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Lipolysis in reduced sodium Kefalograviera cheese made by partial replacement of NaCl with KCl

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Abstract

Kefalograviera cheeses (five trials) of different sodium contents were made from split lots of curd by varying the salting processes, i.e. brine- and dry-salting with NaC1 (control) or a mixture of NaClJ/Cl (3:1 or 1:1, w/w basis). Lipolysis in cheeses was monitored during aging by the acid degree value (ADV) method and gas chromatography (GC). It was found that the ADV of control and experimental cheeses were similar ($P > 0.05$) at all sampling ages (5, 25, 60, 90 and 180 days). Moreover, the results of GC showed that there were neither qualitative nor significant ($P > 0.05$) quantitative differences in the levels of individual free fatty acids of the control and experimental cheeses at the age of 90 and 180 days. These findings indicated that the partial replacement of NaCl with KCl in the manufacture of Kefalograviera cheese did not significantly influence the lipolysis during cheese aging.

1. Introduction

As a result of the association of sodium intake primarily with hypertension (Abernethy, 1979; Dillon, 1987; Freis, 1976; Tobian, 1979) but also with osteoporosis (Goulding, Gold & Campbell, 1993) and the incidence of kidney stones (Goulding, 1997), the consumer's concern about sodium in processed foods has increased (Reddy & Marth, 1993). The most frequent estimate of the minimum adult daily requirement for sodium is 200 mg (0.5 g of NaC1), while the average total daily sodium intake by most persons in developed countries is 4 to 5 g (10–12 g of NaCl) (Dillon; IFT, 1980). These quantities, which are 10–35 times greater than the minimum adult requirement (NAS/NRC, 1980a; Shank, Youngmee, Harland, Vanderbeen, Forbes & Prosky, 1982), are regarded as excessive, even dangerous, by many of those responsible for public health (Dillon). A sodium intake of 1100–3300 mg (2.8–8.3 g of NaCl) per day has been recommended as safe and adequate for adults (NAS/NRC, 1980b). Various studies have indicated that an increased intake of potassium, via the diet, exerts a protective effect in individuals with sodium-induced hypertension (Fregly, 1981; Haddy, 1991; Lecos, 1983; Linas, 1991), reduces urinary calcium excretion and potentially protects skeletal mass (Lemann, Pleuss & Gray, 1993).

Some dairy foods, such as natural and processed cheeses, are high in sodium content and attempts to reduce this have been made by the dairy industry and scientific community (Reddy & Marth, 1991). When the salt concentration in cheese is simply reduced, proteolysis, water activity, acidity and bitterness all increase, while firmness and saltiness decrease (Editorial, 1993); abnormal fermentations may also occur (Olson, 1982; Petik, 1987). All of these factors make it difficult to reduce the sodium level in cheese substantially without adversely affecting quality. However, replacing some of the NaCl by KCl helps to address some of the above problems (Editorial). KCl has been the most widely and successfully used partial replacement for NaCl in cheese. Numerous attempts have been made by the scientific community and cheese industry to develop an acceptable low-sodium cheese using NaCl/KCl mixtures (Reddy & Marth, 1991).

In Cheddar cheese, partial replacement (1:1) of NaCl by KCl induced a higher level of lipolysis (Lindsay, Hargett & Bush, 1982). Fitzgerald and Buckley (1985) reported that, not partial, but total substitution of KCl for NaCl enhanced lipolysis rates in Cheddar cheese.

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However, Reddy and Marth (1993) found no significant ($P > 0.05$) differences in lipolysis at a given sampling time among cheeses made with NaCl, KCl or mixtures of these two salts.

Kefalograviera cheese, a traditional Greek cheese, has a high salt content of about 3.4% (Anifantakis, 1991; Katsiari, Voutsinas, Alichanidis & Roussis, 1998), which is equivalent to 1338 mg Na per 100g of cheese, since salt consists of about 39% sodium (IFT, 1980). Recently, we reported (Katsiari et al., 1998) the feasibility of reducing the sodium content in Kefalograviera cheese up to 50% by using mixtures of NaCl and KCl (3:1 or 1:1 w/w) in the salting process without adversely affecting the quality. Our results also indicated that the cheeses made with these NaCl/KCl mixtures exhibited no significant ($P > 0.05$) differences in composition (moisture, fat, protein, total salt), physicochemical (pH, a_w), sensory (appearance, body and texture, flavour, overall quality) or textural properties (force and compression to fracture, hardness, cohesiveness, springiness, guminess, chewiness) the control cheese. The objective of the present study was to compare lipolysis in Kefalograviera cheeses made with the above mixtures of NaCl and KCl with that of cheese made with NaC1 (control).

