EFFECT OF GRAPE VARIETY (*VITIS VINIFERA* L.) AND GRAPE POMACE FERMENTATION CONDITIONS ON SOME VOLATILE COMPOUNDS OF THE PRODUCED GRAPE POMACE DISTILLATE

EFFET DES VARIÉTÉS DE VIGNE (*VITIS VINIFERA* L.) ET DES CONDITIONS DE FERMENTATION DE PULPE DE RAISIN SUR QUELQUES COMPOSÉS VOLATILS DU DISTILLAT PRODUIT À PARTIR DE PULPE DE RAISIN

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Abstract : A grape pomace distillate was produced from 4 Greek white grape varieties (*Vitis vinifera* L.). Pomace was fermented with and without addition of citric acid, acting as an antibacterial agent, during fermentation. Fermented grape pomace was distilled in a traditional copper distillation apparatus.. Five major volatile compounds, including methanol, were measured. Pentanol-3 was used as an internal standard. Flame ionization detector (FID) coupled to capillary gas chromatography was used for determination of five volatile distillate components. The addition of citric acid resulted in the reduction of methanol content by about 15%. All the other components studied did not affect in any appreciable degree.

Résumé : Un distillat de pulpe de raisin a été produit à partir de 4 variétés de vigne grecques . Le moût a fermenté avec et sans l'ajout d'acide citrique, agissant en tant qu'agent antibactirien. Le moût fermenté a été distillé dans un appareillage de cuivre traditionnel de distillation. Cinq composés volatils principaux, y compris le méthanol, ont été mesurés. Le pentanol-3 a été utilisé comme norme interne. Le détecteur d'ionisation de la flamme (FID) couplé avec la chromatographie en phase gazeuse capillaire a été utilisé pour la détermination de cinq composants volatils de distillat. L'ajout d'acide citrique a comme conséquence la réduction du méthanol d'environ 15 %. Les autres composants étudiés n'ont pas été affectés d'une manière appréciable.

Mots-clés : *Vitis vinifera* L., distillat de pulpe de raisin, variétés de raisin, acide citrique.

Key words : *Vitis vinifera* L., grape pomace distillate, grape varieties, citric acid addition.

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INTRODUCTION

Grape pomace distillates are widely consumed in most European wine producing countries. Several names are used for this distillate in different countries. In Italy it is named Grappa, in Portugal bagaceira, in Turkic raki, in Yugoslavia kommovica, in Greece tsipouro etc. Some of grape pomace volatiles originate from the grape, but most of them are developed during pomace fermentation. Aroma profile is influenced by various factors such as environment, soil, climate, grape variety, degree of the grape ripeness, enological methods used for pomace production, pomace fermentation conditions, distillation process, and the aging of the distillate. Grape pomace volatiles belong to different chemical groups such as higher alcohols, esters, aldehydes, ketones, acids, sulfur compounds etc. Some of these compounds are volatile or highly volatile, while others exhibit lower volatility. These aroma compounds exist in a wide concentration range. Some of them are present at high concentrations (hundreds of mg/L), but most of them are found at very low concentration range, from traces to l0 mg/L.

Grape pomace volatiles can be determined by GC/FID. Some times concentration of the volatiles by extraction is necessary before analysis by chromatographic method. Several extraction, -concentration methods have been used, such as liquid-liquid extraction (FERREIRA *et al.*, 1993; HARDY *et al.*, 1969; RAPP *et al.*,1976;USSEGLIO, 1971), liquid-liquid extraction with ultrasound (BOIDRON *et al.*,1988), simultaneous distillation-extraction (ORRIOLS - FERNANDEZ, 1994), and other techniques (GARCIA-JARES et al., 1995; SALINAS *et al.*,1994; SILVA *et al.*,1996; VEMIN *et al.*,1987). These techniques are generally labor-intensive and are characterized by relatively low reproducibility. Sample preparation was mainly concerned to obtain more concentrated samples, but the elimination of interfering substances is also important. The specific advantages and disadvantages of these methods are always considered when selecting the most adequate technique for given condition. Producers of grape pomace distillates have major concern for methanol concentration. Methanol is a toxic volatile constituent of fermented must or grape pomace, produced by the action of the enzyme pectinesterase on grape pectin. In order to reduce the amount of methanol the addition of tartaric acid on the fermented grape pomace was studied (SILVA and MALCATA, 1998).

