Technical Alert

Call for Comment
Expression of the enzymatic units for bromelains

Dear Member,

Call for comment – Enzymatic units for bromelains – due to CHC by COB Thursday, 24 October

Over a number of years the Therapeutic Goods Administration (TGA) and the complementary medicines industry have recognised difficulties in appropriately quantifying the activity of enzymes used as active ingredients in listed medicines. One difficult aspect has been in establishing an appropriate unit for the enzyme activity of the improved ingredient 'bromelains' (Australian Approved Name).
As such the CHC seeks industry feedback on the below proposal to assist in the resolution of this issue.

Bromelain is an enzyme that is extracted from the stem or fruit of the pineapple plant (*Ananas comosus*, family Bromeliaceae). Currently the TGA eBusiness Services (eBS) only allows the quantity of the ingredient to be expressed in *milligrams* (mg). However, based on past consultation with industry, the expression of its content on a 'per mg' basis is unsatisfactory, particularly as the material available commercially is highly variable in terms of enzyme (therapeutic) activity.

There are currently no TGA recognised default standards, nor a TGA compositional guideline, to provide clarity to sponsors as to how to express the biological activity of bromelains. Of the scientific literature the following variety of designations are used: *Martindale* makes reference to 'Rorer' unit of protease activity and Commission E cites 'FIP' units of bromelain activity. Bromelain activity has also been described in terms of 'milk clotting unit' (MCU), 'gelatin digesting unit' (GDU) and 'papain unit' (PU).

Most relevantly papain unit is specified for bromelain by the joint FAO/WHO Expert Committee on Food Additives (JECFA) (Attachment 1) and the Food Chemical Codex (FCC) (Attachment 2). This is also the unit adopted by Health Canada in its monograph for 'stem bromelain' and 'fruit bromelain' (Attachments 3-4).

Previous discussions with the OCM/Industry Consultative Group (OICG) on the use of papain units was not resolved, and industry has indicated that GDU is often used for material supplied in Australia.

A recent TGA review has again recommended that papain unit (PU) be used as the enzymatic unit for bromelains in the eBS. One papain unit is defined as the quantity of enzyme that liberates the equivalent of 1 µg of tyrosine per hour under the conditions of the assay (FCC).

ACTION - Members are asked to provide comment on the TGA proposal to adopt PU as the unit of enzymatic activity for 'bromelains', noting that there is a simple conversion factor that can be applied to quantities expressed as GDU. Your feedback by Thursday, 24 October would be appreciated.

For further information contact: Emma Burchell on 0451 681 663 or Emma.Burchell@chc.org.au

Attachment 1

PROPOSED METHOD FOR SPECIFICATION OF BROMELAIN ACTIVITY

Proteolytic Activity, Plant

(Sourced from "*FAO JECFA Monographs 1: COMBINED COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS...*, http://www.fao.org/docrep/009/a0691e/A0691E07.htm, viewed 5 May 2008)

Scope

This procedure is designed for the determination of the proteolytic activity of papain, ficin and bromelain.

Principle

The assay is based on a 60 min proteolytic hydrolysis of a casein substrate at pH 6.0 and 40°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration; solubilized casein is then measured spectrophotometrically.

Reagents and Solution

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

Citric acid solution (0.05 M): Transfer 10.5 g of citric acid monohydrate into a 1,000-ml volumetric flask, dissolve in about 500 ml of water, dilute to volume with water, and mix. Add 1 drop of toluene as preservative.

Phosphate-cysteine-EDTA buffer solution: 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 to pH 6.0 ±0.1 with 1 N hydrochloric acid or 1 N sodium hydroxide, then transfer into a 1,000-ml volumetric flask, dilute to volume with water, 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 casein or equivalent in 50 ml of Sodium Phosphate Solution, and heat for 30 min in a boiling water bath, with occasional shaking. Cool to room temperature, and with rapid and continuous shaking, adjust to pH 6.0 ±0.1 by the addition of citric acid solution.

Note: *Rapid and continuous agitation during the addition prevents casein precipitation.*

Quantitatively transfer the mixture into a 100-ml volumetric flask, dilute to volume with water, and mix.

Stock standard solution: Transfer 100.0 mg of USP Papain Reference Standard into a 100-ml volumetric flask, dissolve and dilute to volume with Phosphate-Cysteine-EDTA Buffer Solution, and mix.