2. Materials and methods

2.1. Cheese manufacture

Kefalograviera cheese was manufactured from ewes' milk at the pilot plant of the Dairy Research Institute as described in detail by Katsiari et al. (1998).

2.2. Lipolysis

The level of lipolysis was assessed in samples of 5, 25, 60, 90 and 180-day-old cheese by measuring the acid degree value. In addition, determination of individual free fatty acids (FFA) by gas chromatography was carried out in cheeses aged for 40 and 120 days.

2.3. Acid degree value (ADV)

The ADV was determined as described by Deeth and Fitz-Gerald (1976). Samples were prepared by mixing 5 g of cheese with 37.5 ml of 2% sodium citrate at 50°C in a Sorvall Omni-mixer at setting 3 for 1 min, and then at setting 7 for 2 min. The ADV was determined on 35 ml samples of this extract.

2.4. Gas chromatography (GC)

Extraction of cheese lipids, isolation of the FFA and determination of the FFA concentration by GC were performed as described by De Jong and Badings (1990). The sample was prepared as follows: cheese (1.0 g) was ground with anhydrous Na_2SO_4 (3.0 g), and then 0.3 ml H_2SO_4 (2.5 M) and 1.0 ml internal standard solution containing $C_{5:0}$, $C_{7:0}$, $C_{13:0}$ and $C_{17:0}$ (0.5 mg/ml each) were added. This mixture was extracted three times with 3 ml diethyl ether/heptane (1:1, v/v). After each extraction, the solution was clarified by centrifugation (500 × g for 2 min at room temperature), and the upper solvent layer was transferred to a screw-capped tube containing anhydrous Na_2SO_4 (1.0 g). The pooled diethyl ether/heptane extract was applied to a Bond Elut aminopropyl column (2.8 ml, containing 500 mg of silica modified with aminoprolyl groups; Varian, Harbor City, CA, USA), which was conditioned with 10 ml heptane. The neutral lipids were eluted from the column with 10 ml chloroform/2-propanol (2:1, v/v). The FFA were eluted with 10 ml diethyl ether containing 2% formic acid. An injection standard, 500 μg $C_{9:0}$, was added to the solution, in order to check the recovery of the internal standards. A sample (0.5 μl) from this solution was taken for GC determination of the FFA. Two chromatographic injections were made from each cheese extract.

A Shimadzu model GC-17A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), equipped with an on-column injector and a flame-ionization detector (FID) was used with fused silica capillary column (length 15 m, inner diameter 0.53 mm), coated with free fatty acid phase OV-351 (bonded polyglycol–nitroterephthalic, film thickness 1.0 μm; Ohio Valley Specialty Chemical, Inc., Marietta, OH, USA). Direct cold on-column injection took place at 60°C; the injector temperature was raised from 60 to 230°C at a rate of 35°C/min, and then held at 230°C for 40 min. Column oven temperature was programmed from 60 to 70°C at a rate of 1°C/min, after a 2 min hold at 60°C, and then to 220°C at a rate of 10°C/min and a 18 min hold at 220°C. The detector temperature was held at 225°C. The flow rate of carrier gas (helium) was 8.8 ml/min, and the flow rates for hydrogen and air were 50 and 500 ml/min, respectively. The identification of the individual fatty acids of the cheese samples was based on a comparison of the retention times of the unknown FFA with those obtained from known FFA standards (≥99% GC; Sigma, Steinheim, Germany) under identical conditions. The quantitation of the FFA of cheese samples was performed using the internal standardization technique with $C_{9:0}$ as an internal standard and processing the chromatograms with the CLASS-VP™ Chromatography Laboratory Automated Software System (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA).

2.5. Statistical analysis

The data were analysed by One-way Analysis of Variance (ANOVA) to test the differences (at $P < 0.05$)

among the three cheeses at each sampling age using the software Statgraphics (Statistical Graphics Corp., Rockville, MD, USA).

