

ISOLATION AND ENCAPSULATION OF *DESI* GHEE FLAVOUR

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by

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CERTIFICATE

This is to certify that the thesis entitled, “**Isolation and encapsulation of *Desi* ghee flavour**” being submitted by **Ms. Neha Duhan** to the **Indian Institute of Technology Delhi** for the award of ‘**Doctor of Philosophy**’ is a record of bonafide research work carried out by her. She has worked under our guidance and supervision and has fulfilled the requirements for the submission of the thesis. To the best of our knowledge, the results contained in the thesis have not been submitted, in part or full, to any other university or institute for the award of any degree or diploma.

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Neha Duhan

ABSTRACT

Desi ghee (clarified butter fat) has been an integral part of Indian traditional foods and medicine systems. It is highly cherished in food and pharmaceutical industries and consumed widely in Asian and middle east countries. Ghee contains about 65% saturated fatty acid, 32% mono-unsaturated fatty acid, and 3% polyunsaturated fatty acid. Higher intake of saturated fat and cholesterol increases the risk of cardiovascular diseases, obesity and type 2 diabetes. Yet the craving for that natural flavour and aroma associated with *desi* ghee food products still lingers. Instead of avoiding direct consumption of ghee, it would be more practical, significant and scientific to modify structure, texture, and functionality of *desi* ghee while maintaining the acceptability and flavour components comparable with the original product. Isolation and encapsulation of volatile flavour compounds from *desi* ghee for development of low fat and reduced cholesterol food products would be a novel approach to provide the flavour of *desi* ghee to people who cannot consume it owing to health constraints. With this background, the present research was designed to understand the rheological properties of *desi* ghee, isolate and encapsulate its volatile flavour compounds, and estimate the shelf-life stability and sensory quality attributes of the encapsulating powder *desi* ghee flavour.

Rheological characteristics of *desi* ghee were analysed using a dynamic rotational and oscillatory rheometer. Headspace (HS), simultaneous distillation extraction (SDE), sub-critical CO₂ extraction-headspace (SCF-HS) and sub-critical CO₂ extraction-simultaneous distillation extraction (SCF-SDE) were compared for GC/MS analysis of volatile profile of *desi* ghee. Effect of sub-critical CO₂ extraction process on recovery of volatile flavour compounds viz., δ -dodecalactone, δ -tetradecalactone, 3-ethyl-3-methyl heptane, myristic acid (C14:0), palmitic acid (C16:0) and total extraction yield were studied. Encapsulation of the obtained extract was carried out by spray drying. Whey protein concentrate (15 to 22% w/w) and guar gum (0.1 to 0.5% w/w), were used as wall material and binding agent, respectively. The concentration of the ghee extract was varied from 10 to 15% (w/w) to maintain the total solid level in the emulsion within the range of 25 to 38% (w/w). A Box-Behnken experimental design was used for conducting the experiments. Encapsulation efficiency, process yield, solubility index, and flavour retention were measured as responses.

Moisture sorption isotherms of the encapsulated particles were determined at 25, 35 and 45 °C over a water activity range of 0.11 to 0.93 using saturated salt solutions. Guggenheim-Anderson-de Boer (GAB), Brunauer-Emmett-Teller (BET) and Caurie models were fitted to sorption data. Accelerated shelf-life of the encapsulated particles packed in aluminium foil laminated polyethylene package was estimated at 35°C and 90% relative humidity. Stickiness moisture content at which caking was observed, was considered the limiting value for shelf life estimation of the particles based on moisture absorption, while a peroxide value of 0.3 mEq. of O₂ per kg fat was taken as the threshold limit for oxidative rancidity. Sensory evaluation of the encapsulated particles when reconstituted, was carried out using Fuzzy comprehensive modelling.

Results showed that the flow behaviour of *desi* ghee samples tend to shift from pseudo-plastic to Newtonian nature with increase in temperatures and shear rate. Out of four rheological models, Ostwald model was found to be superior in predicting the shear rate-stress data at 18°C, whereas Ostwald-de Waele and Herschel-Bulkley models predicted all the data points over the temperature range of 24 to 30°C. The value of flow behaviour indices ranged from 0.224 to 0.911 over the entire range of temperature, inferring that *desi* ghee samples displayed non-Newtonian shear thinning. The

value of activation energy was found to be 1.98×10^6 kJ.mol⁻¹ over the entire temperature range and the Cox–Merz rule was found to be not applicable to the ghee samples.

