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Effect of acid whey as starter culture on selected physicochemical properties of fermented pork sausage

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ABSTRACT

The aim of the study was to evaluate the effect of acid whey as a starter culture on selected physicochemical properties of fermented pork sausage after ripening (21 days). Four variants of the product have been produced. The first variant (PKS) - cured sausage (2.8%) with starter cultures. Second variant (SSK) – salted sausage (2.8%) with acid whey (5%). Third variant (SKS) – salted sausage (2.8%) with starter cultures. In the fourth variant (PSK), sea salt (2.8%) and acid whey (5%) were used. The research included determination of pH value, water activity, TBARS indicator, analysis of total content of haem pigments and heme iron, determination of color parameters by CIE L * a * b * system and analysis of sausage texture parameters (TPA). The pH values of sausages with acid whey were significantly lower compared to fermented sausages with starter cultures. The highest TBARS index was observed in the SSK (0.612 mg MDA / kg), and the lowest in the PKS sample (0.118 mg MDA / kg). The highest overall content of heme dyes and heme iron was recorded in the PSK test. A higher share of redness was observed in the general tone of color in cured sausages (PKS, PSK) by about 1-2 units compared to salted samples (SSK, SKS). In case of texture, significantly higher values of the hardness parameter were observed in the PSK test as compared to the remaining test samples. Based on the tests, it has been found that it is possible to use acid whey as a starter culture in fermented pork sausage. However, the best results were obtained in the curing sample with acid whey (PSK).

Keywords: acid whey, starter culture, fermented sausage, pork, physicochemical properties

1. INTRODUCTION

Meat fermentation is one of the oldest method of food preservation, known in antiquity (the earliest records from 1500 BC). Fermented sausages are the most popular products in the Mediterranean countries (Italy, Portugal and Spain). This type of products characterize long shelf life and specific flavour and taste. Fermented meat products are produced from whole muscle or from chopped parts of meat (pork, beef) with salt or curing agents additions.

The specificity of products depends on bacterial culture applications. Biochemical and physical changes of fermented meat products, such as degradation of lipids, nitrate reduction, acidification or nitroso-myoglobin formation caused by microbial enzymatic activities. Starter cultures are selected microorganisms (bacteria, moulds, yeasts) added to food to improve its appearance, smell, taste or to prolong the shelf life of product (Holzapfel 2002). The most commonly used starters in meat industry are Lactic Acid Bacteria (LAB) and Coagulase Negative Staphylococci.

Probiotic starter also found application, such as *Lactobacillus casei* (maximum of free amino acids generation and myofibrillar degradation).

Other used bacteria are: *Lb. sakei*, *Lb. plantarum* (improving sensorial characteristics and microbial safety), *S. xylosus* and *S. carnosus* (ability to reduce nitrate) or even yeasts *Penicillium aurantiogriseum* (improve sensory characteristic), *P. chrysogenum* (lower hardness, higher intensity of aroma).

This type of bacteria have positive effect not only on a product but also on a human health. Lactic Acid Bacteria have the ability to reducing the risk of tumours, amount of LDL cholesterol fraction and can also balancing the intestinal flora and stimulation immune system (Ojha et al. 2015, Li et al. 2012).

Acid whey is a yellow-green liquid by-product generated during the production of cottage cheese and has a very high biological value, exceeds that from egg (considered as a reference standard). Whey contains high amounts of B-group vitamins (especially B₂ vitamin) and vitamin A and significant amounts of tryptophan (serotonin precursor) and cysteine (glutathione precursor). Acid whey contains peptides and proteins, among which the most important are α -lactalbumin and β -lactoglobulin, representing 75% of all whey proteins. Except their pro-health properties e.g. prevention of muscular atrophy, anti-carcinogenic effect and increasing bone resistance to fractures, immune-enhancing properties (Smithers 2008, Majewska et al. 2009) acid whey proteins have also among others strong antimicrobial and antioxidant effects (Chatterton et al. 2006; Tong et al. 2000).

Acid whey can be obtained from not only a cow's milk, but from the any type of milk such as goats milk or camels milk (Smithers 2008).

