Published in Journal of Food Chemistry 113:832-4, 2009 – doi: 10.1016/j.foodchem.2008.05.019

Unexpected sources of vitamin C

J. Fredriksen[a], E.B. Løken[a], Å. Borgejordet[b], K. Gjerdevik[c], A. Nordbotten[b]

a Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1046 Blindern, 0316 Oslo, Norway
b Norwegian Food Safety Authority, P.O. Box 383, 2381 Brumunddal, Norway
c National Institute of Nutrition and Seafood Research, P.O. Box 2029 Nordnes, 5817 Bergen, Norway

Abstract

Sausages are quite common in the Norwegian diet. Presently the vitamin C content in sausages is listed as zero in the Norwegian Food Composition Table as well as in several other countries' food composition tables. In the present study, five different kinds of sausages were analysed for relevant nutrients including vitamin C. One composite sample consisting of 15–16 subsamples was analysed for each sausage type. Vitamin C was measured by high-performance liquid chromatography (HPLC) with electrochemical detection as the sum of L-ascorbic acid and dehydro-ascorbic acid. The vitamin C content in the analysed sausages varied from 11 to 40 mg/100 g. This is explained by addition of vitamin C as an antioxidant by the manufacturer mainly to preserve colour. When recalculating vitamin C intakes from the most recent Norwegian national dietary surveys in adults, children and youths using the updated vitamin C values, total vitamin C intake increased by 3–10%. Other processed meat products such as cold cuts may also contain vitamin C as an antioxidant, and a larger impact on vitamin C intake may be seen if also these products turn out to contain considerable amounts of vitamin C. When planning analytical projects for food composition databases, care should be taken to include vitamin C for products where this vitamin may have been added for antioxidant purposes.

1. Background

Sausages of various kinds are quite common at Norwegian dinner tables and as fast foods sold at various fast food outlets. In the most recent Norwegian dietary surveys (Dahl, Johansson, Julshamn, & Meltzer, 2004; Øverby, Lillegaard, Johansson, & Andersen, 2004), the average intakes of sausages were 18 and 11 g per day for adult men and women, respectively, while the average daily intakes among children at 4, 9 and 13 years of age were 18, 22 and 21 g, respectively. Sausages and mashed potatoes are a popular combination. So far, the mashed potatoes have been assumed the only source of vitamin C in this dish, since the content of vitamin C in sausages is listed as zero in the most recent version of the Norwegian Food Composition Table MVT-06 (Norwegian Food Safety Authority, Directorate of Health and Social Affairs & University of Oslo, 2006) as well as in several other food composition tables in Europe. The main ingredients in Norwegian sausages are typically a mixture of beef and pork (or turkey and chicken), starch, often milk, salt and spices. The table values for sausages in MVT-06 are derived from recipe calculations based on information from the meat industry and from analyses performed in 1991.

In the present study, a selection of meat products from the main manufacturers on the Norwegian market was analysed for relevant nutrients to update and supplement existing data in the Norwegian food composition database. Here, we present the results for vitamin C in five types of sausages.

2. Materials and methods

2.1. Sampling and sample preparation

Samples of the three most commonly consumed sausages (Frankfurters, meat sausages and grill sausages) and two low-fat varieties made by the food company holding the dominating market shares for sausages in Norway were bought from grocery stores in the Bergen area (Western Norway). The sampling was carried out during a period of 1 year (November 2004 and February, May and August/September 2005) to cover possible seasonal variations in nutritional composition. For each type of sausage, four packages with different production dates containing 400 or 500 g each from the same brand name were collected at each sampling occasion. For the low-fat grill sausage, three instead of four packages were collected on one of the sampling occasions. Dates of purchase and expiration dates were collected for each of the sausage packages (n = 79). The sausages were kept frozen at _80 _C until all samples had been collected. Monthly checks showed that the temperature in the freezer varied between _80 _C and _82 _C. Composite samples of each of the five types of sausages to be analysed for vitamin C, each consisting of 15–16 sub samples, were prepared in August/September 2005 (Table 1).

Table 1: Sampling and content of vitamin C in five types of Norwegian sausages

Type of sausage	Seasons (n)	Sub samples in composite sample (n)	Analyzed samples (n)	Vitamin C (mg/100g)
Meat sausage, smoked	4	16	1	40
Frankfurter (wiener)	4	16	1	33
Grill sausage	4	16	1	30
Grill sausage, low fat	4	15	1	11
Chicken & turkey sausage	4	16	1	29

After homogenisation in a Braun food processor, test portions of all composite samples were analysed for vitamin C immediately after sample preparation. Due to the large sample size, no special precautions were made to prevent oxidation.

