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Model Studies on Retention of Added Volatiles during Breadcrumb Production

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Breadcrumb samples were prepared with a range of volatile compounds at known concentrations. The retention of these volatiles was assessed via solvent extraction and quantification by gas chromatography−mass spectrometry. Volatile loss during processing was shown to be substantial and dependent upon the compound's vapor pressure. The influence of initial concentration levels on the retention of volatiles was linear within the bounds of the experimental concentrations (0−300 mg/kg). Comparison of volatile concentration at various stages throughout the production process (by headspace analysis) showed that the greatest losses occurred during the processing stages that involved heat, namely, microwave heating and drying. The production of samples by freeze drying showed an increased average retention of 17% as compared to fluidized bed drying and flat bed drying, which showed the highest volatile losses.

KEYWORDS: Flavor; volatile release; microwave heating; drying; APCI-MS

INTRODUCTION

Breadcrumb is a widely used commodity within the food industry, particularly in the processed meat sector where herb-flavored crumb coatings are common. The loss of aromatic, volatile compounds during processing has significant implications for the food industry (*1*). First, the quality of a flavor is determined by the compounds present, as well as their respective concentrations within the mixture. Alteration of the aroma balance through processing can detrimentally affect the quality of the perceived flavor. Second, although the amount of flavor added to breadcrumbs is small, the cost is relatively high, and so, a greater retention during processing will lead to economic gains.

The conventional way of reducing flavor loss during processing is to encapsulate the volatile compounds so that release during processing is minimized (*2*). While this approach can be successful, it involves a separate encapsulation step for the flavor, adding costs and decreasing flexibility in the choice of available flavors. However, it is also possible to improve the retention of volatile compounds by understanding the factors responsible for retention (and loss) in a specific food system and then optimizing those factors for maximum retention. Typically, volatile release occurs via mass transfer of molecules from their initial phase location (aqueous, lipid, solid, etc.) into the gas phase (*3*). The rate of this release is dependent on many factors such as temperature, pressure, specific physicochemical properties of a volatile (*4*, *5*), and its affinity for its surroundings within the food matrix (*6*, *7*). One popular method of increasing retention is to reduce the molecular mobility of volatile compounds by interactions with macromolecules present in food at the processing stage where loss occurs. Examples of binding molecules used are starch (*8*), lipid (*9*), or some other carrier (*10*). The success of these retention methods relies on the volatile compounds being released when matrix properties change. This is most often achieved through hydration of the matrix when the product is subjected to cooking or placed in the mouth for eating. While retention during processing is important, it is imperative that the aroma that does survive the process can be released on eating, and a successful approach will balance these two factors.

This study used three compounds (i.e., anethole, carvone, and α-pinene) typical of the herb flavor found in crumbs as marker compounds to understand volatile loss during the breadcrumb production process with respect to both the food matrix and the properties of the individual volatiles involved.

MATERIALS AND METHODS

Standard Sample Production. Crumbs were formulated as detailed. Any addition of volatile solutions was compensated for by reduction of water added to the dough to keep water addition constant. All components used to produce breadcrumb samples were supplied by Griffith Laboratories (Somercotes, Derby, United Kingdom). Anethole, carvone, α-pinene, ethyl octanoate, ethyl hexanoate, and 2-isobutyl 3-methoxypyrazine were obtained from Firmenich SA (Geneva) (purity 99% or above).

The ingredients (wheat flour, 61.9%; concentrate, 4%; cystine hydrochloride anhydrate, 0.004%; disodium dihydrogen diphosphate, 0.32%; acetic acid, 0.08%; bread fat, 0.4%; and water, 33.3%) were accurately weighed and added to a Kenwood stainless steel mixing bowl and mixed for 1 min (speed setting 5) to form a dough. The following protocol was then used.

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Step 1: Volatiles were directly added into a well in the dough; this was beaten for 4 min and then allowed to rest at room temperature for 10 min. Step 2: A 300 g sample of dough was formed into a cylinder and placed in an 800 W microwave for 3 min. Step 3: The bread was allowed to cool for 1 h. Step 4: The cylinder was then broken by hand and placed in a grinder for 1 min. Step 5: Batches of chopped bread (100 g) were dried in a preheated fluidized bed drier (PRL Engineering, Clwyd, United Kingdom) at 105 °C for 4 min with the blower speed set to 5. All samples were allowed to cool before vacuum sealing in foil bags for storage at −80°C (NB, stage 5 was altered for the drying method experiment; see a later section for details).