Grape pomace volatiles analyzed by direct injection of the samples with a gas chromatograph (GC) equipped with FID (EC, 2870/2000).

The effect of citric acid on the fermentation of grape pomace has not been studied up until now. To that effect, methanol and 4 other major volatile compounds (acetaldehyde, ethyl acetate, 2-methyl-butanol and 3-methylbutanol) contained in fermented grape pomace derived from 4 of the most popular Greek grape varieties were studied. It was also studied the effect of citric acid addition during the fermentation process of grape pomace.

MATERIALS AND METHODS

I - SAMPLES

Four plots of four white grape varieties (Moschato of Limnos, Moschato of Patras, Savatiano and Asyrtiko), cultivated in private industrial vineyards of Greece were selected. Samples of 150 kg of grapes of each variety were collect. Sampling was performed by randomly collecting grapes from various places of the experimental plots, according to the FAO/WHO (1986) recommendations. Grapes were crushed in a pilot horizontal press machine and the must was removed. Samples of 40 kg pomace from each variety were collected. Each sample was divided in two lots (20 kg each) so eight lots were prepared. Half of them, one from each sample, were fermented by means of indigenous yeast flora. The second half of the grape pomace lots were independently sprayed with aqueous solution of citric acid (commercial grade - 20 g/L) prior to storage for fermentation. The addition of acid reduces pH and increases acidity that limits bacteria growth and in turn the formation of methanol is retarded. (Silva and Malcata.,1998). As soon the fermentation was ended distillation of the pomace was followed.

II - DISTILLATION

The distillation process of fermented grape pomace from red grape varieties realised with the small copper alembics of 130 L, which traditionally used. The fermented raw material is transferred to the distillation pot up to the 3/4 of its capacity in order to be distilled. Before the beginning of heating, the copper alambic is hermetically closed with dough in order to prevent any vapour leakage. When the temperature reaches 80–90 °C, the liquid spirit starts to run and collected in glass bottles.

The first 0.2–1 L of the distilled product, corresponding to the beginning of the distillation procedure, is removed as « head ». It usually presents a very high alcoholic title, 90 % vol. approximately. Then, and for about 3.5 h, a pure spirit, distilled to levels lower than 87 % vol., is collected into glass bottles. This distillate is commonly called first distillation. The distillation product is subsequently obtained until 5 % vol., and it is led back to the alembic for a second distillation. The distillation of grape pomace is being done without the use of any aromatic plants or seeds. In this case, the aroma of the spirit is deriving exclusively from the raw material, the fermentation process. The «tails»' (distilled spirit below 5 % vol.)

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used for the next first distillation process with the new lot of the fermented grape pomace. Collected distillate from the 1st distillation was redistilled. Distillation products were collected as six equal volume fractions for both the 1st and 2nd distillation.

In the end, the various lots of the second distillation spirit are mixed and placed, before the dilution with water, into oak barrels and left to age one year.

III - CHEMICAL ANALYSIS

Standards (chromatographic grade) of volatiles studied (methanol, acetaldehyde, ethyl acetate, 2-methyl-butanol and 3-methyl-butanol) were purchased from Merck, Switzerland.

A 5-mL sample of each varietal grape pomace distillate was mixed with 50 µL of the internal standard solution (50 g of pentanol-3 per L of ethanol). A gas chromatograph (Hewlett Packard 5890 series II) equipped with FID has been used for the ana1ysis of aroma compounds. Samples of 0.2 µL were injected into the gas chromatograph. The injector of the gas chromatograph was maintained at 200°C and operated under split mode. Elution was achieved in a 30m x 0.25 mm i.d. x 0.2-µm capillary INNOWAX cross-linked polyethylene glycol column.

The oven temperature program was as follows: 40 °C for 5 minutes, a linear ramp from 40 °C to 200 °C at 3 °C / min, and 200 °C for 20 min. Detection was by FID at a temperature of 200 °C. Helium was used as the carrier gas at a split ratio of 1:60. The flow rate of the carrier gas was 2 mL/min. Air pressure was adjusted to 119 kPa (air). Chromatographic runs were carried out in triplicate, and their average was used as a single data point in the results section. The average coefficient of variation for the triplicate assays was 1.1-1.6 %.

