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Preformulation Studies on *LACTUCA SATIVA* as Nutraceuticals Granules

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Graphical abstract

Abstract

The utilization of natural products for their pharmaceutical and nutraceuticals purposes have high value in prevention and treatment of myriad illness. In pharmaceutical industry the formulation of natural products into an effective and stable dosage form necessitate evaluation of the processing techniques required and the properties of the product obtained. Therefore the objective of the present study was to prepare and evaluate lettuce leaves *Lactuca sativa* granules in a view to develop solid dosage forms. Lettuce leaves were washed with water, one batch was dried in open air and the other one in oven and grinded into fine powder. Dried powdered lettuce leaves were granulated without and with polyvinylpyrrolidone (PVP) at various water concentrations. The prepared granules were evaluated for its particle size distribution, bulk density, tapped density, hausners index, Carr's index, friability, flowability and water absorption. The influence of water concentration, PVP addition and drying methods on the granule properties were also investigated. The results obtained showed that lettuce granules possess good flow properties and can be used as a candidate for the formulation of solid dosage forms particularly tablets.

Keywords: Preformulation; *Lactuca Sativa*, granules; nutraceuticals; flow properties

Abstrak

Penggunaan produk-produk semulajadi untuk tujuan farmaseutikal dan nutraseutikal mempunyai nilai yang tinggi dalam membasmi dan merawat pelbagai penyakit. Dalam industri farmaseutikal, formulasi produk-produk semulajadi ke dalam bentuk dos yang stabil dan berkesan memerlukan penilaian terhadap aspek pemprosesan yang diperlukan dan ciri-ciri produk yang diperolehi. Justeru, objektif kajian ini adalah untuk menyediakan dan mengkaji butiran dari daun salad (Lactuca sativa) secara keseluruhan bagi menghasilkan produk dalam bentuk dos pepejal. Daun salad dibersihkan dengan air, sebahagian dikeringkan dengan udara sekeliling dan sebahagian dikeringkan dalam ketuhar seterusnya dikisar menjadi serbuk halus. Serbuk daun salad kering dijadikan butiran dengan menggunakan polyvinylpyrrolidone (PVP) dan tanpa PVP pada kepekatan air yang pelbagai. Butiran-butiran yang telah disediakan dikaji dari segi penyebaran saiz partikel, ketumpatan pukal, ketumpatan pejal, index hausners, index Carr's, kebolehkecilan, kebolehaliran dan penyerapan air. Pengaruh kepekatan air, penambahan PVP dan cara pengeringan ke atas ciri-ciri butiran turut dikaji. Hasil yang diperolehi menunjukkan butiran daun salad menjurus kepada ciri-ciri alir dan boleh digunakan sebagai bahan untuk formulasi bentuk pepejal terutamanya kapsul.

Kata kunci: Pra-formulasi; *Lactuca Sativa;* butiran; nutraseutikal; sifat aliran

1.0 INTRODUCTION

Traditional medicine is well known science world-wide for the treatment of various diseases. Formulations composed from plant parts for curing some common illnesses are well known since a long time. Most of these formulations are processed in a way that does not take into consideration the basic principle that should be followed during formulation and processing of such traditional dosage forms. Further some of these formulations are conducted in non-hygienic conditions, which could result in serious defects in the formulation.[1-2] In recent years, consumers have begun to look at food not only for basics nutrition, but also for health benefits. Development of nutraceuticals necessitates further investigation on plants that have some therapeutic values beside its nutritional value in order to present them in a formulation that can be safely and effectively applied and used.[3]

Garden lettuce *Lactuca sativa*, belonging to the Asteraceae family, is an important leafy vegetable known for its medicinal properties. It is grown mostly as cold weather crop but can also be

grown in spring or early summers. A monthly mean temperature of 13-16 °C is considered ideal for its growth.[4] in many countries; it is typically eaten cold, raw, in salads, sandwiches, hamburgers, tacos and in many other dishes. Lettuce is a fat free, low calorie food and is good for a well balanced diet. It is a valuable source of vitamin A and folic acid.[5]

The food substances used as nutraceuticals contain antioxidants, prebiotics, probiotics, certain photochemical, and pectin, which lower cholesterol, or fiber, and reduce the risk of colon cancer too.[6-7]

