Food Sci. Technol. Res., **12** (4), 256–260, 2006

Flavor Release of Diacetyl and 2-Heptanone from Skimmed and Full Fat Milk under Mouth Conditions

Sachiko ODAKE[1]*, Saskia M. Van RUTH[2] and Ryozo AKUZAWA[1]

[1] *Nippon Veterinary and Life Science University, 2–27–5 Sakai, Musashino, Tokyo 180–0022, Japan*
[2] *Institute of Food Safety, PO Box 230, 6700 AE Wageningen, The Netherlands*

Received February 8, 2006; Accepted August 12, 2006

To gain insight into the process of retronasal olfaction, flavor release from skimmed milk and full fat milk (containing 3.75% fat) was investigated using a model mouth system with a screw plunger. Large differences were determined in the quantity of flavor released using two different methods: with screw plunger movement, which represents "retronasal" flavor release, and without the screw plunger, which represents "orthonasal" flavor release. The screw plunger motion accelerated the release of diacetyl and 2-heptanone in both skimmed and full fat milk. The amount of diacetyl released was not influenced by the fat content of the milk in either case (with or without screw plunger operation), while the amount of 2-heptanone released was lower in full fat milk both with and without screw plunger motion. The influence of fat content on the amount of flavor released was explained by the lipophilicity of the flavor components, and mass transfer also contributed to release of the hydrophilic flavor compound.

Keywords: flavor release, milk, model mouth, log P, partition coefficient

Introduction

The release of flavors from food during mastication plays an important role for the consumer in deciding the degree of satisfaction and the acceptability of a food. The perception induced by flavors released during mastication is called "retronasal olfaction" (Fig. 1). This is distinguished from "orthonasal olfaction", which occurs before putting food into the mouth, and in which flavors from the food pass through the nostrils. The importance of studying flavor release from the viewpoint of retronasal olfaction was reviewed by Overbosch (1991). In Odake's questionnaire survey (2002), panelists who showed low acceptability of milk noted that the smell during consumption was the most predominant negative aspect. Consequently, it is important to investigate retronasal perception of flavor compounds during milk consumption, taking account of flavor release behavior.

The processes which occur during mastication are complex, with many variations taking place over a very short period of time. These include changes in food temperature, composition, and shape, along with salivation and variations in breath flow and chewing speed. One effective method for elucidating these complex aspects of retronasal flavor release is the use of model mouths or mouth simulators (Piggott, 2001). The first model mouth which focused on the aspect of flavor release under mouth-like conditions was reported by Lee (1986). The simple device consisted of a sample bottle containing 2-mm metal or glass balls, which was shaken from side to side. Lee reported that the degree of flavor release agreed with the sensory intensity. About a decade after this work, many groups devised model mouths mimicking the motion of mastication. The mouth-mimicking systems differed for each group: impeller rotation (Naßl *et al.,* 1995), magnetic bar stirring (Elmore and Langley, 1996 and Bakker *et al.,* 1998), or the use of a cutting blender (Roberts and Acree, 1996) or a screw plunger (Van Ruth *et al.,* 1994 and Odake *et al.,* 1998 and 2000).

This study was performed to investigate flavor release from milk in the mouth. A model mouth system with a screw plunger, which was reported to achieve sufficient mixing of various viscous emulsion samples (Odake *et al.,* 1998), was used. Two flavor compounds, diacetyl and 2-heptanone, discussed in previous studies (Overbosch *et al.,* 1991 and Odake *et al.,* 1998), were selected for study with the aim of clarifying release behavior in terms of lipophilicity.

Materials and Methods

Milk Raw milk was taken from a Holstein cow at Fuji Animal Farm (a farm affiliated to the Nippon Veterinary and Life Science University, Fujigamine, Yamanashi Prefecture) on 15 August and 15 September 2005. Skimmed milk was prepared from the raw milk by centrifuging (KUBOTA7930, Tokyo, Japan) at 4000 rpm for 15 minutes. Full fat milk was prepared by homogenizing (H-30G, Sanmaru Machinery Co., Ltd., Shizuoka, Japan) the raw milk at 14.7 MPa for 2 minutes after heating to 50℃. The fat content of the milk sample was measured

* To whom correspondence should be addressed.
E-mail: odake@nvlu.ac.jp

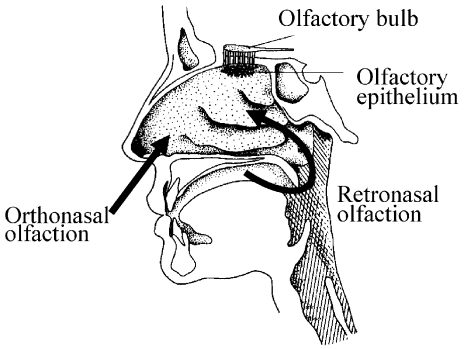

Fig. 1. Retronasal and orthonasal olfaction.

