

ANNEXURE I A

PERMISSION FOR CONDUCTING RSM

ANNEXURE-III-A

DEPARTMENT OF FOODS AND NUTRITION
FACULTY OF FAMILY & COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002 - INDIA



Phone : } 0265-2795526
Tele. } 0265-2795522 [Ext.33]
Grams : } "HOMSCIENCE"

Date: 21/07/2022

No. F. C. Sc./ FND /

To,
Dr. Atanu H. Jana
Professor & Head,
Department of Dairy Processing & Operations
Anand Agricultural University, Anand.

Subject: Seeking permission and guidance for conducting Using Response Surface Methodology

Respected Sir,

I am Kanchi Baria, a Doctoral Research Scholar in the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, Vadodara. The title of my research is "Sports Nutrition: Impact of Nutritional Intervention on Performance" under the guidance of Prof. Komal Chauhan, Professor, Department of Foods and Nutrition, The Maharaja Sayajirao University of Baroda.

One of the objectives of my study is to develop *Nutribars* using **Response Surface Methodology** in order to replenish and supplement the gap in the macronutrients consumption as per the dietary requirement of athletes and the trainers involved in fitness training. With this regard, I request you to provide allow me to use the software, 'Response Surface Methodology', under the guidance of the expert, Dr. Amit Patel, Assistant Professor of your esteemed Institute.

Moreover, I am applying for a study leave to conduct the research study, and permission form you are instrumental for the study leave application. Sir, I hereby also request you to grant me the permission for the same. A request letter, seeking for the permission will reach you soon through the Guide and the Head, Prof. Mini Sheth. I humble request you to also permit me through the reply of this mail for accelerating the leave formalities.

Thanking you and waiting for your positive response.

Thanking you.

Sincerely,

Kanchi Baria

Research Fellow, Assistant Professor,
Email: Kanchi@baria-fn@msubaroda.ac.in
M: +91 7228821182

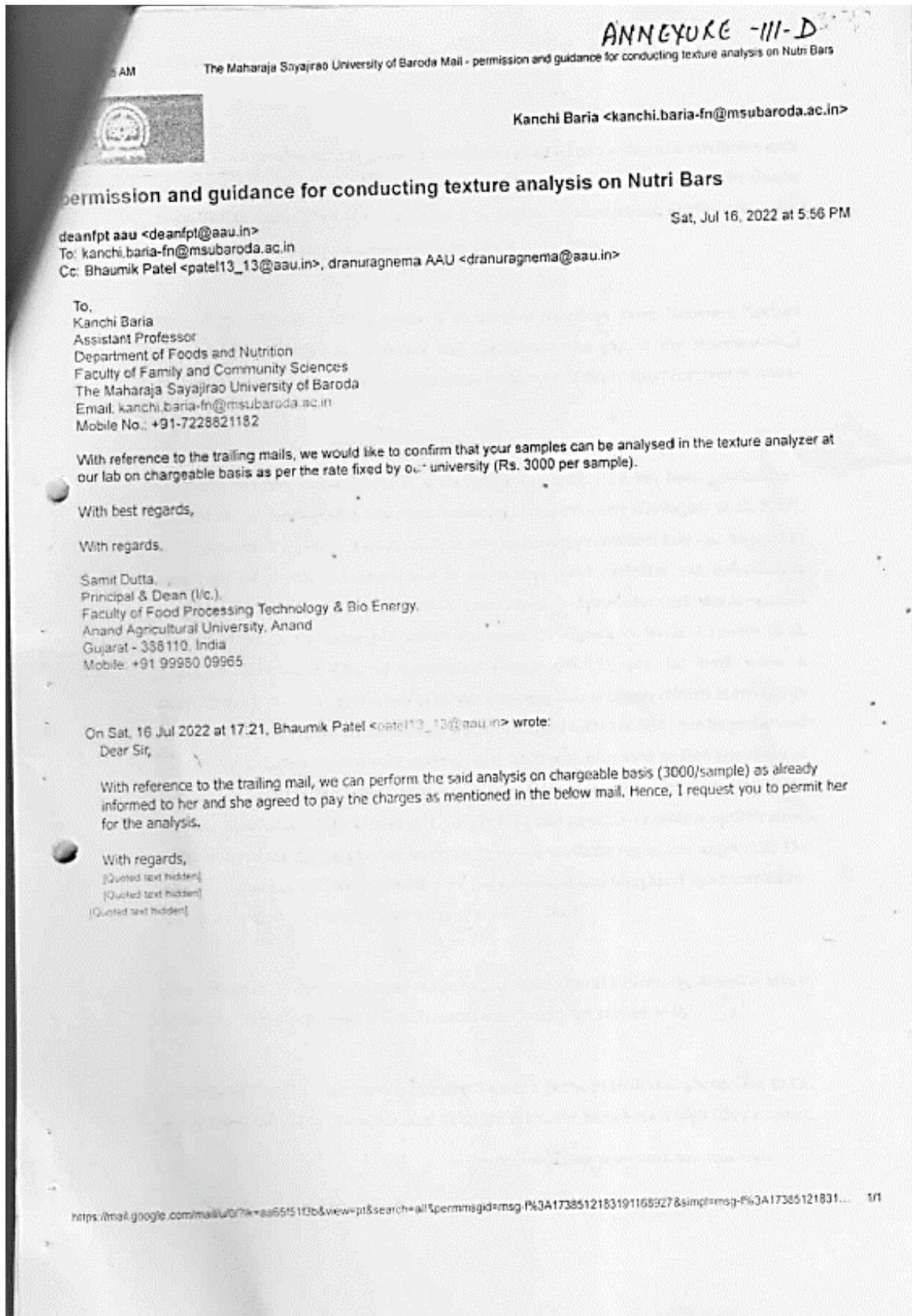
Professor. Komal Chauhan
Professor and Guide
Email: komal.chauhan-fn@msubaroda.ac.in
M: +91 9898790340

Through

Prof. Mini Sheth
Professor and Head (I/C)
Department of Foods and Nutrition,
Faculty of Family and Community Sciences,
The Maharaja Sayajirao University of Baroda

ANNEXURE II

PERMISSION FOR UTILIZING FACILITY TO CONDUCTIVE TESTURE PROFILE ANALYSIS



ANNEXTURE III A

ETHICAL COMPLIANCE CERTIFICATE



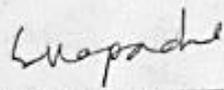
Institutional Ethics
Committee for Human
Research
(IECHR)

FACULTY OF FAMILY AND COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

Ethical Compliance Certificate 2021-2022

This is to certify that Kanchi Baria's study titled, "Sports Nutrition: Impact of Nutritional Intervention on Performance" from Department of Foods and Nutrition has been approved by the Institutional Ethics Committee for Human Research (IECHR), Faculty of Family and Community Science, The Maharaja Sayajirao University of Baroda. The study has been allotted the ethical approval number IECHR/FCSc/PhD/2022/5.