3. Results and discussion

3.1. Acid degree value

The extent of lipolysis in the cheese, expressed as acid degree value (ADV), at different sampling ages is shown in Table 1. The ADV in all cheeses increased continuously during aging. The rate and extent of lipolysis in the control cheese was similar to that of Kefalograviera cheese (control) made by Katsiari and Voutsinas (1994). As may be seen from Table 1, the ADV of cheeses salted with the NaCl/KC1 mixtures were not significantly ($P > 0.05$) different from that of the control cheese at all sampling ages. This finding is in accordance with the results of Reddy and Marth (1993) who reported no significant differences ($P > 0.05$) in the levels of total FFA, measured by the cooper soap method, of Cheddar cheeses made with NaC1, KCl, or various mixtures of these two salts. However, Aly (1995) found that the concentrations of total volatile fatty acids (expressed as ml 0.1 M NaOH per 100 g cheese) increased significantly ($P < 0.01$) in UF Feta-type cheese by partially replacing NaCl with KC1.

The change of mean ADV of all cheeses, i.e. control and experimental, during aging, is presented in Fig. 1. Up to 90 days, i.e. the ripening time of Kefalograviera cheese, the polynomial correlation coefficient was significantly high ($R^2 = 0.9997$), indicating that the ADV may be a good index of Kefalograviera cheese ripening.

3.2. Gas chromatography

Chromatographic separation of underivatized FFA (Fig. 2) allowed the quantitation of all major fatty acids in one run. Although acetic acid is not produced by lipolysis, it was included in this study because it is extracted with FFA (Lesage, Voilley, Lorient & Bezard, 1993) and contributes to the final flavour of Gruyere cheese (Zerfiridis, Vafopoulou-Mastrogiannaki & Litopoulou-Tzanetaki, 1984), a cheese related to Kefalograviera but having half the salt content. The mean concentrations of individual FFA for Kefalograviera cheeses are presented in Table 2. Acetic and palmitic acids were the dominant FFA found in all cheeses at both sampling ages. These results are in agreement with those of Zerfiridis et al. who reported that acetic and palmitic were the dominant short- and long-chain fatty acids, respectively, in commercial Greek Gruyere cheese made from cow's milk and salted only with NaCl. Acetic and palmitic acids made up 32 to 38% and 14 to 16%, respectively, of total FFA present in all Kefalograviera cheeses (Table 2). Butyric and myristic acids were also found in considerable quantities, while the levels of oleic and linoleic acids were low in all cheeses. The experimental cheeses had higher, but not significantly ($P > 0.05$) different, concentrations of acetic acid at both sampling ages, especially at 90 days. It should be noted that short chain FFA are not all derived from lipolysis, but some are from breakdown of amino acids. Thus, acetic acid is produced, in addition to lactose fermentation, mainly from the degradation (oxidative deamination or decarboxylation) of the amino acids alanine and serine, isobutyric acid from valine, isovaleric acid from isoleucine and valeric acid from leucine (Hanspach, 1981).

Table 2 shows that there were neither qualitative nor significant ($P > 0.05$) quantitative differences in the levels of individual FFA of control and experimental cheeses at both sampling ages. These results agree with those of Fitzgerald and Buckley (1985) who reported that the FFA profile of Cheddar cheese, salted with a 1:1 mixture of NaCl/KC1, was very similar to that of

Table 1
Acid degree value (meq KOH/100 g fat) of Kefalograviera cheese[a,b] made with NaCl or mixtures of NaCl and KCl during aging

Age of cheese (days)	Cheese[c]		
	A	B	C
5	0.58	0.58	0.58
25	0.99	1.01	0.96
60	1.43	1.42	1.40
90	1.59	1.57	1.54
180	1.84	1.74	1.86

[a] Means in each row without a letter did not differ significantly ($P > 0.05$).
[b] Means of five trials.
[c] Cheese: A, salted with NaC1 (control); B, salted with 3:1 (w/w) mixture of NaCl and KC1; C, salted with 1:1 (w/w) mixture of NaC1 and KCl.

