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METHOD FOR DETERMINATION OF PROVITAMIN A IN MEAT BASED SAMPLES

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Abstract

The method described in this paper was adapted for determination of provitamin A from meat based products that were supplemented with plant oils contain carotenes. The first step consisted in grease saponification with alcoholic KOH solution and antioxidant, for 30 minutes. Acetone and hexane were used for the extraction of carotenes several times. Finally the absorbance was determined at 450 nm. Quantification was realised with a calibration curve prepared using 5 µg/ml β-carotene solution. The method was tested for determination of β-carotene in three samples of meat products supplemented with sea-buckthorn (Hippophae rhamnoides) oil. For comparison non supplemented meat products was considered too. The provitamin A content ranged between 0.109 and 0,218 mg/100g and after one week between 0.079 and 0.195 mg/100g in the product tested. Different values were obtained according to the preparation technology.

Keywords*: meat products, provitamin A, sea-buckthorn oil.*

INTRODUCTION

Vitamin A is the generic name applied to a group of fat soluble compounds with biological activity that includes: retinol (alcohol), retinal (aldehyde), retinoic acid (carboxylic acid) and pro-vitamin A carotenoids such as ß-carotene. Vitamin A is an essential compound needed in small amounts by humans for the normal functioning of the visual system; for growth and development; for maintenance of epithelial cellular integrity, immune function, and reproduction (Hatchcock, 1997).

Human body necessary of vitamin A is provided by animal food as preformed retinol (mainly as retinyl ester) and by fruits and vegetables as pro-vitamin A carotenes. Preformed vitamin A in animal foods occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells (Castermiller, 1998).

Preformed vitamin A is found almost exclusively in animal products, such as milk and dairy products, liver and fish liver oils (especially), egg yolk, while pro-vitamin A carotenes are found in green leafy vegetables, yellow vegetables and yellow and orange noncitrus fruits (Booth et al., 1992).

Preformed vitamin A is also used to fortify processed foods, which may include sugar, cereals, condiments and oils (Rodriguez-Amaya, 1997). Food fortification is one of the nutritional interventions used to improve the dietetic intake of the population and reduce the consequences of micronutrient deficiencies.

Meat and meat products are poor in vitamin A, except for liver and other organ meats, but are rich in saturated fats, that are cholesterol suppliers. Most of the processed meat products contain high amounts of fat, which are related to chronic diseases such as obesity and cardiovascular heart diseases. For this reason meat industry is interested in reduction of saturated fatty acids and cholesterol in processed meat products, so that a partial substitution of animal fat with vegetable oils is taking into account. Vegetable oils are characterized by a high level of mono- and polyunsaturated fatty acids, therefore some of these, such as olive oil, may be considered as a substitute for animal fat in processed meat products (Jiménez-Colmenero, 2007).

Our research considering the enrichment of some meat based products with sea-buckthorn oil, which has a high biological value due to a favorable mix of predominantly MUFA and PUFA and naturally occurring antioxidants including carotenes and vitamin E. The amount of provitamin A expressed as β-carotene was analysed in four samples of such meat products obtained by different preparation technologies using added sea-buckthorn oil.

The aim of the reported research was to adapt an experimental model easy to apply in laboratory for determination of provitamin A, a method suitable for such complex matrices as meat products, which contain varied proportions of water, meat and fats.

For more complicated matrices (presence of fats or proteins), sample preparation can be performed using various procedures, such as direct extraction, enzymatic hydrolysis or saponification followed by extraction with organic solvents. Traditionally, fat-soluble vitamin analysis in complex matrices is performed by alkaline saponification, in the presence of antioxidants, followed by liquid extraction with organic solvents such as hexane, petroleum ether, diisopropyl ether, chloroform (Mendoza et al., 2003; Jedlička et al., 2005). Saponification is used to facilitate extraction by releasing carotenoids from the sample matrix (Salo-Väänänen et al., 2000). Retinoids are thermo labile, photosensitive, and easily attacked by oxidants: oxygen, trace metals, daylight, excessive heat, therefore both the presence of an antioxidant and working in dark colour containers are necessary.

The absorbance of the obtained carotene extract is finally measured at 450 nm then quantification is realized with a calibration curve prepared with β-carotene solution.

MATERIALS AND METHODS

Three samples of meat based products containing sea-buckthorn oil (S1, S2, S3) and one sample with no supplement added (C), used as control, were analysed. The supplemented samples were obtained by different preparation technologies using the same proportion of sea-buckthorn oil.

The samples were also analysed after seven days of chilling storage at 4-6°C.

The determination method is based on Romanian standard 13058-91 which was adapted to a complex matrix such as meat products. The amount of carotene was spectrophotometrically determined and quantified as β-carotene.

Samples preparation. Weighted samples (1 to 5 g) were finely grounded in the presence of 1 g of ascorbic acid as antioxidant. After homogenization, the samples were transferred in tubes with 20 ml alcoholic KOH solution in order to perform a grease saponification. For this purpose the tubes were placed into 80˚C water bath for 30 minutes with 200 rpm agitation.

