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Preharvest Aminoethoxyvinylglycine Plus Postharvest Heat Treatments Influence Apple Fruit Ripening after Cold Storage

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Abstract. **The impact of heat plus aminoethoxyvinylglycine (AVG) treatments alone or in combination on ripening of four apple cultivars has been studied. A solution of AVG was applied to 'Lodi', 'Senshu', 'Redchief Delicious', and 'Red Fuji' apple trees ≈4 weeks before normal harvest at 124 g·ha^{-1} a.i. After harvest, half of each group of control and AVG-treated fruit was heated at 38 °C for 4 days and then stored at 4 °C for 30 days. After cold storage, AVG and heat individually suppressed ethylene production of 'Senshu' and 'Redchief Delicious' but not of 'Lodi' or 'Red Fuji'. The combination of AVG with heat treatment reduced ethylene production the most consistently in each cultivar except 'Lodi', suggesting some additive effect of the treatments. The respiration rate after cold storage was not consistently affected by any treatment. AVG alone and with heat maintained firmness of 'Lodi', AVG plus heat maintained it in 'Senshu', but neither 'Redchief Delicious' nor 'Red Fuji' firmness responded to the treatments. AVG-treated 'Lodi' and 'Redchief Delicious' fruit, heated fruit of all cultivars, and AVG plus heat in all had lower titratable acidity than controls after cold storage. Although there were no effects of any treatment on fruit soluble solids concentration, the combined treatment increased the soluble solids:titratable acidity ratio of all cultivars, although heat or AVG alone had no consistent effects. Total ester production by 'Redchief Delicious' peel tissue after cold storage was reduced 44% by AVG and 70% or more by heat and AVG plus heat. There were no differences in peel alcohol acyltransferase activity among the treatments, supporting the hypothesis that substrate availability was the limiting factor for ester synthesis in treated fruit. Overall, heat plus AVG treatment did not provide any advantage over each alone for maintaining apple fruit quality during short-term cold storage.**

Techniques that slow ripening of apple are valuable tools that can maintain fruit quality during cold storage. Aminoethoxyvinylglycine (AVG) inhibits the pyridoxal phosphate-linked enzyme aminocyclopropane synthase (ACS) activity (Capitani et al., 2002) essential in the ethylene biosynthetic pathway (Yang and Hoffman, 1984) and is commercially used to stop fruit drop as a preharvest application (Greene, 2005, 2006; Greene and Schupp, 2004; Stover et al., 2003). In addition to inhibiting fruit drop and ethylene production, AVG was found to delay apple fruit ripening, maintain fruit firmness, and inhibit aroma volatile production after harvest and cold storage (Autio and Bramlage, 1982; Bramlage et al., 1980; Drake et al., 2005; Halder-Doll and Bangerth, 1987; Mir et al., 1999; Stover et al., 2003).

Heat treatment after harvest has shown potential for inhibiting ripening and extending cold storage life. In climacteric fruit, heat might act through its effect on enzymes involved in the synthesis of ethylene (Atta-Aly, 1992; Klein, 1989; Yu et al., 1980). Although heat treatment itself inhibits ripening, after the treatment, ethylene production may recover to equal or higher levels than those of control fruit (Klein, 1989; Klein and Lurie, 1990). Heat treatments have decreased firmness loss and maintained a higher soluble solids:titratable acidity ratio in apples after regular cold storage (Klein and Lurie, 1992; Lurie and Klein, 1992; Porrit and Lidster, 1978; Saftner et al., 2002, 2003), a change sensed by taste panels who indicated that heated apples were crisper and sweeter than unheated ones after cold storage (Lurie and Nussinovitch, 1996). Fallik et al. (1997) found that volatile production was first inhibited but eventually recovered to even higher levels than nonheated fruit after 6 weeks of cold storage. However, the response to heat treatment may be cultivar-specific (Shao et al., 2007).

Although there are numerous reports about the effects of heat or AVG alone on apple ripening, nothing has been reported about their combined effect after cold storage. Based on apple response to each treatment alone, the increase in ethylene production often observed after heat treatment and subsequent removal from cold storage may be suppressed by the persisting effect of AVG and thus modify apple ripening. The objective of this study was to determine if AVG plus heat treatment would additively or synergistically affect fruit ripening of four apple cultivars more than either treatment alone.

