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Airborne Particulate Contaminants During Refrigeration of Meat in Controled Medium

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Abstract.The transfer of contamination through the airborne route is one of the most significant areas of high-care food production. The food industry specially the manufacturing of the chilled meat products strive for lower levels of the air contamination, therefore lot of experimental and numerical studies considers the concentration of airborne particulate contaminants, such as different species of food spoilage microorganism.

In a food processing system concerned with efficient and duration preservation of primary agricultural produce post-harvesting/post-slaughtering, low carbonylic compounds as potential volatile metabolites generated by the micro flora present in the modified atmosphere ***CO_2/O_2; N_2/O_2***, etc. of refrigeration areas for wholesale or pre-packaged fresh meat are of major interest together with other risk factors of consumer food safety.

Some low aliphatic ketones with high volatility can be seized by smelling even at freezing temperatures in an advanced stage of accumulation.

In this paper we suggest a guick method for the measurement of their concentration expressed as ***MEK*** starting with values of ***1 µg/m^3*** atmosphere (refrigeration environment) through periodical and/or continuous sampling through aspiration from the proximity of meat surface both pre-packed and stored in standard cells in modified ***atmosphere (25% CO_2; 75% O_2) (25% N_2; 75% O_2)*** in an ethanol solution of salicylaldehyde ***10% (CAS 90-02-8)*** when it makes up a colorimetrable yellow-orange compound. Assessment can be done continuously in parallel with comparing the colour with a potassium dichromate scale (qualitatively) and also by reporting it to a sampling curve previously prepared (quantitatively). The specificity of the method is affected only by the presence in the refrigeration atmosphere of other toxic volatile metabolites of ketonic nature (acetone, acetoin, 2,3-butane dione).

Keywords: food safety, airborne particulate contaminants, low aliphatic ketones, volatile metabolites, refrigeration.

INTRODUCTION

The risk is higher when air is contaminated with eventually foodborne pathogen microorganisms and spores. The risk of contamination derive prior to plant surfaces that includes both product – contact and non – product contact surfaces. Airborne contamination should be occurred by indirect contact by means of airborne particles which can be represented by spoilage or pathogene microorganisms.

Microbial flora of naturally contaminated meat (air) is similar to that of meat stored under gas permeable ***"films"*** (pillows). Major compounds identified during chilling of meat and meat preparations as a result of microbiological development are volatile metabolites ***(acetoin, diacetyl, 3 – methyl butanol, 2 – methyl propanol, propionic, butyric, hexanoic acid, m*ethanethiol *as such and their ethyl esters, dimethyl sulphide, dimethyl disulphide).***

The first two lower carbonylic compounds, known as final product of metabolism of *Brochothrix thermosphacta* microorganism were detected in early stages of storage to others who were reported as metabolites of *Pseudomonas spp*. Simultaneously with chemical changes (biochemical) occurred is observed and the appearance of additional unwanted odor ***("odor off").***

Edible films can be made from any one of a variety of compounds including pectins, starch derivatives, collagen, gelatin, proteins, polysaccharides, lipid, and seaweed extracts ***(Krochta et al. 1994, Ben and Kurth 1995).***

The properties of edible films are similar to those of synthetic packaging films in that they act as barriers against moisture, oxygen, oil, and solute migration, improve mechanical handling of certain foods, retain volatile flavor compounds, and bind specific food additives ***(Ben and Kurth 1995).***Several studies have demonstrated that the incorporation of additives, such as food-grade antimicrobials, into edible coatings may provide additional barriers/hurdles to reduce the incidence of pathogenic or spoilage bacteria on the surfaces of meat or raw meat products ***(Meyer et al. 1959, Siragusa and Dickson 1992, 1993, Baron 1993, Cutter and Siragusa 1996, Fang et al. 1996). Siragusa and Dickson (1992, 1993)*** determined that the antimicrobial activity of lactic and acetic acids was greater when immobilized by calcium alginate gels than when the acids were applied alone. Previously, we have demonstrated that nisin activity was greater against the Gram-positive meat spoilage organism, *Brochothrix thermosphacta*, when immobilized in an edible gel than when applied directly to beef surfaces ***(Cutter and Siragusa 1996).*** Ultimately, the incorporation of antimicrobials with edible packaging materials may provide additional safety and shelf-life measures for raw meat products. The following study was conducted to determine if immobilization of nisin in an edible gel was a more effective delivery system for a bacteriocin into ground beef than direct application.

