THE DETERMINATION OF CARBOXIMETHYL-CELLULOSE AS A MICRO-INGREDIENT IN SAUSAGES SUCH AS WÜRSTEL

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SUMMARY

A method has been proposed to determine the presence of sodium carboximethyl-cellulose (CMC) in sausages-type meat products (würstel) prior to its separation from the other components of the matrix such as lipids and proteins. This is followed by the formation of a cetil-pyridine chloride complex with 0,2 of NaCl. This complex is absorbed on a celite column and successively eluted with a hot solution of 30% sulphuric acid. A part of this eluate is than reacted with 2,7-Naphthalendiol in concentrated sulphuric acid. The absorption rate of the resulting coloured compound is spectrophotometrically measured at 540 nm. The lowest revelation point for this method is approximately 50 ppm, and therefore inferior to 0,03% (300 ppm) the limit allowed by law in Italy for skinless würstel. Nevertheless, a linear instrumental response can be obtained within a concentration interval of 50 to 1600 ppm. A satisfactory recovery percentage was achieved, with valued exceeding 90%. If the quantities of CMC found are close to the limits established by law (within ± 10% of the determined analytical value), it is necessary to consider the rate of recovery and the substitution rate of CMC.

INTRODUCTION

The Italian law upon the food additives permits a maximum dosage of 0,03% of sodium carboximethyl-cellulose (CMC) in numerous food products. Among these is the viennese sausage, commonly known as würstel, marked as "skinless". The value is the amount that can be expected to pass into the meat mixture when CMC is utilised as a "separation agent" on the internal lining of the casing.

Considering the outstanding water retention properties of CMC, it could be used directly in the meat mixture. However, in order to achieve effective results, quantities notably higher than the legal limit must be employed.

Among the different analytical methods available (4,5,6), the most precise and specific is the one employed by Graham in 1971 (4). This method, however, has never been applies to meat products but to food products of a different nature (mayonnaise, ice cream, ecc.), in which the quantity of CMC allowed by Italian law is approximately 10 times greater than that which may be used in viennese sausages. It was determined, therefore, to study a method that would consent the evaluation of concentrations on the order of several tenths of ppm to establish if the quantities of CMC found could be attributed to the normal release from the casing or if additions had been made directly to the meat mixture during the processing phase.

The ethereal bond on a molecule of CMC is broken by utilising headed (approx. 100°C) sulphuric acid concentrate forming glycolic acid, which is subsequently degraded to formaldehyde by eliminating the carbon dioxide and water. The formaldehyde condenses with two molecules of 2,7-naphthalendiol, bonding in the ortho position with respect to one of the OH groups, the latter forming: 2,2',7,7'-tetrahydroxy-1,1'-dinaphthylmethane.

This compound was gradually oxidized always in the presence of sulphuric acid concentrate, to a derivative with a probable quinoidal structure that is responsable for the appearance of the typical red-purple colour determined spectrophotometrically at 540 nm (2). The method is performed in essentially three phases:

a) sample "clean up" in which the lipidic fraction is eliminated from the meat matrix using extraction with diethyl ether in Soxhlet and the protein fraction is eliminated utilising enzymatic digestion with papain;
b) CMC complexation with cetylpyridinium chloride (CPC), using a 0,2 M concentration of NaCl that allows a selective precipitation from other polysaccharides which may be present and also complexed with the CPC;
c) in this final phase a portion of the eluate (1 ml) is reacted with 2,7-naphthalendiol in sulphuric acid concentrate and left in a boiling water bath for about 3 hours.

In presence of CMC in the eluate is developed the expected coloration. The estimated detection limit calculated on the original sample is equal to approximately 50 ppm and, therefore, inferior to the amount established by legislative provisions currently in force (300 ppm). This resulted in a linear instrumental response of a concentration range between 2,5 and 80 microgramms per reaction tube, which corrisponds to a range of 50-1600 ppm when referring to the original sample.

