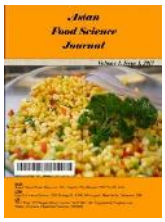

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Production and Evaluation of Soybean Tempeh to Use as a Ready to Eat Meal in Egyptian Hotels

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Production and evaluation of soybean tempeh for use as a new food category (as appetiser, salads and crisps) in Egyptian hotels.
Study Design: Study soybean tempeh was prepared by fermenting soybean seeds with *Rhizopus oligosporus* ATCC 22959 during 96 hours fermentation period. Sensory evaluation was carried out on this food as deep frying and stewing products.
Place and Duration of Study: Microbiology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
Methodology: Production of soybean tempeh by pretreatments for soybean seeds (soaking, dehulling, washing and cooking) and fermentation with *Rhizopus oligosporus* and fermentation for 96 hours, with chemical and microbiological evaluation of the production stages as well as microbiological, chemical and sensory evaluation of the final product.
Results: Compared the chemical analysis for soybean seeds and soybean tempeh found increasing protein and decreasing fat, carbohydrate, ash and phytic acid. Microbiological and

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chemical evaluations were carried out during solid state fermentation process and storage period at refrigerator temperatures. During the fermentation process, microbiological load and chemical changes were monitored. The content of moisture %, ash %, lipid and carbohydrate were decreased but nitrogen, protein and total amino acid, were increased to give the highest figures after 72 hr. fermentation period. During storage period at refrigerator temperature. The nitrogen, protein, lipid, carbohydrate and ash were little or not change was observed in the samples. Sensory evaluation of deep-frying and stewing fermented tempeh manufactured from soybean it could be concluded that the deep-fried soybean tempeh was more susceptible than stewing soybean tempeh. The highest score of the most sensory characteristics was recorded by deep-fried soybean.

Conclusion: This study was undertaken as part of efforts to introduce tempeh technology in Egypt was and to evaluate the combined effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on some antinutritional factors of soybean. As well as fermentation improved and changing the characteristics of soybeans, which increases the antioxidants and decreased anti-nutritional factors. The deep-fried tempeh was more acceptable than stewing tempeh.

Keywords: Tempeh; soybean; Rhizopus oligosporus; fermentation; sensory evaluation; microbiological load.

1. INTRODUCTION

Fermented foods play an important economic role in developing countries as well as making rich the protein requirements. For preparation of fermented food, microorganisms are used.

Tempeh is a traditional Indonesian fermented food made from soybean through fermentation with *Rhizopus oligosporus*. It is manufactured with dehulled, soaked and cooked soybeans inoculated by fungal. The cultured soybeans are bound together by newly grown thick white mycelia to form a cake when thinly sliced and deep fried in oil, tempeh obtains a crispy golden crust making it suitable for marinades, as a snack or staple food [1]. The fermentation of tempeh completely change the soybeans to produce a new flavour, aroma, texture and also increase the nutritional value. Tempeh also contains strong antioxidants which are highly reactive free radicals with oxygen [2]. Dinesh Babu et al. [3] reported that fermentation of soybean improved digestibility by reduction of anti-nutritional factors: tannin and phytase in addition to the production of acids which inhibit the production of pathogenic bacteria and this is particularly important in the manufacture of food designated for specially targeted groups such as infants and old aged ones. Moreover, soy products are free of saturated fat implicated in many health problems and particularly heart-related problem.

Tempeh is now quickly becoming more popular all over the world, as people look for ways to increase their use of soybean, and enjoy its delicious taste.

Rhizopus oligosporus is a food-grade fungus that has been widely used in solid state fermentation -substrate bioconversion systems to produce value-added food products [4].

Jelen et al. [5] found that key odorants in tempeh after five days of fermentation included pyrazine, sulfides, aldehydes and 8-carbon-alcohols and ketones.

Tempeh is a popular meat substitute as the fermentation gives it meat-like nutritional and textural properties. Therefore, tempeh intake has been reported to decrease the effects of chronic degenerative diseases. Tempe and Mineral Availability 191 including cancer, coronary disease, osteoporosis, and menopausal symptoms, due to its high antioxidative activity [6] and the decreasing effects of plasma low density lipoprotein (LDL) levels [7]. Reyes et al. [8] found that the solid state fermentation of soybean with *Rhizopus oligosporus* increased the protein content of tempeh flour (21.7%) and decreased lipids (-38.4%), carbohydrate (-3.5%) and phytic acid (-58.3%).