Prof Mini Sheth
Member Secretary
IECHR


Prof Shagufta Kapadia,
Chairperson
IECHR

ANNEXURE III B**LETTER FOR CHANGE OF TITLE OF THE THESIS**

Estd. 1949
Accredited Grade 'A+' by NAAC

Academics section**THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA**

Head Office, Maharaja Fatehsinghrao Gaekwad Marg, Fatehgunj,
Vadodara – 390 002, Gujarat, INDIA

☎: office-academics@msubaroda.ac.in ✉: dr-academic@msubaroda.ac.in

ACA7/387

Date: 3/08/2023

To,
The Dean, Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda,
Vadodara.

Subject: Letter for Change of title of the thesis - Ph.D. in Foods and Nutrition,
under the Faculty of Family and Community Sciences - case of **Kanchi
Kishorsinh Baria**

Reference: Application No. FFCSc/132 dated 10/05/2023

FACULTY OF FAMILY &
COMMUNITY SCIENCES

05 AUG 2023

Sir,

In pursuance of the SR-21 dated 31/07/2019 and recommendations of the Research Advisory Committee meeting held on 04/05/2023, the change of title of the thesis provided to the **Kanchi Kishorsinh Baria** with following credentials is hereby accepted & noted:

Name of the candidate	Kanchi Kishorsinh Baria
Date of Registration of Ph.D.	FoFCSC/2/157, 24/05/2014
Department	Foods and Nutrition
Faculty	Family and Community Sciences
Status of permission	Permitted to change of title of thesis
Faculty Research Committee by Circulation	04/05/2023 11:30 AM
Original Title	Sports Nutrition : Impact of Nutritional Intervention on Performance
New Title	Development, Optimization and Characterization of Protein-Energy Bars Using Response Surface Methodology

Further actions as per rules in this matter will be taken up by this office. You are requested to take note of the same and inform to the concerned research scholar accordingly.

[Signature]
Dy. Registrar (Academics)
For Registrar (Offg.)

Copy for information and necessary action to:

1. P.S. to Vice-Chancellor / Registrar (Offg.)
2. Head, Dept. of Foods and Nutrition, Faculty of Family and Community Sciences
3. Prof. Komal Chauhan (Guiding Teacher)
4. Kanchi Kishorsinh Baria –through the Head and the Dean, Faculty of Family and Community Sciences.
5. Section Exam.

[Signature]
DEAN
FACULTY OF FAMILY &
COMMUNITY SCIENCES
VADODARA

ANNEXURE IV**DETAILS OF CO-ROTATING TWIN SCREW EXTRUDER****Extruder Machine**

The extruder used for this experiment was laboratory Co-Rotating Twin-Screw Extruder (Basic Technology Pvt. Ltd (BTPL lab model) made, Model EB-10).

Components and Features of Extruder Machine

Component	Description
Material of Construction	Stainless steel for hygiene purposes
Drive System	Main drive: 7.5 HP variable speed motor (440 V, 3 phase, 50 Hz) with Siemens/ABB frequency drive. Torque limiter coupling on output shaft of worm-reduction gear.
Extruder Barrel	Two parallel co-rotating intermeshing screws, driven by drive assembly. Self-cleaning due to rotation in the same direction.
Feeder	Co-rotating feeder with Siemens Frequency Controller variable speed. Rated capacity controlled by knob on control panel. Calibration chart for feed rate.
Heating Arrangement	Three electric heaters at feeding, kneading and die sections. Temperature sensors connected to temperature controller on control panel.
Extruder Die	Die plate fixed with a screwed nut. Opening size fixed at 3 mm.
Cutting Knife	Automatic cutting knife driven by variable speed AC motor controlled by frequency controller. Safety guard provided.
Panel Board	Stand-alone control panel displaying screw speed, barrel temperature, feed rate, feed temperature and cutter rpm.

Water Circulation	Water jacket connected to extruder barrel for cooling. Circulation starts when heater temperature exceeds set limit.
Inching	Bypass switch provided for direct motor drive application for cleaning purposes. Dies must be removed before using inching device.
Emergency Stop	Emergency stop switch on control panel for immediate shutdown in case of foreign object entry into the barrel. Red color for visibility.

Operational Parameters for Crispies Production Using Twin Screw Extruder

Parameters	Specifications
Barrel Temperature	110°C
Extruder screw speed	350 rpm
Feeder speed	12 rpm
Cutter speed	2629 rpm
Torque	10.68
Diameter of the die	3 mm

ANNEXURE V. A**PROXIMATE COMPOSITION OF RAW MATERIALS FOR THE PREPARATION OF THE P-E BARS**

(Values Per 100g Edible Portion)

IFCT Code	Food Name	Proximate Principles and Dietary Fibre						
		Moisture (g)	Carbohydrate (g)	Protein (g)	Crude Fat (g)	Crude Fiber (g)	Ash (g)	Energy (Kcal)
(INDIAN FOOD COMPOSITION TABLES, 2017)								
H012	Groundnuts (<i>Arachis hypogea</i>)	6.97	17.27	23.65	39.63	10.38	2.11	520
E017	Dates, dry, dark brown (<i>Phoenix dactylifera</i>)	11.14	74.91	2.45	0.35	8.95	2.20	320
EO57	Raisins, dried, black (<i>Vitis vinifera</i>)	19.69	71.29	2.57	0.34	3.92	2.19	306
H009	Gingelly seeds, black (<i>Sesamum indicum</i>)	4.51	10.29	19.17	43.10	17.16	5.78	508
Agarwal et al., 2023								
--	Chia seeds	4.43	42.10	24.20	40.20	34.4	4.80	486
Product Label								
--	Chocolate Chips (Amul)	--	60.00	5.00	31.00	--	--	541

ANNEXURE V. B**PRODUCT SPECIFICATION OF LIQUID MALT EXTRACT**

j.k.malt products pvt. ltd.

PRODUCT SPECIFICATION Malt Extract Liquid	
1] Physical Appearance	Light Brown Viscous Liquid
2] Taste	Sweet
3] Flavour	Characteristic Malty
4] Total Solids (Min)	77.0 %
5] Acidity (As Lactic Acid) (Max)	1.2%
6] Protein (On Dry Basis) (Min.)	4.0%
7] Total Reducing Sugar (on Dry basis) (Min.)	60%
8] Colour [in EBC]	Min. 20
9] pH (of 10% of Solution)	5.0 - 6.5

ANNEXURE VI

ESTIMATION OF MOISTURE

The technique recommended by AOAC, 21st Edn., 2019, 925.10; Cha, 32.1.03; Vol II; Pg: 1. was utilized to ascertain the moisture content.