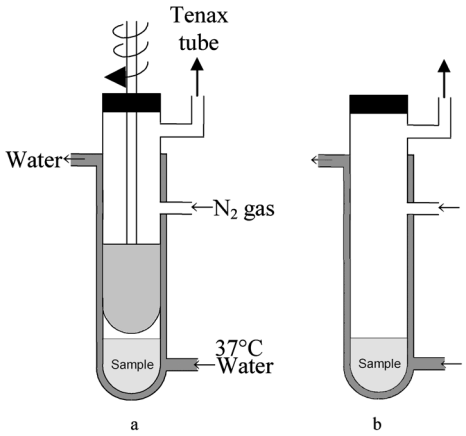

Fig. 2. Model mouth system.
a and b: Schematic diagrams with and without screw plunger, respectively.

by the Gerber method (Roginski, 2002). Non-fat solids in the milk were measured by the AOAC method (2000). The milk fat globule distribution of the full fat milk was determined using a laser scattering particle size distribution analyser (LA-500, HORIBA, Ltd., Kyoto, Japan).

Flavor compounds Diacetyl (>97% purity, Merck-Schuchardt, Hohenbrunn, Germany) and 2-heptanone (>98% purity, Sigma, St. Louis, MO, USA) were used without further purification to represent water-soluble and oil-soluble flavor compounds, respectively. Both compounds were added at 20 ppm concentration to the milk samples, and the experiment was conducted after leaving the flavored milk to equilibrate at 4℃ for 24 hours. All experiments were finished within two days of milking at the farm.

Model mouth apparatus The model mouth shown in Fig. 2 was used, with the sample flask kept at 37℃ during the experiment. The milk sample (5.0 mL) was put in the sample flask and the released flavors were trapped on Tenax TA (poly (2,6-diphenyl-*p*-phenylene oxide), 60/80 mesh, 180 mg), via a glass tube 178 mm length and 4 mm i. d. (Gerstel, Mülheim an der Ruhr, Germany) for 2 minutes under a flow of purified nitrogen gas at a flow rate of 100 mL/min. The experiment was carried out with and without the plunger being connected. When connected, the plunger moved vertically at 1.5 Hz with a stroke of 20 mm, and simultaneously rotated at 86 rpm. These conditions were chosen to approximate real throat movement, based on a preliminary experiment in which the throat movements of 24 panelists were observed during milk-swallowing (average age of panelists 23.8, male-to-female ratio=1: 1). Operation with plunger movement represents the "retronasal" type of flavor release, and that without plunger movement the "orthonasal" type.

GC-MS analysis After desorption from Tenax TA using a thermal desorption/cold trap device (TDS2, Gerstel), the flavors were analyzed by GC-MS (6890GC-5973MSD, Agilent Technologies, Inc., Palo Alto, CA, USA) with DB-WAX (30 m length, 0.25 mm i.d. and 0.25 μm film thickness, Agilent Technologies). Column pressure was 116.0 kPa. The oven temperature was held at 40℃ for 3 min and programmed to increase to 240℃ at a rate of 8℃/min followed by further holding at 240℃ for 10 min. Each result was the mean of results obtained using 2 samples (15 August and 15 September), and the data were analyzed by analysis of variance (ANOVA, 3-way layout) and Tukey's test with significant difference level at $p<0.05$ (EXCEL Toukei Ver. 5.0, ESUMI Co., Ltd., Tokyo, Japan).

Diacetyl and 2-heptanone dissolved in methanol (>99.5% purity, Merck-Schuchardt) at 50% concentration were injected (0.02 μL and 0.05 μL) onto Tenax TA and thermally desorbed as mentioned above, and calibration curves were obtained to allow calculation of the amounts of the flavor compounds present. The measurements were duplicated, and the calibration curves for the amounts of the volatile compounds injected [x] (μg) versus the peak areas of the total ion chromatogram [y] were as follows: $[y]=2.293\times10^8[x]$ for diacetyl ($r^2=0.982$, $p<0.05$) and $[y]=3.118\times10^8[x]$ for 2-heptanone ($r^2=0.980$, $p<0.05$).