The whole *Lactuca sativa* plant has been used in the treatment of stomach problems to stimulate digestion, to enhance appetite and relieve inflammation [8] via the anti-inflammatory activities of triterpene lactones.[9] *Lactuca sativa* gives protection against *D*-galactose-induced oxidative stress and reduces accumulation of lipofuscin granules.[10] *Lactuca sativa* is known to be rich in antioxidants viz. quercetin, caffeic acid, vitamin C, carotenoids, and phytols.[11-12] The major components present in *Lactuca sativa* extract are 15-oxalyl and 8-sulfate conjugates of the guaianolide sesquiterpene lactones, lactucin, deoxylactucin, and lactucopicrin.[13] The antioxidant activity of *Lactuca sativa* has been reported to prevent chronic diseases related to oxidative stress such as cancer. Antioxidant activity of lettuce leaves has been reported that would prevent chronic diseases related to oxidative stress such as cancer.[14] In addition to antioxidant, antibacterial and antiviral effect has been observed in its extract which make it easily accessible source for food supplement or in pharmaceutical industry.[15-16-17]

Lettuce leaves are found to have antioxidant, heamatinic, hypnotic stomachic, galactagogue, improve appetite, purify the blood, burning sensation, headache, trouble of nose, in scabies, leucoderma, ophthalmia, liver diseases.[18] Lactucarium ("Lettuce Opium") is a mild opiate-like substance that is contained in all types of lettuce. Sesquiterpenoid lactones were isolated from lettuce include constitutive components of latex such as lactucin, guanine-type sesquiterpene glycoside, lactusidec ($C_{21}H_{28}O_9$) along with lactuside A, macrocliniside A and lactupicrin.[19-20-21] Immune detection revealed multiple protein types in young leaves of Lettuce.[22-23]

Anticonvulsant and sedative-hypnotic effects have been mentioned for the leaves of plant, [24] the study of effect on behavioral and locomotor activity has shown no noticeable response and no lethality was observed up to the dose of 6g/kg.[25-8] Moreover, the exposure of lettuce leaves to cytotoxic, carcinogenic and mutagenic elements of Cadmium has been assessed and provided no mutagenic and genetic instability were found. [26-24-27-28]

Considering the varied beneficial activities reported in the traditional system of medicine as well as the recent reports, *Lactuca sativa* was selected plant to prepare and evaluate several Preformulation studies for pharmaceutical and nutraceutical industry as granules in a view to develop solid dosage form.

■2.0 EXPERIMENTAL

2.1 Materials

Fresh garden lettuce leaves were purchased from the local market. Polyvinylpyrrolidone (PVP) was obtained from BDH Chemical (Poole, England). Double distilled water was used throughout the study. All chemicals and reagents were of analytical grade.

2.2 Methods

2.2.1 Drying of Lettuce Leaves

Lettuce leaves were properly cleaned with water, one batch was dried in open air and the other in oven at 50 °C for 6 hours.

2.2.2 Preparation of Granules

Lettuce leave granules were prepared without PVP and with various concentrations of PVP (1.25 and 2.5% dry addition). Water (in different concentration was added gradually to the dried leaves powder with continuous wet massing. Following liquid addition the wet mass was sized into granules in oscillating granulator (Erweka, Germany) at speed 3 using 1mm screen.

The distance between screen and rotor was kept constant. The obtained granules were dried in open air for one batch and in oven at 40 °C for hours for another batch.

2.2.3 Evaluation of Granules

The prepared granules were evaluated for its particle size distribution, bulk density, tapped density, hausners index, Carr's index, friability, flowability and water absorption (2, 14).

2.2.4 Particle Size Measurement with Sieve Method

The particle size distribution of all samples was determined by sieve analysis (Ritsch, Strasse, Germany) using the sieve No. 14, 18, 25, 35, 60, 120 in a series. The samples were subjected to vibration using sieve vibrator (Ritsch, Strasse, Germany) for certain period of time.

2.2.5 Angle of Repose

The static angle of repose, a, was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel.

The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

$$\text{Tan } a = 2h/D$$

2.2.6 Granule Friability

The granule friability was determined in a friabilator (Germany), at a speed of 25 rpm for 10 min, by subjecting 10 g of granules together with 200 glass beads (mean diameter 4 mm) to falling shocks. The glass beads were then removed and the weight of granules retained was determined after vibrating for 5 min (Retsch, Germany).

