Journal of Chromatography, 325 (1985) 450–455
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 643

Note

Liquid chromatography of tomato paste serum with sample preparation by ultrafiltration

STEVEN E. COOK and THOMAS C. PINKERTON*
Department of Chemistry, Purdue University, West Lafayette, IN 47907 (U.S.A.)
and
GEORGE SADLER and PHILIP E. NELSON
Department of Food Sciences, Purdue University, West Lafayette, IN 47907 (U.S.A.)
(Received February 11th, 1985)

Thermal browning reactions caused by heating food products during processing and storage lead to changes in the color, odor, and flavor of foods. In tomato products, these undesirable effects, as well as methods for evaluating product quality, have been extensively investigated[1–5]. Because the formation of hydroxymethylfurfural (HMF) results from thermal browning reactions[6], one method used to measure the degree of thermal abuse to foods is the quantification of HMF. Allen and Chin[7] have described a procedure to quantify HMF in tomato paste by using high-performance liquid chromatography (HPLC) of the serum obtained from the ultracentrifugation of tomato paste at 96 000 rpm. Access to a centrifuge that can attain these velocities may be impossible for some investigators; therefore, other methods for easily obtaining tomato paste serum for HPLC analysis are desirable. An alternative to ultracentrifugation is ultrafiltration. Blanchard[8] reported that the ultrafiltration of blood plasma through centrifuge membrane cones acts to deproteinate a plasma sample prior to HPLC analysis. This method removes more than 99% of the proteins from a plasma sample.

The objective of this investigation was to utilize ultrafiltration centrifuge membrane cones to prepare a tomato paste serum filtrate that can be injected directly onto a reversed-phase HPLC column for quantification of HMF. Also, the chromatographic profiling of unknown tomato paste serum components is achieved by gradient elution.

EXPERIMENTAL

Ultrafiltration

CF-25 Centriflo™ membrane cones (Amicon, Danvers, MA, U.S.A.) were used for filtration of tomato paste samples, in conjunction with a PR-J International centrifuge with a 7-in. diameter rotor (International Equipment, Needham, MA, U.S.A.).

CF-25 filtration cones were prepared by soaking in deionized distilled water for 1 h followed by centrifugation at 2000 rpm (800 *g*) for at least 10 min. Excess

0021-9673/85/$03.30

water was then decanted and the empty cones were spun for an additional 15 min. After dispensing 8–9 g of tomato paste per cone, the cones were spun for 30 min to yield a clear serum filtrate.

Chromatographic apparatus

The liquid chromatographic system consisted of a LC9533 ternary gradient chromatograph, a LC9523 variable-wavelength detector (IBM, Danbury, CT, U.S.A.); and a HP3390A integrator (Hewlett Packard, Palo Alto, CA, U.S.A.). The column was a 10 cm × 4.6 mm I.D. 3-μm Microsorb C_{18} (Rainin, Woburn, MA, U.S.A.).

Chromatographic procedure

Multicomponent qualitative profiles. To 100 μl of tomato paste serum filtrate were added 100 μl of deionized distilled water, and 10 μl of this diluted serum were injected onto the C_{18} column, followed by gradient elution. The solvent gradient consisted of an initial state of 100% water at 0 min, held for 1.5 min; a 6% per min linear gradient for 30 sec to 3% methanol in water, held for 4 min; a 4.3% per min linear gradient for 4 min to 20% methanol; a 1% per min linear gradient for 10 min to 30% methanol; and finally a 5.0% per min linear gradient for 14 min to 100% methanol. A flow-rate of 0.9 ml/min was maintained throughout the elution with optical detection at 284 nm (0.200 a.u.f.s.).

HMF quantification. The standard addition method was employed to quantify HMF in the serum filtrate of tomato paste stored for seven months at 100°F. Standard addition samples were prepared by adding 0.00, 0.025, 0.050, 0.075, and 0.100 ml of a 40.3 μg/ml HMF stock solution to five 0.1-g weighed aliquots of tomato paste serum filtrate, containing an unknown amount of endogenous HMF. Appropriate volumes of deionized distilled water were added to these solutions in order to impart an equal dilution to each standard. The final concentrations of HMF added to the serum filtrate were 0, 9.22, 18.4, 27.9, and 38.8 ppm, respectively. Each standard was injected onto the C_{18} column at least three times. Optimum solvent conditions were as follows: solvent gradient initial state 3.0% methanol in water at 0 min; 1.2% per min linear gradient for 2.5 min to 100% water, with sample injection at 30 sec into program; elution held at 100% water for 4.5 min; followed by a 6% per min gradient for 30 sec to 3% methanol in water. The flow-rate was maintained at 0.9 ml per min with detection at 284 nm (0.200 a.u.f.s.). A calibration curve was constructed by plotting the relative areas under the HMF peaks *versus* the concentration of HMF added to serum filtrate.

