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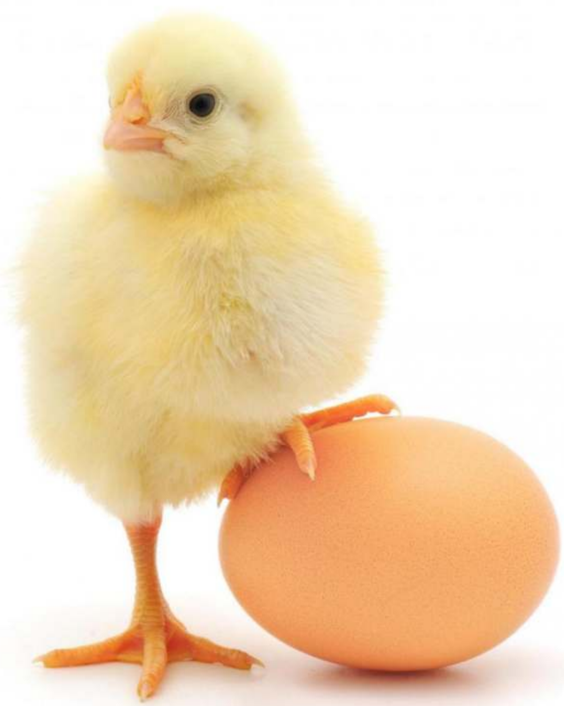

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Assessment of the Antioxidant Potential of Food Ingredients in Fresh, Previously Frozen and Cooked Chicken Patties

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Abstract: Antioxidants were added (concentration range 0-4%) to fresh, minced chicken meat. Antioxidant potential was assessed through TBARS and colour measurements. Optimum test ingredient concentrations determined were; aloe vera (AV) (1.0%), fenugreek (Fen) (0.01%), ginseng (Gin) (0.05%), mustard (Mtd) (0.25%), rosemary (RM) (0.1%), sage (S) (0.1%), soya protein (SP) (0.01%), tea catechins (TC) (0.01%) and whey protein concentrate (WPC) (2%). The optimum concentration of each test ingredient was incorporated into fresh, previously frozen and cooked chicken patties. Control meat (low vitamin E) was divided into 11 groups. Optimum concentrations of test ingredients were added (groups 1-9). BHA/BHT was group 10, control (group 11) and dietary vitamin E supplemented meat (group 12). In fresh meat, BHA/BHT was the most effective antioxidant, while RM was the most effective among the food ingredients tested. In previously frozen meat, Vitamin E, TC, S, BHA/BHT and RM were effective antioxidants. In cooked chicken patties, BHA/BHT was the most effective antioxidant, while TC were the most effective among the food ingredients tested. Antioxidants (BHA/BHT, RM, Vitamin E, TC and S) were more active in patties formed from previously frozen meat than patties formed from fresh meat and were most active in cooked patties. Thus, increasing the oxidative stress on the meat product, increased the effectiveness of added antioxidants. Overall, TC, RM and S had the best antioxidant potential in fresh, previously frozen and cooked chicken patties.

Key words: Antioxidant, patties, TBARS, chicken

Introduction

Apart from microbial spoilage, lipid oxidation is the primary process by which quality loss of muscle foods occurs (Buckley *et al*., 1995). Several studies have indicated that lipid oxidation can be controlled or at least minimized through the use of antioxidants (Gray *et al*., 1996). Antioxidants are compounds capable of delaying, reducing or preventing auto-oxidation processes (Shahidi and Wanasundara, 1992). Synthetic antioxidants were widely used in the meat industry but consumers concern over their safety and toxicity pressed the food industry to find natural sources of antioxidant (Monahan and Troy, 1997). Therefore, the importance of screening naturally occurring alternatives, which are safe, effective as dietary supplements or as processing aids, and relatively cheap, is increasing (Tang *et al*., 2001). The plant kingdom offers a range of natural phenolic compounds, among which α-tocopherol (vitamin E) is best known as one of the most efficient naturally occurring liposoluble antioxidants (Mallet *et al*., 1994). α-Tocopherol has demonstrated strong antioxidant activity in a wide range of meats when elevated levels of α-tocopheryl acetate were supplemented in the diet of poultry; chicken (O'Neill *et al*., 1999) turkey (Higgins *et al*., 1998) and duck (Russell *et al*., 2003).

Some of the major active compounds reported are flavonoids and related compounds in plant extracts such as phenolics in spices and herbs (Rajalakshmi and Narasimhan, 1995). Herbs and spices occupy a special position in foods as traditional food ingredients and hence are appropriately used directly for their antioxidant characteristics (Jadhav *et al*., 1995).

