

A PHYTOCHEMICAL STUDY OF *Allium sativum* – W.S.R TO ALLIIN CONTENT

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ABSTRACT

Allium sativum has attracted the interest of many researchers due to its wide range of therapeutic effects with minimal adverse reactions. Its role in promoting the female reproductive health can be well understood from the fact Acharya Kashyap in his text has described a full chapter *Lashuna Kalpadhyaya* mentioning that the woman consuming *Lashuna* will not suffer from diseases of *kati*, *shroni* (*pelvis*), *gramyadharma janya rogas* (sexually transmitted diseases) and infertility. Its effects are mainly attributed to its chemical constituents like Allicin, Ajoene and certain other sulphur compounds etc. In the present study, bulbs of *Allium sativum* were dried and in controlled temperature and fine powder was made. It was filled in

capsules and clinical trial was done in the patients of Hypomenorrhoea. In context of this, a phytochemical study of dried powder of *Allium sativum* was done and various chemical constituents of garlic have been investigated to support its pharmaco-therapeutic actions as per clinical study. As Alliin is the main alkaloid present in garlic, special attention was given to it. As the concentration of Alliin varies according to methods of processing, its loss on drying was assessed by the phyto-chemical analysis.

KEYWORDS: *Lashuna* (*Allium sativum*), Garlic, Alliin, Thio-2-propene-1-sulfinic acid S-allyl ester, Allicin, Ajoene, phytochemical analysis, Yamuna Safed (G-1).

1. INTRODUCTION

Allium sativum i.e *Lashuna* is a bulbous herb which has been used in India as a food item, spice and medicine since centuries. Garlic is one of the earliest cultivated plants, which is well mentioned in the Bible and in the literature of Ancient Israel (The Talmud), Egypt (Codex Ebers) and India (Vedas and Purans, Charak Samhita). Chinese people strongly believe that Garlic prolongs longevity.^[1]

The role of garlic in prevention of cardiovascular diseases, dyslipidemia, cancer etc. is well known. Also its insecticidal, nematicidal, rodenticidal and antifungal activity has been acclaimed by many researchers. *Acharya Charaka* has asserted that each and every substance present on our earth is useful in combating illness when applied with proper planning for a specific purpose (Shastri Kashinath and Chaturvedi Gorakhnath, 1989).

CHEMISTRY

The main chemical constituent of intact garlic is the amino acid Alliin, an alkyl derivative of cysteine alkyl sulfoxide, which may varies from 0.2 to 2.0% fresh weight.^[2] On an average, garlic cloves contain ~0.8% Alliin. A pure dehydration process, with no loss of ingredients, would result in a 2–2.5 mg/g Alliin content in the powder. However, garlic powders contain only 1% Alliin at most, indicating that more than half of the Alliin is lost during dehydration. The efficacy of its chemical constituents chiefly depends on the processing involved in preparation of its various forms like powder, extract and oils etc. Crushing, chewing or cutting (or exposing dehydrated, pulverized garlic to water) of garlic cloves release the vacuolar enzyme Allinase that rapidly lyses the cytosolic cysteine sulfoxide^[3] to form sulfenic acid (R-SOH)^[4] which immediately condenses to form Allicin: the compound which produces the odor of freshly cut garlic. Garlic contains at least 100 sulfur-containing compounds basic to medicinal uses.^[5] Allicin represents 70-80 % of the total thiosulfinates. Other thiosulfinates formed include allyl-ss(o)-methyl (6-16% of total), methyl-ss(o)- allyl (3-9% of total), trans-1-propenyl (0.2-0.4% of total), trans-1-propenyl-ss (o)-methyl+methyl-ss(o)- trans-1-propenyl (0.1-2.5% of total) and methyl-ss(o)methyl (2% of total) thiosulfinates.^[6]

The thiosulfinates released from crushed garlic are reactive molecules and undergo a number of transformations, depending on the temperature, pH and solvent conditions. Allyl-s-thiosulfinates (Allicin) are the least stable of the eight thiosulfinates.^[4] The principal

