

Effect of garlic (*Allium sativum* L.) on the physicochemical, microbiological and sensory properties of chili sauce

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Abstract

Garlic or scientifically known as *Allium sativum* L. is known to have natural antimicrobial properties that help to prolong the shelf life of food products. Previously most studies focused on the effect of garlic as antimicrobial agents and seldom continued to study up to the physicochemical and sensory properties of chili sauce. The aim of the present study is to evaluate the effects of different percentages of garlic (3%, 5% and 10%) on the physicochemical, microbiological and sensory properties of chili sauce product. The physicochemical properties such as pH, total soluble solids, viscosity and colour as well as microbiological activities of *Bacillus cereus*, yeast and mould were evaluated. The growth of microorganisms in the spiked samples of chili sauce was continuously examined for 30 days of the experimentation period. The acceptance of chili sauce formulated with different percentage of garlic was determined by untrained panellists using affective test. Incorporation of garlic to the chili sauce had increased the pH, total soluble solids and viscosity with the increasing percentage of garlic added. The colour of chili sauce increased in term of lightness and yellowness while showed less redness at a higher percentage of garlic. The effectiveness against *B. cereus*, yeast and mould were identified in the sauce contain 10% garlic and maintain within the acceptable level for up to 30 days of the experimental period. Sensory evaluation showed that the addition of up to 10% garlic also did not affect the acceptance level ($p > 0.05$) of the chili sauce. The presents study showed that garlic has the potential to replace chemical preservative that normally being used in making chili sauce.

1. Introduction

Garlic (*Allium sativum* L.) has great medicinal importance and is extensively used due to its strong odour, appetizer property and bitter taste, which contributes flavours to food consumed. It should be consumed fresh for its effectiveness for human health (Daka, 2011), but some researchers claim that in some situations garlic of higher temperature extract can provide better protection against free radicals and infections than fresh garlic (Kwon *et al.*, 2006; Jastrzebski *et al.*, 2007; Queiroz *et al.*, 2009). Ethanolic extracts of garlic showed the presence of many secondary metabolic such as saponins, tannins, alkaloid and steroids (Arekemase *et al.*, 2013) which commonly known to possess antimicrobial activity. A study on the antibacterial effect of garlic extract showed that it exhibited excellent antibacterial activity against *Escherichia coli*, *Salmonella enterica serovar* Typhi, *Staphylococcus aureus* and *B. cereus* (Chand, 2013). The

presence of allyl group in garlic essential oils especially diallyl monosulfide, diallyl disulphide, diallyl trisulfide and diallyl tetrasulfide were identified to give good antimicrobial activity against *S. aureus*, *Pseudomonas aeruginosa* and *E. coli* (Casella *et al.*, 2013).

The growing demand on the variety of chili sauces in the market has inspired food industries to create many types of formulations of the product. However, ordinary properties of chili sauce subjected to contamination by bacteria and fungi, cause undesirable reactions such as deterioration in flavour, odor, colour, sensory and textural properties. The potential of microbial growth in chili sauce is a major concern due to some microorganisms may cause food-borne illness. Although synthetic antimicrobials are approved in many countries, due to consumer's awareness and concern towards the health effects of chemical additives in foods has increased demand for natural additives. One of the ways to inhibit the growth of undesirable microorganism is by

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direct incorporation of bioactive agents into food that can lead to a short-term reduction of bacterial populations (Hanusová et al., 2009).

Certain food products contain preservatives in order to delay the growth of microorganisms, to maintain the nutritional value and to prolong the shelf life and quality of the products (Liu et al., 2014; Ding et al., 2015). The most commonly used preservative in chili sauce is sodium benzoate to inhibit the growth of mould, yeast and some bacteria (Zengin et al., 2011). Thai Agricultural Standard (2008) has listed the allowed quantity of bacteria species present in chili sauce including *E. coli*, *S. aureus*, *B. cereus*, *Clostridium perfringens* and *Salmonella* spp. The possibility of chili sauce to be spoiled by yeast and mould is quite high due to the ability of these organism to grow in a broad-ranging of pH and moisture content. The same properties also found in *B. cereus* which is known to have capability of proliferating in a wide range of environments include soil, water and both raw and processed products (Linback et al., 2004; Fangio et al., 2010), thus make it significance to observe for its existence and safety level. Report of isolation of *B. cereus* group found in many studies including several starchy food products and ready-to-eat food products (Fangio et al., 2010; Samapundo et al., 2011). The addition of starch hydrocolloids as a thickening agent in chili sauce may increase the potential for microbial growth especially those without the addition of preservative.