The friability was calculated as $\{(I_{wt} - F_{wt})/I_{wt}\}*100$.
Where I_{wt} is initial weight and F_{wt} is final weight of the granules

2.2.7 Bulk and Tapped Densities

The bulk volume (Vo) of 50 g granules was recorded in a 100 ml measuring cylinder as well as the volume after taping till constant volume. Bulk and tapped densities in g/ml were calculated as 50/Vo and $50/V_f$, respectively.

2.2.8 Hausners Index

This was calculated as the ratio of tapped density to the bulk density of the samples.

2.2.9 Carr's Index

The compressibility index (C %) was calculated from the bulk and tapped density using the equation C % = $\{(\rho_f - \rho_i) / \rho_f\}$ *100
Where ρ_i is the bulk density and ρ_f is the tapped density.

2.2.10 Water Absorption

A sample of 5 g granules was put on the top of filter paper and placed in the funnel then 20 ml distilled water was added to the sample. The excess water was filtered and the amount of water absorbed was calculated in time interval of 5, 15, 30, 45, 60 min. The actual water absorption was calculated by subtracting the weight of the wet filter paper and the weight of the sample.

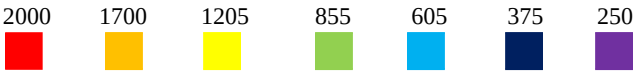
3.0 RESULTS AND DISCUSSION

The properties of air dried and oven dried lettuce leaves granules prepared without and with PVP are listed in table 1 and 2 respectively. The results obtained showed that the friability of oven dried granules ranged between 0 and 0.7% indicating good granule friability whereas much higher friability was observed for air dried samples granulated without PVP and with 1.25% PVP. However, increasing PVP concentration to 2.5% resulted in granule friability similar to those formulations proceed in oven. The angle of repose in both air dried and oven dried granules was about less than 30° for formulations prepared at lower water concentration indicating good flow properties but it started increasing as water concentration increased or when PVP was included in the formulation.

The Hausners index and Carr's index of air dried and oven dried granules was found to be 1 to 1.27 and below 15 respectively suggesting excellent flowability of the granules.

Particle size analysis of air dried and oven dried lettuce granules prepared without PVP and with PVP at various water concentrations is shown in Figure 1 to Figure 12. The results showed that as water concentration increased and no PVP involved, the air dried granules fraction between 855 to 2000 µm increased. Similar results were obtained for oven dried granules However, incorporation of PVP resulted in increased air dried as well as oven dried granules fraction between 855 and 2000 µm at much lower water concentrations.

Figure legend particle size analysis (µm) of lettuce powder granulated at different water concentrations (air drying).



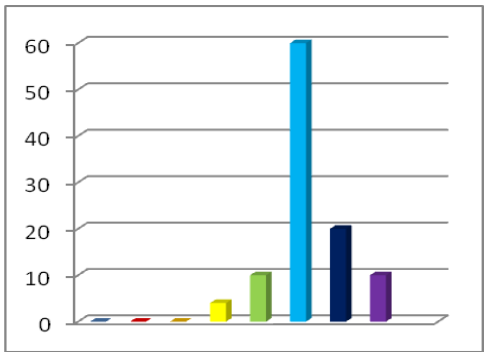


Figure 1 27% degree of repose for no PVP (air drying)

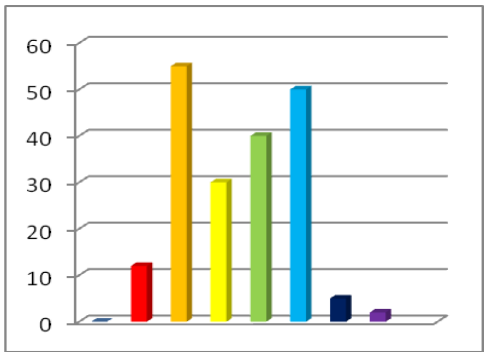


Figure 2 60% degree of repose for no PVP (air drying)

