

MenaQ7 Crystals®: Ingredient innovation rationale

Any industry that rests on its laurels will stagnate. A requirement for industry growth as well as commercial and consumer appeal is ingenuity in conceiving new technology that allows for significant innovative improvements.

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NattoPharma of Oslo, Norway, has initiated a project to create MenaQ7® brand of vitamin K2 that can significantly impact the international market for vitamin K2 – and specifically its derivative menaquinone-7 – in direct response to the growing importance placed by the medical community of this vitamin supplementation for bone and cardiovascular health. The market for natural vitamin K2 is already significant, approaching five tons in the US, and 1.5 tons in EU – and with this it has not even begun to realize its potential. Asia, with the exception of India, has only begun promoting vitamin K2 for health purposes, and the smaller but developing markets in South America, such as Brazil, have yet to begin. However, growing epidemiological evidence indicates that a burgeoning percent of the global population, which otherwise appears healthy, is deficient in vitamin K2; this has far-reaching consequences for overall health, well-being and economies of many countries.

For these reasons, we evaluated the commercial landscape and K2 offerings and found them lacking. First and foremost, NattoPharma has set out to produce the highest quality and consistency menaquinone-7, a challenge that has plagued many K2 manufacturing processes. Many of the existing products have allergens and contaminants such as soy and antifoaming agents as well as impurities carried over from the fermentation process. The new MenaQ7 fermentation-process starting materials and excipients are soy free and hypoallergenic. In addition, MenaQ7 complies with EU substantial equivalence and US GRAS Compositions including stability, safety, preclinical and clinical research data.

What is probably the most significant technological breakthrough in the new generation of MenaQ7 is

a proprietary multistep process of purification, condensation and crystallization of fermentation-derived K2. This innovative process leads to an end product that is more than 95% pure natural menaquinone-7 or MK-7, with less than 0.5% (traces) of menaquinone-6 (MK-6), a marker of natural menaquinone-7 technology (note: MK-6 is not present in a synthetic MK-7 product). The all-natural MenaQ7 Crystals are also free of other menaquinones, e.g. MK-3, MK-4, MK-5, MK-8 etc. which is important for a higher rate of gastrointestinal absorption, bioavailability to target tissues, e.g. bone and blood vessels, and may also contribute to safety of K2.

There are three more uniquely significant factors that define the new MenaQ7 Crystals.

1. The pure MenaQ7 Crystals can be used directly in food, food supplements, premixes and multivitamins without the customary excipients – resulting in much desired space and weight savings in finished vitamin K2-containing products. In other words, it is more economical, manufacturing-wise and financially because you use less to obtain desired amounts.

Parameter	Description
Source	<i>Bacillus licheniformis</i>
Chemical name	Menaquinone-7
Systematic name	1,4-Naphthalenedione, 2-(3,7,11,15,19,23,27-heptamethyl-2,6,10,14,18,22,26-octacosahpta-onyl)-3-methyl-, (all-E)-
CAS No.	2124-57-4
Molecular weight	648
Chemical formula	C ₅₄ H ₈₀ O ₂
Appearance	Crystals, oil, granule or powder
Solubility	Insoluble in water
Color	Pale yellow
Odor	None
Taste	Characteristic
Storage	Store in tightly closed containers in cool place Exposure to light may deteriorate K2 activity
Shelf life	Expected up to 3 years

Table 1 – General description of MenaQ7® Crystals active component.

- MenaQ7 Crystals are 100% defined for their composition and impurities in their Drug Master File (DMF) documentation which allows use of MenaQ7 Crystals in pharma compositions.
- MenaQ7 Crystals can be reconstituted in oil, e.g. medium-chain triglycerides of safflower oil, sunflower oil and coconut oil or mixed with appropriate carriers for obtaining triturated powder form.

