

## Improving the oxidative stability of pork by antioxidant type phytonutrients

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## ABSTRACT

The objective of this study was to determine the influence of supplementations with 3.5 or 7.5 mg dihydroquercetin (experimental groups D1 and D2) or with 0.255 or 0.545 g dry distilled rose petals (experimental groups R1 and R2)/kg/d added as to pig's combined feed on the parameters of lipolysis expressed by acid value; lipid hydroperoxides expressed by peroxide value, lipid oxidation secondary products expressed by 2-tiobarbituric acid reactive substances (TBARS), pH and L\*, a\*, b\* colour characteristics in m. Longissimus lumborum et thoracis, m. Semimembranosus, backfat and leaf fat stored 24 h and 7d at 2±1°C, or 315 d at -18±1°C. A total of 120 pigs were randomly divided into five groups – a control (C) and four experimental (D1, D2, R1 and R2) each fed 45 d prior to harvest with shown above levels of phytonutrients enriched diets. More pronounced effects were determined (P≤0.05) at frozen storage compared to chilled storage. The oxidative and colour stabilities of chilled (2±1°C) and frozen (-18°C) pork are comparatively higher when pig's diet was supplemented with 3.5 mg dihydroquercetin or 0.255 g dry distilled rose petals/kg/d. The conclusion was made can the supplementation of pig's combined feed (finisher) with 3.5 mg dihydroquercetin or 0.255 g dry distilled rose petals/kg/d is a promising strategy to increase the oxidative stability of lean pork or fat and stabilized pork meat colour without deleterious changes of meat acidity.

**Keywords:** pigs; dihydroquercetin; dry distilled rose petals; feed supplementation; muscles; fat; colour; lipolysis; lipid oxidation;

## 1. INTRODUCTION

Numerous strategies exist to modify the composition of pork by altering protein content, vitamins, fats and fatty acid composition [1]. Further, additional technologies in breeding practices increase the productivity of pigs and impact pork quality, specifically, indoor versus outdoor rearing [2], use of various muscle glycogen-reducing diets [3] and dietary enrichment with vitamin E [4], conjugated linoleic acid [5], tuna oil [6], grape pomace [7] and astaxanthin [8]. Falowo et al. [9] specifically describe the positive effects of feed supplementation with natural and synthetic antioxidants on the inhibition of the oxidative degradation of meat. In addition, newly discovered plant-based substances such as Siberian larch dihydroquercetin (*Larix sibirica* Ledeb) [10], extract of distilled rose (*Rosa damascena* Mill.) petals [11], the goji berry (*Lycium barbarum*) dried fruits and pumpkin powder [12] and others have been investigated for benefiting food stability.

Dihydroquercetin (DHQ) is capable of donating an electron and as such, inhibits hydroxyl radicals [13]. DHQ is a powerful antioxidant that deactivates alkylperoxyl and superoxide radicals, reduces haemolysis induced by phospholipase C and inhibits superoxide produced by xanthine oxidase [14]. Dihydroquercetin

is a proven antioxidant, capillary protector with hepatoprotective, gastroprotective, anti-inflammatory, antisclerotic, radioprotective, anti-coagulant, anti-inflammatory properties and inhibits the LDL-cholesterol oxidation in blood serum [13]. Dihydroquercetin is often used for the prevention of oxidative stress and is well accepted as a treatment for select carcinomas, and cardiovascular and liver diseases [13]. DHQ's anti-radical activity occurs at a concentration of about 0.0001-0.00001% in the absence of mutagenic activity [10].

A by-product of rose oil production is distilled rose (*Rosa damascena* Mill) petals. This product contains a wide range of flavonol glycosides and polyphenol with strong antioxidant capacity [11]. The addition of distilled rose (*Rosa Damascena* Mill) petal extracts improves colour stability of the canned strawberries' beverage [15]. The dietary supplementation of dry rose (*Rosa damascena* Mill) petals or dihydroquercetin in chicken meat cuts improves their quality [16].

Therefore, objective of this study was to determine the influence of two sources of dihydroquercetin and dry distilled rose petals on the oxidative and colour stability of chilled (2±1 °C) and frozen (-18 °C) pork.

## 2. MATERIALS AND METHODS

This experiment was designed using ARRIVE guidelines and performed in accordance to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes [17], Directive 2010/63/EC [18], Directive 2008/120/EC [19], Recommendation 2007/526/EC [20],

Regulation (EC) No 1099/2009 [21] and such as Bulgarian Veterinary Activity Act [22] and Ordinance No 20 of 1 November 2012 [23]. The experiment was approved by the Scientific Ethics Committee.

2.1. Dietary treatments and study design.

2.1.1. Combined feed.

Animals were fed an ad libitum grower diet to 60 kg live weight and a finisher to 110 kg. Feed was prepared at the Agricultural Institute, Shumen, Bulgaria. Ingredient composition and the energy values of the diets are presented in Table 1, and their chemical composition in Table 2.

Table 1. Ingredient composition and the energy value of the two fodder mixtures.

Components	Grower <sup>1</sup> Used in the period of adolescence (live weight 20 - 60 kg)	Finisher <sup>1</sup> Used during the fattening period (live weight 60 – 110 kg)
Maize, %	15.00	13.00
Barley, %	25.00	10.00
Wheat, %	27.00	50.00
Wheat bran, %	8.00	7.00
Vitamin/mineral premix Bio-con- centrate-14 <sup>1</sup> , %	25.00	–
Vitamin/mineral premix Bio-con- centrate-16 <sup>1</sup> , %	–	20.00
<b>Total:</b>	<b>100.00</b>	<b>100.00</b>
Digestible energy, MJ	13.46	13.72
Metabolizable energy, MJ	12.92	13.18

<sup>1</sup>Bio-concentrates BK14 and BK16 included in the formulations of grower and finisher basal diets were supplied by Vasil Kostov Feed Factory, village Lyuben Karavelovo, Varna District, Bulgaria.

Table 2. Proximate compositions of two diets used.

Item	Grower Used in the period of adolescence (live weight 20 - 60 kg)	Finisher Used during the fattening period (live weight 60 – 110 kg)
Moisture, %	17.10±0.67	15.70±0.57
Dry matter, %	82.90±0.73	84.30±0.68
Organic substances, %	78.22±0.74	79.91±0.69
Crude protein, %	15.75±0.83	15.02±0.54
Crude lipids, %	2.81±0.52	2.42±0.56
Crude fibers, %	4.79±0.91	3.84±0.87
Ash, %	4.68±0.38	4.39±0.32
Nitrogen free extract (NFE), %	54.87±1.01	58.63±1.22
Lysine, %	0.80±0.09	0.72±0.08
Calcium, %	1.31±0.31	1.26±0.28
Phosphorus, %	0.85±0.14	0.31±0.13

2.1.2. Powdered dihydroquercetin isolate.

DHQ preparation from Siberian larch (*Larix sibirica* Ledeb) with purity ≥ 96% was secured from Flavitalife Bio JSCo (Sofia, Bulgaria).

2.1.3. Dry distilled rose petals.

Rose distillation (*Rosa damascena* Mill.) was supplied by Bulatarts Productions (Skobelevo village, Bulgaria). Raw material was pressed and air dried (60 °C, 6 hrs). Dry pressed distilled rose petals (DDRP) were finely powdered (< 0.4 mm) prior to use as a dietary additive.

2.1.4. Study design.

The study was performed using late finishing Danube white pigs raised at the Animal Production Experimental Farm at the Agricultural Institute, Shumen, a division of the Agricultural Academy in Sofia, Bulgaria. One hundred and twenty animals of

equal size and age (male: female = 60: 60) were randomly assigned to five treatment groups. Treatment groups included: control pigs fed a normal diet (C); pigs fed a normal diet supplemented with 3.5 mg DHQ/kg/d (D1); pigs fed a normal diet supplemented with 7.5 mg DHQ/kg/d (D2); pigs fed a normal diet supplemented with 0.255 g DDRP/kg/d (R1); and pigs fed a normal diet supplemented with 0.545 g DDRP/kg/d (R2).

2.1.5. Growing and feeding of pigs.

Thirty-five days old pigs were group fed a growing pig diet until reaching an average weight of 33 ± 0.65 kg. During the finishing period, they were reared according to the requirements of Ordinances No 21 of 14 December 2005 [24]. Diet composition is shown in Table 1. DHQ and DDRP supplementation began at an average live weight of 72 kg. Pigs were fed individually twice daily and diets were weighed separately for each pig. Phytonutrients were weighed and dosed individually for each pig according to live weight and expected increase from the previous weighing. Supplements were mixed with diets and fed each morning. Supplementations occurred over 40 d until harvest. Ad libitum water was provided.

2.1.6. Harvesting.

Pigs were blocked by treatment and assigned to one of five harvest groups. Animals were transported and harvested at a commercial abattoir (Unitemp Ltd, Village of Voyvodinovo, Municipality Maritsa, District Plovdiv, Bulgaria) according to Regulations (EC) No 1/2005 [25], approved procedures from the Bulgarian Food Safety Authority (Ordinance No 16 of 3 February 2006 [26]). After 18 hrs of lairage pigs were harvested in compliance with the requirement of Art. 9, par. 3 of Ordinance No 15 of 8 May 2009 [27]. The pigs were electrically stunned with the following exsanguination. The carcasses were scalded, dehaired and eviscerated. Carcasses were cooled for 24 hrs to 4 - 7 °C.