Glucose plays a major role in the selection of alteration microorganisms dominant for chilled meat ***(2-7°C)*** (*Pseudomonas fragi, Ps. Lundensis şi Ps. Fluorescens*). It is considered that in their development carbohydrate is bioprocessed in a less accessible substrate (***gluconate***) later as backup ***extracellular power***. While depletion of glucose pseudomonas converts amino acids, generating ***"adulterated" characteristic smell of meat.***

Microbial flora of naturally contaminated meat (air) is similar to that recorded in meat stored in foliated systems [film and/or pillows (inert or controlled atmosphere)] gas permeable. As a result of metabolites development occurring microbial volatile (wide range of organic compounds) as products the metabolism of grame positive microorganism as late volatile metabolites. Glucose, amino acids as nutritional, environmental parameters (temperature, pH, controlled and / or modified atmosphere composition, storage/refrigeration duration, etc.) have a major, decisive role in the emergence and proliferation of these metabolites.

In our continuing concerns about the optimization of storage parameters (conditions) in fresh chilled of food products this paper extends the range of volatile metabolites measurements formed under aerobic and / or anaerobic conditions for packed fresh meat in modified and / or chilled atmosphere.

MATERIALS AND METHODS

Materials, reagents

- pig (pulp) fresh chilled in the temperature range ***1 - 15°C*** and for ***1 - 30*** days in normal and / or modified (controlled) atmosphere: ***CO_2*** or ***N_2 100%; CO_2/O_2 (25/75 $^v/_v$); N_2/CO_2 (50/50 $^v/_v$); N_2/CO_2 (70/30 $^v/_v$);***

- reagents (selective presentation) with analytical purity (Sigma Aldrich) in accordance with the method of determining. Salicylaldehyde (***CAS 90 – 02 – 8***), diacetyl (2,3 – butanedione) (***CAS 431 – 03 – 8***) ***p.f. = 88°C*** (standard).

Equipment

-***Spectrophotometer UV – VIS (λ = 470 nm);***

-Watertight enclosure ***50/50/40 cm*** with static and/or dynamic operating, possibility of guided power for biological material and/or component of modified/controlled atmosphere (***N_2, CO_2, O_2, air***) and continuous or intermittent monitored prelation from operating atmosphere.

-Pure gas generators (***N_2, CO_2, O_2***) (Cole Parmer).

-Gas chromatograph.

Colorimetric determination of ***carbonyl metabolites*** with ***salicylaldehyde***
methyl ethyl ketone reacting in alkaline medium, at hot, like all ketones, with salicyl aldehyde, forming a yellow-orange colored compound

Reagents

1.sodium bisulphite, ***2%*** solution in water;
2.potassium hydroxide, ***63,6%*** solution;
3 salicilaldehyde, ***10%*** solution in alcohol (will prepare the determination required before);
4.stock standard solution form methyl ethyl ketone.

Weigh a ***50 mL*** flask**,** containing approximately ***20 mL*** distilled water. Add ***2-3*** drops of methyl ethyl ketone; reweigh the flask and bring to volume. Calculate as the amount of methyl ethyl ketone (***MEK***) containing ***1 mL*** of solution after the relationship:

$$mg\ MEK\ pure = (B - A) \cdot 20 - C,\ where:$$

A = flask weight with distilled water, expressed ***g***;

B = flask weight with distilled water and ***MEK***, expressed ***g***.

5. Working standard solution. Dilute the stock solution, such as ***1 mL*** containing ***0,1 mg MEK***.

Its drawn the calibration curve chart, scoring on abscissa the concentration, and the ordinate the values of respective optical absorption.

It sucks air through two microabsorbante mounted in series, each containing ***3 mL*** solution of sodium sulphite or distilled water with a flow ***to 0,05—0,1 mL/minute***.