Application of four isolation techniques followed by GC/MS analysis i.e., HS-GC/MS, SDE-GC/MS, SCF-HS-GC/MS, and SCF-SDE-GC/MS could identify a total of 129 nos. of volatile compounds in *desi* ghee. The volatile compounds identified were mainly lactones, aldehydes, ketones, esters, acids, hydrocarbons, and alcohols. Among the four analytical approaches, HS-GC/MS, SDE-GC/MS, SCF-HS-GC/MS, and SCF-SDE-GC/MS could individually detect 5, 41, 4, and 110 nos. of compounds, respectively. The key volatile flavour compounds such as δ -dodecalactone, δ -tetradecalactone, and δ -octadecalactone could be detected in the concentration of 121.3, 69.34 and 130 ng.g⁻¹, respectively. Minimum and maximum values of extraction recovery of δ -dodecalactone, δ -tetradecalactone, 3-ethyl-3-methylheptane, myristic and palmitic acid were found to be 0-288.22, 0-503.12, 0-142, 0-1894.86, and 73.6-4270 ng.g⁻¹, respectively. The extraction yield was observed to vary from 1.83-17.14 % (w/w). Extraction pressure had a positive effect on the extraction recovery of all the five flavour compounds. Optimum SC-CO₂ extraction parameters with respect to maximum recovery of δ -dodecalactone, δ -tetradecalactone, 3-ethyl-3-methyl-heptane, and total extraction yield, and minimum recovery of myristic and palmitic acid in the extract were found to be pressure = 7 MPa, temperature =10°C, and time =66 min.

The optimum combination of whey protein concentrate, guar gum and ghee extract for maximum process yield, encapsulation efficiency, solubility index and flavour retention were found to be 15% (w/w) ghee flavour, 15.76% (w/w) whey protein concentrate and 0.1% (w/w) guar gum. At this optimum combination, the encapsulation efficiency, process yield, solubility and flavour retention for δ -dodecalactone and δ -tetradecalactone were predicted to be 53.24%, 79.03%, 81.46%, 66.38%, and 78.32%, respectively, which were observed to be very close to the respective measured values.

The sorption isotherms reflected Type II BET sorption characteristics at 25, 35 and 45°C. GAB and Caurie models were found superior in predicting the moisture adsorption of encapsulated particles over the entire range of water activity. The monolayer moisture content values calculated by the best-fitted GAB and Caurie models were found to be 0.055, 0.052 and 0.047, and 0.053, 0.051, and 0.049 kg per kg dry solids at 25, 35 and 45 °C, respectively. Stickiness moisture content of the ghee flavour containing powder was observed to be 0.80 kg per kg dry solids. Out of two criteria viz., caking and oxidative rancidity, occurrence of caking was observed to take place prior to rancidity. The predicted shelf life of the powder was found to be 298.98 days while the actual shelf life was observed to be 288 days. The sensory quality attribute ranking of the encapsulated particles was: *aroma* > *taste* > *colour*.

In conclusion, the ghee flavour encapsulated with whey protein concentrate and guar gum could be utilized for the formulation of low fat and reduced cholesterol based food products. Also, the encapsulated ghee flavour could be utilized as an additive or ingredient in formulation of various food products such as mayonnaise, spread, etc., as and when desired to provide the taste and flavour of *desi* ghee. Also, the study would improve food safety and quality of *desi* ghee widening its value addition and utilization pattern in food and pharmaceutical industries.

Keywords: *Desi* ghee; Flavour; Rheology; Volatile isolation; Subcritical fluid extraction; Flavour encapsulation; Spray encapsulation; Encapsulation efficiency; Moisture sorption isotherms

