selected for a survey study by the combined gpc–lc technique. Figure 5 shows chromatograms of 1- and 6-year-old rum samples from the same distillery. There is an obvious increase in the concentration of lower molecular weight uv absorbing components in the older rum, while the main peak at about 350 molecular weight remains relatively constant. These differences may be due to variables in the aging process, including the age and history of the barrel, as well as the temperature of the aging area. One possible explanation for these differences could be that the main peak of the 1-year rum at about 350 molecular weight could come primarily from extraction from the barrel, while the lower molecular weight components in the aged rums come from slow extraction and side reactions. To complicate matters, the age of the samples can be misleading if it is not realized that the stated age on the bottle reflects the youngest rum used and not necessarily the age of the entire sample. A 1-year rum, then, could contain various amounts of older rums blended together to give a particular desired taste.

The chromatograms in Figure 6 show 1-year rum samples from different distilleries. The differences in the chromatograms apparently represent the differences in the aging and blending processes.

The 6-year-old rum sample was selected for further characterization by preparative gpc and subsequent liquid chromatography. Fractions were collected in 5-ml volumes as shown in Figure 7. These fractions were blown to dryness with a stream of dry nitrogen and reconstituted to 100 μl. A 5-μl aliquot was then analyzed by partition chromatography. Chromatograms of these fractions and the total rum sample are shown in Figure 8. Fraction 3 with an upper molecular weight of approximately 500 was the highest molecular weight fraction that could be chromatographed under these conditions. By using combined gpc–lc methods, some 60 peaks have been separated from the 6-year-old rum. Similar results have been obtained for aged bourbon samples. Once the relationship between the gpc profile and the aging process has been established, liquid chromatography can be used to isolate those specific compounds of primary importance in the aging process.

CONCLUSIONS

The use of combined gpc–lc for the characterization of complex flavor mixtures has been established. By this technique it should be possible to better determine the identity of those compounds responsible for the odor and flavor of essential oil. In addition, it should be possible to determine the contribution of various climatic and geographic variables to the characteristics of the oil should this be possible. These techniques should also prove valuable in determining the effect of various processing steps on the quality of the final product.

In the analysis of alcoholic beverages, the data presented would suggest that this combined liquid chromatographic approach would prove useful in determining such factors as the influence of barrel age, barrel history, temperature of the aging area, blending, and other variables on the flavor of the final product.

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Automated Colorimetric Measurement of Free Arginine in Peanuts as a Means to Evaluate Maturity and Flavor

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This paper includes a review of published information and presents new information relative to the adaptation of the Sakaguchi reaction for measuring free arginine content of raw (green and cured) and roasted peanuts. Emphasis is on the automation of the method for research and industrial use in the peanut industry so that several hundred samples can be analyzed daily. The role of free arginine in flavor development and its relationship to maturity are discussed.

Based upon the work of Newell (1967), Mason *et al.* (1968, 1969) discussed the nonvolatile components of peanuts and their importance in typical and atypical roasted flavor. They pointed out that changes in free arginine were very dramatic and that its concentration was inversely correlated with maturity.

Their work was accomplished with ion-exchange chromatography which is excellent for identification and quantitation of amino acids but too time-consuming for

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routine studies with large numbers of samples. Izumi (1965a,b) reported work in which he improved upon reproducibility of the Sakaguchi reaction for measuring free arginine. Young (1970) and Young and Mason (1972) used the "New Sakaguchi Method" of Izumi to determine the level of free arginine in Oklahoma grown peanuts and thereby established the degree of immaturity in freshly harvested and cured peanuts. The method was tested under field conditions and found to be an accurate measure of maturity. This method was limited to about 12 analyses per working day per technician. More recently, Young (1972) has shown that the application of such an analytical method to field samples might provide considerable economic gains for the peanut farmer.

