Food Chemistry 122 (2010) 60–64

Contents lists available at ScienceDirect

Food Chemistry

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

Partition of selected food preservatives in fish oil–water systems

Hongyuan Cheng [a,*], Alan Friis [a], Torben Leth [b]

[a] Food Production Engineering, National Food Institute, Technical University of Denmark, Søltofts Plads, Building 227, DK-2800 Lyngby, Denmark
[b] Food Chemistry, National Food Institute, Technical University of Denmark, Mørkhøj Bygede 19, Building A, 2860 Søborg, Denmark

ARTICLE INFO

Article history:
Received 23 June 2009
Received in revised form 16 November 2009
Accepted 26 January 2010

Keywords:
Food preservative
Partition coefficient
Fish oil
Sorbic acid
Benzoic acid

ABSTRACT

The partition coefficients (K_{ow}) of benzoic acid and sorbic acid in systems of fish oil (sand eel)–water, fish oil–buffer solution, rape oil–water and olive oil–water were experimentally determined in a temperature range from 5 to 43 °C and pH from 4.5 to 6.5 °C. The dimerization of benzoic acid in fish oil–water system was observed at 25 °C. Two modifications have been made to the Nordic Food Analysis Standard for the determination of sorbic acid by HPLC. The experimental results show that the K_{ow} of benzoic acid and sorbic acid in fish oil–buffer system is ca. 100 times lower than that in fish oil–water system. The K_{ow} values of benzoic acid and sorbic acid in fish oil and water system decrease with increasing system pH values. The partition coefficients of plant origin and fish origin oils are in the same order of magnitude even though their molecular structures are very different.

1. Introduction

Food preservatives are commonly used in food production to preserve, improve and extend food shelf life. Today, foods are often produced in one area and shipped elsewhere for further processing or consumption. In the food distribution and transportation process, preservatives play a significant role in inhibiting spoilage micro-organisms and extending food shelf life. However, the use of food preservatives should always be under control and monitoring, despite the recognised requirement for them, which is regulated through the positive list and monitored regularly by National Food Administrations and Laboratories (An Expert Report, 2006; WHO, 2000). After all, consumers wish to eat minimum amount of preservatives (or alien chemicals) in any food products. Organic or ecologic foods have become an important part in the stock of many supermarkets. The exact quantitative properties of the preservatives are the most crucial information in production applications and toxicological safety evaluations.

Partitioning of food preservatives in a food system is one of the most important properties in the use of preservatives (Davidson & Branen, 2005; Lueck, 1980). In a food system, preservatives dissolve only in water and lipid phases, not in hydrocarbon, protein and fibre. As all micro-organisms survive only in a water environment, the concentration of a preservative in the water phase is directly related to the micro-organism control in the food system. The amount of a preservative dissolved in the lipid phase could be treated as a "loss" for the preservative. It is therefore desirable to know the partitioning of a preservative between oil and water phases in practical applications.

Benzoic acid and sorbic acid are widely used as preservatives in food, medicine, cosmetic and other applications (An Expert Report, 2006). In practice, the upper benzoic acid concentration allowed in food is 0.1% (wt.) in USA and 0.015–0.5% (wt.) in the European Commission (Kirk-Othmer, 2001; WHO, 2000). Sorbic acid is generally recognised as safe (GRAS) for use in food under US ingredient regulation in the *Code of Federal Regulations*. The limit of addition of sorbic acid is generally 0.2% (wt.) permitted by applicable US FDA Food Standards of Identity (Kirk-Othmer, 2001).

The partition coefficients of benzoic acid and sorbic acid in different systems have been investigated by researchers (Gooding, Melnick, Lawrence, & Luckmann, 1955; Lubieniecki-von Schelhorn, 1964, 1967a, 1967b; Sofos, 1989). However, a systematic investigation of the partition coefficients of these food preservatives in any fish oil systems has not been seen. Especially, these investigations often do not take into account real food situations, i.e., no food components involved in aqueous phase. The experimental investigation of partitioning of preservatives in fish oil–water systems is fundamentally valuable for applications in seafood products.

