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Stability of α-tocotrienol and α-tocopherol in salami-type sausages and curing brine depending on nitrite and pH

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ABSTRACT

We studied the stability of the valuable vitamer nutrients α-tocotrienol and α-tocopherol and options for their protection in salami-type sausages (blended with α-tocotrienol-rich barley oil) and curing brine. Four different sausage formulations were produced containing nitrite curing salt; nitrite curing salt and ascorbic acid (300 mg/kg); nitrite curing salt and carnosic acid (45 mg/kg); or sodium chloride. Initial vitamer contents (100 mg/kg) did not decrease significantly during ripening and decreased only slightly during storage. Ascorbic acid and carnosic acid were found to be effective in preserving the vitamers in fresh sausages. Freeze-drying of sausages resulted in a significant loss of vitamers (97%), particularly after 14-day storage at room temperature, even in the presence of shielding gases. The vitamer content in the curing brine decreased with decreasing pH in the presence of nitrite. A nitrite concentration of 136 mg/L at pH 4 resulted in significant loss (90%) of the vitamers. Sufficient stability of the vitamers in salami-type sausage and curing brine can be achieved by processing, formulation, and storage conditions.

1. Introduction

Vitamin E is necessary for vital functions in humans and animals; mammals require vegetable dietary sources for their intake of this bioactive compound because they cannot synthesize it (Kamal-Eldin & Appelqvist, 1996). Vitamin E can be divided into two classes comprising eight tocochromanol vitamers, the tocopherols (α-, β-, γ-, and δ-tocopherol) and tocotrienols (α-, β-, γ-, and δ-tocotrienol). The difference between α-tocotrienol and the better-known α-tocopherol is found in the three double bonds at the side chain of the tocotrienols, and this is also true of the other corresponding vitamers. While palm oil is the most prevalent source of tocotrienols, oil from barley, more precisely oil from its low-cost by-product, brewer's spent grain, has also been reported to be extraordinarily rich in tocotrienols, particularly α-tocotrienol. The properties of spent grains were extensively studied by Bohnsack, Ternes, Büsing, and Drotleff (2011). Vitamin E has an excellent antioxidizing effect and has therefore been classified as a stabilizer for oils and fats (Al-Saqer et al., 2004; Seppanen, Song, & Csallany, 2010). Various studies indicate that the generally often disregarded tocotrienols exhibit antiproliferative, anti-inflammatory, anticancer, and cholesterol biosynthesis suppressive effects which cannot be achieved by tocopherols (Aggarwal, Sundaram, Prasad, & Kannappan, 2010); e.g., tocotrienols attain a cholesterol level lowering effect at micromolar concentrations (for humans, about 50 mg mixed tocotrienols per day) (Qureshi, Sami, Salser, & Khan, 2002). These positive food technological and health promoting effects can be used selectively by enhancing foods with tocotrienols.

Salami-type sausages are one of the favorite meat products in Europe. The mean fat content of raw sausages is 35%, with 80 mg cholesterol/100 g (Souci, Fachmann, & Kraut, 2008). Between manufacturing and consumption, lipid oxidation may cause degradation of the desired unsaturated fatty acids. Supplementation of sausages with tocotrienols can help to enhance protection against oxidation and create commercially viable quality products with additional nutritional value.

Some previous research was based on treatment of ground meat with vitamin E (α-tocopherol), which reduced pigment and lipid oxidation (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2002; Mitsumoto et al., 1991). Other researchers compared the effectiveness of vitamin E (α-tocopherol) following direct addition to animals' diet (Mitsumoto, Arnold, Schaefer, & Cassens, 1993) and found that endogenous application of vitamin E improved pigment and lipid stability much better than exogenous.

