

## UPTAKE EFFICIENCY OF NATURAL ANTIOXIDANTS INTO CHICKEN BREAST MEAT

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**Abstract.** The efficiency of natural antioxidant uptake into fresh and frozen chicken breast meat was investigated. Uniform pieces of chicken breast fillets (4 × 2 × 2 cm) were immersed for increments between 0 and 60 min in solution containing extracts of natural antioxidants of rosemary, small red bean, sunflower seed, and ginger at 200mg total phenolic equivalent. Moisture uptake and antioxidant (phenolic content) diffusion into different layers of chicken breast fillets were monitored. The moisture uptake was found significantly highest (P<0.05) in fresh meat samples immersed in small red bean extract followed by rosemary from 30 minutes onwards. The major variance in moisture uptake by previously frozen samples was observed in chicken breast treated with rosemary. The diffusion of phenolic compounds into the meat layers of frozen samples increased in order of: cut muscle tissue> fascia membrane side> the core. Fresh and frozen breast fillets dipped into rosemary solution had the highest total phenolic content over immersion time, while the highest penetration of phenolic content was found in frozen samples. Rapid absorption of phenolic content in fresh meat was in the first 20 min and the frozen meat was at 60 min. These finding proved that all antioxidants were capable of improving moisture uptake and phenolic content in chicken fillets when dipped in a natural antioxidant solution, suggesting that penetration of a particular solution into chicken meat may be very useful to increase water-holding capacity, decrease oxidation process and improving texture Since most marination solution that employed in commercial meat processing contain sever phenolic compound.

**Keywords:** chicken breast, immersed, total phenolic content, antioxidant, lipid oxidation.

### Introduction. Formulation of the problem

A number of different approaches can be used to apply antioxidants to a piece of meat. These approaches include wet applications such as: Immersion through soaking or dipping (with or without tumbling), or injection; or rubbing on as a dry application [1-3]. The basis of any wet application process is the capacity of animal muscles to retain water and any water-soluble material when penetrating into muscle [3,4]. The immersion process involves submerging meat for a limited time into a liquid containing additives, with the potential to facilitate absorption of water allow for the functionality of the additive [3,5]. The diffusion rate of a solution into meat, and its retention, is dependent on the gross characteristics of the meat, such as fat content [6]; the

content and location of connective tissue [7]; and the muscle myofibril content [4]. Nunez-Gonzalez [4] reported that muscle myofibrillar proteins are principally responsible for water retention by meat. The process of immersing meat in any solution will cause the uptake of water and increase the weight of a piece of meat. More importantly, the process of immersion can alter the structure of muscle tissue to increase tenderness, juiciness and to enhance the flavour of meat [8,9].

In addition, several studies have shown the progress of uptake of active ingredients into different layers of meat, using phosphate, sodium chloride and a fluorescent dye to monitor the diffusion [9-11]. Xiong and Kupski [10] reported that the diffusion rate of phosphate into the chicken meat was depth dependent, where the rate of phosphate uptake was higher at the outer layer, followed at progressively lower rates at the

middle and inner layers when chicken breast fillets were dipped with different concentrations of phosphate. Offer and Trinick [12] argued that the penetration of total phenolics into meat occurs by capillary force when water penetrates into the narrow spaces within the muscle tissue, which allows muscle fibres to expand and swell. The ability of meat to absorb any solution was found to be correlated with immersion time [8]. Furthermore, penetration of a particular solution into chicken meat is dependent on the portion of the chicken carcass, with chicken breast having a greater ability to absorb a solution than the thigh or drumstick [13]. Although several studies have been carried out to evaluate the effect of the dipping process with antioxidants on characteristics of chicken meat, the amount of moisture uptake and penetration of antioxidant ingredients as mediated by various antioxidants is not well documented. Especially, the kinetic process that contributes to the penetration of the antioxidant solution into the core of the chicken breast fillet during immersion is not well understood. Such knowledge is of practical importance since it can assist to establish the optimum schedule for the dipping process and gives an indication of the diffusion of an antioxidant solution into the different meat layers.