Lipid oxidation in meat products appears to be accelerated by cooking (Wu and Sheldon, 1988) and freezing (McCarthy *et al*., 2001b). Therefore, there is a great need for effective antioxidants in cooked and frozen meat products. This is especially important with the modern trends towards convenient, pre-cooked, frozen muscle-based foods. The acceptance by consumers of these products can be diminished by oxidation (Ruenger *et al*., 1978). Poultry meat is particularly susceptible to oxidative deterioration due to its relatively high content of polyunsaturated fatty acids, with oxidation often determining the shelf life of pre-cooked, refrigerated ready-to-eat products (Igene and Pearson, 1979). While numerous studies in the literature exist with respect to investigating single ingredients as effective antioxidants in muscle food systems, relatively few studies exist whereby various antioxidants have been compared against each other at determined optimum concentrations under controlled experimental conditions.

The objectives of this study were (1) to screen nine food ingredients for potential antioxidant activity in fresh chicken patties and (2) to investigate the antioxidant activity of these ingredients at their optimum concentration, in fresh, previously frozen and cooked chicken patties and compare their activity against strong synthetic and natural antioxidant sources in the forms of BHA/BHT and vitamin E, respectively.

Materials and Methods

Chemicals and Reagents: All Chemicals used were "AnalaR" grade obtained from Sigma Chemical Co., Steinheim, Germany; Wardle Chemicals Ltd., Green Lane, Wardle, Nr. Nantwich, Cheshire; British Drug Houses, Poole, England, Merck, Darmstadt, Germany; or Oxoid Unipath Ltd., Basingstoke, Hampshire, England. α-Tocopheryl acetate used in the chicken diets was obtained from Roche Products Ltd., Welwyn Garden City, Hertfordshire, U.K.

Screening Ingredients: Aloe Vera in a liquid form was obtained from Pro-Ma Systems, Calgary, Alberta, Canada. Fenugreek powder and dried sage (freshly ground) were purchased from Natural Foods, Paul St., Cork, Ireland. Ginseng and tea-catechins powders were obtained from Kingnong Natural Plant Products Company, Changsha, Hunan, China. Mustard powder was obtained from UFL Foods Inc., Ontario, Canada. Soya protein concentrate (500E) was obtained from Protein Technologies International, St. Louis, MO., U.S.A. Whey protein concentrate was supplied by Dairygold, Mitchelstown, Co. Cork, Ireland. Rosemary powder (Oxyless clear) was supplied by Guinness Chemicals Ltd., Portlaoise, Co. Laois, Ireland.

Vitamin E Feeding Trial: Day-old Cobb chickens (n=300) were distributed over 6 pens (5 x 4m) (n=50). Floors were bedded with sawdust on cardboard and each pen had a 250-watt infra red bulb suspended overhead. Groups were assigned to either a low (10g α-tocopheryl acetate/tonne finished diet) or high (400g α-tocopheryl acetate/tonne finished diet) vitamin E diet for six weeks prior to slaughter. Food and water were available *ad libitum*. Feed was manufactured in the feed mill in Teagasc, Moorepark Research Centre, Fermoy, Co. Cork. Cereals were ground through a 3 mm screen before mixing. The vitamin-trace mineral mix was premixed through 10 kg wheat before addition to the mixer. Feed was stored in pre-weighed 25 kg sacks, stamped with ID number and date of manufacture, and closed by sewing. All feed was prepared at the beginning of the trial. All bags were stored in the dark at 10-14^{o}C for the duration of the trial.

Preparation of Chicken Patties for the Antioxidant Screening Trial: Fresh chickens were obtained from Cappoquin Chickens, Cappoquin, Co. Waterford for the initial screening trial and processed immediately. Chickens were skinned and thigh and breast meat removed by hand. This meat was minced (Mainca, C/ Jaume Ferran s/n, Granollers, Barcelona, Spain) in a ratio of 1:1 (breast: thigh) using a 5mm-mincing plate. Antioxidants were added at various concentrations (range 0-4%) and mixed into the meat thoroughly by hand. Meat was then formed into patties weighing 125g and 100g for lipid oxidation and colour analyses, respectively. These were over-wrapped with cling-film [6000-8000 $cm^3/m^2/24$ hours at standard temperature and pressure (STP)] and stored in a display cabinet (Criosbanc Refrigeration, Via Montegrotto, Padova, Italy) at 4^{o}C for 10 days.