Thus, this study was aimed to determine the effect of garlic constituents on the physicochemical, microbiological and sensory properties of chili sauce. The use of garlic in chili sauce indirectly can contribute to various benefits including human health, functional foods and dietary supplement.

2. Materials and methods

2.1 Sample materials

Garlic (*Allium sativum* L.) and dried chilies were purchased from the local market at Kuala Nerus, Terengganu. The raw materials purchased were kept at room temperature ($\pm 25^{\circ}\text{C}$) for further use. Other ingredients used in making chili sauce include sugar, modified corn starch, salt, acetic acid (vinegar), carboxymethyl cellulose (CMC) and water.

2.2 Preparation of chili sauce

The primary ingredients in making chili sauce including water (61-72%) depending on the amount of garlic used), dried chilies (13%), sugar (10%) and modified corn starch (1.7%). The dried chilies were

washed, cut into small pieces and removed the seeds before blanching for 10 mins at 100°C . It was then drained and washed again to remove any remaining seeds and other impurities. Then, the dried chilies and garlic were blended together with water using a blender until having a smooth texture of puree. The puree was then poured into a pan and brought to boil at $92\pm 3^{\circ}\text{C}$ for 20 mins before added with salt and CMC. The puree was continuously stirred evenly and brought to boil for another 15 mins at a lower temperature ($84\pm 3^{\circ}\text{C}$). After all the ingredients have dissolved, the modified corn starch was added and the mixture was cooked for at least 2 mins. Finally, the acetic acid (0.7% of the total amount) was added into the mixture before removing from heat. It was followed by hot filing into sterilized bottles, screwed caps and cooling at room temperature for further analysis.

2.3 Physical properties measurement

The physical properties including pH, total soluble solids, colour and viscosity were measured at room temperature. pH and total soluble solids were measured using a pH meter (Eutech instrument) and a refractometer (Hand refractometer, Milwaukee, MA871) respectively. The colour of the sauces was measured using Hunterlab colourimeter (Konika Minolta). Triplicate measurements were recorded on the value of L, a and b. The parameter 'L' is a measure of brightness/whiteness using scale from 0 to 100 (white if L=100, black if L=0). While, parameter "a" indicates redness varies from -a to +a (-a=green, a=red). Lastly, parameter "b" indicates the measurement of yellowness that varies from -b to +b (-b=blue, +b=yellow). The viscosity of the sauces was analysed using a Brookfield digital viscometer with no 4 spindles at 10 rpm. A precise measurement was obtained by adjusting positions as well as the setting of the viscometer. Results of the viscosity were recorded in unit mPas. All the measurement for the above analysis were conducted in triplicates and the data were recorded as mean \pm standard deviation.

2.4 Microbiological analysis

Microbial growth mediums such as Potato dextrose agar (PDA) and Mannitol egg yolk polymyxin agar (MYP) were prepared as acquired standard procedure. Samples of chili sauce that contain different amount of garlic (0%, 3%, 5% and 10%) were stored for a period of 30 days at room temperature. The growth of colonies was counted periodically for every 6 days of the experimental period. For yeast and mould count, 25 g of chili sauce was mixed with 225 mL BPW and homogenized using stomacher. A serial dilution of chili sauce was prepared ranging from 10^{-1} to 10^{-6} dilution of the original sample. Then, 0.1 mL of sample from each

dilution was spread onto PDA plate and incubated at 25°C for 5 days followed by colony counting. Measurement of *B. cereus* was in accordance with Beuchat and Brackett (1991) using bacteria spiking method. *B. cereus* ATCC 11778 were grown in a nutrient broth at 37°C for 24-48 hrs. Isolation of pure culture was used to prepare cell suspensions. 0.5 McFarland Standard were tested spectrophotometrically with a 1 cm light path set at 600 nm (Zapata and Ramirez-Arcos, 2015). For bacteria spiking, 245 g of chili sauce was inoculated for 24 hours with 5 ml of a 10⁻¹ dilution of *B. cereus* strain. Then, 25 g of the spiked sauce was mixed with BPW and homogenized using stomacher. A serial dilution ranging from 10⁻¹ to 10⁻⁶ dilution was prepared and 1 ml sample was spread onto MYP agar plate. The plate was then incubated at 30°C for 24 hours to allow the bacteria growth and calculate the colony. The result of microbial analysis was evaluated at day 0, 6, 12, 18, 24 and 30. Microbial counts were transformed to log values of the number of colony (CFU/g) and subjected to analysis of one-way ANOVA using SPSS software version 20.