Petri dishes were first cleansed with distilled water, then dried in an electric oven at 105°C for four hours and subsequently cooled for thirty minutes in desiccators.

Afterward, the Petri dishes were weighed and appropriately labeled.

Three replicates of a 5g sample were weighed following the established standard.

These samples, along with the Petri dishes, were subjected to heating in an electric oven at 105°C for four hours.

Upon removal from the oven, the samples were allowed to cool to room temperature for thirty minutes. Subsequently, both the samples and the Petri dishes were weighed and the observations were recorded.

The moisture content was expressed as a percentage, calculated using the following formula:

$$\text{Moisture (\% by weight)} = \frac{\text{loss in weight of the sample (g) } (W_2 - W_3)}{\text{Weight of sample taken (g) } (W_2 - W_1)} \times 100$$

Where,

W_1 = Initial weight of the sample and Petri dish (g)

W_2 = Final weight of the sample and Petri dish after drying (g)

W_3 = Weight of the sample taken

ANNEXURE VII**ESTIMATION OF PROTEIN BY KJELDAHL METHOD**

The method described by (AOAC, 21st Edn., 2019, 984.13, Cha,4.2.09, Vol I, Pg: 31), was used to determine the protein content.

- A digestion flask was filled with one gram of each sample, which included 1.25 g of copper sulfate and 7 g of sodium sulfate.
- The sample was mixed with 12.5 ml of concentrated sulfuric acid and placed inside the digester for a duration of eight hours or until a clear solution was achieved.
- 30 ml of distilled water were added to a flask containing the cooled digest.
- 4% boric acid and 40% alkaline (NaOH) were the reagents used in the automated Kjeldhal apparatus distillation process.
- The mixture was pipetted and allowed to distil for nine minutes.
- After that, the samples had been diluted with 0.1 N-hydrochloric acids (HCL) and five drops of methyl red dye were added to the conical flask as an indicator.
- The outcome will be a light pink. The titrate valve was noted, then the following formula was used to get the percentages of nitrogen and protein,

$$\% \text{ Nitrogen} = \frac{CBR \times \text{Normality of } H_2SO_4 \times \text{Moles of nitrogen} \times DF}{\text{Weight of sample}} \times 100$$

Where,

D.F. Dilution Factor

CBR = Corrected Burette Reading: Normality of Acid (HCL)=0.1

Moles of Nitrogen 0.014

Total protein is equal to % Nitrogen x Protein Factor.

ANNEXURE VIII

ESTIMATION OF TOTAL ASH

Calculating the amount of ash involved following the steps by, AOAC, 21st Edn., 2019, 923.03; Cha 32.1.05; Vol II; Pg: 2.

- In short, after being cleaned with distilled water, the crucibles were dried in an electric oven for 30 minutes and then chilled in a desiccator was weighed after being labeled for three duplicates.
- For every sample, three grams were weighed and recorded.
- To carbonize the sample, the crucibles were set on a heated plate inside a fume hood.
- The crucibles were heated to 550°C for five hours in a cool muffle furnace.
- Following the five hours, the muffle furnace was turned off and after three hours, the temperature was allowed to drop to 150°C before being moved to desiccators for a half-hour of cooling.

The formula below,

$$\% \text{ Ash} = \frac{\text{Weight of crucible with ash} - \text{weight of empty crucible}}{\text{Weight of sample}} \times 100$$

ANNEXURE IX

ESTIMATION OF CRUDE FAT

(AOAC, 21st Edn., 2019, 2003.05; Cha, 4.5.05; Vol I; Pg: 41)

Crude fat content was determined using above mentioned method with little modification.

Weighing the Flour Sample:

- Accurately weigh 1 gram of the flour sample using an analytical balance.

Preparing the Sample:

- Transfer the weighed flour sample to a 50 ml beaker.
- Addition of Hydrochloric Acid:
- Add approximately 10 ml of concentrated hydrochloric acid to the sample in the beaker.

Heating on Water Bath:

- Heat the mixture on a water bath until it reaches a gentle boil.

Cooling and Transfer:

- Allow the mixture to cool to room temperature.
- Quantitatively transfer the cooled mixture to a Mojonnier flask using 10 ml of alcohol.

Solvent Extraction:

- Add 25 ml of diethyl ether and 25 ml of petroleum ether to the Mojonnier flask containing the mixture.
- Thoroughly mix the contents by shaking or swirling.
- Allow the mixture to stand until two layers (upper ethereal layer and lower aqueous layer) separate completely.

Transfer of Upper Layer:

- Carefully transfer the upper ethereal layer to a pre-weighed aluminum dish.

Repeat Extraction:

- Repeat the extraction process twice more using 15 ml of each solvent (diethyl ether and petroleum ether) each time.

Distillation:

- Distill off the solvents completely from the ethereal layer on a water bath.

Oven Drying:

- Transfer the dried residue in the aluminum dish to an oven preheated to $100\pm 1^{\circ}\text{C}$.
- Dry the residue in the oven for 1 hour.

Cooling and Weighing:

- After drying, cool the dish containing the residue in a desiccator to room temperature.

Weigh the dish containing the dried residue (Dish + Fat weight).

Calculating Fat Content:

- Calculate the weight of fat by subtracting the initial weight of the dish from the weight of the dish with the fat residue.

Calculate the percentage of fat in the flour sample using the formula:

$$\text{Percentage Fat (\%)} = (\text{Weight of Fat} / \text{Weight of Sample}) \times 100$$

ANNEXURE X**ESTIMATION OF CRUDE FIBER**

- The amount of crude fiber was calculated using the (AOAC, 21st Edn., 2019, 2003.05; Cha, 4.5.05; Vol I; Pg: 41) technique and the formula below,
- Two grams of the defatted sample were heated in 100 milliliters of a solution containing 1.25% HCl to produce 50 milliliters.
- Filter paper was used to filter the solution and after that, water was used to wash it until it was no longer acidic.
- After transferring the residue to a beaker, 100 ml of a 1.25% NAOH solution was added.
- The mixture was then heated to create 50 ml of solution.
- After that, the residue was filtered using ash less filter paper with a predetermined weight.
- The leftover material was dried in an electric oven, weighed and set ablaze at 550 °C in a muffle furnace using a known-weight crucible.
- After cooling, weigh the results.

$$\% \text{ Crude Fibre} = \frac{((W_2 - W_1) - (W_3 - W_1))}{W} \times 100$$

Where,

W = weight of the sample

W1 crucible dish weight

W2 is the sample weight prior to ignition

W3 is the sample weight following ignition.

