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Menaquinone-7 Retention in Whole-Soybean Natto Fermentation Powder: A Formulation Framework for Vitamin-K-Dependent Bone Metabolism

Yassine Igounda^{1z}, Hugo Wallace², Aiden Kennedy³

^{1,2,3} National Laboratory of Medicine LLC

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Abstract: Menaquinone-7 (MK-7) is a long-chain vitamin K2 form produced during natto fermentation and directly participates in vitamin-K-dependent γ -carboxylation of osteocalcin. We characterized MK-7 retention in a whole-soybean natto powder produced by controlled solid-state fermentation, low-temperature post-ripening, low-temperature drying and temperature-limited pulverization. Three optimized process conditions contained 26.8-48.5 μg MK-7/g together with 1.52-2.62% γ -PGA and 1,160-1,680 FU/g natto kinase. Human studies span a useful sequence of endpoints. Natto feeding increased circulating MK-7 and carboxylated osteocalcin [1], and a one-year natto exposure study in premenopausal women changed bone-formation markers [2]. Longitudinal Japanese data linked habitual natto intake with more favorable BMD change at selected sites [3]. In early postmenopausal women, 360 $\mu\text{g/day}$ MK-7 improved osteocalcin carboxylation without changing one-year bone-loss rates [4]. A three-year trial of 180 $\mu\text{g/day}$ reported less decline at selected skeletal sites [5]. In osteopenic women receiving calcium and vitamin D, 375 $\mu\text{g/day}$ produced a marked osteocalcin response and a one-year tibial microarchitecture signal [6], but the three-year analysis did not show significant between-group BMD or microarchitecture differences [7]. The NVTIA® matrix therefore offers a measured MK-7 exposure that can be linked first to osteocalcin carboxylation and then, where appropriate, to longer-term skeletal endpoints.

Keywords: *menaquinone-7; vitamin K2; natto; osteocalcin; bone mineralization; whole-soybean fermentation; MK-7 stability; fermented soybean*

1. Introduction

Vitamin K-dependent carboxylation gives osteocalcin its full mineral-binding capacity. MK-7 is especially relevant to fermented-soy nutrition because natto can deliver much higher menaquinone-7 exposure than many unfermented foods. Human intervention work has therefore used circulating MK-7 and carboxylated or undercarboxylated osteocalcin as proximal markers before considering structural bone outcomes.

For a powdered natto ingredient, that biology has to be connected to process control. The MK-7 concentration of the finished material depends on the fermentation matrix and subsequent heat exposure. An enzyme-focused process can retain high nattokinase activity while producing much lower MK-7 and γ -PGA. The product-development question is therefore whether the process preserves the intended multi-component natto profile, not simply whether fermentation occurred.

Several studies sharpen the exposure side of this question. Natto-derived MK-7 has a long plasma half-life and produces sustained extrahepatic vitamin-K responses compared with shorter-chain forms [8]. Low-dose daily MK-7 has also improved osteocalcin γ -carboxylation in healthy adults [9], and supplementation can improve extrahepatic vitamin-K status without materially changing thrombin generation in healthy subjects [10].

Population studies add broader context. Regional differences in natto intake have been associated with circulating vitamin K2 and hip-fracture patterns [11], and meta-analytic work has assessed vitamin K2 supplementation across postmenopausal osteoporosis trials [12]. Reviews of vitamin K and bone health emphasize that biochemical response and structural response occur on different time scales [13]. Natto itself is a multi-component food, so composition reviews are useful when MK-7 is interpreted inside the whole fermented matrix [14].

2. Materials and Methods

2.1 Formulation and process design

Food-grade yellow soybeans were soaked to 58-64% moisture, cooked/sterilized at 115-121°C, cooled and inoculated with *Bacillus subtilis* subsp. natto. Fermentation was conducted at 37-41°C for 18-28 h using a 2-4 cm bed. Optimized batches underwent post-ripening at 8-16°C, followed by vacuum low-temperature drying or freeze drying to moisture $\leq 6\%$ and water activity ≤ 0.35 . Pulverization was controlled so that material temperature did not exceed 25°C.

2.2 Analytical characterization

Three optimized conditions were compared with variants that omitted post-ripening, used 60°C hot-air drying, used liquid fermentation focused on nattokinase, represented conventional freeze-dried natto, used 25°C post-ripening, used a 6 cm fermentation bed, or allowed high-temperature pulverization. MK-7 was quantified by HPLC, γ -PGA by HPLC or colorimetry, and nattokinase as FU/g.

2.3 Human-evidence contextualization

The human evidence was organized from exposure biomarkers to structural endpoints. Natto feeding and MK-7 supplementation studies were included when dose, duration, population and vitamin-K-dependent bone measures were described [1]-[7]. Additional pharmacokinetic, vitamin-K-status and review literature was used to interpret why osteocalcin can change before BMD or microarchitecture [8]-[14].