NEW MENAQ7 CRYSTALS: MANUFACTURING PROCESS

The new MenaQ7 Crystals are manufactured according to current good manufacturing practices (cGMPs). The strain used in the production of the new MenaQ7 brand of menaquinone-7 is *Bacillus licheniformis*, a non-toxicogenic and non-pathogenic strain. The strain has been well characterized by employing gross morphological characters, biochemical reactions and by 16S RNA.

The menaquinone-7 is prepared by submerged fermentation using *B. licheniformis* as the producing strain and chickpea flour (chickpea flour is flour made from dried chickpeas or garbanzo beans, and is also known as gram flour) and dextrin as carbon and nitrogen sources. The chickpea is used in the manufacture instead of soy beans to minimize potential for allergens from legumes. The menaquinone in the fermentation broth is spray dried and extracted with hexane with no detectable residual solvent. The menaquinone-7 is extracted in vegetable oil and subject to process of purification, concentration and crystallization to obtain the MenaQ7 Crystals.

The purification process leads to crystals that are the pure physical form of vitamin K2 (Figure 1). The final commercial product is used in the form of crystals diluted either in MCT oil or in a powder mixture containing non-animal, vegetable ingredients. The resulting products of advanced MenaQ7 Crystals are characterized by:

- No less than 95% content of only *trans* MK-7 with less than 0.5% MK-6;
- No other detectable menaquinones;

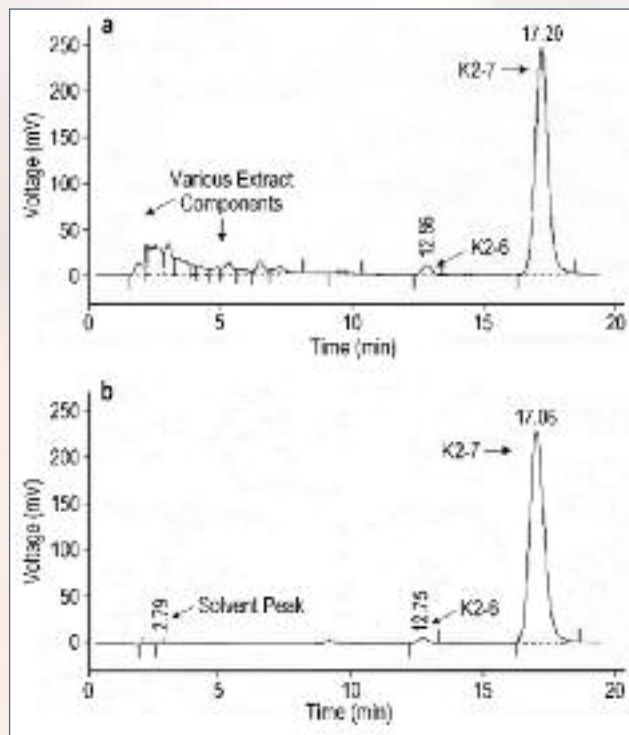


Figure 1 – Chromatograms of
 a. Extract of natural vitamin K2-7 oil, and
 b. Pure natural vitamin K2-7 crystals (MenaQ7 Crystals).

- Solvent under the detectable levels;
- Improved stability profile in storage and in finished supplement and food preparations;
- The product is protected by granted patents for use as dietary supplements and functional food, for all cardiovascular health claims in US, EU and Canada.

Based on these characteristics it is implicit that MenaQ7 Crystals will have an improved gastrointestinal absorption rate, higher rate of activating target proteins, e.g. osteocalcin and MGP and an increased bioavailability of K2-dependent proteins activating target receptors in the body.

