Extraction & Qualitative Analysis of Carnosine from Intestine of Fish, Chicken and Goat

Joel Massey[1], Dr. Swati Bande[2]
[1]*M.Sc. Student, M.M College of Arts, N.M. Institute of Science, H.R.J. College of Commerce, Bhavan's College (Autonomous), Andheri (W), Mumbai-400058.*
[2]*Asst. Professor, M.M College of Arts, N.M. Institute of Science, H.R.J. College of Commerce, Bhavan's College (Autonomous), Andheri (W), Mumbai-400058.*

***Absract*–Carnosine is a dipeptide synthesized in the body from □-Alanine and L-Histidine. It's found in high concentrations in red muscles, and also in the heart, liver, kidneys and gastro-intestinal tract. Carnosine was discovered by Russian chemist Vladmir Gulevich. Like, carnitine, carnosine is composed of root word *carn*, meaning "flesh"", alluding to its prevalence in meat. The study "Extraction & Qualitative Analysis of Carnosine from Intestine of Fish, Chicken and Goat" focuses on organic solid waste management (here, intestines), thereby reducing the amount of waste being thrown into local dump-yards which might result in decrease in number of bacteria being accumulated in the region and might also reduce the putrid smell. Carnosine is a very beneficial compound as it has many applications ranging from anti-inflammatory, antioxidant & anti-glycating to curing neurological disorders, stress & insulin sensitivity. The study focuses on the most inexpensive extraction method of carnosine & obtaining carnosine in the natural stable form and it's detection using Nuclear Magnetic Resonance (NMR) and to also try minimize the use of lab-made carnosine in carnosine based products.**

***Keywords*–Carnosine, Organic Solid-Waste Management, Fish Chicken & Goat, Quality Analysis, Nuclear Magnetic Resonance (NMR).**

I. INTRODUCTION

In India, 51.14% of the total meat produced is contributed by poultry meat (A. Singh 2023). Large volumes of solid waste are produced by the chicken industry, including sawdust for bedding, excreta, feed, feathers, intestines, bones, hatchery trash, mortality waste, medications, leftover pesticide residue, disinfectants for chicken houses, etc. (Singh *et al.,* 2018). Therefore, there are serious environmental issues associated with the waste produced at different phases of chicken production (Sims and Wolf, 1994; Singh *et al.,* 2018). Chapman and Werth *et al.* also discussed the potential risks on environment associated with the waste generated by meat processing industries (Chapman 1996; Werth *et al.,* 2014). In 1900, Russian scientist V.S. Gulewitch made the initial discovery of carnosine while searching Liebig's meat extract for unknown nitrogen-containing non-protein molecules (Gulewitch, 1900).

During the last two decades, a great deal of research has been done on carnosine, and it has been shown to have a number of benefits over the digestive system (as zinc-carnosine, or polaprezinc), immunomodulation, and anti-glycating properties, as well as benefits in cataract and other degenerative diseases (I. Rashid, 2007; D. L. Price, 2001). Carnosine also possesses properties such as neurotransmission and antioxidant properties which are beneficial in the scientific field (S. Fleisher-Berkovich, 2009). However, there have never been any documented serious adverse effects of carnosine supplementation in healthy individuals with intact carnosinase activity (Marios, 2010). Carnosine is now widely recognised as a potent antioxidant (Aydin, 2010; Shen, 2010; Hipkiss, 2009). Carnosine, as a zinc-chelate (Polaprezinc), has shown actions against ROS production, with notably well-studied effects in the small intestinal mucosa.

When carnosine is combined with other popular antioxidants including coenzyme Q10, vitamin C, vitamin E, selenium, ginkgo biloba, and vitamin B complex, it shows signs of neuroprotection, particularly against Alzheimer's disease. When comparing patients treated with donepezil + placebo to those treated with the antioxidant combination with the cholinesterase inhibitor, mental test evaluation showed a significant clinical improvement. Antioxidant activity and homocysteine decrease are thought to be combined to provide the advantage (Artioli, 2010). The anti-inflammatory effects of carnosine, including a decrease in

cytochrome C and caspase-3, are thought to be caused by this ROS-quenching action of the amino acid (Stvolinsky, 2009).

Certain aldoses or aldehyde molecules connect to proteins (or to DNA) during the glycation process that occurs with ageing, resulting in cross linking, or aberrant protein-to-protein or protein-to-DNA linkages. Carbonyl groups help to facilitate this process. These aberrant proteins may then build up to form AGEs (Advance Glycation End-products), which may subsequently react with free radicals to produce age-related chronic degenerative illnesses. In addition to the antioxidant characteristics mentioned previously, carnosine has been shown to have strong anti-glycating qualities that disrupt the glycation processes at several stages (Stvokinsky, 2009; Boldyrev, 2010). Malondialdehyde (MDA) and protein carbonyl (PCO) groups, which are known to be implicated in glycation, are known to be reduced by carnosine supplementation (Aruoma, 1989).

