Bromelain and Capsaicin

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Work completed under Dr. Bruce Patterson for use in MCB 181L
MCB 492, Spring 2012

Research was completed to look into the inhibition of bromelain via capsaicin. Since bromelain is measured in milk clotting units (MCU) and gelatin dissolving units (GDU), this inhibition was studied through milk clotting and jello gelatin hardening. Two assays were developed in which pineapple is used as the source of bromelain, and chilies as the source of capsaicin.

BROMELAIN

KEY POINTS:

- Optimal temperature: 50 – 60^{oC}
- Deactivation temperature: >65^{oC}
- Optimal pH: 4.5 - 5.5
- Measured in:
    - MCU (milk clotting units)
    - GDU (gelatin dissolving units)

  where 1 GDU = 1.5 MCU ("Bromelain")
- Potent products (such as pineapple) that contain bromelain have approx. 2,000 MCU/
- Can buy it in pills of 3,000 GDU ("Find")
- Highest concentration of bromelain in stem of pineapple ("Bromelain")
- Destroys proteins through lysis of peptide bonds (Wong)
    - Tends to break peptide bonds that follow the amino acids lysine, alanine, tyrosine and glycine - therefore, the degree to which a protein may be broken down by bromelain depends on the amounts of these amino acids in the protein ("Bromelain")

OTHER USES:

- Meat tenderizer
- Anti-inflammatory (reduces symptoms of arthritis)
- Helps digestive problems
- May reduce ill effects of some types of chemotherapy
- Anticoagulant by breaking down fibrin
- Thins mucus (may benefit asthmatics to reduce effects of chronic bronchitis)
- May trigger changes in white blood cells (immune system improvement)
- Speed wound healing
- Enhances effect of antibiotics ("Bromelain")

OTHER PROTEASES FOUND IN FRUITS LIKE BROMELAIN:

Protease	**Fruit Source**	**Availability**	**Peak Seasonality**
Bromelain	Pineapple	All year	All year
Papain	Papaya	All year	June - Sept
Actinidin	Kiwi	Sept - Mar	Sept - Dec
Ficin	Fig	June - Nov	June - Nov
(Name not found)	Guava	Nov - April	Nov – April

* *Pineapple is the only source of the above listed protease that is both available and harvested year-round.* ("Dole", "CUESA")

CAPSAICIN

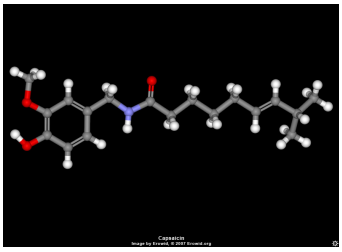


Figure A: molecular structure of capsaicin (Erowid)

KEY POINTS:

- Active component in chilies ("Capsaicin")
- Colorless and odorless
- Hydrophobic (Erowid)
- Measured in scoville heat units (SHU)
- Strong irritant, especially to mucus membranes
- Most highly concentrated in the white pith of a chili, which is located in the inner wall ("Capsaicin")
- It is known that *some* chemical in chili destroys proteolytic enzymes and this chemical is presumed to be capsaicin (Schwarcz).

OTHER USES:

- Relieves pain associated with shingles
- Relief of minor pain associated with arthritis
- Relief of minor pain associated with muscle pain
- Reduces inflammation and itching associated with psoriasis
- Decreases insulin release in humans, leading to reduced blood sugar
    - Affects on insulin are species specific
- Inhibits growth of some types of cancer cells through promotion of apoptosis
- Aids in coagulation (Eustice)

INTERESTING FACTS:

- Production of capsaicin is a mechanism used by chilies that protects its seeds from mammals, which eat, crush, and ruin the seeds because of their tough molars
- Birds are not affected by capsaicin – this makes sense because birds can eat the seeds without ruining them, fly large distances, and poop. This disperses the seeds and promotes the growth of more chilies ("Capsaicin").