RESULTS AND DISCUSSION

The ultrafiltration of tomato paste with centrifuging, ultrafiltration membrane cones yields 0.2–0.4 ml of serum filtrate. This volume of serum is sufficient for several HPLC injections; however, to quantify HMF in serum by the standard addition method described, it is necessary to pool the filtrate from at least two cones, since a minimum of 0.5 ml of serum is required to make up the standard addition samples. The reproducibility of the serum preparation was studied by centrifuging a tomato paste sample in four cones followed by a one-fold dilution of the resulting filtrate

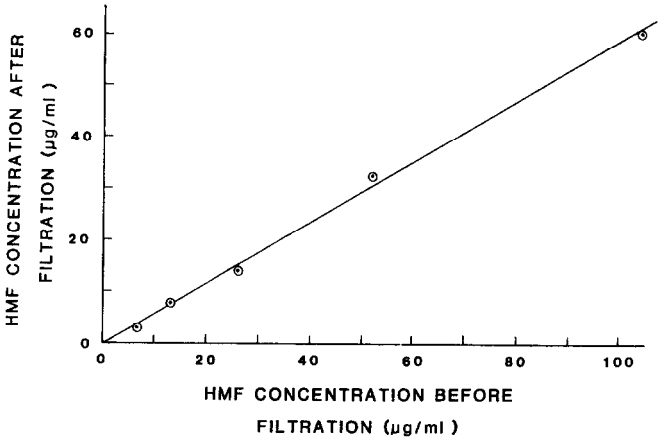

Fig. 1. Comparison of the concentrations of HMF before and after filtration through centrifuge membrane cones of five 0.400-ml aliquots of aqueous HMF solutions.

from each cone prior to injection onto the liquid chromatographic system. Comparison of HMF peak areas (concentration of *ca.* 40 ppm in the filtrate) resulted in a coefficient of variation of 4.94%. Centriflo membrane cones can be successfully reused after cleaning with a 0.1% solution of sodium hydroxide and storage in a 0.1% solution of sodium azide, in accordance with the manufacturer's recommendation; however, after prolonged use or neglect in cleaning and storage, the cones become clogged and ineffective.

Binding of HMF to the cone membrane was investigated by filtering 0.400-ml

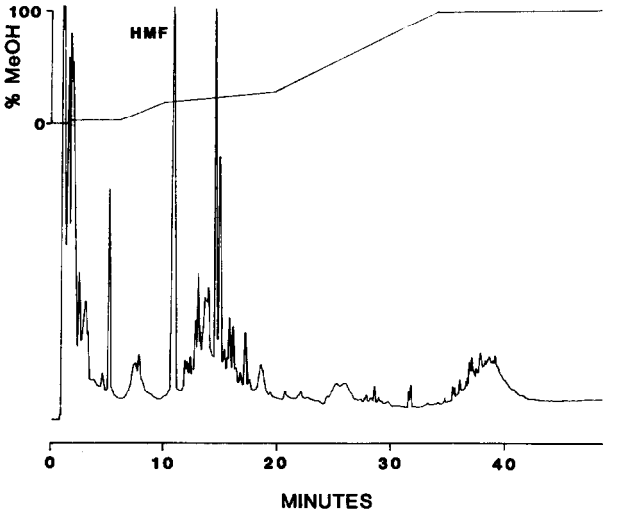

Fig. 2. HPLC separation of serum filtrate from tomato paste stored for seven months at 100°F resulting in multicomponent chromatographic profiles. Solvent conditions are described in the Experimental section. Flow-rate, 0.9 ml/min; detection, 284 nm; 0.200 a.u.f.s.

aliquots of aqueous HMF solutions ranging in concentration from 6.481 μg/ml to 103.7 μg/ml and comparing the HMF concentration before and after filtration. A curve of HMF concentration after filtration *versus* HMF concentration before filtration was linear with a slope of 0.5960, a y intercept of -0.4380 μg/ml, and a correlation coefficient of 0.9986 (Fig. 1). This implies that binding occurs, but that the degree of binding is directly proportional to the concentration of HMF in the filtered samples. Evaluation of heat damage to food products relies upon relative HMF concentrations obtained by comparison of damaged and undamaged food samples; therefore, the observed binding of HMF to the centrifuge cone ultrafiltration membranes does not interfere with the estimation of thermal abuse to tomato paste samples by this standard addition HMF quantification.

