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Effects of spearmint (*Mentha spicata* L.) infusion in drinking water during rabbit fattening on the microbial and physicochemical qualities of the end meat product

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Abstract

The objective of the present work was to evaluate the effects of the administration of spearmint (*Mentha spicata* L.) infusion into drinking water (0, 5, and 10 g.L^{-1}) in rabbits during 28 days of fattening on the microbiological and lipid stability of the end meat product, as well as the evaluation of meat and carcass quality. The rabbits were sacrificed, and the quality of the carcass and meat was evaluated. Once the meat was obtained, burger patties were made, which were then subjected to microbiological and physicochemical evaluation through the application of various treatments for 14 days. The results did not show an effect on the quality of the carcass; however, for the infusion treatment with 5 g of spearmint, the previous portion corresponding to the skeletal muscle, *longissimus dorsi*, was greater than the control treatment. Regarding the meat quality, the hardness parameter of the texture profile analysis was higher in the control and treatment with 5 g of spearmint. The microbiological analysis on day 0 showed that there was no growth of *Staphylococcus* in the treatments with spearmint infusion as compared to the control; the enterobacterial count at day 7 was higher in the control group than in the treatments with spearmint infusion; and on day 14, the total viable count was higher in control than in the treatments with spearmint infusion. In the physicochemical analysis, only the colour (L*, a*, and b*) showed significant differences in the parameters at 0 and 14 days for the control. In conclusion, the spearmint infusion in drinking water for fattening rabbits could influence the physicochemical and microbiological characteristics of the end meat product, and could be considered as an alternative for improving the lipid and microbiological stability of rabbit meat products.

Introduction

Rabbit meat has been considered a functional food due to its nutritional importance, which also explains the fact that its preference among people who consume meat has increased. Rabbit meat has low calorie content, triglycerides, cholesterol, sodium, and significant amounts of both monounsaturated and polyunsaturated fatty acids (Dalle-Zotte and Szendrő, 2011; Escribá-Pérez *et al.,* 2019). Due to this, its consumption is increasing with a world production estimated close to 1,482,000 tons per year (Krupová *et al.,* 2020) with China being the world's largest producer (849,150 tons per year), followed by South Korea (172,680 tons per year), Egypt (65,602 tons per year), Italy (54,397 tons per year), Spain (50,552 tons per year), and France (48,396 tons per year) (Cullere and Dalle Zotte, 2018). The main problems for rabbit production is that, generally, the production units are traditional and subsist in rural communities (Falcone *et al.,* 2020), despite the fact that this species is relatively easy to handle, having a short gestation period, high prolificacy, and high feed conversion capacity (Cullere and Dalle Zotte, 2018). In addition, research related to the innovation and development of rabbit meat products is limited, due to the fact that it is considered as a much smaller animal as compared to farm animals that are more commonly consumed (Li *et al.,* 2018). The preference in the consumption of rabbit meat by consumers is related to the final presentation of the product, either as carcass or processed meat, as well as the conditions of its production and slaughter method. A world per capita consumption of about 0.19 kg of total meat consumption is estimated (Szendrő *et al.,* 2020).