2.2. Chemical analysis

The chemical analyses were preformed by the National Institute of Nutrition and Seafood Research in Bergen, Norway. Vitamin C (determined as the sum of dehydro-ascorbic and Lascorbic acid) was extracted from the sample after addition of 5% meta-phosphoric acid containing ethylenediaminetetraacetic acid (EDTA) and dithiothreitol (DTT). DTT reduces dehydro-ascorbic acid to ascorbic acid in addition to stabilising ascorbic acid. The solution was centrifuged using a Kubota centrifuge model 2010 (Kubota Co., Tokyo, Japan) and the upper phase, which contains vitamin C, was collected. The compounds in the water solution were separated by high-performance liquid chromatography (HPLC). The concentration of ascorbic acid was determined by electrochemical detection using a Hewlett Packard electrochemical detector model 1049A (Hewlett Packard Co., Waldbronn, Germany) at 0.6 V and external standard calibration. Integration of peak height was performed with TotalChrome software (PerkinElmer, MA, USA). The method has been validated and accredited for foods and is based on the Hewlett Packard procedure ‘‘Analysis of selected vitamins with HPLC and

electrochemical detection" (Gratzfeld-Hüsgen, Schuster, & Häcker, 1992) and modified as described by Wilson and Shaw (1987). The HPLC system consisted of a Merck Hitachi LaChrom pump model L-7100KB (Merck Hitachi, Tokyo, Japan). The column used was ODR Hypersil (C18) 5 lm, 250 _ 4.6 from Hewlett Packard, and the flow rate was 0.8 ml. The sample injection volume was 20 ll using a Gilson model 434 autoinjector (Gilson, WI, USA). The mobile phase consisted of sodium acetate, EDTA, tetrabutyl ammonium hydrogen sulphate and water. A minimum of two parallels was analysed for each composite sample. The limit of quantification was 0.1 mg/100 g wet weight, and the measurement range was 0.1–300 mg/100 g wet weight. The measurement uncertainty (u) was 6% (u = 1*RSD–based on the control chart (n = 20)). The recovery was 90–110% calculated from laboratory proficiency test (LPT) results and standard addition to samples (the analysed values of the reference materials fell within ±5% of the certified value). The reliability of the analytical method was further controlled by analysis of certified reference materials, i.e. NIST-SRM-1846 (infant formula) and BCR-CRM-383 (haricot beans). The laboratory is accredited by Norwegian Accreditation for vitamin C analysis and participates regularly in LPTs. For vitamin C, the laboratory has participated in LPTs organised by FAPAS (York, UK) and Bureau InterProfessionnel d'Etude Analytique (BIPEA) (Gennevilliers, France). All results were accepted according to normal procedures for proficiency testing schemes.

2.3. Statistical analysis

The statistical analyses were done using the Statistical Package for the Social Sciences version 14.0 (SPSS Inc., IL, USA). The remaining number of days before the expiration date for the collected packages of the low-fat grill sausages was compared with the number of remaining days before the expiration date for each of the other sausage types using independent-samples t-test. All p-values are two-sided, and a 5% level of significance was used.

3. Results and discussion

The analysed content of vitamin C in the five sausage types varied between 11 and 40 mg/100 g of sausage (Table 1). These findings are explained by the addition of vitamin C as an antioxidant additive by the manufacturer mainly to preserve colour. Vitamin C is widely used as a colour retention agent in various processed meat products (Ahn & Nam, 2004; The Codex Alimentarius Commission, 2007). Authorisation and use of food additives in foodstuffs are harmonised at European level (The European Union, 2007), and food additives are stated on the package label by an E number and/or by the additive's chemical name. From the package labels of the sausages included in the present study it was evident that vitamin C and/or ascorbate in the form of the E numbers E300 (ascorbic acid) or E301 (sodium ascorbate) had been added to all the analysed sausages. The manufacturer of the sausages included in the project informed that they added 0.042% vitamin C to their sausages, equaling 42 mg vitamin C/100 g sausage. These amounts correspond quite well to the vitamin C levels analysed in the sausages in the present study except for the low-fat grill sausage for which the analysed value was lower than for the other sausages. According to the manufacturer, the same amount of vitamin C was added to all of the analysed sausages. However, because of the very small amount of vitamin C compared to the total amount of sausage meat, the added vitamin C might not be completely evenly distributed in all of the sausage meat. Recently produced sausages may also be likely to retain more of the added vitamin C than sausages closer to the expiration date. The expiration date was by the manufacturer set to 31 or 32 days after the production date for all of the sausages. As shown in Table 2, the low-fat

grill sausage had fewer remaining days before the expiration date at the time of purchase than the other sausages. This difference was statistically significant for the regular grill sausages and for the smoked meat sausages. Hence, the vitamin C content of the sausages may have been influenced by how recently the sausages were produced, but this will need further investigation.

Table 2: Remaining days before expiration date for low fat grill sausages compared with the other sausage types.