Therefore, this **study aimed** to determine the readiness by which antioxidant ingredients penetrate chicken breast fillets using the immersion by dipping method.

In order to investigate the efficiency of antioxidant uptake into chicken breast meat the following experiments were devised: both fresh and frozen breast meat samples were dipped into four natural antioxidant solutions and deionised water (control) for 60 min to establish moisture uptake.

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### Research materials and methods

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Whole chicken carcasses of Ross breed were obtained from a single supplier in the UK (Faccenda Group Ltd. (Brackley, UK), where all animals fed the same base diet and slaughtered at the same day. The slaughtered age ranged from 36–41 days with an average carcass weight of 1.7 kg.

Ground rosemary (*Rosmarinus officinalis*), ginger (*Zingiber officinale* Roscoe), dry small red bean seeds (*Phaseolus vulgaris*) and sunflower seeds (*Helianthus annuus*) were obtained from various commercial sources. Dried and ground rosemary and ginger were obtained from Gekruidvof (Hoogstraten, Belgium), small red bean seeds from Bob's Red Mill (USA) and sunflower seeds from Suma (Elland, UK).

Chemical materials such as butylated hydroxytoluene (BHT); Acetone, Folin-Ciocalteu and sodium carbonate anhydrous obtained from Fisher Scientific (Loughborough, UK).

#### Preparation of samples

The experiments were conducted on both fresh and frozen chicken carcasses. As such, whole carcasses of chickens that were obtained from the supplier were

divided into two equal batches; one batch was used as fresh meat and the other batch of whole carcass of chickens were frozen at -20°C for two months. For the fresh samples, the whole carcasses were obtained on the day that the experiments commenced. While for the frozen samples, the carcasses were defrosted for 24 h, at refrigerated temperature (4°C). For both the fresh and thawed samples, the breast fillets were removed, and trimmed from all external fat. After which they were cut into a cuboid shape of approximately 4 × 2 × 2 cm which equated to approximately 23.59 ± 0.68 g of meat in weight. One side of the cuboid retained the fascia membrane while the remaining longitudinal sides were cut through the muscle tissue.

#### Preparation of Natural Antioxidant Extracts and analysis

The extraction of natural antioxidants from rosemary (ROS), small red bean (SRB), sunflower seeds (SFS) and ginger (GGR) was optimised and analysed to determine the anti-oxidative capacity of the extracts. The ROS and GGR were supplied as powders, whereas the SRB and SFS were supplied whole. Hence, SRB and SFS were freeze dried (Edwards Modulyo freeze dryer, Sussex, UK) and then ground to a powder in a commercial coffee grinder (DeLonghi KG49, Treviso, Italy), after which the SRB and SFS powders were passed through an 80 and 20 mesh sieve respectively.

Antioxidant extracts of ground rosemary, ginger, and sunflower seeds were produced employing aqueous ethanol (absolute ethanol: water at 80:20 v/v) according to the method described by Selani et al. [14]. Ten grams of the powdered samples were accurately weighed and mixed with 100 ml aqueous ethanol. The mixture was gently shaken in an orbital shaker (HS 501 digital, IKA laborotechnik, Staufen, Germany) for 48 h in a dark place at ambient temperature (20°C). The extracts were passed through a cheesecloth before being filtered through Whatman® No. 1 filter paper. The filtrates were then concentrated in a water bath at 60°C to remove excess solvent until a volumetric reduction of 90% was obtained. The concentrated extracts were stored at -20°C, until analyses for calibration and subsequent use. Small red bean seeds were also extracted with absolute ethanol: water at 80:20 v/v in a similar manner. The total phenolic content in extracts was determined according to the method described by Singleton and Rossi [15].