Extraction of β-carotene. After cooling, the saponified samples were transferred in separatory funnels and the extraction of β-carotene was performed several times with portions of 5 ml acetone each, till the last extract became colorless. The acetonic extracts were collected in another separatory funnel, and then 15 ml hexan were added and moderate stirred. Two layers are separated after a couple of minutes: the inferior layer, consists in acetone and water, was removed. The clear extract of β-carotene was collected in a volumetric flask and the volume was adjusted to 20 ml with hexane.

Quantification. Finally the absorbance was measured at 450 nm. Quantification was realized with a calibration curve prepared with 5 μg/ml β-carotene solution. The limit of quantification was 0.2 μg/ml β-carotene.

Statistical analysis was performed using statistical package ANOVA.

RESULTS AND DISCUSSIONS

Characterization of the analytical method

The amount of provitamin A was determined according to the described method in sample S2, which registered 0.211 mg/100g fresh weight β-carotene.

To ensure that the developed method is suitable for provitamin A analysis, some performance characteristics were determined: the precision expressed as repeatability and reproducibility within-laboratory, sensitivity.

The precision (variability) of an analytical procedure is usually expressed as the standard deviation (S) or coefficient of variation (=

relative standard deviation, RSD) of a series of measurements.

Repeatability expresses the precision under the same operating conditions over a short interval of time.

In order to establish these parameters five determinations were performed by the same analyst, on portions of 3g each of the same sample (S2) in the same conditions, at short time intervals.

The results (Table 1) allowed us to calculate the standard deviation, so the determined amount of β-carotene is 0.2186 ± 0.0079 mg/100 g F.W.

Table 1. Determination of repeatability as expression of precision of analytical procedure

Repetition	Provitamin A (β-carotene) mg /100g	Standard deviation	RSD %
1	0.211	0.00792	3.6251
2	0.225		
3	0.215		
4	0.213		
5	0.229		

Reproducibility (within-laboratory) expresses the precision obtained with the same method on identical test material under different conditions (execution by different analysts, with the same or different equipment, in the same laboratory, at different times).

For determination of this parameter the analysis were performed by three analysts using the same sample (S2), the same protocol, the same equipment (table 2), in the same day. In this case, the standard deviation was higher, so that the calculated amount of β-carotene was 0.2216 ± 0.0115 mg/100 g F.W.

Table 2. Reproducibility determination

Analyst	Provitamin A (β-carotene) mg/100g	Standard deviation	RSD %
A1	0.234	0.01159	5.2287
A2	0.211		
A3	0.220		

Sensitivity is a measure for the response of the instrument or of a whole method to the concentration of the analyte or property, e.g. the slope of the analytical calibration graph. We plotted the calibration graph in 10 points of the reference standard (β-carotene) (Figure 1). The slope must be constant on the working range and the value of correlation coefficient R^2 must be close to 1.

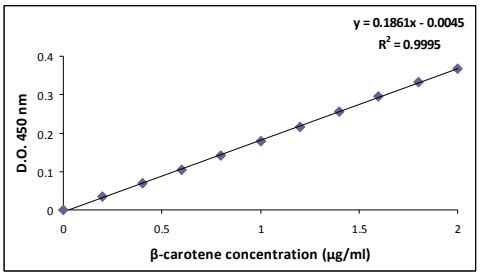

Figure 1. The calibration graph for provitamin A (β-carotene) determination

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected. The calibration graph reveals value 0.2 µg/ml β-carotene as limit of detection for our determination method.

Determination of provitamin A in the samples of meat based products supplemented with sea-buckthorn oil

The adapted method was tested on three samples of meat products enriched with provitamin A by adding sea-buckthorn oil. The control consisted in the same type of meat product without oil supplementation.

Table 3. Provitamin A (β-carotene) content in the samples of meat products supplemented with sea-buckthorn oil

Sample type	Provitamin A (β-carotene) mg/100g	
	Fresh sample	After 7 days chilling storage
C	0.003	0.007
S1	0.146	0.127
S2	0.218	0.195
S3	0.109	0.079

The obtained results (Table 3) revealed that the control sample (C) registered only 0.003 mg β-carotene/100 g F.W., value which indicates that such meat products contain almost no provitamin A. The amounts of provitamin A determined in the samples supplemented with sea-buckthorn ranged between 0.109 and 0.218 mg β-carotene/100 g F.W., depending on the preparation technology used.

As result of preserving the meat products by chilling storage at 4-6°C temperature, a decreasing of the amount of provitamin A registered in all the analysed samples after seven days. However, a differentiation was observed regarding the decreasing of the β-carotene content: sample S3 sample registered 27.52% loss of β-carotene, while in the sample S2 the loss was only 12.84% of β-carotene.

CONCLUSIONS

The results of the tests indicate that the performance characteristics of the method are according to the requirements for its practical application, therefore this method can be a practical procedure suitable for determination of provitamin A in complex matrices such as meat products.

Usually the meat based products contain no provitamin A (β-carotene). However, as result of supplementation of meat based products with sea-buckthorn oil, varied amounts of provitamin A (β-carotene) were determined in the sample analysed. The provitamin A content ranged between 0.109 and 0,218 mg/100g according to different preparation technologies applied.

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