In this work, the partition coefficients of benzoic acid and sorbic acid in fish oil–water and fish oil–buffer solution systems were determined experimentally using a classic "shake-flask method". The fish oil–buffer solution system is designed to simulate a real food aqueous phase situation. The temperature and pH value effects on the partition coefficients of the two preservatives were

* Corresponding author. Tel.: +45 45252728; fax: +45 45884922.
E-mail address: hoch@food.dtu.dk (H. Cheng).

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doi:10.1016/j.foodchem.2010.01.070

also investigated. For comparison with oils of plant origin, the partition coefficients of the preservatives in rape oil–water and olive oil–water systems were also experimentally determined at 25 °C. Because benzoic acid often forms a dimer in nonpolar solvent, where the partition coefficient is not constant and varies linearly with solute concentration in solvent (Prausnitz, Lichtenthaler, & de Azevedo, 1999), the effects of systems at different initial concentrations on partition coefficients were also investigated at 25 °C, to observe the dimerization in the studied system for benzoic acid and sorbic acid.

2. Materials and method

2.1. Partition coefficient and measurement method

A substance may distribute itself between two immiscible liquid solvents coexisting in a system. In the limit of zero concentration, the equilibrium ratio, K, of solute concentrations in the two phases, α and β, is defined as the distribution coefficient or partition coefficient (Lide, 2009).

$$K = x_2^{\alpha}/x_2^{\beta} \tag{1}$$

where x_2^{α} and x_2^{β} are solute 2 mol fraction in α and β phase, respectively. Eq. (1) can be expressed with Henry's constant in very dilute solution condition. At phase equilibrium,

$$\mu_2^{\alpha} = \mu_2^{\beta} \tag{2}$$

where μ_2^{α} and μ_2^{β} are the chemical potential of solute 2 in α and β phase, respectively. At dilute solution condition, Eq. (2) gives (Prausnitz, Lichtenthaler, & de Azevedo, 1999):

$$K = \frac{x_2^{\alpha}}{x_2^{\beta}} = \frac{H_{2,\beta}}{H_{2,\alpha}} \tag{3}$$

where $H_{2,\alpha}$ is Henry's constant for solute 2 in phase α, and $H_{2,\beta}$ is Henry's constant for solute 2 in phase β. At constant temperature and pressure, for dilute solutions, the partition coefficient is a constant, independent of composition. The Eq. (3) is frequently called the Nernst distribution law.

A simple classic extraction procedure, "shake-flask method", is widely used in partition coefficient measurements (Sangster, 1989). In the method, a small amount of solute is dissolved in one phase, equilibrium partition is obtained by agitation, the phases are separated and one or both phases are analysed for solute. The Nernst distribution law is applicable in this system. In this investigation, the shake-flask method was used to determine the partitioning of preservatives in different systems.

2.2. Materials and experiment procedure

In this work, fish oil (sand eel) was supplied by Skagen A/S, Skagen, Denmark. Benzoic acid (>=99.5%), sorbic acid (>=99.5%), methanol (99.9%), H_2KPO_4 (99%), K_2HPO_4 (99.5%) were purchased from Fluka, Sigma–Aldrich Chemie, GmbH, Steinheim, Germany. Rape oil and olive oil were purchased from Coop Trading A/S, Alberslund, Denmark. A water bath (Compatible Control Thermosats, CC1, manufactured by Peter Huber, Kältemaschinenbau GmbH, Offenburg, Germany) was used to carry out the experimental work. The temperature control deviation of the water bath was ±0.1 °C over the experimental temperature range. The temperature control system, pH meter and all pipettes used in the experimental work were calibrated. The temperature range selected to carry out measurements was 5–43 °C, which is a likely temperature range for exposure in a food production and service chain.

