UNIVERSITI PUTRA MALAYSIA

PRODUCTION AND PROPERTIES OF SHELF-STABLE SPRAY-DRIED POWDER FROM ENZYME-TREATED SOURSOP (Annona muricata L.) FRUIT

CHANG LEE SIN

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By

CHANG LEE SIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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May 2017

Chair: Prof. Hasanah Mohd Ghazali, PhD
Faculty: Food Science and Technology

In Malaysia, the production of soursop (*Annona muricata* L.) fruit has increased dramatically due to a strong consumer demand because of its nutritional and health protective values. However, one of the apparent features of this fruit is that it softens easily after harvested due to a high respiration rate, and this leads to a short shelf life. Thus, in this study, fresh soursop fruit was preserved by spray-drying it into a shelf-stable powder that may serve the food industry as a value-added intermediate product, and also in the health industry as a food supplement. In the first part of the study, commercially available enzyme preparations, Pectinex® Ultra SP-L (pectinase), Fungamyl® 800 L (α-amylase) and Celluclast® 1.5 L (cellulase) (Novozymes, Denmark), were used in a pre-treatment step to liquefy soursop mesocarp and 1.5 % (v/w) pectinase was selected as the primary enzyme because it produced soursop puree effectively. Besides, when pectinase was combined with cellulase, a liquefied puree with a significant ($p \leq 0.05$) reduction of viscosity of up to 50 % within a shorter incubation time (75 minutes) was produced. Apart from determining many physicochemical properties such as pH, titratable acidity, total soluble solid, sugar, and organic acid profiles, the volatile compounds of soursop fruit and puree were compared using a zNose (Ultrafast GC, USA). Principal Component Analysis (PCA) results indicated six important volatile compounds generated the highest total variance (92.9 %) which classifies the aroma profiles into three groups: raw soursop fruit, soursop fruit treated with pectinase, and liquefied puree from combined enzyme treatment. Two ester compounds, namely methyl hexanoate and methyl *trans*-2-hexenoate, were identified as the major volatile compounds present in soursop.

In an attempt to optimize the production conditions for soursop powder, Response Surface Methodology (RSM) was applied to study the effect of cellulase concentration (0 to 2 % v/w) at fixed pectinase concentration (1.5 % v/w), the addition of maltodextrin (20 to 40 % w/w), and spray-drying inlet temperature (130 to 160 ºC) on the properties of spray-dried soursop powder. Results showed that the polynomial model was significantly fitted ($p \leq 0.05$) for process yield, moisture content, water activity, hygroscopicity, and stickiness. However, the bulk density, true density, porosity, particle size and water adsorption index did not fit significantly ($p > 0.05$) into the model. The color of the spray-dried soursop powder appeared slightly creamy, most probably due to the combination of soursop puree (cream) and maltodextrin (white). The glass transition temperature (T_g) was found to range between 46.53 and 58.25 °C, indicating the spray-dried soursop powder is an amorphous material. Surface morphology of powder, viewed by Scanning Electron Microscopy (SEM), showed that the particles exhibited the general morphology of amorphous powder: spherical shape and possessed a continuous wall (crust) without surface cracks. Based on multiple responses optimization of the process, puree that is pre-treated with 1.3 % (v/w) cellulase and incorporated with 37 % w/w maltodextrin and then spray-dried at an inlet temperature of 156 ºC may be transformed into soursop fruit powder that had optimal physicochemical properties.

At the optimum spray drying conditions, the soursop powder obtained had residual stickiness when stored. Thus, the effect of addition of different types [tricalcium phosphate (TCP) and calcium silicate (CS)] and concentrations (0 - 1.5 % w/w) of anticaking agents, and storage temperatures (conventional storage at 25 ± 1 ºC and accelerated storage at 38 ± 1 ºC) on properties of the powder heat-sealed in aluminum laminated polyethylene (ALP) pouches was examined. Statistically, results showed the addition of either of the anticaking agent significantly ($p \leq 0.05$) increased the process yield of powder. The physicochemical properties of the powder were also significantly ($p \leq 0.05$) affected by the presence of an anticaking agent, and by storage time and temperature. The critical moisture content (X_c) for the powder was ranged from 0.069 to 0.072 g H_2O/g ds. Kinetic modelling for color change (ΔE) showed zero order degradation reaction. The lowest kinetic constant was recorded for 1.5 % CS which had the highest activation energy (Ea) (17.26 kJ/mol), indicating powder added with CS had less tendency to undergo color change. Powder added with 1.0 % TCP or 1.5 % CS were optimal for the production of soursop powder, and for estimation of shelf life.

