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Effect of cooking on the concentration of Vitamins B in fortified meat products

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Abstract

B vitamins fortification of meat products is useful to compensate the loss of these compounds occurring during the heat treatment. The objective of this study was to evaluate the influence of heat treatments on the B vitamins concentration in fortified meat products. A rapid and reliable method for the simultaneous determination of Vitamins B_1, B_6 and B_{12} in homogenized boiled ham and in various fortified burgers was set up. Extraction procedure and HPLC method ensure low detection limits, good sensitivity and resolution. Results showed that cooking processes caused a decrease in the B vitamins content both in mild (70–90 °C) and severe (120 °C) conditions. Performing a fortification of 25 $\mu g\ g^{-1}$ the residual concentration of B vitamins after cooking allow to reach the recommended daily allowance, thus suggesting that B vitamins fortification of meat product is an useful practice.

Keywords: Fortified products; Boiled ham; Vitamins degradation; HPLC analysis

1. Introduction

Vitamins are a broad group of essential constituents of food required for the normal growth, self-maintenance and functioning of human and animal bodies [1]. These compounds can be classified in two main groups: water-soluble and fat-soluble vitamins. Among water-soluble vitamins, the B group including thiamin (Vitamin B_1), pyridoxine (Vitamin B_6) and cyanocobalamin (Vitamin B_{12}) are the most important. Each member of the B-complex has a unique structure and performs unique functions in the human body [2]. In particular, they play different specific and vital functions in metabolism, and their lack or excess produces specific diseases. The main source of vitamins for human beings is from foods [3]. B avitaminosis are usually attributed to nutritional deficiency, to competition between bacteria, to disturbances of the in intestinal flora and the excessive exposure to sunrays [4]. The loss of the naturally present vitamins in foods is mainly due to processing and storage [5].

Vitamins of the B group are easily excreted from human body with biological fluids. Therefore, they cannot be accumulated and the hypervitaminosis risk is practically absent [6] while the deficiency, especially of Vitamin B_{12} in the elderly, is more common [7]. B group vitamins are present in a lot of foods (see Table 1): yeast, mackerel, wheat flour, porridge oats, milk, powder milk, peas, liver, veal and pork [8].

Meat, especially pork, has long been recognized as a good source of B vitamins [9–12]. A serving of pork meat provides the daily thiamine requirement [13] and is one of the main sources of niacin in the Italian diet [14]. The nutritional value of meat products depends on the nature of the meat used, on the simultaneous presence of other ingredients, and on the method of preparation [15]. In particular, the cooking conditions strongly affect the concentration of trace elements and B vitamins in meat. Losses in both trace elements and B vitamins can occur during cooking processes so the amounts of these nutrients actually ingested with meat is greatly variable [15]. In fact, cooking can cause considerable losses in some essential nutrients, such as hydrosoluble vitamins, due to their high solubility and thermal instability [16]. Losses can also be the consequence of slaughtering methods [17].

To overcome the problems of deficiency food fortification with B vitamins is a quite common practice in western countries, particularly in the infant formulae. Fortification of meat formulae with vitamins is intended to compensate the loss of these

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Table 1
Content of B vitamins in hamburger raw and after cooking on grill, non-fortified and fortified (25 μg g^{-1}) with vitamins

Sample	Vitamins	Concentration (raw) (μg g^{-1})	Concentration (grilled) (μg g^{-1})
Cow meat	B_1	Not detected	Not detected
	B_6	Not detected	Not detected
	B_{12}	1.79	1.50
Boiled ham	B_1	3.18	1.90
	B_6	0.56	0.38
	B_{12}	0.54	0.42
Fortified cow meat	B_1	17.22	9.40
	B_6	18.29	14.10
	B_{12}	18.54	15.40
Fortified boiled ham	B_1	18.98	14.00
	B_6	22.50	16.50
	B_{12}	18.50	10.70

compounds occurring during the heat treatment. The question arise if this fortification is really effective; as the addition of vitamins could only results in an increased degradation during processing.

Traditional methods for B vitamins determination require the analysis of each vitamin individually by widely different methods, including colorimetric, fluorimetric, spectrophotometric and titrimetric techniques. Method selection depends on the accuracy and sensitivity required and on the interferences due to sample matrix [18]. To determine B vitamins the extraction steps usually consist of an acid hydrolysis and an enzymatic treatment [19–21]. A solid-phase extraction (SPE) technique using disposable C18 columns was described by Consiglieri and Amendola [22].

