EFFECT OF TEMPEH ADDITION ON *IN VITRO* OXIDATIVE PROCESSES DURING THE DIGESTION OF BEEF PATTIES

J. Taylor[1], A. E. D. Bekhit[1], M. Schultz[2] and M. R. Chandraratne[3*]

[1] Department Food Science, University of Otago, Dunedin, New Zealand

[2] School of Medicine, University of Otago, Dunedin, New Zealand

[3] Department of Chemical Engineering, Abu Dhabi Men's College, Higher Colleges of Technology, Abu Dhabi, UAE

Abstract – The stomach is considered as a bioreactor where both pro-oxidative and anti-oxidative compounds from the diet interact under low pH and at a relatively high temperature of 37°C. Meat is susceptible to oxidative reactions as it contains both the catalyst (iron) and the substrate (lipid) of lipid peroxidation. Consumption of beef with an antioxidant was hypothesized to limit lipid peroxidation in a model stomach system. Tempeh could be successfully incorporated into a beef patty to provide a source of antioxidant to limit *in vivo* lipid oxidation.

The aim of this research was to determine the extent of *in vitro* oxidative processes occurring in beef patties containing tempeh (10% addition level), compared to controls, during the digestion with gastric fluid from healthy and patients with stomach ulcers.

The *in vitro* digestion results showed no significant difference between control and 10% tempeh patties for TBARS. As there is an apparent antioxidant effect for tempeh which was negated by the high PUFA content in 10% tempeh patties. The use of tempeh can be successful in delivering high PUFA without concerns of higher oxidative processes. The addition of antioxidant source with meat containing meals is important to reduce oxidative processes during digestion. The need for this antioxidant seems to be crucial for patients on PPI treatment.

Key Words – Polyunsaturated fatty acid (PUFA), Human gastric fluid, Proton pump inhibitor (PPI) medication

I. INTRODUCTION

Red meat has been consumed by humans for thousands of years. Compared to plant foods, red meat is rich in protein, fat, minerals such as iron, zinc and selenium and many vitamins [1].

In recent years, there has been negative consumer reaction to red meat not only due to its saturated fat content but also due to the causal link between red meat consumption and the incidence of colorectal cancer. Concurrently, consumers have also become aware of the importance of dietary antioxidants and there has been an extensive research on the benefits and the dietary requirements of antioxidants. The effects of free radicals which can cause oxidation *in vivo* and are involved in many pathologies including carcinogenesis can be mitigated by antioxidants [2].

Meat contains haem and free iron as well as fat which cause lipid peroxidation resulting in the generation of free radicals during digestion [3, 4]. The stomach is regarded as a perfect bioreactor where the interaction of many food components, such as lipids, proteins and carbohydrates in the presence of enzymes are constantly being mixed at a temperature of 37°C, under conditions of low pH and presence of dissolved oxygen. Under these conditions different reactions could occur which lead to increased lipid oxidation especially in lipid rich foods [3]. Lipid hydroperoxides which catalyse myoglobin oxidation in foods can also be generated during digestion, particularly in gastric fluid which has a low pH and contains absorbed oxygen [3].

Recently, several studies have focussed on oxidation and antioxidative effects occurring in model stomach systems during the digestion of food. There have been several recent studies focussed on oxidation of meat using simulated digestion and the effects of dietary antioxidants on this process [3, 4, 5]. The inclusion of an antioxidant source into a meat product would conveniently provide antioxidant in the meal.

Reactive oxygen species (ROS) have been implicated in pathogenesis of gastric disorders and increased lipid peroxidation has been observed experimentally in gastric ulcers [6, 7, 8].

The gastric fluid of a patient normally taking proton pump inhibitor (PPI) medication may have an accumulation of hydrogen peroxide, hydroxyl radicals and catalytic transition ion metals than the gastric fluid of a healthy individual [6,8].

The processed meat industry commonly uses non meat extenders to decrease costs, and to bind water to reduce cooking losses. Predominantly these extenders have been textured soy protein, soy flour or soy protein isolates [9]. Tempeh has recently become popular amongst vegetarians for the meat like flavour profile it provides despite not being a meat product. Tempeh has been used as a meat extender, but its antioxidant benefits have not been explored in meat.