ANNEXURE XI

ESTIMATION OF CARBOHYDRATES

Subtract the sum of the weights of moisture, protein, fat and ash from the total weight of the sample to determine the weight of carbohydrate:

$$\text{Carbohydrate (g/100g)} = 100 - (\text{Ash} + \text{Moisture} + \text{Crude fibre} + \text{Protein} + \text{Fat})$$

ANNEXURE XII

ENERGY (KCAL)

Food Labeling – Requirements for FDA Regulated products, by James L. Vetter, E. M. Melran, Ed., AIB International. Manhattan, K.S, 2007, Pavithraa & Mageshwari (2021).

ANNEXURE XIII

INFORMED CONSENT-SEMI TRAINED PANEL



The Maharaja Sayajirao University of Baroda
Faculty of Family and Community Sciences
Department of Foods and Nutrition

INFORMED CONSENT FORM

**Research Topic: SPORTS NUTRITION: IMPACT OF
NUTRITIONAL INTERVENTION ON PERFORMANCE**

**Title Revised: DEVELOPMENT, OPTIMIZATION AND
CHARACTERIZATION OF PROTEIN- ENERGY BARS USING
RESPONSE SURFACE METHODOLOGY**

Name of Investigators: Guide: Prof. Komal Chauhan

Student: Ms. Kanchi Baria

INFORMATION ABOUT THE PRODUCT “NURTIBARS”

I, **Kanchi Baria**, pursuing my PhD from the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, am conducting research on the sensory attributes and acceptability of Nutribars for my study in the area of Sports Nutrition with the title “Sports Nutrition: Impact of Intervention of Performance”.

Food bars are snacks with good sensory and nutritional qualities because they offer a portable and useful source of carbohydrates with varying amounts of protein and micronutrients for use around any physical activity under particular circumstances (athletes, school-age children, etc.) or in a busy lifestyle. The market's selection of snack items, including potato chips, extruded goods, chocolate bars and trail mixes, cannot provide the needs of a balanced diet. Moreover, many products are unhealthy for customers, particularly school-age children. The need for nourishing snacks has increased as customers' understanding of health and nutrition has grown. This has led food manufacturers to create food bars that combine convenience and nutrition.

For training purpose, you are requested to taste the Nutribars and fill up the google form, which will be sent to you via WhatsApp. The forms have to be filled each time you taste the Nutribars for its quality parameters.

The developed product contains cereals, millet, pulse protein isolate, dairy protein concentrate, nuts and dry fruits (Groundnuts, almonds, raisins and sesame seeds) dates, honey, malt and fat.

Thus, I would request you to: Taste the given products and give feedback for its quality parameters.

COSTS

The study requires your time and cooperation. The cost incurred will be borne by the investigator and there is no financial compensation for your participation in the study.

RIGHT TO WITHDRAW

The participant's cooperation and compliance would be highly appreciated; however, the participant has full right to withdraw from the study at any point of time. We hope you will take part for the entire study period, as we need all the information to draw correct conclusions.

INVESTIGATOR'S STATEMENT

I have described the research plan, the study's objectives and the potential advantages and disadvantages of participating. The participants had a chance to talk about the steps and ask any additional questions.

Signature of Investigator: _____

Date:

Prof. Komal Chauhan
Research Guide
Ph: +91 98987 90340

Ms. Kanchi Baria
Research Scholar
Ph: 0722 882 1182

ANNEXURE XIV

**SUBJECT CONSENT FORM- SEMI TRAINED PANEL
FOR SENSORY EVALUATION AMONG THE SEMI-TRAINED
PANEL**



The Maharaja Sayajirao University of Baroda
Faculty of Family and Community Sciences
Department of Foods and Nutrition

Research Topic: **SPORTS NUTRITION: IMPACT OF NUTRITIONAL INTERVENTION ON PERFORMANCE**

Name of Investigators: **Guide: Prof. Komal Chauhan**
Student: Ms. Kanchi Baria

SUBJECT CONSENT FORM

Name:

Age:

Sex:

Phone number:

Email Id:

DECLARATION:

Allergy (if any): _____

Cold/ flu: _____

- I confirm that I have read and understood the above information and have/had the opportunity to ask questions.

- I understand that my participation in this study is voluntary and I am free to reject being a part of this study, without giving a reason.
- I understand that the ethics committee and the regulatory authorities will not need my permission to look at my health records and my identity will not be revealed in any information released to third parties or published.
- I agree not to restrict the use of any data or results that arise from this study provided such a use be only for scientific purpose(s).
- I understand the study will involve multiple times of collection of data, in terms of tasting the product and give marks for its quality.
- I understand that the medical expenses will not be borne by the research investigators from any allergic reactions arising due to consumption of the Nutribars, unless proven.
- I agree to consume the Nutribars as offered.
- I voluntarily agree to participate in the community health study conducted by the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda.

Date:

Signature of the Participant

ANNEXURE XV

**COMPOSITE SCORE CARD FOR THE SENSORY
EVALUATION- SEMI TRAINED PANEL**

**Research Topic: SPORTS NUTRITION: IMPACT OF NUTRITIONAL
INTERVENTION ON PERFORMANCE**

**Revised Title: DEVELOPMENT, OPTIMIZATION AND
CHARACTERIZATION OF PROTEIN- ENERGY BARS USING RESPONSE
SURFACE METHODOLOGY**

NAME:

DATE:

PRODUCT NAME:

- You are given a sample of the Nutribar (Protein-Energy Bars). You are expected to evaluate the following characteristics, rate the Nutribars according to the highest possible score specified in the Scorecard and then taste the product.

Quality	Possible Score	Sample score
1. Appearance	10	
2. Taste	15	
3. Flavour	10	
4. Texture	20	
5. Mouthfeel	10	
6. Serving size	15	
7. Absence of defect	10	
8. Overall score	10	
9. Total	100	

Comment:

Signature:

ANNEXURE XVI

HEDONIC SCORE CARD FOR THE SENSORY EVALUATION- SEMI TRAINED PANEL

Research Topic: SPORTS NUTRITION: IMPACT OF NUTRITIONAL INTERVENTION ON PERFORMANCE

Revised Title: DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF PROTEIN- ENERGY BARS USING RESPONSE SURFACE METHODOLOGY

NAME:

DATE:

PRODUCT NAME:

- You are given a Nutribar (Protein-Energy Bars). Taste the bar and check (✓) how much you like or dislike each of the characteristic of the bar.

Degree of preference	Appearance	Taste	Flavour	Texture	Serving size
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like or dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Comment:

Signature:

ANNEXURE XVII

ESTIMATION OF NITROGEN CONTENT

AOAC, 21st Edn, 2019, George, W., & Latimer, J. (2019). Official method of analysis of AOAC by AOAC International, The National Institute of Food Technology, Entrepreneurship and Management, Thanjavur, Coimbatore, Tamil Nadu.

