

Self-contained Food Sample Homogenisation Filter Bag for Microbial Analysis

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ABSTRACT

The complexity of food materials owing to the diverse matrices and biochemical composition poses challenge to microbiologists especially to identify the microbial contamination at low level. The present study describes the development and evaluation of a ready to use self-contained food sample homogenisation bag (All-In-Bag) with the required sterile diluent and an in-built filter for subsequent clarification of the homogenate for microbiological analysis. Three-ply non-foil laminate comprising outer alumina oxide coated polyester film, middle nylon and inner polypropylene layers were used for the outer layers while non-woven polypropylene sheet with of 50 μ to 100 μ size porosity was sandwiched between the laminated sheets to restrain the food debris but allow the microbial cells to pass through across along with the diluent. The homogenisation bag along with the diluent was sterilised by thermal (retort) processing with F_0 value (lethality value) of 12 to ensure the sterility of diluent during storage. The effectiveness of the All-in-Bag for the homogenisation of different food sample matrices for microbiological analysis was compared with BagPage®+ bag. All-in-Bag withstood the shearing action during sample paddling in the bag mixer/stomacher and no significant difference was observed for both aerobic plate count. Spike and recovery of *E. coli* from the different food matrices indicating absence of interference for microbial recovery in newly developed All-in-Bag. The All-in-Bag, the first of its kind with 12 months shelf life does away with the requirement of sterile diluent preparation and additional steps for the clarification of the homogenate and thus making microbial food quality analysis easier in places with limited resources.

Keywords: Food sample preparation; Homogenisation filter bag; Microbiology analysis; Stomacher bag

1. INTRODUCTION

Food materials are complex in nature with matrices of varied texture, granularity and biochemical composition. This complexity poses challenge to microbiologists especially when screening for low levels of specific microbes in the samples having particulate matter of high viscosity, high fat content or due to formation of macromolecular networks upon addition of water⁴. Sample preparation, a prerequisite step for food microbiological analysis is aimed to achieve homogenisation and separation of target microorganisms from the food matrices³. Homogenisation is a very important sample preparation step in food microbiological analysis that significantly influences the enumeration and detection of the microbial load from diverse food matrices like meat^{15,23}, frozen vegetables²², and egg²⁷. General rules of sample preparation for the initial suspension and subsequent dilutions for microbiological examination have been described for food and feed products¹¹.

Initial suspension of food samples for microbiology analysis is mainly done by mixing along with diluents by hand stirring, warring blender²⁷, pulsifier¹⁴, and paddle-type processor (stomacher) where in the food sample and diluents added into a sterile homogeniser bags are vigorously pounded on outer surfaces by paddles moment²⁹. Paddle type homogeniser cause very negligible temperature rise during homogenisation and

do not necessitate cleaning and sterilisation of used blenders/homogeniser cups²⁴. Significantly higher levels of microbial recovery has been reported from food homogenates prepared with paddle type processor compared with other methods and thus a favoured method for preparing suspensions for food microbiological analysis when compared to other homogenisation methods^{19,21,25,26,28,29}.

Conventional homogeniser bags used with the paddle-type processor are two to three -ply polymer laminates heat sealed at three sides and one side left open for addition of food samples and diluents for sample homogenisation²⁴. Samples drawn from the initial suspension after homogenisation through sterile pipettes are used for subsequent inoculation in microbiological media. However, the pipetting is often constrained by blockage with food particles and fatty materials from homogenised samples. Hence the homogenate has to be either left aside for settling of larger particles or necessarily transferred to another container with filter thereby making this conventional homogenisation bag less user-friendly. The use of disposable pre-filters before pipetting greatly improved the clarity of food suspension, improving the ease of subsequent liquid handling²⁰. To make the homogenisation bag more user friendly, a filter sheet was sandwiched between the two outer layers thereby dividing the bag into 2 chambers; sample is put into one of the two chambers and the resulting filtered extract is taken out by a pipette from another chamber and named 'filter bag'¹³. Nevertheless there is a requirement for the preparation

of sterile diluent separately for use during homogenisation, which becomes a challenge in resource poor settings for the routine microbiological analysis. Advances in molecular technologies and automated instrumentation have provided many opportunities for improved detection and identification of microorganisms; however, the upstream sample preparation steps needed to implement these advances to foods have not been adequately addressed³.