Preparation of Chicken patties for Comparison of Optimum Antioxidant/Food Ingredient Concentrations against Natural (Vitamin E) and Synthetic (BHA/BHT) Antioxidant Controls: For the subsequent concentration comparison trials, chickens reared at Teagasc Moorepark Research Centre were divided into three batches. One batch was processed immediately as described above. Two batches were frozen at 20^{o}C for 7 days, thawed at 4^{o}C over 12 hours, and processed as described above. One of these batches was assessed in the raw state (previously frozen) while the other was cooked on a gas hotplate (Gico Spa, Vazzola (Tv), Italy), for 5 min. on each side or until an internal temperature of 70^{o}C was reached. All three batches of patties were over-wrapped with cling-film (6000-8000 $cm^3/m^2/24$ hours at STP) and stored in a display cabinet (Criosbanc Refrigeration, Via Montegrotto, Padova, Italy) at 4^{o}C for 9 days.

Within each batch, the meat from chickens fed basal levels of vitamin E was divided into 11 groups. The optimum ingredient concentration (determined from the screening trial) was added to groups 1-9. Synthetic antioxidant, BHA/BHT (Sigma) was added to group 10. Group 11 was the experimental control while group 12 represented vitamin E supplemented meat. All batches were mixed by hand.

Determination of Oxidative Stability: Oxidative deterioration was assessed using the 2-thiobarbituric acid distillation method of Tarladgis *et al.* (1960) as modified by Ke *et al.* (1977). This procedure was carried out in duplicate for each of 3 patties from each group on days 0, 2, 4, 6, 8 and 10, for the initial screening trial, and on days 0, 3, 6 and 9 for the optimum concentration comparison trials.

Assessment of Colour Stability: Surface patty colour (Hunter L, a, b) was measured using a Minolta CR-300 colorimeter (Minolta Camera Co., Chuo-Ku, Osaka 541, Japan). Raw and cooked patties were over-wrapped in cling-film (6000-8000 $cm^3/m^2/24$ hours at STP) and 6 readings were taken for each of 2 patties on days 0, 2, 4, 6, 8 and 10 for the screening trial, and on days 0, 3, 6 and 9 for the optimum concentration comparison trials.

Statistical Analysis: For TBARS, there were three samples for each concentration and each sample was analyzed in duplicate. For colour measurements two samples at each concentration were analyzed and six readings were taken from each sample. Data was analyzed using a repeated measure model with a SPSS 8.0 for windows software package (SPSS, Chicago, IL). The level of statistical significance, unless stated otherwise, was $p<0.05$.

Results

Screening Trial of Natural Ingredients in Chicken: Due to the large volume of data generated during the screening trial, only the optimum concentrations identified for each of the test antioxidants have been presented (Table 1). The antioxidant potential of these ingredients was determined through TBARS measurements. In general, the concentration range used for each test ingredient screened ranged from 0 to 1%. However, in the case of Fen, Mtd and WPC, the concentrations screened ranged from 0 to 2%, 0 to 2.5% and 0 to 4%, respectively. Moreover, doubling of test ingredient addition rates (0, 0.01, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 4.0%) was used in patty manufacture to assess ingredient performance more effectively. Optimum test ingredient addition rates, based on antioxidant activity, were determined as follows; AV (1.0%), Fen (0.01%), Gin (0.05%), Mtd (0.25%), RM (0.1%), S (0.1%), SP (0.01%), TC (0.01%) and WP (2%).

Comparison of the oxidative stability of chicken patties containing the optimum concentration of test food ingredients: Based on preliminary screening trials, the optimum concentrations of test ingredients were incorporated into fresh, previously frozen and cooked chicken patties. The effect of optimum food ingredient concentrations on the oxidative stability of patties manufactured from fresh chicken meat held under chilled conditions are shown in Fig. 1. All ingredients screened, with the exceptions of AV (1.0%) and Fen (0.01%) were effective as antioxidants having lower TBARS values than the control during refrigerated display. TBARS values tended to decrease in the order: Fen > AV > Gin > Control > Mtd > S > TC > vitamin E > WPC > SP, RM > BHA/BHT. Results showed that BHA/BHT was the most effective antioxidant, while rosemary was the most effective among the food ingredients tested. However, the TBARS values for BHA/BHT were not significantly different to those for RM,

Table 1: Optimum concentrations of the antioxidants determined in fresh chicken patties held under chilled (4°C) display for 10 days

Food Ingredients	Concentration Range Screened	Optimum Concentration Determined
Aloe vera	0 - 1.0%	1.00%
Fenugreek	0 - 2.0%	0.01%
Ginseng	0 - 1.0%	0.05%
Mustard	0 - 2.5%	0.25%
Rosemary	0 - 1.0%	0.10%
Sage	0 - 1.0%	0.10%
Soya protein	0 - 1.0%	0.01%
Tea catechins	0 - 1.0%	0.01%
Whey protein	0 - 4.0%	2.00%

TC or vitamin E meat or control meat on days 0, 3 and 9 of refrigerated display.