PRECAUTIONS:

- Gloves
    - Any bit of contact can irritate the skin and contact with any mucus membranes such as in the eye, nose, and mouth will cause intense burning
- Facemask
    - Prevents inhalation of any powder containing capsaicin

SOURCES OF CAPSAICIN USED:

- Dried whole Habanero Chili Peppers
    - Had to crush using mortar and pestle
    - Approx. 100,000 - 350,000 SHU
- Hot New Mexico Chili Pepper
    - Provided already ground

- Approx. 3,500 – 8,000 SHU (“Scoville Scale”)

MILK

Milk is composed of butterfat globules in a water-based solution (“Milk”). To understand this structure, it is best to first look at the basic structure of a micelle, as shown in Figure B. Micelles are typically made of phospholipids, which have polar heads and nonpolar, or greasy, tails. When put into an aqueous solution, the polar heads have partial charges that interact with the partial charges of water. This pushes the greasy tails together, forming a bubble-like structure where the greasy parts are shielded from the partially charged aqueous solution on the outside. In milk, micelles are comprised of butterfat triglycerides (Figure C), phospholipids, and proteins known as caseins (“Milk”).

There are four types of caseins including alpha-s1, alpha-s2, beta, and kappa, which will be referred to as k-casein from here on out. The important thing to know here is that the first three caseins are hydrophobic, and therefore readily precipitate in aqueous solution such as that of milk. K-casein, however, is soluble in milk (Bowen). As a result, hydrophobic caseins are wrapped in k-casein, “shielding” them from the aqueous surroundings. This stabilizes the micelle and keeps it soluble in aqueous solution. The exact conformation of the casein proteins in these micelles is unknown, but all proposed models show the micelles that are formed with casein aggregates covered in k-casein. Two of the proposed models can be viewed below in Figures D and E (Goff).

Now that the structure of milk is established, we can go into how it clots. Rennet is the general name for any enzymatic preparation that acts to clot milk. In mammals, rennet is produced in the stomach comprised mainly of pepsin, lipase, and chymosin, which is also known as rennin (“Rennet”). Chymosin cleaves k-casein. Since k-casein is what keeps the micelle stable in aqueous solution, destruction of this protein leads to destabilization of the micelle. Greasy casein proteins are now able to precipitate, resulting in clotting of the milk (Bowen).

Chymosin is an important enzyme for mammals because of its ability to clot milk in the stomach. Clotting in the stomach makes it possible for milk to stick around longer due to its increased thickness. It makes sense that this would be beneficial for mammals because the longer milk sticks around, the longer the body has a chance to absorb as many nutrients as possible. This is especially true in the stomachs of young mammals whose diet is comprised entirely of their mother’s milk. As a result, chymosin is present in the highest quantities in very young mammals. Consequently, traditional cheese-making methods involve the use of chymosin obtained by the slaughter of young calves (“K-casein”). For obvious reasons, this method does not go over well with many religious and activist groups. In addition to this opposition, it is difficult to obtain enough calf stomachs to make all cheese this way. This leads us to the discovery of other proteases that can be used as rennet including, but not limited to, enzymes found in fig, ivy, soybeans, and certain molds, bacteria, fungi, and yeasts.

Through our own research on jello dissolving and the bromelain in pineapple, it was discovered that bromelain is not only measured in gelatin dissolving units but also in milk clotting units. This led us to the study of the clotting of milk via bromelain as rennet. We have been led to believe that the protease bromelain acts just like these other proteases by cutting k-casein and interfering with its ability to stabilize the micelle.

Although all rennets have the same activity of cutting k-casein, each may do so in its own way. In other words, different proteases may cut the k-casein at different spots, but all result in the coagulation of milk ("K-casein"). These differences may lead to differences in clotting times where the breakdown of k-casein is the rate-limiting step. Times may also vary depending on the type of milk, pH, and temperature ("K-casein"). Because of these other variables, it is difficult to determine reproducible clotting times for bromelain. This makes it hard for us to develop an assay in which clotting times are consistent with every trial. As a side note, methods have been used to determine the rate of clotting through k-casein degradation tracking, as a way to quantify milk clotting by way of rennet. One common way this has been done is through labeling k-casein in micelles with a fluorochrome compound known as fluorescein isothiocyanate, or FITC. This allows researchers to detect clotting through protease activity alone. It also allows researchers to see if low levels of clotting are occurring that may not be visible to the naked eye ("K-casein").

