J Food Sci Technol (May–June 2010) 47(3):295–299
DOI: 10.1007/s13197-010-0046-9

ORIGINAL ARTICLE

AFSTI
Empowering the Food Professional

Role of oxidative stress and the activity of ethylene biosynthetic enzymes on the formation of spongy tissue in 'Alphonso' mango

Nagamani J. E. · Shivashankara K. S. · Roy T. K.

Revised: 27 July 2009 / Accepted: 2 August 2009
© Association of Food Scientists and Technologists (India), Mysore

Abstract Spongy tissue formation in 'Alphonso' mangoes (*Mangifera indica* L) is a major national problem leading to loss for farmers and traders. Spongy tissue is whitish sponge like tissue formed near the seed with insipid taste and off odour. Lipid peroxidation of membranes as studied by malondialdehyde formation was significantly higher in spongy tissue. Activities of antioxidative enzymes like superoxide dismutase, catalase, peroxidase and polyphenol oxidase were lower in spongy tissue. Among the antioxidative enzymes, activities of catalase and peroxidases were severely reduced leading to membrane damage in spongy tissue. A significant reduction in 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and accumulation of ACC was also observed in spongy tissue. However, ACC synthase activity in spongy tissue was more compared to healthy tissue. Results indicate that the membrane peroxidation leading to lower activity of ACC oxidase might lead to the formation of spongy tissue in 'Alphonso' mango.

Keywords Mango · Alphonso · Spongy tissue · Malondialdehyde · Antioxidative enzymes · Aminocyclopropane-1-carboxylic acid oxidase

Nagamani J. E. · Shivashankara K. S. · Roy T. K.
Division of Plant Physiology and Biochemistry,
Indian Institute of Horticultural Research,
Hessaraghatta lake Post, Bangalore - 560 089, India

Shivashankara K. S. (✉)
E-mail: shiva@iihr.ernet.in

Introduction

Spongy tissue in 'Alphonso' mango is the formation of whitish sponge-like tissue near the stone. The fruit pulp remains unripe due to physiological and biochemical disturbances caused by various factors during ripening of fruits (Katrodia and Seth 1988). External symptoms are not apparent at the time of picking or at the ripe stage. The fruit with spongy tissue exhibits varying degree of tissue breakdown and contains more starch, acid and has off-odour.

Numerous factors are known to influence the incidence and extent of disorder. Some of the probable causes cited are lower calcium content (Gunjate et al. 1979), higher fruit weight (Joshi and Limaye 1986), high soil temperature (Katrodia and Seth 1988), excessive tree vigour (Katrodia and Rane 1981), post-harvest exposure of fruits to sunlight (Gunjate et al. 1982) and lower fruit transpiration rate (Shivashankar and Mathai 1999). Spongy tissue also showed lower activities of α-amylase, glutamate dehydrogenase, glutamate oxaloacetate transaminase, peroxidase, catalase, superoxide dismutase, pectin methyl esterase and higher activities of invertase and ascorbic acid oxidase (Gupta et al. 1985, Selvaraj et al. 2000). Higher expression of alcohol dehydrogenase has been reported in spongy tissue indicating the anaerobiasis (Vasanthaiah et al. 2006). Recently, bacterial infection was reported to be a factor responsible for the initiation of spongy tissue (Janave and Sharma 2008). Lower availability of oxygen may alter the antioxidative systems and ethylene biosynthesis in the affected tissues. Even though the lower activities of antioxidative enzymes were reported its relationship with the membrane peroxidation and ethylene biosynthesis was not studied.

In mango, a small but notable peak of ethylene is produced during ripening in the cultivars 'Haden', 'Kent', 'Manila' and 'Ataulfo' (Cua and Lizada 1990, Lopez-Gomez and Gomez-Lin 1992). The incidence of spongy tissue is considered to be a ripening disorder, which may be due

to lower availability of ethylene due to the lower activities of ethylene forming enzymes. Lower ethylene production may also be due to the membrane damage caused by the generation of reactive oxygen species. In this study we compared the activities of ethylene biosynthetic and antioxidative enzymes in spongy tissue, matured unripe tissue, $3/4^{th}$ ripened and fully ripened healthy tissues of 'Alphonso' mangoes to identify the role of these enzymes in spongy tissue development.