The present study was designed to investigate the oxidative processes (as indicated by thiobarbituric acid reactive substances (TBARS)) during the digestion of beef patties by gastric fluid from healthy individuals and patients on PPI medication. Furthermore the effect of inclusion of tempeh on TBARS was investigated.

To achieve this goal, the present research determined the extent of *in vitro* oxidative processes occurring in beef patties treated with tempeh (10% addition level) (Figure 1) and controls (untreated control patties and 10% breadcrumb extended patties) at 1 and 6 days of display at 4°C during digestion using gastric fluid from healthy individuals and patients on PPI medication.

II. MATERIALS AND METHODS

The study was approved by the Southern District Health Board. Human gastric fluid (HGF) was collected from 28 patients during their scheduled endoscopy appointments at the Dunedin Hospital gastroenterology ward. Of these participants eight were taking PPI medication prior to the endoscopy. All the participants signed a consent form before the fluid was collected. The HGF were filled into sterile biological sample containers, stored on ice and later frozen at -80°C until analysis.

Samples containing no tempeh (Control), 10% w/w breadcrumb and 10% w/w tempeh were prepared (Table 1). The samples were obtained from the displayed patties at 1 and 6 days of storage at 4°C. Burger patties were cooked until centre temperature of 75°C was reached, cut into thin pieces and were snap frozen in liquid nitrogen. The frozen pieces were pulverized, filled into 50 ml Falcon tubes, flushed with nitrogen gas and frozen at -80°C until analysis.

Table 1 Formulation of burger patty treatments

Treatment	Lean meat %	Fat %	Tempeh %	Breadcrumb %	Salt %
Control	89	10	-	-	1
Control + 10% breadcrumb	79	10	-	10	1
Control + 10% tempeh	79	10	10	-	1

Figure 1. Beef patty with 10% w/w tempeh

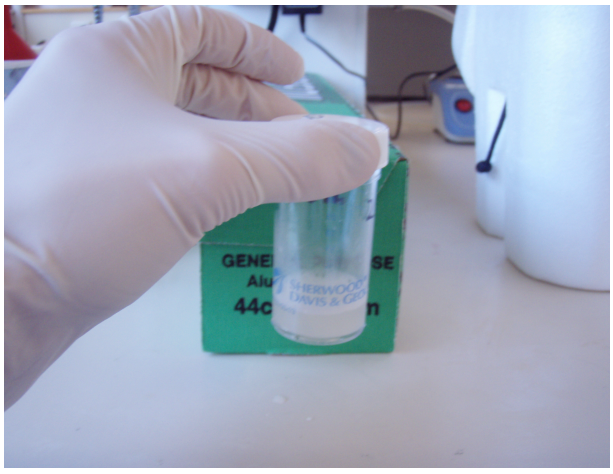

Figure 2. Gastric fluid sample

Burger patty powders (0.33g) from samples were individually weighed into glass kimax tubes. One ml of gastric fluid was added into tubes containing each of the three treatments to obtain 1:3 w/v

sample to gastric fluid ratio. The tubes were wrapped in tin foil and shaken at 37°C for 180 min in an orbital shaker incubator set at 180 rpm. After shaking the digested samples were immediately placed on ice. Distilled water (up to nine ml) was added to dilute the samples to a level suitable for TBARS analysis.

One ml of the gastric fluid homogenate was added in duplicate into 15 ml falcon tubes and tested using the TBARS assay. Results were expressed as milligrams of malondialdehyde/100g of meat (mg MDA/100g meat).

III. RESULTS AND DISCUSSION

The TBARS in samples digested by HGF from patients normally taking PPI medication was higher than those digested by HGF from healthy individual (Figure 3). In the stomach iron from meat is expected to increase TBARS by catalysing lipid peroxidation [4].