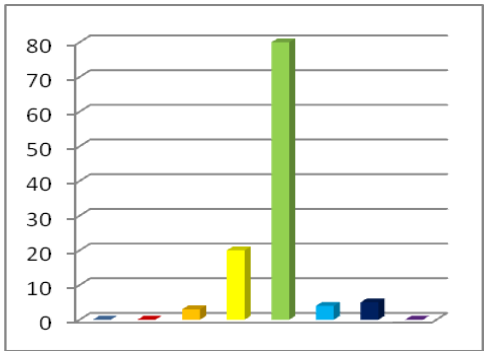


Figure 3 30% degree of repose for 1.25% PVP (air drying)

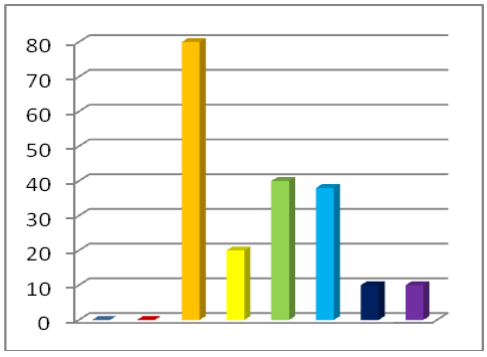


Figure 4 60% degree of repose for 1.25% PVP (air drying)

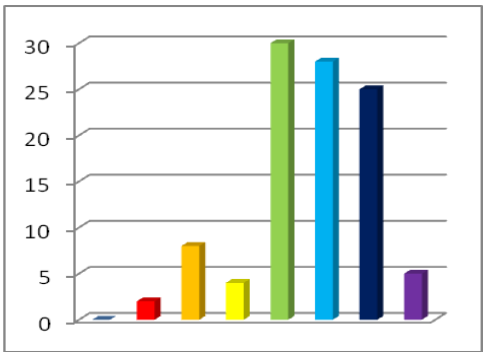


Figure 5 30% degree of repose for 2.5% PVP (air drying)

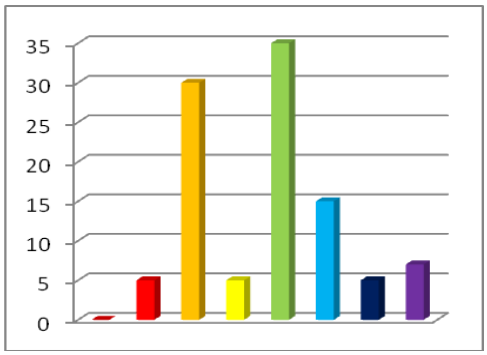


Figure 6 60% degree of repose for 2.5% PVP (air drying)

Table 1 Influence of formulation parameters on the properties of dried lettuce leaves granules (air drying)

Water (%)	PVP (%)	Bulk density (g/cc)	Tapped density (g/cc)	Hausners index	Carr's index	Friability (%)	Angle of repose (°)
27	0	0.27	0.72	2.67	62.5	6.71	27.02
50	0	0.58	0.58	1	0	27.3	26.1
55	0	0.53	0.54	1.02	1.85	47.2	27.47
60	0	0.44	0.46	1.05	4.35	29	26.57
30	1.25	0.42	0.46	1.1	8.7	41.4	26.57
40	1.25	0.3	0.33	1.1	9.09	48.2	27.47
50	1.25	0.27	0.29	1.07	6.9	50.2	29.25
60	1.25	0.36	0.36	1	0	22.8	28.37
30	2.5	0.3	0.3	1	0	0.4	25.17
40	2.5	0.28	0.31	1.11	9.68	1	25.64
50	2.5	0.29	0.3	1.03	3.33	0.2	28.81
60	2.5	0.26	0.29	1.12	10.34	0.1	33.42

Table 2 Influence of formulation parameters on the properties of dried lettuce leaves granules (Oven drying)

Water (%)	PVP (%)	Bulk density (g/cc)	Tapped density (g/cc)	Hausners index	Carr's index	Friability (%)	Angle of repose (°)
27	0	0.29	0.34	1.17	14.71	0.6	25.17
50	0	0.23	0.25	1.09	8	0.7	25.17
55	0	0.22	0.28	1.27	21.43	0.6	34.61
60	0	0.22	0.23	1.05	4.35	0	29.25
30	1.25	0.14	0.16	1.14	12.5	0	33.42
40	1.25	0.23	0.25	1.09	8	0	36.13
50	1.25	0.24	0.26	1.08	7.69	0	36.87
60	1.25	0.19	0.21	1.11	9.52	0	30.96
30	2.5	0.14	0.15	1.07	6.67	0	26.57
40	2.5	0.15	0.18	1.2	16.67	0.2	28.37
50	2.5	0.19	0.19	1	0	0	28.81
60	2.5	0.25	0.25	1	0	0	33.21