ANNEXURE XVIII

DETERMINATION OF PEROXIDE VALUE

(Association of Official Analytical Chemists (AOAC), 2019).

- Chemical preparation must Pour 90 milliliters of concentrated acetic acid into areagent flask after measuring it.
- Pour 60 ml of chloroform into the same reagent flask and stir to mix.
- To combine the chemicals, give the flask a shake.
- The acetic acid and chloroform combination at a ratio of 3:1 is now ready for use and for making 1% starch solution Pour 50 ml of distilled water into a 100 ml beaker, set the beaker on a hot skillet and bring the water to a boil.
- Consider pour the 0.5g of weighed starch solution into the water that is boiling.
- To dissolve the starch in the water, now stir the mixture using a clean glass rod while it is boiling.
- Boil and stir until a clear solution are achieved.
- Immediately use filter paper to filter the solution once the starch has been dissolved.
- The 1% starch solution is now ready for use after 30 minutes of filtering the solution and transferring it to an appropriate container.
- The manufacture of 0.01N Sodium Thiosulfate is required.
- Mix thoroughly by shaking after dissolving 0.25g of sodium thiosulfate in 80ml of distilled water and adding 0.02g of sodium carbonate.
- If needed, heat the mixture to thoroughly dissolve the sodium thiosulfate and then allow it cool to room temperature.

- To get the final volume of 100ml, add enough distilled water. Add potassium dichromate to the prepared solution to standardize it.
- The process of making saturated potassium iodide (KI) solution was Pour 2 milliliters of distilled water into the test tube, then gradually add KI until the bottom of the test tube is filled with undissolved KI.
- Saturated KI is prepared for usage.
- Preparing the samples was taking around 0.5-2 milliliters of the oil sample that was extracted from millet and records its weight in an Erlenmeyer flask.
- 30 milliliters of the acetic acid-chloroform combination should be measured, added to the flask and shaken to combine the mixture with the sample.
- After adding one milliliter of saturated potassium solution, shake for a minute. To ensure a thorough mixing. add 30 milliliters of distilled water to the container and mix for a minute.
- Titration: Place 0.01N sodium thiosulfate in a burette and record the burette's first reading.
- As an indication, add 0.5 milliliter of 1% starch solution.
- After mixing by shaking and rotating the flask, begin the titration.
- Titration until the entire black hue is gone.
- Once the solution turns white, stop titrating.
- The last burette reading should be noted.
- To get the peroxide value in meq/kg, utilize the equation below.

$$\text{Peroxide value (meq of O}_2\text{/ Kg fat)} = \frac{S \times N \times 1000}{W}$$

Where, S = ml of sodium thiosulphate (blank corrected)

N= normality of sodium thiosulphate solution

W= weight of sample taken

ANNEXURE XIX

DETERMINATION OF FREE FATTY ACIDS

AOAC, 21st Edn, 2019, George, W., & Latimer, J. (2019). Official method of analysis of AOAC by AOAC International, The National Institute of Food Technology, Entrepreneurship and Management, Thanjavur, Coimbatore, Tamil Nadu.

Five gram of sample pre-weighed in a test tube was mixed with 10 ml of extraction mixture (Isopropanol: Petroleum ether: 4N Sulphuric acid in 40:10:1 proportion). Subsequently, petroleum ether (6 ml) and water (4 ml) were added to test 2 tube and stoppered. Then the contents were shaken vigorously for 15 s. The two layers were allowed to separate for 10 min undisturbed and an aliquot (usually 10 ml) of the upper layer was withdrawn gently and transferred to small flask (50 ml). After addition of 6 drops of 1% methanolic phenolphthalein, the solution was titrated with the 0.02 N methanolic potassium hydroxide solution. Blank using water instead of sample was run simultaneously to obtain blank titration.

$$\text{FFA (as \% Oleic acid)} = \frac{N \times T}{P \times W}$$

Where, T= Titre volume in ml,

N= Normality of KOH,

W= Weight of sample taken in g,

P= Aliquot of the upper layer

ANNEXURE XX

DETERMINATION OF ACID VALUE

AOAC, 21st Edn, 2019, George, W., & Latimer, J. (2019). Official method of analysis of AOAC by AOAC International, The National Institute of Food Technology, Entrepreneurship and Management, Thanjavur, Coimbatore, Tamil Nadu.

ANNEXURE XXI

DETERMINATION OF WATER ACTIVITY

The water activity was measured after tempering samples at 25°C temperature. It was measured using Rotronic Hygroskop Model: Hygrolab-3 (M/s. Rotronicag, Switzerland) connected to a sensing element (aw-DIO) with a measuring range of 0-100 per cent relative humidity (RH).

The sample was filled in a dish/cup up to the brim to the maximum possible extent and the dish was kept in an air tight chamber taking care that the sample does not touch the diaphragm of the sensor. The measuring chamber was conditioned to the temperature of the sample. The chamber was then closed in a manner so as to ensure metallic contact between the two parts. The values of RH and temperature were noted from the display panel when it reached the equilibrium and a constant reading was observed (Pardo et al., 2004).

ANNEXURE XXII

TEXTURE PROFILE ANALYSIS

Texture of bars was determined according to Nadeem et al. (2012) with certain modification. The 3-Point Bending Rig (HDP/3PB) using 50 kg load cell on TA - HDi texture analyser (make: Stable Micro Systems, Surrey, UK) shown in Figure 3.15 was used to assess textural quality (hardness and fracturability) of the energy bar samples. The bars were bended to determine structural characteristics present inside or on the surface. Samples for bend test were placed centrally on heavy duty plate form under three point bend rig probe. Both the load cell and probe were calibrated before test. Hardness measurement of bars by bending involved plotting force (N) versus time (sec). The maximum force (N) was used as an index of hardness for the bend test.

The settings of texture analyser for the study were as follow:

Mode:	Measure force in compression
Option:	Return to start
Pre-Test Speed:	1.0 mm/s
Test Speed:	1.0 mm/s
Post-Test Speed:	10.0 mm/s
Distance:	44 mm
Trigger Type:	Auto
Data Acquisition Rate:	500 pps

Test Set Up

Two adjustable supports of the rig base plate were placed at suitable distance (44 mm) apart so as to support the sample. The distance was noted and kept constant throughout the study. The base plate was then secured onto the Heavy-Duty Platform. The Heavy-Duty Platform was manoeuvred and locked in a position that enabled the upper blade to be equidistant from the two lower supports. The sample was placed centrally over the supports just prior to testing. The test was run by using Texture Expert Exceed software version 2.64 after setting all the parameters as given above. After breakdown of the product due to the pressure developed by the probe, the results for the two properties (hardness and fracturability) were noted. Then the pieces of product were removed and test was run for the second sample.