Log P calculation Log P values of diacetyl and 2-heptanone were calculated using the ACD/Log P DB program (2003).

Results

Properties of the milk samples Measurement of the fat content by the Gerber method showed that the fat content of the skimmed milk was 0.00±0.00%, and that of the full fat milk was 3.75±0.07%. The contents of non-fat solids in the skimmed and full fat milk were 9.5±0.0% and 8.4±0.1%, respectively. The fat globule distribution of the full fat milk is shown in Fig. 3. The median diameter was 1.15±0.00 μm.

Effect of plunger movement The amounts of diacetyl and 2-heptanone released from the skimmed and full fat milk samples are shown in Fig. 4. Large differences were determined in the quantities of flavors released using the two methods: with plunger movement (which represents "retronasal" flavor release), and without ("orthonasal" flavor release). The amounts of diacetyl and 2-heptanone released from both skimmed and full fat milk with plunger motion were larger than those released without plunger motion. The significant difference between the amounts released with and without plunger motion was calculated using ANOVA ($p<0.01$). The amount of diacetyl released

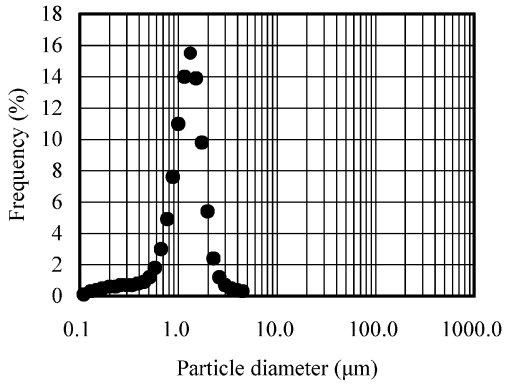

Fig. 3. Particle size distribution of full fat milk.

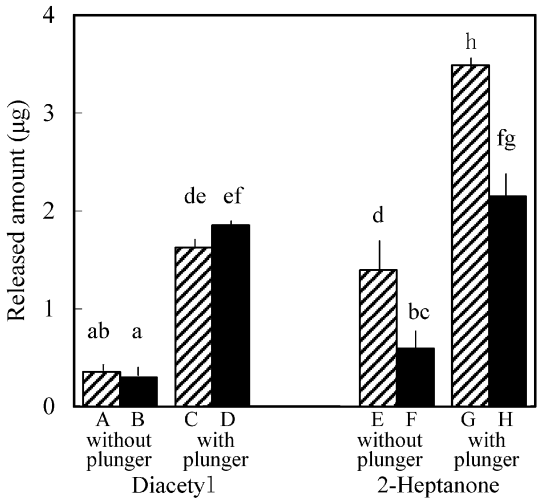

Fig. 4. Amounts of diacetyl and 2-heptanone released from skimmed and full fat milk.
Skimmed milk, Full fat milk.
The different small letters indicate the significant differences by Tukey's test at $p<0.05$.

from skimmed milk with plunger motion was 5 times larger than that without plunger motion, and in the case of 2-heptanone, 3 times larger. The amount of diacetyl released from full fat milk with plunger motion was 6 times larger than that without plunger motion, and in the case of 2-heptanone, 4 times larger. These differences were confirmed to be significant by Tukey's test with $p<0.05$ (A-C, B-D, E-G, and F-H in Fig. 4).

Effect of fat content The effect of fat content was different for diacetyl and 2-heptanone; the significant difference between the amount released from skimmed milk and that from full fat milk was obtained by ANOVA ($p<0.01$). In the case of diacetyl, the amount released from skimmed milk without plunger motion was greater than that from full fat milk. In contrast, the amount released from skimmed milk with plunger motion was less than that from full fat milk. In both cases, no significant difference was found between skimmed milk and full fat milk by Tukey's test (A-B and C-D in Fig. 4). For 2-heptanone, the amounts released from full fat milk (with and without plunger motion) were smaller than those released from skimmed milk—40% without plunger motion and 60% with plunger motion—and they were significantly different ($p<0.05$) by Tukey's test (E-F and G-H in Fig. 4).

Effect of flavor compounds The amount of 2-heptanone released was higher than that of diacetyl in every case. The differences in the amounts of the two compounds released were significant at $p<0.01$ (ANOVA). The amounts of diacetyl and 2-heptanone released were significantly different in all operations (A-E, B-F, and C-G in Fig. 4) except for full fat milk with plunger motion as shown in Fig. 4 D-H.

