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Imaging and image analysis of freeze-dried cellular solids of gellan and agar gels

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ABSTRACT

The fabricated gels are a form of novel structured food made by incorporation of nutritious ingredients. Agar and gellan gels having nutrients like ferrous sulfate, sucrose, mango pulp, whey protein concentrate and flaxseed powder were subjected to freeze-drying followed by image analysis of the cellular structure. The indices analyzed were the maximum/minimum dimensions, area and perimeter of cells, and the thickness of cell walls. Based on these primary data, other derived indices like roundness, equivalent diameter, elongation and compactness were determined. These nutrients affected the gel structure. Cells with smaller sizes were observed for sugar (98–296 μm) and mango pulp (115–433 μm) incorporated gels. The agar gels produced cells with a higher cell wall thickness (2.8–8.8 μm) compared to that of gellan gels (2.4–6.3 μm). The roundness values were between 0.539–0.666 and 0.453–0.728 for nutrient-enriched gellan and agar gels. Freeze-drying offered closed type cellular structure having five non-equal sides.

1. Introduction

Many common synthetic and edible materials belong to the category of cellular solids. The classification of the structure of cellular solids is possible in terms of a few variables, such as open or closed cells, cell size distribution, thickness of cell wall and cell shape. Various processing technologies are employed to produce cellular solids. For example, baking of fermented dough produces bread (Lazaridou et al., 2007), extrusion cooking of moistened cereal flour gives expanded corn balls (Bhattacharya, 2011) and frying of pulse batter drops offers a crisp snack (Bhat and Bhattacharya, 2001). The products made from hydrocolloid dispersions may include several sponges, obtained due to the drying of gels. Many food products also exist as sponges, in which bubbles are interconnected to give a porous network with a continuous gas phase; examples include bread, cakes, wafers and extruded products.

Low-density cellular solids exist in nature, and are also commercially produced. Different aspects of their structure and associated properties have been investigated (Gibson and Ashby, 1999). Hydrocolloid based cellular solids have many applications, one of which is in the creation of dried texturized fruits. The different air-incorporated food products contain void cells, pores and cavities having different sizes and shapes. However, the analysis of the cellular solids obtained from dried gels is scarce while detailed image analysis is a rare phenomenon. Thus, scope exists to use dried hydrocolloid based food gels for using as an absorbent/cushioning material. Further, these products can supply health-benefiting nutrients having a long shelf-life. Nutrient-enriched gels are thus possible to prepare with hydrocolloids in which an iron source can be introduced, sugar for imparting a sweet taste, mango pulp for offering an attractive natural feel in addition to serving as a source of health benefitting omega 3 and 6 fatty acids through a natural source like flaxseed flour. However, the authors have not come across any study wherein the image analysis of the nutritious gels has been investigated after freeze drying. Hence, the objective of the present investigation is to conduct imaging and image analysis of the cellular solids obtained from freeze-drying of gellan and agar gels along with selected nutrients/ingredients such as $FeSO_4$, sugar (sucrose), mango pulp, flaxseed powder and whey protein concentrate.

2. Materials and methods

2.1. Materials

Agar powder (Loba Chemie, Mumbai, India) and gellan powder (HiMedia, Mumbai, India) were used as the gelling agents. Whey protein concentrate (WPC) was procured from Mahaan Foods,

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Coimbatore, Tamil Nadu, India. Sucrose (cane sugar) and Flaxseeds were procured from a local super market and after cleaning, were ground to fine flaxseed powder (FSP) and sugar powder (average particle size was less than 100 μm) by using a laboratory model grinder. Ferrous sulfate (Merck, Mumbai, India) was used as the iron source.

2.2. Methods

2.2.1. Gel preparation and freeze-drying

The agar and gellan gels were prepared as indicated by Sharma and Bhattacharya (2014). The 1% (w/w) gellan gel was prepared along with $FeSO_4$ (0.05%), mango pulp (10%), sucrose (10%), FSP (5%) and WPC (5%). The nutrients/ingredients and their proportions were selected by conducting preliminary studies to obtain a good set food gel having acceptable taste, texture and integrity. The ranges of nutrients/ingredient studied in preliminary studies were: gellan and agar (0.5–1.5%), $FeSO_4$ (0–0.10%), mango pulp (0–10%), sucrose (0–20%), FSP (0–5%) and WPC (0–10%). Agar gels (1%, w/w) were also prepared in the same manner. All gels were freeze-dried for 16 h at a temperature of −24 °C by employing a pilot plant model freeze-dryer (Model # Lyodryer-LT-5S, Lyophilization Systems, NY, USA) at a shelf temperature starting from −40 °C progressing towards 10 °C. The basis of selecting additional two formulations 1 and 2 is the preliminary studies aimed at developing nutritious gels. Formulation 1 (agar/gellan: 1%, $FeSO_4$: 0.05%, sucrose: 10%, mango pulp: 10%, FSP: 5% and WPC: 5%) was meant for common people while formulation 2 (agar/gellan: 1%, $FeSO_4$: 0.05%, FSP: 5% and WPC: 5%) was for diabetic people. All experiments were repeated twice.