Diluted standard solutions: Pipet 2, 3, 4, 5, 6 and 7 ml of Stock Standard Solution into a series of 100-ml volumetric flasks, dilute each to volume with Phosphate-Cysteine-EDTA Buffer Solution, and mix by inversion.

Test solution: Prepare a solution from the enzyme preparation so that 2 ml of the final dilution will give an absorbance in the Procedure between 0.2 and 0.5. Weigh the sample accurately, transfer it quantitatively to a glass mortar, and triturate with Phosphate-Cysteine-EDTA Buffer Solution. Transfer the mixture quantitatively into a volumetric flask of appropriate size, dilute to volume with Phosphate-Cysteine-EDTA Buffer Solution, and mix.

Procedure

Pipet 5 ml of Casein Substrate Solution into each of a series of 25 x 150 mm test tubes, allowing three tubes for the enzyme unknown, six for a papain standard curve, and nine for enzyme blanks. Equilibrate the tubes for 15 min in a water bath maintained at 40 ±0.1°. At zero time, rapidly pipet 2 ml of each of the Diluted Standard Solutions, and 2-ml portions of the Test Solution, into the equilibrated substrate, starting the stopwatch at zero time. 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. **(Caution:** *Do not use mouth suction).* Mix each tube immediately by swirling.

Prepare enzyme blanks containing 5.0 ml of Casein Substrate Solution, 3.0 ml of Trichloroacetic Acid Solution, and 2.0 ml of one of the appropriate Diluted Standard Solutions or the Test Solution.

Return all tubes to the water bath, and heat for 30.0 min allowing the precipitated protein to coagulate completely. Filter each mixture through Whatman No. 42, or equivalent, filter paper, discarding the first 3 ml of filtrate. The subsequent filtrate must be perfectly clear. Determine the absorbance of each filtrate in a 1-cm cell at 280 nm with a suitable spectrophotometer, against its respective blank.

Calculation

One papain unit (PU) is defined in this assay as that quantity of enzyme that liberates the equivalent of 1 µg of tyrosine per h, under the conditions of the assay. Prepare a standard curve by plotting the absorbances of filtrates from the Diluted Standard Solutions against the corresponding enzyme concentrations, in mg/ml. By interpolation from the standard curve, obtain the equivalent concentration of the filtrate from the Test Solution. Calculate the activity of the enzyme preparation taken for analysis as follows:

$$PU/mg = (A \times C \times 10)/W$$

in which

A is the activity of USP Papain Reference Standard, in PU per mg,
C is the concentration, in mg per ml, of Reference Standard from the standard curve, equivalent to the enzyme unknown,
10 is the total volume, in ml, of the final incubation mixture, and
W is the weight, in mg, of original enzyme preparation in the 2-ml aliquot of Test Solution added to the incubation mixture.

ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e.pdf

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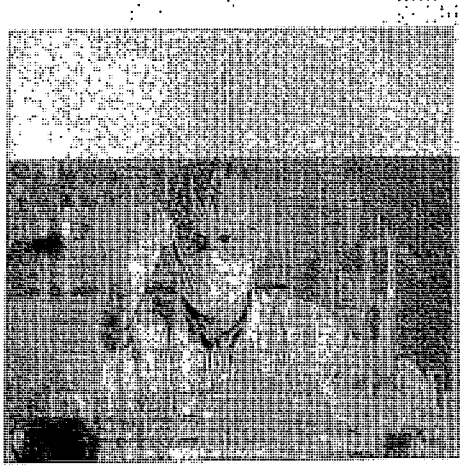
FAO JECFA Monographs 1



COMBINED COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

All specifications monographs from the 1st to the 65th meeting (1956–2005)

Volume 4

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2006

Attachment 2

Calculate the activity for each *Phytase Reference Preparation*:

$$(A_R \times f)/(30 \times R \times E) = FTU/g,$$

in which A_R equals the corrected absorbance of the *Phytase Standard Solution*; f equals the total dilution factor of the reference preparation; 30 equals the incubation time, in min; R equals sample weight, in g; E equals average of D factors; W equals the weight of potassium dihydrogen phosphate, in g; and MW equals the molecular weight of potassium dihydrogen phosphate, 136.09 (g/mol).

PLANT PROTEOLYTIC ACTIVITY

Application and Principle This procedure is used to determine the proteolytic activity of papain, ficin, and bromelain. The assay is based on a 60-min proteolytic hydrolysis of a casein substrate at pH 6.0 and 40°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration; solubilized casein is then measured spectrophotometrically.

