EFFECTIVENESS OF DIFFERENT PLANT SOURCES IN IMPROVING THE SHELF-LIFE OF CHICKEN MEAT PATTIES

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Abstract – Aim of the study was to evaluate the effect of kiwi fruit pulp, lemon juice and olive leaves extract on chicken meat quality along a six-day refrigerated storage. Fresh chicken breast meat was ground and assigned to four batches corresponding to four treatments: Control (C), fresh kiwi fruit pulp (K), freshly squeezed lemon juice (L) and Olive Leaves extract (OL). Control group had no supplementation, whereas the other treatments were supplemented with 2% (w/w) K, L or OL. Patties were stored at 4±1 °C under fluorescent light illumination for a six-day shelf-life trial. Analyses were conducted at days 0, 3 and 6 of storage, including drip losses, pH, L*a*b* colour values, lipid oxidation (TBARs) and microbial growth (Total viable count, total Coliforms, Enterobacteria and *Pseudomonas*). Overall results indicated that K improved meat shelf-life mainly through bacteriostatic action against spoiling and phatogenic microorganisms, without negatively affecting the other meat quality traits. Differently, K antioxidant activity on fresh meat should be further investigated.

Key Words – Chicken breast meat, Kiwifruit, Lemon, Olive leaves

I. INTRODUCTION

A growing interest towards alternative food additives mainly derived from plants has been taking place in the last decades. Phenolic compounds, besides playing an effective antioxidant role as free radical scavengers, chelators of metal ions and oxygen radicals quenchers (1, 2), can also have antimicrobial action against certain bacterial species. Kiwi fruit (*Actinidia deliciosa*) is very popular because of its high contents of vitamins C and E (2). In the meat industry, it started being appreciated and studied for its content of actinidin, a tenderizing enzyme member of the cysteine protease family (3). In addition, recent *in vitro* studies displayed a certain antibacterial activity of kiwi fruit against various Gram-positive and Gram-negative strains (3) as well as antioxidant activity, which was attributable to its polyphenols content (2). Lemon (*Citrus limon*) could be another interesting plant source for the meat industry. Its extract displayed effectiveness in reducing lipid oxidation in turkey meat, which was mainly due to citric acid action (4), together with an antimicrobial activity against *Campilobacter jejuni*, *Salmonella enteritidis* (5), and *Vibrio parahaemolyticus* (6). Olive (*Olea europaea L.*) leaves extract was found to be a potent lipid antioxidant, mainly due to its rutin, catechin and luteolin contents (1) and to enhance color stability in beef and pork meats (7). Based on the above-mentioned considerations, the aim of the present study was to test the effectiveness of a direct inclusion of such plant sources in the chicken meat, aiming to improve its shelf-life. In this study meat color, pH, drip loss, lipid oxidation and microbial growth, were considered.

II. MATERIALS AND METHODS

About 10 kg of breast meat from chickens slaughtered the day before were purchased from a local supplier. Kiwi fruits (var. Hayward) selected on the basis of a similar ripening degree and lemon fruits were purchased in a local market, whereas a commercial dried olive leaves extract titrated in oleuropein (7%) was purchased from Nutraceutica Srl (Monterenzio, Bologna, Italy). In the laboratory

of the Department of Animal Medicine, Production and Health (University of Padova, Italy), lemons were manually squeezed and filtered to remove seeds. Kiwi fruits were peeled and homogenized to obtain a pulp. Then, meat was ground through a type 22 meat mincer (ABO Srl, VA, Italy) and assigned to four batches weighing about 3 kg/each and corresponding to four treatments: Control (C) with no supplementation, kiwi fruit pulp (K), lemon juice (L) and olive leaf extract (OL) supplemented at 2% (w/w) inclusion level. The minced meat of each batch was further manually homogenized and shaped into No. = 68 patties/treatment (56 patties weighing 40 g and 12 patties weighing 20 g). Shelf-life lasted 6 days with analyses conducted at day 0, 3 and 6 of refrigerated storage. Sixty patties/treatment were used for TBARs (thiobarbituric acid reactive substances) analysis (8 patties/day of analysis), pH and L*a*b* color values measurements (8 patties/day) and microbiological analysis (4 patties/day). Patties were individually wrapped with plastic film to minimize direct air contact and laid into polystyrene trays. The remaining 8 patties/treatment were used for drip loss calculations; with this purpose, only the tray was wrapped with plastic film. Finally, all patties were stored at 4±1 °C under fluorescent light illumination for a six-day shelf-life trial. At each day of analysis, patties assigned to TBARs determination were collected from the refrigerator and stored at -40 °C until analysis. Subsequently, they were defrosted overnight at +4 °C and TBARs analysis was performed according to the procedure described by Botsoglou *et al.* (1994) (8). For each patty, pH (portable pH-meter FG2-Five Go™, Mettler Toledo, Greifensee, Switzerland) and CIE L*a*b* colour values (X-Rite, Co., Neu-Isenburg, Germany) were measured twice. Total Viable Count (TVC) was measured according to ISO 4833:2004; Enterobacteria counts were measured according to ISO 17604:2003 and ISO 21528-2:2004. ISO 4831:2006 and 4832:2006 were both adopted for total Coliforms determination, whereas *Pseudomonas* counts were measured according to internal procedure (MI 025 rev 1 2009). Patties were placed into disposable sterile bags containing 180 ml of sterile buffered peptone water and homogenized with a Colworth Stomacher 400 Circulator (Seward Ltd, Worthing, West Sussex, UK). Decimal logarithmic scale dilutions were included in specialized bacterial growth media and incubated according to the times and temperatures specified by the above-mentioned procedures. Results were expressed as CFU/g meat. When no colonies were detected, the value <10 was considered. Data were analyzed using the general linear model procedures (PROC GLM) of the statistical software SAS (2006) package (version 9.3) for Windows. A one-way ANOVA tested the effect of the treatment on the studied variables. Least square means were obtained using Bonferroni test. The differences were considered significant when $P<0.05$.

