Federation of Malysia

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MS 1372 (1995) (English): SPECIFICATION FOR SATAY SAUCE

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MALAYSIAN STANDARD

MS 1372 : 1995

SPECIFICATION FOR SATAY SAUCE

Price : RM20

SIRIM STANDARDS & INDUSTRIAL RESEARCH INSTITUTE OF MALAYSIA

This Malaysian Standard, which had been approved by the Food and Agricultural Industry Standards Committee and endorsed by the Board of the Standards and Industrial Research Institute of Malaysia (SIRIM) was published under the authority of the SIRIM Board in August, 1995.

SIRIM wished to draw attention to the fact that this Malaysian Standard does not purport to include all the necessary provisions of a contact.

The Malaysian Standards are subject to periodical review to keep abreast of progress in the industries concerned. Suggestions for improvements will be recorded and in due course brought to the notice of the Committees charged with the revision of the standards to which they refer.

The following references relate to the work on this standard:
Committee reference : SIRIM 481/2/14
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CONTENTS

Committee representation

The Food and Agricultural Industry Standards Committee under whose supervision this Malaysian Standard was prepared, comprises representatives from the following Government Ministries, trade, commerce and manufacturer associations and scientific and professional bodies.

Federal Agricultural Marketing Authority
Federation of Malaysian Consumer's Association
Federation of Malaysian Manufacturers
Malaysian Agricultural Research and Development Institute
Ministry of Agriculture
Department of Agriculture
Malaysian Oil Palm Grower's Council
Rubber Research Institute of Malaysia
Universiti Pertanian Malaysia

The Working Group on Sauces which prepared this Malaysian Standard consists of the following representatives:

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Encik Mazlan Duaji	Federation of Malaysian Manufacturers
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FOREWORD

This Malaysian Standard was prepared by the Working Group on Sauces, under the authority of the Food and Agricultural Industry Standards Committee.

It was felt necessary that this standard be drawn up to ensure product quality where manufacturers can adopt strict quality control specifications, and to use it for consumer protection and as a reference in trade, thus create a quality image for the product.

This Malaysian Standard was based on the current practices existing in the industries concerned.

This standard is to be used in conjunction with:

MS 1120, 'Methods of sampling and test for sauces'.

SPECIFICATION FOR SATAY SAUCE

1. Scope

1.1 This Malaysian Standard Specification prescribes the requirements and method of testing for satay sauce.

2. Definition

2.1 Satay sauce is the product consisting of ground or milled roasted, clean, sound, mature groundnuts also known as peanuts (Arachis hypogaea L.) kernels from which the seed coats have been removed, with the addition of spices, edible salt (sodium chloride), and starch.

NOTE. After this, wherever the word 'groundnuts' appears in this standard, it should also be referred to as 'peanut' under unless otherwise specified.

3. Requirements

3.1 The satay sauce shall be the product made from the ingredients specified in Clause 2.1 and shall be partially grainy in texture.

3.2 Raw materials used for the manufacture of satay sauce shall conform to the appropriate Malaysian Standards.

3.3 Satay sauce shall not contain any non-nutritive sweetening substances.

3.4 It shall not contain any added colouring substance.

3.5 It shall not contain any preservative except benzoic acid or sodium benzoate and sulphur dioxide or sulphites at a level permitted by the current Malaysian Food Regulations.

3.6 It may contain permitted stabilizer and permitted antioxidant.

3.7 It shall be free from any foreign matter, and from any other objectionable taste and odour.

3.8 The aroma and taste shall be characteristic of satay sauce.

3.9 Satay sauce shall comply with the requirements in Table 1.

Table 1. Requirements for satay sauce

Property	Requirements	Method of test refer to *MS 1120 (Clause)
Oil content, %, w/w, max.	15.0	Appendix A of this standard
Total nitrogen, %, w/w, min.	1.0	8
Total solids, % w/w, min.	30.0	3
Aflatoxins, ppb, max.	20.0	Appendix B of this standard
pH	≤ 4.5	5
Salt %, w/w, max.	2.0	7
Mould count (Howard), % max. of total fields examined	50	17

4. Hygiene

4.1 The product shall be processed and packed under hygienic conditions in premises licensed in accordance with the Public Health Legislations currently in force in Malaysia.