The effect of the optimum concentration of antioxidants on the oxidative stability of patties manufactured from previously frozen chicken meat and held under chilled conditions is shown in Fig. 2. TBARS values for previously frozen chicken meat were 3-fold higher than those for the corresponding fresh chicken meat. TBARS values tended to decrease in the order: Fen > WPC > Gin > AV > BHA/BHT > Mtd > control > RM > S > TC > vitamin E. Vitamin E, TC, S and RM were effective as antioxidants giving lower TBARS values than the control over the storage period. Vitamin E was the most effective antioxidant in previously frozen chicken meat, having significantly ($p<0.05$) lower TBARS values than all other treatment groups on days 6 and 9. TC and RM had lower ($p<0.05$) TBARS values than the control on days 3, 6 and 9, while S had lower ($p<0.05$) TBARS values than the control on day 9. BHA/BHT was not effective as an antioxidant in patties formed from previously frozen chicken meat, having higher TBARS values than the control on day 3, and significantly ($p<0.05$) higher on days 6 and 9.

The effect of the optimum concentration of antioxidants on the oxidative stability of cooked patties manufactured from previously frozen chicken meat and held under chilled conditions is shown in Fig. 3. TBARS values for cooked chicken meat were 10-fold higher than those of previously frozen chicken meat and 30-fold higher than those of fresh chicken meat. All test ingredients, with the exceptions of Gin (0.05%) and AV (1%) were effective as antioxidants giving lower TBARS values than the control during refrigerated display. TBARS values tended to decrease in the order: AV > Gin >control > SP > Fen > Mtd > WPC > RM > S > vitamin E > TC > BHA/BHT. Results showed that BHA/BHT was the most effective antioxidant, while TC was the most effective among the food ingredients tested. TBARS values for BHA/BHT and TC were not significantly different from each other throughout the refrigerated display period. TBARS values for patties formed from control meat were

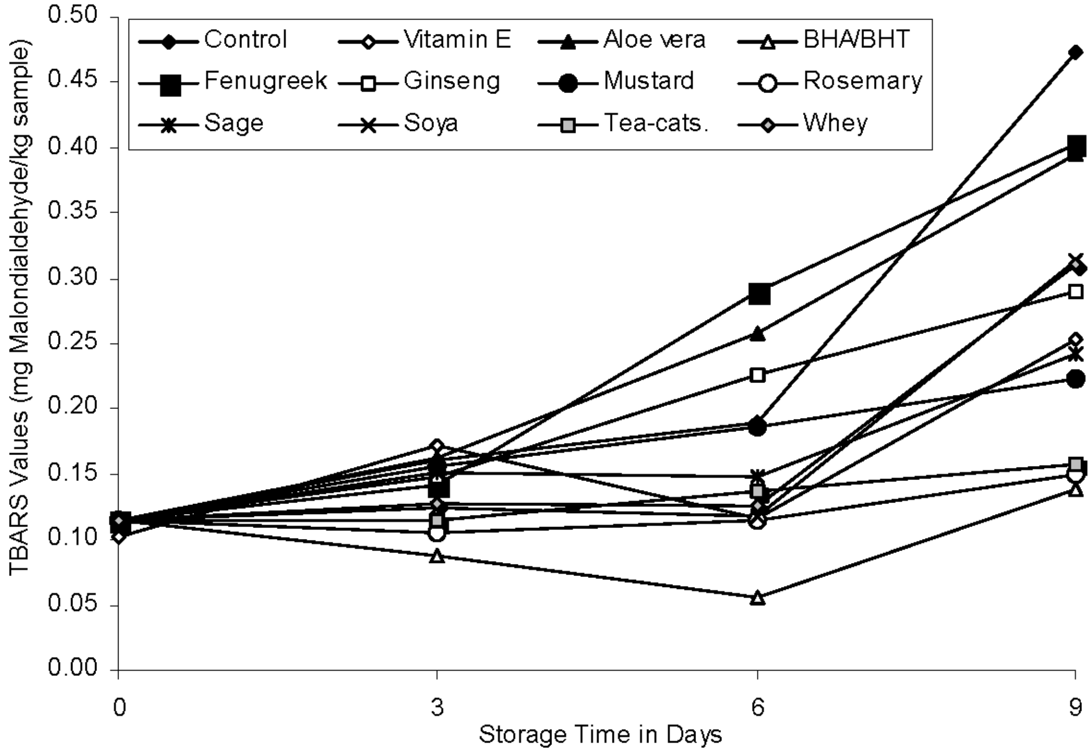

Fig. 1: A comparison of the effect of antioxidants on the oxidative stability of fresh chicken meat stored under refrigerated display conditions (4°C) for 9 days