2.2. Muscle and fat sampling.

After chilling, meat samples from m. Longissimus thoracis et lumborum (hereafter referred to as m. Longissimus lumborum, LL) were collected between 12 - 13 ribs. The lumbar portion of and m. Semimembranosus (SM) muscle two adipose tissue depots - backfat (spinal subcutaneous fatty tissue, BF) and leaf fat (soft adipose tissue from chest cavity, LF) were dissected. Separated muscles and fat from each forequarter were vacuum packed and stored at 2 ± 1 °C. Samples for analysis were taken initially at 24 h post-mortem and after 7 d of storage. Additional muscles and fat samples from the right forequarter were vacuum packed and frozen quickly at an air temperature of -35 ± 1 °C and an air flow rate 2 ± 1 m/s. Frozen samples were stored at -18 ± 1 °C. Samples were analysis after 315 d (9 months) of storage.

2.3. Sample analyses.

Analyses were performed in the laboratory of the Department of Meat and Fish Technology from Technological Faculty of University of Food Technologies (Plovdiv, Bulgaria).

2.3.1. Lipid extraction

Total lipids were extracted from muscle and adipose tissue samples in the dark using the polar and non-polar solvent methods as outlined by Bligh & Dyer [28]. Extracted lipids were stored at 2 ± 1 °C in the dark.

2.3.2. Acid value.

Approximately one gram of extracted total fat was used for determination of free fatty acids expressed as acid value (AV).

Lipolytic changes in the fraction of the total lipids extracted from the samples were determined using the procedure described by Zhang et al. [29]. The principal of this titration method is based on the measurement of the number of carboxylic acid groups in lipids, such as a liberated during lipolysis free fatty acids. The AV was presented as the mass of potassium hydroxide (KOH) in mg needed for neutralization of one g of fatty tissue. The analysis were conducted in triplicate.

### 2.3.3. Peroxide value.

Primary lipid oxidation products were determined by the peroxide value (POV) using a Camspec M 550 double-ray UV-VIS spectrophotometer (Spectronic Camspec Ltd, Leeds, United Kingdom) and methods outlined by Yi et al. [30]. Specifically, the improved ferrous oxidation-xylenol orange (mFOX) method was used where the absorbance measurements of colored complex of ferric ions formed by the oxidation of ferrous ions with the reaction of peroxides in the presence of xylenol orange (3, 3'-bis [N,N'-di{carboxymethyl}-amino methyl]-o-cresolsulfonephthalein) are determined at 650 nm. POV was estimated using a standard curve. The analysis was conducted in triplicate.

### 2.3.4. TBARS.

Secondary lipid oxidation products, expressed as free malondialdehyde (MDA) were determined using TBARS as an indicator [31]. Absorbance measurement was performed at 532 nm on a double-lane scanning UV-VIS spectrophotometer Camspec M 550 (Spectronic Camspec Ltd, Leeds, United Kingdom). TBARS were calculated as mg of malonaldehyde/kg of meat. MDA results per kg of meat. Samples were run in triplicate; measures within a treatment and replication were averaged.

### 2.3.5. pH analysis.

The pH of the samples was measured potentiometrically [32] using a calibrated digital pH meter (Microsyst 2004; Microsyst Ltd, Plovdiv, Bulgaria), equipped with a temperature and combined pH electrode using a 10 g sample diluted with 90 ml distilled water. Measurements were conducted in triplicate.

### 2.3.6. Instrumental colour measurements.

Colour of the muscle and fat samples was determined according to lightness (L\*), redness (a\*), and yellowness (b\*) values using a Konica Minolta colorimeter CR-410 (CR-400, Konica Minolta, Inc., Tokyo, Japan) with a 2° standard observer and D65 illuminant daily of simulated retail display. The colorimeter was calibrated using a standard white tile covered in the polyvinylchloride film [33]. The samples were measured after unpacking and subsequence blooming for 30 min at 4 °C. Measurements were collected on all samples. Samples were averaged from measurements collected at three locations.

## 2.4. Statistical analyses.

A two factor analysis with replications was used to evaluate the effect of type and concentration of phytonutrients and storage

time on different traits (pH, L\*, a\*, b\*, AV, POV, TBARS) using statistical software (SPSS version 12.0, SPSS, Thailand) package with an ANOVA. The overall analysis was conducted including all traits in the model (type and concentration of phytonutrients (fixed), time of storage (fixed) and pH, L\*, a\*, b\*, AV, POV, TBARS (covariates). The analyses were conducted for two types of muscles - m. Longissimus lumborum, m. Semimembranosus, as well as backfat and lean fat.

**Table 3.** The percent of variation in pH, L\*, a\*,b\*, AV, POV, TBARS modelled against concentration of phytonutrients and storage time.

Significant predictors (Phytonutrient type and concentration and storage time)	R <sup>2</sup> (%)
m. Longissimus lumborum	
pH	5.56
L *	10.63
a *	27.39
b *	6.14
AV	26.67
POV	71.22
TBARS	25.43
m. Semimembranosus	
pH	37.58
L *	30.51
a *	12.10
b *	68.92
AV	30.54
POV	71.58
TBARS	44.33
Backfat	
pH	66.11
L *	38.16
a *	27.25
b *	12.62
AV	38.01
POV	77.34
TBARS	57.95
Leaf fat	
pH	1.70
L *	42.12
a *	35.16
b *	13.58
AV	30.54
POV	75.05
TBARS	56.79

Comparison of the values of the various indicators was done by the Student and ANOVA t-test of all the experimental samples with the controls on the one hand and inside the factor levels (low and high concentration of the supplemented antioxidant type phytonutrient) for statistically significant differences at the probability  $P < 0.05$  or  $P < 0.01$  respectively (Table 3). When the significant effect was found ( $p < 0.05$ ), the Duncan New's Multiple Rank test was used to compare the mean values.

## 3. RESULTS AND DISCUSSION

### 3.1. Acid value.

Irrespective of muscle or adipose tissue, feed supplementation (DDRP and DHQ) did not affect significantly the AV after nearly one year of frozen storage (Table 4). One possible reason is reduction of enzyme-catalyzed lipolysis [34] as well as water activity of the sarcoplasm [35] after formation of

microscopic ice crystals in the meat fibers due to rapid freezing [36]. On the other hand, the relatively low levels of AV [37] after storage may be a consequence of free fatty acids in initiation reactions and further development of lipid oxidation [38]. This would be further reduced by rapid freezing immediately after harvest. Similar results were found in both muscles indicating that

DDRP and DHQ supplementation slightly impact lipolytic changes in muscle tissue at early postmortem stages (Table 4).

Significant ( $P < 0.05$ ) differences, though small, were detected in the AV of adipose tissues (Table. 4) after 24 hrs storage at  $2 \pm 1$  °C. The difference between lowest and highest AV value varies from 0.20 mg KOH/g in the leaf fat to 0.26 mg KOH/g in the backfat. Compared to controls, the AV of backfat was lower ( $P < 0.05$ ) in group (D1), followed by the group (R2). Feed supplementation with 0.252 g of DDRP/kg/d and 7.5 mg of DHQ/kg/d did not contribute to the AV reduction (24 hrs post mortem) in backfat and the leaf fat after storage.

After 7 days refrigerated storage at  $2 \pm 1$  °C (Table 4), AV in LL samples from C, D2 and R1 groups did not differ significantly ( $P > 0.05$ ). For the same studied period AV in D1 and R2 were 8 and 20% higher ( $P < 0.05$ ) than controls, respectively. AV in SM lipids, group C and D1 were not differed significantly, whereas R1 and R2 were lower than controls by 3 - 4%. One possible explanation is comparatively high initial pH of studied muscles ( $> 6.1$ ) (Table 5) which does not allow higher free fatty acids contentment during storage [39]. In comparison, the AV of SM lipids in D2 pigs was approximately 10% less than controls.

In contrast, after 7 d of storage at  $2 \pm 1$  °C, both types of adipose tissue had lower ( $P < 0.05$ ) AV for all experimental groups compared to controls (Table. 4). This reduction was most pronounced in R1 backfat and D1 leaf fat, with 0.12 and 0.18 mg KOH/g, respectively. According to Gandemer [40] the factors storage time and temperature greatly effect on the lipases activity and lipolysis of adipose tissue. It seems that lipolytic changes are not significantly affected by the type of phytonutrients used as supplemented in pig's diet [41].

### 3.2. Peroxide value.

DHQ and DDRP supplementation had a little effect on the POV in LL and SM samples after 24 hrs and after 7 d of storage at  $2 \pm 1$  °C (Table 4). The POV of refrigerated stored samples was quite low and varied between 0.68 and 0.86 meqv O<sub>2</sub>/kg. These data argue that 7 days storage period is too short and lipid oxidation processes are probably in its` initial phase. In addition, there is relatively low amount of intramuscular fats and triglycerides in the two muscles studied [42]. It is known that oxidation generally begins with phospholipids and if the meat was stored intact, the oxidation occurred in short term refrigerated storage is weak [43].

