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Effects of cooking methods on thiamin and riboflavin contents of chicken meat

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Broiler meat (light and dark) was cooked by roasting, braising, deep-frying and microwave methods and cooking yield, moisture, fat, thiamin and riboflavin contents of the meat, before and after cooking), were determined. Light and dark muscles differed significantly in moisture, fat, thiamin and riboflavin contents. Generally, moisture and fat contents of broiler meat were significantly decreased by cooking, but the fat content of the dark muscles showed no significant change when cooked by microwave. All cooked meats were significantly lower ($p < 0{\cdot}05$) in thiamin content than raw meat. Cooking had no significant effects on riboflavin content of dark meat on fresh weight or dry weight fat-free basis (FW and DWF), but in light meat, the riboflavin content decreased significantly (DWFF) when cooked by frying. While thiamin retention (DWFF) ranged from 28 to 64%, riboflavin was fairly well retained (46–94%) in cooked broiler meat. Differences were observed in the retentions of both vitamins of cooked meats suggesting that there were differences in the effects of the four cooking methods evaluated.

INTRODUCTION

Chicken is an important item in the diet of the Saudi. This is indicated by a high *per capita* consumption record of 28·3 kilograms per person consumed in 1989 (Anon., 1989). The increasing production of chickens and their potential use in restaurants and food service operations implies the need for more detailed information regarding their quality and nutrient retention. Chicken is comparable to red meats and fish in composition and nutritive value. Like all lean meats, it is a good source of B-vitamins. Mountney (1976) reported that chicken meat is a moderately good source of riboflavin and thiamin. The nutritional composition of chicken is influenced by many variables such as breed, feed, age, method of production, sex and cooking method. Singh & Essary (1971) reported differences in amounts of thiamin and riboflavin found in breast and thigh meats from 8 and 10 week broilers according to age, sex and part examined.

Cooking causes significant reductions in values of nutrients of chicken flesh. However, effects can vary between cooking methods (Demby & Cunningham, 1980). Of all the nutrients present in chicken meat, water-soluble vitamins and minerals are most susceptible to loss or destruction during cooking. Several workers have studied the effects of cooking methods on vitamin contents of chicken. Spencer (1973) noted that thiamin was unstable to heat, but riboflavin was somewhat more stable. Lang (1970) reported that while riboflavin was fairly heat-stable, thiamin was heat-labile; also Lund (1973) noted that thiamin was one of the most heat-labile vitamins. According to Klein *et al.* (1984), heat processing of meat results in vitamin losses as high as 40% for more labile vitamins such as thiamin. Harris & Von Loesecke (1960) indicated that thiamin was significantly reduced (up to 40%) in chicken, while riboflavin was fairly well retained (80–90%) in cooked poultry.

Cooking methods have different effects on the nutritional values of chicken. Demby & Cunningham (1980) reported retention levels for thiamin and riboflavin in stewed chicken as 54·5 and 92·7% respectively. They also reported retention mean values for thiamin and riboflavin in baked chicken of 73·1 and 90·1, respectively. Roasting at 177°C reduced the thiamin content in breast muscle an average of 28·9% and in dark meat an average of 34·3% (Robertson *et al.*, 1966). The effect of microwave cooking on the retention of water-soluble vitamins has been the subject of several research reports. Results range from no difference between microwave and conventional method to microwave cooking giving better retention (Demby & Cunningham, 1980). Harris & Karmas (1975) reported no essential changes in nutrients caused by microwave cooking.

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A number of analytical methods have been proposed for water-soluble vitamin determination (Finglas & Faulks, 1987). HPLC methods offer an attractive alternative to the more time-consuming chemical or microbiological assays because of their increased specificity, sensitivity and short time needed for analysis, although the initial capital cost and subsequent costs are relatively high (Bertelsen *et al.*, 1988). Because of the importance of chicken in the Saudi diet, this study was aimed at investigating the effects of different methods of cooking on thiamin and riboflavin levels in chicken meat using HPLC methods.