Rzepakowska et al. (2017) pointed out that organic acid whey could be a good source of new LAB strains a new potential starter culture for food industry. They proved that organic whey comprises a large number of microorganisms and a great variety of microbial group, especially LAB (*Lb. plantarum* and *L. fermentum* species) (Rzepakowska et al. 2017).

Previous research with acid whey addition has shown positive effects on sensory properties, microbiological quality and psychochemical quality parameters of fermented pork or beef products (Wójciak et al. 2015a, Wójciak et al. 2015b).

The aim of this study was to check the possibility of using acid whey as a new starter culture for fermented pork sausage production with comparison to other commonly used in meat industry starters.

2. MATERIALS AND METHODS

2. 1. Preparation of acid whey

Acid whey was obtained from a local organic farm breeding dairy cattle breeding and a producer of organic dairy products.

2. 2. Starter cultures

BESSASTART starter cultures were used - starter cultures for controlled acceleration of raw sausage maturation. The preparation included the following bacterial strains: *Staphylococcus xylosum*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*.

2. 3. Manufacturing of sausage

Pork meat from Wielka Biała Polska bred in the organic system was pre-minced meat. The meat was salted (sample SSK and SKS) or cured (sample PKS and PSK) and chilled at 4 °C for 24h. After this time, pork backfat and meat were minced on a wolf (ø 10 mm) and other ingredients were added: starter cultures, acid whey 5%, glucose 0,6%, water 5%. Four samples were prepared (Table 1): sample with curing mixtures and starter cultures (PKS), sample with sea salt and acid whey (SSK), sample with sea salt and starter cultures (SKS), and finally sample with curing mixtures and acid whey (PSK).

Samples have been mixed up and afterwards this were stuffed into fibrous casings (ø 58 mm) and matured for 21 days at 16 °C and 75-80% relative humidity. Next, matured sausages were vacuum-sealed and stored at 4 °C. The tests were carried out immediately after production.

Table 1. Variants of fermented sausages.

PROBE	SEA SALT (%)	CURING MIXTURE (%)	ACID WHEY (%)	GLUCOSE (%)	WATER (%)
PKS	-	2.8	-	0.6	5
SSK	2.8	-	5	0.6	-
SKS	2.8	-	-	0.6	5
PSK	-	2.8	5	0.6	-

2. 4. pH determination

pH value was measured according to PN-ISO 2917:2001^[22]. The samples had been homogenized in distilled water by the IKA ULTRA-TURRAX T25 Basic homogenizer (Staufen, Germany) and pH was measured using a pH meter CPC-501 (Elmetron, Zabrze, Germany).

2. 5. Water activity

Water activity was measured according to user's manual for the device – water activity analyser *LabMaster* – a_w at 20 °C.

2. 6. ORP

ORP – oxidation-reduction potential was measured according to the method described by Nam and Ahn (2003) (Nam and Ahn, 2003). The value was determined by pH meter set, equipped with redox electrode and set of the millivolt scale.

2. 7. TBARS value

TBARS value was measured according to the method described by Pikul et al. (1989) (Pikul et al. 1989) for measuring TBA-reactive substances. The values were expressed as mg of malondialdehyde per kilogram of sample [mg MDA/kg].

2. 8. Total haem pigments, haem iron

Total haem pigments, haem iron were measured according to the method described by Hornsey (1965) (Hornsey 1965). The amount of total haem pigments [mg/kg] was calculated by multiplying 640 nm (the absorbance) by 680. Haem iron amount [mg/kg] was calculated by multiplying the amount of total haem pigments by 8.82 and dividing the result by 100.

2. 9. Colour measurement

Instrumental colour measurement was measured according to the method described by Michałowski (1995) (Michałowski 1995) in CIE L^* , a^* , b^* system (L^* - lightness/darkness, a^* - redness/greenness, b^* - yellowness/blues, c^* - chroma and H° – hue angle) with an X-Rite Color® Premiere 8.200 colorimeter (X-Rite Incorporated, Michigan, USA). The instrumental conditions were: a 10° standard observer, illuminant D65 and an 8 mm port size.

