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Use of Oxygen Analyzer for Oxygen Uptake Tests on Encapsulated Citrus Flavors

Primjena analizatora kisika za određivanje utroška kisika pri istraživanju ukapsulirane citrus arome

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Summary

Gas chromatograph with a thermal conductivity detector has been used to measure oxygen uptake in order to probe particle structure and to predict relative storage stability of encapsulated citrus flavors. Alternatively, changes of headspace oxygen concentration over a sample placed in a hermetically closed container can be followed with a cheaper/simpler instrument, oxygen analyzer. Oxygen analyzer is much more compact, requires less maintenance, and is more suitable for routine research use or manufacturing quality control applications than gas chromatograph. We compared critically MOCON HS 750 Oxygen Analyzer (Modern Controls, Minneapolis, Minnesota, USA) vs. gas chromatograph in oxygen uptake tests on blanks and on samples with extremes of low and high percent residual oxygen (encapsulated citrus flavors, bread crumbs and chicken fat). Statistical analysis indicated satisfactory performance of oxygen analyzer without loss of accuracy or precision. Oxygen-analyzer based method is now permanently used in our flavor technology laboratory. The method has also been successfully introduced to and applied by the quality control personnel in a manufacturing plant.

Sažetak

Da bi se ispitala struktura čestice i predvidjela relativna stabilnost skladištenja ukapsuliranih aroma citrusa, primijenjena je plinska kromatografija s detektorom toplinske vodljivosti za mjerenje utroška kisika. Alternativno, promjene koncentracije kisika iznad uzorka smještenog u hermetički zatvorenoj posudi, mogu se pratiti jednostavnijim i jeftinijim instrumentom, analizatorom kisika. Analizator kisika je kompaktniji, lakši za održavanje i mnogo prikladniji za rutinska ispitivanja ili kontrolu kakvoće proizvoda od plinskog kromatografa. Kritički je uspoređen MOCON HS 750 analizator kisika (Modern Controls, Mineapolis, Minnesota, USA) s plinskim kromatografom u pokusima utroška kisika sa slijepim probama i uzorcima s ekstremno malim i visokim postotkom zaostalog kisika (ukapsulirane arome citrusa, mrvice kruha, pileća mast). Statistička analiza pokazala je zadivljujuće značajke analizatora kisika bez smanjenja točnosti (ili preciznosti). Metoda zasnovana na analizatoru kisika sada se stalno koristi u našem laboratoriju za tehnologiju aroma. Postupak je isto tako uspješno uveden i primjenjuju ga u kontroli kakvoće proizvoda u industrijskom pogonu.

Introduction

The key degradation process for citrus used as food flavorings is that of oxidation. For example, limonene, a principal terpene component of citrus oils easily oxidizes via a free-radical mechanism reaction to a 1,2-epoxide. Further oxidation progress eventually leads to off-flavor compounds such as carveol and carvone *(1)*, with low odor thresholds and having odor character described as spicy, pungent, heavy, pine needles-like and turpentine-like (»terpeney«).

We have been particularly interested in protection of citrus oils against oxidation, whether by use of appropriate antioxidant preservatives, by improved encapsulation, or both. To evaluate the relative effectiveness of these treatments, we needed to measure the rate and extent of oxidation. Rather than following the disappearance of the oxidizable reactant(s) or the generation of oxidized product(s), we focused on the measurement of oxygen consumption.

The oxygen uptake test monitors headspace oxygen concentration above a sample in contact with air atmosphere inside a hermetically closed inert vessel exposed to an elevated temperature, a type of testing often done in edible oil and rubber manufacturing industries. Regarding the experimental technique, the Warburg ma-

Table 1. Comparison of gas chromatography and oxygen analyzer for oxygen uptake determination
Tablica 1. Usporedba plinske kromatografije i analizatora kisika za određivanje utroška kisika

