

A Stabilized SOD-Rich Melon Polysaccharide-Protein Complex Preserves Antioxidant Activity and Supports Oxidative-Stress Homeostasis

Bougrain Mouad¹, Sipho Dlamini², Nur Aisyah³
^{1,2,3}National Standards Laboratory SA, Switzerland



DOI : <https://doi.org/10.61796/jmgcb.v3i9.1929>



Sections Info

Article history:

Submitted: July 20, 2026

Final Revised: August 10, 2026

Accepted: August 30, 2026

Published: September 25, 2026

Keywords:

Superoxide dismutase

Cucumis melo

Oxidative stress

Polysaccharide-protein complex

Oral antioxidant

Enzyme stability

ABSTRACT

Objective: Plant-derived superoxide dismutase (SOD) is of interest for nutritional antioxidant use, but oral delivery places the enzyme under several forms of stress, including gastric acidity, heat, moisture, and the composition of the surrounding powder matrix. **Method:** We characterized the BCLC® SOD-Rich Melon Complex, a low-reducing-sugar endogenous polysaccharide-protein preparation in which native *Cucumis melo* soluble polysaccharides are retained with SOD-containing protein fractions without an exogenous prolamins or lipid-soluble coating. Low-temperature, low-oxygen extraction followed by ultrafiltration/diafiltration, controlled pH-ionic-strength complexation, and low-temperature drying yielded optimized extracts containing 34,280–43,620 U/g SOD, 3.7–4.5% reducing sugars, and water activity of 0.29–0.34. **Results:** After 60 min at pH 1.2 and 37°C, SOD retention was 41.7–47.8%, compared with 16.9–38.2% in comparator preparations. After 30 days at 40°C/75% RH, 66.5–72.6% of activity remained. DPPH scavenging was 57.8–63.7%, ABTS scavenging was 65.7–71.6%, and ORAC values were 2,064–2,416 µmol TE/g. Human studies using other SOD-rich melon preparations have also reported changes in oxidative-stress-related outcomes. **Novelty:** The formulation data define a practical basis for human evaluation of this endogenous melon polysaccharide-protein stabilization approach.

INTRODUCTION

Reactive oxygen species (ROS) arise during mitochondrial respiration, inflammatory signaling, environmental exposure, and normal metabolism. At controlled levels they participate in redox signaling; sustained imbalance can instead modify lipids, proteins, nucleic acids, and cell-signaling networks [1]–[3]. Superoxide dismutases act early in this defense system by catalyzing conversion of superoxide anion to hydrogen peroxide and molecular oxygen [1], [2].

Oral use of protein antioxidants creates a separate formulation problem. Low pH, proteolytic digestion, interfacial denaturation, thermal stress, and storage-associated moisture can reduce enzyme function, and small sugars and salts in crude fruit powders can increase hygroscopicity. These constraints make delivery architecture relevant to retained activity [4]. Earlier human and experimental work has used other proprietary melon-SOD preparations and carrier systems [5]–[9].

BCLC® was developed around material already present in melon. Soluble polysaccharides are co-retained with the SOD-containing protein fraction after ultrafiltration/diafiltration; pH and ionic strength are then adjusted to favor an endogenous polysaccharide-protein association. Such complexes are sensitive to pH, ionic strength, biopolymer ratio, and temperature [10], [11]. We measured composition,

acid-phase SOD retention, accelerated storage stability, and in-vitro antioxidant activity, and then considered those results alongside published human studies of SOD-rich melon products.

RESEARCH METHOD

Preparation and stabilization framework

Melon flesh and placental tissue were pre-cooled and homogenized under low-oxygen conditions. Extraction was carried out in food-grade buffer near neutral pH at low temperature. The clarified extract was concentrated with a 10–30 kDa ultrafiltration membrane and diafiltered to reduce low-molecular-weight sugars, organic acids, and free salts. The SOD-enriched retentate was adjusted to pH 6.2–7.2 and ionic strength 10–80 mmol/L, then held at 2–15°C to permit formation of the melon-endogenous soluble-polysaccharide/protein stabilization system. The material was freeze-dried or dried under low-temperature vacuum conditions.

Analytical characterization

SOD activity was measured by the WST-1 method [12]. Total protein was measured by a validated colorimetric protein assay, soluble polysaccharides by the phenol-sulfuric acid method [13], reducing sugars by the DNS method [14], moisture by gravimetric drying, water activity with a water-activity meter, and lipid by solvent extraction. Optimized preparations were compared with ordinary freeze-dried melon juice, an ultrafiltered extract prepared without diafiltration, and an SOD-enriched preparation produced without the endogenous polysaccharide-protein stabilization step.