Materials and methods

Uniform sized 'Alphonso' mango (*Mangifera indica* L) fruits were harvested from orchard and the samples from fruits of different ripening stages were collected. Samples from mature unripe tissue, $3/4^{th}$ ripen tissue, completely ripen healthy tissue, spongy tissue, healthy ripen tissue surrounding the spongy tissue and spongy tissue induced at 40°C (mature unripe fruits were ripened at 40°C in an oven) were collected. About 500 g of tissue from each of the samples were taken for the preparation of acetone powder, which was used for different enzyme assays.

Preparation of acetone powder: The fruit tissue was ground using chilled acetone (–10°C) in a cold room. The fine paste was filtered and squeezed using muslin cloth. The process was repeated 3 to 4 times until the tissue became a white powder. The powder was air dried to remove traces of acetone, packed in polythene bags and stored at –10°C. This acetone powder was used for different enzyme assays.

Estimation of malondialdehyde (MDA) content: MDA was extracted following the procedure of Draper and Hadley (1990) by homogenizing 1 g of fruit tissue with 5 ml of 5% aqueous trichloroacetic acid (TCA) solution and 0.5 ml of methanolic butylated hydroxy toluene (BHT). The resulting homogenate was heated for 30 min in a boiling water bath. Then the sample was cooled and centrifuged at 1000×g for 10 min. The volume of the resulting supernatant was adjusted to 10 ml with distilled water. The assay mixture consisted of 1 ml aliquot of the supernatant sample and 1 ml of saturated thiobarbituric acid (TBA) solution. The tubes were incubated in boiling water bath for 30 min, cooled and the absorbance of the reaction product was assayed spectrophotometrically at 532 nm against a blank solution.

Estimation of superoxide dismutase (SOD) activity: SOD activity was assayed as per the method of Zhanyuan and Bramlage (1994). Acetone powder (0.5 g) was mixed with 10 ml of 50 mM phosphate buffer (pH 7), containing 0.1 ml of 0.3 M $MgCl_2$, and 0.1 ml of 10 mM ethylenediamine tetraacetic acid (EDTA) solution and incubated overnight at 4°C. The mixture was homogenized and centrifuged at 15,000×g for 15 min and the supernatant was used for enzyme assay. SOD activity was estimated by measuring the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT) in presence of riboflavin and methionine and the absorbance of the assay mixture was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme that inhibited the NBT photoreduction by 50 % under assay conditions.

Estimation of catalase activity: Catalase activity was estimated as per the procedure of Masia (1998). Enzyme was extracted from acetone powder using 10 ml of 100 mM chilled sodium phosphate buffer (pH 7) as described earlier by centrifuging at 11,000× g for 20 min at 4°C. The resulting supernatant solution was immediately used for enzyme assay. Catalase activity was recorded at 20°C according to Aebi (1984). Catalase activity was measured by the reduction in absorbance at 240 nm in a mixture containing 1.5 ml of 50 mM sodium phosphate buffer (pH 7), 0.5 ml of 100 mM H_2O_2 solution and 0.3 ml of crude enzyme extract and was expressed as units / g fresh weight.

Peroxidase: Peroxidase activity was assayed as per the procedure of Chan and Yang (1971). Crude enzyme extract from acetone powder using 10 ml of chilled 0.05 M citrate phosphate buffer (pH 6.4) was used for the estimation of enzyme activity. The assay mixture contained 4.5 ml of 50 mM chilled sodium citrate buffer (pH 6.4), 0.2 ml of 0.3% prechilled H_2O_2 solution, 0.2 ml of 1% chilled o-phenyldiamine solution and 0.5 ml of crude enzyme extract. Intensity of the colour produced was read at 450 nm at 1 min interval up to 10 min gainst suitable blank.

Estimation of *polyphenol oxidase (PPO) activity:* Polyphenol oxidase activity was assayed following the method described by Selvaraj and Kumar (1989). Crude enzyme from acetone powder was extracted using 10 ml of 0.05 M citrate phosphate buffer (pH 6.8). Oxidation of pyrogallol was read at 450 nm in 0.05 M citrate phosphate buffer (pH 6.8) media.

