ANTIOXIDANT ACTIVITY OF PEPTIDES ISOLATED FROM DRY AGED LOINS INOCULATED WITH *LACTOBACILLUS* PROBIOTIC STRAINS

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Abstract - The present study deals with the assessment of antioxidant activity of peptides isolated from dry aged loins inoculated with a mixture of two *Lactobacillus* probiotic strains. The peptides were extracted and tested at 0, 60, and 120 days of refrigerated storage. Obtained results pointed out that storage time significantly ($P < 0.05$) influenced the antioxidant activity of peptides in ABTS assay.

Key Words – antioxidant, probiotics, pork loins.

I. INTRODUCTION

In recent years, a large part of the food research is involved in releasing biologically active peptides and determining their *in vitro* and *in vivo* activities. Bioactive peptides has been defined as "components supplied with food (naturally occurring in a product or generated ones), which besides nutritional characteristics positively affect human health". Biologically active peptides have been identified in a variety of foodstuffs, including those of animal origin (e.g. milk, fish, and meat) [1]. Bioactive peptides are typically built of 2-20 amino acid residues, and because of the low molecular weight, they can be absorbed through the intestine into the bloodstream in an intact form and exert various physiological effects or act locally at the site where they occur (gastrointestinal tract section) [2].

Meat from slaughter animals and fish muscle tissue is a valuable source of protein in a diet of many people all over the world, and in addition they may provide a novel source of bioactive peptides. Up-to-date, bioactive peptides of protein hydrolyzates from muscle tissue of slaughter animals and fish having angiotensin convertase enzyme inhibitory effect, antioxidant, antibacterial, and anti-proliferative features, have been identified [3, 4, 5, 6].

The current state of knowledge upon the proteolysis in probiotic meat products is negligible. In recent years, the attempts of using probiotic strains as starter cultures for dry aged meat products and to determine their optimum technological processing conditions, have been made. Erkkilä S. *et al.* [7] - as one of the first ones, suggested the addition of probiotic starter cultures to fermented sausages. However, there are no research on the effect of probiotic bacteria on protein proteolytic conversion in dry aged meat products in the available literature. Peptides that are formed during ageing may affect different biological and physiological functions [8, 9, 10, 11, 12, 13, 14, 15, 16, 17].

Bioactive peptides are normally inactive in the native proteins and may be released during enzymatic proteolysis during meat ageing and digestion in the gastrointestinal tract. Intensive research is carried out on properties of bioactive peptides derived from animal origin foods, such as eggs, fish, seafood, meat and meat products (mainly fermented meat products) [8, 10]. Among them, those with antioxidant activity are widely studied.

Bioactive peptides with antioxidant properties (glutathione, anserine, carnosine) naturally occur in raw meat or can be obtained by hydrolysis of proteins. The antioxidant peptides has been identified in a number of foods, such as casein protein, whey protein, soybean, rice bran, buckwheat proteins, eggs, myofibrillar proteins and other. Antioxidant activity of these peptides is mainly attributed to their ability to chelate transition metals such as cobalt, zinc and copper [18, 19].

This study aimed to determine the antioxidant activity of peptides isolated from dry aged loins inoculated with a mixture of *Lactobacillus* probiotic strains.

II. MATERIALS AND METHODS

Pork loins - *Longissimus thoracis* muscles (n=6) with an average weight of 1.02±0.29 kg were excised at 48 h post mortem from crossbred pigs (Puławska x Polish Landrace) with a body weight of approximately 125 kg at slaughter. Meat came from a local meat plant. Study material consisted of dry aged pork loins inoculated with probiotic strain *Lactobacillus rhamnosus* LOCK900 and *Lactobacillus acidophilus* Bauer assessed immediately after ageing and then after 2 and 4 months of cold storage. Two test variants has been prepared: control without the probiotic (C), sample with a mixture of strains *Lb. rhamnosus* LOCK900 and *Lb. acidophilus* Bauer (RB). Samples C and RB were salted using the curing mixture 2.8% in relation to meat. All samples were then kept in 4°C for 56 h to allow the curing mixture to diffuse. Then the samples from batch RB were inoculated (2 ml/kg) with the mixture of *Lb. rhamnosus* LOCK900 and *Lb. acidophilus* Bauer (1:1) and 1% (w/v) glucose. Subsequently, the loins were hung at 16°C in a disinfected laboratory ageing chamber with a relative humidity of between 70 and 75% for 28 days. After five days of ageing, the samples were subjected to smoking with a cold smoke (30°C). At the end of the ageing period, loins were considered "ready-to-eat". After the completion of ageing each of the loins was divided into three parts, individually vacuum-packed in polyethylene bags (80 mm thick) and stored in a refrigerator at 4 ± 0.5°C.