Pang (1967) had divided peanuts into four maturity classifications (*i.e.*, mature, high intermediate, low intermediate, and immature) and found that peanut butter made with the low intermediate group of peanuts was inferior to those made with mature peanuts. The study of Young and Mason (1972) using a similar classification method for peanut maturity showed this lower intermediate group contained about 50% more free arginine than the mature and high intermediate groups. The immature peanuts which are usually quite small were found to be much higher in free arginine, and Pang scored these lower in flavor than the low intermediate group. Therefore the measurement of free arginine in peanuts appeared to be an adequate measure of both lack of maturity and poor flavor of roasted peanut products.

For the practical application of this basic information to the peanut industry, it will be necessary to analyze large number of samples. Primary attention in this paper deals with the automation of the modified Sakaguchi reaction in order to analyze a sufficient number of samples of peanuts for adequate evaluation of maturity (several thousands per growing season). Desired flavor development in roasted peanuts appears to be a very sensitive function of maturity (Mason *et al.*, 1968, 1969; Newell, 1967) and flavor (Pang, 1967).

MATERIALS AND METHODS

The following is an outline of the reagents, equipment, and procedure developed for the automated determination of free arginine.

Apparatus. The following apparatuses were used: Servall Omni-Mixer with a Time-O-Lite timer; Technicon Auto Analyzer Sampler II; Technicon Variable Speed Proportioning Pump Model 1; Technicon S. C. Colorimeter; Infotronics CRS-110A Digital readout system; Teletype, Model 33 Teletypewriter; and Bristol, Multiple-Point Dynamaster Recorder.

Procedure. While the procedure described below has been applied successfully, additional modifications are being investigated to speed up further the analyses for routine application.

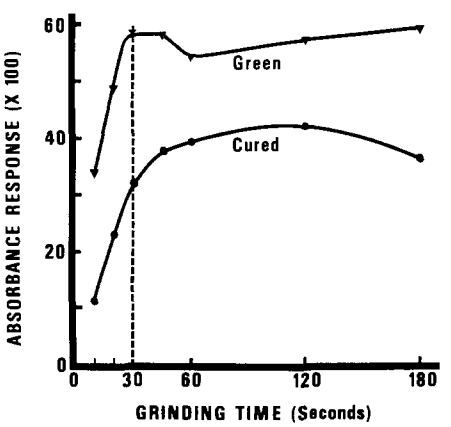

Figure 1. Effect of grind time (seconds) on the analysis of free arginine in green and cured peanuts.

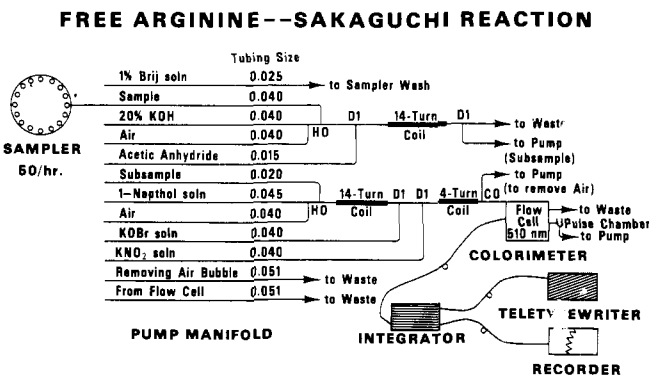

Figure 2. Schematic flow diagram of the manifold used for the automated continuous flow analysis of free arginine.