Hence in this present study, we designed All-in-Bag as self-contained sterile homogenisation bag with the diluent for homogenisation and inbuilt filter to draw clear samples for subsequent microbiological analysis. All-in-bag possessed sufficient thermal, mechanical properties to withstand retorting and paddling action during the homogenisation and barrier properties to facilitate its longer shelf life in room temperature.

2. MATERIALS AND METHODS

2.1 Packaging Materials, Chemicals and Food Samples

In this present study, alumina oxide (AlOx) coated polyester/nylon/cast polypropylene (PET/NY/PPP) laminate of 110 µm thickness and polypropylene filter sheet (75 gsm) were used for the homogeniser bag fabrication. Chemicals and culture media were obtained from Hi-Media laboratory, India. Samples from four different types food; fresh chicken, ready to eat vegetable rice, dehydrated papaya and *sooji halwa* (fat rich food with 15% hydrogenated oil) having distinct matrices sourced from local market and cafeteria were used to evaluate homogenisation process for microbiological analysis with homogeniser bag BagPage®+ from Interscience, France was used for comparative study.

2.2 Bag Design and Construction

Homogenisation bag was designed to be of rectangular shape with 200 mm x 300 mm dimension and constructed with two sheets of PET/NY/PPP laminate with a polypropylene filter sheet sandwiched between them to act as filter to restrain the food debris. The sides and bottom were heat sealed by using high-pressure impulse sealer with 10 mm seal width (Model: HP Impulse Sealer, M/S Sunray Industries Mysore, India) at 3 s sealing time at 160 °C and 10 s dwell time with cold water circulation. The top side was kept open for diluent filling purpose.

2.3 Mechanical and Barrier Properties of the Bag

The PET/NY/PPP laminated sheet and non-woven polypropylene sheet were cut into required sizes of 25.4 mm x 100 mm for tensile strength and percentage of elongation, 50mm x 50 mm for tear strength and 25.4 mm x 100 mm size for the seal strength of homogenisation bag and analysed using universal testing machine (UTM) with 1 KN load cell (Lloyd instrument Ltd, Steyning Way, UK) as per ASTM D882-02, 2002 method¹. The mechanical properties of the samples were tested with five determinations. The Gas transmission rate (GTR) was tested with, monomeric gas permeability tester (Lyssy L100-5000, Switzerland) and water vapour transmission rate (WVTR) was tested using the PERMATRAN-W® Model 3/33, USA.

2.4 Sterilisation of Diluent

Peptone salt diluent (225 ml) was prepared as per ISO 6887-1 method; and manually filled through the open side of the bag. Headspace air was removed and the open side of the bag was hermetically sealed by an impulse heat sealer as described in 2.2. The sealed pouch looked like a pillow pouch having diluent in both compartments (Fig. 2) and steam sterilised using a steam-air retort (M/s Alpha Steritech, Bangalore, India). The retort was provided with facilities for using compressed air for over-riding pressure and a high-pressure water-circulating pump for cooling. The temperature and pressure of the diluents were continuously recorded during sterilisation processing through temperature monitoring system, which was fixed at the geometric centres of the bags and connected to a monitoring system (Model: E. Val flex, M/s. Ellab, Denmark). The pressure and temperature of the retort were raised progressively with steam and air to increase the temperature up to 121 °C. The processing was carried out to achieve a F_{10}^{121} value of 12. After attaining the required lethality value (F_0), the product temperature was brought down to 40 °C - 45 °C by pressurised cooling (compressed air and water) within 10 minutes. The cooled bags were wiped dry and examined for any visual physical damages. Two bags each were incubated at 35 °C and 55 °C for 7 days and tested for microbial sterility of the diluent by testing the mesophilic, thermophilic aerobic and anaerobic spore growth¹⁸.