Extracted Volatile Concentration Analysis. A sample of crumb (0.1 g) was placed in a polypropylene Eppendorf tube with 0.5 mL of 2-propanol [high-performance liquid chromatography (HPLC) grade, Fisher Scientific, Loughborough, United Kingdom] containing 100 mg/kg octanol (internal standard). The tube was placed in a sonication bath for 1 h at room temperature before centrifugation at 13000 rpm for 3 min. A glass Pasteur pipet was used to remove the supernatant. The sample was spun for a further 3 min, and again, the supernatant was removed. The two supernatants were combined, and the volatile content was analyzed using gas chromatography−mass spectrometry (GC-MS).

GC-MS. A gas chromatograph (GC 8000 series) was connected to a EI mass spectrometer (MD 800, Fisons Instruments, Loughborough, United Kingdom) operated in selected ion mode. A 30 m fused silica column (0.25 mm ID) was used with 1 μm thick DB5 coating (Agilent/J&W Scientific, West Lothian, United Kingdom). Samples (1 μL) were injected through a split injection port (70:1) at 200 °C.

A temperature ramping program was used to resolve the compounds. An initial temperature of 40 °C was held for 1 min and then increased at a rate of 8 °C/min to 140 °C. The temperature was then increased at 1 °C/min to 155 °C. A solvent delay of 6 min was programmed to avoid contamination of the MS with solvent. Calibration of the GC-MS system was carried out by injection of authentic standards. Extraction efficiency was accounted for by the peak areas of the internal standard (octanol).

Volatile Release: Static Equilibrium Headspace. Glass bottles (Schott; 250 mL) were filled with approximately 30 g of crumbs, sealed, and left to equilibrate for a minimum of 6 h at room temperature. The static headspace was then analyzed using atmospheric pressure chemical ionization−mass spectrometry (APCI-MS) (Micromass, Manchester, United Kingdom) fitted with the MS Nose interface. The APCI-MS was used in the positive, selected ion mode to monitor protonated molecular ions (MH^+) at 0.1 s intervals with a corona pin voltage of 4 kV. The sampling flow rate was approximately 5 mL/min. Chromatograms were integrated using Masslynx software to obtain peak heights.

Drying Methods. Crumbs were prepared according to the standard sample protocol except that stage 5 was modified as follows.

Freeze Drying. Samples were preconditioned at −80 °C overnight. Four separate 100 g samples were placed in a Super Modulyo freeze drier (Edwards, Crawley, United Kingdom) set to an internal temperature of −50 °C with a vacuum of 1×10^{-1} mbar applied. No heat was applied. Samples were left under these conditions for 18 h.

Static Bed Drying. The sample (100 g) was placed in a foil tray to form a 1 cm layer. This was placed in a preheated laboratory drying oven set to 105 °C. The samples were dried for 1 h.

Fluidized Bed Drying. This was the same as stage 5 of the standard sample protocol.

Moisture Content. Approximately 3 g of crumbs was placed in a preheated and weighed foil tray and left in an oven heated at 105 °C for 15 h. The differential weight was deemed to be water. All samples were analyzed in triplicate.

Starch Gelatinization. This was measured via differential scanning calorimetry (DSC) (Perkin-Elmer DSC7, Beaconsfield, United Kingdom). Approximately 20 mg of crumb was placed in a stainless steel DSC pan, and excess water was added. The temperature was increased from 40 to 80 °C at a rate of 10 °C/min. The same protocol was followed for raw flour and samples taken throughout the crumb production. The degree of gelatinization in each sample was expressed as the enthalpy under the gelatinization peak at approximately 60 °C.

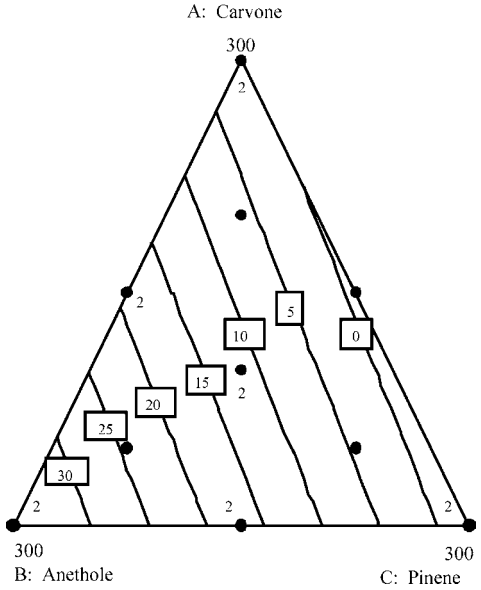

Figure 1. Plot of the volatile recovery of anethole when initial concentration levels were altered. Contour lines and values in rectangular boxes represent postprocessing anethole concentration (mg/kg) plotted against the initial concentration (filled circular markers). The number 2 indicates design points with replication.