For its chemical characteristics, rabbit meat is

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more susceptible to lipid oxidation (Nakyinsige *et al.,* 2014), thus causing a negative impact on flavour, colour, texture, and the nutritional value of the meat and meat products (Shah *et al.,* 2014; Trebušak *et al.,* 2014); the pH value during the post-mortem stage is approximately 5.5 to 6, which makes it susceptible to microbial growth and spoilage (Koné *et al.,* 2016). For this reason, it is necessary to use additives in the preservation of the meat and meat products that can increase microbiological stability, such as nitrites, nitrates, and lactate (Koné *et al.,* 2016). In addition to microbial spoilage, lipid oxidation is another critical factor that must be controlled in the storage of the meat and meat products. Monounsaturated and polyunsaturated fatty acids are more susceptible to lipid oxidation; these molecules are oxygen dependent, which generate a self-catalytic mechanism called auto-oxidation, a reaction which generates undesirable substances that affect the nutritional and sensory quality of the meat, with malonaldehyde being the main product of this reaction (Kumar *et al.,* 2015). This compound is a free radical that can alter biological macromolecules, and could contribute to its toxicity, mutagenic, and carcinogenic properties (Reitznerová *et al.,* 2017). To minimise this effect on the meat and meat products, synthetic antioxidants are used in order to delay lipid oxidation without altering the sensory properties of the product. Butyl hydroxytoluene (BHT) and butyl hydroxyanisole (BHA) is the most commonly used antioxidants (Movileanu *et al.,* 2013); however, these synthetic antioxidants have been investigated due to their possible toxic effects on health (Nieva-Echevarría *et al.,* 2015). Besides, the trend in the consumption of natural products and those with a lower composition of synthetic additives increases the use of natural antioxidants mainly from extracts of botanical sources, as these represent alternatives for the optimisation of shelf life for the meat and meat products. Some of the extracts that have been tested are grapes, ginger, mint, and broccoli, which are used in the forms of aqueous, ethanolic, or methanolic extracts, and applied directly to the meat product, thus delaying the lipid oxidation (Shah *et al.,* 2014). Plants and spices offers wide range of bioactivities, including animal breeding and increased nutrient availability. As compared to some antibiotics or inorganic chemicals, they have low toxicity, are free of unwanted residues, and act as growth promoters in animal diets, including rabbit feed (Dalle-Zotte *et al.,* 2016).

Spearmint (*Mentha spicata* L.) is an aromatic plant often used in the traditional medicine for antispasmodic, stomachic, and diuretic purposes, due to the presence of antioxidants and other phenolic compounds which contribute to its antimicrobial and antioxidant properties (Padmini *et al.,* 2010). Also, it has been used as an additive in ground pork and demonstrated better microbiological stability of the meat. The objective of the present work was to evaluate the effects of the inclusion of spearmint infusion as a beverage during the fattening of rabbits, and to determine the microbial and physicochemical quality of the resulting end meat product.

Materials and methods

Animals and treatments

The investigation was carried out in the Experimental Rabbitry, Institute of Agricultural Sciences, Autonomous University of Hidalgo State (Tulancingo, Hidalgo, México), and was approved by the Animal Care Committee of the Autonomous University of Hidalgo State (protocol no.: CICUA/002/18). Twenty-four Chinchillas crossed with New Zealand rabbits were weaned at 30 days of age, and were housed for 28 days in cages of 90 × 60 × 40 cm. Next, they were randomly distributed in three treatments (eight in each) with three repetitions. Feed was pelletised using a pellet machine (model SKJ120; Shandong, China), fed *ad libitum* with an isoproteic, isoenergetic, and isofibrous diet, and administered *ad libitum* spearmint infusion (5 and 10 $g.L^{-1}$ of spearmint). The infusion was prepared by adding 5 or 10 g of dry ground spearmint to 1 L of water, and filtered using a coffeemaker (Hamilton Beach, Glen Allen, Virginia, USA) at 80°C for 15 min. Spearmint was obtained from a local market in Tulancingo, State of Hidalgo, Mexico. The entire plant was dehydrated at room temperature, kept away from sunlight, milled, and stored dry until used.

Sacrifice of animals

Once the 28 days of fattening concluded, all 24 animals were slaughtered according to NOM-033-SAG/ZOO-2014 (Official Mexican Standard, 2014). The post-mortem weights of the animals were determined before and after slaughter, the length of each animal was measured from the first vertebra until the last caudal vertebrae, and the hip diameter of each animals was measured. The caudal extremities were cut between the distal epiphysis of the tibia and the tarsus, and the warm carcass was weighed and stored in the cold room for 24 h at 4°C (Blasco *et al.,* 1993, Ouhayoun and Dalle-Zotte, 1996).

