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INVESTIGATION OF BACTERIOSTATIC ACTIVITY OF RAFINED LIQUID SMOKE

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Key words: RLS — Refined Liquid Smoke, aerophilic, mesophilic microflora strains, model medium, nutrient broth.

The bacteriostatic activity of the smoke flavouring has been investigated. It has been found that the Refined Liquid Smoke does not influence the bacterial growth in doses below 200 ppm.

The liquid smoke flavourings are used more frequently now in the meat processing — instead of the traditional smoking, because they contain usually less carcinogenic substances, are easier to distribute and they allow for higher yielding of meat products. In Poland the new liquid smoke flavouring of domestic origin has been accepted to be used in limited scope — and it was necessary to examine its bacteriostatic activity.

To investigate the influence of the Rafined Liquid Smoke on the growth of the aerobic microflora, common in meat products, the model medium, imitating the sausage enviroment, has been used. It has been examined also the influence of fractions of RLS on the growth of monocultures of selected strains, being representative for the microflora of sausages.

EXPERIMENTAL PROCEDURE

MATERIALS

Refined Liquid Smoke — RLS — (product of the factory at Rzepedź) — confectioned, 5%-solution in the emulsion of gelatine.

Fractions of RLS:

Phenolic fraction:	phenol and homologues	— 24.3%
	guaiacol	— 29.5%
	syringol	— 37.0%

Acidic fraction:	acetic acid, homologous compounds	— 30.0%
	cycloten and homologues	— 25.0%
	phenols	— 40.0%
Neutral fraction:	ketones and other oxy-compounds	— ~ 30.0%
	hydrocarbons	— ~ 30.0%

Edible gelatine powder (product of the gelatine factory, Puławy)

Bacterial cultures: *Escheridia Coli* (without name), received from Agricultural University, Warsaw, *Clostridium sporogenes* 512 (museal strain from the Institute of Hygiene), *Streptococcus faecalis* L36/838 (received from Microbiologic Dpt of the Institute of Hygiene)
Gram-negative, lactose- and gelatinase-positive rods, isolated by authors from spoiled meat conserves, culture of saprophitic, mesophilic aerobs, isolated from cured, minced pork-meat

Media: — Nutrient broth (liquid) — Wytwórnia Surowic i Szczepionek
— Thioglicolate Medium (TM) — Diphco
— Plate Count Agar (PCA) — Oxoid
— Iron Sulphite Agar (ISA) — Oxoid
— Dilution-fluid (acc. to Polish Standard PN-73/A-82054)
— Model-medium: dilution fluid 180 ml
minced, cured pork meat 20 g
distributed into Erlenmayer's flasks of 250 ml capacity.
Sterilized at 120 ± 1°C — 20 min.
Equipment — Droplette — type BA6013 (Colworth)

METHODS

Scheme of experiments
Refined Liquid Smoke
confectioned form
microbiological investigation of the gelatine
microbiological investigation of 5% of RLS in gelatine emulsion
Bacteriostatic activity of RLS: the influence on the autholysis-effect
the influence on the growth of microflora, isolated from minced, cured meat,
the influence on the lethal effect of the thermal processing and on the growth on heat-treated cultures
Fractions of RLS: The influence on the lethal effect of the thermal processing (microflora isolated from cured meat) and on the growth on heat-treated cultures.
The influence on the growth of *Str. faecalis, E. coli, Clostr, sporogenes,* G(-)rods, lactose- and gelatinase-positive.

MICROBIOLOGICAL EXAMINATION OF GELATINE

After usual sampling procedure it has been prepared the 10% emulsion, of which 1 ml was transfered into 3 tubes with the nutrient broth (9 ml each) and mixed by pipeting. 0.1 ml of this solution has been transfered on the surface of PCA plates. Inoculation — 18 h. at 37°C.

MICROBIOLOGICAL EXAMINATION OF GELATINE-EMULSION OF RAFINED LIQUID SMOKE

The confectioned form of RLS has been investigated 3 times during 3 months. It has been transformed into liquid in the waterbath at 37°C, assayed in the nutrient broth in the proportion: 1 ml emulsion RLS to 9 ml of medium. Incubation 37°C, 48 h.

The influence of RLS on the autholysis-effect of mesophilic, aerobic cultures has been observed by using inoculated Petri dishes with addition of 20, 40, 60 and 80 ppm of RLS — after 24, 48 and 72 h incubation at 37°C.

Investigation of the influence of RLS on the bacterial growth in the model medium has been conducted by applying doses 20, 40, 60 and 80 ppm — at 37°C, at incubation period of 3 h., 5 h., 17 h.

The experiments have been repeated at 30°C and 5°C — during 24 and 72 h. cultivation.

It has been examined also the influence 100 ppm and 200 ppm of the smoke flavouring contents on the bacterial count at 30°C and 5°C — 24 h. and 72 h. The inoculum: $10 - 20 \times 10^6$ of microorganisms in 1 ml.