2.5 Sensory evaluation

A total of 30 untrained panellists were assigned hedonic scale from one (dislike extremely) to seven (like extremely) to evaluate their acceptance on appearance, colour, odor, taste, viscosity and overall acceptability. Significant differences between samples were analysed using Tukey HSD (Honestly Significant Different) multiple comparison at 95% confidence level.

3. Results and discussion

3.1 Physical properties measurement

The pH value of chili sauce was in the range from 3.56 to 3.96 and complied with the required standard that shall not exceed 4.5 (Thai Agricultural Standard, 2008). Generally, the acidic properties of chili sauce were contributed by the vinegar as one of the ingredients used in chili sauce. pH value for chili sauce in this study (Table 1) was comparable with commercial chili sauce which was in between 3.43 to 3.93 (Gamompilas *et al.*, 2011), depending on the amount of garlic incorporated into the formulations. The higher the amount of garlic in the formulation, the higher the pH value. Fresh garlic is acknowledged to have slightly acidic properties with pH is in between 6.0 to 6.4. These properties of garlic contributed to significantly increase of pH in the sauce. Total soluble solids (TSS), were in the range from 33.77 to 39.90 and fulfilled the minimum requirement of TSS set by Malaysian Food Act (1983) and Regulation (1985) that TSS in chili sauce should not less than 25%. The higher the percentage of garlic added, the higher the value TSS and viscosity of the sauce. The present of total

sugars (4%) and carbohydrates (29%) in fresh garlic (Puranik *et al.*, 2012) contributed to higher TSS in the chili sauce. Furthermore, high TSS can be associated with the increase in viscosity of chili sauce. Past research by Gamompilas *et al.* (2011) revealed that the sauce with the lowest TSS had the lowest consistency which also closely related to viscosity. The intensity of colour properties in chili sauce is presented in Table 2. The lightness (L*) of chili sauce increased significantly as the amount of garlic was increased. This is possibly due to the whitish colour of fresh garlic added in the formulation. As previously reported, the lightness of fresh garlic itself gave L* value of 62.55 (Fante and Noreña, 2012). Meanwhile, increasing of garlic in sauce decreased the redness (a*) due to the mixing of carotenoid pigments such as capsanthin and capsorubin in red chili (Arimboor *et al.*, 2014) and white yellow pigment in garlic (Hye *et al.*, 2014). Chili sauce prepared without addition of garlic showed significantly lower in yellowness (b* value) compared to other formulations without garlic.

3.2 Microbiological analysis

Table 1. pH, total soluble solids and viscosity of chili sauce with different percentage of garlic

Amount of garlic (%)	pH	Total soluble solid (°Brix)	Viscosity (mPas)
0	3.56±0.26 ^d	33.77±0.06 ^d	10539.33±64.58 ^c
3	3.70±0.31 ^c	35.63±0.15 ^c	10768.00±85.14 ^c
5	3.83±0.17 ^b	37.53±0.06 ^b	11589.00±123.89 ^b
10	3.96±0.12 ^a	39.90±0.17 ^a	13271.00±206.21 ^a

Values are expressed in mean ± SD for three replication (n=3). Value with different superscript letter are significantly different (p<0.05)

Table 2. Colour analysis score of chili sauce with different percentage of garlic

Amount of garlic (%)	Colour		
	L*	a*	b*
0	34.25 ± 0.32 ^c	27.66 ± 0.24 ^a	17.92 ± 0.98 ^c
3	37.72 ± 0.15 ^b	26.18 ± 0.61 ^b	22.42 ± 0.26 ^b
5	38.10 ± 0.05 ^b	26.14 ± 0.48 ^b	22.87 ± 0.13 ^b
10	40.42 ± 0.28 ^a	24.83 ± 0.27 ^c	25.54 ± 0.33 ^a

Values are expressed in mean ± SD for three replications (n=3). Value with different superscript letter are significantly different (p<0.05)

The number of colonies for yeast and mould and *B. cereus* increased by days, even though with the addition of garlic with 3% and 5% (Tables 3 and 4). However, a reduction of yeast and mould and *B. cereus* count was observed with 10% garlic started from day 6 to day 30. No detection of the microbe observed from day 6 to day 18 (<1.0 x 10²), however, the microbial growth slightly increased again at day 24 to day 30. Higher detection in

Table 3. Yeast and mould count (CFU/g) in chili sauce with different percentage of garlic for five weeks of experimental period

Day of storage	Yeast and mould count (CFU/g)			
	0% garlic	3% garlic	5% garlic	10% garlic
0	2.7×10^3	2.1×10^3	3.2×10^3	1.7×10^3
6	1.7×10^4	1.6×10^4	8.0×10^3	$<1.0 \times 10^2$
12	1.7×10^5	1.4×10^5	1.2×10^4	$<1.0 \times 10^2$
18	6.3×10^6	2.1×10^6	1.2×10^5	$<1.0 \times 10^2$
24	1.0×10^7	5.3×10^6	1.1×10^5	2.0×10^2
30	5.1×10^7	2.1×10^7	1.2×10^6	2.0×10^2

< - plates with no colony, estimation plates with 1-29 colonies.