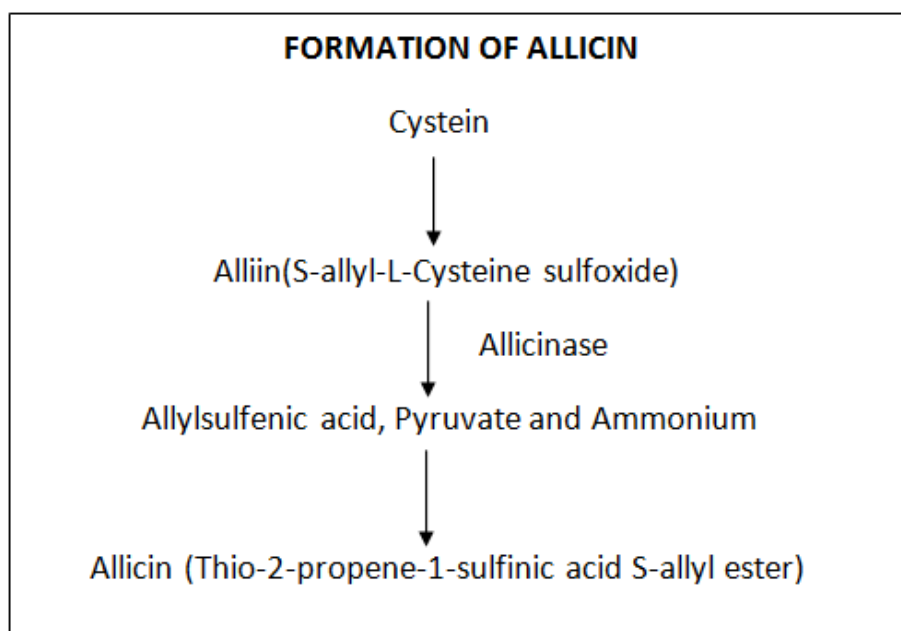
transformation products after incubation of thiosulfinate in water are diallyl trisulfide, diallyl disulfide and allyl methyl trisulfide.^[4]

ALLIIN AND ALLICIN

The health benefits related to garlic have been attributed to sulphur compounds particularly to thiosulfates and among these substances, Allicin (diallylthiosulfinate) is the most abundant which is found in fresh garlic (approximately 0.4% by fresh mass).^[7]

Allicin is a product of enzymatic reaction when raw garlic is crushed or chopped. The enzyme, Allinase, stored in a separate compartment in garlic, combines with a compound called Alliin and produces Allicin. However, the preservation of Allicin is difficult as it is very unstable and reactive in nature. Once it is generated, it readily changes into other compounds such as allylsulfides, vinylidithins, and ajoenes.^[8]

Crushed raw garlic is high in Allicin, containing ~3.7 mg/g). Although Allicin is often emphasized in dehydrated powder, many preparations contained no Allicin, possibly reflecting its instability.



STANDARD PARAMETERS AS PER AYURVEDIC STANDARDS

As per Ayurvedic Pharmacopoeia of India Part-1, Volume -3, the following standards should be followed to ascertain the purity and efficacy of *Allium sativum*.

IDENTITY, PURITY AND STRENGTH

Foreign matter	Not more than 2 Per cent
Total ash	Not more than 4 Per cent
Acid-insoluble ash	Not more than 1 Per cent
Alcohol-soluble extracts	Not less than 2.5 Per cent
Water-soluble extracts	Not less than 60 Per cent
Volatile Oil	Not less than 0.1 Per cent

THIN LAYER CHROMATOGRAPHY (T.L.C.)

T.L.C. of the alcoholic extract on Silica gel 'G' plate using n-Butanol : Isopropanol Acetic acid: Water (3 : 1: 1 : 1) shows under UV (366 nm) two fluorescent zones at Rf. 0.58 and 0.72 (both light Blue). On exposure to Iodine vapour nine spots appear at Rf. 0.18, 0.26, 0.34, 0.38, 0.46, 0.58, 0.72, 0.77 and 0.93 (all Yellow). On spraying with Ninhydrin reagent and heating the plate for ten minutes at 110°C seven spots appear at Rf. 0.26, 0.38, 0.46, 0.58, 0.67, 0.72 and 0.93 (all Pink). On spraying with Vanillin-Sulphuric acid reagent and heating the plate for ten minutes at 110°C seven spots appear at Rf. 0.26, 0.38, 0.46, 0.58, 0.67, 0.72 and 0.93 (all Grey).