#### Experimental treatments

Five treatments were prepared, one control (deionised water) and four natural antioxidant treatments. Stock solutions for each of the natural antioxidants (rosemary (ROS), small red bean (SRB), sunflower seeds (SFS), and ginger (GGR)) were made by mixing ethanolic extracts of each antioxidant at 200 ppm (based on total phenolics: GAE). The sectioned fillets of fresh and thawed separately were immersed in the five respective solutions for 10, 20, 30 and 60 min under ambient conditions. The conditions of these

treatments were determined to ensure that immersed fillets would have a range of moisture uptake and phenolic concentration. After that, meat samples were removed from the solution, drained from the extraction liquid and dab-dried using a paper towel and moisture uptake were measured. After that all samples were immediately frozen at -20°C to aid in slicing for measuring phenolic penetration as illustrated in Figure 1. Each treatment was conducted in triplicate. Four longitudinal slices of chicken breast meat ranging from 5 mm thickness were removed from each side of fresh and frozen dipped meat samples as described in figure 1.

#### Determination of total phenolic content in meat

The phenolic content was extracted from the chicken meat according to the method described by Naveena et al. [1] with slight modifications. Approximately 2g of individual slices (the fascia membrane facing part of the chicken breast fillet; longitudinal cuts along of the chicken breast fillet and the core of the chicken breast fillet) were weighed and homogenized in 10 ml of aqueous acetone (acetone: water at 70:30 v/v) using a homogenizer (Silverson Machines Ltd., Chesham, UK) for 30 sec at high speed. The mixture was gently shaken in an orbital shaker (HS 501 digital, IKA labor Technik, Staufen, Germany) for 4 h in the dark at ambient temperature

(23°C) and then refrigerated at 4°C for 24 h after which. The extracts were filtered through Whatman® No. 1 filter paper. Total phenolic content was assessed by the Folin-Ciocalteu reagent as reported by Singleton and Rossi [15]. In brief, 0.5 ml of appropriate extracts and standard solutions were transferred and mixed with 30 ml of distilled water in 50 ml test tubes. To that, 2.5 ml of Folin-Ciocalteu reagent were initially added, after 1 min, 7.5 ml of 20% of sodium carbonate (20 g dissolved in 100 deionised water) was added, and the volume was adjusted to 50 ml using deionised water. All samples were incubated with the standard for 2 h at 23°C. The absorbance at 760 nm was recorded after colour development against a blank by using spectrophotometer (Beckman, DU640 spectrophotometer, Fullerton, CA). The total phenolic content was expressed as mg GAE/1ml extract, followed by conversion of this value to mg GAE/100 g of dry weight.

#### Moisture uptake analysis

Portions of chicken breast meat as described before were weighed before and immediately following dipping. The dipped meat samples were drained from the extraction solution and dab-dried before being re-weighed. Each treatment was conducted in triplicate. Moisture uptake was calculated using the following formula:

$$\text{Moisture uptake (\%)} = \left[ \frac{\text{weight of dipped meat (g)} - \text{weight of meat before dipping (g)}}{\text{weight of meat before dipping (g)}} \right] \times 100$$

