

INVESTIGATION INTO ANTIOXIDANT PROPERTIES OF SUPERCRITICAL CO<sub>2</sub>-EXTRACTSLisitsyn A.B.<sup>1</sup>, Semenova A.A.<sup>1</sup>, Gundyreva M.I.<sup>1</sup>, Kharitonova A.A.<sup>2</sup>, Lepeshkov A.G.<sup>3</sup><sup>1</sup> V.M.Gorbatov All-Russian meat research institute, Moscow, Russia<sup>2</sup>VNII of Physical chemistry of RAN, Russia, <sup>3</sup> NPTs ER "GORO", Rosotv-na-Donu, Russia

## Background

Extracts of spicy-aromatic raw materials find wide use in meat industry as the substitutes for natural spices. Not long ago The Scientific and Production Center of ecological sources (NPTs ER) "GORO" (the town of Rostov-na-Donu) developed and put in commercial practice a new technology of spicy-aromatic raw materials processing - a supercritical (SC) CO<sub>2</sub> extraction allowing to obtain extracts, the composition of which significantly differs from the composition of the extracts obtained by conventional methods. In supercritical extracts are widely and in great varieties are presented: terpene compounds, as well as waxes, pigments, high-molecular saturated and non-saturated fatty acids, alkaloids, vitamins and phytosterenes, many of which possess biological, antimicrobial and antioxidant activity.

In the papers of Tipsrisukond N. et al. [1,2] the data on the study of antioxidant activity of SC-CO<sub>2</sub> extracts *Piper Nigrum* are presented. These studies have shown that they are suitable for imparting flavor to meat products and are the best for retarding the fats oxidation as compared to traditional commercial extracts *Piper Nigrum*. However, no studies of SC-CO<sub>2</sub>-extracts of other spices, having high antioxidant activities have been carried out.

## Objectives of investigations

The objective of this investigation was a comparative evaluation of the antioxidant properties of SC-CO<sub>2</sub> extracts on the basis of determination of the total amount of antioxidants in the preparation and studying the retardation of oxidative deterioration of the meat raw materials.

As the objects of investigations were chosen the extracts of *Piper Nigrum*, *Rosmarinus Officinalis*, *Salvia officinalis* and *Thymus serpyllum*, as produced in NPTs ER "GORO". The samples of the extracts were non-fractionated oily liquids with wax-like inclusions. The extract *Piper Nigrum* featured a high level of terpenoid compounds (not less than 70%). The extract *Rosmarinus Officinalis* contained not less than 8% of terpenoids, 25% waxes, 50% lipids, 9% steroid compounds. The extract *Salvia Officinalis* contained not less than 20% of terpenoids, 8% of waxes, 9% lipids, 2-7 % of steroid compounds, 0.15% of vitamin E. The extract *Thymus serpyllum* had a considerable amount of terpenoids (not less than 20%, among them thymol - up to 60%) and waxes (up to 10%).

Trimmed fat pork and pork raw fat with the mass fraction of fat 48.8 ± 3.8 and 95.8 ± 2.5, respectively, that are at high degree are susceptible to the development of oxidative processes were chosen as meat raw materials. Chilled raw materials were comminuted in the chopper through the plate with the diameter 3mm, then the extract of rosemary, sage or thyme at 0.006% was introduced and mixed. The sample without any extract was a control. All the samples were stored during 5 and 7 days (for fat pork and pork raw fat, respectively) at 4°C. The signs of oxidative spoilage were studied on the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> days of storage (for fat pork) and for the pork raw fat - on the 1<sup>st</sup>, 4<sup>th</sup> and 7<sup>th</sup> days of storage.

## Methods

The following methods of investigations were used in the work:

- determination of the mass fraction of fat and indices of oxidative spoilage (acid /AN/, peroxide /PN/ and thiobarbituric acid number /TBA/) according to common procedures [3];
- determination of total amount of antioxidants - by the method of cumol test [4];

The method of cumol test is based upon the model reaction of the liquid-phase oxidation of hydrocarbon with the molecular oxygen. The reaction is carried out under conditions where the process of oxidation of hydrocarbon goes with constant velocity for a long time. When the antioxidant, being the inhibitor of the oxidation process, is introduced into the reaction, the reaction rate is significantly retarded and as the inhibitor is consumed, increases to the value of the rate of the non-inhibited reaction. According to the time of consumption of the inhibitor (induction period,  $\mu$ , ) and the depth of the reaction (the amount of absorbed oxygen,  $\Delta O_2$ ), concentration of the analyzed antioxidant was determined  $[InH]_0$ . For this purpose the following relationships were used:

$$\tau = \frac{2[InH]}{W_i} \quad \frac{\Delta O_2}{[RH]} = \frac{K_i}{K_s} \ln(1 - t/\tau)$$

where  $W_i$  - velocity of inducing,  $[RH]$  - concentration of hydrocarbon being oxidized,  $K_i$  - constant of the velocity of continuation of the chain,  $K_s$  - constant of inhibition, determined from plot of the relationship  $O_2 = f(t)$ ,  $t$  - time of the experiment (30-60 min).

The amount of the absorbed oxygen during oxidation was measured on gas metric plant. As an oxidizing hydrocarbon - cumol (isopropylbenzene) was used, as the initiator - dinitril of azoisobutyric acid. The reaction was carried out at 60°C.

## Results and discussion

Results of the determination of total amount of antioxidants in the extracts by the method of cumol test are presented in Table 1.

