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Free radical interactions between raw materials in dry soup powder

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ABSTRACT

Interactions at the free radical level were observed between dry ingredients in cauliflower soup powder, prepared by dry mixing of ingredients and rapeseed oil, which may be of importance for quality deterioration of such dry food products. The free radical concentrations of cauliflower soup powder, obtained by electron spin resonance (ESR) spectroscopy, rapidly become smaller during storage (40 °C and relative humidity of 75%) than the calculated concentrations of free radicals based on the free radical concentrations of the powder ingredients used to make the soup powder and stored separately under similar conditions. Similarly, free radical concentrations decreased faster when any combination of two powder ingredients (of the three major ingredients of the soup powder) were mixed together and stored at 50 °C for 1 week than when each powder component was stored separately. Furthermore, yeast extract powder was found to play a key role when free radical interactions between powder ingredients occurred. The incubation of rapeseed oil with powder ingredients at 45 °C for 24 h, indicated the ability of cauliflower powder to increase the concentration of hydroperoxides in rapeseed oil, while yeast extract powder was found to prevent this hydroperoxide formation.

1. Introduction

Intermolecular free radical reactions have been shown to occur in irradiated freeze-dried systems at temperatures as low as −196.15 °C (Copeland, Sanner, & Pihl, 1968). The level of these intermolecular free radical reactions has further been found to depend strongly on the degree of contact between the components in dry mixtures (Henriksen & Sanner, 1967). Free radicals from oxidizing lipids have been shown to transfer to proteins and amino acids and these interactions may initiate the reactions leading to protein degradation (Schaich, 2008). The free radical transfer between lipids and proteins has been studied by ESR spectroscopy, both directly, in dry systems, and in emulsions, by using the spin trapping technique. When only dry systems are considered, the free radical transfer has been observed, in both lyophilized emulsions containing protein and oxidizing lipid and systems consisting of crystalline protein mixed with oxidizing fatty acid (Huang, Yu, Hua, & Qiu, 2006; Saeed, Fawthrop, & Howell, 1999; Saeed, Gillies, Wagner, & Howell, 2006; Schaich & Karel, 1976).

On the other hand, free radicals may transfer from proteins to lipids and initiate lipid oxidation. Incubation of linoleic acid with bovine serum albumin radical has been shown to change the ESR signal shape and intensity compared to that of the BSA radical incubated alone or with stearic acid. At room temperature these interactive reactions occur within minutes, and the incubation of linoleic acid with BSA radical has further been shown to promote the formation of lipid hydroperoxides and conjugated dienes (Østdal, Davies, & Andersen, 2002). Furthermore, free radicals may transfer from proteins to other biomolecules, such as other proteins and peptides (Schaich, 2008; Østdal et al., 2002). In general, the reactions of protein radicals may lead to protein cross-linking, scission and further oxidation reactions (Gardner, 1979; Østdal, Andersen, & Davies, 1999). Hence, the free radical interactions in food systems may be important in determining the stability and shelf-life of dry foods.

Our previous study showed a remarkable decrease in the free radical concentration of dry cauliflower soup powder during storage under accelerated conditions of 40 °C and 75% relative humidity, while no significant lipid oxidation was observed (Raitio, Orlien, & Skibsted, 2011). It is noted, however, that the free radical concentrations of different dry food systems have often been observed to increase during storage and this increase has been related to lipid oxidation (Nissen, Månsson, Bertelsen, Huynh-Ba, & Skibsted, 2000; Stapelfeldt, Nielsen, & Skibsted, 1997; Thomsen, Lauridsen, Skibsted, & Risbo, 2005). As in our study of cauliflower soup powder (Raitio et al., 2011), a decrease in free radical concentrations during storage was observed for dry cappuccino powder. The decrease in the levels of free radicals in cappuccino powder

Abbreviations: ESR, electron spin resonance; CP, cauliflower powder; WP, whey protein extract; YE, yeast extract; POV, peroxide value.

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was explained by an increase in molecular mobility in the powder, caused by moisture adsorption, allowing radicals to combine, reaching a lower steady state-concentration (Becker, Madsen, & Skibsted, 2009). The decrease in free radical concentrations of cauliflower soup powder was not related to increased molecular mobility as no significant changes in water activity or water content of cauliflower soup powder were observed during the accelerated storage. The other analyses indicated that protein degradation reactions, including Maillard reactions and protein oxidation, were important for quality deterioration of the cauliflower soup powder during storage (Raitio et al., 2011). As the reactions leading to decreased concentrations of free radicals in cauliflower soup powder may have significance in triggering the reactions leading to quality deterioration, the objective of the present study was to characterize the nature of the reactions occurring between different dry ingredients of cauliflower soup powder, and between dry ingredients and rapeseed oil. Very little is known about the stability of dry food systems similar to cauliflower soup powder, prepared by dry mixing of ingredients and rapeseed oil. Information on the interacting reactions may help us to protect such dry food systems from quality degradation.