Other studies investigated the effect of α-tocopherol on meat products. Hoz, Arrigo, Cambero, and Ordóñez (2004) showed that lipid oxidation of dry fermented sausages which had been enriched with α-tocopherol by dietary vitamin E supplementation was lower

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than that of sausages without this addition. Additional studies investigated the stability of α-tocopherol (enhanced by dietary supplementation of pigs) in salami-type sausages with various results: while Harms, Fuhrmann, Nowak, Wenzel, and Sallmann (2003) reported no change in vitamin E concentration in the sausages over eight weeks of storage, Sammet et al. (2006) showed that the α-tocopherol content decreased during ripening and storage. Thus far, only Rosli, Babji, Aminah, Foo, and Abd Malik (2006, 2010) have studied the stability of tocotrienols in meat products (beef burgers, beef frankfurters, and chicken frankfurters). They showed that added α-tocopherol and α-tocotrienol were reduced by about half after 6 months of storage and that the effect of processing, cooking, and frozen storage could influence the vitamin E stability and content in meat products. The maximum temperature during the manufacturing process of salami-type sausages typically is not elevated above room temperature, and an anaerobic environment is formed during ripening; both conditions may provide a protective function against oxidation. In this respect, salami-type sausage is a reasonable matrix for supplementation with tocotrienols. However, the use of nitrite-containing curing salt is mandatory for salami-type sausages. Nitrite is known to have a prooxidant effect, but nitrite and its secondary products may also be effective antioxidants in combination with other ingredients. In the presence of myoglobin, nitric oxide-myoglobin can be formed, which has been shown to inhibit lipid oxidation (Kanner, Harel, & Rina, 1991). The simultaneous presence of nitrite and tocotrienols in meat matrix leads to complex reaction conditions; under these circumstances the fate of the tocotrienols in salami-type sausages is hard to predict. Therefore, comprehensive studies on tocotrienol degradation processes are needed in order to lay the foundations for the development of a customized production technology which protects α-tocotrienol and its health benefits for consumers.

One may assume that the addition of other antioxidants like ascorbic acid and carnosic acid, a rosemary ingredient, would help to prevent the oxidative degradation of α-tocotrienol. Ascorbic acid is often used as a curing additive, and it is known for its protective properties in food (Chauhan, Ramteke, & Eipeson, 1998; Liao & Seib, 1988). Niki, Tsuchiya, Tanimura, and Kamiya (1982) reported that vitamin C regenerates vitamin E by transferring a hydrogen atom to a vitamin E radical. Furthermore, the antioxidant properties of rosemary extracts have often been reported, and the outstanding antioxidant activity of carnosic acid is well documented (Richheimer, Bernart, King, Kent, & Beiley, 1996; Schwarz & Ternes, 1992). In the present study, we aimed to investigate the effectiveness of these two antioxidants for the preservation of α-tocotrienol.

Another method to prolong the shelf life of meat is drying. Nowadays, meat is dried for use in instant products, often by freeze-drying. However, the literature contains only marginal information about antioxidants in freeze-dried products. Wilkinson, Senecal, and Faustman (2001) showed that thiobarbituric acid reactive substances (TBARS) levels in freeze-dried meats treated with mixed tocopherols were lower than in controls, but there are no reports on the stability and/or protection of tocotrienols in freeze-dried meat.

Another conventional way to cure meat is to use a brine containing sodium chloride and/or nitrite. In order to produce hams or bacon, curing solution is often used to perfuse the meat after slaughtering or is injected into the meat, up to 20% of the meat weight (Gareis & Kabisch, 2011). This makes curing solution a potential medium for transferring tocotrienols into the meat. Spicy, acidic curing brines are used to give the meat a certain taste or make it more tender. It is also known that in acidic milieu, nitrite acts primarily as an oxidant, and that nitric oxide can be formed (Skibsted, 2011). However, no information is available about the direct influence of nitrite and pH on tocotrienols and tocopherols in brine. The present investigations were intended to investigate the stability of tocochromanols in brines with different concentrations of nitrite curing salt at different pH values.

