

## **EVALUATION OF METHODS USED FOR DETECTION OF FORMALIN IN MILK**

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### **ABSTRACT**

Seven colorimetric methods were conducted to detect formalin in either in water solutions, or in milk containing different concentration of formalin (1-1000 ppm). The methods used were : Hehner's test (1) ; Leech's test (2) ; Shrewsbury and Knapp's test (3) ; Nash's reagent (4) ; Chromotropic acid method (5) , Schryver's test (6) and phenylhydrazin potassium ferrocyanid test (7). It was noticed that methods (4) and (5) were more sensitive than others for detecting formalin in water solutions, whereas the most sensitive methods to detect formalin in milk samples were (2), (4) and (5). On the other hand, with water-base solution, methods (1), (2) and (3) failed to detect either low or high formalin concentrations, while methods (6) and (7) failed only to detect the lower concentrations. The same trend was obtained with milk samples except method (2) which was sensitive for all concentrations.

Methods (4) and (5) were selected to detect the presence of formalin in the whey of acid coagulation cow's milk (with sulphuric or acetic acid), and in the distillate obtained by indirect distillation of milk samples. The results obtained by using method (4) were always less than the actual polluted concentrations and represent ~ 58-62% in the case of acid whey and 37-52% in the indirect distillation method. Examination of the presence of formalin in acid whey by using method (5) to detect it, however, more accurate results were obtained by using indirect distillation method compared with method (4) (53-70% vs. 37-52%, respectively )

### **INTRODUCTION**

Milk quality plays an important role in the cheese processing to get proper cheese texture and flavour. So, because of an improper system used for collecting milk in some milk production areas, the milk quality becomes out of control. Some milk collectors and processor illegally use many milk preservatives, such as formalin. Since this material is very toxic and hence, very harmful for consumers health. Many official organizations completely prohibited using formalin as a preservative in dairy industry and, particularly, in cheese making.

The quantitative determination of formaldehyde is a commonly encountered problem in analytical chemistry. Von Schalm (1983) succeeded in analyzing milk for the determination of formaldehyde by applying a reversed phase 4 PLC-system. On the other hand, Gamal El-Den (1970) found that recovery of formalin in 20% of original volume of distillate was 31% and 32% in polluted buffalo's and cow's milk samples, and Celano (1985) found that the chromotropic acid method is suitable to detect formaldehyde in

whey. Brunn and Klostermeyer(1983)used an amino acid to determine N-epsilon-methyl lysine (NML) formed by the reaction of formaldehyde with lysin. Therefor, it was thought appropriate to examine the sensitivities of the different available methods and their modifications being used for detecting formalin to find out the most comvenent and the cheapest and accurate on to be used for detecting the milk adulteration with such preservative.

## MATERIALS AND METHODS

### **I. Formalin qualitative tests:**

There were seven colorimetric methods being selected to detect the presence of formalin in polluted water-base or milk samples. The methods were selected from the literature as the most accurate qualitative methods. They were indicated as follows:

- 1- Hehner test: as described by Pearson (1970) and A.O.A.C (1980).
- 2- Leech test: according to Snell and Snell (1953).
- 3- Shrewsbury and Knapp's test: as described by Pearson (1970).
- 4- Nash's reagent: according to Snell *et al.* (1961).
- 5- Chromotropic acid test. as described by Bansal and Singhal (1990).
- 6- Schrgver's test: according to Pearson (1970).
- 7- Phenylhydrazin and potassium ferrocyanid test: as described by Snell *et al.* (1961).

### **ii. Formalin quantitative tests:**

Nash's reagent and chromotropic acid methods were applied by two different ways to determine, the formalin contents in polluted milk samples as indicated below:

(1) In filtrate after acidifying the milk samples as described by Bansal and Singha (1990). 10 ml aliquot of milk was diluted with an equal vol of distilled water, heated to 60°C, and acidified with 5% (1 N)sulphuric acid to pH 4.6 (in the low of concentration of formalin in milk, the dilution may decrease). The vol. was made up to 50 ml and filtered, through a Whatman No. 42. (This method was modified by acidifying the sample with 10% acetic acid to pH 4.6).One ml of the filtrate (which produced from two ways) was added to 5 ml of chromotropic acid reagent in test tube, incubated in boiling water bath for 15 min. The tubes were then cooled to room temperature and absorbance was recorded at 580 nm against blank of sample with reagent by using spectronic 21D. This method was modified as follows: two ml of the filtrate were added to 2 ml of Nash's reagent in a test tube, incubated in boiling water for 3 min. The contents were then cooled to room temperature and absorbance was recorded at 412 nm against the sample with reagent blank.