3. Results

Table 1. Multi-component profile of optimized and comparator natto powders.

Group	MK-7 ($\mu\text{g/g}$)	γ -PGA (%)	Nattokinase (FU/g)
Opt-A	34.6	2.18	1450
Opt-B	48.5	2.62	1680
Opt-C	26.8	1.52	1160
C1	30.2	1.84	1070
C2	22.7	1.55	640
C3	7.2	0.35	1580
C4	24.5	1.36	980

C5	31.5	1.88	960
C6	27.9	1.65	1010
C7	33.5	2.12	1240

3.1 Formulation performance

The optimized powders contained 26.8, 34.6 and 48.5 µg MK-7/g. The same batches contained 1.52-2.62% γ-PGA and 1,160-1,680 FU/g nattokinase. The liquid-fermented nattokinase comparator reached 1,580 FU/g enzyme activity but only 7.2 µg MK-7/g and 0.35% γ-PGA. A high enzyme value therefore did not imply preservation of the broader natto composition.

3.2 Process contrasts

High-temperature drying produced 22.7 µg MK-7/g. Conventional freeze-dried natto contained 24.5 µg/g, omission of low-temperature post-ripening produced 30.2 µg/g, and high-temperature pulverization produced 33.5 µg/g. The optimized 26.8-48.5 µg/g range reflects fermentation, post-ripening and thermal management acting together rather than one isolated unit operation.

3.3 Human evidence synthesis

The most consistent human response is biochemical. Natto intake increased circulating MK-7 and γ-carboxylated osteocalcin, with lower undercarboxylated osteocalcin at higher MK-7 exposure [1]. In premenopausal women, frequent natto intake over one year was associated with higher bone-specific alkaline phosphatase and lower undercarboxylated osteocalcin, while bone stiffness did not clearly change [2]. In 334 early postmenopausal women, 360 µg/day MK-7 improved osteocalcin carboxylation but did not alter the one-year rate of bone loss [4].

3.4 Mechanistic alignment

Longer randomized trials address a harder endpoint. In 244 healthy postmenopausal women, 180 µg/day MK-7 for three years improved vitamin K status and reduced age-related decline in BMD/BMC at the lumbar spine and femoral neck, with favorable bone-strength indices [5]. In osteopenic women taking calcium and vitamin D, 375 µg/day strongly reduced ucOC and produced a one-year tibial trabecular microarchitecture signal [6]. The three-year analysis did not show significant between-group BMD or microarchitecture differences [7].

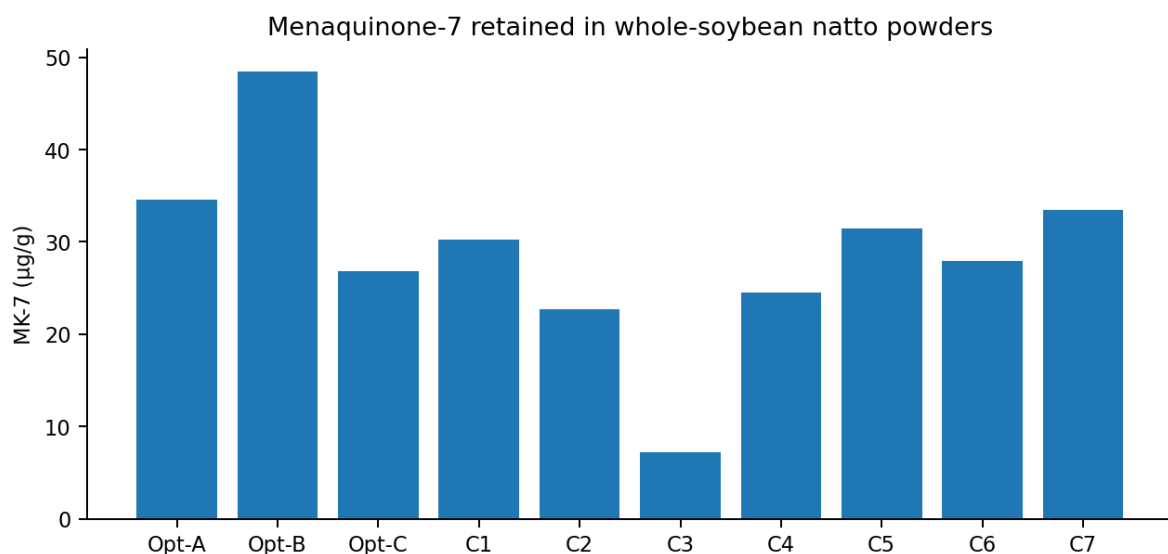


Figure 1. MK-7 content across optimized and comparator whole-soybean natto powders.