Reagents and Solutions

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

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

Phosphate–Cysteine–EDTA Buffer Solution 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 to pH 6.0 ± 0.1 with 1 N hydrochloric acid or 1 N sodium hydroxide, then transfer into a 1000-mL volumetric flask, dilute to volume with water, 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 casein (United States Biochemical Corp., Catalog No. 12840, or equivalent) in 50 mL of *Sodium Phosphate Solution*, and heat for 30 min in a boiling water bath, with occasional agitation. Cool to room temperature, and with rapid and continuous agitation, adjust to pH 6.0 ± 0.1 by the addition of *Citric Acid Solution*.

[NOTE—Rapid and continuous agitation during the addition prevents casein precipitation.]

Quantitatively transfer the mixture into a 100-mL volumetric flask, dilute to volume with water, and mix.

Stock Standard Solution Transfer 100.0 mg of USP Papain Reference Standard into a 100-mL volumetric flask, dissolve, and dilute to volume with *Phosphate–Cysteine–EDTA Buffer Solution*, and mix.

Diluted Standard Solutions Pipet 2, 3, 4, 5, 6, and 7 mL of *Stock Standard Solution* into a series of 100-mL volumetric flasks, dilute each to volume with *Phosphate–Cysteine–EDTA Buffer Solution*, and mix by inversion.

Test Solution Prepare a solution from the enzyme preparation so that 2 mL of the final dilution will give a ΔA in the *Procedure* between 0.2 and 0.5. Weigh the sample accurately, transfer it quantitatively to a glass mortar, and triturate with *Phosphate–Cysteine–EDTA Buffer Solution*. Transfer the mixture quantitatively into a volumetric flask of appropriate size, dilute to volume with *Phosphate–Cysteine–EDTA Buffer Solution*, and mix.

Procedure Pipet 5 mL of *Casein Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing three tubes for the enzyme unknown, six for a papain standard curve, and nine for enzyme blanks. Equilibrate the tubes for 15 min in a water bath maintained at 40° ± 0.1°. Starting the stopwatch at zero time, rapidly pipet 2 mL of each of the *Diluted Standard Solutions*, and 2-mL portions of the *Test 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 enzyme blanks containing 5.0 mL of *Casein Substrate Solution*, 3.0 mL of *Trichloroacetic Acid Solution*, and 2.0 mL of one of the appropriate *Diluted Standard Solutions* or the *Test Solution*.

Return all tubes to the water bath, and heat for 30.0 min, allowing the precipitated protein to coagulate completely. Filter each mixture through Whatman No. 42, or equivalent, filter paper, discarding the first 3 mL of filtrate. The subsequent filtrate must be perfectly clear. Determine the absorbance of each filtrate in a 1-cm cell at 280 nm, with a suitable spectrophotometer, against its respective blank.

Calculation One papain unit (PU) is defined in this assay as that quantity of enzyme that liberates the equivalent of 1 µg of tyrosine per h under the conditions of the assay.

Prepare a standard curve by plotting the absorbances of filtrates from the *Diluted Standard Solutions* against the corresponding enzyme concentrations, in mg/mL. By interpolation from the standard curve, obtain the equivalent concentration of the filtrate from the *Test Solution*.

Calculate the activity of the enzyme preparation taken for analysis as follows:

$$PU/mg = (A \times C \times 10)/W$$

in which A is the activity of USP Papain Reference Standard, in PU per mg; C is the concentration, in mg/mL, of Reference Standard from the standard curve, equivalent to the enzyme unknown; 10 is the total volume, in mL, of the final incubation mixture; and W is the weight, in mg, of original enzyme preparation in the 2-mL aliquot of *Test Solution* added to the incubation mixture.

PROTEOLYTIC ACTIVITY, BACTERIAL (PC)

Application and Principle This procedure is used to determine protease activity, expressed as PC units, of preparations derived from *Bacillus subtilis* var. and *Bacillus licheniformis* var. The assay is based on a 30-min proteolytic hydrolysis of casein at 37° and pH 7.0. Unhydrolyzed casein is removed by filtration, and the solubilized casein is determined spectrophotometrically.

Reagents and Solutions

Casein Use Hammarsten-grade casein (United States Biochemical Corp., Catalog No. 12840, or equivalent).

Tris Buffer (pH 7.0) Dissolve 12.1 g of enzyme-grade (or equivalent) tris(hydroxymethyl)aminomethane in 800 mL of water, and titrate with 1 N hydrochloric acid to pH 7.0. Transfer into a 1000-mL volumetric flask, dilute to volume with water, and mix.

