*Food Chemistry* **25** (1987) 31–39

# Cryoscopic Approach to Water Activity Measurement of Non-Liquid Foods: Application to Cheese

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(Received 18 February 1986; accepted after revision 6 November 1986)

*ABSTRACT*

*The initial freezing points (fp) of aqueous cheese extracts were related ($r = 0{\cdot}99$) to the water activity ($A_w$) of the starting non-extracted cheeses by the equation $A_w = 1{\cdot}0162 + 0{\cdot}0981\,fp$. Comparisons of the cryoscopic procedure for $A_w$ evaluation in cheese with a reference method (thermocouple psychrometer) show that differences between the methods were, in the majority of cases, lower than 0·02 $A_w$ units and, consequently, the cryoscopic approach may be used in the cheese industry as a quality control technique. Cryoscopic measurements gave better estimates of $A_w$ than chemical analysis.*

## INTRODUCTION

Water activity ($A_w$) and freezing point (fp) depression are colligative properties of aqueous solutions related by the equation:

$$A_w = \exp(\mathrm{fp}/103)$$

The $A_w$ of aqueous solutions, liquid foods and covering solutions of canned foods has been calculated from freezing point measurement (Ferro Fontán & Chirife, 1981; Lerici *et al.*, 1983; Alzamora & Chirife, 1983). Also, $A_w$'s have been calculated from reported data on the initial freezing point of some solid foods like fruits, vegetables and meats (Chirife & Ferro Fontán, 1982) but no direct cryoscopic approach to $A_w$ measurement of non-liquid food systems has been accomplished.

In most foods, $A_w$ is depressed mainly, and almost solely, by the aqueous concentration of low molecular weight solutes; in solid foods such solutes may be extracted with water under standard conditions to obtain an aqueous diluted solution quantitatively related to their concentration in the aqueous phase of the original product; the diluted aqueous phase may be easily separated from non-soluble compounds and subjected to freezing point measurement by cryoscopy.

Freezing point measurement is a routine operation in the quality control of the dairy industry to detect adulteration of milk with added water, and high performance accurate apparatus (cryoscopes) are used for this purpose. In cheese manufacture, $A_w$ is an important physico-chemical factor intrinsically related to the curd ripening and to the quality, storage stability and safety of the ripe product (Rüegg & Blanc, 1981; Hardy, 1984; Choisy *et al.*, 1984; Weber & Ramet, 1984; Veillet-Poncet, 1984; Rüegg, 1985).

The aim of the present paper was to test if there is a relationship between the $A_w$ of cheese and the freezing point of a diluted aqueous cheese extract, to allow the determination of the $A_w$ of such non-liquid food systems.

## MATERIALS AND METHODS

### Cheese samples

Assorted cheese samples were purchased from the market. The rinds were removed when necessary and the internal portions were ground.

The Spanish cheese samples were collected from May 1982 to May 1984. Their $A_w$'s were measured gravimetrically by a simplified version (Marcos *et al.*, 1985*a*) of the proximity equilibration cell method developed by McCune *et al.* (1981) and were also subject to other immediate physical and chemical analyses. The remaining ground material was placed into airtight containers and stored at −24°C for further analysis. All the results have been reported (Marcos *et al.*, 1985*b*).

Samples of imported cheese varieties were used for immediate freezing point and $A_w$ measurements without frozen storage.

### Preparation of aqueous cheese extracts for cryoscopy

Ground cheese samples stored at −24°C from 1 to 3 years were thawed overnight at room temperature and each thawed sample was mixed thoroughly. To 25 g of ground cheese (thawed or unfrozen) were added 75 ml of deionized water and the mixture was blended, centrifuged at 2000 rpm/5 min and then left to stand at refrigeration temperature for 30–60 min to cold-harden the upper layer of fat.

### Freezing point measurement

The fp of the aqueous cheese extract of solutes was measured on the intermediate phase (between the bottom pellet of protein and other insoluble matter and the solidified fat of the upper layer) in a standard cryoscope (original Gerber) with a calibrated cryoscopic thermometer with scale (from +0·40 to −1·23°C) readings to 0·01°C which enable expression of the $A_w$ to the nearest fourth decimal figure, if desired.