CHEMICAL CONSTITUENTS

Volatile Oil contains Allyl Disulphide and Diallyl Disulphide. It also contains Allin, Allicin, Mucilage and Albumin.

PERCENTAGE OF ALLIN AND ALLICIN CONTENT

There are many research works related to the yield. On an average, it is standardized to 1.3 percent Alliin or 0.6 percent Allicin yield.^[9]

According to another study- the main chemical constituent of intact garlic is the amino acid Alliin, an alkyl derivative of cysteine alkyl sulfoxide, which may varies from 0.2 to 2.0% fresh weight.^[10]

The most abundant sulfur compound in garlic is Alliin (S-allylcysteine sulfoxide), which is present at 10 mg/g in fresh garlic or 30 mg/g dry.^[11]

Garlic contains not less than 0.2% of Alliin (the main constituent present in the garlic), calculated on the dried basis.^[12]

STABILITY OF ALLICIN AND ALLICINASE

Different findings are available related to stability of Allicin and Allcinase in various researches. The important ones are summarized as following-

- The main thiosulfanate Allicin has a half-life of up to 16 hours at room temperature or two and a half days when kept as a juice or crushed form.^[13]
- Allicin is completely formed in 0.3 min at 310 K and, at room temperature, its half-life ranges from 10 days to few hours depending in the solvent in which it is dissolved.^[14]
- The half-life of Allicin (concentration of 0.1-0.4mg/ml) at room temperature is 10 days in 1mM citric acid (pH 3), 4 days in water, 48 h in methanol or chloroform, 24 h in ethanol, 24 h in hexane and 3 h in ether.^[15]
- The Allinase enzyme responsible for Thiosulfanate conversion becomes inactivated below a pH of 3.5 or with heating.^[13]

PROPERTIES AND ACTION

RASA	:	Madhura, Katu
GUNA	:	Guru, Picchila, Sara, Snigdha, Tikshana
VIRYA	:	Ushna
VIPAKA	:	Katu
KARMA	:	Balya, Cakshushya, Deepana, Hridya, Kaphahara, Medhya, Raktadosha hara, Vata hara, Vrishya, Varnya, Pitta dushanakara, Bhagnasandhanakara, Rasayana, Jantughna, Kanthya and Asthi Mansa Sandhanakara

2. MATERIALS AND METHODS

2.1 Materials

Fresh garlic bulbs were purchased for the purpose of this study from the local market near, Gopabandhu Ayurveda mahavidyalaya, Puri, Orisa, India. These were later identified as Yamuna Safed (G-1) variety.

2.2 Processing

Convective hot air-drying is a common process used for garlic dehydration. The same was used for preparing the Trial drug in this study.

Raw Garlic cloves contain approximately 59% water. Total 8 kg of Garlic was processed in two shifts 5kg and 3kg each. The raw bulbs were manually peeled off and uncrushed garlic

cloves hence obtained were weighed and put in stainless steel tray and dried in an oven. The temperature of the oven was set at a low at 40⁰C. The process was continued for a period of 72 hrs with 8 hours at 40⁰C and the rest at room temperature. After complete drying, the garlic cloves were ground and strained through a fine mesh sieve number 60 to obtain the fine powder. Total powder obtained after drying was 3.850 kg. This might be due to 10% loss during the process of drying and pulverizing. After processing, the garlic powder obtained was filled in capsules and stored in containers at room temperature.



2.3 Determination of Alliin

Alliin concentration was determined in the prepared Garlic powder .Analysis was done at Satiata Research and Anatech Pvt. Ltd. (Approved by Food and Drug Administration, Haryana) GLP certified testing laboratory and Government Ayurvedic State Drug Testing Laboratory, Kurukshetra, Haryana, India.

