EFFECTS OF MUSTARD-HONEY, APPLE VINEGAR, WHITE WINE VINEGAR AND KEFIR ACIDIC MARINADES ON THE PROPERTIES OF PORK

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Summary. The present research studied the quality of pork and the technological parameters of the *Longissimus thoracis et lumborum* muscle subjected to ageing with white wine vinegar (pH 3.0), apple vinegar (pH 3.1), mustard-honey (pH 3.9) and kefir marinades (pH 4.5) with the marinating time of one, three and seven days. Mustard-honey and kefir marinades retained their initial pH during the ageing period. As compared to the raw meat samples, a considerable drop in the pH value in the samples treated with apple and white wine vinegar marinades ($P < 0.05$) could be observed after three days of treatment. Electroconductivity of the raw marinated meat increased slightly during the ageing process and there was no significant difference between the marinades. After thermal treatment, electroconductivity differed between marinades on a larger scale, but this difference decreased during ageing. The acidity in apple vinegar and white wine vinegar marinades turned raw samples significantly ($P < 0.05$) lighter. However, the cooked samples treated with kefir marinade remained lighter during the seven-day period ($P < 0.05$) and the samples treated with mustard-honey marinade were the darkest only on the seventh day. Raw mustard-honey marinated samples had a lower redness value ($P < 0.05$), but a higher yellowness value, whereas cooking increased the yellowness considerably. Kefir marinade decreased the yellowness of raw samples ($P < 0.05$), but cooking increased this value close to that of white wine and apple vinegar treated samples. The weight loss of kefir treated raw samples was not remarkable during the ageing period (0.27-1.35%), compared to that of other variants (4.25-8.70%). Thermal treatment had a smaller effect on the mustard-honey treated samples (25.43-27.41%), whereas kefir treated samples lost weight almost at the same level as the samples in two other groups. The cooked samples treated with white wine and apple vinegar turned tougher than compared to the other two marinades. The obtained data demonstrated that immersion in kefir and mustard-honey marinade turned samples softer after cooking.
Keywords: pork; acidic marinade; texture analysis; marinating time; pH; colour

Introduction. Value-added meat products have become globally more and more popular and have also an opportunity for future growth (Bord Bia, 2011; Seong et al., 2012; Food Marketing Institute, 2017). Marinating is one of the most common processes used to increase the value of various meat cuts in the meat industry. Value-added meat can be defined as ready-to-cook products that are pre-cut and pre-marinated, whereas the flavour of the marinade depends on ingredients chosen by personal preferences. Adding value to meat products through marinating is a common practice in the food industry (Marel, 2015). Marinades and their ingredients are essential components for value-added meat products as they improve the ultimate characteristics of meat and finally give a better eating experience for the consumers who are seeking new and exciting flavours and easy-to-prepare products.

Most commercial marinades are based on acidic water-oil emulsions, where spices, salt, sugar and other ingredients are added (Yusop et al., 2010). The purpose of the acidic marination is to improve meat tenderness and enrich it with different flavours. Tenderization and flavouring of treated meat are influenced through marinade pH, which causes the swelling of muscle fibres and connective tissue and increases the extractability of myofibrillar proteins (Berge et al., 2001; Aktaş et al., 2003; Bertram et al., 2004). This process weakens muscle structure and ultimately increases the solubilisation of collagens during cooking (Serdaroğlu et al., 2007). Additionally, soaking meat in an acidic solution affects various meat quality-related traits, such as its water holding capacity, juiciness and colour (Hamm, 1986; Medynski et al., 2000).

Some previous works have studied the marination effect of grapefruit juice (Serdaroğlu et al., 2007), garlic and onion (Kim et al. 2010), dry red wine, kefir, lemon juice and raw pineapple juice (Żochowska-Kujawska et al., 2012), Chinese-style commercial (Yusop et al., 2010; Yusop et al., 2012) and soy sauce (Kim et al., 2014) acidic marinades on turkey, pork, venison and chicken meat and found that marinating increased meat tenderness and juiciness. However, researchers expressed some concern about the colour change in some meat cuts, which decreased the attractiveness of the product. The change in colour may be attributed to the increased binding reaction of myoglobin and myofibrillar protein (Kim et al., 2010).

Therefore, the objective of this research was to investigate kefir, mustard-honey, apple vinegar and white wine vinegar effect of the acidic marinades to the raw and cooked pork *Longissimus thoracis et lumborum* muscle properties during the ageing period.