DHQ and DDRP antioxidant phytonutrients feeding reduced ( $P < 0.05$ ) POV by 17-27% in D2 and R1 leaf fat after 24 hrs and after 7 d of storage at  $2 \pm 1$  °C compared to controls (Table 4). In backfat, the POV reduction was greatest ( $P < 0.05$ ) in R1, followed by R2. The POV decrease in chilled fat can be explained by the strong antioxidant action of both DDRP [11], as well as DHQ [10]. One possible explanation is higher antioxidant accumulation after feed supplementation in pig fats exerting protective effect against oxidation [4].

The use of DHQ and DDRP as feed supplements inhibited ( $P < 0.05$ ) the formation of primary lipid oxidation products during extended (9 months) storage at  $-18 \pm 1$  °C in quick-frozen SM and backfat but was not so effective in frozen LL (Table 4). This is probably due to oxidative stability of the frozen pork during storage at  $-18$  °C resides in an initial protracted lag period [44]. The most pronounced reducing effect on the POV levels in long-

term frozen storage (9 months) was found in R1 and R2, though positive effects were noted in D1. These results demonstrate greater efficacy of phytonutrients added in lower doses against formation of primary lipid oxidation products in both muscle and fat tissue. According to Amaral et al. [45] the main determinants for stability of raw meats to lipid oxidation are content of free ionic iron and myoglobin, ferric reducing ability, PUFA as well as antioxidant content in meat as mentioned above. As the muscle tissue contained more myoglobin and free ionic iron than adipose tissue is expected oxidation to be faster in the LL and SM muscles. Similar results have been reported by Loetscher et al. [1] with poultry supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle.

### 3.3. TBARS.

Variable TBARS results were observed in samples after 24 hrs of storage at  $2 \pm 1$  °C. Samples from the LL did not differ ( $P > 0.05$ ) with respect to TBARS values (Table 4). Conversely, values from the SM and backfat samples were lower ( $P < 0.05$ ) in all treated samples compared to controls. In backfat the only exception is D2 which does not change significantly to C. The lowest ( $P < 0.05$ ) TBARS values were found in D1 samples, followed by R2 and R1. All measured TBARS values up to day 7 ranging from 0.202 to 0.664 and indicate that the meat is fresh [46].

After 7 d of storage, the MDA content of all samples was lower ( $P < 0.05$ ) or unchanged compared to controls (Table 4), except was D2 in LL with 17% increasing ( $P < 0.05$ ). The lowest secondary products of lipid oxidation were observed in D1 backfat. In SM samples, the lowest ( $P < 0.05$ ) TBARS values were found in D1 and R1. Compared to controls, lower TBARS ( $P < 0.05$ ) were found in the four treated groups of frozen samples under long-term storage (Table 4). The only exception was D2 in SM which does not change significantly ( $P > 0.05$ ) to C.

Formation of secondary lipid oxidation products was minimal in LL of D1 pigs, followed by SM of D1 and R1 pigs, backfat of R2 and R1 pigs and leaf fat samples from D1 and D2 pigs. According to Frank [4] dietary supplementation with phytonutrients reaches in flavonoids such as catechin, epicatechin and quercetins increased in vivo concentrations of tocopherols mainly in pig's fat. Our results confirm this suggestion by lower TBARS obtained at 7 d post mortem at  $2 \pm 1$  °C and after 315 d freezing in enriched with DDRP and DHQ backfat, leaf fat and m. Semimembranosus compared to m. Longissimus lumborum.

In conclusion, supplementation with a phytonutrient such as DHQ and DDRP leads to a reduced accumulation of primary and secondary lipid oxidation products during storage in both muscle and adipose tissue. These results indicate that the dietary DHQ and DDRP addition had antioxidant effects on meat lipids and confirmed previous reports [1] with positive effects on oxidative stability of the meat after feed supplementation. This effect is more pronounced in long term versus short term storage.

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**Table 4.** Lipolytic and oxidative changes in pork lipids during different types of storage represented by the AV, POV and TBARS expressed as means ± standard error of the mean (SEM).

	Storage time	<i>m. Longissimus lumborum</i>					<i>m. Semimembranosus</i>					Backfat					Leaf fat				
		(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)
AV, mg KOH/g	24 h 2±1 °C	0.40 ±0.04 <sup>a,y</sup>	0.40 ±0.03 <sup>a,y</sup>	0.42 ±0.05 <sup>a,y</sup>	0.44 ±0.02 <sup>a,y</sup>	0.40 ±0.03 <sup>a,y</sup>	0.50 ±0.05 <sup>a,y</sup>	0.45 ±0.05 <sup>a,y</sup>	0.43 ±0.05 <sup>a,y</sup>	0.44 ±0.03 <sup>a,y</sup>	0.40 ±0.07 <sup>a,y</sup>	0.50 ±0.05 <sup>b,c,y</sup>	0.37 ±0.05 <sup>a,y</sup>	0.63 ±0.04 <sup>d,y</sup>	0.55 ±0.02 <sup>c,y</sup>	0.42 ±0.03 <sup>a,b,y</sup>	0.65 ±0.04 <sup>c,y</sup>	0.45 ±0.05 <sup>a,b,y</sup>	0.55 ±0.05 <sup>b,y</sup>	0.55 ±0.02 <sup>b,y</sup>	0.45 ±0.03 <sup>a,y</sup>
	7 d 2±1 °C	0.65 ±0.05 <sup>a,z</sup>	0.70 ±0.05 <sup>a,b,z</sup>	0.64 ±0.05 <sup>a,z</sup>	0.68 ±0.05 <sup>a,b,z</sup>	0.78 ±0.05 <sup>b,z</sup>	0.73 ±0.03 <sup>a,z</sup>	0.74± 0.04 <sup>a,z</sup>	0.66 ±0.04 <sup>a,z</sup>	0.70 ±0.02 <sup>a,z</sup>	0.71 ±0.03 <sup>a,z</sup>	0.75± 0.01 <sup>c,z</sup>	0.67± 0.03 <sup>a,b,z</sup>	0.74± 0.01 <sup>c,z</sup>	0.63± 0.03 <sup>a,z</sup>	0.72± 0.02 <sup>b,c,z</sup>	0.84 ±0.04 <sup>c,z</sup>	0.64 ±0.04 <sup>a,z</sup>	0.76 ±0.03 <sup>b,z</sup>	0.66 ±0.03 <sup>a,z</sup>	0.76 ±0.06 <sup>b,c,z</sup>
	315 d -18±1 °C	0.10 ±0.02 <sup>a,x</sup>	0.12 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.12 ±0.01 <sup>a,x</sup>	0.12 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.12 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.11 ±0.0 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.12 ±0.01 <sup>a,x</sup>	0.12 ±0.01 <sup>a,x</sup>	0.11 ±0.01 <sup>a,x</sup>	0.12 ±0.01 <sup>a,x</sup>
POV, meqv O <sub>2</sub> /kg	24 h 2±1 °C	0.83 ±0.05 <sup>a,b,x</sup>	0.87 ±0.03 <sup>b,x</sup>	0.79 ±0.02 <sup>a,x</sup>	0.79 ±0.04 <sup>a,x</sup>	0.84 ±0.03 <sup>a,b,x</sup>	0.83 ±0.02 <sup>b,x</sup>	0.83 ±0.03 <sup>b,x</sup>	0.84 ±0.03 <sup>b,x</sup>	0.80 ±0.03 <sup>b,x</sup>	0.68 ±0.03 <sup>a,x</sup>	0.86 ±0.01 <sup>c,x</sup>	0.82 ±0.02 <sup>b,x</sup>	0.83 ±0.01 <sup>b,x</sup>	0.67 ±0.04 <sup>a,x</sup>	0.70 ±0.05 <sup>a,x</sup>	0.88 ±0.03 <sup>c,x</sup>	0.87 ±0.03 <sup>c,x</sup>	0.75 ±0.03 <sup>b,x</sup>	0.69 ±0.02 <sup>a,x</sup>	0.95 ±0.03 <sup>d,x</sup>
	7 d 2±1 °C	0.83 ±0.05 <sup>a,x</sup>	0.78 ±0.02 <sup>a,x</sup>	0.77 ±0.03 <sup>a,x</sup>	0.79 ±0.05 <sup>a,x</sup>	0.75 ±0.04 <sup>a,y</sup>	0.86 ±0.03 <sup>a,x</sup>	0.85 ±0.02 <sup>a,x</sup>	0.86 ±0.03 <sup>a,x</sup>	0.83 ±0.01 <sup>a,x</sup>	0.81 ±0.04 <sup>a,x</sup>	0.87 ±0.01 <sup>b,x</sup>	0.85 ±0.03 <sup>b,y</sup>	0.85 ±0.02 <sup>b,x</sup>	0.74 ±0.05 <sup>a,y</sup>	0.76 ±0.03 <sup>a,x</sup>	0.94 ±0.02 <sup>c,y</sup>	0.93 ±0.02 <sup>c,y</sup>	0.81 ±0.04 <sup>b,x</sup>	0.74 ±0.01 <sup>a,y</sup>	0.92 ±0.04 <sup>c,x</sup>
	315 d -18±1 °C	1.39 ±0.02 <sup>a,y</sup>	1.52 ±0.02 <sup>b,y</sup>	1.48 ±0.03 <sup>b,y</sup>	1.48 ±0.04 <sup>b,y</sup>	1.51 ±0.02 <sup>b,z</sup>	1.51 ±0.02 <sup>b,y</sup>	1.56 ±0.07 <sup>b,y</sup>	1.67 ±0.01 <sup>c,y</sup>	1.47 ±0.02 <sup>b,y</sup>	1.31 ±0.07 <sup>a,y</sup>	1.85 ±0.02 <sup>c,y</sup>	1.79 ±0.03 <sup>b,y</sup>	1.80 ±0.02 <sup>b,y</sup>	1.78 ±0.04 <sup>b,z</sup>	1.71 ±0.01 <sup>a,y</sup>	1.65 ±0.01 <sup>a,z</sup>	1.69 ±0.05 <sup>a,z</sup>	1.80 ±0.04 <sup>b,y</sup>	1.64 ±0.02 <sup>a,z</sup>	1.61 ±0.04 <sup>a,y</sup>
TBARS, mg MDA/kg	24 h 2±1 °C	0.27 ±0.03 <sup>a,x</sup>	0.28 ±0.02 <sup>a,x</sup>	0.31 ±0.02 <sup>a,x</sup>	0.25 ±0.04 <sup>a,x</sup>	0.26 ±0.03 <sup>a,x</sup>	0.60 ±0.05 <sup>c,y</sup>	0.24 ±0.03 <sup>a,x</sup>	0.32 ±0.03 <sup>b,x</sup>	0.26 ±0.02 <sup>a,x</sup>	0.26 ±0.01 <sup>a,x</sup>	0.58 ±0.06 <sup>c,x</sup>	0.20 ±0.02 <sup>a,x</sup>	0.50 ±0.02 <sup>c,x</sup>	0.46 ±0.05 <sup>b,x</sup>	0.42 ±0.01 <sup>b,x</sup>	0.50 ±0.05 <sup>b,x</sup>	0.44 ±0.02 <sup>b,x</sup>	0.47 ±0.02 <sup>b,x</sup>	0.44 ±0.03 <sup>a,b,x</sup>	0.39 ±0.02 <sup>a,x</sup>
	7 d 2±1 °C	0.29 ±0.05 <sup>a,x</sup>	0.34 ±0.02 <sup>a,y</sup>	0.44 ±0.02 <sup>b,y</sup>	0.29 ±0.03 <sup>a,x</sup>	0.32 ±0.03 <sup>a,x</sup>	0.63 ±0.04 <sup>c,y</sup>	0.28 ±0.03 <sup>a,x</sup>	0.38 ±0.03 <sup>b,x</sup>	0.29 ±0.02 <sup>a,x</sup>	0.33 ±0.02 <sup>a,b,y</sup>	0.67 ±0.03 <sup>d,x</sup>	0.23 ±0.04 <sup>a,x</sup>	0.54 ±0.02 <sup>c,y</sup>	0.49 ±0.02 <sup>b,x</sup>	0.45 ±0.03 <sup>b,x</sup>	0.57 ±0.03 <sup>c,x</sup>	0.47 ±0.01 <sup>b,x</sup>	0.49 ±0.02 <sup>b,x</sup>	0.47 ±0.02 <sup>a,x</sup>	0.43 ±0.04 <sup>a,x</sup>
	315 d -18±1 °C	0.49 ±0.03 <sup>c,y</sup>	0.34 ±0.01 <sup>a,y</sup>	0.43 ±0.04 <sup>b,y</sup>	0.40 ±0.01 <sup>b,y</sup>	0.41 ±0.03 <sup>b,y</sup>	0.51 ±0.05 <sup>b,x</sup>	0.37 ±0.06 <sup>a,y</sup>	0.58 ±0.03 <sup>b,y</sup>	0.38 ±0.02 <sup>a,y</sup>	0.43 ±0.03 <sup>c,z</sup>	1.31 ±0.08 <sup>c,y</sup>	1.27 ±0.02 <sup>c,y</sup>	1.06 ±0.03 <sup>b,z</sup>	1.03 ±0.05 <sup>b,y</sup>	0.90 ±0.04 <sup>a,y</sup>	3.07 ±0.05 <sup>d,y</sup>	1.30 ±0.02 <sup>a,y</sup>	1.28 ±0.15 <sup>a,y</sup>	2.10 ±0.03 <sup>c,y</sup>	1.55 ±0.01 <sup>b,y</sup>

