The use of NMR spectroscopy in analyses of selected characteristics of meat products

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Abstract

Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive and non-invasive analytical chemical technique that is also increasingly used in food chemistry. The method allows specification of a broad group of components in foodstuffs characterised by a simple or complex form of matrix, as in meat and meat products. While NMR studies on fresh meat are numerous, NMR studies on meat products are less common and have focused mostly on relaxometric measurements of water mobility and determination of fatty acid chain composition by high-resolution ^{1}H NMR spectroscopy. The results presented in this article represent an original approach in analyses of fermented meat products. Fourier transform nuclear magnetic resonance (FT NMR) for determination of free amino acids and carnosine content applied in this work has been used for the first time, and has proven suitable for the analysis of fermented sausages.

Carnosine, free amino acids, FT NMR, sausages

Introduction

The last decade has seen major progress in the research and development of analytical methods and an accompanying improvement in methods used for the analysis of various food industry products. Analytical approaches and methods that are able to quickly determine the largest number of food ingredients simultaneously with the highest possible sensitivity are currently gaining ground. The study of interactions between these components at the microstructural (atomic and subatomic) level is an extremely interesting area of research and a task for future food research. The analytical methods that meet these requirements include nuclear magnetic resonance (NMR) spectroscopy. One of the method's major advantages is the non-destructive nature of sample analysis, since the magnetic field and radio waves readily penetrate a number of non-ferromagnetic materials and cause no change or degradation in them during measurement. For this reason even valuable biological samples such as DNA, RNA and proteins can be analysed by NMR, avoiding the use of destructive analytical methods. Due to the fact that the vast majority of foods contain large amounts of protons that come from water, fats, carbohydrates and proteins, ^{1}H NMR (Proton Nuclear Magnetic Resonance Spectroscopy) has become one of the methods most frequently used to determine these important food ingredients (Macomber 1998).

The NMR methods have also been applied to the analysis of meat and meat products, particularly to the assessment of water mobility by relaxometry and the fatty acid content by ^{1}H NMR spectroscopy. A substantial part of the studies hitherto conducted in this area consists of NMR analyses of fresh meat, and only a small percentage is devoted to the analysis of complex meat products (Pearce et al. 2011).

The presence of free amino acids in fermented meat products is mainly due to proteolytic activity in the fermentation process of these meat products due to natural and applied microflora. The importance of free amino acids for the consumer's organism lies in their

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ability to bind directly to G-protein-coupled receptors (GPCRs) in the oral and abdominal cavities which allows them to be absorbed more rapidly and reach specific targets in the organism, compared with amino acids bound to the protein structure (San Gabriel and Uneyama 2013). Free amino acids are a group of extractive substances that also affect the sensory characteristics of food products. By interacting with other product ingredients, individual amino acids also make a specific contribution to the taste of meat products (Leggio et al. 2012). Carnosine is a dipeptide (β-alanine-L-histidine) and, at the same time, a bioactive substance that is present in the largest concentrations in the muscle tissues and internal organs (kidneys, lung, liver and brain) of man and animals (in particular pork, beef and poultry). In humans, it may be absorbed in its intact form in the jejunum by means of specific receptor carriers (Gilbert et al. 2008). Scientific studies have shown anti-stress effects of carnosine (Li et al. 2012), its importance to the inhibition of cell death during acute loss of blood supply to the brain (ischemia) (Wang et al. 2013), and its antioxidant activity (Wu et al. 2004).

The aim of this study was to generate new knowledge on the use of Fourier transform nuclear magnetic resonance (FT-NMR) spectroscopy in the analysis of carnosine and free amino acid content during the ripening of fermented (uncooked) sausages.

Materials and Methods

Materials used in this study: Fifteen sausages with Lyocarni RBL-73 starter culture (Sacco, Italy) containing the following microorganisms: *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus curvatus*. Fifteen sausages with a combination of Lyocarni RBL-73 starter culture and *Lactobacillus paracasei* LPC-37 probiotic culture (Danisco, Denmark).

Sausage ingredients: beef (28.7%), pork (33.6%), bacon (33.6%), antioxidant, sodium nitrite and seasoning mixes.

Conditions during the 4-week maturation period: temperature: 16 – 23 °C, relative humidity: 80 – 95%. The analyses were conducted using the 600 MHz VNMRS NMR spectrometer at the Department of NMR and Mass Spectrometry at the Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava.

The acquired spectra were evaluated using the software CHENOMX Suite versions 4.0 and 7.7. The spectra had to be adjusted to the final form in the following steps:

- Set-up of measurement parameters (pH, concentration of the standard)
- Phase correction
- Baseline correction
- Water signal removal
- Deconvolution

Individual compounds from the library of chemical compounds available in the CHENOMX Suite software were entered into the spectra adjusted in this way according to the shape and height of the peaks. The program automatically evaluated the quantitative representation of the compounds according to the shapes and heights of the peaks entered. The resulting values were obtained by a conversion depending on the sample dilution used during the working process. This procedure was taken from Mannina et al. (2012).

Results and Discussion

The alanine content was determined as the highest among the free amino acids over the entire ripening period of 28 days. The total increase in the alanine content during the ripening period was 81.8% (0.28 $mg \cdot g^{-1}$), and its maximum level was detected in 3rd week of ripening (0.35 $mg \cdot g^{-1}$). The least represented amino acid in the samples was valine which reached its maximum concentration in the samples in the last week of ripening (0.03 $mg \cdot g^{-1}$). The highest percentage increase during ripening (96.3%) was found in phenylalanine (Plate V, Fig. 1).