Abu-Salem et al. [9] found Changes in phytic acid, total phenols and trypsin inhibitor were monitored during the pretreatments (soaking, dehulling, washing and cooking) and fermentation with *Rhizopus oligosporus*.

It is often sliced and fried, battered and fried, roasted, grilled, as appetiser and in salad. The

long use of tempeh at all stages of life, without recognised adverse effects, suggests that it poses no undue safety risk at the levels of intake [10].

The purpose of the study was to produce soybean tempeh and to evaluate it's microbial, chemical and Sensory characterisation after the fermentation period as well as during the storage period at refrigerator temperature.

2. MATERIALS AND METHODS

2.1 Raw Materials

The seeds of Soybean *(Glycine max)*, were obtained from Agriculture Research center, Giza, Egypt.

2.2 Microorganisms

Fungal strain, *Rhizopus oligosporus* ATCC 22959 was obtained from Egypt Microbiology culture collection, Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

2.3 Media Used

Nutrient agar medium [11] was used for enumeration of total aerobic mesophilic bacteria. Malt Extract Agar Medium [12] was used for enumeration of total yeast and fungi. Lauryl Tryptose Sulphate Broth [13] was used to enumerate of total and faecal coliform bacteria. Levine's Bile Eosin Methylene Blue Agar (Modified) [13] used for detection of *E. coli*. Baird Parker Agar Base [13], was used to determine the total Staphylococci count. whereas Vogel Johnson agar medium [14] was used for enumeration *Staphylococcus aureus.*

Salmonella Shigella (SS) Agar [14] was used to detect of *Salmonella* sp. and *Shigella* sp.

2.4 Standard Inoculum of *Rhizopus oligosporus*

Standard inoculm of *R. oligosporus* ATCC 22959, suspension was prepared by spore resuspension on 10 ml sterile distilled water from malt slant inoculated with fungal spore and incubated at 30ºC for 7 days,.- One ml of this suspension was contained about $2.8x10^4$ to $5.3x10^4$ spores. The content of fungal slant was used to inoculate of one gram seed in order to ferment of soybean by solid state fermentation according to the method described by Nout and Kiers [10].

2.5 Microbial Evaluation of Fermented Soybean Tempeh

Samples were microbiologically analysed by aseptic transfer of 10 g of homogenised sub-sample into 90 ml in sterile diluents (0.1% peptone water). Serial dilutions were prepared in the same diluents. Different microbial groups were enumerated using the poured plates technique [11]. The microbiological parameters included densities of total bacteria, total Staphylococci, *Staphylococcus aureus, Salmonella* sp, yeast and total fungi whereas the total and faecal coliform content were determined by most probable number using fermentation tubes containing Lauryl sulphate tryptose broth supplemented with inverted Durham's tubes for collection of gas. *E.coli* was detected by streaking a loopful from positive tubes on Levine's Eosin Methylene Blue agar plate. After incubation at 35ºc for 24-48 hr., plates were examined for suspected colonies of *E.coli* which represented as dark centered, flat, with or without metallic sheen.

2.6 Chemical Evaluation

The chemical analysis of soybean seeds and tempeh samples were carried out at the same time using the following methods:

Total amino acids: were determined according to the method of Moore and Stein [15]. Phytate were determined by method described by Ola and Oboh [16], Determination of moisture, ash, crude protein and lipid content were carried out according to the methods reported in AOAC [17]. Carbohydrate content: was calculated using indirect method of Rahman et al. [18] by the equation of carbohydrate content on dry basses (%) = 100% – (% protein+ % fat + %Ash).

2.7 The Sensory Evaluation of Fermented Products

The sensory attributes of different prepared tempeh, treatments were evaluated by a panel group of ten members randomly selected from the staff members of Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Panelists were asked to evaluated appearance, color, odor, taste, mouthfeeling, texture and overall acceptability according to 7 point hedonic scale [19].

