# The Effects of Temperature Abuse and Microbial Quality Changes of Thiobarbituric Acid on Tilapia sous vide during Refrigerated Storage

**Tarig. Abd Elmuniem. Mohamed. Ali[1], Russly.Abdul. Rahman[2], Jamilah. Bakar[3],Mujeeb Alrahman Kabbashi[4], Tarig Abdalrahman Qamar[5]**

1,4. Dep. of microbiology Faculty of medical laboratory science University of science and technology.

2,3. Dep. of Technology Faculty of food science university of Putra Malaysia

5.Dep. of parasitology Faculty of medical laboratory science University of science and technology.

## Abstract

**Background:** The *sous-vide* foods are vacuum packaged in high barrier pouches/packages to extend the shelf life, which provides a favourable growth environment for anaerobic psychrotrophic pathogens, before being subjected to pasteurization treatment. These products require refrigeration at temperatures as low as 3.3°C to prevent spoilage and guarantee microbiological protection. According to National Food Processors Association (NFPA, 1988), manufacturers should assume that temperature abuse will take place at some point during the distribution of a chilled food product. Surveys of retail food stores and consumer refrigeration units have shown that holding temperatures of > 10 °C are common (Daniels, 1991 and Hutton et al., 1991).

**Objective**: To determine the effects of the temperature abuse on Thiobarbituric acid and Microbiological analyses of tilapia *sous vide*during storage.

**Method**: Two different combinations of time and temperature were studied (15 min/70°C, 15 min/90°C). Evaluation of the product during stored attemperature abuse10°Cwas done periodically (0, 3, 7, 14, 21, 28, 35, 42,45days) to study its microbiological condition.

**Result**: The heat treatment at 90°C for 15 min was the most effective during 45 days of storage at 10°C. . Microorganisms such as*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes* were not detected in any of the samples. Figures 3.1 and 3.2 show the results of aerobic mesophile and anaerobic mesophile counts for the control, and samples processed at 70°C and 90°C and stored at 2°C and 10°C.The means for the

mesophiles and anaerobic mesophiles for the control were more than 6-log cfu/g in the first week of storage at 2°C and 10°C

**Conclusions:** The results showed that microbial, physical-chemical, sensory characteristics of *Sous vide* processing to 90°C were not consistently affected by the brief temperature abuse, but *Sous vide* processing to 70°C were affected by temperature abuse. These findings indicate that heat treatment at 90°C better than 70°C and temperature abuse had no consistent effect on tilapia *sous vide*.

**Keywords:** Tilapia *sous vide*. Thiobarbituric acid, Sensory characteristic, Microbiological Quality.

**Corresponding author:** tarig159@hotmail.com

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## Introduction

*Sous vide* has created huge interest in the food service and retail sectors in recent years as one particular form of enhanced cook-chill technology. *Sous-vide* means cooking under vacuum and includes a process where raw foods or half-cooked foods are placed in a plastic pouch or bag, hermetically sealed, and cooked slowly in a water bath at 65–95°C over prolonged time (usually from 1 to 7 h) Zavadlav,2020.The term originally comes from French, meaning simply under vacuum. The vacuum-packed food cooked in that state at low temperature. It also included foods cooked conventionally and simply vacuum packed.The *sous-vide* foods are vacuum packaged in high barrier pouches/packages to extend the shelf life, which provides a favourable growth

environment for anaerobic psychrotrophic pathogens, before being subjected to pasteurization treatment. These products require refrigeration at temperatures as low as 3.3°C to prevent spoilage and guarantee microbiological protection. Sufficient confirmation exists to certificate that temperature abuse is a common occurrence at both the retail and consumer levels. According to National Food Processors Association (NFPA, 1988), manufacturers should assume that temperature abuse will take place at some point during the distribution of a chilled food product. Surveys of retail food stores and consumer refrigeration units have shown that holding temperatures of > 10 °C are common (Daniels, 1991 and Hutton et al., 1991). Thus, it is unlikely to rely on refrigeration for the safety of *sous-vide* processed foods.The aim of this work is to determine the effects of the temperature abuse on microbial, physical-chemical and sensory of tilapia *sous vide*during storage.