Evaluation of meat quality parameters

After 24 h of refrigeration, the cold carcass was weighed; primary cuts were made to separate the head and cranial part, middle part, and caudal part (*longissimus dorsi*) of skeletal muscle, legs, and kidneys; and the legs were dissected to separate the bone and fat, and meat were weighed. The parameters calculated for the consistency of meat were made in *longissimus dorsi*; the pH was calculated with a different potentiometer by HANNA meat instruments, with a blade electrode for penetrating meat; and the colour was measured as indicated by the American Meat Science Association (AMSA, 2012) with a handheld visible spectrophotometer colorimeter (MicrOptix I VRV-300). The water holding capacity was also measured using methods described by Honikel (1987) which involved placing 0.3 g of meat between two pieces of filter paper, and then placed between two plates of Plexiglas weighing 1 kg for 10 min. Later, the paper and sample was measured for water retention capacity by weight difference.

The weight loss by cooking was determined by weighing the *longissimus dorsi* and placing it in a water bath at 70°C for 20 min. The cooking loss was estimated as percentage of weight of the raw sample with respect to weight of the cooked sample.

A texture profile analysis was carried out following the method used by Bourne (1978) on the cooked *longissimus dorsi* to determine the hardness, cohesiveness, resilience, adhesiveness, and elasticity using a Brookfield CT3 texture analyser with an AT3000 probe, whereby a 10 × 10 × 10 mm cube of meat was placed at the base of the texture analyser.

Preparation of the meat product (rabbit burger patties)

The meat corresponding to the legs and arms of the rabbits was stored in freezing conditions at -20°C until further use. The meat was ground with a Torrey meat grinder. Eighty grams of ground meat were taken from each treatment, and 0.2% of common salt was added. Next, three batches of rabbit burger patties were made with two repetitions, corresponding to the days of storage (0, 7, and 14 days) for each treatment. The rabbit burger patties were placed in polystyrene trays, covered with glass rubber, and stored in refrigerator at 4°C to carry out microbiological and physicochemical analyses on different days of storage.

Microbiological analysis

The microbiological analysis was carried out at 0, 7, and 14 days on the rabbit burger patties that had been imbibed with spearmint infusion during fattening. Under aseptic conditions, 1 g of burger patty was weighed and mixed with 9 mL of peptone water (Bioxon®) at pH 7.2 to determine (1) the total viable count in trypticase soy agar (Bioxon®), and (2) the concentration of staphylococci and enterobacteria in staphylococcal agar plates and MacConkey agar plates, respectively, using the most probable number technique suggested by NOM-092-SSA1-1994 (Official Mexican Standard, 1994).

Physicochemical analysis

The lipid stability was evaluated by the means of technique of substances reactive to 2-thiobarbituric acid (TBARS) as suggested by Nam and Ahn (2003). The results were expressed in mg of malonaldehyde per kg of meat (mg MDA.kg^{-1}). The pH was measured at 0, 7, and 14 days of storage, by weighing 10 g of sample with 90 mL of distilled water. The samples were then homogenised, and the pH was measured with HANNA instruments potentiometer. The colour was evaluated at 0, 7, and 14 days using a MicrOptix i-LAB VRV-300 handheld visible analysing spectrophotometer colorimeter, and the colour parameters L*, a*, b*, C, and H were measured according to the manufacturer's instructions.

Statistical analysis

The data were interpreted by an analysis of variance (ANOVA) using the statistical package SPSS 20. The quality of the meat and the carcass was analysed by means of variance analysis with a completely randomised statistical arrangement. The microbiological and physicochemical results of the rabbit burger patties were analysed with three repetitions by means of variance analysis, with a completely randomised arrangement with repetitions over time with a significance value of $p < 0.05$.