Transfer the liquid from the two microabsorbante in a ***25 mL*** graduated cylinder**.** Wash dishes with small portions of sodium sulphite solution and bring the cylinder is completing the final volume of ***15 mL,*** also with a sulphite solution.

From this solution, take a witness in a ***3 mL*** test tube; add ***2 mL*** of ***KOH*** solution (reagent 2) and ***1 mL*** of alcoholic solution of salicylicaldehyde (reagent 3). Mix and take 20 minutes in a water bath, at ***50°C (±2°C).***

The mixture is cooled under the tap and allow ***5-10*** minutes for color development.

Its determined the optical absorption at ***470 nm*** in a cell of ***0.5 cm***. Comparison is made from the witness.

Interpolate the optical absorption values obtained for samples, on the calibration curve chart, determining the quantity of ***MEC*** existing in sample, according to the relation:

$$mg\ MEK/m^3\ air = \frac{Cx5}{V}, \text{ where :}$$

C = amount of methyl ethyl ketone existing in aliquot, expressed ***μg***;
V = volume of air collected from processed atmosphere, expressed ***L***.

RESULTS AND DISCUSSIONS

Biological material (round pig) immediately after slaughter was cut into rectangular pieces with the average size ***40/40/5 cm*** and brought stratified with the possibility of environmental movement on all interfaces.

Storage of meat in modified atmosphere ***(25% CO_2/75% O_2)*** selected gram positive flora spread the ***"smell of cheese"*** by generating lactic acid and volatile fatty acids. Similarities stands for meat with acid pH (under ***5,8)*** in atmosphere with traces of oxygen because less acid-resistant gram positive microorganisms are majority in enriched atmosphere with ***CO_2*** respectively ***CO_2/O_2*** (has major role in packaged meat spoilage in modified atmosphere). The main factor that negative affecting the validity of chilled meat products in controlled atmosphere is odor due to microbial metabolism.

The main metabolites resulting from the consumption of glucose under anaerobic conditions were ***lactic acid*** and ***ethanol***. Not identified gazcromatografic the presence of acetoin but only random small amounts of short- average chain organic acids ***($C_1 – C_4$).***

Under aerobic present flora generates ***acetoin, acetic acid, isobutyric acid, 2 – methyl butyric acid, isovalerianic acid and 3 – methyl butanol***. The main clue to the "macro" scale of spoilage meat is the "***sour***" smell associated with acetoin and to a lesser extent with the acids mentioned.

The real practical interest in later agro preserving in this paper has been systems nominated ***CO_2/O_2 which*** delaying the myoglobin oxidation and ensuring that the present flora metabolism remain predominant anaerobic.

- From the analysis of experimental data for gaseous environment ***CO_2/O_2/N_2 (0/25/75)*** has been noted:after about ***240 hours (9 – 10 days)*** present in the culture medium flora development peak followed by a steady plateau;
- reported to this reference moment begins to decrease the concentration in glucose which is exhausted after approx. ***500 hours (19 – 20 days);***
- similar development record the acetic acid which remains constant the packaging area and after about ***20 days;***
- ethylic alcohol has modest progress its presence is sporadic throughout the determinations;
- lactic acid and acetoin/diacetyl system follows an upward trend with a maximum after approx. ***400 hours (16 – 17 days)*** when it remains constant

Analyzing developments for a controlled atmosphere ***CO_2/O_2/N_2 (0/0/100)*** could observe the following:

- evolution (the appearance and development) gram positive flora cover a route similar regardless of the composition of media;

- ***glucose*** to the maximum development of microorganism remains approximately constant. still aprox. ***100 hour (3 – 4 days)*** then decreases steadily without disappearing from the culture medium;
- ***lactic acid*** is the only metabolite consistently increasing trend similar to previous environmental conditions;
- ***acetic acid*** evolves similar but less than pronounced the above mentioned case law;
- ***acetoin/diacetyl system*** respectively ***ethanol*** are identified at trace levels throughout the test conducted.

