

MEAT THERAPY FOR HYPERTENSION: HYBRID HYDROLYSATE AS ACE INHIBITORY COMPOUNDS

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ABSTRACT - In the Turkish cuisine, beef pastirma is one of the renowned meat products. Pastirma refers to a great variety of muscle-based products in the Anatolian region. Cuts of pastirma were prepared as treated samples, while fresh meat cuts from the same muscle were also used as control samples. Objectives of this study were to determine the antihypertensive activity of peptic hydrolysates from fresh beef and pastirma, and then isolate and purify some potential peptides with angiotensin converting enzyme (ACE) inhibitory activity. The antihypertensive activities were determined using ACE inhibitory activity assay. As a result, the pastirma hydrolysates showed a higher hypertension activity against ACE mechanism when compared to the fresh meat. The reason of high antihypertensive activity perhaps refers to the coexistence of aromatic and sulfuric amino acids (Met, Lue, Try and Phe) and also to acidic amino acids such as (Arg and Lys) on the hydrolysate fragments (peptides). To summarize, pastirma showed a lower value of IC₅₀ (1.02 mg/ml) than in fresh meat (1.46 mg/ml), which means pastirma is more effective than meat against ACE. In short, hydrolysates of pastirma and fresh meat might be used as a model for sourcing ACE inhibitory peptides that serve further nutraceutical purposes. This study reflects the importance of empowering hypertensive people to cope with life related health-issues and in offering nutrition-based treatment options rather than medical alternatives.

Key Words : Bioactive peptide, pastirma, meat protein.

I. INTRODUCTION

Recently, production of food derived physiologically effective peptides for human body is an interesting dietary approach. Theoretically, the main use of meat in cooking remains as a commodity rich in protein, food makes people happy, and exclusively gives a

satisfactory feeling of being full. Conversely, we should explain using scientific means to the local societies the functional importance of meat and meat proteins. Meat diet contains fresh, processed, fermented and cured meat products, which are rich in ACE inhibitory peptides, protein hydrolysatation fragments that inhabit the conversion of a peptide-hormone, angiotensin I to angiotensin II, the latter which in turn causes vasoconstriction and a subsequent increase in blood pressure. Hypertension disease is a major public health for so long that had been treated with some medications. Alternatively, we have to replace the drug use with nutraceuticals to minimize the risk of that disease, and particularly side-effect that may rise from the use of chemical treatments.

Fresh meat cuts and pastirma were implemented for this research. Pastirma is a dry-cured meat product that is normally covered with chemen paste (mashed garlic, fenugreek powder, and red paprika). This sort of cured meat made in the region of Kayseri in and has an attractive exterior and interior appearance, delicious taste, unique smell and muscle-like shape[1]. We aimed by this work to comparatively determine the antihypertensive activity of fresh beef and pastirma protein hydrolysates *in vitro*.

II. MATERIALS AND METHODS

Food types: Cuts of fresh and beef Pastirma

Cuts of pastirma were manufactured about 1 month prior to the experiment and obtained from a local retailer. The pastirma was produced in a factory in Kayseri city using the traditional process. *M. semimembranosus (SEM)*, Top (inside) round muscles were sourced from male cows at 33 months old. The pH values of the

muscles before processing ranged from 5.6. SEM muscles were used 48 h post-mortem. Two groups of muscles were prepared for each experiment: one group was analyzed as fresh meat while the other group was processed into pastirma. Fresh and pastirma samples were sourced from the same animals, and all fresh samples were kept at -80°C until the experiment. The cuts used for pastirma were subsequently covered with a paste of ground spices known as *çemen*, as described by Kaban [2]. Finally, after 4 weeks of processing, pastirma was used for experiment as cured cuts.

Processing samples and digestion process

Samples of each cut type were minced by a meat grinder (Pro-meat W 2000 Grande, Arnica). Fifty grams of each sample were mixed with 130 ml of distilled water. The mixture was then cooked for 30 min at 70°C . Cooked samples were digested with pepsin and trypsin consecutively for 4 hours after adjustment of pH. The samples were boiled to inactivate the enzymes[3]. Subsequently, the samples were cooled down to room temperature and being fractionated and centrifuged and then passed through a cellulose membrane filter ($0.45\mu\text{m}$).