2.5. Microbial Analysis of Food Samples

Food samples described in section 2.1 were homogenised with both newly developed All-in-Bag and commercially available BagPage®+ to evaluate and compare the effectiveness of the All-in-bag for homogenisation and food microbiological analysis. Sample preparation has been done according to applicable parts of ISO 6667-1 2017¹¹. During sample preparation, the upper portion of the bag was cut open using sterile scissors after wiping with alcohol and 25gm of food sample was put in one of the compartments and allowed for mixing-cum-homogenisation in a stomacher®, model BA 6021 (M/s. Seward, UCA House, London, UK) for 3 min¹⁰. After homogenisation, filtered extract was collected from the other compartment for further dilution and plating. The filtrate from the food samples was 10⁻¹ dilution and 10 fold serial dilutions till 10⁻⁵ were prepared by transferring 1 ml of the homogenate to 9 ml of sterile diluent. 1 ml of each dilution was transferred into the sterile Petri dish and 15 to 20 ml plate count agar media was added and gently swirled to achieve uniform mixing of sample with agar media. Plating was done in triplicate and plates were incubated at 30 °C for 72 h¹². Bacterial colonies in the plates were counted manually with colony counter to obtain the aerobic plate count. Mean values of plate count of samples plated from both the bags were recorded for further calculations.

The effectiveness of the filter with respect to specific microorganism, *E. coli* ATCC 10536 (10⁵ cfu/g) when spiked into 100 g of each food samples of different matrix as described in section 2.1 that were otherwise *E. coli* negative. 25 g sample of each spiked food sample was collected and homogenised using BagPage®+ and All-in-Bag separately and homogenised

samples were serially diluted till 10^{-5} in buffered peptone water and plating was done in triplicate using MUG violet red bile agar⁶ and plates were incubated at 35 °C for 24 h. After incubation, bluish fluorescence colonies were observed under UV light and counted. Mean values of plate count of samples plated from both the bags were recorded for further calculations.

2.6 Statistical Analysis

Every analysis 5 samples trials were conducted in duplicate and mean counts for each trial were used to calculate arithmetic means, standard deviations, two-tailed unpaired student's t-test and F test for variation. To evaluate the significance of the results and the variance between the two types of bags; p values below 0.05 were considered as significant t-test statistic value is determined using unpaired two sample t-test formula and the critical value of Student's t-distribution read in t-test table corresponding to the significance level alpha (5%)^{30, 8}.

3. RESULT AND DISCUSSION

3.1 Bag Design and Mechanical Properties

The All-in-Bag described in the present study was designed as a ready to use self contained bag/pouch with the required sterile diluent for homogenisation and an inbuilt filter to separate the particulate and liquid portion in the homogenate. The materials employed/used in the bag were chosen to provide mechanical and thermal properties to withstand the strenuous retort pressure and temperature during thermal processing to sanitise the diluents, provide barrier properties to retain the shelf life of the diluents and the shearing action during sample paddling in the bag mixer/ stomacher. The detailed structure of All-in-Bag layers is given in Fig. 1. The outer layers of the pouch are composed of 3 ply laminated sheet comprising inner polypropylene layer to give adequate seal strength, middle nylon and outer alumina oxide (AlOx) coated polyester film to provide tensile, tear strength and barrier properties. Similar type of clear high-barrier non foil retort packaging films are used for packaging retort processed food for military meals ready to eat (MRE) owing to their transparent nature and possibility for microwave warming before consumption^{7,5}. The non-foil transparent laminate used in this work allowed monitoring of the diluents clarity and extent of homogenisation. Blockage of the pipettes by the residual particulate matter after homogenisation is a major problem observed during the aseptic transfer of the liquid portion to microbiological media. This is addressed by incorporating nylon filter to the homogenisation bags or polyethylene mesh to the filter tips²⁰. We used non-woven polypropylene (75 gsm) sheet with porosity of 50 μ to 100 μ size and sandwiched between the laminated sheets to act as a filter to restrain the food debris but allow the microbial cells to pass through across along with the diluent. The tensile strength, percentage elongation of non-woven polypropylene filter sheet was observed to be 69.19 \pm 0.32 kgf and 111 \pm 8 % respectively and tear strength was 1498 \pm 12 g. Due to porous nature of the filter material, much stress load was not observed during the paddle action of homogenisation. After the sample preparation the non-woven polypropylene was intact without any damage and hence this material found to be suitable for the application.