Texture Analyser

ANNEXURE XXIII

DETERMINATION OF YEAST AND MOULD

All the samples were drawn aseptically and analysed for standard plate count, E. coli count and yeast and mould count during storage. Colonies were counted in viable cell count were express as log colony forming units per gram (\log_{10} cfu/g).

Preparation of Dilutions

Suitable dilutions were selected based on preliminary trials. Sodium citrate buffer solution used for serial dilutions was prepared as described in IS 5403:1999.

One ml of sample was aseptically transferred in to 9 ml sterile Sodium citrate buffer containing test tube and suitable number of dilutions were prepared. One millilitre of suitable dilution was transferred aseptically in sterile petri plates.

The yeast and mould count of the samples were enumerated by suitable dilutions as described above, using was potato dextrose agar as a medium (as specified by ISO 17792, 2006) incubating plates at 25°C for 5 days.

ANNEXURE XXIV

DETERMINATION OF TOTAL COLIFORMS

Energy bar samples were diluted to 1:10 using buffered peptone water as specified by the method IS 5401 (Part 1). A MPN series was prepared by adding a specified amount of sample and decimal dilutions of sample to tubes of MacConkey broth (selective enrichment medium) containing inverted Durham tubes. The tubes were then incubated at $37\pm 1^\circ$ for 24h. Tubes without gas production were incubated up to 48h.

Samples from tubes with gas production was selected for enumeration of positive E. coli colonies on the surface of Eosin methylene blue (EMB) agar. The typical colonies of E. coli are purple in colour with black centre and green metallic sheen after the incubation at $37\pm 1^\circ$ C for 24h.

ANNEXURE XXV**DETERMINATION OF TOTAL BACTERIAL COUNT**

The total plate count was determined as per BIS method (IS 5402: 2002). Suitable dilutions of each sample was transferred (1.0 ml) aseptically into sterile petri plates and thereafter 10 to 15 ml of molten standard plate count agar (SPCA) was added. The plates were incubated in an incubator maintained at $37\pm 0.5^{\circ}\text{C}$ for 48 h and the number of colony forming units (cfu) was noted. All the plates were prepared in duplicate.

ANNEXURE XXVI**ELECTRICITY REQUIREMENT OF 10KG P-E-BARS**

SR. NO	EQUIPMENT	ELECTRICITY	TOTAL WORKING TIME	UNITS (kwh/d)
1	Extruder Motor	8.246	10	1.024
2	OTG	1300	8.33	10.82
<i>Total units</i>				11.89
<i>Lightening etc. @ 15.0 per cent</i>				1.78
<i>Actual electricity requirement for 10 kg P-E Bars</i>				13.67

ANNEXURE XXVII

**SEMI STRUCTURED QUESTIONNAIRE-ACCEPTABILITY TRIAL
QUESTIONNAIRE- ATHELTES, COACHES AND TRAINERS**

1. Name:
2. Gender: Male Female
3. Date of Birth:
4. Age:
5. Phone Number:
6. Email address:

7. What kind of snack would you typically purchase?
 - Fruit
 - Chocolate
 - Chips
 - Other (please specify)

8. If there were healthy bars displayed beside the sweets/chocolate section, would you pick it up instead?
 - Yes
 - No
 - Maybe

9. Have you ever eaten a sports bar before?
 - Yes
 - No

10. What is your main reason for eating sports bars?
 - Snack
 - Meal replacement
 - Pre- or post-workout fuel
 - Convenience
 - Other (please specify)

11. How satisfied are you with the bars you currently consume/ available in the market?
In terms of: (1 = very dissatisfied, 5 = very satisfied)
 - Taste
 - Texture
 - Nutritional value
 - Price
 - Quantity
 - Not applicable/Never Consumed

12. How important are the following factors when/if choosing a sports bar? (On the scale of: 1 = not important to 5 = very important)
- Taste
 - Texture
 - Nutritional value
 - Price
 - Brand
13. What is the most you would be prepared to pay for the bar on a regular basis?
- 50-80 Rs.
 - 80-100 Rs.
 - 100-150 Rs.
 - 150-200 Rs.
 - >200 Rs.
14. Would you be willing to pay more for sports bars if they had additional nutritional benefits?
- Yes
 - No
 - Maybe
15. Do you think only 'Athletic/Sporty/Health Conscious' people would buy these bars?
- Yes
 - No
 - Other
16. Do you exercise regularly or go to the gym?
- Yes
 - No
17. How satisfied are you with the sports bar you just ate? (1 = very dissatisfied, 5 = very satisfied)
- Very dissatisfied
 - Dissatisfied
 - Neither satisfied nor dissatisfied
 - Satisfied
 - Very Satisfied
18. Would you like to purchase this sports bar in the future?
- Yes
 - No
 - Maybe
19. Would you recommend this sports bar to a friend?
- Yes
 - No
 - Maybe
20. Do you have any additional comments or suggestions for improving this sports bar?
- Your answer: _____

~End of the Questionnaire~

ANNEXURE XXVII**HEDONIC SCORE CARD FOR THE SENSORY EVALUATION-
CONSUMER PANEL****Research Topic: SPORTS NUTRITION: IMPACT OF NUTRITIONAL
INTERVENTION ON PERFORMANCE****Revised Title: DEVELOPMENT, OPTIMIZATION AND
CHARACTERIZATION OF PROTEIN- ENERGY BARS USING RESPONSE
SURFACE METHODOLOGY****NAME:****DATE:****PRODUCT NAME:**

- You are given a Nutribar (Protein-Energy Bars). Taste the bar and check (✓) how much you like or dislike each of the characteristic of the bar.

Degree of preference	Appearance	Taste	Flavour	Texture	Serving size
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like or dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Comment:**Signature:**

ANNEXURE XXIX

INFORMED CONSENT- CONSUMER PANEL- ACCEPTABILITY TRIALS.



The Maharaja Sayajirao University of Baroda
Faculty of Family and Community Sciences
Department of Foods and Nutrition

INFORMED CONSENT FORM

**Research Topic: SPORTS NUTRITION: IMPACT OF
NUTRITIONAL INTERVENTION ON PERFORMANCE**

**Revised Title: DEVELOPMENT, OPTIMIZATION AND
CHARACTERIZATION OF PROTEIN- ENERGY BARS USING
RESPONSE SURFACE METHODOLOGY**

**Name of Investigators: Guide: Prof. Komal Chauhan
Student: Ms. Kanchi Baria**

INFORMATION ABOUT THE PRODUCT “NURTIBARS”

I, **Kanchi Baria**, pursuing my PhD from the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, am conducting research on the sensory attributes and acceptability of Nutribars (Protein-Energy Bars) for my study in the area of Sports Nutrition with the title “Sports Nutrition: Impact of Intervention of Performance”.