सार

देसी घी (स्पष्ट मक्खन वसा) भारतीय पारंपरिक खाद्य पदार्थों और चिकित्सा प्रणालियों का एक अभिन्न अंग रहा है। यह खाद्य और दवा उद्योगों में अत्यधिक पोषित माना जाता है और एशियाई और मध्य पूर्व के देशों में व्यापक रूप से खाया जाता है। घी में लगभग 65% संतृप्त फैटी एसिड, 32% मोनो-असंतृप्त वसा अम्ल और 3% पॉलीअनसेचुरेटेड फैटी एसिड होता है। संतृप्त वसा और कोलेस्ट्रॉल के अधिक सेवन से हृदय रोगों, मोटापा और टाइप 2 मधुमेह का खतरा बढ़ जाता है। फिर भी देसी घी खाद्य उत्पादों से जुड़े प्राकृतिक स्वाद और सुगंध की लालसा अभी भी है। घी की प्रत्यक्ष खपत से बचने के बजाय, यह मूल उत्पाद के साथ तुलनीयता और स्वाद घटकों को बनाए रखते हुए देसी घी की संरचना, बनावट और कार्यक्षमता को संशोधित करने के लिए अधिक व्यावहारिक, महत्वपूर्ण और वैज्ञानिक होगा। कम वसा और कम कोलेस्ट्रॉल वाले खाद्य उत्पादों के विकास के लिए देसी घी से अस्थिर स्वाद यौगिकों का अलगाव और इनकैप्सुलेशन उन लोगों के लिए देसी घी का स्वाद प्रदान करने के लिए एक महत्वपूर्ण दृष्टिकोण होगा जो स्वास्थ्य बाधाओं के कारण इसका उपभोग नहीं कर सकते हैं। इस पृष्ठभूमि के साथ, वर्तमान शोध को देसी घी के रियोलॉजिकल गुणों को समझने, इसके अस्थिर स्वाद यौगिकों को अलग करने, इनकैप्सुलेट करने और इनकैप्सुलेटिंग पाउडर देसी घी स्वाद की शेल्फ-लाइफ स्थिरता और संवेदी गुणवत्ता विशेषताओं के अनुमान के लिए डिज़ाइन किया गया था।

देसी घी की रियोलॉजिकल विशेषताओं का विश्लेषण गतिशील घूर्णी और दोलक रियोमीटर का उपयोग करके किया गया। Headspace (HS), simultaneous distillation extraction (SDE), sub-critical CO₂ extraction-headspace (SCF-HS) और sub-critical CO₂ extraction-simultaneous distillation extraction (SCF-SDE) को देसी घी के अस्थिर प्रोफ़ाइल के विश्लेषण के लिए GC/MS के साथ तुलनात्मक जाँच के लिए उपयोग किया। देसी घी के वाष्पशील स्वाद यौगिकों (δ -dodecalactone, δ -tetradecalactone, 3-ethyl-3-methyl heptane), myristic acid (C14: 0), palmitic acid (C16: 0) और कुल yield की वसूली पर sub-critical CO₂ निष्कर्षण प्रक्रिया का प्रभाव अध्ययन किया गया। प्राप्त अर्क को स्प्रे सुखाने की प्रक्रिया से संपुटित किया गया था। Whey protein concentrate (15 से 22% w/w) और Guar Gum (0.1 से 0.5% w/w), का उपयोग क्रमशः दीवार सामग्री और बाध्यकारी एजेंट के रूप में किया गया था। 25 से 38% (w/w) सीमा के भीतर कुल ठोस स्तर को बनाए रखने के लिए पायस में घी की एकाग्रता 10 से 15% (w/w) थी। प्रयोग-संचालन के लिए Box-Behnken डिज़ाइन का उपयोग किया। प्रतिक्रिया के रूप में एनकैप्सुलेशन दक्षता, प्रक्रिया उपज, घुलनशीलता सूचकांक और स्वाद प्रतिधारण को मापा गया।

अच्छता कणों का sorption isotherm 25, 35 और 45 °C पर निर्धारित किया, जिसमें संतृप्त नमक समाधानों का उपयोग करके 0.11 से 0.93 की जल गतिविधि रेंज शामिल की। Guggenheim-Anderson-de Boer (GAB), Brunauer-Emmett-Teller (BET) और Caurie model को डेटा के लिए फिट किया गया। Aluminium foil laminated polyethylene package में पैक किए एनकैप्सुलेटेड कणों का त्वरित शेल्फ-जीवन 35 °C और 90% सापेक्ष आर्द्रता पर अनुमानित किया गया। चिपचिपाहट नमी की मात्रा जिस पर केकिंग देखी गई थी, उसे नमी अवशोषण के आधार पर कणों के शेल्फ जीवन के आकलन के लिए सीमित मान माना गया जबकि peroxide value का सीमित मान 0.3 mEq.O₂ प्रति किग्रा वसा, oxidative rancidity के लिए लिया गया। मजबूत और कमजोर गुणवत्ता वाले गुणों का पता लगाने के लिए स्किम दूध के साथ पुनर्गठित कणों का संवेदी मूल्यांकन fuzzy comprehensive modelling से किया गया।