Materials

Raw material. Pork *Longissimus thoracis et lumborum* (LT) muscles were obtained fresh from farm slaughterhouse that is a member of the Estonian Pig Breeding Association. Pig carcasses were cooled down after slaughtering in the cooler house at 4 °C for 12 hours. Totally 8 LT muscles were dissected from randomly selected commercially produced pig carcasses. Samples were inspected visually and any remaining external fat and facias (connective tissues) were physically removed. The weight of trimmed muscles ranged between 2,913 and 3,406 g and their pH between 5.32 and 5.49 (Table 1). Meat samples were vacuum-packaged and cold-stored (4 °C) in the meat laboratory until testing.

Table 1. **Characteristics of *Longissimus thoracis et lumborum* (LT) muscles**

Traits	LT			
	1	2	3	4
Weight, g	2,913	3,141	3,010	3,406
pH	5.37	5.38	5.32	5.49
Electroconductivity, mS/cm	11.14	7.63	5.45	1.95
Minolta lightness L^*	51.85	55.44	51.27	50.47
Minolta redness a^*	5.16	5.15	7.07	4.87
Minolta yellowness b^*	4.44	5.29	4.92	3.44

Marinades. Appropriate marinade formulas had been developed in preliminary trials. All marinade ingredients were bought from the retail store.

The main ingredients of mustard-honey marinade (pH 3.9) were mustard Põltsamaa Strong (Põltsamaa Felix Ltd.) and local honey in the proportion of 30.7 and 36.8 g/100 g, respectively (Table 2). Rapeseed oil Olivia (Scanola Baltic Ltd.) was added (24.6 g/100 g) into the marinade as a liquefying agent. The second marinade with a pH 3.1 consisted of 5% apple vinegar (JAPS M.V.M. Ltd.) 58.4 g/100 g and onion 29.2 g/100 g. For the third marinade white wine vinegar with the concentration of 6% (Vilux SN) was diluted with distilled water to ensure that its acidity was at the same level as in apple vinegar. The proportion of other ingredients used (Table 2), were 52.3 and 10.5 g/100 g, respectively. Despite the dilution, the acidity of this marinade remained the lowest (pH 3.0). The fourth marinade was less acidic (pH 4.5) as it consisted mainly of kefir (77.8 g/100 g) (Valio Estonia Ltd) and onion (15.6 g/100 g).

All marinades were seasoned with salt (3.3-6.1 g/100 g) and black pepper (0.3-0.5 g/100 g). As the sweetness of mustard-honey marinade increased with honey, 3.1-5.8 g/100 g of sugar was added to the other marinades.

Garlic and onions were peeled and ground with Defort DSJ-200 (SBM Group, Austria). All ingredients were weighed with the precision balance KERN EW 4200-2NM (KERN & SOHN GmbH, Germany) and mixed homogeneously.

Table 2. **Marinade pH and ingredient quantities (g/100 g)**

Ingredients	Marinade			
	kefir	mustard-honey	apple vinegar	white wine vinegar
pH	4.5	3.9	3.1	3.0
Mustard (31%)		30.7		
Honey		36.8		
Oil		24.6		
Apple vinegar (5%)			58.4	
White wine vinegar (6%)				52.3
Distilled water				10.5
Kefir (2.5%)	77.8			
Onion‡ / garlic†	15.5‡	2.3†	29.2‡	26.0‡
Salt	3.3	5.2	6.1	5.5
Sugar	3.1		5.8	5.2
Black pepper (grounded)	0.3	0.4	0.5	0.5

Ageing with marinade. Marinades were prepared on the day of the trial and stored at 4 °C until required. 20 g of the marinade was used per 100 g of a sample using immersion method, soaked samples individually in a plastic bag. Pork LT muscles were cut across the muscle grain with a thickness of 3 cm. Bags with samples aged with marinade at 4 °C up to 7 days. The raw and cooked samples were analysed on the first, third and seventh day of ageing with marinade. Control samples were not marinated, and they were tested immediately after other samples were placed into the marinade. Four series of experiments were conducted using fresh meat and samples with four different marinades – mustard-honey, apple vinegar, white wine vinegar and kefir marinade.

Cooking. Alto-Shaam 300 TH-III Halo Heat (Alto-Shaam, Inc., Menomonee Falls, WI, USA) oven was used for the heat treatment of the samples, where samples were cooked with hot air at 140 °C. The samples were placed on a baking tray lined with baking paper and a temperature probe was inserted. Logger Lite 1.8 (Vernier, Beaverton, OR, USA) software was used to ensure that all the samples were cooked to the internal temperature of 75 °C.

Methods

Marinated raw and cooked meat samples were analysed for pH, electroconductivity, colour, weight loss and shear force. The pH-value of heat-treated meat was not determined.

pH measurement. The pH of raw meat samples was determined with a pH-meter Testo 205 (Testo SE & Co, Lenzkirch, Germany) before the marination and that of the marinated samples on the first, third and seventh day of the treatment. The device was calibrated with standard solutions pH 4.0 and 7.0 before the utilization. The probe was cleaned with distilled water and paper tissue after each measurement to avoid deviations. pH was measured from the 24 slices per muscle.