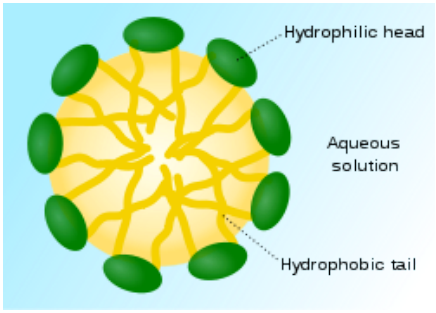


***Figure B**: Basic structure of a "typical" micelle ("Rennet")*

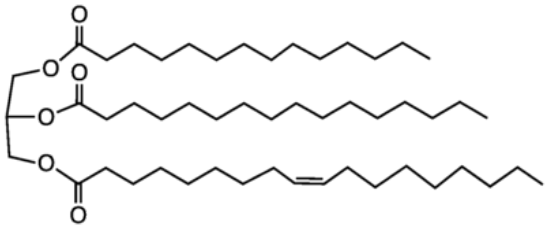


***Figure C**: butterfat triglyceride ("Milk")*

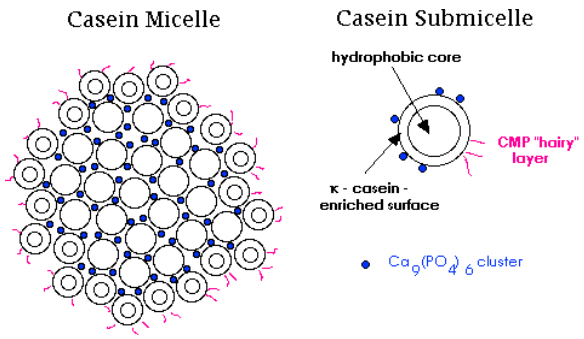


***Figure D:** Proposed structure of casein conformation in micelles in which micelle is formed by sub micelles that are covered in k-casein (Goff)*

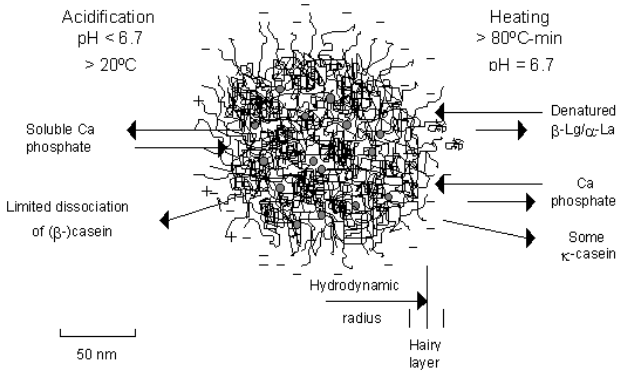


***Figure E**: Proposed structure of casein conformation in micelles in which caseins are interlinked (Goff)*

JELLO GELATIN

Gelatin is derived from the collagen in animal bones and skin ("Gelatin"). Collagen has a helical structure similar to that of DNA, with weak hydrogen bonds holding together amino acid chains. However, collagen fibers are comprised of a triple helix, in contrast to the double helix seen in DNA ("Collagen"). Placing jello gelatin powder in hot water dissolves the powder, and weak hydrogen bonds holding together the protein chains are broken. Addition of cold water to the dissolved gelatin, followed by further cooling causes the broken chains to come back together, but this time water is incorporated into the structure ("Gelatin"). This gives finished gelatin its wiggly-jiggly form.