5. Packaging and labelling

5.1 The product shall be packed in containers which shall be suitably lacquered and can be hermetically sealed or other suitable containers. The containers shall be properly sealed.

5.2 Each container shall be marked legibly and indelibly or a label shall be attached to the container, with the following information:

5.2.1 Name of the product.

5.2.2 Name and address of the manufacturer and/or packer or the owner of the rights of manufacture or packing or the agent of any of them.

5.2.3 Minimum net content.

5.2.4 List of ingredients in descending order of proportions used in the product, as per labelling requirements stipulated in the current Malaysian Food Regulations.

5.2.5 Code number or marking device indicating date, month and year of manufacture.

* MS 1120, 'Method of sampling and test for sauces'.

5.2.6 Date of expiry.

5.2.7 Country of origin.

5.3 Each container may, by arrangement with the Standards and Industrial Research Institute of Malaysia be marked with the Certification Mark of SIRIM, provided the product conforms to the requirements of this Malaysian Standard.

6. Legal requirements

6.1 The product shall in all other aspects comply with the requirements of the legislations currently in force in Malaysia.

7. Sampling and testing

7.1 Representative samples of the product shall be drawn and tested according to the method prescribed in *MS 1120.

7.2 Test for determining oil content shall be carried out according to the method prescribed in Appendix A.

7.3 Test for determining aflatoxins shall be carried out according to the method prescribed in Appendix B.

7.4 Other tests shall be carried out according to the method specified in Table 1. For this type of satay sauce, the pH is measured directly by a previously standardized electrode of a pH meter.

8. Quality of reagents

8.1 Unless otherwise specified, chemicals used in analysis shall be of a recognized analytical reagent quality, i.e. they shall not contain impurities which affect the results of the tests.

8.2 Distilled water, or water of at least equivalent purity shall be utilized. Solutions shall be, where necessary, freshly prepared and filtered.

9. Compliance

9.1 When on testing, each of the samples is found to conform to the requirements specified in this Malaysian Standard, the lot batch or consignment from which the samples have been drawn shall be deemed to comply with this standard specification.

* MS 1120, 'Methods of sampling and test for sauces'.

Appendix A

Determination of oil content

A1. Apparatus

A1.1 **Soxhlet extraction apparatus**

A1.2 **Drying oven**, maintained at 100 ± 2°C.

A1.3 **Extraction thimbles**.

A1.4 **Desiccator**, charged with any efficient desiccant.

A2. Reagent

A2.1 **Petroleum ether**, boiling point 40°C to 60°C.

A3. Procedure

A3.1 Weigh accurately 2 g to 3 g of sample directly into an extraction thimble.

A3.2 Cover the sample with some glass wool.

A3.3 Place the thimble in the extractor, fitted with a pre-weighed round-bottom flask.

A3.4 Extract the sample with 200 ml petroleum ether for 4 h in a fume chamber.

A3.5 Distil off the solvent in the flask.

A3.6 Dry the flask containing the extracted oil at 100 ± 2°C in the oven for 1 h.

A4. Calculation

A4.1 Oil, % by weight = $100 \dfrac{(W_2 - W_1)}{W_3}$

where,

W_1 is the weight, in grammes, of the extraction flask;

W_2 is the weight, in grammes, of the flask plus the extracted oil;

W_3 is the weight, in grammes, of sample.

Appendix B

Determination of aflatoxin

B1. Apparatus

B1.1 Waring blender

B1.2 Chromatographic tubes, 22 mm x 300 mm with Teflon stopcock, reservoir type (250 ml) (for 50 g samples).

B1.3 Wrist-action shaker, Burrell, or equivalent.

B1.4 Extractors, 500 ml g-s Erlenmeyers or 11 (12 qt) stainless steel pail.

B2. Reagents

B2.1 Benzene - acetonitrile mixture, 98 + 2, prepared from ACS solvents stored in glass.

B2.2 Boiling chips - SiC (Carborundum Co,)

Float off fine and extraneous matter with H_2O, wash with acetone, and dry.

B2.3 Diatomaceous earth, hyflo super-cel.