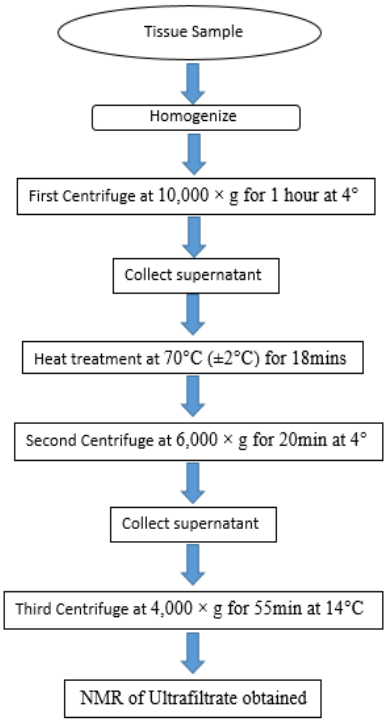
Hence, this study primarily focuses on a time efficient carnosine extraction technique with minimal impurities in the end-product and also enlightens us on ways to reduce load on solid waste management. Hence, the main goal of this study is to establish a qualitative estimation of carnosine in the intestines of fish, chicken and goat, thereby utilizing natural carnosine instead of lab-made carnosine in cosmetics product and also helps to reduce the load on organic solid waste management.

II. MATERIALS AND METHODOLOGY

Extraction method as described by Maikhunthod and Intarapichet (2005)-

1. Sample preparation: Intestine samples are cut open and cleaned thoroughly to remove any undigested food material and cut into small pieces to facilitate easy homogenization.
2. Homogenization: 100gm of sample was homogenized in 100ml of distilled water (D/W) to obtain 100% homogenate.
3. Filtration: The 100% homogenate is filtered through mesh to remove any solid matter of tissue clump (if any).
4. Centrifugation: The homogenate is put through series of centrifugation process in Cold Centrifuge (Remi Cooling Microfuge) using eppendorf tube as described below-

a) The first centrifugation step involves rotation of 10,000 × g for 1 hour at 4° and collect the supernatant.
b) Keep the supernatant in water bath at 70°C (±2°C) for 18mins.
c) Collect the supernatant and centrifuge at 6,000 × g for 20min at 4°C and collect the supernatant.
d) Centrifuge the supernatant at 8,000 × g for 28min at 14°C.
e) The product obtained is impure carnosine.



Qualitative Analysis: The obtained product is analysed using 1H Proton NMR to detect Carnosine and the result is inferred with the help of carnosine chemical structure and Elementary Organic Spectroscopy by Y. R. Sharma.

1H NMR Spectroscopy:
1H NMR spectra were obtained using a NMR500-vnmrs500 at 499.85MHz 1H frequency in solvent deuterium oxide (D2O). The radiofrequency pulse was manipulated with PROTON (s2pul) pulse sequence (in fish & goat sample) and PRESAT pulse sequence (in chicken sample).

The spectra of chicken sample was obtained at 25°C and the spectra of fish & goat was obtained at room temperature, because of trial of different pulse sequence between the samples. The spectral width was set at 8.012 kHz with acquisition time of 2.045 sec. The relax delay between pulses was set at 2 sec,

and the total time of experiment was set between 5min to 9min according to need & pulse sequence.

III. OBSERVATION & RESULT

The following Table I. shows the average amount of supernatant received after each centrifuge step of the samples: The initial amount of homogenate (in eppendorf tube) for each sample is 18ml.

Supernatant obtained↓	Fish (mL)	Chicken (mL)	Goat (mL)
After 1st centrifuge at 10,000 × g × 1hr at 4°C	15.4	14	12
After 2nd centrifuge at 6,000 × g × 20min at 4°C	11	11	7.9
After 3rd centrifuge at 4,000 × g × 55min at 14°C	8	10	7.2

Table I. Quantity of Supernatant obtained after each centrifuge step.