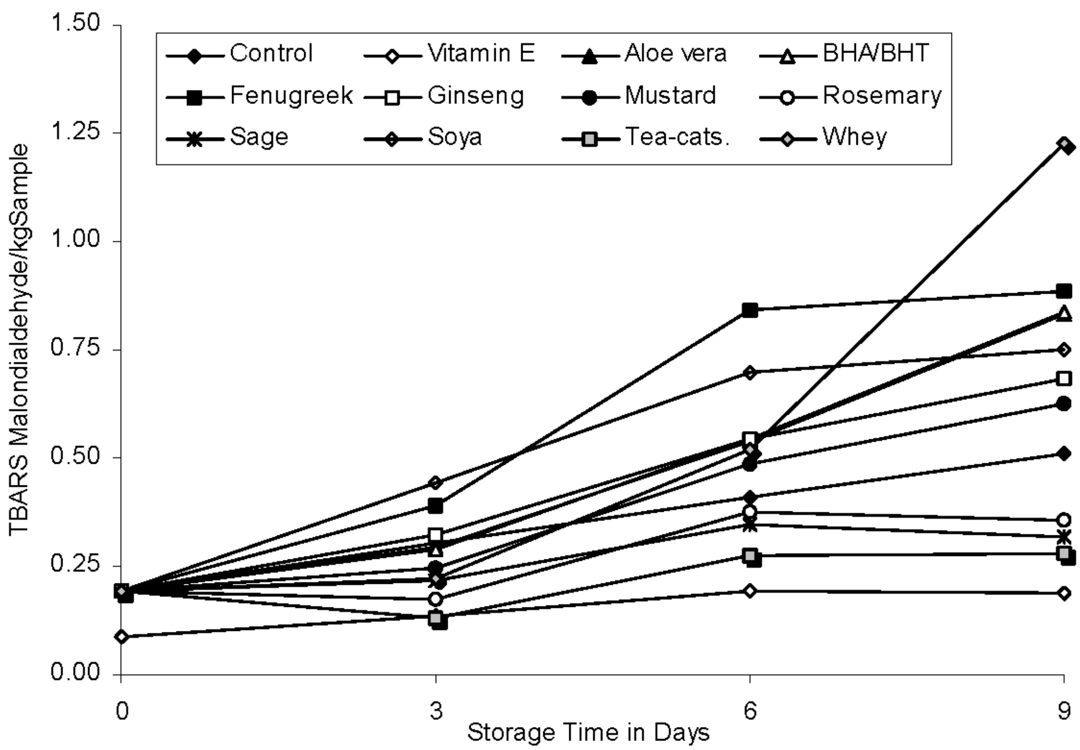

Fig. 2: A comparison of the effect of antioxidants on the oxidative stability of patties formed using frozen chicken meat and held under retail display conditions (4°C) for 9 days

significantly ($p<0.05$) higher than those for patties containing RM, S, TC, vitamin E meat and BHA/BHT from day 3 onwards.

Comparison of the colour stability of chicken patties containing the optimum concentration of test food ingredients: The colour (Hunter L,a,b values) of patties formed from fresh and previously frozen chicken meat, as well as cooked patties were measured on days 0, 3, 6 and 9 of refrigerated display. There were no trends observed for Hunter 'b' values (data not shown).
There were no significant trends observed for Hunter 'L' values of patties formed from fresh chicken meat. Overall, Hunter 'L' values tended to decrease in the order: BHA/BHT >Fen > WPC > Gin > vitamin E > AV > Mtd > RM > S > SPC > TC > Control (Fig. 4). There were no significant trends observed for Hunter 'L' values of patties formed from previously frozen chicken meat. However, the Hunter 'L' values were higher than those observed for fresh chicken patties for all treatments (Fig. 4). Overall, Hunter 'L' values for previously frozen chicken patties tended to decrease in the order: Fen > BHA/BHT > WPC > Gin > vitamin E = AV = Mtd = RM > SPC > control > S > TC. Hunter 'L' values were generally higher than those observed for fresh patties but lower than those observed for previously frozen patties. Overall, Hunter 'L' values for cooked chicken patties tended to decrease in the order: BHA/BHT > Fen > WPC > Gin > vitamin E = AV > Mtd = RM > SPC > S > control > TC (Fig. 4). Hunter 'L; values were higher for patties containing vitamin E supplemented meat compared to control meat for fresh, previously frozen and cooked trials.
The effect of the optimum concentrations of antioxidants on Hunter 'a' values of patties manufactured from fresh chicken meat after refrigerated display is shown in Fig. 5. In fresh meat, the control sample had the highest average Hunter 'a' values throughout the display period. Hunter 'a' values decreased in the order: control > RM > SP = Vitamin E > BHA/BHT = Fen > Mtd > AV > TC > Gin > WPC > S.
Patties manufactured from previously frozen chicken meat containing Fen, vitamin E, and SP had higher Hunter 'a' values than control patties (Fig. 5). Hunter 'a' values decreased in the order: Fen > Vitamin E > SP > AV > Mtd > Control > Gin > BHA/BHT > S > TC > RM .> WPC. There were no definite trends in the Hunter 'a' values of previously frozen patties compared to fresh patties.
In cooked patties, Hunter 'a' values were higher than the control for all ingredients tested after refrigerated display. Hunter 'a' values for all cooked patties were significantly ($p<0.001$) lower than those for fresh and previously frozen patties (Fig. 5). Mean Hunter 'a' values for cooked patties decreased in the order: Mtd > Vitamin E > RM > BHA/BHT > TC > SP > Fen > AV > WPC > S > Gin > Control. Hunter 'a' values for cooked patties containing AV, S, WP, Gin and control meat were negative after refrigerated display for 9 days, indicating that these samples were devoid of any degree of redness.