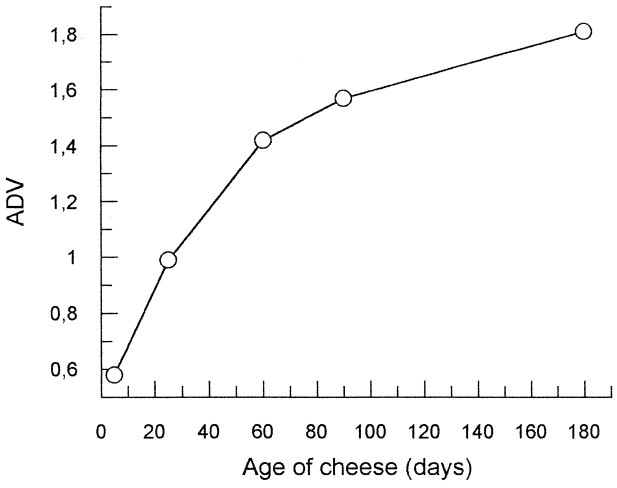

Fig. 1. Relationship between ADV and Kefalograviera cheese aging.

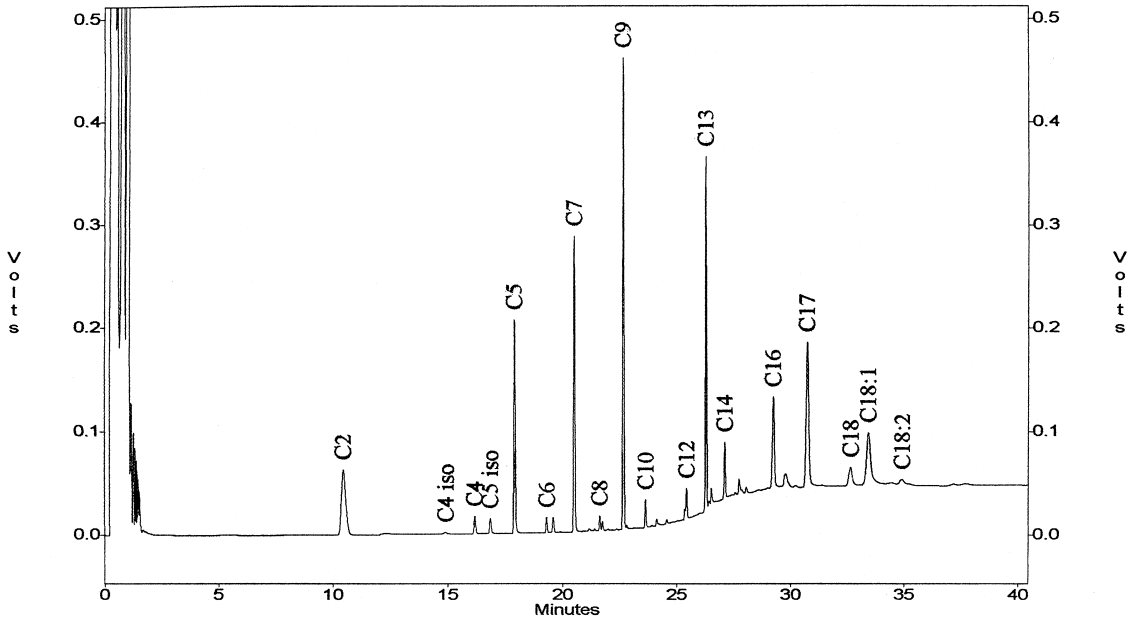

Fig. 2. Gas chromatogram of FFA extracted from a Kefalograviera cheese (180-day-old) spiked with internal FFA standards C_5, C_7, C_9, C_{13} and C_{17}.

Table 2
Free fatty acids (mg/100 g) of Kefalograviera cheese[a,b] aged for 90 and 180 days

Fatty acid	Age of cheese (days)					
	90			180		
	A[c]	B	C	A	B	C
$C_{2:0}$	63.1	76.3	77.3	79.5	82.3	89.9
$iC_{4:0}$	8.72	9.09	9.09	9.09	9.09	9.09
$C_{4:0}$	15.2	15.9	15.4	16.4	15.9	16.7
$iC_{5:0}$	10.1	11.4	12.1	10.4	10.6	10.9
$C_{5:0}$	2.25	2.52	3.03	3.80	3.03	3.79
$C_{6:0}$	11.6	11.1	11.1	12.1	12.4	11.9
$C_{8:0}$	8.09	7.58	7.58	8.08	7.58	7.85
$C_{10:0}$	11.3	11.0	11.2	12.7	11.9	12.3
$C_{12:0}$	8.68	9.09	8.68	9.60	8.68	9.60
$C_{14:0}$	14.4	13.6	13.8	17.3	14.7	15.9
$C_{16:0}$	31.0	30.5	28.9	34.5	32.4	34.9
$C_{18:0}$	12.7	12.6	11.9	14.0	12.4	13.4
$C_{18:1}$	6.63	5.25	4.88	7.00	6.75	7.88
$C_{18:2}$	0.63	0.50	0.67	0.75	0.75	0.75
Total	204	216	216	235	229	245