The Nuclear Magnetic Resonance (NMR) reports of each of the sample i.e. intestines of fish, chicken and goat are as follows:

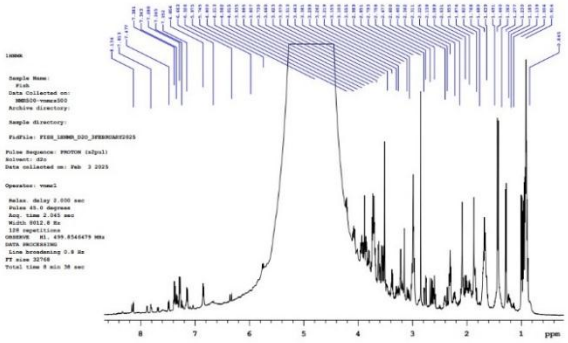


Fig (a). NMR report of intestine sample of fish

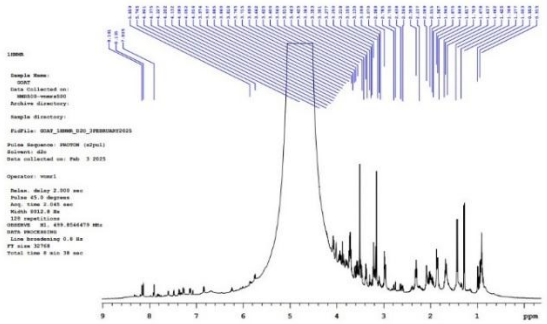


Fig (b). NMR report of intestine sample of goat

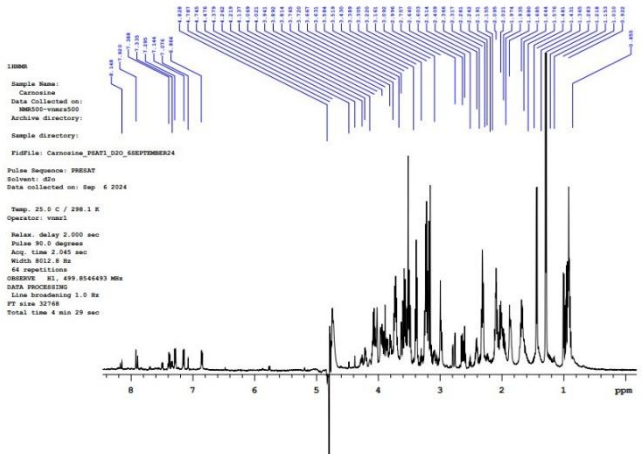


Fig (c). NMR report of intestine sample of chicken

IV. DISCUSSION

There are many methods which are used worldwide for extraction of carnosine from biological tissues. One such method is traditional method which includes aqueous or alcoholic solvent extraction from the homogenized tissue. Efficient extraction method is a much needed solution which will open the doorways to maximizing carnosine yield while preserving its chemical structure.

This study's cold centrifugation method for extraction of carnosine when compared with techniques such as high-voltage pulsed electric fields (PEF), which focuses on enhancing the cell membrane permeability for isolation of compounds showed a positive comparison i.e. the extraction of carnosine was successful in both the techniques (Robin *et al.*, 2022; Zhu *et al.*, 2016).

Similarly, along with his coworkers, Seung-Ki Kim,

demonstrated extraction of carnosine using water and ethanol. These researcher applied response surface methodology (RSM) for determination of optimal extraction conditions focusing on chicken muscle tissues (Kim *et al.*, 2014). This study of Seung-Ki Kim when set side by side with this study procured positive results for carnosine extraction, showing the efficacy of cold centrifugation technique in extracting carnosine.

Chan *et al.* also explored three different methods of extraction of carnosine from cow muscle viz. unheated, 60°C and 100°C. They determined that heating the muscle to 100°C resulted in a boost in its extraction & antioxidant activity (Chan *et al.*, 2006). This result equates with the study conducted, as both shows presence of carnosine at the end of the extraction.

V. CONCLUSION

The extraction and qualitative analysis of carnosine from the intestines of fish, chicken and goat is new field of study with wider potential applications in food science, biochemistry, nutraceuticals and sustainable resource management. The naturally occurring dipeptide carnosine (β-alanyl-L-histidine) is known to be high in concentration in vertebrate brains and skeletal muscles. But this study revolves around the potential of intestines, usually considered a low-economic-value by-product in the meat and fish processing industries, as a new source of carnosine.

This study conclude that, although in different amounts, carnosine can be detected in the intestines of all three animal species: fish, chicken and goat. Among these, chicken intestines may have higher carnosine levels. A number of physiological characteristics, including the shorter lifespan and faster metabolism of chicken, may support this theory by increasing the turnover and accumulation of dipeptides like carnosine.

Chicken intestines might present a more varied result. Depending on the species of chicken and its diet, environmental conditions, and health status, the levels of carnosine extracted may differ considerably. Some species might demonstrate moderate carnosine content, likely due to their high-protein diet and active metabolism. This variability points to the importance of species selection in optimizing chicken-based sources of carnosine. In contrast, goat intestines might yield lower levels of carnosine in comparison. This may be related to the digestive physiology of ruminants, in which extensive microbial fermentation and enzymatic activity may degrade dipeptides like carnosine or reduce their accumulation.