Food bars are snacks with good sensory and nutritional qualities because they offer a portable and useful source of carbohydrates with varying amounts of protein and micronutrients for use around any physical activity under particular circumstances (athletes, school-age children, etc.) or in a busy lifestyle. The market's selection of snack items, including potato chips, extruded goods, chocolate bars and trial mixes, cannot provide the needs of a balanced diet. Moreover, many products are unhealthy for customers, particularly school-age children. The need for

nourishing snacks has increased as customers' understanding of health and nutrition has grown. This has led food manufacturers to create food bars that combine convenience and nutrition.

For training purpose, you are requested to taste the Nutribars (Protein-Energy Bars). The forms have to be filled each time you taste the Nutribars (Protein-Energy Bars) for its quality parameters.

The developed product contains cereals, millet, pulse protein isolate, dairy protein concentrate, nuts and dry fruits (Groundnuts, almonds, raisins and sesame seeds) dates, honey, malt and fat.

Thus, I would request you to: Taste the given products and give feedback for its quality parameters.

COSTS

The study requires your time and cooperation. The cost incurred will be borne by the investigator and there is no financial compensation for your participation in the study.

RIGHT TO WITHDRAW

The participant's cooperation and compliance would be highly appreciated; however, the participant has full right to withdraw from the study at any point of time. We hope you will take part for the entire study period, as we need all the information to draw correct conclusions.

INVESTIGATOR'S STATEMENT

I have described the research plan, the study's objectives and the potential advantages and disadvantages of participating. The participants had a chance to talk about the steps and ask any additional questions.

Signature of Investigator: _____

Date:

Prof. Komal Chauhan
Research Guide
Ph: +91 98987 90340

Ms. Kanchi Baria
Research Scholar
Ph: 0722 882 1182

ANNEXURE XXX

**SUBJECT CONSENT FORM- CONSUMER PANEL- ACCEPTABILITY
TRIALS**



The Maharaja Sayajirao University of Baroda
Faculty of Family and Community Sciences
Department of Foods and Nutrition

Research Topic: **SPORTS NUTRITION: IMPACT OF NUTRITIONAL
INTERVENTION ON PERFORMANCE**

**Revised Title: DEVELOPMENT, OPTIMIZATION AND
CHARACTERIZATION OF PROTEIN- ENERGY BARS USING
RESPONSE SURFACE METHODOLOGY**

Name of Investigators: **Guide: Prof. Komal Chauhan**
Student: Ms. Kanchi Baria

SUBJECT CONSENT FORM

Name: _____ **Age:** _____ **Sex:** _____

Phone number: _____

Email Id: _____

DECLARATION:

Allergy (if any): _____

Cold/ flu: _____

- I confirm that I have read and understood the above information and have/had the opportunity to ask questions.

- I understand that my participation in this study is voluntary and I am free to reject being a part of this study, without giving a reason.
- I understand that the ethics committee and the regulatory authorities will not need my permission to look at my health records and my identity will not be revealed in any information released to third parties or published.
- I agree not to restrict the use of any data or results that arise from this study provided such a use be only for scientific purpose(s).
- I understand the study will involve multiple times of collection of data, in terms of tasting the product and give marks for its quality.
- I understand that the medical expenses will not be borne by the research investigators from any allergic reactions arising due to consumption of the Nutribars (Protein-Energy Bars), unless proven.
- I agree to consume the Nutribars (Protein-Energy Bars) as offered.
- I voluntarily agree to participate in the community health study conducted by the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda.

Date:
Participant

Signature of the

ANNEXURE XXXI

The 3D graph in the figure 4.2.1 shows the effect of Crispies (A) and WPC-80 (B) on Appearance. The response dips at mid-levels of both variables but increases at higher levels due to positive quadratic effects. The interaction term AB negatively affects appearance, while BC shows a positive interaction. In the figure 4.2.2, 3D surfaces plot shows how Crispies (A) and WPC-80 (B) affect appearance. The response decreases at mid-levels of both variables but improves when either Crispies or WPC-80 is higher. The contour plot suggests a rapid appearance change in this range. The plot in the figure 4.2.3 depicts the interaction between Crispies (A) and SPI (C) on appearance. Appearance decreases at mid-levels of both factors but increases at higher levels. The contour plot shows that lower SPI levels lead to reduced appearance scores with moderate changes across the range.

The 3D graphs as shown in the figure 4.2.4, depicts that as the Crispies (A) and SPI (C) increase, the appearance score improves slightly but remains within a moderate range (7.2–8.0). Higher SPI levels combined with Crispies show a more stable improvement, indicating mild interaction between the two factors. The graph in the figure 4.2.5 shows the appearance score of P-E bars out of 10 on a composite score card, where WPC-80 and SPI significantly impact the score, with notable quadratic effects of both variables and their interactions, indicating an optimal range for enhancing appearance. The graph in the figure 4.2.6 however, with scores out of 9.0, highlights a similar trend but reflects a lesser impact of WPC-80 and a stronger interaction between WPC-80 and SPI, showing a balanced range for optimizing appearance on the hedonic scale. Both graphs emphasize the need for precise ingredient balance to achieve higher sensory scores.

The figures 4.2.7 and 4.2.8 the set of graphs represents the response surface for taste scores of P-E bars on a composite scorecard out of 15 and a 9-point hedonic scale, influenced by Crispies (A) and WPC-80 (B). The optimal taste is achieved when Crispies and WPC-80 are balanced, showing a significant quadratic effect for WPC-80. The second set shows the taste score on a 9-point hedonic scale, where both Crispies and WPC-80 have notable effects, though Crispies exhibit a stronger influence. In both cases, an optimal range of ingredients is crucial for improving taste.

The pair of graphs in figures 4.2.9 and 4.2.10, analyses taste in terms of Crispies (A) and SPI (C), where Crispies have a greater influence on taste optimization, with minimal interactions between Crispies and SPI. The same pattern is reflected on both the 15-point and 9-point scales, but the composite score highlights stronger interactions, whereas the 9-point hedonic scale shows a narrower range of taste variation based on ingredient levels. The graph in the fig. 4.2.11 shows the response surface for taste scores out of 15, where WPC-80 and SPI levels significantly affect the taste of P-E bars. A clear interaction between the two ingredients is observed, with an optimal range that enhances taste scores. The graph in the fig. 4.2.12 presents a similar trend using a 9-point hedonic scale, reflecting that moderate levels of both WPC-80 and SPI contribute to improved taste, though the response is more nuanced and gradual compared to the composite scorecard. Both emphasize the importance of balance for optimal taste.