RESULTS AND DISCUSSION

Competition and Linearity of Volatile Behavior. The purpose of this work was to understand the behavior of individual volatiles when present in a mixture with other volatile compounds in terms of possible competition/displacement effects. Three volatile compounds were added to the samples in differing ratios with the total volatile concentration remaining constant (300 mg/kg). Samples were then analyzed to determine the concentration of volatiles postprocessing.

Individual volatiles within the mixture showed a linear relationship between initial and extracted volatile concentration. This linear trend was also observed for headspace release data determined from the same samples. The results for anethole are illustrated in **Figure 1** (all relationships also held for carvone and pinene). It can be observed that changing the initial concentration of carvone and pinene had no effect on the amount of anethole retained. This was purely dependent on the initial amount of anethole added to the sample. This relationship indicates that within the concentration range used, no overloading of the capacity of the dough to entrap or bind the volatile compounds occurred. If saturation occurred, then the extracted volatile concentration would remain the same even if the initial concentration was further increased. The linearity is probably due to the high ratio of possible volatile retention sites as compared with the low levels of volatile compounds present in this multiphasic sample. These may include localized areas of volatile binding molecules such as fat, protein, and starch. Alternatively, volatiles may be bound within the matrix structure itself, for example, as a gas at low levels within an air bubble. The linearity of the response also indicated that there was no competition between the volatiles. The amount of volatile retained/released was neither increased nor decreased by the presence of other volatile molecules.

Volatile Loss. It is ineviatble that a certain amount of volatile will be lost during processing (*1*). Under the process conditions used in this study, the recovery of anethole was approximately 10% of the initial loading concentration and this percentage dropped to 3% for carvone and 2% for pinene. These are

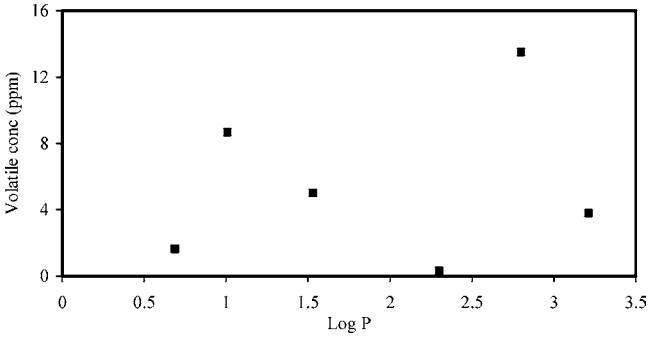

Figure 2. Postprocessing concentration of volatiles in crumbs as a function of their Log *P* values. Volatiles were added at 150 mg/kg prior to processing. Each value is the mean of eight replicates. The average coefficient of variance is 7%.

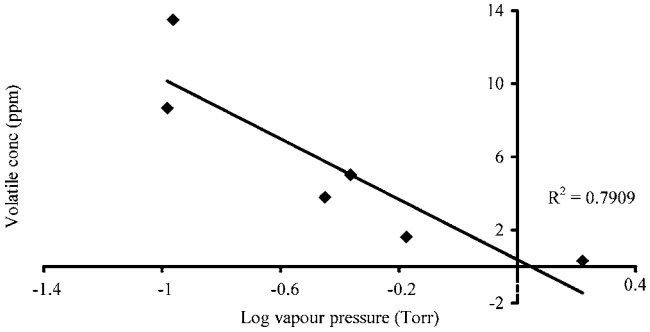

Figure 3. Postprocessing concentration of volatiles in crumbs as a function of their vapor pressure. Volatiles were added at 150 mg/kg prior to processing. Each value is the mean of eight replicates. The average coefficient of variance is 7%.

substantial losses and would have a significant impact on the economics of flavored crumb production as well as the flavor quality of the end product.