3. OBSERVATION: The Findings were as following-

SR. NO	PROTOCOL	OBSERVATION
1.	Texture	Brown capsule containing Yellow powder
2.	Colour	Yellowish powder in side capsule
3.	Odour	Strong garlic odour
4.	Taste	Spicy
5.	pH	6.2
6.	Water soluble extract	70.06%
7.	Moisture content	11.18%
8.	Acid soluble ash	0.49%
9.	Total phenolic content	0.71
10.	Total ash	5.44%
11.	Physical Microscopy and Staining test	No viable organism, animal tissue or insect body part is detected in the sample

Alliin Content in prepared Garlic powder-

Alliin Content	3.37mg/gram																																																																																								
 <p>GOVERNMENT AYURVEDIC STATE DRUG TESTING LABORATORY (In the Campus of Sh. Krishna Govt Ayurvedic college, Umari Road , Kurukshetra) E-MAIL:- sdtharyana@gmail.com</p> <p>Certificate of Analysis</p> <p>Drug License No.: 5-ISM (HR) DTL Certificate No.: Allium Sativum/17042017/001</p> <p>FORM 13A [See rule 163 (5)]</p> <p>Certificates of tests or analysis by Government Analyst/ Person in-charge of testing under section 33H of the Drugs and Cosmetics Act, 1940</p> <ol style="list-style-type: none"> Names, from whom received : Dr. ANJANA TAK Ph.D Scholar, Gam puri, Utkal University, Orissa Serial No and date of memorandum no. : —NA— Name of sample : Allium Sativum (Lashuna) Dried Powder in Cap. Date of receipt : 17/04/2017 Names of ingredients purporting to have been used in the preparation of the sample : NA Condition of seals on the package : Intected Results of test or analysis <table border="1"> <thead> <tr> <th>Sr.no.</th> <th>Protocol</th> <th>Observation</th> <th>Specification / Remarks</th> </tr> </thead> <tbody> <tr> <td>1.</td> <td>Uniformity of Weight</td> <td>500 mg</td> <td>500 mg average</td> </tr> <tr> <td>2.</td> <td>Texture</td> <td>Brown capsule containing Yellow powder</td> <td></td> </tr> <tr> <td>3.</td> <td>Colour</td> <td>Yellowish powder in side cap.</td> <td></td> </tr> <tr> <td>4.</td> <td>Odour</td> <td>Strong Garlic odour</td> <td></td> </tr> <tr> <td>5.</td> <td>Taste</td> <td>Spicy</td> <td></td> </tr> <tr> <td>6.</td> <td>pH</td> <td>6.2</td> <td></td> </tr> <tr> <td>7.</td> <td>Water Soluble Extract</td> <td>70.06 %</td> <td></td> </tr> <tr> <td>8.</td> <td>Moisture Content</td> <td>11.18%</td> <td></td> </tr> <tr> <td>9.</td> <td>Acid Insoluble Ash</td> <td>0.49 %</td> <td></td> </tr> <tr> <td>10.</td> <td>Total Phenolic Content</td> <td>0.71</td> <td></td> </tr> <tr> <td>11.</td> <td>Total Ash</td> <td>5.44 %</td> <td></td> </tr> <tr> <td>12.</td> <td>Physical Microscopy and Staining Test</td> <td>No, viable organism, Animal tissue Or insect body part is detected in the sample.</td> <td>Absent</td> </tr> </tbody> </table> <p>Results of test or analysis with protocols of test or analysis applied as per Ayurvedic, Siddha, Unani Pharmacopoeial in house developed standards.</p> <p>In the opinion of the undersigned, the sample referred to above is of standard quality/is not of standard quality as defined in the Act or the rules made thereunder for the reasons given below</p> <p>Analyst: <i>Harish</i> Date: <i>17/04/2017</i> Analyst: <i>R. Karan</i></p> <p>Signature of the Person in-charge of testing In-Charge Govt. Ayurvedic State Drug Testing Laboratory Sh. Krishna Govt. Ayurvedic College & Hospital Kurukshetra, Haryana</p>	Sr.no.	Protocol	Observation	Specification / Remarks	1.	Uniformity of Weight	500 mg	500 mg average	2.	Texture	Brown capsule containing Yellow powder		3.	Colour	Yellowish powder in side cap.		4.	Odour	Strong Garlic odour		5.	Taste	Spicy		6.	pH	6.2		7.	Water Soluble Extract	70.06 %		8.	Moisture Content	11.18%		9.	Acid Insoluble Ash	0.49 %		10.	Total Phenolic Content	0.71		11.	Total Ash	5.44 %		12.	Physical Microscopy and Staining Test	No, viable organism, Animal tissue Or insect body part is detected in the sample.	Absent	 <p>Satiata Research & Anatech Pvt. Ltd. (Approved by Food and Drugs Administration, Haryana)</p> <p>(GLP Certified Testing Laboratory)</p> <p>CERTIFICATE OF ANALYSIS</p> <p>Lic. 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Total liability of our institute is limited to the invoiced amount. This report is not to be reproduced wholly or in part and cannot be used as evidence in the court of law and should not be used in any advertising media without our special permission in writing. Sample drawn and submitted by the party for Analysis unless otherwise stated. <p>Plot No. 264, 1st & 2nd Floor, HSIIDC, Barwala - 134118, Panchkula, Haryana. Ph. 01733-256 570, E-mail: satiataresearch@gmail.com, info@sralabs.com, website: www.sralabs.com</p>	Name of Sample	: Allium Sativum (Lashuna) Dried powder capsules	A.R. No.	: SA/FP-170417/052	Batch No.	: N.M.	Batch size	: N.M.	D/M	: N.M.	D/E	: N.M.	Original Manufacturer's	: N.M.	Sample submitted by	: Dr. Anjana Tak, Ph. D. Scholar, Gam Puri, Utkal University, Orissa	Mfg. Lic. No.	: N.M.	Ref. No. (As in test req. Slip)	: N.M.	Sample Qty	: 20 Capsules	Ref. No. (As given by party)	: N.M.	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4. DISCUSSION