2. 10. Texture parameters

Samples were deformed twice between two parallel surfaces of the endurance apparatus Instron 4302. Texture parameters were measured according to the method described by Bourne (2002) – hardness, cohesiveness and gumminess.

2. 11. Statistical analysis

The obtained results from two independent studies in three replications were analysed by the KYplot 5.0 statistical program. The significance of the differences between mean values were calculated at a significant level of $p < 0.05$ using the T-Tukey's range test.

3. RESULTS AND DISCUSSIONS:

3. 1. Determination of pH-value, water activity (a_w), ORP and TBARS value

The effect of physicochemical characteristics on fermented sausages with bacteria culture and acid whey were shown in Table 2. The addition of acid whey significantly changed the pH

value of fermented sausage. pH value of sample with curing agent and acid whey addition was significantly ($p < 0.05$) lower than compared to other samples (4.98). The analysis of the water activity (a_w) revealed, that the highest a_w value was observed in the sample with curing agent and starter cultures (0.912) and the lowest for sample with curing agent and acid whey (0.883). The curing agent with acid whey addition significantly reduced the a_w and pH values of sausage. This could be probably a result of LAB presence in acid whey. Lactic Acid Bacteria, such as *L. plantarum* produced (from carbohydrates) lactic acid, which lowers pH value of meat products (Wójciak et al. 2015a, Kaban and Kaya 2009). In both samples with acid whey addition, values of these parameters were lower compared to samples with starter culture. This may be an effect of low acidifying capability culture starter presents such as *S. xylosus* (Chawla et al. 2009). It is also possible, that temperature during maturing process was too low for full growth of starter culture. Rzepkowska et al. (2017) proved that 25 individual LAB strains form organic acid whey had the ability to produce β -galactosidase, the enzyme responsible for the hydrolysis of lactose to glucose and galactose monosaccharides. The ability of bacteria to decompose disaccharides contributes to health benefits, proper flavour and safety of fermented meat. The optimal temperature for *S. xylosus* is at 20-30 °C (Rzepkowska 2017 et al., Essid et al. 2007).

In context of the ORP value, it can be stated that significant ($p < 0.05$) difference occurred between samples containing starter cultures and acid whey. Samples containing acid whey were characterized by higher values of oxidation-reduction potential than other samples. Especially, the sample with sea salt and acid whey had higher (101.53) ORP value compared to sample with curing agent and acid whey.

Table 2. pH, water activity, ORP [mV] and TBARS [mg/kg] values of fermented sausages (means \pm standard deviations)

Samples \ Parameters	PKS	SSK	SKS	PSK
pH	5.66 \pm 0.11 ^A	5.3 \pm 0.15 ^B	5.69 \pm 0.09 ^A	4.98 \pm 0.03 ^C
a_w	0.912 \pm 0.01 ^A	0.891 \pm 0.00 ^B	0.895 \pm 0.00 ^B	0.883 \pm 0.01 ^B
ORP [mV]	62.52 \pm 15.34 ^A	101.53 \pm 23.43 ^B	63.77 \pm 6.75 ^A	97.88 \pm 3.16 ^B
TBARS [mg MDA/kg]	0.118 \pm 0.01 ^A	0.612 \pm 0.33 ^B	0.230 \pm 0.03 ^A	0.231 \pm 0.40 ^A

Sample: PKS – curing agent and starter cultures, SSK – sea salt and acid whey, SKS – sea salt and starter cultures, PSK – curing agent and acid whey. Means with different superscript letters are significantly different ($p < 0.05$). Means \pm standard deviation.