**Materials and Methods**

Red Tilapia fillet (*Oreochromis Niloticus*) was purchased from Pasar Borong, Selangor. Fillets from tilapia were cut into portions of 100g; 3 g ofpalm oil, 0.3 g of salt, and 0.2g of black pepper were added.Each portion was packaged in laminated plastic boilable pouches which made from nylon(Polyamides) laminated withPolyethylene (PE). The pouches were heat-sealed using a vacuum sealing machine (VacMasterSVP-40, Kansas City, USA). Heat processing was carried out in a water bath (Memmert, Germany). The heating profiles of vacuum packed foods were obtained by locating a thermocouple (E-Val Flex with 16 channels, Ellab, Denmark) in the geometric centre of the sample. Two different combinations time and temperature treatments were tested: 90 °C for 15 min, and 70 °C for 15 min. After heat processing, products were immediately chilled using iced water. After cooling,the pouches were stored at 10°C for up to 45 days. Samples were taken from raw fish, immediately after cooling and after 3, 7, 14, 21,28,35,42 and 45 of storage. Experiments were carried out. The following microbiological analyses and Thiobarbituric aciddeterminations were made in each experiment.All analyses were performed in four replicates.

**2.1 Thiobarbituric acid (TBA)**

The Thiobarbituric acid test was used to assisting the extent of lipid oxidation in samples over the storage time and between types of samples, following the distillation method described by Tarladgis et al. (1960) (Pikul, Leszczynski, and Kummerow, 1983).10g of samples were homogenized with 50ml distilled water and the mixture was then washed into a distillation flask with 47.5 ml of distilled water. The pH of the mixture was adjusted by 2.5 ml of (4 N) hydrochloric acid to reach pH 1.5. The flask was heated by mantel heater until 50ml of distillate was collected. Five ml of distillate was pipetted into screw cap tube; 5 ml of 0.288% (w/v) TBA reagent in 90% glacial acetic acid were added. Stopper was the tubes, shaken well and heated in the boiling water for 35 minutes. A blank tube was prepared in the same way using 5 ml of distilled water and 5 ml of TBA reagent. All tubes were cooled in running water for 10 minutes and the absorbance was measured against blank at 538 nm by UV/VIS spectrophotometer (Genesys 20). TBA value was expressed as milligrams of malondialdehyde per kilograms of sample which equal to 7.8 D.

**Microbiological analyses**

Ten grams of Tilapia fillet were weighed aseptically and homogenized in a Stomacher (Stomacher, UK) for 2 minutes with 90 ml of sterile peptone water (0.1% peptone). Further decimal dilutions were made with the same diluent. The total number of Mesophilic aerobic microorganisms was determined on Plate Count Agar (PCA, Merck) following the pour plate method, incubated at 30 °C for 72 h (ICMSF, 1978). Mesophilic anaerobic were determined in PCA incubated under anaerobic conditions at 30 °C for 72 h (ICMSF, 1978).The presence of *Listeria Monocytogenes* was investigated by the following procedure: a 25 g sample was homogenized with 225 ml of *Listeria* Enrichment Broth (LEB, Merck) in a Stomacher. The enrichment broth was incubated at 30 °C for 48 h. LEB cultures were streaked onto Palcam agar and the plates were then incubated at 37 °C for 48 h and analyzed for the presence of *Listeria* colonies (Mossel, Corry, Struijk, & Baird, 1995; Varnam & Evans, 1996).Total psychrotrophs were determined on plate count agar (PCA, Merck), and incubated at 4°C for 7 days, following the pour plate method (ICMSF, 1978). Total anaerobic psychrotrophs were determined

on TSA (Triptose Soya Agar, Merck), and incubated under anaerobic conditions at 4°C for 7 days.

### 2.3 Statistical analysis

Analysis of variance was performed using the one way ANOVA Minitab version 14. The factors were storage time (0, 3,7,21,28,35,42 and 45 days) and treatment method (90 °C for 15 min, and 70 °C for 15 min). The graph drawings were performed using JMP version 9.0 (SAS Institute Inc. USA). Tukey is multiple range tests was performed for several comparisons. Significance was established at $p<0.05$ level.