परिणामों से पता चला कि देसी घी के नमूनों का प्रवाह व्यवहार तापमान और कतरनी दर में वृद्धि के साथ छद्म प्लास्टिक से न्यूटनियन प्रकृति में स्थानांतरित हो जाता है। चार रियोलॉजिकल मॉडल में से, 18°C में Ostwald model को कतरनी दर-तनाव डेटा की भविष्यवाणी करने में बेहतर पाया गया, जबकि Ostwald और Herschel-Bulkley model ने 24 से 30°C के तापमान रेंज पर सभी डेटा बिंदुओं की भविष्यवाणी की। तापमान के पूरे रेंज पर प्रवाह व्यवहार सूचकांकों का मान 0.224 से 0.911 तक था, जो कि देसी घी के नमूने की गैर-न्यूटोनियन कतरनी पतलेपन का प्रदर्शन करते हैं।

सक्रियण ऊर्जा का मान पूरे तापमान रेंज में $1.98 \times 10^6 \text{ kJ.mol}^{-1}$ पाया गया और Cox-Merz नियम घी के नमूनों पर लागू नहीं होगा, पाया गया था। HS-GC/MS, SDE-GC/MS, SCF-HS-GC/MS और SCF-SDE-GC/MS तकनीकों का अनुप्रयोग कुल 129 यौगिकों की पहचान कर पाया।

देसी घी में वाष्पशील यौगिकों की पहचान में मुख्य रूप से लैक्टोन, एल्डीहाइड, कीटोन, एस्टर, एसिड, हाइड्रोकार्बन और अल्कोहल थे। चार विश्लेषणात्मक दृष्टिकोणों में, HS-GC/MS, SDE-GC/MS, SCF-HS-GC/MS, और SCF-SDE-GC/MS व्यक्तिगत रूप से क्रमशः 5, 41, 4 और 110 यौगिकों का पता लगा सकते हैं। प्रमुख वाष्पशील स्वाद यौगिकों जैसे कि δ -dodecalactone, δ -tetradecalactone, और δ -octadecalactone को क्रमशः 121.3, 69.34 और 130 ng.g^{-1} की एकाग्रता में पाया गया है। δ -dodecalactone, δ -tetradecalactone, 3-ethyl-3-methylheptane, myristic acid और palmitic acid के निष्कर्षण रिकवरी के न्यूनतम और अधिकतम मूल्य क्रमशः 0 to 288.22, 0 to 503.12, 0 to 142, 0 to 1894.8, और 73.6 to 4270 ng.g^{-1} पाए गए। निष्कर्षण की पैदावार 1.83 to 17.14% (w/w) देखी गई। निष्कर्षण दबाव का सभी पांच यौगिकों के निष्कर्षण वसूली पर सकारात्मक प्रभाव पड़ा। इष्टतम SC-CO₂ निष्कर्षण पैरामीटर δ -dodecalactone, δ -tetradecalactone, 3-ethyl-3-methylheptane की अधिकतम वसूली के संबंध में, और कुल निष्कर्षण उपज, और अर्क में myristic acid और palmitic acid की न्यूनतम वसूली पाया गया। दबाव = 7 MPa, तापमान = 10 ° C, और समय = 66 मिनट।

अधिकतम प्रक्रिया उपज, एनकैप्सुलेशन दक्षता, घुलनशीलता सूचकांक और स्वाद प्रतिधारण के लिए 15% (w/w) घी स्वाद, 15.76% (w/w) whey protein concentrate, और 0.1% (w/w) guar gum केंद्रित किया गया। इस इष्टतम संयोजन में, एनकैप्सुलेशन दक्षता, प्रक्रिया उपज, विलेयता और स्वाद प्रतिधारण δ -dodecalactone और δ -tetradecalactone के लिए क्रमशः 53.24%, 79.03%, 81.46%, 66.38%, और 78.32% होने की भविष्यवाणी की गई थी, जिन्हें संबंधित मूल्यों के बहुत करीब देखा गया।