RESULTS AND DISCUSSION

Results obtained, concerning the concentrations of grape pomace distillates volatiles in the studied four Greek grape varieties, are presented in figures 1, 2, 2a, 3, 4, 5 and 6. Differences in ethanol content for pomace fermented without addition of citric acid and for pomace fermented with addition of citric were less than 1.5 %vol. Methanol ranged from 3 045 mg/L to 6 277 mg/L for pomace fermented without addition of citric acid (figure 1) and 2 226 mg/L to 5 490 mg/L, for pomace fermented with addition of citric acid (figure 2). BAUMES *et al.* (1986) reported a concentration of methanol in grape spirits from 5300 mg/L to 15 900 mg/L, KANA *et al.* (1991) reported methanol concentration from 700 to 1770 mg/L, DANILATOS and HARVALA (1981) found methanol concentration 180-800 mg/L and SOUFLEROS (1987)

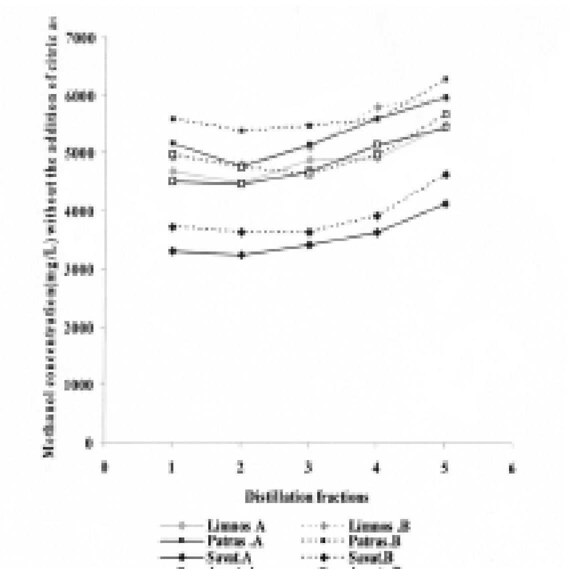

Fig. 1 - Methanol content in distillate from grape pomace from Greek white grape varieties, fermented without citric acid (mg/L).

A. first distillation B. second distillation.

Contenu en méthanol dans le distillat de pulpe des variétés grecques de raisin blanc grec, fermentées sans acide citrique (mg/L).

A. Premier distillation B. Deuxième distillation.

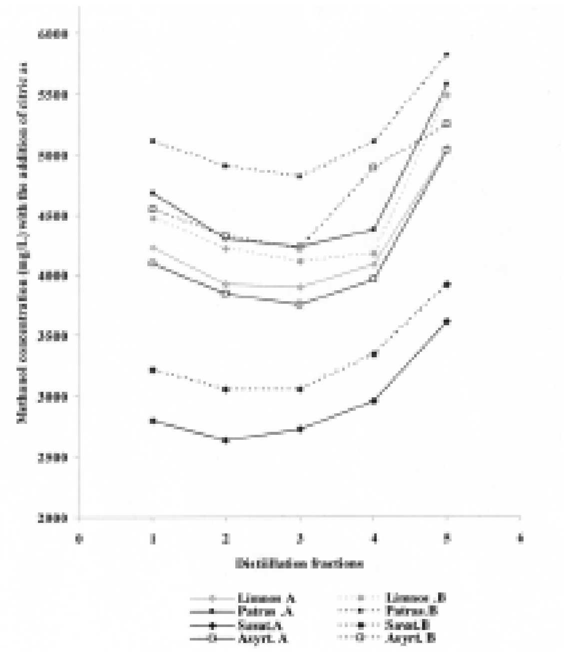

Fig. 2a - Methanol content in distillate from grape pomace from Greek white grape varieties, fermented with citric acid (mg/L)

A. first distillation B. second distillation.

Contenu en méthanol dans le distillat de pulpe de raisin des variétés grecques de raisin blanc , fermenté avec de l'acide citrique (mg/L).

