# Furan and 5-hydroxymethylfurfural removal from high- and low-moisture foods



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**Furan and 5-hydroxymethylfurfural removal from high- and low-moisture foods**

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**ABSTRACT**

The possibility to remove furan and 5-hydroxymethylfurfural (HMF) from meat sauce and biscuits by means of the application of vacuum treatments was studied. These foods were chosen because differing in moisture and fat contents. Three different pressure levels (i.e. 4, 12 and 19 kPa) were applied for increasing lengths of time. Results showed that the vacuum treatments were ineffective in removing HMF from both food types, as well as furan from the biscuits, unless this food was preliminary hydrated at high water activity values. On the contrary, the vacuum treatments allowed furan to be removed from the high moisture food. In particular, 67% furan removal from the meat sauce was achieved by applying 12 kPa for 10 min. Sensory analysis results showed that meat sauce subjected to such a treatment presented the same odor intensity of the untreated sample. The results clearly showed that the post-process vacuum treatment could represent a reliable strategy to mitigate the furan levels in high moisture foods.

*Keywords:* Furan, 5-Hydroxymethylfurfural, Meat sauce, Biscuits, Sensory properties, Vacuum

*Highlights*

Vacuum treatments removed furan, but not HMF, from high moisture, low fat food, i.e. meat sauce

Short time vacuum treatments did not affect the meat sauce sensory properties

Furan and HMF could be removed from low moisture food by vacuum treatment

## 1. Introduction

Furan and 5-hydroxymethylfurfural (HMF) are heterocyclic compounds that are formed in a variety of heat-treated commercial foods (Maga, 1979; Morales, 2009; EFSA, 2011) where they can significantly contribute to the sensory properties. In particular, HMF can be formed as intermediate in the Maillard reaction, which occurs when reducing sugars are heated in the presence of amino acids or proteins, or by thermal dehydration of a sugar under acid conditions (Mauron, 1981; Kroh, 1994). Even more pathways of furan formation in model systems and foods have been elucidated, involving carbohydrates, amino acids, carbohydrate-amino acid mixtures, vitamins, polyunsaturated fatty acids and carotenoids as precursors (Becalski & Seaman, 2005; Crews & Castle, 2007; Fan, Huang, & Sokorai, 2008; Limacher, Kerler, Conde-Petit, & Blank, 2007; Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008, Owczarek-Fendor et al., 2012; Perez-Locas & Yaylayan, 2004; Senyuva & Gokmen, 2007).

Although furan has been classified as “possibly carcinogenic to humans” (IARC, 1995) and HMF was supposed to induce genotoxic and mutagenic effect in bacterial and human cells and promote colon cancer in rats (Monien, Engst, Barknowitz, Seidel, & Glatt, 2012), the risk associated with the furan and HMF exposure has not been elucidated yet with certainty (EFSA, 2011). Nevertheless, due to their widespread presence in foods, furan and HMF have generated great concern, and a number of strategies are reported in the literature to keep their levels as low as reasonably achievable (Crews & Castle, 2007). However, only a limited number of them finds practical application at the industrial level. A limiting factor to their exploitation is that the formation of these heat-induced toxicants is concomitant with the development of color, flavor and texture. Therefore it is difficult to minimize their generation without compromising the sensory acceptability of the food. Mitigation of furan and HMF levels in food can be achieved by means of preventive or removal interventions (Anese & Suman, 2013). The former are aimed to minimize furan and/or HMF formation during the heating process, by means of the decrease of precursor concentration or formation rate; the removal interventions are aimed to move away or decompose the already formed molecules in the finished product. Among the removal strategies, the vacuum technology has been already studied as a tool to remove furfural, HMF and acrylamide from different foods (Anese, Suman, & Nicoli, 2010; Quarta & Anese, 2012; Zhaoyang, 2008). According to the results of these studies the efficacy of the vacuum treatment greatly depends on the molecule nature as well as on the food composition and physical state. By applying a same combination of temperature, pressure and time conditions, higher levels of furfural were removed from coffee as compared to HMF, due to differences in the chemical and physical properties of the two molecules. Moreover, acrylamide removal by vacuum treatments was not possible from dry foods, such as coffee and biscuits, due to viscosity contrains that limit molecule diffusion through the matrix. On the contrary, food hydration before the vacuum process allowed the molecule to be effectively removed.