The thermal processing effect in the presence of the RLS has been investigated in the following experimental conditions: 73°C — 5 min, 80°C — 10 min (in the water bath) and 20, 40, 60, 80, 100 and 200 ppm of the RLS. The enumeration of the bacterial count has been done directly after heating and after 24 and 72 hours of cultivation at 30°C and 5°C. The inoculum — $10—20 \times 10^6$ in 1 ml.

The sensitivity of the monocultures to the RLS and its fractions has been investigated in the presence of 200 ppm of the smoke flavouring in the medium. 250 ml capacity flasks filled with 180 ml of suitable liquid medium have been inoculated with 0.5 ml 24 h incubated bacterial culture. *E coli*, *Streptococcus faecalis*, lactose-positive rods have been assayed on the nutrient broth; *clostridium sporogenes* has been cultivated in the Thioglicolate Medium. The inoculum-above 100×10^3.

Counting of microorganisms

The number of microorganisms has been determined by using the microcolony's technique "Droplette" (T.BA6013). In all the described experiments the nutrient broth has been used as the cultivation medium (at 40-45°C) except the case of *Clostridium sporogenes*, where Thioglicolate Medium has been used — and the plates, have been incubated in the anaerostats filled with natural gas.

RESULTS AND DISCUSSION

By microbiological examination of the gelatine powder in the tubes with nutrient broth, containing the samples of gelatine powder as well as on the surface of plate count agar an intensive growth of microorganisms has been observed.

The 5% solution of RLS in 10% gelatine emulsion, placed into tubes with nutrient broth did not cause any visible turbidity after 24 h incubation at 37°C. By using agar plates any growth of microorganisms hasn't been observed. The microbiological examination of the confectioned form of RLS has been repeated three times (every month) with negative result.

The autholysis effect, shown in Tab. 1, has been observed at first on the plates, in which the concentration of RLS was the lowest (20 ppm), while the same effect with 80 ppm of RLS in the medium has been observed after 72 h.

Table 1. The influence of RLS doses on the autholysis effect of mesophylic, aerobic cultures

Concentration of RLS in PCA medium (ppm)	Incubation (37°C)		
	24 h	48 h	72 h
20	„Lawn-growth"	++++	++++
20		++++	++++
40		+++	++++
40		+++	++++
60		++	++++
60		++	++++
80		+	++++
80		+	++++

Eeffctiveness of the autholysis is marked with „+" signs.
The conclusions about the bacteriostatic effect of the RLS were based on the time and intensity of appearing of fields of lysis on the bacterial lawn.

It could be explained by the bacteriostatic influence of RLS on the intensity of the microorganisms growth, which was slower at the higher concentration of the smoke flavourin. The similar phenomenon has been observed by Pulvertaft and Lamb [3] who investigated the influence of phenol concentration on the autholysis effect in *E. coli* cultures.

INFLUENCE OF THE RLS ON BACTERIAL GROWTH

In all the above experiments under the conditions desribed in the Experimental procedure any differences were not noticed between the number of microorganisms in the cultures with several concentrations of RLS and the control tubes — after the observation periods at the temperature 37°C, 30°C and 5°C. These results are in conformity with those obtained by Rojowska [5] during the investigation about influence of another liquid smoke flavouring on the microflora of sausages (this smoke flavouring was produced according to another technology, but is components pattern was very similar), where the number of microorganisms in the raw sausages with and without smoke flavouring — were on the same level.

The results of the thermal processing cultures in the model-medium in the presence of different concentrations of RLS and its fractions are demonstrated in Tab. 2, 3 and 4. The Table 2 shows that the lethal action

Table 2. The lethal effect of the heating and RLS on the mesophilic culture (80°C — 10 min model-medium)

Concentration of RLS in the model-medium (ppm)	Total count of bacteria per ml before heating	Total count of bacteria per ml after heating $\bar{x}$	t
0 (control culture)	9.05×10^5	4.4×10^2	
200	9.05×10^5	3.5×10^2	6.38

$t_0 = 4.303$, $\alpha - 0{,}05$ $\nu = 2$

Table 3. The influence of RLS fractions (200 ppm) on the number of microorganisms in the mesophilic-aerophilic cultures after thermal processing at 73°C — 10 min and 24 h cultivation at 5°C

Fraction of RLS	Heated cultures		Non-heated cultures	
	$\bar{x}$	t	$\bar{x}$	t
Control	2.36×10^4		3.35×10^7	
Phenolic fraction	2.68×10^4	0.1513	2.09×10^7	1.4233
Neutral fraction	2.28×10^4	0.0320	2.70×10^7	0.7793
Acidic fraction	2.58×10^4	0.1075	2.10×10^7	1.3332

$t_0 = 2.447$, $\alpha - 0.05$, $\nu = 6$

Table 4. The influence of RLS fractions (200 ppm) on the number of microorganisms in the monocultures after cultivation for 24 h at 5°C