### Water activity measurement of imported cheese varieties

The SC-10 Thermocouple Psychrometer/MT-3 Nanovolt-Thermometer System (supplied by Decagon Devices, Inc., Pullman, WA, USA) was used to measure the $A_w$ of the imported cheese varieties. The operation and calibration of the instrument was carried out according to the instructions from the manufacturer.

### Statistical analysis

Linear regression by the method of least squares was used to calculate the coefficient of determination ($r^2$) and the equation of best fit between paired measures of the variables $x$ (fp of the aqueous phase of the cheese extract in °C) and $y$ ($A_w$ of non-extracted cheese).

## RESULTS AND DISCUSSION

### Relationship between the freezing points of cheese extracts and the water activity of non-extracted cheeses

The initial freezing points (fp) of the aqueous phase of some diluted extracts of individual samples of several cheese varieties, together with the corresponding $A_w$'s experimentally determined at 20°C between 1–3 years earlier on ground (unextracted) cheese samples, are shown in Table 1.

The $A_w$ ranged from near to 1·00 to near 0·90 (i.e. covered the $A_w$ spectrum of most cheese varieties and also the more critical $A_w$-range from the technological and microbiological points of view). The freezing points ranged from about −0·20 to near the lowest limit of the cryoscopic thermometer scale (−1·23).

It is evident that the depression of both $A_w$ and fp increased from fresh, unripened products to the more ripened and strongly salted varieties.

The correlation coefficient ($r = 0{\cdot}99$) between the freezing point of cheese

**TABLE 1**
Water Activity ($A_w$) of Cheese Samples, Freezing Point (fp) of Cheese Extracts, $A_w$ Calculated from the fp of the Extract and Difference from Measured $A_w$

| *Sample number*[a] | *Cheese variety* | $A_w$ *exp.*[a] | *fp (°C) of extract* | $A_w$ *cal.*[b] | *Difference* $A_w$ *cal − exp* |
|---|---|---|---|---|---|
| 16 | Whey cheese | 0·997 | −0·19 | 0·998 | +0·001 |
| 1 | Cuajada | 0·995 | −0·18 | 0·999 | +0·004 |
| 239 | Fresh cheese | 0·974 | −0·43 | 0·974 | 0·000 |
| 19 | Processed cheese | 0·969 | −0·49 | 0·968 | −0·001 |
| 237 | Majorero | 0·963 | −0·54 | 0·963 | 0·000 |
| 52 | Bola | 0·964 | −0·60 | 0·957 | −0·007 |
| 33 | Manchego | 0·947 | −0·74 | 0·944 | −0·003 |
| 48 | Mahón | 0·923 | −0·91 | 0·927 | +0·004 |
| 230 | Blue | 0·914 | −1·02 | 0·916 | +0·002 |

[a] From Marcos *et al.* (1985*b*).
[b] $A_w = 1{\cdot}0162 + 0{\cdot}0981$ fp (see Fig. 1).

extracts and the measured $A_w$'s of cheeses was highly significant and, consequently, the equation of the best fit line (Fig. 1):

$$A_w = 1{\cdot}0162 + 0{\cdot}0981\,\text{fp}$$

may be used to calculate the $A_w$ of the unextracted cheese. Table 1 shows that the differences between the calculated and measured $A_w$'s were, in general, lower than 0·005 $A_w$ units. Ferro Fontán & Chirife (1981) reported that $A_w$ values calculated from freezing point measurements were not very different from values measured at 25°C and that, in the majority of cases, the