## Results and Discussion

### Thiobarbituric acid (TBA)

Lipid oxidation is one of the main reasons of quality deterioration in raw and processed meat products throughout the refrigerated or frozen storage. The development of rancidity and oxidative deterioration in food occur by Lipid oxidation. Fish have a high content of polyunsaturated fatty acids (PUFA), which is highly susceptible to lipid oxidation during processing and cooking. TBA is a simple technique used in fish analysis to determine lipid oxidation (Panpipat & Yongsawatdigul, 2008). The TBA value of raw red tilapia fillet before processing was (0.66±0.01 mg MDA/kg). Figures 4.15 and 4.16 show the means of TBA values for the control and samples treated at 70°C and 90°C during the 45 days of storage. For the control, TBA values increased significantly ($p<0.05$) during storage at 2°C and 10°C. Increments started from the first week of storage and proceeded to the end of 45 days of storage. For samples with and without vinegar processed at 70°C, the TBA value increased significantly ($p<0.05$) during storage at 2°C, an increase started from day 28 then proceeded to the end of storage. For *sous vide* samples with and without vinegar processed at 70°C, the TBA value started to increase significantly ($p<0.05$) during storage at 10°C, and the increase started from day 21 then proceeded to the end of storage.samples observed a slight increase during storage at 2°C.For samples processed at 90°C, the TBA value significantly ($p<0.05$) increased during the storage time at 10°C. The TBA values of samples treated at 90°C and stored at

2°C and 10°C were lower ($p<0.05$) than those cooked at 70°C and stored at 2°C and 10°C. According to Gittleson et al. (1991), the TBARS of *sous vide* processed salmon slightly increased during the refrigerated storage (12 weeks), although not at sufficient levels to detect rancidity. Schormüller, (1968) defined fish containing TBARS value lower than, 3 mg MDA/kg as very good quality, good between 3 and 5 mg MDA/kg, marketable between 5 mg and 8 mg MDA/kg and spoiled at over 8 mg MDA/kg. According to these authors, the probable thermal breakdown of primary oxidation compounds is likely to occur, consequently, that their assessment would not provide an accurate tool for assessing lipid damage progress Diaz et al. (2011).TBARS of 0.5-2 mg MDA/kg have been described in *sous vide* meat-based dishes (Grigioni *et al.,* 2000; Smith & Álvarez, 1988; Vaudagna *et al.,* 2002; Wang *et al.,* 2004). These results are in accordance with that of Wang (2004) who reported that TBA values increased throughout 7 weeks of storage in *sous vide* chicken wings. Sato and Hegarty (1971) claimed that any process causing disorder of the muscle membrane system, like grinding, cooking, and deboning, causes exposure of the labile lipid components to oxygen, which accelerates the oxidative rancidity.

According to Fernandez-Lopez (2008), the TBA values can increase during storage due to the amount of residual oxygen in the pouch after vacuum packaging and the permeability of the packaging material.

