LWT - Food Science and Technology 45 (2012) 277–288

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Complexation with starch for encapsulation and controlled release of menthone and menthol

Hagar Ades, Ellina Kesselman, Yael Ungar, Eyal Shimoni*

Faculty of Biotechnology and Food Engineering, Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Haifa 32000, Israel

ARTICLE INFO

Article history:
Received 13 October 2009
Received in revised form
28 July 2011
Accepted 25 August 2011

Keywords:
V-amylose
Aroma
Stability tests
Controlled release

ABSTRACT

A platform of starch complexes for the oral release of aroma substances by salivary fluids was studied. Menthone, menthol and limonene were used as model flavor compounds for complexation with starches with different amylose content. Complexes were characterized by X-ray diffraction (XRD), differential scanning calorimetry (DSC), dynamic light scattering (DLS), and atomic force microscopy (AFM). Aroma retention was tested under pH, temperature and storage challenges. Furthermore, the kinetic of aroma release in simulated saliva fluids (SSF) was also tested. Both menthone and menthol form V-amylose complexes in a 'food grade' process while limonene does not form such complexes efficiently. Complexation yield was higher, with more included aroma and less free core content, as the amylose content increased. The complexes had melting temperature of over 90 °C. Digestion results suggest that the complexes can release the aroma in the oral cavity.

1. Introduction

The encapsulation of flavor ingredients is very attractive and widely investigated in food science (Baranauskienė, Bylaitė, Žukauskaitė, & Venskutonis, 2007; Bruckner, Bade, & Kunz, 2007; Hambleton, Debeaufort, Beney, Karbowiak, & Voilley, 2008). Complexation of aroma or volatiles can improve food flavoring by reduction of evaporation, and control their release during storage and application. It is well known that starch is able to form inclusion complexes with small molecules, especially nonpolar molecules with a non-dominant polar group (Jouquand, Ducruet, & Le Bail, 2006; Kuge & Takeo, 1968; Nuessli, Putaux, Le Bail & Buléon, 2003; Rutschmann & Solms, 1990a; Rutschmann & Solms, 1990b). Since starch is widely used component in food, inclusion based on starch is of major interest. Moreover, salivary α-amylase can hydrolyze the starch–aroma complex and release the entrapped aroma compound while chewing. Early researches using the complex showed the potential of controlled release by enzymatic hydrolysis (Holm et al., 1985). The structure of amylose complexes formed with a wide range of small molecules has been investigated mainly by X-ray and electron diffractions (Buléon, Delage, Brisson, & Chanzy, 1990; Cardoso et al., 2007; Conde-Petit, Escher, & Nussli, 2006; Le Bail, Rondeau, & Buléon, 2005; Oguchi, Yamasato, Limmatvapirat, Yonemochi, & Yamamoto, 1998; Takeo & Kuge, 1969), differential scanning calorimetry (DSC) (Karkalas, Ma, Morrison, & Pethrick, 1995; Le Bail, Rondeau, & Buléon, 2005; Nuessli, Putaux, LeBail, & Buléon, 2003; Tapanapunnitikul, Chaiseri, Peterson, & Thompson, 2008)and solid-state nuclear magnetic resonance (NMR) (Rondeau-Mouro, Le Bail, & Buleon, 2004; Tozuka et al., 2006; Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2009). Atomic force microscope - AFM was also used to characterize the complex structure and dimensions (Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005; Tang & Copeland, 2007). The amylose–lipid complex is described as a single left handed α helix, also known as V-amylose. Several polymorphs were identified differing in glucose units per turn. So that the molecular dimensions of the complex polymorphs are varying with external diameter of 1.36–1.62 nm and inner diameter of 5.4–8.5 Å (Cardoso et al., 2007; Immel & Lichtenthaler, 2000; Oguchi et al., 1998; Tozuka et al., 2006; Takeo et al., 1969). The ligand can be trapped either inside or between the helixes (Jouquand et al., 2006; Rondeau-Mouro et al., 2004). Differences between polymorph are manifested in unit cell size as can be calculated from X-ray diffraction. Some evidences relate differences in unit cell to the characteristics of the ligand molecule (Conde-Petit et al., 2006; Le Bail et al., 2005). Other evidences relate the helical cavity dimensions to the ligand content (Rutschmann & Solms, 1990a, 1990b; Tozuka et al., 2006). Differences in the complex properties were identified between different complex structures and different ligands (Biais, Le Bail, Robert, Pontoire, & Buléon, 2006; Nuessli et al., 2003; Osman-Ismail & Solms, 1972; Rutschmann & Solms, 1990a). Other important issues