TCA Solution Dissolve 18 g of trichloroacetic acid and 19 g of sodium acetate trihydrate in 800 mL of water in a 1000-mL volumetric flask, add 20 mL of glacial acetic acid, dilute to volume with water, and mix.

Substrate Solution Dissolve 6.05 g of enzyme-grade tris(hydroxymethyl)aminomethane in 500 mL of water, add 8 mL of 1 N hydrochloric acid, and mix. Dissolve 7 g of *Casein* in this solution, and heat for 30 min in a boiling water bath, stirring occasionally.

Cool to room temperature, and adjust to pH 7.0 with 0.2 N hydrochloric acid, adding the acid slowly, with vigorous stirring, to prevent precipitation of the casein. Transfer the mixture into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Sample Preparation Using *Tris Buffer*, prepare a solution of the sample enzyme preparation so that 2 mL of the final dilution will contain between 10 and 44 bacterial protease units.

Procedure Pipet 10.0 mL of the *Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing one tube for each enzyme test, one tube for each enzyme blank, and one tube for a substrate blank. Equilibrate the tubes for 15 min in a water bath maintained at 37° ± 0.1°.

Starting the stopwatch at zero time, rapidly pipet 2.0 mL of the *Sample Preparation* into the equilibrated substrate. Mix, and replace the tubes in the water bath.Add 2 mL of *Tris Buffer* (instead of the *Sample Preparation*) to the substrate blank. After exactly 30 min, add 10 mL of *TCA Solution* to each enzyme incubation and to the substrate blank to stop the reaction. Heat the tubes in the water bath for an additional 30 min to allow the protein to coagulate completely.

At the end of the second heating period, shake each tube vigorously, and filter through 11-cm Whatman No. 42, or equivalent, filter paper, discarding the first 3 mL of filtrate.

[NOTE—The filtrate must be perfectly clear.]

Determine the absorbance of each sample filtrate in a 1-cm cell, at 275 nm, with a suitable spectrophotometer, using the filtrate from the substrate blank to set the instrument at zero. Correct each reading by subtracting the appropriate enzyme blank reading, and record the value so obtained as A_U.

Standard Curve Transfer 100.0 mg of L-tyrosine, chromatographic-grade or equivalent (Aldrich Chemical Co.), previously dried to constant weight, to a 1000-mL volumetric flask. Dissolve in 60 mL of 0.1 N hydrochloric acid. When completely dissolved, dilute the solution to volume with water, and mix thoroughly. This solution contains 100 µg of tyrosine in 1.0 mL. Prepare three more dilutions from this stock solution to contain 75.0, 50.0, and 25.0 µg of tyrosine per mL. Determine the absorbance of the four solutions at 275 nm in a 1-cm cell on a suitable spectrophotometer versus 0.006 N hydrochloric acid. Prepare a plot of absorbance versus tyrosine concentration.

Calculation One bacterial protease unit (PC) is defined as that quantity of enzyme that produces the equivalent of 1.5 µg/mL of L-tyrosine per min under the conditions of the assay.

From the *Standard Curve*, and by interpolation, determine the absorbance of a solution having a tyrosine concentration of 60 µg/mL. A figure close to 0.0115 should be obtained. Divide the interpolated value by 40 to obtain the absorbance equivalent to that of a solution having a tyrosine concentration of 1.5 µg/mL, and record the value thus derived as A_S.

Calculate the activity of the sample enzyme preparation by the equation

$$PC/g = (A_U/A_S) \times (22/30W)$$

in which 22 is the final volume, in mL, of the reaction mixture; 30 is the time, in min, of the reaction; and W is the weight, in g, of the original sample taken.

PROTEOLYTIC ACTIVITY, FUNGAL (HUT)

Application and Principle This procedure is used to determine the proteolytic activity, expressed as hemoglobin units on the tyrosine basis (HUT), of preparations derived from *Aspergillus oryzae* var. and *Aspergillus niger* var., and it may be used to determine the activity of other proteases at pH 4.7. The test is based on the 30-min enzymatic hydrolysis of a hemoglobin substrate at pH 4.7 and 40°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilized hemoglobin in the filtrate is determined spectrophotometrically.

Reagents and Solutions

Hemoglobin Use Hemoglobin Substrate Powder (Sigma Chemical Co., Catalog No. H2625) or a similar high-grade material that is completely soluble in water.