Materials and Methods

Treatments and harvest. Experiments were carried out at the University of Kentucky Horticultural Research Farm in Lexington, KY. Eight mature trees each of 'Lodi/M7', 'Senshu/M26', 'Redchief Delicious/M7', and 'Red Fuji/M7a', one tree per row, were selected. Four randomly chosen trees were sprayed to runoff with a solution of AVG (ReTain; Valent Biosciences, Libertyville, IL) containing 500 μl·L^{-1} Silwet L-77 (Helena Chemical Co., Collierville, TN) as a surfactant at the commercial rate of 124 g·ha^{-1} a.i. (Commercial Tree Fruit Spray Guide, 2003) at 32, 28, 31 and 35 d before harvest, respectively. All fruit of control and AVG-treated trees were harvested at the beginning of control fruit ripening based on starch indices and ethylene production.

Fruit were equilibrated at room temperature (21 ± 0.5 °C) for 3 h immediately after harvest. A subsample of six fruit from both treatments was set aside for initial firmness, starch index, titratable acidity, and soluble solids measurements. Fruit from the same treatment but different trees were pooled. Half of the pooled fruit from each treatment was placed directly into 4 °C storage, and the remaining half was heat-treated in trays placed in an incubator at 38 °C for 4 d inside plastic bags to reduce weight loss (Fallik et al., 1997). A pan of water was also placed in the incubator to maintain a high relative humidity. After the heat treatments concluded, the fruit were equilibrated at room temperature for 3 h then placed into 4 °C storage. Ethylene levels in the cold storage unit were monitored but were never evident above the lowest limits of detectability. Fruit from the four treatments (control, AVG, heat, and heat plus AVG) were stored for 30 d and then removed and ripened at room temperature for 5 d. At 1 ('Lodi' only) or 5 d, cortex sections of each fruit were taken after measuring ethylene production and respiration and frozen at –80 °C. For volatile analyses, peel was also collected from the same 'Redchief Delicious' fruit and frozen at –80 °C. There were five to eight replicate fruit per treatment for each cultivar and treatment after cold storage.

Ethylene production and respiration rate. Ethylene production and respiration rate of five to eight replicate fruit from each treatment group were assessed 1, 3, and 5 d after removal from cold storage. Ethylene production was quantified by weighing and then placing individual fruit in sealed 2-L glass jars and withdrawal of 0.2 mL headspace samples after 4 h. Samples were analyzed with a gas chromatograph (HP 5890; Agilent Technology, Wilmington, DE) equipped with a flame ionization detector (FID) and an alumina capillary column (AT-Alumina Plot GC Column, 30 m, 0.53 cm i.d.). Temperatures were

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35, 175, and 125 °C for oven, injector, and FID detector, respectively. Respiration rate was measured on Day 1 for 'Lodi' and Day 5 for the other cultivars by taking 5-mL samples from the headspace and measuring each sample with an O_2/CO_2 analyzer (Model ZR 892 HS; IL Instruments Inc., McHenry, IL).

Starch index and cortex firmness. Starch index (SI) and cortex firmness were measured after harvest, and cortex firmness was also measured at 1 ('Lodi' only) or 5 d after removal from cold storage from five to eight replicate fruit of each treatment group using the same fruit as for ethylene production. To assess starch degradation, fruit were cut in half perpendicular to the stem–blossom axis, the halves were soaked in iodine solution (0.1% iodine, 1% potassium iodide in water), and the SI determined. The SI was rated on a visual scale of 1 to 9, in which 1 = the entire cut surface stained (high starch content) and 9 = no staining (no starch) (Cowgill et al., 2003). For 'Fuji', a 1 to 6 scale was used. After removing a disk of skin from opposite sites on the equatorial plane of the fruit, cortex firmness (N) was measured using a press-mounted penetrometer (Model DF M10; John Chatillon & Sons, Inc. Greensboro, NC), and mean firmness per fruit was derived.