Recently, HPLC procedures have been set up to determine thiamine and riboflavin in infant formulae (liquid and powder infant milk) [3,5,21,23,24], but no references are available for the homogenized meat. In the present work, a study on the presence of Vitamins B_1, B_6 and B_{12} in raw and cooked homogenized boiled ham and burger fortified with B vitamins, was carried out. A rapid procedure for vitamins extraction, previously applied for powder infant milk [23], was used for meat products and coupled with a fast HPLC analytical procedure able to quantify in a single run thiamine, pyridoxine and cyanocobalamine. The objective of this study is to evaluate the influence of heat treatments on the concentration of B vitamins in fortified meat products, to establish the usefulness of this practice.

2. Experimental

2.1. Chemicals and reagents

Thiamine–HCl, pyridoxine–HCl, cyanocobalamine and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Glacial acetic acid, methanol and acetonitrile HPLC grade were obtained from Merck (Darmstadt, Germany); sodium octanesulfonate was from Fluka (Switzerland); triethylamine was from Aldrich (Steinheim, Germany).

2.2. Food samples preparation

Boiled ham, minced meat, maize starch and rice flour were purchased in a local market.

Homogenized meat: eighty grams of boiled ham was weighed and mixed with 43 ml of hot water, 5 g of maize starch and 1.6 g of precooked rice flour. All the ingredients were blended to obtain an homogeneous preparation and sealed in 100 ml glass pots. In fortified samples 25 μg g^{-1} of the B vitamins were added. The pots were put for 5, 10 and 15 min in boiling water or in autoclave at 120 °C for 20 min.
Burger: two typologies of burgers were prepared, one consisted of cow meat and the other of boiled ham minced, respectively, non-fortified and fortified with B vitamins (25 μg g^{-1}).

Cooking was performed placing the burgers on an aluminium foil using an electric plate. Cooking time was 15 min, 7.5 min per side of the burgers. The internal temperature was monitored during cooking introducing a digital thermometer (Oregon Scientific), that was inserted in the geometric centre of each burger. The core temperature at the end of the cooking time was 68–70 °C.

2.3. Vitamins B_1, B_6 and B_{12} extraction protocol

The procedure described by Albalà-Hurtado et al. [23] was followed with few modifications. Boiled ham and minced meat: 8.0 g of sample were accurately weighed into a 50 ml centrifuge tube. Then 1 g TCA solid were added. The mixture was thoroughly shaken and centrifuged for 10 min at 3000 rpm to separate the two phases. Subsequently, 3 ml of 4% TCA were added to the obtained solid residue, mixed and centrifuged, solid phase was discarded and the two acid extracts were combined and placed at −20 °C for 10 min. Acid extracts were centrifuged for 5 min at 4000 rpm and placed at −20 °C for 5 min to facilitate the outcrop of the fat, which was eliminated by a spatula. The acid extracts were again centrifuged and filtered through a 0.45 μm filter before the HPLC analysis.

2.4. HPLC analysis

A liquid chromatograph (SPD-M10A Shimadzu, Kyoto, Japan) equipped with diode array detector was used to record chromatograms and to calculate peak areas. The HPLC column used was a Luna 5 μm C_8 (250 mm × 4.6 mm) from Phenomenex (USA). The mobile phase system was as described by Lam et al. [26] for determination of water-soluble vitamins in multivitamin preparations. Phase A was octanesulfonic acid 5 mM, triethylamine 0.5%, acetic acid 2.4% and methanol 15%; phase B was acetonitrile 100%. The solutions were filtered through Nylon 0.45 μm before use. Analyses were carried out isocratically (90% A and 10% B) at room temperature at a flow-rate of 1 ml min^{-1} and with a 20 μl loop. A total

run time of 17 min is necessary for the elution of the three vitamins.

Standards of thiamine, pyridoxine, cyanocobalamine were prepared in 2.4% (v/v) aqueous acetic acid solution. All standard solutions and all samples were filtered through a 0.45 μm membrane, protected from light and stored at 4 °C.

Recovery was tested by the standard addition procedure; two spiking levels (25 and 12.5 μg g^{-1}) were used for each vitamin.