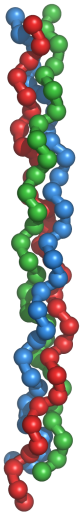


Figure F: *Collagen triple helix ("Collagen")*

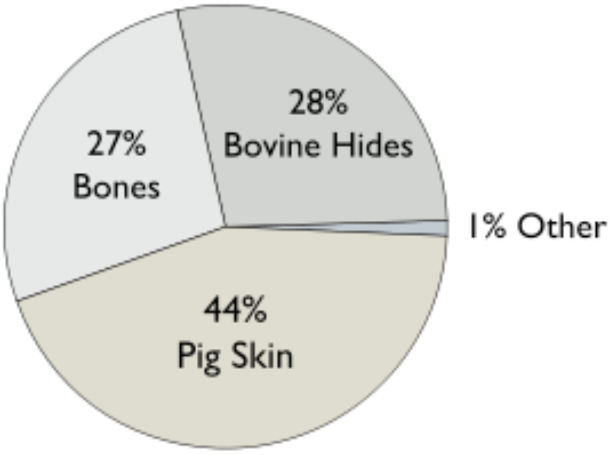


Figure G*: Materials used in gelatin production ("Gelatin")*

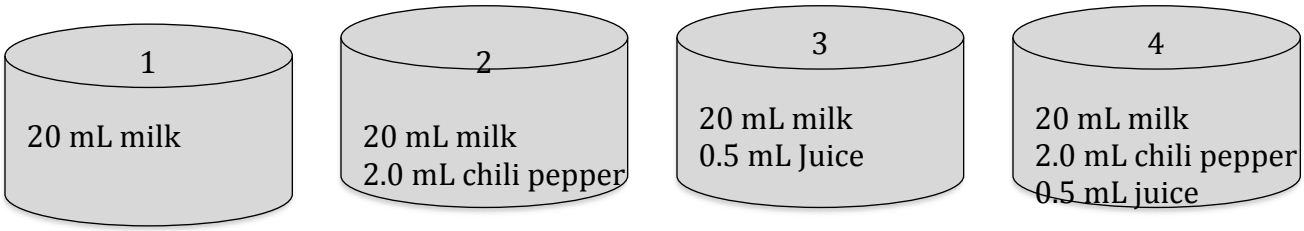
Milk Assay

MATERIALS:

- Whole milk
- Freshly cut pineapple
- Five 20 mL beakers
- Ground Hot New Mexico Chili Powder
- Hot plate
- Mortar and pestle
- Cheese cloth
- Micropipette
- Pipet tips

PROPOSED METHOD (students will develop this themselves):

(1) Obtain four 20 mL beakers and label as necessary
(2) Grind pineapple using mortar and pestle
(3) Place cheese cloth over extra beaker and pour mashed pineapple into pit made with the cloth to filter. Squeeze the cloth to get out any extra juice.
(4) Fill each beaker with the materials as seen below. Use micropipette to measure the small amount of juice.



(5) Immediately place beakers on hot plate set to lowest setting
(6) Watch beakers and record any observations made including time of clotting

BEAKER PUPOSES:

(1) Control – Does milk clot on its own with heat?
(2) Control – Does ground chili cause milk to clot with heat?
(3) Experiment – Does bromelain in pineapple cause milk to clot with heat?
(4) Experiment – Does capsaicin in ground chili pepper inhibit the effects of bromelain in pineapple on the clotting of milk?

TYPICAL RESULTS:

Beaker #	**Contents**	**Observations**
1	20 mL milk	Did not clot
2	20 mL milk 2.0 mL ground chili pepper	Did not clot

3	20 mL milk 0.5 mL pineapple juice	Thick, heavy clotting after approx. 4 – 6 min.
4	20 mL milk 0.5 mL pineapple juice 2.0 mL ground chili pepper	Smaller clotting after 17 – 22 min.

SOME TECHNICAL ASPECTS ABOUT THE MILK ASSAY

POSITIVE THINGS TO KNOW:

- Adding ground Hot New Mexico Chili Pepper directly to milk showed slowed effects of bromelain.
- Adding more ground Hot New Mexico Chili Pepper directly to milk results in slower clotting times.
- Adding smaller amounts of bromelain and larger amounts of capsaicin each trial has lead us closer to developing an effective number of each to use so that the experiment is consistently reproducible.

"NEGATIVE" THINGS TO KNOW:

- Crushing dried whole habanero peppers and adding directly to milk did not show any inhibition of bromelain.
- Capsaicin should not be extracted into oil for use in this assay because oil and milk do not mix.
- Capsaicin extraction in alcohol did not show as much inhibition of bromelain as did adding chili pepper directly to the milk, and was set aside as a method.

OTHER NOTES:

- pH of milk is 6.4 – 6.8 ("Milk"). Note that this is not the optimal pH of bromelain.
- 1 g rennet can coagulate 2 – 4 L milk ("Rennet").