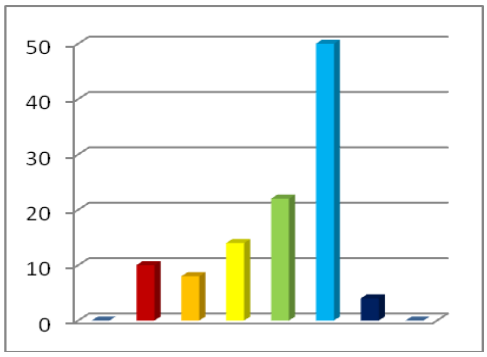


Figure 7 27% degree of repose for no PVP (oven drying)

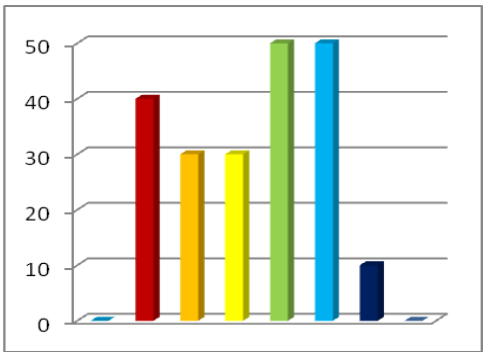


Figure 8 50% degree of repose for no PVP (oven drying)

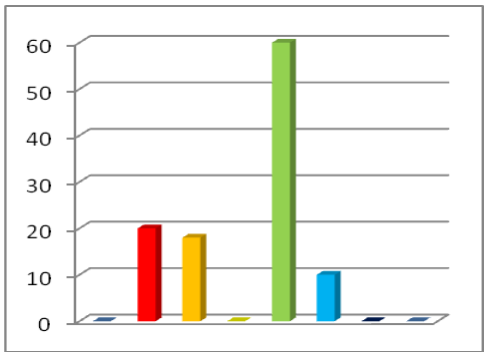


Figure 9 30% degree of repose for 1.25% PVP (oven drying)

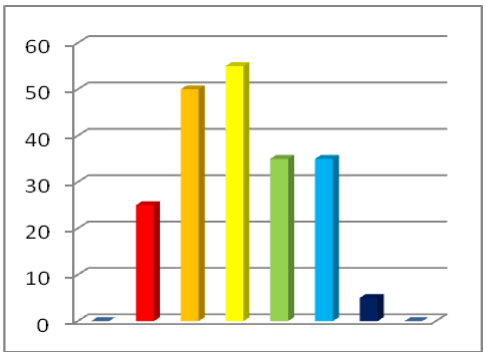


Figure 10 60% degree of repose for 1.25% PVP (oven drying)

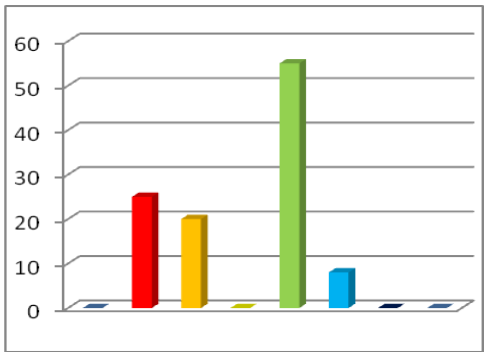


Figure 11 30% degree of repose for 2.5% PVP (oven drying)

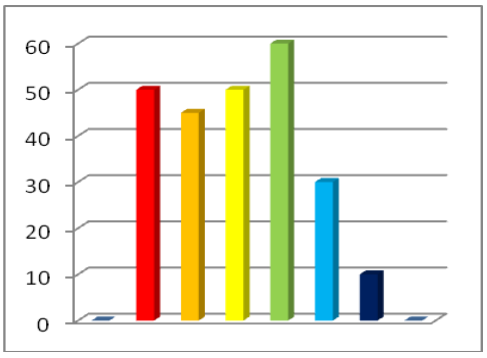


Figure 12 60% degree of repose for 2.5% PVP (oven drying)

Weight-size distributions of granules as measured by U.S Standard Sieves for different weights of granules and at different times are listed in table 3 to 10. It was found that average diameter of the granules decreased as the time of sieving increased for different weights to be sieved. However for 350 gm granules to be sieved there was no significant decrease in average diameter with time as there was with 50g, 150g, and 250g. It could be attributed to the load of granules per unit area of sieves influences the sieving.