MATERIALS AND METHODS

Twenty-four locally produced and dressed broilers, each weighing 1000 g (±50 g) without giblets, were bought from a local supermarket. Chickens were divided randomly into four groups, six in each. The wings were removed by a cut through the shoulder joint at the proximal end of the humerus. The leg portion (dark muscle) was obtained by cutting through the junction of the thigh muscles with the pelvic girdle to the hip joints disjointing the femur. The breast was obtained by cutting on each side of the vertebral column beginning at the mid-point of the sternal ribs until the breast portion was completely freed from the back portion, then split down to right and left portions. The right legs and breast portions for each group were pooled separately as dark and light muscles to provide the raw controls. The remaining left legs and breast portions for each group were prepared for cooking. Four cooking methods were evaluated; roasting, braising, deep-frying and microwave methods.

Roasting. Meats were cooked, uncovered in a dry heat oven model NMK, Barbecue King Ltd, Reading, UK.

Braising. Meats were placed in braising pans and, after water was added, covered with aluminium foil then heated in the above oven.

Frying. Meats were placed in a frier rack and cooked in an AEG Electrical Fried Type EWK 0037 Model FT 102.

Temperature was set at 175°C for the three cooking methods and the samples were cooked to the desired internal end point temperature (85°C). The cooking times required until the internal temperature of the meat reached 85°C were 45, 40 and 15 min, for roasting, braising and frying, respectively.

Microwave cooking. Meats were cooked uncovered for 15 min per kg at the high (100%) power level setting in a Tappan microwave oven, model 65-4787-10/01174.

The cooked samples were allowed to cool to room temperature (24°C). Weights of meats were recorded prior to and after cooking. From raw and cooked pooled samples the bones were removed, then lean and skin (edible portion) was ground three times in a meat grinder. Ground samples were frozen as rapidly as possible and stored frozen at −20°C until required for analysis.

Cooking yield. Cooked sample weight was divided by the raw weight and multiplied by 100 to give the percentage of cooking yield.

Crude fat and moisture. These analyses were carried out according to procedures outlined by the AOAC (1984).

Thiamin and riboflavin analysis by HPLC. Samples were prepared in duplicate following the procedure of Fellman *et al.* (1982). The thiamin in the filtrate was derivatized into thiochrome by adding 5 ml, 1% potassium ferricyanide in 15% NaOH. In order to concentrate and filter the two vitamins, the extract was passed through an activated C18 Sep-Pak cartridge (Waters Associates, Milford, MA), and then eluted with 4 ml of 50% aqueous methanol.

Filtered samples were analysed on a Waters Associates Model 501 Solvent delivery system, a U 6 K injector and a 30 cm × 3·9 mm Bondapak C18 column isocratically eluted with 37% methanol/0·01 M phosphate buffer, pH 7·0. The detector settings for the two vitamins were 360 nm, excitation filter, and 425 nm, emission filter, for thiamin as thiochrome and 450 nm and 530 nm for riboflavin using the 420 fluorescence detector of Waters Associates. The data were quantified by the maxima 820 baseline of Waters Associates.

Vitamin retention on cooking. Retention of the two vitamins was expressed on a fresh weight basis using average data for each muscle type. Retentions on the dry, fat free basis were calculated according to the formulas given by Cooksey *et al.* (1990).

Statistical analysis. The main effects of muscle type and cooking methods on moisture, fat and vitamin (B_1 and B_2) contents were subjected to analysis of variance using the Statistical Analysis System package (SAS, 1986). Significant differences between treatment means were determined using Duncan's New Multiple Range Test (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Results for moisture and fat content of raw and cooked meats from light and dark muscles are summarized in Table 1. Statistical analysis showed that there were significant differences ($P < 0·05$) between light and dark muscles for moisture and fat content. Moisture values of the light meat were generally higher than those of dark meat, but fat values of the light meat were lower than those of dark meat. Literature data showed that the percentages of moisture of broiler meat from light and dark muscles ranged from 62·87 to 64·00% (Twining *et al.*, 1977), 72·2 to 75·2% (Hamm *et al.*, 1980) and 63·2 to 75·4% (Demby & Cunningham, 1980). Our results of 65·16 to 70·42% are generally in agreement with the literature.