Shelf life of powder optimized under the conditions obtained above was estimated based on the moisture sorption isotherm (MSI) of soursop powder which was determined gravimetrically. Results indicated that the equilibrium relative humidity (ERH), the type of anticaking agents, and storage temperatures had significant ($p \leq 0.05$) effects on the equilibrium moisture content (EMC) of soursop powder. The Guggenheim, Anderson, and de Boer (GAB) model which was used to fit sorption data reflected a sorption curve which followed Type III Brunauer's classification. The monolayer moisture content (M_o) of the powder varied from 0.0221 to 0.0243 g H_2O/g ds. The longest shelf life was predicted for powder treated with 1.5 % TCP (316 days). Prediction of shelf life using the kinetic model was reasonably adequate as the calculated mean relative percent deviation modulus was less than 10 % (2.3 - 6.9 %). Hedonic test of five sensory attributes, namely aroma, color, mouthfeel, taste, and overall acceptability, for all reconstituted soursop drink showed mean scores that were higher than six from a maximum of nine, indicating high acceptability.

Overall, the production of shelf-stable soursop powder was made possible by having a spray drying feed that was puree-like that had low viscosity due to enzymatic treatment of fruit pulp. Soursop powder produced by spray drying exhibited general properties and morphology of an amorphous powder. The addition of food additives demonstrated anticaking effect by reducing moisture adsorption of powder to prevent caking phenomenon. Both anticaking agents prolonged the shelf life of powders possibly by forming a protective barrier on the sample particle to improve powder stability over time.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN DAN PENCIRIAN SERBUK KERING-SEMBUR DARIPADA BUAH DURIAN BELANDA (*Annona muricata* L.) YANG TERAWAT ENZIM

Oleh

CHANG LEE SIN

Mei 2017

Pengerusi: Prof. Hasanah Mohd Ghazali, PhD
Fakulti: Sains dan Teknologi Makanan

Pengeluaran buah durian belanda (*Annona muricata* L.) telah meningkat secara mendadak di Malaysia disebabkan permintaan pengguna kerana nilai khasiat buah ini. Walau bagaimanapun, salah satu ciri buah ini adalah ia melembut dengan mudah kerana ia menpunyai kadar respirasi yang tinggi menyebabkan jangka hayat yang pendek. Jadi, dalam kajian ini, buah durian belanda ditransformasi menjadi serbuk yang stabil dengan teknik pengeringan sembur supaya menjadikan produk pertengahan yang mempunyai nilai tambah yang sihat untuk industri makanan. Dalam bahagian pertama, enzim komersial iaitu Pectinex® Ultra SP-L (pectinase), Fungamyl® 800 L (α-amilase) dan Celluclast® 1.5 L (selulase) (Novozymes, Denmark), telah digunakan dalam langkah pra-rawatan untuk mencairkan mesokarpa durian belanda dan 1.5 % (v/w) pectinase telah dipilih sebagai enzim utama kerana ia menghasilkan puri durian belanda dengan berkesan. Selain itu, apabila pectinase digabungkan dengan selulase, puri yang signifikan ($p \leq 0.05$) lebih cair dengan pengurangan kelikatan sebanyak 50 % dihasilkan dalam jangka pengeraman yang lebih pendek (75 minit). Selain menentukan ciri-ciri fizikokimia seperti pH, keasidan tertitrat, jumlah larut pepejal, gula, dan profil asid organik, kompaun meruap daripada buah dan puri dibandingkan dengan menggunakan zNose (Ultrafast GC, US). Keputusan Principal Component Analysis (PCA) menunjukkan bahawa terdapat enam sebatian meruap utama menghasilkan jumlah varians yang paling tinggi (92.9 %) untuk mengklasifikasikan profil aroma kepada tiga kumpulan: buah durian belanda, puri dihasilkan daripada pectinase, dan puri cair yang dihasilkan daripada gabungan enzim. Dua kompaun ester, iaitu metil hexanoate dan metil *trans*-2-hexenoate, adalah kompaun meruap utama yang terdapat dalam buah durian belanda.