Soluble solids concentration, titratable acidity, and soluble solids:acid ratio. Soluble solids concentration (SS) was determined on a fresh sample from each fruit using an automatically temperature-compensated hand refractometer (Model 10430; Reichert Scientific Instruments. Buffalo, NY). For titratable acidity (TA), ≈15 g of frozen cortex cut from the apples used for the analyses described previously was used. Samples were thawed, macerated with a mincer/chopper, and filtered through two layers of cheesecloth separated by a layer of Miracloth (Calbiochem, EMD Biosciences Inc., La Jolla, CA). One milliliter of each sample was mixed with 14 mL of deionized water and titrated to pH 7.0 with 0.1 N NaOH. Results were expressed as mg malic acid/100 mL. For calculating the soluble solids:titratable acidity ratio (SS:TA), TA was recalculated as g malic acid/100 mL.

Aroma volatile production. Volatile production was measured on peel of three individual 'Redchief Delicious' apples per treatment according to Hamilton-Kemp et al. (2003). Frozen peel tissue (9 g) was thawed in 30-mL glass jars sealed with Teflon-lined plastic screw caps containing a three-layer septum. Samples were equilibrated in a water bath to 26 °C for 1.75 h and then placed at ambient laboratory temperature. The headspace in the bottle was sampled for 15 min by solid phase microextraction (SPME) using a 100-μm poly(dimethylsiloxane) fiber. The SPME fiber was removed and injected into a gas chromatograph (GC) (Model Hewlett Packard 5890 Series II; Agilent Technology) equipped with a DB-5 column (60 m × 0.32 mm i.d., 1 μm film thickness) and a FID detector. Volatiles were desorbed in the GC injection port for 5 min. Conditions were as follows: injection port temperature, 220 °C; FID detector, 240 °C; initial oven temperature, 35 °C held for 5 min and then increased to 184 °C at 2 °C/min; injector splitless for 5 min. A modified splitless injection port was used so that both the septum and inlet purges were interrupted during SPME injections. Volatiles were identified from retention times matching those of authentic standards and are reported as area units (AUs) for each compound.

Alcohol acyl-CoA transferase activity. Alcohol acryl-CoA transferase (AAT) activity of peel tissue was assayed from three individual 'Redchief Delicious' apples per treatment. For this, 3 g of frozen tissue was pulverized and then homogenized in 6 mL of extraction solution [0.1 M potassium phosphate, 1 mM ethylenediaminetetraacetic acid, 0.1% (w/v) Triton X-100, and 1% (w/v) polyvinylpolypyrrolidone]. The homogenate was centrifuged at 25,000 × *g* for 20 min at 4 °C. The supernatant was recovered and assayed using the method from Echeverria et al. (2004b). AAT activity was measured spectrophotometrically (Model Cary 50 Bio; Varian Analytical Instruments, Walnut Creek, CA) by following the production of the yellow thiophenol product from 5,5′-dithiobis (2-nitrobenzoic acid) reacting with free CoA through an increase in absorbance at 412 nm over time. AAT activity was expressed as $mU \times mg\ protein^{-1}$ where U is the increase in one unit of absorbance per minute. Total protein content of the enzyme extract was determined spectrophotometrically at 595 nm using the Coomassie Plus™ Protein Assay Kit (Pierce, Rockford, IL) following the manufacturer's instructions and using bovine serum albumin (Fisher Scientific, Fair Lawn, NJ) as a standard.

Statistical analysis. Each experiment was conducted using a completely random design. All data were subject to analysis of variance. Means were compared with Fisher's protected least significance difference ($P = 0.05$) using SAS Version 9.1 software (SAS Institute Inc., Cary, NC).

Results and Discussion

At harvest, AVG-treated fruit were more firm than control fruit and had more starch, except for 'Red Fuji' (Table 1). Thus, most AVG-treated fruit were at a less advanced stage of ripening when the heat treatment was applied. There were few differences in SS or TA between treatments across cultivars.

After 30 d in cold storage, AVG suppressed ethylene production during ripening of 'Senshu' and 'Redchief Delicious' but not 'Lodi' or 'Red Fuji' (Fig. 1). 'Lodi' had the highest ethylene production of all cultivars and 'Red Fuji' the least. Autio and Bramlage (1982) evaluated AVG on several cultivars and found that 'McIntosh', the earliest cultivar with the highest ethylene production, responded less to AVG than the others. They suggested that some early cultivars may have greater ACS activity and therefore might need greater concentrations of AVG to delay ripening. Byers (1997), working with cultivars having different harvest dates, suggested that there might not be clear early-versus-late cultivar responses to AVG, although ethylene production levels versus the AVG response was not directly addressed.