Table 2. Published human and mechanistic evidence relevant to the study question.

Study	Population/design n	Exposure	Endpoints	Main observation
Tsukamoto et al. [1]	8 men sequential natto exposure; plus 134 adults by intake	Natto with 775- µg MK- 1,765 7/100 g	Serum MK-7, carboxylated/un- dercarboxylated osteocalcin	Higher MK-7 and carboxylated osteocalcin; lower ucOC at

	frequency				higher MK-7 exposure
Katsuyama et al. [2]	73 healthy premenopausal women; 1 year	Natto from none to 3 times/week	Bone stiffness, BAP, ucOC		Higher BAP and lower ucOC in frequent-intake group; stiffness unchanged
Ikeda et al. [3]	944 women; 3-year cohort	Habitual natto intake	BMD at spine, hip, forearm		Higher natto intake associated with more favorable femoral-neck/radius BMD change in postmenopausal women
Emaus et al. [4]	334 early postmenopausal women; randomized double-blind	360 µg MK-7/day for 12 months	BMD, cOC, ucOC		Improved osteocalcin carboxylation; no between-group BMD loss difference
Knapen et al. [5]	244 healthy postmenopausal women; randomized placebo-controlled	180 µg MK-7/day for 3 years	BMD/BMC, bone strength, osteocalcin		Improved vitamin K status and reduced decline at selected skeletal endpoints
Rønn et al. [6,7]	148/142 osteopenic postmenopausal women; randomized placebo-controlled	375 µg MK-7/day + calcium/vitamin D	ucOC, HRpQCT	DXA,	Strong ucOC reduction; 1-year tibial microarchitecture signal, but 3-year BMD/microarchitecture differences were not sustained

4. Discussion

4.1 Process–bioactivity coupling

Whole-soybean fermentation preserved MK-7 together with nattokinase and γ -PGA. The liquid-fermented enzyme comparator makes the matrix effect visible: 1,580 FU/g nattokinase coexisted with only 7.2 µg MK-7/g and 0.35% γ -PGA. This is why a batch specification for an MK-7-oriented product should report MK-7 directly instead of inferring it from enzyme activity.

4.2 Translational interpretation

Osteocalcin carboxylation is the clearest proximal human endpoint. It responds to natto and to purified or supplemental MK-7 across multiple study designs [1], [2], [4]–[7], and pharmacokinetic work shows sustained exposure from MK-7 [8]. This gives a formulation-specific trial a biologically close marker that can be measured well before a BMD change would be expected.

4.3 Evidence heterogeneity

Structural bone outcomes vary more. Knapen et al. reported selected three-year benefits at 180 µg/day

[5], while the osteopenia trial maintained a strong osteocalcin response without significant three-year BMD or microarchitecture differences [7]. One-year data were likewise biochemically positive without a clear BMD effect [4]. Baseline bone status, calcium/vitamin D co-supplementation, skeletal site, dose and duration therefore need to be treated as design variables rather than sources of narrative inconsistency.

4.4 Whole-matrix relevance

The whole-soybean matrix offers a second research layer. γ -PGA can influence calcium availability, and human crossover data show that 60 mg γ -PGA can increase fractional calcium absorption under defined conditions [15]. That pathway is distinct from MK-7-dependent osteocalcin carboxylation. A whole-natto trial can measure both exposures, but the primary vitamin-K hypothesis should remain centered on MK-7 and osteocalcin.

4.5 Standardization and research design

The observed 26.8-48.5 μ g MK-7/g range is wide enough that powder mass alone would give an imprecise exposure description. Batch-defined MK-7 content should therefore be used to calculate the daily dose. That approach is consistent with the broader vitamin-K literature, where dose and circulating exposure are important determinants of extrahepatic response [8]-[10].

5. Study Considerations and Future Directions

A formulation-specific study can first establish the relation between finished-powder dose, circulating MK-7 and osteocalcin carboxylation. Bone-turnover markers can be added over a medium-term intervention. BMD or site-specific microarchitecture should be reserved for sufficiently long studies and interpreted with menopausal status, baseline vitamin-K status and calcium/vitamin-D intake recorded prospectively. The broader whole-matrix hypothesis can be explored separately by quantifying γ -PGA exposure and calcium handling [15].

6. Conclusion

Controlled whole-soybean natto fermentation with low-temperature post-ripening and downstream thermal control retained 26.8-48.5 μ g MK-7/g while preserving 1.52-2.62% γ -PGA and 1,160-1,680 FU/g nattokinase. Human studies consistently show that MK-7 exposure improves osteocalcin carboxylation, whereas BMD and microarchitecture responses depend more strongly on population and study design. Batch-defined MK-7 exposure therefore provides a direct route from NVTIA® process control to formulation-specific studies of vitamin-K-dependent bone metabolism.

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