Table 1. Effect of muscle type on moisture and fat contents of cooked and raw broiler meat*

Treatment	% Moisture ± standard deviation				% Fat ± standard deviation			
	Cooked		Raw		Cooked		Raw	
	Light muscles	Dark muscles	Light muscles	Dark muscles	Light muscles	Dark muscles	Light muscles	Dark muscles
Roasting	65·35^a ± 0·01	62·92^b ± 0·14	68·48^a ± 1·01	67·16^b ± 0·39	7·03^b ± 0·05	11·94^a ± 0·73	10·60^b ± 0·11	16·89^a ± 0·04
Braising	64·58^b ± 0·05	65·36^a ± 0·13	69·42^a ± 0·07	65·80^b ± 0·12	7·52^b ± 0·10	12·29^a ± 0·08	8·99^b ± 0·08	13·84^a ± 0·46
Frying	59·52^b ± 0·12	62·39^a ± 0·23	68·21^a ± 0·43	65·16^b ± 0·03	8·51^b ± 0·09	12·78^a ± 0·22	12·43^b ± 0·02	16·24^a ± 0·51
Microwave	63·74^b ± 0·10	64·51^a ± 0·02	70·42^a ± 0·10	67·05^b ± 0·85	7·84^b ± 0·06	14·57^a ± 0·12	9·16^b ± 0·14	14·47^a ± 0·29
Mean	63·30^a	63·80^a	69·13^a	66·29^b	7·72^b	12·90^a	10·29^b	15·36^a

* Means in the same row, within raw or cooked treatment groups for % moisture or % fat followed by different superscript letters are significantly different ($p \leq 0·05$).

Data in the literature for fat content varied widely. Hamm *et al.* (1980) found a range of 0·70–8·53%; Demby & Cunningham (1980) reported 10·2–14·1% and Twining *et al.* (1977) reported 12·60–14·36%. Our results of 8·99–16·89%, confirmed the variations in fat content between parts and also indicated differences between samples of the same part.

The results of moisture and fat content of cooked tissues are presented in Table 2. Significant differences were observed between the cooked light and cooked dark muscles in the four different cooking methods. Wider significant differences in moisture and fat content were observed between the raw and cooked meats as indicated in Table 2. These differences were largely caused by loss of moisture and fat during cooking. Meats cooked by frying and roasting lost more weight than those cooked by braising and microwave. These differences may also reflect moisture and fat absorbed by braised and fried meats from the cooking water and fat, respectively.

Roasting tends to produce lower cooking yield in light meat and dark meat (66·84% and 77·65%, respectively) than braising which produces 78·77% and 85·82% for light and dark meats, respectively. This is due to moisture evaporation (uncovered meat) and the extended cooking time used. Frying gave an average cooking yield of 61·31% and 73·58% while microwave cooking resulted in cooking yields of 74·76% and 84·98%, for light and dark meats, respectively.

The mean values of cooking losses found in this study are in general agreement with the cooking losses of 22–29% reported by Matthews & Garrison (1975) for broiler roasting. Cunningham & Lee (1975) compared cooking losses of broilers on roasting, microwave and deep-fat frying. They reported that losses were 21·6%, 23·6% and 45·5%, respectively.

Tables 3 and 4 show the analytical results for thiamin and riboflavin in raw and cooked meats from light and dark muscles. Generally, dark muscles appeared to contain significantly ($P < 0·05$) more thiamin and riboflavin than light muscles. Thiamin values are about the same and in the range of 0·08 to 0·15 and 0·11 to 0·20 mg/100 g reported by Bertelsen *et al.* (1988), and 0·4 to 1·4 and 0·5 to 2·11 μg/g reported by Demby & Cunningham (1980), for light and dark chicken meats, respectively. Our thiamin mean values (1·66 and 1·92 μg/g) are higher than those (0·051 and 0·069 mg/100 g) reported by Ang & Hamm (1982) and 0·051 and 0·063 mg/100 g reported by Ang (1986).

Also, riboflavin values are close to values (0·132–0·140 mg/100g) reported by Ang (1986) and values (0·128–0·217 mg/100 g) reported by Ang & Hamm (1982) and those (0·212–0·288 mg/100 g) reported by Singh & Essary (1971) for chicken meats. These

Table 2. Effect of cooking on moisture and fat contents, cooking yield and cooking loss of broiler meat*