<sup>a, b, c</sup> Means in the same row for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

<sup>w, x, y, z</sup> Means in the same column for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

**Table 5.** Changes in the pork pH during different types of storage expressed as means ± standard error of the mean (SEM)

pH	<i>m. Longissimus lumborum</i>					<i>m. Semimembranosus</i>					Backfat					Leaf fat				
	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)
45 min	6.25 ±0.08 <sup>a,z</sup>	6.21 ±0.02 <sup>a,z</sup>	6.19 ±0.04 <sup>a,z</sup>	6.15 ±0.06 <sup>a,y</sup>	6.18 ±0.08 <sup>a,z</sup>	6.24 ±0.03 <sup>a, z</sup>	6.20 ±0.04 <sup>a,z</sup>	6.18 ±0.05 <sup>a</sup>	6.16 ±0.05 <sup>a,z</sup>	6.17 ±0.04 <sup>a,z</sup>	6.89 ±0.05 <sup>a,z</sup>	6.91 ±0.04 <sup>a,z</sup>	6.99 ±0.07 <sup>a,z</sup>	6.95 ±0.05 <sup>a,z</sup>	6.98 ±0.06 <sup>a,z</sup>	6.87 ±0.06 <sup>a,z</sup>	6.81 ±0.05 <sup>a,z</sup>	6.89 ±0.05 <sup>a,z</sup>	6.84 ±0.06 <sup>a,z</sup>	6.86 ±0.05 <sup>a,z</sup>
24 h 2±1 °C	5.71 ±0.02 <sup>b,x</sup>	5.79 ±0.03 <sup>c,x</sup>	5.62 ±0.02 <sup>a,w</sup>	5.90 ±0.04 <sup>d,x</sup>	5.72 ±0.05 <sup>b,c,x</sup>	5.70 ±0.03 <sup>b, x</sup>	5.60 ±0.04 <sup>a,w</sup>	5.69 ±0.05 <sup>a,b,w</sup>	5.71 ±0.01 <sup>b,w</sup>	5.61 ±0.02 <sup>a,w</sup>	5.82 ±0.03 <sup>d,x</sup>	5.68 ±0.02 <sup>b,y</sup>	5.55 ±0.01 <sup>a,w</sup>	5.73 ±0.01 <sup>c,y</sup>	5.66 ±0.10 <sup>b,c,x</sup>	6.52 ±0.05 <sup>a,x</sup>	6.59 ±0.04 <sup>a,b,x</sup>	6.51 ±0.06 <sup>a,x</sup>	6.56 ±0.02 <sup>a,x</sup>	6.62 ±0.01 <sup>b,x</sup>
7 d 2±1 °C	5.90 ±0.05 <sup>a,y</sup>	5.89 ±0.04 <sup>a,y</sup>	5.88 ±0.02 <sup>a,y</sup>	5.91 ±0.01 <sup>a,x</sup>	5.90 ±0.01 <sup>a,y</sup>	6.01 ±0.03 <sup>a,b,y</sup>	5.98 ±0.03 <sup>a,b,y</sup>	6.00 ±0.04 <sup>a,b,y</sup>	6.03 ±0.01 <sup>b,x</sup>	5.99 ±0.01 <sup>a,y</sup>	5.65 ±0.02 <sup>c,w</sup>	5.54 ±0.04 <sup>a,b,x</sup>	5.60 ±0.02 <sup>b,x</sup>	5.50 ±0.06 <sup>a,x</sup>	5.58 ±0.02 <sup>a,b,x</sup>	5.61 ±0.03 <sup>b,c,y</sup>	5.57 ±0.01 <sup>a,b,x</sup>	5.60 ±0.01 <sup>b,c,w</sup>	5.55 ±0.03 <sup>a,w</sup>	5.63 ±0.03 <sup>d,w</sup>
315 d -18±1 °C	5.84 ±0.03 <sup>b,y</sup>	5.73 ±0.03 <sup>a,x</sup>	5.78 ±0.03 <sup>a,x</sup>	5.92 ±0.05 <sup>c,x</sup>	5.73 ±0.03 <sup>a,x</sup>	5.96 ±0.04 <sup>b, y</sup>	5.88 ±0.03 <sup>a,x</sup>	5.88 ±0.02 <sup>a, x</sup>	6.06 ±0.01 <sup>c,y</sup>	5.91 ±0.01 <sup>a,b,y</sup>	6.66 ±0.03 <sup>a,y</sup>	6.67 ±0.03 <sup>a,y</sup>	6.70 ±0.03 <sup>a,y</sup>	6.68 ±0.08 <sup>a,y</sup>	6.67 ±0.05 <sup>a,y</sup>	6.64 ±0.05 <sup>a,y</sup>	6.68 ±0.04 <sup>a,y</sup>	6.69 ±0.03 <sup>a, y</sup>	6.65 ±0.04 <sup>a,y</sup>	6.69 ±0.02 <sup>a,y</sup>

<sup>a, b, c</sup> Means in the same row for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

<sup>w, x, y, z</sup> Means in the same column for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

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**Table 6.** Changes in the instrumental colour characteristics of m. *Longissimus lumborum*, m. *Semimembranosus*, backfat and leaf fat during different types of storage expressed as means ± standard error of the mean (SEM).