Understanding the nature of the loss of volatiles in terms of whether the volatiles were altered chemically during processing, hence losing their odor characteristics, or just simply lost by volatilization was required. The headspace of samples of dough and finished crumbs were compared using APCI-MS in full scan mode. This gives a fingerprint of ions present in the headspace, and the presence of different compounds would be shown by new ions. These may be caused by oxidation (ion mass increase) or by breakdown (ion mass decrease). No differences in ion profile were observed, but an overall decrease in intensity was found in the finished crumbs relative to the dough sample; hence, volatilization seemed to be the driving factor in volatile loss during processing.

Every volatile has individual physicochemical properties, which impact on that volatile's behavior. Two commonly quoted properties are that of hydrophibicity (Log *P*) and vapor pressure. A series of volatile compounds was selected to provide a range of values for these properties. **Figure 2** shows the extracted volatile concentration from crumbs as a function of the compound's Log *P*. No correlation was found, which is in contrast to the conclusions found for pure starch (*11*), although breadcrumb is a multiphasic system rather than a single polysaccharide. If losses were related to the retention of volatiles by lipid (*12*, *13*), then one would expect increased retention with increased hydrophobicity. However, the random scatter plot of extracted volatile concentrations against Log *P* (**Figure 2**) showed random scatter implying that the retention of volatiles was not lipid-related. However, there was a good correlation ($R^2 = 0.79$) between the vapor pressure value of a volatile and its concentration postprocessing (**Figure 3**). The influence of a volatility measure (air−water partition coefficient) on the retention time of compounds during microwave heating has been reported previously (*14*). Utilization of a range of volatiles also allowed the investigation of further factors. No trend was found between the retention of a volatile and its molecular weight. The relationship found previously (*15*), where increasing the molecular weight of esters showed an increase in volatile retention, is probably due to associated changes in other physical properties as molecular weight increases. Comparison is also difficult due to the ester systems containing a single carrier (e.g., glucose and maltose) rather than a real food matrix. Functional groups and molecular shape also showed no correlation with retention values giving an indication that volatile retention was not a result of the amylose−volatile complex formation (*8*, *16*−*20*).

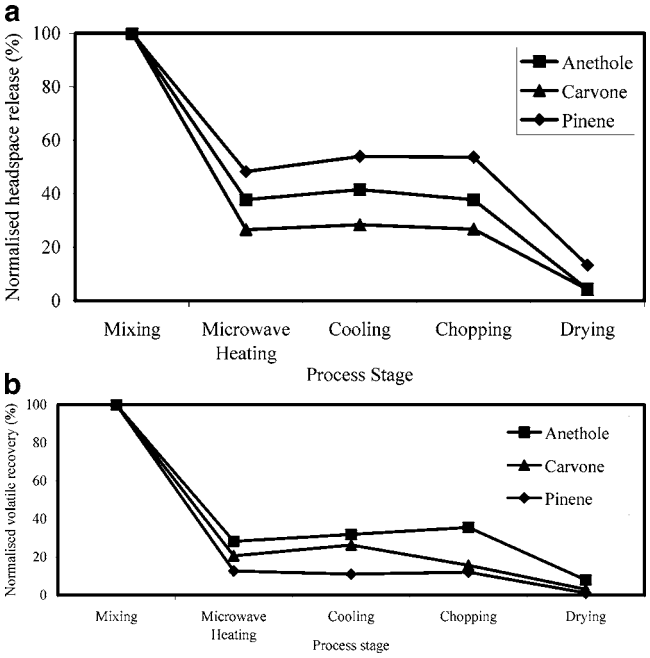

Figure 4. (**a**) Normalized static headspace release of three volatiles from samples taken after each stage of crumb production. Each value is the mean of four replicates. See the text for details of processing stages. (**b**) Normalized recovery concentration for three volatiles of samples taken at each stage of crumb production. Each value is the mean of four replicates. See the text for details of processing stages.

Volatile Loss during Processing. Sample production consists of several processing stages. Key areas of the production process may be identified where volatile losses are high and where changes to the process might reduce losses. The influence of heat energy on the release of volatiles has been demonstrated (*21*), so microwave heating (*22*) and the loss of volatiles by steam distillation (*21*, *23*) are potentially stages for loss.

