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# ESTIMATION OF LACTOSE IN CHEESE

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## Summary

Methods used to date for estimating lactose in cheese are not specific. It is shown that this leads to considerable errors, results in some cases being several times the true figure. A method for chromatographic separation of the lactose from other reducing substances prior to colorimetric determination is described.

## Introduction

The methods reported to date for the quantitative determination of the lactose content of cheese are affected by the presence of other reducing substances and thus the results quoted are of little value. This became apparent in a study of the disappearance of lactose from Cheddar cheese using a modification of the phenol-sulphuric acid method of Barnett and Tawab (1957) in which the apparent lactose content increased considerably as the cheese matured. Similar results have been reported by O'Connor (1967) with the copper reduction method of Swartling and Mattsson (1953) and would also be expected, because of its lack of specificity, from the anthrone method of Richards (1959).

The object of this study was to develop an analytical method which is specific for lactose.

## Experimental

Since specific reagents for the colorimetric determination of lactose are not available it was necessary to seek a technique for isolating lactose from the other reducing substances and then quantitatively estimating it with the phenol-sulphuric acid method.

The following techniques were investigated.

### Thin-layer chromatography

Good separation of lactose from galactose and glucose was obtained using silicagel plates (Mercks H.R. grade) with a formic acid-di-isopropyl ether-isopropanol-water mixture as developing solvent and Blankophor CE, incorporated in the plate, as visualizer. Zones containing lactose were scraped from the plates and the quantity of lactose measured using the modified phenol-sulphuric acid colorimetric method outlined later in this paper.

However, because of bad tailing of spots caused by contamination of the extracts with other water-soluble substances from the cheese, this method was abandoned. The contamination was more marked with matured cheese. In addition, the

preparation of extracts of sufficient concentration was difficult and time-consuming.

### Yeast fermentation

The method of Gillet (1965) for determination of lactose in ice-cream which involves a preliminary fermentation with yeast (*Saccharomyces cerevisiae*) to remove interfering reducing sugars was applied to cheese.

In experiments using both washed bakers' yeast and pure strains of *Saccharomyces cerevisiae*, under various conditions, glucose was utilized but galactose remained unfermented, possibly due to a deficiency in the enzymic make-up of the strain of yeast available to us.

### Column chromatography

Column chromatography proved the most satisfactory method of separation. The sample was macerated with water and the macerate deproteinized by the technique of Gillet. A final separation was then made by a modification of the chromatographic technique of Whistler and Durso (1950). This is a system whereby a mixture of mono-, di- and trisaccharides are separated by chromatographic adsorption on charcoal and displaced by water, 5% ethanol and 15% ethanol in succession. We found that water-soluble contaminants in the cheese extract are either permanently adsorbed on the charcoal or are displaced by the water. Lactose in the ethanol fraction was estimated colorimetrically.

## Method

### Reagents

- Charcoal — Barneby-Cheney YF6
- Celite 545 — Johns Manville, acid washed
- Phosphotungstic acid — 20% solution (w/v) in distilled water
- Ethanol, carbonyl free — absolute ethanol distilled from 2,4-dinitrophenylhydrazine + H<sub>2</sub>SO<sub>4</sub>, dried over CaSO<sub>4</sub> and redistilled.
- Phenol — 5% solution (w/v) analytical reagent in distilled water.

### Preparation of columns

A 1:1 mixture by weight of the charcoal and celite is made into a slurry with distilled water and poured into a 1 cm diameter sintered-base column having three discs of Whatman No. 541 filter paper fitted above the sinter. The length of the settled material should be about 7 cm. A

vacuum of about 25" Hg is applied to the lower end of the column to obtain a reasonable flow rate. The columns are not suitable for re-use.

#### Preparation of extract

Five grams of a representative sample of the cheese is weighed into a macerator jar, 40 ml of distilled water added and the mixture macerated for 1 minute at 6500 R.P.M. (MSE top-drive macerator.) The macerate is washed into a 100 ml volumetric flask with water, 5 ml 20% phosphotungstic acid solution and 2 ml concentrated HCl added and the volume adjusted to 100 ml with distilled water, mixed and filtered through Whatman No. 4 filter paper.

#### Separation

An 80 ml aliquot of the deproteinized extract is passed through the prepared column and followed immediately with 400 ml distilled water.

The receiver is changed and lactose displaced from the column with 200 ml 15% ethanol solution. The volume of eluate is measured.

15% ethanol is used as eluting solvent instead of the 5% ethanol recommended by Whistler and Durso since there are no trisaccharides present and good recovery of lactose can be achieved with a much smaller volume of solvent.

#### Estimation

Modification of the method of Barnett and Tawab (1957).

