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GARLIC POWDER PREPARATION METHODOLOGY TO IMPROVE ALLICIN CONTENT

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ABSTRACT: Background: Garlic has been used in world culinary art as well as in herbal medicine since long back. Allicin, an active moiety in garlic, is truly recognized as heart of garlic. But allicin is highly unstable and undergoes formation of numbers of sulfur-containing compounds with reduced allicin content. The major problem associated with dosage form development is instability of allicin. **Objective:** The aim of the present research work was to obtain a stable garlic powder from the garlic bulbs with maximum allicin content with prolonged physical and chemical stability of the garlic powder. The target was to improve household storage for culinary purpose as well increased active drug content proclaimed for long term utilization in dosage form design. **Methodology:** The principles of microwave drying technology and the flower drying technology along with some modifications have been successfully utilized for preparing, processing and preserving garlic powder to improve its medicinal and culinary acceptance. **Results:** The allicin content was found to be increased (2533.7 µg/gm) as compared aqueous garlic extract and garlic powder prepared by air drying. Garlic powder was found to be free-flowing and long-lasting. **Conclusion:** We can conclude that garlic-the king of healing herbs can be utilized fruitfully for antimicrobial formulations, development, and optimization and as food additive in the culinary art.

INTRODUCTION: In the last few years, people have begun to realize the importance of garlic and have acknowledged the fact in the literature. Dating back over 5,000 years ago, to the early Egyptians and Sumerians, this magical herb was quickly established as the most adaptable and proficient food widely used for both its culinary and medicinal benefits.

Garlic contains many beneficial components, and over the last 10 to 15 years an enormous amount of research has been published on the properties of various breakdown components that are formed when fresh garlic is cut, crushed, chopped or processed.

Of these well-known and categorized sulfur-containing chemicals, it is allicin that stands out head and shoulders above the rest as showing the most exciting, interesting, and beneficial properties. Allicin is truly recognized as heart of garlic. Allicin is diallyl thiosulfinate, allyl sulfide. The most crucial and reactive part of allicin is sulfur-sulfur bond coupled with oxygen molecule. This is very reactive bond which gives allicin its amazing

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properties as an antibiotic or antimicrobial. But alliin is highly unstable and undergoes formation of numbers of sulfur-containing compounds¹. Alliin is produced upon tissue damage from the non-proteinogenic amino acid alliin (S-allyl cysteine sulfoxide) in a reaction that is catalyzed by the enzyme alliinase. Being a thiosulfinate, alliin is a reactive sulfur species (RSS) and undergoes a redox-reaction with thiol groups in glutathione and proteins that is thought to be essential for its biological activity².

Alliin normally loses its beneficial properties within hours because it begins to react with other garlic components as soon as the clove is crushed. Alliin is a relatively unstable molecule that spontaneously decomposes into a group of odoriferous compounds like sulfides, including ajoene and dithiins. Alliin is said to be highly volatile, poorly miscible with water and has the odors of freshly crushed garlic³. Alliin is converted to allyl sulfides, which are largely responsible for the typical garlic odor⁴. Alliin and enzyme alliinase are quite heat-stable, but alliin is not, which is converted to several other sulfur compounds, such as diallyl sulfide, diallyl disulfide, *etc.* Alliin and alliinase are also stable when dry⁵. Garlic is a proven antimicrobial agent can be explored for its antimicrobial potentials, but garlic never found place in clinical trials due to instability of alliin, an antimicrobial component present in garlic bulbs. The composition and the effect of its bioactivity depend on the garlic strain, age, storage condition, method of processing and consumption⁶.

The major drawbacks found during our laboratory studies were the problem of moisture grabbing by the garlic powder prepared by any reported method which in turn was hindrance in taking the powder for further dosage form optimization studies. Moreover it also affected the yield of alliin. The physical stability for long term storage of garlic powder needed a focus first, which in turn would help to improve the chemical stability in terms of alliin content of garlic powder prepared. So improvement in the physical and chemical stability of alliin became the major target of the present work before actually going for the formulation, development, and optimization. Our previous study reported in article⁷ presents the microwave-

assisted process for the production of stable garlic powder with modifications in reported literature⁸. Various reports regarding the beneficial biological activities of alliin, such as antimicrobial effect, antioxidant effect, suppression of cholesterol biosynthesis, anticancer activity and anti-inflammation effect, have emerged since its discovery⁹. However, unfavorable chemical properties of alliin, such as high reactivity and instability, were also revealed, raising questions regarding alliin being really an active, beneficial component of garlic¹⁰.