Discussion

Comparison of the oxidative stability of chicken patties containing the optimum concentration of test food ingredients: All ingredients screened in this trial, with the exceptions of AV and Fen, proved to be effective antioxidants in fresh chicken patties. In a similar study to the one presented here, O'Shea *et al.* (1998) compared the effect of food ingredients on the oxidative stability of beef patties. These authors reported TC, RM and S to be effective in lowering the TBARS numbers compared to the control in beef samples. However, Mtd and WPC were also effective antioxidants in beef. Similarly, Formanek *et al.* (2001) reported that extracts of *Rosmarinus officinalis* proved to be as effective as a BHA/BHT synthetic antioxidant in fresh minced beef. However, O'Shea *et al.* (1998) found that beef patties containing TC, RM, S, Mtd and WP were comparable to vitamin E supplemented beef and beef patties containing BHA/BHT in terms of their antioxidant potential.
Our results for previously frozen chicken patties are in agreement with the findings of Galvin *et al.* (1998) who reported that dietary α-tocopheryl acetate increased the vitamin E levels in chicken meat and thus improved the oxidative stability of chicken meat during refrigerated and frozen storage. In a similar study and using the same ingredients, McCarthy *et al.* (2001b) found TC and S to be effective antioxidants in fresh and previously frozen pork patties. Interestingly, TC and S behaved very differently in previously frozen pork compared to chicken meat systems, however, RM behaved in the same way for both types of meat.
O'Shea *et al.* (1998) compared the effect of the same food ingredient samples on the oxidative stability of cooked beef patties. These authors reported that all ingredients tested produced lower TBARS values compared to control beef. TC resulted in lower TBARS values compared to patties containing vitamin E supplemented meat or BHA/BHT. This is in agreement with the findings of this study. Similarly, McCarthy *et al.* (2001a) found that cooked pork patties containing TC had the lowest TBARS values. O'Shea *et al.* (1998) observed that Mtd and WPC resulted in lower TBARS values than patties containing vitamin E supplemented meat or BHA/BHT for beef. TBARS values for BHA/BHT were lower than those for vitamin E supplemented meat. TBARS values for patties containing RM and S were comparable to those containing vitamin E supplemented meat.
In general, TBARS numbers were lowest in fresh raw