The aim of the present study was to investigate the possibility to physically remove furan and HMF from foods differing in their chemical composition. To this purpose meat sauce and biscuits with different water and fat contents were chosen. Although the highest furan and HMF concentrations were found in coffee products, jarred foods and cereal products may also contribute to the furan and HMF contents of the diet (EFSA, 2011). Samples were subjected to vacuum treatments consisting in the application of different pressures for increasing lengths of time and subsequently analyzed for their furan and HMF concentrations. As the vacuum treatment may cause loss of volatile compounds, the effect of this technology on meat sauce and biscuits sensory properties was also evaluated.

## 2. Materials and methods

### *2.1. Sample preparation*

Commercial short dough biscuits and meat sauce were chosen for experiments on furan and HMF removal, by virtue of the differences in their chemical composition, i.e. water and lipid content. Their average compositions reported in the labels are shown in Table 1.

Previously hydrated biscuits were also considered. To this purpose, weighed Petri dishes containing the whole biscuits (approximately 10 g) were introduced in vacuum desiccators saturated with water vapors. Samples were left in the desiccators for the time (about 48 h) necessary to reach the desired water activity. After hydration, samples were immediately subjected to the experiments at low pressure.

*2.2. Vacuum treatments*

Experiments were carried out by using an apparatus consisting of an oven (5Pascal, VS-25 SC, Trezzano S/N, Milano, Italy), connected to a vacuum pump (BOC Edwards, E2M40, Crawley, West Sussex, UK). The samples, previously weighed (approximately 10 g) in aluminum dishes, were introduced in the oven once the desired temperature was reached. Afterwards, the vacuum pump was immediately switched on. The time needed to achieve the desired vacuum ranged from 20 to 40 s depending on the set pressure value and the water content of the samples. In all cases, computation of treatment duration started once the set pressure value was achieved. Treatments were carried out at pressures of 4, 12 and 19 kPa at 30 °C or 60 °C for 10, 30 and 60 min. After the treatments, the samples were immediately removed from the oven, and stored at -18 °C until the analyses were performed.

*2.3. Analytical procedures*

*2.3.1. Furan concentration*

Furan determination was carried out by combining SPME and GC-MS analysis according to slight modifications executed on the method of Bianchi, Careri, Mangia, and Musci (2006). SPME experiments were performed with a 85 µm carboxen-polydimethylsiloxane (CAR-PDMS) fiber (Supelco, Bellfonte, PA, USA). Aliquots of 2 g of samples were added with 2 mL NaCl 20% (w/w) water solution of $d_4$-furan (internal standard with a concentration equal to 30 µg/kg) and were placed in 20 mL sealed vials. Incubation time and temperature of the fiber were 5 min and 40 °C, respectively. The fiber was then exposed to the headspace of the vial operating under the optimized extraction conditions, i.e. extraction temperature equal to 40 °C and extraction time equal to 20 min. A constant magnetic stirring was always applied. Desorption was carried out at 270 °C for 2 min. Two fiber blanks were run between each sample to avoid potential "memory effects". An ultra Thermo TRACE GC (Thermo Scientific, Waltham, MA, USA) equipped with a DSQ II detector (Thermo Scientific, Waltham, MA, USA) was used for GC-MS analysis. Helium was used as the carrier gas at a flow rate of 1 mL/min; the gas chromatograph was operated in splitless mode with the PTV injector maintained at 270 °C and equipped with a PTV multi-baffled liner (i.d. 1.5 mm, Thermo Scientific, Waltham, MA, USA). A Rxi-5ms (5% diphenyl 95% dimethylpolysiloxane) (30 m x 0.25 μm, 0.5 μm) capillary column (Thermo Scientific, Waltham, MA, USA) was used. The following GC oven temperature program was applied: 40 °C for 5 min, 15 °C/min to 300 °C. Transfer line and source were maintained at 270 °C and 200 °C, respectively. The mass spectrometer was operated in selected-ion monitoring mode (SIM) by recording the current of the following ions: *m/z* 68 and 39 for furan and *m/z* 72 and 42 for $d_4$-furan. The corresponding ion ratios were used to confirm the identification of the analyte. A dwell time of 50 ms was used for all the ions. Preliminarily, full scan EI data were acquired to determine appropriate masses for SIM under the following conditions: ionization energy: 70 eV, mass range: 35-150 amu, scan time: 3 scan/s. All the analyses were performed setting the electron multiplier voltage at 1500 V. Signal acquisition and elaboration were performed using the software Xcalibur (Thermo Scientific, Waltham, MA, USA).