MATERIALS

Preparation of the celite column

In the bottom of a 250 ml glass chromatography column, a 1 cm layer of glasswool is placed, on the top of which the celite, suspended in boiling water, is deposited. The column is filled with distilled water and the valve is closed. After 5 minutes, the water is then allowed to pass through celite to form a compact layer with a thickness of 1-2 cm. The contents of the column are washed with H_2SO_4 concentrate, then with tap water, then with 0,1 M sodium bicarbonate, and finally with distilled water until a 2 ml aliquot of eluate give no colour when tested with phenol-H_2SO_4. After eliminating any possible air bubbles in the celite layer, 20 ml of a 5% solution is added to the column, 15 ml is then allowed to pass through, leaving the column covered with a thin layer of this solution.

Phenol/sulphuric acid reagent

An 80% phenol solution is prepared in distilled water and kept in the refrigerator until used. This solution is utilised as follows: 50 microliters are added to a 2 ml test aliquot of eluate, mixing carefully, 5 ml of sulphuric acid are then added directly to the surface of this liquid.

If oligosaccharides, polysaccharides or their derivates are present in the eluate, the development of a yellow colour is observed, that, in cases where this is pertinent, can be determined spectrophotometrically at 490 nm. However, in our case, it indicates that the column requires subsequent washing.

Washing solution

A 0,01 M NaCl aqueous solution 100 mg/l of CPC is added to, when the salt is completely dissolved, and diluted to mark with water.

2,7-naphthalendiol reagent

50 mg of 2,7-naphthalendiol is dissolved in 100 ml sulphuric acid contentrate, thoroughly stirred, and left in an amber-coloured bottle in the dark for at least 18 hours. It is then thermostatically maintained at 28°C for 6 hours. This reagent must be kept in the refrigerator.

Acetate-EDTA-cysteine-papain buffer

A solution of 0,1 M sodium acetate is prepared, the pH is raides to 5,5 using acetic acid, then 2 g/l of EDTA and 1 g/l hydrochloride cysteine are added. If necessary, the pH is re-adjusted using acetic acid or NAOH. Just prior to use, 25 ml is pipetted for each sample to be tested, 2 g of 3,2 U/mg papain is added and poured in the solution containing the sample. This buffer solution must be kept in the refrigerator.

Standard solutions preparation

To prepare the standards, stock solutions with concentrations of not more than 0,2% are suitable. The standard solutions were diluted to obtain various concentrations from 0,25 to 8 mg/100 ml (from 50 to 1600 ppm on the original sample).

Equipment and glassware

- GIBERTINI mod. E42 analytical scales, precise to 0,1 mg;
- METTLER technical scales, precise to 0,1 g;
- 10-speed OSTERIZER mixer;
- set of Soxhlet extractors, complete with double-boiler;
- thermostatic bath;
- 250 ml glass columns for flash chromatography;
- JASCO mod. 7800 spectrophotometer with video and glass cuvettes with a thickness of 1 cm;
- glassware standardized for laboratory use.

Special reagents

- 3,2 U/mg papain - FLUKA CHEMIE A.G. - Buchs (CH);
- cetylpyridinium chloride - MERCK - Darmstadt (D);
- 2,7-naphthalendiol - MERCK - Hoehenbrunn (D);
- celite 535 - FLUKA CHEMIE A.G. - Buchs (CH).

METHOD

Sample drying

10 g of sample is weighed in a clock glass and dried out for at least 12 hours in a vacuum over at 60°C.

Extraction of the lipidic fraction

The dried sample is extracted in Soxhlet using diethyl ether for at least 4 hours. The ether is then completely evaporated and this fatless material is finely ground in a mortar.

Enzymatic digestion

The ground material is carefully trasferred to a 250 ml short-necked flask in which 75 ml or distilled water is added. The contents are stirred for 5 minutes, 6 g of NaCl is then added and the flask is placed in a boiling water bath for approximately 10 minutes stirring occasionally. In the flask, cooled to about 70°C, 25 ml of the sodium/acetic acid acetate buffer solution containing papin is added. The flask is hermetically closed with a glass stopper and placed in a 70°C water bath for approximately 16 hours.