The manufacturing process employed in the production of New MenaQ7 Crystals ensures that the potential for contamination or introduction of impurities is low, and each batch manufactured is tested to ensure that the stringent purity criteria are met. Processing aids, such as

Five distinguishing characteristics of MenaQ7® Crystals

- A minimum 95% purity standardized for menaquinone-7, 100% *trans* form, no other forms of menaquinone present except for 0.5% or less admixture of menaquinone-6 – a marker of natural fermentation product.
- Screened out other menaquinones except for minute amount of MK-6, which provides better bioavailability of MK-7 and better safety profile.
- A proprietary non-soy based fermentation product unique to NattoPharma.
- Available Drug Master File.
- Supported by clinical research and patents granted and pending.

solvent (removed by vacuum evaporation) and buffer salts used in the manufacturing process are all food-grade quality and comply with specifications described in the 5th Edition of Food Chemicals Codex.

NEW MENAQ7 CRYSTALS: REGULATORY STATUS

In the US the new MenaQ7 Crystals form is considered a dietary supplement, since menaquinone-7 containing products in the United States are regulated under the Dietary Supplement Health and Education Act (DSHEA, 1994). In addition, New MenaQ7 meets the specifications as self-affirmed GRAS to be used as a nutrient [21 CFR§170.3(o) (20)] at maximum use levels of up to 10 µg/serving in specific foods (Fats & Oils; Milk Products; Cheese; Frozen Dairy; Confectionary & Frosting; Beverages Type I – Non-alcoholic; Seasonings & Flavors, Soups) resulting in the 90th percentile all-user estimated intake of 36.51 µg menaquinone-7/person/day.

The new effort is to utilize MenaQ7 in so-called medicinal food. The medicinal food is distinguished from regular food by the higher dose of menaquinone-7 allowable per serving based on the three-year clinical study of MenaQ7 among healthy postmenopausal women in preserving bone mass and strength and the cardiovascular health.

In EU MenaQ7 Crystals meets the specifications outlined recently by the European Commission for European Food Safety Authority (EFSA). In 2008, in response to a request to deliver a scientific opinion on the safety of "vitamin K2" as a source of vitamin K for nutritional purposes to foodstuffs, EFSA examined the safety of vitamin K2. After considering all the data on specifications, manufacturing, anticipated intake, bioavailability, metabolism and toxicology, the EFSA Panel concluded that the use of menaquinone-rich (primarily menaquinone-7) edible oil in foods for the general population (including food supplements) and in foods for particular nutritional uses, other than baby foods and infant formula, at the use levels of 10 µg/serving is not of safety concern.

In Japan the menaquinone-7 rich natto is considered under The Food for Specified Health Use (FOSHU) regulations, i.e.

incorporation of natto in the daily diet could help bone health. The New MenaQ7 may comply with FOSHU regulations in Japan, and most likely with TGA regulations in Australia. The efforts to establish MenaQ7 in various countries in view of tight regulatory environment is supported because of the long-term clinical studies conducted on a healthy population.

NEW MENAQ7 CRYSTALS: EFFECTIVE BIOLOGICAL MECHANISM

Menaquinone-7 is a co-substrate for the enzyme gamma-glutamyl carboxylase in the post-translational synthesis of gamma-carboxyglutamic acid (Gla) from glutamic acid (Glu) residues in the nascent Gla-protein molecules, e.g. osteocalcin, playing a critical role in bone and metabolic health. Vitamin K2 is also an effective cofactor for production of blood coagulation proteins and active matrix Gla-protein (MGP) for elasticity and prevention of calcification of blood vessels. Other functions of vitamin K2 include suppression of the inflammation, and prevention of free-radical pathology.

It was not until the three-year breakthrough study of MenaQ7 completed in May 2012 that there has been a clinical study showing that supplemental vitamin K, especially menaquinone-7, improves bone mineral density, bone strength and cardiovascular health (Vermeer C. *et al.*, 2012 VitaFoods Presentation). The breakthrough study was a double-blind randomized clinical trial evaluating the results of a three-year regular intake of MenaQ7 in a daily dose 180 µg by a group of 244 healthy post-menopausal Dutch women, 55 to 65 years old, randomly assigned to receive daily either MenaQ7 or identical looking placebo capsules.