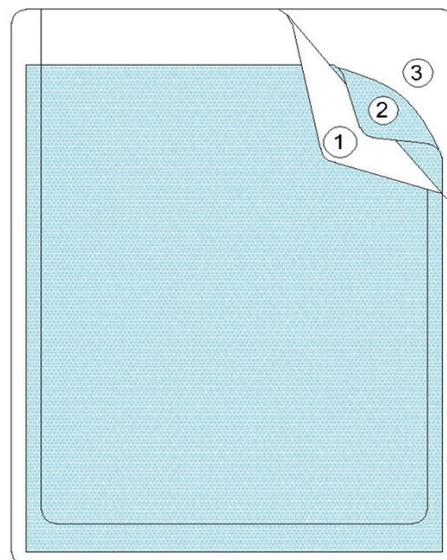


Figure 1. The rectangular shape homogeniser bag includes a first flexible laminated sheet (1), a second non-woven polypropylene sheet (2) permeable to liquid but not to solid matter, and a third flexible laminated sheet (3). The second sheet (2) is sandwiched between the first (1) and third sheet (3), and edges of three sides of all the sheets are heat sealed.

Polypropylene filter has been extensively used in liquid-solid filtration due to sleekness of surface, good resistance to acids and alkalis and low moisture absorption^{9,31}.

We compared the present All-In-Bag with commercially available BagPage®+ from Interscience, a full-page irradiated, nonwoven multilayer filter bag of 80 micron thickness for intrinsic mechanical properties and functional properties like sample filtration after the food sample blending. As the present All-In-Bag is designed to be self-contained with diluents, steam sterilisation processing was required; hence it was made with three layer, 110 μ thickness, heat resistant polymer laminate with better mechanical properties. The All-In Bag demonstrated 76%, 9.4% and 27% higher tensile, tear and seal strength respectively than the commercially available BagPage®+ filter bag (Table 1). The higher seal strength 4.0 \pm 0.43 kg/10 mm achieved in the present bag helped withstanding internal pressure during sterilisation along with the diluents. These mechanical properties of the present All-In-Bag is analogous to the foil based retort pouches with seal strength of 3.96 kg/10 mm used by us earlier for retort processing of tender jack fruit curry¹⁷. The barrier properties provided by outer ply of the laminated sheet play an important role in achieving longer shelf life of the diluent. The non-foil laminate material has water vapour rate 0.4 g/m²/day and gas transmission rate 0.25 ml/m²/day. Gas transmission rate of retort pouch materials is an important parameter that determines the chemical deterioration of packed product, especially oxidative spoilage. The present packaging material exhibited lower GTR on par with similar packaging materials used for microwave-sterilised mashed potato preservation even though the bag contained peptone salt diluent solution free from fat and very less

Table 1. Mechanical properties of packaging materials

Parameter	BagPage®+	All-In-Bag
Total thickness (µm)	80±1	110±2
Tensile strength (kgf)	416±8.8	733±43
Elongation at break (%)	80.7±11.46	46.83±6
Tearing strength (g)	108±8	116±8
Seal strength (kg/10 mm)	3.15±0.33	4.0±0.43
Gas transmission rate (ml/m ² /day)	--	0.25±0.01
Water vapor rate (g/m ² /day)	--	0.40±0.01
Total migration (mg/kg)	--	45±0.51

Values are presented as mean ± SD, (n=5)

prone to oxidative spoilage³². The slight weight loss observed during the storage is offset by the provision of a secondary; foil based 110 µ packaging material (PET/Aluminium foil/Nylon/Cast Polypropylene) was provide to accommodate 5 numbers of All-In-Bags. The use of polypropylene with less elongation properties in the inner ply of the laminate reduced the percentage of elongation at break against BagPage®+ filter bag and is required to retain the shape of the bag structure during thermal processing and sample blending. The All-In-Bag was simple, easy to fabricate, economical, and safe to use as well as it can be done in any dimension.