III. RESULTS AND DISCUSSION

The inclusion of kiwi fruit, lemon and olive leaves extract to chicken meat patties significantly affected all meat quality traits (Table 1). Already at day 0 of storage, L exhibited the lowest meat pH ($P<0.001$), which was attributable to its citric acid content. K and OL presented intermediate values, probably due to the presence of ascorbic acid and phenolic compounds. An identical situation was observed at day 3 of storage, whereas at day 6 all treatments exhibited statistically different pH values (6.10 *vs* 6.00 *vs* 5.93 *vs* 5.72 for C, K, OL and L meat patties, respectively). Also meat color was significantly affected by treatments ($P<0.001$). Meat patties belonging to L group always exhibited the highest lightness (L*) and the lowest redness (a*), which were attributable to the denaturing effect of the citric acid. Differently, yellowness (b*) of OL-treated meat patties was always higher than the other groups ($P<0.001$), as a direct consequence of the green/yellow-colored extract. The denaturing effect of citric acid determined greater fluids leakage from muscle fibers increasing drip loss; in fact compared to C, the L meat patties exhibited the highest drip loss throughout the storage ($P<0.05$). Lower pH values, combined with lightening ability of the lemon juice and the highest drip loss, could have further predisposed the meat of the group L to the light scattering, resulting in paler patties.

The very low initial TBARs values of all chicken meat patties denoted the freshness and the good quality of the meat used, as well as the optimal handling procedures, which did not promote the oxidation of lipids. Despite this, the addition of OL to chicken meat patties caused an immediate lipid oxidation. In fact, already at day 0, OL meat patties

had the highest MDA content (P<0.001), which almost doubled at day 6 of shelf-life (Table 1). This finding is in contrast with other studies, which denoted a certain effectiveness of olive leaves extracts as antioxidant in beef and pork meat (7). Probably, the inclusion of dried extract chosen for this study, instead of the use of a fluid solution, could have played a negative role in the antioxidant compounds availability or action. The L addition determined a certain oxidation degree at day 0, which was higher than in C group (0.16 *vs* 0.07 mg MDA kg/meat for L and C groups, respectively; P<0.05). However, TBARs values did not change along the storage, thus confirming the results of Contini *et al*. (2014) (4) on lemon protective effect against oxidation.

Table 1. Effect of plant sources inclusion on drip loss (%), pH, L*a*b* colour values and lipid oxidation (mg MDA/ kg meat) of chicken meat patties

	Treatments				SE	Sign.
	C	K	L	OL		
No.	24	24	24	24		
pH:						
Day 0	6.22^{A}	6.02^{B}	5.70^{C}	6.04^{B}	0.01	***
Day 3	6.12^{A}	5.97^{B}	5.67^{C}	5.95^{B}	0.01	***
Day 6	6.10^{A}	6.00^{B}	5.72^{D}	5.93^{C}	0.01	***
L* value:						
Day 0	52.9^{B}	54.4^{B}	58.0^{A}	44.9^{C}	0.4	***
Day 3	52.0^{C}	55.2^{B}	60.3^{A}	45.6^{D}	0.4	***
Day 6	52.9^{B}	53.9^{B}	59.8^{A}	44.7^{C}	0.3	***
a* value:						
Day 0	0.03^{B}	-0.99^{Cb}	-0.36^{BCa}	1.41^{A}	0.15	***
Day 3	1.39^{A}	0.25^{B}	0.16^{B}	1.70^{A}	0.17	***
Day 6	0.71^{B}	0.42^{BC}	0.00^{C}	1.49^{A}	0.12	***
b* value:						
Day 0	21.1^{B}	21.0^{B}	20.8^{B}	24.6^{A}	0.4	***
Day 3	20.8^{B}	20.3^{B}	20.2^{B}	23.6^{A}	0.4	***
Day 6	19.1^{C}	20.3^{B}	20.0^{BC}	22.9^{A}	0.2	***
Drip loss:						
Day 0	0.75^{AB}	0.37^{B}	1.18^{A}	0.65^{AB}	0.16	*
Day 3	1.10^{B}	1.81^{Ab}	2.45^{Aa}	2.25^{Aab}	0.14	***
Day 6	1.84^{C}	2.18^{BCc}	3.60^{Aa}	2.89^{ABb}	0.17	***
MDA:						
Day 0	0.07^{C}	0.09^{BCb}	0.16^{Ba}	0.29^{A}	0.01	***
Day 3	0.09^{B}	0.12^{ABb}	0.16^{ABab}	0.23^{Aa}	0.02	**
Day 6	0.16^{B}	0.13^{B}	0.19^{B}	0.41^{A}	0.04	***