Electroconductivity measurement. LF-Star CPU (Matthäus GmbH & Co. KG, Eckelsheim, Germany) was used to measure the electroconductivity of the meat samples by placing two metal electrodes into the sample and estimating the conductivity (mS/cm) between them. Due to the dry texture of the cooked control samples, measurements were not taken and therefore, 24 slices were analyzed per muscle.

Colour measurement. The surface colour of the marinated raw and cooked meat was estimated using Minolta Chroma Meter CR-400 (Konica Minolta Inc., Tokyo, Japan) set-up with an 11 mm diameter aperture and D65 illuminant which was calibrated against a white surface. Lightness (L^*), redness (a^*) and yellowness (b^*) were determined according to Lab colour model. Measurements were taken from the raw marinated meat and the cooked samples after they had been cooled down to room temperature. 25 slices of the meat samples were used per muscle and for each sample, 10 measurements were taken at different locations.

Marinated samples weight measurement. Meat loses liquid and soluble substances, but it can also absorb marinade ingredients during immersion. All samples were cleaned from excessive marinade with a paper towel and weighed before cooking. The initial weight of the blotted samples was obtained before marinating.

Weight changes were calculated during marination process as follows:

$$z = \frac{(a - b)}{a} \times 100$$

where z - marination loss (g/100 g); a - raw meat weight (g); b - marinated sample weight (g).

Cooking loss measurement. The cooking loss is defined as the loss of liquid and soluble substances of meat during thermal treatment. Samples were blotted before and after cooking with a paper towel and their weights were recorded instantly.

The calculation of the cooking loss was carried out as follows:

$$t = \frac{(c - d)}{c} \times 100$$

where t - cooking loss (g/100 g); c - marinated sample weight (g); d - cooked sample weight (g).

Texture measurement. Muscle shear force (N) was estimated with the TA.XT plus (Stable Micro System Ltd., Surrey, UK) analyser following the Warner-Bratzler methodology (Savell et al., 2013). Core samples were obtained by a drill, which was fitted with an 11 mm diameter tube. Raw samples were toughened by cooling them down to -2 °C and the cooked meat was drilled at room temperature parallel to the muscle fibre. Texture analyser fitted to the crosshead speed of 120 mm/min and load cell of 50 N was used. 26 slices of the meat samples were used per muscle and 8 repeated shear force measurements were made for each sample.

Statistical analysis. Statistical analyses were performed with the statistical package SAS 9.4 (SAS Inc., Chicago, U.S.A.).

Effects of marinade type and marinating time to the samples' weight change, electroconductivity and pH were studied by two-factor repeated measures analysis of variance following the model:

$$Y_{ijkl} = \mu + M_i + A_j + M_i{*}A_j + L_k + \varepsilon_{ijkl},$$

where Y_{ijkl} - dependent variable; μ - model intercept; M_i - marinade effect (mustard-honey, apple vinegar, white wine vinegar and kefir; i = 1-4); A_j - marinating time effect (1st, 3rd and 7th day; j = 1-3); $M_i{*}A_j$ - marinade and marinating time interaction effect; L_k - random effect of muscle considering the relationship between repeated measurements made on the same muscle (k = 1-4); ε_{ijkl} - random error.

Effects of the thermal treatment, marinade type and marinating time on the sample colour and texture parameters were studied by a three-factor repeated measures analysis of variance following the model:

$$Y_{ijklmn} = \mu + T_i + M_j + A_k + T_i{*}M_j + T_i{*}A_k + M_j{*}A_k + T_i{*}M_j{*}A_k + L_l + P_m(L_l) + \varepsilon_{ijklmn},$$

where Y_{ijklmn} - dependent variable; μ - model intercept; T_i - treatment effect (raw / cooked, i = 1, 2); M_j - marinade effect (mustard-honey, apple vinegar, white wine vinegar and kefir; i = 1-4); A_k - marinating time effect (0th, 1st, 3rd ja 7th day; j = 1-4); $T_i{*}M_j$, $T_i{*}A_k$, $M_j{*}A_k$, and $T_i{*}M_j{*}A_k$ - treatment, marinade and marinating time interaction effects; L_l - random effect of muscle considering the relationship between repeated measurements made on the same muscle (l = 1-4); $P_m(L_l)$ - random effect of sample (nested to muscle) considering the relationship between repeated measurements made on the same sample (m = 1-10 and m = 1-8, respectively on colour and texture analysis); ε_{ijklmn} - random error.

Results are presented as least-square means with standard errors (se), and estimated effects and differences were considered statistically significant at $P \leq 0.05$.