*2.3.2. HMF concentration*

HMF was determined by HPLC according to the slightly modified method of García-Villanova, Guerra-Hernández, Martínez-Gómez, and Montilla (1993). Briefly, 1 or 5 mL of water Milli Q (Millipore, Italy) respectively were added to 1 g of meat sauce or ground biscuit into a 100 mL centrifuge tube. The sample was mixed with Polytron (Polytron PT-MR 3000, Kinematica AG, Littau, Switzerland) at 3200 x g for 2 min and clarified with 0.5 mL each of Carrez I and Carrez II solutions. The resulting mixture was centrifuged at 9500 x g for 15 min at 4 °C (Beckman, Avanti Centrifuge J-25, Palo Alto, CA, USA) and subsequently filtered through a 0.45 µm membrane filter before the HPLC analysis.

A HPLC system Varian Pro Star (model 230, Varian Associates Ldt., Walnut Creek, CA, USA) equipped with a Varian Pro Star photodiode array detector (model 330, Varian Associates Ldt., Walnut Creek, CA, USA) was used. A Econosil C18 column (Alltech, Deerfield, IL, USA), 250 mm length, 4.6 mm internal diameter, 10 µm granulometry was used. Injection volume was 20 µL and the mobile phase, delivered at a flow rate of 1 mL/min, consisted of 90% water and 10% methanol (Carlo Erba, Milano, Italy) in isocratic conditions. The detection wavelength was 280 nm. The external method was used for the determination of HMF content. The linearity of the HPLC method used was tested in the concentration range of 1-150 mg/kg by means of HMF (Sigma-Aldrich, Milano, Italy) standard diluted with distilled water. Peak integration was performed by the Software Chromatography Star IC (5.3 version).

*2.3.3. Total solid content*

Total solid content was determined by gravimetric method by drying the samples under vacuum (1.3 kPa) to constant weight (AOAC, 1995). As respect to the official method, drying was carried out at 75 °C instead of 100°C, to avoid losses due to non-enzymatic browning and pyrolysis reactions.

*2.3.4. Water activity*

Water activity ($a_w$) was determined by means of a dew-point measuring instrument (AQUA LAB, Decagon, Pullman, WA, USA) at 25 °C.

*2.3.5. Sensory analysis*

The procedure described by Manzocco and Lagazio (2009) was followed. A panel of ten Italian assessors was selected. Judges were aged between 18 and 60 years and approximately balanced between males and females. They all had a minimum of 2 years of experience in discrimination and descriptive sensory methods. For sensory testing, 5 g of sample were served in 50 mL capacity odorless plastic cups at ambient temperature. Samples were indicated by a three-digit code and submitted to the panel paired with a reference (untreated) sample. Assessors were asked to sniff the samples after the reference one and evaluate the intensity of odor, differentiating the treated sample from the reference one on a 9-cm unstructured scale anchored with “high”. Due to meat sauce and biscuit persistent flavor only two samples were evaluated each session and assessors evaluated samples twice on different sessions.

*2.4. Statistical analysis*

Analyses were carried out at least twice on two replicated experiments. Results are presented as mean value ± SD. Coefficients of variation, expressed as the percentage ratio between the standard deviations and the mean values, were lower than 18 for furan, 15 for HMF, and 1 for total solid content and $a_w$.

Analysis of variance was carried out with significance level set to $P<0.05$ (STATISTICA for Windows, 5.1, Statsoft Inc., Cary, NC, USA). The Tukey procedure was used to test for differences between means.

