Acta Univ. Sapientiae, Alimentaria, **2**, 1 (2009) 81–88

Influence of the microwave heating on the water soluble vitamin and D-amino acid content of meat

J. Csapó[1,2]
email: csapo.janos@ke.hu

Sz. Salamon[2]
email: salamonszidonia@sapientia.siculorum.ro

É. Varga-Visi[1]
email: visi@ke.hu

Zs. Csapó-Kiss[1]
email: csapo.janosne@ke.hu

[1]University of Kaposvár,
Faculty of Animal Science,
Guba S. u. 40, 7400 Kaposvár, Hungary

[2]Sapientia–Hungarian University of Transylvania,
Csíkszereda Campus, RO-530104, Libertatii 1., Miercurea-Ciuc

Abstract. The authors examined the vitamins B_1, B_2 and C content of meatballs and also the D-aspartic acid and D-glutamic acid content plotted against the cooking time. The vitamin B_1 mixed in 50 mg/100 g concentration to the normal-sized meatball changed minimal in 10 minutes, but during 20 minutes baking, 70% decomposed. The vitamin B_2 seems to resist more against microwave treatment since its concentration in 10 minutes reduced from 50 mg/100 g to 43 mg/100 g, and after 20 minutes of microwave treatment it reduced to 35 mg/100 g. The vitamin C content of the meatballs (50 mg/100 g initial concentration), applying the two methods of our own was 20–22 mg/100 g after

Key words and phrases: meat, microwave treatment, vitamins, vitamin B_1, vitamin B_2, vitamin C, D-aspartic acid, D-glutamic acid

10 minutes and 13–14 mg/100 g after 20 minutes. No significant differences were found in the vitamin content of inside and outside of the meatball. The D/L aspartic acid ratio was 0.048 in the non-treated sample, the D/L glutamic acid ratio was 0.027. This ratio concerning the aspartic acid increased to 0.069 in 10 minutes, 0.151 in 20 minutes, and concerning the glutamic acid increased to 0.042 in 10 minutes, and 0.047 in 20 minutes.

1 Introduction

Several articles were published in the scientific press on the harmful effects of microwave and heat treatment concerning the vitamin content of foods [1, 2, 3, 4, 5, 7, 12, 10, 16]. Several articles are also known according to which microwave and heat treatment can promote the amino acid racemisation [14, 15]. In the case of baby-foods such an account was given that due to microwave treatment D-allo-hidroxiproline was formed in a great contentration [13]. Japanese researchers also presented that the solutions of L-amino acid due to microwave treatment racemised completely which was used to produce D-amino acid from L-amino acid [8, 9]. Since, as far as we know, no such investigations were done in Hungary, we have set ourselves the task to analyse the changes of water-soluble vitamins B_1, B_2 and C under the influence of microwave treatment. From the same samples the greatest proportion of protein, the aspartic acid's and the glutamic acid's D-enantiomers were also determined examining the effects of microwave treatment on the racemisation of amino acids.

2 Material and methods

Preparation of meatballs 5 kg non-fatty pork chop was minced on a laboratory grinding mill, it was made homegeneous, and then 600 g of the homogenised material was used for our experiments. For the minced meat as much vitamins B_1, B_2 and C was given in 1% concentration that 100 g of the meatball made of the homogenised material consisted of 50 mg vitamin. The meatball enriched with vitamins were treated in microwave oven for 2, 5, 10, 15 and 20 minutes on 750 W energy, then after cooling down the samples were equalized with laboratory homogeniser, and the vitamin content and also the D-amino acid content was analysed from these homogenised samples. The experiments were completed, parallel with the homogenised sample, in the inner and outer layer of the meatball.

Vitamin determination Before the determination of vitamins B_1, B_2 and C 50 ml extraction mixture was added to 5 g homogenised sample, which was assembled from the water solution of 800 ml 1% KH_2PO_4 and 200 ml metanol. After making the mixture the pH was adjusted to 3. The samples were treated in ultrasonic bath for 5 minutes for 3 times with 2 minutes intervals, then were filtered with the help of Büchner funnel and water jet pump. The material remained on the filter was washed with 2×5 ml extraction mixture, then the filted material was vacuum dried, centrifuged for 10 minutes in 5000 g, and 20 μl from the supernatant was injected to the 250×4 mm RP-18 column purospher of the MERCK Hitachi LaChrom HPLC. The separation of the vitamins was made with pH=2.8; 0.04 M and pH=2.8; 0.02 M phosphate puffer and acetonitril according to the following gradient *(Table 1).*

Table 1: Gradient used for the separation of vitamins B_1, B_2 and C

Time	**0.04 M phosphate buffer**	**0.02 M phosphate buffer**	**Acetonitril**
0	100	0	0
2	100	0	0
3	0	98	2
8	0	88	12
12	0	83	17
16	0	83	17
16.1	0	50	50
26.0	0	50	50
26.1	100	0	0
30.0	100	0	0

The detection was made on 254 nm with LaChrom UV-detector. The flow rate was 1 ml/min. Parallel with the HPLC determination the vitamin C content was determined with the traditional 2,6-dichlorophenol-indophenol reaction.

D-amino acid determination The determination of the D-amino acid content of the meatballs was made with HPLC after the 6 M HCl hydrolysis, and after OPA/TATG precolumn derivatization with fluorescent detection besides 325 nm extinction and 420 nm emission wavelength [6, 11].

