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journal homepage: www.elsevier.com/locate/foodres

Mixture design approach as a tool to study *in vitro* flavor release and viscosity interactions in sugar-free polyol and bulking agent solutions

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ARTICLE INFO

Article history: Received 11 May 2011 Accepted 10 August 2011

Keywords: Critical concentration (c*) Experimental mixture design Flavor release Matrix-flavor-interactions PTR-MS Viscosity

ABSTRACT

Flavor release from mixtures of maltitol, erythritol, polydextrose and oligofructose dissolved in water at concentrations of 43% (w/w) was investigated to analyze possible interactions between the viscous matrix and the volatiles using an experimental mixture design in combination with data analysis and response modeling tools. The dynamic release of four flavor compounds (cis-3-hexenol, benzaldehyde, ethyl butanoate and butyl isovalerate) from the matrices at 37 °C was determined by headspace analyses using proton-transferreaction mass spectrometry (PTR-MS). The aim of the present study was to understand the complex release mechanisms from high-viscosity polyol and bulking agent solutions for the intention of conducting further studies on flavor release from sugar-free confectionary products. Each of the non-volatiles had a significant effect (p < 0.05) on flavor release with regression coefficients (R^2) between 0.72 and 0.93 for the release parameters I_{max} , t_{y_2} , and $\Delta c/\Delta t$, and 0.99 for viscosity. However, the magnitude of influence varied between the bulking agents and polyols due to their different molecular weights. A clear correlation between viscosity of the solutions and flavor release was found, revealing complex matrix-volatile interactions in the highviscosity solutions. The release of all investigated flavor compounds decreased when the viscosity of the solutions increased. Therefore, it is assumed that the flavor release is significantly influenced by the nonvolatiles when a critical concentration (c*) is exceeded. Interactions between the sugar substitutes were found to affect the viscosity of the matrices, whereas flavor release was not affected by interactions between the polyols and bulking agents investigated.

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1. Introduction

One of the major nutritional problems today is the consumption of high quantities of sucrose, which is considered to be at least partially responsible for diabetes and obesity (World Health Organization, 2003). The challenge to the food industry is therefore to redesign traditional foods to include particular nutritional properties without neglecting consumer demands. Thus, in order to receive widespread acceptance of such novel foods, the flavor of these products should reflect those of the traditional products. One promising way to achieve a low calorie sugar-free food product is via the reduction or omission of sucrose in products typically containing high amounts of sugar. In most foods, however, the reduction or removal of sugar by any single polyol causes readily detectable losses in appearance, texture, mouthfeel, and flavor perception, especially in complex food systems like confectioneries (Ronda, Gomez, Blanco, & Caballero, 2005; Zoulias, Oreopoulou, & Kounalaki, 2002). Therefore, to gain the consumers' acceptance, the texture and release of flavor compounds must be adapted to those of conventional sugar-containing food products by combining different polyols, bulking agents, and sweeteners.

In agreement with Goubet, Le Ouere, and Voilley (1998), who reviewed the retention of flavor compounds by carbohydrates, the retention and release mechanisms in food matrices is a complex phenomenon in which several factors contribute. The presence of carbohydrates has an influence on the gas-liquid partitioning and consequently the headspace concentration of the flavor compounds, leading to a reduced or enhanced flavor release compared to in pure water (Friel, Linforth, & Taylor, 2000; Goubet et al., 1998; Hollowood, Linforth, & Taylor, 2002; Nahon, Harrison, & Roozen, 2000; Philippe et al., 2003; Rabe, Krings, & Berger, 2003; Roberts, Elmore, Langley, & Bakker, 1996; Voilley & Bosset, 1985; Voilley, Simatos, & Loncin, 1977). Contradictory results were also reported for the influence of texture on the flavor release of solutions or gels. Some researchers reported no influence of texture on flavor release (Hansson, Andersson, & Leufvén, 2001; Lethuaut et al., 2005), others a decrease in flavor intensity and/or headspace concentration of the flavor compounds when the viscosity of a solution or a gel increased (A. B. Boland, Buhr, Giannouli, & van Ruth, 2004; De Roos, 2003; Mestres, Kieffer, & Buettner, 2006). The latter release patterns in thickened and

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^{0963-9969/\$ –} see front matter 0 2011 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodres.2011.08.005

gelled foods have been attributed to the thickener itself, which provides a barrier to diffusion, as well as to interactions between the flavor compounds and the thickener. Additionally, Boland, Delahunty, and van Ruth (2006) reported a decrease in flavor perception when the rigidity of either gelatin or pectin gels increased, while the *in vitro* release suggested enhanced release for gelatin and decreased release for pectin gels, respectively.

In our previous study on low-viscosity solutions, the higher volatile and less polar compounds were retained more readily in the presence of the non-volatiles at concentrations of 20% (w/w), while the least volatile and most polar compound *cis*-3-hexenol showed an increased flavor release compared to water. It was found that the polarity and relative volatility of the flavor compounds influenced flavor release and also the molecular weight of the volatiles was assumed to have an effect on flavor release, as has been previously reported in literature (Goubet et al., 1998). On the other hand, no correlation between flavor release and the viscosity of the solutions was apparent (Siefarth et al., 2011).

The aims of the present study were to characterize the flavor release from specific high-viscosity polyol and bulking agent solutions (maltitol, erythritol, polydextrose, and oligofructose, respectively) at a total dry matter content of 43% (w/w).