Table 1

Extract	Total amount of antioxidants	
	In M/kg	In % to weight of specimen
Extract of <i>Salvia officinalis</i>	0.140	3.10
Extract of <i>Rosmarinus officinalis</i>	0.070	1.60
Extract of <i>Thymus serpyllum</i>	0.018	0.40
Extract of <i>Piper Nigrum</i>	0.003	0.07

Maximum amount of antioxidants (3.1%) was in the extract of sage, minimum (0.07%) – in the extract of black pepper. It is generally agreed that natural extracts with the content of antioxidants not less than 0.1% may be considered as a food additive with antioxidant properties. Based on this the investigations on meat raw materials were carried out with the use of the extracts *Rosmarinus officinalis*, *Salvia officinalis* and *Thymus serpyllum*.

In the control samples of the meat raw materials (without extracts) the values of the indices of oxidative spoilage were higher, than in the experimental samples, which indicated the antioxidant properties of all the investigated extracts.

In case of the addition of the extracts both lower values of the indices of oxidative spoilage and their slower growth were observed during storage. The extract *Salvia officinalis* has shown the highest antioxidant properties. The values of AN and PN for the samples of fat pork with this extract were lower, than for other samples. And the value of TBA for the samples with the extract *Salvia officinalis* and the extract *Rosmarinus officinalis* though different during the first three days of storage, on the 5<sup>th</sup> day were practically the same. The results of the investigations on the determination of AN, PN and TBA in the samples of fat pork during storage are presented in Figs 1, 2 and 3. Similar results were obtained for the samples of raw fat.

Results of the investigation of the fat pork and raw fat have shown that as far as the effectiveness of retardation of oxidative processes is concerned, the extracts can be ranged as follows: *Salvia officinalis* > *Rosmarinus officinalis* > *Thymus serpyllum*. It should be noted that antioxidant properties of the first two differed insignificantly. It is clear, that antioxidant ability of the extracts depended on the total content of antioxidants in them, as determined by the method of cumol test. In Table 2 correlation coefficients of these values are presented.

Table 2

Samples	Coefficients of correlation between the contents of antioxidants and absolute change		
	AN	PN	TBA
Fat pork	-0.991	-0.644	-0.773
Raw pork fat	-0.990	-0.890	-0.674

The most pronounced correlation dependence was observed between total contents of antioxidants and the value of AN, characterizing accumulation of free fatty acids as a result of hydrolytic decomposition of fats.

The quantity of antioxidants in the extracts is mainly dependent on the kind of spicy-aromatic raw materials and conditions of extraction. Antioxidant properties, as shown by the extract *Salvia officinalis*, in our opinion, can be explained by a higher content of terpenoid compounds and the presence of vitamin E.

The use of the method of cumol test allows to predict antioxidant effectiveness of the extracts in meat raw materials, and also to use the index determining the total contents of antioxidants as a standardized characteristic of supercritical CO<sub>2</sub> extracts.

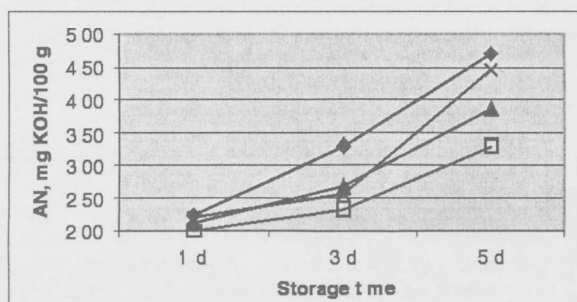


Fig. 1 Change of AN in fat pork

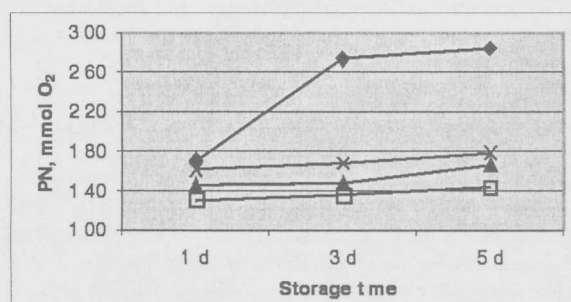


Fig. 2 Change of PN in fat pork

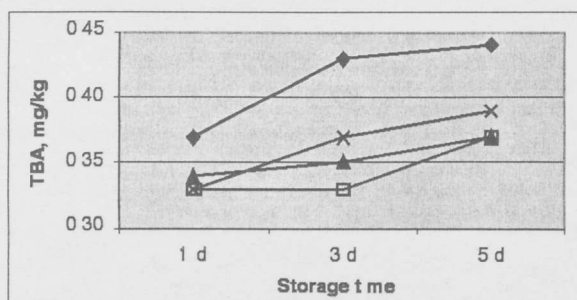


Fig. 3 Change of TBA in fat pork

Symbols:  
 ♦ – control (without extracts)  
 □ – *Salvia officinalis*  
 ▲ – *Rosmarinus officinalis*  
 × – *Thymus serpyllum*

## References

1. Tipsrisukond N. et al. Antioxidant activity of essential oil and oleoresin of black pepper from supercritical carbon dioxide and conventional extractions in ground pork. // 43<sup>th</sup> International Congress of meat science and technology, New Zealand, 27<sup>th</sup> August – 1<sup>st</sup> September, 1997, 466 pp.
2. Tipsrisukond N. et al. Antioxidant effects of essential oil and oleoresin of black pepper from supercritical carbon dioxide extractions in ground pork. // J. Agric. Food Chem., - 1998, 46, p.4329-4333
3. Zhuravskaya N.K. et al. Investigation and meat and meat products quality control. - M. Agropromizdat, 1985, 296 pp.
4. Tsepalov V.F. et al. Natural antioxidants: express method of analysis and prospects of use as food additives. Collection. Food ingredients. - M., 2000, pp. 7–8.