Evaluation of results The results were evaluated with the help of Microcall origin programpackage.

3 Results

Evaluating the results of our experiments for determining vitamins B_1, B_2 and C and for separating vitamins B_1, B_2 and C we can ascertain that the separation of the mentioned vitamins is good, no overlaying emerged disturbing the determination. *Table 2* shows the formation of dry material, vitamins B_1, B_2 and C content plotted against microwave treatment.

Table 2: Formation of dry material, vitamins B_1, B_2 and C content plotted against microwave treatment

Baking time (minutes)	vitamin B_1 (mg/100 g)	vitamin B_2 (mg/100 g)	vitamin C (HPLC) (mg/100 g)	vitamin C (titration) (mg/100 g)
0	49.02	49.14	49.07	48.76
2	48.89	47.82	41.24	39.66
5	48.73	45.97	34.34	29.60
10	48.79	43.22	22.70	19.44
15	32.26	35.55	16.72	14.96
20	14.57	34.90	12.84	13.79

Evaluating the data of *Table 2,* which shows the formation of vitamin content plotted against baking time, we can state that the vitamins B_1, B_2 and C content of the untreated sample is around 49 mg/100 g, so the recovery of the added vitamins is almost 100% in the raw, untreated sample. No significant changes were experienced until the 10^{th} minute, after 10 minutes of treatment in the 15^{th} minute, the vitamin content decreased to 32, in the 20^{th} minute to 15 mg/100 g. In the case of vitamin B_2 the concentration decreased to 43.2 in 10 minutes, and in 20 minutes to 34.9 mg/100 g. It seems that the vitamin B_2 bears the long lasting microwave treatment better than the vitamin B_1, in 5–10 minutes no considerable decomposition is to be expected concerning the vitamins. The situation is completely different concerning vitamin C, which decreases to 41.2 in 2 minutes, 34.3 in 5 minutes, 22.7 in 10 minutes, 16.7 in 15 minutes and 12.8 mg/100 g in 20 minutes. It seems that vitamin C is much

more sensitive to microwave treatment than vitamins B_1 and B_2. It is clearly demonstrated in the table that there is no significant difference between the vitamin content determined by HPLC and the classic method determined by titration, so in the case of meatballs for analysing the added vitamin C content both the HPLC and the titration method is convenient.

The effect of the microwave treatment on the D-Asp and D-Glu content of the meatballs is shown in *Table 3.*

Table 3: The effect of microwave treatment on the D-Asp and D-Glu content of meatballs

Baking time (minutes)	**D/L-Asp**	**D/L-Glu**
0	0.0480	0.0270
2	0.0699	0.0376
5	0.0664	0.0380
10	0.0689	0.0417
15	0.1439	0.0442
20	0.1506	0.0466

The D/L aspartic acid ratio of the untreated meatballs was found 0.048, and the D/L glutamic acid ratio 0.027. This initial hydrolysis resulted very likely due to the protein hydrolysis, derivatization and during the determination of D-amino acids. Under the influence of 2 minutes of treatment in the case of aspartic acid it decreases to 0.07, concerning glutamic acid to 0.038 and this ratio is not practically likely to change until 10 minutes of treatment. Major racemisation results at the aspartic acid between the 10 and 15 minutes - treatment, and the maximum of the D/L aspartic acid ratio is reached at 20 minutes treatment with 0.151. The D/L ratio of the glutamic acid after 15 minutes of treatment is 0.044 and after 20 minutes is 0.047. It seems so that racemisation of the aspartic acid is much more considerable than of the glutamic acid, under our applied circumstances. In the case of 20 minutes of microwave treatment we did not get any considerable amount of D-amino acids neither concerning aspartic acid nor glutamic acid, but in the case of exceeding the ten minutes treatment 15% of the aspartic acid, 4–5% of the glutamic acid changed into D-enantiomers.

Parallel to our previous experiments we analysed the vitamin content of

the meatballs' outer 1 mm thick layer and its inside, and also its D-amino acid contents. We ascertained that the water soluble vitamin content of the skin is a little higher than that of the inner parts, which is probably due to the movement of the water taking the vitamins and then evaporating on the surface. Considering D-amino acids in the case of the aspartic acid on the influence of 10 minutes of treatment no significant difference was experienced between the skin and the inner parts, neither concerning aspartic acid, nor glutamic acid, but in the 20^{th} minute it seems that the surface of the meatball contains substantially more D-aspartic acid and slightly more D-glutamic acid than its inner parts.

From our researches we can come to the conclusion that due to the impact of microwave treatment used in households, out of the 3 water soluble vitamin analysed by us, vitamins B_1 and B_2 hardly changes, but vitamin C can decrease about 20% even at two minutes of microwave treatment. 10 minutes of microwave treatment destroys more than half of vitamin C. Concerning D-amino acids, 10 minutes of microwave treatment just slightly increases the amount of D-amino acids, while due to a longer microwave treatment the amount of D-amino acids can be considerable. Further on we would like to measure the change in the concentration of vitamin B_6 and B_{12} during microwave treatment, and we would also like to complete the microwave treatment with other sorts of food-products and analyse its effects on the vitamin and D-amino acid content.

4 Acknowledgements

Authors are grateful to the Sapientia Foundation, Institute of Research Programs for the financial support.

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Received: August, 2008