The major problem associated with the reported method of preparation of garlic powder was the problem of moisture grabbing by the prepared garlic powder moreover alliin content was also low concerning reported alliin content (should not be less than 0.45%)¹¹. So, the formulation and development using the said powder was the tedious issue due to problem of moisture which in turn had an impact on stability and content of alliin the major antimicrobial constituent present in the garlic powder. The major focus of the updated method for the preparation of garlic powder in present research work was to maintain suitable surroundings for enzymatic fermentation and by stabilizing the resulting product with the aid of silica beads used during the further microwave drying process. The flower drying technology generally involves the use of microwave and the silica gel which was beneficially utilized during the processing of garlic powder¹².

MATERIAL AND METHODS: The garlic cloves were purchased from the local market, grown in Nashik, Maharashtra, India. C₁₈ Sep pak cartridges were obtained from Waters (India) Private Limited, Mumbai, Maharashtra, India. All the reagents and chemicals used were of analytical grade and were procured from S.D. Fine-Chem Ltd., Mumbai, India. Scientific Microwave System-Catalyst™ System was used for microwave drying process.

Modified (Updated) Microwave-Assisted Preparation of Garlic Powder: Garlic bulbs were made into thin slices which were then triturated in mortar and pestle. Slices were spread on petri plate evenly and were just moistened with the 5% citric acid solution and 5% sugar solution to maintain the acidic condition and fermentation environment

which helps in enzymatic conversion of precursor allin to allicin¹³. The mixture was allowed to react for 30 min in ambient conditions. It was then subjected to microwave drying cycles⁸ at 350, 280, 210, 140 watts for 3, 3, 9, 3 min respectively with slight modifications in microwave powers and power cycles applied as cited in the literature⁸. Due to presence of citric acid solution, the samples were still wet after first drying cycle, so it was subjected to two successive drying cycles.

The second cycle was run with the desiccant silica gel bids kept over the partially dried garlic slices with the beaker of water kept in the microwave oven^{12, 14}. Successive five runs were taken at 210 watts with the garlic slices fully covered with the silica bids desiccant. The temperature throughout the process was monitored to be below/up to 30-degree centigrade, literature reveals, fermentation process speeds up between 25 to 35 °C and approaches maximum at 30 °C¹³. Due to the significant instability of allicin, synthesis procedures are usually performed at temperatures from 0°C to 25°C¹⁵.

Preservation Method for Garlic Powder: As the color of the triturated slices turns slightly brownish the silica bids were separated manually and dried slices were mixer grinded by adding slight amount of additive like cornflour. One of the batches was grinded without adding additive corn flour. This batch was prepared for comparative evaluation. The grinded powder was sieved through sieve number # 26, which was preserved in suitable container with prepared powder covered with the desiccant silica bids for garlic powder stabilization till further use.

Infrared (IR) Spectroscopic Examination of Garlic Powder: The IR spectra of garlic powder samples with and without additives were recorded using Fourier Transform Infra-Red spectrophotometer (Shimadzu FT/IR 84005) with diffuse reflectance principle. Sample preparation involved mixing the sample with potassium bromide (KBr), (~2 mg sample in 100 mg KBr) triturating in glass mortar and finally placing in the sample holder. The spectrum was scanned over a frequency range 4000-400 cm⁻¹. Silica bids were separated at the time of IR estimation **Table 1, Table 2, Table 3 and Fig. 1, Fig. 2**.

Content Estimation of Allicin: The content of allicin was estimated using technique of Solid Phase Extraction (SPE) optimization and UV absorbance measurements¹⁶. One gm of the fine garlic powder was soaked in 10 ml of cold (refrigerated) distilled water. The garlic solution was filtered through Whatman no. 41 filter papers under vacuum. The filtrate was used for content estimation. C₁₈ Sep pak 100 mg cartridges were conditioned by rinsing with 10 ml methanol and then equilibrated with 10 ml of water. 1 ml of the garlic powder extract solution was introduced to the top of a cartridge and eluted with varying volumes of cold distilled water (2 ml), ethanol (3 ml), methanol (3 ml), methanol/ water (2:3 v/v), (3 ml), methanol/ water (1:1 v/v)(3 ml) and methanol/ water (3:2 v/v) (3 ml) after adjusting the flow rate to about 1-2 ml min⁻¹ manually using rubber sucking bulb. The fractions were collected in test tubes placed in an ice bath and their absorbance against eluting solvent was measured at 240 nm and 254 nm in a 1cm quartz cuvette using a UV-VIS spectrophotometer. Absorbance is the averages of triplicate determinations. Results are shown in **Table 4**.