Results

Physical properties of raw and cooked marinated meat.

Core pH of raw marinated meat. White wine vinegar and apple vinegar marinades with higher acidity decreased the initial raw meat pH within one day of ageing by 0.35 and 0.41 ($P > 0.05$) (Table 3). The pH of these samples remained at the same level throughout the next two days of ageing. However, considerable absorption of white wine vinegar and apple vinegar marinades took place after the third day of ageing, when the pH of the samples decreased significantly ($P < 0.05$), being 4.89 and 4.80, respectively.

The immersion of meat samples in kefir and mustard-honey marinades had no considerable effect ($P > 0.05$) on sample pH during the ageing period. The differences in marinades were clearly expressed within an ageing period, being influenced by the initial marinade pH.

Table 3. **Least square means and standard errors (se) of the pH value in the core of control and marinated raw meat samples during the ageing period at 4 °C**

Marinade	Marinating period (days)			
	0†	1	3	7
Kefir	5.38^{ABa}	5.39^{Aa}	5.39^{Aa}	5.42^{Aa}
Mustard-honey	5.35^{Aa}	5.35^{Aa}	5.32^{Aa}	5.31^{Ba}
White wine vinegar	5.40^{Ba}	5.04^{Bb}	5.06^{Bb}	4.89^{Cc}
Apple vinegar	5.41^{Ba}	4.98^{Bb}	4.99^{Bb}	4.80^{Dc}
se	0.04	0.05	0.05	0.05

† Control samples were not marinated, and they were tested immediately before the samples were placed into the marinade. Different capital letters in columns and lowercase letters in rows indicate a significant difference of at least 0.05.

Electroconductivity.

Raw samples. Although the electroconductivity of the samples in the control group varied from 6.20 to 7.04 mS/cm, there was no significant (P > 0.05) difference between the raw meat samples (Table 4). Differences between the raw marinated samples were the largest on the third day, but their treatment did not have a considerable effect on electroconductivity. During the treatment electroconductivity increased in all marinade types, showing significant differences only in raw mustard-honey samples on the first and seventh day. The reason for the increase in electroconductivity was presumably the swelling of meat and the denaturation of muscle protein, which activated marinade absorption and increased the moisture content of the muscle. The highest increase in electroconductivity was found in mustard-honey and apple vinegar samples during the first three days (3.62 and 3.88 mS/cm, respectively).

Cooked samples. Heat treatment slightly decreased the electroconductivity in samples marinated with mustard-honey and kefir during the ageing period (P > 0.05), whereas the electroconductivity of white wine and apple vinegar treated samples increased, although on a smaller scale (Table 4). The highest value of electroconductivity was determined for mustard-honey samples at the first day of ageing (9.50 mS/cm). The only significant difference (P < 0.05) between the samples treated with mustard-honey and white wine vinegar marinades was recorded on the first day of ageing.

Table 4. **Least square means and standard errors (se) of electroconductivity (mS/cm) in control and meat samples aged with marinade**

Marinade	Raw marinated				Cooked marinated ‡		
	Marinating period (days)				Marinating period (days)		
	0†	1	3	7	1	3	7
Kefir	7.04^{Aa}	9.53^{Ab}	10.50^{Ab}	11.50^{Ab}	7.78^{ABa}	6.03^{Aa}	7.33^{Aa}
Mustard-honey	6.53^{Aa}	10.15^{Ab}	11.73^{Abc}	12.34^{Ac}	9.50^{Aa}	8.55^{Aa}	8.65^{Aa}
White wine vinegar	6.20^{Aa}	10.35^{Ab}	10.88^{Ab}	12.15^{Ab}	6.28^{Ba}	6.95^{Aa}	6.60^{Aa}
Apple vinegar	6.40^{Aa}	10.28^{Ab}	11.80^{Ab}	12.15^{Ab}	6.85^{ABa}	7.58^{Aa}	7.30^{Aa}
se	1.07	1.24	1.24	1.24	1.47	1.47	1.47

† Control samples were not marinated, and they were tested immediately before the samples were placed into the marinade. Different capital letters in columns and lowercase letters in rows indicate a significant difference of at least 0.05. ‡ Due to the dry texture of the cooked control samples, measurements were not taken.

*Minolta lightness L**

Raw samples. The lightness L^* value of the untreated raw meat samples ranged from 51.09 to 52.66 (P < 0.05). The results in Table 5 indicate the influence of the marinade pH on meat colour. Due to a higher level of denaturation of the pigment-protein myoglobin, both vinegar marinades with the lowest pH values (3.0 and 3.1) changed the samples significantly lighter than compared to the control samples (P < 0.05). These samples retained their lightness L^* within the seven-day ageing period (P > 0.05). The lightness L^* of the mustard-honey and kefir marinated samples with a higher pH (3.9 and 4.5, respectively) increased less in comparison with the control group. Further treatment with these marinades increased the muscle L^* value significantly up to the third day of ageing (P < 0.05), after that, the change was not considerable.