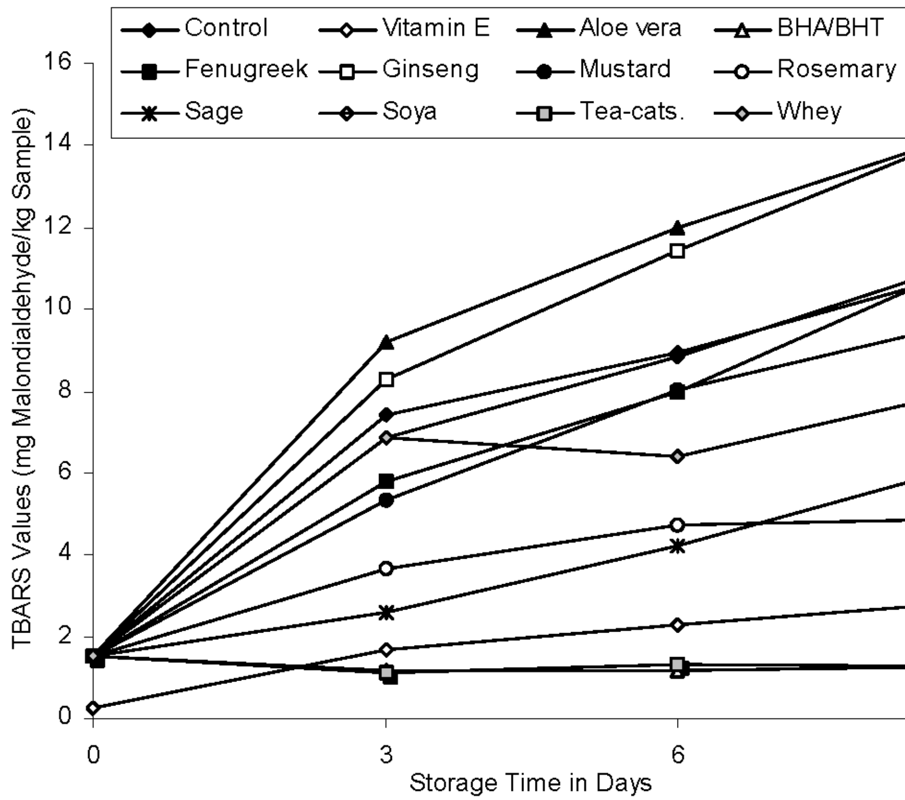

Fig. 3: A comparison of the effect of antioxidants on the oxidative stability of chicken patties cooked and held under retail display conditions (4°C) for 9 days

chicken meat. Frozen storage resulted in increased TBARS numbers. This was previously observed in our laboratory by McCarthy *et al.* (2001a) in pork meat using the same food ingredients and the same methodologies. Cooking resulted in a 10-fold increase in TBARS values compared to previously frozen samples. When meat is cooked and exposed to atmospheric conditions, it oxidizes rapidly (Ahn *et al.*, 1993). Vitamin E supplementation results in chicken meat being protected against oxidation during frozen storage and cooking (Sheehy *et al.*, 1993). This is in agreement with this study which had lower TBARS values in vitamin E supplemented meat than control meat in all trials. In this study, S, RM, and TC, had antioxidant activity comparable to that of dietary vitamin E and BHA/BHT (combined) for fresh and previously frozen chicken meat, while only TC was as effective as vitamin E and BHA/BHT for cooked chicken meat.

Comparison of the colour stability of chicken patties containing the optimum concentration of test food ingredients: There were no definite trends observed for Hunter 'L' values in fresh, previously frozen or cooked chicken patties in the presence or absence of added antioxidants. This is in agreement with McCarthy *et al.* (2001b) who reported no significant differences in Hunter 'L' values for different antioxidants in fresh and previously frozen pork patties. Hunter 'L' values for previously frozen patties were higher ($p>0.05$) than those observed for fresh chicken patties for all treatments and higher ($p>0.05$) than or equal to those for cooked chicken patties. Hunter 'L' values were higher, but not significantly so, for patties containing vitamin E supplemented meat compared to control meat for fresh, previously frozen and cooked trials.

In fresh meat, the control sample had the highest average Hunter 'a' values throughout the display period compared to all other treatments. However, in a similar study on fresh pork patties, McCarthy *et al.* (2001b) identified S and SP as being the most effective test ingredients in terms of meat redness as they had significantly ($p<0.05$) higher Hunter 'a' values than the control and other test ingredients on days 0 and 6. The study by McCarthy *et al.* (2001b) was carried out in our laboratory, using the same food ingredients and the same methodologies as this study, yet the food ingredients behaved very differently in terms of meat redness values (Hunter 'a' values).

Patties manufactured from previously frozen chicken meat containing Fen, vitamin E, and SP had higher Hunter 'a' values than control patties. In a similar study on pork patties, McCarthy *et al.* (2001b) also found Fen

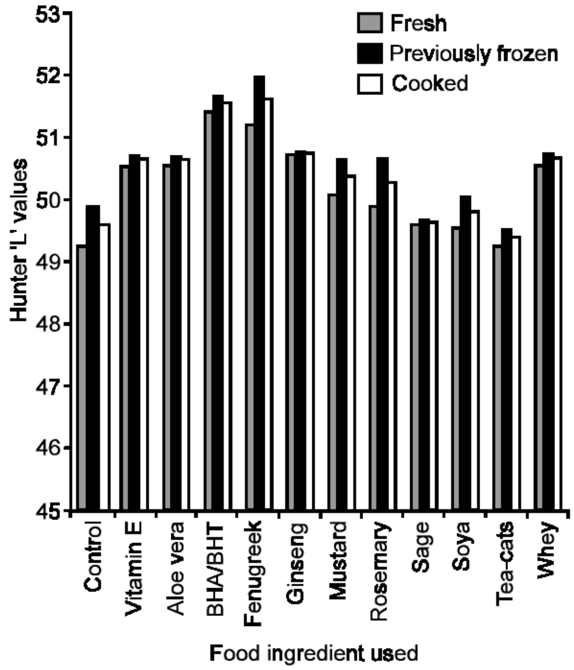

Fig. 4: A comparison of the effects of antioxidants on the Hunter 'L' values of fresh, previously frozen and cooked chicken patties stored under display conditions (4°C) for 9 days