Peanuts (30 g of green in the pod, or 20 g of shelled cured or roasted) are ground for 30 sec in 200 ml of a 2% trichloroacetic acid (TCA) solution (Figure 1) using a Servall Omni-Mixer. A timer is used to standardize the grinding time. This blended mixture is transferred to a beaker and allowed to stand 10–15 min before decanting and filtering approximately 75 ml through Whatman No. 2 folded filter paper. The 20-ml sample cup is partially filled with the filtrate and placed on the analyzer. Samples are analyzed at the rate of 50/hr using a manifold which is constructed as shown in Figure 2. The tubing size (Figure 2) determines the portion of chemical used in the analyses. To the sample is added 20% KOH with the addition of dry acetic anhydride (ACS grade). The solution is mixed *via* a coil and subsampled at room temperature of 21–23°. The acetylated sample and 1-naphthol solution (0.01% 0.00059 *M*, in 10% aqueous potassium hydroxide, prepared daily) are mixed (room temperature) and KOBr [concentrated potassium hypobromite (0.0116 *M*) (0.62 ml of bromine in 100 ml of 5% potassium hydroxide) which is stored at 4° is diluted 10× with 5% potassium hydroxide before using] is added to develop the color. Color is stabilized by the addition of the 1% KNO_2 methanol–water (1:1) solution with mixing. The absorbance of a portion of this sample is read at 510 nm using the flow cell colorimeter. Standard arginine solutions (1 g/1000 ml with 5, 10, 20, 30, 40, and 50 ml/100 dilutions) are used routinely to adjust and standardize recorder response. The linear electronic signal is transmitted to an integrator for measuring and the output is recorded by integrator–teletypewriter and recorder. Recorder response level was adjusted to correspond to the levels of free arginine (μg/ml) reported by Young and Mason (1972). Results are expressed as arginine maturity index (AMI) values by measuring peak height and/or by calculation from the integrator output. The addition of an in-lab mini-computer for further increased efficiency is anticipated.

RESULTS AND DISCUSSION

Peanut samples blended with solvents such as methanol, methanol–chloroform–water, methanol–water, water, and citric acid solutions did not give a very clear filtrate or free filtration that was considered acceptable for the automation of the Sakaguchi reaction. Grinding the samples for 30 sec with 2% TCA solution which was allowed to stand several minutes before filtering gave the best results with a clear filtrate and ease of filtration. Also, the grinding time was short enough to prepare large numbers of samples daily. The 2% TCA solution also extracted some interfering compounds as previously noted (Young

and Mason, 1972) with the perchlorate extracts. Since the percentage of these substances (glutamic acid, aspartic acid, phenylalanine, and peptides) appears to be rather constant over the range studied, it has not been necessary to add a correction factor to maturity values. It was necessary for the solution to stand several minutes after grinding in order to avoid a milky filtrate which interfered with the reading of the developed color. The filtrate could be held up to 48 hr at 21° without a significant decrease in the arginine readings if no evaporation occurred.

Several precautions pointed out by Young and Mason (1972) were not critical with the described equipment. For example it was not necessary to carefully protect the KOH solution against CO_2 absorption or keep the extraction mixture cold. It was still necessary to prepare the 1-naphthol solution daily. Since the alcohol addition for preservation of color gave a cloudy solution, it was necessary to modify this step. A solution of 1.0% KNO_2 in a 1:1 mixture of methanol–water gave excellent results.

During the adaption of the Sakaguchi reaction to continuous flow equipment, the precision and accuracy previously reported (Young and Mason, 1972) was maintained with an analysis rate of 30/hr. During the analyses of peanut samples, some precision has been sacrificed by increasing the rate to 50/hr. This rate gave a standard deviation of 2.72 (range 22–54) for duplicate analysis of 268 cured peanut samples (Young *et al.*, 1972) which is about twice the previously reported values of Young and Mason (1972).

Present data (Young *et al.*, 1972) indicate that samples with an AMI value of greater than 35 (the least mature) almost always produced bitter peanuts, but additional investigations of the factors affecting the relationship are needed. Samples with a 30–35 value often contained a mixture of good and bitter-flavored peanuts. Peanuts below 30 (the most mature) usually produced a desirable or acceptable roasted peanut.

Additional studies are now in progress on methods of sampling green peanuts from the field so that the method can be routinely adapted to advise farmers of appropriate digging dates for maximum quality and best economic returns.

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