3.2 Validation of Sterilisation

The retort process was aimed to achieve F0 value (lethality value) of 12 to ensure the sterility of diluent during storage. The lethality value was calculated based *Geobacillus stearothermophilus* spores with D value of 1.5 min at 121 °C². The retort and internal temperatures of the diluent in the All-In-Bag were recorded through real time temperature monitoring systems during steam sterilisation. The come up time was 7 min to reach sterilisation temperature of 121 °C and the retort was maintained at 121 °C for holding period to achieve 12 lethality values. As the bag contained only diluent, the heat penetration profile was close to that of the retort with convection heat penetration profile and just 2 min difference was observed. The heat penetration observed during come up time was more rapid when compared to that during our egg curry preservation process where conduction heat penetration profile was observed due to the solid nature of the food¹⁶. Retort temperature and heat penetration profiles of diluent with process lethality are presented in Fig 3. The PET/Nylon/cast Polypropylene laminate along with polypropylene filter layer of All-In-Bag were found to be withstanding the sterilisation temperatures and thus no physical changes or damages such as de-lamination or seal opening were observed after sterilisation. Similar type of retort pouches were used earlier for microwave assisted thermal processing of potato³². The sterility of the diluent was ensured by microbial testing and no microbial growth was observed even after 7 days of incubation at 35 °C and 55 °C under aerobic and anaerobic conditions in bromocresol purple dextrose broth for aerobic organisms and cooked meat media for anaerobic microorganisms respectively¹⁸.

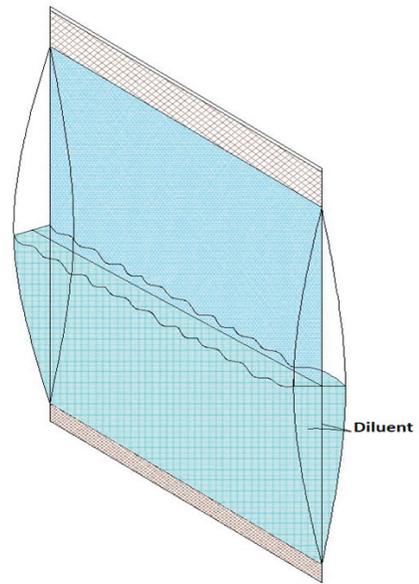


Figure 2. Illustrates an overall view of self- and self-contained homogeniser bag with filter and diluent. Any one of the compartments of the bag to receive and hold food samples for homogenisation. During homogenisation the sample extract is filtered through the non-woven polypropylene sheet and reaches to the other compartment; from there clear sample solution can be directly pipetted out for microbiological examination.

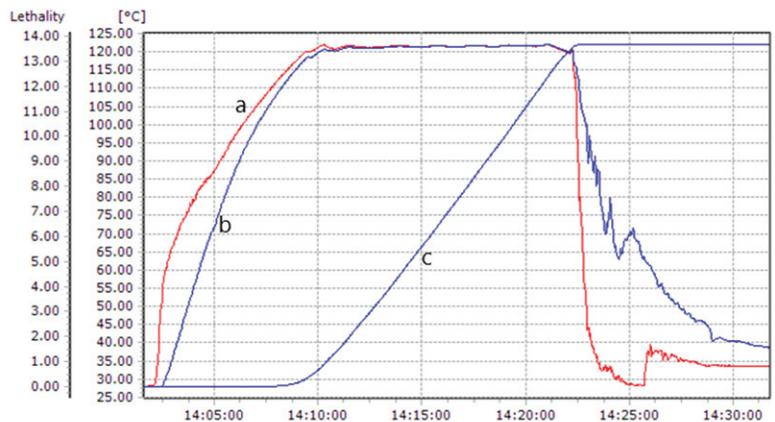


Figure 3. Heat penetration profile during steam sterilisation of diluent from (a) probe placed inside the retort to monitor the retort process temperature, (b) Probe placed inside the bag with diluent and (c) cumulative lethality value of the diluent temperature calculated from the data logger.