Under changes of environmental storage (refrigeration) ***(5°C) [O_2 (0 – 60%); CO_2 (0 – 60%)] a*** samples of pork following conclusions can be drawn to glucose bioprocessing in various volatile metabolites.

- to small percentage of ***O_2*** to ***CO_2*** in storage environment stands reduced presence of ***lactic acid*** but while increased with favoring aerobic conditions;
- ***CO_2*** favored the presence of ***lactic acid***,
- ***O_2*** and ***CO_2*** does not encourage excessive formation of ***acetoin*** and ***diacetyl*** the full range of modified atmosphere;
- formation of ***acetic acid*** is clearly favored over the entire range of composition ***O_2/CO_2*** of modified atmosphere;
- bioprocessing glucose and development of gram positive flora gradient is encouraged throughout the range of composition ***O_2/CO_2.***

It was found that ***MEK(1)*** share in this conditions ***(table 1)*** is dependent on the size of the meat samples (higher with increasing the contact interface with the environment).

Maintaining constant the duration, refrigeration temperature and dimensions of biological material, controlled modification the composition of direct contact atmosphere influence the amount of ***MEK*** determined ***(table 2).*** ***CO_2*** does not favor the formation of diacetyl or ***MEK***. Gradient bioprocessing of proteins and / or lipid as majority nutrients and development of microbial is favored by ***O_2/CO_2*** ratio evolution. Depending on this report takes place in proportion that aerobic and anaerobic transformation. In this sense in the storage atmosphere will be found and their specific volatile metabolites (acetic acid, ethyl alcohol, acetic aldehide, diacethyl).

Tab. 1

Influence of pig sample size (pulp) fresh chilled (10°C) for 20 days in normal atmosphere (80% N_2; 20% O_2) on emissions of MEK

Nr. Crt.	Geometrical dimensions of meat sample (cm)	Content in MEK (mg/m^3 medium)
1	40/40/5	194,14
2	40/30/5	206,26
3	40/20/5	214,39
4	40/20/5	226,76

Tab. 2

Influence of contact medium composition of pig sample (pulp) with dimensions 40/40/5 cm fresh chilled (10°C) for 20 days on emissions of MEK

Nr. Crt.	Composition of contact environment (% v/v)	Content in MEK (mg/m^3 medium)
1	vacuum ($5 \cdot 10^{-2}$ mm col. Hg)	140,26
2	CO_2/O_2 (20/80)	128,32
3	N_2/CO_2 (50/50)	117,15
4	N_2/CO_2 (60/40)	106,26

Random determinations made under aerobic/anaerobic specific conditions have confirmed their presence at the lower limit of detection.Temperature and time (***5-30*** days) of refrigeration ***(1 - 15°C)*** also affects the amount of ***MEK*** proportion recovered ***(table 3, 4)*** while maintaining constant composition of refrigeration environment (anaerobe/aerobe) of storage time and size of meat samples investigated.

Tab. 3

Influence of refrigeration temperature on emissions of MEK from pig sample (pulp) fresh chilled with dimensions 40/40/5 cm in normal atmosphere (80% N_2; 20% O_2) and 20 days shelf life

Nr. Crt.	Refrigeration temperature (°C)	Content in în MEK (mg/m^3 medium)
1	1	131,84
2	5	170,42
3	8	179,36
4	10	192,14
5	15	209,56

Tab. 4

Influence of storage duration (refrigeration) on emissions of MEK from pig sample (pulp) fresh chilled with dimensions 40/40/5 cm in normal atmosphere (80% N_2; 20% O_2) and refrigeration temperature 10°C.

Nr. Crt.	Refrigeration range (days)	Content in MEK (mg/m^3 medium)
1	5	76,56
2	10	169,42
3	15	202,42
4	20	212,39
5	30	220,22

[1] Exprimarea semnifică totalitatea compusilor carbonilici inferiori mentionati cuantificati ca metil etil cetonă)

CONCLUSION

The method is suitable for rapid assessment by static and/or dynamic flow storage (refrigeration) of the most different kinds of meat by the effectiveness, reproducibility, speed.

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