Effect of interactions in ANOVA The main variables (plunger movement, fat content, and flavor compound) all produced significant differences ($p<0.01$) by ANOVA as shown above. Among two-factor interactions, "plunger movement×flavor compound" and "fat content×flavor compound" were significant at $p<0.01$, and the interaction "plunger movement×fat content" was not significant. With regard to the three-factor interaction "plunger movement×fat content×flavor compound", the result showed significance at the level $p<0.01$.

Discussion

Conduction of the experiment with screw plunger motion represents "retronasal" flavor release, while that without screw plunger motion represents "orthonasal" flavor release. The screw plunger motion was found to accelerate the release of flavors in both diacetyl and 2-heptanone from both skimmed and full fat milk. The same effect was observed in other food samples such as French beans, bell peppers, and leeks (Van Ruth *et al.*, 1995), and emulsion samples (Odake *et al.*, 1998) with the same type of model mouth system. The screw plunger motion mimics the processes that occur in real eating, such as mixing food with the tongue and swallowing food by link motion of the tongue and palatum molle.

The fat content influenced the release of diacetyl and 2-heptanone differently. The two flavor compounds are ketones which display opposing behaviors with regard to water and fat/oil. The log P value indicates the degree of hydrophobicity or hydrophilicity using the octanol-water partition coefficient, P. A compound with a higher log P value is more hydrophobic, partitioning more in the oil phase than in the water phase. In contrast, compounds with a lower log P value are less hydrophobic, partitioning more in the water phase than in the oil phase. The calculated values of log P were −1.33 for diacetyl and 1.97 for 2-heptanone, which means that diacetyl is relatively hydrophilic and 2-heptanone relatively lipophilic. Therefore, in full fat milk, diacetyl tends to be distributed in the water phase and 2-heptanone in the fat globules.

Roberts and Pollien (2000) reported that the behavior of flavor compounds released from milk, measured in the static headspace, allowed separation of the compounds into 3 groups, according to their determined lipophilicity, as follows. Compounds in the first group (group A in Fig. 5) were not influenced by the milk component (fat and non-fat solids); diacetyl and 2,3-pentanedione were

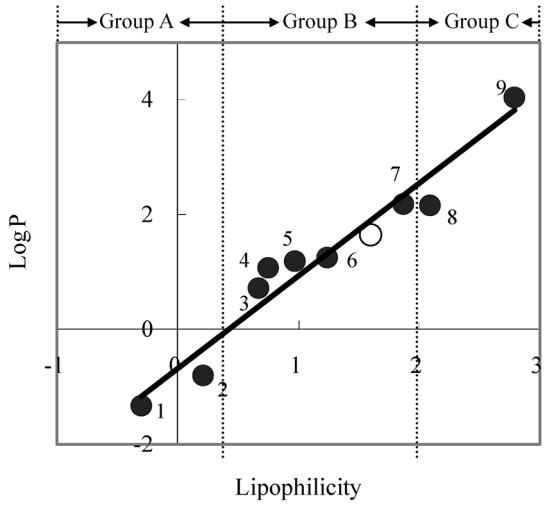

Fig. 5. Relationship between lipophilicity and log P.
Lipophilicity was obtained from Roberts and Pollien (2000) and log P was calculated using ACD/Log P DB (2003).
The relationship between lipophilicity and log P was approximately expressed by a linear equation as follows: log P = 1.61 × lipophilicity − 0.68 (r^2 = 0.947, p < 0.0005) using values of 9 compounds.
● Compounds measured by Roberts and Pollien (2000): **1**, diacetyl; **2**, 2,3-pentanedione; **3**, 2-methylpropanal; **4**, 3-methyl-2-butenal; **5**, guaiacol; **6**, 3-methylbutanal; **7**, 4-ethylguaiacol; **8**, 1-octen-3-one; **9**, β-damascenone.
○ 2-Heptanone interpolated into the linear equation using log P = 1.97.
The compounds of group A were not influenced by milk component (fat or non-fat solids); the compounds of group B showed a reduction in volatility with milk fat content but not with non-fat solids content; the compounds of group C showed a reduction in volatility with non-fat solids and a much greater reduction with fat content (Roberts and Pollien, 2000).