Gradient elution profiles of serum filtrate from tomato paste stored for seven months at different temperatures are shown in Figs. 2 and 3. Differences between the profiles are apparent: the most significant is the variation in the amount of HMF. Evidence for the identity of the HMF peak was supported by stopped-flow manual wavelength scanning and retention time correlation with standard HMF. The results suggest that the profiles can be used to group and identify tomato pastes stored under similar conditions. Furthermore, additional investigation of components that vary markedly with different processing and storage environments may provide information about chemical changes occurring in tomato products.

A tomato paste sample that was stored at 100°F for seven months was used to demonstrate the ability to quantify HMF in the serum filtrate. Because of the heterogeneous nature of tomato paste and the inherent large proportion of solids to serum, standard addition was accomplished by adding HMF to the serum isolated after filtration rather than to the paste prior to filtration. This ensured that all the added HMF was reproducibly and rapidly incorporated into the serum portion of

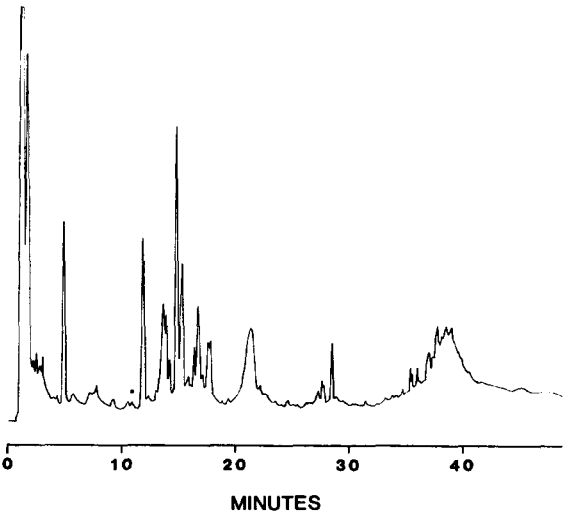

Fig. 3. HPLC separation of serum filtrate from tomato paste stored for seven months at 40°F resulting in multicomponent chromatographic profiles. Solvent conditions are described in the Experimental section. Retention time of HMF denoted by square (■). Flow-rate, 0.9 ml/min; detection, 284 nm; 0.200 a.u.f.s.

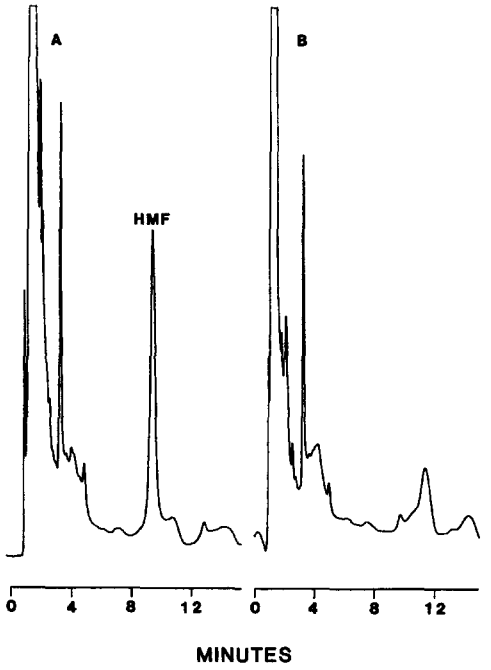

Fig. 4. HPLC separation used for quantification of HMF in the serum filtrate of tomato paste. (A) Paste stored for 7 months at 100°F. (B) Paste stored for seven months at 73°F. Solvent conditions are described in the Experimental section. Flow-rate, 0.9 ml/min; detection, 284 nm; 0.200 a.u.f.s.

the tomato paste. Separations carried out under essentially isocratic conditions (near 100% water), with only subtle gradient changes in solvent composition, were found to provide good reproducibility and resolution for HMF quantification (Fig. 4). Components observed to have longer retention times than HMF in the gradient elution profiles had capacity factors in the quantitative separations large enough to allow sample injections at intervals of *ca.* 15 min, without interference to the HMF band. After 15 to 20 injections, the column was cleaned by eluting these strongly retained components with a gradient to 100% methanol. The standard addition curve produced from the quantitative procedure was linear, with a correlation coefficient of 0.995, a slope of 24 000 area units/ppm HMF added, and a y intercept of 982 000 area units, giving an HMF concentration of 40.9 ppm in the serum filtrate from the paste sample stored at 100°F for seven months.

This brief study demonstrates that the usefulness of ultrafiltration with centrifuging membrane cones as a sample clean-up method prior to liquid chromatographic analysis is not limited to common biological matrices such as blood plasma, but also can be successfully extended to samples as unusual as tomato paste.

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