This brand new three-year study of

Ten attractive features and benefits of new MenaQ7 Crystals

1. **Crystals stand for consistency:** Well defined no less than (NLT) 95% purity menaquinone-7, consistent yield from fermentation, with virtually no other constituents of the fermentation process which may interfere with absorption and bioavailability of menaquinone-7.
2. **Crystals epidemiology and safety:** Nature produces only one kind menaquinone-7, the *trans* form, which is the only safe and effective form of this vitamin. The pure *trans* menaquinone-7 is assured in crystals. No need for excipients eliminates potential allergens and unsafe additives.
3. **Crystallization space saving in food and food supplements:** The real estate is at a premium in multivitamin tablets and premix blends, with high purity material, e.g. crystals of vitamin C, B12 and B6 in high demand by manufacturers to provide for a smaller size of the tablet.
4. **Incorporation in pharma products:** Crystals wouldn't have the hurdles of incorporation in pharma products. Such combination products need the drug master file (DMF).
5. **Crystals analytical:** The strict regulatory norms can be easier fulfilled working with high purity crystals with purity and impurities profile defined 100% in the drug master file (DMF).
6. **Transport, warehousing and handling:** The high purity translates to compact size and negligible weight for transportation and handling.
7. **EFSA, GRAS, Kosher, Halal, etc:** All certifications and safety studies are valid for the menaquinone-7 crystals, creating numerous market opportunities.
8. **Efficacy:** Peer-review published preclinical and clinical studies on MenaQ7®.
9. **Intellectual property:** International patents granted and pending on MenaQ7®.
10. **HACCP:** The HACCP-certified manufacture facility gives added advantage to MenaQ7 Crystals.

MenaQ7 is a "breakthrough" study because it shows for the first time clinically statistically significant protection of the vertebrae and the hip (femoral neck) against osteoporosis and cardiovascular deterioration. One of the most important findings from the study was that clinically relevant improvement became evident no sooner than after two and three years of MenaQ7 supplementation. This finding explains for the first time why shorter studies e.g. 12 months, typically failed to show benefits of vitamin K on bone health and cardiovascular health. Establishing a correlation between length of administration and efficacy of menaquinone-7 intake is a clinically significant breakthrough established in this three-year study of MenaQ7.

The MenaQ7 trial additionally showed for the first time substantial benefits in

preventing age-related stiffening of arteries resulting in statistically significant increase of the pulse wave velocity (PWV) in the placebo group, but not in the MenaQ7-group. Previously the positive effects on bone and vascular health have been demonstrated only with a pharmacological dose of the synthetic form of vitamin K, of up to 45 mg per day. The MenaQ7 study shows for the first time in the history of vitamin K evaluation the positive health effects with a "nutritional" dose of vitamin K (180 µg/day for three years), e.g., a dose that can be obtained from a healthy Western-type diet. The dose of 180 µg is higher than current recommended daily dose of vitamin K of approximately 90 µg. This higher dose resulting in clinical benefits and no side effects in the course of the three year study may attest to safety of MenaQ7 and open discussion for new guidelines for supplemental dose of vitamin K2.

The discussion of an effective dose of vitamin K2 is particularly relevant in view of recent epidemiological studies suggesting that most adults, apparently healthy, may be sub-clinically K2 deficient – which results in 10-40% of K2-dependent proteins not carboxylated and biologically

inactive. In addition, the emerging understanding of vitamin K2 biological mechanism indicates that carboxylation of proteins may only be a step in activation of K2 dependent proteins. The bioavailability of activated protein to the target receptor, e.g. in osteoblasts/osteoclasts or endothelial cells may depend on multiple steps in activation of K2 dependent protein. The current generation of MenaQ7 in the form of crystals may provide an effective method in activating K2-dependent proteins.

Nattopharma's distinguished New MenaQ7 Crystals are now ready for marketing partners to formulate their own innovative health products for a consistently growing marketplace that desires effective, top-quality tools to manage their healthy lifestyles.