**(2) In the distillate of milk samples by using micro-Kjeldahle flask as described by A.O.A.C. (1980).** 20 ml of sample were transferred into tarred 30 ml micro-Kjeldahle flask. 2 drop of antifoaming agent (Tween 80) were added, and the flask was connected to condenser, heating the flask with micro heater, collecting 4 ml distillate in 12-14 min. Then (I) place 1 ml distillate in a test tube + 1 ml H<sub>2</sub>O and 2 ml of Nash's reagent, heat in boiling water for 3 min.,

then cool to room temperature. The absorbance was recorded at 412 nm against the sample with reagent blank. (II) place 0.5 ml distillate in a test tube + 0.5ml H<sub>2</sub>O and 5 ml of chromotropic acid reagent, heat in boiling water for 15 min., then cool to room temperature. The absorbance was recorded at 580 nm against the sample with reagent blank.

## RESULTS AND DISCUSSION

### I. Formalin detection:

The results were obtained by applying seven colorimetric methods with water-base samples and polluted milk samples could be summarized as follow:

#### I- Presence of formalin in watery samples:

It could be noticed from table 1 that these methods could be divided into three groups according to the level of their accuracy as a formalin detector. First groups which included the methods 1, 2 and 3 were of the lowest efficiency, since they failed to detect either the lower or higher concentrations of formalin in water-base solutions. A part of these results might be explained by the results obtained by Pearson (1970), who found that with Hahn's methods (1), the violet coloration does not appear because of the absence of tryptophane or its shortage in the watery tested samples. Second group of the moderate efficiency included methods (6) and (7). The results obtained in table (1) illustrated that these methods were suitable to detect the high concentrations of formalin only, but they were inconvenient for detecting the low concentrations. The third group, which contained the most sensitive methods as they succeeded to detect even the formalin traces. This group included method (4) and (5).

**Table (1): Detection of formalin in watery solutions containing 1-1000 ppm by using various colorimetric methods.**

Test	Formalin concentration (ppm)															Notes	
	1	2	3	4	5	10	20	30	40	50	60	70	80	90	100		1000
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
IV	+	+	+	++	++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	Yellow colour
V	+	+	+	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Violet colour
VI	-	-	-	-	-	-	-	-	+	+	+	+	++	++	++	+++	Yellow colour
VII	-	-	-	-	-	-	-	-	-	+	+	+	++	++	++	+++	(-) rose colour (+) yellow colour

I- Hahn's test II-Leech's test III- Shrewsbury and Knapp's test IV-Nash's reagent  
 V- Chromotropic acid method VI- Schryver's test and VII- phenylhydrazin potassium ferrocyanid

#### 2. Presence of formalin in polluted milk sample:

In this part of the study the same previously mentioned methods were used to detect formalin in polluted milk samples with the same formalin concentration. Data presented in table (2) indicated that all of the examined

methods have certain accuracy to detect formalin in milk samples, but it's obvious that these methods could be divided into two different groups according to their sensitivities. First group could detect only the high level of formalin (> 10 ppm). This group contained the methods (1), (3), (6) and (7), but the results obtained showed that method (6) could detect less formalin concentration if the reaction time extended to one hour instead of 30 min. The second group, which is more accurate included methods (2), (4) and (5), and it's obvious that by using methods (4) and (5) the reaction with formalin doesn't need the presence of any components to stimulate the reaction as they gave the same results obtained in water-base solution. Results also indicated that methods (1), (2) and (3) gave positive reaction with polluted milk samples (table 2), whereas their reactions were negative with water-base samples (table 1). This change might be due to the presence of certain substances found in the milk which catalyse the reaction between formalin and test's reagent. The most sensitive results were obtained by method (2).