Estimation of Aminocyclopropane-1-carboxylic acid (ACC) content: Estimation was done as per the method of Lizada and Yang (1979). ACC extract was prepared by homogenizing 5 g of fruit tissue with 10 ml of cold 5% sulphosalicylic acid and centrifuged at 10,000×g for 10 min. The resulting supernatant solution was taken for ACC estimation and the assay was carried out in 25 ml of Erlenmeyer flasks. The assay mixture consisted of 3 ml of crude ACC extract and 0.3 ml of 10 mM $HgCl_2$ solution; the reaction vessels were then sealed with rubber serum stoppers and kept in ice. Again 0.3 ml of cold mixture of 5% sodium hypochlorite – saturated NaOH solution was injected through stoppers using 1 ml syringe fitted with a 25-gauge needle. Reaction mixtures in flasks were agitated on a shaker for 2.5 min after which, 100 µl of gas samples were withdrawn for ethylene determination. Ethylene was assayed on a gas chromatograph (HP 5890, USA) equipped with a porapack column and a flame ionization detector. The temperature of the column was maintained at 80°C. The amount of ethylene formed corresponds to the quantity of ACC in the tissue and was determined by using internal standards.

Estimation of ACC synthase activity: ACC synthase activity was assayed as per the method by Sitrit et al.

(1987). Enzyme extract was prepared by suspending 2.5 g of acetone powder in 50 ml of extraction buffer (50 mM, pH 7.2) and incubated overnight in cold conditions. The extract was homogenized using a prechilled mortar and pestle, filtered through 4 layers of muslin cloth and centrifuged at 10,000×g for 10 min at 4°C. The resulting supernatant solution was used as crude enzyme extract. All the steps in preparation of the enzyme extract were carried out at 0–4°C. ACC synthase enzyme was precipitated from the supernatant solution by slowly adding ammonium sulphate salt up to 90% saturation, letting the mixture stand for 1 h and centrifuging as above. The supernatant was discarded and the pellet obtained was dissolved in 4 ml of 10 mM potassium phosphate buffer (pH 7.2) containing 0.1 mM dithiothreitol (DTT) and 2 µM pyridoxal phosphate and dialysed overnight against the same solution. The dialysate was clarified by centrifugation and was used as enzyme extract. The assay mixture contained 3 ml of enzyme extract, 0.25 ml of 40 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 8.2) and 0.25 ml of 200 nM S-adenosyl methionine (SAM) solution in 25 ml of Erlenmeyer flasks. The flasks were sealed with rubber serum cap and kept for incubation at 30°C for 1–2 h. Then the reaction was stopped by adding 0.3 ml of 10 µM $HgCl_2$ solution followed by the addition of 0.3 ml cold 5% NaOH-sodium hypochlorite solution and again incubated for 2 h. The ACC formed was assayed by the method of Lizada and Yang (1979). Enzyme activity is expressed as µg of ACC synthesized.

Estimation of ACC oxidase activity: ACC oxidase activity was assayed as per the method by Henryk (1989). For the preparation of enzyme extract, 0.5 g of acetone powder was incubated overnight in 20 ml of 25 mM HEPES – tris buffer (pH 7.5) in cold condition, and was homogenised using a prechilled mortar and pestle. The extract was filtered through muslin cloth then centrifuged at 10,000×g for 10 min. Extraction and subsequent steps were carried out at 4°C. The supernatant was used for enzyme assay. ACC oxidase activity was determined by measuring the conversion of administered ACC to ethylene *in vivo*. The assay mixture consisted of 4 ml of enzyme extract, 0.1 ml of 1.55 mM sorbital solution and 0.25 ml of 1 mM ACC in 25 mL of Erlenmeyer flasks. The flasks were sealed with rubber serum cap and shaken constantly at 27°C for 1–2 h. The ethylene formed was estimated on a gas chromatograph equipped with a porapack column and a flame ionization detector. The temperature of the column was maintained at 80°C. Ethylene was measured every 2 h and the flasks were aerated after each sampling and resealed.

Statistical analysis: Data were statistically analysed by completely randomized design method using statistical analysis package of Microsoft Office Excel.