NEXT STEPS:

The milk assay ended with a final experiment where clear inhibition of bromelain by capsaicin was observed, and specific details were quantified so that the assay could be repeated (Table 8). There was, however, some clotting seen in the control beakers when no bromelain was present. It is believed that this clotting could be attributed both to prolonged heat as well as a possible unknown substance in the chili pepper that could alter the pH of the milk. Next steps should include completing the experiment where no hot plate is used (kept at room temperature), and a buffer is added to each beaker to eliminate pH as a confound variable. Further research should be completed to determine if it is indeed

capsaicin that affects bromelain, or if it is some other chemical in chilies. From the literature we found so far, it has only been presumed that capsaicin is the chemical (Schwarcz).

TABLES FROM OUR MILK CLOTTING STUDIES

TABLE 1: *Milk Clotting with Ground HOT NEW MEXICO CHILI PEPPER Added Directly to Milk*

Milk	Pineapple	Ground Chili Pepper	Outcome
25 mL	Approx. 4 cm^3	1.5 mL	Clotting (grain-like) – 12 min.
25 mL	Approx. 4cm^3	1.0 mL	Clotting (grain-like) – 10 min.
25 mL	Approx. 4 cm^3	0.5 mL	Clotting (small chunks) – 9 min.
25 mL	Approx. 4 cm^3	0.1 mL	Heavy clotting (thick chunks) – 5 min.
25 mL	Approx. 4 cm^3	-	Heavy clotting (thick chunks) – 5 min.
25 mL	-	1.5 mL	No clotting after 12 min.
25 mL	-	-	No clotting after 12 min.

TABLE 2: *Milk clotting using dried HABANERO Added Directly to Milk*

Milk	Pineapple	Habanero	Outcome
25 mL	Approx. 2 cm^3	1.5 mL	Clotted at 7 min
25 mL	Approx. 2 cm^3	1.0 mL	Clotted at 7 min
25 mL	Approx. 2 cm^3	0.5 mL	Clotted at 7 min
25 mL	Approx. 2 cm^3	0.1 mL	Clotted at 7 min

25 mL	Approx. 2 cm^3	-	Clotted at 6 min
25 mL	-	1.5 mL	Did not clot
25 mL	-	-	Did not clot

TABLE 3: 4/12/2012 – *Milk Experiment with CHILI PEPPER Extract After a 2-Day Extraction in Oil*

Cup #	**Pineapple**	**Milk (mL)**	**Ratio of Extract**	**Amount of Extract**	**Outcome**
1	Approx. 2 cm^3	20	1:1	1 mL	Clotted at 9 min.
2	Approx. 2 cm^3	20	1:2	1 mL	Clotted at 6 min.
3	Approx. 2 cm^3	20	1:5	1 mL	Clotted at 9 min.
4	Approx. 2 cm^3	20	1:10	1 mL	Clotted at 9 min.
5	Approx. 2 cm^3	20	1:15	1 mL	Clotted at 8 min.
6	pprox. 2 cm^3	20	1:20	1 mL	Clotted at 8 min.

**Ratios are written in the format ground chili pepper: oil.*

TABLE 4: *Milk Experiment with HABANERO Extract After a 2-Day Extraction in Alcohol*

Cup #	**Pineapple (cm^3)**	**Milk (mL)**	**Ratio of Extract**	**Amount of Extract (mL)**	**Outcome**
-	-	-	1:1	(none)	-
-	-	-	1:2	(none)	-
1	Approx. 2	25	1:5	1	Clotted at 9 min

2	Approx. 2	25	1:10	1	Clotted at 9 min
3	Approx. 2	25	1:15	1	Clotted at 8 min
4	Approx. 2	25	1:20	1	Clotted at 7 min
5	Approx. 2	25	-	-	Clotted at 7 min

**Ratios are written in the format ground chili pepper: alcohol.*

TABLE 5: *Milk Experiment with CHILI PEPPER Extract after a 2-Day Extraction in Alcohol*

Cup #	**Pineapple (cm^3)**	**Milk (mL)**	**Ratio of Extract**	**Amount of Extract (mL)**	**Outcome**
-	-	-	1:1	(none)	-
1	Approx. 2	20	1:2	1	Clotted at 8 min.
2	Approx. 2	20	1:5	1	Clotted at 7 min.
3	Approx. 2	20	1:10	1	Clotted at 6 min.
4	Approx. 2	20	1:15	1	Clotted at 6 min.
5	Approx. 2	20	1:20	1	Clotted at 6 min.