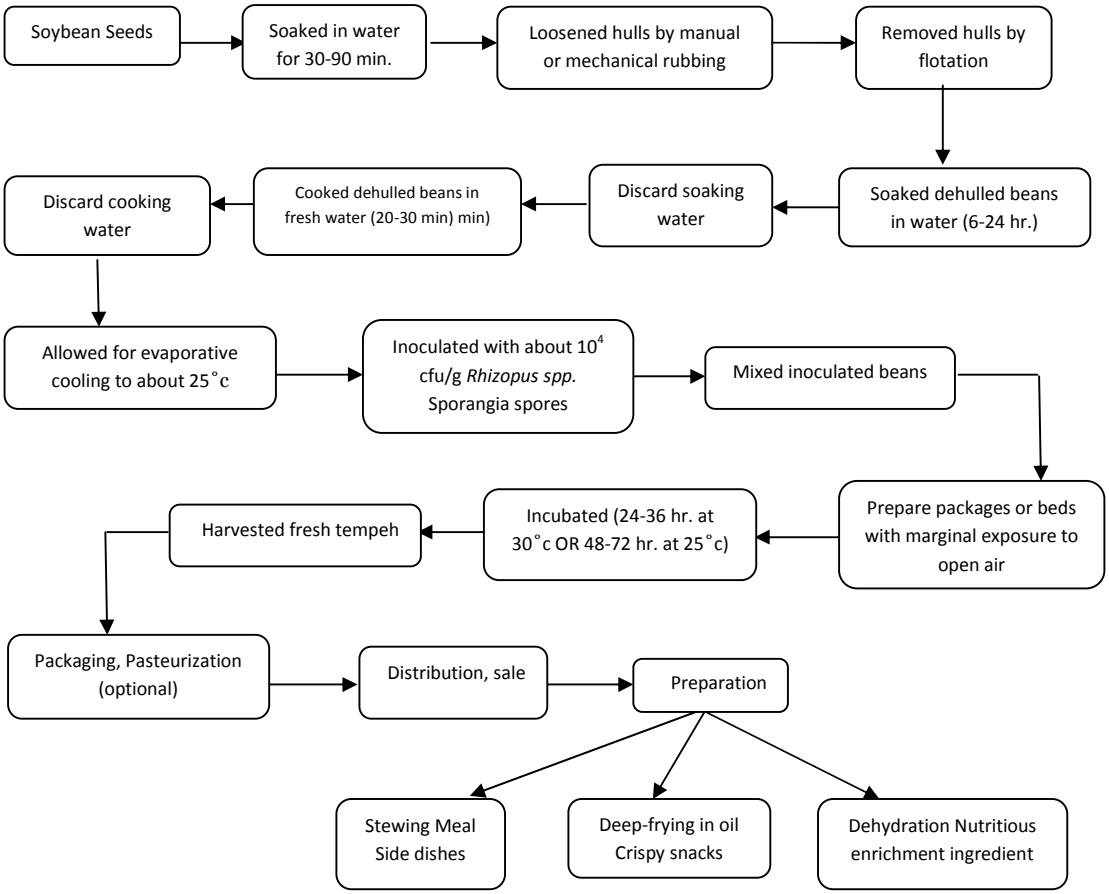

Fig. 1. Flowchart of tempeh production

2.8 Statistical Analysis

The Statistical Analysis System [20] was used to carry out means values, in addition to an overall analysis of variance (ANOVA), LSD (0.05) test, Duncan's multiple range.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Soybean Seeds and Tempeh

Data in Table 1 show that only slight or no change in ash% and decrease in carbohydrate % during the soaking about (2.4%) and cooking process about (0.2 & 0.5%) respectively. Whereas the fermented tempeh increased the crude protein content about 6.8% and decreased the fat, carbohydrate and phytic acid about 11%, 1.1% and 60.6%, respectively, comparing to raw seeds. Also, Egounlety and Aworh [21] found that cooking dehulled soybean decreased phytic acid about 30.7% than raw seeds. Phytic acid and total phenols were decreased during fermentation in soybean. Abu-Salem et al. [9] found Fermentation of soybean with *R. oligosporus* starter culture decrease in phytic acid content 19.6% and 44.2% from total phenolic compounds.