3.3. Sample Preparation and Microbial Analysis

The suitability of the All-In-Bag for the homogenisation of different food sample matrices for microbiological analysis was studied by examining 4 different food products such as fresh chicken, vegetable rice, *sooji halwa* and dehydrated papaya fruit. Fresh chicken comprises fibrous muscle tissue with fats and require proper clarification after blending. Vegetable *pulav* is a heterogeneous solid food product and contain rice, spices and chopped vegetables. *Sooji halwa* is another food

matrix that is pasty and greasy in nature and contains vegetable fat, sugar and semolina. We selected dehydrated very low moisture food, papaya fruit that require 10 min soaking for softening before homogenisation. Studies on the release of microorganisms from most of the food matrices recommend 15 s to 1 min of stomaching for routine microbiological analysis²⁴. However, we followed a uniform homogenisation time of 3 min for all samples as per ISO 7218:2007 otherwise recommended for microbial release from muscle and fat rich samples that resulted in very good homogenation with the newly developed All-in-Bag. As *sooji halwa* contained 15% hydrogenated oil, it was treated as fat rich processed food sample and 0.2g Tween 80 was added along with the bag diluent to improve emulsification and avoid adherence to the bag surface during homogenisation as recommended by ISO 6887-1 method¹¹. Five samples were homogenated for the aerobic plate count (APC) and *E. coli* count for each of the four food types both in All-In-Bag and BagPage®+ filter bags and the result from all the samples and provided as log 10 value of mean count and standard deviation calculated from repeated duplicate analysis of 5 sample results. Similar counts without significant difference was observed for aerobic plate count in samples plated from both the bags and recovery of *E. coli* from the spiked samples was 97.83 % to 98.72 % which indicated the absence of interference for microbial recovery in All-in-Bag (Table 3).

3.4 Statistical Analysis

Unpaired two sample t-test is used to compare the means of two bag samples results to see any significant difference between them. In an experiment, t-test is used to derive whether or not any difference seen between methods. A test for equality of these methods means was also performed on the transformed data by using the Student's t-test. The p-value result was not significant at p<0.05. This critical value exceeds the calculated value; therefore hypothesis H0 is not rejected. No significant difference in the performance of both the bag types was observed (Tables 2 & 3). Statistical analysis of the arithmetic mean and standard deviation was calculated and the variance estimated by using the F-test at a 0.05 significance level. The F-test showed no significant difference between the means of the results obtained from the BagPage and All-In-Bag sample preparations (Tables 2, 3).

Table 2. Aerobic plate count and statistical analysis

Food samples	Aerobic plate count log ₁₀ cfu/g	
	BagPage®+	All-In-Bag
Fresh chicken	5.54±0.017	5.55±0.022
Vegetable rice	2.19±0.015	2.18±0.011
<i>Sooji halwa</i>	2.17±0.013	2.19±0.020
Dehydrated papaya fruit	1.72±0.034	1.76±0.0.042
t-test for two independent (p-value)	0.988	
F Ratio of Variances	0.984	

values are presented as mean ± SD, (n=10) p>α (0.05) – Not significant

Table 3. Spiked and recovery of *E.coli* in different food matrix

Food samples	Spiked level log ₁₀ cfu/g	BagPage®+		All-In-Bag	
		log ₁₀ cfu/g	% of recovery	log ₁₀ cfu/g	% of recovery
Fresh chicken	4.47	4.42	98.72	4.40	98.28
Vegetable rice	4.47	4.40	98.28	4.38	97.83
Sooji halwa	4.47	4.38	97.83	4.39	98.05
Dehydrated papaya fruit	4.47	4.39	98.05	4.40	98.28

T test for two independent (p-value) = 0.728 p>α (0.05) – Not significant, F Ratio of Variances =1.066

4. CONCLUSION

All-In-Bag developed in this study is the first of its kind self-contained sample homogenisation bag that is ready to use, with sterile food sample diluent and filter to draw fluid samples free from solid particles for microbiological analysis. The three ply laminate and filter along with the sealing process used in the construction of the bag enabled it to withstand the thermal process conditions, provided barrier properties to retain the self-life of the diluents and withstand the paddle action of paddle type processor (Stomacher®). Comparative microbial enumeration from different food samples along with commercially available BagPage®+ revealed similar plate counts, thus proving its ease of use and utility in routine food microbiological analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of paper.

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