FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Guidance for Thermal Inactivation of Hepatitis A virus in Berries

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1 Summary

- Food Standards Australia New Zealand (FSANZ) and the Ministry for Primary Industries have developed the following guidance for the inactivation of hepatitis A virus in berry fruits.

- MPI and FSANZ recommend cooking food to 85°C for 1 minute to inactivate hepatitis A virus but recognise that the extent of virus inactivation is influenced by the food matrix.

- Should alternative, generally lower, processing temperatures be required, this report presents guidelines temperature/time equivalents for the inactivation of hepatitis A virus based on available experimental data and according to the product characteristics (e.g. pH and sugar content).

- In the absence of data for all berry types, manufacturers should apply the temperature/time equivalent guidelines closely corresponding to the characteristics (e.g. pH and sugar content) of their products.

- The D-values and temperature/time equivalents described in this document are guidelines only. Manufacturers should conduct a proper risk assessment taking into consideration the likely types and levels of microbial hazards present in their raw materials, characteristics of their own products in terms of effect on the hazards, and the treatment parameters required to inactivate any hazards that are reasonably likely to occur.

2 Background

The thermal stability of hepatitis A virus is highly dependent on the food matrix. For example, FSANZ[1] states that complete inactivation of hepatitis A virus has been observed in shellfish when heated to 85°C for 3 minutes while maintaining a commercially acceptable product. Similarly, heating milk and cream to 85°C for 30 seconds is sufficient to cause a 5 $\log_{10}$ reduction in hepatitis A virus titre.

The United States *Centers for Disease Control* (US CDC) have reported that hepatitis A virus is inactivated at temperatures of 85°C for 1 minute, based on studies using laboratory media (US CDC 2004). This is consistent with the World Health Organization's statement on hepatitis A virus inactivation (WHO 2000). Notwithstanding this general guidance, there remains uncertainty regarding the level of reduction of hepatitis A virus that would be specifically achieved in berry products (Codex 2012, Baert *et al* 2009 and Baert 2013).

MPI and FSANZ are aware that food processors may be cooking their berry products at various temperatures and times, and guidance on temperature/time combinations for determining inactivation has been requested.

[1] FSANZ – Agents of Food Borne Illness (http://www.foodstandards.gov.au/publications/Pages/agentsoffoodborneill5155.aspx)

3 Published guidance on hepatitis A virus thermal inactivation in berry products

MPI and FSANZ are not aware of published D values[2] or temperature/time equivalence tables for thermal inactivation of hepatitis A virus in berry fruits.

4 Guidelines for thermal inactivation in berries

MPI and FSANZ recommend cooking food to 85°C for 1 minute to inactivate hepatitis A virus but recognise that the extent of virus inactivation is influenced by the food matrix.

The following guidelines based on available experimental data should be considered when evaluating the processing parameters required for thermal inactivation in berries with different product characteristics (pH and sugar content) and alternative temperatures.

There have been a small number of studies as described in recent reviews (Baert *et al* 2013, Sanchez *et al* 2015), although all are limited in that thermal resistance studies were carried out in capillary tubes, not commercial manufacturing operations, and used tissue culture adapted hepatitis A virus strains and may not be representative of wild-type viruses. Notwithstanding these limitations, the studies provide useful guidance for determining adequate temperature-time combinations for inactivation of hepatitis A virus in berry fruits.

Based on available experimental data, the variables with the greatest influence on thermal resistance of hepatitis A virus in berries are the concentration of sugar (measured as °Brix) and pH. Normal characteristics of berry fruits are:

- natural sugar content of berries = 5°Brix (5% wt/wt)
- pH of various berries: 2.3 to 4.5, (ICMSF, 2005; FDA, 2012)

Fruit	pH
Blackberry	3.0 – 4.5
Blueberry	3.2 – 3.7
Cranberry	2.3 – 2.7
Raspberry	2.9 – 3.7
Redcurrant	2.9
Strawberry	2.3 – 3.9

The level of hepatitis A virus in contaminated berries reported internationally is largely unknown due mainly to limitations in laboratory methods.

A target 6-log reduction would be considered to provide satisfactory assurance for control of the hazard. Lower log-reductions may be applicable if improved methods and the availability

[2] Time required for a 1 $\log_{10}$ reduction in concentration of viral particles

of more robust data could provide stronger assurances on the microbiological status of the fruit being processed.