3.2 Microbiological and Chemical Composition of Soybean Tempeh during Fermentation Period

The results show that a tempeh-like food product could be obtained through fermented soybean by fermentation it with *Rhizopus oligosporus* ATCC 22959 using solid state fermentation for 96 hr fermentation period. Mycelia of the fungi began to develop after the first 24 hr of fermentation binding the soybean seeds together. The microbiological quality of products was evaluated during the fermentation period. The microbiological analysis were recorded in Table 2 and express the log numbers of cell forming unit (cfu)/g, for the initial load of total

aerobic mesophilic bacteria, staphylococci, yeast and mould were 8.48, 6.0, 8.0 and 8.0 for soybean. During the fermentation period the log numbers of total aerobic mesophilic bacterial count, total staphylococci and yeast were increased gradually during the fermentation to record the highest value after 72, 48 and 96 hr being 9.94, 8.4 and 9.7 respectively, whereas the log number of mould in soybean tempeh was decreased from 8.0 to 7.0 at the end of fermentation. The log number of total coliform count of soybean tempeh ranged from 3.6 to 5.7 during the first 48 hr of fermentation, then not observed after 72 hr. also, True fecal coliform were detected during the first 24 hr and 24 – 48 hr. Both *E.coli* and *Salmonella* group were not detected during the fermentation period Moreover, *Staphylococcus aureus* was not detected in the tempeh products, so tempeh could be consumed safety. This may be due to the antibacterial activity of *Rhizopus oligosporus*. In this respect, Kiers et al. [22] stated that *Rhizopus oligosporus* can produce certain compounds that interfere with the adhesion of *E.coli* to small intestinal brush- border membranes. At the end of production period (96 hr), all bacterial and fungal loads recorded the lowest values of log number count, whereas the highest figure of yeast was observed on soybean tempeh. Also, yeast has been detected on commercial and traditional tempeh products.

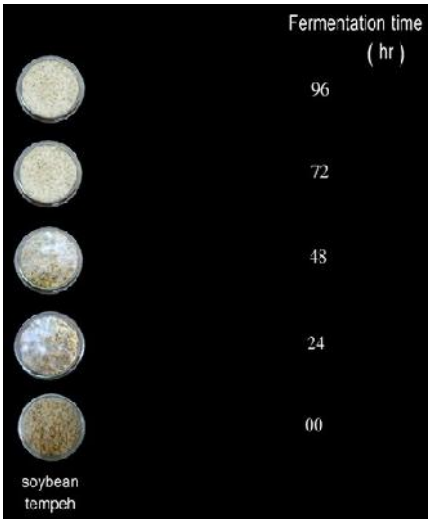

Fig. 2. Fermentation period on the soybean tempeh manufacture by *Rhizopus oligosporus* ATCC 22959 using solid state fermentation process

Table 1. Chemical composition of soybean seeds (dry weight basis) and tempeh as affected by different processing methods

Components %	Treatments			
	Raw seeds	Soaked seeds	Dehulled cooking seeds	Tempeh products
Protein	38.50	38.50	38.50	41.10
Fat	18.00	18.12	18.00	16.00
Ash	2.50	2.50	2.44	2.25
Total carbohydrate	41.00	40.88	40.76	40.55
Phytic acid	1.70	1.69	1.42	0.67

Table 2. The log count of some microorganism groups during 96 hr of the solid state fermentation for soybean tempeh production

Fermentation time (hr)	(log_{10}cfu/g) Log number of cell forming unit (cfu)/g					
	Aerobic mesophilic bacteria	Total Staphylococci	Total coliform	True fecal coliform	Yeast	Mould
0	8.48^{d}	6.00^{e}	3.60^{c}	-	8.00^{f}	8.00^{a}
24	9.64^{b}	8.34^{b}	6.04^{a}	+	8.30^{d}	7.80^{b}
48	9.08^{c}	8.40^{a}	5.70^{b}	+	9.32^{c}	7.60^{c}
72	9.94^{a}	6.81^{c}	0.00	-	9.61^{b}	7.34^{d}
96	6.78^{e}	5.40^{d}	0.00	-	9.70^{b}	7.00^{f}