**Ratios are written in the format ground chili pepper: alcohol.*

TABLE 6: *Milk clotting with Ground HOT NEW MEXICO CHILI PEPPER Added Directly to Milk*

Milk (mL)	**Pineapple (cm^3)**	**Ground chili pepper (mL)**	**Outcome**
25	Approx. 2	1.5	Clotted at 8 min.
25	Approx. 2	1.0	Clotted at 7 min.
25	Approx. 2	0.5	Clotted at 6 min.
25	Approx. 2	0.1	Clotted at 6 min.
25	Approx. 2	-	Clotted at 6 min.

TABLE 7: *Milk Clotting With Fresh Pineapple Juice to Determine Minimum Amount Needed for Observable Clotting*

PURPOSE: Can we use fresh pineapple juice to effectively clot milk, and if so, what is the minimum amount needed to do so?

OUTCOME: Clotting was achieved with juice. At least 0.25 mL were needed for clotting, but clotting was much more dramatic and took half the time with 0.5 mL juice.

Beaker #	Amount Pineapple	Amount Milk	Outcome
1	5.0 mL	20 mL	Started showing signs of fine clotting at 1 min., heavy clotting at 4.5 min.
2	1.0 mL	20 mL	Heavily clotted at 5 min.
3	0.5 mL	20 mL	Heavily clotted at 5 min.
4	0.25 mL	20 mL	Started showing sign of clotting at 8 min., clotted at 9 min., but was not as dramatic as beaker #3
5	0.05 mL	20 mL	Started showing signs of clotting at 9 min., light clotting by 12 min.
6	0.01 mL	20 mL	Started showing signs of clotting at 10 min., light clotting by 12 min.
7	-	20 mL	Began to bubble at 12 min.

TABLE 8: *Milk Clotting With Minimum Fresh Pineapple Juice to Determine Amount of Ground New Mexico Chili Powder Needed for Observable Inhibition of Clotting*

Beaker #	Amount Pineapple	Amount Milk	Amount Chili Powder	Outcome
1	0.5 mL	20 mL	2.0 mL	Clotting after 22 min.
2	0.5 mL	20 mL	1.5 mL	Clotting at 14 min.
3	0.5 mL	20 mL	1.0 mL	Clotting at 13 min.
4	0.5 mL	20 mL	0.5 mL	Clotting at 12 min.
5	0.5 mL	20 mL	0.1 mL	Clotting at 5 min.
6	0.5 mL	20 mL	-	Clotting at 4 min.
7	-	20 mL	2.0 mL	Clotting after 22 min.
8	-	20 mL	-	Thickening after 22 min.

Jello Assay

PURPOSE: How much ground chili pepper is needed to inhibit 0.5 mL pineapple juice from clotting milk?

OUTCOME: It took 18 minutes longer to clot in the presence of 2.0 mL ground chili pepper than with no ground chili pepper present.

MATERIALS:

- Gelatin Packages
- Water
- Hot Plate
- Ice
- Freshly cut pineapple
- Disposable plastic cups

2X RECIPE:

(1) Obtain 89 mL boiling water.
(2) Obtain 60 mL water at room temperature and add ice until volume increases to 150 mL.
(3) Add one standard package of jello gelatin powder to boiling water and mix until dissolved.
(4) Add ice water to jello mixture. Thoroughly mix and remove any unmelted ice.

→ Recipe was derived from basic "quick setting" recipe on Jello box. Twice the amount of Jello powder was found to be better due to shortened hardening time.

SOME TECHNICAL ASPECTS ABOUT THE JELLO ASSAY

POSITIVE THINGS TO KNOW:

- Heating the pineapple in boiling water for 2-3 minutes allowed the gelatin to harden when put in the fridge
- The more thoroughly mixed the juice is in the gelatin, the more liquid the gelatin will appear after hardening time.
- Using a 2x concentration of gelatin mixture allows for the hardening of the gelatin to better occur in a 30 – 60 minute time period.

