The Pulsifier®

An Improved Instrument for the Preparation of Food Samples for Microbiological Analysis

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Introduction

Micro-organisms, including pathogenic bacteria, attach with varying affinities to the surface of food samples. Conventional paddle-type food sample processors process samples by crushing until a homogenate e.g. a puree has been produced. The Pulsifier®, Figure 1 (Microgen Bioproducts Ltd, Surrey, UK) provides an alternative technology for the processing of food samples for microbiological analysis. Rather than beating or crushing food samples the Pulsifier® produces a combination of shock waves and intense stirring using a rapidly oscillating ring, Figure 2. The resultant shock waves and intense agitation liberate micro-organisms whilst leaving the food sample essentially intact. Due to the minimal food matrix destruction, the resultant "pulsificate" is easy to pipette, filter and free of EIA and PCR inhibitors.

Trials were performed to determine the efficiency of the "pulsification" process using a number of important food pathogens (***E.coli*** O157, ***Salmonella* spp** and ***Listeria monocytogenes***) for a range of soft and hard food types.

Figure 1. *Pulsifier®, showing transparent observation window and programming key pad.*

Materials and Methods

Food Samples:

Separate batches of frozen peas, minced beef and potato powder were purchased from retail supermarkets and 25gm samples were examined to ensure that they were free from the target organisms. The absence of ***Listeria monocytogenes*** and ***Salmonella* spp** was confirmed using the methods described in BS 5763: Part 18 and Part 4 respectively. The absence of ***E.coli*** O157 was confirmed by enrichment in mTSB+n for 6 hours at 42°C, followed by immunomagnetic separation and culture on CT-SMAC which was incubated at 37°C for 24 hours.

Enumeration of Key Pathogens:

In the case of ***Salmonella* spp** and ***E.coli*** O157, four 25gm samples of each food type were inoculated with approximately 10^2 cells/ gm. The samples were placed into 225ml of an appropriate pre enrichment medium after which 2 were placed in the Pulsifier® for 60 seconds and 2 placed in a Seward Stomacher® 400 for 60 seconds.

Figure 2. *Pulsifier®, showing oscillating ring.*

The evaluation of the recovery of ***Listeria monocytogenes*** was performed using four 10gm samples of each food type. Each sample was processed as previously described with the exception that samples were diluted in 90ml Buffered Peptone Water after which they were processed using either the Pulsifier® or Stomacher® as described previously.

To determine the release of the respective pathogens after processing using the Pulsifier® or Stomacher®, plate counts were performed in the following manner:

- ***Salmonella* spp**: 0.1ml samples were plated onto XLD at T=0 and after 18 hours incubation at 37°C. Plates were incubated at 37°C for 24 hours.
- ***E.coli*** O157: after incubation of the pre enrichment broth for 6 hours at 42°C (pea and beef homogenates) and 37°C (potato homogenate), 0.1ml samples were removed and plated onto CHROMagar® 0157 and incubated at 42°C or 37°C for 24 hours.
- ***Listeria monocytogenes***: after samples were processed using the Pulsifier® or Stomacher®, they were incubated at 20°C for 1 hour after which 0.1ml samples were removed and plated onto PALCAM agar and incubated at 37°C for 48 hours.

After incubation, plates containing < 150 colonies were selected and mean colony counts of the specific pathogens performed.

Results:

Analysis of both the initial (T=0) and final (T=18) plate counts for ***Salmonella* spp** (Table 1.) demonstrated that there was no significant difference between the counts obtained at either time using either the Pulsifier® or Stomacher®.

The counts performed after 6 hours pre enrichment for ***E.coli*** O157 and ***Listeria monocytogenes*** after 1 hour pre enrichment are found in Table 2. Once again, no significant difference in counts was detected.

Sample	Method	Log_{10}cfu/gm (T=0)	Log_{10}cfu/gm (T=18)
Peas	Pulsifier®	3.13	7.58
	Stomacher®	3.08	7.17
Minced Beef	Pulsifier®	3.13	9.48
	Stomacher®	3.21	9.63
Potato	Pulsifier®	2.82	9.67
	Stomacher®	3.04	9.68

Table 1. *Recovery of* ***Salmonella* spp** *after stomaching or pulsification.*

Sample	Organism	Method	Log_{10}cfu/gm
Peas	***E.coli*** O157	Pulsifier®	4.90
		Stomacher®	5.65
Minced Beef	***E.coli*** O157	Pulsifier®	5.29
		Stomacher®	5.63
Potato	***E.coli*** O157	Pulsifier®	4.13
		Stomacher®	3.89
Peas	***Listeria monocytogenes***	Pulsifier®	3.54
		Stomacher®	3.68
Minced Beef	***Listeria monocytogenes***	Pulsifier®	3.79
		Stomacher®	3.76
Potato	***Listeria monocytogenes***	Pulsifier®	3.55
		Stomacher®	3.45

Table 2. *Recovery of* ***E.coli*** *O157 and* ***Listeria monocytogenes*** *after stomaching or pulsification.*

Conclusion:

Analysis of the data resulting from this evaluation demonstrated that there was no significant difference in the counts obtained with either stomaching or pulsifying samples.

This data confirms the observations of other workers who have determined that the Pulsifier® is equally as good as the Stomacher® for the recovery of bacteria from a wide variety of food samples (1,2).

In addition, the Pulsifier® was found to offer a number of additional significant advantages over the Stomacher® including:

Figure 3. *Pulsified meat demonstrating minimal destruction of the food matrix.*

- **Cleaner samples (Figure 3):**
  - Allows the supernatant to be easily drawn off.
  - Allows more accurate pipetting and less clogging.
  - Reduces the need for filter bags.
  - Facilitates the use of filtration methods (2), automatic platers and Petri Film®.
- **Less destruction of the sample:**
  - Lower incidence of bag breakages with hard foods.
  - Minimal release of food enzymes which may effect bacterial viability.
  - Minimal release of PCR and EIA inhibiting substances.

References:

1. Fung D.Y.C., A.N. Sharpe, B.C. Hart and Y.Liu. (1998) The Pulsifier: A new instrument for preparing food suspensions for microbiological Analysis. J Rapid Methods Automation in Microbiol **6**: 43 – 49.
2. Sharpe A.N, E.M. Hearn and J. Kovacs – Nolan (2000) Comparison of Membrane Filtration and Hydrophobic Grid Membrane Filter Coliform and Escherichia coli Counts in Food Suspensions Using Paddle-Type and Pulsifier Sample Preparation Procedures. J Food Protection **63**: 126 - 130.