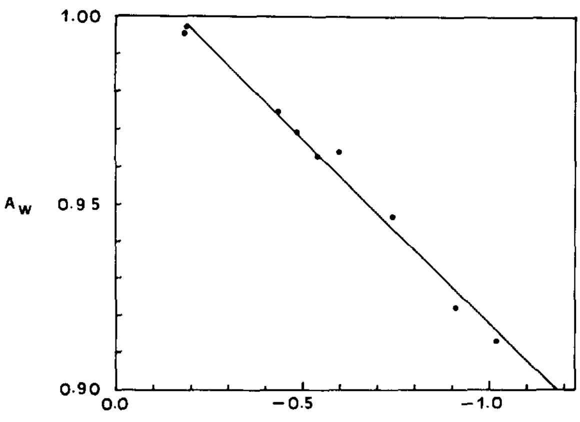


**Fig. 1.** Freezing point (fp) of aqueous cheese extracts versus water activity ($A_w$) of cheese. Equation of best fit $A_w = 1{\cdot}0162 + 0{\cdot}0981$ fp (coefficient of determination $r^2 = 0{\cdot}9863$).

difference in the high $A_w$ range is not likely to be larger than about 0·01 $A_w$ units, a level of accuracy that may be considered acceptable in most food-related applications.

In Fig. 1 it can be seen that there is a 'death zone' between 0·0°C and about −0·2°C because the slope intercept of the *y* axis is above $A_w = 1{\cdot}00$ and, consequently, if the measured freezing point of the extract is above −0·2°C, the equation of best fit may give an $A_w$ greater than 1·00; in such a case, the freezing point of the extract can be corrected by the 'minimal' dilution factor '4' (= 100 g cheese slurry/25 g cheese) to apply the equation:

$$A_w = \exp((\text{fp} \times 4)/103)$$

## Relationships between physical and chemical parameters

Table 2 shows the moisture content of the experimental cheese samples as well as of some major $A_w$- (and fp)-lowering components in cheese. It has been shown that in fresh cheeses the depression of water vapor pressure is determined almost solely by the presence of sodium chloride and, consequently, their $A_w$ can be predicted from NaCl molality in total moisture of the product (Marcos *et al.*, 1981) or graphically determined from the percentages of cheese moisture and salt (Marcos & Esteban, 1982). Another practical equation derived from the NaCl concentration (Marcos, 1987) is as follows:

$$A_w = 1 - 0{\cdot}005\,65\ \text{g NaCl}/100\ \text{g } H_2O$$

This relationship may be useful in predicting the $A_w$ at any point in the cheese after any period of brining, according to data reported by Guinee &

**TABLE 2**

Some Compositional Data[a] of Cheese Samples in Table 1

| *Sample number* | *Cheese variety* | *Moisture* (g/100 g cheese) | *Ash* | *NaCl* |
|---|---|---|---|---|
| 16 | Whey cheese | 79·8 | 2·2 | 0·15 |
| 1 | Cuajada | 85·7 | 1·4 | 0·61 |
| 239 | Fresh cheese | 50·9 | 3·9 | 1·86 |
| 19 | Processed cheese | 54·3 | 5·3 | 1·12 |
| 237 | Majorero | 51·5 | 4·1 | 2·04 |
| 52 | Bola | 42·0 | 4·5 | 2·04 |
| 33 | Manchego | 40·2 | 5·2 | 2·60 |
| 48 | Mahón | 36·0 | 5·9 | 3·91 |
| 230 | Blue | 43·8 | 5·3 | 3·79 |

[a] From Marcos *et al.* (1985*b*).

**TABLE 3**
Freezing Point (fp) of Cheese Samples in Table 1 Calculated from Physical and Chemical Parameters

| *Sample number* | *Cheese variety* | *fp* (*a*) | *fp* (*b*) | *fp* (*c–e*) | *Difference* | |
|---|---|---|---|---|---|---|
| | | | | | [*b* − *a*] | [(*c*–*e*) − *a*] |
| 16 | Whey cheese | −0·3 | −0·3 | −0·1*c* | 0·0 | −0·2 |
| 1 | Cuajada | −0·5 | −0·1 | −0·4*c* | −0·4 | −0·1 |
| 239 | Fresh cheese | −2·7 | −2·7 | −2·2*c* | 0·0 | −0·5 |
| 19 | Processed cheese | −3·2 | −3·3 | −1·2*c* | +0·1 | −2·0 |
| 237 | Majorero | −3·9 | −3·9 | −3·4*d* | 0·0 | −0·5 |
| 52 | Bola | −3·8 | −4·5 | −5·5*d* | +0·7 | +1·7 |
| 33 | Manchego | −5·6 | −6·0 | −7·1*d* | +0·4 | +1·5 |
| 48 | Mahón | −8·3 | −7·8 | −9·8*d* | −0·5 | +1·5 |
| 230 | Blue | −9·3 | −9·0 | −9·5*e* | −0·3 | +0·2 |