"NEGATIVE" THINGS TO KNOW:

- If the gelatin is not stirred between each pour into the disposable cups, the gelatin will not be evenly dispersed and gelatin will not harden.
- If extra ice is not removed from the mixture after thorough mixing, gelatin will not harden or will take three times as long to harden.
- Capsaicin inhibits the effects of bromelain to a very mild extent. Instead of making it continue to harden to full extent, it merely allows the gelatin to reach a higher viscosity.

OTHER NOTES:

- The 1:2 extraction of capsaicin and 1:1 extraction of capsaicin were the best candidates for further study.
- If given enough time, fresh pineapple will eat through already hardened gelatin.
- It takes at least 3 mL of fresh pineapple to inhibit jello hardening.

NEXT STEPS:

The jello assay was halted with a final experiment to see if capsaicin could be extracted into alcohol in order to both make it more concentrated as well as soluble in gelatin. The experiment showed promise with higher concentrations of capsaicin, but dramatic inhibition was never observed. If one were to work to continue development of this assay, starting points may be to find a more concentrated source of capsaicin. Heating pineapple to inhibit the effects of bromelain should also be considered as a prospect. As with the milk assay, further research should be completed to determine if capsaicin is indeed the chemical in chilies that acts on bromelain, or if is some other component of the chili. This research should also look to find the mechanism through which this inhibition acts.

TABLES FROM OUR JELLO HARDENING STUDIES

TABLE 9: *Determining How Much Triton x 100 Can be Used Without Interfering With Jello Hardening*

PURPOSE: Is it possible to use detergent to extract capsaicin without itself interfering with the hardening of jello?

OUTCOME: The maximum detergent that can be used is too small to hold an effective amount of capsaicin.

Triton x100	Jello	Outcome
1.0 mL	20 mL	Thick film on top of cup, but liquid beneath
0.9 mL	20 mL	
0.8 mL	20 mL	Very soft gel with about 1 mL liquid still present – gel falls apart upon moving the cup
0.7 mL	20 mL	
0.6 mL	20 mL	
0.5 mL	20 mL	No liquid present, but jello pulls away from sides of the cup upon moving - very little liquid present
0.4 mL	20 mL	
0.3 mL	20 mL	No liquid present, but jello is very soft formed
0.2 mL	20 mL	
0.1 mL	20 mL	
0.0 mL	20 mL	Completely hardened jello that stays solid in cup even when tipped upside down

TABLE 10: *Basic Jello Experiment with 25 mL of 2x Jello Recipe*

PURPOSE: Does using twice the amount of gelatin powder make for a shorter hardening time?

OUTCOME: Compared to previous trials, using twice the gelatin makes the jello harden faster.

Cup #	Pineapple	Outcome
1	Approx. 4 cm^3 Fresh	After 1 hour – complete liquid
2		
3	Approx. 4 cm^3 Cooked	After 25 minutes – hardened to firm consistency. Stayed inside cup when held upside down.
4		
5	(None)	After 25 minutes – hardened to firm consistency. Stayed

6	(None)	inside cup when held upside down.

TABLE 11: *Jello Experiment with 25 mL of 2x Jello and Ground HOT NEW MEXICO CHILI POWDER Added Directly to Jello*

PURPOSE: Does adding ground Hot New Mexico Chili Powder to gelatin inhibit affects of bromelain on the gelatin?

OUTCOME: No signs of inhibition were observed, and this method was ruled out.

Cup #	Pineapple	Chili powder	Outcome
1	Approx. 4 cm^3 Fresh	1 mL	After 1 hour – complete liquid
2			
3			
4	(None)	1 mL	After 25 minutes – hardened to firm consistency. Stayed inside cup when held upside down.
5			
6			

TABLE 12: *Jello Experiment Using HABANERO Extract after 1-Day Extraction in Alcohol and 25 mL of 2x Jello*

PURPOSE: Can we make the capsaicin from habanero more concentrated by extracting in alcohol (for one day) and therefore more effective in the inhibition of bromelain?