E. coli and Salmonella group were not detected

Values with the same letter in the same column have no significant different (P= 0.05)

Fig. 3. Soybean tempeh

With respect to chemical composition of soybean tempeh during 96 hr fermentation period, results in Table 3 indicated that during the fermentation process, moisture content decreased from 42.7 to 37.6% which was similarly reported by Maduka et al. [23]. This could be attributed to evaporation during the fermentation. Also, on dry basses lipid of soybean tempeh content decreased from 18.0 to 15.0% but nitrogen content increased from 6.21 to 6.79%. This may be due to use a part of fatty acid by *Rhizopus oligosporus* ATCC 22959 as carbon source [24]. Maduka et al. [23] recorded that both nitrogen and protein content increased about 1.39 fold in bread fruit tempeh. Fadahunsi and Sanni [25] also reported an increase in protein content during the fermentation of food substrates. The carbohydrate content decreased from 40.76 to 40.35% after 72 hr. Also Maduka et al. [23] noticed that the carbohydrate content of bread fruit tempeh decreased from 29.31% to 24.69% after 60 hr fermentation period. This may be due to breakdown of carbohydrate by some microorganisms into simple sugar for energy. The results also show that the total amount of amino acids of soybean tempeh increased proportionally to fermentation time and recorded the highest value after 72 hr being 274. The corresponding figures of total free amino acids of these products becomes 2.25 folds of the control, this might be due to fungal hydrolysation of protein into amino acids and peptides. Handoyo and Morita [26] noticed that the total free amino acids of soybean fermented by *Rhizopus oligosporus* increased about 3-10 folds than control.

3.3 Evaluation of Soybean Tempeh Storage at Refrigerated Temperature

Manufactured fresh soybean tempeh was storage at refrigerate temperatures for 8 weeks as preserved methods, samples were taken periodically every week for determining the count of different microorganisms group using the selective media. Data presented in Table 4 show that the log numbers of aerobic mesophilic bacteria were increased gradually to record the highest figure after 8 weeks storage period, also, the highest values of total staphylococci were obtained after 8 weeks storage period. *Staphylococcus aureus* was not detected during the storage period. No remarkable increase of log number of mould count was observed during storage period at refrigerator temperature, whereas the highest log number of yeast count (10.78) was attained after 2 weeks. Also, Ashenafi [27] noticed that most products had counts of > 10^6 cfu/g after 7 days of cold storage. The log number of total coliform count was recorded only after 7 and 8 weeks at refrigerator temperature being 4.04 and 3.7, respectively, due to contamination. True fecal coliform, *E.coli* and salmonella group were not detected at all storage period, tempeh could be consumed safely.

Data presented in Table 5 clearly show that the moisture percentage of soybean tempeh was decreased during the storage period to reach 32.32% after 8 weeks at refrigerator temperature. The nitrogen, protein, lipid, carbohydrate and ash were little or not change was observed in the samples. The total amino acids were increased after the first week of storage period. Then decreased gradually to reach the lowest value after 8 weeks storage period. The amount of amino acids was ranged from 274.7 mg/100 g in zero time production of tempeh (control) before storage to 265.1 mg/100 g after 8 weeks storage period.

Fig. 4. Fried soybean tempeh

3.4 Sensory Evolution of Soybean Tempeh

Sensory evaluation of deep fried and stewing fermented tempeh manufactured from soybean was carried out by ten man panel. Based on their assessment a correlation of the following attributes: appearance, colour, texture, taste, odour, mouth feeling and the over all acceptability of deep fried soybean tempeh are 13.59%, 13.28%, 12.66%, 11.09%, 7.81% and 8.59%, respectively. It could be inferred that the over all acceptability of this product greatly depends on 13.59% and least on 7.81%. Table 6 shows the sensory panels mean score of deep-fried and stewing soybean tempeh products. The latter product recorded the lowest parameters with significantly differences between them. Generally, it could be concluded that the deep-fried soybean tempeh was more susceptible than stewing soybean tempeh and recorded the over all acceptability being 6.4 and 1.7, respectively.