To a 1 ml aliquot of eluate in a test-tube 1 ml of 5% phenol solution is added with mixing; 5 ml concentrated  $H_2SO_4$  (A.R. grade, s.g. 1.84) is added and the solution shaken for 1 minute. After cooling to room temperature and shaking for a further 1 minute the tube is allowed to stand till any gas bubbles formed have settled out. The optical density at 490  $m\mu$  is measured against a reagent blank. (Unicam SP.500 spectrophotometer, 1 cm glass cuvettes). The lactose content is determined by reference to a standard curve prepared from water solutions of pure lactose containing from 10 to 100  $\mu g$  lactose/ml water (Fig. 1).

#### Recovery

Tests were made on the recovery of lactose from water solutions and from cheese samples to which known amounts of lactose had been added prior to maceration. Recovery varied between 90 and 104% from water solution and between 88 and 91% from cheese (see Table 1).

TABLE 1  
Recovery of Lactose

Lactose added mg.	Lactose recovered	
	from water solution mg.	from cheese mg.
1.00	1.04	
2.50	2.24	2.27
5.00	4.81	4.39

A further check on recovery in the separation stage was made by comparing the results for total

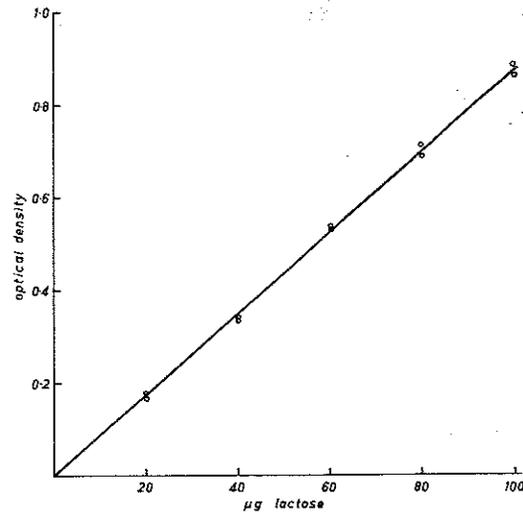


Fig. 1.

reducing sugars content (expressed as % lactose) of several cheeses obtained by applying the modified phenol-sulphuric acid colorimetric technique (a) to an untreated water extract and (b) to each fraction from the chromatographic separation and summing their individual reducing sugars content.

These two results were found to be in good agreement (see Table 2).

TABLE 2  
Comparison of Results Obtained by (a) & (b)  
(see text).

	(a)	(b)
Total reducing Sugars (as % lactose)	0.57	0.57
	0.66	0.61
	0.21	0.18
	0.15	0.13
	0.84	0.79
	0.81	0.76

#### Reproducibility

The results of replicate analyses on each of two cheeses shown in Table 3 indicate that the reproducibility of the method is satisfactory.

TABLE 3  
Reproducibility of Lactose Estimations

Lactose %	
Cheese 1	Cheese 2
0.0186	0.324
0.0210	0.286
0.0192	0.308
0.0205	0.344

#### Results

The results of analyses of several Cheddar cheeses after overnight pressing are given in Table

4. Those in column 1 were obtained by applying the phenol-sulphuric acid colorimetric technique to crude water extracts and indicate total reducing substances, while those in column 2 were obtained by applying the same colorimetric method to lactose fractions chromatographically isolated from the extracts.

**TABLE 4**  
**Lactose Content of Cheddar Cheeses After Overnight Pressing.**

Cheese	Lactose %	
	1	2
V.1. 30/5/67	0.57	0.30
V.2. 30/5/67	0.66	0.48
V.1. 31/5/67	0.24	0.02
V.2. 31/5/67	0.46	0.04
V.1. 6/6/67	0.21	0.03
V.2. 6/6/67	0.15	0.05
V.1. 14/6/67	0.81	0.48
V.2. 14/6/67	0.94	0.66
V.1. 21/6/67	0.84	0.72
V.2. 21/6/67	0.63	0.46

It can be seen that use of crude extracts leads to considerable error, in some cases giving results which are several times higher than the actual lactose level. The compounds responsible for the high results will include galactose, glucose, short-chain fermentation by-products and also amino

acids liberated during protein breakdown. The variation in the ratio of actual lactose content to total reducing substances (expressed as lactose) is due to differences in the metabolic pathways of the starter organisms and to differences in the salt content of the cheese.

#### Conclusion

The chromatographic method for determination of lactose has been shown to be superior to previously published methods as it eliminates interference from other reducing substances which are invariably present in cheese. The method gives good recovery, is reproducible and is easy to apply.

#### Acknowledgements

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