Headspace analysis of samples after each processing stage was performed to give an indication of the product's release properties (**Figure 4a**). A large decrease in volatile release was shown between stages 1 and 2. The release of volatiles of course depends strongly on the state of the matrix as well as the amount of volatile present in the matrix. However, there was a good correlation between headspace analysis and the actual amount of an aroma compound present at any particular stage (**Figure 4b**). Decreases in volatile release and content were observed during the processing stages that involved heat (2 and 5), but little change was observed as a result of the other processing stages. Volatile loss during microwave heating outweighed those

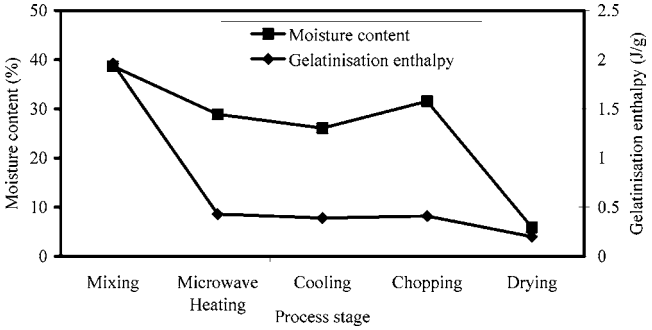

Figure 5. Changes in the moisture content and the state of starch through breadcrumb manufacture. See the text for details of processing stages.

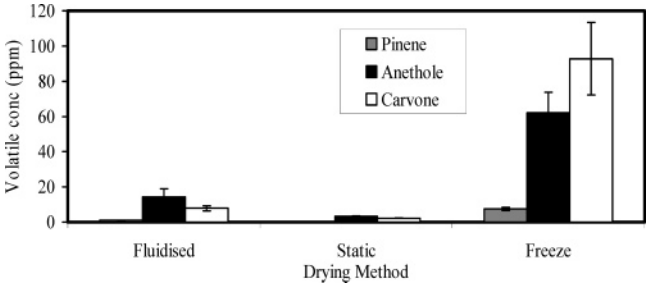

Figure 6. Effect of drying method on volatile concentration postprocessing (mg/m^3). All volatiles were added at 200 mg/kg prior to processing. Each value is the mean of four replicates, and error bars show the standard deviation.

shown during the drying stage, although if the values are considered as percentage losses during the stage, and not normalized to the dough sample, then both heating stages show similar severities in volatile loss, for example, 79 and 80% loss of carvone were shown at stages 2 and 5, respectively. The loss of volatiles during microwave heating is slightly higher than those quoted (*14*).

Throughout the process, alteration of matrix factors such as moisture content and the state of the starch occurs. Water loss also occurred during heat application stages (see **Figure 5**), although the majority was lost during the final drying stage. This water may act to steam strip the volatile compounds from the sample matrix (*23*). Water loss and thus steam distillation of volatiles during the microwave heating stage were relatively low, probably due to the water being consumed in the starch gelatinization process; hence, the losses shown during this stage are caused by the effect of added heat energy. The enthalpy data in **Figure 5** show that gelatinization of the starch occurred predominantly during stage 2 (microwave heating), when heat energy was applied to the product in the presence of excess water. Only a small change in gelatinization enthalpy occurred during stage 5 (drying).

Drying Methods. Having identified that heat was a major factor in the loss of volatiles during processing, experimentation was undertaken to understand the effect of different drying methods on volatile retention. The methods available in industry vary largely in the severity of their action. This severity is usually linked to the regimes of temperature and time of the drying method. For example, freeze drying requires a long time (approximately 15 h for these samples) but is carried out at low temperatures as it works on the basis of sublimation of ice. Static bed drying uses a thin layer of sample with the addition of heat energy (105 °C) for 1 h. Under fluidized bed conditions (still at the same temperature), the sample requires only 5 min to dry to the same moisture content. **Figure 6** shows that there was a significant increase in volatile concentration in freeze-dried crumbs as compared to the crumbs dried by the other methods ($P < 0.01$). The largest difference was shown by carvone with a 9-fold increase in retention in freeze-dried crumbs as compared to fluidized bed-dried crumbs. Crumbs produced by static bed drying showed the lowest volatile concentration. These observations correlate with knowledge of the time/temperature regimes of each of the drying methods as the highest volatile concentration was observed for the freeze-dried sample (no heat addition). The difference between the other two heat-dependent methods was probably due to the length of the drying time used in each case. Overall differences between the drying methods are in agreement with other comparative studies (*24*, *25*) on the basis of process severity.

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