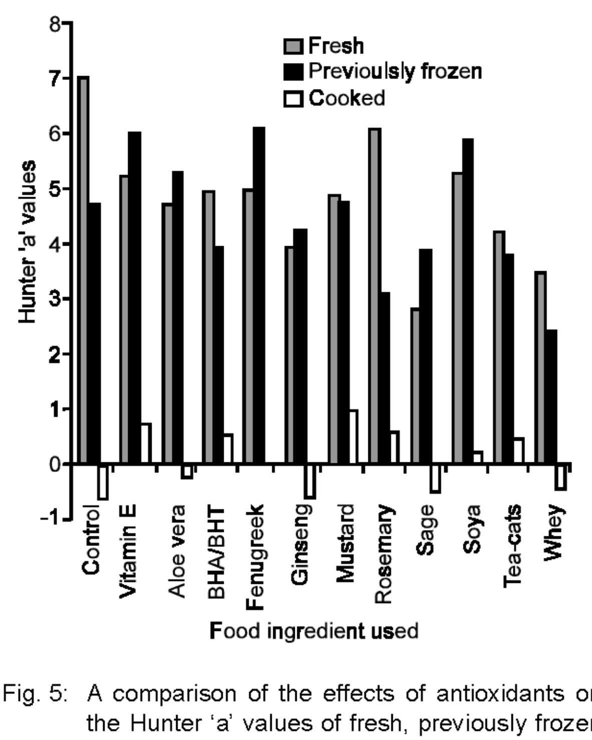

Fig. 5: A comparison of the effects of antioxidants on the Hunter 'a' values of fresh, previously frozen and cooked chicken patties stored under display conditions (4°C) for 9 days

to be the most effective food ingredient tested in terms of maintaining the red colour of patties manufactured from previously frozen meat. This finding is in contrast to that for fresh meat patties, in which the food ingredients tested had very different effects on meat redness in chicken and pork.

Patties manufactured from previously frozen chicken meat containing Fen, vitamin E, and SP had higher Hunter 'a' values than control patties. In a similar trial, McCarthy *et al.* (2001a) found that cooked pork patties containing Fen and WP had higher Hunter 'a' values than the control patties on day 9. However, in contrast to this study on chicken meat, all other food ingredients tested by McCarthy *et al.* (2001a) had higher Hunter 'a' values than the control. This may be due to the ingredients reacting differently in the two meat systems. In fresh chicken patties, control meat had the highest Hunter 'a' values throughout the storage period. This indicates that the chicken meat used was of a high quality and that it maintained it's fresh colour when displayed under retail conditions. However, when the meat was 'stressed' by freezing or cooking prior to display, the Hunter 'a' values of the control meat decreased. The addition of antioxidants had a greater effect on the meat when 'stressed' compared to fresh meat. For previously frozen chicken meat, the addition of Fen, Vitamin E, SP, AV and Mtd all resulted in patties with higher Hunter 'a' values than control meat. When the chicken patties were cooked prior to retail display storage, control meat had the lowest Hunter 'a' values of all the patties studied.

Conclusion: All food ingredients screened were effective at reducing TBARS numbers compared to control meat, with the exceptions of AV and Fen. In fresh meat, BHA/BHT was among the most effective antioxidant in fresh, previously frozen and cooked chicken patties. RM was the most effective food ingredient in fresh patties while TC was the most effective food ingredient in cooked patties. Increasing the 'stress' on the chicken patties increased the effectiveness of added antioxidants. TBARS values in previously frozen chicken meat were 3-fold higher than those for the corresponding fresh meat. TBARS values for cooked chicken meat were 10-fold higher than those for previously frozen meat and 30-fold higher than those for fresh meat. Overall, TC, RM and S had the best antioxidant potential in all chicken patties.

Hunter 'L' values for cooked chicken patties, manufactured with vitamin E supplemented meat, were significantly ($p<0.05$) lower than all other treatment groups on all sampling days. Hunter 'a' values for all cooked chicken patties were significantly ($p<0.001$) lower than the corresponding fresh and previously frozen chicken patties on all sampling days.

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