* Corresponding author. Tel.: +972 4 8292484; fax: +972 4 8293399.
E-mail address: eshimoni@tx.technion.ac.il (E. Shimoni).

0023-6438/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2011.08.008

for a controlled release platform are the stability of the complex and the release profile of the ligand. Early researches imply that the complex is stable and could retain aroma for 100 days (Wyler & Solms, 1982) and even one year (Wulff, Avgenaki, & Guzmann, 2005). Stability tests showed retention of the ligand upon heating to 80 °C (Wulff et al., 2005). In this research three aromas were used as guest molecules: menthone, menthol and limonene. All have similar methyl-cyclo-hexane structure but different functional group. This structural difference between the aroma molecules is expressed mostly in hydrophobicity. The three aroma molecules have similar molecular weight of 136–156 g/mol, and therefore a very similar molecular volume of 157–177 Å^3. Each one of the three aromas is considered non water soluble. Menthol has the higher water solubility, 1.3 g/L, menthone has less water solubility, 0.8 g/L, and limonene is the least soluble in water, 0.013 g/L. The poor water dispersability may impair the interaction with starch in aqueous system (Tapanapunnitikul et al., 2008). Differences are observed in vapor pressure as well, with menthol and menthone having a similar vapor pressure and limonene having one order of magnitude higher vapor pressure. These small differences in structure may affect the inclusion mode and stability. Evidences for the complexation of menthol with starch were identified (Biais et al., 2006; Nuessli et al., 2003; Osman-Ismail & Solms, 1972) and maximum content of 8 g menthol/100 g amylose was recorded (Schmidt & Maier, 1987). The amount of menthol seems to decrease in the order amylose, corn starch and amylopectin (Maier, Moritz, & Rummler, 1987). The formation of menthone-amylose inclusion compounds was widely investigated, mostly for its structural properties (Biais et al., 2006; Le Bail et al., 2005; Osman-Ismail & Solms, 1972; Rutschmann & Solms, 1990b; Rondeau-Mouro et al., 2004). Amylose or HACS (High Amylose Corn Starch) menthone complexes had melting temperature of 109–119 °C according to DSC (Nuessli et al., 2003; Tapanapunnitikul et al., 2008) and maximum menthone content was recorded as 7.6 ± 3 g/100 g (Tapanapunnitikul et al., 2008). Some attempts to evaluate limonene interaction with starch are found in the literature. Rutschmann, Heiniger, Pliska, and Solms (1989) calculated maximal binding capacity between limonene and potato starch to be 0.5 g limonene/100 g starch. The aim of the present study is therefore to determine the best inclusion mode for aroma substance and determine the release profile of the flavor material from the complex, in particular for its enzymatically controlled release in the oral cavity. In order to follow this goal, complexes of starch with different amylose content with limonene, menthone or menthol were produced. The inclusion characteristics, inclusion ability, yield and free core content, of the complexes, was evaluated. The inclusion complexes properties were characterized and the retention ability in different conditions and release profile in saliva like fluids were evaluated.

2. Materials and methods

2.1. Materials

Amylose (Type III from potato), Corn starch (25 g amylose/100 g starch), Waxy corn starch (≤1 g amylose/100 g starch), α-Amylase (53.7 unit/mg solid, from *Aspergilus oryzae*) was purchased from Sigma–Aldrich Chemical Company, St. Louis MO, USA. High Amylose Corn Starch, HACS (70 g amylose/100 g starch) was purchased from National Starch, Bridgewater NJ, USA. Aroma chemicals were D-limonene (purity ≥97, Sigma–Aldrich Chemical Company, St. Louis MO, USA), L-menthol (purity >99%, Merck KGaA, Darmstadt, Germany), and Menthone (purity> 97%, Sigma–Aldrich Chemical Company, St. Louise, USA). All other reagents were of analytical grade.