EFFECT OF HEAT AND DRYING ON ALLICIN CONTENT

The efficacy of garlic and garlic products is supposed to be related to the Allicin content. Many studies have been conducted to study the Allicin content of garlic samples dried by hot air-drying and freeze-drying were compared with the Allicin content of fresh garlic. In a study conducted by C. Ratti et al, 2007^[16], it was noticed that the average Allicin content in fresh garlic was found to be 0.3% of the wet garlic approximately. Allicin values after 40 and 50 °C hot air-drying were close to those for fresh garlic. Moderate air temperatures (40 and 50 °C) allowed a better Allicin retention than a higher temperature (60 °C). It is clear from this data that the Allicin content decreases with an increase of hot air-drying temperature.

Freeze-dried whole cloves at 20 °C presented a higher Allicin content (about 14%) than fresh garlic. This may be due to the differences in the sample preparation for HPLC analysis between fresh and dried garlic samples. However, the fact that whole cloves freeze-dried at 20 °C showed the highest Allicin content when compared to other treatments may be due not only to the low temperatures during freeze-drying but also due to the fact that no cutting of the sample was done.

REASONS FOR ALLIIN CONTENT FOUND RATHER THAN ALLICIN

Allicin is a highly unstable compound. In several studies it is noticed that by the heat exposure in hot air-drying, Allinase gets partly inactive or denatured and the remaining capacity to produce Allicin is therefore reduced. Statistics confirmed that Allicin content of garlic slices was significantly affected ($p < 0.05$) by hot air-drying temperature.^[17]

Though there are certain other methods of processing in which Allicin content can be retained, like Freeze Drying Method. Freeze-dried whole cloves at 20°C presented a higher Allicin content (about 14%) than fresh garlic.^[18]

In the present study Garlic powder was prepared by peeled raw garlic cloves and without crushing drying them in shade. This does not allowed Allinase to transform all the Alliin present in it to Allicin and other Allyl thiosulfinates.

INTERPRETATION OF OBSERVATION

- As in this present study Alliin content was 3.37mg/gram about 0.33%, it seems to be less than the expected value. But the trial drug fulfills the minimum criteria of efficacy though, as it should not contain Alliin less than 0.2%. 123-a The lesser content might be because of the dehydration technique or the time gap between drug manufacturing and analysis.
- Due importance is given to Allicin and it is held responsible for significant biological effects on cardiovascular, immune and hepato-biliary system; but the findings in this study indicates that it is not necessary to retain Allicin for its favorable effects. Although Allicin is considered the major antioxidant and scavenging compound, studies are showing that other compounds may play stronger roles.^[131]
- So, it can be inferred that some other compounds beside Alliin and Allicin like Sulphur & non sulphur compounds, Saponins, Protein fractions etc might be actually responsible for its health benefits. More researches are needed from different perspectives regarding the biological activities and action mechanisms, along with the bioavailability analysis.