Treatment	% Moisture ± standard deviation		% Fat ± standard deviation		Cooking yield (%)	Cooking loss (%)
	Cooked	Raw	Cooked	Raw		
Light muscles:						
Roasting	65·35^b ± 0·01	68·48^a ± 1·01	7·07^b ± 0·05	10·60^a ± 0·11	66·84	33·16
Braising	64·58^b ± 0·05	69·42^a ± 0·07	7·52^b ± 0·10	8·99^a ± 0·08	78·77	21·23
Frying	59·52^b ± 0·12	68·21^a ± 0·43	8·51^b ± 0·09	12·43^a ± 0·02	61·31	38·69
Microwave	63·74^b ± 0·10	70·42^a ± 0·10	7·84^b ± 0·06	9·16^a ± 0·14	74·78	25·22
Mean	63·30^b	69·13^a	7·72^b	10·29^a		
Dark muscles:						
Roasting	62·92^b ± 0·10	67·16^a ± 0·39	11·49^b ± 0·73	16·89^a ± 0·04	77·65	22·35
Braising	65·36^b ± 0·13	65·80^a ± 0·12	12·29^b ± 0·08	13·84^a ± 0·46	85·82	14·18
Frying	62·39^b ± 0·23	65·16^a ± 0·03	12·78^b ± 0·22	16·24^a ± 0·51	73·58	26·42
Microwave	64·51^b ± 0·02	67·05^a ± 0·85	14·57^b ± 0·12	14·47^a ± 0·29	84·78	15·02
Mean	63·80^b	66·29^a	12·90^b	15·36^a		

* Means in the same row, for % moisture of % fat followed by different superscripts letters are significantly different ($P \leq 0·05$).

Table 3. Effect of muscle type on thiamin content (μg/g) of cooked and raw broiler meat*

Treatment	Fresh weight basis (FW)				Dry weight, fat -free basis (DWFF)			
	Cooked		Raw		Cooked		Raw	
	Light muscles	Dark muscles	Light muscles	Dark muscles	Light muscles	Dark muscles	Light muscles	Dark muscles
Roasting	$0.91^b \pm 0.002$	$1.12^a \pm 0.03$	$1.51^b \pm 0.09$**	$1.92^a \pm 0.03$	$3.28^b \pm 0.01$	$4.47^a \pm 0.24$	$7.22^b \pm 0.66$	$12.1^a \pm 0.50$
Braising	$0.88^a \pm 0.08$	$0.99^a \pm 0.08$	$1.09^a \pm 0.10$	$1.21^a \pm 0.04$	$3.15^b \pm 0.03$	$4.43^a \pm 0.37$	$5.07^b \pm 0.47$	$5.94^a \pm 0.07$
Frying	$1.76^b \pm 0.05$	$2.17^a \pm 0.03$	$2.31^b \pm 0.17$	$2.61^a \pm 0.05$	$5.50^b \pm 0.15$	$8.74^a \pm 0.12$	$11.9^b \pm 0.64$	$14.0^a \pm 0.06$
Microwave	$1.35^b \pm 0.12$	$1.63^a \pm 0.09$	$1.73^b \pm 0.05$	$1.94^a \pm 0.07$	$4.75^b \pm 0.41$	$7.90^a \pm 0.40$	$8.50^b \pm 0.35$	$10.6^a \pm 1.01$
Mean	1.22^a	1.48^a	1.66^a	1.92^a	4.17^b	6.39^a	8.18^a	10.7^a

* Means in the same row, within cooked or raw treatment groups on a fresh weight or a fat-free weight basis followed by different superscript letters are significantly different ($P \leq 0.05$).
** Means ± standard deviation.

differences may be partially explained by differences in processing, analytical procedures and other factors. Singh & Essary (1971) reported differences in amounts of riboflavin and thiamin found in breast and thigh meat from broilers according to age, sex and part examined. According to Singh & Essary (1969) there was some vitamin loss in the thaw drip. Also, as reported by Ang & Hamm (1982) there are differences between vitamin extractions due to the method of preparing the meat sample.

Since the percentages of fat and moisture varied significantly between light and dark meats, and in some instances between birds, it was thought that the variations of vitamin values expressed on a wet weight basis could be due in part to the variations in moisture and fat content. Therefore, additional statistical analyses of the data were done on a fat-free dry weight basis (Tables 3 and 4), to show the variations without the influence of moisture and fat. However, the analyses as shown in Tables 3 and 4 strongly suggest that the variations in levels of thiamin and riboflavin in broiler meat were related to type of muscles and not just due to the variations in moisture and fat contents.