This study concludes that an efficient path for the exploitation of animal low-economic-value by-products is highlighted by the effective extraction and qualitative analysis of carnosine from the intestines of fish, chicken and goats and detection using NMR spectroscopy. In addition to supporting environment friendly slaughterhouse waste management techniques, this strategy tries to create new avenues for the creation of natural goods that promote health. These techniques when improved and developed further, possess a greater chance that they will be included into the food and pharmaceutical sectors, supporting international initiatives for resource efficiency and sustainability.

VI. REFERENCES

[1] A. A. Boldyrev. Carnosine as a natural antioxidant and geroprotector: from molecular mechanisms to clinical trials, Rejuvenation Res., 2010.
[2] A. Aydin. Effect of carnosine against thioacetamide-induced liver cirrhosis in rat Peptides, 2010.
[3] A. R. Hipkiss. Carnosine and its possible roles in nutrition and health, Advances in Food and Nutrition Research, 2009.
[4] Bussayarat Maikhunthod, Kanok-Orn Intarapichet. Heat and ultrafiltration extraction of broiler meat carnosine and its antioxidant activity, Meat Science, 71(02): 364- 374, 2005.
[5] Chan, K. M., Decker, E. A., and Means, W. J. Extraction and activity of carnosine, a naturally occurring antioxidant in beef muscle, Journal of Food Science, 58(1): 1-4, 1993.
[6] Chapman, L.S. Soil and solid poultry waste nutrient management and water quality, Poultry Science, 75: 862-866, 1996.
[7] D.L. Price. Chelating activity of advanced glycation end-product inhibitors, MeSH terms Substancesiol. Chem, 2001.
[8] Dr. Amandeep Singh. Livestock Production Statistics of India. Vet Extensions, 2023.
[9] G. Artioli. Role of beta-alanine

supplementation on muscle carnosine and exercise performance, Medicine & Science in Sports & Exercise, 2010.

[10] Gulewitch WS, Amiradzibi S. Uber das carnosin, eine neue organische Base des Fleischextraktes. Ber Deutsch Chem Ges B33: S1902–S1903, 1900.

[11] I. Rashid. Carnosine and its constituents inhibit glycation of low-density lipoproteins that promotes foam cell formation in vitro, FEBS Letters, Experimental and Molecular Biology Journal, 2007.

[12] Kim Seung-Ki, Kwon Dodan, Kwon Da-Ae, Paik In Kee, Auh Joong-Hyuck. Optimizing carnosine containing extract preparation from chicken breast for anti- glycating agent, Korean Society for Food Science of Animal Resources, 34(01): 127- 132, 2014.

[13] Marios Kyriazis. Anti-ageing potential of carnosine: approaches toward successful ageing; Drug Discovery Today: Therapeutic Strategies (Science direct), 7(3-4): 45-49, 2010.

[14] O.I. Aruoma. Carnosine, homocarnosine and anserine: could they act as antioxidants in vivo? The Biochemical Journal, 1989.

[15] Purnima Singh, Tanmay Mondal, Rachana Sharma, N. Mahalakshmi, Mokshta Gupta. Poultry Waste Management, International Journal of Current Microbiology and Applied Sciences, 7(08), 2319-7706, 2018.

[16] Robin A, Ghosh S, Gabay B, Levkov K, Golberg A. Identifying critical paramters for extraction of carnosine and anserine from chicken meat with high voltage pulsed electric fields and water, Innovative Food Science and Emerging Technologies, 76, 2022.

[17] S. Fleisher-Berkovich. Inhibitory effect of carnosine and N-acetyl carnosine on LPS-induced microglial oxidative stress and inflammation Peptides, 2009.

[18] S.L. Stvolinsky. Biological activity of novel synthetic derivatives of carnosine, Cell Molecular Neurobiology, 2009.

[19] Sims T.J., Wolf C. D. Poultry waste management: agricultural and environmental issues, Advances in Agronomy, 52: 1-83, 1994.

[20] Werth, S.J., Schusterman, E.G., Peterson, G.B., Mitloehner, F.M. Air: Confined Animal Facilities and Air Quality Issues, Encyclopedia of Agriculture and Food Systems, 1: 283-292, 2014.

[21] Y. Shen. Carnosine protects against permanent cerebral ischemia in histidine decarboxylase knockout mice by reducing glutamate excitotoxicity, Free Radical Biology and Medicine, 2010.

[22] ZHU Jun-ying, ZHAO Li-ming, JIANG Li-hua, QIU Yong-jun, XIA Quan-ming. Factors affecting the extraction of carnosine/anserine from chicken breast, Science and Technology of Food Industry, (17): 215-219, 2016.