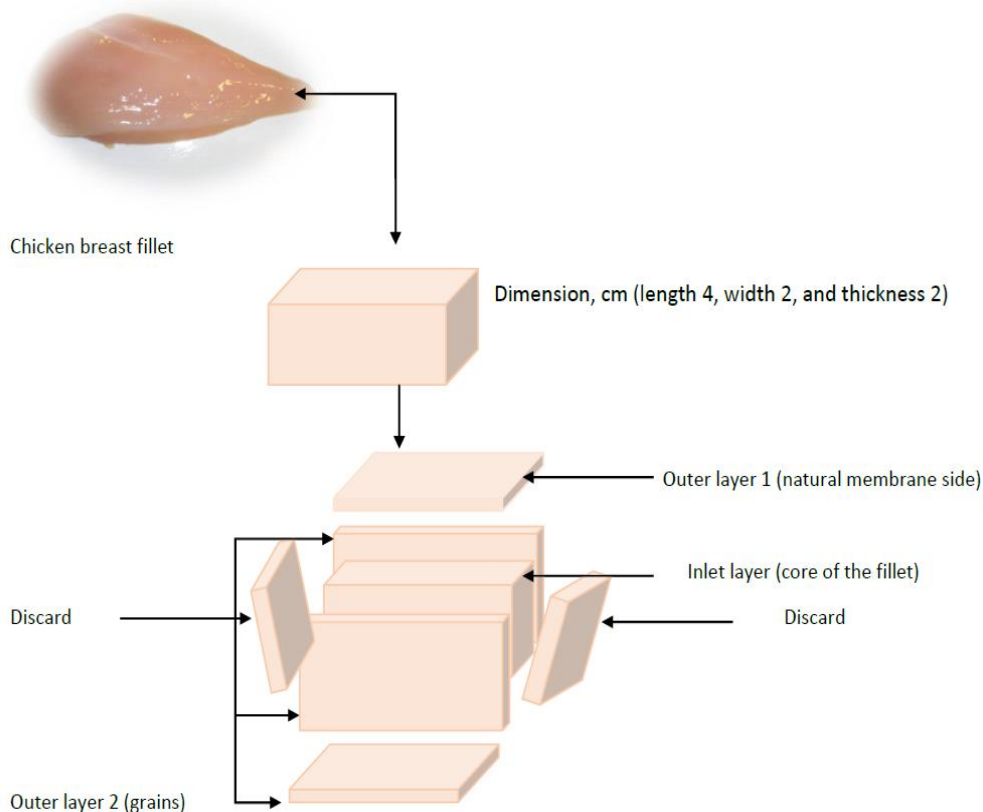


Fig.1 Illustration of the sample diffusion for monitoring total phenolic penetration into the chicken breast fillet

### Statistical analysis

The data of dipping fresh and frozen chicken meat were analysed separately. A  $6 \times 5 \times 3$  factorial design was used for treatments (rosemary, ginger, sunflower seed, small red bean, and non-treated), dipping times (0, 10, 20, 30 and 60 minutes) and chicken breast fillets layers (Outer layer 1, outer layer 2 and inlet layer) as main factors and interaction between them. Penetration of total phenolic content was analysed by using three-way analysis of variance ANOVA, while moisture uptake and TBARS values was analysed by using two-way analysis of variance ANOVA by using the GenStat statistical software (Edition 17<sup>th</sup>, VSN International Ltd). The experiment was conducted in triplicate. Tukey's test was used to identify the significant differences between means, and the significance level of all data was set at ( $P < 0.05$ ).

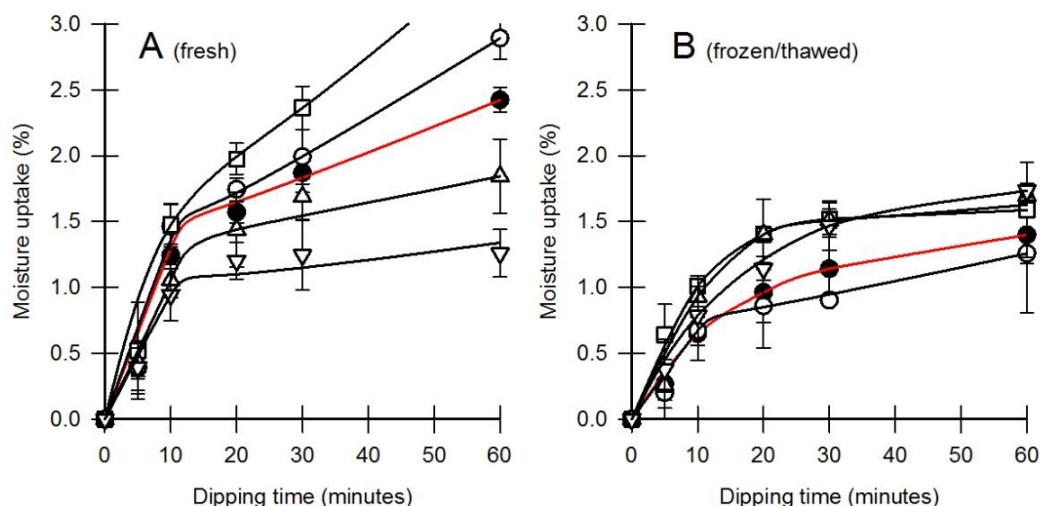
## Results of the research and their discussion