Heat alone had no consistent effect on ethylene production after cold storage, suppressing it in 'Senshu' and 'Redchief Delicious' but having no impact with 'Lodi' or 'Red Fuji' (Fig. 1). Shao et al. (2007) also observed variation in the impact of prestorage heat on ethylene production among cultivars. Although heat can repress ethylene production during application of the treatment, production may recover if fruit are subsequently held at room temperature (Klein, 1989; Klein and Lurie, 1990). The key ethylene biosynthetic enzyme, aminocyclopropane oxidase (ACO), is more sensitive to heat than ACS (Atta-Aly, 1992; Klein, 1989), and increasing levels of ACO mRNA and protein have been seen during recovery from heat treatment (Lurie et al., 1996). This may explain why heat treatment alone was less effective than AVG alone in suppressing ethylene production after cold storage. The combination of AVG with heat treatment reduced ethylene production the most consistently in each cultivar except 'Lodi', suggesting some additive effect of the treatments.

The respiration rate after cold storage was not consistently affected by any treatment. AVG reduced respiration rate in 'Redchief

Table 1. Fruit characteristics of 'Lodi', 'Senshu', 'Redchief Delicious', and 'Red Fuji' apples 1 d after harvest.[z]

Treatment	Firmness (N)	Starch index	Soluble solids (%)	Titratable acidity (mg/100 mL)
		Lodi		
Control	61 ± 1	2.4 ± 0.3	9.9 ± 0.4	797 ± 27
AVG	75 ± 1	1.8 ± 0.2	9.1 ± 0.2	665 ± 69
		Senshu		
Control	61 ± 1	6.9 ± 0.7	10.0 ± 1.0	331 ± 40
AVG	69 ± 1	4.3 ± 0.8	9.8 ± 1.6	330 ± 24
		Redchief Delicious		
Control	70 ± 1	4.8 ± 0.6	11.6 ± 0.4	211 ± 19
AVG	76 ± 1	3.5 ± 0.3	10.2 ± 0.4	212 ± 11
		Red Fuji		
Control	67 ± 1	2.4 ± 0.2	12.3 ± 0.4	385 ± 18
AVG	67 ± 1	3.4 ± 0.3	13.1 ± 0.1	399 ± 27

[z] Values are mean ± SE of six replicate fruit. Firmness in Newtons (N); starch index from 1 to 9 except for Fuji from 1 to 6 with 1 high and 6 or 9 no starch; titratable acidity as mg malic acid/100 mL.
AVG = aminoethoxyvinylglycine.

