

Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-Dec-2023
Document Type: Dietary Supplement Monographs
DocId: GUID-793DF200-085E-4456-8AE4-8FDB8B341837_2_en-US
DOI: https://doi.org/10.31003/USPNF_M16575_02_01
DOI Ref: ib1hj

© 2025 USPC
Do not distribute

Add the following:

▲Bromelain

Bromelain

CAS RN[®]: 37189-34-7; UNII: ZLM4P8929R.

DEFINITION

Bromelain is a purified proteolytic substance derived from the stem of *Ananas comosus* (L.) Merr. sensu lato (Fam. Bromeliaceae). Bromelain, prepared as directed in the Assay, contains NLT 1100 USP Papain Units/mg and NLT 85% and NMT 115% of the labeled amount of bromelain activity in USP Papain Units/mg. It may contain suitable added substances as carriers.

1 USP Unit of papain activity is that which releases the equivalent of 1 µg of tyrosine from a specified casein substrate under the conditions of the Assay.

ASSAY

• PROCEDURE

[NOTE—The *Casein substrate solution*, *Standard stock solution*, *Standard solutions*, and the *Sample solution* should be used on the same day of the preparation.]

0.05 M sodium phosphate solution: Transfer 7.1 g of anhydrous dibasic sodium phosphate into a 1000-mL volumetric flask, dissolve in about 500 mL of water, dilute with water to volume, and mix. Add 1 drop of toluene as a preservative.

0.05 M citric acid solution: Transfer 10.5 g of citric acid monohydrate into a 1000-mL volumetric flask, dissolve in about 500 mL of water, dilute with water to volume, and mix. Add 1 drop of toluene as a preservative.

Buffer solution: Phosphate-cysteine-ETA buffer prepared as follows. Dissolve 7.1 g of anhydrous dibasic sodium phosphate in about 800 mL of water, and then dissolve in this solution 14.0 g of disodium EDTA dihydrate and 6.1 g of cysteine hydrochloride monohydrate. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to pH 6.0 ± 0.1, then transfer into a 1000-mL volumetric flask, dilute with water to volume, and mix.

Trichloroacetic acid solution: Dissolve 30 g of trichloroacetic acid in 100 mL of water.

Casein substrate solution: Disperse 1 g (moisture-free basis) of Hammarsten grade casein¹ in 50 mL of 0.05 M sodium phosphate solution, and heat in a boiling water bath for 30 min with occasional agitation. Cool to room temperature and, with rapid and continuous agitation, adjust with 0.05 M citric acid solution to pH 6.0 ± 0.1. [NOTE—Rapid and continuous agitation during the addition prevents casein precipitation.] Quantitatively transfer the mixture into a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard stock solution: Transfer 100.0 mg of [USP Papain RS](#) into a 100-mL volumetric flask, dissolve, and dilute with *Buffer solution* to volume, and mix.

Standard solutions: Pipet 2, 3, 4, 5, 6, and 7 mL of *Standard stock solution* into a series of 100-mL volumetric flasks, dilute each flask with *Buffer solution* to volume, and mix by inversion.

Sample solution: A solution of bromelain prepared so that 2 mL of the final dilution will give an absorbance between 0.2 and 0.5 in the *Analysis*. To prepare, transfer an amount of papain, accurately weighed and equivalent to about 100 mg of [USP Papain RS](#), into a 100-mL volumetric flask and add *Buffer solution* to dissolve. Dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Use within 30 min after preparation.

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: Vis

Analytical wavelength: 280 nm

Analysis

Samples: *Standard solutions* and *Sample solution*

Pipet 5 mL of *Casein substrate solution* into each of a series of 25-mm × 150-mm test tubes, allowing 3 tubes for the *Sample solution*, 6 for a papain standard curve (*Standard solutions*), and 9 for the *Blanks*. Equilibrate the tubes for 15 min in a water bath maintained at 40° ± 0.1°. Starting at zero time, rapidly pipet 2 mL of each of the *Standard solutions* and 2-mL portions of the *Sample solution* into the equilibrated substrate. Mix each by swirling, stopper, and place the tubes back in the water bath. After 60.0 min, add 3 mL of *Trichloroacetic acid solution* to each tube. Immediately mix each tube by swirling.

Prepare 9 enzyme blanks containing 5.0 mL of *Casein substrate solution*, 3.0 mL of *Trichloroacetic acid solution*, and 2.0 mL of either the appropriate *Standard solution* or the *Sample solution*.

Return all tubes to the water bath and heat for 30.0 min, allowing the precipitated protein to coagulate completely. Pass each mixture through filter paper of medium pore size,² discarding the first 3 mL of filtrate. The subsequent filtrate must be perfectly clear.

Prepare a standard curve by plotting the absorbances of filtrates from the *Standard solutions* against the corresponding concentrations of [USP Papain RS](#), in mg/mL. By interpolation from the standard curve, obtain the equivalent concentration of the filtrate from the *Sample solution*.

Calculate the activity of the enzyme preparation (A_p), in USP Papain Units/mg, in the portion of Bromelain taken:

$$A_p = (A \times C \times 10)/W$$

A = activity of [USP Papain RS](#) (USP Papain Units/mg)

C = concentration of [USP Papain RS](#) from the standard curve (mg/mL)

W = weight of the original enzyme preparation in the *Sample solution*

Calculate the percentage of the labeled amount of bromelain activity in the portion of Bromelain taken:

$$\text{Result} = (A_p/L) \times 100$$

A_p = activity of the enzyme preparation, previously determined

L = label claim of the bromelain enzyme (%)

Acceptance criteria

Activity: NLT 1100 USP Papain Units/mg

Labeled potency: 85%–115% of the labeled amount of bromelain activity in USP Papain Units/mg

SPECIFIC TESTS

• [pH \(791\)](#)

Sample solution: 1 in 100

Acceptance criteria: 3.5–6.0

• [Loss on Drying \(731\)](#)

Analysis: Dry under vacuum at 60° for 4 h or by using an automatic infrared moisture analyzer at 90°–105°.

Acceptance criteria: NMT 7.0%

• [Articles of Botanical Origin \(561\)](#), [Methods of Analysis, Total Ash](#): NMT 6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool place.

• **LABELING:** The label states the bromelain activity in USP Papain Units/mg.

• [USP Reference Standards \(11\)](#)

[USP Papain RS](#) ▲ (USP 1-Dec-2023)

¹ J12840 Casein, Hammarsten, Ultrapure, Thermo Scientific (<https://www.alfa.com/en/catalog/J12840/>), or equivalent.

² Whatman No. 42 (www.cytivalifesciences.com/en/us/about-us/our-brands/whatman), or equivalent.

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
BROMELAIN	Maria Monagas Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. 50(6)

Current DocID: GUID-793DF200-085E-4456-8AE4-8FDB8B341837_2_en-US

DOI: <https://doi.org/10.31003/USPNF.M16575.02.01>

DOI ref: [ib1hj](#)