## 3. Results and discussion

Fig. 1 shows furan concentrations of meat sauce samples subjected to treatments at 4, 12 or 19 kPa and 30 °C for increasing lengths of time. The vacuum treatment caused a significant decrease in furan concentration. In particular, after 10 min the removal varied from 54% to 67% depending on the pressure applied. As expected, the lowest removal was achieved by carrying out the vacuum treatment at the highest pressure (19 kPa). By prolonging the time, no significant or slight further removal was observed. Similar results were obtained by carrying out the vacuum treatments at 60 °C instead of 30 °C (data not shown). By contrast, no changes of HMF concentration were observed in the meat sauce samples subjected to the vacuum treatments. In fact, the HMF concentrations of the vacuum treated meat sauce samples, ranging from 77 ± 9 $mg/kg_{dm}$ to 104 ± 16 $mg/kg_{dm}$, were not significantly different from that of the control sample (66 ± 11 mg/kg$_{dm}$). The diffusion rates of furan and HMF through the food matrix are supposed to be different due to their different molecular weight (Goubet, Le Quere, & Voilley, 1998). By virtue of its lower molecular weight, furan would diffuse through the matrix and reach the meat sauce surface faster than HMF. As a result, in our experimental conditions, only furan was removed from the meat sauce, while HMF was mostly retained.

Table 2 shows the moisture and $a_w$ values of the meat sauce samples subjected to treatments at 4, 12 or 19 kPa and 30 °C for increasing lengths of time. It can be observed that the lower the pressure and the longer the time, the greater the moisture and $a_w$ decrease. As expected the minimum moisture and $a_w$ values (i.e. 70.8% and 0.969) were obtained by applying 4 kPa for 60 min. It is noteworthy that the 10 min treatments, which allowed a great furan loss to be achieved, did not cause significant moisture and $a_w$ changes as compared with the control sample.

The effect of the vacuum treatments on furan and HMF levels in biscuits was also investigated. No significant changes in furan and HMF concentrations were found in biscuits subjected to the vacuum treatments at 4, 12 or 19 kPa and 30 °C for 10 min (Fig. 2). These results are in agreement with previous findings showing that these molecules cannot be removed from dry matrices, due to the viscosity constrain which limits the molecules diffusion through the matrix (Roos & Karel, 1991). Moreover, the high lipid content of the biscuits would contribute to hurdle the molecules diffusion (Van Lancker, Adams, Owczarek, De Meulenaer, & De Kimpe, 2009).

Additional trials were carried out on biscuits hydrated to water content and activity respectively of 16.9% ± 0.7 and 0.819 ± 0.002 prior the vacuum treatment. The hydration step caused a 94% decrease in furan concentration, while no differences in HMF levels were found between the hydrated and non-hydrated biscuits. The subsequent vacuum treatment at 4 kPa and 30°C for 10 min did not caused any appreciable further furan loss, while it allowed 50% HMF reduction to be achieved (data not shown). Following this treatment biscuits with moisture and $a_w$ values of 12.7 ± 0.1 and 0.721 ± 0.003 were obtained. Such values are far away from the desired initial ones (moisture, 3.0% ± 0.1; aw, 0.196 ± 0.003), which can be achieved only by prolonging the vacuum treatment, to the detriment of the food sensory properties.

In order to study the effects of the vacuum treatments on meat sauce and biscuit quality, sensory analysis by sniffing of the treated samples compared with the control ones was performed. Fig. 3 shows the odor intensities of meat sauce and biscuits samples undergone the vacuum treatments at 4, 12 or 19 kPa and 30°C for 10 min. It can be observed that the meat sauce subjected to 4 kPa was perceived with lower odor intensity than both the reference (untreated) sample and those treated at higher pressures. Moreover, meat sauce samples undergone the treatments at 12 or 19 kPa were not judged different from the control one. No significant differences in odor perception among the biscuits were found. It can be inferred that the glassy state as well as the high lipid content of this food product contributed to hurdle molecule mobility; therfore not only furan and HMF removal but also flavor release were negligible. By increasing the length of the vacuum treatment at 12 kPa, meat sauce samples were perceived progressively with lower intensity than the reference one, especially after 60 min of treatment (Fig. 4). In the case of biscuits, a significant decrease in odor perception was found only in the 60 min treated sample.

## 4. Conclusions

The results of this study confirmed previous findings in that furan and HMF removal cannot take place in dry foods such as biscuits, due to viscosity constrains. Therefore, a hydration step of the dry food to high $a_w$ prior the vacuum treatment is necessary to allow the molecules to be removed. It is noteworthy that the vacuum treatment of hydrated foods favors not only toxicants escape but also the release of flavor compounds. By contrast, the application of the vacuum treatment effectively removed furan from the meat sauce having a high moisture content. In fact, under the process conditions adopted in the present work (i.e. 12 kPa for 10 min), this technology led to an efficient reduction of the undesired molecule without affecting the food sensory properties and its overall quality. In the light of these results, the application of vacuum treatments for furan mitigation in high-moisture, low fat food formulations could be a reliable strategy for the industrial exploitation.