[a] Means in each row and at the same age without a letter did not differ ($P > 0.05$) significantly.
[b] Means of three trials with duplicate determinations.
[c] Symbols as in Table 1.

the control cheese; however, total replacement of NaCl with KCl in Cheddar cheese, increased lipolysis extensively. Furthermore, Reddy and Marth (1993) found no qualitative differences in the even-numbered carbon chain fatty acids liberated after 36 weeks of ripening of Cheddar cheese salted with NaC1, KCl or various mixtures (2:1, 1:1, 1:2 and 3:4, w/w basis) of these two salts. These authors, however, reported that, with exception of stearic acid, which was released in about equal proportions in all cheeses, the control cheese made with NaC1 consistently had slightly lower levels of FFA than the cheeses made with comparable contents of various NaCl/KC1 mixtures. The slower rate of FFA production in the control cheese was attributed to the inhibitory effect of NaCl on lipolysis. Lindsay et al. (1982) also reported that Cheddar cheeses, salted with a mixture (1:1) of NaCl/KCl, had higher contents of individual FFA than those salted only with NaCl.

Generally, all FFA increased slightly between 90 and 180 days, with the exception of isobutyric and caprylic acids which remained almost constant and the isovaleric acid which decreased (Table 2). Zerfiridis et al. (1984) reported that, generally, short chain fatty acids (C_2–C_6) increased throughout ripening of Greek Gruyere cheese, whereas longer chain fatty acids from C_8 to C_{18} increased up to 90 days and decreased upon further aging (180 days), except $C_{18:1}$ and $C_{18:2+3}$ which increased throughout ripening.

The mean values for total FFA content in the control cheese were 204 and 235 mg/100 g of cheese at 90 and 180 days, respectively (Table 2). The total FFA content (mg/100 g) in the experimental cheeses ranged from 216 to 229 (cheese B) and from 216 to 245 (cheese C) at 90 and 180 days, respectively. Generally, the total FFA contents in the experimental cheeses were slightly higher than that in the control cheese. However, these differences were not significant ($P > 0.05$) and were mainly due to the higher content of acetic acid found in the former cheeses than in the latter. These results are in agreement with those of Lindsay et al. (1982) and Reddy and Marth (1993) who reported that matured

Cheddar cheeses made with NaCl/KCl mixtures had higher concentrations of total FFA than those made with comparable levels of NaCl only.

4. Conclusion

The results of the present study indicate that an up to 50% reduction of sodium in Kefalograviera cheese, achieved by partial replacement of NaCl with KCl, did not significantly affect lipolysis, as measured by the ADV method and gas chromatography, during cheese aging.

References

Abernethy, J. D. (1979). Sodium and potassium in high blod pressure. *Food Technology*, *33 (12)*, 57–59, 64.

Aly, M. E. (1995). An attempt for producing low-sodium Feta-type cheese. *Food Chemistry, 52*, 295–299.

Anifantakis, E. M. (1991). *Greek cheeses:A tradition of centuries*. Athens: National Dairy Committee of Greece.

Deeth, H. C., & Fitz-Gerald, C. H. (1976). Lipolysis in dairy products: a review. *Australian Journal of Dairy Technology, 31*, 53–64.

De Jong, C., & Badings, H. T. (1990). Determination of free fatty acids in milk and cheese: Procedures for extraction, clean up, and capillary gas chromatographic analysis. *Journal of High Resolution Chromatography, 13*, 94–98.

Dillon, J. C. (1987). Cheese in the diet. In A. Eck, *Cheesemaking: Science and technology* (pp. 499–512). New York: Lavoisier Publishing Inc.

Editorial (1993). KC1 spells quality in low-salt cheese. *Dairy Foods, 94*(3), 72.