### Moisture uptake

The proportion of moisture uptake due to dipping chicken breast meat into various antioxidant solutions in fresh and frozen meat samples increased progressively as the dipping time progressed (Figures 2A and 2B respectively). The absorption of moisture between treatments of fresh meat was not significantly different ( $P > 0.05$ ) over the first 20 min of the dipping process, however from 30 minutes onwards the highest absorption of moisture uptake was observed in fresh meat samples dipped in SRB followed by ROS solution. However, moisture uptake in fresh breast samples treated with SFS and GGR was markedly less (Figure 2A). On the other hand, breast meat samples that were previously frozen showed very little diversion with regards to weight

from the CON and each other during the entire dipping process (Figure 2B). The only major variance in moisture uptake by previously frozen samples was observed in chicken breast treated with ROS.

The moisture content is one of the most important indicators to evaluate meat quality. Dipping meat into a solution can increase moisture content. Hosseini and Mehr [16] found a high correlation between moisture content and solute uptake in beef meat. Dipping chicken meat in the GGR solution leads to increased moisture content which enhanced the hydrophilic properties [17]. Similar findings were observed in the current study, where the dipping process increased moisture uptake of chicken breast fillets (Figure 2). The percentage of moisture uptake in chicken meat was similar to those reported by Yusop et al. [5], who found that the uptake of a marinade by chicken breast fillets dipped for 30, 60, 120 and 180 min ranged from 2.63–2.92%. In the current study, the moisture uptake was dependent on the dipping time in accordance with results previously published by Yusop et al. [5]. Our results suggest that the antioxidant solution entry into the chicken meat is a dynamic process during which compounds gradually diffuse into the meat tissue. The greatest moisture uptake was observed in fresh meat. Furthermore, the reduction of moisture uptake among the frozen samples could be due to the changes in muscle fibre structure during the freezing periods that limits the ability of meat to take up and retain moisture. The lower moisture uptake in the fresh samples when dipped in the ethanolic extracts of sunflower and ginger is surprising and suggest a hitherto unknown mechanism that reduces the uptake of water into chicken breast fillets by these extracts.



**Figure 2. The effect of dipping time on the antioxidant solution uptake by 'A' fresh chicken fillets, and 'B' frozen and thawed chicken fillets.**

The data shown are the average and standard deviation of three independent samples (Means  $\pm$  SED;  $n=3$ ).

● – control; ○ – Rosemary extract; □ – small red bean extract; △ – sunflower seed extract; ▽ – ginger extract

A possible explanation might be that in the ethanolic extract various hydrophobic constituents were extracted from both the ginger and sunflower that blocked the uptake of water. Both ginger and sunflower are known to contain hydrophobic compounds that could facilitate such an affect [18,19].