Table 4. *B. cereus* count (CFU/g) in chili sauce with different percentage of garlic for five weeks of experimental period

Day of storage	<i>B. cereus</i> count (CFU/g)			
	0% garlic	3% garlic	5% garlic	10% garlic
0	1.6×10^4	2.0×10^3	1.8×10^3	5.5×10^2
6	1.5×10^4	1.0×10^4	9.2×10^3	$<1.0 \times 10^2$
12	1.6×10^5	1.7×10^5	1.4×10^4	$<1.0 \times 10^2$
18	1.2×10^6	9.7×10^5	2.8×10^5	$<1.0 \times 10^2$
24	6.7×10^6	2.0×10^6	1.1×10^6	1.0×10^2
30	2.1×10^7	3.2×10^6	1.7×10^6	1.0×10^2

< - plates with no colony, estimation plates with 1-29 colonies.

Table 5. Acceptance score of chili sauce with different percentage of garlic

Sensory Attributes	Formulation (percentage of garlic)			
	0%	3%	5%	10%
Colour	6.20 ± 0.17^a	5.77 ± 0.82^a	5.57 ± 0.90^a	4.77 ± 1.48^b
Aroma	5.33 ± 0.88^a	5.57 ± 0.97^a	5.70 ± 0.92^a	5.63 ± 1.03^a
Viscosity	5.67 ± 0.96^a	5.60 ± 0.89^a	5.53 ± 1.01^a	5.33 ± 0.84^a
Sweetness	5.00 ± 0.79^a	5.13 ± 0.82^a	5.23 ± 0.94^a	5.20 ± 0.71^a
Sourness	5.13 ± 0.65^a	5.17 ± 0.75^a	5.27 ± 1.05^a	5.03 ± 0.62^a
Taste	5.13 ± 0.78^a	5.30 ± 0.84^a	5.43 ± 1.01^a	5.43 ± 0.77^a
Overall acceptance	5.17 ± 0.95^a	5.33 ± 0.88^a	5.40 ± 1.10^a	5.33 ± 0.84^a

Value with different superscript letter in the same row is significantly different ($p < 0.05$)

the initial day was due to spiking of *B. cereus* culture at day 0. Microbial growth's pattern in this study was quite similar with Sihombing *et al.* (2014), who reported that 10% garlic extract as the most effective to inhibit 100% growth of *B. cereus* using diffusion agar method. Thus, the colony counts for 10% garlic fulfilled the minimum standard for fungi and *B. cereus* due to less than 30 colonies observed in the chili sauce and the values stated were only based on estimation. The presence of sulfur compounds in garlic probably the main reason for antimicrobial effects in chili sauce. Crushed garlic dissolved in water could release sulfur compounds mainly allicin which react as an antimicrobial agent (Amagase, 2006). Meanwhile, ajoene compounds in garlic possibly act as antifungal due to the ability of ajoene in breaking the cell walls of fungi (Irkin and Korukluoglu, 2007).

3.3 Sensory evaluation

Sensory evaluation of chili sauce showed no significant difference in mean scores for most sensory attributes (Table 5). Addition of garlic from 3% to 10% have not influenced overall acceptability of the sauce.

However, the addition of 10% of garlic was identified to affect colour acceptance which was significantly lower than 0%, 3% and 5%. This might be due to whitish colour perceived by panellists when a high amount of garlic was added compared to the other samples. This study showed that the addition of garlic up to 10% in chili sauce was acceptable for most sensory attributes in terms of aroma, viscosity, sweetness, sourness, taste and overall acceptance.

4. Conclusion

Chili sauce produced in this study fulfilled the minimum requirement of Malaysian food legislation in terms of pH and TSS. The increment in the quantity of garlic has influenced the pH, viscosity, colour and antimicrobial effects of chili sauce. Addition of 10% garlic was found to be the most effective in inhibiting the growth of yeast and mould and bacteria of *B. cereus*. Sensory evaluation indicated that the addition of up to 10% garlic in chili sauce did not affect the overall acceptability of chili sauce except for colour. This study is expected to provide useful information on the beneficial effects of garlic as a natural preservative in

food products and hence promote good health in preventing various chronic diseases.

Conflict of Interest

We declare that we have no conflict of interest.

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