A. Premier distillation B. Deuxième distillation .

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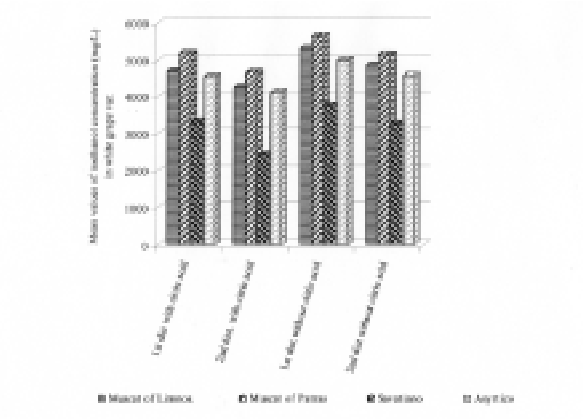

Figure 2b - Histogram of mean values of methanol concentration of the 4 grape varieties studied for 1st and 2nd distillation (with addition of citric acid and without addition of citric acid).

Histogrammes des valeurs moyennes de la concentration en méthanol des 4 variétés de raisin étudiées pour la 1ère et 2e distillation (avec addition d'acide citrique et sans addition d'acide citrique).

respectively 504-840 mg/L. It has to be noted that according to the present EC regulations (EC 1576/1989) the permitted maximum for methanol concentration in alcoholic beverages is 10 000 mg/L.

Pomace from pressed grapes takes advantage of spontaneous anaerobic fermentation during storage in which yeasts convert water-soluble carbohydrates to alcohols, esters, carboxylic acids, and aldehydes. The increased acidity limits the activity of bacteria and reduces the formation of methanol by inhibition of enzymes implicated in pectin degradation attack (SILVA and MALCATA, 1998).

It is known that microbial attack of the fermenting grape pomace increases pectinesterase production and accordingly methanol content of the produced distillate (SILVA and MALCATA, 1998). Different methods have been used for prevention of microbial attack. In Spain, for bagaceira production, tartaric acid was used for pH reduction and concomitant obstruction of microbial multiplication (SILVA and MALCATA, 1998). For the same reason citric acid, instead of tartaric, is used by Greek distillers to reduce methanol content and improve flavour of traditional grape pomace distillates. Due to lack of studies concerning the addition of citric acid during fermentation, samples of grape pomace were fermented with and without citric acid addition. From the results the following values were found.

Methanol content in all samples of pomace fermented with addition of citric acid (figure 2) was lower than that in the samples fermented without addition of citric acid by 3.6 up to 16.36 % with a mean value of about 15 %. The highest methanol content was found in the variety Muscat of Patras. It was followed by Muscat of Limnos and Asyrtiko and the least methanol had Savatiano. In the beginning of distillation (fraction 5) methanol concentration was a bit higher than in the rest of the fractions. It is interesting that methanol is eluted in rather similar concentration during the whole distillation process.

Taking into consideration mean values for methanol it can be seen (figure 2b) that in all cases methanol content in samples of 2nd distillation was higher than in corresponding samples of 1st distillation. Also, distillates from samples with addition of citric acid had lower methanol than the corresponding samples derived from grape pomace fermented without addition of citric acid.

Acetaldehyde (figure 3) ranged from 87 mg/L to 1 290 mg/L for pomace fermented without addition of citric acid. SOUFLEROS and BERTRAND (1987) found acetaldehyde 60.0 to 400.8 mg/L in tsipouro from Greek grape varieties and DANILATOS and HARVALA (1981) 130 to 1380 mg/L also in Greek tsipouro. SILVA *et al.*, (1998) reported concentration of acetaldehyde in Bagaceiras between 179-671.9 mg/L. Pomace fermented with addition of citric acid did not resulted in any appreciable difference in the concentration of acetaldehyde and the other congeners studied. The higher concentration of acetaldehyde (figure 3) had grape pomace distillates from grape varieties of Asyrtiko followed by Muscat of Patras, Muscat of Limnos and Savatiano. Based on Savatiano, the concentration of acetaldehyde in Asyrtiko was 290 % higher, in Muscat of Patras 220 % higher and in Muscat of Limnos 148 % higher. In the beginning of distillation (fraction 5) acetaldehyde concentration, as in the case of methanol, was a bit higher than