The study design included three factors affecting the stability of α-tocotrienol and α-tocopherol (for comparison): (i) different sausage formulations, (ii) freeze-drying, and (iii) the curing brine conditions.

The results of the comprehensive study should help in the application of tocotrienols or barley oil, which is extraordinarily rich in α-tocotrienol, to salami-type sausages and curing brines, thus making it possible to exploit the food technological and health benefits of these compounds.

2. Materials and methods

2.1. Sausages

2.1.1. Brewer's spent grain extract preparation

The brewer's spent grain for this study was obtained from a single manufacturer (Leiber GmbH, Bramsche, Germany). Previous research showed that α-tocotrienol levels differed by only 10% in several types of brewer's spent grain including that from two different breweries (Bohnsack et al., 2011). As in that study, the dried brewer's spent barley grain was sieved to obtain the richest tocotrienol fraction with a particle size <500 μm. In 300–400 g portions, the sieving fraction was given into an extraction thimble and placed in a soxhlet apparatus, where it was extracted for 8 h with 96% ethanol. The extraction solvent was evaporated with a rotary evaporator under vacuum at 45 °C. To ensure the static value of the oily barley extract, it was analyzed immediately and standardized by addition of α-tocotrienol standard substance (Davos Life Science, Singapore) so that every sausage batch had the same initial value of 100 mg/kg. For this, the vitamer content of the extract was determined (mean: 248.2 mg α-tocotrienol/kg and 87.4 mg α-tocopherol/kg), and the calculated difference to the 100 mg α-tocotrienol/kg (wet weight) in the final product (corresponds to a mean content of 200 mg α-tocotrienol/kg dry weight (Fig. 1)) was added as an oily standard substance under continuous stirring. The procedure was repeated for each sausage production. The standardized extract was stored at −20 °C until use for sausage production.

To determine the α-tocotrienol (and other tocochromanols) in barley oil, 250 mg was weighed into a 5-mL volumetric flask and filled to volume with *n*-hexane. After extraction into hexane for 2 min, the solution was used for HPLC determination.

2.1.2. Sausage—manufacture

The sausages were manufactured in three replications. Sausages were produced by a butcher of the Institute of Food Quality and Food Safety, University of Veterinary Medicine Hannover, Foundation, Germany. The barley oil was emulsified with water and soy protein isolate (Pro-Sana, Halle, Germany) (10:6:1, *w/w/w*). All sausages contained pork meat (40%), beef (35%), dorsal pork fat (25%), standardized barley oil (3%) in emulsion, starter culture BFL-F05, BactoFlavor® (0.25%) (Chr. Hansen, Pohlheim, Germany), spice mixture (0.2%), and dextrose (0.25%). Samples were taken to determine the blank value of the meat and fat (total mean: 1.3 mg α-tocotrienol/kg wet weight and 6.8 mg α-tocopherol/kg wet weight (corresponds, with the extract content, to a mean content of 25 mg α-tocopherol/kg dry weight (Fig. 2)).

Sausages were produced by the same basic recipe but with special additives: firstly, 1.5% nitrite curing salt (0.9% $NaNO_2$ in curing salt); secondly, 1.5% nitrite curing salt and 300 mg/kg ascorbic acid; thirdly, 1.5% nitrite curing salt and 45 mg/kg carnosic acid; and fourthly, 1.5% sodium chloride without nitrite. Three batches were produced of every formulation. The amount of sodium nitrite curing salt added was within the legally allowed level (≤ 150 mg/kg). After spiking, the sausages contained 100 mg/kg α-tocotrienol before ripening. The sausages were ripened for four weeks under usual conditions (a slow decrease in relative humidity from 96% to 84%, a slow decrease in temperature from 22 to 15 °C, and 3 × 10 min smoking on days 3, 6, and 11). At the end of ripening the sausages were stored for 30 days at 4–6 °C. This temperature was chosen to simulate the conditions in a commercial refrigerator. Each batch was