Results and discussion

Results are presented in Table 1. Lipid peroxidation was more in spongy tissue (2.32 µg of MDA) when compared to artificially induced spongy tissue (1.80 µg) and healthy tissue (1.44 µg). MDA content of mature unripe tissue (0.48 µg), 3/4th ripen tissue (1.16 µg) was also lower than the completely ripen healthy tissue (1.44 µg) indicating that the lipid peroxidation of cell membrane increases with the increase in ripening. Higher lipid peroxidation of membranes was observed during fruit ripening (Keista et al. 1998, Kumar et al. 1990) due to generation of free radicals. Higher MDA in spongy tissue indicate higher lipid peroxidation of membranes when compared to normal healthy ripe tissue.

Activities of antioxidative enzymes: Activities of SOD and catalase were significantly lower in spongy tissue (23.0 and 1.0 U/g tissue, respectively) when compared to the healthy ones (64.2 and 2.5 U/g tissue). A significant reduction in the activity of peroxidase was also observed in spongy tissue (0.06 U/g) as compared to healthy tissue (0.30 U/g). Lower activity of PPO was observed in spongy tissue (1.06 U/g) when compared to the healthy tissue (1.66 U/g) but the extent of reduction was less when compared to other enzymes. Activity of all the antioxidative enzymes increased with ripening. The induced spongy tissue showed slightly higher activity PPO when compared to the natural spongy tissue.

Table 1 Malondialdehyde (MDA) and ACC contents and the activities of antioxidative and ethylene biosynthetic enzymes in six types of tissues from 'Alphonso' mango

Tissues	SOD, U/g fw	Peroxidase, U/g fw	Catalase, U/g fw	PPO, U/ 100g fw	MDA, µg /100 g fw	ACC content, µg /g fw	ACC-OA, µl C_2H_4/h/Kg fw	ACC-SA, µg ACC/h/Kg fw
MU	29.92^{c}	0.12^{d}	0.65^{e}	1.06^{c}	0.48^{f}	0.42^{a}	0.0474^{e}	0.0180^{a}
3/4th R	63.84^{a}	0.40^{b}	1.80^{c}	1.20^{b}	1.16^{d}	0.14^{d}	0.2769^{a}	0.0114^{bc}
CR	64.16^{a}	0.30^{c}	2.48^{b}	1.66^{a}	1.44^{d}	0.12^{d}	0.1780^{b}	0.0094^{d}
HSS	63.68^{a}	0.42^{a}	3.03^{a}	1.19^{b}	1.55^{c}	0.13^{d}	0.1797^{b}	0.0098^{d}
SP	23.01^{d}	0.06^{e}	1.00^{d}	1.06^{c}	2.32^{a}	0.34^{b}	0.0612^{d}	0.0129^{b}
ISP	37.66^{b}	0.03^{f}	1.08^{d}	1.20^{b}	1.80^{b}	0.18^{c}	0.0812^{c}	0.0100^{cd}

MU = Mature Unripe tissue, 3/4th R = 3/4th Ripen tissue, CR = Completely ripen tissue, HSS = Healthy tissue surrounding spongy tissue, SP = Spongy tissue, ISP = Induced spongy tissue, SOD = Superoxide dismutase, PPO = Polyphenol oxidase, ACC = 1-Aminocylopropane-1 carboxylic acid, ACC-OA = ACC oxidase, ACC-SA = ACC synthase. Values followed by same superscript in a column are not significantly different at p = 0.01

Increased activities of peroxidase and catalase were observed during the ripening of mango (Keista et al. 1998). This was associated with the climacteric raise in ethylene production (Masia 1998). However, lower activities of peroxidase and catalase (Gupta et al. 1985), higher activities of PPO (Selvaraj et al. 2000) and peroxidase (Lima et al. 1999) were reported in spongy tissue when compared to the healthy tissue. Our results indicated that the activities of antioxidant enzymes increase during fruit ripening. However, in spongy tissue the activity was significantly reduced indicating that the ripening process was inhibited in the spongy tissue. This could be due to lower ethylene production in the spongy tissue.