The graph in the fig. 4.2.13 shows the flavor score out of 10 for P-E bars on a CSC, where both Crispies and WPC-80 levels have a notable impact, with WPC-80 exhibiting a quadratic effect, indicating an optimal range for maximizing flavor. The fig. 4.2.14, using a 9-point hedonic scale, shows a similar trend, emphasizing the influence of WPC-80 for flavor enhancement. The graphs in the figures 4.2.15 and 4.2.16 analyze the influence of Crispies and SPI on flavor. Crispies have a stronger impact on flavor optimization in both scoring systems, with quadratic effects contributing significantly to the flavor response. Optimal ingredient balance is critical in improving flavor scores across both scales. The fig. 4.2.17 shows the flavor score out of 10 on a CSC for P-E bars influenced by WPC-80 and SPI levels. The interaction between WPC-80 and SPI indicates a balanced range optimizing flavor, with notable quadratic effects of WPC-80. The fig. 4.2.18 presents the flavor score out of 9 on a hedonic scale, highlighting a similar trend, where a moderate combination of WPC-80 and SPI improves the flavor perception. Both graphs demonstrate that optimizing the levels of WPC-80 and SPI is key to achieving the best flavor results in the P-E bars.

The surface graph (fig. 4.2.19) shows an upward trend as Crispies and WPC-80 levels increase, improving the texture score. However, beyond a certain point, a downward trend appears, indicating that exceeding optimal levels negatively affects texture. The contour lines indicate a region of maximum texture score where the ingredients are balanced. The quadratic effect of WPC-80 is evident, suggesting that fine-tuning its

level is essential for achieving a higher texture score. The graph in the fig. 4.2.20 similarly shows an upward trend in texture scores with increased Crispies and WPC-80 but with a more gradual curve due to the 9-point hedonic scale. The downward trend suggests that too much of these ingredients causes texture to deteriorate. The contour lines again depict a concentrated optimal range for texture, emphasizing the need for precise adjustments of these ingredients to achieve the best sensory quality. Third Graph in the figure 4.2.21 depicts Crispies and SPI levels which show a clear upward trend that leads to improved texture. However, the downward curve signifies that excessive levels reduce the texture score. The contour lines illustrate a more stable texture region where the ingredients are balanced. The quadratic effect of SPI is critical in ensuring the optimal texture of the product. Again, the figure 4.2.22 shows a gradual upward trend as Crispies and SPI increase, leading to improved texture, but like the other graphs, the downward trend reflects overuse of these ingredients. The contour lines indicate an optimal range for these variables, showing how a balanced level of Crispies and SPI is key for maintaining ideal texture on the 9-point scale. The quadratic effect of SPI demonstrates the importance of precise optimization for better sensory acceptability.

The surface graph in the fig. 4.2.23 shows an upward trend in texture as both WPC-80 and SPI levels increase, reaching an optimal point before the texture score begins to decline. The quadratic effect of both ingredients is evident, particularly for WPC-80. The contour lines indicate a concentrated optimal range for texture, showing rapid changes in the score as ingredient levels shift slightly. The graph in the fig. 4.2.24 shows a similar upward trend in texture scores on the 9-point hedonic scale. The graph highlights the importance of balancing WPC-80 and SPI, as the texture improves with their increase, but excessive levels lead to a downward curve. The contour lines suggest a specific region where the optimal texture is achieved, with sharp transitions indicating that careful ingredient optimization is necessary for maintaining texture quality.

The serving size response surface graphs highlight the influence of Crispies, WPC-80 and SPI on P-E bars. In the fig. 4.2.25 (CSC out of 15), serving size scores decrease as WPC-80 increases, while Crispies have a stabilizing effect, with contour lines indicating an optimal balance. The graph in figure 4.2.26 (9-point scale) shows a similar downward trend for WPC-80, with tighter contour lines suggesting rapid serving size changes with ingredient variations. In the fig. 4.2.27 (CSC out of 15), increasing

Crispies and SPI results in improved serving size, with a more stable response indicated by the contours. The fig. 4.2.28 (9-point scale) shows a similar upward trend but emphasizes the need for balance, as excessive levels of either ingredient led to score reductions. The surface graph (fig. 4.2.29) shows a general upward trend in serving size scores as WPC-80 increases, while SPI has a lesser but noticeable impact. The contour lines depict a region where moderate levels of both ingredients achieve optimal serving size, indicating that balancing WPC-80 is key to improving scores. The graph in the figure 4.2.30 mirrors the trend seen in the first one, with serving size scores increasing as WPC-80 rises. SPI also contributes to this effect. The contour lines indicate that small variations in WPC-80 have a more significant impact on serving size, requiring precise optimization to achieve the best results.

The response surface graphs for mouthfeel scores of protein-energy bars demonstrate the effects of Crispies, WPC-80 and SPI on sensory perception. In the fig. 4.2.31, as Crispies and WPC-80 increase, an upward trend in mouthfeel scores is seen, with the contour lines indicating an optimal balance between both ingredients. The graph in the fig. 4.2.32 shows a more stable mouthfeel response as Crispies and SPI increase, with a moderate effect on mouthfeel improvement. The figure 4.2.33 highlights the interaction between WPC-80 and SPI, where mouthfeel improves with moderate levels of both ingredients, but declines if either is excessively high, as shown by the downward trend and tight contour lines. Balancing these ingredients is crucial for optimal mouthfeel.

The response surface graphs for the absence of defects score in protein-energy bars, influenced by Crispies, WPC-80 and SPI, provide insights into optimizing ingredient levels. In the fig. 4.2.34, increasing Crispies and WPC-80 improves the absence of defects score, with contour lines showing a stable region where these ingredients are balanced. The fig. 4.2.35 reveals a similar trend with Crispies and SPI, indicating that moderate increases in both ingredients enhance the absence of defects. Although the fig. 4.2.36, showing WPC-80 and SPI, highlights a critical interaction between these ingredients. Moderate levels of both optimize the absence of defects, with contour lines indicating a clear, stable region of optimization. Balancing these ingredients is key to minimizing defects in the product.

The response surface graphs for the overall score of the protein-energy bar, influenced by Crispies, WPC-80 and SPI, illustrate the interactions between these ingredients. In the fig. 4.2.37, as Crispies and WPC-80 levels rise, there is an initial upward trend in the overall score, followed by a downward trend if the levels exceed optimal limits. The contour lines highlight an optimal range for balance. The fig. 4.2.38 shows similar trends for Crispies and SPI, where moderate levels yield higher overall scores, with a downward trend at excessive levels. The fig. 4.2.39 shows that both WPC-80 and SPI contribute to the overall score, with contour lines indicating a stable region of optimization. Proper balancing of these ingredients leads to the highest overall scores, as indicated by the contour patterns.