Taking into account the solubility of benzoic acid and sorbic acid in water (Kirk-Othmer, 2001), 2.7001 g benzoic acid and 0.9995 g sorbic acid were dissolved in 1000 ml distilled water, separately, as stock solutions. The mole fractions of benzoic acid and sorbic acid in the stock solutions were respectively 3.978×10^{-4} (2.7001 g/l) and 1.603×10^{-4} (0.9995 g/l). At the set-up temperature, 50 g benzoic acid (or sorbic acid) solution and 50 g of fish oil were mixed for four hours with a magnetic stirrer in a water bath. At the experimental concentrations, the preservatives are at very dilute concentrations in aqueous and oil phases, where Henry's law is applicable. After 4 h, the magnetic stirrer was stopped. The samples then stood in the same water bath at the same temperature for 16 h to allow the phase separation and phase equilibrium. The temperature conditions were set at 5, 15, 25, 35 and 43 °C. The oil phase was transferred out from the sample flask by a disposable pipette and the aqueous phase was carefully moved out by a syringe with a needle. In using the syringe to access the aqueous phase sample, a very small amount of air was injected out from the syringe to avoid a tiny oil droplet entering the syringe before starting to suck the aqueous phase sample into the syringe. After obtaining the aqueous phase sample, the pH value of the sample was measured at room temperature. The samples taken from aqueous phase were directly applied to HPLC analysis with suitable dilution. The oil phase samples were treated according to Nordic Committee on Food Analysis method (NMKL, 1997) and analysed by HPLC.

To create conditions similar to a real food environment, a buffer solution instead of water was employed to study the partitioning of the preservatives between fish oil and buffer solution phase. The buffer solution was prepared by dissolving 2 g H_2KPO_4 and 2 g K_2HPO_4 in 2000 ml water at 25 °C (1 g/l for H_2KPO_4 and 1 g/l for K_2HPO_4). The pH of the buffer solution was 6.82; HCl and NaOH were used to adjust the pH value of the buffer solution. Benzoic acid (0.5003 g) and sorbic acid (0.5005 g) were dissolved in 1000 ml distilled water as stock solutions. The mole fractions of benzoic acid and sorbic acid were respectively 7.370×10^{-5} (0.5003 g/l) and 8.030×10^{-5} (0.5005 g/l) in the new stock solutions. The new system, i.e., fish oil–buffer solution, creates a buffer environment to reflect the characteristics of a real food system. The same experimental equipment and procedure were applied to measure the partitioning of benzoic acid and sorbic acid in the new system.

2.3. Benzoic acid and sorbic acid concentration determination

The concentration of a preservative in oil and aqueous phase was determined by HPLC. A Nordic Committee on Food Analysis method: "benzoic acid, sorbic acid and *p*-hydroxybenzoic acid esters. Liquid chromatographic determination in foods" (NMKL, 1997), was applied. The method was validated in a collaborative study by 11 laboratories, according to the IUPAC-1987 protocol. For liquid samples a reproducibility standard deviation between 1.4% and 7% was found for benzoic and sorbic acid and a recovery of 100% for both preservatives. The detection limit was 5 mg/l or lower.

The NMKL method was modified slightly by extracting with pure methanol instead of a mixture of water–methanol to improve the extraction of sorbic acid, and a filtration step of extraction was excluded, since all the extracts were very clean. The reason for using pure methanol is because the solubility of sorbic acid in anhydrous methanol solution is 12.9 g/100 g solvent, and it drops to 1.9 g/100 g solvent in 50 wt.% methanol solution (Kirk-Othmer, 2001). The solubility of benzoic acid in the methanol–water solution is also decreased from 71.5 in anhydrous methanol solution to 13.9 g/100 g in 50 wt.% methanol solution (Das, Das, Bose, & Kundu, 1978).

The aqueous phase samples of the partitioning experiments were analysed directly by HPLC, while the oil phase samples were