Sample containing sea salt and acid whey were characterized by significantly ($p < 0.05$) higher TBARS value compared to other samples. TBARS parameter was used to evaluate the extent of secondary oxidation substances which reacted with thiobarbituric acid which, in turn determine the content of malondialdehyde (MDA – by-products of lipid oxidation). Lipid oxidation is unfavourable process contributes to, in effect of oxidation unsaturated fatty acids, deterioration of smell, flavour and toxic compounds generation which may be harmful for

consumers. In order to delay the oxidation process, strong antioxidants such as: alpha-tocopherol, lycopene or rosemary are added. The increase of TBARS value could have the effect on formation of peroxides (Ferioli et al. 2008). Research indicates antioxidant properties of acid whey proteins (Chawla et al. 2009, Wen-qiong et al. 2013, Madureira et al. 2007). Although, present study shows that acid whey combined with sea salt demonstrated low antioxidant ability in TBARS parameter context. A similar statement appeared in the Lee et al. (2006) study. Pork sausage with salt addition had higher level of hydroperoxides – may have been an accelerating oxidation factor (Lee et al. 2006).

3. 2. Determination of total haem pigments and haem iron

There were no significant ($p < 0.05$) differences of total haem pigment content and haem iron values between fermented sausage samples. Total haem pigment value was between 69.02 and 89.76. Haem iron value was between 6.09 and 7.92.

Table 3. Total haem pigments and haem iron content of fermented sausages (means \pm standard deviations)

Samples	Parameters	Total haem pigments [mg/kg]	Haem iron [mg/kg]
PKS		84.32 \pm 0.96 ^A	7.44 \pm 0.08 ^A
SSK		85.00 \pm 28.85 ^A	7.50 \pm 2.55 ^A
SKS		69.02 \pm 2.40 ^A	6.09 \pm 0.21 ^A
PSK		89.76 \pm 2.88 ^A	7.92 \pm 0.25 ^A

Sample: PKS – curing agent and starter cultures, SSK – sea salt and acid whey, SKS – sea salt and starter cultures, PSK – curing agent and acid whey. Means with different superscript letters are significantly different ($p < 0.05$). Means \pm standard deviation.

3. 3. Colour measurement

For L* value significant ($p < 0.05$) differences between samples were noted. The highest lightness was noted for sample containing curing agent and acid whey. For a* value significant ($p < 0.05$) differences between samples were noted. The highest redness was noted for sample containing curing agent and starter culture. Such high difference can be an effect of nitrite. Nitrite is a chemical substance which, after reduction to NO can react with myoglobin in meat and convert to nitrosylmyoglobin. After thermal treatment occur nitrosohemochrome appears – stable, attractive red colour of meat products (Alahakoon et al. 2015). *Staphylococcus xylosum* and *Staphylococcus carnosus* can convert metmyoglobin into nitrosylmyoglobin. Microorganisms such as *S. xylosum* or *S. carnosus* have nitrate reductase enzyme, which allows to reduce nitrate to nitrite. The obtained nitrite was reduced to nitric oxide, which reacts with myoglobin. The result of this reaction is occurring unstable nitrosylmyoglobin (bright-red colour of meat before thermal treatment). Due to the ability of *Staphylococcus* strains to convert nitrate to nitrite they were added in combination with vegetables, which are rich source of nitrate (alternative meat curing methods). In Li et al. (2013) study it is stated that *S. xylosum* can

convert myoglobin to nitrosylmyoglobin without any nitrate or nitrite addition compared to the *P. pentosaceus* strain (Li et al. 2013; Bosse et al. 2016]. Other research, states also that Lactic Acid Bacteria (LAB) such as *L. plantarum*, *L. sakei* and especially *L. fermentum*, have high ability to reduce nitrite and are good starter cultures for fermented meat productions (Li 2016). However Rzepkowska et al. (2017) proved that *Lb. fermentum* S8, S10 and *Lb. plantarum* S11, S16 isolated from organic acid whey were able to carry out denitrification (Rzepkowska et al. 2017). Therefore, similar technology effect on colour formation could be achieved by using acid whey as starter culture.

Sample contains sea salt and starter cultures had significantly ($p < 0.05$) higher b^* values than other samples. For C^* (chroma) values significant difference between samples was noted. Sample with curing agents and acid whey addition had the lowest value of C^* colour parameter compared to the other samples. Sample containing curing agents and starter culture had significantly the highest C^* values of all samples. Value of hue angle parameter (h°) was significantly lowest in sample with curing agents and starter culture addition.