Acetate Buffer Solution Dissolve 136 g of sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$) in sufficient water to make 500 mL. Mix 25.0 mL of this solution with 50.0 mL of 1 M acetic acid, dilute to 1000 mL with water, and mix. The pH of this solution should be 4.7 ± 0.02.

Substrate Solution Transfer 4.0 g of the *Hemoglobin* into a 250-mL beaker, add 100 mL of water, and stir for 10 min to dissolve. Immerse the electrodes of a pH meter in the solution, and while stirring continuously, adjust the pH to 1.7 by adding 0.3 N hydrochloric acid. After 10 min, adjust the

Attachment 3

Health Canada Santé Canada

STEM BROMELAIN

This monograph is intended to serve as a guide to industry for the preparation of Product Licence Applications (PLAs) and labels for natural health product market authorization. It is not intended to be a comprehensive review of the medicinal ingredient.

Notes

- Text in parentheses is additional optional information which can be included on the PLA and product label at the applicant's discretion.
- The solidus (/) indicates that the terms and/or the statements are synonymous. Either term or statement may be selected by the applicant.

Date July 5, 2012

Proper name(s)

Stem bromelain (IUBMB 1992)

Common name(s)

- Stem bromelain (IUBMB 1992)
- Pineapple stem bromelain (IUBMB 1992)
- Bromelain (IUBMB 1992)

Source material(s)

Pineapple (*Ananas comosus* (L.) Merr. var. *comosus* (Bromeliaceae)) stem (USDA 2011)

Route(s) of administration

Oral

Dosage form(s)

- The acceptable pharmaceutical dosage forms include, but are not limited to capsules, chewables (e.g. gummies, tablets), liquids, powders, strips or tablets.
- This monograph is not intended to include foods or food-like dosage forms such as bars, chewing gums or beverages.

Use(s) or Purpose(s) Statement(s) to the effect of:

Digestive enzyme

Dose(s)

Subpopulation(s)

Adults ($\geq$ 19 years)

Quantity(ies)

Dose information must include the quantities of both the enzyme preparation and its enzymatic activity:

- Providing up to 1500 mg per day enzyme preparation, not to exceed 540 mg per dose (Kerkhoffs et al. 2004; Walker et al. 2002; Singer et al. 2001; Klein and Kullich 2000; Gutfreund et al. 1978); and
- Enzyme activity providing up to 1.3 x 10^8 FCC PU per day, not to exceed 4.5 x 10^7 FCC PU per dose (Glade et al. 2001; Gutfreund et al. 1978).

Notes

- One papain unit (PU) is defined as that quantity of enzyme that liberates the equivalent of 1 µg of tyrosine per hour under the conditions of the assay (FCC 8).
- One gelatin digestion unit (GDU) is approximately equivalent to 1.5 x 10^4 FCC papain unit (1 GDU $\approx$ 1.5 x 10^4 FCC PU).
- For multi-ingredient products containing both papain and bromelain (fruit and/or stem), the combined proteolytic activity should not exceed the maximum proteolytic activity of 2.0 x 10^7 FCC PU per day (as per the NHPD *Bromelain, Fruit* monograph).

Directions for use

Take with food/meal.

Duration of use

For prolonged use, consult a health care practitioner.

Risk information Statement(s) to the effect of:

Caution(s) and warning(s)

- If you are pregnant of breastfeeding, consult a health care practitioner prior to use.

- If you have a gastrointestinal lesion/ ulcer, are taking an anticoagulant/ blood thinner, anti-inflammatory or antibiotic, or are having surgery, consult a health care practitioner prior to use (Martindale 2011; Brinker 2010; Blumenthal et al. 2000).

Contraindication(s)

No statement required.

Known adverse reaction(s)

- Hypersensitivity/allergy has been known to occur, in which case discontinue use (Martindale 2011; Brinker 2010; Murray and Pizzorno 2006; Blumenthal et al. 2000; Baur and Fruhmann 1979).
- Nausea, vomiting, and diarrhoea have been known to occur, in which case discontinue use (and consult a health care practitioner) (Martindale 2011; Brien et al. 2006; Blumenthal et al. 2000).

Non-medicinal ingredients

Must be chosen from the current NHPD *Natural Health Products Ingredients Database* and must meet the limitations outlined in the database.