Untuk mengoptimumkan keadaan penghasilan serbuk durian belanda, Metodologi Response Surface (RSM) telah digunakan untuk mengkaji kesan kandungan selulase (0 – 2 % v/w) pada kandungan pectinase yang tetap (1.5 % v/w), penambahan

maltodekstrin (20 – 40 % w/w) dan suhu pengeringan sembur (130 – 160 °C) terhadap sifat-sifat serbuk durian belanda. Hasil kajian menunjukkan bahawa model polinomial adalah bersignifikan ($p \leq 0.05$) terhadap hasil proses, kandungan kelembapan, aktiviti air, hygroscopicity, dan kelekitan. Walau bagaimanapun, ketumpatan pukal, kepadatan sebenar, keliangan, saiz zarah dan index serapan air tidak bersignifikan ($p > 0.05$) untuk model polinomial. Warna serbuk durian belanda adalah berkrim mungkin disebabkan oleh gabungan puri (krim) dengan maltodekstrin (putih). Suhu transition (T_g) adalah di antara 46.53 dan 58.25 °C, menunjukkan serbuk durian belanda adalah bahan amorfus. Morfologi permukaan serbuk diperiksa menggunakan Mikroskop Imbasan Elektron (SEM) menunjukkan bahawa zarah mempamerkan morfologi umum serbuk amorfus yang bentuk bulat dan mempunyai dinding berterusan dengan ketiadaan permukaan yang retak. Berdasarkan keputusan pengoptimuman, puri yang ditambah dengan 1.3 % (v/w) selulase digabungkan dengan 37 % w/w maltodekstrin dan dikering sembur pada suhu 156 °C dapat menghasil serbuk durian belanda yang mempunyai ciri-ciri fizikokimia optimum.

Pada keadaan pengeringan sembur yang optimum, serbuk durian belanda yang dihasil mempunyai sifat kelekitan apabila disimpan. Oleh itu, kesan penambahan ejen anticaking [fosfat trikalsium (TCP) dan kalsium silikat (CS)] dan kandungan (0 – 1.5 % w/w), dan suhu penyimpanan (penyimpanan konvensional pada 25 ± 1 °C dan penyimpanan memecut pada 38 ± 1 °C) ke atas sifat-sifat serbuk dibungkus dalam aluminium berlapis polietilena (ALP) telah dikaji. Keputusan statistik menunjukkan bahawa penambahan egen anticaking meningkatkan hasil proses secara signifikan ($p \leq 0.05$). Sifat-sifat fizikokimia serbuk dijejaskan secara signifikan ($p \leq 0.05$) dengan penambahan egen anticaking, tempoh dan suhu penyimpanan. Kandungan lembapan kritikal (X_c) adalah di antara 0.069 dan 0.072 g H_2O /g ds. Model kinetik untuk perubahan warna (ΔE) menunjukkan tindak balas degradasi sifar. Pemalar kinetik terendah dicatatkan pada serbuk ditambah dengan 1.5 % w/w CS yang mempunyai tenaga pengaktifan yang paling tinggi (Ea) (17.26 kJ/mol), menunjukkan serbuk ditambahkan dengan CS kurang mengalami perubahan warna. Serbuk ditambah dengan 1.0 % w/w TCP dan 1.5 % w/w CS adalah kandungan optimum untuk menghasilkan serbuk durian belanda dan anggaran jangka hayat.