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**Figure Captions**

**Fig. 1**. Furan concentration of meat sauce samples subjected to vacuum treatment at 4, 12 or 19 kPa and 30 °C for increasing lengths of time.

**Fig. 2**. Furan and HMF concentrations of biscuits subjected to vacuum treatment at 4, 12 or 19 kPa and 30 °C for 10 min. Different letters indicate significant difference ($P<0.05$).

**Fig. 3**. Odor intensity of meat sauce and biscuit samples subjected to vacuum treatment at 4, 12 or 19 kPa and 30 °C for 10 min. Different letters indicate significant difference ($P<0.05$).

**Fig. 4**. Odor intensity of meat sauce and biscuit samples subjected to vacuum treatment at 12 kPa and 30 °C for increasing lengths of time. Different letters indicate significant difference ($P<0.05$).

**Table 1**

Average composition of commercial biscuits and meat sauce, as reported in the respective labels.

| Food component | Meat sauce (g/100 g) | Biscuits (g/100 g) |
|---|---|---|
| Protein | 5.0 | 8.0 |
| Carbohydrate | 6.6 | 56.6 |
| Fat | 5.0 | 19.9 |
| Water | 80.7 | 3.0 |
| Fiber | 0.0 | 11.0 |
| Other minor ingredients | 2.7 | 1.5 |

**Table 2**

Moisture and $a_w$ values of meat sauce samples subjected to vacuum treatments at 4, 12 or 19 kPa and 30 °C for increasing lengths of time.

| Vacuum treatment | | Moisture (%) | $a_w$ |
|---|---|---|---|
| Pressure (kPa) | Time (min) | | |
| Control | | 80.9±1.9[a] | 0.988±0.002 [a] |
| 4 | 10 | 80.5±0.2[a] | 0.986±0.001[a] |
| | 30 | 76.9±3.5[b] | 0.981±0.001[b] |
| | 60 | 70.8±1.6[c] | 0.969±0.003[c] |
| 12 | 10 | 82.3±0.2[a] | 0.991±0.002[a] |
| | 30 | 80.4±0.2[a] | 0.987±0.002[a] |
| | 60 | 76.2±2.7[b] | 0.979±0.002[b] |
| 19 | 10 | 81.7±0.4[a] | 0.990±0.000[a] |
| | 30 | 80.5±0.7[a] | 0.987±0.001[a] |
| | 60 | 77.7±2.6[b] | 0.982±0.001[b] |

Data are the mean of two repetitions on two replicated samples ± sd

[a,b,c] Means with the different letter in the same column are significantly different ($P<0.05$) by Tukey test.