#### **Total phenolic content**

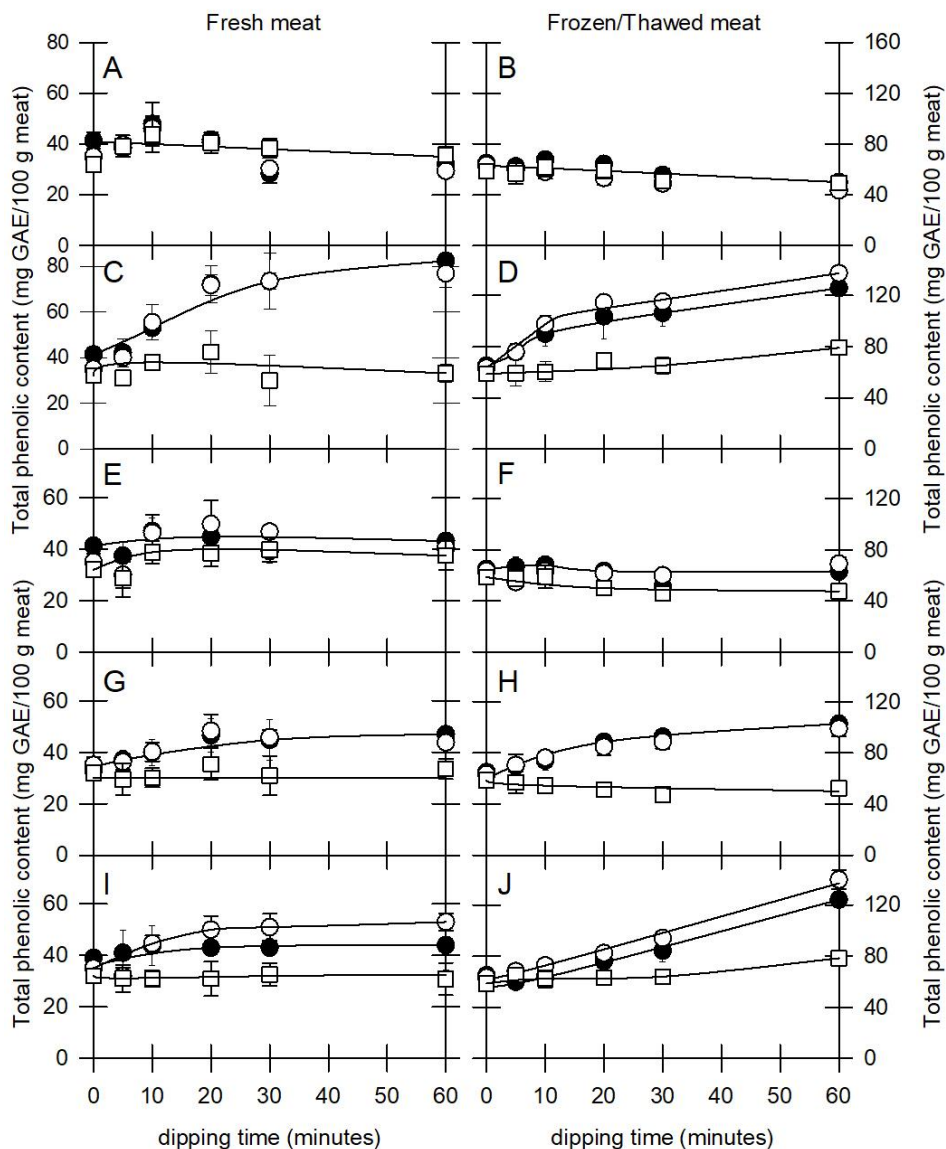
The total phenolic content in the core of fresh chicken breast samples showed a significant change over time regardless of treatment (Figure 3). Furthermore, no significant changes over time were observed when the chicken breast samples were exposed to deionised water (CON) regardless of tissue location (Figure 3A). The outer layer of fresh chicken breast tissue that is directly covered by the membrane showed very little variation from the CON samples when dipped in a variety of antioxidant solutions, with the exception of the ROS dipping solution. Over time, the samples exposed to the ROS dipping solution doubled its total phenolic content. The same observations were made on the cut side outer layer of the chicken breast tissue (Figure 3).

In general, frozen meat samples were found to have significantly higher ( $P < 0.05$ ) penetration of antioxidants than those in fresh meat when compared to the samples dipped into deionised water (CON) (Figure 3). The diffusion of phenolic compounds into the meat layers of frozen samples increased in order of: outer layer2 > outer layer1 > the core of fillets. Moreover, either fresh or frozen breast fillets dipped into ROS solution had the highest total phenolic content compared to other treatments over dipping time, while the highest penetration of phenolic content was found in frozen samples; however, frozen meat in ROS solution absorbed higher total phenolic content than those in fresh meat.

In addition, during the dipping time Figure 3 shows, that total phenolic content was highest in fresh meat samples dipped in ROS at 60 min and SRB at 10 min, while meat samples dipped into SFS, GGR and BHT had the highest rate of penetration at 20 min. On the contrary, as shown in Figure 3, all dipped frozen meat samples with except SRB had a highest total phenolic penetration at 60 min. At 60 min, frozen meat samples had the highest total phenolic when dipped in GGR followed by ROS, BHT, SFS, SRB, and CON, respectively.

