Question: Do you have any temp/time study for the inactivation of bromelain in pineapple? Trying to use frozen pineapple instead of canning for a meat product.

Response:

In summary, the literature suggests that incubation at 80°C for at least 8 minutes should completely inactivate the bromelain in frozen pineapple (Jutamongkon and Charoenrein, 2010). About 80% of the activity would likely be lost by incubating at 70°C for 15 minutes, while less than half of the activity would be lost by incubating at 60°C for 30 minutes (Hale et al., 2005).

However, the thermal stability of bromelain is dependent on the pH; it is more stable at pH values near its isoelectric point (4.6 for fruit bromelain) than at lower pHs (Sriwatanapongse et al., 2000). At a pH of 3.4, shorter incubations/ lower temperatures (60° for 25 minutes reduced activity by 90%, while 67°C for 5 minutes completely inactivated the activity) appear to be sufficient to inactivate the enzyme.

Here are the details:

- Bromelain is a **mixture** of cysteine proteases extracted from pineapple (Ramli et al., 2018).
- Bromelain itself has attracted interest in recent years for medical and commercial uses; most studies are therefore more interested in how to **maintain** its activity in **relatively purified states** than to ensure its destruction. A lot of the commercial bromelain does not come from the fruit but from other parts of the pineapple plant (which might be considered waste products).
- Different sources of bromelain (**stem** vs. **fruit** vs. **leaf** vs**. peel** bromelain) from pineapple may differ in activities and inactivation parameters. Fruit bromelain has been reported to have "higher proteolytic activity" than stem bromelain (Koroleva et al., 2021).
- Most forms have their greatest enzymatic activity at around 50-60°C (Sarkar et al., 2017).
- Bromelain may exhibit different activity depending on the substrate (casein, synthetic peptide, etc.) and also depending on pH and how concentrated it is (commercial preparations can be concentrated and may be more stable). Polyphenols and other components of the fruit may bind and stabilize bromelain.

Temperature inactivation of fruit bromelain has been discussed in the following papers:

- Jutamongkon and Charoenrein (2010) looked at the thermal stability of bromelain from pineapple juice extracted from the fleshy part of pineapple and buffered at pH 8.0 and found "The temperature stability profiles as a function of different time intervals showed higher retention of enzyme activity at low temperature. **Incubation at 40°C showed no loss of fruit bromelain activity up to 60 min, whereas at 50°C almost 83% of activity remained. Incubation at 80°C for 8 min caused almost complete activity loss.** Thermal inactivation of fruit bromelain in the temperature range 40-80°C was described by a first-order model." See Figure 2 from that paper for a graph of the data.
- This paper and others calculated and compared activation energies for inactivation of bromelain from various sources, concluding that bromelain from pineapple fruit is more stable at a higher temperature than bromelain from pineapple stems (Jutamongkon and Charoenrein, 2010).
- Another paper (Hale et al., 2005) looked at the stability of "natural" bromelain from fresh pineapple (mostly fruit bromelain) in water (pH not specified): "Slightly more than half of the original proteolytic activity remained after 30 min incubation at 60°C (60% and 53% vs. the Z-Arg-Arg-pNA and Bz-Phe-Val-Arg-pNA substrates, respectively). Activity against these 2

substrates ranged from 9% to 22% of original after 15 min incubation at 70°C. All proteolytic activity was consistently lost when bromelain solutions were heated at 100°C for 1 to 10 min.

- An older paper (Sriwatanapongse et al., 2000) looked at the effect of temperature on bromelain activity in pineapple juice at pH 3.4 and found residual activity could more easily be inactivated, with ~complete inactivation within 20 minutes when treating at temperatures of 63°C or higher (see Figure 2 in that paper).
- The Koroleva paper (Koroleva et al., 2021) looked at **stem** bromelain's stability at different temperatures (see graph below). Although they didn't look at early timepoints, 30 minutes at 80 degrees appears to be more than sufficient eliminate all activity.
- Another paper (Ramalingam et al., 2012) looked at thermal stability of bromelain from **fruit pulp extract vs. leaf extract:** "Both (crown leaf and fruit pulp bromelain) were active when incubated at 50ºC for 15 minutes. **At higher temperatures, the enzyme was gradually inactivated. The complete inactivation was observed for crown leaf at 70°C and the fruit pulp bromelain showed complete inactivation at 80°C.**
- Another paper (Silvestre et al., 2012) reported that inactivation of **peel** bromelain occurred at 90°C. Peel bromelain was also shown to require higher activation temperatures/length of time for inactivation in another study (Zhou et al., 2021).

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