Subsamples of loins were taken randomly at 0, 60 and 120 days of refrigerated storage to analyse peptides content and their antioxidant properties. The peptides were extracted following the method developed by Mikami et al. [20] with slight modifications. Pork loin samples (1 g) were homogenized in 100 mL of water for 2 min at 4°C. The homogenates were mixed with 7,5% trichloroacetic acid in the ratio 1:1. After 30 minutes at 4°C, samples were filtrated. The filtrates of pork loin extracts were stored at -20°C until analysis. The concentration of water soluble peptides has been determined according to the Lowry method [21] (λ= 750 nm). The results are shown in mg peptides/g product. Antioxidant properties of peptides has been tested by the ABTS method. This method of antioxidant activity assessment is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, carotenoids, hydroxycinnamates, and plasma antioxidants. The pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6sulfonic acid) ($ABTS^{+\cdot}$) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity [22]. Antioxidant activity of peptides has been verified though $ABTS^{+\cdot}$ extinction according to Re *et al.* [22] spectrophotometer method. The results are shown in mg Trolox/mg of peptides. The experiment was carried out in three replicates. All measurements were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). To assess the significance of the experimental factors on all the characteristics studied a two-way analysis of variance (ANOVA) using the SPSS software package version 22.0 for Windows (SPSS, Inc., Chicago, IL, USA) was carried out. When a significant F-value was found, Tukey's post hoc test was used to determine the source of significance set at a confidence level $P < 0.05$.

III. RESULTS AND DISCUSSION

The changes in generation and $ABTS^{+\cdot}$ extinction of peptides of dry-aged loins during vacuum storage are shown in Table 1. Statistical analysis displayed that storage time significantly influenced the antioxidant activity of peptides in ABTS assay ($P < 0.05$), while content of peptides has been affected by neither treatment nor storage time.

The results of peptides content determination during refrigerated storage of loins (Table 1) showed no significant ($P < 0.05$) differences in their concentration during the entire storage period. It can be assumed that the reduction in their content in inoculated loins after two months of storage, and subsequent increase after 4 months (which has not been observed in the C sample) could be caused by a different range of the distribution of these products using bacterial enzymes. This may result from unknown synergy mechanisms of probiotic strains.

Table 1
Antioxidant properties of water-soluble peptides of dry-aged organic loins (mean±standard deviation) stored at 4 ± 0.5°C

Storage time (days)	Treatment	
	C	RB
Content of peptides		
0	4.04 ± 0.21^{Aa}	2.97 ± 0.17^{Aa}
60	4.62 ± 0.24^{Aa}	2.48 ± 0.11^{Aa}
120	4.17 ± 0.17^{Aa}	2.91 ± 0.18^{Aa}
ABTS		
0	0.96 ± 0.12^{Ba}	0.90 ± 0.06^{Ba}
60	1.49 ± 0.03^{Ba}	2.30 ± 0.11^{Ba}
120	2.18 ± 0.09^{Ba}	3.20 ± 0.13^{Ca}

^{a,b,c} Means within row with different superscripts under the same storage time are statistically different ($P < 0.05$)
^{A,B,C} Means within column with different superscripts under the same treatment are statistically different ($P < 0.05$)

Statistically significant (P <0.05) increase (of about 2.29 mg Trolox/mg peptide) in the antioxidant activity of the peptides were found in the sample RB after 120 days of storage. Thus, the researchers confirmed the antioxidant action of isolated peptides. In the available literature it is the first publication confirming the biological activity of peptides produced in dry aged meat products inoculated with probiotic strains as starter cultures. Antioxidative properties of peptide are related to their amino acid composition, structure and hydrophobicity. The exact mechanism underlying the antioxidant activity of peptides is not fully understood, but various studies have shown that they are lipid peroxidation inhibitors, free radical scavengers and transition metal ion chelators. The results of this study confirm the antioxidant activity analysis of the peptides *in vitro*. Antioxidant peptides have also been derived from meat products by Broncano et al. [5]. They defined low molecular weight compounds in fermented sausages Chorizo. The antioxidant properties of the fractions isolated from extracts of chorizo were tested for their ability to neutralize free radicals by DPPH radical scavenging assay. The compounds primarily responsible for the antioxidant activity of the chorizo extract are included in the most hydrophilic fractions, expected to be the natural dipeptide carnosine together with Phe-Gly-Gly and L-carnitine. *In vitro* assays based on chemical reactions are widely used in quantifying antioxidative effectiveness of whole food, partially purified peptides, and/or individual peptides isolated from food mixtures in preventing oxidative processes occurring in the human body as well as in food systems during storage. Even though these chemical assays give an insight to the potential biological activity of these food-derived antioxidants, further analysis such as investigating the fate of peptides during GI digestion as described previously, their permeability through cellular membranes, as well as their *in vivo* stability and reactivity has to be conducted in order to confirm their biological efficacy. On the other hand, when considering applications of antioxidative compounds to control oxidative rancidity in complex food systems, their physical location is also an important parameter to be considered.

IV. CONCLUSION

In dry aged meat products antioxidative peptides can be produced due to the action of microbes and endogenous proteolytic enzymes. The probiotic strains inhibit proteolysis and oxidation changes in dry aged pork loins. The use of probiotic bacteria positively affected the antioxidant activity of peptides. Nevertheless, the present study has to be considered as preliminary and further research is needed to purify and characterize the peptides that can exert antioxidant activity *in vitro* and then, to determine their potential *in vivo* antioxidant activity.

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