3. Results and discussion

3.1. Set up of the HPLC method

A chromatogram of the mixture of vitamins B standards and the profile of the extract obtained from a non-fortified boiled ham are shown in Fig. 1 (panels A and B), respectively. The extraction procedure facilitated the fat separation from boiled ham extract and increased the recovery and improved reproducibility. The developed procedure give an excellent linearity ($r > 0.999$; $r^2 > 99.9\%$) for all the vitamins. The mean recoveries obtained were satisfactory for both the fortification levels (25 and 12.5 μg g^{-1}) used for each vitamin in homogenize boiled ham. The recoveries were always higher than 83% for thiamine, 77% for pyridoxine and 73% for cyanocobalamin, respectively. The sensitivity was similar to that described in the literature with a limit of detection of 0.05 μg ml^{-1} for all the three analytes.

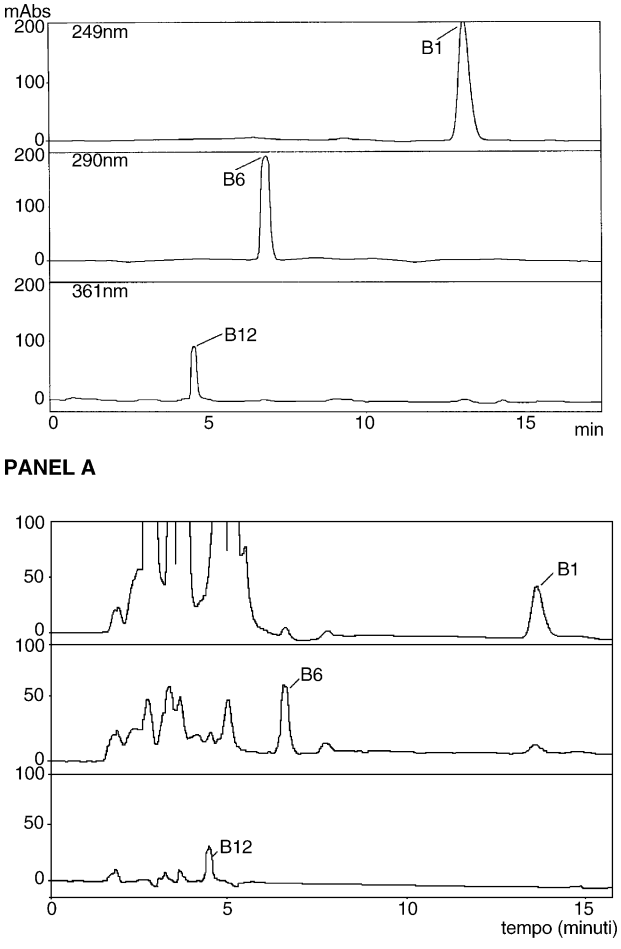

Fig. 1. Chromatograms of: (panel A) the mixture of B vitamins standards at a concentration of 100 ppm and (panel B) the extract from homogenized boiled ham. The B_1, B_6 and B_{12} vitamins are highlighted.

The developed analytical protocol prove to be reproducible, with an intraday variation below 5%; there were no overlapping among peaks and the chromatographic run is faster than the methods reported in the literature (Albalà-Hurtado to 40–60 min.). Moreno and Salvadò [1] obtained the separation of B vitamins using as mobile phase ammonium acetate (solvent A) and methanol (solvent B). The separation of the B vitamins is limited, in fact Vitamins B_1 and B_6 show a retention time at 2.8 and 2.5, respectively. Vinas et al. [5] used acid and enzymatic pretreatments before analyses performed using acetonitrile–phosphate buffer as mobile phase. The method was applied to the determination of nine vitamins. The separation of the B vitamins is satisfactory, but the method is time consuming both during extraction and determination (Vitamin B_{12} has a retention time of 27 min).

The method here used for B vitamins determination was simpler than the traditional method. In fact without application of acid hydrolysis and enzymatic treatment and without derivatization, necessary for fluorometric detection, we obtained satisfactory results and a good peaks separation. Moreover, the determination of the three B vitamins was carried out simultaneously.

3.2. Vitamins B in fortified boiled ham

The amount of B vitamins detected in the boiled ham was quite small. Pyridoxine and cyanocobalamine are present in small concentrations (0.56 and 0.69 μg g^{-1}, respectively) compared to the thiamine (3.18 μg g^{-1}).