Cooked samples. Thermal treatment increased the lightness L^* value of the samples aged with kefir marinade as compared with the control group, being significantly higher on the seventh day of ageing (P < 0.05). Contrariwise, the lightness L^* value decreased when the samples were treated with mustard-honey marinade, being the lowest also on the seventh day of ageing (P < 0.05). Although, white wine vinegar and apple vinegar turned the meat lighter, cooking these samples did not alter the lightness L^* significantly (P > 0.05) during the ageing with marinade. The difference in lightness L^* between the samples treated with kefir and mustard-honey marinade was significantly different within the marinating period (P < 0.05), being the highest on the seventh day.

Table 5. **Least square means and standard errors (se) of lightness *L** in the control and meat samples aged with marinade**

Marinade	Raw marinated				Cooked marinated			
	Marinating period (days)				Marinating period (days)			
	0†	1	3	7	0†	1	3	7
Kefir	51.09^{Aa}	55.58^{Ab}	57.40^{Abc}	58.14^{Ac}	73.26^{a}	75.42^{Aab}	74.03^{Aab}	75.89^{Ab}
Mustard-honey	52.66^{Ba}	53.34^{Bac}	56.71^{Ab}	54.88^{Bbc}	73.26^{a}	69.98^{Bab}	69.60^{Bab}	67.31^{Bb}
White wine vinegar	52.21^{ABa}	69.00^{Cb}	67.60^{Bb}	67.70^{Cb}	73.26^{a}	71.66^{Ba}	70.85^{Ba}	70.45^{Ca}
Apple vinegar	51.66^{ABa}	70.15^{Cb}	68.67^{Bb}	68.64^{Cb}	73.26^{a}	71.12^{Ba}	71.27^{ABa}	70.49^{Ca}
se	0.88	1.10	1.07	1.07	1.69	1.3	1.3	1.3

† Control samples were not marinated. Different capital letters in columns and lowercase letters in rows indicate a significant difference of at least 0.05.

*Minolta redness a**

Raw samples. The redness *a** of the untreated raw meat varied from 4.99 to 5.92, which decreased significantly after the samples were processed with marinade (Table 6). Redness *a** was influenced by the composition of the marinade, where the redness *a** value of the mustard-honey marinade was the lowest during the ageing period ($P < 0.05$). The redness *a** value of the samples treated with apple vinegar marinade was slightly lower compared to the two remaining group of samples.

Cooked samples. The thermal treatment of cooked marinated samples decreased the redness *a** as compared with the control group, but the differences between marinades were not expressed during the marinating period, except in samples treated with kefir on the seventh day of ageing ($P < 0.05$).

Table 6. **Least square means and standard errors (se) of the redness *a** in the control and meat samples aged with marinade**

Marinade	Raw marinated				Cooked marinated			
	Marinating period (days)				Marinating period (days)			
	0†	1	3	7	0†	1	3	7
Kefir	5.53^{Aa}	2.59^{Ab}	2.31^{Ab}	2.22^{Ab}	4.14^{a}	2.22^{Ab}	2.25^{Ab}	1.64^{Ab}
Mustard-honey	5.74^{Aa}	1.13^{Bb}	0.94^{Bb}	1.26^{Bb}	4.14^{a}	2.32^{Ab}	2.11^{Ab}	2.33^{ABb}
White wine vinegar	5.92^{Aa}	2.13^{ACb}	2.33^{Ab}	2.38^{Ab}	4.14^{a}	2.95^{Aab}	2.60^{Ab}	2.63^{Bb}
Apple vinegar	4.99^{Ba}	1.56^{BCb}	1.68^{Ab}	1.84^{ABb}	4.14^{a}	2.96^{Aab}	2.47^{Ab}	2.46^{ABb}
se	0.58	0.40	0.39	0.39	0.33	0.46	0.46	0.46

† Control samples were not marinated. Different capital letters in columns and lowercase letters in rows indicate a significant difference of at least 0.05.

*Minolta yellowness b**

Raw samples. The treatment of raw meat with mustard-honey, apple vinegar and white wine vinegar marinades increased the yellowness *b** value of the samples (Table 7). In case of samples aged with the mustard-honey marinade the yellowness *b** value increased significantly up to the third day, whereas in apple vinegar and white wine vinegar samples, the *b** value increased during the whole period of ageing. The samples treated with kefir marinade, retained their yellowness *b** value on the first day of ageing, after which the value increased from 4.17 to 5.54 ($P < 0.05$). Further ageing of the samples in the kefir marinade raised the yellowness *b** value (6.47).