**Table (2): Detection of formalin in polluted cow's milk with different concentration by using various colorimetric methods.**

Test	Formalin concentration (ppm)															Notes	
	1	2	3	4	5	10	20	30	40	50	60	70	80	90	100		1000
I	-	-	-	-	-	-	-	-	-	-	±	±	+	+	++	+++	Yellow ring then formed purple ring under yellow ring
II	+	+	+	+	+	+	+	+	+	++	++	++	++	++	++	+++	Violet layer in the acid side
III	-	-	-	-	-	-	-	-	-	±	±	+	+	+	++	++	Violet layer in curd side
IV	+	+	+	+	+	+	+	+	+	++	++	++	++	++	+++	+++	Yellow colour
V	+	+	+	+	+	+	+	+	+	++	++	++	++	++	+++	+++	Violet layer in acid side
After 30 min. VI	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	++	Yellow colour
After 1 hr	-	-	-	-	-	-	±	±	+	+	+	+	+	++	++	++	Yellow colour
VII	-	-	-	-	-	-	-	-	-	-	±	±	±	+	+	++	Rose colour (-) Yellow colour (+)

I- Mahner's test II-Leech's test III- Shrewsbury and Knapp's test IV-Nash's reagent  
V- Chromotropic acid method VI- Schryver's test and VII- phenylthiazin potassium ferrocyanid

Therefore, the most sensitive methods (Nash's and Chromotropic, (4), and (5) were selected to carry out further studies in the next part of this work to determine the formalin concentrations.

**II. Formalin determination in milk samples:**

Several researches indicated that formalin may react differently according to the type of media. In this part the formalin was detected in the filtrates obtained by acidifying the polluted milk at pH 4.6 with either 5% sulphuric acid or 10% acetic acid. The formalin concentration was also traced in the distillate obtained by the indirect heating of polluted samples.

**1. In acid filtrate:**

**a. Sulphuric acid filtrate:**

Table (3) showed the results obtained when the formalin concentrations were determined in the filtrates obtained after acidification of the milk sample with 5% sulphuric acid, followed by filtration through Whatman paper No. 1. Both methods (4 and 5) were used for the determination of formalin in the filtrate. Formalin concentrations were 2, 5 and 10 ppm.

**Table (3): Determination of formalin in the acid filtrate of cow's milk (pH 4.6) as obtained by 5% sulphuric acid by using methods (4) and (5).**

Replicate	Actual formalin concentration (ppm)					
	2		5		10	
	4	5	4	5	4	5
1	1.2	Burned	2.6	Burned	6.4	Burned
2	1.3	" " "	2.8	" " "	6.8	" " "
3	1.0	" " "	3.0	" " "	6.4	" " "
4	1.2	" " "	3.0	" " "	6.1	" " "
5	1.1	" " "	2.8	" " "	6.8	" " "
6	1.2	" " "	3.2	" " "	6.4	" " "
7	1.3	" " "	2.9	" " "	6.3	" " "
8	1.0	" " "	2.6	" " "	6.2	" " "
9	1.2	" " "	3.2	" " "	6.4	" " "
10	1.2	" " "	3.0	" " "	6.6	" " "
X±SE	1.17±0.106	-	2.91±0.213	-	6.44±0.232	-
Efficiency coefficient	58.50%	-	58.20%	-	64.40%	-

Mean values of 10 replicated.

Results given in table (3) showed that method (5) gave burnt color after being added to the samples filtrates and during the reaction time. So, the method failed to determine formalin content in the sulphuric acid filtrate, which might be due to the reaction between sulphuric acid and chromotropic acid in the presence of milk organic constituents, such as lactose or whey proteins. On the other hand, by using Nash's reagent (Method 4) the reaction and the development of the color increased as formalin concentration increased. These results were always less than the actual polluted concentration and represent 58.50, 58.20 and 64.40%, respectively as percentages of the actual tested concentration (2, 5 and 10 ppm) in the same order. These results didn't reflect the free actual formalin present, since they gave almost close percentages with the three different formalin concentration, but the most proper explanation that a part of the formalin reacted with milk casein and lift in the precipitate, but this part supposed to be equal to the three examined concentrations, and these is a part of formalin which might be

distracted or converted to another substances, and consequently didn't react with Nash's reagent.