The penetration efficiency of antioxidant compounds into the subsequent layers of chicken breast fillets was monitored in order to determine the optimal time taken to achieve a desired phenolic content. Several past studies have used phosphate, sodium chloride and fluorescent dyes to monitor the

diffusion of marinade into different layers of meat [9-11]. However, the principal aim of this study was to establish the appropriate time of exposure to an antioxidant containing solution for subsequent experiments. In the current study, the penetration level of total phenolics into chicken breast fillets mostly depended on antioxidant type and dipping time. Since the penetration rate of total phenolic into the core of the fillet was lower than what was found to be present in the outer layers; it was established that the uptake of various natural antioxidants was not instantaneous. It is evident that the antioxidant solution that overcomes the physical barriers or restrictions in the chicken muscles was limited to approximately 2 to 2.5 mm on each side during the dipping time. Xiong and Kupski [10] reported similar finding that the diffusion rate of phosphate into the chicken meat layers was higher in the outer layer, followed middle and inner layer when chicken breast fillets dipped with different concentration of phosphates. The penetration of antioxidant solutions and total phenolics into meat is apparently facilitated by a capillary force that can deliver water into the core of muscle and expand muscle fibres [12]. Xiong and Kupski [10] observed swollen muscle fibres in the chicken muscle tissue after meat was marinated with different phosphate marinades that caused a rapid extension of the myofibril matrix of the muscle and disintegration of the actomyosin complex. Naveena and Mendiratta [17] pointed out that samples dipped into GGR extract at 3%, for 24 h had a higher muscle fibre diameter compared to the non-treated samples with mean values 76.90 and 80 microns, for non-treated and treated samples, respectively. Furthermore, in our study, best penetration of total phenolic was observed in fresh meat and frozen meat at 20 min and 60 min, respectively. This result was in contrast to those reported by Xiong and Kupski [11] who found that optimal penetration of phosphate was detected at 5 min of dipping time. Moreover, fillets of frozen and fresh dipped in ROS solution were found to have the highest levels of total phenolic compounds. However, the mechanisms behind this remain unknown but could be due to the molecular weight of phenolic compounds present in ROS, which could have a relatively high diffusivity. This result suggested that the ROS solution penetrated into the chicken fillets more rapidly while the diffusion in the deep layer of fillets was much slower. The dynamic process that contributed in term of penetration of the antioxidant solution into the core of the chicken breast fillet during immersion could be due to capillary forces [12].



**Figure 3. Uptake of antioxidants from natural sources as measured in gallic acid equivalents (GAE) in fresh and frozen-then-thawed chicken breast meat.**

Graphs A and B represent the control samples (dipped in deionised water only) for fresh and frozen/thawed samples respectively; C and D represent samples dipped in a solution containing rosemary extract at 200 ppm GAE for fresh and frozen/thawed samples respectively; E and F represent samples dipped in a solution containing 'small red bean' extract at 200 ppm GAE for fresh and frozen/thawed samples respectively; G and H represent samples dipped in a solution containing sunflower seed extract at 200 ppm GAE for fresh and frozen/thawed samples respectively; I and J represent samples dipped in a solution containing ginger extract at 200 ppm GAE for fresh and frozen/thawed samples respectively. ●, GAE presence in the outer 5 mm of the fascia membrane facing part of the chicken breast fillet; ○, GAE presence in the outer 5 mm of the longitudinal cuts along of the chicken breast fillet; □, GAE presence in the core of the chicken breast fillet sample after the outer layers (5 mm each) were removed. The data shown are the average and standard deviation of three independent samples (Means  $\pm$  SED; n=3).

### Conclusion

All antioxidant extracts were capable of improving moisture uptake in chicken fillets when dipped in a natural antioxidant solution. The absorbed moisture increased with increasing dipping time. Breast fillets dipped with either SRB or ROS solutions were observed to have a higher moisture uptake than any of

the other treatments. The results also suggested that dipping meat into various antioxidant solutions had a positive effect on penetration of phenolic content. Rapid absorption of phenolic content occurred in fresh meat was in the first 20 min; while a similar absorption in frozen meat took 60 min. The penetration of phenolic content was higher in both fresh, and frozen

chicken breast fillets dipped in ROS solution. Penetration of phenolic content was greater at the fascia membrane followed by the cut muscle tissue.

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