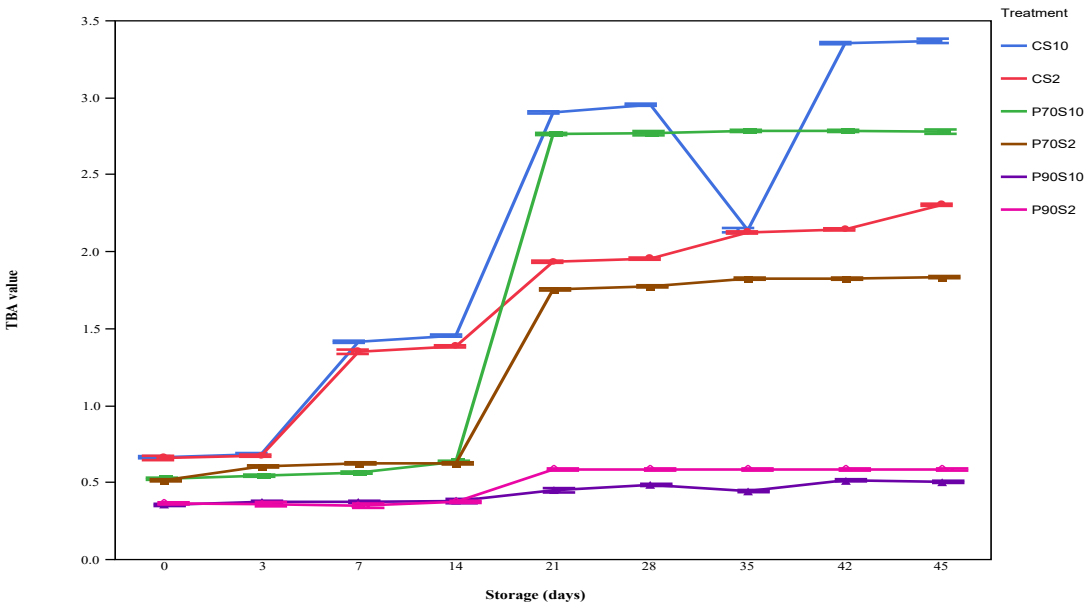



**Figure: 3.1 Effect of storage time on TBA of *sous vide* products and control.**

## Microbiological analyses

The raw tilapia had beginning total mesophile and anaerobic mesophile counts of 4.8±0.0 log cfu/g. Microorganisms such as*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes* were not detected in any of the samples. Figures 3.1 and 3.2 show the results of aerobic mesophile and anaerobic mesophile counts for the control, and samples processed at 70°C and 90°C and stored at 2°C and 10°C.The means for the mesophiles and anaerobic mesophiles for the control were more than 6-log cfu/g