**b. Acetic acid filtrate:**

Acetic acid as an organic acid was used to coagulate milk samples. Results obtained in table (4) showed that methods (5) also failed again to determine accurately formalin in the filtrate and that might be due to that this reaction couldn't take place at pH 4.6 since it gave the same result with H<sub>2</sub>SO<sub>4</sub>. On the other hand, method (5) gave a good reaction with different concentrations of formalin, as the recovery percentages were 61.5, 63.4 and 62.8%, respectively for 2, 5 and 10 ppm.

**Table (4): Determination of formalin in the acid filtrate of cow's milk (pH 4.6) as obtained by 10% sulphuric acid by using methods (4) and (5).**

Replicate	Actual formalin concentration (ppm)					
	2		5		10	
	4	5	4	5	4	5
1	1.2	Burned	3.4	Burned	5.6	Burned
2	1.2	" " "	3.2	" " "	6.8	" " "
3	1.3	" " "	3.4	" " "	6.4	" " "
4	1.2	" " "	3.1	" " "	6.2	" " "
5	1.2	" " "	3.2	" " "	6.4	" " "
6	1.2	" " "	3.2	" " "	6.4	" " "
7	1.3	" " "	3.2	" " "	6.2	" " "
8	1.4	" " "	3.0	" " "	5.6	" " "
9	1.1	" " "	3.0	" " "	6.8	" " "
10	1.2	" " "	3.0	" " "	6.4	" " "
X±SE	1.23±0.082	-	3.17±0.149	-	6.28±0.413	-
Efficiency coefficient	61.50%	-	63.40%	-	62.80%	-

**2. In sample distillate:**

To avoid the possible reaction between the sulphuric or acetic acid with chromotropic acid or the at pH 4.6, this part was planned to determine the formalin in distillate, which obtained by the indirect heating of polluted milk samples.

Results in table (5) showed that both of (4) and (5) methods gave positive reactions in the distillate of polluted milk samples with 2, 5 and 10 ppm. Date obtained from method (4) were lower than that obtained from the same reagent but in acid filtrate samples (37.5, 55.4 and 51.3% vs. 58.5, 58.2 and 64.4%), and this might due to the destruction of some of formalin by heating during the distillation of milk samples.

Chromotropic acid method (5) was more accurate than Nash's method (4) even with the lowest formalin content (2 ppm). The recovery coefficients were 53.0%, 73.6% and 70.1%, respectively for 2, 5 and 10 ppm. It's also obvious that method (5) which failed to give positive reaction

with inorganic acid filtrate, gave more accurate results with indirect heat distillate even when compared with method (4).

**Table (5): Determination of formalin in the distillate of known polluted cow's milk sample by using methods (4) and (5).**

Replicate	Actual formalin concentration (ppm)					
	2		5		10	
	4	5	4	5	4	5
1	0.5	0.8	2.9	3.8	4.9	7.3
2	0.9	0.9	2.8	3.9	5.5	6.8
3	0.6	0.7	2.7	3.6	4.8	6.9
4	0.8	0.9	2.7	3.1	5.4	6.9
5	0.7	1.1	2.6	3.7	4.8	6.4
6	0.5	1.2	2.9	3.7	5.4	7.3
7	0.6	1.3	2.8	3.8	4.9	6.8
8	1.0	0.8	2.6	3.9	5.4	7.3
9	0.8	1.5	2.8	3.5	4.8	7.4
10	1.1	1.4	2.9	3.8	5.4	7.0
X±SE	0.75±0.207	1.06±0.280	2.77±0.116	3.68±0.239	5.13±0.309	7.01±0.314
Efficiency coefficient	37.5%	53.0%	55.4%	73.6%	51.3%	70.1%

We recommended to use methods (4) and (5) as the most faster methods to detect the presence of formalin in milk samples of normal acidity, and use method (4) only with high acidic samples since method (5) failed to detect formalin in acid whey which might be explained by the reaction between chromotropic acid with organic or inorganic acid.