Acid-phase stability

Samples were suspended at 1 mg/mL in pH 1.2 medium at 37°C. Residual SOD activity was measured after 30, 60, and 120 min after neutralization to the analytical pH. Activity at time zero was defined as 100%.

Accelerated storage stability

Sealed samples were held at 40°C and 75% relative humidity and tested at baseline and during storage. The 30-day retention value served as the primary comparative index.

Antioxidant assays

DPPH and ABTS radical-scavenging assays were performed at 1 mg/mL using established assay principles [15], [16]. ORAC was measured with the fluorescein-based oxygen radical absorbance capacity method described by Ou et al. [17] and expressed as μmol Trolox equivalents per gram.

Published human evidence context

PubMed/PMC and journal sources were searched through August 2026 using combinations of 'Cucumis melo', 'melon concentrate', 'superoxide dismutase', 'oxidative stress', 'randomized', 'placebo', 'photoprotection', and 'skin'. Randomized controlled trials and other human intervention studies were prioritized. Studies using gliadin-

protected SOD or multi-ingredient matrices were treated as clinical context for melon-derived SOD rather than as formulation-matched evidence.

RESULTS AND DISCUSSION

Results

Composition and powder quality

SOD activity in the three optimized preparations was $43,620 \pm 1,180$, $38,740 \pm 1,060$, and $34,280 \pm 970$ U/g, respectively. Reducing sugar contents were $3.7 \pm 0.2\%$, $4.5 \pm 0.2\%$, and $4.1 \pm 0.2\%$, and water activities were 0.31 ± 0.01 , 0.29 ± 0.01 , and 0.34 ± 0.01 . Ordinary freeze-dried melon juice contained $25.4 \pm 1.1\%$ reducing sugar and had a water activity of 0.51 ± 0.01 . The optimized powders were lower in both reducing sugar and water activity than the ordinary freeze-dried juice.

Acid-phase SOD retention

After 60 min in pH 1.2 medium, the optimized preparations retained $47.8 \pm 2.1\%$, $44.6 \pm 1.9\%$, and $41.7 \pm 1.8\%$ of initial activity. Ordinary freeze-dried melon juice retained $16.9 \pm 1.2\%$; the no-diafiltration comparator retained $29.7 \pm 1.6\%$; and the preparation without the stabilization step retained $38.2 \pm 1.7\%$.

Accelerated stability

At 30 days under $40^\circ\text{C}/75\%$ RH, SOD retention was $72.6 \pm 2.5\%$, $70.2 \pm 2.4\%$, and $66.5 \pm 2.2\%$ in the optimized preparations. Comparator retention values ranged from $42.1 \pm 2.0\%$ to $58.6 \pm 2.3\%$.

In-vitro antioxidant performance

The optimized reference preparation showed DPPH scavenging of $63.7 \pm 2.1\%$, ABTS scavenging of $71.6 \pm 2.3\%$, and an ORAC value of $2,416 \pm 82$ $\mu\text{mol TE/g}$. The other optimized preparations followed the same direction, while comparator values were consistently lower.

Table 1. Selected composition and quality indices of optimized BCLC® SOD-melon preparations

Preparation	SOD activity (U/g)	Protein (%)	Soluble polysaccharides (%)	Reducing sugar (%)	aw
F1	$43,620 \pm 1,180$	21.7 ± 0.8	15.1 ± 0.6	3.7 ± 0.2	0.31 ± 0.01
F2	$38,740 \pm 1,060$	18.4 ± 0.7	12.5 ± 0.5	4.5 ± 0.2	0.29 ± 0.01
F3	$34,280 \pm 970$	19.5 ± 0.6	10.8 ± 0.5	4.1 ± 0.2	0.34 ± 0.01

Table 2. Selected human studies providing clinical context for SOD-rich melon supplementation

Study	Design	Population / exposure	Key relevance
Milesi et al., 2009 [5]	Randomized, double-blind, placebo-controlled	70 healthy adults; 140 IU SOD/day; 4 weeks	Stress, fatigue and quality-of-life outcomes
Carillon et al., 2014 [6]	Randomized, double-blind, placebo-controlled	61 adults; 10 mg melon concentrate (140 U SOD)/day; 12 weeks	Stress and fatigue outcomes; registered RCT
Saby et al., 2020 [7]	Randomized, double-blind, placebo-controlled human exercise study	Healthy subjects during standardized physical training	Oxidative-stress and CRP measures
Koike et al., 2022 [9]	Double-blind randomized placebo-controlled	46 women aged 50–80 years; 6 months	Oxidative and inflammatory biomarker context