Fraction RLS / Name of the strain	Control		Phenolic fraction		Neutral fraction		Acidic fraction	
	$\bar{x}$	t	$\bar{x}$	t	$\bar{x}$	t	$\bar{x}$	t
Streptococcus faecalis L36/838	9.13×10^5		7.6×10^8	0.3965	3.27×10^5	2.9698	7.28×10^5	0.5721
Rods lac+, gel +	1.84×10^6		6.55×10^5	1.2724	6.39×10^5	1.2761	2.73×10^5	1.6916
Clostridium sporogeues 512	5.70×10^4		1.50×10^5	1.0437	2.80×10^4	1.2933	2.55×10^4	1.6967
Escheridia coli *	6.14×10^5		5.92×10^5	0.0571	7.08×10^5	0.3244	6.27×10^5	0.0360

of heating at 80°C during 10 min is more effective in the medium, containing 200 ppm of RLS, the difference being statistically significant. The higher lethal effect of the thermal processing on the bacterial contamination in sausages, produced with addition of liquid smoke flavouring has been observed also by other authors [5].

This phenomenon has been observed in the case of counting of microorganisms immediately after thermal processing; after 24 and 72 h of cultivation at 5°C the difference between the number of bacteria in experimental and control flasks equalized. Probably the RLS presence prolonged the lag-phase period of bacteria after the thermal-shock. The concentrations of the RLS lower than 200 ppm — did not give any positive results in these experiments. Stanescu and Laslo [6] investigating various methods of conservation of meat products — obtained also similar results of the traditional smoking: the sausages after smoking were showing very low microbial contamination, which increased after storage at 4°C during a few days. The short duration of the bacteriostatic influence of the various liquid smoke flavourings on the microflora of sausages has been noticed also by Hattowska [1].

Considering the results, which did not allow to provide conservating influence of the RLS doses on the meat products in the practice, the authors tried to establish the influence of the separated fractions of RLS on the growth of microflora after heating at 73°C. The 3 containing the results, shows that any influence, statistically significant, has not been discovered. Those results were rather unexpected, because the bacteriostatic influence of phenol and phenol-homologues has been known since long time [3]. Olsen [4], investigating the properties of various

distilled fractions of the "Scansmoke" (liquid flavouring) — has noticed some inhibition of the growth of *Staphylococcus aureus* culture as the result of the influence of the phenols fraction, boiling at lower temperatures.

The results of the investigation about influence of fractions of RLS on the monocultures of selected strains, being representative of the microflora of sausages are shown in Tab. 4.

The inhibition effect of the neutral fraction on the growth of *Streptococcus faecalis* (ab. 35.8%) could be explained by the presence of formaldehyde in this fraction, which is known as the component of strong bacteriostatic effect [2, 3].

CONCLUSIONS

1. The 5% solution of RLS in the gelatine emulsion (commercial form) is sterile.

2. The presence of 20-200 ppm of RLS and its fractions in the medium did not influence the number of microorganisms in culture of mesophilic, aerobic cultures under the described experimental conditions.

3. The addition of 200 ppm RLS prolonged the lag-phase period after thermal-shock, but did not influence the bacterial-count in the cultures after 24 h and 72 h cultivation at 5°C.

4. 200 ppm of neutral fraction in the nutrient broth caused statistically significant drop of the number of *Streptococcus faecalis* L 36/838. It has not been found dependence between the fractions of RLS and growth of other strains, used in the experiments.

5. On the basis of the above results one can conclude that using of RLS in organoleptically acceptable doses (< 20 ppm) does not show any positive effect on the shelfstability of the meat products.

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BADANIE BAKTERIOSTATYCZNYCH WŁAŚCIWOŚCI RAFINATU DYMU WĘDZARNICZEGO (RLS)

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Streszczenie

Badano jałowość formy konfekcjonowanej rafinatu dymu wędzarniczego (Rafined Liquid Smoke) oraz jego frakcji na wzrost mikroflory wyizolowanej z mielonego, peklowanego mięsa i wybranych szczepów drobnoustrojów. Stwierdzono nieznaczny wpływ obecności 80 ppm RLS w podłożu na opóźnienie efektu lizy bakterii tlenowych (tab. 1), brak wpływu dawek od 20 do 200 ppm na wzrost mikroflory tlenowej wyizolowanej z peklowanego mięsa, przedłużenie lag-fazy w kulturach mieszanych poddanych pasteryzacji (tab. 2) oraz wyrównanie liczebności kultur uprzednio ogrzewanych w RLS i jego frakcjach po 24 i 72 h hodowli w temperaturze 5 i 30°C (tab. 3). Ponadto odnotowano nieznaczne obniżenie liczebności Str. faecalis w hodowli z dodatkiem frakcji neutralnej RLS (tab. 4).

Wobec faktu, że najwyższa organoleptycznie akceptowana dawka RLS wynosi 20 ppm, a dowody słabo zaznaczonej bakteriostatycznej aktywności preparatu zebrano w doświadczeniach przy znacznie wyższych jego poziomach (80-200 ppm), nie można traktować RLS jako czynnika przedłużającego trwałość produktów mięsnych.