Table 4. The L^* , a^* , b^* values of fermented sausages (means \pm standard deviations)

Samples Colour parameter	PKS	SSK	SKS	PSK
L^*	56.14 \pm 3.15 ^A	58.64 \pm 1.34 ^{AB}	57.94 \pm 6.34 ^{AC}	63.35 \pm 2.9 ^{DBC}
a^*	11.85 \pm 1.32 ^A	8.23 \pm 1.21 ^B	8.83 \pm 2.47 ^{BC}	8.84 \pm 1.33 ^{BC}
b^*	8.76 \pm 0.78 ^A	8.46 \pm 0.35 ^A	9.54 \pm 0.89 ^{AB}	7.63 \pm 0.78 ^A
C^*	14.75 \pm 1.42 ^A	11.82 \pm 1.08 ^{BD}	13.13 \pm 1.68 ^{AD}	11.69 \pm 1.47 ^{CD}
h°	36.54 \pm 2.34 ^A	46.03 \pm 3.2 ^B	48.09 \pm 9.76 ^B	40.95 \pm 2.3 ^{AB}

Sample: PKS – curing agent and starter cultures, SSK – sea salt and acid whey, SKS – sea salt and starter cultures, PSK – curing agent and acid whey. Means with different superscript letters are significantly different ($P < 0.05$). Means \pm standard deviation.

3. 4. Texture parameters

Significant ($p < 0.05$) differences in some texture parameters were noted. The highest ($P < 0.05$) hardness (38.89 N) was observed for sample with curing agent and acid whey additions (PSK). In context of springiness parameter, significantly ($p < 0.05$) higher value was obtained for sample SKS compared to other samples. Sea salt was added to meat to increase the water binding capacity and hydration of proteins which leads to improvement of texture of meat products (Desmond 2006). Lactic Acid Bacteria can generate lactic acid, with reduces the pH of environment. Low value of pH parameter affects the reduction of water holding capacity and protein coagulation which leads to the binding of the structure (Kaban and Kaya 2009). It can be concluded, that combination of sea salt and acid whey with LAB in it, has positive effect on springiness parameter of tested sample. Sample with sea salt and starter cultures had significantly lower value of cohesiveness parameter in comparison with other samples. Significant ($p < 0.05$) difference at gumminess parameter was noted. Sample with curing agent and acid

when addition had significantly higher (24,94 N) value of gumminess parameter when compared to other samples.

Table 5. Texture parameters of fermented sausages (means \pm standard deviations)

Parameters Samples	hardness [N]	springiness [mm]	cohesivess	gumminess [N]
PKS	18.40 \pm 10.29 ^A	0.68 \pm 0.05 ^A	0.58 \pm 0.10 ^A	11.1 \pm 7.86 ^A
SSK	15.43 \pm 1.17 ^A	0.71 \pm 0.02 ^{AB}	0.57 \pm 0.05 ^A	8.69 \pm 0.41 ^A
SKS	9.18 \pm 3.45 ^A	0.60 \pm 0.08 ^A	0.50 \pm 0.08 ^{AB}	4.70 \pm 2.33 ^A
PSK	38.89 \pm 4.28 ^B	0.66 \pm 0.05 ^A	0.63 \pm 0.02 ^A	24.94 \pm 2.66 ^B

Sample: PKS – curing agent and starter cultures, SSK – sea salt and acid whey, SKS – sea salt and starter cultures, PSK – curing agent and acid whey. Means with different superscript letters are significantly different ($P < 0.05$). Means \pm standard deviation.

4. CONCLUSIONS

Based on the tests results, it had been found, that it is possible to use acid whey as a starter culture in fermented pork sausage. The best results were obtained in the curing sample with acid whey (PSK). PSK sample characterized the lowest pH and aw values compared to the other samples. This sample was characterized by the highest L* parameter (lightness) from all samples. Redness of PSK sample was not significantly different from samples with sea salt and curing agent and sea salt with acid whey addition.

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