Sorption Isotherm 25, 35 और 45 °C पर type-II BET विशेषताओं को प्रतिबिंबित करता है। GAB और Caurie मॉडल पानी की गतिविधि की पूरी सीमा पर अतिक्रमित कणों की नमी सोखने की भविष्यवाणी में श्रेष्ठ पाए गए। सर्वश्रेष्ठ फिट किए गए GAB और Caurie मॉडल द्वारा गणना की गई monolayer नमी सामग्री मान क्रमशः 25, 35 और 45 °C पर 0.055, 0.052 और 0.047 और 0.053, और 0.049 किलोग्राम प्रति किलोग्राम सूखे ठोस पाए गए। घी स्वाद युक्त पाउडर की चिपचिपाहट नमी की मात्रा 0.80 किलोग्राम प्रति किलोग्राम सूखे ठोस पदार्थों में देखी गई। दो मानदंडों में से, सीकिंग और ऑक्सीडेटिव रैंसिडिटी, रैंकिंग से पहले होने के लिए कॉकिंग की घटना देखी गई। पाउडर का अनुमानित शेल्फ जीवन 298.98 दिन पाया गया जबकि वास्तविक शेल्फ जीवन 288 दिन माना गया। संवेदी कणों की संवेदी गुणवत्ता विशेषता रैंकिंग थी: सुगंध > स्वाद > रंग।

अंत में, घी स्वाद मट्टा प्रोटीन ध्यान और ग्वार गम के साथ कम वसा और कम कोलेस्ट्रॉल आधारित खाद्य उत्पादों के निर्माण के लिए इस्तेमाल किया जा सकता है। इसके अलावा, घी के स्वाद को विभिन्न खाद्य उत्पादों जैसे मेयोनेज़, स्प्रेड आदि के निर्माण में एक योज्य या घटक के रूप में उपयोग किया जा सकता है, जब और जब चाहें देसी घी का स्वाद और स्वाद प्रदान करने के लिए। इसके अलावा, इस अध्ययन से खाद्य सुरक्षा और देसी घी की गुणवत्ता में सुधार होगा और खाद्य और दवा उद्योगों में इसके मूल्यवर्धन और उपयोग के पैटर्न में सुधार होगा।

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LIST OF ABBREVIATIONS

%	: Percent
ANOVA	: Analysis of variance
FSSAI	: Food Safety and Standards Authority of India
NDDDB	: National Dairy Development Board
AOAC	: Association of Official Analytical Chemists
BC	: Before Christ
BET	: Brunauer-Emmett-Teller
BIS	: Bureau of Indian Standard
°C	: Degree celsius
cm	: Centimeter
SC-CO ₂	: Super-critical Carbon dioxide
eg.	: example
EMC	: Equilibrium moisture content
Eq.	: Equation
Ev	: electro Volt
Fig.	: Figure
g	: gram
g/L	: gram per litre
G'	: Storage modulus
G''	: Loss modulus
GAB	: Guggenheim-Anderson-de Boer
GC/MS	: Gas chromatography mass spectroscopy
GRAS	: Generally recognized as safe
HPLC	: High performance liquid chromatograph
IFT	: Institutes of Food Technologists
IS	: Indian Standard
K	: Kelvin
kg	: kilogram
kg.cm ⁻²	: kilogram per centimeter square
kPa	: kilopascal
kW	: kilo watt
kWhr	: kilo watt hour
µm	: micro meter
µL	: micro Litre
mg	: milli gram

m/z	: mass to charge ratio
Meq	: milli equivalent
MPa	: mega Pascal
Min	: minute
Mol	: Mole
MSI	: Moisture sorption isotherm
MS	: Mass Spectroscopy
MUFA	: Monounsaturated fatty acid
no.	: number
O ₂	: Oxygen
°C.min ⁻¹	: Degree Celsius per minute
Pa	: Pascal
Pa.s	: Pascal second
PUFA	: Polyunsaturated fatty acid
<i>R</i> ²	: Coefficient of determination
RI	: Retention Index
<i>RMSE</i>	: Root mean square error
rpm	: rotation per minute
s	: Second
SCE	: Sub critical CO ₂ extraction
SCF	: Sub critical fluid
SDE	: Simultaneous distillation extraction
SPME	: Solid phase micro extraction
<i>w</i>	: weight
<i>w_b</i>	: wet basis
τ	: shear stress
γ	: shear rate
η	: viscosity