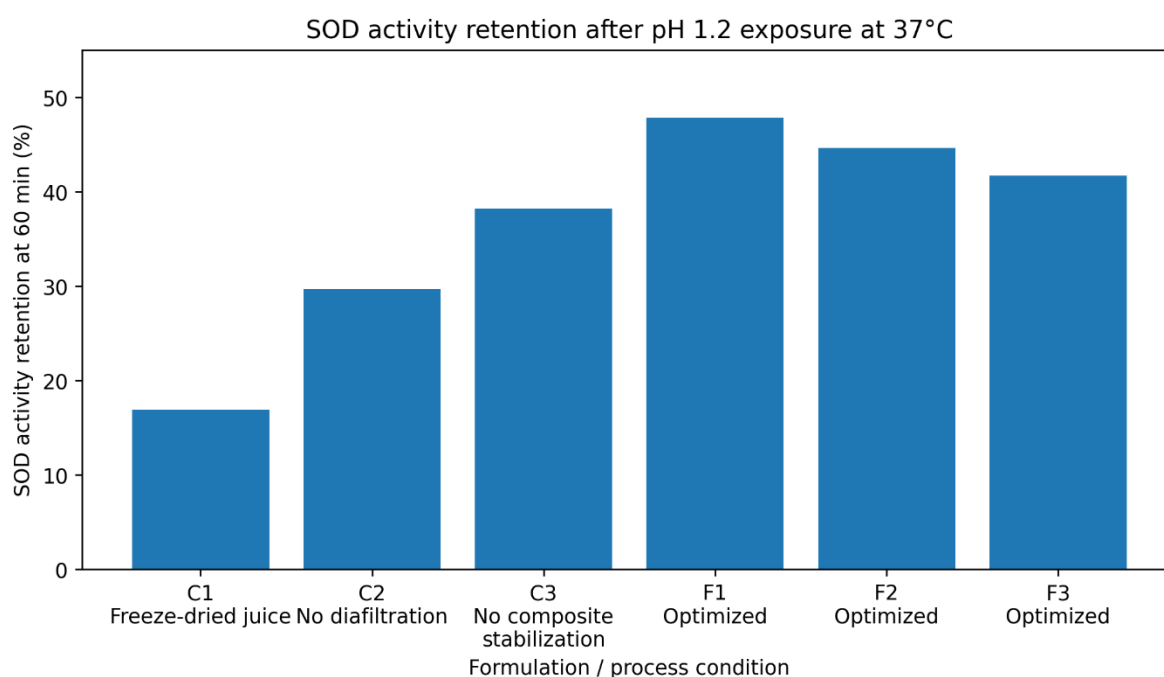


Figure 1. SOD activity retention after 60 min in pH 1.2 medium at 37°C. Values are mean retention percentages from the defined experimental conditions.