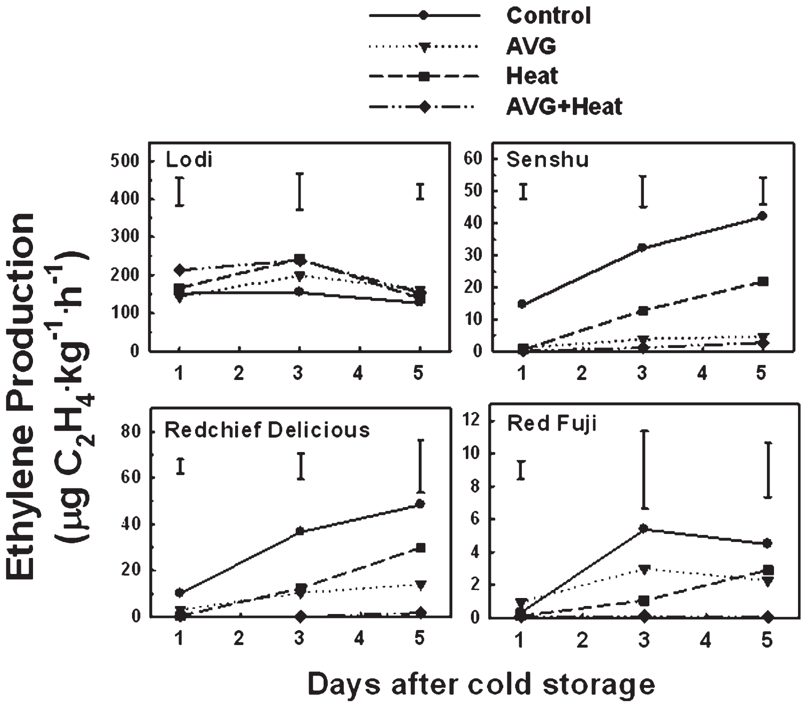

Fig. 1. Effect of aminoethoxyvinylglycine (AVG) and/or heat treatment on ethylene production of 'Lodi', 'Senshu', 'Redchief Delicious', and 'Red Fuji' apples ripened at room temperature for 5 d after 4 weeks of cold storage at 4 °C. Each data point is the mean of five to eight fruit. Vertical bars indicate the least significant difference (LSD) at $P = 0.05$.

Table 2. Effect of AVG and/or heat treatment on fruit characteristics of 'Lodi', 'Senshu', 'Redchief Delicious', and 'Red Fuji' apples ripened at room temperature for 1 ('Lodi') or 5 ('Senshu', 'Redchief Delicious', 'Red Fuji') d at ambient temperature after 4 weeks of cold storage.[z]

	Fruit characteristic				
Treatment	Respiration (mg CO_2/kg^{-1}·h^{-1})	Firmness (N)	Soluble solids (%)	TA (mg malic acid/100 mL)	SS:TA
		Lodi			
Control	27	27	9.0	770	11.2
AVG	25	36	9.1	587	15.5
Heat	32	31	9.2	558	16.9
AVG + heat	31	40	9.4	516	18.5
LSD	4	6	NS	105	5.5
		Senshu			
Control	22	58	10.5	298	35.1
AVG	20	63	9.9	251	41.6
Heat	2	63	10.8	242	44.6
AVG + heat	1	65	10.1	202	52.6
LSD	2	5	NS	53	13.1
		Redchief Delicious			
Control	24	69	11.1	240	46.6
AVG	20	70	11.9	168	71.5
Heat	16	70	11.4	134	86.5
AVG + heat	21	66	10.6	150	72.2
LSD	2	NS	NS	41	14.7
		Red Fuji			
Control	11	69	13.8	373	37.7
AVG	12	67	14.6	316	46.4
Heat	10	66	12.6	192	63.9
AVG + heat	1	67	13.9	280	50.4
LSD	2	NS	NS	61	7.8

[z] Cortex firmness is in Newtons (N). For calculating the soluble solids (SS):titratable acidity (TA) ratio, TA was first converted to g malic acid/100 mL. Means are from five to eight replicate fruit with significant differences among treatments within a cultivar by least significant difference (LSD) at $P = 0.05$. NS indicates no significant differences among means.
AVG = aminoethoxyvinylglycine.

Delicious' only, heat treatment reduced it in 'Senshu' and 'Redchief Delicious', and the combined treatment reduced it in 'Senshu', 'Redchief Delicious', and 'Red Fuji' (Table 2). Klein and Lurie (1990) observed that respiration rate was unaffected by heat, although ethylene was reduced. Although heat can repress respiration during the treatment (Porrit and Lidster, 1978), Saftner et al. (2002) measured respiration rate of previously heated fruit that were similar to or higher than those of control apples. Shao et al. (2007) observed a general reduction in respiration rate after heat treatment and cold storage, although the magnitude of the reduction varied among cultivars.

AVG alone and with heat maintained firmness of 'Lodi', AVG plus heat maintained it in 'Senshu', but neither 'Redchief Delicious' nor 'Red Fuji' firmness responded to the treatments (Table 2). Heat treatment alone had no effect on firmness. AVG-treated fruit have often, but not always, been more firm than control fruit after cold storage (Bangerth, 1978; Drake et al., 2005; Stover et al., 2003), possibly as a result of variation among cultivars and specific storage temperature (Autio and Bramlage, 1982; Bramlage et al., 1980). The effectiveness of AVG in reducing firmness loss during storage of 'McIntosh' and 'Red Delicious' was reduced when storage temperature was 3.3 °C versus 0 °C (Autio and Bramlage, 1982), perhaps explaining some of the variation noted here with fruit stored at 4 °C. Heat treatment has yielded variable responses on firmness loss in prior studies (Klein and Lurie, 1992; Lurie and Nussinovitch, 1996; Saftner et al., 2002, 2003; Shao et al., 2007; Tu and De Baerdemaeker, 1997). For 'Lodi' and 'Senshu', the combined treatment was more effective than heat treatment alone in reducing firmness loss with results similar to or better than AVG alone, suggesting that there could be an additive effect of the treatments for some cultivars.