For comparative evaluation content of allicin from aqueous garlic extract and air-dried garlic powder was also performed. The content estimation of aqueous garlic extract was performed by the same procedure as that of the above method. Garlic bulbs were crushed in a mortar and pestle. 20 mg crushed garlic was weighed and dissolved in 20 ml of distilled water and the solution was mixed properly. Then the solution was filtered through Whatman filter paper (grade 41) to get aqueous garlic extract. In another procedure the garlic bulbs were chopped into thin slices and were air-dried for the number of days the slices took to get completely dry. The dried slices were then grinded into fine powder and the sample was taken for content estimation. Content estimation of garlic powder prepared by air-drying method was performed by the same procedure as that of the above method. Comparative evaluation of allicin content of garlic by all the three methods is shown in **Table 5**.

Flow Properties of Garlic Powder: Parameters noted for observing the flow properties of the prepared garlic powder were bulk density, tapped density, angle of repose, compressibility index

(Carr's Index), Hausner's ratio¹⁷. The results of flow properties along with certain predictions for freshly prepared samples are shown in **Table 6**. The results are averages of triplicate determinations¹⁸.

RESULTS AND DISCUSSION:

Preparation and Preservation of Garlic Powder:

During the present research work it was observed that garlic powder prepared by said method could be stored for longer. Corn flour could be the alternative to talcum powder as it itself is a food component. The problem of grabbing moisture by the prepared powder over the time of storage could be avoided. The garlic powder could be handled well during further usage during formulation, development, and optimization of dosage form. The prepared powder was characterized by IR spectroscopy for its authenticity, content was estimated in terms of allicin, an active antimicrobial constituent, and prepared powder was also evaluated for flow properties. The results are discussed in further sections.

IR Spectroscopic Examination of Garlic Powder:

IR interpretation was performed taking into consideration general group frequencies and group frequencies reported for the Allicin Infrared spectrum as reported in literature^{19, 20, 21, 22, 23}. The common group frequencies for various functional groups present in allicin and its precursor alliin are as shown in **Table 1**. Thiosulfinates can be distinguished from sulfoxides by infrared spectroscopy since they have a characteristic S=O band at about 1078 cm^{-1} compared to 1030-1060 cm^{-1} in sulfoxides²⁴.

TABLE 1: FUNCTIONAL GROUPS AND GROUP FREQUENCIES WITH ALLICIN AND ITS PRECURSOR

Functional groups	Structural Representation	Group Frequencies cm^{-1}
Alkene	C=C	1680-1620 cm^{-1}
Terminal Vinyl	-CH	3095-3075 cm^{-1}
Alkane	-CH ₂	1485-1445 cm^{-1} , 1465 cm^{-1}
Thiol group	CH ₂ -S	710-685 cm^{-1}
Disulfides	C-S	705-570
Disulfides	S-S	620-600
Thiosulfinates	S=O	1078 cm^{-1}
Primary Amine	-NH	3400-3380 cm^{-1}
Carboxylic Group	-COOH	3000 - 2500 cm^{-1}
Sulfoxide	S=O	1050 cm^{-1}

Another study reports a band in the IR spectrum originating from S=O valence vibrations at 1087

cm^{-1} (indicative of allicin)²⁵. The IR spectra for garlic powder prepared by updated method are shown in **Fig. 1 & 2**, and respective peak information is provided in **Table 2 and 3** for, with and without additive batches respectively.

In the present updated method of preparation of garlic powder, the functional groups and group frequencies with allicin and its precursor **Table 1** were comparatively observed in batches with and without additives. The IR spectrum of the garlic powder prepared in the present study indicates that there is no appreciable change in the positions of the characteristic bands of the functional groups present in allicin structure. Group frequencies for alkenes were observed at 1635.64 cm^{-1} , terminal vinyl group at 3043.65 cm^{-1} , alkane group at 1456.26 cm^{-1} , thiol group at 650.01 cm^{-1} , thiosulfinates at 1060.85 cm^{-1} . Since there is no change in the nature and position of the bands in the prepared garlic powder by updated method, it can be concluded that the drug maintains its identity without going any chemical interaction with the additives used for improving its physical stability.