Jangka hayat serbuk yang dioptimumkan dalam keadaan yang diperolehi di atas dianggarkan berdasarkan isoterma kelembapan penyerapan (MSI) yang ditentukan secara gravimetrik. Keputusan menunjukkan bahawa kelembapan keseimbangan relatif (ERH), jenis egen anticaking, dan suhu penyimpanan mempamerkan kesan signifikan ($p \leq 0.05$) terhadap kandungan kelembapan keseimbangan (EMC) serbuk durian belanda. Model Guggenheim, Anderson, dan de Boer (GAB) telah digunakan untuk menyesuaikan data penyerapan dan menunjukkan bentuk sigmoidal yang mengikuti klasifikasi Brunauer jenis III. Kandungan lembapan monolayer (M_o) serbuk adalah di antara 0.0221 dan 0.0243 g H_2O/g ds. Jangka hayat yang paling lama telah dianggar dalam serbuk ditambah dengan 1.5 % w/w TCP (316 hari). Jangka hayat dianggar menggunakan model kinetik adalah sesuai sebab min relative peratus sisihan modulus adalah kurang daripada 10 % (2.31 - 6.93 %). Ujian hedonik untuk lima sifat deria iaitu aroma, warna, perasaan dalam mulut, rasa, dan penerimaan keseluruhan untuk semua minuman durian belanda menunjukkan skor min lebih daripada enam, menunjukkan penerimaan yang tinggi.

Secara keseluruhan, serbuk kering sembur durian belanda boleh dihasil dengan puri yang mempunyai kelikatan yang rendah dengan menggunakan rawatan enzim. Serbuk durian belanda yang dihasilkan dengan operasi pengeringan sembur mempamerkan sifat umum dan morfologi serbuk amorfus. Penambahan aditif makanan menunjukkan anticaking berkesan dengan mengurangkan penyerapan kelembapan untuk mengelakkan fenomena caking. Kedua-dua ejen anticaking memanjangkan jangka hayat serbuk mungkin dengan membentuk pelindung pada sampel zarah untuk meningkatkan kestabilan serbuk.

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I certify that a Thesis Examination Committee has met on 22 May 2017 to conduct the final examination of Chang Lee Sin on her thesis entitled "Production and Properties of Shelf-Stable Spray-Dried Powder from Enzyme-Treated Soursop (*Annona muricata* L.) Fruit" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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2D	Two-dimensional
3D	Three-dimensional
°	Degree
° C	Degree celcius
° Brix	Degree Brix
%	Percentage
θ	Day
∞	Infinity
λ	Lambda
±	Plus minus
+	Positive
-	Negative
~	Approximate
ΔE	Color change
ΔC_p	Heat capacity changes
µg	Microgram
µL	Microliter
µM	Micromole
µm	Micrometer
α	Alpha
ABTS	3-ethylbenzothiazoline-6-sulphonic acid
ALP	Aluminum laminated polyethylene
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ASLT	Accelerated shelf life testing

Aw	Water activity
BD	Bulk density
BET	Brunauer-Emmett-Teller
BOPP	Biaxially oriented polypropylene
BOPP/MCPP	Metallized co-extruded biaxially oriented polypropylene
C	Chroma
CCD	Central Composite Design
C_g	Monolayer adsorption constant
cm	Centimeter
cm^3	Centimeter cube
Cp	Centipoise
CO_2	Carbon dioxide
d	Diameter
DE	Dextrose Equivalency
db	Dry basis
ds	Dry solid
DSC	Differential Scanning Calorimetry
E	Mean relative percent derivation modulus
Ea	Activation energy
e.g.	Example gratia (for example)
EMC	Equilibrium moisture content
Eq.	Equation
ERH	Equilibrium relative humidity
et al.	And others
FAO	Food and Agriculture Organization
FAU-F	Fungal Alpha-Amylase unit
FDA	Food and Drug Administration