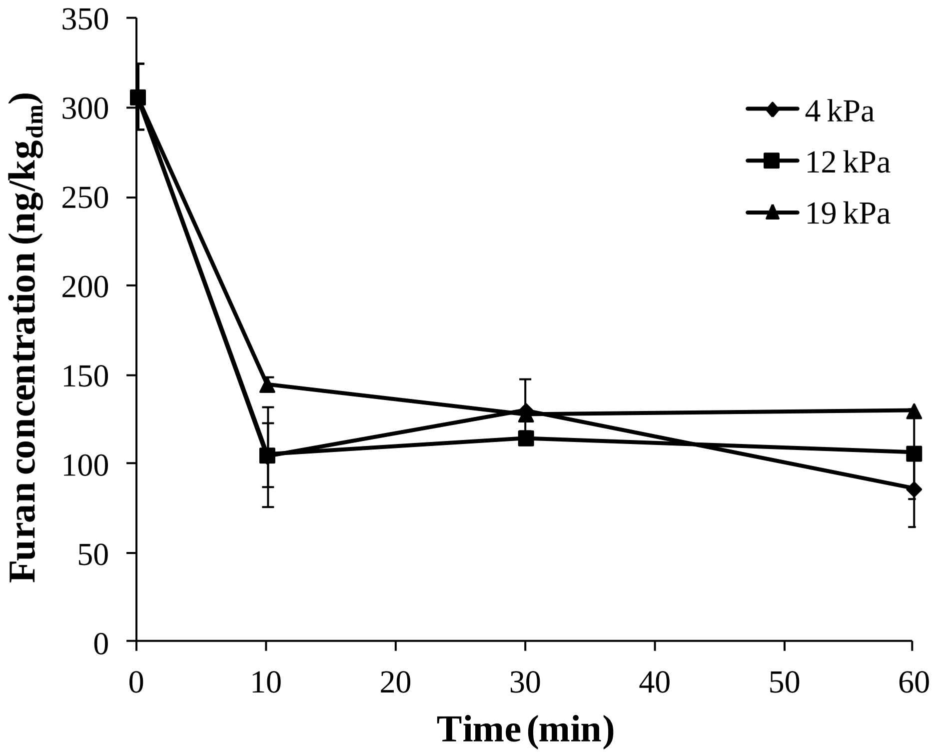
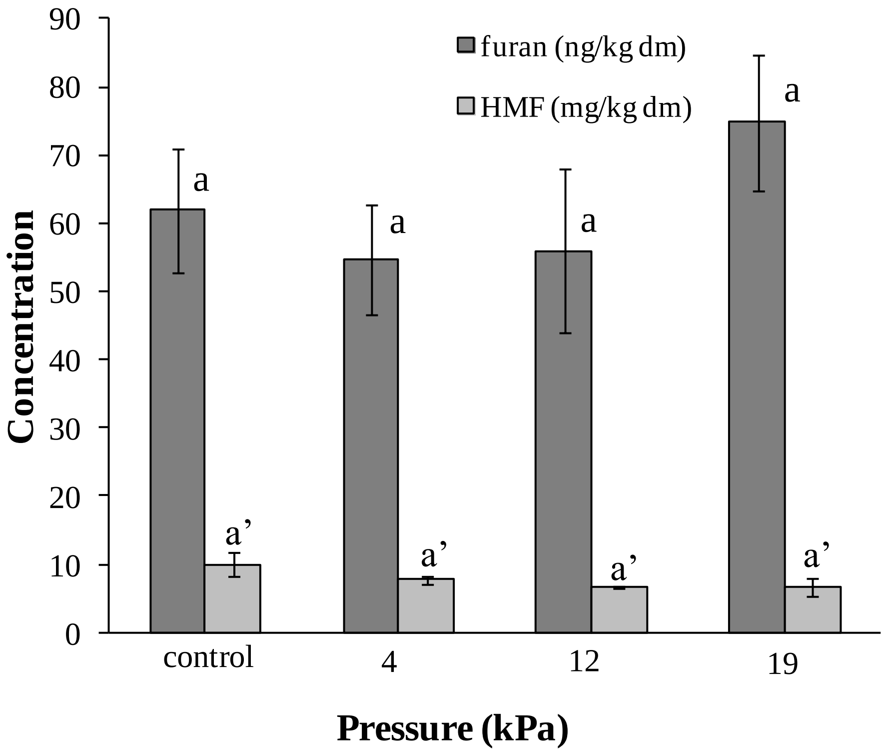

350
300
250
200
150
100
50
0
Furan concentration (ng/kg$_{dm}$)
0
10
20
30
40
50
60
Time (min)
4 kPa
12 kPa
19 kPa

**Fig. 1.**



90
80
70
60
50
40
30
20
10
0
furan (ng/kg dm)
HMF (mg/kg dm)
a
a
a
a
a’
a’
a’
a’
Concentration
control
4
12
19
Pressure (kPa)