Elimination of interfering substances

If the presence of pectins and/or alginates are presumed, 10 ml of 0,1 M calcium chloride is added and incubated for 15 minutes at 37°C in order to provocate the precipitation of these substances. 1 g of celite 535 is added, stirring thoroughly, and after 5 minutes the contents are filtered though a fiberglass filter into another 250 ml flask. What remains in the first flask is recovered with 5 aliquots of 20 ml of distilled water which is then filtered into the second flask. If it is certain that these interfering substances are not present, this phase may be bypassed and requires only the addition of 100 ml of distilled water.

CMC-CPC compound formation

2 g of CPC and another 1 g of celite are added and left to incubate at 45°C for 15 minutes. This is allowed to cool to 30°C and again filtered through a fiberglass filter into a 800-1000 ml baker, rensing the flask with 10 aliquots of 25 ml of the washing solution, which is then poured over the glassfiber filter. Distilled water is added to the beaker to make the volume up to 600 ml in order to obtain a final NaCl concentration equal to 0,2 M, with which a selective preparation of the CMC-CPC compound can be achieved. The contents of the beaker are thoroghly stirred and placed in a double-boiler at 35°C for approximately 30 minutes.

Filtration on celite column

The contents of the beaker are poured onto a celite column, verifying that the filtration speed is 15-20 ml/min. and pouring the first 250 ml back onto the column. When the filtration is completed, the beaker are rinsed with 5 aliquots of 50 ml of the washing solution, which is then poured onto the column. The column is subsequently washed; first with 200 ml of a 0,5 M sodium sulphate solution to remove ulterior interfering substances (gum, guar, ecc.) if present, and then with several ml of washing solution until a 2 ml aliquot does not give a colorazion with the phenol/sulphuric acid reagent. If it is certain that the above-mentioned substances are not present it is possible to bypass washing the column with sodium sulphate.

Dissociation of the CMC-CPC compound and eluation of the CPC from the celite column

When the filtration is completed, the extraction is performed with 10 aliquots of 20 ml of hot (90-100°C) 30% su

phuric acid. The eluate is collected in a 200 ml flask, left to cool and, made up to mark with 30% sulphuric acid.

Spectrophotometric measure

1 ml of eluate is transferred in duplicate to a 300 x 25 mm test-tube in which 9 ml of the reagent 2,7-naphthalendiol/sulphuric acid concentrate is added. Using extreme caution, the test-tube is agitated, closed with a metal top and placed in a boiling water bath for about 3 hours. Prior to cooling under running water for 5 minutes, the spectrophotometric measure is taken at 540 nm against blank constituted of 9 ml reagent and 1 ml of 30% sulphuric acid.

RESULTS AND DISCUSSION

A calibration curve was obtained utilising the average values of 6 determinations, employing six series of standards in duplicate to verify the riproducibility and linearity of the method. The latter, particular, is respected for the whole range of contentrations concerned (Tab. 1).

In a few samples, cenrtainly free of CMC, standards of CMC in an aqueous solution were added. The spectrophotometric measure of these were confronted with those of standard solutions having equal concentrations. These additions were calculated in a way to remain in proximity of the values established by law. In order to accomplish this, various aliquots were pipetted from a stock solution having a 0,2% concentration to obtain quantities of 2, 4, 12 and 16 mg of CMC per 100 of sample. The samples were subjected to the entire procedure, whereas the standards only the last two phases, that is, the incubation in boiling water for 3 hours and the spectrophotometric measure at 540 nm. The results obtained revealed satisfactory CMC recovery percentages from the meat matrix ranging from 88,6% to 94,7% based on the amount of the addition, with an average recovery of 91,4% (Tab.2). The recovery percentages lower with the increase of CMC added. The evaluation of the average recovery percentages was obtained considering a sieries of 20 determinations for each addition. This tendency is probably due to the fact of being in the proximity in the spectrophotometric response curve, of a critical point in which the Lambert-Beer law beings to lose validity. The degree of substitution (DS) is an important parameter to consider in the quantitative evaluation of CMC. When this rises, an increase in the response intesity (7) is observed in order to a major number of carboximethilic groups present on the cellulosic molecule, and therefore, a greater quantity of glycolic acid, and consequently, of formaldehyde produced during the incubation in hot sulphuric acid concentrate. It is, therefore, necessery to utilise calibration curve obtained with standards that have the same DS as the CMC present in the sample. Still, this is not always possible because, in the majority of cases, unknown samples are utilised, and as a result, this determination becomes indispensable: these are several documented, valid analytical methods available for this porpose (1) (3).