Molecular weight vs SDS-PAGE

Extraction of proteins from fresh meat and pastirma cuts obtained from the same muscle were electrophoresed on SDS-PAGE patterns to estimate the impact of processing on the proteins structures. Proteins were extracted in different buffers such as 1 M sucrose, 2 M urea, low ionic strength buffer (WSP), and high ionic strength buffer (Guba Starub-ATP).

Determination of the antihypertensive activity

To check the biological functions against ACE, normally *in vitro* a value called IC_{50} is checked. The samples were treated with ACE and its substrate (hippuryl-histidyl-leucine) in the case of availability of the peptide. The resultant is hippuric acid that spectrophotometrically determined at 280nm, which is released if there is no complete inhibition for ACE.

III. RESULTS AND DISCUSSION

The development of products containing ingredients which are presumed to promote health such as peptides to minimize some diseases is an intellectual keynote in recent years. Meat eaters (carnivore) who suffer from high blood pressure disease would be more consistent and comfort in case we can provide scientific validation that meat is healthy and provide peptides that have a positive physiological effect beyond its nutritional values. Chronically ill people will appropriate if we can replace medication drugs with nutraceuticals with a better explained mechanism. The concept here to find nutrients as alternatives for medications to treat people without any side effect, a treatment based on natural nutrients rather than chemicals supplementations. Few publications are available in relation to this research. Most of the research was carried out on chicken, porcine and fish, but the bovine was neglected so far. so the main unique difference is that the meat type and product. This research emphasizes on beef and dry-cured beef product called pastirma that is produced in Turkey. Bovine product had been chosen because in general there are very little information about the bioactive peptides derived from beef and beef products.

SDS-PAGE and protein structure

We carried out this experiment in order to evaluate the changes in the protein structure in relation to the course of processing. In previous publication we mentioned that if meat dry-cured, there must be many biochemical and biological changes accrue during processing [1]. Such changes take place on protein and fat structures, but what amazes us here is that protein may provide peptides that serve as functional compounds. From that point a comparative qualitative , quantitative and analytical experiments on beef and pastirma were carried out. Figure 1 shows the differences in the structure of proteins extracted from fresh meat

and pastirma. Apparently, major proteins were degraded because of the dry-curing process, many factors have contributed to those changes, including, salt treatment, drying, pressing, and finally chemen treatment. We would remiss if we do not mention about the proteolytic enzymes that may the meat itself provides or that may come from garlic and chemen paste.

ACE inhibition ratio and biological value

Precise bioactive peptides inhibit ACE actions of mechanism and have potentials as nutraceutical for treating hypertension, those peptides sourced from meat products are under focus. Recently, tremendous food peptides that positively work against ACE have been identified but the bioavailability of these peptides on human trials has not completely been reported. However, inhibition of ACE action of mechanism was evaluated by checking the absorbance of hippuric acid that released as a end-product of ACE activity (Inhibition ration = $C - S / C - B \times 100$)[3]. The higher the absorbance of the acid, the higher the ACE activity, meaning that the lower the peptides potentiality. As results, hydrolysates of fresh beef and pastirma showed a remarkable inhibition rate against ACE, an enzyme elevates blood pressure in humans. Beef and pastirma showed inhibition ratio against ACE by 82.9 and 78.7%, respectively (Figure 2). Results revealing that having cooked meat that metabolized in the intestinal tract is a source of a tremendous antihypertensive peptides.

IC₅₀ values as antihypertensive index

The concept here is that meat provides hybrid peptides that their effectiveness tested at reducing hypertension. Medications based on chemical structure may have harmful side effects if used for a long period. Deficiency in consumption of meat products rich in proteins may motivate the etiology of the hypertension in humans. The concentration of ACE inhibitors required to inhibit 50% of ACE activity was defined as the IC₅₀. Exclusively sufficient, in

this study both meat and pastirma were found to have not only nutritional utility but also medical value, because proteolysis on meat generated a substantial number of peptides that have nutrafunctional roles and some of which have strong ACE inhibitory activity. Hydrolyzed proteins from beef and pastirma showed 1.46 and 1.02 mg/ml as reflection to IC₅₀ value, respectively (Figure 3). Apparently, this also demonstrates that meat proteins might lead to better nutraceutical therapy that minimizes hypertension and might aid in finding the most effective approaches for meeting the needs of all hypertension patients. Clearly, results suggest that beef and pastirma are rich in hybrid peptides that may provide physiologically functional peptides, thus lowering blood pressure, and normalizing associated biochemical and physiological changes. Thus, beef and pastirma have a nutraceutical value that effects positively on meat-eating society.