Results and discussion

Spearmint infusion into drinking water for fattening rabbits did not affect the quality of the carcass with respect to the control group (Table 1). Other botanical sources used in the fattening of rabbits have shown similar results; for example, Alagawany *et al.* (2016) added garlic and turmeric to the diets of rabbits without affecting the quality of the carcasses, and Koné *et al.* (2016) added extracts of plants and essential oils to the diets of rabbits without affecting the characteristics of the carcasses. On the other hand, Cardinalli *et al.* (2015) increased the live weight of rabbits selected for sacrifice by adding an aqueous extract of oregano and rosemary, and Peiretti *et al.* (2013) added tomato marc to rabbit feed, thus increasing the live weight at slaughter.

The results obtained with respect to the yields of the carcasses of rabbits that imbibed spearmint infusion during fattening showed that in the treatment with 5 g of spearmint, the previous part corresponding to the *longissimus dorsi* was greater

Table 1. Quality of rabbit carcasses that imbibed spearmint infusion in drinking water during fattening.

Parameter	Control	Spearmint		E.E.
		5 g	10 g	
Weight (g)	2023.13	2040.00	2017.50	74.14
Length (cm)	27.38	28.56	30.00	0.86
Hip circumference (cm)	25.25	26.31	25.63	0.574
Carcass length (cm)	31.375	33.75	31.25	1.20
Carcass hip circumference (cm)	24.44	22.25	24.56	1.26
Warm carcass weight (g)	1111.88	1101.25	1123.00	41.98

than the control treatment and the treatment with 10 g of spearmint (Table 2). The *longissimus dorsi* of the rabbit carcass is one of the parts that give more weight to the carcass due to the percentage of meat present in this muscle section (Barrón *et al.,* 2005). Similar results were obtained by Omer *et al.* (2015) by adding a mixture of garlic, onion, and lemon extracts to rabbit feed during fattening.

With respect to the meat quality, pH, weight loss by cooking (WLC), water holding capacity (WHC), adhesiveness, resilience, cohesiveness, and elasticity did not show significant differences between treatments and control; however, hardness was higher in the control and treatment with 5 g of spearmint (Table 3). Dal Bosco *et al.* (2012) reported no significant differences in the texture of rabbit meat consuming olive during fattening; Rotolo *et al.* (2013) reported no significant differences in the texture of rabbit meat in which oregano was added to the diet; and Meineri *et al.* (2010) reported that there were no variations in the meat texture of rabbits consuming chia seeds.

Table 2. The yield of rabbit carcasses that imbibed spearmint infusion in drinking water during fattening.

Parameter ($g.kg^{-1}$)	Control	Spearmint		E.E.
		5 g	10 g	
Cold carcass weight (g)	1077.50	1090.00	1090.00	39.68
Yield	55.10	53.93	55.63	0.80
Empty body weight (g)	1878.88	1886.00	1900.38	71.24
Skin	163.72	185.05	166.53	5.87
Leg	17.60	19.26	24.62	0.92
Lung	8.16	9.20	9.18	0.75
Heart	3.75	3.98	4.43	0.34
Gastrointestinal tract	154.78	171.95	154.83	13.70
Spleen	0.87	0.88	0.92	0.19
Liver	45.27	46.46	45.60	2.27
Bladder	1.67	3.16	2.01	0.23
Kidney	7.23	8.09	7.14	0.44
P. previous	244.46[ab]	251.61[a]	239.70[b]	3.10
P. media	112.96	111.74	107.89	3.51
P. later	204.33	202.57	211.97	5.13
Leg	324.23	322.75	320.84	4.518
Head	101.00	96.18	108.68	3.63
Meat	734.50	747.06	754.59	12.90
Bone	296.62	187.88	197.81	45.10
Fat	11.0	16.80	11.82	4.12

[ab] Indicates significant differences using the Tukey's test ($p < 0.05$).

Table 3. Quality of rabbit meat that imbibed spearmint infusion in drinking water during fattening.