5. CONCLUSION

Though due importance is given to Allicin and it is held responsible for significant biological effects on cardiovascular, immune and hepato-biliary system; but the findings in this study indicates that it is not necessary to retain Allicin for its favorable effects. So, it can be inferred that some other compounds (Sulphur, non sulphur compounds, Saponins, Protein

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REFERENCES

1. Srivastava KC, Bordia A, Verma SK. Garlic (*Allium sativum*) for disease prevention. South Afr J Sci, 1995; 91: 68-77.
2. Lutomski J. Components and biological properties of some *Allium* species. Poznan: Institute of the Medicinal Plants, 1987; p.1-58.
3. Stoll A, Seebeck E. Über den enzymatischen Abbau des alliins und die Eigenschaften der Allinase. Helv Chim Acta, 1949a; 32: 197-205.
4. Block E. The organosulfur chemistry of the genus *Allium* -Implication for the organic chemistry of sulfur. Angew Chem Int Ed Engl, 1992; 31: 1135-78.
5. Cai XJ, Uden PC, Block E, Zhang X, Quimby BD, Sullivan JJ. *Allium* chemistry: identification of natural abundance organoselenium volatiles from garlic, elephant garlic, onion and Chinese chive using headspace gas chromatography with atomic emission detection. J Agric Food Chem, 1994; 42: 2081-4.
6. Lawson LD, Wang ZJ, Hughes BG. Identification and HPLC quantitation of the sulfides and dialk(en)yl thiosulfinates in commercial garlic products. Planta Med, 1991b; 57: 363-70.
7. Iberl, B.; Winkler, G.; Müller, B.; Knobloch, K. Quantitative determination of allicin and alliin from garlic by HPLC. Planta Medica, 1990; 56: 320-326.
8. Iberl, B.; Winkler, G.; Müller, B.; Knobloch, K. 1990. Products of allicin transformations: Ajoenes and dithiins, characterization and their determination by HPLC. Planta Medica, 1990; 56: 202-211.
9. Journal of Complementary and Integrative Medicine, Potential Health Benefits of Garlic (*Allium Sativum*): A Narrative Review Peter B. Bongiorno, Patrick M. Fratellone, and Pina LoGiudice, 2008; 5(1):
10. Lutomski et al., 1968; Kabelik, 1970; Michahelles, 1974; Lutomski, 1983a, b; 1987.
11. Lawson, 1998 L.D. Garlic: a review of its medicinal effects and indicated active compounds. In: L.S. Lawson and R. Bauer, Editors, Phytomedicines of Europe: Chemistry and Biological Activity, ACS Symposium Series 691, American Chemical Society, Washington, D.C, 1998; pp. 176-209.

12. Vineet Singla*, Jai Deep Bajaj, RadhikaBhaskar, Bimlesh Kumar* “Garlic: A ConciseDrug Review with Probable Clinical Uses” Int. J.Drug Dev. & Res., October-December 2012; 4(4): 9-17.
13. Pedraza-Chaverri J, Medina-Campos ON, Segoviano-Murillo S. Effect of heating on peroxynitrite scavenging capacity of garlic. Food and Chemical Toxicology, In Press, Corrected Proof, Available online 24 October 2006.
14. H. Fujisawa, K. Suma, K. Origuchi, H. Kumagai, T. Seki, T. Ariga, Biological and Chemical Stability of Garlic-Derived Allicin, Journal of Agricultural and Food Chemistry, 2008; 56(11): 4229-4235.
15. Lawson LD. Bioactive organosulfur compound of garlic and garlic products: role in reducing blood lipids. In: Kinghorn AD, Balandrin MF, editors. Human medicinal agents from plants. Washington: American Chemical Society, 1993; p.306-30. (ACS Symposium Series, n. 534).
16. C. Ratti, M. Araya-Farias, L. Mendez-Lagunas & J. Makhoulf (2007) Drying of Garlic (*Allium sativum*) and Its Effect on Allicin Retention, Drying Technology, 25: 2, 349-356, DOI: 10.1080/07373930601120100.
17. Pezzutti, A.; Crapiste, G.H. Sorptional equilibrium and drying characteristics of garlic. Journal of Food Engineering, 1997; 31: 113–123.
18. Ratti, C. Hot-air and freeze-drying of high-value foods: A review. Journal of Food Engineering, 2001; 49: 311 – 319. [Crossref], [Web of Science ®]