included in this group. The second group (group B), including 2-methylpropanal, 3-methylbutanal, guaiacol and 4-ethylguaiacol, showed a reduction in volatility with higher milk fat contents but not with higher contents of non-fat solids. The compounds of the last group (group C), such as β-damascenone and 1-octen-3-one, decreased in volatility in the presence of higher concentrations of non-fat solids but showed a much greater reduction in the presence of increased fat contents. They used values of lipophilicity measured using the same system reported by Piraprez *et al.* (1998). Comparing the lipophilicity measured by Roberts and Pollien (2000) and the log P values calculated using software (Fig. 5), the relationship was approximately expressed by the linear equation log P = 1.61 × lipophilicity − 0.68 (r^2 = 0.947, p < 0.0005). Interpolating log P as being 1.97, the lipophilicity of 2-heptanone was estimated at 1.66. Thus, 2-heptanone belongs to the second group, i.e. compounds whose volatility was influenced only by fat, not by non-fat solids. The non-fat solids contain protein, sugars, minerals, vitamins, and organic acids.

Some results concerning flavor compound-milk protein interactions and binding have been reported. Guichard and Langourieux (2000) showed that 7% and 20% of 2-heptanone bound to 2% and 3% β-lactoglobulin aqueous solutions, respectively. They mentioned that hydrophobic interactions in the central cavity of β-lactoglobulin were the main reason for the binding of flavor compounds. Since the content of β-lactoglobulin is 0.2–0.4% in skimmed milk (Eigel *et al.*, 1984), it can be estimated that the possibility of binding of 2-heptanone to β-lactoglobulin is approximately 10% of that obtained in the results of Guichard and Langourieux. It was reported that no binding took place between diacetyl and milk proteins (whey protein concentrate, α-lactalbumin, β-lactoglobulin, or milk powder) using dynamic headspace analysis and solid-phase microextraction analysis (Fabre *et al.*, 2002).

The difference in the amounts of diacetyl and 2-heptanone released from skimmed milk was explained by the partition coefficient difference of the air-water phase of the two compounds. Since skimmed milk is considered to be an aqueous solution containing non-fat solids, the air-water partition coefficients (K_{aw}), reported by Overbosch *et al.* (1991) as K_{aw} = 0.0016 for diacetyl and K_{aw} = 0.0116 for 2-heptanone, can be applied. Based on these values, it is expected that diacetyl should be retained more efficiently by skimmed milk than 2-heptanone, resulting in higher headspace concentrations of 2-heptanone. In full fat milk, fat globules act as reservoirs for 2-heptanone, as mentioned above using log P values, so 2-heptanone is retained to a greater extent in full fat milk compared to skimmed milk. In contrast, the presence of milk fat did not affect the retention of diacetyl, which was reflected by the statistically identical results for the amounts of diacetyl released by skimmed milk and full fat milk.

From the results showing increasing release ratios, it is noted that mouth movements effectively caused an increased release of diacetyl compared to 2-heptanone in both skimmed milk and full fat milk. This is thought to be due to the fact that the release of diacetyl is more limited by mass transfer. The mechanism of mass transfer under dynamic conditions is mathematically predicted by the penetration theory (Van Ruth and Roozen (2004)). The penetration theory takes into account that the boundary layers are often not completely stagnant and that there is also mass transport by eddy diffusion. Partitioning of diacetyl in the water phase was dominant in full fat milk, and the mixing action by the screw plunger allowed fast renewal of diacetyl concentrations at the air/water interphase. The effect of mastication was also greater for full fat milk than for skimmed milk. This shows a similar aspect of mass transfer not from the aqueous phase but from the oil phase. The fat droplets were sufficiently mixed by the screw plunger to have a chance of being exposed to the air phase, and consequently, transportation of diacetyl from oil to air was effective.

The effect of mastication changed the balance of the flavor compounds between no-chewing conditions and chewing conditions. The proportional change in flavors was observed as the ratio of hydrophilic flavor increase

with chewing motion. From this result, it can be concluded the flavor profile perceived by retronasal olfaction differs from that perceived by orthonasal olfaction.

Conclusion

Flavor release from milk during consumption was investigated using a model mouth system with a screw plunger. The screw plunger motion represents the action of the tongue and the palatum molle, and this was found to accelerate the release of flavors. The amount of the hydrophilic flavor compound, diacetyl, that was released was not influenced by the amount of fat in the milk samples, regardless of whether simulation of mastication was carried out. The amount of the hydrophobic flavor compound, 2-heptanone, that was released was found to decrease when the fat content of the milk increased, both with and without mastication. The release behavior of diacetyl and 2-heptanone was explained by partitioning and mass transfer, and the effect of mastication was a change in the proportion of the released flavors.