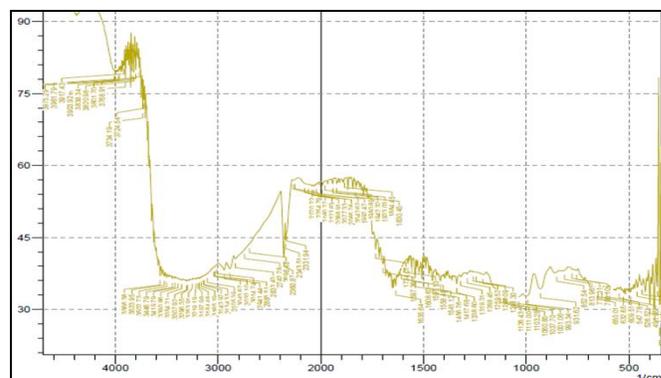


FIG. 1: GARLIC POWDER PREPARED BY CITRIC ACID AND GLUCOSE 5% SOLUTION EACH WITH CORN FLOUR USED FOR GRINDING

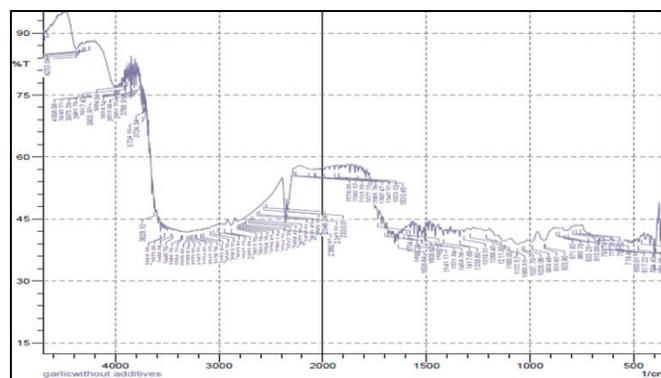


FIG. 2: GARLIC POWDER WITHOUT ADDITIVE

TABLE 2: GARLIC POWDER PREPARED BY CITRIC ACID PLUS GLUCOSE 5% SOLUTION WITH CORNFLOUR USED FOR GRINDING

Functional groups	Structural Representation	Group Frequencies Reported	Observed cm^{-1}
Alkene	C=C	1680-1620 cm^{-1}	1635.64
Terminal Vinyl	-CH	3095-3075 cm^{-1}	3043.65
Alkane	-CH ₂	1485-1445 cm^{-1}	1456.26
Thiol group	CH ₂ -S	710-685 cm^{-1}	650.01
Disulfides	S-S	620-600	609.51
Thiosulfinates	S=O	1078 cm^{-1}	1060.85

TABLE 3: OBSERVED GROUP FREQUENCIES FOR GARLIC POWDER WITHOUT ADDITIVE

Functional groups	Structural Representation	Group Frequencies Reported	Observed cm^{-1}
Alkene	C=C	1680-1620 cm^{-1}	1635.64
Terminal Vinyl	-CH	3095-3075 cm^{-1}	3082.25
Alkane	-CH ₂	1485-1445 cm^{-1}	1456.26
Thiol group	CH ₂ -S	710-685 cm^{-1}	719.45
Disulfides	S-S	620-600	617.22
Thiosulfinates	S=O	1078 cm^{-1}	1060

There are no stretching frequencies observed for primary amine and carboxylic group which are the functional groups present in alliin precursor allin, indicates the completion of the reaction.

Estimation of Allicin Content in Garlic Powder Prepared by Updated Method: UV readings were taken at 240 nm and 254 nm. Absorbance readings are as follows as per given in **Table 4**. The ratio was obtained by dividing absorbance at 240nm by absorbance at 254 nm. Ratio that falls within range 1.4-1.5 was selected. That is given specifically for alliin. Hence, the respective values were

considered for content estimation. The concentration of alliin for SPE chromatography elutes that gave an absorbance ratio ($A_{240 \text{ nm}} / A_{254 \text{ nm}}$) of 1.4-1.5 was calculated. The extinction coefficient for alliin in water is 145.4 (E 1 cm cell @ 240 nm). The concentration found was 253.37 ($\mu\text{g/ml}$) *i.e.* 2533.7 micrograms per gram of garlic powder. Absorbance readings are averages of triplicate determinations. Thus, concentration was depicted by equation below.

$$C (\mu\text{g/ml}) = ((\text{Absorbance at 240 nm}) (10000)) / E1 \text{ cm}$$

TABLE 4: CONTENT ESTIMATION OF UPDATED MICROWAVE DRIED GARLIC POWDER

Sample	Absorbance (Mean \pm S.D*)		Ratios 240/254
	At 240 nm	At 254 nm	
Water	3.684033 \pm 0.51215	2.516967 \pm 0.32495	1.4636795
Ethanol	6.799233 \pm 0.156418	6.782467 \pm 0.159162	1.002471962
Methanol	0.646533 \pm 0.004957	0.566733 \pm 0.002359	1.140807047
Methanol: Water (2:3) v/v	0.124433 \pm 0.004704	0.064267 \pm 0.00195	1.93618809
Methanol: Water (1:1) v/v	0.222343 \pm 0.010888	0.131233 \pm 0.006813	1.694261352
Methanol: Water (3:2) v/v	0.079533 \pm 0.000208	0.0511 \pm 0.000819	1.556418787

*S.D- Standard deviation.