Actually, this problem doesn't exist, in that the CMC utilised as an additive in the food industry normally has a DS of between 0,7 and 0,9, a range that doesn't create significant variations in the spectrophotometric response at 540 nm (Fig. 1).

The data of commercial würstel samples, taken from various points-of-sale, are resported in Tab.3.

The analytically found concentration is reported for all samples, comparing it to the theoretical concentration, applying the average recovery rate of 91,4%.

Ulterior studies were carried out on samples certainly free of CMC and stuffed in CMC-treated and non-treated casings. The results are reported in Table 4.

From these data, it is reasonable to assume that the analytically found concentration values up to 100 pm can be attributed to the trasfer from the casings, and that the variations refer to the quantity of CMC employed per dm^2 of casing surface. Values greater than 100 ppm and up to the limits of the law may signify larger quantities present on the casing, utilised to facilitate the removal of the skin.

CONCLUSIONS

The method is specifically for CMC, even in the presence of similar interfering substances such as gum arabic, alginates, pectins, ecc., which may be detected during the spectrophotometric measure phase and eliminated during the preliminary "clean-up" of the sample. Nevertheless, with würstel, it's possible to reduce the preliminary treatment of the sample, in that it is reasonable to suppose that these substances are not present. The principal disadvantage in the proposed method is the length of time required for the analysis. In fact, this method requires vacuum drying and enzyme digestion procedures which necessitate long periods for completion. These analytical phases do not, however, require continous supervision and can be conducted during the night for completion the following day. Still, based on the results obtained, this method has demonstered to correspond completely to the ripetibility characteristics required in a quantitative analysis.

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TABLE 1 : absorpition values of standard solutions

Standard (ppm)	Absorption at 540 nm						Average	St. Dev. %
	1°	2°	3°	4°	5°	6°		
50	0,033	0,041	0,033	0,029	0,029	0,033	0,033	0,44
100	0,045	0,050	0,042	0,050	0,038	0,045	0,045	0,46
200	0,112	0,116	0,093	0,103	0,093	0,077	0,099	1,44
300	0,167	0,137	0,135	0,147	0,155	0,167	0,151	1,41
400	0,191	0,188	0,184	0,192	0,188	0,215	0,193	1,11
800	0,383	0,373	0,372	0,375	0,391	0,405	0,383	1,29
1200	0,689	0,665	0,696	0,661	0,689	0,680	0,680	1,42
1600	0,777	0,772	0,780	0,737	0,739	0,802	0,768	2,53

TABLE 2 : recoveries achivied from würstel stuffed in natural casing and CMC-free;

CMC added (ppm)	(mg)	CMC recoveried (mg)	Average recovery (%)	Standard dev. (%)
200	2	1,83	91,7	1,04
400	4	3,79	94,7	0,94
1200	12	10,88	90,7	2,90
1600	16	14,18	88,6	3,38

TABLE 3 : concentration of CMC found in commercial würstel;

Company	concentration of CMC (ppm) found	recovery of 91,4%
1	53	58
2	1877	2054
3	109	119
4	1158	1267
5	6	6,5
6	n.r.	n.r.
7	251	275
8	91	99
9	93	102
10	148	162
11	n.r.	n.r.
12	75	82
13	150	164
14	789	863
15	40	44
16	34	37
17	812	888
18	507	555
19	144	157
20	113	124
21	229	250
22	101	110
23	188	206
24	71	78
25	55	60
26	144	157
27	1004	1098

TABLE 4 : CMC concentration of würstel stuffed in:
A) treated casing;
B) non-treated casing;

Sample	concentration (ppm) A	B
1	80,9	17,8
2	69,0	23,7
3	94,7	11,8

Fig 1
Absorption values based on degree of sobstitution (DS)