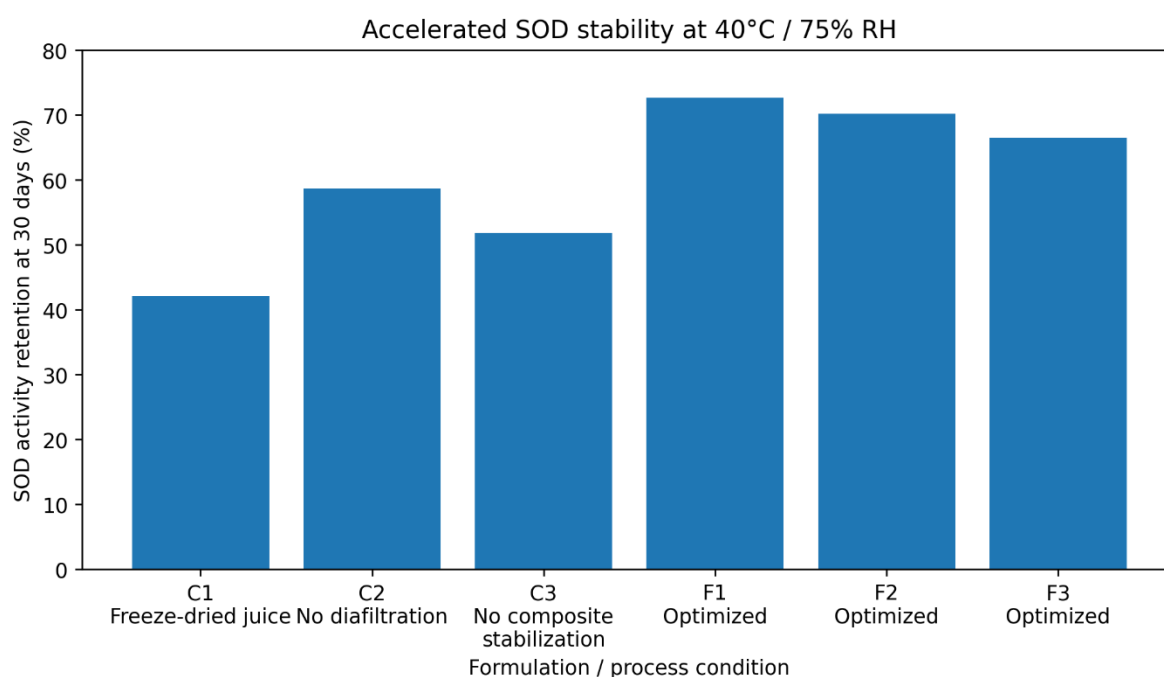


Figure 2. SOD activity retention after 30 days of accelerated storage at 40°C/75% RH.

Discussion

The formulation work separates two issues that are often conflated for oral enzyme ingredients: initial activity and activity that remains after processing or exposure. Diafiltration reduced low-molecular-weight material and reducing sugars, while controlled complexation was followed by higher SOD retention during acid exposure and accelerated storage. Protein-polysaccharide associations are known to depend on the physicochemical environment [10], [11], and oral therapeutic enzymes face additional stability pressures in the gastrointestinal tract [4]. For BCLC®, those considerations are part of the formulation itself rather than an after-the-fact interpretation of antioxidant activity.

Milesi et al. studied 70 healthy adults in a double-blind placebo-controlled pilot trial and reported improvements in perceived stress and fatigue with 140 IU SOD/day from a proprietary melon concentrate [5]. Carillon et al. later tested 10 mg/day of SOD-melon concentrate (140 U SOD) for 12 weeks in 61 adults; lower perceived stress and improvements in fatigue- and quality-of-life-related measures were reported [6]. Neither study used the endogenous-polysaccharide matrix evaluated here.

Redox-oriented outcomes have also been examined in other settings. During a standardized exercise program, Saby et al. reported lower oxidative-stress and C-reactive-protein measures with SOD-rich melon concentrate supplementation [7]. Under hyperbaric oxygen challenge, Muth et al. found lower DNA strand damage and F2-isoprostanes in participants receiving a gliadin-combined plant SOD preparation [8]. Koike et al. studied older women with musculoskeletal discomfort; Melon GliSODin® showed within-group trends toward lower malondialdehyde and d-ROM values, while

between-group differences across the overall endpoint set were modest [9]. Carrier system, dose, physiological challenge, and population all differed across these trials.

For BCLC®, the practical formulation feature is measurable SOD activity in a low-reducing-sugar matrix built from native melon polysaccharides and protein. The optimized preparations retained 41.7–47.8% of initial SOD activity after 60 min at pH 1.2 and 66.5–72.6% after 30 days at 40°C/75% RH. No external prolamin or lipid coating was used. Those measurements distinguish the ingredient platform from the delivery systems used in the human studies above.

Study Considerations and Future Directions

The experiments were designed around ingredient stability, radical-scavenging performance, and physicochemical attributes relevant to an oral SOD ingredient. A prospective BCLC® study can extend this work with plasma redox biomarkers, lipid-peroxidation products, DNA oxidation markers, exercise-related oxidative responses, and pharmacodynamic time courses. The tested material should retain the defined SOD, protein, polysaccharide, and low-reducing-sugar specifications so that any human response can be interpreted against the formulation characterized here.

CONCLUSION

Fundamental Finding : BCLC® preparations contained 34,280–43,620 U/g SOD, 3.7–4.5% reducing sugars, and water activity of 0.29–0.34. After 60 min at pH 1.2 and 37°C, optimized preparations retained 41.7–47.8% of initial SOD activity, and 66.5–72.6% remained after 30 days at 40°C/75% RH. DPPH, ABTS, and ORAC testing also showed substantial in-vitro antioxidant activity. **Implication :** The BCLC® formulation data now define the material and endpoints for formulation-specific human evaluation. **Limitation :** **Human** studies with other SOD-rich melon systems offer relevant clinical models. **Future Research :** Formulation-specific human evaluation of BCLC® is warranted.