Type of sausage	Remaining days to expiration date[a], mean (SD)	N[b]	p-value[c]
Grill sausage, low fat	10.5 (7.3)	15	NA
Chicken & turkey sausage	12.4 (8.5)	16	0.51
Frankfurter (wiener)	14.1 (4.6)	16	0.11
Meat sausage, smoked	15.9 (4.1)	16	0.02
Grill sausage	17.0 (5.0)	16	0.01

NA; not applicable

[a] Expiration date was by the manufacturer set to 31-32 days after the production date for all of the sausages

[b] Number of purchased sausage packages

[c] Independent-samples t-test, remaining days before expiration date for the low fat grill sausages compared with each of the other sausage types

When estimating the nutritional composition of sausages using recipe calculation, information of the added amount of vitamin C from the manufacturers seems in most cases to be an acceptable alternative when chemical analysis is not possible. However, considerable variation in vitamin C content must be expected.

The addition of ascorbic acid to sausages is not intended to enhance the nutritional quality of the product, and is thus not used in marketing. However, the added amount is large enough to be a potential source of vitamin C, in particular among those with a high intake of sausages. When recalculating the intake of vitamin C in the Norwegian national dietary surveys with the new analysed values for sausages, mean vitamin C intake increased by 3–10% in the various age and sex groups. Since vitamin C is added to some cold cuts and other meat products as well, meat products may turn out to be unexpected, but significant sources of vitamin C in high consumers. Among 14 entries for similar sausages identified in the Swedish (The National Food Administration, 2008), Danish (Møller, Saxholt, Christensen, Hartkopp, & Hess Ygil, 2005) and Finnish (National Public Health Institute of Finland, 2008) food composition tables on the Internet, only three sausages had a table value above zero (range 24–75 mg/100 g). The vitamin C values for these three sausages were all analytical values. For five of the sausages for which the vitamin C content was listed as zero the reference indicated that the value was based on recipe calculation, four were considered logical zeros and two values did not have a reference. In general, only nutrients that are expected to be present in the product in question are analysed due to economic constraints. As shown, food items not usually considered to contain a specific nutrient may still provide a considerable amount if this nutrient has been added for technological purposes.

4. Conclusion

When planning analytical projects for food composition databases, care should be taken to include nutrients that may have been added for technological purposes as the added amounts may

be large enough to be of potential nutritional interest. The results from the present study show that for vitamin C, information from the manufacturer concerning the added amount may be an acceptable alternative if chemical analysis is not possible, although considerable variation in vitamin C content must be expected.

Acknowledgements

The project was funded by the Norwegian Food Safety Authority and the Directorate for Health and Social Affairs.

References

Ahn, D. U., & Nam, K. C. (2004). Effects of ascorbic acid and antioxidants on color, lipid oxidation and volatiles of irradiated ground beef. *Radiation Physics and Chemistry*, 71, 149–154.

Dahl, L., Johansson, L., Julshamn, K., & Meltzer, H. M. (2004). The iodine content of Norwegian foods and diets. *Public Health Nutrition*, 7(4), 569–576.

Gratzfeld-Hüsgen, A., Schuster, R., & Häcker, W. (1992). Analysis of selected vitamins with HPLC and electrochemical detection. Hewlett Packard application note.

Møller, A., Saxholt, E., Christensen, A.T., Hartkopp, H.B., & Hess Ygil, K. (2005). Danish Food Composition Databank, revision 6.0. Available from http://www.foodcomp.dk/.

National Public Health Institute of Finland (2008). Fineli Food Composition Database Release 9. Available from http://www.fineli.fi/index.php?lang=en.

Norwegian Food Safety Authority, Directorate for Health and Social Affairs & University of Oslo (2006). The Norwegian Food Composition Table 2006. Available from http://www.norwegianfoodcomp.no/.

Øverby, N. C., Lillegaard, I. T., Johansson, L., & Andersen, L. F. (2004). High intake of added sugar among Norwegian children and adolescents. *Public Health Nutrition*, 7(2), 285–293.

The Codex Alimentarius Commission (2007). Codex General Standard for Food Additives (GFSA) Online Database. Available from http://www.codexalimentarius.net/gsfaonline/index.html.

The European Union (2007). Authorised food additives: general scheme. Available from http://europa.eu/scadplus/leg/en/lvb/l21067.htm.

The National Food Administration (2008). The National Food Administration's food database, version ''06/03/2008". Available from http://192.121.81.11/livsmedelsok/sok.aspx?lang=2.

Wilson, C. V., & Shaw, P. E. (1987). High performance liquid chromatographic determination of ascorbic acid in aseptically packaged orange juice using ultraviolet and electrochemical detectors. *Journal of Agriculture and Food Chemistry*, 35, 329–331.