*a* fp = 103 ln $A_w$ exp.; from Table 1.
*b* fp = 103 ln (1·0162 + 0·0981 fp aqueous cheese extract); from Table 1.
*c* fp = 103 ln (1–0·00565 g NaCl/100 g $H_2O$); data from Table 2.
*d* fp = 103 ln (1·0234–0·007 g Ash/100 g $H_2O$); data from Table 2.
*e* fp = 103 ln (1·0076–0·0079 g Ash/100 g $H_2O$); data from Table 2.

**TABLE 4**
Water Activity ($A_w$) and Some Compositional Data for Major Spanish Cheese Varieties[a]

| *Cheese variety* | $A_w$ | *Moisture* | *Ash* | *NaCl* |
|---|---|---|---|---|
| | | (*g/100 g cheese*) | | |
| Whey cheese | 0·997 | 74·5 | 1·9 | 0·27 |
| Cuajada | 0·995 | 81·9 | 1·3 | 0·32 |
| Burgos (type) | 0·994 | 54·0 | 2·7 | 0·54 |
| Fresh from goat's milk | 0·984 | 57·4 | 3·4 | 1·51 |
| Gallego | 0·967 | 46·6 | 3·5 | 1·67 |
| Tetilla | 0·956 | 39·1 | 3·8 | 1·52 |
| Manchego | 0·945 | 37·5 | 4·6 | 2·39 |
| Majorero | 0·942 | 45·3 | 5·0 | 2·14 |
| San Simón (pear shaped) | 0·933 | 34·0 | 4·0 | 2·28 |
| Roncal | 0·919 | 29·4 | 4·8 | 2·37 |
| Blue from ewe's milk | 0·906 | 44·0 | 5·3 | 4·15 |
| Cabrales | 0·887 | 41·8 | 5·8 | 3·70 |
| Mahón | 0·881 | 31·7 | 6·8 | 4·29 |

[a] Averages of six samples of each variety except Manchego cheese (eight samples); data from Marcos *et al.* (1985*b*) and Marcos (1987).

Fox (1983) on salt/moisture diffusion in cheese during the initial salting period.

In most natural cheeses ripened by bacteria, the $A_w$ is related to the ash/moisture quotient (Marcos *et al.*, 1985*b*) by the equation (Marcos *et al.*, 1983):

$$A_w = 1{\cdot}0234 - 0{\cdot}007\ \mathrm{g\,Ash/100\,g\,H_2O}$$

and in blue-veined varieties ripened by internal moulds is related by the equation (Marcos, 1987):

$$A_w = 1{\cdot}0076 - 0{\cdot}0079\ \mathrm{g\,Ash/100\,g\,H_2O}$$

A simple nomograph for the estimation of the $A_w$'s of cheeses ripened by bacteria or internal mould from ash-in-cheese moisture has also been developed (Marcos, 1987).

The initial freezing point of cheese may be calculated from physical and chemical parameters by the equation:

$$\mathrm{fp} = 103 \ln A_w$$

where $A_w$ can be measured experimentally or replaced by any of the empirical equations previously mentioned, as shown in footnotes to Table 3.