B2.4 Silica gel for thin layer chromatography. Any silica gel that meets following test may be used (test each shipment). (Macherey-Nagel GHR (Macherey, Nagel & Co., P.O. Box 307, D516 Duren, Germany, distributed by Brinkmann Instruments, Inc.), Applied Science Adsorbosils-I or -5, and Mallinckrodt Silic AR 4G or 7G have been found satisfactory). Prepare TLC plates, spot, develop, and observed as in Clause B6.1 and Clause B6.2.

Place on same origin spot, from solutions of standards, 10 ng each of aflatoxins B_1, M_1, and G_1, 2 ng each of aflatoxins B_2, 1 μg sterigmatocystin, and 50 ng each of ochratoxins A and B. Repeat application of test spots to give $\geq$ 3 test spots evenly spaced across plate. Place origin spots of individual mycotoxins adjacent to spots of multiple mycotoxins. Develop plate and examine. Individual mycotoxins of interest must be separated from each other and appear in the following R_1 sequence: Aflatoxins M_1, G_2, G_1, B_2, and B_1, ochratoxins B and A, and sterigmatocyetin. Time for solvent front to travel 12 cm must be $\leq$ 1.5 h.

On second plate, place number of origin spots containing amount of each mycotoxin which is barely visible under UV illumination employed, plus adjacent spots containing twice these amounts to serve as guide. Develop and observe. Store in the dark in clean air for $\geq$ 18 h. Disappearance of one or both spots after storage is evidence of exceesive fading, and silica gel fails tests.

Note special requirement for silica gel to be used in aflatoxin methods for paper background separation.

B2.5 Sodium sulphate, anhydrous granular ACS grade.

B2.6 Solvent - ACS grade in glass : Acetonitrile, acetone, alcohol benzene, $CHCl_3$, hexane, HOAc, MeOH, and petroleum ether

Ether (anhydrous, ≤ 0.01% alcohol).

B2.7 Aflatoxin standards

For aflatoxin standards received as dry films or crystals. To container of dry aflatoxins B_1, B_2, G_1, or G_2, add volume benzene-C H_3CN, calculated to give concentration of B μg/ml to 10 μg/ml. For aflatoxin M_1, use CH_3CN_3. Use label statement of aflatoxin weight as guide. Vigorously agitate solution for 1 min on Vortex shaker and transfer without rinsing to convenient size g-s flask. (Dry films on glass are not completely recoverable because of absorption. Continued contact with solvent may result in slow dissolution. Do not transfer dry aflatoxin for weighing or other purposes unless facilities are available to prevent dissemination of aflatoxins to surroundings due to electrostatic charge on particles.

For aflatoxin standards received as solutions

Transfer solution to convenient size g-s flask. Dilute, if necessary, to adjust concentration to 8 μg/ml to 10 μg/ml.

B2.7 Resolution reference standard

Prepare resolution reference standard by mixing aflatoxin B_1, B_2, G_1 and G_2, solutions, to give concentration at final dilution with benzene-CH_3CN_2(98 + 2).

B2.8 Silica gel for column chromatography

E. Merck (Darmstadt) Silica Gel 60, 0.063-0.2 mm for 50 g samples. Activate by drying for 1 h at 150°C. Add H_20, 1 ml/100 g, seal, shake until thoroughly mixed, and store 15 h in air-tight container.

B2.9 Silica gel for thin layer chromatography

Silica gel, Clause B2.4, should also meet following test: Prepare aflatoxin free extract of commodity being tested as in Clause B4. Dissolve in 500 μl benzene-CH_3CN (98 + 2). Place 10 μl on origin spot. To this spot, add, from solutions of standards, 10 ng each of aflatoxins B_1 and G_1 and 2 ng each of aflatoxins B_2 and G_2. Repeat application of test spots to give ≥ 3 test spots evenly spaced across plate. Develop plate and examine. The 4 aflatoxins must be separated from each other in clearly defined spots and separated from non-aflatoxin fluorescent material in extract of commodity being examined.

B2.10 Benzene-alcohol-water developing solvent

Prepare enough for several analysis. Shake benzene-alcohol-H_2O (46 + 35 + 19) in separator and let stand overnight at ≤ 22°C. Then store the 2 layers that separate in separate g-s containers. Warm gently before use if they appear cloudy. Alternatively, use benzene-alcohol-H_20 (40 + 6 + 3) for top layer and (40 + 27 + 20) for bottom layer.