2.2.2. Microstructure

A sharp blade was used to cut the freeze-dried gels vertically and the exposed cross-section was used to capture the images. The microstructure of the samples was examined by employing a scanning electron microscope (SEM) (Model # 435VP, Leo Electron Microscopy, Cambridge, UK). The cross-sections of the freeze-dried samples were mounted on metal stubs along with double-adhesive conducting tapes, and were coated with a thin film of gold employing a sputter coater. Microscopic examination was conducted at an accelerating voltage of 15 kV at magnifications of 100 and 500× to observe the internal morphology. Representative photomicrographs are presented here.

2.2.3. Imaging and image analysis

The photomicrographs, obtained during SEM exposures, were analyzed using the image analysis software (IMAGEJ 1.45s, National Institutes of Health, Maryland, USA). The two dimensional basic parameters like maximum and minimum dimensions, surface area of cells (S), thickness of the cell wall, Feret diameters (F_{max} and F_{min}) and perimeter (P) were obtained from the same software. The shape parameters like roundness, equivalent diameter, elongation and compactness were calculated using Eqs. (1)-(4) (Russ, 2006; Dhanalakshmi and Bhattacharya, 2014). Each photomicrograph was represented by 1024 × 768 pixels and the dimensional equivalent for each pixel was 3.125 μm for 100×. The size of each stored photomicrograph was 819 kb. The sizes of the sample scanned in SEM were about 6–8 mm in length and 6 mm in height. The 500× photomicrographs were used for measuring the thickness of cell wall; the perpendicular views of the cell walls were captured by adjusting the instrument settings. We randomly selected 5–10 cells for measurements of imaging indices in each photomicrograph while 10 photomicrographs were employed to have a minimum of 50 set of values. Duncan's multiple range test (DMRT) was used to examine the statistical significance at a probability (p) of 0.01.

3. Theory

The size of the cells was characterized by the thickness of cell wall and equivalent circular diameter (Eq. (1)) while the shape was reported in terms of roundness, elongation and compactness (Eq. (2)-(4)) (Russ, 2011). The equivalent circular diameter (Eq. (1)) of an irregular shaped cell was the diameter of a circle of equivalent area. The value of equivalent circular diameter offers a simple and easily comparable parameter to characterize the size of cell.

$$\text{Equivalent circular diameter} = \sqrt{\frac{4S}{\pi}} \tag{1}$$

The Feret diameter was used to calculate the shape related parameters. The Feret diameter is a measure of the size of an object along a specified direction. The minimum Feret diameter (F_{min}) is important when passing through a sieve (for powdery material) while the maximum Feret diameter (F_{max}) is useful for estimating the length of an elongated object. The roundness (Eq. (2)) is an indication of the extent of the circular shape, and a circle assumes a value of 1 and the deviation from the circularity is denoted by its deviation from 1.

$$\text{Roundness} = \frac{4S}{\pi F_{max}^2} \tag{2}$$

The elongation is a ratio of the maximum and minimum Feret diameters which accounts for the ratio of length and breadth of the cell. For a circular body, the elongation is 1 while for a thread like structure, the value of elongation increases markedly in a quadratic manner as $F_{max} \to \infty$ and $F_{min} \to 0$ to show a marked increase in elongation.

$$\text{Elongation} = \frac{F_{max}}{F_{min}} \tag{3}$$

Table 1
Cell characteristics of freeze-dried gellan gels obtained by image analysis.

Gelling agent	Additive	Thickness of cell wall (μm)		Major dimension of cells (μm)		Minor dimension of cells (μm)		Perimeter of cells (μm)		Area of cells (μm²)	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Gellan	–	3–6	5.2 ± 1.7^{c}	219–600	399 ± 142^{b}	144–474	288 ± 131^{c}	600–1843	1167 ± 471^{b}	24,844–223,506	101,648 ± 77,984^{c}
	$FeSO_4$	2–8	4.9 ± 2.0^{ac}	330–851	590 ± 198^{c}	129–679	378 ± 197^{c}	819–2526	1742 ± 667^{c}	453,721–143,105	198,330 ± 156,966^{d}
	Pulp	3–6	4.3 ± 1.0^{b}	115–433	305 ± 137^{a}	70–291	220 ± 96^{b}	314–1371	956 ± 450^{b}	6338–96,436	60,549 ± 40,044^{b}
	Sugar	1–3	2.4 ± 0.9^{a}	98–296	225 ± 80^{a}	55–223	164 ± 65^{a}	267–970	696 ± 268^{a}	4229–52,041	32,180 ± 18,326^{a}
	Flaxseed	2–8	5.3 ± 3.0^{c}	279–1508	729 ± 486^{d}	247–1143	556 ± 404^{d}	888–4633	2295 ± 1525^{c}	54,141–1,353,477	434,631 ± 55,065^{e}
	WPC	2–5	4.2 ± 1.7^{b}	253–615	437 ± 163^{b}	177–428	320 ± 105^{c}	734–1719	1305 ± 466^{b}	35,244–188,291	119,607 ± 70,385^{c}
	Formulation 1	2–7	3.7 ± 2.0^{b}	144–325	234 ± 84^{a}	127–211	175 ± 39^{a}	459–987	729 ± 254^{a}	14,385–53,828	34,125 ± 18,134^{a}
	Formulation 2	4–8	6.3 ± 1.4^{d}	131–308	274 ± 118^{a}	96–273	172 ± 66^{a}	394–1170	753 ± 285^{a}	9883–93,926	41,106 ± 31,761^{a}

Values in the same column with different superscripts differ significantly at $p = 0.01$.