The results obtained from water absorption experiment are shown in figure 13 to 16. The result indicated that the best water absorption was obtained for air dried and oven dried granules prepared without PVP at 55% and 60% concentration respectively and the amount absorbed was retained by the granules for almost one hour. However with addition of PVP the best water absorption was achieved at 50% (for dried samples) and 40% (for oven dried samples).

■4.0 CONCLUSION

The findings of the study have provided wide range of Preformulation basic information in order to make *Lactuca sativa* in granules solid dosage form at different conditions, with considerable low cost natural source. Moreover, it concludes that lettuce granules possess good flow properties and can be applied and used as a potential candidate for the pharmaceutical and nutraceutical formulation of solid dosage forms particularly tablets and granules.

Table 3 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 50 g; Time= 5 min). Average diameter = 65324.75/100 = 653.25 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings (µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	9.97	19.94	24027.7
18/25	855	10.1	20.2	17271
25/35	605	10.63	21.26	12862.3
35/60	375	10.47	20.94	7852.5
60/120	187.5	8.83	17.66	3311.25
		50	100	65324.75

Table 4 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 50 g; Time= 15 min). Average diameter = 63559.075/100 = 635.59 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings (µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	9.07	18.15	21870.75
18/25	855	10.49	20.98	17937.9
25/35	605	10.25	20.51	12408.55
35/60	375	10.06	20.13	7548.75
60/120	187.5	10.11	20.23	3793.125
		49.98	100	63559.075

Table 5 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 150 g; Time= 5 min). Average diameter = 65028.525/100 = 650.29 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings (µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	29.35	19.73	23774.65
18/25	855	29.91	20.11	17194.05
25/35	605	31.29	21.04	12729.2
35/60	375	31.69	21.31	7991.25
60/120	187.5	26.49	17.81	3339.375
		148.73	100	65028.525

Table 6 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 150 g; Time= 15 min). Average diameter = 63082.975/100 = 630.83 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings (µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	27.67	18.52	22316.6
18/25	855	30.64	20.51	17536.05
25/35	605	28.96	19.39	11730.95
35/60	375	29.51	19.75	7406.25
60/120	187.5	32.61	21.83	4093.125
		149.39	100	63082.975

Table 7 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 250 g; Time= 5 min). Average diameter = 64946.25/100 = 649.46 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings (µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	50.17	20.08	24196.4
18/25	855	49.85	19.94	17048.7
25/35	605	51.55	20.63	12481.15
35/60	375	51.19	20.49	7683.75
60/120	187.5	47.13	18.86	3536.25
		249.89	100	64946.25

Table 8 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 250 g; Time= 15 min). Average diameter = 63663.6/100 = 636.64 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings (µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	47.89	19.16	23087.8
18/25	855	50.88	20.36	17407.8
25/35	605	48.97	19.6	11858
35/60	375	48.58	19.44	7290
60/120	187.5	53.57	21.44	4020
		249.89	100	63663.6

Table 9 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 350 g; Time= 5 min). Average diameter = 65388/100 = 653.88 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings (µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	70.5	20.21	24353.05
18/25	855	69.47	19.91	17023.05
25/35	605	74.25	21.28	12874.4
35/60	375	72.58	20.8	7800
60/120	187.5	62.11	17.8	3337.5
		348.91	100	65388

Table 10 Weight-size distribution of granular lettuce leaves powder as measured by US standard Sieves (Weight of granules = 350 g; Time= 15 min). Average diameter = 64287.825/100 = 642.88 µm

Sieve Number (Passed/Retained)	Arithmetic Mean Size of Openings(µm)	Weight Retained on smaller sieve	% Retained on Smaller sieve	Weight size
1	2	3	4	2 X 4
14/18	1205	69.14	19.77	23822.85
18/25	855	69.71	19.93	17040.15
25/35	605	70.1	20.04	12124.2
35/60	375	69.97	20.01	7503.75
60/120	187.5	70.82	20.25	3796.875
		349.74	100	64287.825