B3. Preparation of sample

Peanut butter and peanut meal need no preparation for extraction unless they contain large particles, in which case reduce by milling. Use hammer mill, rotary cutter, or disk (burr) type mill for meals. Grind raw and roasted peanuts and peanut butter with pieces of peanuts to paste with disk (burr) type mill before extraction.

B4. Extraction

Weigh 50 g prepared samples into 500 ml g-s Erlenmeyer. Add 25 ml H_2O, 25 g diatomaceous earth, and 250 ml $CHCl_3$, and secure stopper with masking tape. Shake 30 min on wrist action shaker and filter through fluted paper. If filtration is slow, transfer to buchner precoated with approximately 5 mm layer diatomaceous earth, and use light vacuum. (Use vacuum filtration only for slow filtering samples since evaporation of $CHCl_3$ is rapid, resulting in concentration of extract). Collect first 50 ml portion $CHCl_3$ filtrate and proceed as in Clause B5.

B5. Column chromatography

Place ball of glass wool loosely in bottom of 22 mm x 300 mm chromatographic tube and add approximately 5 g anhydrous Na_2SO_4 to give base for silica gel. Add $CHCl_3$ until tube is 1/2 full; then add 10 g silica gel, Clause B2.8. Wash sides of tube with 20 ml $CHCl_3$ and stir to disperse silica gel. When rate of settling slows, drain some $CHCl_3$ to aid settling, leaving 5 cm to 7 cm above silica gel. Slowly add 15 g anhydrous Na_2SO_4. Drain $CHCl_3$ to top of Na_2SO_4. Add 50 ml sample extract to column, elute at maximum flow rate with 150 ml hexane followed by 150 ml anhydrous ether, and discard. Elute aflatoxins with 150 ml MeOH-$CHCl_3$ (3 + 97), collecting this fraction from time of addition until flow stops.

Add few boiling chips to eluate, evaporate nearly to dryness on steam-bath, and quantitatively transfer residue to vial with $CHCl_3$. Add 2 to 3 boiling chips and evaporate, preferably under stream of nitrogen. Seal vial with hollow polyethylene stopper and cap. Save for TLC.

B6. Thin layer chromatography

B6.1 Preparation of plates

Weigh 30 g silica gel, Clause B2.9, into 300 ml g-s. Erlenmeyer, add amount of H_2O recommended by manufacturer shake vigorously $\leq$ 1 min, and pour into applicator. Adjust amount of H_2O to obtain best consistency of slurry for spreading, as required by batch-to-batch variation in silica gel. Immediately coat five 20 cm x 20 cm glass plates with 0.25 mm thickness of silica gel suspension, and let plates rest undisturbed until gelled (approximately 10 min). Adjust thickness of spread (and weight gel) to 0.5 mm, if necessary, to provide good resolution of aflatoxins and tightness of spots. Dry coated plates $\geq$ 2 h at 80°C or $\geq$ 1 h at 110°C, and store in desiccating cabinet with active silica gel desiccant until just before use. To prepare plate for chromatography, scribe line 16 cm from bottom edge as solvent stop: scribe lines approximately 0.5 cm in from each side or remove 0.5 cm gel from each side to prevent edge effects.

B6.2 Preliminary thin layer chromatography

(This step may be omitted when approximate aflatoxin content is known). Uncap vial containing sample extract, add 200 μl benzene-CH_3CN (98 + 2), and reseal with polyethylene stopper. Shake vigorously to dissolve, preferably with Vortex shaking machine. Puncture polyethylene stopper to accommodate needle of 10 μl syringe. In subdued incandescent light and as rapidly as possible, spot 2 μl, 5 μl, and two 10 μl spots on imaginary line 4 cm from bottom edge or TLC Plate. Keep vial for quantitative analysis. On same plate, spot 2 μl, 5 μl, and 1 μl aflatoxin standards. Spot 5 μl standard used on top of one of the two 10 μl sample origin spots as internal standard. Spot at least one 5 μl resolution reference standard, to show whether adequate resolution is attained.