in the first week of storage at 2°C and 10°C. The means for mesophile counts for samples processed at 70°C were less than 4-log cfu/g during the first two weeks, then increased to reach 6-log cfu/g after six weeks of storage at 2°C, but samples stored at 10°C reached 6-log cfu/g by the second week of storage. Anaerobic mesophile counts for samples processed at 70°C were 6log cfu/g after three weeks storage at 2°C and after two weeks storage at 10°C.

**Figure: 3. 1 Mesophilic aerobic count of *sous vide* products and control during storage at 2 and 10°C.**

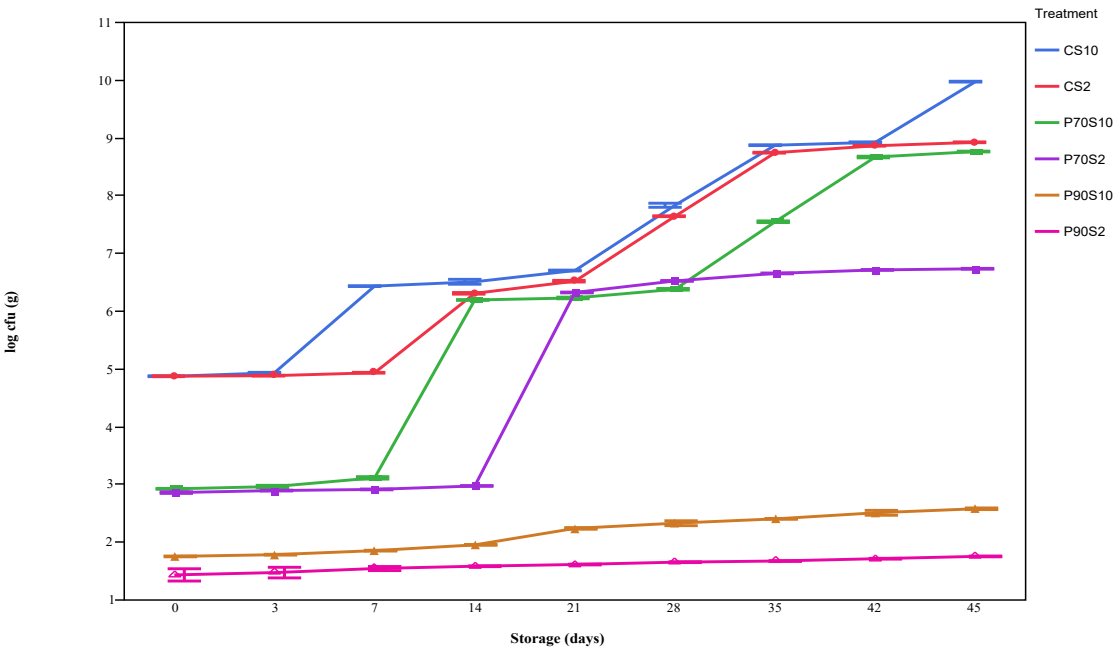
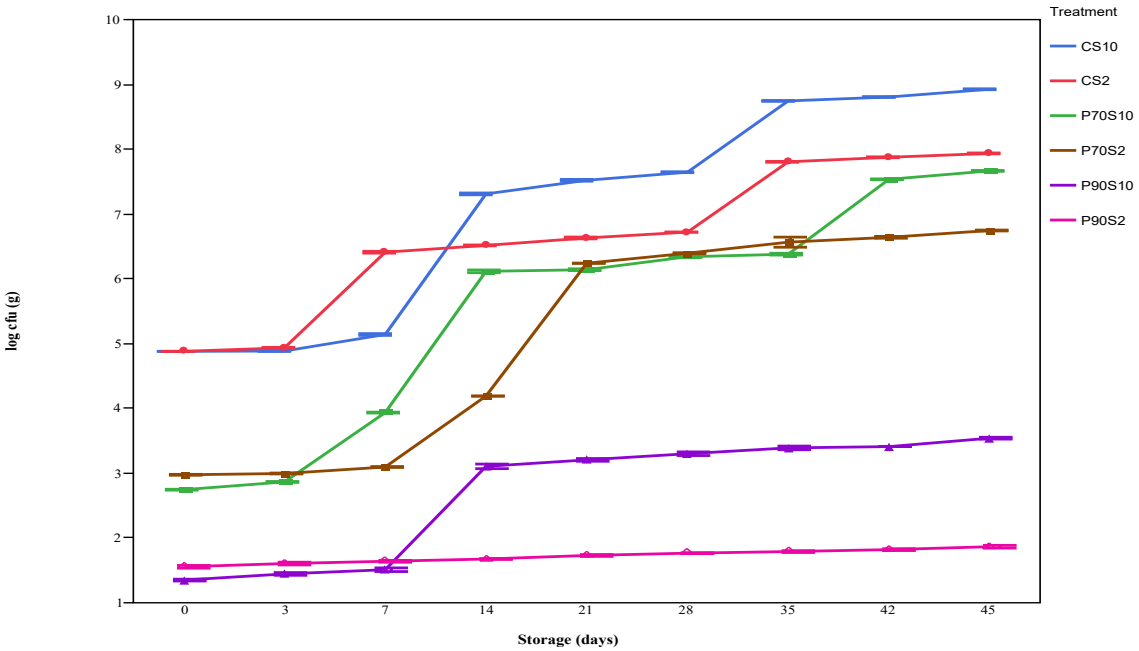