4.1 NATURAL FRUITS (NO ADDED SUGAR)

Inactivation of hepatitis A virus has been described for some fruits with no added sugar and a natural pH (Deboosere et al 2010) (Table 1), and different time-temperature combinations calculated to provide some guidelines for a 6-log reduction.

Table 1 Calculated time required for 6-log reduction of HAV at 3 temperatures in natural Strawberries, Raspberries and Bilberries[3].

Fruit	Natural pH	Time required for 6-log reduction (minutes)		
		65°C	70°C	75°C
Strawberries	3.35	18	9	6
Raspberries	3.05	9	6	4
Bilberries	2.87	12	7	5

As these calculations are based on a mathematical model specific to this particular temperature range validated with a limited number of experimental data, it is not possible to predict the thermal inactivation of hepatitis A virus in berries for temperatures outside the limits of this table.

It is recommended for other fruits not included in this table that the manufacturers measure the pH of their fruits, take into account other factors such as the natural sugar content, and apply the guidelines corresponding to the closest characteristics.

4.2 GUIDELINES FOR PROCESSED FRUITS

The effect of added sucrose (sugar) and pH on $D_{85°C}$ values for inactivation of hepatitis A virus in strawberry mashes is described in Table 2 (Deboosere *et al* 2004).

Products with added sugar at less than 28°Brix would achieve a greater level of hepatitis A virus inactivation at 85°C than that indicated in Table 2.

Deboosere *et al* (2004) then described D-values observed in fruit mashes at different temperatures and concentrations of added sugar (Table 3).

Again, manufacturers should apply the guidelines corresponding to the closest characteristics of their products and ensure that products are heated to the selected temperature (at the slowest heating point) and held for the corresponding time.

[3] Extrapolated from linear portion of inactivation model.

Table 2 Calculated time required for 4, 5 and 6-log reductions of hepatitis A virus at 85°C in strawberry mashes at different levels of sugar and pH.

Sucrose concentration (°Brix)	pH	$D_{85°C}$ (minutes)	Time required for 4-log reduction (minutes)	Time required for 5-log reduction (minutes)	Time required for 6-log reduction (minutes)
28	3.8	0.96	4	5	6
40	3.3	1.04	5	6	7
40	3.8	2.37	10	12	15
40	4.3	2.78	12	14	17
52	3.8	4.98	20	25	30

Table 3 D-values for hepatitis A virus in fruit mashes at different temperatures and concentrations of added sugar.

Sucrose concentration (°Brix)	D value (95% confidence interval) at different temperatures			z value (°C)
	80°C	85°C	90°C	
28	1.22 (1.17, 1.27)	0.96 (0.69, 1.56)	0.32 (0.21, 0.68)	21.41
52	8.94 (8.59, 9.32)	4.98 (4.50, 5.58)	3.00 (2.65, 3.45)	21.10

4.3 GUIDELINES FOR OTHER THERMAL PROCESSES: FREEZE-DRYING

Freeze-dried berries are commonly used by the food industry in diverse products (e.g. cereals, muesli bars, chocolate products, bakery goods, ice creams) as the freeze-drying process maintains the organoleptic qualities of the fruits. Freeze-drying usually involves initially freezing the fresh berries, followed by heating the berries under reduced pressure to sublimating the ice.

Only one study has investigated the effects of freeze-drying on inactivation of hepatitis A virus (Butot *et al* 2009). Using experimentally inoculated fresh berries, freeze-drying at 55°C for 10 hours in the product reduced the numbers of hepatitis A virus on most of the products by less than 1 $\log_{10}$ unit (as determined by real-time RT-PCR).

Therefore, in the absence of other available data, it is recommended that manufacturers add a supplementary heating step after the freeze-drying process. For example, Butot *et al* (2009) suggests that heating freeze-dried berries for 20 min at 120°C inactivates infectious hepatitis A virus.

Note: The D-values and temperature/time equivalents described above are guidelines only. Manufacturers should conduct a proper risk assessment taking into consideration the likely types and levels of microbial hazards present in their raw materials, characteristics of their own products in terms of effect on the hazards, and the treatment parameters required to inactivate any hazards that are reasonably likely to occur.

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