Parameter	Control	Spearmint		E.E.
		5 g	10 g	
pH	5.12	5.07	5.02	0.08
WLC	14.90	15.77	15.92	1.17
WHC	40.40	40.34	45.63	2.79
Adhesiveness	0.06	0.09	0.06	0.02
Resilience	0.16	0.14	0.17	0.01
Cohesiveness	0.40	0.43	0.44	0.02
Elasticity	3.05	2.30	2.42	0.30
Hardness	565.05^{ab}	853.88^{a}	519.23^{b}	105.40

^{ab} Indicates significant differences using the Tukey's test ($p < 0.05$). WLC: weight loss by cooking; WHC: water holding capacity.

The colour of the meat showed an effect on parameter L*, with lower values in the treatments of 5 and 10 g of spearmint infusion, with respect to the control group (Table 4). Peiretti *et al*. (2013) showed significant differences in the parameters L*, a*, and b* in the meat of rabbits fed with tomato marc; on the

Table 4. Colour of the rabbit meat that imbibed spearmint infusion in drinking water during fattening.

Parameter	Control	Spearmint		E.E.
		5 g	10 g	
L*	56.30^{a}	53.75^{b}	54.55^{ab}	0.55
a*	4.05	4.10	4.41	0.56
b*	-11.75	-12.14	-11.87	0.94
C	12.39	13.30	12.85	0.99
H	0.22	0.25	0.25	0.20

^{ab} Indicates significant differences using the Tukey's test ($p < 0.05$).

Table 5. Effects of the spearmint infusion for the fattening of rabbits, and on the microbiological quality at different days of storage.

Spearmint ($g.L^{-1}$)	TVC (Log_{10} $CFU.g^{-1}$)	Enterobacter (Log_{10} $CFU.g^{-1}$)	*Staphylococcus* (Log_{10} $CFU.g^{-1}$)
Day 0			
0	1.45 ± 0.10	1.13 ± 0.10	0.70 ± 0.00^{a}
5	1.60 ± 0.10	1.01 ± 0.10	0.00 ± 0.00^{b}
10	1.53 ± 0.10	0.89 ± 0.10	0.00 ± 0.00^{b}
Day 7			
0	2.69 ± 0.10	1.96 ± 0.04^{a}	0.15 ± 0.10
5	2.55 ± 0.10	2.45 ± 0.04^{b}	0.24 ± 0.10
10	2.62 ± 0.10	2.45 ± 0.04^{b}	SC
Day 14			
0	3.03 ± 0.14^{a}	2.04 ± 0.10	0.15 ± 0.08
5	2.88 ± 0.14^{ab}	1.88 ± 0.10	0.00 ± 0.08
10	2.15 ± 0.14^{b}	1.82 ± 0.10	0.00 ± 0.08

^{ab} Indicates significant differences using the Tukey's test ($p < 0.05$). TVC: total viable count.

Table 6. Physicochemical analysis of burger patties made from rabbits that imbibed spearmint infusion during fattening.