**TABLE 5**

Freezing Point (fp) of Major Spanish Cheese Varieties Calculated from Physical and Chemical Parameters from Table 4

| *Cheese variety* | *fp* (*a*) | *fp* (*b–d*) | *Difference* (*b–d*) − *a* |
|---|---|---|---|
| Whey cheese | −0·3 | −0·2 (*b*) | −0·1 |
| Cuajada | −0·5 | −0·2(*b*) | −0·3 |
| Burgos (type) | −0·6 | −0·6(*b*) | 0·0 |
| Fresh from goat's milk | −1·7 | −1·7(*b*) | 0·0 |
| Gallego | −3·5 | −3·0(*c*) | −0·5 |
| Tetilla | −4·6 | −4·7(*c*) | +0·1 |
| Manchego | −5·8 | −6·6(*c*) | +0·8 |
| Majorero | −6·2 | −5·7(*c*) | −0·5 |
| San Simón (pear shaped) | −7·1 | −6·3(*c*) | −0·8 |
| Roncal | −8·7 | −9·8(*c*) | +1·1 |
| Blue from ewe's milk | −10·2 | −9·5(*d*) | −0·7 |
| Cabrales | −12·4 | −11·1(*d*) | −1·3 |
| Mahón | −13·1 | −14·0(*c*) | +0·9 |

*a* $\mathrm{fp} = 103 \ln A_w$.
*b* $\mathrm{fp} = 103 \ln (1 - 0{\cdot}00565\ \mathrm{g\,NaCl/100\,g\,H_2O})$.
*c* $\mathrm{fp} = 103 \ln (1{\cdot}0234 - 0{\cdot}007\ \mathrm{g\,Ash/100\,g\,H_2O})$.
*d* $\mathrm{fp} = 103 \ln (1{\cdot}0076 - 0{\cdot}0079\ \mathrm{g\,Ash/100\,g\,H_2O})$.

**TABLE 6**
Water Activity ($A_w$) of Imported Cheese Varieties Determined by the Cryoscopic and the Psychometric Methods

| *Cheese variety* | *fp extract* | $A_w$ *cryoscopy*[a] | $A_w$ *psychrometry* | *Difference Cry–Psy* | *fp cheese*[b] |
|---|---|---|---|---|---|
| Processed cheese | −0·45 | 0·972 | 0·973 | −0·001 | −2·8 |
| Camembert | −0·42 | 0·975 | 0·972 | +0·003 | −2·9 |
| Gruyère (processed) | −0·54 | 0·963 | 0·963 | 0·000 | −3·9 |
| Chaumes | −0·50 | 0·967 | 0·963 | +0·004 | −3·9 |
| Brie | −0·49 | 0·968 | 0·962 | +0·006 | −4·0 |
| Emmenthal | −0·32 | 0·985 | 0·959 | +0·026 | −4·4 |
| Havarti | −0·47 | 0·970 | 0·956 | +0·014 | −4·7 |
| Gouda | −0·62 | 0·955 | 0·939 | +0·016 | −6·5 |
| Roquefort | −1·22 | 0·897 | 0·890 | +0·007 | −12·0 |

[a] $A_w = 1{\cdot}0162 + 0{\cdot}0981$ fp aqueous cheese extract.
[b] fp $= 103 \ln A_w$ (psychrometric method).

Results in this Table show that the physical approach (based on the fp of cheese extracts) yielded a better approximation than did compositional data. Obviously, when average data are used (Tables 4 and 5), the differences of the chemical approach are clearly reduced (see Table 5).

## Comparison of the cryoscopic procedure for $A_w$ evaluation in cheese with a reference method

Imported cheese varieties were used in a comparative parallel trial carried out with the cryoscopic method and a thermocouple psychrometer, used as a reference method for $A_w$ measurement. Results in Table 6 show that differences between both methods were, in the majority of cases, lower than 0·02 $A_w$ units and, consequently, the cryoscopic approach may be used in the dairy industry as a quality control technique for cheese manufacture.

## ACKNOWLEDGEMENTS

The authors are grateful to Professor P. F. Fox, Department of Dairy and Food Chemistry, University College, Cork, Ireland, for helpful assistance in the preparation of the manuscript. Supported by the Comisión Asesora de Investigación Cientifica y Técnica (Research Project No 1022/84), Ministerio de Educación y Ciencia, Madrid, Spain.

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