FW	Fresh weight
g	Gram
GAB	Guggenheim-Anderson-de Boer
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrophotometry
h	Height
hr	Hour
H	Hydrogen
H °	Hue angle
H_m	Monolayer molar sorption enthalpies
H_n	Multilayer molar sorption enthalpies
H_1	Bulk liquid molar sorption enthalpies
HDPP	High-density polypropylene
HPLC	High Performance Liquid Chromatography
HP-SPME	Headspace Solid-Phase Microextraction
i.e.	Id est (that is)
IU	International Unit
IUB	International Union of Biochemistry
IUPAC	Internaltion Union of Pure and Applied Chemistry
k	Kinetic constant
K	Kelvin
K_g	Multilayer adsorption constant
Kp	Permeability of packaging
kcal	Kilocalories
kg	Kilogram
kJ	Kilojoule
kV	Kilovolt

l	Length
L	Liter
LCMS	Liquid Chromatography-Mass Spectrometry
LLDPE	Linear low density polyethylene
m	Meter
M_0	Monolayer moisture content
m^2	Meter square
m^3	Meter cube
mg	Milligram
min	Minute
mL	Milliliter
mol	Mole
mm	Millimeter
MSI	Moisture sorption isotherm
MW	Molecular weight
n	Reaction order
N	Nitrogen
NA	No available information
ND	Not detected
nm	Nanometer
No.	Number
O	Oxide
p	Probability
Pa	Pascal
PCA	Principal Component Analysis
PET	Polyethylene terephthalate
PGNU	Polygalacturonase unit

PP	Polypropylene
ppm	Part per million
psig	Per square inch gauge
PTFE	Polytetrafluoroethylene
Q	Heat of adsorption
QDA	Quantitative Descriptive Analysis
R	Universal gas constant
R^2	Determination coefficient
R^2 adj	Adjusted R-square
RE	Relative error
RH	Relative humidity
RID	Refractive Index Detector
rpm	Rate per minute
RSEX	Reflectance Specular-Excluded
RSM	Response Surface Methodology
s	Second
SAW	Surface Acoustic Wave
SD	Standard deviation
SEM	Scanning Electron Microscopy
SSR	Sum of square residual
t	Time
T	Temperature
TA	Titratable acidity
TD	True density
TEs	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity
T_g	Glass transition temperature

TS	Total solid
$T_{surface}$	Temperatures of particles surfaces
TSS	Total soluble solid
USDA	United State Department of Agriculture
UV	Ultraviolet
v	Volume
w	Weight
wd	Width
WAI	Water adsorption index
wb	Wet basis
W_s	Dry weight
WSI	Water solubility index
x	Times
X_c	Critical moisture content
X_i	Initial moisture content

CHAPTER 1

INTRODUCTION

Annona muricata L. is commonly known as soursop in English and *durian belanda* in Malay. It is fairly a large fruit with a generally heart-shaped to oval with dark green, rough and thin skin (Figure 1). The flesh is a white cottony pulp with soft pithy core connecting the juicy pulp (Nakasone and Paull, 1998). In Malaysia, the production of soursop fruit has increased from 887.4 metric tonnes in 2011 to 1483.7 metric tonnes in 2015 (Fruit Crops Statistic, 2015). In recent years, soursop has received considerable attention from the public because of the nutritional and health protective values of this fruit. Numerous studies have been done concerning the nutritional and health-benefit properties. Umme *et al.* (1997) reported that a hundred gram of soursop contains 20.9 mg of ascorbic acid and 7.59 g/ of sugar which indicates soursop is nutritionally high in carbohydrates and contains significant amounts of vitamins. Soursop also has many therapeutic properties such as antibacterial, anticancer, sedative, and other properties (Asprey and Thornton, 1995). Soursop fruit is recognized as one of the plant based therapies in United States National Center for Complementary and Alternative Medicine (NCCAM). A study by Diaz *et al.* (2012) showed 40.5 % of breast cancer patients out of 149 respondents agreed that soursop radically improved their health status. High antioxidant content (6.09 ± 0.13 µM TEAC/g) in soursop fruit is able to reduce the risk of heart diseases and prevent cardiovascular diseases (De Souza *et al.*, 2012b). This suggests soursop can be processed and incorporated into human diet in order to improve the nutritional status of consumer.