Cooked samples. Contrary to the raw samples, the yellowness *b** value of the meat treated with apple vinegar and white wine vinegar marinade did not change significantly within the ageing period. However, the yellowness *b** value of the mustard-honey samples increased significantly as compared to the control group ($P < 0.05$) and remained at about the same level during the ageing period.

Table 7. **Least square means and standard errors (se) of the yellowness *b** in the control and meat samples aged with marinade**

Marinade	Raw marinated				Cooked marinated			
	Marinating period (days)				Marinating period (days)			
	0†	1	3	7	0†	1	3	7
Kefir	4.20^{ACa}	4.17^{Aa}	5.54^{Ac}	6.47^{Ac}	11.82^{a}	11.60^{Aa}	11.53^{Aa}	11.13^{Aa}
Mustard-honey	4.62^{ABa}	10.30^{Bb}	11.93^{Bc}	11.68^{Bc}	11.82^{a}	16.41^{Bb}	17.33^{Bb}	17.03^{Bb}
White wine vinegar	4.80^{Ba}	8.88^{Cb}	9.40^{Cbc}	10.16^{Cc}	11.82^{a}	12.10^{Aa}	12.22^{Aa}	12.39^{ACa}
Apple vinegar	3.95^{Ca}	9.12^{Cb}	9.72^{Cbc}	10.37^{Cc}	11.82^{a}	12.48^{Aa}	12.36^{Aa}	12.77^{Ca}
se	0.25	0.41	0.38	0.38	0.73	0.52	0.52	0.52

† Control samples were not marinated. Different capital letters in columns and lowercase letters in rows indicate a significant difference of at least 0.05.

Weight loss

Marination loss. Within the seven days of ageing, the meat immersed in the marinades with the lowest pH values (white wine vinegar and apple vinegar) lost 7.15 and 8.70 g/100 g, respectively (Table 8). However, the samples treated with mustard-honey marinade lost most of the weight (6.02 g/100 g) during the first three days, whereas further weight loss proved to be smaller. The samples treated with kefir marinade are at variance with the others as they lost the least weight, most of it on the first day of ageing (1.35 g/100 g).

Cooking loss. The thermal treatment of the samples marinated with different marinades had no significant ($P > 0.05$) effect on the weight loss within the ageing period. However, some variation between the marinades could be observed. The cooking loss of the samples treated with mustard-honey was significantly lower throughout the whole ageing period (25.43-27.41 g/100 g), compared to the samples soaked in other marinades. The highest cooking loss was registered for the samples marinated in white wine vinegar on the seventh day of ageing (39.07 g/100 g).

Table 8. **Least square means and standard errors (se) of the weight losses (g/100 g) of meat samples aged with marinade**

Marinade	Marinating period (days)					
	1	3	7	1	3	7
	Marination loss			Cooking loss		
Kefir	1.35^{Aa}	0.27^{Aa}	0.50^{Aa}	33.57^{Aa}	34.24^{Aa}	32.58^{Aa}
Mustard-honey	4.67^{Ba}	6.02^{Bab}	6.56^{Bb}	27.41^{Ba}	25.43^{Ba}	26.62^{Ba}
White wine vinegar	4.25^{Ba}	5.52^{Ba}	7.15^{Bb}	36.77^{Aa}	37.59^{Aa}	39.07^{Ca}
Apple vinegar	5.25^{Ba}	6.31^{Ba}	8.70^{Cb}	35.72^{Aa}	38.15^{Aa}	34.06^{Aa}
se	0.74	0.74	0.74	1.70	1.70	1.70
Different capital letters in columns and lowercase letters in rows indicate a significant difference of at least 0.05.						

Shear force

Raw samples. Marinating had no remarkable effect on the shear force of the meat within the ageing period (Table 9). Initially, on the first and third day of ageing, least force was used to shear the samples treated with kefir marinade, but by the seventh day, the differences between marinades had levelled off.

Cooked samples. The samples marinated in increased acidity turned out tougher after cooking. The shear force of the samples treated with apple vinegar and white wine vinegar marinades increased until the third day of ageing. The toughening effect was the greatest for apple vinegar samples. However, the samples marinated in white wine vinegar achieved the same level on the seventh day of ageing. Contrary to the marinades with low pH, both the kefir and mustard honey marinades turned meat samples slightly softer after the seven days of ageing ($P > 0.05$).