Since flavor release from complex food matrices is difficult to predict, characterization of specific release mechanisms in simple model systems was of particular interest in the present study. An experimental mixture design was chosen as a tool to determine the effects of each non-volatile compound and their interactions on flavor release and viscosity of the solutions through statistical analysis. A correlation of viscosity and flavor release pattern in high-viscosity solutions containing sugar surrogates is discussed in detail. The dry matter content of 43% (w/w) of the solutions was chosen to provide viscous solutions that can be further adapted for applications in sugar-free food products. It was previously reported in the literature that there is a concentration for any given hydrocolloid at which individual polymer chains interpenetrate and start to form an entangled network. This concentration is termed the point of random coil overlap (c*) and is associated with an abrupt increase in viscosity (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). It was found that above this concentration the perceived flavor intensity decreases steadily with increasing polymer concentration (Baines & Morris, 1987; Cook, Hollowood, Linforth, & Taylor, 2003). According to the literature, a decrease in flavor release should be obvious when c* is exceeded. Thus, the present study on 43% (w/w) polyol and bulking agent solutions should help to understand the complex release mechanisms from sugar-free and sugar-reduced food matrices.

Finally, this fundamental knowledge on release mechanisms from simple model systems will aid in adapting the flavor of sugar-free and sugar-reduced food products to the flavor of conventional sugarcontaining foods.

2. Materials and methods

2.1. Chemicals

Four flavor compounds exhibiting a berry flavor note were chosen among different chemical classes to investigate the flavor release from various aqueous solutions. The compounds (*Z*)-hex-3-en-1-ol (*cis*-3-hexenol), benzaldehyde and ethyl butanoate were obtained from Fluka (Neu-Ulm, Germany), and butyl 3-methylbutanoate (butyl isovalerate) from Aldrich (Steinheim, Germany). All compounds had a purity of at least 98% and were used without further treatment. Maltitol was obtained from Cargill Inc. (Minneapolis, MN), erythritol from Jungbunzlauer AG (Basel, Swiss), polydextrose from Danisco (Litesse® Ultra[™], Copenhagen, Denmark) and oligofructose P95 (fructo oligosaccharides) from BENEO-Orafti (Tienen, Belgium). The polymerization degree (DP) of polydextrose is highly variable and up to 100. According to the supplier, about 90% of the polydextrose molecules exhibit a DP between 3 and 30 with an average DP of 12 (Lahtinen et al., 2010). Oligofructose P95, which is produced from natural sources by extraction, has a DP ranging from 2 to 8 and consists of $\beta 1 \rightarrow 2$ linkages of fructose and glucose, respectively.

2.2. Sample preparation

Aqueous solutions of combinations of maltitol, erythritol, polydextrose and oligofructose were prepared at final concentrations of 43% (w/w) according to the experimental D-optimal mixture design described in Section 2.5 (Design Expert, v. 6.0.10, Stat Ease Inc., Minneapolis, MN) as a tool to study the interactions within sugar-free polyol and bulking agent solutions and to limit the number of experiments.

The substances were weighed (accuracy \pm 0.01 g) in a beaker, dissolved in tap water and evaporated under heat to the required dry matter content of 43% (w/w). Tap water was chosen as a reference due to its routine utilization in general food production.

Each solution was divided into two parts: one aliquot was used for the headspace measurements and the other aliquot was utilized to determine the viscosity. The combined flavor compounds were mixed with an aliquot of 25 mL of the individual solutions and subsequently equilibrated at room temperature for 15 min. This period reflected a common equilibrium time for each sample prior to headspace measurements and was determined in preliminary analyses. The purity of the compounds was checked regularly via gas-chromatography flameionization detection (GC-FID) analysis and PTR-MS fragmentation patterns. To provide optimum mass spectrometric detection to accommodate for compound-specific sensitivities of the instrument different initial volatile concentrations for each flavor compound were chosen as follows: 2.2 mg L⁻¹ for ethyl butanoate, 4.5 mg L⁻¹ for *cis*-3-hexenol and benzaldehyde, and 6.7 mg L⁻¹ for butyl isovalerate.

All experiments were carried out in duplicate or triplicate and the solutions were in each case freshly prepared prior to measurement. The relative deviations of the headspace measurements were within 10% except for butyl isovalerate, which showed higher deviations of up to 30%. The accuracy of the viscosity measurements was within 5%.

2.3. Headspace analysis by proton-transfer-reaction mass spectrometry (PTR-MS)

Headspace analyses were conducted using a high sensitivity proton-transfer-reaction mass spectrometer (hs-PTR-MS; Ionimed Analytik GmbH, Innsbruck, Austria) (Hansel et al., 1995). PTR-MS is a soft, sensitive and efficient method of chemical ionization particularly suitable for real-time measurements. Thus, it is often used in food research to monitor the flavor release from various food matrices in real time (Buettner et al., 2008; Mestres, Moran, Jordan, & Buettner, 2005).

25 mL portions of the equilibrated solutions were filled individually into 100 mL beakers that were each placed into a 180 mL perfluoroalkoxy (PFA) beaker (AHF Analysentechnik AG, Tuebingen, Germany) and closed with an air-tight lid containing two connection ports for headspace flushing and sampling. During the measurements the samples were continuously stirred with a magnetic stirrer at 550 rpm using a Teflon-coated magnetic stirrer and maintained at a temperature of 37 ± 1 °C to simulate body temperature conditions. The headspace above each solution was flushed with 1000 mLmin^{-1} humidified zero-air (i.e. air free of volatiles), 370 mL min⁻¹ of which was drawn into the inlet of the PTR-MS instrument. The inlet line and the PTR-MS reaction chamber were maintained at 60 °C. A constant drift voltage of 400 V and a reaction chamber pressure of 2.2 mbar were employed. A schematic of the headspace sampling set-up, as well as some further details on the set-up and measurements has been reported elsewhere (Siefarth et al., 2011). The headspace concentration of the compounds from each of the aqueous solutions was monitored

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