Fitzgerald, E., & Buckley, J. (1985). Effect of total and partial substitution of sodium chloride on the quality of Cheddar cheese. *Journal of Dairy Science, 68*, 3127–3134.

Fregly, M. J. (1981). Sodium and potassium. *Annual Review of Nutrition, 1*, 69–93.

Freis, E. D. (1976). Salt, volume and the prevention of hypertension. *Circulation, 53*, 589–595.

Goulding, A. (1997). Sodium and calcium metabolism. IDF Bulletin no. 322, (pp. 33–36). International Dairy Federation, Brussels.

Goulding, A., Gold, E., & Campell, A. J. (1993). Salty foods increase calcium requirements and are a potential risk factor for osteoporosis. IDF Newsletter no. 138. (pp. 16–19). International Dairy Federation, Brussels.

Haddy, F. J. (1991). Roles of sodium, potassium, calcium, and natriuretic factors in hypertension. *Hypertension 18* (Suppl. III), 179–183.

Hanspach, I. (1981). *Untersuchngen uber freie fettsauren als aromasubstanzen verschiedener kasesorten*. PhD dissertation, Justus Liebig Universitat, Giessen.

IFT (1980). Dietary salt—A scientific status summary by the Institute of Food Technologists' expert panel on food safety and nutrition and the committee on public information. *Food Technology, 34*(1), 85–91.

Katsiari, M. C., & Voutsinas, L. P. (1994). Manufacture of low-fat Kefalograviera cheese. *International Dairy Journal, 4*, 533–553.

Katsiari, M. C., Voutsinas, L. P., Alichanidis, E., & Roussis, I. G. (1998). Manufacture of Kefalograviera cheese with less sodium by partial replacement of NaCl with KC1. *Food Chemistry, 61*, 63–70.

Lecos, C. (1983). Potassium—keeping a delicate balance. *FDA Consumer*, 17 (1), 21–23.

Lemann Jr, J., Pleuss, J. A., & Gray, R. W. (1993). Potassium causes calcium retention in healthy adults. *Journal of Nutrition, 123*, 1623–1626.

Lesage, L., Voilley, A., Lorient, D., & Bezard, J. (1993). Sodium chloride and magnesium chloride affected by ripening of Camembert cheese. *Journal of Food Science, 58*, 1303–1306.

Linas, S. L. (1991). The role of potassium in the pathogenesis and treatment of hypertension. *Kidney International, 39*, 771–786.

Lindsay, R. C., Hargett, S. M., & Bush, C. S. (1982). Effect of sodium/potassium (1:1) chloride and low sodium chloride concentrations on quality of Cheddar cheese. *Journal of Dairy Science, 65*, 360–370.

NAS/NRC (1980a). *Toward healthful diets*. Food and Nutrition Board, National Academy of Sciences. Washington, DC: National Research Council.

NAS/NRC (1980b). *Recommended dietary allowances,* Food and Nutrition Board, National Academy of Sciences (9th ed.). Washington, DC: National Research Council.

Olson, N. F. (1982). Effects of sodium reduction on natural cheeses. *Dairy Field, 165* (6), *48*, 50–78.

Petik, S. (1987). Reduced sodium cultured dairy products. *Cultured Dairy Products Journal*, *22* (1), 12–14, 23.

Reddy, K. A., & Marth, E. H. (1991). Reducing the sodium content of foods: A review. *Journal of Food Protection, 54*(2), 138–150.

Reddy, K. A., & Marth, E. H. (1993). Lipolysis in Cheddar cheese made with sodium chloride, potassium chloride or mixtures of sodium and potassium chloride. *Milchwissenschafi, 48*, 488–493.

Shank, F. R., Youngmee, K. P., Harland, B. F., Vanderbeen, J. E., Forbes, A. L., & Prosky, L. (1982). Perspective of Food and Drug Administration on dietary sodium. *Journal of the American Dietetic Association, 80*, 29–35.

Tobian, L. (1979). The relationship of salt to hypertension. *The American Journal of Clinical Nutrition, 32*, 2739–2748.

Zerfiridis, G., Vafopoulou-Mastrogiannaki, A., & Litopoulou-Tzanetaki, E. (1984). Changes during ripening of commercial Gruyere cheese. *Journal of Dairy Science, 67*, 1397–1405.