VI. CONCLUSIONS

Feeding the world with healthier meat to minimize some diseases is highly valuable and ideally theme. Current food research requires that the health benefit attributed to a meat component be derived from its “nutritive value”. Results indicate pastirma process along with the enzymatic hydrolysatation generated peptides that inhibited ACE activity *in vitro*. Results suggest major proteins were degraded such as MHC (200kDa) and many enzymes including: β-galactosidase, Phosphorylase B, Lactate Dehydrogenase, Trypsinogene and that is because of the pastirma making process. Apparently, proteins in Turkish meat have remarkable antihypertensive activities, also the results seem promising for peptide isolation that is currently ongoing using chromatographic techniques. We found it is not necessarily to process meat to elevate its biological activities. Although the salt level is high in pastirma still it could provide peptides with great ACE inhibitory activity.

Further isolation and identification studies are considered and *in vivo* studies are proposed for the future using spontaneously hypertensive rats as models.

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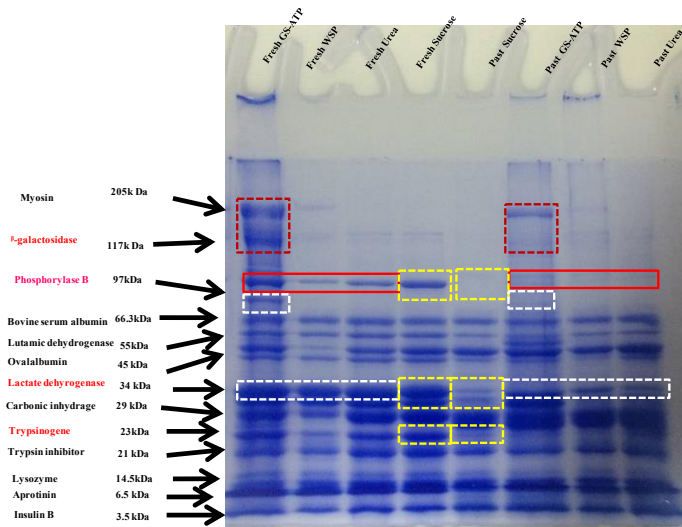


Figure 1. SDS-PAGE patterns of proteins extracted from fresh beef cuts and pastirma.

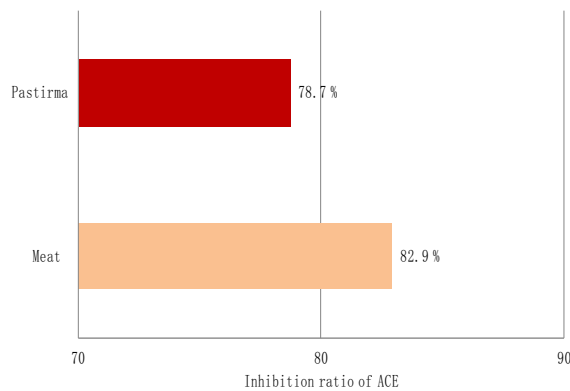


Figure 2. ACE inhibition ratio of fresh meat and pastirma samples that were hydrolyzed with pepsin and trypsin.

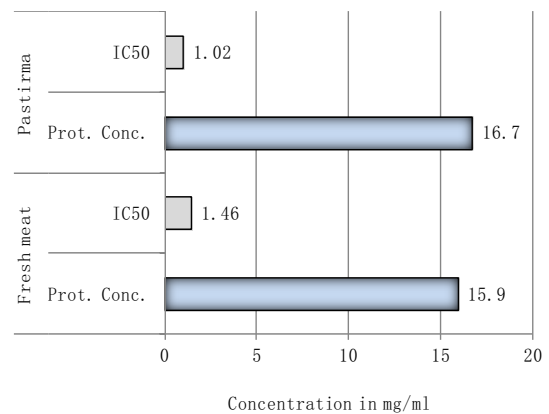


Figure 3. IC₅₀ values against ACE versus protein content values in hydrolysates from fresh meat and pastirma of *M. semimembranosus* (SEM).