Feature	Gas Chromatography	Oxygen Analyzer *(7,8)*
Bench space	ca. 1 m	< 30 cm
Portability	Permanently installed	Portable, 6 kg with case
Cost	ca. $ 15.000	ca. $ 8.000
Hardware	Matched dual columns, oven, carrier gas supply and purifier	Injection port, optional charcoal filter
Sample size	0.25 mL	5–6 mL preferred (2–10 mL), sensitive to injection rate
Resampling	Yes/multiple possible	No/suggest multiple samples
Sensor	Thermal conductivity detector	Zirconia cell, heating block
Resolution	0.0001 %	0.1 % at > 10 % 0.01 % at 1–9.99 %
Warmup time	ca. 1 h	20 min
Test duration	ca. 3 min	ca 0.5 min
Calibration	Automated	Ambient air injection
Cleaning/purging	Overnight program	30-min automated cycle
Analyst	Highly trained	General training
Versatility	Broad	Narrow/dedicated
Autom. sampling	Available	Possible

nometric method was used in earlier works on orange oil oxidation *(2)* and to compare stability of encapsulated citrus flavors *(3,4)*. Gas chromatography (GC) has been previously applied by us to probe the encapsulate particle structure and to compare stability of encapsulated citrus oils *(5-6)*; in some of our own unpublished work, we have also extended the application to liquid citrus oils and to the prediction of storage stability of the encapsulates. Unlike methods used in lipid chemistry which make repeated measurements over time until certain pre-determined uptake value is reached, we have fixed the time/temperature exposure conditions and compared uptakes attained after such standardized treatment.

The purpose of the present work is evaluation of the oxygen analyzer instrument as a simpler, cheaper and faster alternative to GS for measurement of oxygen uptake in research and quality control applications.

Materials and Methods

Food sample containing 2–5 g oil (e.g. 5–20 g of an encapsulate) is placed in a 60 mL glass serum bottle No. 223554 (Wheaton Scientific, Milville, NJ) and the bottle firmly closed with a rubber stopper/septum. Blanks consist of empty stoppered bottles (air). Initial measurements of fraction of oxygen for the untreated blanks are performed by sampling and analyzing ambient air. Blank and sample bottles are placed in a thermostat kept at 60 °C. Standard incubation time is 1 week; however, we had to depart from that in this work due to the test schedule. Following the treatment, bottles are allowed to cool to room temperature before analysis.

Our initial method *(5)* employed solid-gas partition chromatography and a headspace sample size of 250 μL. A pair of matched, 6.35 mm dia. stainless steel Supelco columns of 90 or 150 cm length are used, packed with a solid molecular sieve type 5A of 0.177/0.250 mm granulation. The instrument is Perkin Elmer Sigma 300 GC with a thermal detectivity detector and an automatic integrator/recorder. With oven temperature at 45 °C, oxygen separates well from nitrogen in ca. 2.5 min. Ratio of area of oxygen peak vs. total peak area corresponds to the volume fraction of oxygen in the analyzed headspace sample.

In the alternative test method, a 6 mL headspace sample is injected at an even rate, ca. 1 mL/s, into the port of the oxygen analyzer Mocon HS-750 (Modern Controls, Inc., Minneapolis, MN). The instrument contains a zirconia galvanic cell sensor and promptly displays oxygen content in percent or ppm units on an analog display; its main use is in packaging testing. The main features of the two instruments are summarized in Tab. 1. GC is calibrated automatically while the oxygen analyzer requires periodic checking by injecting air, verifying that the display actually reads in the 20.6–20.8 % range and adjusting if necessary (instead of air, a custom-made gas mixture can be used for this purpose).

For Table 2, various available food samples were used to cover a wide range of residual percent oxygen values. During the initial GC analyses in Tarrytown, NY (TT), blanks used were intact and oven-treated for 3.5 days, respectively. Samples of different lemon and orange oils, encapsulated by several methods shown, have been treated in the same fashion, together with the blanks. Bread crumbs contained hydrogenated soybean oil shortenings from different suppliers, with and without rosemary extract antioxidant added. Bread crumb samples and a single sample of ckicken fat preserved with BHA had been previously treated for several weeks and were expected to have sustained oxidative damage with the accompanying decreased values of fraction of residual oxygen in the headspace.

After the air trip to Minneapolis, oxygen analyzer was used (MOCON, MPS) on both the intact bottles and on some previously sampled bottles, as indicated. Instrument display was adjusted to 20.7 % using repeated 10 mL ambient air injections.

