Title: Menaquinone content of pork - **NPB #14-100**

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Industry Summary:

1. **Objectives:**
a. Measure the amounts of vitamin K_2 (also known as menaquinones) in representative samples of fresh pork and processed pork products commonly consumed in the U.S. diet.
b. Identify fresh pork cuts that contain the highest content of vitamin K_2 to target those cuts that will provide the highest vitamin K intakes.

2. **How research was conducted:** Nationally representative samples of processed pork products were obtained from the USDA, as part of the National Food and Nutrition Analysis Program (NFNAP). In addition, fresh-cut pork products including chops, chops with bone, back ribs with bone, shoulder blade with bone, tenderloin and St. Louis style cut ribs, were purchased from multiple retail outlets,. Pork samples were then analyzed for vitamin K_2 using state-of the art technology.

3. **Research findings:** All pork products analyzed contained measurable amounts of vitamin K_2. The vitamin K_2 content of processed pork products increased with their fat content. The total vitamin K_2 content of sausage is three to five times higher than the minimum amount of vitamin K recommended in the diet. Among the fresh pork cuts, St. Louis-style spareribs contained about twice the vitamin K_2 content as pork chops and pork tenderloin.

4. **What these findings mean to the industry**: Fresh pork cuts and processed pork products are a rich dietary source of vitamin K_2. There is considerable scientific and consumer interest in vitamin K_2 with respect to their potential protective effect on heart disease risk. However, currently we know very little about the biological activity of vitamin K_2. If it is established that vitamin K_2 has biological activity, pork will have an important dietary role in vitamin K nutrition.

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Keywords: fresh pork, processed pork products, vitamin K, menaquinones, food composition data

Scientific Abstract:

Vitamin K food values in current food composition tables are generally limited to phylloquinone (vitamin K_1), which is plant-based. As such, fresh meat cuts and processed meat products are not considered significant contributors to dietary vitamin K intakes.
The purpose of this study was to measure phylloquinone (PK; vitamin K_1) and 10 forms of menaquinones (MK; vitamin K_2) in fresh-cut and processed pork products. Fresh-cut pork products (chops, chops with bone, back ribs with bone, shoulder blade with bone, tenderloin and St. Louis style cut ribs; n=5 per cut) and bacon (n=4) were purchased from retail outlets in the New England region. In addition, nationally representative samples of processed pork products (n=28) were obtained as part of the National Food and Nutrition Analysis Program (NFNAP). All samples were analyzed by HPLC (PK and MK4), followed by APCI-LC/MS (MK5-MK13). PK was below the limit of detection (0.2 µg PK/100 g) in all fresh pork cuts and in the range of below the limit of detection to 2.1±0.5 µg PK/100 g in processed pork products. In contrast, all fresh pork cuts and processed pork products contained MK4, MK10 and MK11 (range: 68.7±18.1-164.8±0.5 µg MK/100 g for fresh pork cuts; 35.1±11.0-534±89.0 µg MK/100g for processed pork products). Pork sausage contained the highest concentrations of MK10 (274-615 µg/100 g). The total vitamin K (PK + MK) content of processed pork products were strongly associated with fat content (r=0.935). Moreover, PK + MK contents were stable during cooking as demonstrated through comparing uncooked and cooked products. In summary, pork and processed pork products are an unexpected rich dietary source of MK forms that are currently unaccounted for in the assessment of dietary vitamin K. Very little is known about the biological activity of dietary menaquinones but given their abundance in pork products, more research is required to determine their contribution to the role of vitamin K in human health.

Introduction:

Vitamin K (VK) is necessary for normal blood clotting and synthesis of VK-dependent proteins, which have emerging protective roles in multiple chronic diseases. Dietary sources of VK are found in two natural forms: phylloquinone (PK) and menaquinones (MK). PK, the most abundant form of VK in the diet, is present mainly in dark green vegetables. MK are generally synthesized by bacteria found in human gut and fermented foods. The MK differ in structure from PK in their side chain, and are designated by the number of repeating chemical units, i.e. MKn. MK with up to 13 units have been identified. Menaquinone-4 (MK4) cannot be synthesized by bacteria, but can be converted from dietary PK. Whereas PK is widely distributed in food supply, MK forms are limited to animal products and fermented foods.

The current U.S. guidelines for adequate intakes of VK are 90 and 120 µg/day for women and men, respectively. These guidelines are termed adequate intakes (AI) because there were insufficient data available to generate a precise dietary recommendation for VK. Recent reports from Europe attribute unique heart health benefits to MK forms obtained from the diet. Because MK forms have not been systematically analyzed in U.S. foods nor have MK intakes been estimated in the U.S. population, these observations have yet to be substantiated in the U.S.

Guided by preliminary data indicating that pork products may be a dietary source of MK forms, the purpose of this study was to apply LC/MS technology to the measurement of PK and MK forms in representative samples of fresh and processed pork products commonly consumed in the U.S. diet.

Objectives:

a. Precisely quantify the amounts of menaquinones, also known as vitamin K_2, using state-of-the-art technology in representative samples of fresh pork and processed pork products commonly consumed in the U.S. diet. Food samples have already been collected as part of the USDA National Food and Nutrient Analysis Plan (NFNAP).

b. Determine the variability of menaquinone contents within foods by analyzing multiple aliquots within a given sample and across different samples.

c. Identify the tissue and organs in fresh pork that contain the highest content of menaquinones to target those cuts that will provide the highest vitamin K intakes.