Of all the nutrients present in chicken meat, water-soluble vitamins are the most susceptible nutrients to loss or destruction during cooking. Four of the most commonly used cooking methods were evaluated to determine their effects on the thiamin and riboflavin values of broiler meat. Tables 5 and 6 show the statistical comparisons of vitamin data obtained from raw and cooked meats and the vitamin retentions when chicken meat is cooked by the four different methods. Significant differences were found ($P \leq 0.05$) between raw and cooked meats, except in riboflavin content of light muscles when cooked by either braising or frying (Table 6). Studies of nutritional quality of cooked meats by Klein *et al.* (1984), Spencer (1973), Harris & Von Loesecke (1960) and Lang (1970) showed that thiamin was unstable to heat, but riboflavin was somewhat more stable. According to Cooksey *et al.* (1990), cooked roasts were significantly lower in thiamin content (dry, fat-free basis) and thiamin retention (true and dry, fat-free basis) than raw roasts ($P \leq 0.05$). The true retentions of thiamin and riboflavin in the cooked light and dark meats of broilers, when calculated on the basis of raw and cooked weights, were between 39·9 and 71·5% and 70·1% and 126%, respectively (Tables 5 and 6). In a review of factors affecting composition of chicken meat, Demby and Cunningham (1980) reported that percent retentions of thiamin and riboflavin in cooked chicken ranged from 44 to 80%; and 79 to 117%, respectively. These results are generally in agreement with the literature.

Thiamin and riboflavin contents are expressed as μg/g and mg/100 g of meat as consumed, but do not take into account weight losses during cooking. True retention, as defined by Murphy *et al.* (1975), is an adjusted value which includes weight changes. How-

Table 4. Effect of muscle type on riboflavin content (mg/100 g) of cooked and raw broiler meat*

Treatment	Fresh weight basis (FW)				Dry weight, fat-free basis (DWFF)			
	Cooked		Raw		Cooked		Raw	
	Light muscles	Dark muscles	Light muscles	Dark muscles	Light muscles	Dark muscles	Light muscles	Dark muscles
Roasting	$0.25^b \pm 0.01$	$0.40^a \pm 0.03$	$0.17^b \pm 0.01$**	$0.33^a \pm 0.03$	$0.83^b \pm 0.03$	$1.59^a \pm 0.11$	$0.81^b \pm 0.07$	$2.07^a \pm 0.18$
Braising	$0.17^b \pm 0.02$	$0.40^a \pm 0.02$	$0.16^b \pm 0.03$	$0.34^a \pm 0.01$	$0.60^b \pm 0.07$	$1.79^a \pm 0.09$	$0.74^b \pm 0.12$	$0.65^a \pm 0.03$
Frying	$0.11^b \pm 0.02$	$0.35^a \pm 0.04$	$0.14^b \pm 0.01$	$0.24^a \pm 0.06$	$0.34^b \pm 0.06$	$1.40^a \pm 0.17$	$0.70^b \pm 0.07$	$1.31^a \pm 0.31$
Microwave	$0.17^b \pm 0.01$	$0.37^a \pm 0.02$	$0.11^b \pm 0.01$	$0.30^a \pm 0.06$	$0.61^b \pm 0.04$	$1.78^a \pm 0.10$	$0.52^b \pm 0.07$	$1.61^a \pm 0.42$
Mean	0.17^b	0.38^a	0.14^b	0.30^a	0.60^b	1.64^a	0.69^b	1.66^a

* Means in the same row, within cooked or raw treatment groups on a fresh weight or a fat-free dry weight basis followed by different superscript letters are significantly different ($P \leq 0.05$).
** Means ± standard deviation.