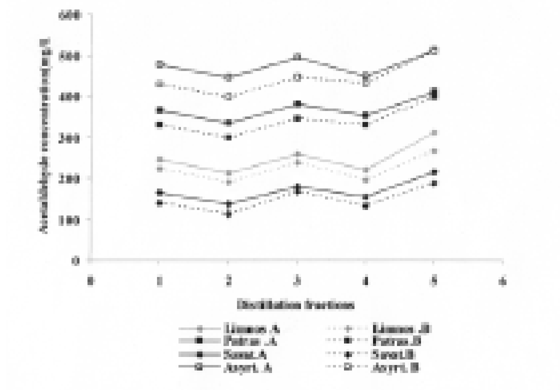

Fig. 3 - Acetaldehyde production of grape pomace distillate from four Greek white grape varieties (mg/L).
A. first distillation B. second distillation

Production d'acétaldéhyde de distillat de pulpe de raisin des quatre variétés grecques de raisin blanc (mg/L).
A. Premier B. Deuxième distillation.

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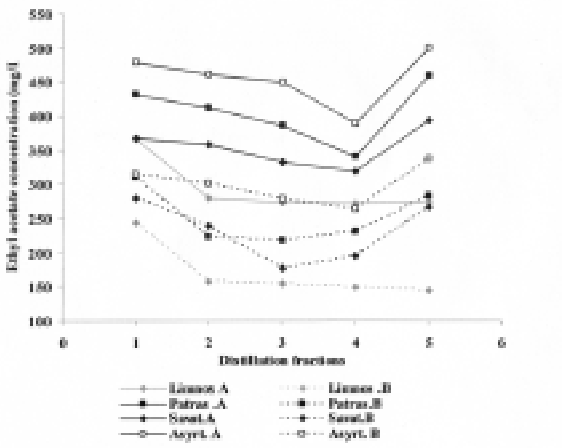

Fig. 4 - Ethyl acetate production of grape pomace distillate from Greek white grape varieties (mg/L).
A. first distillation B. second distillation.

Production d'acétate d'éthyle de distillat de pulpe de raisin des variétés grecques de raisin blanc (mg/L).

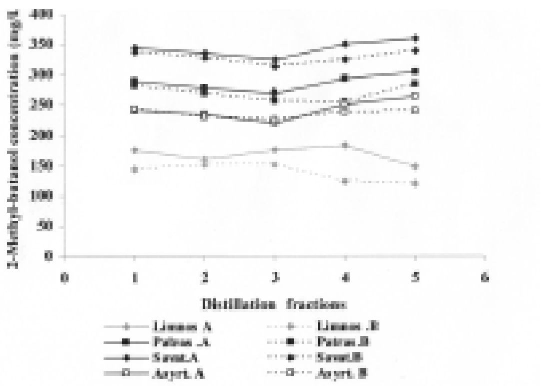

Fig. 5 - 2-methyl-butanol production of grape pomace distillate from Greek white grape varieties (mg/L).
A first distillation B. second distillation.

Production de 2-méthyl-butanol du distillat de pulpe de raisin des variétés grecques de raisin blanc (mg/L).
A. Première distillation. B. Deuxième distillation