**Figure: 3.2 anaerobic mesophilic count of *sous vide*products and control during storage at 2 and 10°C.**



Figures 3.3 and 3.4 show the results for psychrotrophic and anaerobic psychrotrophic counts for control, and samples processed at 70°C and 90°C and stored at 2°C and 10°C. The means for the aerobic psychrotrophs and anaerobic psychrotrophs for the control were more than 6-log cfu/g in the second week throughout the storage at 2 and 10°C. The means for the psychrotrophs counts for the samples processed at 70°C reached 6-log cfu/g after six weeks of storage at 2°C and 10°C. The means for the aerobic psychrotroph and anaerobic psychrotroph counts for samples processed at 90°C were less than 2-log cfu/g throughout the storage time at 2°C and 10°C.

**Figure: 3.3 Psychrotrophic aerobic count of *sous vide* without vinegar products and control during storage at 2 and 10°C.**

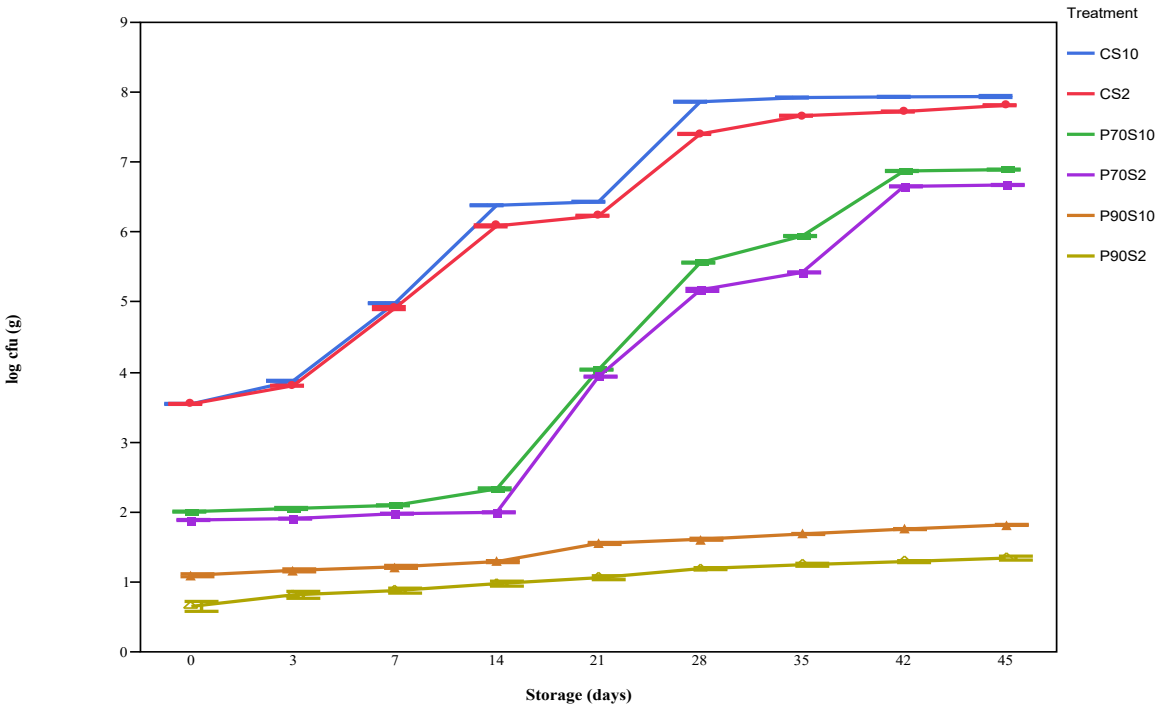
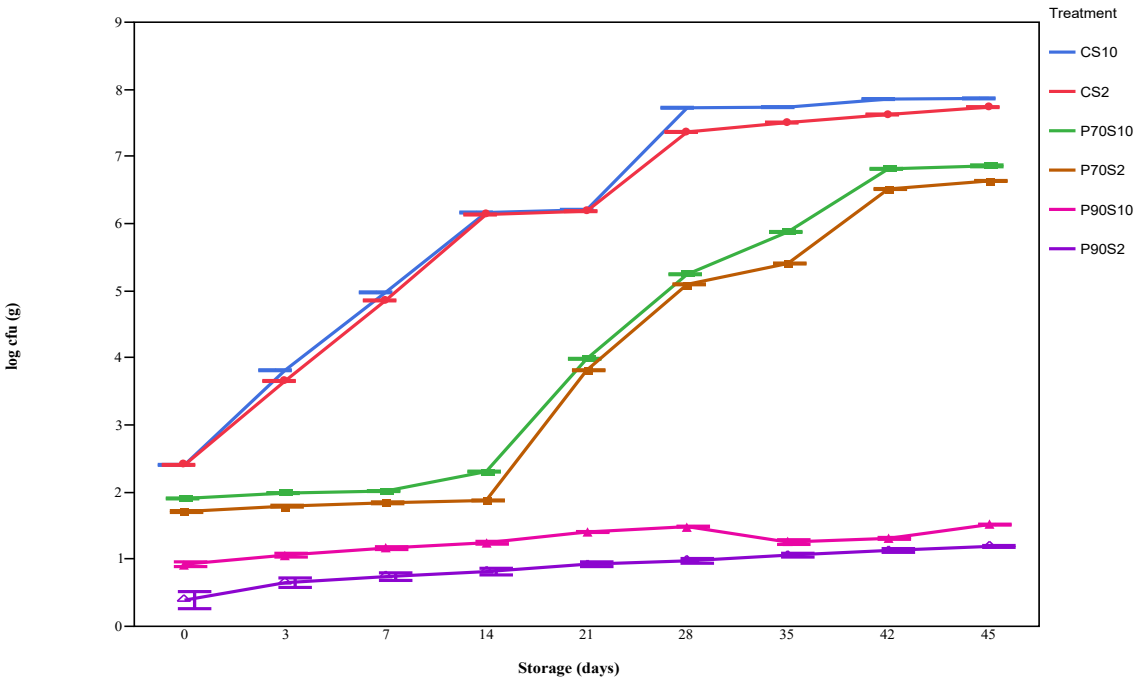