Due to the presence of volatiles in headspace samples, an optional longer injection port was used which contains activated charcoal absorption tube. This is a recommended configuration that prevents sensor contami-

Table 2. Fraction of residual oxygen in untreated blanks (05/29), and in treated samples of bread crumbs, chicken fat with BHA and encapsulated citrus flavors, measuremed with gas chromatograph (GC) and oxygen analyzer (MOCON)

Tablica 2. Frakcija zaostalog kisika u neobrađenim slijepim probama (05/29) i obrađenim uzorcima s komadićima kruha, pileće masti s BAH i ukapsulirane citrusne arome mjerene plinskim kromatografom (GC) i analizatorom kisika (MOCON)

Location	>	TT		TT		MPS		TT	
Date	>	05/25		05/29		05/30		06/04	
Method	>	GC		GC		MOCON		GC	
Sample	*m/g*								
Air		20.98		21.01	20.98	20.7	(adjustment)		
Blanks		20.85	20.95	20.41		20.2		–	
		20.92		20.41		20.2		20.49	
		20.93		20.43		20.2		20.45	
		20.94		–		–		20.23	
		21.07	20.93	20.32		20.3		20.42	
		20.94		20.39		–		20.32	
		20.93		–		–		20.30	
		20.94		20.37		–		20.42	
Bread crumbs									
Control	10	13.79	14.94			13.6	14.7	15.94	16.76
	15	13.08	14.35			13.2	14.4	15.07	16.28
D31	10	14.40	–			14.6	–	16.49	16.65
	15	13.06				13.4		15.37	
D31 + rosemary	10	15.08	15.64			14.8	–	16.44	17.04
	15	14.27	15.88			15.3	–	17.53	17.38
B	10	17.17	13.42			17.7	13.4	18.96	15.64
	15	14.27	15.76			15.6	15.8	16.00	17.53
B + rosemary	10	14.91				14.7		17.34	
	15	15.22				15.1		18.20	
Chicken fat	10	9.30				10.7		12.63	
	15	5.24				6.8		8.93	
Encapsulated citrus flavors									
Glass extrusion									
Lemon 1	10			19.59	19.53	19.4	19.4	19.98	19.59
				–	–	–	19.4	19.32	19.61
Orange 1	10			19.86	19.96	19.8	–	–	19.96
				19.90	–	19.8	–	–	19.73
Spray drying									
Lemon 2	10			18.00	17.93	–	17.2	18.20	17.92
				17.86	17.87	17.6	–	18.38	17.88
Orange 2	10			14.39	–	–	–	14.55	14.38
				–	14.46	14.3	14.2	14.34	15.57
Lemon 3	5			18.09	–	17.5	17.2	18.25	18.12
				–		–		17.98	
Molecular inclusion									
Lemon 3	5			20.13	–	19.9	20.0	20.45	20.20
				–		–		20.10	

nation and danger of fire or explosion. Manufacturer's literature *(7,8)* cites sample size in 2–10 mL range. After a few trials, we chose 6 mL as an optimum size for this application. Chart recorder was connected to the instrument, and the continuous output was monitored during extreme samples' changes in both high-low and low-high directions. In all instances, the instrument response was fast and uniform.

Following the return trip, GC test was conducted in TT on samples with different histories, as shown. This series of measurements included some bottles that were not subjected to air travel.

Data analysis

Standardized baseline values were calculated for the GC method from the control blanks for the initial base time, for the treated samples and for the treated traveled blanks. For oxygen analyzer samples, it was assumed that the instrument would have read 20.7 % value for the initial air. The raw data was then compared to their respective baseline means as follows:

GC	Oxygen analyzer
20.94 % – test date value (»fresh«)	20.7 % – value
20.94 % – traveled	(fresh-traveled)

Changes from base line were then compared between the two methods as follows:

Mean (GC change – Oxygen analyzer change), i.e.
Mean [(20.94 % – fresh) – (20.7 % – value)].

Analogous comparison was made for traveled samples.

None of the differences between the two methods are significant at the 95 % level.

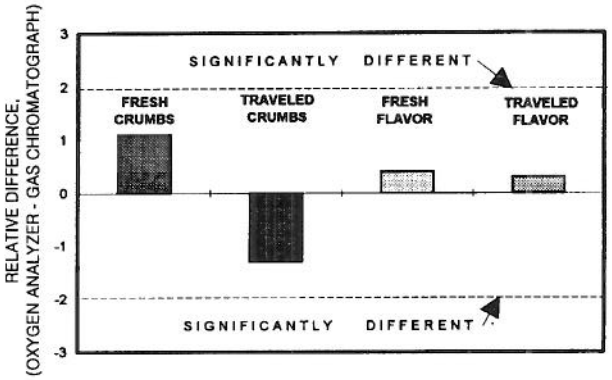

Fig. 1. Differences in fraction of residual oxygen results between oxygen analyzer and gas chromatograph for treated samples of bread crumbs and encapsulated citrus flavors, pooled results. Results marked »fresh« indicate intact samples that were first analyzed by the method indicated. Results marked »traveled« indicate repeated sampling and measurement on those samples that had been analyzed previously and have undergone air transport. See also Table 2. and the text for further details on sample history. Displayed bands of significant difference have been calculated from pooled standard deviations for all four group of samples shown.