TABLE 5: COMPARATIVE ESTIMATION OF ALLICIN CONTENTS

Garlic Sample	Alliin Content ($\mu\text{g/gm}$)
Microwave-assisted garlic powder	2533.7
Aqueous garlic extract	55.2132
Freshly prepared garlic powder (Air-dried)	139.5115

Comparative estimation of alliin content by updated method, aqueous garlic extract, and air-dried garlic powder are shown in **Table 5**. The content found in updated microwave-assisted garlic powder with the aid of silica bids is more as

compared to aqueous garlic extract. Use of citric acid and sugar solutions are found to help in complete conversion of alliin precursors into alliin. Moreover control of temperature conditions also has found to help in the stability of alliin.

From these results it has been concluded that the degradation of alliin in aqueous garlic extract occurs more rapidly than the microwave-assisted garlic powder prepared by maintaining fermentation and temperature conditions. Alliin degrades earlier in aqueous garlic extract due more

water content. It can be said that garlic powder prepared by said methodology is more suitable for preparation of various dosage forms than aqueous garlic extract or air-dried garlic powder provided presence of aqueous environment is avoided. The present research indicates that the methodology aids in the stability of Allicin.

Flow Properties of Garlic Powder: The garlic powder samples flow characteristics were found to be free-flowing throughout the study indicating the physical stability of the sample prepared and stored by the said method in the present research work.

TABLE 6: FLOW PROPERTIES OF GARLIC POWDER UPDATED METHOD

Formulations	Bulk Density (g/ml)	Tapped Density (g/ml)	Angle of Repose ($^{\circ}$)	Compressibility Index (%)	Hausner's Ratio
F1	0.58±0.01*	0.98±0.009	25.91±0.71	40.53±2.11	1.68±0.05
F2	0.63±0.02	0.83±0.00	25.94±1.07	23.33±2.88	1.30±0.04
F3	0.69±0.02	0.90±0.00	27.61±0.68	23.17±3.02	1.30±0.05

*All values are-Mean ± Standard deviation.

Highlights of Present Method: On the basis of IR interpretation study, content estimation and yield of allicin and flow properties evaluation it was observed that the garlic powder prepared by taking certain precaution and method updates, we reach to the conclusion that said method of preparation of garlic powder is advantageous as compared to previously reported methods. Garlic slices cut should be very thin as it is believed that alliin (Allicin precursor) and alliinase (Enzyme responsible for reaction of allin to allicin) are kept in separate compartments of the cells and can only combine once these compartments have been ruptured. Trituration to rupture the cell packets is necessary. Acidic and fermentation environment needs to be maintained.

The mixture should be allowed to react for a while to proceed for completion of reaction before taking the mixture for microwave drying cycles. The temperature should be maintained between 25 to 30-degree centigrades to speed up the fermentation process. Use of silica bids speeds up the drying process. Use of slight amount of additives like cornflour or talcum powder aids up the grinding process after microwave drying process which otherwise may turn to damp wet mass during grinding process.

Use of silica bid layer and additive help to improve the flow properties and physical stability of the

Results for freshly prepared garlic powder by updated methodology are shown in **Table 6**. Flow properties of the garlic powder prepared by updated method using cornflour as additives and silica bids in the microwave drying process were found to be satisfactory to improve physical stability of the powder sample. The angle of repose was found to be good for all the three batches. Compressibility index of the updated method was obtained in poor to fairly acceptable range which could be modified during dosage form design and optimization with suitable excipients.

prepared garlic powder and also help to improve the storage life of the sample which may turn into agglomerates over the time otherwise. The garlic powder prepared by updated microwave-assisted method is satisfactory method to improve physical stability and yield of allicin content.

CONCLUSION: Garlic powder prepared with updated microwave drying method is found to be physically stable free-flowing with increased allicin content. With this we can conclude that the king of healing herbs could have great potentials for antimicrobial formulations, development, and optimization and as food additive preserved by the said method can last longer for utilizations in the culinary art.

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CONFLICT OF INTEREST: Nil

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