in the rest of the fractions. Differences between the first and the fifth fraction were in the order of 25-35 %. Ethyl acetate (figure 4) ranged from 1 531 mg/L to 4 996 mg/L without the addition of citric acid. SOUFLEROS and BERTRAND (1987) found ethyl acetate from 257 to 1 197 mg/L in tsipouro from Greek grape varieties and DANILATOS and HARVALA (1981) from 300 to 580 mg/L also in Greek grape pomace distillates. NYCANEN and NYCANEN (1991) reported concentration of ethyl acetate in brandy between 20-284 mg/L, in rum between 24-243 mg/L and in whisky between 4-148 mg/L. Fermented pomace from grape varieties of Muscat of Limnos, Savatiano and Asyrtiko had higher and very similar concentration of ethyl acetate (from 310-326 mg/kg), followed by Savatiano (190.7 mg/kg). In the beginning of distillation (fraction 5) ethyl acetate concentration was higher than in the 4th fraction from 8.5-18 %. A small increase of ethyl acetate concentration that is noted between 3rd and 1st fraction was in the range of experimental error and it was not evaluated. 2-methyl butanol (figure 5) ranged from 101 mg/L to 360 mg/L for grape pomace fermented without addition of citric acid. SOUFLEROS and BERTRAND (1987) found 2-methyl-butanol from 216 to 490.5 mg/L in tsipouro from Greek grape varieties. Silva et al (1998) reported concentration of2-methyl-butanol in bagaceira between 165.8-255.3 mg/L. The higher concentration of 2-methyl-butanol (figure 5) had spirit produced from pomace of grape varieties of Savatiano followed by Muscat of Patras, Asyrtiko and Muscat of Limnos. Based on Muscat of Limnos concentration of 2-methyl-butanol, the concentration of Savatiano was 108 % higher, Muscat of Patras 75 % higher and of Asyrtiko 46 % higher. In the beginning of distillation (fraction 5) 2-methyl-butanol concentration was higher than in the 4l fraction from 2.8-8.5 %. rest of the fractions. In the rest of the fractions 2-methyl-butanol had similar concentrations. The amount of 3-methyl-1-butanol (figure 6) in spirit from grape pomace fermented without addition of citric acid was from 143 mg/L up to 456 mg/L. SOUFLEROS and BERTRAND (1987) found 3-methyl -butanol from 916 to 1727 mg/L in spirit from Greek grape varieties. SILVA et al. (1998) reported concentration of 3-methyl-butanol in bagaceira between 400,2-685.2 mg/L. Nycanen and Nycanen(1991) reported concentration of 3-methyl- butanol in whisky between 410-475 mg/L, in Cognac between 960-1 250 mg/L and in armaqnac 990-1 025 mg/L. Initial concentrations of 3-methyl-butanol (fraction 5) were higher than that of fraction 3 from 0.8-7.6 % (figure 6). In the rest of fractions the concentrations of3-methyl-butanol were rather similar. The higher concentration of 3-methyl-butanol had Asyrtiko and Muscat of Limnos (423 and 405 mg/kg respectively), followed by Savatiano 367 mg/kg and Muscat of Patras 247 mg/kg. Based on Muscat of Patras concentration of 3-methyl-butanol, the concentration of Asyrtiko and Muscat of Limnos were higher by 71 % and 64 % respectively and of Savatiano was higher by 48, 5 %.

CONCLUSIONS

It can be seen that application of citric acid during grape pomace fermentation resulted in a decrease of methanol by 8-20.9 %. Taking into consideration the deleterious effect of high methanol content of grape pomace distillates it seems that addition of citric acid during pomace fermentation improves the quality of produced grape pomace distillates. Citric acid had not any effect on the concentration of the other tsipouro volatiles studied. Concerning differences in concentration of distillate volatiles between first and second distillation the

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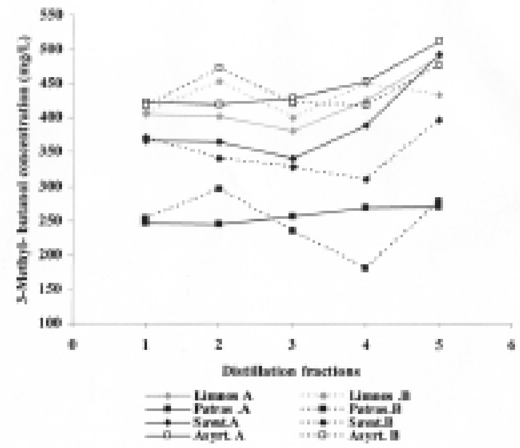

Fig. 6 - 3-methyl-butanol production of grape pomace distillate from Greek white grape varieties (mg/L).
A. first distillation B. second distillation.

Production de 3-méthyl-butanol de distillat de pulpe de raisin de variétés grecques de raisin blanc (mg/L).
A. Première distillation. B. Deuxième distillation

following can be seen from figures 2a, 3, 4, 5, and 6. Methanol and ethyl acetate concentrations were higher in the second distillation for grape pomace fermented with and without addition of citric acid. For the rest of volatiles studied (acetaldehyde, 2-methyl-butanol and 3-methyl-butanol) differences between first and second distillation were rather small and random.

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