Acknowledgements The authors gratefully acknowledge Morinaga Milk Industry Co., Ltd., for analysis of the fat globule distribution in milk. This study was supported by Grant-in-Aid for Scientific Research (B) No. 17300239 from JSPS, Japan and High-Tech Research Center Project (2004–2008), from MEXT, Japan.

References

ACD/Log P DB (2003), version 8.0, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com.

AOAC (2000). Official methods of analysis of AOAC International, 17th ed., Chapter 3, p.33.

Bakker, J., Boudaud, N. and Harrison, M. (1998). Dynamic release of diacetyl from liquid gelatin in the headspace, *J. Agric. Food Chem.* **46**, 2714–2720.

Eigel, W.N., Butler, J.E., Ernstrom, C.A., Farrell, H.M., Harwalkar, V.R., Jenness, R. and Whitney, R. McL. (1984). Nomenclature of proteins of cow's milk, *J. Dairy Sci.* **67**, 1599–1631.

Elmore, J.S. and Langley, K.R. (1996) Novel vessel for the measurement of dynamic flavour release in real time from liquid foods, *J. Agric. Food Chem.* **44**, 3560–3563.

Fabre, M., Aubery, V. and Guichard, E. (2002). Comparison of different methods: Static and dynamic headspace and solid-phase microextraction for the measurement of interactions between milk proteins and flavour compounds with an application to emulsions, *J. Agric. Food Chem.* **50**, 1497–1501.

Guichard, E. and Langourieux, S. (2000). Interactions between β-lactoglobulin and flavour compounds, *Food Chem.* **71**, 301–308.

Lee, W.E. III (1986). A suggested instrumental technique for studying dynamic flavor release from food products, *J. Food Sci.* **51**, 249–250.

Naßl, K., Kropf, F. and Klostermeyer, H. (1995). A method to mimic and to study the release of flavour compounds from chewed food, *Z. Lebensm. Unters. Forsch.* **201**, 62–68.

Odake, S., Roozen, J.P. and Burger, J.J. (1998). Effect of saliva dilution on the release of diacetyl and 2-heptanone from cream style dressings, *Nahrung* **42**, 385–391.

Odake, S., Roozen. J.P. and Burger, J.J. (2000). Flavor release of diacetyl and 2-heptanone from cream style dressings in three mouth model systems, *Biosci. Biotechnol. Biochem.* **64**, 2523–2529.

Odake, S., Shimamura, T. and Akuzawa, R. (2002). Difference in perception and intake of milk among female adults in different occupations and study fields and children (boys & girls) in rural Yamanashi prefecture, Japan, *ITE Lett. Batt. New Tech. Med.* **3**, 608–611.

Overbosch, P., Afterof, W.G.M. and Haring, P.G.M. (1991). Flavor release in the mouth, *Food Reviews Inter.* **7**, 137–184.

Piggott, J.R. and Schaschke, C.J. (2001). Release cells, breath analysis and in-mouth analysis in flavour research, *Biomolecular Engineering* **17**, 129–136.

Piraprez, G., Hérent, M.-F. and Collin, S. (1998). Determination of the lipophilicity of aroma compounds by RP-HPLC, *Flavour Fragr. J.* **13**, 400–408

Roberts, D.D. and Acree, T.E. (1995). Simulation of retronasal aroma using a modified headspace technique : Investigating the effect of saliva, temperature, shearing, and oil on flavor release, *J. Agric. Food Chem.* **43**, 2179–2186.

Roberts, D.D. and Pollien. P. (2000). Relative influence of milk components on flavour compound volatility, "Flavour Release", ed by Roberts, D.D. and Taylor, A.J., American Chem. Soc., USA, pp. 321–332.

Roginski, H. (2002). Encyclopedia of dairy sciences, Academic Press, UK, pp. 36 and 1585.

Van Ruth, S.M., Roozen, J.P. and Cozijnsen, J.L. (1994). Comparison of dynamic headspace mouth model systems for flavour release from rehydrated bell pepper cuttings, "Trends in Flavour Research", ed by Maarse, H. and Van der Heij, D.G., Elsevier Science, pp. 59–64.

Van Ruth, S.M., Roozen, J.P. and Cozijnsen, J.L. (1995). Volatile compounds of rehydrated French beans, bell peppers and leeks, *Food Chem.* **53**, 15–22.

Van Ruth, S.M. and Roozen, J.P. (2004). Delivery of flavours from food matrices, "Food Flavour Technology", ed by Taylor, A.J., Academic Press, Sheffield, pp. 167–184.