$^{a, b}$ or *: Means within row with different superscripts differ $P \leq 0.05$

$^{A, B}$ or **: Means within row with different superscripts differ at $P \leq 0.01$; *** at $P \leq 0.001$

The addition of K to chicken meat patties did not significantly improve nor worsen their oxidative status, when compared to the C patties. However, along the storage time the MDA values increased more in C than in K patties. This result suggests a possible long-term antioxidant effect of the kiwi fruit, which could be exerted by its phenolic compounds as well as by vitamin C and E contents. A longer shelf-life needs to be tested to confirm the antioxidant power of the kiwi fruit pulp.

Table 2. Effect of plant sources inclusion in chicken meat patties on Total Viable Count (TVC), Total Coliforms Count (TCC), *Enterobacteria* and *Pseudomonas* measured at days 0, 3 and 6 of shelf-life and expressed as CFU/g meat

	Treatments				SE	Sign.
	C	K	L	OL		
No.	12	12	12	12		
TVC:						
Day 0	2725	2850	3700	2425	417	NS
Day 3	2600^{B}	3300^{B}	8100^{Aa}	4050^{ABb}	842	**
Day 6	152500^{B}	11375^{B}	6100^{B}	1335000^{A}	96066	***
TCC:						
Day 0	102^{ab}	300^{a}	$<10^{b}$	225^{ab}	51	**
Day 3	77	200	<10	202	46	*
Day 6	177	<10	<10	252	60	*
Enterobacteria:						
Day 0	<10	<10	<10	<10	0	-
Day 3	80	82	177	32	71	NS
Day 6	37000^{ABa}	$<10^{Bb}$	$<10^{Bb}$	44000^{A}	7056	***
Pseudomonas:						
Day 0	6975^{B}	2625^{C}	100^{C}	14075^{A}	630	***
Day 3	28200^{A}	12375^{B}	4975^{C}	31000^{A}	1024	***
Day 6	31000^{A}	975^{C}	9575^{B}	31000^{A}	839	***

$^{a, b}$ or *: Means within row with different superscripts differ $P \leq 0.05$

$^{A, B}$ or **: Means within row with different superscripts differ at $P \leq 0.01$; *** at $P \leq 0.001$

Total Viable Count (TVC) was low at day 0, without any statistical difference among treatments (Table 2). At day 3, L meat patties exhibited the highest TVC count (P<0.01), probably due to the development of an acid-tolerant bacterial flora. At day 6, OL meat patties showed the highest TVC (P<0.001) compared to the other three experimental

groups, thus suggesting a selecting activity of the olive leaf extract. In literature (9) (10) it has been demonstrated that olive phenolic compounds of the aqueous extract exerted an antimicrobial activity against *B. cereus, S. aureus, E. Coli, P. aeruginosa, C. albicans,* and *C. neoformans*, not confirmed in the present study. Overall, total Coliforms counts were characterized by low values along the shelf-life study. Enterobacteria counts did not differ among treatments at days 0 and 3, whereas kiwi fruit and lemon showed an efficient antimicrobial activity at day 6 (<10 CFU/g meat), confirming previous studies on kiwi (3) and lemon properties (5, 6) against foodborne pathogens. On the contrary, olive leaf extract demonstrated to be ineffective towards these spoilage microorganisms. *Pseudomonas* count was affected by the treatments in all the three storage times ($P<0.001$). At day 0, *Pseudomonas* count of OL meat patties was the highest (14075 CFU/g *vs* 6975, 2625 and 100 CFU/g meat exhibited by C, K and L, respectively) and this high count was maintained at 3 and 6 days, with values similar to the C group. On the contrary, lemon juice and kiwi fruit pulp exerted a significant antibacterial action against *Pseudomonas* ($P<0.001$) both at 3 and 6 days of storage. It's remarkable to stress that whereas lemon showed a strong short-term bacteriostatic efficacy against *Pseudomonas*, kiwifruit proved a more effective long-term bacteriostatic capacity along the storage period.

IV. CONCLUSION

Kiwi fruit pulp revealed to be the most effective supplementation in improving the shelf-life of meat, mainly through bacteriostatic action. Differently, its antioxidant activity in meat should be further investigated.

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