### REFERENCES

- A O A.C. (1980). Official methods of analysis 13<sup>th</sup> Ed., Association of official analytical chemists, Washington, DC.
- Bansal, A. and O.P. Singhal (1990). Colorimetric estimation of formalin in milk. Brief communications of the XXIII International Dairy Congress, Montreal, Oct. 8-12, 1990 Vol. 1 C.F.: D.S.A. 53 (1): 4852.
- Brunu, W. and H. Klostermeyer (1983). Detection and determination of protein bound formaldehyde IN epsilon-methyllysine as indicator. Zeitschrift-Fuer Lebensmittel Untersuchung und forschung: 176(2): 108-112. 51 Ref.
- Celano, G (1985). Verification of Donilenko's methods for the detection of formaldehyde in milk and milk products. Rivista d'izootecnia-e-veterinaria, 13(2): 114-116.5 ref. C. F. DSA, 48 ( 7): 4234.
- Gamal El-Den, A. M. (1970). Some treatment used in Domiatti cheese milk and their detection in the final products. M. Sc. Thesis, Fac. Agric, Al-Azhar Univ., Egypt.

- Pearson, D. (1970). The chemical analysis of food. 6<sup>th</sup> Ed. J. & A. Churchill, London.
- Snell, D. Foster and C. T. Snell (1953). Colorimetric methods of analysis. Vol. III, 3<sup>rd</sup> Ed., Van Nostrand Company.
- Snell, D. Foster; C. T. Snell and C. A. Snell (1961). Colorimetric methods of analysis. Vol. IIIA, 3<sup>rd</sup> Ed., Van Nostrand Company.
- Van Schalm, K. J. (1983). Determination of traces of formaldehyde in milk as the 2, 4 dinitrophenylhydrazone by HPLC. Neth. Milk Dairy J., 37: 59-64.

تقييم للطرق المستخدمة في الكشف عن الفورمالين في اللبن  
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استخدمت سبع طرق لونية للكشف عن وجود الفورمالين في كلا من الوسط المائي أو في عينات اللبن الملوثة وانتي تحتوي على نسب متصاعدة من الفورمالين من ١ جزء في المليون وحتى ١٠٠٠ جزء في المليون.

الطرق المستخدمة كانت (١) اختبار Hehner (٢) اختبار Leech (٣) اختبار Shrewsbury & Kanapp (٤) اختبار Nash (٥) طريقة حامض الكروماتروبك (٦) اختبار Schyver (٧) اختبار فينيل هيدرازين بوتاسيوم فيروسيانيد.

لوحظ ان الطرق (٤) ، (٥) كانت الأكثر حساسية عند استخدامها ككاشف عن الفورمالين فسي العينات المائية بينما كانت الطرق الأكثر حساسية في حالة اللبن الملوثة بالفورمالين هي (٢) ، (٤) ، (٥). من ناحية اخرى فشلت الطرق (١) ، (٢) ، (٣) في الكشف عن وجود الفورمالين فسي عينات الماء الملوثة سواء في حالة تلوثها بتركيزات منخفضة او مرتفعة ، بينما فشلت الطرق (٦) ، (٧) فقط فسي حالة التركيزات المنخفضة . عند الكشف عن وجود الفورمالين في عينات اللبن الملوثة لوحظت نفس النتائج فيما عدا ان الطريقة (٢) اعطت نتائج ايجابية مع كل التركيزات.

اختبرت الطرق (٤) ، (٥) الأكثر حساسية لتنفيذ الجزء الثاني من البحث لتتبع وجود الفورمالين في الشرش الناتج عن تخمير عينات اللبن الملوثة او في المنقطر المتحصل عليه من التسخين الغير مباشر لعينات اللبن الملوثة.

القيم المتحصل عليها باستخدام الطريقة (٤) كانت تمثل نسب منخفضة من القيم الفعلية المضافة للعينات حيث كانت في المدى من ٥٨-٦٢% في حالة عينات الشرش الناتج عن التخمير بينما كانت اقل دقة في حالة المنقطر حيث كانت في المدى من ٣٧-٥٢% ، لم يتم التحصل على نتائج باستخدام الطريقة (٥) مع عينات الشرش بينما كانت النتائج المتحصل عليها باستخدامها في حالة المنقطر كانت اكثر دقة مقارنة بالطريقة (٤) (٥٣-٧٠% مقارنة بـ ٣٧-٥٢% على التوالي).