Specifications

- The finished product must comply with the minimum specifications outlined in the current NHPD *Compendium of Monographs*.
- Details of the manufacturing of the enzyme at the raw material stage should include fermentation medium and the isolation process of the medicinal ingredient.
- The specifications must include testing for enzymatic activity of the medicinal ingredient at appropriate stages of formulation and manufacturing using the assay outlined in the current Food Chemicals Codex (FCC):
  PLANT PROTEOLYTIC ACTIVITY.
- Where published methods are not suitable for use, manufacturers will use due diligence to ensure that the enzymes remain active to the end of the shelf life indicated on the product label.

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Canada

Attachment 4

Health Canada Santé Canada

FRUIT BROMELAIN

This monograph is intended to serve as a guide to industry for the preparation of Product Licence Applications (PLAs) and labels for natural health product market authorization. It is not intended to be a comprehensive review of the medicinal ingredient.

Notes

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- The solidus (/) indicates that the terms and/or the statements are synonymous. Either term or statement may be selected by the applicant.

Date July 5, 2012

Proper name(s)

Fruit bromelain (IUBMB 1992)

Common name(s)

- Fruit bromelain (IUBMB 1992)
- Pineapple fruit bromelain (IUBMB 1992)
- Juice bromelain (IUBMB 1992)

Source material(s)

Pineapple (*Ananas comosus* (L.) Merr. var. *comosus* (Bromeliaceae)) Fruit (USDA 2011)

Route(s) of administration

Oral

Dosage form(s)

- The acceptable pharmaceutical dosage forms include, but are not limited to capsules, chewables (e.g. gummies, tablets), liquids, powders, strips or tablets.
- This monograph is not intended to include foods or food-like dosage forms such as bars, chewing gums or beverages.

Canada

Use(s) or Purpose(s) Statement(s) to the effect of:

Digestive enzyme

Dose(s)

Subpopulation(s)

Adults ($\geq$ 19 years)

Quantity(ies)

Dose information must include the quantities of both the enzyme preparation and its enzymatic activity:

- Providing up to 600 mg per day enzyme preparation, not to exceed 300 mg per dose (Kerkhoffs et al. 2004; Walker et al. 2002; Singer et al. 2001; Klein and Kullich 2000; Gutfreund et al. 1978); and
- Enzyme activity providing up to 2.0 x 10^7 FCC PU per day, not to exceed 1.0 x 10^7 FCC PU per dose (Glade et al. 2001; Gutfreund et al. 1978).

Notes

- One papain unit (PU) is defined as that quantity of enzyme that liberates the equivalent of 1 µg of tyrosine per hour under the conditions of the assay (FCC 8).
- One gelatin digestion unit (GDU) is approximately equivalent to 1.5 x 10^4 FCC papain unit (1 GDU $\approx$ 1.5 x 10^4 FCC PU).
- For multi-ingredient products containing both papain and bromelain (fruit and/or stem), the combined proteolytic activity should not exceed the maximum proteolytic activity of 2.0 x 10^7 FCC PU per day.

Direction(s) for use

Take with food/meal.

Duration of use

For prolonged use, consult a health care practitioner.

Risk information Statement(s) to the effect of:

Caution(s) and warning(s)

- If you are pregnant or breastfeeding, consult a health care practitioner prior to use.

- If you have gastrointestinal lesions/ulcers, are taking anticoagulant agents, anti-inflammatory agents or antibiotics or before having surgery, consult a health care practitioner prior to use (Martindale 2011; Brinker 2010; Blumenthal et al. 2000).

Contraindication(s)

No statement required.

Known adverse reaction(s)

- Hypersensitivity/allergy has been known to occur, in which case discontinue use (Martindale 2011; Brinker 2010; Murray and Pizzorno 2006; Blumenthal et al. 2000; Baur and Fruhmann 1979).
- Nausea, vomiting, and diarrhea have been known to occur, in which case discontinue use (and consult a health care practitioner) (Martindale 2011; Brien et al. 2006; Blumenthal et al. 2000).

Non-medicinal ingredients

Must be chosen from the current NHPD *Natural Health Products Ingredients Database* and must meet the limitations outlined in the database.

Specifications

- The finished product must comply with the minimum specifications outlined in the current NHPD *Compendium of Monographs*.
- Details of the manufacturing of the enzyme at the raw material stage should include fermentation medium and the isolation process of the medicinal ingredient.
- The specifications must include testing for enzymatic activity of the medicinal ingredient at appropriate stages of formulation and manufacturing using the assay outlined in the current Food Chemicals Codex (FCC):
  PLANT PROTEOLYTIC ACTIVITY.
- Where published methods are not suitable for use, manufacturers will use due diligence to ensure that the enzymes remain active to the end of the shelf life indicated on the product label.

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