Table 9. **Least square means and standard errors (se) of the shear force in the control and marinated meat samples during the ageing period**

Marinade	Raw marinated				Cooked marinated			
	Marinating period (days)				Marinating period (days)			
	0†	1	3	7	0†	1	3	7
Kefir	13.15^{a}	13.80^{Aa}	14.87^{Aa}	17.38^{Aa}	29.99^{a}	30.75^{ABa}	29.75^{ABa}	27.75^{Aa}
Mustard-honey	13.15^{a}	18.79^{BCb}	16.84^{Aab}	16.52^{Aab}	29.99^{a}	28.30^{Aa}	28.57^{Aa}	28.07^{Aa}
White wine vinegar	13.15^{a}	16.05^{ACab}	19.08^{Ab}	19.61^{Aab}	29.99^{a}	31.82^{ABab}	34.79^{BCab}	36.59^{Bb}
Apple vinegar	13.15^{a}	18.12^{ACa}	18.43^{Aa}	18.67^{Aa}	29.99^{a}	34.80^{Bab}	38.39^{Cb}	37.57^{Bb}
se	2.23	2.23	2.23	2.23	2.25	2.23	2.23	2.23
† Control samples were not marinated. Different capital letters in columns and lowercase letters in rows indicate a significant difference of at least 0.05.								

Discussion

Meat pH is possibly the most important factor affecting the colour of fresh and cooked meat (AMSA, 2012). Marinades with the lowest pH affected greatly meat samples core ultimate pH value during storage at 4 °C. Kim et al. (2010) showed that treating pork samples with garlic and onion juice marinade decreases pH of the meat within the first three days of ageing ($P < 0.05$), after which the pH of the samples remained stable. A similar result was also obtained by Serdaroğlu et al. (2007), who demonstrated that increased citric acid concentration decreases turkey breast pH during a 24-hour storage. Yousop et al. (2010), on the contrary, found that marinade uptake in chicken breast fillets was greater at higher marinade pH levels (≥3.8), leaving core pH unchanged.

Bendall and Wismer-Pedersen (1962) showed that muscle protein denaturation during storage takes place if the pH values of pork decrease step by step. The immersion of the meat in acidic marinade caused the absorption of marinade between the muscle fibres, which induced the swelling of muscle fibres and accelerated the proteolytic weakening of muscle structure (Berge et al., 2001; Aktaş et al., 2003; Bertram et al., 2004).

Aguilera and Stanley (1999) brought out that the negative aspect related to the immersion of meat in the acidic solution is dewatering of the product, making it tougher. On the other hand, low pH can increase product life by reducing microbial development (Pathania et al., 2010) and with the effective antioxidant activity of garlic and onion help to preserve meat even longer (Kim et al., 2010).

Compared to the non-marinated control group, the colour parameters of raw meat samples aged with marinade were mainly affected by the ingredients of the marinade and marinade pH, as the notable colour changing the effect of the marinades appeared already on the first day of ageing, but not later. However, these findings do not correspond entirely to the Kim et al. (2010) results, who established that acidic marinades can significantly increase lightness, redness and yellowness of the pork during a seven-day storage, but no effect on redness was not found related to pH concentration of the treatments. The difference in findings can be explained that only ingredients Kim et al. (2010) used in their study were onion and garlic.

The current study showed that raw samples treated with low pH marinades turned out significantly lighter compared to the kefir and mustard-honey treated samples. A significant change in pork lightness was found also by Kim et al. (2010), where muscles turned lighter within 7 days, marinated in onion and garlic juice. Aktaş and Kaya (2001) immersed beef steaks in citric acid (0.5%) and that resulted also paler. Lower pH intensifies the denaturation of muscle proteins and increases light reflection. Wismer-Pedersen (1959) showed that variations in muscle structure may also affect light reflectance and that the extent of the denaturation of muscle proteins differs in normal and in a pale colour meat (Kim et al., 2010).

The redness of the meat samples decreased instantly after raw samples were treated with marinade, contrary, to the yellowness of the raw meat samples, which increased. Both colour parameters were probably affected by the ingredients colour of the marinade. Staining effect of the marinade was observed also by Kim et al. (2014), who confirmed that the colour parameters of the raw chicken breasts may be affected due to staining effect of the marinade.

Thermal treatment not affected marinated meat colour parameters at the same level as in raw marinated meat. Lightness, redness and yellowness obtained from the ingredients of the marinades not affected cooked meat colour during ageing with marinade.

Marinade pH had no notable effect on the lightness, redness and yellowness of the cooked meat samples. Yusop et al. (2010) showed that lower marinade pH produces significantly lighter chicken fillets. Marinade with lower pH produced lighter muscles also in Hinkle (2010) research with beef cuts. However, Serdaroğlu et al. (2007) did not find a clear relationship between the lightness of cooked turkey breast and the marinade pH. Yusop et al. (2010) and Serdaroğlu et al. (2007) concluded that marinade pH did not affect cooked poultry meat redness and yellowness.