Parameter	Storage time								
	Day 0			Day 7			Day 14		
	Control	**5 g spearmint**	**10 g spearmint**	**Control**	**5 g spearmint**	**10 g spearmint**	**Control**	**5 g spearmint**	**10 g spearmint**
pH	6.02 ± 0.05	5.97 ± 0.05	6.04 ± 0.05	6.11 ± 0.15	6.21 ± 0.15	6.10 ± 0.15	6.92 ± 0.16	7.02 ± 0.16	7.34 ± 0.16
L*	49.52 ± 1.31[a]	44.82 ± 1.31[ab]	44.11 ± 1.31[b]	43.77 ± 1.95	44.08 ± 1.95	48.36 ± 1.95	32.53 ± 2.34[b]	53.53 ± 2.34[a]	47.01 ± 2.34[a]
a*	-0.90 ± 0.32[a]	-1.04 ± 0.32[a]	-2.31 ± 0.32[b]	-1.89 ± 0.26	-1.63 ± 0.26	-1.19 ± 0.26	2.87 ± 0.46[b]	3.25 ± 0.46[ab]	4.72 ± 0.46[b]
b*	8.813 ± 0.58[b]	11.38 ± 0.58[a]	11.68 ± 0.58[a]	8.81 ± 0.83	8.89 ± 0.83	9.47 ± 0.83	-1.48 ± 1.01[b]	3.18 ± 1.01[a]	-0.45 ± 1.01[b]
C	8.88 ± 0.57[a]	11.48 ± 0.57[a]	11.95 ± 0.57[b]	9.03 ± 0.83	9.06 ± 0.83	9.58 ± 0.83	4.62 ± 0.72	4.89 ± 0.72	6.20 ± 0.72
H	0.18 ± 0.02[a]	0.26 ± 0.02[a]	0.28 ± 0.02[b]	0.21 ± 0.02	0.21 ± 0.02	0.20 ± 0.02	0.16 ± 0.03	0.10 ± 0.03	0.13 ± 0.03
TBARS (mg MDA.kg^{-1})	0.19 ± 0.10	0.06 ± 0.10	0.22 ± 0.10	0.64 ± 0.10	0.40 ± 0.10	0.54 ± 0.10	0.75 ± 0.15	0.37 ± 0.15	0.46 ± 0.15

[ab] Indicates significant differences using the Tukey's test ($p < 0.05$).

other hand, the meat colour of rabbits fed with plant extracts and essential oils as an additive in the food was not affected. Koné *et al.* (2016) and Kovitvadhi *et al.* (2016) fed rabbits with *Lythrum salicaria* as an additive without affecting the colour parameters of the meat.

The effect of the infusion of spearmint in rabbits on the microbiological quality of the meat product is shown in Table 5. It was observed that on day 0 there was no growth of staphylococci in the treatments with spearmint infusion as compared to the control. The enterobacterial count at day 7 was greater in the control group than in the treatments with spearmint infusion. On day 14, the total viable count was higher in control than in the treatments with spearmint infusion. Rabbit meat contains enough nutrients to facilitate microbial growth even at refrigeration temperatures, and has intrinsic and extrinsic factors (Koné *et al.,* 2016); besides, the physiological status of the animal at slaughter and the ultimate pH of 6 (Nakyinsige *et al.,* 2014) affect the microbial growth rate. Omer *et al.* (2015) added a mixture of garlic, onion, and lemon as an additive in rabbit feed to improve the microbiological quality of the meat and demonstrated a decrease in the total viable count; likewise, Mancini *et al.* (2016) reported a lower total bacterial count in rabbit burger patties supplemented with turmeric powder and ascorbic acid at day 0 of storage in refrigeration. Koné *et al.* (2016) reported a lower concentration of mesophilic bacteria in the meat of rabbits fed with plant extracts and essential oils, stored under refrigeration and aerobic conditions. On the other hand, Soultos *et al.* (2009) reported positive effects in the meat of rabbits fed with oregano essential oil in the food, showing a lower concentration of the total viable account as compared to the control treatment.

The physicochemical analysis of burger patties made with meat from rabbits that imbibed spearmint infusion during fattening is shown in Table 6. The pH did not show significant differences between treatments during the different analytical days; however, statistical differences were observed in parameters L*, a*, and b* at 0 and 14 days, and parameters C and H were higher in the control and 5 g of the spearmint treatment at day 0; meanwhile the malonaldehyde concentration showed no significant differences.

Conclusion

The use of botanical ingredients as natural sources of antioxidants and antimicrobials in rabbit meat has been increasing. The present work suggested that the infusion of spearmint into drinking water for fattening rabbits could influence the physicochemical and microbiological characteristics of the end meat product. Nevertheless, more research is needed to investigate the effects of the presence of phytochemical compounds in spearmint on the feeding of rabbits, and the characteristics of the end meat product.

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