As expected, cooking have a negative effect on B vitamins concentration in boiled ham: after 5 min of cooking at 100 °C all vitamins were not detectable. These results are in good agreement with data reported in literature [15,25]. Lombardi-Boccia et al. [15] measured B vitamins concentration before and after cooking among meats of different species. They found a strong decrease of B vitamins: a loss of 75% for thiamine, 42% for riboflavin and 35% for niacin in pork; a loss of 98% for thiamine, 65% for riboflavin and 63% in veal.

Interestingly, Prodanov et al. [25] performing a similar work on legumes, found a less pronounced decrease of B vitamins: 25% for thiamine and 32% for niacin, suggesting that the legume matrix has a protective effect on B vitamin

The following step of this work consisted in the monitoring of the extent of vitamin degradation on B vitamin fortified products. Samples of homogenized boiled ham added of 25 μg g^{-1} of the B vitamins were investigated. The pots were boiled for 5, 10 and 15 min. Results are summarised in Fig. 2. A strong reduction of 49, 33 and 51% of Vitamins B_1, B_6 and B_{12}, respectively, was obtained after 5 min of treatment, while prolonging the thermal treatments up to 15 min no further degradation was detectable. Data also show that thiamine and cyanocobalamine were more susceptible than pyridoxine to the thermal degradation. When a more severe treatment, namely 120 °C for 20 min was performed

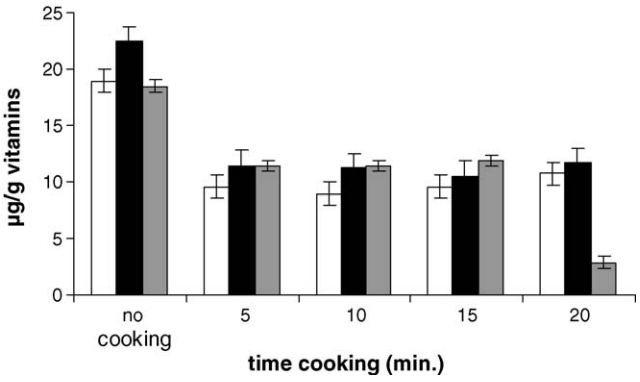

Fig. 2. Effect of heat treatments on the concentration B group vitamins. Homogenized boiled ham was fortified by addition of 25 $\mu g\,g^{-1}$ of Vitamins B_1, B_6 and B_{12} and cooked in glass pots at 100 °C for 5, 10 and 15 min or in autoclave at 120 °C for 20 min. White bars, Vitamin B_1; black bars, Vitamin B_6; gray bars, Vitamin B_{12}.

the extent of degradation remain constant for Vitamins B_1 and B_6 while the Vitamin B_{12} (loss of 84.4%) was almost completely degraded also from the fortified sample.

A different thermal treatment, i.e. grill cooking, was performed using burgers made of cow meat and boiled ham, respectively, non-fortified and fortified with B vitamins (25 $\mu g\,g^{-1}$).

Data obtained before and after grilling are reported in Table 1. After cooking on grill, thiamine was degraded of 40% in boiled ham and fortified cow meat; the degradation was slower (26%) in fortified boiled ham. The pyridoxine was more stable of thiamine; it showed degradation values from 32% (boiled ham) to 23% (fortified cow meat). Data also show that cyanocobalamine in this test was more stable than severe treatment (120 °C) described previously, in fact it showed degradation values from 42% (fortified boiled ham) to 17% (fortified cow meat).

These data show that the extent of B vitamin degradation depends, beside the molecular nature, also on the type of thermal treatment and on the food matrix.

4. Conclusions

This study contributes to update the literature data about the B vitamins content of homogenized boiled ham and burgers of boiled ham and cow meat. Extraction procedure and HPLC method used for determination of B vitamins in meat ensure low detection limits, good sensitivity and resolution within an analysis time of 17 min. Cooking, in general, produced a decrease in the B vitamins content both in mild (70–90 °C) and severe (120 °C) conditions. In fact, after 5 min of cooking at 100 °C all B vitamins analyzed were not detectable in the homogenized boiled ham without fortification. A reduction of B vitamins after only 5 min of treatment was observed also in fortified product. Severe treatment degraded nearly completely Vitamin B_{12}. However, considering the residual amount of B vitamins after cooking, it can be concluded that the fortification of meat product is an useful procedure to increase their dietary intake.

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