The colour differences between different marinades were not as obvious as in raw marinated meat samples. However, a minor decrease was found in redness of the cooked marinated meat samples compared to the control group. Contrary, Yusop et al. (2010) asserted that the redness of cooked chicken breast fillets increased during the ageing period. Kim et al. (2014) concluded that brown colour of the soy sauce reduce lightness and increase redness and yellowness as result of staining of the cooked chicken breasts. Authors explained this effect with Maillard reaction, which affects the colour of the marinated meat. An effect was not noticed in the current study, except in the cooked samples immersed with mustard-honey marinade, where yellowness increased significantly. As honey is a well-known source of the sugars, which is one of the key ingredients of the Maillard reaction, then the intensification in yellowness occurred in cooked samples treated with mustard-honey marinade.

Several studies have reported marinade absorption during the immersion of beef (Önenç, et al., 2004) and poultry meat (Serdaroğlu et al., 2007; Yusop et al., 2010; Yusop et al., 2012; Kim et al., 2014), while a loss in weight of the marinated pork samples was observed in the current study. As reported by Aktaş et al. (2003) acid marinades increase water binding properties of muscle proteins by moving pH value away from the isoelectric area. Our study did not confirm this theory as the presence of salt in marinades might have reduced the effect. The meat samples aged with kefir marinade with the lowest salt content (3.3 g/100), almost retained its initial weight during the immersion process. However, other marinades with a higher salt content (5.2, 5.5 and 6.1 g/100 g) lost more weight. Also, marinade immersion process into the muscle may require sufficient time to initiate intramuscular changes.

Meat samples aged with mustard-honey marinade differentiated from others, when thermal treatment was used. The lowest cooking loss was caused probably due to caramelization of the sugars in mustard-honey marinade by covering the surface of the meat with the coating which improved moisture retention. This theory confirms also higher electroconductivity of the cooked pork aged with mustard-honey marinade. Despite added sugar, other marinades had no such effect.

Contrary to the expectations that acidic marinades weaken the structure of meat due to swelling, the increased involvement of cathepsins in proteolysis and the conversion collagen into gelatine when cooking meat at low pH (Berge et al., 2001), the current study showed an increased shear force of cooked samples immersed in marinades with low pH. However, excessive concentrations of acetic acid and other acidic components in marinade make the meat dry (Maailma toiduainete …, 2006). Santos et al. (2012) concluded also that marinating at high pH improve also sensory properties of the pork. Aktaş and Kaya (2001) was not found a correlation between the penetrometer values and citric acid concentration either. They also stated that, in order to achieve a tenderizing effect, long immersion time and longer absorption of acid solutions are necessary.

Dairy products, including kefir, contain calcium, which activates calpain enzymes in meat and may decrease shear force (Marques et al., 2010) of the cooked meat, as was observed in the current study. Lawrence et al. (2003, 2004) studied the effect of calcium solutions on beef and found increased its tenderness. Żochowska-Kujawska et al. (2012) aged wild boar meat with kefir addition within four days and reported a similar effect. A comparable effect to the tenderness was found also when the meat samples were treated with mustard honey marinade. Honey contains enzymes like proteases, which can be as meat tenderizing agent during the ageing process (Rossano et al., 2012). Meat tenderness may also be affected by the optimal pH in the range of 3.5-5.0 for cathepsin enzymes activity (Burke and Monahan, 2003). Burke and Monahan (2003) speculated that a decrease in meat pH in an acid marinade may boost proteolytic attack by these enzymes, but the current study could not confirm that the shear force of the meat samples immersed in marinades with low pH (3.0 and 3.1) increased.

Conclusion. The study showed that low pH white wine vinegar and apple vinegar marinades (≤3.1) led to a decrease in the meat pH value of immersed pork slices. The low pH in marinades made the raw marinated meat turn paler, but did not affect pork redness and yellowness. Still, related to the decreased redness and increased yellowness of pork, a staining effect was observed. Higher salt concentration in marinades is a possible reason for the increased marination loss under acidic conditions. Ageing with marinades for up to seven days had a notable effect on the pH, electroconductivity and marination loss of pork only.

The colour of the cooked product was mainly affected by the Maillard reaction, but compared to non-marinated pork, a decrease in redness was observed. Another reaction - caramelization - increased the yellowness of cooked pork aged with mustard-honey marinade. This process also facilitated the retention of water inside mustard-honey threated pork, which made it juicier. Our results suggest that kefir and mustard-honey marination within the ageing period may decrease the shear force of cooked pork and this effect may be associated with the presence of enzymes in the ingredients.

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Received 27 February 2018
Accepted 12 April 2018