Colour parameters		m. <i>Longissimus lumborum</i>					m. <i>Semimembranosus</i>					Backfat					Leaf fat				
		(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)	(C)	(D1)	(D2)	(R1)	(R2)
L*	24 h 2±1 °C	43.57 ±0.70 <sup>a,x</sup>	46.47 ±0.16 <sup>c,x</sup>	46.34 ±0.29 <sup>c,x</sup>	48.89 ±0.27 <sup>d,x</sup>	45.06 ±0.86 <sup>b,x</sup>	41.22 ±0.62 <sup>a,x</sup>	44.21 ±0.24 <sup>c,x</sup>	46.01 ±0.23 <sup>d,x</sup>	45.75 ±0.24 <sup>d,x</sup>	43.15 ±0.06 <sup>b,x</sup>	73.91 ±0.25 <sup>d,x</sup>	73.10 ±0.11 <sup>c,x</sup>	72.59 ±0.23 <sup>b,x</sup>	73.30 ±0.19 <sup>c,x</sup>	70.52 ±0.30 <sup>a,x</sup>	78.97 ±0.29 <sup>d,x</sup>	76.42 ±0.24 <sup>b,x</sup>	76.58 ±0.32 <sup>b,x</sup>	77.20 ±0.13 <sup>c,x</sup>	75.57 ±0.18 <sup>a,x</sup>
	7 d 2±1 °C	43.17 ±0.56 <sup>a,x</sup>	50.01 ±0.29 <sup>d,z</sup>	48.10 ±0.55 <sup>c,y</sup>	48.67 ±0.67 <sup>c,x</sup>	44.92 ±0.43 <sup>b,x</sup>	41.05 ±0.34 <sup>a,x</sup>	47.97 ±0.27 <sup>d,z</sup>	46.27 ±0.64 <sup>c,x</sup>	45.52 ±0.71 <sup>c,x</sup>	43.28 ±0.38 <sup>b,x</sup>	79.34 ±0.37 <sup>a,y</sup>	79.25 ±0.25 <sup>a,y</sup>	79.09 ±0.54 <sup>a,y</sup>	79.24 ±0.53 <sup>a,y</sup>	79.01 ±0.49 <sup>a,y</sup>	79.80 ±0.25 <sup>a,y</sup>	79.71 ±0.30 <sup>a,y</sup>	79.62 ±0.46 <sup>a,y</sup>	79.63 ±0.37 <sup>a,y</sup>	79.59 ±0.44 <sup>a,y</sup>
	315 d -18±1 °C	46.33 ±0.21 <sup>a,y</sup>	47.93 ±0.31 <sup>b,y</sup>	50.09 ±0.57 <sup>c,z</sup>	50.92 ±0.28 <sup>c,y</sup>	54.41 ±0.28 <sup>d,y</sup>	44.54 ±0.68 <sup>a,y</sup>	45.82 ±0.44 <sup>b,y</sup>	47.22 ±0.50 <sup>c,y</sup>	48.85 ±0.23 <sup>d,y</sup>	50.71 ±0.18 <sup>c,y</sup>	79.69 ±0.43 <sup>a,b,y</sup>	79.64 ±0.21 <sup>b,y</sup>	79.15 ±0.27 <sup>a,b,y</sup>	79.18 ±0.40 <sup>a,b,y</sup>	79.06 ±0.22 <sup>a,y</sup>	80.02 ±0.42 <sup>a,y</sup>	79.93 ±0.29 <sup>a,y</sup>	79.84 ±0.31 <sup>a,y</sup>	79.73 ±0.30 <sup>a,y</sup>	79.77 ±0.25 <sup>a,y</sup>
a*	24 h 2±1 °C	15.70 ±0.11 <sup>c,z</sup>	13.61 ±0.04 <sup>c,y</sup>	12.87 ±0.16 <sup>a,y</sup>	15.03 ±0.07 <sup>d,z</sup>	13.39 ±0.08 <sup>b,z</sup>	13.15 ±0.07 <sup>d,x</sup>	12.54 ±0.06 <sup>b,x</sup>	11.79 ±0.32 <sup>a,y</sup>	12.32 ±0.11 <sup>b,y</sup>	12.76 ±0.18 <sup>b,x</sup>	3.74 ±0.55 <sup>c,x</sup>	3.16 ±0.49 <sup>a,x</sup>	3.17 ±0.32 <sup>a,x</sup>	3.19 ±0.12 <sup>a,x</sup>	3.49 ±0.38 <sup>b,x</sup>	4.24 ±0.14 <sup>b,x</sup>	4.04 ±0.31 <sup>a,b</sup>	4.15 ±0.22 <sup>c,b</sup>	3.81 ±0.11 <sup>a,x</sup>	3.62 ±0.35 <sup>a,c,x</sup>
	7 d 2±1 °C	13.71 ±0.17 <sup>b,x</sup>	13.08 ±0.47 <sup>b,x</sup>	12.13 ±0.13 <sup>a,x</sup>	13.60 ±0.25 <sup>b,y</sup>	12.05 ±0.38 <sup>a,x</sup>	13.31 ±0.27 <sup>c,x</sup>	12.81 ±0.48 <sup>b,c,x</sup>	11.05 ±0.28 <sup>a,x</sup>	11.77 ±0.41 <sup>a,x</sup>	12.63 ±0.29 <sup>b,x</sup>	5.49 ±0.22 <sup>b,z</sup>	4.88 ±0.26 <sup>a,y</sup>	4.77 ±0.29 <sup>a,y</sup>	4.86 ±0.31 <sup>a,y</sup>	4.83 ±0.25 <sup>a,y</sup>	5.83 ±0.18 <sup>b,z</sup>	5.41 ±0.19 <sup>a,z</sup>	5.42 ±0.14 <sup>a,z</sup>	5.43 ±0.17 <sup>a,z</sup>	5.34 ±0.21 <sup>a,z</sup>
	315 d -18±1 °C	15.35 ±0.21 <sup>c,y</sup>	12.91 ±0.25 <sup>b,x</sup>	12.56 ±0.3 <sup>b,x,y</sup>	11.88 ±0.11 <sup>a,x</sup>	12.77 ±0.13 <sup>b,y</sup>	14.27 ±0.12 <sup>c,y</sup>	13.71 ±0.11 <sup>d,y</sup>	12.03 ±0.44 <sup>a,z</sup>	12.71 ±0.06 <sup>b,z</sup>	13.31 ±0.07 <sup>c,y</sup>	4.54 ±0.20 <sup>a,y</sup>	4.50 ±0.19 <sup>a,y</sup>	4.30 ±0.21 <sup>a,y</sup>	4.41 ±0.43 <sup>a,y</sup>	4.39 ±0.19 <sup>a,y</sup>	4.77 ±0.35 <sup>a,y</sup>	4.82 ±0.28 <sup>a,y</sup>	4.86 ±0.16 <sup>a,y</sup>	4.74 ±0.34 <sup>a,y</sup>	4.91 ±0.20 <sup>a,y</sup>
b*	24 h 2±1 °C	3.72 ±0.05 <sup>a,x</sup>	4.39 ±0.05 <sup>c,x</sup>	4.24 ±0.14 <sup>c,x</sup>	5.07 ±0.09 <sup>d,y</sup>	3.93 ±0.20 <sup>b,x</sup>	3.03 ±0.05 <sup>a,x</sup>	3.05 ±0.01 <sup>a,x</sup>	4.53 ±0.09 <sup>d,y</sup>	4.06 ±0.04 <sup>b,x</sup>	4.22 ±0.04 <sup>c,y</sup>	3.67 ±0.21 <sup>a,x</sup>	4.52 ±0.10 <sup>c,x</sup>	4.31 ±0.48 <sup>b,c,x</sup>	4.23 ±0.17 <sup>b,x</sup>	4.40 ±0.14 <sup>b,c,x</sup>	4.48 ±0.32 <sup>a,x</sup>	4.92 ±0.18 <sup>b,x</sup>	4.95 ±0.25 <sup>b,x</sup>	4.91 ±0.23 <sup>b,x</sup>	4.93 ±0.09 <sup>b,x</sup>
	7 d 2±1 °C	3.54 ±0.12 <sup>a,x</sup>	5.48 ±0.23 <sup>c,y</sup>	5.34 ±0.13 <sup>c,z</sup>	4.55 ±0.22 <sup>b,x</sup>	3.90 ±0.28 <sup>a,x</sup>	3.02 ±0.26 <sup>a,x</sup>	5.11 ±0.38 <sup>c,y</sup>	4.16 ±0.35 <sup>b,x</sup>	4.68 ±0.40 <sup>b,y</sup>	4.00 ±0.32 <sup>b,x</sup>	4.87 ±0.33 <sup>a,y</sup>	5.49 ±0.35 <sup>a,b,y</sup>	5.68 ±0.38 <sup>b,y</sup>	5.50 ±0.21 <sup>b,y</sup>	5.61 ±0.13 <sup>b,y</sup>	6.19 ±0.27 <sup>a,y</sup>	6.70 ±0.10 <sup>b,z</sup>	6.60 ±0.10 <sup>b,y</sup>	6.69 ±0.11 <sup>b,z</sup>	6.63 ±0.11 <sup>b,z</sup>
	315 d -18±1 °C	4.31 ±0.01 <sup>a,y</sup>	5.51 ±0.06 <sup>d,y</sup>	5.17 ±0.02 <sup>c,y</sup>	5.20 ±0.04 <sup>c,y</sup>	4.57 ±0.23 <sup>b,y</sup>	4.13 ±0.08 <sup>a,y</sup>	5.72 ±0.06 <sup>b,z</sup>	6.70 ±0.03 <sup>c,z</sup>	6.39 ±0.26 <sup>d,z</sup>	6.01 ±0.04 <sup>c,z</sup>	5.64 ±0.13 <sup>a,z</sup>	5.66 ±0.25 <sup>a,y</sup>	5.60 ±0.06 <sup>a,y</sup>	5.69 ±0.23 <sup>a,y</sup>	5.72 ±0.10 <sup>a,y</sup>	6.11 ±0.23 <sup>a,y</sup>	6.01 ±0.24 <sup>a,y</sup>	6.07 ±0.17 <sup>a,y</sup>	5.99 ±0.20 <sup>a,y</sup>	6.09 ±0.19 <sup>a,y</sup>