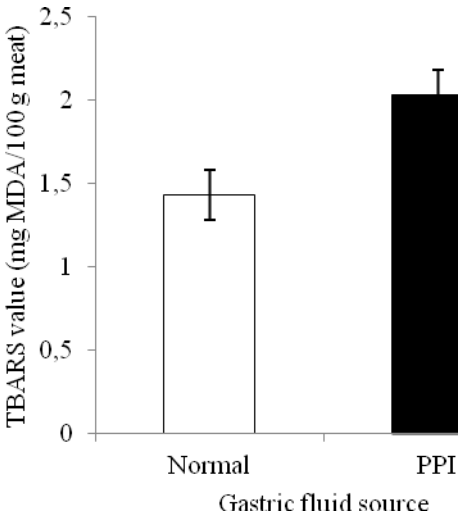

Figure 3. Effect of gastric fluid from healthy individuals and PPI patients on mean values of TBARS expressed as mg malondialdehyde MDA/100g of cooked beef patties

For the treatments there were no significant differences between healthy and PPI patients for TBARS with the exception of 10% breadcrumb which was significantly ($p < 0.05$) higher than tempeh treated samples (Figure 4). The lack of significant differences may be due to the large differences between display times which were pooled in the analysis which was evident from the significant ($p < 0.05$) differences observed with display time (Figure 5).

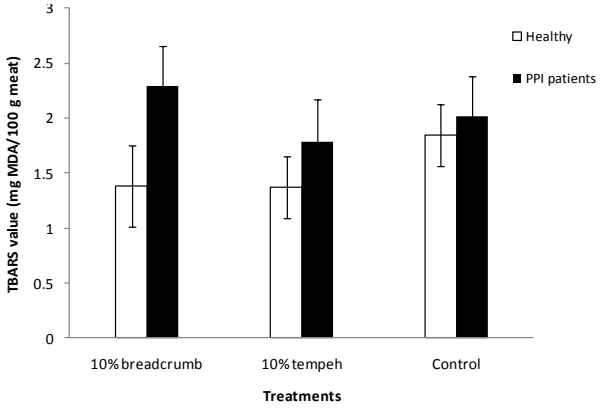

Figure 4. Effects of addition of breadcrumb (10% w/w) and tempeh (10% w/w) on mean values of TBARS during digestion using HGF from healthy individual and patients on PPI. TBARS is expressed as mg MDA/100g of cooked beef patties. Error bars are the standard error of the difference (SED).

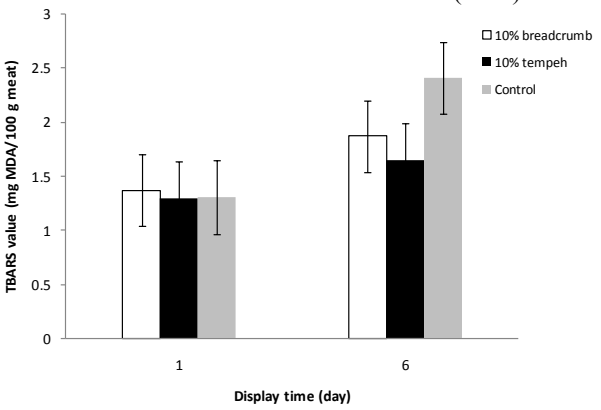

Figure 5. Effect of the addition of breadcrumb (10% w/w) and tempeh (10% w/w) on mean values of TBARS expressed as mg MDA/100 g of cooked beef patties at days 1 and 6 of display at 4°C. Error bars are the SED.

The 10% tempeh patties digested at display day 6 are not significantly different from patties digested at display day 1 whilst the other treatments at day 6 are significantly higher (Figure 5). There were no significant ($p < 0.05$) differences in TBARS between the three treatments tested after digestion (Figure 6). The mean values for TBARS in Figure 5 include samples from day 1 and day 6 display time. The 10% tempeh patties digested at day 6 were not significantly different from patties digested at display day 1 whilst the other treatments at day 6 were significantly higher.