**Fig. 2**.

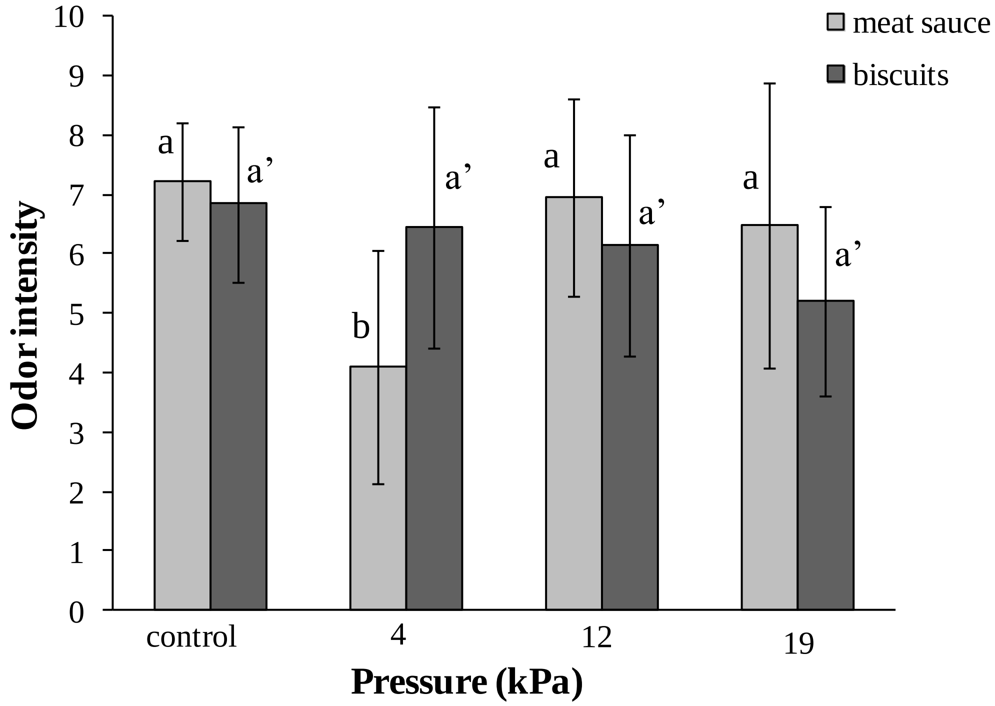

10
9
8
7
6
5
4
3
2
1
0
Odor intensity
meat sauce
biscuits
a
a’
b
a’
a
a’
a
a’
control
4
12
19
Pressure (kPa)

**Fig. 3.**

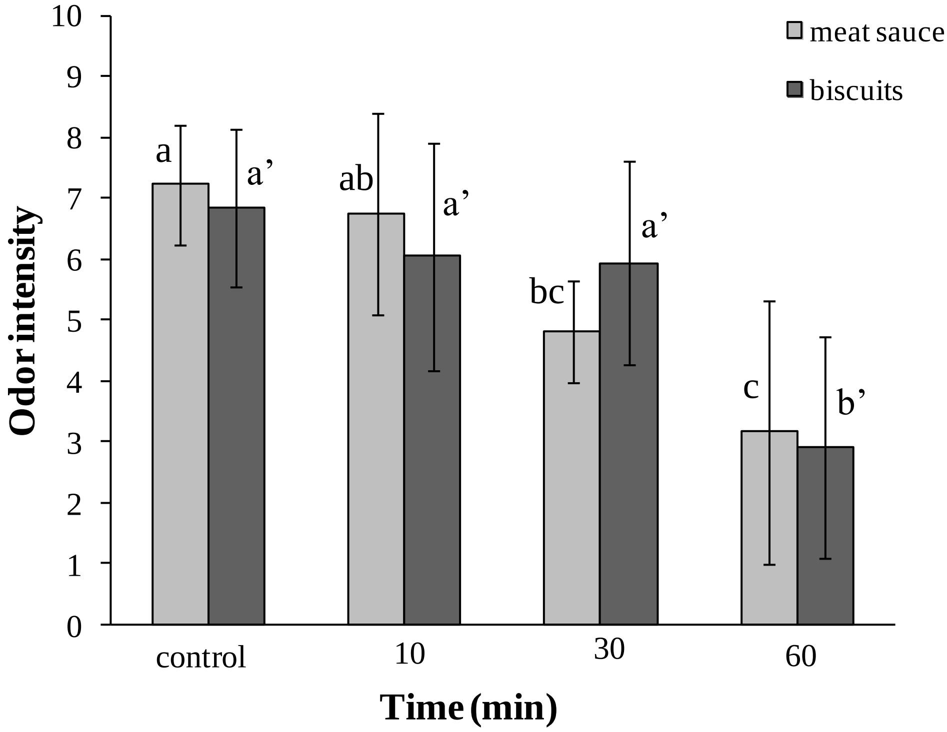

meat sauce
biscuits
Odor intensity
10
9
8
7
6
5
4
3
2
1
0
a
a’
ab
a’
bc
a’
c
b’
control
10
30
60
Time (min)

**Fig. 4.**