Place 50 ml acetone-$CHCl_3$ (1 + 9) in trough of unlined developing tank. If tank is other than Thomas-Mitchell, use volume to provide solvent depth of approximately 2 cm. Composition of acetone-$CHCl_3$ can be varied from (5 + 95) to (15 + 85) to compensate for variations in silica gel and developing conditions. Use only one plate per tank, placing trough near one side to permit maximum exposure of coated surface to tank volume. Immediately insert plate into tank and seal tank.

Develop plate 40 min at 23°C to 25°C or until aflatoxins reach R_1 0.4 - 0.7. Adjust develop ment time to compensate if different developing temperature is used. Adjust developing solvent if developing time is > 90 min. Remove from tank, evaporate solvent at room temperature and illuminate plate from below by placing it flat, coated side up, on longwave UV lamp in darkened room, or view plate in Chromato-Vue cabinet, or illuminate from above. (If illumination requires looking directly at lamps, protect eyes with UV-absorbing filter, such as Eastman Kodak Co. 2A). Observe pattern of 4 fluorescent spots of resolution reference standard. In order of decreasing R_f, they are B_1, B_2, G_1, and G_2. Note small colour difference (bluish fluorescence of 'B' contrasted with slightly green 'G' aflatoxins). Examine patterns from sample for fluorescent spots having R_f close to those of standards and similar appearances. From this preliminary plate, estimate suitable dilution for quantitative TLC analysis. In final calculation, take into account amount of extract used for preliminary TLC.

B6.3 Quantitative thin layer chromatography

If preliminary plate shows that new concentration of sample extract is required, evaporate to dryness on steam-bath and redissolve in calculated volume benzene-CH_3CN (98 + 2).

Spot successively 3.5 μl, 5.0 μl and two 6.5 μl portions of sample extract. All spots should be approximately same size and $\leq$ 0.5 cm diameter. On same plate, spot 3.5 μl, 5.0 μl and 6.5 μl aflatoxin standards, corresponding to aflatoxins observed on preliminary plate. Spot 5.0 μl of each standard used on top of one of the two 6.5 μl sample origin spots as internal standard. Spot at least one 5 ml resolution reference standard, to show whether adequate resolution is attained. Proceed as in Clause B6.2.

B6.4 Interpretation of the chromatogram

Four clearly identifiable spots should be visible in resolution reference standard.

Examine pattern from sample spot containing internal standard for aflatoxin spots. R_1 values of aflatoxins used as internal standards should be the same as or only slightly different from those of respective standard aflatoxins spots. (Since spots from sample extract are compared directly with standard aflatoxins on same plate, magnitude of R_1 is unimportant. These may vary from plate to plate).

Compare sample patterns with pattern containing internal standard. Fluorescent spots in sample thought to be aflatoxins must have R_1 values identical to and colour similar to aflatoxin standard spots when unknown spot and internal standard spot are superimposed. Spot from sample and internal standard combined should be more intense than either sample or standard alone.

Compare fluorescent intensities of B_1 spots of sample with those of standard spots and determine which sample portion matches one of the standards. To aid in the determination, move plate away from lamp to attenuate UV light so that any particular pair of spots can be compared at extinction. Interpolate if intensity of sample spot is between those of two of the standard spots. If spots of smallest portion of sample are too intense to match standards, dilute sample and rechromatograph. Compare B_2, G_1 and G_2 spots in same manner.

Calculate concentration of aflatoxin B_1 in μg/kg from formula:

$$(S \times Y \times V)/(X \times W)$$

where,

S is the aflatoxin B_1 standard equal to unknown in μl;

Y is the concentration of aflatoxin B_1 standard in/μg/ml;

V is the final dilution of sample extract in μl;

X is the sample extract spotted giving fluorescent intensity equal to S (B_1 standard) in μl;

W is the sample applied to column (10 g if 50 ml $CHCl_3$ extract is used) in g.

If final extract dilution does not represent 10 g, calculate correct sample weight and substitute. The 50 ml aliquot of $CHCl_3$ extract of peanut butter removed for analysis in Clause B4 usually contains 5 ml to 6 ml fat which adds to volume. Thus, 45 ml aliquot of $CHCl_3$ has been removed and extract actually represents 9 g starting materials, instead of 10 as for low-fat materials.

Calculate aflatoxins B_2, G_1 and G2 similarly.

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