AVG-treated 'Lodi' and 'Redchief Delicious' fruit, heated fruit of all cultivars, and AVG plus heat in all cultivars had lower TA than controls after cold storage (Table 2). There were no effects of any treatment on fruit SS. Heat treatment has also produced inconsistent results on these characteristics in other studies, although a reduction in TA with no influence on SS has been noted (Klein and Lurie, 1992; Porrit and Lidster, 1978; Tu and De Baerdemaeker, 1997). As a result of its impact on TA, the combined treatment increased SS:TA of all cultivars, although heat or AVG alone had no consistent effects. Apples with higher SS:TA ratio were described as sweeter and less tart by taste panelists (Lurie and Nussinovitch, 1996), suggesting that this response to the combined treatments could result in greater acceptability by consumers.

Although 12 major volatiles were produced by 'Redchief Delicious', 11 straight and branched-chain esters and one alcohol, the eight esters listed in Table 3 accounted for over 90% of the total esters produced by control fruit. Total ester production at 5 d after cold storage was reduced 44% by AVG and 70% or more by heat and AVG plus heat. Total alcohols, or hexanol because it was the only one detected, were also reduced by the treatments. 2-Methylbutyl acetate, butyl hexanoate, and butyl-2-methylbutanoate exhibited the same response profile as that of total esters. Ethyl butanoate and hexyl acetate were reduced equally by all of the treatments.

Table 3. Effect of AVG and/or heat treatment on volatile production of 'Redchief Red Delicious' apples.^{z}

		Area units × 10^{-3}/g fresh weight			
Compound	Control	AVG	Heat	AVG + heat	LSD
Total esters	1,231	1,087	418	301	122
Total alcohols	7	4	3	2	2
2-Methylbutyl acetate	232	130	27	37	97
Butyl hexanoate	230	156	55	40	65
Hexyl hexanoate	199	183	68	45	42
Ethyl butanoate	144	59	61	64	68
Hexyl acetate	108	35	6	12	54
Ethyl-2-methylbutanoate	101	71	36	43	56
Hexyl-2-methylbutanoate	77	373	125	40	65
Butyl-2-methylbutanoate	64	35	11	5	21

^{z}Fruit were ripened at room temperature for 5 d after 4 weeks in cold storage at 4 °C. Means are from three replicate fruit with significant differences within rows by the least significant difference (LSD) at $P = 0.05$. AVG = aminoethoxyvinylglycine.

Ethyl-2-methylbutanoate and hexyl hexanoate were reduced by heat and AVG plus heat but not AVG. Unique among the treatments, AVG increased hexyl-2-methylbutanoate above controls, whereas heat had no effect. Butyl acetate, butyl butanoate, and hexyl propionate were also detected at very low levels and were reduced by the treatments as well (data not shown).

Reductions in volatile production resulting from AVG or heat treatment immediately after harvest or after short-term cold storage have been reported (Mir et al., 1999; Saftner et al., 2002, 2003). Fallik et al. (1997) found that volatile production of heated apples eventually returned after several weeks of cold storage. Given that heated fruit had ethylene production values similar to those of AVG-treated apples but lower total ester production, the inhibition of volatile production by heat may be independent of ethylene production. After cold storage, there were no differences in AAT activity, which averaged 156 ± 18 mU/mg protein ($P > 0.05$) across treatments, results suggesting that substrate availability was most limiting for ester synthesis (Argenta et al., 2004; Berger and Drawert, 1984; Echeverria et al., 2004a), because treated fruit had less total alcohols. Heat treatment and AVG did not interact to influence volatile production with the combined treatment the same as that of heat alone.

Overall, combining preharvest AVG application with postharvest heat treatment resulted in no clear additive or synergistic effects on most apple-ripening characteristics. Based on the present results, heat plus AVG treatment did not prove to be a commercially desirable alternative to either alone for maintaining apple fruit quality during short-term cold storage.

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