REFERENCES

- [1] J. M. McCord and I. Fridovich, “Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein),” *J. Biol. Chem.*, vol. 244, no. 22, pp. 6049–6055, 1969.
- [2] I. Fridovich, “Superoxide radical and superoxide dismutases,” *Annu. Rev. Biochem.*, vol. 64, pp. 97–112, 1995, doi: 10.1146/annurev.bi.64.070195.000525.
- [3] H. Sies, “Oxidative stress: A concept in redox biology and medicine,” *Redox Biol.*, vol. 4, pp. 180–183, 2015, doi: 10.1016/j.redox.2015.01.002.
- [4] P. Lapuhs et al., “Engineering strategies for oral therapeutic enzymes to enhance their stability and activity,” *Adv. Exp. Med. Biol.*, vol. 1148, pp. 151–172, 2019, doi: 10.1007/978-981-13-7709-9_8.
- [5] M. A. Milesi, D. Lacan, H. Brosse, D. Desor, and C. Notin, “Effect of an oral supplementation with a proprietary melon juice concentrate (Extramel) on stress and fatigue in healthy people: A pilot, double-blind, placebo-controlled clinical trial,” *Nutr. J.*, vol. 8, art. no. 40, 2009, doi: 10.1186/1475-2891-8-40.
- [6] J. Carillon, C. Notin, K. Schmitt, G. Simoneau, and D. Lacan, “Dietary supplementation with a superoxide dismutase-melon concentrate reduces stress, physical and mental fatigue in

- healthy people: A randomised, double-blind, placebo-controlled trial,” *Nutrients*, vol. 6, no. 6, pp. 2348–2359, 2014, doi: 10.3390/nu6062348.
- [7] M. Saby, A. Gauthier, S. Barial, L. Egoumenides, and B. Jover, “Supplementation with a bioactive melon concentrate in humans and animals: Prevention of oxidative damages and fatigue in the context of a moderate or eccentric physical activity,” *Int. J. Environ. Res. Public Health*, vol. 17, no. 4, art. no. 1142, 2020, doi: 10.3390/ijerph17041142.
- [8] C. M. Muth, Y. Glenz, M. Klaus, P. Radermacher, G. Speit, and X. Lerverve, “Influence of an orally effective SOD on hyperbaric oxygen-related cell damage,” *Free Radic. Res.*, vol. 38, no. 9, pp. 927–932, 2004, doi: 10.1080/10715760412331273197.
- [9] M. Koike, M. Nagao, Y. Iwase, K. Kaneko, M. Ishijima, and H. Nojiri, “Clinical efficacy of Melon GliSODin® for the treatment of aging-related dysfunction in motor organs—A double blind, randomized placebo-controlled study,” *J. Clin. Med.*, vol. 11, no. 10, art. no. 2747, 2022, doi: 10.3390/jcm11102747.
- [10] C. Schmitt and S. L. Turgeon, “Protein/polysaccharide complexes and coacervates in food systems,” *Adv. Colloid Interface Sci.*, vol. 167, nos. 1–2, pp. 63–70, 2011, doi: 10.1016/j.cis.2010.10.001.
- [11] S. N. Warnakulasuriya and M. T. Nickerson, “Review on plant protein-polysaccharide complex coacervation, and the functionality and applicability of formed complexes,” *J. Sci. Food Agric.*, vol. 98, no. 15, pp. 5559–5571, 2018, doi: 10.1002/jsfa.9228.
- [12] A. V. Peskin and C. C. Winterbourn, “A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1),” *Clin. Chim. Acta*, vol. 293, nos. 1–2, pp. 157–166, 2000, doi: 10.1016/S0009-8981(99)00246-6.
- [13] M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, “Colorimetric method for determination of sugars and related substances,” *Anal. Chem.*, vol. 28, no. 3, pp. 350–356, 1956, doi: 10.1021/ac60111a017.
- [14] G. L. Miller, “Use of dinitrosalicylic acid reagent for determination of reducing sugar,” *Anal. Chem.*, vol. 31, no. 3, pp. 426–428, 1959, doi: 10.1021/ac60147a030.
- [15] W. Brand-Williams, M. E. Cuvelier, and C. Berset, “Use of a free radical method to evaluate antioxidant activity,” *LWT-Food Sci. Technol.*, vol. 28, no. 1, pp. 25–30, 1995, doi: 10.1016/S0023-6438(95)80008-5.
- [16] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, “Antioxidant activity applying an improved ABTS radical cation decolorization assay,” *Free Radic. Biol. Med.*, vol. 26, nos. 9–10, pp. 1231–1237, 1999, doi: 10.1016/S0891-5849(98)00315-3.
- [17] B. Ou, M. Hampsch-Woodill, and R. L. Prior, “Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe,” *J. Agric. Food Chem.*, vol. 49, no. 10, pp. 4619–4626, 2001, doi: 10.1021/jf010586o.

Bougrain Mouad

National Standards Laboratory SA, Switzerland

Sipho Dlamini

National Standards Laboratory SA, Switzerland

Nur Aisyah

National Standards Laboratory SA, Switzerland