OUTCOME: experiment showed some promise but should be repeated with less pineapple and more concentrated extract (at lease 2:1)

Cup #	Ratio of Extract	Pineapple	Amount of Extract	Outcome
1	1:1	Approx 2 cm^3	1 mL	After 5 hours – still liquid Slightly more gelatinous than others
2	1:2	Approx 2 cm^3	1 mL	After 5 hours – still liquid Slightly less gelatinous than 1:1
3	1:5	Approx 2 cm^3	1 mL	After 5 hours – still complete liquid
4	1:10	Approx 2 cm^3	1 mL	After 5 hours – still complete liquid
5	1:15	Approx 2 cm^3	1 mL	After 5 hours – still complete liquid
6	1:20	Approx 2 cm^3	1 mL	After 5 hours – still complete liquid

**Ratios are written in the format ground chili pepper: alcohol.*

TABLE 13: *Basic Jello Experiment Using Boiled Pineapple and 25 mL of 2x Jello*

Cup #	Pineapple	Amount of Pineapple	Amount of Jello	Outcome
1	Approx. 4 cm^3 Fresh	1.5 mL	25 mL	After 4 hours – liquid
2		3.0 mL	25 mL	After 4 hours – liquid
3	Approx. 4 cm^3 Boiled (mashed)	2.5 mL	25 mL	After 4 hours – complete solid Does not pour when held upside down
4		3.0 mL	25 mL	After 4 hours – complete solid Does not pour when held upside down
5	Approx. 4 cm^3 Boiled (chunks)	2 – 2 cm^3 chunks	25 mL	After 4 hours – complete solid Does not pour when held upside down

TABLE 14: *Jello Experiment Using HABANERO Extract After 2-Day Extraction in Alcohol*
and 25 mL of 2x Jello

PURPOSE: Are the results of the previous experiment of inhibiting the affects of bromelain via heat reproducible?

OUTCOME: Yes, it has consistently been modeled that heating pineapple stops the affects of bromelain on gelatin.

Cup #	Ratio of Extract	Pineapple	Amount of Extract	Outcome
1	1:1	Approx. 2 cm^3	1 mL	After 6 hours – slightly gelled
2	1:2	Approx. 2 cm^3	1 mL	After 6 hours – liquid
3	1:5	Approx. 2 cm^3	1 mL	After 6 hours – liquid
4	1:10	Approx. 2 cm^3	1 mL	After 6 hours – liquid
5	1:15	Approx. 2 cm^3	1 mL	After 6 hours – liquid
6	1:20	Approx. 2 cm^3	1 mL	After 6 hours – liquid

**Ratios are written in the format ground chili pepper: alcohol.*

TABLE 15: *Jello Experiment Using HOT NEW MEXICO CHILI PEPPER Extract after a 2-Day Extraction in Alcohol and 25 mL of 2x Jello*

Cup #	Ratio of Extract	Pineapple	Amount of Extract	Outcome
1	1:1	Approx. 2 cm^3	1 mL	After 6 hours – slightly gelled
2	1:2	Approx. 2 cm^3	1 mL	After 6 hours – liquid
3	1:5	Approx. 2 cm^3	1 mL	After 6 hours – liquid
4	1:10	Approx. 2 cm^3	1 mL	After 6 hours – liquid
5	1:15	Approx. 2 cm^3	1 mL	After 6 hours – liquid
6	1:20	Approx. 2 cm^3	1 mL	After 6 hours – liquid

**Ratios are written in the format ground chili pepper: alcohol.*

TABLE 16: Jello Experiment Using HOT NEW MEXICO CHILI PEPPER Extract After a
7-Day Extraction in Alcohol in a 1:1 Ratio (chili pepper: alcohol)

PURPOSE: Can we make the capsaicin from chili pepper more concentrated by extracting in alcohol (for seven days) and therefore more effective in the inhibition of bromelain?

OUTCOME: Experiment showed promise, but not complete inhibition.

PURPOSE: Can we make the

Cup #	Amount Jello	Amount Pineapple	Amount Extract	Outcome
1	20 mL	3 mL	None	Completely liquid after 1.5 hours
2	20 mL	3 mL	5 mL	Completely liquid after 1.5 hours
3	20 mL	3 mL	8 mL	Slightly more viscous than control after 1.5 hours
4	20 mL	3 mL	10 mL	Consistency of hair gel after 1.5 hours – mix of gelled and liquid parts

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