Materials & Methods:

Fresh-cut pork products were purchased from various regional retail outlets. Five samples each of various fresh-cut pork cuts (chops, chops with bone, back ribs with bone, shoulder blade with bone, tenderloin and St. Louis style cut ribs) were selected. Bone was removed where necessary prior to homogenization. In addition, representative samples of processed pork were obtained from the USDA Nutrient Data Laboratory, as part of the National Food and Nutrition Analysis Program (NFNAP). The infrastructure of NFNAP incorporates a nationally-representative sampling approach that uses approved analytical methods and a rigorous quality assurance scheme.

The PK and MK4 contents of each sample were determined by established HPLC procedures, which have the sensitivity to detect PK and MK4 concentrations as low as 0.2 μg/100 g. The MK5-MK13 contents of samples were determined by APCI-LC/MS, which is more suited for measurement of the menaquinones. All samples were analyzed in duplicate. The assay was repeated if the coefficient of duplicates was >15%.

Results:

Our analysis of fresh pork cuts (**Table 1**) and processed pork products (**Table 2**) demonstrate that pork contains large amounts of menaquinones. As expected, pork was not a good dietary source of PK. In contrast, all fresh pork cuts and processed pork products contained MK4, MK10 and MK11, which to the best of our knowledge has not been previously reported. The fresh pork cuts also contained MK9, which was not detected in any of the processed pork products.

The total vitamin K content of processed pork products was strongly associated with fat content (r=0.935). Moreover, PK + MK contents of the processed pork products were stable during cooking (Table 2). These results were presented at the 2015 FASB Summer Research Conference "Molecular, Structural, & Clinical Aspects of Vitamin K and Vitamin-K Dependent Proteins" (Chicago IL). Per the policy of FASEB, no published abstract is available.

Table 1. Vitamin K contents of fresh-cut pork (μg/100 g)

Fresh-cut pork	n	PK	MK4	MK5-8	MK9	MK10	MK11	MK12-13
Tenderloin	5	ND*	3.7±1.3	ND	0.4±0.2	0.6±0.3	67.5±6.2	ND
Pork chops, boneless	5	ND	5.3±2.8	ND	2.6±1.2	9.2±7.0	51.7±14.6	ND
Pork chop with bone	5	ND	10.1±5.5	ND	4.1±2.3	26.6±15.0	34.0±5.0	ND
Pork back ribs with bone	5	ND	9.7±4.4	ND	3.6±1.4	12.0±4.7	79.5±12.4	ND
St. Louis-style spareribs with bone	5	ND	12.8±7.1	ND	13.9±9.1	43.6±28.0	94.5±28.7	ND
Shoulder blade Boston with bone	5	ND	5.0±4.0	ND	4.8±3.8	8.8±7.0	109±14.4	ND

*ND – not detectable; the lower limit of detection: PK and MK4: 0.2 μg/100 g; MK5: 0.4 μg/100g; MK6: 0.4 μg/100g; MK7: 0.5 μg/100g; MK8: 0.6 μg/100g; MK12: 0.8 μg/100g; MK13: 0.8 μg/100g.

Table 2. Vitamin K contents of processed pork products (μg/100 g)*

Food	n	Total VK (PK + MK4 + MK10+MK11)	Fat (g/100g)
Kielbasa, unprepared	3	408±90.7	26.4±3.8
Kielbasa, gilled	2	534±89.0	31.7±4.8
Kielbasa, pan-fried	3	475±97.0	28.3±2.5
Pork Sausage, regular fat, unprepared	6	355±41.1	23.5±2.0
Pork Sausage, regular fat, pan-fried	6	383±82.9	27.9±4.4
Pork Sausage, reduced fat, pan-fried	2	325±28.1	18.8±0
Canadian Bacon, unprepared	1	52.0±1.8	3.16
Canadian Bacon, cooked**	4	35.4±11.0	3

* Vitamin K content was correlated to fat content (r=0.935)
**Fat content was estimated by manufacturer.

Discussion:

A recent review commissioned by the International Life Sciences Institute concluded that "there is merit for considering both MK and PK when developing future recommendations for VK intake". Emerging evidence suggests novel physiologic functions for VK beyond the vitamin's role in hemostasis. Underlying mechanisms are not well characterized, though several are postulated to be unique to MK forms.

We have demonstrated for the first time that fresh pork cuts and process pork products contain large amounts of specific MK forms, specifically MK forms with longer side chains. Interestingly, these same longer chain MK forms in pork have been identified in human liver, but the origin of these MK forms have been a source of speculation. It is biologically plausible that pork may be an important dietary source of VK forms that are absorbed and stored in liver. However, data on metabolism and function of these longer chain MK forms obtained from the diet are almost non-existent and we need these absorption data to demonstrate the dietary contributions of fresh pork to VK status, hence human health.

Should future research demonstrate that MK forms have biological function, fresh pork cuts and certain processed pork products will be considered a rich dietary source of VK, and can be used in various diet plans to increase overall VK intake.