Figure 1: Soursop fruit

One of the apparent features of soursop fruit is it softens and bruises easily, and releases an unpleasant strong smell when ripe (Janick and Paull, 2008; Allen, 1967). These are associated with a rapid respiration rate of soursop fruit that has a double sigmoidal growth pattern (two maximum respiratory), making it highly susceptible to physical damages, thus soursop is very perishable and has a short shelf life (Worrell *et al.*, 1994). Hence, raw soursop should be processed into intermediate products such as puree, which can then be value-added into commercial products such as jam, jelly, sweets and beverages (De Souza *et al.*, 2012a; Gratão *et al.*, 2007; Umme *et al.*, 1999). Soursop puree is usually prepared by blending fruit pulp (without seed) with distilled water at a ratio of 1:2 (Onyechi *et al.*, 2012; Abbo *et al.*, 2006; Umme *et al.*, 1997). However, excessive energy is required to remove water during further processing such as drying operation to produce powder. Thus, incorporation of enzymatic method has become an important and essential part of processes used in the modern food industries because water is often not incorporated either during puree or juice production.

In the food processing industry, enzymatic mash treatment is a well-known modern process to obtain higher yield of juice extraction, increase press capacity, improve clarification and liquefaction, and reduce viscosity (Cerreti *et al.*, 2016; Lima *et al.*, 2015; Sagu *et al.*, 2014; Schols *et al.*, 2009). Food grade pectinase hydrolyses pectin and produced low viscosity juice that brings extra advantages for the subsequent filtration process (Croak and Corredig, 2006; Demir *et al.*, 2001). The addition of another cell wall degrading enzyme, cellulase, enhances the breakdown of cell wall matrix that is composed of cellulose and hemicellulose, thus releasing more juice. For optimum liquefaction of fruit pulp to produce low-viscosity puree, preliminary studies must always be conducted to determine the optimal conditions (contact time, concentration of enzyme, temperature of incubation and pH) for reaction which depends on the degree of liquefaction required and the type of fruits (Landbo and Meyer, 2004).

Spray drying is a commercial and well known drying technique used to transform liquid raw material into a solid product (Bhandari *et al.*, 2008). This operation is favorable because the outcome of spray drying process is fine particles with free flowing properties. Spray drying has been widely used in the production of fruit powders (Patil *et al.*, 2014; Ferrari *et al.*, 2012; Chin *et al.*, 2010). In order to obtain spray-dried products with better characteristics and process yield, it is important to optimize the drying process. Response Surface Methodology (RSM) is applied as a statistical method to design the experiment and optimize responses (Myers and Montgomery, 1995). In addition, an anticaking agent such as tricalcium phosphate or calcium silicate is often incorporated into the powders which are then packed in suitable packing materials such as aluminum laminated polyethylene (ALP) in order to further improve the stability of the powder.

In this study, soursop puree was produced from soursop pulp with the aid of enzymes in order for liquefaction to take place. The most effective enzyme in the preparation of soursop puree which can be used as a base feed of spray drying process was then selected. For effective powder production, the effects of maltodextrin concentration and spray drying inlet temperatures on powder production were examined by the RSM experiment design. The effect of packing the powder in heat-sealed aluminum laminated polyethylene (ALP) pouch on storage stability was also evaluated. In addition, the effects of types and concentrations of anticaking agents, and storage time and temperature were investigated and kinetic modeling was determined. The powder which showed the best storage stability was selected to predict the potential shelf life. Thus, the specific objectives of this study were:

1. To prepare soursop puree that is suitable to be used as spray drying feed through enzymatic liquefaction of soursop pulp.
2. To investigate the effect of concentration of cellulase, maltodextrin level, and inlet temperature on process yield and physicochemical properties of the spray-dried soursop powder.
3. To optimize spray drying inlet temperature, carrier and feed concentration for the preparation of soursop fruit powder.
4. To investigate the effects of anticaking agents, storage time, and temperature on process yield and physicochemical properties of spray-dried soursop powder.
5. To predict the shelf life and sensory quality of spray-dried soursop powder packed in aluminum laminated polyethylene pouch using an accelerated shelf life testing (ASLT) approach.

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