second cycle, panelists were not able to detect a difference in firmness the samples. The firmness scores decreased with repeating freeze-thaw cycle number which directly correlated to the results from the texture analyzer, whereas the firmness scores of the SF samples from the first and second freeze-thaw cycles were not significantly different (p>0.05). A high correlation between the instrument measurements and sensory evaluation was found.

Conclusion

Freezing rates and freeze-thaw cycle number have a significant effect on both texture and pectin structure of mangoes where fast freezing lead to high quality products. Multiple freeze-thaw cycles also significantly affected the texture of the mango samples, especially for the slow freezing rate.

In addition, the increase in depolymerization and solubility of the cell wall pectin content during the freezing and thawing process resulted in the degradation of cell structure and cell wall damage. The collapsed cell walls and tearing of the tissue resulted in a large drip loss further leading to loss of firmness in the ripe mango samples. Therefore, on the basis of this study, fast freezing at -80 °C is recommended as the optimum freezing condition for the preservation of optimal texture quality in frozen mangoes. In addition, changes in the temperature of frozen mangoes during storage, transportation and consumer handling should be avoided or kept to a minimum.

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