Lower ethylene forming enzyme activity was reported in spongy tissue (Selvaraj et al. 2000). Our results indicate that, ACC content was more in the unripe stage (0.42 μg/g fw) followed by spongy tissue (0.34 μg/g fw), heat induced spongy tissue (0.18 μg/g fw) and decreased with ripening (0.14 and 0.12 μg /g fw at 3/4th and fully ripen stage respectively). Similar trend was reported by Hoffman and Yang (1980) in avocadoes and banana. They reported that the level of ACC is determined by the rate of ACC synthesis relative to its rate of utilization or degradation. In avocado and banana there was rapid accumulation of ACC during the onset of ripening and also as the fruit becomes overripe. Higher ACC content in spongy tissue shows that the formation of spongy tissue takes place at the initiation of fruit ripening process.

ACC oxidase activity increased rapidly from unripe stage (0.0474 μl C_2H_4 h^{-1} kg^{-1} fw) to the 3/4th ripe stage (0.2769 μg C_2H_4 h^{-1} kg^{-1} fw) indicating the onset of climacteric peak of ethylene production at the initiation of ripening process. As a result of this, ACC content drops from unripe stage to the 3/4th ripe stage. A reduction in the activity was observed from 3/4th ripe stage to completely ripe stage (0.1780 μg C_2H_4 h^{-1} kg^{-1} fw). A reduction in ACC oxidase activity was observed in natural spongy (0.0612 μl C_2H_4 h^{-1} kg^{-1} fw) and induced spongy tissue (0.0812 μl C_2H_4 h^{-1} kg^{-1} fw) when compared to healthy tissue. The activity was not affected in the healthy tissue from affected fruit (0.1797 μl C_2H_4 h^{-1} kg^{-1} fw.). Lower activity of ACC oxidase leads to decreased rate of ethylene production and the accumulation of ACC content in spongy tissue.

ACC synthase activity, measured as ACC produced, decreased gradually from mature unripe stage (0.0180 μg ACC h^{-1} kg^{-1} fw) to 3/4th ripe stage (0.0114 μg ACC h^{-1} kg^{-1} fw) and to completely ripe stage (0.0094 μg ACC h^{-1} kg^{-1} fw). ACC synthase activity of spongy tissue (0.0129 μg ACC h^{-1} kg^{-1} fw) was in between mature unripe stage to 3/4th ripe stage. After heat treatment ACC synthase activity in induced spongy tissue (0.010 μg ACC h^{-1} kg^{-1} fw) was slightly lower than that of natural spongy tissue (0.0129 μg ACC h^{-1} kg^{-1} fw). This further indicates that the incidence of spongy tissue occurs during the initiation of ripening process. Results also indicate that ACC synthase was not much affected by the formation of spongy tissue. Similar results were reported during the heat treatment of mangoes by Keista et al. (1998).

A 5 fold increase in protein content during ripening has been reported in mango (Castrillo et al. 1992). Due to increased concentration of protein the activity of ACC oxidase enzyme per unit fruit tissue increased markedly at the 3/4th ripen stage and completely ripen stage. However there was a reduction in the activity of ACC synthase indicating that the climacteric peak in ethylene evolution is probably due to increased activity of ACC oxidase. Therefore the inhibition of ACC oxidase in spongy tissue might result in insufficient ethylene production leading to ripening disorder.

Conclusion

The spongy tissue formation occurs at the initiation of ripening process and this disorder is mainly due to the lower activity of ACC oxidase leading to lower ethylene production. Higher lipid peroxidation of membranes due to lower activities of antioxidative enzymes has further contributed to the loss of membrane integrity and formation of sponginess in the tissue.

Acknowledgement The work was carried as a part of NATP-CGP scheme of ICAR.

References

Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105:121–126

Castrillo M, Kruger NJ, Whatley FR (1992) Sucrose metabolism in mango fruit during ripening. Plant Sci 84:45–51

Cua AU, Lizada MCC (1990) Ethylene production in the 'carabao' mango fruit during maturation and ripening. Acta Hort 269:69–175

Chan HT, Yang HY (1971) Identification and characterization of some oxidizing enzymes of the MC farlin cranberry. J Food Sci 36:169–173

Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 186:421–431

Gunjate RT, Tare SJ, Rangawala AD, Limaye VP (1979) Calcium content in Alphonso mango fruits in relation to occurrence of spongy tissue. J Mah Agric Univ 4:159–161