<sup>a, b, c</sup> Means in the same row for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

<sup>x, y, z</sup> Means in the same column for individual muscle or fat with different superscript letters differ significantly (P≤0.05).

### 3.4. pH value.

No differences were detected between the pH values of LL across all experimental groups studied either at 45 min post mortem or after 7 d of storage at  $2 \pm 1^\circ\text{C}$  (Table 5). Similar results were also determined for SM. No differences in fat pH at 45 min post mortem were detected, though the pH of backfat and leaf fat was closer to 7.00 than muscle tissue. Similar results were reported by and Bertol et al. [7] after grape pomace supplementation of pigs. After long-term frozen storage, differences in pH value of backfat or leaf fat did not differ from controls (Table 5). However, compared to 45 min post mortem pH values both backfat and leaf fat was lower ( $P < 0.05$ ). This could be explained by the more rapid lipolysis in the triglycerides of adipose tissue during frozen storage.

After 24 hrs small but statistically distinct ( $P < 0.05$ ) pH values were found in refrigerated muscles stored at  $2 \pm 1^\circ\text{C}$ . For example, in LL the lowest pH values ( $P < 0.05$ ) were found in muscle of D2 pigs, whereas muscle from D1 and especially R1, the pH was higher than controls. One possible reason for rapid post-slaughter pH decline occurred after animal stress, both prior to and during processing [47]. However, the largest difference was just 0.28 units (Table 5) and was within the pH limits recommended by Warriss [48]. Similar results were found in the SM. The greatest decrease in SM pH at 24 hrs post mortem was detected in D1 and R2 pigs.

pH values at 45 min and 24 hrs of the both examined muscles do not perform deviations due to stress and were within the pH limits recommended by Warriss [48]. All samples can be characterized as normal (RFN - reddish pink, firm and normal exudative meat). This conclusion is in good agreement with data characterizing the meat colour.

After 315 days frozen storage pH in five studied LL and SM samples were found significantly different ( $P < 0.05$ ) (Table 5). Compared to controls pH in LL of D1 pigs after long time frozen (315 d) storage was significantly ( $P < 0.05$ ) lower. The pH in LL samples of D2 group did not differ to controls, whereas pH of group R1 was slightly higher ( $P < 0.05$ ). After 315 days frozen storage the pH values in SM of groups D1, D2 and R2 were found lower ( $P < 0.05$ ), compared to the controls while pH of group R1 was slightly higher ( $P < 0.05$ ). The pH values of both adipose tissue samples for five studied groups stored 24 hrs and 7 days of pigs were found significantly different ( $P < 0.05$ ). The pH in fat decreased with 1.2 - 1.3 units during 7 d of cold storage. After 7 days cold storage of backfat lowest pH values ( $P < 0.05$ ) was found in D1, R1 and R2 groups. At the same time, pH of D2 backfat was statistically indistinguishable ( $P > 0.05$ ) compared to the controls. The difference between lowest and highest pH level was 2.65% (Table 5). Similar results were found in pH of leaf fat (Table 5). After 7 d of cold storage pH in D1 and R1 groups was lower ( $P < 0.05$ ), whereas in D2 and R2 samples pH was statistically indistinguishable ( $P > 0.05$ ) compared to controls. The difference between the lowest and highest pH in cold stored leaf fat was 0.08 units or 1.43% (Table 5). Usually, during the long time frozen storage the protein buffer systems desaturate releasing hydrogen ions and the content of water-soluble compounds increased. As a result pH decreases [49]. On the other hand, many researchers [50] did not found significant changes in pH or

established increasing and note that pH changes during long time frozen storage were correlated with type of the muscle and the animals' breed. Our results confirmed this study by different trends established in pH during refrigeration or frozen storage.

### 3.5. Colour characteristics.

Feeding DHQ and DDRP increased ( $P < 0.05$ ) increased the brightness ( $L^*$ ) and yellowness ( $b^*$ ) values and reduced the redness ( $a^*$ ) in both refrigerated and frozen storage (Table 6). The colour brightness was influenced by the storage period but not on the used feed supplement.

At 24 hrs post mortem the highest ( $P < 0.05$ )  $L^*$  and  $b^*$  values were recorded in the LL of R1 pigs and in the SM of D2 pigs (Table 6). At the same time,  $a^*$  values were the highest ( $P < 0.05$ ) in the LL and SM of D2 pigs, respectively. After 7 days refrigerated storage, brightness and yellowness increased ( $P < 0.05$ ) in the LL and SM of D1 (Table 6). Neethling et al. [51] attributed the increase of  $b^*$  values to the increase of metmyoglobin in muscle tissue. Our results confirmed previous results established that denaturation of globin in the muscle pigment, makes myoglobin more susceptible to auto-oxidation during chilled or frozen storage residues to changes in the meat color [49]. The lower activity of metmyoglobin-reducing enzymes, reduced redox stability of oxymyoglobin, physical processes related water freezing in the surface layer effects on chilled/frozen meat colour [50] by reducing  $a^*$  value and increasing colour yellowness ( $b^*$ ), too. One possible reason for a slightly decrease in  $a^*$  value in SM and LL from R1 and D1 pigs could be increased antioxidant content in the tissues after dietary feed enrichment which would protect myoglobin from oxidation. Similar results were reported [7] after pigs feed enrichment with grape pomace. The lowest redness ( $P < 0.05$ ) in the SM and LL of R2 and D2 pigs (Table 6) showed the importance of feed supplement concentration and confirmed previous results demonstrated that antioxidant effect depends on the type and the quantity of phytonutrient, and even pro-oxidant effect [1].

After long-term frozen storage, the LL and SM had the highest ( $P < 0.05$ )  $L^*$  values in R2 pigs, while  $b^*$  were the greatest in the LL of D1 pigs and the SM of D2 pigs (Table 6). At the same time,  $a^*$  values were the lowest ( $P < 0.05$ ) the LM of R1 pigs and SM of D2 pigs. Similar variations in the instrumental colour of the LL have been reported for chilled pork [7]. Differences were noted ( $P < 0.05$ ) in the color of adipose tissue compared to the muscle tissue (Table 6). Both the backfat and leaf fat were ( $P < 0.05$ ) brighter and less ( $P < 0.05$ ) red. The greatest ( $P < 0.05$ ) decrease in brightness of the backfat and leaf fat was in R2 pig carcasses after 24 hrs storage at refrigerated temperatures. Storage, frozen or refrigerated increased  $L^*$  values of fat but were not affected by antioxidant pig feeding (Table 6). A decrease ( $P < 0.05$ ) in redness ( $a^*$ ) and an increase in yellowness ( $b^*$ ) were recorded in chilled backfat and leaf fat in all experimental groups compared to controls (Table 6). Redness and yellowness were not different across treatments for backfat and leaf fat. These results indicate that the pigs feed supplementation with antioxidant type phytonutrients did not effect on colour characteristics in quick-frozen backfat and leaf fat (Table 6).