**Figure: 3.4 anaerobic psychrotrophic count of *sous vide* without vinegar products and control during storage at 2 and 10°C.**




Uncooked fish may be contaminated by a variety of factors including the water environment and temperatureand has been extensively reported (ICMSF, 1996; González-Fandos, 2004). Notwithstanding the fact that the primary counts of the mesophile and psychrotrophic populations gradually increased throughout the storage, they were significantly lower in the productswere significantly lower in the products that were subjected to a relatively more extreme heat treatment at 10°C.Therefore, the microorganisms might not have been entirely deactivated at the temperatures used in the heat treatment and, consequently, organisms might only have suffered thermal injury and recover during the period of storage. This might result in a public health issue, in conditions of temperature abuse conditions (10°C) or when only subjected to less severe treatments (González-Fandos, 2004).

Mesophile counts were significantly ($p<0.05$) lower in samples stored at 2°C when matched with those stored at 10°C. Mesophile counts were significantly ($p<0.05$) lower in samples with vinegar stored at 2°C when matched with those stored at 10°C.

Mesophile counts reached 6-log cfu/g for samples without vinegar processed at 70°C after 21days of storage at 2°C, and after 14 days when stored at 10°C. The psychrotroph counts were less than mesophile counts. While psychrotrophs developalbeit relatively slowly, at refrigeration temperatures (<5°C) with the best being approximately 25°C milder treatments can cause harm to microorganisms resulting from additional nutritionalneeds that areessential for the growth of microorganisms (Montville, 1997; González-Fandos, 2004). Furthermore, various environmental factors with unfavourable values such as oxygen concentration, causes a significant increase in the minimum growth temperature.Although the damaged cells may still be sustainable, albeit negatively, recovery through the process of normal enumeration is affected three fold: First, there is a considerable increase in lag times, even with the use of optimal recovery media. Second, on occasion, in comparison to completely viable cells the generation times might escalate under similar essential and non-essential conditions (Mossel *et al.,* 1995; (González-Fandos, 2004). Finally, the revival of incapacitated populations may be affected by the incubation temperature. Furthermore, it should be noted that non-sporeforming bacteria harmed by heat have a more limited range of growth compared to fully vital ones (Thomas, Reinbold, & Nelson, 1963; González-Fandos, 2004). Consequently, the major distinction between mesophile and psychrotroph populations is that damaged cells cannot recover at low temperatures. Of interest is that determinations of psychrotrophs generally require a minimum of one week incubation at 7°C for completion, whereas visible colonies of psychrotrophs appear quicker after incubation at 20-25°C for a few hours before the temperature is reduced to 5°C (Mossel *et al.,* 1995; González-Fandos, 2004). Consequently, for foods with a prolonged shelf life, this might provide adequate time for these organisms to grow in the chill environment after adjusting. However, the qualitative composition of the microbiotamay may change noticeably due to the refrigeration, as microorganisms temperature needs differ (Farkas, 1997). Notwithstanding that the primary interest in *sous vide* products stored at appropriate refrigeration temperatures (<5 °C) relate to psychrotrophic bacteria, the majority of studies conducted on the microbiological quality of *sous vide* products evaluate mesophile counts and not psychrotroph counts (Carlin *et al.,* 1999; Guerzoni, Gianotti, & Lopez, 1999; Simpson *et al.,* 1994).

However, these findings do not agree with Gonzalez-Fandos et al. (2005) who reported *sous vide* salmon slices mesophile counts of more than 6-log cfu/g after 45 days of storage and similarly with Rosnes et al. (1999) who was working with *sous vide* salmon treated at 70°C for 15 min and storage at 2°C and 10°C. The psychrotroph counts were less than the mesophile counts throughout the storage at 2°C and 10°C. These low psychrotroph results were also found by Gonzalez-Fandos et al. (2005) and Rosnes et al. (1999) working with *sous vide* salmon slices.

Mesophilic microorganisms were found to be present more than other groups. According to Carlin et al. (2000), mesophilic microorganisms, which resist the pasteurization temperature, grow even under refrigerated storage and cause spoilage at abuse temperatures. The effect of vinegar in *sous vide* processed tilapia fillets was to delay microbiological growth and spoilage. An increase in the number of mesophilic microorganisms depends on the storage temperature and the type of *sous vide* processed tilapia, and may also have been due to initial contamination differences.

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