Slika 1. Razlike u rezultatima zaostalog kisika između analizatora kisika i plinskog kromatografa

Referring to Fig. 1, GC fresh: Mean [GC change – Oxygen analyzer change]

GC fresh readings are lower than oxygen analyzer readings, both for crumbs and for flavors.

GC traveled: Mean [GC traveled change – Oxygen analyzer change]

GC traveled readings are higher than the oxygen analyzer readings for crumbs, and slightly lower than the oxygen analyzer readings for the flavors, respectively.

Time/travel effect: Mean [GC fresh – GC traveled] = – 2.2 %.

Due to the time/travel effect, GC readings for food samples have increased by 2.2 % (significant at the 95 % level). However, the mean difference for the blanks does not agree with this 2.2 % increase:

Mean blank fresh = 20.94 %
Mean blank traveled = 20.38
Difference = 0.56 % *decrease.*

Discussion

Above observations may be explained by different effects of sampling for the two methods and by the driving forces on oxygen concentration equilibration between the headspace and external air (imperfect sealing). In blanks, removal of only 0.25 mL from a full 60 mL, headspace with a thinner syringe causes only small pressure differential, with little or no puncture channeling in the stopper. By contrast, removal of a 6 mL oxygen analyzer sample from the reduced headspace of ca. 40–50 mL does create vacuum in the bottle. Compounded with the pressure changes in the aircraft during the return trip, the ambient air may have begun to penetrate into the headspace through a larger syringe-formed perforation in the stopper causing increased oxygen content.

In support of this explanation, combined time/travel changes are higher in crumbs samples where oxygen had been more depleted than in the flavor samples that had much lower oxygen depletion. From Fig. 1, while neither fresh crumbs' difference or traveled crumbs' difference is statistically significant by itself, the difference between them (2.4 % > 2.0 %) is indeed significant. That is not the case with the flavor samples.

The introduction of the oxygen analyzer method made it much easier for us to bring the oxygen uptake testing to the manufacturing plant as a possible quality control (QC) tool. During large-scale trials with spray-dried lemon flavor, finished product was repeatedly sampled and analyzed by the QC personnel in the plant laboratory.

Table 3. Analyst-to-analyst variation in measurements of fraction of residual oxygen in untreated and treated blanks (quadruplicates) and treated samples of spray-dried lemon flavor representing a commercial lot (triplicates). Measurements performed by plant quality control personnel using oxygen analyzer method.

Tablica 3. Razlike u rezultatima dobivenim s analizatorom kisika, koje su proveli tvornički kontrolori kakvoće određujući udjele zaostalog kisika u obrađenim i neobrađenim slijepim probama (kvadriplikati) i obrađenim uzorcima s aromom limuna nanesenom metodom raspršivanja (triplikati)

Analyst	Untreated (initial) blank	Final (treated) blank	Spray-dried lemon flavor		
			Sample #5	Sample #12	Sample #20
T.I.	20.6	19.6		17.7	17.1
	20.5	19.8		17.7	17.2
	20.7	19.8		17.5	17.2
	20.6	19.8			
A.B.	20.6	19.8	17.3		17.1
	20.6	19.8	17.6		17.0
	20.6	19.8	17.6		17.1
	20.5	19.6			
D.F.	20.7	19.7	17.4	17.4	
	20.5	19.8	17.5	17.6	
	20.6	19.7	17.5	17.5	
	20.6	19.7			

Results of the partial-block test are presented in Tab. 3. Without any preliminary training, good repeatability was achieved, and analyst-to-analyst variations were not significant. There is a slight genuine sample-to-sample variation related to the changing lemon oil content in the encapsulated flavor.

Conclusion

In conclusion, oxygen analyzer method yields closely comparable results to the GC method. Reasonable explanation exists for the observed differences found in the conditions atypical for normal testing. Due to its simplicity, ease of use and lower cost, oxygen analyzer method is now being permanently used as the only oxygen uptake test in our flavor technology laboratory. Finally, we also demonstrated test suitability for potential routine QC applications in the manufacturing environment.

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