In summary, the addition of DHQ and DDRP to feed of growing-fattening pigs does not have a major effect on colour

characteristics of muscle and adipose tissue during their frozen and chilled storage. Changes in the colour characteristics of muscle tissue were more pronounced than fats. In backfat and leaf

fat the influence of freezing and cooling has a profound effect on quality parameters than the addition of the phytonutrient.

#### 4. CONCLUSIONS

This study showed that dietary supplementation of growing pigs with DHQ and DDRP results in reduction of primary and secondary lipid oxidation products during storage LL and SM muscles and adipose samples from backfat and leaf fat depots. This most pronounced effect for LL and SM was found after 3.5 mg DHQ/kg/d and 0.255 g or 0.545 DDRP/kg/d supplement. For short term chilled storage both used supplements decreased lipid oxidation in adipose tissue. In long term frozen storage greatest oxidation stability was observed after feeding low levels of DHQ (3.5 mg/kg/d) for muscle tissue and high levels of DHQ (7.5 mg DHQ/kg/d) for adipose tissue.

Both used concentrations of DDRP showed positive effect in muscle and adipose tissue oxidative stability after 315 days of storage at  $-18 \pm 1$  °C.

Irrespective of muscle type or adipose tissue depot, dietary supplementation of DHQ and DDRP to finishing pigs did not affect the AV of fast-frozen pork after 315 days of storage at  $-18 \pm 1$  °C. After 7 d of storage at  $2 \pm 1$  °C in chilled muscles and fats positive impact against oxidation was found after DDRP and 3.5 mg DHQ/kg/d supplementation.

Despite the higher used concentration of DHQ (7.5 mg/kg/d) the effect of supplementation on oxidative stability in muscle and adipose tissue for short term chilled storage was insufficient.

Reduction of AV in backfat was most pronounced in pigs supplemented with 3.5 mg DHQ/kg/d as well as in leaf fat from pigs fed 0.255 g DDRP/kg/d.

DHQ and DDRP feeding slightly influenced the pH of pork but did not have major effects on colour characteristics of lean or fat, regardless of the type of storage used.

In summary, we can conclude that the tested concentrations of DHQ and DDRP as pigs' feed supplements show a little impact on pH changes of pork. It is most pronounced in the rigor mortis at 24 hours post mortem. Compared to controls, the supplements and their concentrations differ by the muscle and adipose tissue type.

Supplementation of pigs with DHQ or DDRP is a promising strategy to increase the oxidative stability of lean pork or fat and stabilized pork meat colour without deleterious changes of meat acidity.

#### 5. REFERENCES

- Loetscher, Y.; Kreuzer, M.; Messikommer, R.E. Oxidative stability of the meat of broilers supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle, and effects on performance and meat quality. *Poultry Sci.* **2013**, *92*, 2938-2948, <https://doi.org/10.3382/ps.2013-03258>.
- Pugliese, C.; Bozzi, R.; Campodoni, G.; Acciaioli, A.; Franci, O.; Gandini, G. Performance of Cinta Senese pigs reared outdoors and indoors. 1. Meat and subcutaneous fat characteristics. *Meat Sci.* **2005**, *69*, 459-464, <https://doi.org/10.1016/j.meatsci.2004.09.001>.
- Tikk, K.; Tikk, M.; Karlsson, A.H.; Andersen, H.J. The effect of a muscle-glycogen-reducing finishing diet on porcine meat and fat colour. *Meat Sci.* **2006**, *73*, 378-385, <https://doi.org/10.1016/j.meatsci.2005.12.015>.
- Frank, J. Beyond vitamin E supplementation: An alternative strategy to improve vitamin E status. *J. Plant Physiol.* **2005**, *162*, 834-843, <https://doi.org/10.1016/j.jplph.2005.04.017>.
- Thiel-Cooper, R.L.; Parrish Jr.F.C.; Sparks J.C.; Wiegand, B.R.; Ewan, R.C. Conjugated linoleic acid changes swine performance and carcass composition. *J. Anim. Sci.* **2001**, *79*, 1821-1828, <https://doi.org/10.2527/2001.7971821x>.
- Jaturasitha, S.; Srikanchai, T.; Chakeredza, S.; ter Meulen, U.; Wicke, M. Backfat characteristics of barrows and gilts fed on tuna oil supplemented diets during the growing-finishing periods. *Asian-Austrel. J. Anim. Sci.* **2008**, *21*, 1214-1219, <https://doi.org/10.5713/ajas.2008.70456>.
- Bertol, T.M.; Ludke, J.V.; Campos, R.M.L.; Kawski, V.L.; Cunha Junior, A.; de Figueiredo, E.A.P. Inclusion of grape pomace in the diet of pigs on pork quality and oxidative stability of omega-3 enriched fat. *Ciê. Rural.* **2017**, *47*, e20150358, <https://doi.org/10.1590/0103-8478cr20150358>.
- Pogorzelska, E.; Godziszewska, J.; Brodowska, M.; Wierzbicka, A. Antioxidant potential of *Haematococcus pluvialis* extract rich in astaxanthin on colour and oxidative stability of raw ground pork meat during refrigerated storage. *Meat Sci.* **2018**, *135*, 54-61, <https://doi.org/10.1016/j.meatsci.2017.09.002>.
- Falowo, A.B.; Fayemi, P.O.; Muchenje, V. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. *Food Res. Int.* **2014**, *64*, 171-181, <https://doi.org/10.1016/j.foodres.2014.06.022>.
- Fomichev, Y.; Nikanova, L.; Lashin, A. The effectiveness of using dihydroquercetin (taxifolin) in animal husbandry, poultry and apiculture for prevention of metabolic disorders, higher antioxidative capacity, better resistance and realisation of a productive potential of organism. *Agric. Food.* **2016**, *4*, 140-159.
- Shikov, V.; Kammerer, D.R.; Mihalev, K.; Mollov, P.; Carle R. Antioxidant capacity and colour stability of texture-improved canned strawberries as affected by the addition of rose (*Rosa damascena* Mill.) petal extracts. *Food Res. Int.* **2012**, *46*, 552-556, <https://doi.org/10.1016/j.foodres.2011.04.004>.
- Bulambaeva, A.A.; Vlahova-Vangelova, D.B.; Dragoev, S.G.; Uzakov, Y.M.; Balev, D.K. Development of new functional cooked sausages by addition of goji berry and pumpkin powder. *Am. J. Food Technol.* **2014**, *9*, 180-189, <https://doi.org/10.3923/ajft.2014.180.189>.
- Artem'eva, O.A.; Pereselkova, D.A.; Fomichev, Yu.P. Dihydroquercetin, the bioactive substance, to be used against pathogenic microorganisms as an alternative to antibiotics. *Agricul. Biol.* **2015**, *50*, 513-519, <https://doi.org/10.15389/agrobiol.2015.4.513eng>.
- Chen, Y.; Deuster, P. Comparison of quercetin and dihydroquercetin: antioxidant-independent actions on erythrocyte and platelet membrane. *Chem.-Biol. Interac.* **2009**, *182*, 7-12, <https://doi.org/10.1016/j.cbi.2009.06.007>.
- Mollov, P.; Mihalev, K.; Shikov, V.; Yoncheva, N.; Karagyozov, V. Colour stability improvement of strawberry beverage by fortification with polyphenolic copigments naturally occurring in rose petals. *Innov. Food Sci. Emer. Technol.* **2007**, *8*, 318-321, <https://doi.org/10.1016/j.ifset.2007.03.004>.