Sausages with an addition of probiotic culture were found to have a similar volume representation of free amino acids as sausages containing a starter culture. However, a higher increase in phenylalanine (96.9% – the highest among the amino acids determined) was observed during the course of ripening and phenylalanine was the dominant amino acid in 3rd week of ripening (0.42 $mg \cdot g^{-1}$).

As a result of the deamination of amino acids, the concentrations of certain free amino acids fell during the 4th week of ripening. This caused the phenylalanine level to decrease to 0.33 $mg \cdot g^{-1}$, and alanine again became the slightly dominant free amino acid (0.34 $mg \cdot g^{-1}$). The least represented was valine with a concentration of 0.04 $mg \cdot g^{-1}$ in the last week of ripening (Plate V, Fig. 2). The carnosine content in the samples with a starter culture grew from an initial 0.17 $mg \cdot g^{-1}$ to 1.64 $mg \cdot g^{-1}$ in the last 4th week of ripening (an increase of 89.6%), caused by its release from protein structures during the course of ripening. The carnosine content in samples with a probiotic culture was 0.19 $mg \cdot g^{-1}$ in the initial stage of ripening and 1.40 $mg \cdot g^{-1}$ in the final stage (an increase of 86.4%) (Plate V, Fig. 3). Similar results were reported by Mora et al. (2007) and Purchas and Busboom (2005).

Conclusions

Although a routinely used method in organic synthesis, proteomics and metabolics, Fourier Transform Nuclear Magnetic Resonance (FT NMR) spectroscopy is less common in food product analysis. Given the present state of our knowledge, this method has been used in the analysis of specific type of meat products with a high fat content (43 – 50%) for the first time in our study. The extraction method used, with 5% perchloric acid, can be improved in the future to achieve a better yield of the parameters studied.

The obtained results were only partly comparable with similar studies by other authors since they represent an original approach to the determination of amino acid and carnosine content in meat products by the NMR.

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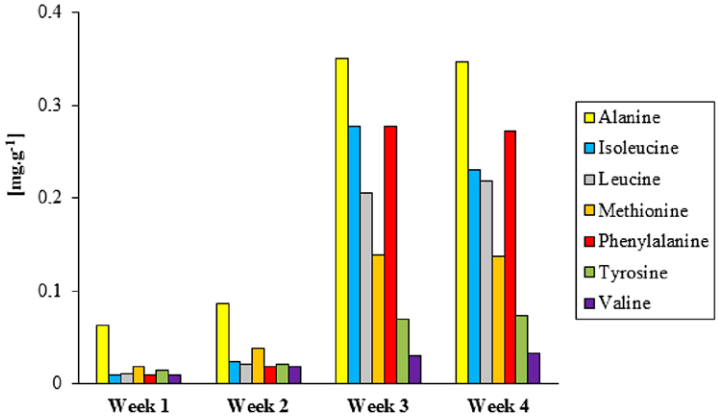

Fig. 1. Concentrations of free amino acids in sausages containing a starter culture

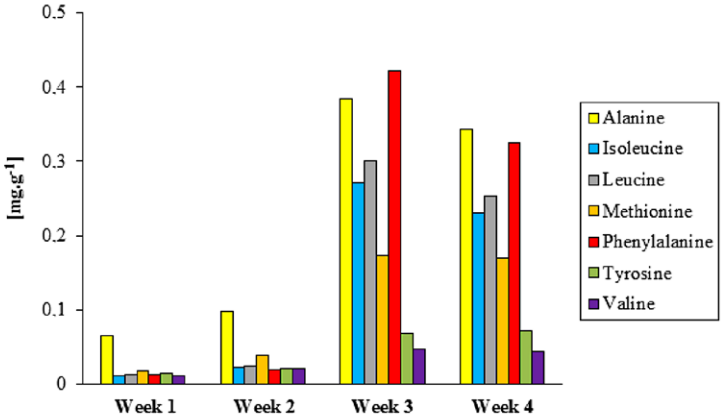

Fig. 2. Concentrations of free amino acids in sausages containing a probiotic culture

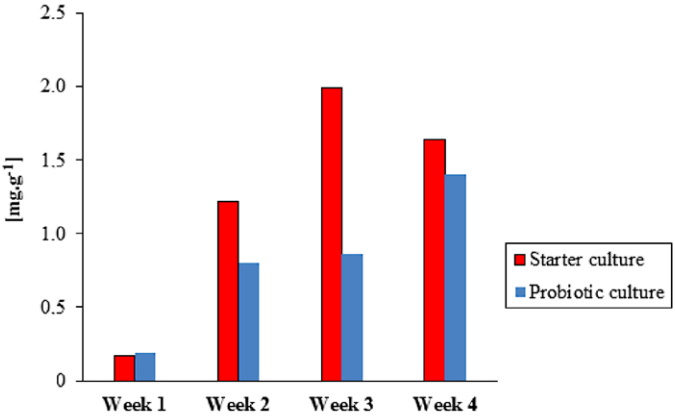

Fig. 3. Carnosine concentrations in samples containing a starter culture and a probiotic culture