Jelen et al. [5] found that frying process of tempeh induced the increase of the main key odorants such as 2-acetyl1-pyrroline, 2-ethyl-3,5-dimethylpyrazine, dimethyl trisulfide, methional, 2-methylpropanal, and (E,E)- 2,4-decadienal.

Table 3. Some chemical composition of soybean tempeh during 96 hr fermentation period of the solid state fermentation process

Fermentation time (hr)	% Moisture	% Ash	% Nitrogen	% Protein	% Lipid	% Carbohydrate	Total amino acids mg/100 g
0	42.70	2.44	6.21	38.30	18.00	40.76	122.20
24	41.30	2.40	6.32	39.50	17.50	40.60	199.30
48	40.50	2.35	6.57	41.10	16.00	40.55	258.40
72	38.40	2.25	6.79	42.45	14.95	40.35	274.70
96	37.60	2.25	6.79	42.45	15.00	40.30	269.20

Table 4. The log count of some microorganism groups during 8 weeks storage period of soybean tempeh at refrigerator temperature (4°C)

Storage period (weeks)	(log_{10}cfu/g) Log number of cell forming unit (cfu/g)				
	Aerobic mesophilic bacteria	Total staphylococci	Total coliform	Yeast	Mould
1	7.43^{h}	5.90^{e}	-	9.78^{f}	8.00^{f}
2	8.76^{g}	5.98^{h}	-	10.08^{a}	8.30^{d}
3	10.21^{f}	6.00^{g}	-	10.20^{b}	8.04^{e}
4	10.28^{e}	6.18^{f}	-	10.29^{c}	8.64^{a}
5	10.48^{d}	6.30^{a}	-	10.10^{d}	8.30^{d}
6	10.79^{c}	6.45^{b}	-	9.89^{e}	8.48^{b}
7	10.89^{b}	7.00^{c}	4.04^{a}	9.60^{g}	8.30^{d}
8	11.08^{a}	7.48^{d}	3.70^{b}	9.48^{h}	8.45^{c}

Staphylococcus aureus, true fecal coliform, E.coli and salmonellae group were not detected at all storage periods. (-) = not detected.

Values with the same letter in the same column have no significant different (P = 0.05)

Table 5. Some chemical composition of soybean tempeh during 8 weeks storage period at refrigerator temperature (4°C)

Storage temperature (ºC)	Storage period (weeks)	Chemical composition						
		% Moisture	% Ash	% Lipid	% Nitrogen	% Protein	% Carbohydrate	Total amino acids (mg/100 g)
Refrigerator temperature (4˚C)	1	38.40	2.25	14.95	6.79	42.45	40.35	281.60
	4	35.10	2.25	14.95	6.77	42.30	40.50	276.20
	8	32.32	2.25	14.95	6.71	41.95	40.85	265.10
Tempeh before storage (control)		38.40	2.25	14.60	6.59	41.20	41.95	274.70

Table 6. Sensory panels Mean score of deep-fried and stewing soybean tempeh

Parameters	Deep-fried soybean tempeh	Stewing soybean tempeh
Appearance	8.7[a]	4.5[c]
Color	8.5[a]	4.4[c]
Texture	8.1[a]	2.2[d]
Taste	7.1[b]	2.3[c]
Odor	5.0[c]	2.4[b]
Mouth feeling	5.5[b]	2.1[d]
Over all acceptability	6.4[b]	1.7[b]

The means with the same letters in the same column are not significantly different (p = 0.05)

4. CONCLUSION

This study was undertaken as part of efforts to introduce tempeh technology in Egypt was and to evaluate the combined effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on some antinutritional factors of soybean. As well as microbiological and chemical evaluation to the optimal stage of production and storage with reference to the high content of amino acids, by improved fermentation and changing the characteristics of soybeans, which decreases the content of tempeh from phytic acid as an anti-nutritional factors. The deep-fried tempeh was more acceptable than stewing tempeh. The highest score of the most sensory characteristics was recorded by deep-fried soybean, for this tempeh can be used in Egyptian hotels as a source of protein for vegetarians, as Appetiser, salads and crisps.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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