16. Balev, D.; Vlahova-Vangelova, D.; Mihalev, K.; Shikov, V.; Dragoev, S.; Nikolov, V. Application of natural dietary antioxidants in broiler feeds. *Jour. Mn. Agric. Balk.* **2015**, *18*, 224-232.
17. EC. European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes Strasbourg, March 18, 1986. *Eur. Treaty Ser.* **1986**, *123*, 1-11. Available online: <https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/123>.
18. EC. Council Directive 2010/63/EC on the Protection of Animals used for Scientific Purposes. *Off. J. Eur. Union.* **2010**, *L276*, 33-79, Available online: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF>.
19. EC. Council Directive 2008/120/EC laying down Minimum Standards for the Protection of Pigs. *Off. J. Eur. Union.* **2009**, *L47*, 5-13, Available online: <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32008L0120>.
20. EC. Commission Recommendation 2007/526/EC on Guidelines for the Accommodation and Care of Animals used for Experimental and Other Scientific Purposes. *Off. J. Eur. Union.* **2007**, *L197*, 1-89, Available online: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:197:0001:0089:EN:PDF>.
21. EC. Regulation EC No 1099/2009 on the Protection of Animals at the Time of Killing. *Off. J. Eur. Union.* **2009**, *L303*, 1-30, Available online: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:303:0001:0030:EN:PDF>.
22. BG. Bulgarian Veterinary Activity Act. In force since May 2, **2006**. Published in *State Gazette* No. 87 of November 1, **2005**, last amendment *State Gazette* No. 24 of March 22, **2019**, Available online: <https://www.lex.bg/laws/ldoc/2135512300>. (In Bulgarian)
23. BG. Ordinance No 20 of 1 November 2012 for Minimum Requirements for the Protection and Welfare of Test Animals and Requirements to the Objects for Use, Growing and/or their Delivery. In force since January 1, **2013**. Published in *State Gazette* No. 87 of November 9, **2012**, last amendment *State Gazette* No. 9 of February 2, **2016**, Available online: <https://www.lex.bg/bg/laws/ldoc/2135820878>. (In Bulgarian)
24. BG. Ordinances No 21 of 14 December 2005 on Minimum Requirements for the Protection and Welfare of Pigs. In force since May 1, **2006**. Published in *State Gazette* No. 5 of January 17, **2006**, last amendment *State Gazette* No. 64 of August 8, **2006**, Available online: <https://www.lex.bg/bg/laws/ldoc/2135515372>. (In Bulgarian)
25. EC. Regulations EC No 1/2005 on the Protection of Animals during Transport and Related Operations. *Off. J. Eur. Union.* **2005**, *L3*, 1-44, Available online: <https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32005R0001>.
26. BG. Ordinance No 16 of 3 February 2006 for Protection and Human Relationship at Growing and use of Livestock. In force since May 1, **2006**. Published in *State Gazette* No. 18 of February 28, **2006**, last amendment *State Gazette* No. 61 of July 28, **2017**, Available online: <https://www.lex.bg/bg/laws/ldoc/2135517353>. (In Bulgarian)
27. BG. Ordinance No 15 of 8 May **2009**. In force since May 19, 2009. Published in *State Gazette* No. 37 of May 19, **2009**, last amendment *State Gazette* No. 16 of February 22, **2019**, Available online: <https://www.lex.bg/bg/laws/ldoc/2135633001>. (In Bulgarian)
28. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911-917, <https://doi.org/10.1139/o59-099>.
29. Zhang, Q.Y.; Wu, J.F.; Ma, P.H.; Cai, J.; Zhang, Y.T. Acid value determination and pre-esterification of crude *Euphorbia lathyris* L. oil. *World J. Eng. Technol.* **2015**, *3*, 70-75, <https://doi.org/10.4236/wjet.2015.3.2007>.
30. Yi, G.; Haug, A.; Nyquist, N.F.; Egelanddal, B. Hydroperoxide formation in different lean meats. *Food Chem.* **2013**, *141*, 2656-2665. <https://doi.org/10.1016/j.foodchem.2013.05.041>.
31. Botsoglou, N.A.; Fletouris, D.J.; Papageorgiou, G.E.; Vassilopoulos, V.N.; Mantis, A.J.; Trakatellis, A.G. Rapid sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food and feedstuff samples. *J. Agric. Food Chem.* **1994**, *42*, 1931-1937, <https://doi.org/10.1021/jf00045a019>.
32. Young, O.A.; West, J.; Hart, A.L.; van Otterdijk, F.F.H. A method for early determination of meat ultimate pH. *Meat Sci.* **2004**, *66*, 493-498, [https://doi.org/10.1016/S0309-1740\(03\)00140-2](https://doi.org/10.1016/S0309-1740(03)00140-2).
33. AMSA. American Meat Science Association Section VIII: Guidelines, Instrumental Meat Color Measurement. In: *Meat Colour Measurement Guidelines*, 2nd ed.; Hunt M., King A., Eds.; American Meat Science Association, Champaign, IL, USA, 2012; pp. 45-52. Available online: [https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012\\_12\\_meat\\_clr\\_guide.pdf?sfvrsn=d818b8b3\\_0](https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012_12_meat_clr_guide.pdf?sfvrsn=d818b8b3_0).
34. Wood, J.D.; Enser, M.; Fisher, A.V.; Nute, G.R.; Sheard, P.R.; Richardson, R.I.; Hughes, S.I.; Whittington F.M. Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.* **2008**, *78*, 343-358, <https://doi.org/10.1016/j.meatsci.2007.07.019>.
35. Dave, D.; Ghaly, A.E. Meat spoilage mechanisms and preservation techniques: A critical review. *Am. J. Agric. Biol. Sci.* **2011**, *6*, 486-510, <https://doi.org/10.3844/ajabssp.2011.486.510>.
36. Huan, Z.; He, Sh.; Ma, Y. Numerical simulation and analysis for quick-frozen food processing. *J. Food Eng.* **2003**, *60*, 267-273, [https://doi.org/10.1016/S0260-8774\(03\)00047-5](https://doi.org/10.1016/S0260-8774(03)00047-5).
37. James, S.J.; James, Ch. Quality and safety of frozen meat and meat products, Part III. Quality and safety of frozen foods. In: *Handbook of frozen food processing and packaging*, 2<sup>nd</sup> ed.; Sun, D.W. Ed.; CRC Press, Taylor & Francis Group: Boca Raton, London, New York, USA, 2012; pp. 303-324, <https://doi.org/10.1201/b11204>.
38. Zhu, S.M.; LeBail, A.; Chapleau, N.; Ramaswamy, H.S.; de Lamballerie-Anton, M. Pressure shift freezing of pork muscle: Effect on colour, drip loss, texture, and protein stability. *Biotechnol. Prog.* **2004**, *20*, 939-945, <https://doi.org/10.1021/bp034338j>.
39. Buscailhon, S.; Berdagué, J.L.; Gandemer, G.; Touraille, Ch.; Monin, G. Effect of initial pH on compositional changes and sensory traits of French dry-cured hams. *J. Muscle Foods* **1994**, *5*, 257-270, <https://doi.org/10.1111/j.1745-4573.1994.tb00535.x>.
40. Gandemer, G. Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products. *Meat Sci.* **2002**, *62*, 309-321, [https://doi.org/10.1016/s0309-1740\(02\)00128-6](https://doi.org/10.1016/s0309-1740(02)00128-6).
41. Cava, R.; Ruiz, J.; Ventanas, J.; Antequera, T. Oxidative and lipolytic changes during ripening of Iberian hams as affected by feeding regime: extensive feeding and alpha-tocopheryl acetate supplementation. *Meat Sci.* **1999**, *52*, 165-172, [https://doi.org/10.1016/S0309-1740\(98\)00164-8](https://doi.org/10.1016/S0309-1740(98)00164-8).
42. Fernandez, X.; Monin, G.; Talmant, A.; Mourot, J.; Lebret, B. Influence of intramuscular fat content on the quality of pig meat - 2. Consumer acceptability of m. Longissimus lumborum. *Meat Sci.* **1999**, *53*, 67-72, [https://doi.org/10.1016/S0309-1740\(99\)00038-8](https://doi.org/10.1016/S0309-1740(99)00038-8).

43. Mercier, Y.; Gatellier, P.; Renerre, M. Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. *Meat Sci.* **2004**, *66*, 467-473, [https://doi.org/10.1016/S0309-1740\(03\)00135-9](https://doi.org/10.1016/S0309-1740(03)00135-9).
44. Andersen, H.J.; Skibsted, L.H. Oxidative stability of frozen pork patties. Effect of light and added salt. *J. Food Sci.* **1991**, *56*, 1182-1184, <https://doi.org/10.1111/j.1365-2621.1991.tb04729.x>.
45. Amaral, A.B.; Silva, M.; Lannes, S.C. Lipid oxidation in meat: mechanisms and protective factors – a review. *Food Sci. Technol.* **2018**, *38*, 1-15, <https://doi.org/10.1590/fst.32518>.
46. Hasty, J.L.; van Heugten, E.; See, M.T.; Larick, D.K. Effect of vitamin E on improving fresh pork quality in Berkshire-and Hampshire-sired pigs. *J. Anim. Sci.* **2002**, *80*, 3230-3237, <https://doi.org/10.2527/2002.80123230x>.
47. Channon, H.A.; D'Souza, D.N.; Dunshea, F.R. Diet composition and slaughter age up to 24 weeks have minimal impact on pork eating quality of loin steaks and silverside roasts from female pigs. *Meat Sci.* **2018**, *135*, 94-101, <https://doi.org/10.1016/j.meatsci.2017.09.005>.
48. Warriss, P.D. Chapter 10. *Meat Science: An introductory text*, 1st ed.; CABI Publishing, New York, NY, USA, 2000.; pp. 209–228, <https://doi.org/10.4102/jsava.v71i4.731>
49. Leygonie, T.J.; Britz, L.C.; Hoffman, C. Impact of freezing and thawing on the quality of meat: Review. *Meat Sci.* **2012**, *91*, 93-98, <https://doi.org/10.1016/j.meatsci.2012.01.013>.
50. Daszkiewicz, T.; Kubiak, D.; Panfil, A. The effect of long-term frozen storage on the quality of meat (*Longissimus thoracis et lumborum*) from female roe deer (*Capreolus capreolus* L.). *J. Food Qual.* **2018**, *2018*, <https://doi.org/10.1155/2018/4691542>.
51. Neethling, N.E.; Hoffman, L.C.; Britz, T.J.; O'Neill, B. Influence of carbon monoxide on the colour stability of defrosted yellowfin tuna (*Thunnus albacares*) muscle stored under aerobic and anaerobic conditions. *J. Sci. Food Agric.* **2015**, *95*, 1605-1612, <https://doi.org/10.1002/jsfa.6991>.

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