2.2. Preparation of starch-aroma complexes

HACS, corn starch and waxy corn starch where defatted using the Soxhlet method with petroleum ether as solvent, the solvent was than vaporized by drying in a vacuum oven at 80 °C for about 12 h. Defatted HACS, defatted corn starch, defatted waxy corn starch or amylose was dispersed in 0.1 mol/L KOH (600mg/100 ml) preheated to 90 °C by vigorous stirring. The suspension was cooled to 60 °C and aroma compound was added, 60 mg for menthol or 60 μl for limonene and menthone. After stirring for 30 min, the suspension was acidified to pH ~4.8, using phosphoric acid 2 g/100 g. The mixture was then held for 24 h at 60 °C. Suspension was then centrifuge (15,000×*g*, 30 min). The pellet was washed twice with ethanol/water mixture (50/50 v/v) and re-centrifuged. The pellet was then freeze-dried.

2.3. Quantification of aroma compounds

2.3.1. Aroma extraction from solutions

Eight hundred μl of the supernatant containing the aroma were transferred into another vial. The aroma was extracted by 1.2 ml of hexane for menthone or ethyl acetate for menthol and limonene. The suspension was vigorously shaken by vortex (REAXtop, Heidolph, Germany) for 10 s and re-centrifuged (1600×*g*, 1 min, 4° C) for complete separation. The supernatant containing the aroma and the solvent was collected and aroma content was quantified by gas chromatography, GC.

2.3.2. Gas chromatography

Gas chromatography analysis was performed on a Hewlett–Packard 5890 Gas Chromatograph; Avondale, PA, USA, using SP^{TM}-2560 fused silica capillary column [100 m × 0.25 mm (i.d.) with 0.2 μm film thickness; Supelco inc., Bellefonte, PA < USA]. The temperature programming was: for menthone detection 75 °C for 5 min, than ramped at 10° C/min to 180 °C and maintained for 15.5 min; for menthol detection 180 °C maintained for 25 min; for limonene detection 130 °C for 2 min, than ramped at 4° C/min to 180 °C and maintained for 5 min. The nitrogen carrier gas flow rate was 2.4 ml/min, hydrogen flow to the detector was 25 ml/min, air flow was 400 ml/min and the flow of nitrogen makeup gas was 45 ml/min. Injection temperature was 250 °C and detection temperature was 250 °C. Peaks were identified using HP GC ChemStation software. Standards for the aroma were used in order to identify the peaks.

2.3.3. Determination of total aroma content in complexes

Total aroma content was determined by disassembly of the complex. The complex (15 mg) was incubated with 2 ml of hydrolysis solution in a 2 ml glass vial sealed by a screw cap covered with an aluminum foil. For the determination of menthone content hydrolysis solution was KOH 2 mol/L. Incubation in KOH solution could fully dissolve the complex and gave a clear suspension. While extracting with ethyl acetate, KOH reacted with the solvent pH descended and complexation occurred again. For that reason KOH solution could not be used for determination of total menthol or limonene content. Alternative hydrolysis solution contained α-amylase (1000 u/ml, phosphate buffer pH 7.2), so that the starch in the sample went through enzymatic hydrolysis and any aroma included in the complex could be released. After incubation for 4 h at 37 °C in continues mixing (12 rpm), the samples were cooled and centrifuged (4600 rpm, 1 min, 4 °C). Full enzymatic digestion confirmed by determination of the reducing hemiacetal groups (Rick & Stegbauer, 1974). The determination of hemiacetal groups was performed by adding 100 μl of reagent containing 10, 3 and 5 g dinitrosalicylic acid, 16 g sodium hydroxide and 300 g potassium