2. Materials and methods

2.1. Chemicals

$BaCl_2 \cdot 2H_2O$ was purchased from Sigma–Aldrich (Steinheim, Germany); HPLC grade chloroform was from Lab-Scan (Dublin, Ireland). Analytical grade methanol and $FeSO_4 \cdot 7H_2O$ were from Merck (Darmstadt, Germany). NH_4SCN was from Riedel-De-Haën (Seelze, Germany). Food-grade refined rapeseed oil was used and its fatty acid composition was determined in our earlier study (Raitio et al., 2011) to be: palmitic acid (5%), stearic acid (2%), oleic acid (62%), linoleic acid (21%) and linolenic acid (11%).

2.2. Selection of ingredients for accelerated storage tests with ESR spectroscopy

The selections of dry powder ingredients, to be used in accelerated storage tests, were made with an ESR spectrometer by determining the initial level of free radicals in each dry ingredient of cauliflower soup powder. The ESR measurements were done by placing each ingredient in a fused quartz tube with 4 mm inner diameter and 1 mm wall thickness (710-SQ-250M, Wilmad Glass, Buena, NJ, USA). The exact sample mass and height in the ESR tube were measured, from which the sample density was calculated and used to correct the results. An ESR spectrometer (MiniScope MS 200, Magnettech, Berlin, Germany) equipped with X-band microwave supply was used. The parameters for ESR determinations were: centre field 3337.73 G, sweep width 79.36 G, sweep time 120 s, modulation amplitude 6000 mG and microwave power 1.58 mW. The software, Analyses (Magnettech), was employed to obtain the amplitudes of ESR lines and the area under the ESR spectra by double integration. The free radical impact of each dry ingredient of cauliflower soup powder was determined as its mass proportion in the dry soup ingredients multiplied by its relative ESR line height or area. The ESR signals of dry ingredients were suggested to be additive, i.e. the sum of their free radical impacts corresponded to the height and the area observed with fresh cauliflower soup powder prepared from these ingredients by mixing. The final selections of ingredients were based on the requirement that the free radical impact of each selected powder ingredient on the calculated ESR spectra of soup powder needs to be more than 5%, calculated from both ESR line amplitude and area under the ESR line. For confidentiality reasons, the results from the initial selection tests are not shown.

2.3. Stability of cauliflower soup powder ingredients during storage

To follow the relative free radical concentrations within particular dry powder ingredients of cauliflower soup powder, the selected cauliflower soup powder ingredients (ca. 70 g) were stored as cauliflower soup powders, as in our previous study (Raitio et al., 2011), in closed 110 ml polypropylene containers in the climate cupboard (Climacell, Planegg, Germany) at 40 °C and 75% relative humidity for up to 12 weeks. At 0, 2, 4, 8, and 12 weeks of storage, the ingredient package was removed from the climate cupboard and, after allowing the sample to cool to room temperature, the sample inside the package was thoroughly mixed and the appropriate powder amount required for ESR analysis was removed from the container and the package was closed and again placed in the climate cupboard. The storage tests on ingredients were done in duplicate. The dry ingredients used in accelerated storage stability tests were yeast extract (YE), cauliflower powder (CP), and whey protein extract (WP). According to data provided by manufacturers, the YE was spray-dried yeast extract obtained by autolysis of *Saccharomyces cerevisiae*; CP was composed of hot air-dried fresh cauliflower florets, and WP was spray-dried whey protein concentrate. The compositions, provided by manufacturers for YE, CP and WP, are presented in Table 1.

2.4. Two-powder system

The two-powder systems were prepared by mixing YE, CP and WP together in a glass beaker to obtain YE + CP, YE + WP, and CP + WP powders (1:1, w:w). 8.0 g (±0.1 g) of a two-powder system or powder ingredient alone were stored in a 30 ml closed amber glass bottle in an oven at 50 °C for up to 7 days. A slightly higher temperature for this experiment was selected in order to decrease the reaction time. The free radical concentrations of each powder and two-powder system were determined using ESR spectrometry, as described in Section 2.2 at the storage times of 0, 1, 4 and 7 days. The calculated free radical concentrations of two-powder systems were obtained from the free radical concentrations of each powder ingredient stored alone at each particular time of storage. It was again supposed that the free radical concentration of any two-powder system, prior to any reaction, was the sum of the free radical concentrations of powder ingredients relative to their mass proportion in the two-powder system. In addition to the ESR analysis presented in Section 2.2, the microwave frequency of each spectrum was recorded with an external frequency meter (Agilent 53150A, Santa Clara, CA, USA) for the determination of *g*-values. Software analysis (Magnettech) was employed to determine the *g*-values.

2.5. Powder–rapeseed oil system

2 g (±0.01 g) of either YE or CP were mixed with 20 g (±0.02 g) of rapeseed oil in a 50 ml closed bottle at 45 °C for 24 h with a speed of 80 u/min in a water bath (Unitronic OR, Selecta, Barcelona,

Table 1
Compositions (%) of yeast extract (YE), cauliflower powder (CP), and whey protein extract (WP). Information provided by manufacturers; other non-specified components were present.

	Water	Proteins	Carbohydrates	Fat
Yeast extract (YE)	2–6	53–61	3–6	–
Cauliflower powder (CP)	7	25	61	2
Whey protein extract (WP)	5	30	53	3