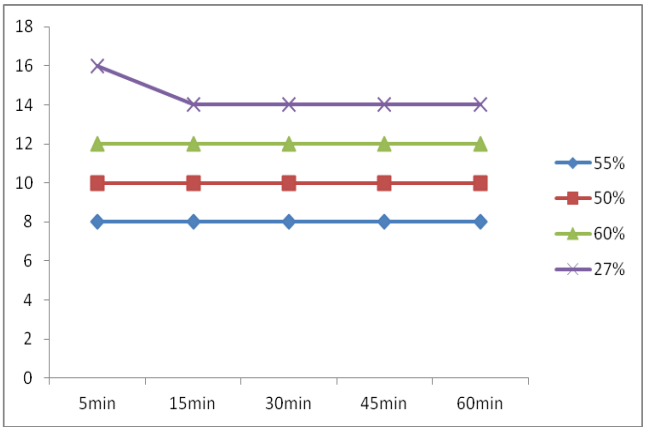


Figure 13 Influence of water concentration on water absorption of lettuce granules. No PVP (air drying)

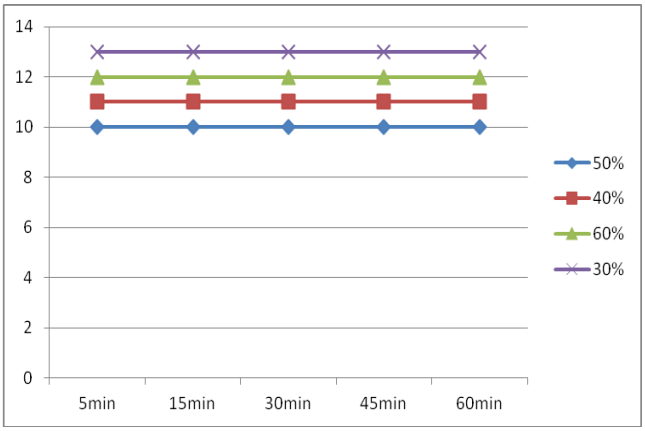


Figure 14 Influence of water concentration on water absorption of lettuce granules. With PVP (air drying)

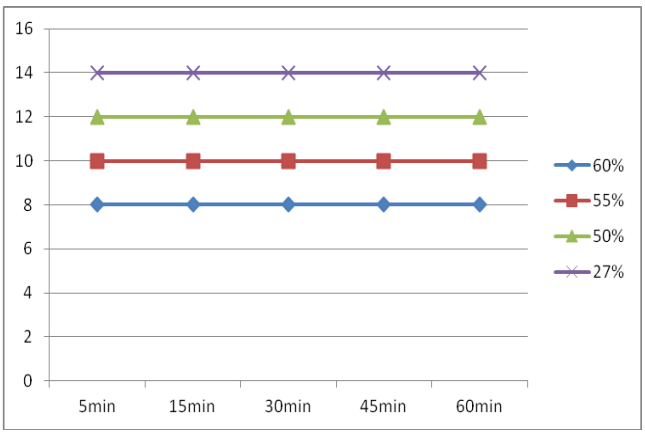


Figure 15 Influence of water concentration on water absorption of lettuce granules. No PVP (oven drying)

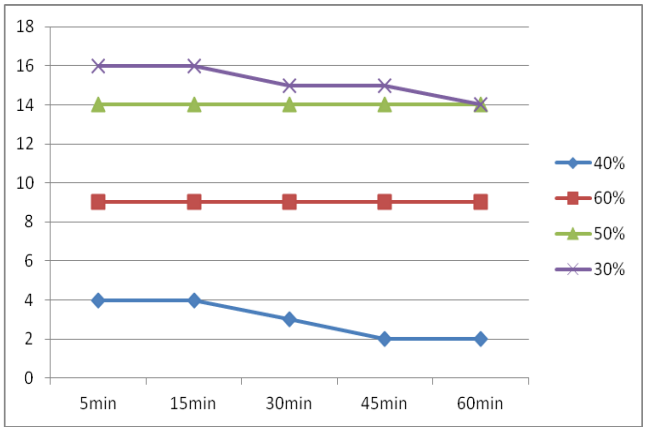


Figure 16 Influence of water concentration on water absorption of lettuce granules. With PVP (oven drying)

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