The PUFA concentration in the crude fat in 10% tempeh, 10% breadcrumb and control were 2.99, 4.28 and 10.21%, respectively. As the 10% tempeh patties, did not show higher TBARS than control and 10% breadcrumb treatments during *in vitro* digestion it is likely that the tempeh provided some antioxidant effect.

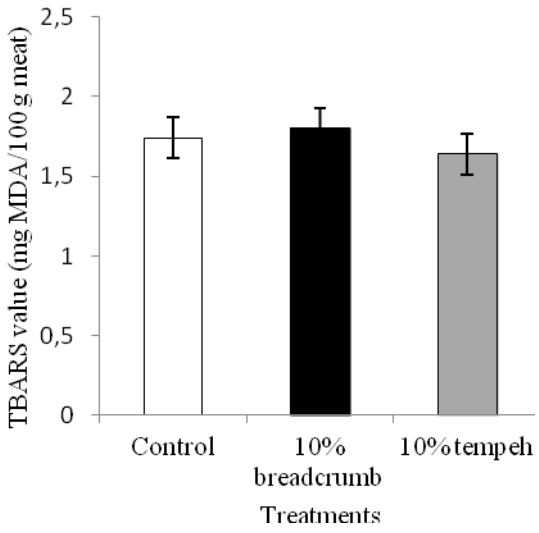

Figure 6. Overall main effects of addition of breadcrumb (10% w/w) and tempeh (10% w/w) on mean values of TBARS expressed as mg MDA/100 g of cooked beef patties. Error bars are the SED.

TBARS in digested control and 10% breadcrumb was increased after 6 days of storage but not 10% tempeh samples. The digestion with HGF from PPI group had higher TBARS compared with healthy individual group in 10% breadcrumb treatment only.

IV. CONCLUSION

Patties containing tempeh which had higher PUFA were expected to have increased TBARS especially after heating and digestion due to their high polyunsaturated fatty acid content. Lack of significant difference in TBARS between control and tempeh patties and lack of difference between day 6 tempeh patties and fresh patties indicates that there is an antioxidant effect occurring with tempeh during digestion.

The addition of antioxidant source with meat containing meals is important to reduce oxidative processes during digestion. The need for this antioxidant seems to be crucial for patients on PPI treatment.

REFERENCES

1. Higgs, J.D. (2000). The changing nature of red meat: 20 years of improving meat quality. Trends in Food Science and Technology 11: 85-95.
2. Kanner, J. (2007). Dietary advanced lipid oxidation endproducts are risk factors to human health. Molecular Nutrition & Food Research 51: 1094-1101.
3. Kanner, J. & Lapidot, T. (2001). The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. Free Radical Biology and Medicine 31: 1388-1395.
4. Gorelik, S., Lapidot, T., Shaham, I., Granit, R., Ligumsky, M., Kohen, R. & Kanner, J. (2005). Lipid peroxidation and coupled vitamin oxidation in simulated and human gastric fluid inhibited by dietary polyphenols: Health implications. Journal of Agricultural and Food Chemistry 53: 3397-3402.
5. Gorelik, S., Ligumsky, M., Kohen, R. & Kanner, J., (2008). The stomach as a "bioreactor": When red meat meets red wine. Journal of Agricultural and Food Chemistry 56: 5002-5007.
6. Das, D., Bandyopadhyay, D., Bhattacharjee, M. & Banerjee, R.K. (1997). Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. Free Radical Biology and Medicine 23: 8-18.
7. Naya, M. J., Pereboom, D., Ortego, J., Alda, J. O. & Lanas, A. (1997). Superoxide anions produced by inflammatory cells play an important part in the pathogenesis of acid and pepsin induced oesophagitis in rabbits. Gut 40: 175-181.
8. Das, D. & Banerjee, R.K. (1993). Effect of stress on the antioxidant enzymes and gastric ulceration. Molecular and Cellular Biochemistry 125: 115-125.
9. Singh, P., Kumar, R. & Sabapathy, S.N. & Bawa, A.S. (2008). Functional and edible uses of soy protein products. Comprehensive Reviews in Food Science and Food Safety 7: 14-28.