Gunjate RT, Walimbe BP, Lad BP, Limaye VP (1982) Development of internal breakdown in Alphonso mango by post harvest exposure of fruits to sunlight. Sci Cult 48:188–190

Gupta DN, Lad BL, Chavan AS, Salvi MJ (1985) Enzyme studies on spongy tissue: a physiological ripening disorder in Alphonso mango. J Mah Agric Univ 10:280–282

Henryk P (1989) The rate of ethylene production related to 1-aminocyclopropane-1-carboxylic acid formation and ethylene forming enzyme activity in ripening apples. Fruit Sci Rep 16:141–154

Hoffman EN, Yang SF (1980) Changes of 1-aminocyclo propane 1-carboxylic acid content in ripening fruits in relation to their ethylene production rates. J Am Soc Hort Sci 105:492–495

Janave MT, Sharma A (2008) Spongy tissue development in Alphonso mango: association with *Staphylococcus xylosu.s* Eur J Plant Pathol 122:335–348

Joshi GD, Limaye VP (1986) Effects of tree location and fruit weight on spongy tissue occurence in Alphonso mango. J Mah Agric Univ 11:104–109

Katrodia JS, Rane DA (1981) Effect of tree vigour in relation to spongy tissue development in Alphonso mango fruit. Haryana J Hort Sci 10:151–154

Katrodia JS, Sheth IK (1988) Spongy tissue development in mango fruit of cv. Alphonso in relation to temperature and its control. Acta Hort 231:827–834

Keista S, Chidtragood S, Klein JD, Wurie S (1998) Ethylene synthesis in mango fruit following heat treatment. Postharvest Biol Technol 15:65–72

Kumar S, Patil BC, Sinha SK (1990) Cyanide resistant respiration is involved in temperature rise in ripening mangoes. Biochem Biophys Res Comm 168:818–822

Lima LC, Chitarra AB, Chitarra MIF, Silva EB, Michalczuk L (1999) Enzymatic activity changes in spongy tissue: A physiological ripening disorder of 'Tommy Atkins' mango. Acta Hort 485:255–258

Lizada MCC, Yang Y (1979) A simple and sensitive assay for 1-Aminocyclo propane-1-carboxylic acid. Anal Biochem 100: 140–145

Lopez-Gomez R, Gomez-Lin MA (1992) Changes in mRNA and protein synthesis during ripening in mango fruit. J Plant Physiol 141:82–87

Masia A (1998) Superoxide dismutase and catalase activities in apple during ripening and post harvest and with special reference to ethylene. Physiol Plant 104:668–672

Selvaraj Y, Edward Raja M, Rawal RD (2000) Biochemical studies on internal breakdown-a ripening disorder in mango fruits. Indian J Hort 57:183–188

Selvaraj Y, Kumar R (1989) Studies on fruit softening enzyme and polyphenoloxidase in ripening mango (*Mangifera indica* L) fruit. J Food Sci Technol 26:218–222

Shivashankar KS, Mathai CK (1999) Relationship of leaf and fruit transpiration rates to the incidence of spongy tissue disorder in two mango cultivars. Sci Hort 82:317–323

Sitrit Y, Blumenfeld A, Riov J (1987) Ethylene biosynthesis in tissues of young and mature avocado fruits. Physiol Plant 69: 504–510

Vasanthaiah HKN, Ravishankar KV, Shivashankara KS, Lalitha A, Narayanaswamy P, Prasad TG, Mukunda GK (2006) Cloning and characterization of differentially expressed genes of internal breakdown in mango (*Mangifera india* L.) cv. Alphonso. J Plant Physiol 163:671–679

Zhanyuan D, Brumlage WJ (1994) Superoxide dismutase activities in senescing apple fruit. J Food Sci 59:581–584

RENEWAL OF AFST(I) MEMBERSHIP AND SUBSCRIPTION TO JOURNALS

AFST(I) members, who have not renewed their membership/subscription for the year 2009-10 may kindly do so immediately to enable us to give the print order for the required number of copies of the journals viz., *Journal of Food Science and Technology/Indian Food Industry.*

Address for correspondence:

AFST(I), CFTRI Campus, Mysore - 570 020, INDIA

Please update your AFST(I) Membership data to ensure prompt delivery of journals and various communications emanating from AFST(I) headquarters.