Table 5. Effect of cooking method on thiamin content (μg/g) of boiler meat*

Treatment	Fresh weight basis (FW)			Dry weight, fat-free basis (DWFF)		
	Cooked	Raw	Retention (%)	Cooked	Raw	Retention (%)
Light muscles:						
Roasting	$0.90^b \pm 0.003$	$1.51^a \pm 0.09$**	39.9	$3.28^b \pm 0.01$	$7.22^a \pm 0.66$	30.4
Braising	$0.88^b \pm 0.08$	$1.09^a \pm 0.10$	63.6	$3.15^b \pm 0.30$	$5.07^a \pm 0.47$	48.9
Frying	$1.75^b \pm 0.05$	$2.31^a \pm 0.17$	46.7	$5.50^b \pm 0.15$	$12.0^a \pm 0.64$	28.2
Microwave	$1.35^b \pm 0.12$	$1.74^a \pm 0.05$	58.0	$4.75^b \pm 0.41$	$8.50^a \pm 0.35$	41.8
Mean	1.22^b	1.66^a	52.0	4.17^b	8.19^a	37.3
Dark muscles:						
Roasting	$1.12^b \pm 0.04$	$1.92^a \pm 0.03$	45.3	$4.47^b \pm 0.24$	$12.1^a \pm 0.50$	28.8
Braising	$0.99^b \pm 0.08$	$1.21^a \pm 0.04$	70.2	$4.43^b \pm 0.37$	$5.94^a \pm 0.07$	64.0
Frying	$2.17^b \pm 0.03$	$2.61^a \pm 0.03$	61.2	$8.74^b \pm 0.12$	$14.0^a \pm 0.06$	45.8
Microwave	$1.64^b \pm 0.10$	$1.95^a \pm 0.07$	71.5	$7.91^b \pm 0.40$	$10.6^a \pm 0.01$	63.5
Mean	1.48^b	1.92^a	62.0	6.40^b	10.7^a	50.5

* Means in the same row, followed by different superscript letters are significantly different ($P \leq 0.05$).
** Means ± standard deviation.

ever, comparison of the thiamin and riboflavin contents of the meat on a dry, fat-free basis is a procedure which allows the determination of vitamin destruction and loss with drip during cooking. On a dry, fat-free basis the raw meat had a higher vitamin content. Approximately 36–71% and 6–54% of thiamin and riboflavin in the broiler meats was destroyed and/or drip lost during cooking, respectively.

Thiamin and riboflavin are likely to be lost into the drip during cooking because of the water-soluble nature of the vitamins and this is greater in light muscle. This is accompanied by some heat destruction of the thiamin throughout the muscles. Thiamin and riboflavin retention would appear to be marginally higher in dark meat, probably resulting from reduced cooking loss. The results of the present study confirmed the different retention values for thiamin and riboflavin due to the different cooking methods. These findings should be useful to dietitians, food services and the general public who are concerned about the nutritive values of cooked chicken meat.

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Table 6. Effect of cooking method on riboflavin content (mg/100 g) of broiler meat*

Treatment	Fresh weight basis (FW)			Dry weight, fat-free basis (DWFF)		
	Cooked	Raw	Retention (%)	Cooked	Raw	Retention (%)
Light muscles:						
Roasting	$0.23^a \pm 0.01$	$0.17^b \pm 0.01$**	90.4	$0.83^a \pm 0.03$	$0.81^a \pm 0.07$	68.5
Braising	$0.17^a \pm 0.02$	$0.16^a \pm 0.03$	83.7	$0.60^a \pm 0.07$	$0.74^a \pm 0.12$	63.9
Frying	$0.16^a \pm 0.02$	$0.14^a \pm 0.01$	70.1	$0.34^b \pm 0.06$	$0.72^a \pm 0.07$	45.8
Microwave	$0.17^a \pm 0.01$	$0.11^b \pm 0.01$	116.0	$0.61^a \pm 0.04$	$0.52^a \pm 0.07$	87.7
Mean	0.17^a	0.14^a	84.5	0.6	0.69^a	66.5
Dark muscles:						
Roasting	$0.40^a \pm 0.02$	$0.33^b \pm 0.03$	94.1	$1.59^a \pm 0.11$	$2.07^a \pm 0.18$	59.6
Braising	$0.40^a \pm 0.02$	$0.34^b \pm 0.01$	126.2	$1.79^a \pm 0.09$	$1.65^a \pm 0.03$	93.1
Frying	$0.35^a \pm 0.04$	$0.24^a \pm 0.06$	107.3	$1.40^a \pm 0.17$	$1.31^a \pm 0.30$	78.6
Microwave	$0.37^a \pm 0.02$	$0.30^a \pm 0.06$	104.8	$1.78^a \pm 0.01$	$1.61^a \pm 0.42$	94.0
Mean	0.38^a	0.30^b	108.0	1.64^a	1.66^a	81.3

* Means in the same row, followed by different superscript letters are significantly different ($P \leq 0.05$).
** Means ± standard deviation.

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